Patricia Vit · Silvia R. M. Pedro
David Roubik *Editors*

Pot-Honey

A legacy of stingless bees

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This book is dedicated to our families,
friends, colleagues—past, present, future–
observers of stingless bee life,
and stingless bee keepers

Foreword

The stingless bees are one of the most diverse, attractive, fascinating, conspicuous, and useful of all the insect groups of the tropical world. This is a formidable and contentious claim but I believe it can be backed up. They are 50 times more species rich than the honey bees, the other tribe of highly eusocial bees. They are ubiquitous in the tropics and thrive in tropical cities. In rural areas, they nest in a diversity of sites and are found on the flowers of a broad diversity of crop plants. Their role in natural systems is barely studied but they almost certainly deserve that hallowed title of keystone species. They are popular with the general public and are greatly appreciated in zoos and gardens. The chapters of this book provide abundant further evidence of the ecological and economic importance of stingless bees.

Given their extreme interest, then it follows that this group must have been the subject of a huge body of scientific research. Unfortunately, this is not the case. Although the stingless bees contain 50 times as many species as the honey bees, the latter have been the subject of perhaps 50 times as much research effort, as estimated by published papers. We have squandered this precious natural heritage by our lack of attention, and in our failure we have limited our use of this resource. But this book starts to address that failure.

The chapters of this book summarize much of the current knowledge of stingless bees and also provide new findings. The diversity of species, behaviors, and the wide geographic range is explored in the Part I. The close relationships between humans and stingless bees through history is the topic of the chapters of Part II. The importance of stingless bees in agricultural and natural ecosystems derives from their flower visitation behavior and resulting pollination; this is the focus of the third part. The final two parts provide reviews and original research on the use and properties of the products of the hives of stingless bees, in particular the honey.

Stingless bees are an ancient source of sweetness and medicine for many indigenous people in the tropics, from the nomadic hunters and gatherers of northern Australia to the mighty Mayan empire of Central America. But modern commercial exploitation of this product has been hampered partially by a lack of information on its properties and composition. A strength of this book is the focus on “pot-honey,” honey derived from the pots of stingless bees, as opposed to the comb of honey bees. Perhaps now stingless bee honey will move from locally available and start to be seen in the global marketplace. Indigenous peoples may not have knowingly used stingless bees as pollinators of their crops, but certainly these industrious insects would have played an important role. Stingless bees also have an important role to play in education. These harmless and fascinating animals can be used in schools and universities, public gardens, and zoos, as case studies in ecological interactions. These bees may even have economical value as pets. Housing a colony of these bees in a city apartment provides an opportunity for urban dwellers to have some contact with nature.

This book is one of the few specifically devoted to stingless bees. Let us hope that it stimulates a generation of further research so that the enormous potential of this group can be realized.

Brisbane, Australia Tim A. Heard

Foreword

Yes, we can

We live in a time when bees seem to become scarce in relation to their former numbers engaged in pollination and honey production. Our time is also one of competition and upset between different kinds of bees. First, in the nineteenth century, *Apis mellifera* invaded the Americas and Australia. That was large-scale invasion. And in the twentieth century and afterwards, we saw the invasion, in a larger scale, of the African *A. mellifera scutellata* in the tropical and subtropical Americas, and there was also a strong decline in the numbers of the meliponine bees.

We, the friendly breeders of stingless bees, must in some way make them recover at least some parts of the areas already nearly lost. For doing so, we must improve and increase our breeding of stingless bees such as *Scaptotrigona* and *Melipona*, good for pollination. In other words we must as soon as possible improve MELIPONICULTURE and also increase the number of colonies engaged in different projects. We are not against any bee properly bred and cared for. However, we must also protect meliponiculture.

For doing so, we must improve our breeding experience in MELIPONICULTURE. This is quite possible, since in Nature, in Africa, in some places *A. mellifera* and the native meliponines are present after millions of years of coexistence. However, now in parts of tropical America, *A. mellifera scutellata* seems to be still gaining ground, becoming generally the dominant bees. In such a situation it is important to publish papers about the best ways of helping the Meliponini to survive and also to let people know more about their life history and their potential in pollination and in other fields.

I am glad to send my congratulations to the authors of the articles here published and for those who organized this initiative.

Some efforts like this one are needed from time to time, for promoting the survival of stingless bees. I would say: yes, we can save them. We really can.

São Paulo, Brazil — Paulo Nogueira-Neto

Introduction

Just as variety is the spice of life, it is also the source of honey. It doesn't matter which kind of honey. There is surely variety, and that explains many of honey's attributes. An average honey taken from a bee colony living within tropical forest contains 50 plant products. Most are nectar or pollen, and some are from the storage containers or food pots, from which this volume takes its name. A few compounds, such as hydrogen peroxide, honey's valuable antibiotic, form within the honey itself, while others derive from plants or the bees themselves. Now, what is there to explain about pot-honey?

Here is a scholarly and lively collection of facts and important insights from people across the world to answer that question. It is explained, as it should be, by a journey across cultures, continents, scientific exploration, and time—a representative sample of knowledge, studies, and applications, some ancient and others nascent. For instance, as we develop analytical techniques both for sequencing honey-making bee genes and reliably defining and characterizing honey, we are exploring ways to market honey and protect the environment it comes from. This is only the beginning. Our human repertoire of honey uses and cultivation techniques can be matched with cultures from Australia to Argentina, from Mexico to Ivory Coast, and from India and Indonesia. This enterprise proffers revelations that few other culinary/linguistic/tribal/cultural/scientific studies can offer.

To begin with, honey from insects is a novel feat. As humans, we have a fondness for this food (and drink—as explained herein) that is deep. At the peak of social evolution in insects there is honey. It seems curious that certain bees, wasps, and ants, truly social with long-lived colonies of a queen and workers, are the sole manufacturers of honey on the planet. Yet we take them for granted. There is not long to study some of these unique and natural honeys, before their makers waver on the edge of extinction, and then are no more. Why? Because they are denizens of the tropics and the world's remaining wildlands.

Most honey comes from bees, but not the bumble bees or the honey bees. The tropical and stingless honey-making bees, the Meliponini, are the original and still the predominant makers of honey. Those stingless bees are not a close relative of *Apis*, the stinging honey-bee of wide renown. Biology of the two kinds of honey-making bees diverged some 100 million years ago, now revealed in biogeographic and molecular information that provides conclusive evidence.

The stingless bees invented honey. Not so many years ago, books on bee keeping would lay down the theme that there are only four honey bees on earth, then describe methods for bee keeping, and mead making, candlemaking and honey extraction, mostly in the temperate zone and since the Middle Ages. That pattern of presentation is now obsolete. We now contemplate there being a dozen living honey bee species. With the stingless bees, formerly "known" to contain about 200 species, we are surpassing 500 well-codified individual ways of being stingless bees—some actually larger than any honey bee—and many having powerful defense methods. With more exploration of tropical forests and other remote areas, such as the vast Australian "Outback," the number will soon eclipse that figure.

Stingless bee honey is unique not only for its origin in the rich vegetation of native environments but also for its unusual degree of sweetness, sourness, acidity, and a host of other qualities that we have studied. One of them is "medicinal value." Another feature is the resin or "propolis" that is a part of the entire nesting home of a stingless bee colony. It is definitely an important ingredient in biology and food. Some stingless bees protect and, in turn, are fed and nurtured by bugs. The bugs feed on plant phloem and provide sugars and sustenance to a few species of meliponine bees. Another factor is the microbes. The rainy tropical forests in which stingless bees thrive, as well as some of the dry and hostile regions they can exist in, challenge the procurement and storage of concentrated sugar in a nest. If the predators do not locate this rich resource, the microbes and micro-predators most certainly will. Yet stingless bees survive. We find they are protected in multiple ways, by behavior and nesting habits, and their health in the environment has a long history of compatibility, if not co-option, with other organisms and many plant materials.

How many kinds of honey exist in the world? Take the number of stingless bee species, multiply this by the number of seasons in the tropical or subtropical year (wet and dry, for the most basic), and then multiply this by a number including combinations of 20–50 pollen types. Of course, in an environment that has fewer flowering plant species, or where invasive honey bees are taking many of the flowers that the two bee groups compete for, that number is reduced. Indeed, a traditional scientific application of pollen study to the honey of bees has been in the identification of a single, predominant resource in a honey sample. Such "unifloral" honey is an economic standard, verified clearly by pollen identified in the honey, which permits commercialization and unquestionable legitimacy. Other kinds of honey are difficult to categorize in such a straightforward way. They are the flavor of the tropics. They come in too many varieties for superficial scrutiny, other than to state that they are diverse. A connoisseur would notice the difference. "Native honeys," as we find them, are a remarkable kaleidoscope of bouquet, aroma, flavors, aftertaste, and even texture. Such sensorial adventure begins with both botanical and entomological origin, often with an added benefit from their matrix of human cultural experience, in which they are embedded.

From a human point of view, stingless bees in Asia (Indonesia and Malaysia) are “the bees that remove sticky substances form their legs,” the “galo galo”, or the “flute bees” with the long, tubular nest entrance, or the “beer bees,” whose fermenting honey encourages the production of alcohol, in a container of bee nests and water. Much the same is true for Africa, and the Australian stingless bees have a multitude of uses and metaphors attached to them. In the American tropics, they are frequently the garden bees—those kept close at hand for a case of sore throat, or a home remedy conferring stamina or at very least, well-being. A remarkable dose of needed sweetness, with which to surrender all pessimism and doubt.

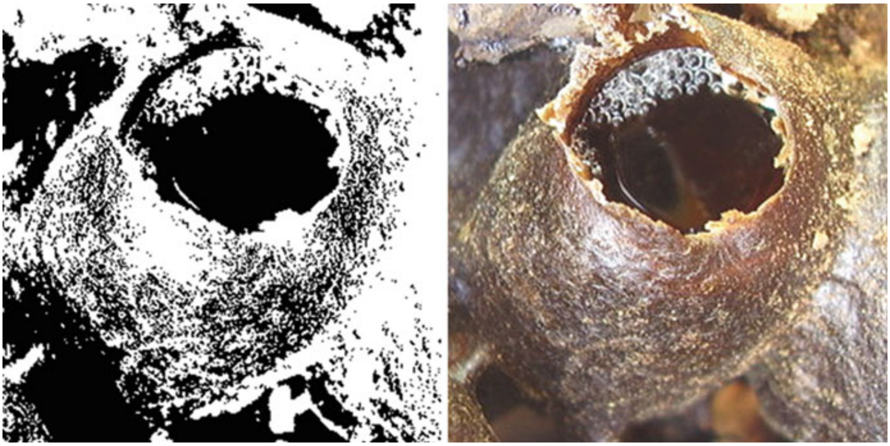
On the other hand, an astringent tang in the back of the throat and a near convulsion of shock with sweetness combined with something nearly its opposite is familiar to those of us who have consumed buckwheat honey. It is a monofloral honey that honey bees produce in Asia, where *Apis cerana* and *Fagopyrum* (Polygonaceae) are native. It is heavily laced with phenolic compounds. This general quality is perhaps the rule, rather than the exception, among the stingless bee honeys in our increasingly homogenized and monofloral world. However, the herbicide-treated and cleared plantations and orchards have given stingless bees, and other bees, a pasture that is more or less uniform, and it has flowers for only a part of the year. Its honey may be harvested, and appreciated, as something fairly novel. But it is far from natural.

Still basically unknown, despite multicultural and multigeographic recognition, are the honey and other so-called “hive products” of most stingless bees. Like the perfumed essences emitted by orchids and many flowers, they may soon vanish forever. They are, first and foremost, the most biodiverse products that nature has to offer. What are they worth, both scientifically and culturally? Further, how much have we, and the myriad other species that interact with them lost, if they are neglected, abused, and consigned to extinction? These are essential and pressing questions that we hope the reader will pursue with us.

Honey is a rare element of science and nature. What components or synergisms explain each mechanism of action? Is the greater water content of stingless bee honey a defect in quality, as would be recognized in *A. mellifera* honey, or an important medicinal factor? Sugar and water hold the invisible (and visible, with pollen grains) structure of honey—to arrange metals, secondary metabolites, microbes, chemical residues and final products, after processing by the bees in their nests. Genuine and false honey are simple comparisons, seen immediately by what is present and what is lacking. Honey is used as food, and as our cosmetics and medicines. The little bubbles in pot-honey suggest that ethanol is in the stingless bee storage pots, but in very low concentration. Modern technology has a wide range of applications to discern whether chemical compounds such as unique flavonoids, organic acids, or oxidative reactions in honey influence the immune system or interfere with cancer onset and progress. The Meliponini introduce the reader to a fascinating world of the woodland bees and their cerumen pots, in which honey and pollen are kept. Our well-known 94-year-old mentor—admiring the first stingless bee he saw alive *Trigona (Tetragonisca) angustula* Latreille—said that this bee was special “because it is small, gentle, pretty, in Panama often nests in cavities in buildings in towns, makes excellent honey and does not visit filth.” Dr. Michener was correct. Biodiversity and similar admiration for the local species of meliponines are found in the following chapters describing stingless bees from Australia, Venezuela, French Guiana, Guatemala, Costa Rica, Argentina, and Mexico. Two chapters examine the possible roles of microorganisms living with stingless bees, and consider whether fermentation is a mutualistic interaction between yeasts and bees. Strategies in communication by stingless bees to locate, collect and process food in competitive niches are developed in two chapters. Historical views communicate the high valuation of stingless bees and their pot-honey, medicinal uses by Mayans, entomological descriptions in the oldest Brazilian report, and melittology and *Melipona* bee scientific heritage, which has a legacy of at least 4000 years. Afrotropical stingless bees are treated from a taxonomic perspective used by traditional healers, naturalists and systematists. Conservation of stingless bees is presented as a challenge in Africa and Mexico, where human disturbance and habitat fragmentation propel Meliponini and many organisms toward depletion or extinction. Pollen spectra and plant use by stingless bees for food and nesting are surveyed, with new details and analytical techniques. The sensory descriptions of pot-honey are accompanied with chapters on physicochemical analysis of pot-honey from bees in Australia, Bolivia, Brazil, Colombia, Guatemala, Mexico, and Venezuela—including microbial, nutritional, and metal composition—an electronic nose, non-aromatic organic acid profiles, and Nuclear Magnetic Resonance. The flavonoid studies show that meliponine pot-honey from Venezuela, Australia, Brazil, and Bolivia is richer in flavonoid glycosides than *A. mellifera* honey. Bioactivity of pot-honey considers antioxidant value, cancer prevention and therapy, and antibacterial properties of Latin American and Thai pot-honey, and a review on immunological properties of bee products. Propolis collected by stingless bees from Bolivia, Philippines, Thailand, and Venezuela also is characterized. A closing chapter on major initiatives of production, and marketing in some parts of Brazil, moves our attention toward sustainable economics and principles that would benefit with increased commercial availability and consumption of pot-honey.

Human emotion and reaction to pot-honey indicate the evolution of natural contact between bees and our species. Sensory attributes of color, taste, texture, odor, and aroma are explored in detail. Pot-honey, as a healthy product, may someday follow millennia-old Traditional Chinese Medicine in the patterns of human response, ecology and cultural use.

The inimitable Professor Camargo left a generous contribution placed here as a seminal chapter of this book. His authentic respect for the local names and cultural uses of the bees were instrumental in producing that which authors heard as a call to offer their insights and research findings.



Future generations may have more ideas than time to further develop the science of pot-honey and decipher the messages carried, in monastic silence, by the bee chefs within their cerumen alchemist cauldrons.

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To the stingless bees and the stingless bee-keepers of the world,
and for the pot-honey and meliponiculture that have evolved.

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Part I
Origin, Biodiversity and Behavior of the Stingless Bees (Meliponini)

Chapter 1
The Meliponini

Charles D. Michener

1.1 Introduction

The stingless bees are a primarily tropical group of over 500 species (and possibly 100 more as yet undescribed). The pot-honey that they produce is the main subject of this book. Given that bees are so well known for their stings, stinglessness among bees seems rather sensational. The term “stingless bee” requires some examination, however. First, all male bees are completely stingless; the sting is a modified ovipositor, a structure found only in females. Second, the parts of the sting of stingless bees are actually present, much reduced and modified and not functional for stinging. Third, there are various other groups of bees whose females have reduced and nonfunctional stings. For example, females of the common bee genus *Andrena* have stings that are too small to be used as stings, and the very different bee genus *Dioxys* and its relatives have the most reduced stings of all bees, smaller than those of the “stingless bees.” Nonetheless, the term stingless bees is well established for the tribe Meliponini and we will use it for this group of primarily tropical bees.

The stingless bees, like the well-known honey bees (tribe Apini, genus *Apis*) and unlike the thousands of species of other bees, live in more or less permanent colonies made up of workers (modified females) and usually only one female reproductive, the queen, for each colony. Thus females appear in two castes, workers and queens. Of the many kinds of bees, the stingless bees are the only ones that have long-term (sometimes called permanent) colonies, morphologically different worker and queen castes, and also reduced stings (so cannot sting).

To clarify the position of bees within the order Hymenoptera: there is a large group within that order in which the ovipositor no longer functions to place eggs, and is typically modified into a sting. Members of this group are called the aculeate

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Hymenoptera or the Aculeata, which includes the bees, ants, and wasps. One major group of Aculeata consists of those with the pronotum short, not reaching the tegulae but forming a rounded lobe below each tegula. These were long called the superfamily Sphecoidea, the sphecoid wasps and the bees. More recently and correctly they are called the Apoidea, the apoid wasps and the bees. The bees, technically the Apiformes or Anthophila, are an apparently monophyletic group of the Apoidea. They differ from the apoid wasps in that they no longer sting prey to feed their larvae but depend instead on other foods, nearly always pollen, as their major protein source, and they have at least some branched or plumose hairs and commonly other structures that may facilitate pollen collecting as well as nectar gathering (Michener 2007; Engel 2011).

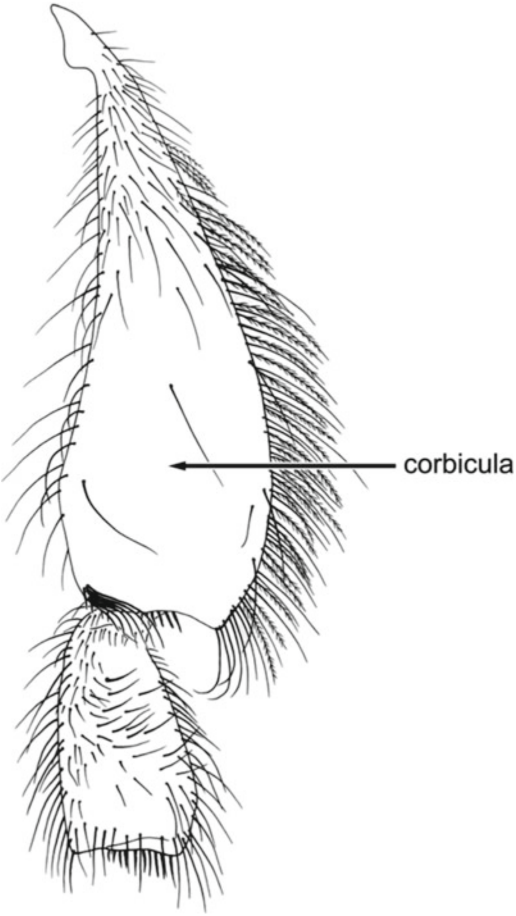
The bees are divided into several families (seven according to Michener 2007), one of which is the Apidae, which includes the large subfamily Apinae, within which is the tribe Meliponini. Recognition of the Meliponini is usually easy, although a few other groups of bees resemble that tribe superficially. A bee collector in tropical America, who may be taking the common stingless bees from the collecting net with fingers, will occasionally be surprised by a sting from a similar looking bee of the tribe Tapinotaspidini, usually of the genus *Paratetrapedia*. The Meliponini belongs to a monophyletic group of four tribes (Apini, Meliponini, Bombini, and Euglossini) known as the corbiculate bees because their females have a corbicula (Fig. 1.1) on each hind tibia (except that queens of the first two tribes listed lack corbiculae, as do workers of a few species that live by taking carrion or by robbing nests of other stingless bees). The corbicula is a large smooth area, often concave, margined by fringes of long hairs. It is used to carry pollen or sometimes other substances into the nest. The Meliponini can be differentiated from all other bees by the lack or weakness (relative to other veins) of the submarginal crossveins and the second recurrent vein in the forewing (Fig. 1.2). As in the Apini, the hind tibial spurs are absent (Fig. 1.1).

The beginner, seeking to recognize stingless bees, should know that while some Meliponini of the genus *Melipona* are as large as or even larger than the common honey bee (*Apis mellifera* Linnaeus), the great majority are much smaller. Perhaps the smallest is a Madagascar species of *Liotrigona* whose workers are as small as 1.8 mm in length. Many particulars about Meliponini can be learned from Nogueira-Neto (1953, 1970, 1997), Roubik (1989, 2006), and Wille (1983).

1.2 Classification

Some earlier authors (e.g., Lepeletier de Saint-Fargeau 1836; Dalla Torre 1896) placed all Meliponini in a single genus, *Melipona*. Others (e.g., Smith 1854; Michener 1944; Schwarz 1948) recognized two major genera, *Melipona* for the species now placed in that genus and *Trigona* for all the rest of the Meliponini except a few robber species commonly placed in a separate genus. *Trigona* in this broad sense is very diverse, not monophyletic, containing species with different relationships to *Melipona*, and it becomes evident that it should be broken up into smaller and more homogeneous units.

Fig. 1.1 Outer side of hind tibia and basitarsus of worker of *Trigona (Trigona) amalthea* (Olivier) showing the corbicula and the lack of tibial spurs, as well as the lack of the auricle (and pollen press) found in *Apis* (prepared by Sara Taliaferro, based on Michener 2007)



Several groups were named as subgenera of *Trigona* but in 1946 and thereafter Moure elevated subgeneric groups to the status of genera and described various new genera. The genus-group names, i.e., generic and subgeneric names, are listed below.

The status of many names is unsettled; Moure's followers consider nearly all the named supraspecific taxa as genera while others (Michener 1990, 2007; Sakagami 1975) place many, rather subjectively, as subgenera of a moderate number of genera. The authors of different chapters of this book show different opinions on some such matters. For example, *Austroplebeia australis* is the same species that in another chapter is called *Trigona australis*.

While the Meliponini are found in all parts of the tropical zone except many Pacific islands, no genus occurs throughout that zone. For our purposes, there are three tropical regions in the world: the American tropics (= Neotropics), sub-Saharan African (= Afrotropical region), and the Indoaustralian (= Austroasian) region. For convenience the meliponine taxa are listed below, for each of these three regions. The number of

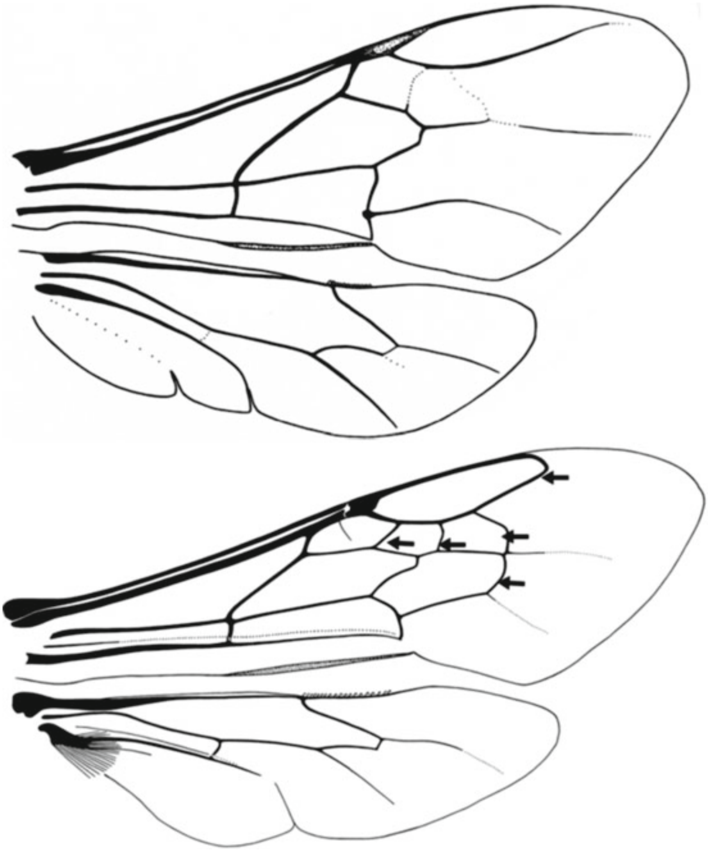


Fig. 1.2 Wings of *Melipona fasciata* Latreille (*above*) and *Euglossa cordata* (Linnaeus) (*below*). The latter shows the wing venation of most bees, with *arrows* marking the vein segments that are weak or absent in the Meliponini (prepared by Sara Taliaferro, based on Michener 2007)

species shown in parentheses after each taxon must be viewed with some caution because distinct new species must exist, and especially because in the Meliponini there appear to be numerous cryptic species not yet recognized. The number of species listed is derived, with some adjustments, from Camargo and Pedro (2007) for the Americas, from Eardley (2004) for Africa plus Pauly et al. (2001) for Madagascar, and from Rasmussen (2008) for the Indoaustralian region. Synonymous names shown in the lists below after equal (=) symbols are of two types. Some are absolute synonyms. Others are synonymized by judgment. An example of the latter is *Celetrigona* which can be used for a distinct group which is here included in *Trigonisca*.

Regardless of possible deficiencies in the lists, they clearly show the great diversity of stingless bees in the American tropics (over 400 species) where, in many localities, they are the most abundant bees, hence presumably the most important pollinators. They also show the much smaller and less diverse meliponine fauna in Africa, with that of the Indoaustralian region intermediate.

Neotropical Meliponini are found northward to Cuba and the states of Tamaulipas and Sonora in Mexico, and southward to Buenos Aires Province, Argentina. The species are listed by Camargo and Pedro (2007) and identification of species is facilitated by keys and descriptions in numerous revisional papers such as Schwarz (1948) and many excellent revisions by Camargo and his associates, such as Camargo and Pedro (2009), as well as by regional studies such as Schwarz (1938) for Guyana and Ayala (1999) for Mexico. The genus-group taxa are listed below; subgenera are indented, and as indicated above the number of species is shown in parentheses.

Cephalotrigona Schwarz 1940 (5)
Lestrimelitta Friese 1903 (20)
Melipona Illiger 1806 (= *Micheneria* Kerr, Pisiani and Aily 1967, *Michmelia* Moure 1975, *Melikerria* Moure 1992, and *Eomelipona* Moure 1992) (72)
Meliwillea Roubik, Lobo and Camargo 1997 (1)
Nannotrigona Cockerell 1922 (10)
Nogueirapis Moure 1953 (3)
Oxytrigona Cockerell 1917 (11)
Paratrigona Schwarz 1938 (= *Aparatrigona* Moure 1951) (34)
Paratrigonoides Camargo and Roubik 2005 (1)
Partamona Schwarz 1939
  Parapartamona Schwarz 1948 (7)
  Partamona Schwarz 1939 s.str. (= *Patera* Schwarz 1938) (32)
Plebeia Schwarz 1938
  Plebeia Schwarz 1938 s.str. (= *Mourella* Schwarz 1946 and *Friesella* Moure 1946) (42)
  Scaura Schwarz 1938 (= *Schwarzula* Moure 1946) (7)
  Schwarziana Moure 1943 (2)
Scaptotrigona Moure 1942 (= *Sakagamilla* Moure 1989) (22)
Trichotrigona Camargo and Moure 1983 (= *?Frieseomelitta*) (1)
Trigona Jurine 1807
  Duckeola Moure 1944 (2)
  Frieseomelitta Ihering 1912 (16)
  Geotrigona Moure 1943 (21)
  Tetragona Lepeletier and Serville 1828 (= *Ptilotrigona* Moure 1951 and *Camargoia* Moure 1989) (19)
  Tetragonisca Moure 1946 (4)
  Trigona Jurine 1807 s.str. (= *Amalthea* Rafinesque 1815, *Aphaneura* Gray 1832, and *Alphaneura* Gray 1832) (32)
Trigonisca Moure 1950 (= *Celetrigona* Moure 1950, *Dolichotrigona* Moure 1950, and *Leurotrigona* Moure 1950) (43)

Frieseomelitta, *Duckeola*, and *Tetragonisca*, along with the genus *Trichotrigona*, may constitute a genus *Frieseomelitta*, separate from *Trigona*; their separation from *Trigona* is indicated by the phylogenetic study of Rasmussen and Cameron (2010). The same study shows *Lestrimelitta* among the species of *Plebeia*, making the latter paraphyletic. These matters should be investigated further.

Sub-Saharan or Afrotropical Meliponini are found from Senegal, Niger, and Eritrea on the north to KwaZulu-Natal Province, South Africa, and the whole of Madagascar on the south. The species are listed and revised by Eardley (2004). The genus-group taxa are listed below; subgenera are indented.

Cleptotrigona Moure 1961 (1)
Dactylurina Cockerell 1934 (2)
Hypotrigona Cockerell 1934 (4)
Liotrigona Moure 1961 (9)
Meliponula Cockerell 1934
  Axestotrigona Moure 1961 (2)
  Meliplebeia Moure 1961 (= *Pebeiella* Moure 1961 and *Apotrigona* Moure 1961) (7)
  Meliponula Cockerell 1934 s.str. (1)
Plebeina Moure 1961 (1)

Indoaustralian or Australasian Meliponini are found from India to Taiwan and the Caroline Islands (perhaps introduced) and from southeastern China to New South Wales, Australia. The species are listed by Rasmussen (2008). Identification to the genus and subgenus levels should be facilitated by the keys of Moure (1961) and Michener (2000, 2007). Identification to the species level is made possible by revisional works such as, for the Asian region, Schwarz (1937, 1939) and Sakagami (1975, 1978), and for Australia, Dollin et al. (1997). The genus-group taxa are listed below (with some advice from the late S.F. Sakagami).

Austroplebeia Moure 1961 (9)
Heterotrigona Schwarz 1939
  Geniotrigona Moure 1961 (3)
  Heterotrigona Schwarz 1939 s.str. (3)
  Sundatrigona Inoue and Sakagami 1995 (= *Trigonella* Sakagami and Moure 1975) (2)
Homotrigona Moure 1961 (4)
Lepidotrigona Schwarz 1939 (12)
Lisotrigona Moure 1961 (4)
Lophotrigona Moure 1961 (1)
Odontotrigona Moure 1961
  Odontotrigona Moure 1961 s.str.(1)
  Tetrigona Moure 1961 (5)
Papuatrigona Michener and Sakagami 1990 (1)
Pariotrigona Moure 1961 (1)
Platytrigona Moure 1961 (6)
Tetragonula Moure 1961
  Tetragonilla Moure 1961 (4)
  Tetragonula Moure 1961 s.str.(32)

1.3 Biology

All stingless bees live in colonies, as already indicated, consisting of dozens to tens or hundreds of thousands of workers, and usually only one queen. At any one time a few to many males may or may not be present in such a colony. Contrary to honey bees (*Apis*), males are usually similar to workers in size and appearance and queens, quite different.

Major works exist on the biology of stingless bees, including such matters as nest construction and resultant structures, defense, foraging, reproduction, caste, and sex determination, as well as culture (meliponiculture) by humans, uses of their honey and cerumen (a combination of plant resin with bee wax) importance as pollinators, etc. Schwarz (1948) undertook the great task of presenting and summarizing everything then known about meliponine biology. Other good book-length accounts of meliponine biology and importance to humans are by Nogueira-Neto (1953, 1970, 1997); the last in particular contains a very extensive list of publications on the biology of stingless bees. A review article covering the same fields is by Wille (1983).

1.3.1 Reproduction

There is no solitary phase in meliponine life history; colony life is continuous. When a colony is dividing, workers from the parent colony fly to a new site and prepare it as a nest, carrying construction materials and food there in repeated trips. A nest entrance of the form characteristic of the species is often or always constructed first. Eventually a new, often unmated, young queen flies to the new nest from the parent colony. The queen soon mates, sometimes within the new nest. For some time (weeks or even months) workers continue to fly back and forth carrying materials from the parent nest to the new one, until eventually such contact ceases and the new colony becomes independent. Wille and Orozco (1975) described the events in the founding of a new colony of *Partamona orizabaensis* (Strand) (originally identified as *Trigona cupira* Smith) in which interchange continued for 6 months. During this process as well as at other times many males, often from other colonies, assemble nearby or hover near the nest entrances, presumably attracted by pheromones produced by young queens.

1.3.2 Foraging

At a nest entrance workers can constantly be seen carrying pollen, nectar, or construction materials into the nest. The foods go into pots, usually made of rather soft cerumen. Pollen and honey (made from the nectar) are placed in separate pots, not mixed. Of course it is this honey, in pots, that is the main subject of this book. Communication for the collection of food by various species is summarized by Aguilar-Monge (2004) and in this book, in Chap. 12.

The above is written as though all stingless bees, like most other bees, collect their foods (nectar and pollen) from flowers and carry the foods to the nest where the larvae are fed. A few stingless bees deviate from this pattern. Some are known to visit scale insects (Coccidae) and collect their wax and honeydew. Nests of *Plebeia (Scaura) timida* Silvestri are in cavities of living plants and contain scale insects that provide a domestic source of honeydew (Camargo and Pedro 2002;

Camargo 2008); this bee collects only pollen, not nectar, from flowers. Species of *Plebeia* subgenus *Scaura* have enlarged hind basitarsi with which they collect pollen from leaves or other flat surfaces onto which they have drifted from flowers above (Camargo and Pedro 2002). Some and perhaps most meliponines will occasionally rob from damaged nests of the same or other species, carrying away honey, pollen, provisions from brood cells, and construction materials. Species of the genera *Lestrimelitta* in the Neotropics and *Cleptotrigona* in Africa carry such behavior to the extreme; they do not visit flowers but live by mass robbing of nests of other species of stingless bees, from which they carry food and nest-making materials to their own nests (Sakagami et al. 1993; Portugal-Araújo 1958). *Trichotrigona*, known from only one locality, may also live by robbing, apparently by individuals solitarily entering host nests (Camargo 2008). *Trichotrigona* nests contain no food storage pots, the host apparently providing for that need.

Carrion is sometimes visited by stingless bees for the liquid or bits of solid material. Three species, however, the group of *Trigona (Trigona) hypogea* Silvestri, do not collect from flowers, have reduced corbiculae, and their protein source is carrion rather than pollen (Roubik 1982). Of course "honey" from such bees (or from those that use feces for construction materials) is not appropriate for human consumption.

Many stingless bees, especially small species, are attracted to perspiration of humans and other animals. People in most tropical areas are well aware of these pestiferous insects. More should be learned about the very minute bees (1.8–3.3 mm in length), particularly of the genera *Trigonisca*, *Hypotrigona*, *Liotrigona*, *Lisotrigona*, and *Pariotrigona*. Some of these bees can be frequent pests on perspiring humans but, although they carry pollen, they are not very commonly seen on flowers. In Southeast Asia bees of the last two genera listed above are not commonly attracted to perspiration but are attracted to eyes and collect tears of mammals (including humans), birds, and reptiles (Bänziger et al. 2009). Tears are high in protein and appear to be a significant source of food for these bees. Behavior of the minute bees of other continents should be investigated further.

1.3.3 Nests

Data on the nest structure of many species is provided by Wille and Michener (1973). An account of nest structures, their evolution and variability, as well as their functions in defense, temperature control, and the like is given by Roubik (2006). For nest construction, stingless bees secrete wax from the dorsal surface of the abdomen, and collect gum and resin or propolis from vegetation. Rich sources include secretions around cut or broken branches and gum secreted as a result of biting off bark and young shoots by the bees themselves. Such damage to citrus trees by *Trigona* (*Trigona*) is well known. Mixtures of these materials for nest construction are called cerumen. Certain species, and for certain parts of the nest, such cerumen is supplemented with mud, feces of vertebrates, probably bits of carrion, etc.

Various combinations of these materials appear to be used to produce the hard and tough, hard and brittle, to soft and pliable cerumens used in construction of the various sheets, pillars, pots, cells, etc. of the nest.

Nest sites vary widely. Many species use hollows, usually in tree trunks or large branches. Such hollows, usually caused by rot, are favored if they have small entrances that can be narrowed and if any extra openings can be closed by the bees' construction activities. Some species appear to prefer cavities of other kinds, for example in limestone cliffs or in constructs by humans (Bänziger et al. 2009, 2011). Thus some species, especially small forms, are common in villages or towns where their nests are frequent in cavities between walls of buildings or in other sorts of man-made cavities. Examples are *Trigona (Tetragonisca) angustula* (Latreille) and *Tetragonula fuscobalteata* (Cameron). Such species may not have a preference for the types of cavities found in buildings; they may merely tolerate a wider variety of locations and cavity sizes and shapes than do most species. For the Meliponini as a whole, the cavities used vary from huge in the trunk of a forest tree for a large species with large colonies to the abandoned burrow of a cerambycid beetle for a small colony of a minute species of *Trigonisca*.

Other species nest in the ground, perhaps in cavities resulting from rotting of large roots or from activities of rodents, ants, or other animals. Probably the bees enlarge and modify such cavities, but there is no evidence that the bees ever start at the surface and dig a nest cavity in the ground.

Some species, however, do make their own nest cavities within exposed nests of ants or termites. Workers from a parent bee colony construct a typical nest entrance projecting from a termite or ant nest, and then dig to construct a cavity and nest, keeping it constantly lined to exclude the hosts from the growing bee nest inside the host's nest. Such behavior seems to have originated independently in diverse groups of Meliponini. Arboreal termites (*Nasutitermes*) are the hosts for *Plebeia (Scaura) latitarsis* (Friese) in the Neotropical region (Wille and Michener 1973); arboreal leaf nests of ants (*Camponotus*) are hosts for *Paratrigona peltata* (Spinola) in Costa Rica while ants (*Crematogaster*) are the hosts for *Heterotrigona (Sundatrigona) moorei* (Schwarz) in Thailand and Sumatra (Sakagami et al. 1989).

Some Meliponini do not nest in preformed cavities or in nests of other social insects, but they make their own "cavities" by constructing exposed walls surrounding a space in which they live. For example, some species of *Partamona* make nests against walls, cliffs, or tree trunks. Such a nest looks as though someone had thrown a large glob of mud against a vertical surface, but of course the bees constructed the nest by carrying mud, wax, cerumen, etc. Other species construct nests, sometimes very large, by building on or around small tree branches so that the nest is exposed on all sides. An excellent example is *Trigona (Trigona) corvina* Cockerell, whose thick, hard nest walls consist largely of bees' feces full of pollen exines (Roubik and Moreno Patiño 2009).

While the nests of stingless bees are rather diverse in structure, they all follow a basic pattern shown in Fig. 1.3. They are the most complex of bee nests. The heart of the nest, usually more or less in the center of the nesting cavity, is the brood chamber, containing the brood cells in each of which one bee is reared from egg to

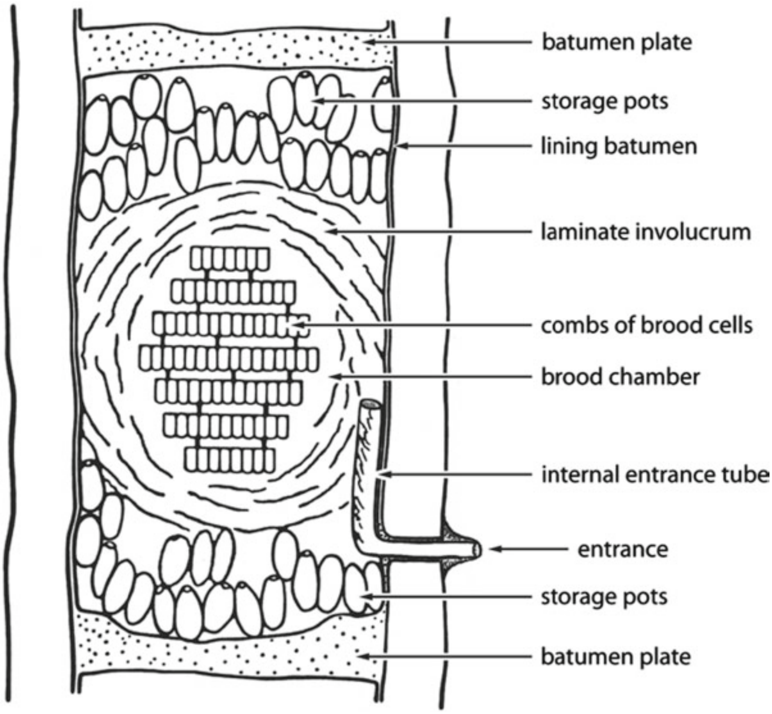


Fig. 1.3 Diagram of a stingless bee nest in a hollow tree trunk with parts labeled (modified from Nogueira-Neto 1970). The elongate food storage pots shown are unusual; they are more often irregularly spherical (prepared by Sara Taliaferro, based on Michener 2007)

emergent adult. Thereafter the cell is destroyed. The cells, which open upward (or laterally in *Dactylurina*) are provisioned, an egg is laid in each (normally by the queen), after which the cell is closed; there is no progressive feeding of the larva. The cells are commonly arranged to form a stack of horizontal combs, sometimes joined to form a broad spiral. In *Dactylurina*, however, cells are in vertical combs arranged much as in *Apis*. And in scattered taxa among the Meliponini the comb arrangement is to varying degrees lost so that cells are in clusters. It is the species with cells in clusters that utilize small and irregular cavities, sometimes with the brood cells dispersed in different subcavities.

Workers and males are reared in similar cells in the same cluster or comb; queens come from a few larger irregular brood cells, except in the genus *Melipona* in which queens are produced in ordinary brood cells among the cells producing other castes. In that genus the queens are unusually small; there is no evidence that they receive any special treatment during development and they are produced (and destroyed) in considerable numbers. This leads to the conclusion that the female castes are determined genetically in *Melipona* whereas in other Meliponini the larger amounts of food provided in their large cells appear to produce queens.

Surrounding the brood chamber is the involucrum. It is frequently laminate, that is, made up of several layers with or without spaces between them in which bees can move about. The involucrum is absent in some species that have brood cells in clusters rather than combs. Outside the involucrum, in one or more clusters or even in a partial layer, are the food pots where honey and pollen are stored. Of course the honey pots and their contents are the main topic of this book. The pots vary among species in size and shape (unusually elongate in Fig. 1.3) but are always much larger than brood cells. Surrounding the whole nest, that is outside the storage pots, is a layer of batumen, which is hard gray, brown, or black material, often with a thin, brittle outermost layer that breaks if disturbed, allowing rapid exit of many bees for defense. In a cavity batumen may include a single lining layer often less than a millimeter thick that smooths irregularities in the wood or soil walls. To close off excess space the batumen may form a thick layer. For example in a long hollow in a tree trunk, strong batumen plates above and below the nest may close off the nest area from other parts of the hollow (Fig. 1.3). The strong and usually laminate outside walls of exposed nests are batumen; in part of the nest laminate batumen may grade into the laminate involucrum. An entrance tube, usually opening in the nest outside the involucrum, extends to the outside world by an entrance that varies widely among species and, except for exposed nests, is usually the only outside indication of the presence of a nest.

It may be that scarcity of suitable nesting cavities has been a limiting factor for Meliponini. Since small and irregular cavities are more frequent than larger cavities that can be appropriately closed off, it is not surprising that minute size appears to have arisen repeatedly among stingless bees. Or perhaps small size characterized some ancestral Meliponini. Often small size is accompanied by brood cells in clusters, not surrounded by an involucrum. However, brood cells of *A. australis* (Friese) are in large clusters, with an involucrum, in rather large cavities (Michener 1961).

1.3.4 Defense

Defense is a significant function of stings in many aculeate Hymenoptera, but of course not for stingless bees. Strong nest structure, difficult to penetrate, must be important. Attacks on intruders by worker stingless bees, however, cannot be ignored. Especially in species that construct exposed nests, workers can swarm out of the nest in large numbers. They get into the hair, eyes, ears, and sometimes under clothing. They crawl about, bite, are sticky, and some say they have offensive odors. Particularly objectionable are species of *Oxytrigona* (they do not have exposed nests). From enlarged mandibular glands they bite a liquid containing formic acid into the skin. The result is severe pain (hence the name fire bees) and long-lasting lesions.

Defense against parasitic and predaceous arthropods must also be important for stingless bees. That the nests are completely sealed except for small and easily guarded entrances suggests that natural enemies have played a role in the evolution of meliponine nesting behavior. Of course foraging workers are subject to the usual predators of flying insects and floral visitors.

1.4 History and Phylogeny

The fossil record for bees is very incomplete. Nonetheless a few fossil Meliponini have been found. The oldest, and it may also be the oldest fossil bee, is the Late Cretaceous (about 70 million years ago, Mya) *Cretotrigona prisca* (Michener and Grimaldi) from New Jersey amber. This species is surprisingly similar to *Trigona* (*Trigona*) of the American tropics (Michener and Grimaldi 1988; Engel 2000).

Two genera of stingless bees are known from the Eocene (44 Mya) Baltic amber. The species are *Kelneriapis eocenica* (Kelner-Pillault) and *Liotrigonopsis rozeni* Engel. Both species are minute (body length little over 3 mm) and have greatly reduced wing venation like the recent minute Meliponini. Engel (2001a, b) provided a detailed account of these species.

More recent fossil Meliponini include the several species of the extinct genus *Proplebeia* Michener from Miocene (15–20 Mya) amber in the Dominican Republic and southern Mexico (Camargo et al. 2000). Except for *Melipona* which is perhaps introduced, Meliponini no longer exist in the Greater Antilles; perhaps they disappeared during a dry epoch or during subduction of portions of the various islands.

It is noteworthy that Meliponini (*Cretotrigona*) are found at least as early as any fossil bees, yet they have striking derived features that unite the Meliponini and distinguish them from other bees. These characters such as reduced wing venation, reduced sting, etc. must have originated substantially after the bees originated from related wasps. For other synapomorphies of the Meliponini see Michener (2007). Engel (2004) suggests that bees differentiated from the related wasps in the later part of the Early Cretaceous, when flowering plants were becoming dominant, and that by Late Cretaceous the major lineages of bees, of which the Meliponini is one, had been established. Although bees in general probably arose in, and much of their early evolution probably occurred in, xeric areas, the stingless bees, to judge by their present distribution, probably evolved in forested zones.

The fossils of stingless bees from New Jersey and the Baltic region indicate that in the Late Cretaceous and the Eocene Meliponini occurred well outside the modern tropical zone to which they are now almost completely restricted. The fact that the present meliponine faunas of South America and Africa have no genera in common indicates that these genera arose and differentiated after the origin of the South Atlantic Ocean in the Late Cretaceous. Rasmussen and Cameron (2010) estimated dates for various events in meliponine evolution.

Earlier studies of phylogenetic relationships within the Meliponini were summarized by Michener (2007). Several of these studies, based primarily on morphological characters, suffered from utilizing too few characters; different studies gave quite different results. For example the genus *Melipona* is sometimes sister to all the other genera. Other studies place *Melipona* among the other genera. A study by Wille (1979) thoughtfully presented many characters but the basis for his phylogenetic tree is not very clear. Certain authors believed that the Meliponini originated in South America because of the great diversity of the group there now. Wille, however, believed that the tribe probably originated in Africa because of ancestral (plesiomorphic) characters such as a less reduced sting in all the African genera except *Hypotrigona*. Recent molecular work using sequences within gene fragments (Rasmussen and Cameron 2007, 2010) provides more satisfying results in that major clades make good sense geographically. The major division is between, I, the neotropical clade and the Old World clade, which is itself divisible into, II, the African clade and, III, the Indoaustralian clade. The two exceptions are the genera *Austroplebeia* and *Lisotrigona* which fall in clade II although geographically they belong with clade III.

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Chapter 2

Historical Biogeography of the Meliponini (Hymenoptera, Apidae, Apinae) of the Neotropical Region

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The Meliponini have a pantropical distribution (Indo-Australia, the Neotropics and Africa-Madagascar) which includes continental disjunctions unique among the Apidae, revealing a complex history of vicariance events of great antiquity. The trait of disjunction by vicariance permits the inference that Meliponini possibly had their origin on the ancient Gondwanan continent and possess a minimum age near 100 million years (Camargo and Pedro 1992). The oldest known fossil of Meliponini is *Cretotrigona prisca*, from upper Cretaceous New Jersey—USA, c.a. 65–96 Ma (Michener and Grimaldi 1988a, b; Engel 2000).

From a few species (possibly only one that left descendants) which remained isolated in South America, after fragmentation of Gondwana, and final separation of that continent from Africa, came all existing diversity of the Neotropical region,

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Table 2.1 Genera and number of Meliponini species from the Neotropical region (in alphabetical order)

Genus	Number of species
Aparatrigona Moure, 1951	2
Camargoia Moure, 1989	3
Celetrigona Moure, 1950	1
Cephalotrigona Schwarz, 1940	5
Dolichotrigona Moure, 1950	10
Duckeola Moure, 1944	2
Friesella Moure, 1946	1
Frieseomelitta Ihering, 1912	16
Geotrigona Moure, 1943	20
Lestrimelitta Friese, 1903	19
Leurotrigona Moure, 1950	2
Melipona Illiger, 1806	69(+10 ssp.)
Meliwillea Roubik, Lobo and Camargo, 1997	1
Mourella Schwarz, 1946	1
Nannotrigona Cockerell, 1922	10
Nogueirapis Moure, 1953	3
Oxytrigona Cockerell, 1917	8
Parapartamona Schwarz, 1948	7
Paratrigona Schwarz, 1938	29
Paratrigonoides Camargo and Roubik, 2005	1
Partamona Schwarz, 1939	32
Plebeia Schwarz, 1938	38
Proplebeia Michener, 1982†	4
Ptilotrigona Moure, 1951	3
Scaptotrigona Moure, 1942	21
Scaura Schwarz, 1938	5
Schwarziana Moure, 1943	2
Schwarzula Moure, 1946	2
Tetragona Lepeletier and Serville, 1828	13
Tetragonisca Moure, 1946	4
Trichotrigona Camargo and Moure, 1983	1
Trigona Jurine, 1807	32
Trigonisca Moure, 1950	25

† extinct genus

which comprises 33 genera, including one that is extinct, *Proplebeia* (Table 2.1), and 391 nominate taxa at the species-group level, following the recent catalog by Camargo and Pedro (2007b).[1]

Evolution of Neotropical Meliponini, in isolation since the upper Cretaceous, resulted not only in the abovementioned large taxonomic diversity, but also in a great variety in life histories, for example: species with obligate necrophagic habits, species

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[1] The online version http://moure.cria.org.br/catalogue?id=27560, updated on 07 February 2012 by SRM Pedro, includes now 412 species (SRMP, personal note)

Fig. 2.1 *Trigona hypogea*, collecting meat at a dead lizard. Photo: provided by D. Wittmann

that cultivate yeast associated with pollen, species having mutualistic relationships with scale insects, etc., in addition to a wide variety of methods used in nest construction.

The obligate necrophagy habit (Fig. 2.1) is known in three species—*Trigona necrophaga*, *T. hypogea*, and *T. crassipes* (Roubik 1982; Camargo and Roubik 1991), the only bees which do not collect pollen (the corbicula is rudimentary in all of them) nor floral nectar; flesh of dead animals is their only protein source (and supply of lipids, carbohydrates and salts); sugars are obtained from ripe or rotting fruit on the ground, extrafloral nectaries, fallen flowers on the ground, etc. (and, possibly, the glycogen obtained from carcasses serves as a glucose source). Collected carrion is transported in the stomach, and regurgitated in storage pots, in the form of a yellowish or greenish jelly which is broken down (probably under the action of digestive enzymes) and mixed with "honey."

In the storage pots (Fig. 2.2), the proteinaceous paste mixed with honey undergoes the action of the bacteria. In the larval food of *T. necrophaga*, Gilliam et al. (1985) found five species of *Bacillus* with reducing enzymatic activity related to protein and lipid metabolism and hydrolysis of carbohydrates, likely involved in digestion of the animal remains, in addition to production of amino acids and antibiotics. In *T. hypogea*, the pots, after being filled with a proteinaceous substance, mixed with "honey," are sealed and chemical reactions proceed inside them for 12–16 days (Noll et al. 1996). At the end of this maturation period, "honey" is obtained, free of reduced sugars, almost transparent, good tasting, and rich in free amino acids.

The storage of pollen associated with yeast—*Candida* sp.—is only known in species of the genus *Ptilotrigona*, as reviewed by Camargo et al. (1982, *sic* =1992)[2] and Camargo and Pedro (2004). Three species comprise the genus: *Ptilotrigona lurida*,

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[2] SRMP note.

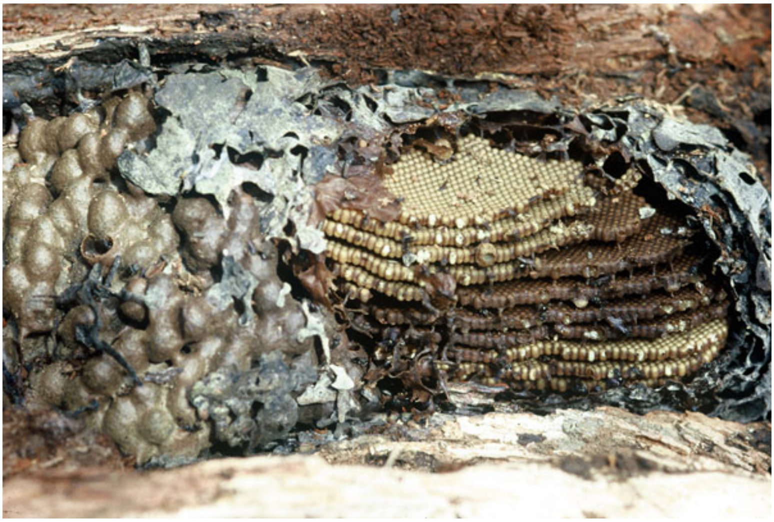

Fig. 2.2 Necrophagous bee nest, *Trigona hypogea* (Itaituba, PA, Brazil); *left*, the storage pots with products derived from meat mixed with "honey". Photo: J.M.F. Camargo

of wide range in Amazonia, *P. pereneae*, endemic to western Amazonia, and *P. occidentalis*, which occurs from northwestern Ecuador to Darién and an isolated population in the area of the Osa Peninsula in Costa Rica (Camargo and Pedro 2004). The studies were made with *P. lurida*, for which dozens of nests were observed (Fig. 2.3).

Pots containing "honey" or sweet liquids are rare or even absent in the nests, while pollen pots, associated with yeast (Fig. 2.4) are found in great number (in one of the three nests studied there was about 3.0 kg of pollen). The activity of yeast promotes the desiccation and stored life of the pollen; it makes pollen dry enough that it can produce a wrinkling and deformation of the pots.

Another interesting aspect, still lacking complementary studies, is that utilization of resins (principally floral resins of the genus *Clusia*), collected by these bees and added to cerumen used for construction of storage pots and brood cells, is that it has bactericidal activity, but no fungicidal effect. The action of such resins can promote the growth of yeast free of bacteria (Lokvam and Braddock 1999; Camargo and Pedro 2004). It is only suggested but not proven, even now, that a part of the sugars, used by bees, may be derived from the metabolic activity of the yeast.

Associations between certain species of Meliponini and free-living phytophagic hemipterans, which make sugar secretions (honeydew), are well known, but their mutualistic associations with sedentary hemipterans, coccids, are known only among species of the genus *Schwarzula* (Camargo and Pedro 2002). Silvestri (1902)

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[3] *sic*, = *Schwarzula timida*. *Scaura timida* was entered by error in the original text (Pedro SRM, personal communication).

Fig. 2.3 Nest of *Ptilotrigona lurida* (Camanaus, AM, Brazil); in the lower portion a large mass of pots can be seen, where the pollen associated with yeast is stored. Photo: J.M.F. Camargo

was the first to suspect mutualism between *Scaura timida*[3] and scale insects, but detailed observations only were made by Camargo and Pedro (2002), who observed dozens of nests of *Schwarzula coccidophila*, residing in galleries excavated by the larva of the moth *Cossula* sp. (Cossidae) in the branches of *Campsiandra angustifolia* (Caesalpiniaceae), on the banks of the Rio Negro, Amazonas state, Brazil. The scale insects (*Cryptostigma* sp.) are found attached to the gallery walls, in the nest interior, where they receive protection and care from the bees (Fig. 2.5), and, in exchange, offer sweet secretions and additional wax the bees use in nest construc-

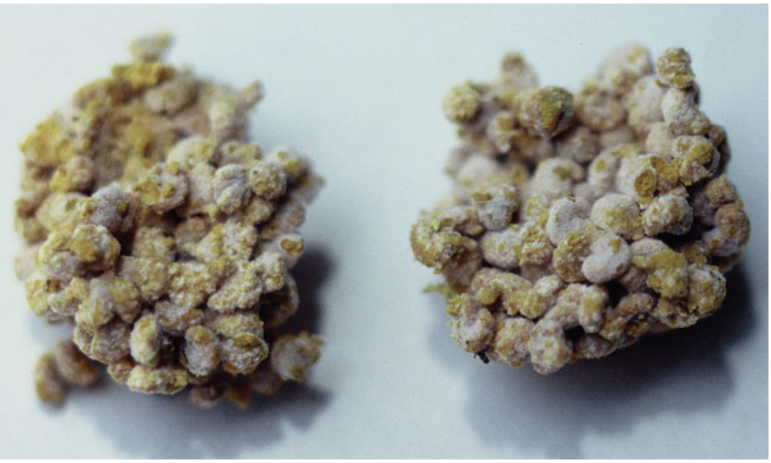

Fig. 2.4 *Ptilotrigona lurida*, closeup of pollen covered with yeast. Photo: J.M.F. Camargo

Fig. 2.5 *Schwarzula coccidophila*, closeup of the scale insects—*Cryptostigma* sp.—in the nest interior, being attended by a bee (Tapurucuara-Mirim, AM, Brazil). Photo: J.M.F. Camargo

tion. The secretions are a subproduct of sap from the plant, on which the scale insects feed. When stimulated by attending bees, the scale insects liberate, through the anus, a small droplet of the sugary liquid, which is ingested by the attendant. These bees are the only known species which have, within their own nest, a permanent source of carbohydrates, in addition to additional wax for building. Only pollen is collected at flowers (Camargo and Pedro 2002).

Another extraordinary behavior is found in *Trichotrigona extranea* (Fig. 2.6), a monotypic genus and until now only known from a single locality, in the middle

Fig. 2.6 Nest of *Trichotrigona extranea*, a bee that does not build storage pots and does not store any kind of food; closeup of brood cells (Samaúma, AM, Brazil). Photo: J.M.F. Camargo

Rio Negro region of Amazonas, Brazil. The colonies are very small, with less than 200 adults, located in small cavities in dead branches (of *Buchenavia suaveolens*); they construct no storage pots and do not store food of any kind. It is likely these bees are cleptobiotic, but not in the manner of *Lestrimelitta*, which robs, during mass raids, the food stores of a host and transfers them to the storage pots of its own nest. Supposedly, the workers (and also possibly the males) of *T. extranea*, individually use and have free access to the food stores of the host species (perhaps of *Frieseomelitta*, very common in the region and sharing nest habits similar to those of *Trichotrigona*; Camargo and Pedro 2007a).

There exists, also, a great diversity in nest architecture, ranging from subterranean, with complex structures for the control of humidity and air circulation, to

Fig. 2.7 Nest aggregation of *Partamona batesi*, in active termite nest (*Nasutitermes acangussu*); endemic in the Tefé region, central Amazonia, Brazil. Photo: J.M.F. Camargo

nests in tree cavities, within the nests of other social insects, such as termites and ants, to exposed arboreal nests. Among these, species of the genus *Partamona* are noteworthy, which are among the most formidable nest builders known, primarily considering the nest entrance structures (Figs. 2.7 and 2.8), conspicuous and richly ornamented, which "facilitate" recognition of the nest and function as true flight targets (several of these species—like *P. batesi*, Figs. 2.7 and 2.8a—construct nests in large aggregations, with the nest entrances very close to each other).

The nest of *P. vicina*, of Amazonas state, is one of the most sophisticated known (Fig. 2.9); the nest entrance structure (Fig. 2.9a) opens upon a wide chamber or vestibule, filled with a structure similar to intertwined roots, constructed with earth and resin (Fig. 2.9b), forming a large labyrinth, where workers stay and constitute

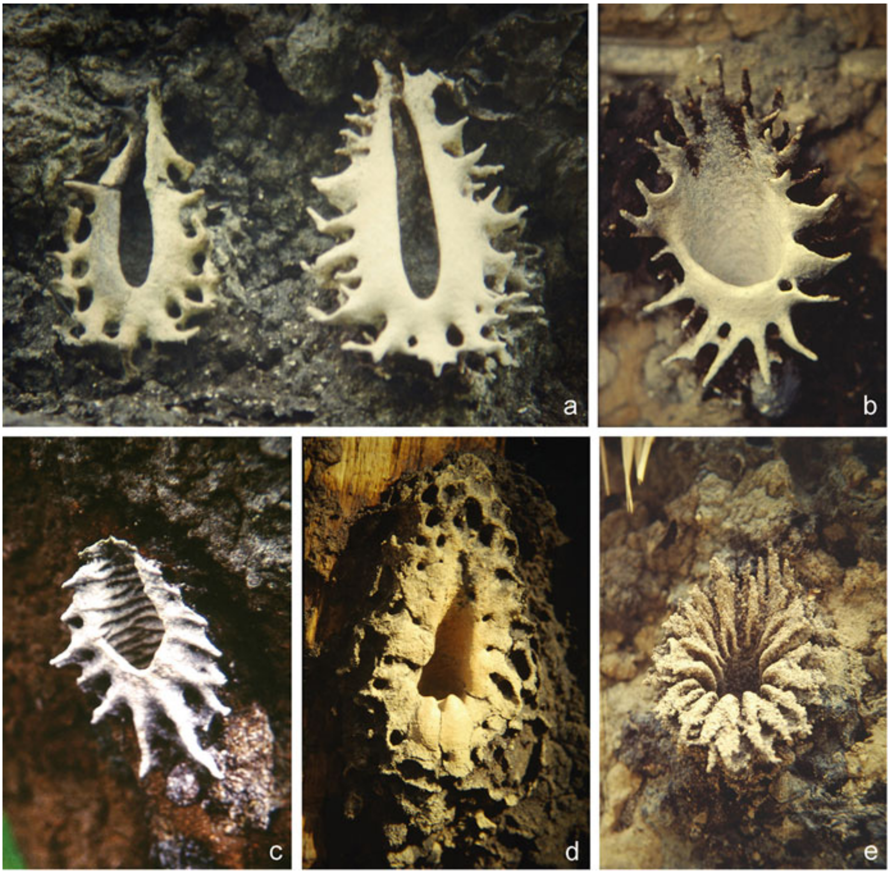

Fig. 2.8 Nest entrances of *Partamona*; (**a**) *P. batesi* (endemic in the Tefé region); (**b**) *P. gregaria* (endemic in the region of lower Tapajós); (**c**) *P. pearsoni* (endemic to north of the Amazon/Negro rivers); (**d**) *P. chapadicola* (endemic to Maranhão—eastern Pará); (**e**) *P. vicina* (of wide Amazonian distribution). Photo: J.M.F. Camargo

the first force of nest defense; the vestibule is connected, through a small tunnel, to a second cavity or atrium (Fig. 2.9c), filled with waxy lamellae, cells and small pots, generally containing an acidic liquid, constituting a typical “false nest.” From this “false nest,” there is a small tunnel leading to the true nest, where the brood and food are located (Fig. 2.9d), and their various satellite chambers—containing honey pots. The entire assemblage of structures and chambers is important in nest defense, against invasions of other insects, primarily cleptobiotic social insects, such as *Lestrimelitta* spp., for example (cf. Camargo and Pedro 2003).

There exists, also, a great diversity in form and size, from the robust *Melipona fuliginosa*, ca. 11.0–13.0 mm in length, to the minuscule *Leurotrigona pusilla*, ca. 2.0 mm in length (Fig. 2.10).

Some species of Meliponini are exploited, economically, since pre-Colombian times. Some native peoples of South America, such as the Kayapós, from southern

Fig. 2.9 Nest of *Partamona vicina*, in active termite nest (*Amitermes excellens*); (**a**) entrance; (**b**) vestibule/labyrinth, where the defense force is located; (**c**) atrium/false nest; (**d**) true nest, with brood cells, food storage pots, etc. (Muçum, Tapajós, PA, Brazil). Photo: J.M.F. Camargo

Pará, Brazil (Fig. 2.11), make varied use of the products from these bees, in food, medicine, ritual, tool making, etc., and also as a model for social organization for their own communities (cf. Posey and Camargo 1985; Camargo and Posey 1990).

The causes of this diversification, especially taxonomic, in the Neotropical region, have been the subject of many speculations. Through the decades of 1960–1970 the postulate of ecological "refuges" emerged. This postulate attempted to associate the known pattern of endemism and speciation in Amazonia with climatic cycles (glacial and interglacial) in the recent quaternary. Although this attempt, a priori, can explain some of the current distribution patterns, it barely touches the problem of the history of the taxa; it only deals with regional fragments of recent history.

Fig. 2.10 Nest of *Leurotrigona pusilla* (Curicuriari, AM, Brazil), in a gallery made by a beetle. This is the smallest known meliponine (body length ca. 2.0 mm). The nest is of ca. 4 cm in length. Photo: J.M.F. Camargo

Fig. 2.11 Kayapó Indians (Gorotire, PA, Brazil), on a trip to collect meliponine nests. These Indians are bee experts. Photo: J.M.F. Camargo

Only recently, some work based on the methods conceived in phylogenetic systematics and vicariance biogeography, involving monophyletic taxa, with large ranges in the Neotropical region, permit access to some periods of evolutionary history/

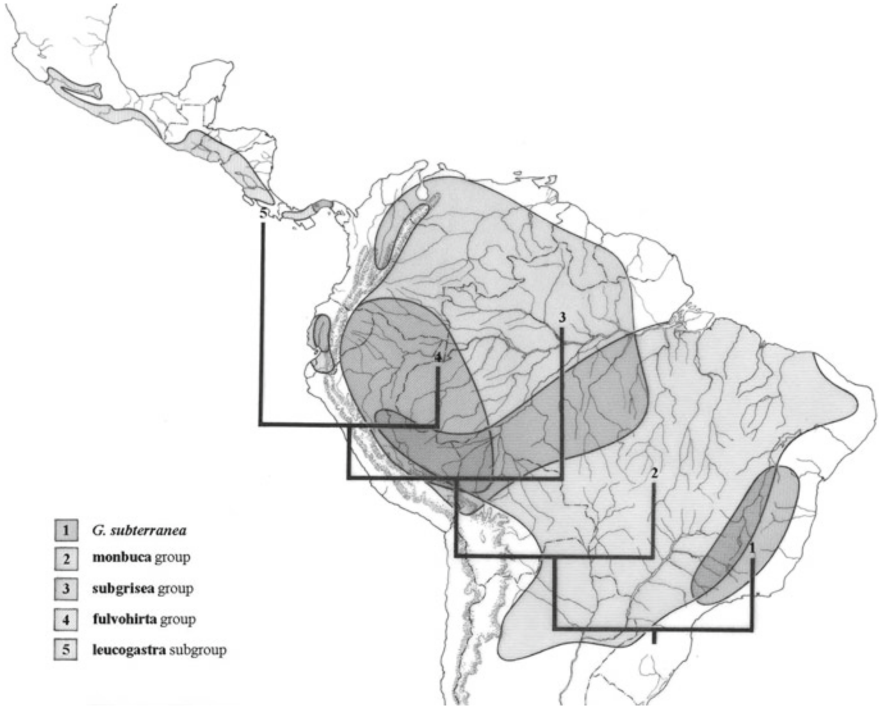

Fig. 2.12 Area and biological cladograms for the subgroups of *Geotrigona* (this is the first area cladogram proposed for Neotropical Meliponini), from Camargo and Moure (1996)

biogeography with great significance in the Neotropical area, permitting, for the first time, integration of space, time and form. The first works on evolutionary biogeography of Meliponini through the viewpoint and protocol of vicariance biogeography were of Camargo and Moure (1996), Camargo (1996) and Camargo and Pedro (2003). The first biological and area cladograms were for the species of the genera *Paratrigona* and *Geotrigona* (Fig. 2.12), and more recently *Partamona* (Fig. 2.13). The results reveal that the species subgroups within each of these genera are notably congruent in terms of biogeographic compartmentalization, that is, when the taxa are placed on the biological cladograms by their respective areas of endemism, the results obtained for the subgroups of the first two genera are the same (particularly in relation to the species of *Partamona*), indicating the same relationships between areas or biogeographic compartments. These results, obviously, suggest a general pattern of biogeographic coevolution in the Neotropical region.

The sequence of events in vicariance/cladogenesis provides, therefore, a definition of a hierarchy in the formation of biogeographic boundaries or geological compartmentalization and barriers, as in Figs. 2.14 and 2.15.

The first great barrier is formed along the alignment of the Madeira/Amazonas Rivers (possibly epicontinental seas related to the Tapajonic transgressions, in the

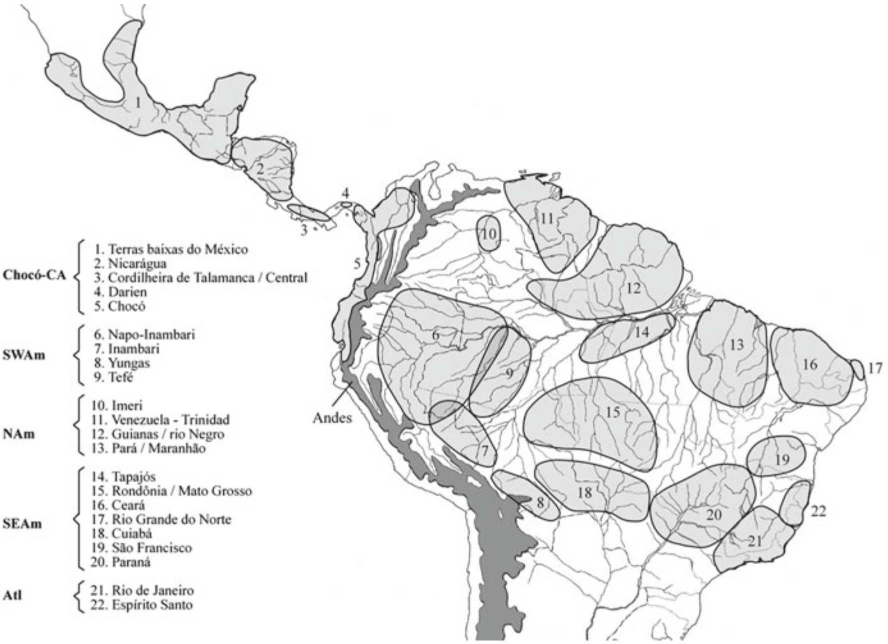

Fig. 2.13 Areas of endemism and biogeographical components, inferred from the species of *Partamona*; Chocó-CA (from northwestern Peru to Mexico); SWAm (a component delimited, on the north, by the alignment of the Uaupés/Negro rivers, on the south, by the Madeira/Mamoré rivers, and on the west, by the Andean mountain range); NAm (north of the Negro/Amazonas rivers); SEAm (area to the south of the Madeira/Amazonas rivers to northwestern Argentina); Atl (Atlantic area, from Bahia to Paraná). See Fig. 1.15a (taken from Camargo and Pedro 2003)

lower Miocene), dividing the Neotropical region into two large compartments: NW–SE (Fig. 2.14a). In the NW compartment a further break occurred (approximately along the line of the Caqueta/Japura rivers, possibly related to the transgression of the Maracaibo seas in the mid Miocene; Fig. 2.14b), separating North Amazonia (NAm) from all of southwestern Amazonia (SWAm) and the north Andean, Central American—Mexico block (Choco-AC). And, finally, a break separating SWAm from the Choco-AC component (Fig. 2.14c), related, possibly, with orogeny of the equatorial Andes, which attained heights greater than 3,000–4,000 m in the Plio-Pleistocene. In the SE component, there is a separation between the southeastern Atlantic region (Atl) and southeast Amazonia (SEAm). The breaks, giving rise to the crown (present) species, may be related to the climatic events of the Pleistocene, as postulated by the proponents of ecological "refuges."

The first image that arises from this biogeographic and geological compartmentalization of the Neotropical region is that Amazonia (Fig. 2.16) is not a single historical unit, and rather, it is composed of three great biogeographic compartments with distinct temporal and phylogenetic relationships (Fig. 2.14, area cladogram).

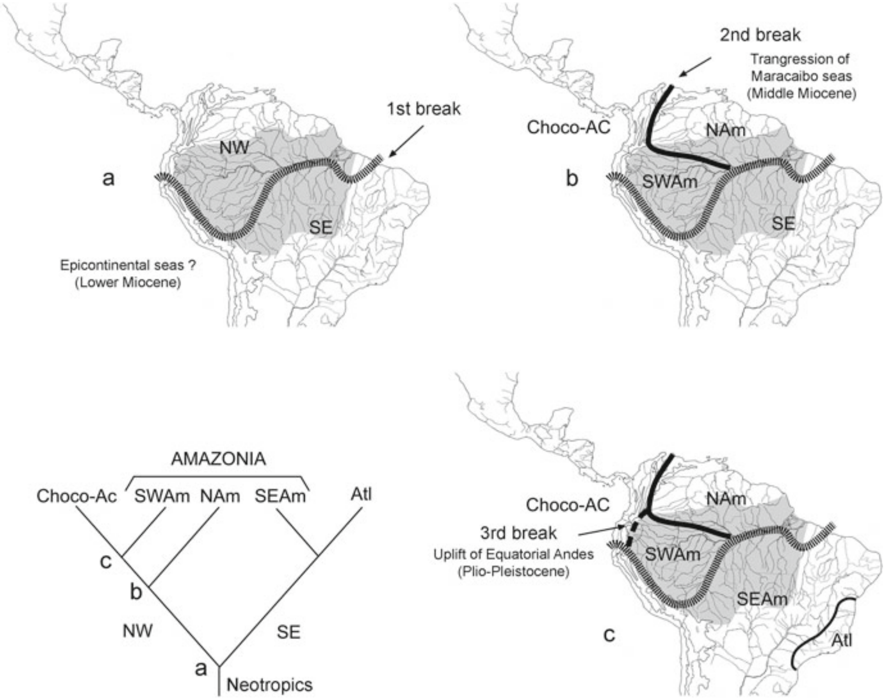

Fig. 2.14 Sequence of events of separation and vicariance in the Neotropical region. The *shaded area* is Amazonia, which, from the biogeographic perspective of vicariance, is not an historical unit, taken from Camargo (2006)

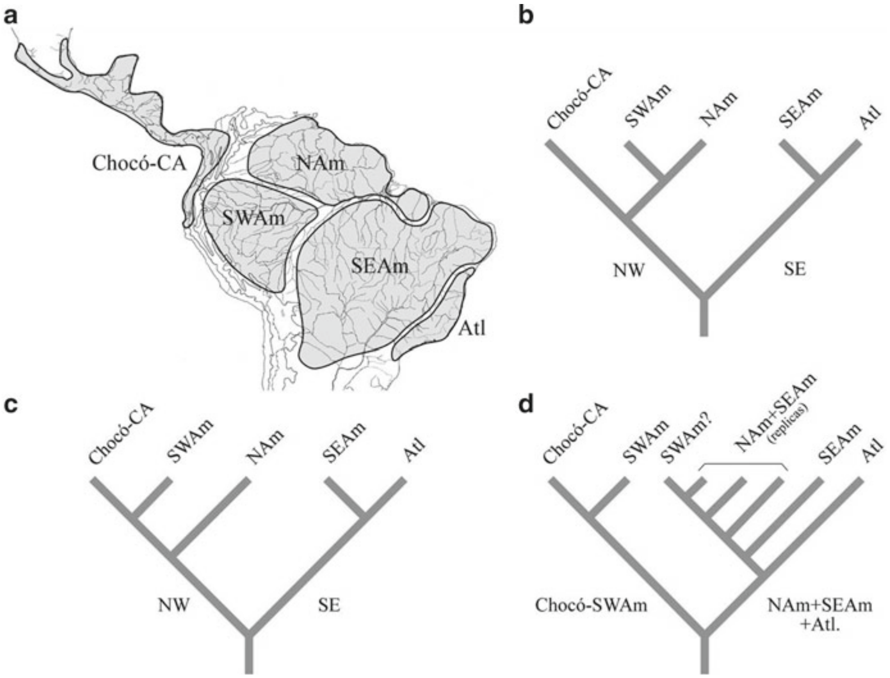

Fig. 2.15 Principal biogeographic elements which unify the Neotropical region ; (**a**) the diverse area cladograms obtained; (**b**) those proposed by Amorim and Pires (1996); (**c**) those proposed by Camargo (1996) and Camargo and Moure (1996); (**d**) those proposed by Camargo and Pedro (2003) (taken from Camargo and Pedro 2003). See legend in Fig. 1.13

Fig. 2.16 The magnificent Amazonian forest (upper Rio Negro region), produced by millions of years of evolution, habitat of many Meliponini and a megadiverse biota, today at the mercy of an irresponsible and uncontrolled devastation. Photo: J.M.F. Camargo

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Chapter 3
Australian Stingless Bees

Megan Halcroft, Robert Spooner-Hart, and Lig Anne Dollin

3.1 Introduction

Stingless bees have been an important part of indigenous Australian culture for centuries; however, modern meliponiculture in Australia is still very much in its infancy (Heard and Dollin 2000). A recent survey showed that interest in stingless bees is growing and Australians are becoming increasingly aware of and concerned about conservation of these species. More community members are keeping hives with this interest in mind (Halcroft, unpublished data). Beekeepers in the northern regions are able to produce honey in small quantities and some multiply hives for profit.

Of the two stingless bee genera in Australia, *Trigona* (*s.l.*) is the most studied. The domestication of *Trigona* (*Heterotrigona*) *carbonaria* colonies began in the 1980s and Dr. Tim Heard conducted ground-breaking work in *T.* (*Heterotrigona*) *carbonaria* husbandry (Heard 1988a,b). As a result, most scientific research has been conducted on this species. Few studies have been conducted on *Trigona* (*s.l.*) pollination efficacy and have mainly used *T.* (*Heterotrigona*) *carbonaria* or *T.* (*Heterotrigona*) *hockingsi* in macadamia nut (*Macadamia integrifolia*) crops. Pollination studies on other horticultural crops are minimal and, as such, anecdotal reports pertaining to crop pollination are cited here.

Austroplebeia have only recently become of interest to beekeepers and hobbyists. A small number of studies have been conducted on aspects of biology of *A. australis* and *A. symei*, as their brood structure and queen/worker interaction

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is more easily observed than that of *Trigona* (*s.l.*), due to reduced nest structures. Recent doctoral research has been conducted (M. Halcroft) to better understand the development of the Australian stingless bee industry, phylogeny of *Austroplebeia*, the biology and behavior of *A. australis*, and to assess the ability of *A. australis* to pollinate crops in greenhouse and field settings. This research is incomplete and ongoing, and therefore, is cited here as unpublished data.

Although Australian stingless bees are not as diverse in size or morphology as Neotropical or Paleotropical species, our bees are proving to be diverse and resilient in their behavior. Their native range is mostly limited to the northern half of the continent; however, *T.* (*Heterotrigona*) *carbonaria* has a distribution that reaches the southernmost range of any stingless bee species (Dollin et al. 1997). *Austroplebeia* occur in some of the most arid areas of Australia, where the climate extremes are harsh and the food resources are often scarce. Australian stingless bees have evolved diverse behaviors to survive under such conditions.

While few scientific studies have been conducted on the behavior of Australian stingless bees, amateur beekeepers often have a wealth of knowledge and their experience is extremely valuable. Communication with experienced beekeepers is of utmost importance when initiating research, and anecdotal accounts are appropriately cited here. While there is great potential for further research on stingless bees in Australia, this chapter aims to provide an overview of current knowledge and suggest areas for further study.

3.2 Indigenous Australians and Their Relationship with Stingless Bees

Indigenous Australians have been collecting the strong, tangy honey from stingless bee nests 1sugarbag for centuries. Hockings (1883) first reports the Australian *Trigona* (*s.l.*) and *Austroplebeia* from his visit to northern regions of Queensland, where local Aboriginal people call these bees "karbi" and "kootchar," respectively. It is unclear which tribal language Hockings refers to in his paper.

There are many different Australian Aboriginal tribes. The Aurukun on Cape York, in far north Queensland, is the homeland for the Wik Mungkan people. In 2003, an industry based on stingless bees and traditional culture was the inspiration for a group of 50 Wik school children, aged between 12 and 16 years. Using the natural resources of their homeland, the sugarbag "may man-pathan" provided the prospect of making real money and building a culturally based business. The children within this indigenous community developed a business plan and become more motivated and engaged in learning (Yunkaporta 2009). Anecdotal accounts of indigenous bee hunting methods are described in bush tales, and these include: placing a fine hair or grass into the terminal abdominal segment of a forager, which is used as a flag to follow it back to the nest; sprinkling foragers with flour to make them easier to see and follow; and bee hunters relying on the loud humming sound of a predatory wasp (*Bembix*) which hovers outside the nest entrance, waiting for foragers to leave (A. Beil, personal communication).

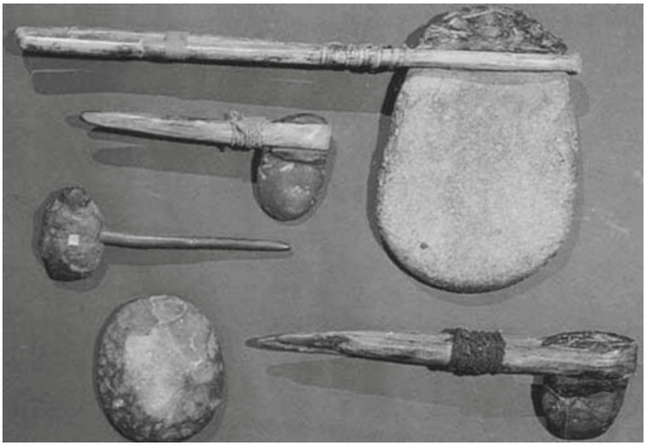

Fig. 3.1 Indigenous Australian axes. Photo: G. Walsh—http://www.hogartharts.com.au

Traditionally, honey “may at” or “may kuyan” is used for medicinal and culinary purposes, while the cerumen “wom” is used as a waterproofing agent for baskets, as a wood preservative, as glue to secure axe heads “thayan” (Fig. 3.1), and for personal and artifact decoration (Rayment 1935; Yunkaporta 2009; Welch 2010). Cerumen has also been found in protective covers, fashioned around ancient rock paintings, to protect them from rain and erosion (Rayment 1935). Pellets of cerumen are used in some rock art, notably in the Kimberley Ranges in Western Australia, to create shapes of humans, dingoes, turtles, and spirit figures on the rock surface (Welch 1995). This collage technique (Brandl 1968) permitted incorporation of organic materials in a normally inert, inorganic rock face. Cerumen and plant resins are extremely amenable to carbon dating because storage of fresh products within hives, and consequent use by indigenous craftsmen, enables accurate estimates of when the collages were created, thus dating the artwork (Bednarik 2002). Interest in indigenous culture and art has increased over the past 20 years and is at a peak in popularity (Artlandish 2010). Cerumen is still used by Australian Aboriginal artists and craftsmen to manufacture hunting tools such as spears “kek” and woomeras “thul,” as well as firesticks “thum pup” and mouth pieces for didgeridoos, a traditional musical instrument (Yunkaporta 2009).

Sugarbag honey can fetch very high prices in comparison to honey bee honey. In 2005, Russell and Janine Zabel commenced a training program in sugarbag harvest and colony transfer within the Aurukun, with the aim to develop a sustainable industry based on sale of sugarbag honey and cerumen (Zabel 2008). An Australian government grant was received to assist development of this new enterprise, which had potential to boost local employment and would be consistent with the rapidly developing ecotourism industry.

In 2010, an industry based on sugarbag is seen as an option for inclusion in a preliminary proposal for the Department of Aboriginal Business Development, in Grafton, in northern New South Wales. This proposal is investigating indigenous land development in the Northern Rivers region using traditional cultures and sustainable practices (Lain 2010). Another initiative is the Thamarrurr Development Corporation proposal to develop a wildlife industry in Wadeye, Northern Territory, including health products containing sugarbag honey (Adlam 2010). Potentially, stingless bees could provide sustainable income for both Australian indigenous and non-indigenous communities through production of honey, cerumen, bee colonies, and pollination service.

3.3 Australian Stingless Bees

There is much needed change regarding classification of the genus/subgenus group name of *Trigona* (*Heterotrigona*), which includes a portion of the native Australian Meliponini. At present, according to Michener (1990), species of *Trigona* (*s.l.*) that occur in the Indoaustralian regions are of the subgenus *Heterotrigona*. Recent molecular studies, and also morphology, suggest this taxonomic classification is incorrect and that Australian species previously named *Trigona* (subgenus *Heterotrigona*) should be changed to the genus *Tetragonula* Moure, 1961 (Rasmussen and Cameron 2007, 2010). There are many species and subgenera to consider in Asia and Australia, with 15 species in Australian comprising two genera. We have chosen to preserve the group name *Trigona* (*Heterotrigona*) in this chapter, until further taxonomic and systematic research is decisive. The bees in Australia are small (<4.5 mm) and black. However, *Austroplebeia* can be distinguished from *Trigona* (*s.l.*) by colored body markings, thoracic shape, and nest architecture.

The highest rainfall areas within Australia occur in the northern, eastern, and far south eastern coasts (BOM 2010a) (Fig. 3.2), resulting in tropical, subtropical, and temperate forest and woodland vegetation. The natural range for Australian stingless bees is in the tropical and subtropical regions of northern Australia, with the exception of *T.* (*Heterotrigona*) *carbonaria*, which has, by far, the southernmost distribution. The temperature threshold for flight activity in *T.* (*Heterotrigona*) *carbonaria* (Heard and Hendrikz 1993) is >18°C, and for *A. australis* >20°C (Halcroft, unpublished data), which means foraging periods are substantially reduced for colonies in the most southerly range of their distribution.

3.3.1 Castes and Genders of the Australian Stingless Bees

As with all stingless bees species there are two castes—queen and worker. All Australian species are thought to be monogynous. However, the incidence of virgin queen imprisonment in queenright colonies of *A. australis* has been observed (MH, personal observation) (Fig. 3.3). Queens can be identified by their long, pale abdomen and short wings (Fig. 3.4). They are usually found on the brood, although extensive nest patrolling is not uncommon in *A. australis* (MH, personal observations).

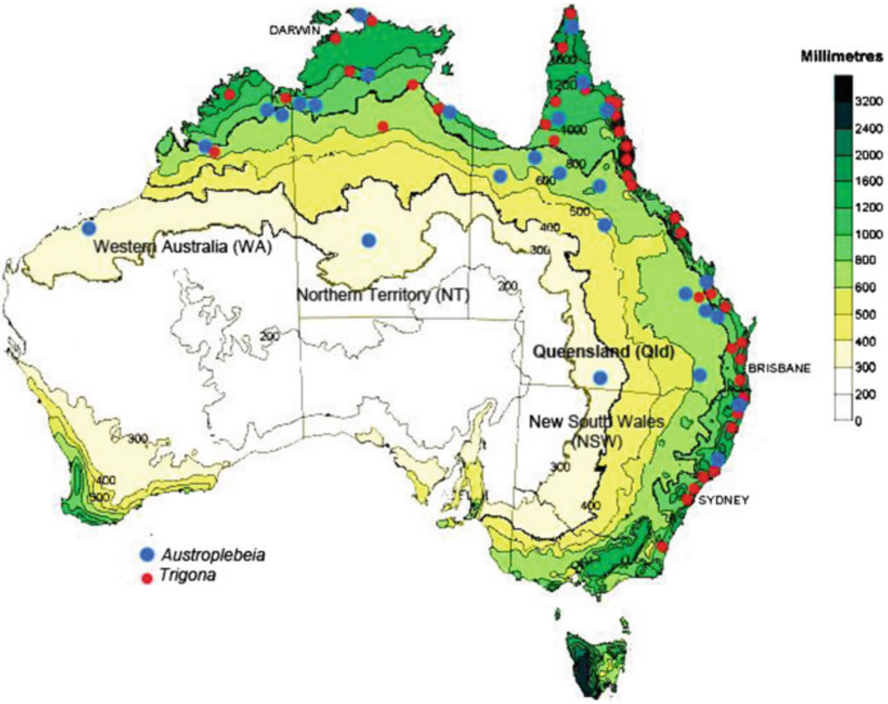

Fig. 3.2 Average annual rainfall charted for Australia, including the reported distribution of Australian stingless bees (Dollin et al. 1997; BOM, 2010a; Dollin, 2010, unpublished data)

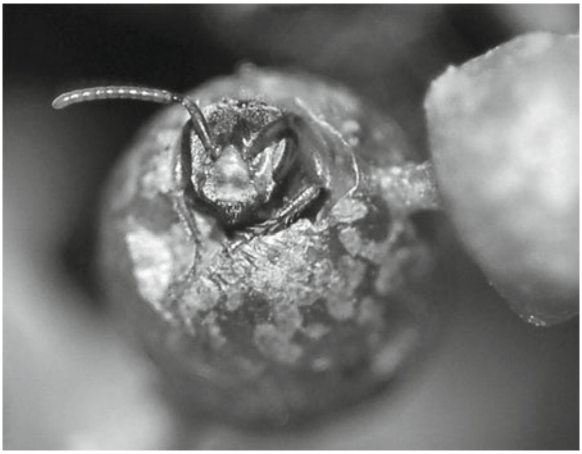

Fig. 3.3 Imprisoned *A. australis* virgin queen. Photo: M. Halcroft

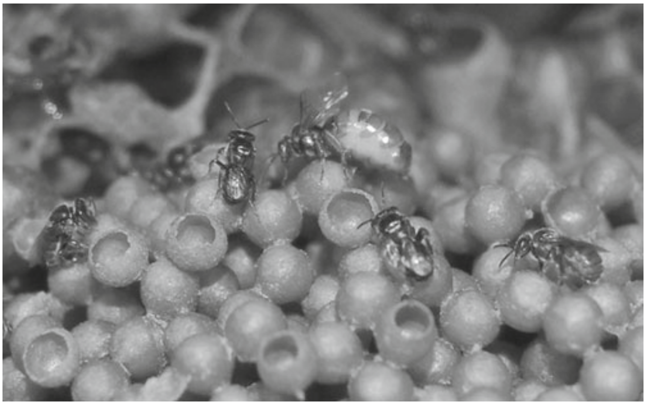

Fig. 3.4 *A. australis* queen with workers. Photo: M. Halcroft

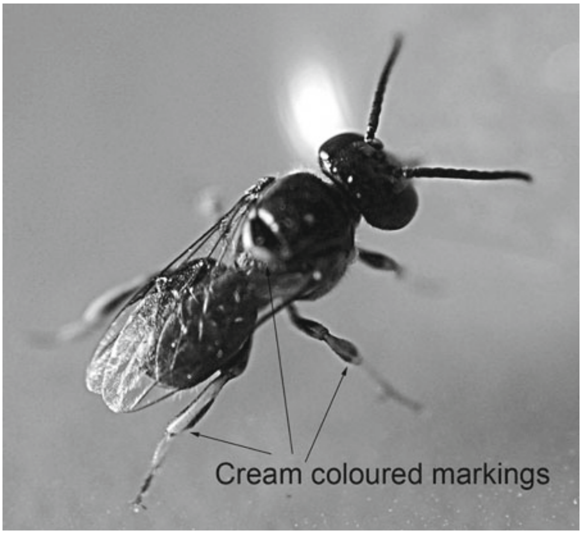

Fig. 3.5 *A. australis* drone showing cream markings on legs and thorax. Photo: M. Halcroft

Trigona (*s.l.*) drones are difficult to identify within the hive, without the aid of a magnifying glass, as they have no defining markings (Dollin 2010a). Their bodies are slightly more slender and the antennae are longer, having one additional segment, compared to females or workers. They frequently form drone swarms outside nests and sometimes aggregate on foliage at night. These aggregations and swarms can be seen for a number of days when conditions are favorable (Klumpp 2007). *Austroplebeia* drones are easier to identify within the nest because the cream-colored markings on their thorax are more pronounced, and they also have markings on the abdomen and legs (Dollin 2010a) (Fig. 3.5). Their apparently slimmer bodies and constant movement of the antennae, as they move, also distinguish them (MH, personal observation). *A. australis* drones also form mating swarms and aggregations, although these are not as large as those of *Trigona* (*s.l.*) (MH, personal observation). *A. australis Au. australis* colonies appear to produce drones in "batches" or "male-producing periods" (MPP) (Velthuis et al. 2005), with drones being present only periodically in a single colony. It is not clear whether drone production is curtailed during periods of resource scarcity.

3.3.2 Brood Production

In the Meliponini, brood production is an elaborate procedure and involves a sequence of interactions between the queen and a group of workers (Sakagami et al. 1973; Sakagami 1982). This temporal sequence is termed the "provisioning and ovipositing process" or "POP" (Sakagami and Zucchi 1963; Michener 1974; Wittmann et al. 1991). Cells are mass-provisioned with a mixture of honey, pollen, and protein-rich secretions from the hypopharyngeal glands (Michener 1974; Silva de Moraes et al. 1996). Some species provision cells successively while others provision synchronously (Sommeijer and Bruijn 1984). Once a cell is provisioned, the queen oviposits and workers seal the cell (operculation) (Drumond et al. 1999). *Trigona carbonaria* constructs and provisions brood cells synchronously, and the queen oviposits in batches (Yamane et al. 1995). *Austroplebeia australis* and *A. symei* construct and provision brood cells in a successive pattern, while the queen does not oviposit in batches (Drumond et al. 1999).

Meliponine queens normally mate only once (Kerr et al. 1962; Michener 1974), returning to the nest with the male genitalia still caught in the vagina (Michener 1974). The incidence of low frequency polyandry has been reported in *Melipona beecheii* and *Scaptotrigona postica*, (Paxton et al. 1999); however, it is thought that most stingless bees are monandrous, including the Australian species (Drumond et al. 2000; Green and Oldroyd 2002). Sperm is stored in her spermatheca. A diploid female is produced when a sperm cell is released to fertilize the egg as it passes through the oviduct. If sperm is not released, the egg is not fertilized and a haploid male is produced (Michener 2000). While drones are normally produced by the queen, laying workers have been reported in some Brazilian species of *Melipona* (Koedam et al. 2005, 2007). Although this is rare in Australian stingless bees (Michener 1974; Drumond et al. 1999; Tóth et al. 2004), *A. australis* and *A. symei* workers have been observed laying small numbers of trophic eggs in queenright colonies. On all recorded occasions the queen consumed those eggs (Drumond et al. 1999). Microsatellite analysis determined that workers were not responsible for drone production in queenright colonies of *A. australis*, *A. symei*, or *T.* (*Heterotrigona*) *carbonaria* (Drumond et al. 2000; Gloag et al. 2007). Drone production has been observed in some queenless colonies (Klumpp 2007; MH, personal observation); however, this has not been studied in sufficient detail.

3.4 Characteristics of Australian Stingless Bees

3.4.1 Austroplebeia

Nine species of *Austroplebeia* are listed in the Zoological Catalogue of Australia (Cardale 1993), and the most commonly domesticated and studied species are *A. australis* and *A. symei*. Species descriptions for this genus are inadequate for effective identification and no working key exists at present. Ongoing research in the areas of molecular, morphological, and morphometric analysis suggests that there are only 3–6 species of *Austroplebeia* in Australia (Halcroft and Dollin, unpublished data). Only one of these, *A. cincta*, occurs outside Australia, in Papua New Guinea (PNG) (Moure 1961; Rasmussen 2008).

Current classification is based mainly on variations in body markings. Mature adult bees are black, with varying levels of cream/yellow markings on the scutellum of their thorax and on their face (Michener 2000). Bees measure between 3.5 and 4.5 mm, and species characteristics are presented in Table 3.1 (Michener 1961; Dollin 2010a).

3.4.1.1 Natural Distribution

Dollin (2010b) found that *Austroplebeia* occurs throughout northern Australia (Fig. 3.2). *A. australis* and *A. symei* have the widest distribution. Specimens currently considered to be *A. symei* have been collected along the east coast from Cape York (11°04′ S) to Kilcoy in Queensland (26°57′ S) as well as the northern areas of the Northern Territory. *Austroplebeia australis* is found coastally, as far south as Kempsey, New South Wales (31.08ºS, 152.82ºE, elevation 10 m) and inland near Inverell, New South Wales (29.46°S, 151.06°E, elevation 584 m) and also occurs in arid regions of inland Queensland. The remaining species are found mainly in northern Queensland, Northern Territory, and Western Australia, with *A. percincta* originally described from an arid region of central Australia (Cockerell 1929).

While *Trigona* (*s.l.*) is commonly found in areas of high rainfall, many *Austroplebeia* thrive in areas that experience low annual rainfall (300–600 mm) and extreme temperature ranges (3–40.5°C) (A. Dollin, 2009, personal communication; BOM 2009). Until recently, it was thought that *Austroplebeia* were more sensitive to low temperatures, resulting in their northerly restricted distribution. Current research has revealed that colonies of *A. australis* are able to survive subzero temperatures, without actively thermoregulating the nest. These colonies were shown to contain developing brood throughout the year (Halcroft, unpublished data).

3.4.1.2 Nest Architecture, Colony Population, and Brood Structure

Similar to *Trigona* (*s.l.*), *Austroplebeia* chooses tree hollows, but cavity diameter is usually smaller. *A. australis* is found in cavities 50–110 mm in diameter (Halcroft,

Table 3.1 Explanation of color markings used to classify species in the genus *Austroplebeia* (Cardale 1993; Dollin 2010a,b,c)

Species name	Native range	Description	Markings
Austroplebeia symei (Rayment 1932)	Qld and NT	4.5 mm, darkest with little or no markings on the face and thorax	
A. australis (Friese 1898) *A. cassiae* (Cockerell 1910)	Qld and NSW	4 mm, four distinct cream markings on the scutellum. Minimal facial markings	
A. cockerelli (Rayment 1930)	NT	3.5–4 mm. Facial markings more extensive but vary in degree. Broad cream markings on thorax, mesothorax narrow stripes each side	
A. essingtoni (Cockerell 1905)	NT		
A. ornata (Rayment 1932)	Cape York, Qld		
A. percincta (Cockerell 1929)	Central NT		
A. websteri (Rayment 1932)	WA		
A. cincta (Mocsary, in Friese 1898)	PNG and possibly Qld	3.5 mm. Distinct facial and thoracic markings	

Dark markings represent cream/yellow markings on black bees

unpublished data). A smaller species found near Normanton, Queensland, may occupy cavities in coolabah (*Eucalyptus coolabah*, Myrtaceae) trees with a diameter of only 35 mm (A. Beil, 2009, personal communication). Some colonies of *A. australis* have been found in narrow tree limb hollows up to 6 m in length (R. Zabel, 2008, personal communication). A recent nest survey conducted in south-east Queensland showed that dead trees comprised over 87% of nest cavities chosen by *Austroplebeia* in that area (M. Halcroft, unpublished data).

Estimates of colony populations in *Austroplebeia* have not been studied in detail; however, recent studies have shown that, within natural nests, brood

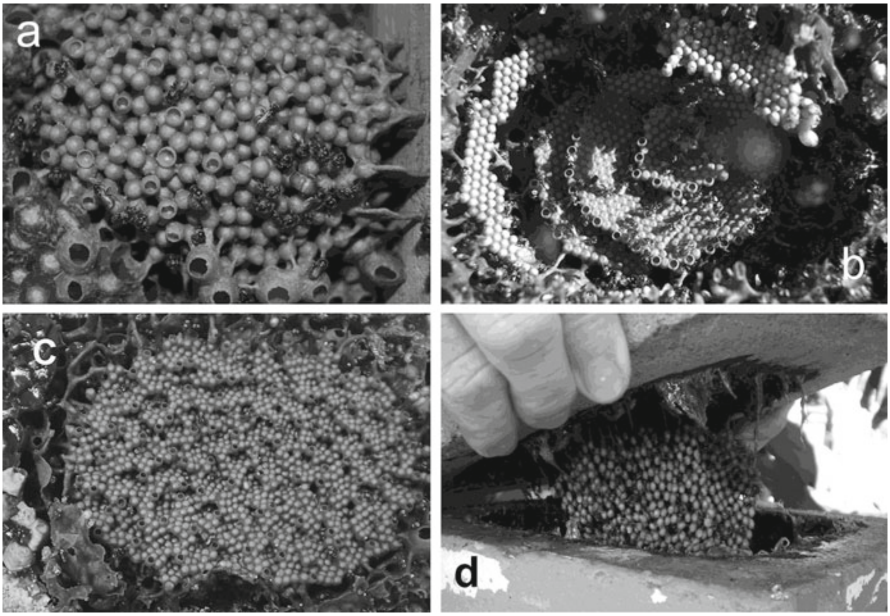

Fig. 3.6 Australian stingless bee brood structures. (**a**) *Austroplebeia australis* (**b**) *Trigona carbonaria*, (**c**) *Trigona hockingsi*, (**d**) *Trigona clypearis.* Photos: (**a**–**b**) M. Halcroft, (**c**–**d**) R. Brito

populations can range from 2,000 to 13,000, averaging of 5,000 (M. Halcroft, unpublished data). All *Austroplebeia* construct spherical brood cells and, with the exception of *A. cincta* (see Table 3.1), make simple cell clusters (Michener 1961; Dollin 2010a) (Fig. 3.6a). Open cells face outwards from the leading edge of the cluster, in irregular directions. Clustered brood cells can be constructed to fit into the narrow, irregular cavities of the smaller trees or large limbs favored by *Austroplebeia*.

The New Guinea species, *A. cincta*, is the only *Austroplebeia* found outside Australia (Moure 1961). Recently, however, some colonies resembling *A. cincta* have been found in Queensland (Dollin 2010a). Nests of these newly discovered colonies have not been examined, and studies are in progress. Unfortunately, no photographs of *A. cincta* nests or brood structures are currently available.

3.4.2 *Trigona (s.l.)*

Identification of Australian *Trigona* (*s.l.*) is very difficult in the field. Some species, especially *T. carbonaria*, can vary considerably in size according to geographic location (Dollin et al. 1997). The largest bee is *T. hockingsi*, measuring approx. 4.5 mm in length, while the smallest is *T. clypearis*, 3.5 mm in length (Klumpp 2007). Species within the carbonaria species group are difficult to separate on their body size or morphology. Thus, nest architecture is an invaluable tool in the accurate identification of species (see "Nest and brood architecture").

The currently described Australian *Trigona* (*s.l.*) are classified into three species groups (Dollin et al. 1997; J. Klumpp, 2010, personal communication; A. Dollin, 2010, personal communication), namely:

- Iridipennis group Sakagami 1978
  - *T.* (*Heterotrigona*) *clypearis* Friese 1908
- Laeviceps group Sakagami 1978
  - *T.* (*Heterotrigona*) *sapiens* Cockerell 1911
- Carbonaria group Dollin et al. 1997
  - *T.* (*Heterotrigona*) *carbonaria* Smith 1854
  - *T.* (*Heterotrigona*) *hockingsi* Cockerell 1929
  - *T.* (*Heterotrigona*) *mellipes* Friese 1898
  - *T.* (*Heterotrigona*) *davenporti* Franck 2004

3.4.2.1 Natural Distribution of *Trigona* (*s.l.*) in Australia

Dollin et al. (1997) report that *T. clypearis* and *T. sapiens* are restricted to the Cape York Peninsula in northern Queensland (18°0′ S–10°56′ S) compared to the carbonaria species group, distributed throughout northern and eastern Australia. The most recently described *Trigona* (*s.l.*), *T. davenporti*, was discovered by Peter Davenport, a local beekeeper who helped to pioneer stingless beekeeping in Australia (Klumpp 2007; Dollin 2010c). So far, this species has only been reported within a restricted area around the Gold Coast in south eastern Queensland (A. Dollin, 2008, personal communication). *T. carbonaria* is the most widely distributed species, occurring along much of the east coast of Australia. It is found as far north as the Atherton Tablelands in Queensland (17°15′ S) and as far south as Bega, in New South Wales (36°40′ S) (Fig. 3.2). *Trigona carbonaria* chooses large tree cavities that may provide superior insulation against the weather extremes experienced in its most southerly locale. Tse (unpublished data) found that both *T.* (*Heterotrigona*) *carbonaria* and *T.* (*Heterotrigona*) *hockingsi* maintain the brood chamber at significantly higher temperatures than the nest cavity or ambient temperature. These studies were not, however, conducted during periods of temperature extremes and further studies would be beneficial to better understand temperature regulation, especially by *T.* (*Heterotrigona*) *carbonaria*.

3.4.2.2 Nest Architecture, Colony Population, and Brood Structure

Tree cavities are the most commonly chosen nest substrate for *Trigona* (*s.l.*) in Australia. They can also be found inside water meter boxes, stone walls, beneath concrete foot paths, and within door and wall cavities. Nest entrance modifications vary, depending on species; however, environmental factors such as weather and predators can also influence those structures (Dollin et al. 1997). *Trigona* (*Heterotrigona*) *carbonaria* often daub the area around the entrance with significant amounts of resin, whereas *T.* (*Heterotrigona*) *hockingsi* and *T.* (*Heterotrigona*) *davenporti* generally leave their entrances unadorned (Dollin 2010a). *Trigona* (*Heterotrigona*) *mellipes*, *T.* (*Heterotrigona*) *sapiens*, and *T.* (*Heterotrigona*) *clypearis* build entrance tubes of varying sizes (Table 3.2), although they do not always do so.

It has been estimated that a strong colony of *T.* (*Heterotrigona*) *carbonaria* has a population of approximately 11,000 workers (Hoffmann, unpublished data). Brood volume can vary 940–3,535 ml in *T.* (*Heterotrigona*) *carbonaria* and 1,100–2,550 ml in *T. hockingsi* (Dollin et al. 1997); however, *T.* (*Heterotrigona*) *hockingsi* is able to build much larger nests if provided with the appropriate nest cavity (A. Dollin, 2010, personal communication). Both *T.* (*Heterotrigona*) *davenporti* and *T.* (*Heterotrigona*) *hockingsi* build brood areas with similar structure; however, *T. davenporti* has a smaller adult population. *T.* (*Heterotrigona*) *mellipes*, *T.* (*Heterotrigona*) *sapiens*, and *T.* (*Heterotrigona*) *clypearis* have much smaller nests and average brood volumes measure 595, 224, and 464 ml, respectively (Dollin et al. 1997).

All Australian *Trigona* (*s.l.*) build elongated, vertically oriented brood cells in regular, or nearly regular, structures (Dollin et al. 1997). There are, however, distinguishing features within these structures that can aid in species identification. *Trigona* (*Heterotrigona*) *carbonaria* (Fig. 3.6b) builds single layers of comb, arranged in a horizontal spiral. Brood cells are constructed on the outer rim of up to three circular spirals, at a time. The spiral formation can be clockwise or counterclockwise. Brood construction can become erratic if the nest is disturbed, e.g., if the tree is felled (A. Dollin, 2010, personal communication). *Trigona* (*Heterotrigona*) *hockingsi* (Fig. 3.6c) builds a regular, horizontal brood structure with hexagonal comb, which is best described as terraced or stepped; it is not in a single layer. Both *T.* (*Heterotrigona*) *davenporti* and *T.* (*Heterotrigona*) *mellipes* build brood comb similar to that of *T.* (*Heterotrigona*) *hockingsi*; however, the brood comb area of *T.* (*Heterotrigona*) *mellipes* is considerably smaller (J. Klumpp, personal communication). Neither *T.* (*Heterotrigona*) *sapiens* nor *T.* (*Heterotrigona*) *clypearis* (Fig. 3.6d) have a hexagonal comb structure because individual cells are arranged irregularly, in horizontal or diagonal layers.

Table 3.2 Comparative description of nest entrance characteristics within *Trigona* and *Austroplebeia* species

Species	Average entrance tube length (mm)	Average nest cavity diameter (mm)	Brood structure
T. (*Heterotrigona*) *hockingsi*	None Seldom smear entrance with resin	145	Horizontal steps/terraces. Hexagonal comb
T. (*Heterotrigona*) *carbonaria*	None Smear entrance with resin +++	198	Flat spiral, single layer. Hexagonal comb
T. (*Heterotrigona*) *mellipes*	16	82	Similar to *T. hockingsi* but smaller
T. (*Heterotrigona*) *sapiens*	6	58	Irregular, horizontal, or diagonal layers. No hexagonal comb
T. (*Heterotrigona*) *clypearis*	28	78	Roughly arranged in diagonal rows No hexagonal comb
A. australis	None to ~20 mm	52–110	Clustered
A. ornata or *cockerelli*	None to ~20 mm	35	Clustered
A. cincta (PNG)	20–80	45	Irregular concentric layers of one cell thickness, with bee space between layers

Trigona species (Dollin et al. 1997; Klumpp 2007), *Austroplebeia* species (Halcroft and Dollin, 2010, unpublished data) and *A. cincta* (Michener 1961)

3.5 Behavior of Australian Stingless Bees

3.5.1 Guard and Forager Behavior

Australian *Trigona* (*s.l.*) colonies usually employ at least 4–5 guards at the entrance (Yamane et al. 1995; MH, personal observation), with higher numbers occurring around the front of the nest on warm days (Klumpp 2007). Guards are not normally aggressive towards human onlookers; however, if the nest is opened workers can become moderately to strongly aggressive (Michener 1961). *Austroplebeia* guards occur in small numbers within the entrance of the nest but they withdraw into the entrance tube if observed too closely. When colonies are opened, workers are not aggressive (Michener 1961), they buzz around the heads of human "predators" and daub their hair with globules of resin until the nest is sealed (MH, personal observation).

Australian *Trigona* (*s.l.*) have evolved mostly in high rainfall areas (Fig. 3.2), which provide consistent, reliable floral resources. *Austroplebeia*, on the other hand, have evolved mainly in arid regions, with evidently unreliable resources (Fig. 3.2). Based on detailed observations, *T.* (*Heterotrigona*) *carbonaria* and *T.* (*Heterotrigona*) *hockingsi* workers appear to be "curious and flighty," whereas *A. australis* and *A. symei* are "shy and cryptic." In 2009 (M. Halcroft, unpublished data) a parallel study was conducted to compare foraging behavior and energy efficiency of three Australian stingless bees: *T.* (*Heterotrigona*) *carbonaria*, *A. australis*, and *A. symei*. The following information is based on this study. When provided with the same floral resources, *T.* (*Heterotrigona*) *carbonaria* sent out nine times as many foragers as *A. australis* and four times as many as *A. symei*. Even when the floral resources were completely depleted, *T.* (*Heterotrigona*) *carbonaria* continued to send foragers from the nest, while *Austroplebeia* colonies ceased to do so. This study also showed that *T.* (*Heterotrigona*) *carbonaria* foragers spend over 30% of their foraging time hovering in close proximity to flowers, before finally alighting to collect pollen or nectar (Fig. 3.7). Conversely, *A. australis* and *A. symei* spend over 90% of their foraging time exploring flowers and collecting pollen and nectar, while only 10% of their time is spent in flight between flowers.

3.5.2 Austroplebeia: Adapted to the Harsh Australian Outback

Floral resources in the Australian outback are often unreliable. Regions may experience periods of drought that can last 1–4 years (BOM 2010b) (Fig. 3.8). Alternatively, they can also experience occasional extensive flooding. Colonies of *Austroplebeia* have presumably evolved and adapted in order to survive such conditions. These behavioral adaptations ensure surviving nestmates exist within the colony after the drought has broken and a long-awaited floral bloom arrives.

Austroplebeia australis is an extremely long-lived worker bee, with a mean maximum worker longevity of 161.4 ± 6.1 days and a maximum longevity of 240 days

Fig. 3.7 *T. carbonaria* forager hovering near a citrus flower. Photo: M. Halcroft

Fig. 3.8 Arid native range of *A. australis*, Tara Queensland. Photo: M. Halcroft

(M. Halcroft, unpublished data). The colonies forgo a "high rate of living" when floral resources are unavailable. Only small numbers of foragers (4 returning/2 min) are recruited during times of limited floral resources, whereas recruitment greatly increases (250 returning/2 min) during floral abundance (A. Beil, personal communication; M. Halcroft, unpublished data). Colonies have also been observed closing their nest entrance with a resin curtain during periods of dearth (MH, personal observation; A. Beil, personal communication), presumably reducing the need to guard the nest entrance.

Many nest sites chosen by *Austroplebeia* are within dead trees (see "Nest and brood architecture"), which provide no canopy protection against frosts in winter or

Fig. 3.9 Typical dead tree chosen by *A. australis* colonies. Colonies in Tara, Qld, being sampled for further studies. Photo: S. Ruttley

searing heat in summer (Fig. 3.9). While the insulation of natural logs is superior to that of most artificial hives, exposed trunks and limbs still allow temperature extremes to penetrate (R. Luttrell, unpublished data). Under such circumstances, it might be expected that *Austroplebeia* has developed thermoregulatory mechanisms. This, however, is, not the case and studies have shown that *A. australis* brood temperatures parallel those of the empty nest cavity and the ambient conditions (M. Halcroft, unpublished data). Prior to the onset of the cold season, colonies begin constructing a layer of involucrum over the brood, on top of which honey pots are built and filled. Those structures provide some level of protection, as the brood beneath remains undamaged. Colonies that have not been prepared for cold exposure suffer chill damage and brood death (MH, personal observation). Brood can survive at temperatures as low as −1°C (although larval development is probably delayed) and as high as 38°C, indicating the possible development of physiological resistance to temperature extremes (Halcroft, unpublished data). *Austroplebeia australis* colonies do not become broodless during the cold winter months, although they build a smaller number of brood cells during this time. The bees may be stimulated to build brood during the winter months when the colony is artificially warmed and provided with supplemental food (Halcroft 2007).

Austroplebeia australis, and possibly other *Austroplebeia* species, have evolved in the unforgiving environmental conditions of arid inland Australia. Their ability to conserve energy through improved foraging efficiency and thermoconformity, and by reducing workers' exposure to high-risk activities and high rates of living, has resulted in a well-adapted and resilient bee species. It is not only capable of surviving conditions most other species could not; it thrives in them.

3.6 The Australian Stingless Bee Industry

The Australian stingless beekeeping industry is still very much in its infancy, especially when compared to many South American countries. However, comparative surveys conducted in 1998 (Heard and Dollin 2000) and 2010 (Halcroft, unpublished data) show the industry is expanding and developing. Information provided below is based upon data compiled in 1998 and 2010.

In recent years there has been growing interest in Australian native bees, especially stingless bees. The honey and other hive products support an industry that has grown from 257 beekeepers in 1998 to 637 in 2010. Half of them owned just one hive and, in 2010, a quarter had less than 3 years of experience. The number of hives owned by the 637 beekeepers totally almost 5,000. Over two-thirds of the beekeepers maintain their hives on suburban blocks, although many of them also live near some form of remnant natural vegetation or "bushland." The most commonly kept bees are *T.* (*Heterotrigona*) *carbonaria*, *T.* (*Heterotrigona*) *hockingsi*, *A. australis*, and *A. symei*. In 2010, all but three survey respondents resided in New South Wales and Queensland.

Enjoyment and conservation were, by far, the most popular reasons for keeping stingless bees. The pollination of nearby vegetable and flower gardens, as well as bushland, was reported to be of considerable benefit. Only eight respondents provided pollination services on a professional basis (see "Pollination").

3.6.1 Colony Production

Australian stingless bee-keepers use a variety of hive designs ranging from a simple, wooden box to a complex, insulated (or even heated), PVC-constructed, cylindrical hive. The most commonly used hive is based on the original Australian *Trigona* (*s.l.*) hive (OATH) design (Dollin 2002; Klumpp 2007) and has a capacity of 6–7 L. Most hives are constructed so that they can be divided into two equal sections. Colony propagation techniques and hive design are discussed briefly here, because these topics are detailed elsewhere (Klumpp 2007; Dollin and Heard 2010; Heard 2010).

Colony propagation of *Austroplebeia* is easier than for *Trigona* (*s.l.*). Small sections of brood containing a queen cell can be removed from an *Austroplebeia* colony

Fig. 3.10 Splitting OATH box with *T. carbonaria* colony. Photo: T.A. Heard

and placed in a small hive, together with food stores and workers (A. Beil, personal communication; MH, personal observation). Queenright colonies with as few as 200 workers can survive and build strong colonies, if provided with the right conditions, which may include supplemental warmth and feeding (MH, personal observation). *Austroplebeia* colonies can also be strengthened during winter if maintained in artificially warmed rooms and provided with supplemental food (Halcroft 2007). *Trigona* (*s.l.*) species, on the other hand, are more particular. Colonies need to be very strong before they are divided for propagation.

Propagation involves dividing the brood mass or inducing colony "budding." The quickest and, therefore, the most popular technique is "splitting," and the success of a division is dependent upon the strength of the mother colony. Colonies (not including the hive or box) should weigh at least 2 kg or 3 kg for those kept in the cooler southern regions (Klumpp 2007). The hive, containing the brood and nest structures, is split horizontally into two sections, and the occupied sections are united with new, empty half-boxes (Heard 1988a) (Fig. 3.10). This results in two half-filled hives, one with a queen (mother colony) and one with several developing queen cells. Colony survival is dependent on adequate worker number for nest repair, foraging, and defense, and the ability of the daughter colony to successfully "re-queen" (Klumpp 2007).

Colony budding is a noninvasive form of hive propagation. An empty hive is attached to the nest entrance of an existing colony via a black polyethylene pipe.

This technique is often used when the nest is located in an inaccessible structure, such as a wall or living tree (Klumpp 2007). An observation lid on top of the hive box is required to monitor progress of the "budded" colony. The colony provisions the attached hive, and after several weeks or, more likely several months, a virgin queen leaves the colony and mates. If successful, she returns to the "front hive" and begins laying eggs within several days. At the same time, the beekeeper intervenes by creating an opening in the connecting tube to allow foragers from the mother colony direct access to the nest. Eventually, the tube is disconnected or the bees close the connection themselves (Klumpp 2007). This technique is ideal for those beekeepers who wish to increase their colony number but are not confident with the splitting technique. It does, however, require considerable patience.

The number of beekeepers involved in hive propagation has doubled. Those practicing hive division have increased colony number eightfold since 1998. More than 8,000 colonies have been produced. The number of beekeepers who sell colonies has doubled, while the number of colonies sold each year has more than quadrupled. Although this development sounds impressive, the overall annual increase in colony number since 1998 is only 9% (Halcroft, unpublished data). The retail value of a strong stingless bee colony in Australia has increased from $AU200 to between $AU350 and $AU450 per hive. Demand is high and many producers report that they are unable to keep up with demand.

3.6.2 Australian Stingless Bee Honey and Other Hive Products

Honey harvesting techniques vary. Often pots are removed from the hive and honey is squeezed through a cloth or sieve. Beekeepers in Queensland and northern New South Wales, where bees can forage all year round, are able to harvest approximately 1 kg/year per hive. Beekeepers who reside in the cooler, southern regions are only able to harvest every 2–3 years, and almost not at all if they are in the Sydney basin or farther south (A. Ashhurst, 2010, personal communication). It is recommended that honey not be harvested if hive propagation is being practiced, because colonies require good stores to rebuild their strength (Dollin 2002; Heard 2010). Beekeepers who produce honey on a large scale utilize a honey super on top of hives. The OATH has a honey super with a capacity of 1.5 L, and the following technique is used by Tim Heard and many other beekeepers.

The honey hive is fitted with a "floorless" super that sits on top of a thin hive ceiling, which separates the main nest from stored honey (Fig. 3.11). The separator provides access for the bees to all nest structures but still allows honey to be stored away from the brood. For easy, non-destructive honey harvest, the super is removed from the hive, exposing the intact pots (Fig. 3.12). Excess pollen stored in the super is cut out before harvest, to reduce contamination and the possibility of fermentation. The super is inverted and pots are pierced with a hand-tool similar to a small bed-of-nails. The super is placed over a plastic tray, into which fresh honey drains. After the honey is completely drained, the super is replaced and the bees can clean

Fig. 3.11 Honey super placed on top of OATH box, with separator in place. Photo: T.A. Heard

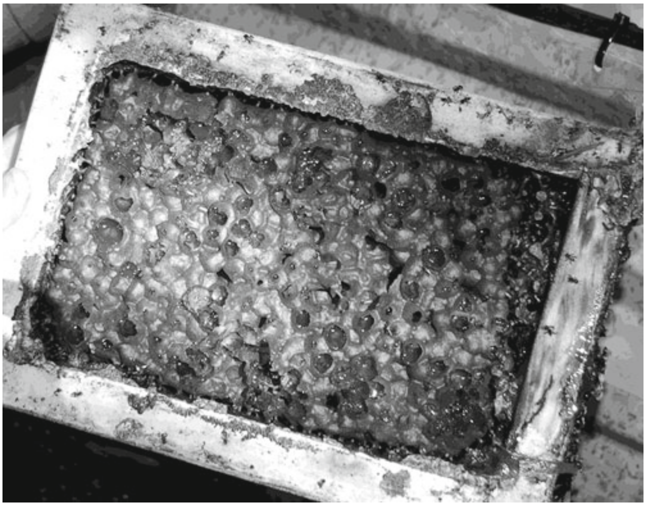

Fig. 3.12 Honey super filled with honey, ready for harvest. Photo: M. Halcroft

and rebuild the pots (Dollin 2002; Heard 2010). The only processing that occurs with sugarbag honey is straining out debris such as cerumen or bees.

Honey harvesting is carried out by a small number of Australian beekeepers and production is low. Although the number of beekeepers has more than doubled over the last decade, overall production of Australian sugarbag honey is <300 kg/year. Of the 63 beekeepers who stated they harvest honey, only five reported selling their product, and they accounted for approximately half of overall production (Halcroft, unpublished data).

Sugarbag honey caters to a "niche market" in Australia and its price is indicative of its rarity. The wholesale price has increased from $AU40/kg in 1999 to $AU70/kg in 2010; however, the retail price remains the same, approximately $AU160/kg (Heard 2010). In comparison, honey bee—*Apis mellifera*—honey sells for only $AU6.50/kg (Shaw 2010). Sugarbag honey is sold in local markets, restaurants, and via Internet, and two producers export to Japan (Halcroft, unpublished data).

Limited research has been conducted on Australian stingless bee honey, but *T. carbonaria* has similar specifications to those of other Meliponine (Persano Oddo et al. 2008). Preliminary studies on antioxidant and antimicrobial activities have shown some promise for nutritional and pharmaceutical uses (Irish et al. 2008; Persano Oddo et al. 2008; Boorn et al. 2010). *Trigona carbonaria* honey has a moisture content of around 26% (Persano Oddo et al. 2008) and should be stored in the refrigerator to avoid fermentation (Heard 2010).

The production of cerumen and resin supplies an extremely small market in Australia. Some beekeepers are able to sustainably harvest around 200 g of cerumen per hive each year. It is sold to "didgeridoo" manufacturers (see "Introduction"), artists, and hobbyists, for $AU5/25 g, which is sufficient to make up to four didgeridoo mouth pieces (Heard 2010).

3.6.3 Pollination

Pollination of commercial crops by stingless bees is rare in Australia and growers of over 35 commercial crops rely heavily on managed honey bee colonies (RIRDC 2007). Free pollination services are also provided by colonies of feral honey bees in Australia, with 40–150 colonies/km^2 present in some surveyed areas (Oldroyd et al. 1997). The Australian honey bee pollination industry is currently strong and reliable (RIRDC 2007); therefore, little funded research has been undertaken on native bees as alternative pollinators. However, the reliability of the honey bee industry is now in question because managed and feral bee populations are declining due to pests and disease, as well as possible pesticide problems. For example, between 2002 and 2006 more than 4,500 colonies died out due to African small hive beetle *Aethina tumida* Murray (Nitidulidae) infestation (Rhodes and McCorkell 2007). Australia is the only major country without varroa mite *Varroa destructor*, but the likelihood of an incursion has raised major concerns about the future reliability of the honey bee pollination industry in this country (RIRDC 2007).

A rapidly expanding almond *Prunus dulcis* (Rosaceae) industry in Australia has resulted in industrial migration of thousands of managed honey bee colonies, transported in from the northern regions for their pollination service. It is estimated that the almond industry requires one-half of all managed honey bees in the eastern states of Australia (RIRDC 2010). This continued development, together with the predicted overall reduction in colony number, is likely to stimulate increasing interest in alternative pollinators in the warmer regions of northern New South Wales and Queensland, from which many honey bee colonies will be taken. There is already a small group of stingless bee-keepers that provide pollination services in these regions.

The Australian stingless bee pollination industry had its beginnings in the late 1980s when it was found that yields of macadamia nut *Macadamia integrifolia* (Proteaceae) grown near remnant native vegetation were noticeably higher than for crops situated in cleared land (Heard 1988a; Heard and Exley 1994). The main pollinators of macadamia are honey bees and stingless bees (Vithanage and Ironside 1986), and presence of these insects is extremely important for maximum seed set (Wallace et al. 1996). Although the temperature threshold for *Trigona* (*s.l.*) flight activity is 18°C (Heard and Hendrikz 1993), resulting in shorter foraging days compared to honey bees (7 vs. 10 h/day, Heard and Exley 1994), *Trigona* (*s.l.*) are superior pollinators of macadamia flowers. Their small bodies are able to make more intimate contact with stigmata while collecting pollen (Heard 1994), thus aiding pollen transfer.

Trigona carbonaria are opportunistic foragers that use group strategies to independently search for resources and rapidly recruit nest mates once rewards are located. Foragers demonstrate floral constancy (White et al. 2001) and resources are harvested, often by groups of bees, until they are depleted (Bartareau 1996). Few studies have been carried out on Australian stingless bee communication. Bartareau (1996) reports that *T.* (*Heterotrigona*) *carbonaria* foragers leave a marker of glandular secretions near the food resource, but do not leave scent trails from the resource to the nest (Nieh et al. 2000). Heard (1987) also demonstrated that *Trigona* (*s.l.*) foragers returned to hives with 100% macadamia pollen, compared to honey bees, carrying only 24%. Interestingly, *Trigona* (*s.l.*) prefer warm flowers (Norgate et al. 2010) and this is demonstrated by their attraction to flowers on outer, sun drenched racemes (Heard and Exley 1994). Macadamia also benefit from varietal interplanting for cross-pollination (Rhodes 1986) as their flowers are mostly self-incompatible and protandrous (providing pollen before stigmata are receptive) (Sedgley et al. 1985).

Heard (1988b) developed a technique whereby colonies could be transferred into artificial hives for use in managed crop pollination. The use of stingless bees for pollination of macadamia has grown since then, and several macadamia farmers have purchased their own hives to improve crop yield. Some growers were originally honey bee keepers, but found it easier to move small *Trigona* (*s.l.*) hives to their macadamia crops, which are often grown on steep slopes. Those farmers have since become reputable stingless bee-keepers in their own right (F. Adcock, personal communication). The demand for stingless bee pollination service by the macadamia nut industry, as well as other crops, is growing. At present, there are not enough hives available to meet this demand (M. Grosskopf, 2010, personal communication). Further effort is required to improve colony propagation. This would ensure that enough colonies are available in the future for suitable stocking rates and satisfactory pollination service (T. Carter, personal communication).

It is estimated that Australian stingless bees have an average flight range of only 500 m (Heard and Dollin 1998). This is advantageous for crop pollination, because bees are more likely to forage within the crop area than to venture farther afield in search of other floral resources, as is often the case with honey bees (Graham 1992). Hive placement is important, and the 15–20 hives per hectare (compared to seven honey bee hives per hectare) should be interspersed throughout the crop if possible, especially if cross-pollination is required (Heard and Dollin; F. Adcock, personal communication; T. Carter, personal communication).

Crops other than macadamia can also benefit from stingless bee pollination. Anderson et al. (1982) showed stingless bees to be effective pollinators of mango (*Mangifera indica*; Anacardiaceae) and anecdotal accounts of increased crop quality and yield have been reported for other crops such as lychee *Litchi chinensis* (Sapindaceae), avocado *Persea americana* (Lauraceae), and watermelon *Citrullus lanatus* (Cucurbitaceae) (T. Carter, personal communication). Although no scientific studies have been conducted on the effectiveness of stingless bees as pollinators in Australian crops other than macadamia and mango, estimates of improved crop yield have been assessed by one beekeeper. Stingless bees have also been introduced into blueberry (*Vaccinium corymbosum*; Ericaceae) and bees are able to collect pollen and nectar more efficiently than honey bees (F. Adcock, S. Maginnity, M. Grosskopf, personal communication). Blueberry flowers are small, with a deep corolla and narrow terminal orifice (Rhodes 2006). Unfortunately, there is no experimental design or statistical analysis associated with these trials. Although the role of stingless bees in pollination of native flora is well documented, their efficacy in horticultural and agricultural crops of Australia needs further study (Heard 1987; Heard 1999; Slaa et al. 2006).

Of the eight beekeepers who reported that they provided pollination services on a professional basis during 2010, only four charged a service fee. One beekeeper charged only $AU10 per hive, while the other three charged $AU35–40 per hive (Halcroft, unpublished data).

3.7 Management Issues

3.7.1 Pests of Australian Stingless Bees

Australian stingless bees seem to be relatively disease-free and no reports of brood disease have been seen. They do, however, suffer from predation, parasitism, and colony infestation. There are many general predators such as flies, ants, spiders, mites, wasps, birds, lizards, toads, and, of course, humans, which are common pests of social bees worldwide. Australia has its own unique species of stingless bee parasites and predators; however, little is known about most species. Usually, strong hives

Fig. 3.13 Syrphid fly adult. Photo: J. Klumpp

are at minimal risk of hive invasion but weakened or newly propagated colonies are vulnerable to attack from pests. It is of critical importance, when managing colonies, that all means of access to the nest cavity are well sealed and that colonies are divided or transferred as quickly and efficiently as possible, to minimize pest infestation.

One of the most serious pests of stingless bee colonies in Australia is the syrphid fly *Ceriana ornata australis* Macquar. *Ceriana ornata* is 12 mm long, with bright orange-yellow and black markings (Fig. 3.13) and is frequently observed hovering near nests during summer (MH, personal observation). This fly has been observed in all regions where stingless bee colonies are present and is seldom observed in areas where stingless bees do not occur (Klumpp 2007). *Ceriana ornata* is most destructive when colonies are divided or damaged. The female lays eggs directly on nest structures if the hive is left open and unattended, or eggs are laid in unsealed joints or cracks in the hive surface. The eggs hatch and the larvae (Fig. 3.14) make their way into the nest cavity and food stores. If fly larval numbers are high the colony will die as stores and immatures are consumed.

The phorid fly *Dohrniphora trigonae* Disney can also cause problems in Australian stingless bees, especially *Trigona* (*s.l.*) species (Disney and Bartareau 1995), similar to phorid fly pests overseas (genus *Pseudohypocera*).

D. trigonae lays its eggs within the colony stores and are most problematic following colony division (Klumpp 2007; Dollin and Heard 2010). These tiny flies (2.5 mm) (Fig. 3.15) enter nests more easily than the larger syrphid fly and can do so in high numbers (Klumpp 2007). Once inside, flies run along the surface of the structures, laying eggs in honey and pollen pots. This pest is less of a problem in Australia than overseas.

Stingless bee predators that are unique to Australia include *Bembix flavipes* Smith and *Bembix musca* Handlirsch (Crabronidae) (Fig. 3.16). These *Bembix* hunt singly and hover outside the entrance, waiting for bees to exit. Once a bee leaves the

Fig. 3.14 Syrphid fly larvae in nest of dead colony. Photo: M. Halcroft

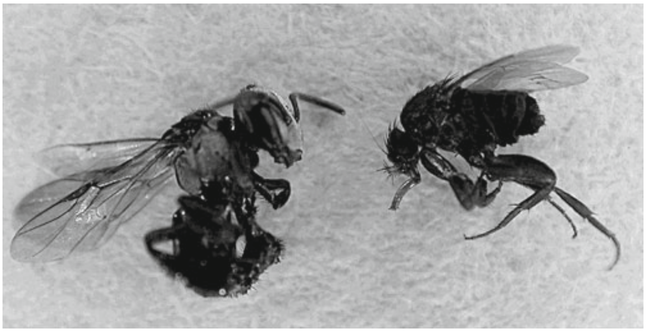

Fig. 3.15 *Trigona* worker (*left*) beside a phorid fly (*right*). Photo: J. Klumpp

Fig. 3.16 *Bembix* wasp. Photo: J. Klumpp

Fig. 3.17 (**a**) Braconid wasp (*right*) lying in wait near *Trigona* foragers. (**b**) Braconid wasp (*left*) preparing to oviposit into *Trigona* forager. Photos: J. Klumpp

nest the wasp swoops from behind the unsuspecting worker and drags it to its own nest (A. Beil, 2009, personal communication). Evans et al. (1982) observed mass provisioning of *B. flavipes* nests with over 25 freshly collected *Austroplebeia*, and *B. musca* provision nests with *T.* (*Heterotrigona*) *carbonaria*. Drones are the main prey during the stingless bee mating season (Evans and O'Neill 2007).

The only known parasitoid of Australian stingless bees is the braconid wasp (*Syntretus trigonaphagus*) (Gloag et al. 2009). *Syntretus trigonaphagus* has only been reported in the Brisbane area. The distribution of its host, *T. carbonaria*, suggests that it may be found more widely along the east coast of Australia. Wasps wait near the hive entrance or on flowers where bees are foraging (Fig. 3.17). When close enough to the posterior of an individual bee the wasp projects her abdomen under and in front of hers and oviposits onto the abdomen of the bee. The hatching larva grows inside the abdomen of the living bee, for an unknown period of time. Parasitized bees are easily identified because the abdomen is 2–2.5 times larger than that of normal bees. The fully developed larva emerges from the bee, which then usually flies away, presumably to die. Gloag et al. (2009) were unable to successfully rear pupae from emerged larvae and it is thought that pupation may take place in the soil (Klumpp 2007).

Australian native beetles in the genus *Brachypeplus* (Coleoptera: Nitidulidae) have been observed in stingless bee hives (MH, personal observation) although it is thought they are not a major problem for strong colonies (A. Dollin, 2010, personal communication). Adult beetles are commonly observed on the outer surfaces of hives. These beetles are smaller and more slender than the worker bees (Fig. 3.18) and are, therefore, able to gain access through unsealed cracks and joints in hives. They lay eggs in inaccessible cracks and crevices within the hive. Beetle larvae have not been observed in high numbers within hives and the main signs of their presence are the accumulation of dry debris in the bottom of the hive and a reduction in worker bee number (MH, personal observation). Little is known about these native beetles and it is unclear what the larvae consume within the stingless bee nests. Pupating beetle larvae have been observed under the transparent lid of *A. australis*

Fig. 3.18 Adults and larva of *Brachypeplus* sp. beside an *A. australis* worker. Photo: M. Halcroft

Fig. 3.19 Adult small hive beetles beside an *A. australis* worker. Photo: M. Halcroft

hives, indicating that these beetles can complete their life cycle within the nest (MH, personal observation).

The African small hive beetle (*Aethina tumida* Murray; Nitidulidae) is a newly introduced honey bee pest in Australia (Fig. 3.19) and can devastate newly divided, or damaged, stingless bee colonies. Adult beetles are frequently found near hives (MH, personal observation) and enter nest openings whenever possible. If left unchallenged, the beetle lays eggs in food stores and brood. The larvae hatch and begin feeding, defecating throughout the nest, and cause hive contents to ferment. Eventually the entire colony collapses into a slimy mass. As with syrphid fly invasion, strong colonies are usually able to remove larvae from an infested nest, but prevention is always better than cure. A strong, undamaged colony can defend against small hive beetle invasion, and studies have shown that *T.* (*Heterotrigona*) *carbonaria* can incapacitate invading adult beetles within 10 min of being introduced to the nest entrance (Greco et al. 2010). Halcroft et al. (2011) showed that *A. australis* was effective in removing or destroying all life stages (eggs, larvae and adults) from hives and that efficiency in entrance defense and invader removal increased with frequency of exposure to beetle invasion. Both *T.* (*Heterotrigona*) *carbonaria* and *A. australis* utilize resin to entomb adult

Fig. 3.20 Adult small hive beetle entombed alive in cerumen while an *A. australis* worker guards the interloper. Photo: M. Halcroft

beetles within the nest (Fig. 3.20). *Austroplebeia australis* later dismembers the remains and removes them from the nest.

Another exotic predator of Australian stingless bees is the cane toad (*Rhinella marina*—formerly known as *Bufo marinus* Linneaus; Bufonidae). Introduced into Australia in 1935 as a biological control agent for the pest cane beetle this highly toxic pest has spread from coastal northern Queensland to the central coast of New South Wales and across northern Australia to Kakadu National Park in Northern Territory (Australian Museum 2010). Cane toads are quite ingenious and may stand on each other's backs in order to reach bee hive entrances. The toad will stay at the hive entrance and consume incoming and outgoing workers until forager numbers dwindle, to the point that the colony may be in danger of perishing (R. Zabel, personal communication) (Fig. 3.21).

3.7.2 Seed Dispersal by Stingless Bees

Corymbia torelliana F. Mueller (Myrtaceae), or cadaghi tree, as it is commonly known, is native to the rainforest margins of the Atherton Tablelands in northern Queensland. This species of Australian gum tree has spread extensively outside its native range as it has been used for plantation timber. Its abundant blooms (Fig. 3.22) and showy gum nuts (Fig. 3.23) have also resulted in it being used in street plantings and parks, especially in the Brisbane area (AWC 2010). Although it is a source of abundant pollen and nectar in spring, it has become a major management problem for some stingless bee-keepers.

Corymbia torelliana seeds are mainly dispersed by gravity; 88% of seeds drop to the ground soon after the fruit opens. However, one or two seeds remain within the gum nut and all are dispersed by *Trigona* (*s.l.*) (Wallace et al. 2008).

Fig. 3.21 Cane toad waiting at the entrance of a stingless bee hive. Photo: R. Zabel

Fig. 3.22 *C. torelliana* flowers are an abundant pollen and nectar source. Photo: J. Klumpp

Resin is produced in the gum nut, behind the valve (Fig. 3.24). When the bee enters the nut to collect resin, the seeds attach to the sticky corbicular load (Fig. 3.25). Seeds are dispersed by bee vectors, or "mellitochory," and may be spread during the flight back to the nest or transported to the nest itself. This may be up to 1 km away from the tree (Klumpp 2007; Wallace et al. 2008; A. Beil, R. Luttrell, J. Klumpp, personal communication). *Trigona* (*s.l.*) are strongly attracted to the resin from *C. torelliana* and the colonies stop normal foraging

Fig. 3.23 The attractive gum nuts of *C. torelliana* make it an ideal amenities tree. Photo: J. Klumpp

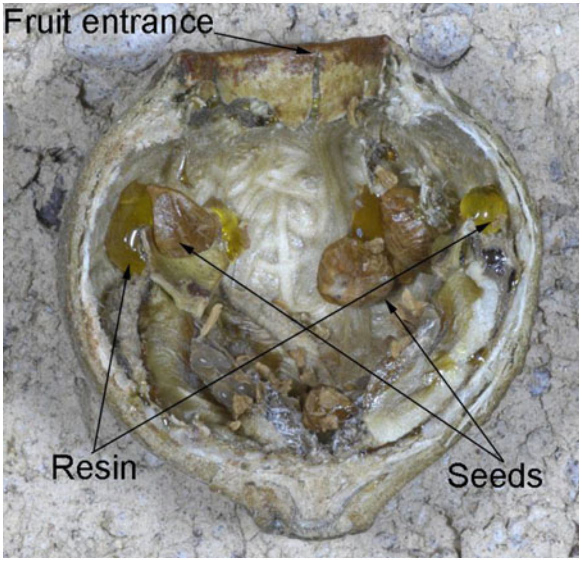

Fig. 3.24 Cross section of *C. torelliana* fruit, showing seeds and resin in close proximity. Photo: R. Luttrell

activity to collect as much of this resource as possible (Klumpp 2007). *Trigona* (*s.l.*) are known to collect and store large amounts of resin, with up to 10% of foragers returning with resin loads (Wallace and Lee 2010). Analysis of the chemical profiles of body surfaces of five *Trigona* (*s.l.*) species showed that 51%

Fig. 3.25 *Trigona* forager on a *C. torelliana* fruit, with a seed adhered to her corbicular load. Photo: R. Luttrell

of these compounds were derived from plant resins. Conversely, *Austroplebeia*, which collect only small amounts of plant resin, had little or no resinous compounds on their bodies (Leonhardt et al. 2010).

As foragers return to the nest some attempt to dislodge seeds on the nest exterior, while others transport seeds directly into the nest cavity. The colony removes some, but not all, of the introduced seeds and these are either disposed of, up to 10 m outside the nest (Wallace and Trueman 1995), or adhere to the sticky surface of the nest entrance (Wallace et al. 2008) (Fig. 3.26). Seeds collect around the entrance (Wallace and Trueman 1995), reducing airflow within the nest. Resin from *C. torelliana* may have a lower melting point than many other plant resins. Collection of the resin and its seed occurs during the hottest months of the year in Australia—December to February (Wallace and Lee 2010); and as temperatures rise, the resin begins to soften. Reports of structural collapse due to seed weight and resin softening are not uncommon, particularly if ambient temperatures exceed 39°C (J. Klumpp, M. Duncan, 2006, personal communication). As a result, some beekeepers remove their hives from *C. torelliana* areas during resin flow to prevent colonies from collecting the resin and seed mixture (Klumpp 2007; T. Carter, 2010, personal communication). While many Australian beekeepers consider *C. torelliana* to be a major management problem, others consider it to be a useful source of pollen, nectar, and resin (Klumpp 2007).

3.7.3 Fighting Swarms

Nest defense is widely reported in stingless bees around the world. Incapacitation of intruders is achieved by biting, resin daubing, chemical repellents, and locking onto the wing or body with their mandibles, thus grounding invaders and rendering them harmless (Roubik et al. 1987; Wittman et al. 1990; Lehmberg et al. 2008; Halcroft

Fig. 3.26 *C. torelliana* seed collection around the entrance of a *Trigona* nest entrance. Photo: R. Luttrell

et al. 2011). Nest defense against conspecifics is, however, more specialized and involves recognition of nestmates from non-nestmates, using recognition cue compounds (Buchwald and Breed 2005). *Trigona carbonaria* demonstrate a collective defense behavior known as a “fighting swarm,” during which time hundreds to thousands of workers, usually from two colonies, become entwined in an aerial battle, to the death. The fight takes place outside the defending nest and may result in the usurpation of the defending colony. In flight, two workers lock together by biting each other and immediately drop to the ground. Sometimes the opponents mistakenly attack their own nestmate and, once they recognize this, the pair will usually unlock mandibles and take to the air again, rejoining the fight. Typically, the combatants remain locked together until death, after which they are dragged away by opportunistic scavengers, such as ants. The battle, which may begin each morning and can last for days, results in a carpet of thousands of dead bees locked together by the mandibles (Fig. 3.27) (Wagner and Dollin 1982; Heard 1996; Klumpp 2007; Dollin 2008; Gloag et al. 2008).

Gloag et al. (2008) found that most “fighting swarms” involved only two colonies. However, there were instances where up to seven colonies were identified

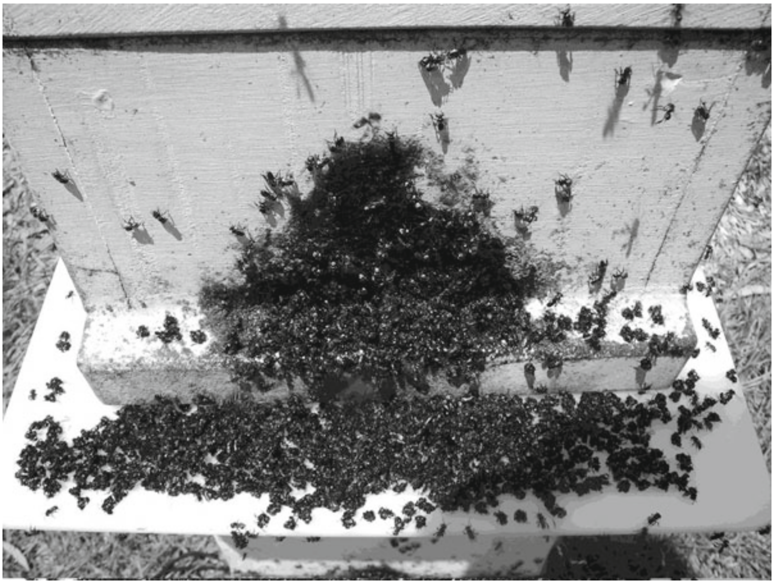

Fig. 3.27 *T. carbonaria* hive entrance showing just a proportion of the coupled bees that will die in battle. Photo: R. Gloag

in a single battle. Alarm pheromones are probably responsible for attracting neighboring colonies into the "fighting swarm." This may potentially increase overall losses within a meliponary or orchard. *Trigona* (*Heterotrigona*) *carbonaria* is the most popular species kept by Australian stingless bee-keepers (Halcroft, unpublished data), and "fighting swarms" are a major management problem. While there are reports of other stingless bee species forming defensive groups, "fighting swarms" are regularly reported in *T.* (*Heterotrigona*) *carbonaria* (ANBees 2010). Colony strength is greatly reduced after a fight and colonies may not be divisible for another season. Gloag et al. (2008) also find that approximately one in five of the paired combatants were nestmates, contributing further to the cost of battle.

Gloag et al. (2008) tested the theory that returning workers may become disorientated, especially when moved into a crop area for pollination. Workers were forced to enter a foreign nest, which quickly provoked a "fighting swarm." In the field, disoriented workers mistakenly entered another nest, thus prompting a "fighting swarm." Management practices that are used to reduce the incidence of "fighting swarm" due to disorientation include: separation of hives by 5 m, positioning hives at different heights and directions, and identification of hives with different colors or symbols (Gloag et al. 2008). Fortunately, colonies involved in "fighting swarms" usually recover (Heard 1996) and in the case of usurpation, the weaker colony may even increase in vigor (Dollin 2008).

More information on fighting swarms can be found at http://www.aussiebee.com.au/video-fighting-swarms-1.html.

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Chapter 4
Stingless Bees from Venezuela

Silvia R.M. Pedro and João Maria Franco de Camargo*

4.1 Introduction

The stingless bees of Venezuela, or "abejas criollas" as known by locals, have aroused the interest of native and foreign people since long ago. According to Rivero Oramas (1972) the first records about the biology of these bees dated 1578 when the Governor Juan Pimentel wrote about the province of Caracas mentioning the use of tree trunk hollows of the "jobo" (*Spondias mombin* L.—Anacardiaceae) as a place for nesting by bees (probably stingless bees). He also commented about the commerce of honey and cerumen, which is always black in that region. In the years 1612–1613 Father Pedro Simón gave more detailed accounts of the bees in the region of Los Llanos. He made observations about the honey stored in pots arranged in clusters, not in combs, the quality of honey and cerumen and their use by native people, and the docile behavior of the bees. Reports about traditional meliponiculture (beekeeping with stingless bees) are even older. Venezuelan Indians kept stingless bees in large calabashes in their houses according to reports of Rodrigo de Bastidas dated from the 1540s (Oviedo 1550 *apud* Crane 1999), who also mentioned the presence of many bees without stinging organs in the wild woods. It is interesting to note that the European bee *Apis mellifera* Linnaeus, 1758 was not present in Venezuela at least until 1866, according to oral information by Prof. Karsten (Gerstaker 1866 *apud* Nogueira-Neto 1962).

Nowadays despite the great diversity of stingless bee species in Venezuela and the traditional meliponiculture widespread in that country, there are few studies dealing with the taxonomic diversity of the Venezuelan native stingless bees.

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The species more intensively reared are known only by their common names, most of them from indigenous origin, and sometimes the same name is applied for different species, or one species can receive different names depending on the region (Rivero Oramas 1972).

On the other hand, there is a field of scientific research that has meaningfully contributed to the improvement of knowledge of the taxonomic biodiversity of the stingless bee fauna from Venezuela: honey and propolis analyses. For the last 25 years, Dr. Patricia Vit and collaborators have been sending Venezuelan specimens of stingless bees, associated with honey and propolis samples, for identification and deposit in the Camargo Collection—RPSP (see Vit 2008). They have been working to create the quality standards of stingless bee honey through determination of their botanical and geographical origins, melissopalynology, biochemical composition, and physicochemical, sensory, and bioactive properties—antibacterial activity, antioxidant capacity, acidity, electrical conductivity, diastase and invertase activities, and levels of ash, nitrogen, flavonoids, hydroxymethylfurfural, reducing sugars, sucrose, and water (e.g., Vit Olivier 1992; Vit and Ricciardelli d'Albore 1994a, b; Vit et al. 1994, 1997, 1998a, b, 2011; Bogdanov et al. 1996; Vit and Pulcini 1996; Vit and Tomás-Barberán 1998; Rodríguez-Malaver et al. 2009; Vit 2005, 2009). Propolis collected from nests of Venezuelan stingless bees has been also analyzed concerning phenolic compounds (e.g., Tomás-Barberán et al. 1993; Vit et al. 1993). The identification was provided mainly by one of the present authors, the late Prof. João MF Camargo, specialist in taxonomy, biology, and biogeography of Meliponini. The bees sampled by Prof. P. Vit have provided valuable information about the meliponine fauna from Venezuela, such as new records of species and geographical records, as well as taxonomic information for future revisions.

The present chapter introduces a preliminary checklist of stingless bees from Venezuela, including common names, geographic records, and studies concerning honey. This is only a preliminary treatment and certainly there are many more species in Venezuela than the ones listed here, considering that we have not studied material deposited in other collections. Other constraints involve the lack of intensive and periodic surveys comprising the large diversity of habitats distributed throughout the Venezuelan territory as well as taxonomic limitations. Nonetheless, this can be useful in future faunistic surveys as well as in taxonomic revisions of Venezuelan Meliponini bees.

4.2 Data Sources

The data were obtained from material studied by the authors, mostly collected by Prof. P Vit (Universidad de Los Andes, Mérida, Venezuela), during the last 25 years, and sent to RPSP (Camargo Collection, housed in the Department of Biology, FFCLRP-USP, in Ribeirão Preto, São Paulo) for identification. Other studied specimens in RPSP from Venezuela were collected by JMF Camargo—who traveled across the states of Merida, Barinas, and Zulia studying nests of Meliponini through

March 2008, accompanied by P Vit. Material has also been collected by RW Brooks and collaborators, D Wittmann, and others. Popular names listed here were obtained from traditional stingless bee-keepers and locals by P Vit when collecting the bees. Some popular names mentioned by Rivero Oramas (1972), such as "bayures" (probably the same as "guayures"), "araguatas," and "mabas," could not be associated with the scientific names of the species. Additional information was obtained from literature, mainly from Schwarz (1932, 1948) and Camargo and Pedro (2007, 2008).

4.3 Diversity and Distribution of Stingless Bees in Venezuela

Species recorded in Venezuela are listed in Table 4.1. Geographical records are listed by states following two-letter abbreviations (*AM* Amazonas, *AP* Apure, *ME* Mérida, etc.). From the 83 species of stingless bees that occur in Venezuela, here listed, 18% have their honeys already analyzed (references in Table 4.1).

Nests of some species are represented in the Fig. 4.1a–f.

The total of 83 species included in 19 genera is certainly an underestimate, mainly considering that material deposited in collections, other than RPSP, was not examined. Also, some genera are currently under revision or need to be revised, and the identity of some species could not be determined for this work. These are mainly *Frieseomelitta*, *Nannotrigona*, *Scaptotrigona*, *Tetragona*, *Tetragonisca* (Fig. 4.1d–f), *Plebeia*, and *Scaura*. Despite the exhaustive revisions by Schwarz (1932, 1948), the taxonomy of *Melipona* and *Trigona* deserves a reevaluation as well. *Melipona* Illiger, 1806 is the most diversified Neotropical stingless bee genus, divided in four subgenera, all represented in Venezuela, and with about 70 known species (Camargo and Pedro 2007, 2008), some of them extensively reared by beekeepers. *Trigona* is also widely diversified with about 32 valid species, besides at least other 10–20 new to science and in some cases there are complexes of different species now frequently identified under the same epithet (e.g., *Trigona fulviventris*, *T. guianae*, *T. fuscipennis*, *T. hypogea*, *T. pallens*).

Some species were only recorded in Venezuela in the literature and we could not confirm the identity of this material. Some of them are certainly misidentifications or junior synonyms of other species [e.g., *Scaptotrigona polysticta* Moure, 1950, *Trigona hyalinata* (Lepeletier, 1836) (probably *T. branneri*), *Trigona spinipes* (Fabricius, 1793) (probably *T. amazonensis*), *Trigona alfkeni* Friese, 1900, *Trigona silvestriana* (Vachal, 1908), *Trigona trinidadensis* (Provancher, 1888) (junior synonym of *T. amalthea*); see Camargo and Pedro (2007, 2008)] and were not included in the Table 4.1. Other names were listed in Table 4.1 with some uncertainty such as *Geotrigona subnigra*, *Lestrimelitta glaberrima*, *Nannotrigona perilampoides*, and *Plebeia fraterna*, recorded in Guárico by Rodríguez-Parilli et al. (2010). These authors also mentioned one unnamed *Friesella*, recorded in Portuguesa, but the material was probably misidentified. *Friesella schrottkyi* (Friese, 1900), the only species of the genus, is restricted to the southern part of Brazil (Camargo and Pedro 2007, 2008). There is no other record of *Friesella* between southern Brazil and Venezuela.

Table 4.1 Stingless bees from Venezuela: Common names, geographic records (by states in Venezuela), and honey analysis

	Scientific names	Common names	Geographic records	Honey analysis
1	*Aparatrigona impunctata* (Ducke, 1916)		AM	
2	*Cephalotrigona capitata* (Smith, 1854)	“abejita,” “negrito”	BO, DA	
3	*Duckeola pavani* Moure, 1963		AM	
4	*Frieseomelitta paupera* (Provancher, 1888)	“guanotica”	AM, AR, BA, BO, FA, GU, MO, NE, PO, ZU	2, 4, 5, 6, 7, 9, 10, 14 [as *Trigona* (*Frieseomelitta*) *nigra paupera*]
5, 6	*Frieseomelitta* spp. (at least two species)	“angelita,” “erica”	AM, BA, BO, MO, TA	2, 4, 5, 6, 9, 10, 11, 12, 13, 14, 15 [as *Frieseomelitta* sp. *aff. varia*, *F. aff. varia* or *F*. sp. group *varia*, *Frieseomelitta officinalis varia*, *Trigona* (*Frieseomelitta*) sp *aff varia, Friesomelitta* (*sic*) *varia*]
7	*Geotrigona subnigra* (Schwarz, 1940)		GU*	
8, 9	*Geotrigona* spp. (at least two species)		AM, LA	
10	*Lestrimelitta glaberrima* Oliveira & Marchi 2005		GU*	
11	*Lestrimelitta maracaia* Marchi & Melo, 2006	“limoncita”	AM	
12	*Melipona (Eomelipona) concinnula* # Cockerell, 1919		BO	
13	*Melipona (Eomelipona) illustris* Schwarz, 1932		AM	
14	*Melipona* (*Eomelipona*) *ogilviei* Schwarz, 1932		AM	
15	*Melipona (Melikerria) compressipes* (Fabricius, 1804)	“guanota”	AM, AP, BA, BO, GU, LA, PO, TA, ZU	1, 2, 3, 4, 5, 6, 9, 10, 12, 13, 14
16	*Melipona (Melikerria) interrupta* (Latreille, 1811)		ZU	

17	*Melipona* (*Melipona*) *favosa* (Fabricius, 1798)	"abejita," "abejita casera," "angelita," "arica," "criollita," "erica," "mabita"	AM, AP, BA, BO, FA, GU, LA, ME, MO, NE, PO, SU, TA	1, 2, 3, 4, 5, 6, 9, 10, 11, 12, 13, 14, 15
18	*Melipona (Michmelia) apiformis*# (Buysson, in Du Buysson & Marshall, 1892)		AR	
19	*Melipona (Michmelia) cramptoni* Cockerell, 1920		AM	
	Melipona fasciata cramptoni duidae# Schwarz, 1932 (junior synonym)			
20	*Melipona (Michmelia) crinita* Moure & Kerr, 1950		AM	2, 4, 5, 6, 14
21	*Melipona (Michmelia) fulva* Lepeletier, 1836		BO	2, 4, 5, 6, 14 (as *Melipona* sp. group *fulva, Melipona sp. aff. fulva*)
22	*Melipona (Michmelia) indecisa*# Cockerell, 1919		AR, LA, TA, YA	
23	*Melipona (Michmelia) lateralis* Erichson, 1848	"ñuriño"	AM, BO, NE, SU	2, 4, 5, 6, 14 [as *Melipona lateralis kangarumensis* Cockerell, 1920]
24	*Melipona (Michmelia) paraensis* Ducke, 1916	"isabitto"	AM, BO	2, 4, 5, 6, 14
25	*Melipona (Michmelia) trinitatis* Cockerell, 1919	"guanota"	AN, DA, MO, NE, SU, ZU	1, 9, 10, 11
26	*Melipona (Michmelia)* sp. 1	"cigarroncito," "sabite," "tobillo morrocoy," "isabitto"	AM	2, 4, 5, 6, 8, 14 [as *Melipona fuscopilosa* Moure & Kerr, 1950]
27	*Melipona* (*Michmelia*) sp. 2		AR, BA	
28	*Nannotrigona melanocera* (Schwarz, 1938)	"moscochola"	AM, AP, AR, DC, PO	
29	*Nannotrigona perilampoides* (Cresson, 1878)		GU*	

(continued)

Table 4.1 (continued)

	Scientific names	Common names	Geographic records	Honey analysis
30	*Nannotrigona schultzei* (Friese, 1901)		AM	
31	*Nannotrigona tristella*# Cockerell, 1922		FA, YA, ZU	
32	*Nannotrigona* sp.	"zamurita"	AP, AR, BA, DA, ME, PO, YA, ZU	2, 4, 5, 6, 14 [as *Nannotrigona* sp. *aff. chapadana* (Schwarz, 1938)]
33	*Oxytrigona mellicolor* (Packard, 1869)		AR, BA, GU, ME, PO, YA, ZU	
34	*Paratrigona anduzei* (Schwarz, 1943)	"abejita," "cortacabello," "españolita," "pegón"	BO, ME, TA	12, 13
35	*Paratrigona pannosa* Moure, 1989		AM	
36	*Paratrigona permixta*# Camargo & Moure, 1994		SU	
37	*Partamona ailyae* Camargo, 1980		AM	
38	*Partamona auripennis* Pedro & Camargo, 2003		AM	
39	*Partamona epiphytophila* Pedro & Camargo, 2003		AM	
40	*Partamona ferreirai* Pedro & Camargo, 2003		BO	
41	*Partamona nigrior* (Cockerell, 1925)		AR, BO, SU	
42	*Partamona pearsoni* (Schwarz, 1938)		AM	
43	*Partamona peckolti* (Friese, 1901)	"pegona," "pegón," "churrusca"	AM, AR, BO, DC, FA, GU, LA, ME, TA, TR, ZU	
44	*Partamona vicina* Camargo, 1980		AM	
45	*Partamona vitae*# Pedro & Camargo, 2003		AM, BO	
46	*Plebeia fraterna*# Laroca & Rodriguez-Parilli, 2009		GU*	
47	*Plebeia goeldiana*# (Friese, 1900)		BO, ME, TR	

48–54	*Plebeia* spp. (at least more seven species)	“mosquito”	AR, BO, GU, LA, NE, TR, ZU	7, 9, 10
55	*Ptilotrigona lurida* (Smith, 1854)		AM, BO	
56	*Scaptotrigona ochrotricha*# (Buysson, in Du Buysson & Marshall, 1892)		AM, AR	2, 4, 5, 6, 14 [as *Scaptotrigona ochrotica* (*sic*=*ochrotricha*)]
57–62	*Scaptotrigona* spp. (at least more six species)	“guaracho,” “isabitto,” “pico,” “sonquette”	AM, AR, NE, ZU	2, 4, 5, 6, 7, 9, 10, 12, 13, 14 [as *S. polystica* (*sic*=*S. polysticta*), *Scaptotrigona officinalis* (*sic*) *depilis*, *Scaptotrigona* sp *aff depilis*, *Scaptotrigona depilis*]
63	*Scaura* sp.	“pegoncito”	BO, TA	7, 9, 10 [as *Scaura aff. latitarsis, Scaura latitarsis, Plebeia* (*Scaura*) *latitarsis*]
64	*Tetragona clavipes* (Fabricius, 1804)	“ajabite,” “ajavitte,” “ajavitta”	AM	8
65	*Tetragona ziegleri* (Friese, 1900)		ME	
66, 67	*Tetragonisca* spp. (at least two species)	“abejita,” “eriquita,” “españolita,” “guayure,” “lambeojitos,” “pañuelita,” “princesita,” “rubita”	BA, GU, LA, ME, PO, YA	2, 4, 5, 6, 7, 8, 9, 10, 14 [as *Trigona* (*Terragonisca*) (*sic*=*Tetragonisca*) *Angustula angustula*, *Trigona* (*Tetragonisca*) *angustula angustula, Tetragonisca angustula angustula, Trigona* (*Frieseomelitta*) *angustula angustula*]
68	*Trigona amalthea* (Olivier, 1789)	“pegón”	AR, BO, GU, LA, ME, SU, TR, YA, ZU	

(continued)

Table 4.1 (continued)

	Scientific names	Common names	Geographic records	Honey analysis
69	*Trigona amazonensis* (Ducke, 1916)		AM	
70	*Trigona branneri* Cockerell, 1912	"pegón"	AM, BO, MO	
71	*Trigona cilipes* (Fabricius, 1804)		AM	
72	*Trigona dallatorreana* Friese, 1900		AM	
73	*Trigona fulviventris* Guérin, 1844		TA, YA	
74	*Trigona fuscipennis* Friese, 1900	"pegón"	AM, GU, TA, ZU	
75	*Trigona guianae* Cockerell, 1910	"pegona," "pegón"	AM, AR, BA, LA, ME, PO, TA, ZU	
76	*Trigona pallens* (Fabricius, 1798)		AM	
77	*Trigona truculenta* Almeida, 1984		AM	
78	*Trigona venezuelana*# Schwarz, 1948		AR, DA, DC, MI, YA	
79	*Trigona williana* Friese, 1900		AM, BO	
80, 81	*Trigona* spp. (at least +2 species)	"pegón"	AM, LA, YA	
82, 83	*Trigonisca* spp. (at least + 2 species)		GU, TR, ZU	

References for honey and propolis analyses: Bogdanov et al. (1996) (1); Vit and Pulcini (1996) (2); Vit et al. (1997) (3); Vit and Tomás-Barberán (1998)(4); Vit et al. 1998a, b) (5, 6); Vit (2009) (7); Vit et al. (2011) (8); Vit Olivier (1992) (9); Vit et al. (1994) (10); Vit and Ricciardelli d'Albore 1994b (11); Tomás-Barberán et al. (1993) (12); Vit et al. (1993) (13); Vit and Ricciardelli d'Albore 1994a (14); Vit (2005) (15); *recorded by Rodríguez-Parilli et al. (2010); #species described from Venezuela. Bees were recorded from AM Amazonas, AN Anzoátegui, AP Apure, AR Aragua, BA Barinas, BO Bolívar, DA Delta Amacuro, DC Distrito Capital (= Distrito Federal), FA Falcón, GU Guárico, LA Lara, ME Mérida, MI Miranda, MO Monagas, NE Nueva Esparta, PO Portuguesa, SU Sucre, TA Táchira, TR Trujillo, YA Yaracuy, ZU Zulia

Fig. 4.1 Nests of stingless bees from Venezuela. (**a**, **b**) *Paratrigona anduzei* (Schwarz, 1943) among roots of epiphyte, Garden of Medicinal Plants, Universidad de Los Andes, Mérida, Venezuela. (**c**) Nest entrances of *Frieseomelitta paupera* (Provancher, 1888) in wall of cement bricks, Trail Peña de La Yuca, Barinas, Venezuela. (**d**) Nest entrance of *Tetragonisca* sp., in the base of a trunk of mango tree, Garden of Medicinal Plants, Universidad de Los Andes, Mérida, Venezuela. (**e**, **f**) *Tetragonisca* sp., nest in artificial cavity of funnel, Food Science Department, Universidad de Los Andes, Mérida, Venezuela; the *arrow* indicates the nest entrance built with cerumen in the open funnel it is possible to see the layers of involucrum protecting the nest. Photos: J.M.F. Camargo

Among the genera with species represented in Venezuela and recently revised, including not only taxonomy but also information about biology, are *Paratrigona* and *Aparatrigona* (Camargo and Moure, 1994), *Geotrigona* (Camargo and Moure, 1996), *Partamona* (Camargo and Pedro, 2003, Pedro and Camargo, 2003), and *Ptilotrigona* (Camargo and Pedro, 2004). *Lestrimelitta* and *Oxytrigona* were partially revised by Marchi and Melo (2006) and Gonzalez and Roubik (2008).

Several species were described from Venezuela and some of them remain known only from the type locality. *Melipona concinnula* Cockerell, 1919 is known only from a single specimen (the holotype) from Rio Mato, Caura District, Bolívar, Venezuela. However, it is possible that *M. ogilviei* is a junior synonym of this species [see comments in Schwarz (1932) and Camargo and Pedro (2007, 2008)]. *Melipona apiformis* (Buysson, *in* Buysson & Marshall, 1892) was originally described in the genus *Trigona* and later included in *Melipona* based on its description (Camargo and Pedro 2007, 2008) and it was recorded only in the type locality (Colonia Tovar, AM, Venezuela). Its true identity, however, remains unknown because the whereabouts of type material is unknown. *Plebeia fraterna* was described by Laroca and Rodríguez-Parilli (2009) from San Juan de Los Morros, Guárico, Venezuela, and is known only from there now.

Other species described from Venezuela are *Melipona indecisa* Cockerell, 1919 from Lagunita de Aroa, Yaracuy (Camargo and Pedro 2007, 2008); *Paratrigona permixta* Camargo & Moure, 1994 from San Rafael, Cumanacoa, Sucre; *Nannotrigona tristella* Cockerell, 1922 and *Trigona venezuelana* Schwarz, 1948 both from Lagunita de Aroa, Yaracuy; *Plebeia goeldiana* (Friese, 1900) from Mérida; *Scaptotrigona ochrotricha* (Buysson, in Du Buysson & Marshall, 1892); and *Melipona apiformis*, described from Colonia Tovar, Aragua. *Melipona fasciata cramptoni duidae* (Schwarz, 1932), junior synonym of *Melipona cramptoni* Cockerell, 1920, was described from the Mt. Duida region, between La Esmeralda and Cerro Duida, Amazonas, near the Orinoco River. According to Camargo and Pedro (2007, 2008), it is possible that *M. cramptoni* is only a dark form of *M. fulva*. Geographic records of these species in Venezuela are listed in Table 4.1.

Although Friese (1900) has included specimens of *Melipona fasciata* Latreille, 1811 from Venezuela in the type series of *M. fuscipes* Friese, 1900 (junior synonym of *M. fasciata*) this species is not present in the Venezuelan stingless bee fauna. Indeed, the type series of *M. fuscipes* was composed of different species and its identity was interpreted by Moure (1971) on the basis of specimens from central Mexico (Morelos). *Melipona fasciata* was also described based on specimens from Mexico (Veracruz) (see notes in Camargo and Pedro 2007, 2008).

Some species are widely distributed in Venezuela, such as *Frieseomelitta paupera* (Fig. 4.1c), *Melipona favosa*, *Partamona peckolti*, *Trigona amalthea*, and *T. guianae*. Most of the species recorded in Venezuela (Table 4.1), however, seem to have a more restricted distribution.

4.4 Biogeographic Patterns of Venezuelan Meliponini

Despite the taxonomic problems and restriction of studied material allied to the lack of consistent surveys in Venezuela, we can make a first attempt to relate the known geographical records of Venezuelan Meliponini with biogeographic patterns already recognized, including other stingless bees (Camargo and Pedro 2003; Camargo 2008; Camargo Chap. 2, this book).

The Venezuelan fauna of stingless bees is quite diversified in terms of biogeographic origins, including predominantly elements from NAm (Amazon region, on north of the Negro and Amazonas rivers) and SWAm (a component delimited, on the north, by the alignment of the Uaupés/Negro rivers; on the south, by the Madeira/Mamoré rivers; and on the west, by the Andean mountain range) components.

Species from Central America (Choco-CA component) that reach Venezuela are *Tetragona ziegleri*, *Trigona fulviventris*, *Trigona fuscipennis*, *Partamona peckolti*, and *Frieseomelitta paupera* (Fig. 4.1c). However, the taxonomic status of some of these must be reviewed. *Oxytrigona mellicolor* is also from Chocó-CA component [as interpreted by Schwarz (1948), followed by Camargo and Pedro (2007, 2008)], although the holotype is from somewhere between Quito and the Napo River (Schwarz 1948), on the eastern side of the Andes; it can be found from Honduras to Esmeraldas, Ecuador, and in Venezuela, in Lagunita de Aroa, Aragua, and other states listed in Table 4.1 (Schwarz 1948; Camargo and Pedro 2007, 2008; Gonzalez and Roubik 2008). *Nannotrigona perilampoides* was only recorded in Venezuela in Guárico by Rodríguez-Parilli et al. (2010), but it was possibly misidentified considering this species is only known from Mexico to Panama (Camargo and Pedro 2007, 2008).

The other two components, Atl (Atlantic area, from Bahia to Paraná, Brazil) and SEAm (area to the south of the Madeira/Amazonas rivers to northwestern Argentina), are apparently not represented in Venezuela except, perhaps, by *Cephalotrigona capitata*, from the SEAm, implying that Amazon River represents an important faunal divisor (geographic barrier) for stingless bees.

Species with more restricted distribution patterns are apparently associated with areas of endemism related with Venezuelan terranes (Venezuela–Trinidad, Imeri, and others not named yet) included in the NAm biogeographic component. They are *Trigona venzuelana*, *Partamona vitae*, *Paratrigona anduzei* (Fig. 4.1a, b), *Paratrigona pemixta*, *Plebeia fraterna*, *P. goeldiana*, *Scaptotrigona ochrotricha*, *Melipona concinnula*, *M. apiformis*, *M. indecisa* (probably junior synonym of *M. apiformis*), *M. ogilviei*, *M. trinitatis*, and *Nannotrigona tristella*.

Other species from the NAm component, but more widely distributed nevertheless, are *Duckeola pavani*, *Partamona nigrior*, *Geotrigona subnigra*, *Lestrimelitta glaberrima*, *Melipona compressipes*, *M. interrupta*, *M. cramptoni*, *M. fulva*, *M. lateralis*, *M. paraensis*, *Paratrigona pannosa*, *Partamona auripennis*, *P. ferreirai*, *P. nigrior*, and *P. pearsoni*. *Melipona favosa* is also widespread throughout Venezuela, Guianas, Trinidad, and Tobago, reaching Colombia (Magdalena), but its wider distribution can be a result of extensive beekeeping and transportation of hives.

Some examples of species from the SWAm component that are represented in Venezuela are *Lestrimelitta maracaia*, *Melipona crinita*, *M. illustris*, *Nannotrigona melanocera*, *N. schultzei*, and *Partamona epiphytophila*.

Many species present in Venezuela have wider distribution in South America occupying two or three of the large components and it is difficult to determine their biogeographic origins. Some examples are:

NAm + SWAm: *Aparatrigona impunctata*, *Ptilotrigona lurida*, *Trigona amalthea*, *T. amazonensis*, *T. branneri*, *T. cilipes* [the material listed from Costa Rica and Panamá by Schwarz (1948):346 is probably another species], *T. dallatorreana*, *T. guianae*, *T. pallens*, and *T. venezuelana*.

NAm + SWAm + SEAm: *Partamon ailyae*, *Partamona vicina*, *Scaura latitarsis* (different species included), *Tetragona clavipes* (different species included), *Trigona hypogea* (different species included), and *T. truculenta*.

In summary, information on distribution patterns must be improved with the increase of surveys in the country and adequate taxonomic treatment of the material sampled, which will allow more precise interpretation of biogeographic patterns.

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Chapter 5
Stingless Bees (Hymenoptera: Apoidea: Meliponini) of French Guiana

Alain Pauly, Silvia R.M. Pedro, Claus Rasmussen, and David W. Roubik

5.1 Introduction

Stingless bees (Hymenoptera: Apoidea; Meliponini) are found worldwide in tropical and subtropical regions (Michener 2007), but are most diverse and numerous in tropical South and Central America, where they often are the most commonly encountered bees. The stingless bees have long played an important role for inhabitants of these areas as the suppliers of excellent honey (Schwarz 1948; Nogueira-Neto 1997; Stearman et al. 2008; Guerrini et al. 2009; Rodríguez-Malaver et al. 2009) and crop pollinators (Heard 1999; Slaa et al. 2006). They are also the focus for scientific research on sociality and colony evolution (e.g., Nieh 2004; Rasmussen and Camargo 2008; Lichtenberg et al. 2010). Further comparative studies are encouraged by a robust phylogeny of the entire group (Rasmussen and Cameron 2007, 2010; Ramírez et al. 2010). The stingless bees have also been of concern for conservation biologists, because most nest in living trees and therefore they may be more susceptible to habitat disturbance than other bees and insects (Brown and Albrecht 2001; Kerr et al. 2001; Samejima et al. 2004; Roubik 2006).

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The lowland forest of French Guiana contains extensive and undisturbed habitats, and the smallest human population density on earth. The land area is little more than 80,000 km^2. No high mountains occur, and the rainfall varies between 4,000 mm annually in the Cayenne and Kaw mountain area, gradually diminishing to half this amount to the south and west. The forests are of white sand soils or of laterites, as well as the extensive mangrove of the coastal and estuarine areas.

In order to conduct research in a given area, it is often essential to have some background on the species encountered in that area. An introduction to the local species is often provided in the form of checklists for families or orders of insects or for smaller units (e.g., genera) in taxonomic revisions or species descriptions. However, no complete ecological perspective is given in such lists, because relative abundances or phenologies of the different species are unknown. In the case of stingless bees, the bees are ecologically active every day and the colonies are perennial (Roubik 1989). For basic information, stingless bee researchers have access to three checklists. Camargo and Pedro (2007, 2008a) and Rasmussen (2008) in their catalogues of stingless bees listed all species from the Neotropical and Indo-Malayan/Australasian region, respectively, including bibliographic references, synonymies, and distribution records based on their literature surveys. Eardley (2004) in a taxonomic revision of the Afrotropical stingless bees provided keys, synonymies, and distribution records for all stingless bees of that region, excluding Madagascar. Much of the information was also included in the later catalogue to the Afrotropical bees, including Madagascar (Eardley and Urban 2010). Distribution records in all three catalogues (Camargo and Pedro 2007, 2008a; Rasmussen 2008; Eardley and Urban 2010) were largely based on the literature, and given the sporadic collection effort of these regions, it is not surprising that several countries are poorly sampled, incompletely known, or that even well-known regions can provide new records of species.

We surveyed the literature and a comprehensive collection of stingless bees from French Guiana to compile a list of all known taxa from that country. Whereas the stingless bee fauna of French Guiana was first studied extensively by Roubik (1979, 1980), century old scattered records (Dominique 1898; Rasmussen et al. 2007) were the first to document the fauna from the country, some even dating to the time of Linnaeus and his students, namely, JC Fabricius who named seven new stingless bee taxa likely collected in French Guiana (primarily Cayenne) and Suriname (Moure 1960; Papavero 1971).

5.1.1 Data Compiled from Preserved Material from Four Collections

Data were compiled from the following sources (collecting sites on Fig. 5.1):

1. Material preserved at the Royal Belgian Institute of Natural Sciences (RBINS), collected by Société Entomologique Antilles Guyane (SEAG) (Brulé et al. 2011), and identified by Silvia RM Pedro: Saut Pararé (4°02 N 52°41′W) à Nouragues, Montagne des Chevaux (4°43′N 52°26′W) à Roura (RN2 PK22), Iracoubo (5°29′N 53°13′W).

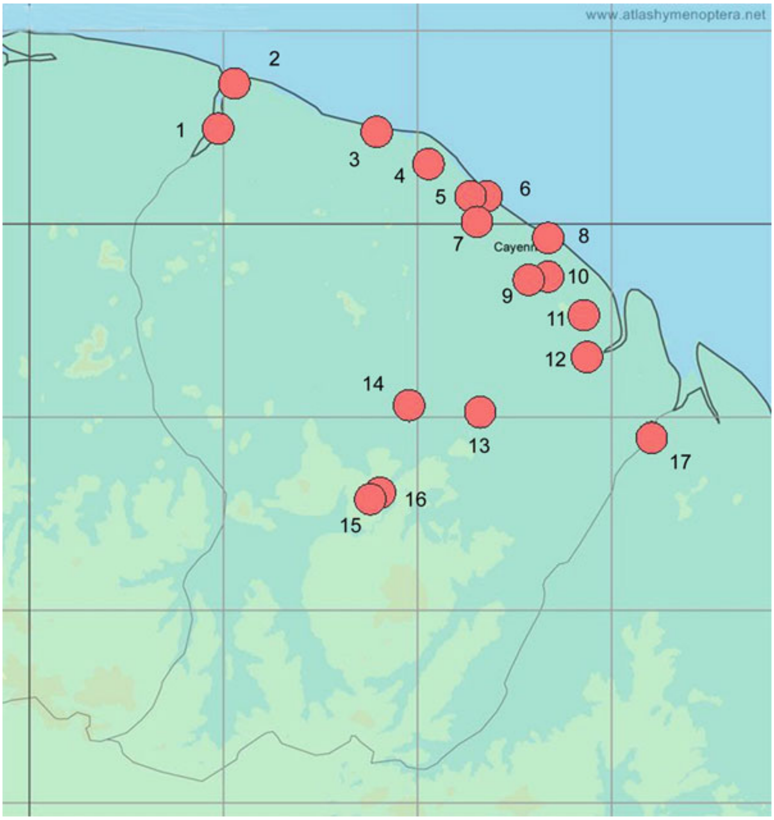

Fig. 5.1 Map of collecting sites in French Guiana (extracted from www.atlashymenoptera.net). (1) Saint Laurent du Maroni, (2) Yalimapo, (3) Iracoubo, (4) Sinnamary, (5) Soumourou, (6) Kourou, (7) Degrad Saramaca, (8) Cayenne, (9) Roura Montagne des Chevaux, (10) Relais Patawa, (11) Kaw, (12) Regina, (13) Saut Pararé, (14) Petit Saut, (15) Mt Galbao, (16) Saul, (17) Saint Georges de l'Oyapock

2. Material preserved at the Royal Belgian Institute of Natural Sciences [RBINS] and identified by Claus Rasmussen: Kaw, Relais Patawa (4°32′N 52°09 W) leg. Y Braet, leg. J Cerda; Kourou (5°09′N 52°39′W) leg. Y Braet; Maroni (5°30′N 54°02′W) (=Saint Laurent du Maroni) leg. Y Braet; Saül (3°37′N 53°12′W) leg. Y Braet; Piste Soumourou (5°09′N 52°44′W) leg. D Faure; Sinnamary, Pointe Combi (5°19′N 52°57′W) leg. P Cerdan; Sinnamary, barrage de Petit Saut (4°04′N 53°03′W) leg. P Cerdan; Yalimapo, Les Hattes (5°44′N 53°57′W), Ecloserie du WWF, leg. R Babin.
3. Material preserved at the [DWR] Collection, Smithsonian Tropical Research Institute, Panamá, collected and identified by David W. Roubik, JMF Camargo, and JS Moure: Kourou-Sinnamary area, in addition to Cayenne, St. Laurent and St. George areas (1976–2009).
4. Material preserved at Division of Entomology, University of Kansas Natural History Museum, Lawrence, Kansas, USA (SEMK). Various localities collected by RW Brooks and identified by JMF Camargo.

5. Material preserved at Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, São Paulo, Brazil (RPSP), identified by JMF Camargo and SRM Pedro. Duplicates of collections a, c, and d are preserved in this institution.

In addition we included literature records (Camargo and Pedro 2005, 2008b, 2009; Moure 1989; Moure and Camargo 1982; Moure et al. 1988; Oliveira and Marchi 2005; Roubik 1980, 1990; Smith Pardo and Engel 2001) and a record of *Celetrigona manauara* collected by R. Snyder and preserved in American Museum of Natural History (AMNH).

5.1.2 A List of Stingless Bee Species Found in French Guiana

Aparatrigona impunctata (Ducke 1916) [DWR, RBINS, RPSP, SEMK]
Camargoia camargoi Moure 1989 [DWR, RBINS, RPSP, SEMK]
Celetrigona manauara Camargo and Pedro 2009) [AMNH]
Cephalotrigona capitata (Smith 1854) [DWR, RBINS, RPSP, SEMK]
Dolichotrigona longitarsis (Ducke 1916) [SEMK]
Duckeola ghilianii (Spinola 1853) [DWR, RPSP]
Duckeola pavani (Moure 1963) [DWR, RPSP]
Frieseomelitta flavicornis (Fabricius 1798) [DWR, RBINS, RPSP, SEMK] (=*Tetragona savannensis* (Roubik 1980))
Frieseomelitta portoi (Friese 1900) [DWR, RBINS, RPSP]
Frieseomelitta sp. A *aff. varia* (Lepeletier 1836) [RBINS]
Frieseomelitta sp. B [RBINS]
Frieseomelitta sp. C [RBINS]
Lestrimelitta glaberrima Oliveira and Marchi 2005 [DWR, RBINS, RPSP]
Lestrimelitta guyanensis Roubik 1980 [DWR, RBINS, RPSP]
Lestrimelitta monodonta Camargo and Moure 1989 [RBINS]
Leurotrigona pusilla Moure and Camargo 1988 *in* Moure et al. 1988 [DWR, RPSP]
Melipona (Eomelipona) bradleyi Schwarz 1932 [RBINS]
Melipona (Eomelipona) ogilviei Schwarz 1932 [DWR, RBINS, RPSP]
Melipona (Eomelipona) puncticollis Friese 1902 [DWR, RPSP]
Melipona (Melikerria) compressipes Fabricius 1804 [DWR]
Melipona (Melikerria) interrupta Latreille 1811 [DWR, RBINS, RPSP]
Melipona (Melipona) favosa Fabricius 1798 [DWR, RBINS, RPSP]
Melipona (Michmelia) captiosa Moure 1962 [DWR, RBINS]
Melipona (Michmelia) fuliginosa Lepeletier 1836 [DWR]
Melipona (Michmelia) fulva Lepeletier 1836 [DWR, RBINS, RPSP]
Melipona (Michmelia) lateralis Erichson 1848 [DWR, RBINS, RPSP, SEMK]
Melipona (Michmelia) melanoventer Schwarz 1932 [DWR]
Melipona (Michmelia) paraensis Ducke 1916 [DWR, RBINS]
Melipona (Michmelia) sp. [RBINS]
Nannotrigona punctata (Smith 1854) [DWR, RBINS, RPSP, SEMK]
Nannotrigona schultzei (Friese 1901) [DWR, RPSP, SEMK]

Nogueirapis minor (Moure and Camargo 1982) [DWR, RBINS, RPSP, SEMK]
Oxytrigona obscura Friese 1900 [DWR, RBINS, RPSP, SEMK]
Paratrigona femoralis Camargo and Moure 1994 [DWR, RBINS, RPSP, SEMK]
Paratrigona pannosa Moure 1989 [DWR, RBINS, RPSP, SEMK]
Partamona auripennis Pedro and Camargo 2003 [DWR, RBINS, SEMK]
Partamona ferreirai Pedro and Camargo 2003 [DWR, RBINS]
Partamona mourei Camargo 1980 [DWR, RBINS]
Partamona pearsoni Schwarz 1938 [DWR, RBINS, RPSP]
Partamona testacea (Klug 1807) [DWR, RBINS, RPSP, SEMK]
Partamona vicina Camargo 1980 [DWR, RBINS, RPSP, SEMK]
Plebeia minima (Gribodo 1893) [DWR, RBINS]
Plebeia mosquito (Smith 1863) [RBINS]
Plebeia sp. 1 [DWR, RBINS]
Plebeia sp. 2 [DWR, RBINS]
Plebeia sp. 3 [DWR, RBINS]
Plebeia sp. 4 [DWR, RBINS]
Plebeia sp. 5 [DWR, RBINS]
Ptilotrigona lurida (Smith 1854) (Fig. 5.2d) [DWR, RBINS, RPSP, SEMK]
Scaptotrigona cf. *depilis* (Moure 1942) [RBINS]
Scaptotrigona fulvicutis (Moure 1964) [DWR, RBINS, RPSP]
Scaptotrigona sp. 1 (gr. *tubiba* Smith 1863) [RBINS]
Scaptotrigona sp. 2 (gr. *tubiba*) [RBINS]
Scaura latitarsis (Friese 1900) [DWR, RBINS, SEMK]
Scaura longula (Lepeletier 1836) [DWR, RPSP]
Scaura tenuis (Ducke 1916) [DWR, RBINS, RPSP]
Tetragona beebei (Schwarz 1938) [DWR, RBINS, RPSP]
Tetragona clavipes (Fabricius 1804) [DWR, RBINS, RPSP, SEMK]
Tetragona dorsalis (Smith 1854) [DWR, RPSP, SEMK]
Tetragona handlirschii (Friese 1900) [DWR, RBINS, RPSP, SEMK]
Tetragona kaieteurensis (Schwarz 1938) [RBINS, SEMK]
Tetragona sp. [RBINS]
Tetragonisca angustula (Latreille 1811) [DWR, RPSP, SEMK]
Trigona branneri Cockerell 1912 [DWR, RBINS, RPSP, SEMK]
Trigona cilipes (Fabricius 1804) (Fig. 5.2c) [DWR, RBINS, RPSP, SEMK] [=*T. mazucatoi* Almeida 1992]
Trigona crassipes (Fabricius 1793) [DWR, RBINS, RPSP, SEMK]
Trigona sp. 1 (gr. *crassipes* (Fabricius 1793)) [DWR, RPSP]
Trigona sp. 2 (gr. *crassipes* (Fabricius 1793)) [RPSP, SEMK]
Trigona sp. 3 (gr. *crassipes* (Fabricius 1793)) [RBINS]
Trigona sp. 1 (gr. *fuscipennis* Friese 1900) [DWR, RBINS]
Trigona sp. 2 (gr. *fuscipennis* Friese 1900) [DWR, RBINS]
Trigona sp. 3 (gr. *fuscipennis* Friese 1900) [RBINS]
Trigona guianae Cockerell 1910 [DWR, RBINS, RPSP, SEMK]
Trigona pallens (Fabricius 1798) (Fig. 5.2a, b) [DWR, RBINS, RPSP, SEMK]
Trigona permodica Almeida 1995 [DWR, RBINS, SEMK]

Trigona sp. (gr. *recursa* Smith 1863) [RPSP, SEMK]
Trigona sesquipedalis Almeida 1984 [DWR, RPSP]
Trigona williana Friese 1900 [DWR, RBINS, RPSP, SEMK]
Trigonisca dobzhanskyi (Moure 1950) [DWR, RPSP]
Trigonisca sp. [DWR, RBINS]

Some common species of stingless bees from French Guiana are illustrated in Fig. 5.2.

Fig. 5.2 Some Meliponini of French Guiana. (**a**) *Ptilotrigona lurida*, (**b**) *Trigona cilipes*, (**c**) *Trigona pallens* (at nest entrance), (**d**) *Trigona pallens*. Photos: Stéphane Brulé

Table 5.1 presents a list of Neotropical genera of stingless bees, including total number of described species and distribution.

Table 5.1 A list of Neotropical genera of stingless bees

Genus	Number of species	French Guiana	Notes on distribution
Aparatrigona Moure 1951	2	1	NW Brazil to Panamá
Camargoia Moure 1989	3	1	E and Central Brazil to French Guiana
Celetrigona Moure 1950	4	1	Bolivia, Peru, Brazil to Guianas
Cephalotrigona Schwarz 1940	5	1	Argentina and S Brazil to Trinidad
Dolichotrigona Moure 1950	10	1	Peru to Mexico and W and N Brazil
Duckeola Moure 1944	2	2	Bolivia and Peru to Guianas
Friesella Moure 1946	1	0	Only found in SE Brazil
Frieseomelitta Ihering 1912	16	5	SE Brazil to Mexico
Geotrigona Moure 1943	21	0	A widespread genus, from Argentina to Mexico, but so far not reported from French Guiana

(continued)

Table 5.1 (continued)

Genus	Number of species	French Guiana	Notes on distribution
Lestrimelitta Friese 1903	21	3	S Brazil to Mexico
Leurotrigona Moure 1950	4	1	S Brazil to Guianas
Melipona Illiger 1806 Including subgenera *Eomelipona* Moure 1992, *Melikerria* Moure 1992, *Melipona*, and *Michmelia* Moure 1975	71[a]	13	Widely distributed, from S Brazil and Argentina to Mexico
Meliwillea Roubik et al. 1997	1	0	Only found in the higher parts of Costa Rica and Panama
Mourella Schwarz 1946	1	0	Only found in S Brazil and south to Argentina, Paraguay, and Uruguay
Nannotrigona Cockerell 1922	10	2	S Brazil and Argentina to Mexico
Nogueirapis Moure 1953	3	1	Amazon region and W Andes from Ecuador to Costa Rica
Oxytrigona Cockerell 1917	11	1	S Brazil (SC) and Paraguay to Mexico
Parapartamona Schwarz 1948	7	0	Endemic to the Andean region (Peru to Colombia) at altitudes between app. 1,400 and 3,400 m
Paratrigona Schwarz 1938	30	2	Widely distributed, Argentina to Mexico
Paratrigonoides Camargo and Roubik 2005	1	0	Narrow endemic from Colombia (Antioquia, Bolívar)
Partamona Schwarz 1939	32	6	Widely distributed, S Brazil (SC) to Mexico
Plebeia Schwarz 1938	40	7	Widely distributed, Uruguay and Argentina to Mexico
Ptilotrigona Moure 1951	3	1	Amazon region and W Andes from Ecuador to Costa Rica
Scaptotrigona Moure 1942	22	4	Widely distributed, S Brazil and Argentina to Mexico
Scaura Schwarz 1938	5	3	SE Brazil to Mexico
Schwarziana Moure 1943	2	0	Restricted to SE and S Brazil, Paraguai, and Argentina
Schwarzula Moure 1946	2	0	SE Brazil to Amazon region (Bolivia, Peru Ecuador, and Brazil)
Tetragona Lepeletier and Serville 1828	13	6	Widely distributed, Uruguay to Mexico
Tetragonisca Moure 1946	4	1	Widely distributed, Argentina, Paraguai, S Brazil to Mexico
Trichotrigona Camargo and Moure 1983	1	0	Narrow endemic from Brazil (Amazonas)
Trigona Jurine 1807	32	14	Widely distributed, Argentina, Paraguai, S Brazil to Mexico
Trigonisca Moure 1950	25	2	Widely distributed, S Brazil to Mexico

Including total number of described species, presence (number of described/number of undescribed species in French Guiana), and notes on the distribution of the genera (based on Camargo and Pedro 2007; 2008a, b)

[a] Or 77, if subspecies are involved

5.1.3 A Unique and Intact Stingless Bee Fauna

We report from our survey all of the genera of stingless bees otherwise expected to be found in French Guiana (Table 5.1), with the exception of *Geotrigona*, a widespread genus of exclusively ground nesting bees. *Geotrigona* can be difficult to locate as they have a shy nest entrance defense, but *Geotrigona subnigra* (Schwarz 1940) was described from Guyana and this and other species of *Geotrigona* could be found with additional collecting in the country. At least two elements are outstanding in this small tropical country—the large number of *Frieseomelitta* and *Duckeola* in the white sand forests and the large number of *Melipona* species, especially in the interior of the country. We surmise that *Trichotrigona* inhabits the southern portion of French Guiana, due to the high number of potential host *Frieseomelitta*—which is the host to parasitic *Trichotrigona extranea* (Camargo and Moure 1983) as neighboring inquilines and thief—apparently never foraging outside its nest (Camargo and Pedro 2007). *Trigona amalthea* (Olivier 1789) was described from Cayenne (Olivier 1789); however, it is not included in the present list because this species has not been collected anywhere near Cayenne or in other parts of French Guiana, despite the intensive surveys in the region during 30 years (DWR). The only record of this species is the lectotype in Kiel collection, presently in the Zoological Museum, University of Copenhagen, Copenhagen Denmark (Camargo and Pedro 2007, 2008a) collected before 1789. It is possible that the type locality is an error; local extinction is another possibility.

The total number of species recorded here from French Guiana is 80 and is the highest number for any of the countries in the Guiana Shield probably in part due to incomplete sampling of the region, and certainly a much lower number than the fauna from better known areas, such as Brazil (178 spp., excluding those undescribed) and Peru (175 spp. including those undescribed, C. Rasmussen, unpublished).

There were still no European bees (*Apis mellifera* (Linnaeus 1758)) in French Guiana and probably not in other South American countries (Bolivia, Ecuador, Venezuela, Guyana, Peru, and Suriname) until the late 1800s (Crane 1999). Fougères Marquis de (1902) reported that in French Guiana most honey was harvested either from natural nests or from hives of stingless bees, but there were apiaries of modern hives, and in Cayenne Mme Cablat's 40 hives gave a colony average of about 40 kg of honey a year. We have no knowledge of stingless bee keeping for honey production. One of us (D.W. Roubik) observed that a beekeeper in Sinnamary, the largest in French Guiana (40 hives), occasionally harvests honey from *Melipona favosa* and *Frieseomelitta flavicornis*—two common savanna-forest edge bees—which he has in small hives at his home. The Africanized honey bee arrived in French Guiana during 1975, and this was the earliest date at which there were honey bees living in the wild (D.W. Roubik, personal observation). The great number of meliponine species recorded from a relatively small area like French Guiana gives an idea of the many sources of honey that must be available there, even though almost no use of them is recorded.

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Chapter 6
Stingless Bees of Guatemala

Carmen Lucía Yurrita Obiols and Mabel Vásquez

6.1 Introduction

In many areas of their tropical distribution, the meliponines are the most common bees and hence are considered to play an important role as pollinators of native and crop vegetation (Slaa et al. 2006). This fact has been taken advantage of by local human populations, who have learned to harvest the honey (Villanueva et al. 2005; Posey 1982).

In Guatemala, as in other Central American countries, the inhabitants of some regions keep a few of the stingless bee species in a traditional way and use the honey and the pollen as a medicine and food source. However, despite their importance these and other bees are at risk due to a combination of factors, including deforestation and presumably competition with nonnative species (Villanueva et al. 2005). In the case of the stingless bees destruction of colonies to extract honey and pollen represents an additional threat.

In this chapter we present an overview of the stingless bee species native to Guatemala, the species richness of the group, their distribution in the country, floral resources visited, stingless bee beekeeping activity, and uses of stingless bee-derived products, particularly honey.

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6.2 Taxonomy and Distribution of Stingless Bees in Guatemala

The bee family Apidae includes the only two groups of highly eusocial corbiculate bees: the tribes Apini and Meliponini. These two tribes are characterized by the presence of a pollen carrying structure on the hind legs called "corbicula." This feature is shared with other corbiculate bees of the same family: the primitively eusocial bumble bees (Bombini) and the mostly solitary orchid bees (Euglossini). From a behavioral point of view Meliponini, like Apini, differ from other eusocial bees in that they form permanent colonies in which queens and workers are morphologically very different, the queen never forages, and neither the queen nor the workers can establish colonies by themselves (Michener 2007).

The Meliponini exhibit a worldwide tropical and subtropical distribution and are the most diverse group of the corbiculate bees, with several hundred species (Rasmussen and Cameron 2010). Different classifications of the group have been proposed. In one classification system, a few genera and many subgenera are recognized, in order to emphasize the relationships between the groups (Michener 2007). In the other classification system many subgenera are elevated to the generic level to stress the full taxonomic diversity of the tribe (Moure 1961; Rasmussen and Cameron 2007, 2010; Camargo and Pedro 2008). Here, we use the classification system proposed by Moure (1961) and Camargo and Pedro (2008). In this system the entire tribe consists of at least 59 genera (Moure 1961; Camargo and Pedro 2008; Rasmussen and Cameron 2007). The greatest diversity of stingless bees is found in the Tropical America where 33 genera have been recognized that include approximately 400 species (Camargo and Pedro 2008).

Here we present an updated list of the stingless bees of Guatemala that has been prepared using the identification key for the Meliponini of Mexico (Ayala 1999) and the specimens included in the entomological Guatemalan Native Bee Collection "Colección de Abejas Nativas de Guatemala" (CANG), of the Biodiversity Research Unit at the Conservation Studies Center (CECON) of the University of San Carlos of Guatemala. In Guatemala the diversity of Apoidea is estimated as at least 500 species (Enríquez et al. 2012), belonging to the families Andrenidae, Apidae, Colletidae, Halictidae, and Megachilidae. Of these the family Apidae has the highest diversity with 227 reported species (Enríquez et al. 2012). Currently, 33 stingless bee species are included in the collection (Table 6.1). Even though this number may increase with additional taxonomic and collecting work, it is not expected to exceed either the Costa Rican richness (50 species, Ortiz 1998) nor the Mexican one (46 species, Ayala 1999). Indeed a bibliographic survey produced a list of approximately 40 species of meliponines already reported for the country (Enríquez et al. 2012), which covers records from the literature (Camargo and Pedro 2008) like *Paratrigona opaca* (Cockerell, 1917), *Geotrigona lutzi* Camargo & Moure, 1996, *Geotrigona terricola* Camargo & Moure, 1996 and *Scaptotrigona wheeleri* (Cockerell, 1913), as well as material from other collections that was not included here.

Table 6.1 List of the Guatemalan stingless bees in the native bee collection (CANG). Departments (geopolitical division), number of collecting sites, and altitudinal ranges where they have been collected (from CANG database)

No.	Stingless bee species	Departments of occurrence	No. of sites	Altitudinal ranges of collecting
1	*Cephalotrigona zexmeniae* (Cockerell, 1912)	AV, CHIQ, SR	4	0–500, 501–1,000, 1,001–1,500
2	*Dolichotrigona schulthessi* (Friese, 1900)	AV, QUE, REU, SM	7	0–500, 501–1,000, 1,001–1,500
3	*Frieseomelitta nigra* (Cresson, 1878)	PR	3	0–500
4	*Geotrigona acapulconis* (Strand, 1919)	G, SR	2	1,001–1,500
5	*Lestrimelitta niitkib* Ayala, 1999	AV, G, PR	5	0–500, 1,001–1,500, 1,501–2,000
6	*Melipona beecheii* Bennett, 1831	AV, BV, CHIQ, QUI, PE, ESC, G, I, JUT, REU, SR, SOL	36	0–500, 501–1,000, 1,001–1,500, 1,501–2,000
7	*Melipona solani* Cockerell, 1912	AV, I, QUI, PE, HUE, QUE, REU, SM	19	0–500, 501–1,000, 1,001–1,500
8	*Melipona yucatanica* Camargo, Moure & Roubik, 1988	HUE, JUT, SAC, SR	5	501–1,000
9	*Nannotrigona perilampoides* (Cresson, 1878)	AV, BV, PE, G, I, JUT, SR, ZAC	16	0–500, 501–1,000, 1,001–1,500, 1,501–2,000
10	*Oxytrigona mediorufa* (Cockerell, 1913)	CHIM, I, QUE, SUCH	4	501–1,000, 1,001–1,500
11	*Paratrigona guatemalensis* (Schwarz, 1938)	AV, SR	4	0–500, 1,001–1,500
12	*Partamona bilineata* (Say, 1837)	AV, BV, CHIQ, G, HUE, I, JUT, JAL, QUE, QUI, REU, SAC, SM, SOL, SUCH	35	0–500, 501–1,000, 1,001–1,500, 1,501–2,000, 2,001–2,500
13	*Partamona orizabaensis* (Strand, 1919)	AV, CHIQ, QUI, QUE, REU, SM, SOL, SUCH	9	0–500, 501–1,000, 1,001–1,500, 2,001–2,500
14	*Plebeia frontalis* (Friese, 1911)	CHIQ, PE, I, ZAC	7	0–500, 1,001–1,500
15	*P. fulvopilosa* Ayala, 1999	CHIQ	1	1,501–2,000
16	*P. jatiformis* (Cockerell, 1912)	AV, G, SR	10	0–500, 1,001–1,500, 1,501–2,000
17	*P. llorentei* Ayala, 1999	AV	2	0–500
18	*P. melanica* Ayala, 1999	BV, CHIQ, QUI	5	1,501–2,000
19	*P. moureana* Ayala, 1999	AV, JUT, SM	5	0–500, 501–1,000
20	*P. parkeri* Ayala, 1999	AV, G, QUE, SM, SR	7	0–500, 501–1,000, 1,001–1,500
21	*P. pulchra* Ayala, 1999	AV, QUI, SR	6	0–500, 1,001–1,500

(continued)

Table 6.1 (continued)

No.	Stingless bee species	Departments of occurrence	No. of sites	Altitudinal ranges of collecting
22	*Scaptotrigona mexicana* (Guérin-Méneville, 1844)	AV, CHIM, QUI, QUE, REU, SM, SR	11	0–500, 501–1,000, 1,001–1,500, 1,501–2,000
23	*S. pectoralis* (Dalla Torre, 1896)	QUI, REU, SM, SR	5	0–500, 501–1,000, 1,001–1,500
24	*Scaura argyrea* (Cockerell, 1912)	AV, PE	11	0–500, 501–1,000
25	*Tetragona mayarum* (Cockerell, 1912)	CHIQ, I	3	0–500, 501–1,000
26	*Tetragonisca angustula* (Latreille, 1811)	CHIM, SUCH, QUE, REU, PR, G, JUT, I, CHIQ, AV	19	0–500, 501–1,000, 1,001–1,500, 1,501–2,000
27	*Trigona corvina* Cockerell, 1913	AV, BV, CHIQ, PR, JUT, I, SUCH, SR, ZAC	23	0–500, 501–1,000, 1,001–1,500, 1,501–2,000
28	*T. fulviventris* Guérin-Méneville, 1844	AV, CHIQ, PE, PR, QUI, G, I, JUT, QUE, SAC, SM, SR, SOL, SUCH, ZAC	31	0–500, 501–1,000, 1,001–1,500, 1,501–2,000
29	*T. fuscipennis* Friese, 1900	AV, CHIM, PR, I, JUT, SUCH	8	0–500, 501–1,000, 1,001–1,500
30	*T. nigerrima* Cresson, 1878	AV, PE, QUE, SR, SOL, SUCH	7	0–500, 1,001–1,500, 1,501–2,000, 2,001–2,500
31	*T. silvestriana* (Vachal, 1908)	AV, I	10	0–500, 501–1,000
32	*Trigonisca maya* Ayala, 1999	PR	1	0–500
33	*T. pipioli* Ayala, 1999	BV, CHIQ	2	501–1,000, 2,001–2,500

AV Alta Verapaz, *BV* Baja Verapaz, *CHIQ* Chiquimula, *CHIM* Chimaltenango, *ESC* Escuintla, *G* Guatemala; *HUE* Huehuetenango, *I* Izabal, *JAL* Jalapa, *JUT* Jutiapa, *PR* El Progreso, *PE* Petén, *QUE* Quetzaltenango, *QUI* Quiché, *REU* Retalhuleu, *SAC* Sacatepéquez, *SM* San Marcos, *SR* Santa Rosa, *SUCH* Suchitepéquez, *SOL* Sololá, *ZAC* Zacapa

The species included in the CANG belong to 17 different genera of those recognized by Camargo and Pedro (2008) for the neotropical region. *Plebeia* and *Trigona* are the most diverse genera with eight and five species, respectively. *Melipona* include three species and *Partamona*, *Scaptotrigona*, and *Trigonisca* two. The remaining 11 genera are represented by a single species each (Table 6.1). The species *Tetragona dorsalis* (Smith, 1854) and *Scaura latitarsis* (Friese, 1900) included in Ayala (1999) now correspond to *Tetragona mayarum* (or *ziegleri*) and *Scaura argyrea*, respectively (Camargo and Pedro 2008). The species cited here as *Trigona silvestriana* has also been interpreted as *T. amalthea* (Olivier, 1789) (Roubik and Moreno 2009; DW Roubik, personal communication). Of the species listed, *Lestrimelitta niitkib* is the only cleptobiotic one.

Most of the species reported are mainly Mesoamerican, with distributions spanning from Mexico to Colombia (Camargo and Pedro 2008). The exceptions are *Trigona nigerrima*, *Trigona silvestriana*, and *Tetragonisca angustula* which are found farther south than Colombia. The presence of *Trigonisca maya* in Guatemala represents a new distributional record, since it was previously reported only in Mexico (Ayala 1999; Camargo and Pedro 2008).

The distributional analysis presented in this work is based on a geopolitical division of the territory. However, in order to provide a more realistic geographic approach we made reference to the altitudinal range and the type of forest where species were collected. Unfortunately, the available information does not represent the actual distribution of species, given that the collecting effort has not been systematic across the country. Indeed, most of the collecting sites correspond to places where the research group has carried out other studies. Nevertheless, the available data show that Meliponini have a wide distribution in Guatemala, since the species have been collected in all but one of the 22 Departments (administrative division equivalent to Province) of the country. The distributional data correspond to 323 unique localities in which at least one of the 33 species recorded has been collected (Fig. 6.1 and Table 6.1).

Some species can be considered more common since they have been collected in more sites (Table 6.1). For instance, *Partamona bilineata* and *Trigona fulviventris* have been collected in 15 Departments at 31 and 35 localities within them, respectively, while *Trigonisca maya*, *Plebeia fulvopilosa*, *P. llorentei*, and *Frieseomelitta nigra* have been collected in one Department and one or two localities. The departments of Alta Verapaz (North Central region), Santa Rosa (Central South), and Chiquimula (East) show the highest diversity, with more species recorded (22, 14, 12, respectively) (Table 6.1). Nonetheless, this result might be biased by the fact that they correspond to areas where a larger collecting effort has been made. Additionally, the collecting sites within them are very localized (Fig. 6.1).

The stingless bee species in Guatemala are found in a wide variety of forests (pine-oak, dry, thornscrub, montane, and moist) at elevations that range from near sea level to as high as 2,353 m in the mountainous areas. The majority of species do not show a very clear distribution in relation to a certain forest type. Indeed, even species for which we have only a few records can be found in very different areas. For instance, *Plebeia pulchra* and *Paratrigona guatemalensis* have been collected

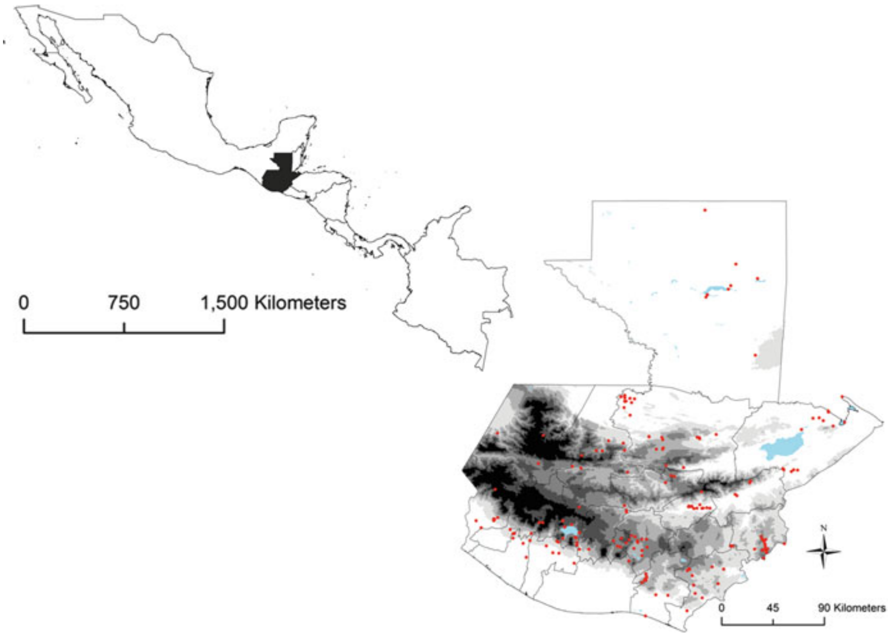

Fig. 6.1 Occurrence localities of the stingless bees in Guatemala. (●) Collecting sites; altitudinal ranges (masl): ☐0–500, ▨501–1,000, ▨1,001–1,500, ▨1,501–2,000, ▨2,001–2,500, ■2,501–4,000

in six and four different sites, respectively, located in moist (department of Alta Verapaz) and montane (Santa Rosa) forests. However, a few species like *Trigona silvestriana* that appear in ten different localities exhibit a distribution restricted to very moist forests (Alta Verapaz and Izabal).

Most species (31) occur between sea level and 1,500 m. Four of these (*Trigonisca pipioli, Oxytrigona mediorufa*, *Geotrigona acapulconis*, and *Melipona yucatanica*) have not been collected at the lowest elevation range (0–500 m), while *Frieseomelitta nigra*, *Trigonisca maya*, and *Plebeia llorentei* were captured exclusively in this altitudinal range. However, according to Ayala (1999) *F. nigra* can be found in altitudes over 1,500 m. Fourteen of these species were also collected up to 2,000 m elevation, and in different kinds of forests. *Plebeia fulvopilosa* and *P. melanica* were restricted to elevations of 1,500–2,000 m, as Ayala (1999) reports. Only four species appeared in the highest altitudinal range, and all of them (*Partamona bilineata*, *P. orizabaensis*, *Trigona nigerrima*, and *Trigonisca pipioli*) exhibit a very wide altitudinal range of distribution and habitat preference, occurring from the lowest to the highest elevations and distributed in different habitat types.

Figure 6.1 indicates that collection is few in the southern coast and in northern part of the country, as well as in the central east and central west regions. Nonetheless, given the wide altitudinal and habitat tolerance of some species we expect that most will appear in these areas in future studies.

6.3 Floral Resources of Stingless Bees

Animal-mediated pollination is an important ecosystem service for sexually reproducing plants. Bees are considered the most important pollinators for major agricultural crops (Klein et al. 2007) and wild plants (Cane et al. 2006). In the tropics, the stingless bees constitute an important portion of the flower-visiting fauna (Lorenzon et al. 2003; Wilms et al. 1996), having been reported as the major pollinators of 18 crops (Slaa et al. 2006; Heard 1999) and contributing to different degrees to the pollination of many others (Heard 1999).

The stingless bee species stored at CANG were collected on flowers from at least 117 different species (data not shown) that belong to 47 plant families, having information about the resource they provide to the bees (polen and/or nectar) only for a small portion (Table 6.2). Most of the visited families (70%) can be considered

Table 6.2 Plant families visited by the stingless bee species in Guatemala. Number of bee species visiting and the number of plant species visited per family (from CANG database)

Plant families visited	No. of visiting bee species	No. of plant species visited	Pollen (P)/nectar (N) source
Asteraceae	21	30	P, N
Zingiberaceae	16	1	P
Fabaceae (Papilionoideae, Mimosoideae, Caesalpinioideae)	13	11	P, N
Bixaceae	11	1	P
Malpighiaceae	11	1	P
Lamiaceae	10	4	P, N
Onagraceae	10	1	P, N
Cucurbitaceae	9	1	P
Poaceae	9	1	
Malvaceae	8	5	P, N
Melastomataceae	6	3	P, N
Rubiaceae	6	4	
Violaceae	6	1	
Solanaceae	5	5	P
Convolvulaceae	4	3	P, N
Commelinaceae	4	2	
Cyperaceae, Piperaceae	4	1	P, N
Cactaceae, Euphorbiaceae	3	3	P
Bignoniaceae, Passifloraceae	3	2	P, N
Bromeliaceae, Salicaceae, Vitaceae, Zygophyllaceae	3	1	
Apocynaceae, Arecaceae, Boraginaceae, Costaceae, Lythraceae,	2	2	
Acanthaceae, Fagaceae, Musaceae, Nyctaginaceae, Orchidaceae	2	1	P, N
Asparagaceae	1	2	
Anacardiaceae, Apiaceae, Brassicaceae, Caryophyllaceae, Myrtaceae, Phytolaccaceae, Ranunculaceae, Rosaceae, Sapindaceae, Verbenaceae	1	1	P, N

occasional floral resources, since only a few species (<5) forage on their flowers. In addition, for each of these families, only a few species were visited (<5 species per family) (Table 6.2). On the other hand, Asteraceae and Fabaceae can be suggested to be an important food source for the stingless bees as the diversity of the visited species within these families was higher (30 and 11 visited species, respectively). Moreover, approximately 60% (21) and 40% (13) of the identified bee species, respectively, have been collected while foraging on their flowers (Table 6.2). Other studies have already reported that the family Asteraceae is one of the main food resources for the stingless bees (Wilms et al. 1996). Other plant families visited by more than five bee species show that seven of them are represented by a single species (Table 6.2). This is the case of "achiote" *Bixa orellana* (Bixaceae), "nance" *Byrsonima crassifolia* (Malpighiaceae), "cardamomo" *Elettaria cardamomum* (Zingiberaceae), and watermelon *Citrullus lanatus* (Cucurbitaceae). These species, all important economic and/or food resources for human populations in Guatemala, were part of a more detailed survey. A palynological analysis was carried out to assess the potential of the stingless bees foraging on their flowers as pollen vectors. For *Elettaria cardamomum* eight of the 16 visiting species are suggested as potential pollinators. In the case of *Bixa orellana* six out of 11 can be considered possible pollinators, and for *Citrullus lanatus* and *Byrsonima crassifolia* six and one stingless bee species, respectively, were detected as potential pollinators (Enríquez 2007). Previous studies had already registered these plants as effectively or occasionally being pollinated by stingless bees in other regions (Slaa et al. 2006; Heard 1999). The flowers of maize *Zea mays* (Poaceae), the only recorded species from the Poaceae family, were visited by nine stingless bee species, but there is no evidence proving that these visiting species are acting as potential pollinators.

Eleven meliponines were collected on less than five plant species, and five were not collected on any flower (Table 6.3). Among the latter *Lestrimelitta niitkib* is not expected to collect pollen (or visit flowers) since it has a cleptobiotic behavior. In Guatemala, this bee has been seen attacking colonies of at least two stingless bee species, *Melipona beecheii* and *Tetragonisca angustula* (CL Yurrita 2011, personal observation). *Trigona fulviventris* is the species that visited the widest array of plants (45) (Table 6.3); nonetheless, it has been documented that sometimes it may not act as a pollinator but rather as a nectar or pollen robber (Barrows 1976; CL Yurrita 2010, personal observation). *Melipona* spp. are capable of buzz pollination (Heard 1999), a feature that makes them potential pollinators of many plants. Finally there is a record of *Partamona orizabaensis* captured on feces.

6.4 Stingless Beekeeping in Guatemala

There is a long tradition of stingless beekeeping, or meliponiculture, in the Mesoamerican region (Kent 1984; Crane 1992; Cortopassi-Laurino et al. 2006) and in the Amazon (Posey 1982; Posey and Camargo 1985), in comparison with other

Table 6.3 Number of plant species visited by the stingless bees (from CANG database)

Stingless bee species	Plant species visited
Plebeia parkeri	5
Scaptotrigona mexicana	5
Scaptotrigona pectoralis	5
Trigonisca maya	5
Trigonisca pipioli	5
Trigona silvestriana	7
Melipona solani	8
Scaura argyrea	8
Tetragona mayarum	8
Partamona orizabaensis	8
Melipona beecheii	13
Trigona nigerrima	14
Cephalotrigona zexmeniae	15
Nannotrigona perilampoides	16
Plebeia jatiformis	17
Trigona fuscipennis	17
Tetragonisca angustula	29
Trigona corvina	29
Partamona bilineata	33
Trigona fulviventris	45
Dolichotrigona schultessi, *Frieseomelitta nigra*, *Melipona aff. yucatanica, Paratrigona guatemalensis*, *Plebeia frontalis*, *P. fulvopilosa*, *P. melanica*, *P. moureana*, *P. pulchra*	<5

The following species were not collected on flowers and were not included in the table: *Geotrigona acapulconis*, *Lestrimelitta niitkib*, *Oxytrigona mediorufa*, *Plebeia llorentei*

regions of the world (Cortopassi-Laurino et al. 2006). This is probably due to the great diversity of meliponines found in Tropical America. In Mesoamerica, stingless bee beekeeping has been culturally important since the precolonial era. Indeed, the Maya codices and some colonial writings record the importance of the stingless bees in the Mayan culture. This importance is revealed by the existence of bee gods (Maya codices) and the rituals of beekeeping and use of hive products documented in the writings of the Bishop Diego de Landa (*apud* Kent 1984). The Mayan region including the Yucatán Peninsula and northern Guatemala and Belize were suggested as a place of intense stingless bee rearing activity in pre-Columbian days, particularly *Melipona beecheii*. Furthermore, this region has been considered the possible place of origin of the practice (Kent 1984; Crane 1992). Thus, both the beekeeping technique and the hive design most commonly employed in the Yucatán Peninsula are considered the original ones (Crane 1992). Nowadays the traditional practice of meliponiculture in the Yucatán Peninsula and in other regions of México (González-Acereto and De Araujo-Freitas 2005), as well as in areas throughout Mesoamerica (Enríquez et al. 2005; Kent 1984), has not changed much over time.

In Guatemala, stingless bee beekeeping is practiced by different ethnic groups across the country. Kent (1984) has documented that the activity takes place in the

Table 6.4 Common names of stingless bees used in Guatemala [modified from Enríquez et al. (2005)]

No.	Scientific name	Folk name
1	*Cephalotrigona zexmeniae*	"congo"
2	*Lestrimelitta niitkib*	"limoncillo"
3	*Melipona beecheii*	"colmena grande," "criolla," "abeja maya," "xuna'n cab," "bichi"
4	*Melipona solani*	"chac chow"
5	*Melipona yucatanica*	"tinzuca"
6	*Nannotrigona perilampoides*	"serenita"
7	*Oxytrigona mediorufa*	"tamagás," "pringador"
8	*Partamona* sp.	"sacar," "cushpun"
9	*Plebeia* sp.	"chelerita," "serenita," "boca de sapo," "sarquita"
10	*Scaptotrigona mexicana*	"magua negro," "congo," "congo negro"
11	*Scaptotrigona pectoralis*	"magua canche," "alazán," "congo canche," "shuruya"
12	*Tetragonisca angustula*	"chumelo," "doncellita"
13	*Trigona fulviventris*	"mandinga," "culo de chucho"
14	*Trigona nigerrima*	"cushusho," "homo," "joloncán"
15	*Trigona silvestriana*	"homo"
16	*Geotrigona acapulconis*	"talnete"

Q'eqchi (Alta Verapaz), Maya-chortí (Jocotán, Chiquimula), and Jacaltec (Jacaltenango, Huehuetenango) areas. Our research group has worked with beekeepers in different regions of the country. The most important group dedicated to rearing the stingless bees are the Ladinos or Mestizos even though the practice is also carried out by Q'eqchí, Chortí, Mam, and Ixil-Quiché populations. For most of these people keeping the stingless bees remains a family tradition inherited for generations, although for others it is a recent activity, initiated as a result of their attendance at workshops carried out by different organizations, including our research group.

People identify at least 16 stingless bee species, some of which have different regional names (Table 6.4). Given the great variety of local names that meliponines receive, we can deduce that they constitute a well-known part of the insect fauna in Guatemala, even if the number of species used in meliponiculture is limited.

Meliponiculture is still practiced in a traditional way in Guatemala. The beekeepers for whom the activity is an inherited family tradition still employ the original techniques (Crane 1992) which involve the use of hollow logs closed at both ends with discs made of wood. Usually the hives are hanging from the roof of houses and less frequently people construct shelters to keep them.

The most important species reared with a honey-harvesting purpose are *Melipona beecheii* and *Tetragonisca angustula*. Another important bee species from which the honey is used is *Geotrigona acapulconis*. However, its nesting behavior (nest constructed deep underground) makes it difficult for people to keep them in hives, and the only way to extract the honey is by destroying the nest. A larger number of bee species are reared with ornamental purposes (because "they are nice"), but eventually their honey can be extracted. Occasionally, people harvest honey from nests kept in their original location without destroying them. This is the case for *Trigona nigerrima* which constructs its nest on tree branches and not in hollows, making it easier to harvest the honey in place. *Scaptotrigona mexicana* and *S. pectoralis* are two species with a special potential in meliponiculture due to the low management requirements and high yields they provide. Finally, the honey of some other species, like that of *Trigona fulviventris*, is avoided due to their anti-hygienic behavior (they collect feces) (M Vásquez 2010, personal observation).

The main product harvested from the stingless bees in Guatemala is the honey, but the pollen and the cerumen are also used. The honey is used mainly for medicinal practices or as an energy supplement, but it is not an important food item, probably due to the small yield. The medicinal properties attributed to the stingless bee honey are very diverse and depend on the species producing it, even though some uses are common to all of them. The honey of *Melipona beecheii* is the most appreciated, probably due to the fact that this species produces larger amounts of honey in comparison with *Tetragonisca angustula* (Vit et al. 2004). Usually the honey is not for sale; if someone in the community needs some, a beekeeper will provide it without any cost. In Guatemala, our research group has undertaken studies aiming to investigate the pollen species content, the antibacterial activity, the physicochemical properties, and the sensory attributes of the honey of nine of the 32 stingless bee species used for honey production (almost 30% of the honey diversity) (Dardón and Enríquez 2008, and Dardón et al., Chap. 28 in this book).

6.5 Final Comments

Given the diversity of stingless bees in Guatemala and the wide distributional range of the majority of the species, promoting the use of the honey as an alternative energetic or medicinal supplement or perhaps as a food complement could be a great opportunity.

Nonetheless, as it has been suggested for other regions (Villanueva et al. 2005), bees like *Melipona* and other species that nest in tree hollows may be at risk in Guatemala. One important reason causing this situation is the loss of nesting sites as a consequence of the high deforestation rate, which reaches 1.53% each year in Guatemala (Tuy et al. 2009). Also, as was pointed out for Yucatán (Villanueva et al. 2005), the stingless bee beekeeping practice itself may be in decline in Guatemala.

Therefore, the potential loss of the stingless bee diversity as well as that of the meliponiculture hampers the use of the great diversity of honeys for medical or food complement purposes. Moreover, the lack of quality standards for the honey prevents the marketing of the product.

It is therefore necessary to promote programs aiming to preserve the species habitats as well as programs to enhance the practice of meliponiculture to transform it in a certifiably hygienic and productive activity. That initiative has to be complemented by continuing studies on honey composition, as well as by educating people on improving meliponiculture techniques.

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Chapter 7
Stingless Bees of Costa Rica

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7.1 Introduction

The keeping of stingless bees (Apidae, Meliponini), or meliponiculture, is carried out in a rustic and traditional way in tropical America by a variety of ethnic groups and rural populations. This practice has been maintained over time in regions of Mexico, Central America, and South America in countries such as Brazil, Venezuela, Colombia, Ecuador, Bolivia, Peru, and Argentina (Mahecha and Nates-Parra 2002; Elizalde et al. 2007; Flores and Sánchez 2010; Jiménez 2011). This is due in part to the cultural value, which this practice holds, but also reflects the interest that has been aroused in consumers of stingless bee honey as a medicinal alternative.

The product of these bees that is mostly used is the honey (De Jong 1999; Aguilar 2010; Herrera and Aguilar 2011) and this has generated much interest in the scientific community due to the results achieved by microbiological tests, which have shown that stingless bee honey has antimicrobial properties (Gonçalves et al. 2005; Aguilera et al. 2009; Vit et al. 2009). Paradoxically, in Costa Rica the growing demand for these products coincides with a decrease in the populations of some species of this group, e.g., *Melipona beecheii* (Villanueva-Gutiérrez et al. 2005; Genaro 2006). This reduction is due to the environmental damage caused by the process of urbanization, the direct consequences of such include loss of forest areas, among other effects. We are thus making scientific progress in recognizing the usefulness of stingless bees in terms of their products and uses in natural folk medicine, as well as pollination services, but at the same time we are losing the natural resource that provides these products and services. This becomes a problem for fulfilling the demands of the market (environmental services of pollination, acquisition of colonies and products such as honey or propolis). Consequently, we must act

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to strengthen the conservation and sustainable use of these bees. This chapter refers to the stingless bees of Costa Rica, with attention to stingless beekeeping, and it has two goals: first, we document the information that exists about the past and current state of meliponiculture in our country, and second we record the bee species as well as the tree species that bees use for establishing nests.

7.1.1 Stingless Beekeeping in Costa Rica

Stingless beekeeping has its origins in the culture of the Maya of the Yucatan Peninsula and this practice spread to other groups in Mesoamerica, extending to the northern part of Costa Rica (southern tip of Mesoamerica, Nicoya, Guanacaste Province) under the influence of the indigenous Nahuatl and Mestizos (Kent 1984). Another study of the pre-Hispanic cultures (Tous 2002) based on ethnographic descriptions of the region known as "La Gran Nicoya"—16th-17th centuries—that among the products obtained from the harvest were honey and cerumen. Honey was used for human consumption; the cerumen was very abundant and used for lighting and silver work , the "lost wax" technique. Tous (2002) also mentions that the practice of trade and exchange with local products, such as honey and cerumen, were of vital importance in the Nicoya Peninsula to resolve situations of scarcity; at the same time these products were used for exchange with other indigenous groups since its redistribution ensured a more diversified access. Kent (1984) mentions that in the Nicoya of the 1500s the indigenous people delivered 55 L of honey every 6 months as a tribute to the priests. The Boruca of the Central Valley (Province of San José) and the Térraba (native of the Atlantic coast, Limón Province) used cerumen on their spears and arrows (Stone and Gabb cited by Kent 1984).

Despite the data mentioned above, there are very few records concerning the use of this natural resource by the indigenous people of Costa Rica. We believe this is because the ethnic groups in Costa Rica suffered from eighteenth century slavery and resettlement to areas far from their original territory (Montoya et al. 2008).

Possibly, as in other Mesoamerican areas, stingless bee keeping in Costa Rica did not play an important role in the religion of indigenous cultures. Yet, an image of a bee found in Costa Rica (De Jong 1999) and other reports cited by Kent (1984) that allude to the use of words such as honeycomb, wax, and honey in the language of the Bribri and Cabécar suggest that the bees had a meaning for them.

It was reported that an abundance of honey and cerumen was produced around the beginning of the twentieth century (Kent 1984; De Jong 1999). Kent (1984), at the beginning of the 1980s in the area of the Central Valley, described the existence of a more advanced meliponiculture. The author mentioned the use of *Tetragonisca angustula* and at least three species of *Melipona*. The greatest numbers recorded of colonies were in the Provinces of Guanacaste and San José with *T. angustula* and *Melipona beecheii*. There are no reports indicating the use of these bees by local indigenous groups during this period.

According to our records, the beginning of the twenty-first century is marked by a widespread use of *T. angustula* (Aguilar 2009; Herrera and Aguilar 2011).

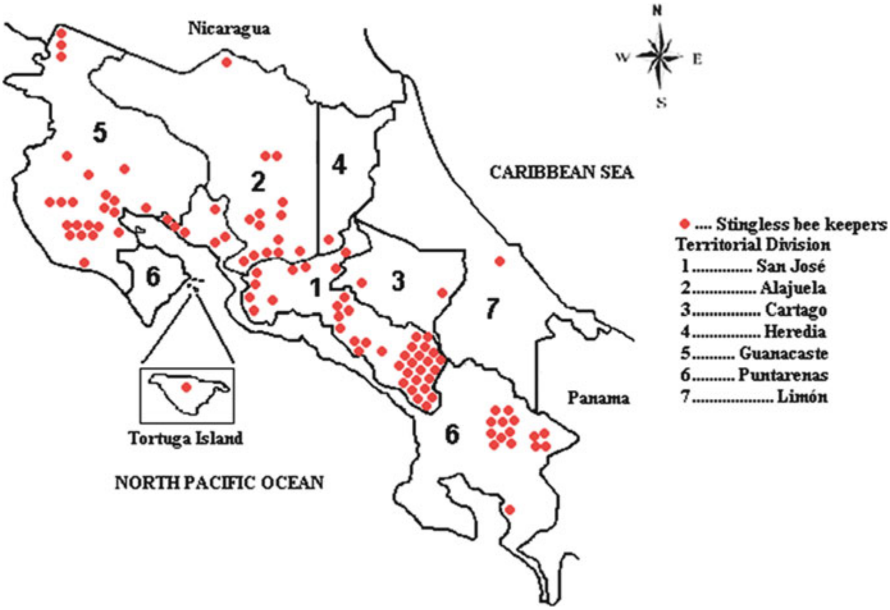

Fig. 7.1 Map of Costa Rica with the location of stingless bee keepers (*red dots*), showing the boundaries of the provinces

A large number of stingless bee keepers are situated in the rural areas of San José, mostly on the Pacific coast rather than the Atlantic region (see Fig. 7.1). Currently in San José Province, in the canton of San José (the capital of Costa Rica), it is very rare to find rational boxes with nests of stingless bees. We have occasionally found boxes with *T. angustula* and *Nannotrigona* in the cantons of Santa Ana, Montes de Oca, Moravia, and Escazú, more frequently in the cantons of Puriscal, Tarrazú, Aserrí, Acosta and Perez Zeledón. In the canton of San José the genus *Melipona* has not been reported in the last three decades, which is linked with the urban development of this area. In addition, we have observed in the remnants of riparian forests, coffee plantations, playgrounds of the urban areas of this and other provinces a variable but important number of nests of *T. angustula*, *T. corvina*, and *Nannotrigona* spp. Other areas belonging to Guanacaste and Puntarenas provinces, for example Santa Cruz, Hojancha, Philadelphia, and Miramar, are known for traditional meliponiculture (De Jong 1999). We have also observed a few stingless bee keepers in Heredia, Cartago, and Limón provinces (see Fig. 7.1). Some of them have received motivation during recent workshops.

7.1.2 Management of Native Stingless Bee Species

A total of 20 different hived or semi-domesticated species have been reported (see Table 7.1) in the provinces of Guanacaste, Puntarenas, San José, Cartago and Heredia (Arce et al. 1994; Ramírez and Ortiz 1995; De Jong 1999; Herrera and Aguilar 2011). It is mainly *T. angustula* that is being kept, followed by *M.*

Table 7.1 Stingless bees of Costa Rica. Nomenclature according to Camargo and Pedro (2007, 2008). Information on domesticated species, common names and distribution is given

Species	Domesticated species	Common name	Distribution (province of Costa Rica)
Cephalotrigona zexmeniae (Cockerell, 1912)	(c)	"tamaga amarillo"	Alajuela, Cartago, Guanacaste, Heredia, Puntarenas, San José
Dolichotrigona schulthessi (Friese, 1900)	ND	ND	Cartago, Puntarenas
Frieseomelitta nigra (Cresson, 1878)	(f)	"ala blanca"	Guanacaste
Frieseomelitta paupera (Provancher, 1888)	ND	"chupaojos"	Guanacaste
Geotrigona chiriquiensis (Schwarz, 1951)	ND	ND	Alajuela, Cartago, Puntarenas, San José
Geotrigona lutzi Camargo & Moure, 1996	ND	ND	Alajuela, Cartago, Guanacaste, Limón, Puntarenas, San José
Lestrimelitta danuncia Oliveira & Marchi, 2005	(c, f)	"jicote limón"	Cartago
Lestrimelitta mourei Oliveira & Marchi, 2005	ND	ND	San José
Melipona beecheii Bennett, 1831	(a, b)	"jicote gato"	Puntarenas, San José
Melipona carrikeri Cockerell, 1919	ND	ND	Guanacaste, San José
Melipona costaricensis Cockerell, 1919	(a)	"jicote barcino"	Guanacaste, Heredia, Limón, Puntarenas, San José
Melipona fuliginosa Lepeletier, 1836	(a, c)	"jicote"	Cartago, Guanacaste, Limón, Puntarenas, San José
Melipona panamica Cockerell, 1912	ND	ND	Costa Rica
Melipona torrida Friese, 1916	ND	ND	San José
Melipona yucatanica Camargo, Moure & Roubik, 1988	ND	ND	Puntarenas
Meliwillea bivea Roubik, Lobo & Camargo, 1997	ND	ND	Cartago, Puntarenas, San José
Nannotrigona mellaria (Smith, 1862)	(d)	ND	Heredia, Puntarenas
Nannotrigona perilampoides (Cresson, 1878)	(a, b)	"chicopipe"	Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas
Nogueirapis mirandula (Cockerell, 1917)	ND	ND	Guanacaste, Puntarenas, San José
Oxytrigona daemoniaca Camargo, 1984	ND	ND	Costa Rica
Oxytrigona mellicolor (Packard, 1869)	(c)	"peladora"	Alajuela, Guanacaste, Limón, Puntarenas, San José
Paratrigona lophocoryphe Moure, 1963	ND	ND	Cartago, Limón
Paratrigona opaca (Cockerell, 1917)	(d)	ND	Limón, Puntarenas, San José
Paratrigona ornaticeps (Schwarz, 1938)	(d)	ND	Cartago, Limón, Puntarenas, San José

Partamona grandipennis (Schwarz, 1951)	ND	ND	Alajuela, Cartago, Guanacaste, Heredia, Puntarenas, San José
Partamona musarum (Cockerell, 1917)	ND	ND	Cartago, Heredia, Limón, San José
Partamona orizabaensis (Strand, 1919)	ND	ND	Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas, San José
Plebeia franki (Friese, 1900)	ND	ND	Costa Rica
Plebeia frontalis (Friese, 1911)	ND	ND	Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas, San José
Plebeia jatiformis (Cockerell, 1912)	(d)	"chupa ojos"	Cartago, Puntarenas
Plebeia llorentei Ayala, 1999[a]	ND	ND	ND
Plebeia minima (Gribodo, 1893)[a]	ND	ND	ND
Plebeia pulchra Ayala, 1999[a]	ND	ND	ND
Plebeia tica (Wille, 1969)	(e)	"bocarena"	Guanacaste, Heredia
Ptilotrigona occidentalis (Schulz, 1904)	ND	ND	Puntarenas
Scaptotrigona luteipennis Friese, 1902	(c,b)	"soncuano"	Costa Rica
Scaptotrigona mexicana (Guérin, 1844)	ND	ND	Cartago
Scaptotrigona (ex. barrocoloradensis)	ND	ND	Guanacaste, Puntarenas, San José
Scaptotrigona pectoralis (Dalla Torre, 1896)	(a)	"soncuano"	Alajuela, Cartago, Guanacaste, Limón, Puntarenas, San José
Scaptotrigona subobscuripennis (Schwarz, 1951)	ND	ND	Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas, San José
Scaptotrigona wheeleri (Cockerell, 1913)	ND	ND	Costa Rica
Scaura argyrea (Cockerell, 1912)	(d)	ND	Cartago, Guanacaste, Puntarenas, San José
Tetragona perangulata (Cockerell, 1917)	ND	ND	Guanacaste, Puntarenas
Tetragona ziegleri (Friese, 1900)	(a,c)	"miel de leche"	Alajuela, Guanacaste, Heredia, Limón, Puntarenas
Tetragonisca angustula (Latreille, 1811)	(a,b,e,d)	"mariola"	Alajuela, Cartago, Heredia, Limón, Puntarenas, San José
Tetragonisca buchwaldi (Friese, 1925)	ND	ND	Puntarenas
Trigona cilipes (Fabricius, 1804)	ND	ND	Costa Rica

(continued)

Table 7.1 (continued)

Species	Domesticated species	Common name	Distribution (province of Costa Rica)
Trigona corvina Cockerell, 1913	ND	ND	Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas, San José
Trigona ferricauda Cockerell, 1917	ND	ND	Cartago, Heredia, Limón
Trigona fulviventris Guérin, 1844	(c)	“culo de buey”	Alajuela, Cartago, Guanacaste, Limón, Puntarenas, San José
Trigona fuscipennis Friese, 1900	ND	ND	Alajuela, Guanacaste, Limón, Puntarenas, San José
Trigona muzoensis Schwarz, 1948	ND	ND	Limón
Trigona necrophaga Camargo & Roubik, 1991	ND	ND	CR
Trigona nigerrima Cresson, 1878	ND	ND	Alajuela, Limón, Puntarenas, San José
Trigona silvestriana (Vachal, 1908)	(c)	“congo”	Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas, San José
Trigonisca atomaria (Cockerell, 1917)	ND	ND	Alajuela, Guanacaste, Puntarenas
Trigonisca discolor (Wille, 1965)	ND	ND	San José
Trigonisca pipioli Ayala, 1999[a]	ND	ND	ND

Sources: (a) Arce et al. (1994); (b) Wagner (1958); (c) Ramírez and Ortiz (1995); (d) Herrera and Aguilar (2011); (e) Aguilar (2009); (f) Aguilar (personal observation); *ND* no data

[a] Species not cited in the catalogue of Camargo and Pedro (2007) but found in the entomological collections (Ayala, personal communication)

beecheii. The breeding of *Frieseomelitta* sp., *T. fulviventris*, *Lestrimelitta* sp., and *Plebeia tica* is less common. *Lestrimellita* sp., a robber bee that does not visit flowers, is not suitable for stingless bee keeping. In addition, *Nannotrigona perilampoides* and *T. angustula* can be considered as alternatives to honey bees for commercial crop pollination in Costa Rica (Slaa et al. 2000).

According to van Veen et al. (1990) meliponiculture in Costa Rica is basically practiced in two ways: (1) maintaining the nests in tree trunks, from which the honey by a lateral opening is extracted, this is typically used for *M. beecheii*; and (2) keeping the colonies in small boxes, pieces of bamboo or hollow logs, common practice with *T. angustula*. As stated by Arce et al. (1994), stingless bee keeping in Costa Rica has been practiced at a low technical level, almost without equipment, and the type of hive mainly used has been the hollow log. They observed that stingless bee keepers kept their colonies in log hives, generally hanging under the roof of their houses.

On the other hand, from 99 interviews carried out during the period 2006–2011, we recorded a total of 720 colonies. Stingless bee keepers with some sort of technical or higher education degree represented the main social group involved (29%, $N=25$), followed by peasants and beekeepers (28%, $N=24$; 27%, $N=23$ respectively). The most commonly kept species was *T. angustula* ($N=523$ colonies). Most beekeepers maintained meliponaries and bees for a long time, an average of 14 years, but ranging from 1 to 81 years. The average number of hives per bee keeper was 3.8 (SD = 5.08), with a maximum of 35 colonies.

In regard to the design and dimensions of the hive boxes, van Veen et al. (1993) recommended for *M. beecheii* a hive with a volume of 10 L, with internal dimensions of 15 cm height, 15 cm width and 45 cm length. For *T. angustula* the recommended box dimensions were 15 cm × 15 cm × 20 cm long, which provided a volume of 4.5 L. In practice we have observed that the stingless beekeepers modify these dimensions according to the species and the size of the colony.

The interest in stingless bees and their honey has increased over the past few years. Today, commerce of this honey in Costa Rica commands high prices; 1 L sells for US$ 20–50 and small containers of 10 ml cost US$ 2–4 due to an increasing interest mostly in its medicinal properties as treatment for cataracts (Aguilar 2007). Finally, the average production was 836 ml of honey/hive/year (SD = 839, $N=37$).

7.1.3 Costa Rican Stingless Bees

According to Roubik (1992) and Griswold et al. (1995) there are 12 stingless bee genera in Costa Rica, with a total of 40–60 described species. However, the latest classification by Camargo and Pedro (2007, 2008) and the revision by Ayala (personal communication) of the entomological collections of University of Costa Rica (UCR), Instituto Nacional de Biodiversidad (INBIO), and the Tropical Beekeeping Research Center (CINAT) of National University (UNA) show that there are 20 stingless bee genera and 58 species present in the country (see Table 7.1, nomenclature as given by (Camargo and Pedro 2007, 2008). If we consider these data and the recent classification of Camargo and Pedro (2007, 2008), Costa Rica possesses approximately 60% of the 33 Neotropical genera of Meliponini. The number of stingless bee species recorded in Table 7.1 is nearly 8% of the known bee fauna of Costa Rica (Griswold et al. 1995). There are about 5000 Neotropical species, and about 800 from Costa Rica. With about 60 species of meliponines, the proportion of total bee species in Costa Rica that are Meliponini is about 8% (see Roubik 2000).

In the tropical wet forests of Costa Rica, higher bee diversity is found at elevations below 500 m (Lobo 1996). Most species occur in the lowland rain forests of the Caribbean and the Osa Peninsula (Hanson 2000). In the Golfo Dulce region, southwestern (Pacific coast) Costa Rica 26 species were identified (Jarau and Barth 2008), which is nearly 54% of the stingless bee species reported for the country. In contrast, stingless bee species richness on the slopes of the Cordillera of Tilarán (Guanacaste province) declines dramatically above 1,000 m, and at altitudes of 700–1,000 m they are rare (Ortiz-Mora and van Veen 1995). The only known stingless bee genus endemic to Central America, *Meliwillea bivea*, is found in the Costa Rican highlands above 1,500 m of elevation (Roubik et al. 1997).

7.1.4 Tree Species Used for Nesting by Stingless Bees

The architecture of stingless bees nests of Costa Rica has been well studied by Wille and Michener (1973), in their work at least nine categories of nesting cavities are described. They find that the cavities in trees can be very variable, but stingless bees nonetheless use them. Owing to the importance for the establishment of nests, it is necessary to identify the species of trees used for nesting. In addition, severe deforestation affects the density of nests and could lead to significant changes in the composition of species; some species may disappear, e.g., *Scaptotrigona pectoralis*, while others could become abundant, e.g., *T. angustula* (Slaa 2003). On this regard, the work carried out in Costa Rica (Berrocal 1998; Arce et al. 2001; Slaa 2003) shows that a total of 36 identified botanical species correspond to timber species of high commercial value, which in turn are sources of nectar and pollen for these bees (see Table 7.2, modified from Aguilar 2001). Furthermore, most of them have multiple uses in our society (forage, wood, shade, crops, pollination, medicinal, etc.) and nowadays are at risk of disappearing. On the other hand, they are suitable for being embedded in tropical agroforestry systems (Aguilar 2001). The latter is a valuable recommendation. If implemented, it would allow the preservation of bee communities (Gordon et al. 2004) and adequate resources for food, protection, and new niches, enabling stingless bees to increase their populations.

7.1.5 Future Trends of the Stingless Bee Keeping in Costa Rica

Stingless bee keeping in Costa Rica is an activity that is present in several regions of the country, especially among the inhabitants of rural areas. The honey produced is used mainly as a medicine and ongoing investigations confirm the indigenous

Table 7.2 Species of trees used by the Costa Rican stingless bees (Apidae, Meliponini) as a substrate to establish nests and importance for the bees

Species tree/substrate	Common name	Bee species	Importance
Acrocomia vinifera[e]	ND	Ts	ND
Anacardium excelsum[a,c,e]	"espavel, rabito"	Sp, Ts, Tan, Mb, Tc	N, 1, 3
Andira inermis[e]	"almendro de montaña"	Mb, Tan	N, 1, 2, 3
Astronium graveolens[e]	ND	Tc	ND
Citrus sp.[e]	"cítricos"	Np	ND
Bombacopsis quinata[e]	"pochote"	Tan, Tc, Tf	N, P, 1, 2, 3
Bravaisia integerrima[a]	"mangle blanco"	Sp, Tan, Np	N, P, 2
Brosimum alicastrum[e]	"ojoche"	Tan, Sp, Tz, Cz, Om, Pf, Tfs, Tf	P, 1, 2, 3
Bursera simaruba[a]	"jiñocuabe"	Mb, Tz, Sp, Tan, Np	P, 1, 2, 3
Cedrela odorata[e]	"cedro amargo"	Tz	P, 1, 2, 3
Clarisia biflora[a,e]	ND	Sp, Om, Tan, Pf	ND
Coccoloba caracasana[e]	"papaturro blanco"	Tz	N, P, 1, 2, 3
Combretum fruticosum[e]	ND	Tfs	ND
Copaifera aromatica[e]	ND	Mb, Fn	ND
Cordia alliodora[a,e]	"laurel"	Tz, Tan, Np	P, N, 1, 2, 3
Diphysa americana[a,c]	"guachipelín"	Tan, Tz, Sp	P, N, 1, 2, 3
Enterolobium cyclocarpum[e]	"guanacaste"	Tz, Tan	P, 1, 2, 3
Ficus sp.[a,e]	"higuerón"	Sp, Mb, Tan	1, 3
Ficus goldmanii[e]	ND	Tan, Tz	ND
Ficus trachelosyce[a]	"higuerón"	Tan	1
Gliricidia sepium[a,e]	"madero negro"	Tan, Tz, Cz, Om, Pf	N, 1, 2, 3
Inga sapindoides[e]	"guaba"	Om	ND
Lonchocarpus costaricensis[c]	"siete cueros"	Np	ND
Luehea seemannii[e]	ND	Tan, Cz	ND
Minquartia guianensis[d]	"manú"	Tz	1, 3
Myrospermum frutescens[a,e]	ND	Cz, Om, Tan, Fn	N, 1, 3
Ocotea veraguensis[e]	"aguacatillo"	Tz	P, 1, 2, 3
Pentaclethra macroloba[d]	"gavilán"	Tan	1, 3
Persea americana[e]	"aguacate"	Tc	ND
Pseudosamanea guachapele[e]	ND	Tan, Tc, Sp, Cz	ND
Psidium guajava[b]	"guayaba, guayabo"	Te.sp, Mb, Sp, Tc, Tan	N, P, 1, 3
Rehdera trinervis[e]	ND	Tan, Np, Pf	ND
Spondias mombin[e]	"jobo"	Tc	N, P, 1, 2, 3
Tabebuia ochracea[a,c]	"corteza amarilla"	Cz, Mb, Tan	N, P, 1, 2, 3
Tabebuia rosea[e]	"roble de sabana"	Tan, Tc	N, P, 1, 2, 3
Terminalia oblonga[e]	ND	Tan, Sp, Om	ND

Modified from Aguilar (2001)

Sources: [a]Berrocal (1998) (for dry tropical forest), [b]Aguilar personal observation, [c]Arce et al. (2001), [d]Rincón (1997) (for premontane humid tropical and humid forest), [e]Slaa (2003) (for tropical dry forest)

N: nectar; P: pollen; ND: no data; 1: used by stingless bees; 2: used by *Apis mellifera*; 3: timber and other uses (according to Arce et al. 2001)

Species code: Sp, *Scaptotrigona pectoralis*; Ts, *Trigona silvestriana* (*amalthea*); Tan, *Tetragonisca angustula*; Mb, *Melipona beecheii*; Tc, *Trigona corvina*; Np, *Nannotrigona perilampoides*; Tz, *Tetragona ziegleri*; Om, *Oxytrigona mellicolor*; Pf, *Plebeia frontalis*; Cz, *Cephalotrigona zexmeniae*; Fn, *Frieseomelitta nigra*; Tfs, *Trigona fuscipennis*; Tf, *Trigona fulviventris*; Te.sp., *Tetragona* sp.

view that honeys from the stingless bees have medicinal potential. According to our most recent data, the number of stingless bee keepers has progressively increased. However, compared to previous studies, the number of colonies per person has declined. This partially is due to the practice, in many cases, of keeping bees as a hobby more than as a source of improvement in family income.

In Costa Rica, nearly 26% of the territory is preserved as national parks and reserves, but these areas are scattered and increasingly becoming isolated. We must continue efforts to preserve stingless bees, learn more about their ecology and populations, which are threatened by the loss of forest areas (Kevan 1999).

Among other weaknesses confronting the successful development of Costa Rican meliponiculture are included: the lack of appropriate collections covering great part of the country, the absence of a good inventory of the existing stingless bee keepers and the fear that many keepers have of dividing nests. When carried out in a careless way, nest division results in parasite attack by phorid flies and eventually in the loss of the colony.

There is an important lack in up-to-date information concerning the use of stingless bees by the natives of Costa Rica, which is noticed due to the few studies performed on meliponiculture after the nineteenth century. Therefore, further research is required in this field and more action should be taken to continue the work initiated by Wille (1961) on the biology, biodiversity conservation and management of stingless bees in Costa Rica.

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Chapter 8
Stingless Bees in Argentina

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8.1 Introduction

Stingless bees in Argentina are found throughout the northern portions of the territory, with the highest diversity in the humid forests of the northeast. Although the knowledge of these bees is deeply rooted in the cultural practices and the use that aboriginal peoples made of them, formal studies of stingless bees in Argentina are scattered over time and rather fragmentary.

The first described species was *Plebeia molesta* (Puls, in Strobel 1868). Later, Holmberg (1887) recorded the habits and characteristics of several species in his article "Viaje a Misiones," but referred to most of them by their vernacular names. The single most extensive account has been that of Silvestri (1902), who also traveled the province of Misiones. He surveyed the fauna recording and characterizing nine species, registered common names, and studied the nesting habits. His material, housed at the University of Portici, Naples, Italy, has been critically examined by Camargo and Moure (1988). Since that date there has been no other comprehensive treatment of Meliponini in Argentina. Other early work, but narrower in scope, consisted in the description of a few new species (Holmberg 1903; Vachal 1904; Schrottky 1911). By the time Schrottky (1913) published his "Distribución de los himenópteros argentinos" 17 species were known to occur. Schwarz, in his

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Table 8.1 Distribution of stingless bees in Argentina. Nomenclature follows Camargo and Pedro (2007)

	Species		
Genus	Northeast	Chaco	Northwest
Cephalotrigona	*capitata* (Smith)[10,12]		
Frieseomelitta	*varia* (Lepeletier)[14]		
Geotrigona	*argentina* Camargo & Moure[4,12]	*argentina*	
Lestrimelitta	*sulina* Marchi & Melo[12], *rufipes* (Friese)[14]	*chacoana* Roig Alsina[13]	*rufipes*[13]
Leurotrigona	*muelleri* (Friese)[14]		
Melipona[a]	*bicolor schencki* Gribodo[9,12], *obscurior* Moure[4,12], *quadrifasciata* Lepeletier[3,12], *quinquefasciata* Lepeletier[3,12]	*orbignyi* (Guérin)[12]	*baeri* Vachal[1,12], *fuliginosa* Lepeletier[11]
Mourella	*caerulea* (Friese)[10,12]		
Nannotrigona	*testaceicornis* (Lepeletier)[7,14]		
Oxytrigona	*tataira* (Smith)[14]		
Paratrigona		*glabella* Camargo & Moure	*glabella*[6,12]
Partamona	*helleri* (Friese)[14]		
Plebeia	*droryana* (Friese)[4,12], *nigriceps* (Friese)[4,12]	*catamarcensis* (Holmberg), *molesta* (Puls)[2,12]	*catamarcensis*[5,12], *wittmanni* Moure & Camargo[12]
Scaptotrigona	*depilis* (Moure)[4,12], *aff. postica* (Latreille)[14]	*jujuyensis* (Schrottky)	*jujuyensis*[8,13]
Schwarziana	*quadripunctata* (Lepeletier)[3,12]		
Tetragona	*clavipes* (Fabricius)[9,14]		
Tetragonisca	*fiebrigi* (Schwarz)[4,12]	*fiebrigi*	*aff. angustula* (Latreille)[14]
Trigona	*spinipes* (Fabricius)[4,12]		*spinipes*
Trigonisca			sp.[14]

Superscript numbers refer to first citation of species for Argentina and nomenclatural updates
[1]Burmeister (1861); [2]Puls, in Strobel (1868); [3]Holmberg (1887); [4]Silvestri (1902) [5]Holmberg (1903) [6]Friese (1908); [7]Bertoni (1911); [8]Schrottky (1911); [9]Schrottky (1913); [10]Schwarz (1948); [11]Moure (1992); [12]Camargo and Pedro (2007); [13]Roig Alsina (2010); [14]Museum specimens
[a]*Melipona titania*, described by Gribodo from La Rioja in 1893, is most probably an erroneous record, since the xeric conditions of La Rioja are extremely different from the tropical conditions of the areas where the species actually occurs (see Camargo and Pedro 2008)

revisionary works on Neotropical meliponines, added further records to the Argentinean fauna, mainly for the province of Misiones; he recorded this province as the southern limit of distribution of several of the species he studied (Schwarz 1932, 1948).

More recently, Almeida and Laroca (1988) studied the single species of *Trigona* present in Argentina, and Camargo and Moure (1994, 1996) described two new species in the genera *Paratrigona* and *Geotrigona*. Camargo and Pedro (2007), in their comprehensive catalog of the Neotropical Meliponini, listed all the known records for every species, updating their systematics, and mentioning for Argentina 22 species in 12 genera. Later additions (Roig Alsina 2010), and a scrutiny of museum specimens, indicate the presence of 33 species in 18 genera (Table 8.1).

When these figures are compared to those of the Neotropical region as a whole (391 species in 32 genera, Camargo and Pedro 2007), the low species richness (8.4%) is evident, but it is striking that over half of the genera (56.2%) are present in the Argentinean fauna. Thirteen of these genera are represented by a single species. This is in agreement with the observation made in Brazil by Biesmeijer and Slaa (2006) that local meliponine assemblages tend to consist of one or a few species of many different genera. In this chapter we compile current information on the systematics, distribution, traditional knowledge, use as a resource, and recent studies on the biology and ecology of meliponines in Argentina.

8.2 Distribution of Stingless Bees in Argentina

Argentina represents in South America a marginal area for the rich tropical fauna of meliponines. This is particularly the case of the northeast, where the Paranaense forest enters the province of Misiones and the northern part of the province of Corrientes (Cabrera and Willink 1973). This area has the highest record of species (Table 8.1), all of which also occur in Brazil and most of them also in eastern Paraguay. A second tropical forest, the Yungas, occurs in the northwestern mountain region of Argentina. The Yungas extends southward, penetrating as a slender wedge in the provinces of Jujuy, Salta, and Tucumán and reaching northern Catamarca (Cabrera and Willink 1973). The fauna of this region is the least surveyed, and the one that may provide new additions to the number of species present in the country.

Between these two regions is the Chaco, a biogeographic unit with xeromorphic forests and savannas (Cabrera and Willink 1973; Prado 1993). Here the precipitations diminish to the west, so the central and western areas have a long, unfavorable, dry season. This region harbors the most distinctive fauna of meliponines in Argentina, although the poorest in number of species (Table 8.1). The Chaco not only occupies north-central Argentina but also western Paraguay, southeastern Bolivia, and the extreme western edge of the state of Mato Grosso do Sul in Brazil (Prado 1993). The distinctiveness of its fauna has been noted by Camargo and Moure (1994, 1996).

The boundaries of the three regions just mentioned are not absolutely distinct when the meliponine fauna is considered, and some species range into neighboring areas. This is the case of *Tetragonisca fiebrigi*, which occurs both in the Chaco and in the Paranaense forest. *Scaptotrigona jujuyensis*, a species characteristic of the Chaco, cohabits in Tucumán with *Trigona spinipes*, a species that does not occur in the xeromorphic central region.

The southernmost records of meliponines in the western hemisphere are represented by four species of *Plebeia* that manage to survive in temperate climates. Two of them are elements of the Chacoan fauna. *Plebeia molesta* was described from San Luis (Strobel 1868), but without indication of whether the province or the city of San Luis was meant. Specimens with sound data come from the northern part of the province of San Luis at 32°30′ S latitude. The second species, *Plebeia catamarcensis*, has been recorded as far south as 31°20′ S latitude in the province of Santa Fe

(Dalmazzo 2010). The two other species are elements of the Paranaense fauna, which extends its range southwards through the gallery forests growing along the Paraná and Uruguay Rivers, reaching the western margin of the Río de La Plata in the province of Buenos Aires. The two species, the identity of which is currently under study, are found as far south as 34°37′ S latitude.

8.3 Traditional Knowledge on Stingless Bee Biodiversity

Stingless bees were exploited and well known by different cultures in northern Argentina before the arrival of Europeans. This knowledge is reflected in the many and accurate names by which different species were known by local people. Holmberg (1887), Silvestri (1902), and Bertoni (1911) recorded Guaraní vernacular names for the bees that they surveyed in Misiones, and Bertoni also in Paraguay. The alimentary customs of the Guaraní people in Misiones have been documented by Martínez Crovetto (1968).

In the early eighteenth century Jesuit missionaries described the abundance of bees and honey in the Chaco region, and the importance of these insects for the Guaycurú people, as well as the uses that they made of the honey and other products of stingless bees (Medrano and Rosso 2010a, b).

Arenas (2003), in a comprehensive ethnographic study of the Wichi and Toba peoples of central Chaco, describes the prominent role that stingless bees have played, and still play presently, in their culture. Nearly all of the species present in the Chaco are individually recognized and have their own names in both ethnic groups. Honey has been important in the production of alcoholic beverages, particularly for festivities, besides being used as a nourishment and as a sweetener for other foods and diluted with water for children. There is an oral tradition regarding which honeys have curative effects for various ailments. Pollen masses and larvae were also consumed, and cerumen was used to mend water containers, as well as in the making of various utensils.

The Quechuan lexicon compiled by Bravo (1975) in the province of Santiago del Estero includes the names of several species of meliponines, although some such names refer to the hives rather than to the bees themselves. Names such as “yana” (*Scaptotrigona jujuyensis*), “ashpamishki” (*Geotrigona argentina*), “tíu simi” (*Melipona* spp.), and “ckella” and “pusquellu” (*Plebeia* spp.) are broadly used nowadays in northern Argentina.

The creole population has also developed their own vernacular names in Spanish, such as “negrito,” “peluquerito,” and “rubita.” Some of them are indicated in Table 8.2.

8.4 Meliponini as a Natural Resource

We present here preliminary results of a survey aimed at knowing which of the many species of stingless bees are exploited or reared nowadays by the local population in northern Argentina. The survey is being carried out in the provinces

Table 8.2 Species of stingless bees exploited in northern Argentina

Region	Species	Common name	Harvested in the field	Kept near dwellings in logs, or rustic hives	Kept in man-made hives
Northeast	*Nannotrigona testaceicornis*		*	*	
	Tetragona clavipes	“borá”	*		
	Tetragonisca fiebrigi	“yateí”	***	**	***
	Scaptotrigona aff. postica	“tapezuá,” “tobuna”	***	**	**
	Melipona obscurior	“mandurí”	**	*	
	Plebeia spp.	“mirim”	*	*	
Chaco	*Geotrigona argentina*	“alpamiski”	*		
	Scaptotrigona jujuyensis	“negrito,” “tapezuá”	***	*	***
	Tetragonisca fiebrigi	“rubiecito”	***	*	***
	Plebeia spp.	“apynguarei,” “shimilo”	*	*	*
	Melipona orbignyi	“moro-moro”	*		
Northwest	*Tetragonisca aff. angustula*	“rubiecito,” “mestizo”	***	*	***
	Scaptotrigona jujuyensis	“yana”	**	*	*
	Plebeia spp.	“pusquello,” “quella”	*	**	**

A higher degree of exploitation is indicated by an increased number of asterisks of Misiones, Chaco, Formosa, Salta, Jujuy, and Tucumán, under a project leaded by INTA (Gennari 2009).

The nests of several species are known to be harvested in the field when they are spotted. This practice includes both species with subterraneous and arboreal habits (Table 8.2). The data in the table reflect the present survey, but other species are known to be collected in the field as they are encountered (e.g., Arenas 2003). Trees are frequently felled to obtain arboreal nests, an undesirable practice. In some cases, logs containing the nests are cut down and then kept near the dwellings, so they can be opened, harvested, and resealed, becoming rustic hives. The survey indicates that this type of extractive exploitation is frequent and widespread. Rustic hives of *Tetragonisca*, *Scaptotrigona*, and, to a lesser extent, *Plebeia* are common in Misiones, Formosa, and Chaco, and less frequent in the northwest.

Although extractive exploitation is a traditional undertaking in rural communities, formal meliponiculture is a recent development in the area. The interest in the use of man-made hives and in the manipulation of the colonies is steadily growing in Argentina, but only a few species are being reared rationally. The most widely cultivated stingless bees are *Tetragonisca fiebrigi*, *T. aff. angustula*, and *Scaptotrigona jujuyensis*. The colonies of *Tetragonisca* are small, and the harvest modest, but these bees are highly esteemed because of the quality of their honey. *Scaptotrigona* are favored because of the relatively large colony size, strength of the colonies, and large honey harvest.

Both governmental and nongovernmental organizations are engaged in projects to promote sustainable beekeeping of stingless bees. The government of the province of Misiones promotes the culture of meliponines (CEDIT 2005) and supports regular meetings of producers of honey of *Tetragonisca fiebrigi*. The national government also promotes projects through several agencies, such as the Consejo Federal de Ciencia y Tecnología, the Secretaría de Ambiente y Desarrollo Sustentable (Meriggi et al. 2008), and the Instituto Nacional de Tecnología Agropecuaria (Gennari 2009). There are several nongovernmental organizations engaged in community-level development of meliponiculture. Some such organizations are the Asociación para la Promoción de la Cultura y el Desarrollo (APCD), in the province of Formosa, which works with the Wichi people. The Fundación Proyungas works to qualify individuals in the management of stingless bees in the northwest (Stamatti 2006; Baquero and Stamatti 2007).

8.5 Recent Studies on Biology and Ecology of Argentine Meliponines

There has been an upsurge of interest in the study of meliponines in Argentina in recent years. Most contributions have been oriented to the study of the pollen resources used by stingless bees. Palynological analyses of honeys and the contents of pollen pots have been carried out in the Northwest for *Tetragonisca aff. angustula* (Flores and Sánchez 2010); in the Chaco region for *Geotrigona argentina* (Vossler 2007a; Vossler and Tellería 2009b; Vossler et al. 2010), for *Tetragonisca fiebrigi* (Cabrera 2007; Vossler 2007a, b, 2011; Vossler and Tellería 2009a), and for

Scaptotrigona jujuyensis (Basilio et al. 2006; Vossler 2007a, b; Vossler and Tellería 2009a; Basilio et al. 2011; Vossler 2011); and in the Northeast for *Tetragonisca fiebrigi* (Fabbio et al. 2007; Dallagnol et al. 2007; Paul et al. 2009, 2011; Flores et al. 2011). Flower visitation has been also used to study the resources harvested by stingless bees. Vossler (2009, 2012) investigated with this methodology six species of Meliponini in the Chaco (*Tetragonisca fiebrigi*, *Scaptotrigona jujuyensis*, *Geotrigona argentina*, *Melipona orbignyi*, *Plebeia catamarcensis*, and *Plebeia molesta*).

Other studies deal with nesting ecology (Basilio et al. 2006; Colleselli et al. 2008; Vossler 2012), management (Achával et al. 2006), medicinal uses of honeys (Zamudio et al. 2011; Kujawska et al. 2012), physicochemical characterization of honeys (Vit et al. 2009, Sgariglia et al. 2010, Salomón et al. 2011, Basilio et al. 2011), antimicrobial properties of honeys (Dallagnol et al. 2007; Sgariglia et al. 2010), and ethnobiology (Zamudio and Hilgert 2012).

8.6 Future Research

Knowledge on the biodiversity of stingless bees in Argentina is not satisfactory. The systematics of some genera, such as *Scaptotrigona* and *Plebeia*, which have several species in the region, is poorly resolved. Some areas have not been adequately surveyed. A more intensive exploration of the Yungas may uncover additional species for the Argentinean fauna. Studies on several aspects of biology, such as nesting behavior, reproduction, caste development, feeding habits, as well as practical matters such as multiplication and management of colonies, and handling of their products, are almost nonexistent for many species. Even for those species that occur in Brazil and have received much attention, their behavior in southern marginal areas may reveal particular issues that merit further study. Undoubtedly, a better knowledge of the fauna will help decide which species can be selected for meliponiculture in specific areas.

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Chapter 9
Mexican Stingless Bees (Hymenoptera: Apidae): Diversity, Distribution, and Indigenous Knowledge

Ricardo Ayala, Victor H. Gonzalez, and Michael S. Engel

9.1 Introduction

Stingless bees (Meliponini) are highly eusocial apine bees restricted to the tropical and subtropical areas of the world but are most diverse in the Western Hemisphere, where about 80% of the nearly 500 known species worldwide are found (Michener 2007; Camargo and Pedro 2007). In the Western Hemisphere, stingless bees occur from Mexico to Brazil and northern Argentina, and also on Caribbean and Pacific Islands, inhabiting a diverse variety of ecosystems, including both humid and xeric lowlands to cloud forests and Páramos in the Andes reaching up to 4,000 m in elevation (Gonzalez and Engel 2004; Nates-Parra 2005; Michener 2007; Camargo and Pedro 2007).

Stingless bees are ecologically, economically, and culturally important. They are considered among the major pollinators of many native and cultivated tropical plants (e.g., Slaa et al. 2006), while pollen, honey, and cerumen of some species have also been used traditionally by indigenous and non-indigenous people in rural areas across the Americas, thus representing an important source of income for these communities (e.g., Nates-Parra 2005; Michener 2007; and references therein). In addition, the shared cultural heritage of these people is integrally tied, in some respects, to the stingless bees which they exploit, representing an inestimable value well beyond modern fiscal concerns. Despite the importance of stingless bees and

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several decades of research, most species hypotheses remain to be tested and many species await discovery. Each species, as recognized by an individual systematist, is a hypothesis subject to rigorous testing when other species, specimens, or characters (morphological or molecular) are discovered (e.g., Wheeler 2004, 2009). A vast majority of stingless bee species have never been tested since they were proposed at the beginning of the nineteenth century by scientists who either had an obsolete species concept or a limited knowledge of their biology, distribution, and morphology, given the specimens and technology available at the time. Stingless bees are characterized by an abundance of cryptic species (i.e., morphologically very similar species), only distinguished by fine morphological characters as well as by differences in nesting biology and distribution (Michener 2007). Moreover, large areas in tropical America have never been or are poorly explored and their stingless bee fauna remains practically unknown.

Likewise, there remains no consensus on the generic and subgeneric classification of stingless bees. Some authors prefer to emphasize the differences, recognizing species or groups of species with unusual characters at the generic or subgeneric rank, resulting in some 60 supraspecific taxa. As in Gonzalez and Griswold (2011), herein we follow Michener's classification for Neotropical Meliponini, except that we recognize at the generic level those taxa he placed as subgenera of *Trigona* Jurine and *Plebeia* Schwarz (Table 9.1). Exploring, discovering, testing species hypotheses, and building sound phylogenies that allow us to understand the evolutionary process and develop stable classifications with predictive values for diverse biological and ecological traits is a dynamic, complex process that requires years of professional training, substantial knowledge of the bees and their environment, time, and an investment in both financial and human capital. Such processes are vital not only to understand the true diversity and evolution of the group but also to promote their conservation and sustainable use.

In Mexico, stingless bees represent a relatively small portion (2.6%) of the highly diverse bee fauna of the country, but the economic, social, and cultural impacts they have are like no other in the world. A large percentage (41.3%) of the comparatively small Mexican stingless bee fauna has been used since pre-Colombian times when compared to other countries with more diverse meliponine faunas (Table 9.2). Mexican stingless bees played a significant role in the religion of the Mayans, one of the most important ancient civilizations of the world; and stingless bees and their products are used for diverse purposes, including managed pollination, folk medicine, art, and honey, cerumen and pollen extraction (Fig. 9.1a–h). Herein, we provide a synopsis of the diversity, biogeography, origin, and traditional uses of the stingless bees in Mexico.

9.2 Diversity

Studies on the Mexican stingless bee fauna started as early as the beginning of the nineteenth century (Latreille 1811; Guérin-Méneville 1844; Bennett 1831; Say 1837; Cresson 1878; Dalla Torre 1896; Friese 1900; Cockerell 1913; Strand 1917; Schwarz

Table 9.1 Stingless bee genera present in Mexico with the total number of species in the neotropics and in Mexico

Genus	Total	Mexico
Cephalotrigona Schwarz	5	3 (2)
Frieseomelitta Ihering	16	1
Geotrigona Moure	21	1
Lestrimelitta Friese	21	2 (1)
Melipona Illiger	70	6 (3)
Nannotrigona Cockerell	10	1
Nogueirapis Moure	4	1[a] (1)
Oxytrigona Cockerell	11	1
Paratrigona Schwarz	34	2[b]
Partamona Schwarz	39	2
Plebeia Schwarz	42	11 (4)
Proplebeia Michener[c]	4	1[d]
Scaptotrigona Moure	22	3 (1)
Scaura Schwarz	7	1
Tetragona Lepeletier de Saint Fargeau & Audinet-Serville	19	1
Tetragonisca Moure	4	1
Trigona Jurine	32	5
Trigonisca Moure	43	5 (2)

A number of endemic species to the country are given in parentheses. Generic classification follows Michener (2007) except by those taxa he placed as subgenera of *Trigona* and *Plebeia* which are herein recognized at the generic level. The approximate number of species in the neotropics is based on Michener (2007), Camargo and Pedro (2007), Ascher and Pickering (2011), and SRM Pedro (personal communication)

[a] One extinct species is known in Miocene Chiapas amber

[b] Camargo and Moure (1994) listed *P. opaca* for Chiapas but we have not seen yet specimens of this species

[c] Extinct, early Miocene Dominican and Chiapas amber

[d] Engel (2004a) recorded an unidentified species in Mexican amber

Table 9.2 Total number of bee species, stingless bees, and species of stingless bees used in some Latin American countries. Bee diversity per country is based on Ascher and Pickering (2011), while estimations for the exploited number of stingless bee species are based on the corresponding citation

Country	Total bee species	Stingless bees (%)	Stingless bees used (%)	Reference
Mexico	1,795	46 (2.6)	19 (41.3)	Herein
Costa Rica	785	58 (7.3)	2 (4.2)	Roubik (2000), Aguilar et al., this volume
Colombia	541	101 (20.0)	17 (16.8)	Nates-Parra (2005)
French Guiana	210	80 (38)	2 (2.5)	Roubik (1979), and Pauly et al., this volume
Peru	688	100 (14.5)	12 (12)	C. Rasmussen (personal communication)
Brazil	1,814	236 (13.0)	21 (8.9)	Crane (1992)

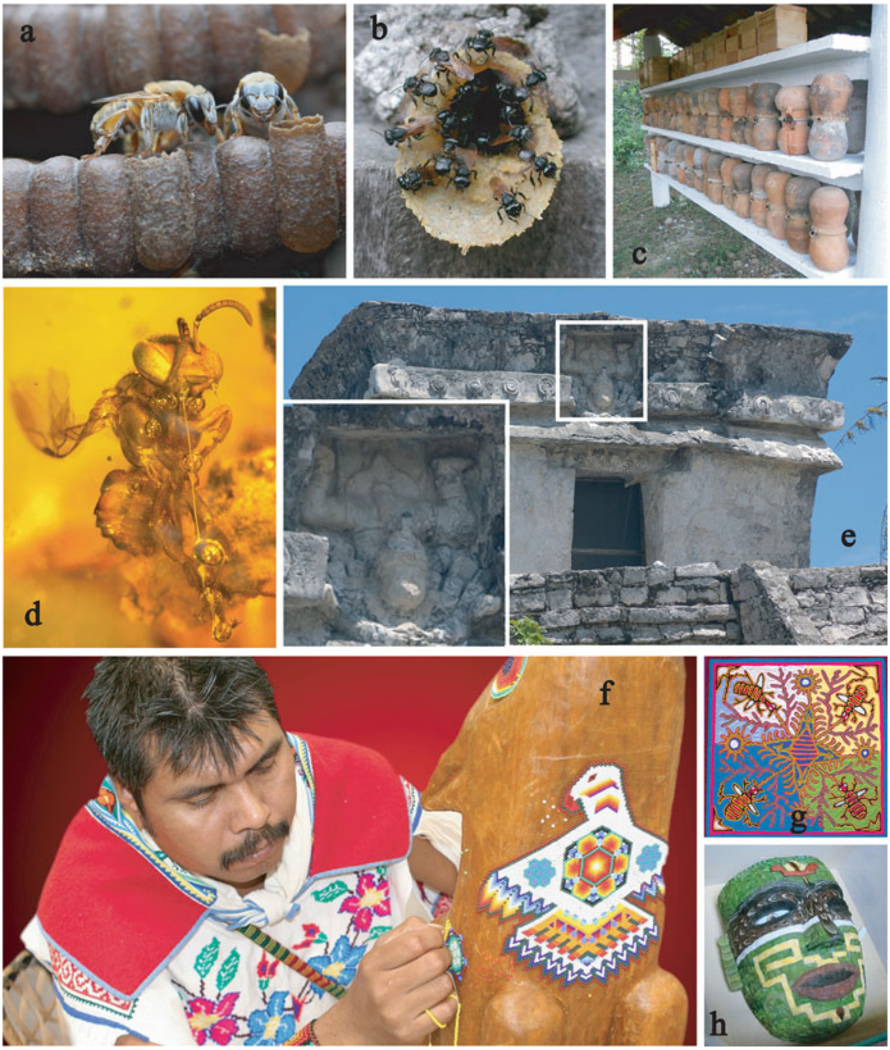

Fig. 9.1 Economic and cultural importance of stingless bees in Mexico. (**a**) Workers of *Melipona beecheii* on a brood comb; (**b**, **c**) nest entrance and managed hives of *Scaptotrigona mexicana*; (**d**) worker of *Nogueirapis silacea* preserved in Early Miocene amber from Chiapas; (**e**) temple of the "descending god" in Tulum, Quintana Roo. Ah-muzen-cab, one of the Mayan gods of bees and honey, is enlarged in the box of the lower left corner; (**f**) Huichol artisan using *Scaptotrigona hellwegeri* cerumen on a piece of wood for his work with chaquira beads; (**g**) Huichol art depicting stingless bees; (**h**) feather art. Photos: (**a-c**) C. Balboa, J. Mérida, M. Guzmán; (**d**) V. Gonzalez; (**e-h**) R. Ayala

1948, 1949; Camargo et al. 1988; Ayala 1988, 1997, 1999; Ayala et al. 1993, 1996), although earlier biological accounts before Linnaean nomenclature or standardized concepts of species were given (e.g., Hernandez 1648; Purchas 1657). The most recent synthesis on the diversity of the Mexican stingless bee fauna is that of Ayala (1999). In that work, the status of species was clarified, and several species, accounting for 36% of the total number of Mexican meliponines known to date, were discovered to Science. Today, 46 species belonging to nearly all extant Neotropical stingless bee genera are known in Mexico (Table 9.3), except *Meliwillea* Roubik et al., endemic to the mountains of Costa Rica and Panama, and the genera *Duckeola* Moure, *Paratrigonoides* Camargo and Roubik, *Schwarziana* Moure, and *Trichotrigona* Camargo and Moure, which occur in South America. *Plebeia*, *Trigona*, *Melipona* Illiger, and *Trigonisca* are the most diverse genera in Mexico with 12, nine, six, and five species, respectively. Because Mexico is located in the northernmost range of the stingless bees in the Americas, it possesses a relatively low number by comparison to that of much smaller countries that are closer to the equator (Tables 9.2 and 9.3). The Pacific Coast, from Guerrero to Chiapas, and southern Veracruz are areas that contain the greatest number of stingless bee species (Fig. 9.2a, b).

An undescribed species of the extinct genus *Proplebeia* Michener, one of the two known extinct stingless bee lineages in the Americas, and a single extinct species of the presently living South American genus *Nogueirapis* Moure, *N. silacea* (Wille 1959) (Fig. 9.1d), are known from the Early Miocene (17–19 myo) Chiapas amber, near Simojovel (Wille 1959; Engel 2004a). The attribution of *N. silacea* to *Proplebeia* by Camargo et al. (2000) and Camargo and Pedro (2007) is based upon a misinterpretation between the former and a true *Proplebeia* species in the Mexican amber fauna. The holotype of *N. silacea* was not examined and their remarks were based upon Wille's account (1959:850, 851). Examination of the holotype of *N. silacea* by MSE reveals it to be a true *Nogueirapis*.

9.3 Distribution

Based on the distribution and type of vegetation in their habitat, Mexican stingless bees can be divided into three large ecological groups: (I) species widely distributed and associated with both tropical deciduous and evergreen forests; (II) species associated with tropical evergreen forest; and (III) endemic species associated with various forest types.

Group I. These species follow three distinct distribution patterns:

1. Wide montane and tropical distribution. *Partamona bilineata* is the only representative of this pattern. This species is present in the Sierra Madre del Sur, from Michoacán to Oaxaca, in the southern slope of the transverse volcanic axis (Eje Volcánico Transversal), Balsas River Basin; it reaches Sinaloa and San Luis Potosí through the Pacific and Gulf slopes. The species occurs in lowlands with tropical deciduous and evergreen forests, and in montane pine-oak forests.

Table 9.3 Stingless bees of Mexico with information on state distribution and uses. Uses: Cerumen used for feather and bead arts (A), honey (H), Meliponiculture (M), pollination (P), cerumen (C).

Stingless bee species	Uses	Distribution
Cephalotrigona eburneiventer (Schwarz, 1948)[a]	H	Colima, Guerrero, Michoacán, Morelos, Puebla
C. oaxacana Ayala, 1999[a]		Oaxaca
C. zexmeniae (Cockerell, 1912)	H	Campeche, Chiapas, Quintana Roo, San Luis Potosí, Tabasco, Tamaulipas, Veracruz, Yucatán
Frieseomelitta nigra Cresson, 1878		Campeche, Chiapas, Colima, Guerrero, Jalisco, Michoacán, Nayarit, Oaxaca, Puebla, Quintana Roo, Sinaloa, Yucatán
Geotrigona acapulconis (Strand, 1919)	H[b]	Chiapas, Estado de México, Guerrero, Michoacán, Morelos, Oaxaca
Lestrimelitta chamelensis Ayala, 1999[a]		Colima, Jalisco, Guerrero, Nayarit
L. niitkib Ayala, 1999		Chiapas, San Luis Potosí, Quintana Roo, Veracruz, Yucatán
Melipona beecheii Bennett, 1831	M, H, C, P	Campeche[c], Chiapas[c], Jalisco, Nayarit, Oaxaca, Quintana Roo[c], San Luís Potosí, Sinaloa, Tabasco, Tamaulipas, Veracruz[c], Yucatán[c]
M. colimana Ayala, 1999[a]	H	Colima, Jalisco
M. fasciata Latreille, 1811[a]	H	Estado de México, Michoacán, Guerrero, Morelos, Oaxaca, Veracruz
M. lupitae Ayala, 1999[a]	H	Michoacán
M. solani Cockerell, 1912		Chiapas
M. yucatanica Camargo et al., 1988		Oaxaca, Yucatán
Nannotrigona perilampoides (Cresson, 1878)	H, P	Campeche, Chiapas, Jalisco[c], Michoacán, Morelos, Nayarit[c], Oaxaca, Puebla, Quintana Roo, San Luis Potosí, Sinaloa[c], Veracruz, Yucatán
Oxytrigona mediorufa (Cockerell, 1913)		Chiapas
Paratrigona guatemalensis (Schwarz, 1938)	H	Chiapas[c], Veracruz
P. opaca (Cockerell, 1917)		Chiapas
Partamona bilineata (Say, 1837)	H[b]	Campeche, Chiapas, Colima, Distrito Federal, Durango, Guerrero, Jalisco, Michoacán, Morelos, Nayarit, Oaxaca, Puebla, Quintana Roo, San Luis Potosí, Sinaloa, Tabasco, Veracruz, Yucatán
P. orizabaensis (Strand, 1919)		Veracruz
Plebeia cora Ayala, 1999[a]		Zacatecas, Sinaloa
P. frontalis (Friese, 1911)		Campeche, Chiapas, Colima, Jalisco, Michoacán, Nayarit, Nuevo León, Oaxaca, Puebla, Quintana Roo, San Luis Potosí, Veracruz, Yucatán

P. fulvopilosa Ayala, 1999[a]		Guerrero
P. jatiformis (Cockerell, 1912)		Chiapas, Quintana Roo, Veracruz
P. llorentei Ayala, 1999		Chiapas, Veracruz
P. manantlensis Ayala, 1999[a]	H	Colima[c], Jalisco[c]
P. melanica Ayala, 1999		Chiapas, Quintana Roo, San Luis Potosí, Veracruz
P. mexica Ayala, 1999[a]		
P. moureana Ayala, 1999		Estado de México, Guerrero, Morelos, Oaxaca, Puebla
P. parkeri Ayala, 1999		Chiapas, Oaxaca, Puebla, Quintana Roo, San Luis Potosí, Veracruz
P. pulchra Ayala, 1999		Chiapas: Hidalgo; Puebla, Quintana Roo, San Luis Potosí, Veracruz
Scaptotrigona hellwegeri (Friese, 1900)[a]	A, H, C, M[b], P	Colima, Durango, Estado de México, Guerrero[c], Jalisco[c], Michoacán[c], Morelos, Nayarit[c], Oaxaca, Puebla, Chiapas
S. mexicana (Guérin-Méneville, 1844)	M, H, C, P	Chiapas, Guerrero, Hidalgo, Morelos, Oaxaca, Puebla[c], San Luis Potosí, Veracruz, Guerrero
S. pectoralis (Dalla Torre, 1896)	H[b]	Campeche, Chiapas, Oaxaca, Quintana Roo, Veracruz, Yucatán
Scaura argyrea (Cockerell, 1912)		Chiapas, Veracruz
Tetragona mayarum (Cockerell, 1912)		Chiapas, Tabasco
Tetragonisca angustula (Latreille, 1811)	M, H, C	Chiapas, Veracruz
Trigona corvina Cockerell, 1913		Campeche, Chiapas, Oaxaca, Quintana, Veracruz
T. fulviventris Guérin-Méneville, 1844		Campeche, Chiapas, Colima, Jalisco, Michoacán, Nayarit, Oaxaca, Quintana Roo, Veracruz, Yucatán
T. fuscipennis Friese, 1900		Campeche, Chiapas, Oaxaca, Puebla, Quintana Roo, Veracruz, Yucatán
T. nigerrima Cresson, 1878	H[b], C[b]	Chiapas, Oaxaca, Tabasco, Veracruz
T. silvestriana (Vachal, 1908)		Chiapas, Campeche y Quintana Roo
Trigonisca azteca Ayala, 1999[a]		Guerrero, Estado de México, Morelos, Puebla
T. maya Ayala, 1999		Quintana Roo, Yucatán
T. mixteca Ayala, 1999[a]		Chiapas, Oaxaca
T. pipioli Ayala, 1999		Chiapas, Jalisco, Quintana Roo, Oaxaca Veracruz, Yucatán
T. schulthessi (Friese, 1900)		Chiapas, Oaxaca

[a] Endemic species

[b] Species that are rarely used for a given purpose are indicated after the abbreviation for that use

[c] Localities where the species is used

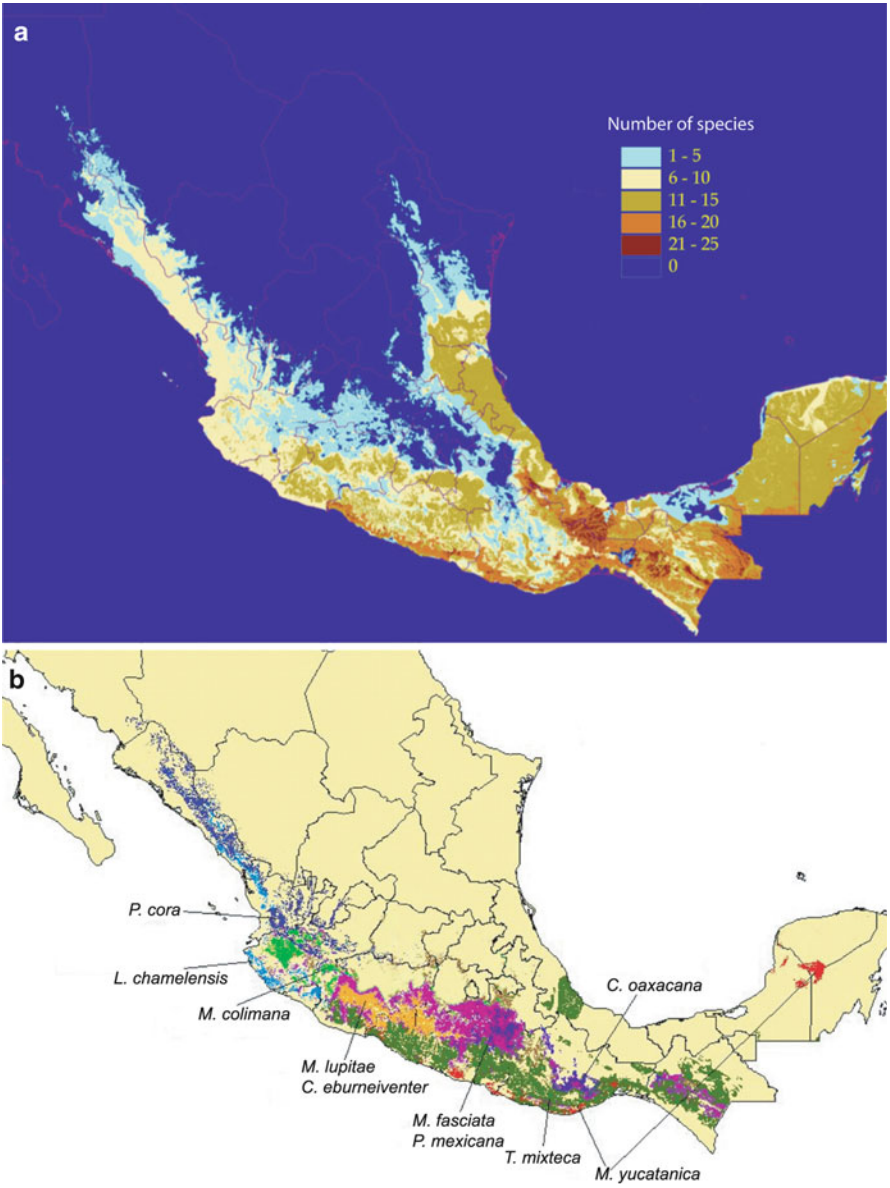

Fig. 9.2 Distribution of stingless bees in Mexico. (**a**) Relative density of the number of species. The *darker* the area, the more species coexist in the same place as indicated by the *color* legend. (**b**) Areas of endemism indicating some of the 16 endemic species of the country (see text for explanation)

2. Wide coastal distribution. Consists of *Melipona beecheii* (Fig. 9.1a), *Plebeia frontalis*, *Trigona fulviventris*, and *Trigonisca pipioli*, which reach Sinaloa and San Luis Potosí through the Pacific and Gulf slopes; they also occur in Chiapas (probably not in the mountains) with a few isolated records from the Balsas River Basin (*M. beecheii* and *T. pipioli*). The species appear to be stenothermic (living only within a narrow temperature range), sometimes reaching areas of transition between tropical and mountain vegetation, including cloud forests. *Plebeia frontalis* is the most widely distributed of the four species, reaching the state of Nuevo León through the Gulf Coastal Plain.
3. Special cases of species with wide distribution. This pattern is exhibited by *Nannotrigona perilampoides* and *Frieseomelitta nigra*. The former species reaches more northern areas (up to 29°N) than the latter, through the Pacific slope (Bennett 1964). It is also present in the southern slope of the transverse volcanic axis, between 1,000 and 1,500 m, primarily in areas with cloud forests. *Nannotrigona perilampoides* is absent from areas with tropical dry or xerophytic vegetation such as those in Chamela, Jalisco (Ayala 1988), and east of the Balsas River Basin. *Frieseomelitta nigra* is found in the Pacific coast, Balsas River Basin, and Yucatán Peninsula but does not reach them through the Gulf coast. Both species seem to have a broad ecological valence that allows them to survive in areas with food resources and nesting sites available year round.

Group II. This group comprises 50% of the Mexican stingless bee species. The following four distribution patterns can be recognized:

1. Species restricted to Chiapas: *M. solani*, *T. mayarum*, and *T. silvestriana*.
2. Species that follow the distribution of the tropical evergreen forest but are absent from Yucatán, reaching central Veracruz or southeastern San Luis Potosí. Species of this group are found in the mountains above 1,000 m, occurring in conifer and cloud forests, such as *P. llorentei*, *P. melanica*, *P. pulchra*, *S. argyrea*, and *T. nigerrima*.
3. Species distributed as above but present throughout the Yucatán Peninsula, occupying drier areas with tropical deciduous forests, such as *L. niitkib* and *S. pectoralis*.
4. Species presumably restricted to the Pacific coast of Chiapas, near Tapachula. Only *Oxytrigona mediorufa* and *Trigonisca schulthessi* are known to exhibit this distribution.

Group III. Thirteen species are endemic to Mexico (Tables 9.1 and 9.3). The distribution of such endemism defines areas that are often disjunct, suggesting possible vicariance events that have resulted in sister or closely related species. The following are the recognized areas with endemisms:

1. Southern half of the Tehuantepec Isthmus. Three species (*C. oaxacana*, *M. yucatanica*, and *T. mixteca*), adapted to tropical deciduous and semi-deciduous forests, are found in that area. *Melipona yucatanica* is also found in southern Yucatán, as well as Belize and Quintana Roo (DW Roubik, personal communication). It is possible that this species is associated with tropical savannah vegetation (Fig. 9.2b).

2. Southern mountains (transverse volcanic axis and Sierra Madre del Sur) with four species (*M. colimana*, *M. fasciata*, *P. fulvopilosa*, and *P. manantlensis*) of insular distribution and present in the mountains between 1,000 and 3,000 m. They appear to be phylogenetically related to those species associated with the tropical evergreen forest from southeastern Mexico and Central America. *Plebeia fulvopilosa* is restricted to the Sierra Madre del Sur in Guerrero; *P. manantlensis* and *M. colimana* are restricted to the mountains of southeastern Jalisco (North of Colima, Volcán Colima, Sierra de Manantlán, and Sierra del Tigre), which represent an isolated group of mountains from the remaining transverse volcanic axis; *M. fasciata* is a montane species widely distributed in Mexico, occurring from the southern slope of the transverse volcanic axis to west of Michoacán, and in the Sierra Madre del Sur, from Guerrero to Oaxaca.
3. Balsas River Basin. *Cephalotrigona eburneiventer*, *P. mexica*, and *T. azteca* occur in the lower basin (Guerrero, Morelos, Puebla, and the central region, east of Michoacán), while *M. lupitae* occurs in the upper basin (Michoacán). The dominant vegetation types of the area are tropical deciduous or semi-deciduous forests and xeric vegetation. The species of this river basin seem to be closely related with those of tropical evergreen forests or deciduous forests from the Tehuantepec Isthmus.
4. Northern Nayarit, southern Sinaloa, and southeastern Zacatecas. *Plebeia cora* occurs in this area, a species presumably closely related to *P. mexica* from the lower basin of the Balsas River (Fig. 9.2b).
5. Pacific Coast between southern Oaxaca and Sinaloa. *Scaptotrigona hellwegeri*, *L. chamelensis*, and *Geotrigona acapulconis* are endemic to this area; the first and last species are also found in the Balsas River Basin and in the mountains up to 2,000 m.

Several species are often found at mid- and high elevations in the mountains. *Melipona fasciata*, *P. bilineata*, and *G. acapulconis* are often found at elevations above 2,000 m. Other species, such as *N. perilampoides*, *T. corvina*, *T. fulviventris*, and *T. fuscipennis*, occur from sea level up to 1,500 m. *Melipona fasciata*, *M. colimana*, *P. fulvopilosa*, and *P. manantlensis* are only found above 1,500 m and only in some areas, such as in the Sierra de Atoyac (southeastern slope of Sierra Madre del Sur in Guerrero); *M. fasciata* is frequently found at elevations around 2,400 m in northern Morelos (southern slope of the transverse volcanic axis) and has been collected at 3,000 m in the Sierra Madre del Sur, Guerrero, the highest elevation record for stingless bees in Mexico.

9.4 Origin of the Mexican Stingless Bees

The extant stingless bee fauna of Mexico seems to be the result of recent migrations of Central or South American taxa during the Pliocene and Pleistocence when the Mexican plateau and its surrounding mountains were already present, such as that described for vegetation and other organisms (e.g., Halffter 1976; Simpson and Neff

1985). If that is the case, then the current areas of endemism likely resulted from vicariance events that occurred during the climatic changes of the Pleistocene (e.g., Toledo 1982), as evidenced by the presence of endemic species or species with disjunct or insular distributions.

The presence of *N. silacea* in Chiapas amber not only indicates that *Nogueirapis*, now known only from Bolivia to Costa Rica, occurred as far north as southern Mexico but also that it must have reached it well before the Central American land bridge was formed during the Pliocene (e.g., Moure and Camargo 1982). Halffter (1978, 1987) suggests that migrations between South and North America during the Oligocene–Miocene transition were possible, yet difficult. However, given that *Cretotrigona prisca* (Michener and Grimaldi) is known from the latest Cretaceous New Jersey amber in North America (Michener and Grimaldi 1988a,b; Engel 2000) and *Proplebeia* from both Dominican and Chiapas amber (e.g., Wille and Chandler 1964; Wille 1977; Greco et al. 2011), alternatively it is possible that *Nogueirapis* is a remnant of a more northern meliponine lineage that inhabited southern Mexico or present-day Guatemala and Honduras (Donnelly 1988), during the latest Cretaceous or Early Tertiary (Michener and Grimaldi 1988b). In other words, it is possible that some Mesoamerican stingless bees may have evolved from otherwise North American lineages, not from extant South American taxa (Michener and Grimaldi 1988a,b; Camargo et al. 2000); also, it is likely that some of those taxa diversified in South America as a consequence of climatic events during the Pliocene and Pleistocene. Certainly, the North American fauna of meliponines, as evidenced by *C. prisca*, suffered considerably from the Chixulub impact (65 Ma) and resulting northern projection of ejecta (Schulte et al. 2010), but remnants may have persisted and move southward during the Early Tertiary. Certainly extensive paleomelittological work needs to be done in additional North American amber deposits (e.g., Eocene Arkansas amber, additional Mexican amber, etc.). In the absence of a phylogenetic hypothesis that includes all stingless bee fossils worldwide, it is difficult to know which taxa evolved from ancient North American lineages, but, given their distribution and diversity, *Cephalotrigona*, *Trigona*, *Nannotrigona*, and *Melipona* seem to be good candidates. Evidence suggestive of this pattern is found in *Melipona*, such as the presence of *M. yucatanica* and *M. lupitae* in Mexico, the diversification of the *fasciata* species group in Mexico and in northern Central America, and the presence of *M. beecheii* Bennett in Mexico, as well as *M. variegatipes* Gribodo in Mexico as well as in some islands of the Caribbean (Camargo et al. 1988). In addition to the Mexican fauna likely being composed of some relics of that tropical North American fauna, there is no doubt that a large part of the Mexican taxa are South American in origin, some lineages of which evolved well before the separation of that continent from Africa.

9.5 Traditional Uses and Indigenous Knowledge

Indigenous knowledge demonstrates how traditional cultures have organized cultural beliefs, linguistic practices, and historical interpretations that have given meaning to their lives. This form of knowledge construction comes directly from experience with the environment, is transmitted through oral tradition, and is based on holistic perspectives of the interconnectedness of all areas of life, as seen by indigenous perceptions of the world (Cajete 2000; Semali and Kincheloe 1999; Ortiz 2009). Such indigenous knowledge may also inform conservation practices (e.g., Posey 1993).

The use of stingless bees by the Mayan people since pre-Colombian times is a good example of ethnobiological knowledge that has been transformed, innovated, and revitalizated. A number of researchers have emphasized the close relationship between the stingless bees and the Mayan culture and how such a practice was almost lost when the Spaniards introduced the Western hive honey bee, *Apis mellifera* Linneaus (e.g., Bennett 1964; Dixon 1987; Labougle and Zozaya 1986; Schwarz 1949). Mayans used honey as a sweetener, antibiotic, and an ingredient of "balché", a culturally important fermented drink still used today. Aztecs also regularly used honey from stingless bees to sweeten and flavor the drink of the gods and one of the most appreciated beverages in the world today: chocolate (Coe and Coe 1996). It is no wonder stingless bees were important, regarded as gifts from the gods, handled with care, or even considered as gods outright, such as "ah-muzen-cab" (Fig. 9.1e), one of the Mayan gods of bees and honey usually appearing landing or taking off in ceremonial temples in the Yucatán Peninsula. *Melipona beecheii*, locally known as xunan kab or *kolil kab* in Mayan, meaning "royal lady", is one of the most culturally and socially important stingless bees in Mexico, and perhaps in the world, given its traditional value for the Mayans, one the most important ancient civilizations of humanity (e.g., Villanueva-G et al. 2005; and references therein). Some works that document the traditional knowledge and use of stingless bees in Mexico, including names in local languages are those of Murillo (1981), Dixon (1987), and González (1983, 1989). Stingless bees are currently used for crop pollination at local scales in Mexico. For example, *S. mexicana* is used in the pollination of avocado [*Persea americana* (Lauraceae)], rambutan [*Nephelium lappaceum* (Sapindaceae)], and coffee [*Coffea arabica* (Rubiaceae)] in Hidalgo, Puebla and Tapachula, Chiapas; *N. perilampoides* is used for pollination of habanero chile [*Capsicum chinense* (Solanaceae)], one of the most piquant (spicy hot) species of peppers; and *Melipona* are used in other more traditional crops, such as tomatoes [*Solanum lycopersicum* (Solanaceae)] (May-Itzá et al. 2008). Also, many towns with ethnic Nahuatl populations around Cuetzalan in northen Puebla have developed and depend almost entirely on stingless beekeeping, particularly *S. mexicana* or "pisilnekmej" (Fig. 9.1b, c); the honey of this species is highly appreciated locally and internationally, and it is estimated that up to two tons of honey are exported each year to Europe, principally Germany (Guzmán et al. 2011).

A total of 19 of 46 known species in Mexico are currently used for crop pollination, crafts, folk art, medicine, honey, pollen, and cerumen some are used more regularly than others, depending on local abundance (Table 9.3). Of the species used, six are endemic and restricted to particular regions. The cerumen of endemic *S. hellwegeri* for feather, strands of yarn, and glass beads (locally known as "chaquiras" or "kuka") arts, developed by the Huichol people from western central Mexico, is a remarkable traditional use of stingless bees. The cerumen, sometimes mixed with pine resin, is spread over a piece of wood onto which feathers, beads, or yarn are pressed (Fig. 9.1f–h). (R Ayala, personal observation).

Mexico has a relatively small number of stingless bee species but they appear more heavily used, when compared to other countries in the Americas. For example, Colombia has at least twice the number of species of Mexico but available information suggests that only a small fraction is regularly exploited (Table 9.2). It is possible that this is a mere coincidence of the technological and cultural advancement of the Mayan and Nahuatl civilizations with the need and availability of the bees in the region. The comparable pre-Colombian civilization in South America was the Incas, but did not have immediate access to stingless bees, because only a few species reach high altitudes in the Andes. Another explanation is that the reduced number of stingless bees may have been the cause of the more exhaustive exploitation, progressively becoming more culturally important with iterative generations. Numerous records indicate that native people in South America (e.g., Colombia: Nates-Parra 2005; Bolivia: Stearman et al. 2008; Brazil: Posey and Camargo 1985; Camargo and Posey 1990) also used stingless bees, but none of them developed such a strong cultural relationship or relied as heavily on stingless bees such as those of the Mayas and Nahuatl, possibly because resources appeared to be limitless; they could sample many more species and as regularly as they pleased. However, archeological records are better preserved and documented in Central America than in the humid, tropical lowlands of South America, where meliponines are especially more diverse and abundant. Also, stingless bees are still poorly studied in most countries of the Americas and their uses poorly documented. Whatever the reason, it is clear that meliponines were, and are, a vital resource for ancient Mexicans and their descendants; for many indigenous groups now pursuing an urban life, stingless bees and their products still play an important role in the material and symbolic artwork that has facilitated their engagement to the regional and national market economies.

9.6 Future Directions

Despite the relatively small number of stingless bee species and several decades of research in Mexico, a significant amount of work remains to be done. For example, the common *M. beecheii* is highly variable morphologically and it is still not clear whether it is composed of several cryptic species (which seems likely to be the case). Conversely, *M. solani*, *M. fasciata*, and *M. belizeae* (Schwarz, 1932) may be the same species. All three species appear to be geographically separated; the first is primarily found in lowlands whereas the second in highlands; the last species is only known from a few old specimens collected in Belize. Also, as discussed by Ayala (1999), similar cases to those described for *Melipona* are likely to be found in *Trigonisca* and *Plebeia* given our limited knowledge on the distribution and variation of these groups. Further studies using molecular characters, such as DNA barcodes may help to test those hypotheses.

Some areas of Mexico need to be explored in more detail to obtain a better understanding of individual species distributions. Records are scarce from the mountains north of Oaxaca, Campeche, the mountains north of Chiapas, areas near to the Guatemalan border, and the mountains north of Puebla.

Alongside this, Mexican amber remains to be explored more fully (Engel 2004a). The study of the extinct Mexican stingless bees will shed light on the evolutionary history and diversification of modern meliponines in the Americas. Indeed, paleomelittological investigation often greatly overturns our preconceived dogmas as they relate to bee diversity, biogeography, or the evolution of particular biological phenomena (e.g., Engel 2004b). Examples include the decreasing disparity and diversity of highly eusocial bees (e.g., Engel 2001a,b; Kotthoff et al. 2011) or the discovery of true honey bees (*Apis* spp.) natively occurring in western North America (Engel et al. 2009). It is exciting to imagine what kind of revelations await in the paleontological record of Mexico and surrounding countries.

Multidisciplinary studies are needed to estimate the economic value of the bee products used in crafts, particularly those employed for the feather and bead arts. We do not know the ecological impact of stingless bee exploitation for crafts and other activities on local bee populations, and whether indigenous people are using colonies in a sustainable fashion for their and the bees' maximal benefit. Special attention to these and other traditional activities related with meliponines, including beekeeping, are critical because such techniques and experiences accumulated by generations can be useful when replicating or promoting them in other countries that do not possess similar indigenous knowledge or tradition. Indigenous knowledge defines indigenous identity and how indigenous people perceive and transmit their understanding of the world (e.g., Ortiz 2009). The ancestral ethnobiological knowledge on stingless bees is an invaluable component of the cultural capital of Mexico and humanity; its preservation ultimately depends on assuring the survival of the bees.

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Chapter 10
The Role of Useful Microorganisms to Stingless Bees and Stingless Beekeeping

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10.1 Introduction

The close relationship between bees and microorganisms is unquestionable (Cano et al. 1994; Gilliam 1997). As in many insects, bacteria, molds, and yeasts seem to play an important role for bee nutrition and protection against harmful microorganisms (Roubik 1989; Gilliam et al. 1990; Gilliam 1997; Mueller et al. 2005; Anderson et al. 2011). The microorganisms are transferred from one bee generation to the next—while associated with their hosts, they find suitable microenvironments in which to live and reproduce (Sachs et al. 2011).

The subject of this chapter has been extensively explored in *Apis mellifera*, from which more than 6,000 microbial strains were isolated and identified (Gilliam 1997). Most studies focus on identification, while a few studies consider biochemical contributions of the microbes (Gilliam 1997; Teixeira et al. 2003; Promnuan et al. 2009; Kroiss et al. 2010). However, the biology and roles of microorganisms associated with bees are still unclear and sometimes controversial (Herbert and Shimanuki 1978; Loper et al. 1980; Standifer et al. 1980; Fernandes-da-Silva and Serrao 2000; Anderson et al. 2011).

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Although stingless bees (Apidae: Meliponini) share many similarities with *Apis mellifera*, this diverse group (Roubik 1989; Michener 2000) still conceals many particularities that have not been explored. Here we discuss the role of non-pathogenic microorganisms in stingless bee colonies and focus on their importance to stingless bee keeping. Our aim is to stimulate further studies on functional aspects of microorganisms associated with stingless bees and their nests or managed hives.

10.2 Known Microorganisms Living in Stingless Bee Colonies

The main microorganisms living in stingless bee colonies are yeasts, molds, and bacteria. However, the knowledge about this biodiversity is very limited, since most of papers only mention their occurrence, not their function. Furthermore, information is available only for a few stingless bee species. Our aim in this section is to present the most common microorganisms, where they live and what they may provide for the host colonies.

10.2.1 Bacteria

Two genera of bacteria have been identified in stingless bee colonies. The most common and always present are from the *Bacillus* genus. Some DNA of this group was even found in fossils of the extinct *Proplebeia dominicana* which is about 20 million years in age (Cano et al. 1994; Camargo et al. 2000). This suggests a very old relationship between bees and *Bacillus*. These microorganisms seem to play an important role by secreting enzymes that cause fermentation and conversion of pollen constituents (Gilliam et al. 1985, 1989, 1990). Apparently, the enzymes have two main functions—pre-digestion of the pollen (softening of the exine wall) before it is ingested and altering the stored pollen so that it is less susceptible to harmful microorganism proliferation. The acetic and lactic fermentations, which occur in pollen and honey, are also realized by these bacteria (Gilliam 1979b).

Besides the apparent function in food digestion, Yoshiyama and Kimura (2009) found strong evidence that *Bacillus* species also secrete antibiotics. By using in vitro inhibition assays, those authors demonstrated that strains of *Bacillus* from the digestive tract of *Apis cerana japonica* inhibit *Paenibacillus larvae*, which cause American foulbrood disease. Similar effects may also be found in stingless bees' *Bacillus*.

A classic study in stingless bee biology indicated that *Melipona quadrifasciata* could not survive without a *Bacillus* species found in the nest (Machado 1971). *Bacillus* was found in stored pollen, brood provisions, digestive tracts of larvae and adult bees, and less abundantly in honey. During 1 month, the study colony was fed with sugar syrup mixed with streptomycin, an antibiotic that killed *Bacillus* species in vitro. After that treatment, the new brood cells were continuously destroyed, and the colony died after 30 days.

The other genus of bacteria recently found in brood cells and nest materials from stingless bee colonies is the actinomycete *Streptomyces* (Promnuan et al. 2009). This genus is well known for secreting antibiotics (Kroiss et al. 2010) and those found in the stingless bees *Trigona* (*Tetragonula*) *laeviceps* and *T. fuscobalteata* showed high inhibitory activity against *Paenibacillus larvae* and *Melisococcus plutonius*, pathogens of *A. mellifera*, responsible for American foulbrood and European foulbrood, respectively.

Recent contributions have clearly demonstrated the potential of the relationships between bees and *Streptomyces* and suggest this kind of relationship may also be found in stingless bees. Kaltenpoth et al. (2006) and Goettler et al. (2007) found a symbiotic relationship between a wasp (*Philanthus triangulum*) and bacteria from the genus *Streptomyces* which live inside antennal glands of female wasps. The bacteria are spread inside brood cells before larval provisioning and secrete nine different antibiotic substances that protect larvae from fungi and other pathogens (Kroiss et al. 2010).

10.2.2 Yeasts

Ten yeast genera are known in stingless bee colonies so far. The most representative are *Candida* and *Starmerella*, which occur very frequently in pollen and honey (Camargo et al. 1992; Rosa et al. 2003; Teixeira et al. 2003). Other genera were found in adult bees, propolis, the colony trash deposit area and, rarely, in the honey (Rosa et al. 2003). Because they are less frequently found in parts of the nest associated with external materials, such as propolis, it can be assumed that they are occasional contaminants from external environment and from plants visited by bees (Lachance et al. 2001a,b; Rosa et al. 2003).

The significance of yeasts and their potential roles to meliponine colonies are similar to the bacterial roles; i.e., they secrete enzymes, which convert substances from stored food and help to conserve it. Alcoholic fermentation is also a process initiated with yeast. It is still unclear how yeasts influence bee nutrition, but the changes seen within stored pollen are striking.

An interesting role of yeasts was described by Camargo et al. (1992). Yeasts of *Candida* genus seem to dehydrate the pollen stored by the stingless bee *Ptilotrigona lurida*. This dehydration process is efficient to avoid spoilage and prevent Phoridae (mainly *Pseudohypocera*) from consuming pollen and causing serious damage to the colony.

10.2.3 Other Fungi

A recent paper has described foragers of *Tetragonula collina* harvesting spores of *Rhizopus* sp. in lieu of pollen (Eltz et al. 2002). The same behavior was also observed

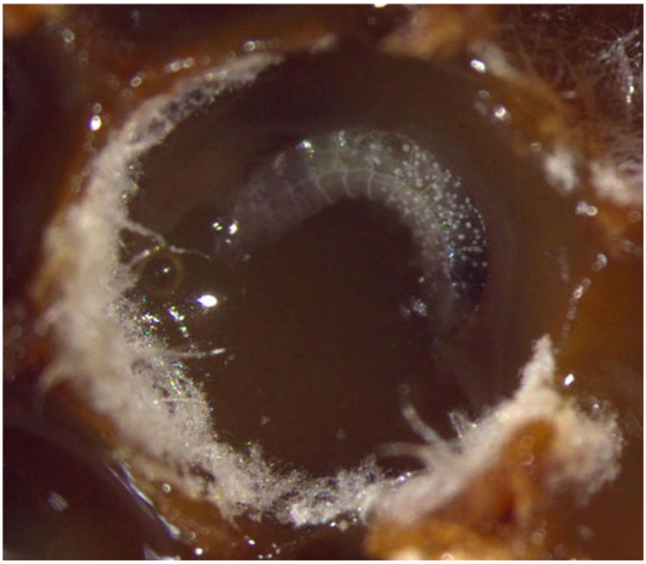

Fig. 10.1 Filamentous fungus growing on the surface of larval food and at the borders of brood cells of *Scaptotrigona depilis*. The larvae eat the fungus as it grows and seem to depend upon this fungus to survive (Menezes 2010). Photo: C. Menezes

in *Partamona* bees (G. Azevedo, cited as personal communication in Oliveira and Morato 2000). Similar observations were also noted by Roubik (1989), Burr et al. (1996), and Oliveira and Morato (2000). They found that workers of stingless bees lick or harvest a mucilaginous mass of spores of stinkhorn species (Fungi, Phaleles). It is still not known what motivates this behavior. The nutritive value of spores is low compared to pollen, but could complement their diet if availability is high and harvest is relatively easy (Oliveira and Morato 2000; Eltz et al. 2002). Indeed, plant trichomes (sometimes called pseudopollen) are harvested from orchid flowers by Neotropical *Partamona*, *Plebeia*, *Melipona*, and *Trigona*, and may have a similar role (Davies 2009).

Another recent paper reports the occurrence of several filamentous fungi isolated from individual workers of *Melipona subnitida* (Ferraz et al. 2008). The bees were already dead from natural causes when collected, and most of those microorganisms must be opportunistic in exploiting the carcasses.

An interesting relationship between a fungus and bees has recently been discovered. A filamentous fungus grows inside brood cells of *Scaptotrigona depilis* at the surface of larval food and is eaten by developing larvae (Fig. 10.1) (Menezes 2010). Apparently, the presence of this fungus was known (Flechtmann and Camargo 1974). It was then considered a disease because the brood of the studied colony presented a high mortality rate. However, recent observations have demonstrated that this fungus is very abundant in healthy colonies of *S. depilis* and also occurs with other stingless bee species, such as *Tetragona clavipes* and *Melipona flavolineata* (Menezes, unpublished data). The fungus proliferates before the egg hatches and grows intensively until the larva reaches 3 days of age. Larvae eat the fungus as it grows and preliminary tests show that the larvae depend on this fungus to survive, because all of them died when fungal growth was inhibited experimentally. We are investigating whether the fungus is providing nutritional benefits or protection against undesirable microorganisms.

10.3 Fermentation and Biochemical Processes

Fermentation is a biochemical process that transforms carbohydrates into other organic substances, providing energy to microorganisms. There are three main categories of fermentation: (1) alcoholic, in which carbohydrates are transformed into alcohol; (2) acetic, when alcohol is transformed into acetic acid; and (3) lactic, in which carbohydrates are transformed into lactic acid and other organic byproducts. Mixed fermentations also occur in nature.

10.3.1 Fermentation of Honey

Stingless bee honey is stored in pots made of cerumen (a mixture of wax and resins). To become honey, nectar undergoes three kinds of change: (1) physical, by the evaporation of a large part of its water, (2) biological, by the fermentation of yeast and bacteria, (3) chemical, when enzymes secreted by cephalic glands are added by the workers, transforming the sucrose of nectar into glucose and fructose (Beutler 1954 *apud* Zucoloto 1975; Nogueira-Neto 1997; Venturieri et al. 2007). Stingless bee honey is different in many ways from the honey of *A. mellifera*. Although its organoleptic and physicochemical characteristics vary according to the bee species and floral resources, we can assume that the main difference is the water content, generally higher than *A. mellifera* honey (Gonnet et al. 1964; Cortopassi-Laurino and Gelli 1991; Vit et al. 2004; Bijlsma et al. 2006; Venturieri et al. 2007; reviewed by Souza et al. 2006).

This relatively abundant water in stingless bee honey allows microorganisms to survive and to be active (Sanz et al. 1995). Additionally, some species of microbes isolated from stingless bee provisions survive under acidic conditions and at high osmotic pressure (Gilliam et al. 1985, 1989, 1990; Rosa et al. 2003; Teixeira et al. 2003).

There is some evidence that stingless bee honey may ferment naturally inside sealed honey pots. It is very common to see foam on the surface of the honey inside honey pots (Souza et al. 2007; Menezes, personal observations), indicating that gas bubbles are escaping from the honey, probably from alcoholic fermentation (Fig. 10.2). In the figure there are evident particles floating on the surface of the honey, which may be yeasts, bacteria, and residue of pollen. If the honey is kept at room temperature after being harvested, this layer of particles increases considerably and the honey becomes more acidic. In addition, fresh honey that was stored

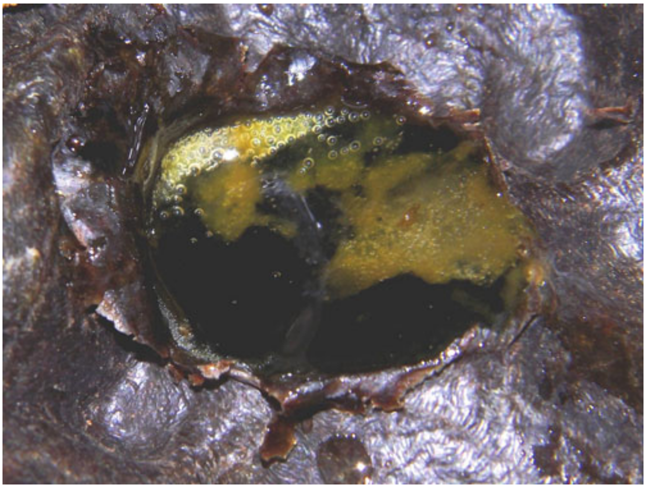

Fig. 10.2 Pot-honey of *Melipona fasciculata*. Foam and floating particles (probably bacteria, yeasts, and pollen grains) are frequently found on the surface of pot-honey which indicates that fermentation naturally occurs. Photo: C. Menezes

recently by the bees is generally not sour, but very sweet (Alves et al. 2007). On the contrary, when honey is harvested from natural colonies living inside tree trunks, or from colonies not managed for long periods, it seems to be more sour (Menezes, personal observation).

The alcoholic fermentation is generally performed by yeasts (Rosa et al. 2003; Teixeira et al. 2003). Sugar molecules are transformed into alcohol and CO_2. Bubbles and foam at the honey indicate alcoholic fermentation. Afterward, under aerobic conditions, certain strains of bacteria can convert alcohol molecules and O_2 into acetic acid and water. This kind of fermentation is generally performed by *Bacillus*, which is common in stingless bee honey (Machado 1971; Gilliam et al. 1985, 1990). In addition, lactic fermentation can also occur, whereupon sugars are converted into lactic acid and water, or other organic molecules. Bacteria are the main agent responsible for this kind of fermentation, although yeasts and other fungi can perform the same function.

In honey from *A. mellifera*, the main biochemical reaction is catalyzed by the enzyme glucose-oxidase, which converts glucose + O_2 + H_2O into gluconic acid and hydrogen peroxide (White et al. 1963; Nogueira-Neto 1997). The gluconic acid is the main acid in honey bee honey and hydrogen peroxide is an important (Burgett 1978), but not the only, anti-microbial substance (Kwakman et al. 2010). The glucose-oxidase enzyme is produced by bee glands, but it is possible that some microorganisms can also produce it (Gilliam 1997).

Many other biochemical reactions occur during honey storage. Workers can add many enzymes to the honey, which are produced by their glands (Costa and Cruz-Landim 2005), but the microorganisms living in honey can also secrete many proteolytic, glycolytic, and lipolytic enzymes, which will convert, ferment, enhance, and/or preserve the honey (Gilliam et al. 1990).

To our knowledge there are only two studies about changes in physicochemical characteristics in stingless bee honey over time. One of them does not allow strong generalizations to other stingless bees, since the studied species was *Trigona hypogea*, a obligately necrophagous stingless bee that does not harvest nectar from flowers, only from fruits and extra-floral nectaries (Noll et al. 1996), and also from homopteran bugs (DW Roubik, personal communication). They observed that there are no changes in amounts of sugar and protein traces in the course of time, but other parameters, such as pH, were not studied. The other study showed that, after harvest, fermentation increases the antioxidant activity of *T. angustula* honey, increases the amount of alcohol, and diminishes the amount of sugar (Pérez-Pérez et al. 2007). Although this is a preliminary study with small sample size and does not represent a natural situation, these results show that fermentation may add important substances to honey.

Due to the high diversity of stingless bees and limited studies on their microorganisms, the honey maturation process is still not understood. Physicochemical analysis of honey in the course of time would be of great value to understand the biological and biochemical processes involved in honey storage by stingless bees.

10.3.2 Fermentation of Pollen

When harvesting pollen, foragers transfer and accumulate pollen grains on their corbicula using nectar and salivary secretions (Herbert and Shimanuki 1978; Leonhardt et al. 2007). Workers return to their colonies with the pollen on their corbicula and leave the pellets inside pollen pots (made of cerumen), which are closed when they are full (Nogueira-Neto 1997). The pollen is stored for about 2 weeks before being consumed (Loper et al. 1980). In honey bees the pollen is stored in the same cells used for brood rearing and then sealed with a drop of honey. Under this condition of storage the pollen is subjected to the action of microorganisms: pollen stored in combs by honey bees is named bee bread; whereas pollen stored in pots by stingless bees is called "saburá" by indigenous people in Brazil (Fig. 10.3). The characteristics of the pollen such as flavor, odor, color, and texture, change considerably after being stored and vary among bee species (Camargo et al. 1992; Souza et al. 2004). A few bee species, such as *Tetragonisca angustula* and *Frieseomelitta varia*, produce dry and relatively sweet fermented pollen. However, other meliponines, such as *Melipona* and *Scaptotrigona*, produce and store moist and sour pot-pollen. Few studies have investigated the transformation process of stored pollen in stingless bees, thus we will base most of our discussion on *A. mellifera*, although even in this bee there is no clear consensus.

The most consistent change during pollen storage in *A. mellifera* is the decrease of pollen pH (from 4.8 to 4.1—Herbert and Shimanuki 1978) caused by lactic acid fermentation (Haydak 1958). Apparently, bacteria of *Streptococcus*, *Bifidobacterium*, and *Lactobacillus* are the main microorganisms responsible for lactic fermentation (Pain and Maugenet 1966; Gilliam 1979b; Vásquez and Olofsson 2009). Yeasts may

Fig. 10.3 Young workers of *Scaptotrigona depilis* feeding on natural fermented pollen “saburá” stored in cerumen pots. Photo: C. Menezes

also ferment pollen, and their population increases after pollen fermentation, supposedly increasing nutritional quality (Pain and Maugenet 1966).

Machado (1971) isolated *Bacillus* from pots of pollen and larval food of *M. quadrifasciata* and verified that stored pollen had more proteins cleaved into free amino acids than did pollen removed directly from the bees’ corbiculae. He found those bacteria in the larval food of 13 more stingless bee species. Gilliam et al. (1990) also studied four species of *Bacillus* in *Melipona* (currently known as the species *M. panamica*) and found that these microorganisms were able to secrete enzymes related to cleavage of lipids, carbohydrates, and proteins. *Bacillus* spp. are known for secreting several extracellular enzymes, antibiotics, and fatty acids, which could act directly on the chemical conversion of pollen and on the control of competing microorganisms that could spoil the pollen. This may explain why *Bacillus* are predominant in pollen and other microorganisms are less abundant (Gilliam et al. 1990). Moreover, some *Bacillus* species are known to ferment glucose when isolated, so pollen fermentation may also be attributed to these microbes.

For a long time it was hypothesized that fermentation increased the nutritional quality and accelerated the digestion of pollen grains. However, this may not be the main function of microbial activity in pollen. Some studies show that the nutritional quality increases (Beutler and Opfinger 1949 *apud* Herbert and Shimanuki 1978; Cremonez et al. 1998) and others demonstrate that the quality can remain the same (Herbert and Shimanuki 1978; Fernandes-Da-Silva and Serrão 2000) or even decrease after pollen storage (Human and Nicolson 2006). Likewise, studies about chemical differences between newly collected pollen and bee bread show that protein content and free amino acids remain the same (Herbert and Shimanuki 1978) or decrease after some time (Standifer et al. 1980; Human and Nicolson 2006). Some kinds of nutrients increase in concentration, like vitamin K (Haydak and Vivino

1950 *apud* Loper et al. 1980), vitamin E (Haydak and Palmer 1938 *apud* Loper et al. 1980), and some fatty acids (Loper et al. 1980). Other vitamins, however, can decrease in concentration, like vitamins C and B6 (Loper et al. 1980). Only the increase of lactic acid and the decrease of starch on bee bread appear to be consistent among the studies (Herbert and Shimanuki 1978).

Moreover, when nutritional quality was tested, results were controversial. Some studies show that longevity increases when workers feed on bee bread, compared to newly collected pollen (Beutler and Opfinger 1949 *apud* Herbert and Shimanuki 1978), in addition to studies that show that bee bread increases the amount of protein in haemolymph (Cremonez et al. 1998) and increases digestibility (Gilliam 1979a), when compared to fresh pollen. Nevertheless, many studies show no significant differences in hypopharyngeal gland development and pollen digestion (Herbert and Shimanuki 1978) when compared to the consumption of bee bread and newly collected pollen in *A. mellifera*. Fernandes-da-Silva and Serrão (2000) also showed that in *S. depilis*, a Brazilian stingless bee, the storage of pollen does not increase nutritional quality for workers. They verified the effect of fermented pot pollen and newly collected corbicular pollen on the development of hypopharyngeal glands and the degree of digestion of pollen grains, and found no significant difference between treatments.

Fermentation may therefore have greater importance in the conservation of stored pot-pollen than in altering its nutritional condition (Herbert and Shimanuki 1978; Fernandes-da-Silva and Serrão 2000). The presence of lactic acid, combined with other microorganism metabolites, could stabilize the stored pollen, preventing the development of other microorganisms that could spoil the pollen (Herbert and Shimanuki 1978; Gilliam 1997), in the same way that this process is used in industrial conservation and stabilization of fermented food, such as cheese, pickles, and wine (Gilliam 1997). There are still no detailed investigations in this area.

Vollet-Neto et al. (unpublished data) verified that newly emerged workers of *S. depilis* are more attracted to fermented pollen stored in pots than newly collected pollen from the corbicula. This behavior could indicate, at first, an instinctive behavior caused by the nutritional advantage in feeding on fermented pollen. However, according to studies of Fernandes-da-Silva and Serrão (2000), who found no nutritional advantage in the processed pot-pollen, the attraction and higher consumption of the fermented pollen could be explained by its strong and distinctive odor, which could attract worker bees. Other parameters to be analyzed include the amount of protein in the haemolymph, nutritional quality of the larval food, and development of immatures, besides studies on chemical composition.

An interesting example described by Camargo et al. (1992) suggests that pollen fermentation can provide additional advantages for stingless bees. They observed intense proliferation of *Candida* on stored pollen of *Ptilotrigona lurida*, an Amazonian stingless bee. These yeasts seem to dehydrate the stored pollen to 13.9% water content (while they found 52.2% pollen water content for *Melipona seminigra* and 24.1% for *Trigona dallatorreana*). These physicochemical changes could prevent the development of undesirable microorganisms that could spoil\ the food. Moreover, they verified that phorid flies (Diptera, Phoridae), parasites that lay their eggs on the larval food and stored pollen of stingless bees, do not lay their eggs on pollen of *P. lurida*, but lay on stored pollen of *Melipona seminigra*. Several other stingless bee species also possess relatively dry stored pollen in nests (e.g., *Frieseomelitta varia*; *Tetragonisca angustula*; Menezes, Cristiano), but the function of dehydration for these species is not known.

In summary, we may assume that the storage of pollen in cerumen pots is associated with inoculation of microorganisms, which promote biochemical changes that alter nutritional quality and enhance digestion and absorption of nutrients, but probably the main function is to prevent spoilage and diseases (Anderson et al. 2011). We still need much more information to draw valid conclusions about the advantages brought about by microorganisms living in pollen.

10.4 Practical Applications for Stingless Bee-Keepers (Problems, Solutions, and Peculiar Products Generated from Fermentation)

Given the above considerations, it is impossible to harvest stingless bee products without including their natural microorganisms (Souza et al. 2009). Therefore, it is very difficult to avoid the consequences, such as fermentation. The use of hygienic procedures while managing, harvesting, and processing stingless bee products considerably reduces the risk of contamination by unnatural microorganisms, from other parts of the nest or from the external environment (Fonseca et al. 2006; Venturieri et al. 2007; Souza et al. 2009).

10.4.1 Proliferation of Microorganisms after Harvesting the Honey

The high water content of most stingless bee honey is a big challenge to stingless beekeeping (Vit et al. 2004; reviewed by Souza et al. 2006). If it is kept at room temperature, honey will ferment after being harvested, even if extremely hygienic procedures are adopted (Nogueira-Neto 1997). Thus, four different solutions, refrigeration, dehydration, pasteurization, and maturation, have been developed by researchers and stingless bee-keepers to increase the post-harvest stability and extend the shelf life of pot-honey (Nogueira-Neto 1997; Fonseca et al. 2006; Alves et al. 2007; Venturieri et al. 2007; Drummond 2010; Contrera et al. 2011).

Refrigeration is the easiest process and preserves the natural characteristics and substances of honey. There are two disadvantages of this method. First is the high cost of storage until sale. Second, if honey was harvested with poor hygienic procedures, pathogens will remain alive in the honey. The honey must be kept refrigerated (approximately 4–8°C) just after harvesting and until consumption (Venturieri et al. 2007).

Honey can be kept refrigerated for long periods, even for years. However, pot-honey produced by different species may behave differently; sometimes off-flavors develop after refrigeration of *Melipona quadrifasciata* honey (P Vit, personal communication).

The dehydration process consists of removal of water from the honey, which can be accomplished by means of ventilation in a dry room (Nogueira-Neto 1997; Alves et al. 2007). Fonseca et al. (2006) describe a method whereby honey is spread upon flat containers in a relatively dry room with a dehumidifier, then bottled when the honey moisture content diminishes to 20% or less, which normally takes up to 3 days. Some advantages are that the honey can be stored at room temperature until consumption, without fermentation, and the natural substances and flavor of honey are not lost, because it has not been heated. A disadvantage is that the honey becomes more viscous than normal for stingless bees, thus becomes very similar to commercial honey bee honey. Crystallization is enhanced, and produces sharp crystals, as observed in some *Melipona* species (P Vit, personal communication).

Pasteurization is a viable option in order to keep honey at room temperature without fermentation and to eliminate pathogenic microbes. The honey should be heated for 15 s at 72°C or 30 min at 63°C (Nogueira-Neto 1997), and bottled just after cooling to room temperature. If the process cannot be done just after harvesting, the honey should be cooled until pasteurization. This process does not kill all microorganisms and spores in the honey, but eliminates pathogens. The disadvantage of this process is that some natural enzymes are lost, like glucose-peroxidase (Nogueira-Neto 1997). Pasteurization offers three great advantages compared to other post-harvest methods. First, it is possible to store the honey at room temperature, without any fermentation. Second, it controls pathogens. Third, the natural flavor and texture of stingless bee honey are maintained (Nogueira-Neto 1997; Venturieri et al. 2007). After opening a bottle, it should be stored under 8°C and should be consumed before 1 year.

In the maturation process, fermentation after harvest will naturally occur at room temperature (Drummond 2010). The honey is kept inside closed bottles, which are opened once a week to release the gases generated by fermentation, and closed again. Honey can also be kept in bottles with lids that allow gas exchange. This process takes up to 3 months or until gas is no longer released. After this period fermentation stops, and the stabilized honey can be bottled. The main advantage of this method is that matured honey does not ferment at room temperature after the process and the costs are very low. The honey becomes more acidic after maturation, and acquires some peculiar odors and aromas (Drummond 2010). Sensory characteristics of matured honey, compared to fresh honey, may be perceived as an advantage or a disadvantage, according to personal tastes and use by the consumer.

The above mentioned possibility has been widely used in Maranhão, Brazil. Although it seems to be an interesting post-harvest alternative to preserve honey, especially for rural communities, it is still very controversial since we remain ignorant regarding its consequences at biochemical and microbial levels. They may provide healthy sub-products for human consumption (Pérez-Pérez et al. 2007), but also conceivably generate toxic substances.

10.4.2 Harvesting Fermented Pollen and Unfermented Pollen

Stingless bee pollen is very nutritious and is an ‘alternative’ healthful food source (Souza et al. 2004). Pollen extracts inhibit oxidizing agents and free radicals, and this property seems to be important in the prevention of various human diseases (Lins et al. 2003; Silva et al. 2006, 2009). However, the only way to harvest stingless bee pollen is by removing it directly from the pollen pots, because pollen traps used for *A. mellifera* do not work for stingless bees (Menezes et al. 2012).

Pot-pollen alone is sour in most stingless bee species. A Brazilian stingless beekeeper, Wilson Melo, who manages more than 600 colonies of *Scaptotrigona* spp. for pollen production, suggests consuming it as a honey-pollen jelly or as a creamy pollen milk shake. Both recipes neutralize the acid from the pollen and produce a pleasant flavor.

Although fermented pollen is relatively easy to harvest, we have developed a method to harvest pollen before fermentation (Menezes et al. 2012). We noticed that if we harvest the pollen a week after it has been stored, it is still sweet and not yet fermented. Because it would be impossible to distinguish fresh from fermented pollen in a bee nest or hive, a solution is moving a strong colony to a different place and replacing it with an empty hive, where the foragers will return from the field and store the pollen in new pots. After a week, the pollen can be harvested and will not be fermented. We tested this method with ten colonies of *S. depilis* and they produced an average of 60 g unfermented pollen in a week. This pollen can be used as it is, stored frozen or dehydrated. Another solution is harvesting the pollen from the honey super every week, so it has yet to ferment. It is important to emphasize that some stingless bees, such as the *Scaptotrigona* species, harvest much more pollen than honey and produce a substantial amount of pollen.

10.4.3 Stingless Bee Mead

A popular beverage since antiquity, consumed by several civilizations like the Chinese, Greeks, Romans, and Vikings (McGovern et al. 2004; Bishop 2005), mead (also known as honey wine) is basically a drink produced with fermented honey and water, which is also produced with pot-honey from stingless bees, known as “balché” by the Mayans (Villanueva et al. 2004). The elaboration of a mead beverage based upon melipona honey is a recent research line from Embrapa Amazônia Oriental, in Belém, Brazil. Preliminary results show that the high acidity of pot-honey needs pH control with calcium carbonate. This procedure allows yeast *Saccharomyces cerevisiae* to better perform its function. Otherwise, the resulting mead will have an unstable and acidic taste, because of its lesser quantity of alcohol.

In order to stop the action of undesirable microorganisms on the fermentation process by *S. cerevisiae*, pasteurization (65°C during 5 min) is performed before the yeast is added. The fermentation process can last from 2 and up to 4 weeks, depending on the proportion of water, honey, acids, and yeast, and should be done under anaerobic conditions allowing CO_2 release. After the fermentation cycle, the mead must be filtered and decanted. A further clarification with bentonite facilitates the precipitation of suspended particles. After bottled and sealed, the mead must be pasteurized (65°C during 5 min), in order to increase its stability and for safety reasons.

10.4.4 Pollen Substitutes for Artificial Feeding

The nutritional base of natural feeding by stingless bees, like in the majority of Apoidea, is nectar and pollen, with few exceptions. Nectar is the source of sugars while pollen, besides carbohydrates, also supplies them with protein, lipids, vitamins, and minerals (Michener 1974). Pollen is stored in pots and undergoes an intense fermentation caused by bacteria and yeasts. These microorganisms seem to be essential to pre-digest and conserve the stored pollen (see the above sections for more details, and also Morais et al. this book).

Honey and pollen substitutes are extremely important to stingless beekeeping, especially during dearth periods and after colony division or artificial multiplication. The nectar is easily substituted by sugar syrup and its acceptance and consumption are very good (Nogueira-Neto 1997), but pollen has been more difficult to substitute and frequently the workers throw it away (reviewed by Vollet-Neto et al. 2010).

The first study on a semi-artificial diet for the substitution of pollen was made by Camargo (1976). She mixed pollen of *Typha dominguensis* with honey and natural pollen from the bee that received the supplementary diet. The artificial food was stored in a glass covered by gauze at temperatures from 28 to 32°C during 10–15 days, leading to fermentation. She concluded that if the pollen substitute is not fermented, the workers reject it. Vollet-Neto et al. (unpublished data) also verified that young workers of *S. depilis* prefer fermented pollen instead of fresh pollen from foragers, and prefer a fermented artificial diet instead of a an unfermented one (Fig. 10.4).

Several pollen substitute formulations were later developed using different ingredients, such as commercial yeasts (*S. cerevisiae*) and soybean extracts (Penedo et al. 1976; Fernandes-Da-Silva and Zucoloto 1990; Pires et al. 2009). For *Scaptotrigona postica*, mixture of 25% commercial yeast and 75% pollen was found to be a good substitute, based on the development of hypopharingeal glands and oocytes (Penedo et al. 1976).

Costa and Venturieri (2009) and Pires et al. (2009) also developed and tested the consumption and nutritional value of pollen substitutes for *M. fasciculata*. They found that soybean extract mixed with sugar, water and about 20% pollen of the same bee species was a good pollen substitute, consumed by workers in a normal colony (Pires et al. 2009). The nutritional value was confirmed by development of worker hypopharyngeal glands and queen oocytes in a laboratory assay with the same bee species (Costa and Venturieri 2009).

Fig. 10.4 Young workers of *Scaptotrigona depilis* are more attracted to fermented food (left plate) than unfermented food (right plate) (Vollet-Neto et al., unpublished). Photo: C. Menezes

Most stingless bee species are not very tolerant of pollen substitutes and, if it is inadequate, workers discard the artificial food in the colony trash pile. However, some species, such as *F. varia*, show the opposite behavior. Foragers of this species are very attracted to artificial food even if offered outside the nest (Vollet-Neto, personal observation). They harvest a large amount and store it inside the nest (Fig. 10.5). Surprisingly, the worker bees were also attracted by food fermented by microorganisms from other stingless bee species.

Although in such a diverse group as Meliponini, generalizations are always difficult, we can conclude that a good substitute for pollen must have characteristics similar to the natural pot-pollen stored in the nest. The main factor to be considered is that a pollen substitute must be fermented, and we conclude that stingless bees prefer a pollen substitute fermented by microorganisms found in pot-pollen of their own species.

10.5 Conclusions

1. The main microorganisms living in stingless bee colonies are yeasts, molds, and bacteria. However, knowledge about this biodiversity is very limited, because most papers only mention their occurrence, not their function.
2. Due to the high diversity of stingless bees and limited studies on their microorganisms, the honey maturation process is still poorly understood. Physicochemical analysis of honey in the course of time would be of great value to understand the biological and biochemical processes involved in honey storage of stingless bees.
3. We may assume that the storage of pollen in cerumen pots is associated with inoculation of microorganisms, which promote biochemical changes that alter

Fig. 10.5 *Frieseomellita varia* storing artificial food. (**a**) Outside the nest, (**b**) inside the pollen pots made of cerumen. Green dye was used in the artificial food to distinguish them from natural pollen inside the nests. Photos: C. Menezes

nutritional quality and enhance digestion and absorption of nutrients, but probably the main function is to prevent spoilage and disease. We still need much more information to draw valid conclusions about the advantages brought about by microorganisms living in pollen.

4. The high water content of most pot-honey is a necessary challenge to stingless bee keeping. If honey is kept at room temperature, it will ferment after being harvested, even if extremely hygienic procedures are applied. Thus, four different solutions, refrigeration, dehydration, pasteurization, and maturation, have been developed by researchers and stingless bee-keepers to increase the post-harvest stability and extend the shelf life of pot-honey.
5. Microorganisms from stingless bees can be very useful for stingless beekeepers because peculiar products may be produced by them, such as mead, honey-pollen jelly, or a creamy pollen milk-shake.

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Chapter 11
Microorganisms Associated with Stingless Bees

Paula B. Morais, Paula S. São Thiago Calaça, and Carlos Augusto Rosa

11.1 Introduction

Evidence for the great biodiversity associated with stingless bees is obtained from the variety of materials and structures used to build their nests. Inside the nest, there are different shapes and arrangements of brood cells and food storage containers. Wax secreted by stingless bees is mixed with plant resins to produce cerumen (Wille and Michener 1973; Michener 1974; Roubik 1983). Honey and pollen are stored in separate cerumen pots (Fig. 11.1). The size and shape of these pots vary among bee species. Stored nectar or ripened honey is found in the extremes of the nest cavity (for storage during heavy flowering periods), while pollen and some honey surround the brood area (Roubik 2006).

Diverse ethnomedicinal properties attributed to stingless bee honeys are known in Brazil, Ecuador, Guatemala, Mexico, and Venezuela (Vit et al. 2004; Mendes and Antonini 2008; Guerrini et al. 2009), where pot-honey is worth up to 20 times more than *Apis mellifera* honey (Nogueira-Neto 1997; Vit et al. 1998).

Most of the studies of the microorganisms associated with stingless bees were carried out with the objective of describing the bacterial and fungal communities associated with these bees. However, data on the functional relationship between

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Fig. 11.1 Honey and pollen of *Melipona quinquefasciata* stored in separate cerumen pots. Photo: P.S. São Thiago Calaça

microorganisms and stingless bees are scarce. Although honey has some distinct properties that inhibit the growth of microorganisms, such as high sugar concentrations and high acidity (Snowdon and Cliver 1996), microbial fermentation has been suspected to contribute to the transformation of pollen into bee bread and in the formation of the honey itself. Microorganisms may also have a role in honey maturation and in the biochemical modification of stored pot pollen. After its collection by bees from flowers, the pollen stored inside meliponine nests becomes biochemically distinct due to fermentation processes, but it is not clear if yeasts or bacteria (or both) are responsible for these processes (Ganter 2006).

The association of microorganisms with honey, pollen, immature, and adult bees is indicative of a functional relationship with these insects. In this chapter, we will discuss the presence of different species of bacteria, molds, and yeasts associated with stingless bees and the possibility of the existence of a symbiotic relationship between these organisms.

11.2 Bees and Microbes

Insects engage in a vast array of symbiotic relationships with a wide diversity of microorganisms, in which some of them benefit the host nutritionally and provide protection from natural enemies (Klepzig et al. 2009). Yeasts, for example, are a food source for insects and are known to be the main source of sterols, vitamins, and protein for adult and larval stages of *Drosophila* (Morais et al. 1995b). The number of symbionts of the ground-dwelling ants and termites is large compared to that of social wasps and bees (Wilson 1971; Kistner 1982). According to Peruquetti (2000), the highly social stingless bees (Apidae, Meliponini) seem to be an exception to this rule. Their nests have many guests, including mites, moths, cockroaches, flies, beetles, fungi, and bacteria, some of which are obligate symbionts (Wasmann 1904; Salt 1929; Nogueira-Neto 1970; Machado 1971; Flechtmann and Camargo 1974; Aponte 1996; Kerr et al. 1996).

Insect species are important vectors of microorganisms, including bacteria, fungi, and protozoans (Starmer and Lachance 2011; Redak et al. 2004; Purcell 1982). For example, the distribution and habitat specificity of yeasts depend primarily on the insect vectors but are also dependent on the substrate composition and the presence of inhibitory compounds (Morais and Rosa 2000; Morais et al. 1995a; Starmer et al. 1976).

Various studies have aimed to characterize the microbial community associated with bees (Gilliam et al. 1984; Gilliam 1997; Inglis et al. 1993; Rosa et al. 1999, 2003; Teixeira et al. 2003). The microbiota of the European honey bee (*Apis mellifera*) has been isolated and identified (Gilliam 1997; Gilliam and Morton 1978; Piccini et al. 2004; Rada et al. 1997). These microbes are believed to help chemical conversion in the intestinal tract, preservation of pollen stored in comb cells, and production of antimycotic substances against the chalkbrood pathogen (Gilliam 1997).

Most of the bacteria isolated from brood combs and hive floors of the honey bee belong to the genera *Bacillus* and *Corynebacterium* (Piccini et al. 2004). Studies on the microbiota of the alfalfa leafcutting bee showed a dominance of fungi (e.g., *Aspergillus niger*, *Penicillium* sp., and *Saccharomyces* sp.) and bacteria (e.g., *Bacillus circulans*, *B. mycoides*, *Enterobacter agglomerans*, and *Pseudomonas* sp.) (Goerzen 1991). Other spore-forming bacteria belonging to the genus *Bacillus* were found to be prevalent in larval populations of two solitary bees (*Centris pallida* and *Anthophora* sp.) (Gilliam et al. 1984, 1990a).

Bacteria of the genus *Lactobacillus* were identified in *A. mellifera* and *A. mellifera scutellata* (Mohr and Tabbe 2006; Jeyaprakash et al. 2003). Recently, a novel bacterial flora composed of lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* was found in the stomach of *A. mellifera* (Olofsson and Vásquez 2008). In contrast, Evans and Armstrong (2006) failed to find *Lactobacillus* species in *A. mellifera*, suggesting that the gut microbial population is not constant even within the same species. Yoshiyama and Kimura (2009) did not find *Lactobacillus* species in the gut of *A. cerana japonica*, but they detected the following gut bacterial groups that had not been found in other *Apis* species: *Staphylococcus saprophyticus* (Firmicutes), *Kocuria* sp., *Tsukamurella tyrosinosolvens*, *Microbacterium* sp. (Actinobacteria), *Sphingomonas melonis*, *Mesorhizobium* sp. (Alphaproteobacteria), *Janthinobacterium* sp. (Betaproteobacteria), *Escherichia coli*, *Pseudomonas* sp., *Providencia alcalifaciens*, *Erwinia tasmaniensis*, and *Moraxella* sp. (Gammaproteobacteria). Honey bees visit flowers of many types, which vary geographically and seasonally. Furthermore, honey bees of different species tend to visit flowers of a particular species. Thus, Yoshiyama and Kimura (2009) suggest that variation of a characteristic gut bacterial flora in *Apis* species is likely to be related to variation in the food source, and this may be also true for other plant pollinators such as meliponines.

The bacteria *Streptomycetes* sp. have also frequently been found in pollen, provisions, and alimentary canals of alfalfa leafcutter bees (*Megachile rotundata*), and these bacteria are considered to be part of the resident microbiota of the bee

(Inglis et al. 1993). *Streptomyces fradiae* was isolated from the hive materials of *A. florea*, and *S. drozdrwiczii*, *S. albidoflavus*, and *S. badius* were isolated from *A. cerana* in Thailand.

According to Promnuan et al. (2009), *Streptomyces* species show a symbiotic relationship with some insects. A unique association between a new *Streptomyces* species and the European beewolf (*Philanthus triangulum*), a solitary hunting wasp, was reported. The beewolf females harbor the *Streptomyces* bacteria in specialized antennal glands and apply them to the brood cell prior to oviposition. The bacteria are taken up by the larva and are also found on the walls of the cocoon. Bioassays indicated that the streptomycetes protect the cocoon from fungal infestation and significantly enhance the survival probability of the larva, possibly by producing antibiotics (Kaltenpoth et al. 2005).

Rosa et al. (1999) found that a killer toxin-producing *Mucor* species was a dominant fungus, together with the yeast *C. batistae*, in nearly 100 nests of the solitary bee, *Diadasina distincta*. This fungus may play a role in pollen maturation because it presents proteolytic and pectinolytic ability that could be combined with the yeast fermentative and lipolytic function for pollen transformation (Rosa et al. 1999).

Inglis et al. (1993) showed that *Candida bombicola* (*Starmerella bombicola*) is frequently found in nectar, pollen, and provisions of the solitary bee *Megachile rotundata*. Rosa et al. (1999) isolated *Candida batistae* from the solitary bees *D. distincta* and *Ptilothrix plumata* in Brazil, and the authors suggested a possible mutualistic interaction between this yeast species and the bees. Pimentel et al. (2005) described two new species of yeasts, *Candida riodocensis* and *Candida cellae*, associated with two solitary bees, *Megachile* sp. and *Centris tarsata*, in the Atlantic rain forest of Brazil.

At this time, the *Starmerella* clade contains more than 40 yeast species, most of which were isolated from bees (Table 11.1). This clade is defined as a single branch in the Ascomycetes that present the common ecological traits of the association with insects and ephemeral flowers. Species belonging to this clade, such as *C. magnoliae*, *C. batistae*, *S. bombicola*, and *S. meliponinorum*, are thought to be involved in a mutualistic relationship with bees (Gilliam 1979a; Inglis et al. 1993; Rosa et al. 1999). In addition to the two *Starmerella* species, *Candida bombi* is common in European bumble bees (Brysch-Heberg 2004). *Candida davenportii*, *C. apicola*, *C. bombi*, *C. powellii*, *C. floricola*, *C. tilneyi*, *C. vaccinii*, *C. sorbosivorans*, *C. magnoliae*, and *C. apis* have been isolated from bees, wasps, substrates that these insects visit and from other insects that visit the same substrates (Lachance et al. 2001a,b; Trindade et al. 2002).

11.3 Bacteria Associated with Stingless Bees and Their Ecological Roles

Bacteria maintain a symbiotic relationship with various groups of bees (Roubik 1989). Although the interior of the nests of stingless bees has a high relative humidity and contains mud and large quantities of feces and other detritus, relatively few

Table 11.1 Some yeast species in the *Starmerella* clade and their association with bees[a]

Yeast species	Bee species or bee substrate of isolation	Locality
Candida apicola	Bee gut *Melipona quadrifasciata*, *M. rufiventris*, *Trigona* spp., and their hives and pollen	Croatia Brazil, Costa Rica, Malaysia
C. apis	Trachea of a bee	UK
C. batistae	Ground nesting solitary bee	Brazil
C. bombi	*Bombus terrestris*, *B. hortorum*, *B. cryptarum*, *Bombus* sp.	France, Germany
C. cellae	*Centris tarsata* (solitary bee)	Brazil
C. davenportii	Dead wasp	UK
C. etchellsii	*Trigona* Unknown bee in *Opuntia* flowers	Costa Rica USA
C. floricola	*Ipomoea* flowers visited by bees	Brazil
C. floris	*Trigona* spp.	Costa Rica
C. geochares	Honey of *T. angustula* and *M. quinquefasciata*	Brazil, South Africa
C. magnolia	Bee gut and pollen (*Apis mellifera*)	Croatia, USA
C. powellii	Unknown bee on *Ipomoea*	Costa Rica
C. riodocensis	Pollen and nectar provision of *Megachile* sp.	Brazil
C. tilneyi	Halictid bee in *Ipomoea carnea*	Costa Rica
Starmerella bombicola	Honey and pollen of *T. angustula*, *M. quinquefasciata*, *M. quadrifasciata*, and *F. varia* *Bombus* sp. *Trigona fulviventris*	Brazil Canada Costa Rica
S. meliponinorum	Honey and pollen of *T. angustula*, *M. quadrifasciata*, *M. rufiventris*, and *F. varia* *Trigona* sp.	Brazil Costa Rica

[a] Data from Lachance (2011)

bacteria are found in the nest, probably due to antibiotic substances in the nest materials and inhibitors produced by the bees themselves to suppress competitors (Roubik 1983). Bacteria present in the bee nests seem to have an important role in pot-honey maybe by inhibiting spoilage bacteria. In the intestinal tract of *M. quadrifasciata*, five different types of *Bacillus* spp. are found, although only one species may maintain a close relationship with the bee because it is found in bee's intestines and also in pot-honey (Cruz-Landim 1996). It is possible that *Bacillus meliponotrophicus* is responsible for a type of pre-digestion of honey and pollen produced by *M. quadrifasciata* (Nogueira-Neto 1997). Machado (1971) has shown that *B. meliponotrophicus* is associated with *Trigona* and *Melipona* but not with *Apis* and *Bombus*, which are phylogenetically related to the stingless bees. In the *M. quadrifasciata* colonies, bacteria are present in high concentrations in larval food and honey pots, where they take part in the fermentation process. The relationship between the bacterial species and the bee is obligatory because the use of antibiotics/streptomycin in the food led to the disappearance of the colony.

Spore-forming bacteria belonging to the genus *Bacillus* were found in some nests of stingless bees *Melipona panamica* (*B. alvei*, *B. circulans*, and *B. megaterium*) and *Trigona necrophaga* (*B. circulans*, *B. licheniformis*, *P. megaterium*, *B. pumilis*, and *B. subtilis*) in Panama (Gilliam et al. 1985, 1990b).

Lactic acid bacteria (probably *Lactobacillus* species) were isolated in high numbers from honey and pollen samples of *T. angustula* and *M. quadrifasciata* (C.A. Rosa, unpublished results). These bacteria likely have a role in the honey maturation of these bees by suppressing spoilage bacteria, as we speculate above.

Two stingless bees, *Tetragourla laeviceps* and *Tetragourla fuscobalteata*, commonly found in the northern region of Thailand, are known to construct nests inside forest trees. Bacterial communities of *T. laeviceps* included *Streptomyces pseudogriseolus*, *S. rochei*, *S. drozdowiczii*, *S. mutabilis*, *S. minutiscleroticus*, *S. albus*, *S. tosaensis*, and *S. malaysiensis*. In contrast, in the *T. fuscobalteata* hives, *S. ambofaciens*, *S. mutabilis*, *S. coalescens*, and *S. violaceoruber* were isolated from brood cells (Promnuan et al. 2009). The ecological role of the bacterial community still needs to be determined.

Although beneficial endosymbiosis has been described in many solitary and colonial insects that vary from obligate and intracellular to facultative and extracellular within the gut lumen (Kikuchi 2009). Anderson et al. (2011) point that virtually nothing is known about beneficial symbionts of bees. Mohr and Tabbe (2006) suggest the existence of cosmopolitan gut bacteria in bees, although Koch and Schmid-Hempel (2011) affirm that bumble bee gut presents a highly specific microflora largely different from bacteria associated with guts of honey bees. Killer et al (2009) described a new species *Bifidobacterium bombi* among gram-positive-staining, anaerobic, non-spore-forming, lactate- and acetate-producing bacteria isolated from the digestive tracts of different bumble bee species (*Bombus lucorum*, *Bombus pascuorum*, and *Bombus lapidarius*). Recent studies on the microbial flora of the honey bee gut have revealed an apparently highly specific community of resident bacteria that might play a role in immune defense and food preservation for their hosts. As pointed by Anderson et al. (2011), honey bees used in agriculture are stressed by a plethora of agricultural chemicals and their associated by products, and this may be a general situation for most bees including wild meliponing, and those antibacterial agents may kill bacterial symbionts resulting in the decline of bee populations as seen for honey bees in part of the world.

11.4 Molds Associated with Stingless Bees

There are few reports on molds associated with stingless bees. Roubik and Wheeler (1982) report the presence of *Stemphylium* (similar to those that decompose wood) in nests of *M. panamica*. Fungal identification was performed by observation of spores and hyphae found in the stomach of a beetle of the genus *Scotocryptus* that inhabits the nests of stingless bees. Gilliam et al. (1990b) reported the presence of a green fungus in the honey of *M. Panamica*. Melo (1996) also reported a dark purple fungus in the cerumen of *M. capixaba*. However, the ecological roles of these fungi have not been determined.

Early mycological studies recognized that certain molds are common saprophytes both on and inside dead honey bees and brood combs and are probably unable to become established within the bee or the hive (Betts 1920). Fungus-associated spoilage of provisions and mortality of honey bees are rare (Batra et al. 1973). Gilliam et al. (1988) showed that only *Ascosphaera apis*, which causes chalkbrood disease, is of economic importance. Egorova (1971) isolated *Aspergillus flavus*, *A. versicolor*, *Mucor alboalter*, *Penicillium granulatum*, *P. solitum*, and *Sporotrichum olivecum* from bee bread. Two studies, Chevtchik (1950) and Pain and Maugnet (1966), did not mention molds in pollen or bee bread (the actual food consumed by bee larvae). However, Gilliam et al. (1989) isolated *Aureobasidium pullulans*, *P. corylophilum*, *P. crustosum*, and *Rhizopus nigricans* (*R. stonolifer*) in pollen and bee bread but not from floral pollen. These authors determined that these isolates may have been introduced by the bees. They noticed that the number of isolates decreased after storage by the bees, and *Mucor* sp., the dominant mold in floral pollen, was not found in corbicular pollen or bee bread. They concluded that, as with yeasts (Gilliam 1979a) and *Bacillus* spp. (Gilliam 1979b), the mold biota of corbicular pollen and bee bread may be the result of microbial inoculation by the bees and chemical changes in pollen that allow some species but not others to survive, as noted by Klungness and Peng (1983).

In the course of a study on pollen diets of three sympatric species of stingless bees *Heterotrigona collina*, *Tetragonnla melina*, and *T. melanocephala* in Sabah, Malaysia, Eltz et al. (2002) observed that large fractions of the foragers of three colonies of *H. collina* collected corbicular loads of fungal spores in lieu of pollen. Collection of spores continued for at least three consecutive days. The spores were brought to germination in the laboratory, and the culture was identified as mold of the genus *Rhizopus*. Their observations represent the first reported case of the collection of *Rhizopus* mold spores in lieu of pollen by bees and a rare case of the collection of fungal spores by bees other than honey bees (*Apis*) (Eltz et al. 2002).

Yeasts and molds are found naturally in honey, according to Gilliam (1997), who argues that microorganisms associated with bees are non-pathogenic and that most of these microorganisms are not yet known. Eltz et al. (2002) affirm that the fungi collection sometimes replaces pollen harvesting in *Apis*, *Trigona*, and *Partamona*. Ferraz et al. (2006) detected *Aspergillus* sp., *A. niger*, *Penicillium* sp., *A. terreus*, *Curvularia* sp., *Monilia* sp., *Nigrospora* sp., *Cladosporium* sp., and *Trichoderma* sp. in “jandaíra” *Melipona subnitida*, which inhabit the semiarid rocky areas of Brazilian Northeast. A species of *Curvularia* was reported as an inhabitant of *Trigona* sp. inhabiting the dry Caatinga ecosystem of Northeastern Brazil (Ferraz et al. 2006). However, the ecological role of these filamentous fungal species in the bee nests has not been determined. Indeed, Gibson and Hunter (2005) noted that the distinction between commensal and mutualistic interactions is often difficult to discern.

11.5 Yeasts Associated with Stingless Bees

Bee nests harbor a diversified yeast microbiota, and their role in biochemistry, nutrition, and physiology of bees has been investigated (Teixeira et al. 2003). According to Gilliam (1997), in social species, yeasts may have an important role in the conversion of pollen into available nutrients. Early studies showed that microbiota of pollen taken directly from flowers, corbicular pollen, and pollen stored in comb cells in the hive (bee bread) are similar. Foraging bees add microbes to pollen during collection and the same species of bacteria and yeasts are found in guts of worker bees and in corbicular pollen (Gilliam 1979a; Gilliam et al. 1984; Gilliam and Prest 1987). These microorganisms may be involved in the metabolic conversion, fermentation, and preservation of the stored food. The conversion of pollen to bee bread has often been postulated to be the result of microbial action, principally a lactic acid fermentation caused by bacteria and yeasts (Haydak 1958).

Yeasts have been isolated from honey bees, stingless bees, and solitary bees (Gilliam 1997; Rosa et al. 2003; Brysch-Heberg 2004). The Amazonian species *Ptilotrigona lurida* maintains mutualistic interactions with an unidentified yeast species that is believed to be responsible for dehydrating and retarding the deterioration of the pollen in the bee nest (Camargo et al. 1992). *Starmerella meliponinorum* was described in association with nests of the eusocial stingless bee, *T. angustula*, and could also be associated with food, both honey and pollen, propolis, detritus, and adult individuals of *M. quadrifasciata*, *M. rufiventris*, *T. angustula*, and *T. fulviventris* (Rosa et al. 2003; Teixeira et al. 2003). *Starmerella meliponinorum* and *C. apicola*, also part of the *Starmerella* clade, have been consistently isolated from *T. angustula* adults, honey, pollen provisions and refuse, *M. quadrifasciata* and *M. rufiventris* in Brazil, and *Heterotrigona Tetragonula* sp. in Malaysia. Therefore, they may have a mutualistic relationship with stingless bees. Most of the described species in the *Starmerella* clade are associated with bees or related habitats (Rosa et al. 2003). Some species in the clade are also found in other environments. In addition to the two *Starmerella* species, *S. bombicola* and *S. meliponinorum*, *C. apicola* and closely related types are found in tropical meliponine bees worldwide (Lachance 2011).

Rosa et al. (2003) showed that the yeast community associated with *T. angustula*, *M. quadrifasciata*, and *Frieseomelitta varia* is specific to these bee species, although the ecological roles of the yeasts have not yet been defined. A large number of other yeast species were isolated from various adults of these three bee species, including *Aureobasidium pullulans*, *Pseudozyma antarctica*, and various species of *Cryptococcus* and *Rhodotorula* that may represent a transient mycota vectored by bees. *Debaryomyces hansenii* was isolated from adults and garbage pellets of *M. quadrifasciata* and from a propolis sample of *T. angustula*. This halotolerant and osmotolerant generalist is a frequent contaminant of human food and usually rare on the phylloplane (Fonseca and Inácio 2006; Kurtzman 2011a, 2011b). It was reported to cause spoilage of *A. mellifera* honey (Snowdon and Cliver 1996). Highly osmotolerant species of *Zygosaccharomyces* were isolated from the honey of *T. angustula*, from an adult *M. quadrifasciata* and from a garbage pellet of *F. varia*. *Zygosaccharomyces machadoi* was isolated from a garbage pellet of *T. angustula*

Fig. 11.2 Ripe honey of *Melipona quinquefasciata*

(Rosa and Lachance 2005). The new species *Zygosaccharomyces siamensis* was isolated from raw honey of *A. mellifera*, *A. dorsata*, and *Tetragonula pagdeni* in Thailand (Saksinchai et al. 2012). These yeasts might act as an agent of pot-honey spoilage for these bees, as argued by Rosa et al. (2003). Other yeasts already isolated from stingless bees are *Hyphopichia burtonii* (Kurtzman 2011a) and *Priceomyces mellissophilus* (Kurtzman 2011b), whereas *M. kunwiensis* and *M. reukaufii* are consistently isolated from *Bombus* bee species (Lachance 2011).

Calaça (2011) reported that the number of yeast cells was higher in unripe pot-honey than in ripe honey of *M. quinquefasciata* (Fig. 11.2) collected in Brazil, which indicates that abundance and diversity of yeasts decreases during honey ripeness. *Candida* sp. MUCL 4571, a new undescribed species sister of *C. apicola*, was the prevalent species in the samples and could have a mutualistic association with this bee.

11.6 A Possible Mutualistic Interaction Between Yeasts and Bees?

High yeast counts in larval provisions suggest that these microorganisms are metabolically active, and that the enzymes they produce may be important for the improvement of the nutritional characteristics of pollen. Both social and solitary bees introduce yeasts into their nests (Gilliam 1997), which possibly bring nutritional benefits to larvae. Bees require nutrients, such as proteins, lipids, and vitamins, from pollen and carbohydrates from nectar (Standifer et al. 1980). Corbicular pollen is transformed into bee bread (comb pollen) through a fermentative process that is carried out primarily by yeasts (Pain and Maugnet 1966) and brings a higher nutritional value and availability of amino acids in the bee bread compared to corbicular pollen (Loper et al. 1980; Standifer et al. 1980).

Gibson and Hunter (2005) defined five stages in the pathway to obligate mutualism: (1) consistent and extended contact; (2) avoidance of lethal harm during contact; (3) coadaptation, leading to increased tolerance; (4) further coadaptation, leading to dependence and/or interdependence; and (5) permanent association. In studies of the association of yeasts and *Chrysoperla* lacewings, Gibson and Hunter (2005) argue that the ease with which the yeasts can be cultured suggests that these two organisms are not interdependent obligate mutualists, as in case of bacterial symbionts (Douglas 1998). Although they could not find evidence that resident yeasts bring nutritional benefits to the lacewings, they were not able to cultivate yeast-free lacewings and, therefore, could not reach a conclusion on the role of yeasts in the interaction. Our own studies on the yeasts associated with the bees *M. quinquefasciata* in Minas Gerais (Southeastern Brazil) and *M. compressipes*, *M. scutellaris*, *Plebeia* sp., *Scaptotrigona polysticta*, and *S. tubiba* in Cerrado ecosystems of Central North Brazil indicate that those yeast strains are very difficult to maintain in culture collections, and various strains die before a complete identification is reached, raising the possibility that association with the bees is important for survival of those yeasts. Further investigation is needed to reach any conclusions on the mutualistic interactions between stingless bees and yeasts.

Records of yeast-insect associations in which the role of the yeasts is not well understood include: green June beetles (Vishniac and Johnson 1990), nitidulid beetles (Lachance et al. 2003), clerid beetles (Lachance et al. 2001a), encyrtid parasitoids (Lebeck 1989), ichneumonid parasitoids (Middeldorf and Ruthmann 1984), fire ants (Ba and Phillips 1996), leafcutting bees (Teixeira et al. 2003), solitary digger bees (Rosa et al. 1999), vespid wasps and bumble bees (Stratford et al. 2002), honey bees (Spencer and Spencer 1997), and the green lacewings in the genus *Chrysoperla* (Hagen et al. 1970; Gibson and Hunter 2005). Although we cannot rule out the possibility that stingless bees are simply vectors for yeasts, Lachance et al. (2011) affirm that the insect vectors appear to be the primary agents responsible for the organization of the yeast communities, a role of great importance for the understanding of yeast ecology in all ecosystems.

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Chapter 12
Stingless Bee Food Location Communication: From the Flowers to the Honey Pots

Daniel Sánchez and Rémy Vandame

12.1 Introduction

Colonies of social insects lack a central control yet they function as a coherent whole, adjusting their activities in response to a changing environment (Seeley 1995; Visscher 1998; Wilson 2000). How such biological systems are organized has been one of the biggest questions raised by researchers in this field. Honey bees have been studied since ancient times. Aristotle noted that honey bees may recruit nestmates to rich food sources (Nieh 1999). It was the Austrian scientist, Karl von Frisch, at the end of World War I, who described a series of behavioral patterns in the honeybee *Apis mellifera* (Hymenoptera: Apidae, Apini) that seemed related to the organization of the colonies of this species (von Frisch 1967). To observe their behaviors inside the colony, he designed a glass-walled hive, which allowed him to notice that some bees were performing particular behaviors which he called dances. These dances apparently had information about where the dancing forager had found pollen or nectar. Von Frisch discovered what it is now known as the honeybee dance language. Later, with his book "The dance language and orientation of bees" published in 1967, von Frisch described in detail the communication behaviors observed in *A. mellifera* and briefly discussed similar behaviors in other insects. Subsequently, other researchers raised the possibility that recruits may orient only to the smells of the food brought back by the explorer. They hypothesized that the dance behavior was actually an experimental artifact, or a behavior that did not convey location information to nestmates (Wenner et al. 1969; Gould and Gould 1988; Wenner 2002). However, subsequent studies provided detailed, convincing evidence that bees can use the spatial information encoded in the dance language

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and that a correct interpretation of this information is beneficial for colony fitness (Robinson 1986; Dyer 2002b; Dornhaus et al. 2006).

Parallel to the research on the honeybee language, a rising interest in unveiling the ultimate and the proximal mechanisms involved in its evolution led researchers to investigate other species, like the stingless bees (Hymenoptera, Apidae, Meliponini). Stingless bees have proven to have mechanisms of communication as remarkable as the honeybee's, although behaviors identical to the honeybee waggle dance have not been observed in studied species. However, stingless bees consist of hundreds of species that display a diversity of behaviors and ecological adaptations. Thus, they deserve to be studied in their own right, given their importance in their respective ecological niches.

In the following pages, the reader is acquainted with elementary knowledge about stingless bee food location communication. First, we give a general view of the topic. Then, several communication mechanisms are described. External and internal factors that affect the communication system in stingless bees are detailed. Finally, as a result of integration of these elements, the food communication systems and their influence on the foods collected become evident. The characteristics of the pot-honey and pot-pollen are of course affected by the food matter thus collected.

12.2 Food Location Communication Systems in Highly Social Bees (Apidae)

After the initial discovery of the honeybee dance, von Frisch turned his attention to the evolutionary origins of this behavior. Because the meliponines (stingless bees) are similar to honeybees, Martin Lindauer, one of von Frisch's students, began to study stingless bee recruitment communication (Lindauer and Kerr 1960; Lindauer 1967). Together with the Brazilian scientist, Warwick Kerr, Lindauer found a wide range of potential recruitment and communication behaviors in the several meliponine species that they studied, including behaviors that were not observed in honeybees: random searching (no location communication) and odor trails, to name two. They hoped to help elucidate the evolution of the *A. mellifera* waggle dance. Whether stingless bee and honey bee recruitment communication derived from a common ancestor or evolved independently is unclear, although molecular evidence suggests that the two groups are not as closely related as once thought (Cameron and Mardulyn 2001). Nonetheless, even if their recruitment communication systems have evolved convergently, they exhibit certain similarities that suggest common pathways, perhaps deriving from traits shared by both groups of bees and similarities in the ecological niches that they occupy.

More recently, it has been demonstrated that the meliponine bees have communication systems as complex, in their own ways, as those described by von Frisch for *A. mellifera* (Dyer 2002a; Nieh 2004). In general, social insects use communication for various purposes, such as to ensure the cohesion of the colony, to warn the presence of danger, to find mates, and to communicate the spatial location of resources, to name a few (Wille 1983; Gould and Gould 1988; Collins et al. 1989; Wilson 2000). With respect to foraging communication systems, the focus of von Frisch's work, it has been observed that highly social bees such as *A. mellifera* and stingless bees have developed sophisticated mechanisms to recruit nestmates to resources such as pollen, nectar, water, resins, and places to establish new colonies (von Frisch 1967; Nieh 2004; Seeley 2010). With these mechanisms, scouts can send recruits to specific sites that offer profitable resources, a process often referred to as "food recruitment". In fact, the arrival of recruits to an advertised food source is the conclusion of a series of processes that occur at various levels of the colony and the individual (Biesmeijer and Slaa 2004). Meliponines are a good model to study the evolution of recruitment because they are a highly diverse taxon and display correspondingly diverse strategies to reach the same goal: recruit nestmates to rich food sources.

12.3 Food Recruitment in Stingless Bees

Stingless bees are a monophyletic group found in tropical and subtropical areas of the world, in America, Asia, Africa, and Australia (Roubik 1989). Unlike honeybees, which consist of approximately 11 species in one genus (*Apis*), stingless bees consist of hundreds of species distributed in 36 genera (Michener 2000). In addition, stingless bees have multiple lifestyles, including necrophagy, and can recruit to resources such as dead animals, nectar sources, and even the food reserves of other bee species (Roubik 1989). Also, stingless bees exhibit a great diversity of behaviors for transferring information about the location of a resource. These range from pheromone trails to the referential coding through sounds (Nieh 2004). Unfortunately, no studies on stingless bees have been conducted as intensively as in *A. mellifera*, so the understanding of their biology is in an early stage compared to what is known in the Apini. Fortunately, the meliponines have recently drawn the attention of researchers in animal communication, since their study could have implications for understanding the evolution of communication within the Apidae.

It is useful at this point to define some key terms for understanding the processes that arise during food recruitment in social bees. An individual is considered a forager if it is collecting resources for the colony. A scout is a forager that leaves the colony to find resources on its own. A forager is considered to be a recruit if it receives information from the scout about the location of a rich food source (von Frisch 1967). Food recruitment is a communication system that refers to a set of behaviors involved in the transfer of information between scouts and recruits; these behaviors are known as mechanisms for information transfer or simply communication mechanisms. The latter explanation is more specific because communication generally occurs through signals whereas information transfer involves both signals and cues. In general, we can classify communication according to where it occurs: inside the colony (recruitment movements, trophallaxis, and sounds) and outside the colony (social facilitation, pheromones). This, however, is not sufficient to understand the complexity that occurs in the communication systems. Biesmeijer and de Vries (2001) proposed the following classification of the individuals involved in food recruitment in order to better understand the phenomenon of communication:

1. Naïve forager: forager without any previous experience in collecting resources.
2. Explorer (also known as a scout): forager using only internal information to search for resources not previously known to it.
3. Recruit: forager using external information, generally from scouts, to find resources not previously known by her.
4. Engaged recruit (also called employed recruit): forager collecting resources in a known location; it does not usually follow external information while collecting resources.
5. Unemployed experienced foragers: individuals that are temporarily idle because the resource they were visiting was depleted.
6. Inspector: individual temporarily idle that periodically revisits depleted food sources expecting to find them profitable again.
7. Reactivated forager: individual that resumes its foraging activities after having received external information on the availability of resources it previously collected.

The information delivered by communication about resources outside the nest along with other information such as weather and the external experiences of foraging outside the nest are jointly referred to as external information (Biesmeijer and Slaa 2004). Thus there are two types of external information according to its source: information from other bees and information from the environment.

The other source of information used by foragers, which has not received sufficient attention yet, is internal information, which can be more precisely defined as the physiological and genetic status of the individual. A bee's experience, genetic variation, age, and hormone levels are examples of internal information (Biesmeijer and Slaa 2004). Although it is not a communication mechanism, internal information has a significant influence on the decision of recruits and experienced bees (Biesmeijer et al. 1998).

The overall strategy that colonies use to gather resources is thus the result of the interaction between the communication system, the conditions inside and outside the colony, and forager internal information. In the end, this results in either the recruitment or non-recruitment of foragers to a specific location.

12.4 External Sources of Information: Mechanisms of Communication and Recruitment

Insects search for and gather food in unpredictable environments (Goulson 1999). This makes it difficult to exploit efficiently those resources. To keep foragers from wasting time and energy in the tasks of resource gathering, highly social bee species have developed organization systems that allow them to make continuous adjustments in the number of individuals performing certain tasks inside or outside the colony. This is achieved through behaviors that enable bees to communicate with each other, establishing the conditions for the colony to survive in cohesion, in addition to providing a competitive advantage, in some cases, over other species that do not communicate or coordinate to the same degree (Dornhaus et al. 2006). Thus, by understanding the mechanisms of foraging communication we will be able to understand more in general about communication systems in social insects.

12.4.1 Mechanisms of Communication Inside the Nest

Successful foragers of most stingless bee species produce sounds and execute particular behaviors inside the nest or hive after returning from a good food source. In some species, these sounds may indicate the distance from the colony to the food source. Lindauer and Kerr (1960), Esch et al. (1965), and Esch (1967) were the first researchers to describe in detail the patterns of dances, the sound pulses, and the trophallactic interactions in colonies of stingless bees, with special attention paid to explorers returning from profitable resources. The general method is based on training bees to a feeder placed at a known distance and direction from the colony and recording the behavior (trophallaxis, dances, and sounds) of the foragers returning to the colony. In fact, this is the same method currently used to investigate possible correlations between a particular behavior and spatial parameters such as distance, direction, and height of stingless bees (Nieh 2004).

12.4.1.1 Behavioral Rituals (Dances) in Meliponini

In several species of recruiting bees, including *Apis* spp. and meliponines, successful foragers display specific behaviors inside the colony to draw the attention of their fellow foragers in order to transfer information related to the site where they discovered resources (Lindauer and Kerr 1960; von Frisch 1967). The dances in *Melipona scutellaris* and *M. quadrifasciata* consist of agitated running and jostling, without any discernible pattern that can be associated with the location of resources found by the scouts (Hrncir et al. 2000). In other species, like *M. panamica* (Nieh 1998a) and *M. beecheii* (Sánchez and Vandame, unpublished data) the returning foragers display both clockwise and counterclockwise turns while emitting sounds. But so far, no dance similar to the honeybee waggle dance has been described in stingless bees. It has been shown that variations in the intensity of the dance of *Apis* and some meliponine species are related to the quality of the resource (Aguilar and Briceño 2002; Dyer 2002a; Nieh et al. 2003b). However, the recruitment "dance" movements of meliponines apparently do not communicate the polar coordinates of resources (distance and direction) as the dance of *Apis* does (Nieh 2004). In studies with *M. panamica*, Nieh (1998a) found no effects of food distance, direction, or height on forager movement patterns inside the nest. In the species *M. scutellaris* and *M. quadrifasciata*, Hrncir et al. (2000) also found no clear correlation between the dances observed in these species and any parameter of the resource's location. This suggests that meliponines are unable to encode direction, distance, or height in recruitment dance movements. Similarly, bumble bee foragers evidently do not communicate resource location and instead forage individually after being activated by the return of a successful forager (Dornhaus and Chittka 2004). Thus, the recruitment dance of meliponines appears to work as a mechanism to alert potential recruits about the presence of a highly profitable resource.

12.4.1.2 Sounds

The pioneering work of Esch et al. (1965) and Esch (1967) suggested that the stingless bee species *M. quadrifasciata* and *M. seminigra* were able to communicate the distance at which the resource was located through sound pulses inside the colony, produced by the flight muscles of successful scouts. Other work has shown similar results, describing in *M. panamica* the production of sound pulses by successful explorers; for instance, the duration of individual pulses correlated well with the distance at which the resource is found (Nieh and Roubik 1998). Moreover, they distinguished sound pulses produced during unloading food (trophallaxis) and pulses produced after unloading food (during the dance) in *M. panamica*. While the duration of the first type of pulses correlated negatively with food quality, the duration of the second type of sound correlated positively with the distance of the resource from the hive. That is, *M. panamica* may be able to communicate through sound pulses both the quality of the resource and its distance. However, the pulse durations were highly variable and thus it is unclear if they could provide the level of precise information observed in how recruits find the indicated food sources. Thus, this area requires further investigation. In a different species, *M. quadrifasciata*, no clear correlation has been found between the recruitment sound pulses and any parameter of the resource's location (Hrncir et al. 2000), although they were correlated with the quality of the food source (Hrncir et al. 2004). Thus, there are many aspects of recruitment communication in the genus *Melipona* that require further study, including the possibility of significant interspecific variation in communication mechanisms. In addition, it is necessary to conduct experiments where the sounds recorded in the colony are played back with high fidelity in order to see whether there is any effect of recruitment to a specific distance.

12.4.1.3 Trophallaxis

When a successful honey bee forager enters the colony, it can produce recruitment dances to attract potential recruits, some of which extend their proboscis to make contact with the mandible of the explorer. When the forager stops dancing it begins to share the collected nectar with her nestmates, resulting in a trophallactic interaction. Trophallaxis thus refers to the exchange of liquid food between individuals of the same colony (Wilson 1971). Trophallactic contact is a primary form of information transfer. It can give information about the quality and odor of food resources. Trophallaxis is believed to have evolved with the need to communicate. However, not all the bees that receive nectar follow the dancer, and vice versa. The bees that both follow the dance and get nectar, on the other hand, receive more information about the resource the explorer just visited. Many of these bees follow to receive the forager's dance information and may decide to visit the resource (Farina and Nunez 1995; Stabentheiner 1996; Wainselboim and Farina 2000; De Marco and Farina 2003).

12.5 Mechanisms of Communication Outside the Nest

Foragers have to make decisions about where and when to explore new places in search of resources. They can make decisions based on innate behavior, their experience, or their interactions with other bees through communication mechanisms. These interactions can occur, as previously stated, inside the nest or outside the nest. Social facilitation and pheromone deposition are mechanisms of communication outside the nest that have been observed in several species of meliponines.

12.5.1 Social Facilitation

In stingless bees, the phenomenon of social facilitation occurs when the behavior of executers influences the behavior of observers (Slaa and Hughes 2009). Social facilitation has also been studied in vertebrates, in which it seems to be one of the most important mechanisms to learn how to gather food, how to build nests, etc. (Wilson 2000). In social vertebrates, social facilitation provides further advantages: it makes it easier to find and handle resources and improves both the recruitment of nestmates and the collection of food, which may additionally reduce the individual probability of being preyed upon (Galef 1976; Burger and Gochfeld 1992; Galef and Giraldeau 2001). Social insects other than stingless bees also exhibit social facilitation, which has been shown to influence decisions about where to gather resources. In social bees, there are two types of social facilitation: local inhibition (foragers avoid places already occupied by other individuals) and local promotion (foragers are attracted to and learn about rewarding resources based upon the presence of other individuals already performing a task). Both have been described in meliponine species (Slaa 2003). Experience and learning also play an important role in the development of these two types of social facilitation. For example, the selection of patches of resources, or even the selection of individual flowers within a patch, can be guided by the physical presence of other bees on the basis of prior learning, modulating the final decision.

12.5.2 Pheromonal Signaling

Several sources of olfactory information can influence bees' orientation: the smell of the resource itself, pheromones and potentially locale odors (Aguilar and Sommeijer 2001; Nieh 2004; Arenas et al. 2007; Barth et al. 2008). Even though resource odors, such as floral scents, have proven to be very important in guiding foragers little has been studied regarding the importance of locale odors (the odors of the environment immediately surrounding the rewarding food source).

Pheromones are mixtures of chemical compounds secreted externally by bees. They convey critical information about many aspects of the status of the individual or of the colony. Pheromones used in recruitment are mainly secreted in glands located in the abdomen, head, and in the legs. In addition to the diversity in the chemical composition of pheromones in stingless bees, there is also a great variation among species in the way they are deposited. These behavioral differences in the ways of depositing pheromones may, in part, be adaptations to the different ecological needs of each species.

12.5.2.1 Complete Pheromone Routes

Some meliponine species can deposit an odor trail extending from the nest to the food source. Successful foragers lay a pheromone trail upon their return from the food source to the nest by depositing pheromone droplets on vegetation (Lindauer and Kerr 1960; Kerr et al. 1981). In some species, the distance between the marks ranges 1–8 m (Nieh 2004). In this way direction and distance to the food source are communicated.

12.5.2.2 Incomplete Pheromonal Routes

Some species leave incomplete pheromone trails that extend from the food source to part of the distance towards the nest. In this case, successful foragers deposit pheromone droplets nearby the advertised resource, but not all the way back to the nest, up to 8 m from the target in *M. rufiventris* and *M. compressipes* and up to 27 m in *Trigona spinipes* (Nieh 2004). By doing this, foragers signal the direction where the resource is located, but not the distance. Such partial odor trails appear to provide partial guidance for a swarm of foragers that is recruited at the nest and guided towards the food source.

12.5.2.3 Polarization of Pheromone Trails

This is an interesting behavior observed in *T. spinipes* and *T. hyalinata* and that may occur in other species (Nieh et al. 2003a, 2004). Basically, foragers deposit larger amounts of pheromones as they reach the resource, thus decreasing towards the nest. In this way recruits can determine with high precision where the food is located, because this is indicated with the highest concentration of pheromones.

12.5.2.4 Odor-Marking the Resource

This strategy refers to the deposition of pheromones on the resource itself. This behavior is frequently found together with pheromone trails, either complete or incomplete. *Melipona panamica* and *M. favosa*, however, only odor-mark the resource, without laying any pheromone trail (Nieh 1998b; Aguilar and Sommeijer 2001).

12.5.2.5 Aerial Pheromones

This is a hypothesis not tested rigorously to date (Kerr 1994). It refers to the releasing of pheromones during the flight back to the resource from the nest, creating a sort of tunnel filled with pheromones that recruits follow as they fly to the food.

12.6 Effect of Internal Information on Communication Systems

The decision to continue or to stop visiting a resource depends on a balance between external and internal information. However, the food recruitment process, as studied until recently, only considered the information from the scout bees and the nutritional needs of the colony to describe the phenomenon, without considering the internal status of recruits. In fact, the influence of internal factors, such as age and experience, has been little studied in meliponines. However, we do know that there are several behavioral stages that scouts and recruits go through, depending upon their experience with resources previously visited. These experiences in turn largely determine the effect that recruitment information will exert upon foragers (Biesmeijer and de Vries 2001). More detailed investigations revealed that naïve bees follow most of the information conveyed by scouts, contrary to experienced bees, which only need an indication that the resource is available once again (Biesmeijer et al. 1998). Other internal sources of information, such as individual's hormone levels, genetic load and experience, affect decisions about what foragers do and where and when to collect resources (Biesmeijer et al. 1998; Robinson 1998; Johnson et al. 2002). The genetic variability among individuals within a colony may give rise to different preferences: some honey bees have a tendency to collect pollen while others prefer nectar (Robinson and Page 1989; Page et al. 1995). Thus food recruitment information may have different influences on the recipients.

12.7 Efficiency and Accuracy of Communication Systems

The purpose of the recruitment systems is to concentrate foragers into a profitable resource trying to bring the majority of recruits to the exact site, preventing their spread in areas where there may be no resources to exploit (Sánchez et al. 2004). To achieve this goal, communication between individuals must be efficient. Efficiency in the context of communication may be defined as the amount of time and energy that explorers use to be "understood" by recruits. The cost of communication should therefore be much less than the energy gained by retrieving the resource, i.e., it must be profitable to communicate. The accuracy of the communication systems is part of their efficiency, and can be defined as the ability of recruits to choose the resource over other non-communicated alternatives (Sánchez et al. 2004). Choosing only one option is therefore the end result of the transfer of information made through the communication systems. Evaluating the accuracy is thus a practical way to measure the adaptation of communication systems in evolutionary time (Towne and Gould 1988).

12.8 Concluding Remarks

Previous studies on the accuracy of the communication system of *A. mellifera* focused on the waggle dance, in an attempt to find an adaptive explanation of this behavior in relation to the size of resource patches that *A. mellifera* foragers visit and their distribution in time and space (Towne and Gould 1988; Weidenmuller and Seeley 1999). However, we now know that additional factors, such as social facilitation, are an essential part of bee foraging communication systems. In fact, more recent studies with stingless bees have revealed high accuracy, even greater than that observed in *A. mellifera*, where bees are allowed to use all means and modalities of communication (Schmidt et al. 2003; Sánchez et al. 2004). However, communication mechanisms are not the only factors that affect accuracy. There is evidence that experience changes the decision making in bees (Sánchez et al. 2007) inexperienced bees being more accurate than experienced ones. Thus, it seems to be more appropriate to study recruitment systems from a multimodal perspective that incorporates information about individual forager experiences to understand the evolution of communication in highly social bee species.

The characteristics of the pot-honey, the pot pollen, and the cerumen the colonies generate are the result of decisions made by the foragers and the resources within the flight range of foragers. For some species that are highly efficient at recruiting nestmates, like *S. mexicana* (Sánchez et al. 2004), it is expected that the pot-honey they produce is less nectar-diverse than that produced by a less efficient bee, like *Tetragonisca angustula* (Aguilar et al. 2005), provided they occur in the same spot. Pot-honey characteristics may thus be inherently different between stingless bee species depending upon the specific recruitment mechanisms used by each bee species.

In this chapter, we briefly explained some of the processes involved in the organization of the foragers, which are the responsible for bringing resources to the colony. Those resources become the goods that beekeepers obtain from their colonies and that make stingless bees so appreciated by rural farmers, their families and until recently considered a delicacy in many international *cuisines*.

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Chapter 13
On the Diversity of Foraging-Related Traits in Stingless Bees

Michael Hrncir and Camila Maia-Silva

13.1 Introduction

When thinking about bees and flowers, frequently an image of a balmy spring-meadow where honey bees, and sometimes maybe a bumble bee, peacefully buzz from flower to flower almost automatically pops up in our minds. Yet, as so often, nature is much more realistic than our soft-focus-lens imagination, for there is tough competition for available food in the insects' world. Thus, our romantic summer-meadow is far from being an amicable place, but rather resembles a free cold buffet at which all invited and uninvited guests, each one equipped with his/her particular little vicious tricks and strategies, struggle to get the major portion.

Due to the rich diversity of both flowering plants and flower-visiting insects, the tropics have been an ideal evolutionary playground to develop a spectacular diversity of plant–insect, plant–plant, and insect–insect interactions, governed by the continuous struggle for survival and successful reproduction. Plants, on the one hand, have evolved a fascinating variety of floral shapes, flowering traits, and phenological strategies in order to prevail in the inter- and intraspecific competition for pollinators (Bawa 1983; Frankie et al. 1983; Waser 1983; Caruso 2000). Flower-visiting insects, on the other hand, have developed a no less impressive diversity of strategies and mechanisms aiming at maximising the exploitation of floral foraging bonanzas (Johnson 1983; Roubik 1989; Goulson 1999).

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In virtually all tropical habitats, the most abundant flower visitors are bees, in particular the eusocial corbiculate bees: the stingless bees (Apidae, Meliponini), bumble bees (Apidae, Bombini), and honey bees (Apidae, Apini) (Roubik 1989; Bawa 1990; Biesmeijer and Slaa 2006). In contrast to solitary insects, which collect food for their individual and direct fitness, foragers of social insect colonies gather food to guarantee the successful rearing of the brood and to satisfy the energetic demands of all non-foraging colony members (Wilson 1971; Michener 1974; Jarau and Hrncir 2009). The survival of a bee colony, therefore, largely depends upon the success of the foragers in collecting carbohydrates (usually nectar) and proteins (usually pollen). Both these food items are stored within the nest to insure a constant food supply, thus preventing potentially fatal colony-weakening during periods of resource scarcity.

Most stingless bees are generalist foragers, and even those species with a relatively low niche breath usually collect at a wide array of food plants (Wilms et al. 1996; Ramalho 2004; Biesmeijer and Slaa 2006). Thus, and due to the fact that tropical habitats are frequently shared by several dozen meliponine species, diet overlap in terms of food sources used is considerable. The generalised utilisation of common resources among stingless bees results in both interference and scramble competition between species, which reduces not only the foraging efficiency at food patches but also diminishes the pollen and nectar harvest of colonies (Johnson 1983; Johnson and Hubbell 1974; Roubik 1980; Roubik et al. 1986; Wilms and Wiechers 1997; Biesmeijer et al. 1999a; Nagamitsu and Inoue 2005; Maia-Silva et al. 2010a). Thus, selective pressure to maximise food collection led to the evolution of a rich variety of foraging strategies among meliponine bees that differ according to variation in different foraging-related traits, among them morphology, foraging strategy, aggressiveness, and recruitment efficiency (Lindauer and Kerr 1958; Johnson 1983; Roubik 1989; Biesmeijer et al. 1999a; Biesmeijer and Slaa 2004; Nieh 2004; Willmer and Stone 2004; Nagamitsu and Inoue 2005; Barth et al. 2008; Hrncir 2009; Jarau 2009). With the present chapter, we want to give a brief overview of some of this diversity found among stingless bees shaped by the competition for food.

13.2 Food Niches

If we want to understand the diet breath of stingless bees, why they collect at particular plant species while ignoring others, we need to differentiate between a species' fundamental food niche and its realised food niche (Biesmeijer and Slaa 2006). The fundamental niche, on the one hand, is the ecological niche occupied by a species in the absence of competitors. Its breath is determined by both the morphological and physiological characteristics of the respective organism. A species' realised niche, on the other hand, is determined through the interactions with other organisms and, thus, depends on the competitor-community of the respective habitat. In the following, we discuss some morphological traits, tongue length, body colour, and size, which putatively play a major role for the separation of fundamental food niches among stingless bees. Further, concerning the realised food niche, we discuss how differences in foraging strategy with regard to aggression, recruitment ability, and recruitment precision may influence dominance relationships at a feeding site and, thus, the partitioning of resources.

13.3 The Fundamental Food Niche: Tongue Length as Predictor of Flower Choice

A major trait for the segregation of stingless bee species in order to reduce competition for food is their morphology. At least since Charles Darwin (1859) it has become clear that the body shape of a bee species is adapted to the plants at which it collects floral resources. In "The Origin of Species" (1859), Darwin wrote: "The tubes of the corollas of the common red and incarnate clovers (*Trifolium pratense* and *incarnatum*) do not on a hasty glance appear to differ in length; yet the hive-bee [honey bee; authors' note] can easily suck the nectar out of the incarnate clover, but not out of the common red clover, which is visited by humble-bees [bumble bees; authors' note] alone; so that whole fields of the red clover offer in vain an abundant supply of precious nectar to the hive-bee". More recent, detailed studies investigating possible correlations between bee morphology and flower choice corroborate Darwin's observations indicating in both stingless bees and bumble bees a morphological matching between tongue length and corolla depth of the preferentially visited flowers (Heinrich 1976; Pleasants 1983; Harder 1985; Johnson 1986; Nagamitsu and Inoue 1998) (Fig. 13.1). Yet, as demonstrated for bumble bees, the relationship between glossa length and corolla depth is not a straight one: long-tongued bees are able to collect nectar at flowers with both long and short corollas, whereas short-tongued species are restricted to shallow flowers. Consequently, species with a long glossa, hypothetically, have access to nectar in a greater diversity of food plants (larger fundamental food niche breath) than those with a short glossa (Heinrich 1976; Harder 1985; Johnson 1986).

Increasing corolla depth raises the energetic costs of foraging due to an increase in probing time. Probing time comprises, in essence, two components: access time, which increases linearly with corolla depth, and ingestion time, which increases with corolla depth only in those flowers that are deeper than the bee's glossa due to a reduced nectar uptake rate (Harder 1983, 1985). Thus, given that bee species with long tongues have the choice to collect nectar from flowers with both shallow and long corollas, why should they bother feeding at deep flowers, thereby unnecessarily increasing their foraging costs? In an investigation of 13 bumble bee-visited plant species, Harder (1985) demonstrated that the average 12-h sugar production was positively correlated with corolla depth. This elevated offer of sugar, and, consequently, energetic gain, putatively is the crucial incentive for bees to visit deep-flower plants as long as the net energetic profit of nectar collection remains positive. Thus, when available, bees should preferentially feed from flowers with corollas approximately as deep as their glossae (Harder 1985).

The high sugar reward of several deep flowers certainly is interesting for most nectar-feeding animals, and several species evolved strategies to circumvent the elevated energetic costs associated with probing time. Several bee species, for

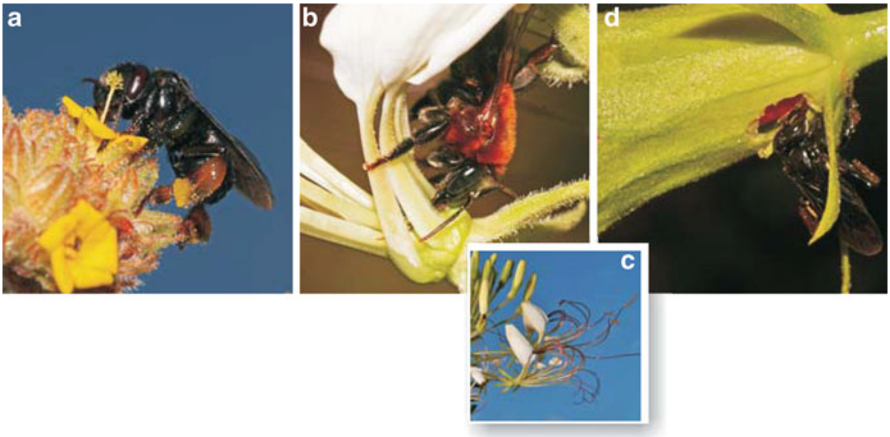

Fig. 13.1 Bee morphology, nectar feeding, and illegitimate flower-visits. Since floral morphology determines the accessibility to floral resources, stingless bees with different tongue length should specialise on different plant species. (**a**) Example of bee tongue-flower-matching: *Trigona spinipes* collecting nectar at *Waltheria rotundifolia* (Malvaceae). (**b**) Example of an illegitimate flower-visit: *Melipona subnitida* collecting nectar at *Tarenaya spinosa* (Capparaceae), which is pollinated by bats. (**c**) Flowers of *Tarenaya spinosa*: note the protruding stamina. (**d**) Example of nectar robbing: *Trigona spinipes* collecting nectar through a hole at the flower-base of *Hibiscus* sp. (Malvaceae). Photos: M. Hrncir

instance, easily enter the flowers designed for larger animals, such as bats or humming birds, without even getting anywhere close to the plant's reproductive units (Heard 1999) (Fig. 13.1). The extremists among these illegitimate flower-visitors are bees who steal nectar and pollen by entering the flowers through piercing or biting (Wille 1963; Inouye 1980; Roubik 1982) (Fig. 13.1). Among the Meliponini, species of the genus *Trigona* have brought this larceny-technique to perfection. Through goal-directed mass-recruitment, these bees are able to activate a large number of nestmates to profitable food patches and, subsequently, defend them against other flower-visitors. Thus, after perforating a flower, and recruiting additional foragers to the food source, the bees aggressively dominate the flower patch, repelling other bees or even hummingbirds through joint attacks. The detrimental effect of these robbers for the plants, therefore, is not so much the damaging of the floral structures, but the fact that they prevent potentially effective pollinators from visiting the flower (Roubik 1982; Heard 1999).

13.4 The Fundamental Food Niche: Body Colour, Body Size, and Thermal Tolerance

In addition to the, since Darwin well-established, relation between flower morphology and bee tongues, two morphological traits, related to thermal tolerance, are considered responsible for the spatio-temporal separation of niches among bee species: body size and colouration (Biesmeijer et al. 1999a, b; Pereboom and Biesmeijer 2003).

Tropical and subtropical bees, such as the Meliponini, are constrained by high ambient temperatures and heat production when foraging (Heinrich 1993; Biesmeijer et al. 1999a; Pereboom and Biesmeijer 2003). Due to the production of excess temperature when flying, many bees are exposed to the danger of overheating, some even operating close to their lethal limit. In full sunlight, generally, small bees heat up and cool down more rapidly than large bees (Fig. 13.2), but, in contrast to large bees, they will not attain excessively high body temperatures due to an elevated convective heat loss (Digby 1955; Pereboom and Biesmeijer 2003) (Fig. 13.2). Large species, therefore, run a higher risk of overheating than small species when foraging in sunshine. Here, body coloration comes into play. Physically, temperature excess and overheating are proportional to absorptivity (radiation absorbed by an object). Consequently, since absorptivity is lower for light than for dark colours (pale-coloured insects: 63–86%; dark-coloured insects: 71–117%[1]; Digby 1955), pale-coloured bees heat up more slowly in full sunlight than dark-coloured bees (Digby 1955; Pereboom and Biesmeijer 2003) (Fig. 13.2).

Stingless bees show both a spatial and temporal segregation concerning sunlit flower-patches in compliance with the thermal characteristics assigned to body size and colouration (Fig. 13.3). Meliponine species of similar size, but differing in body colour, partition patches of the same floral resource according to sunlight incidence.[2] In consequence of differential evaporation, inter-patch differences in illumination result in more concentrated nectar in sunlit flower patches as compared to shaded patches (Willmer and Corbet 1981; Biesmeijer et al. 1999a, b). Consequently, light-coloured Meliponini, which favour sunlit patches, collect more concentrated nectar from the same plant species and at the same time of day as do dark-coloured species that prefer the shaded patches (Biesmeijer et al. 1999b) (Fig. 13.4).

Concerning the temporal partitioning of floral resources among bee species, it has been repeatedly demonstrated that large Meliponini start foraging earlier during the day than smaller species (Fig. 13.5). The first stingless bees to initiate foraging early in the morning are species of the genus *Melipona*, some of which start their activity even before sunrise and at low ambient temperatures (de Bruijn and Sommeijer 1997; Pereboom and Biesmeijer 2003; Teixiera and Campos 2005; Maia-Silva et al. 2010a, b). Their capacity to fly at low temperatures is putatively related to their larger body size as compared to other stingless bee species. Due to their elevated mass of thoracic flight muscles (responsible for heat production),

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[1] The explanation for this apparent absorptivity in excess of 100% probably lies in the site of absorption. Heat produced is carried away by conduction and convection to the air, and by conduction to the underlying body of the insect and to the other cooling surfaces (radiation being very slight). Where the surface is highly absorbing, the heat is produced at the surface where it will readily be carried away; but where the surface absorbs little of the heat, more radiation will be available for absorption throughout the thickness of the thorax. In this case, as cooling is only at the outer surface, the inside will be hotter than the outside" (Digby 1955, pp 287–288).

[2] In an experimental study on the foraging choice of the sympatrically occurring dark-coloured *Melipona costaricensis* (former: *M. fasciata*) and light-coloured *M. beecheii*, the dark species clearly preferred shaded food patches and avoided sunlit ones (Biesmeijer et al. 1999a) (Fig. 13.3).

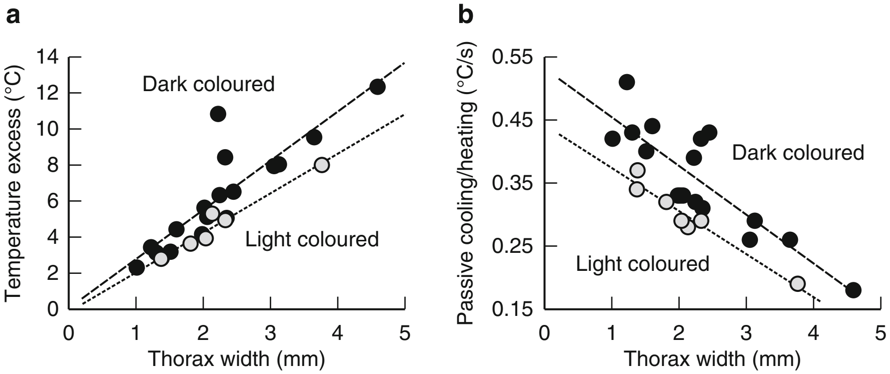

Fig. 13.2 The importance of body size and colouration for heat gain and heat loss of stingless bee foragers. Scatter plots showing the correlation between body temperature (thorax width) and temperature excess (maximum difference between thoracic and ambient temperature) (**a**) as well as passive cooling/heating (cooling constant *K*) (**b**) of 24 species of stingless bees. Light-coloured bees (*grey-filled circles*) have a lower temperature excess and cool down (and warm up) less rapidly than dark bees (*black-filled circles*) of similar size. *Dashed lines* indicate linear regressions for light-coloured and dark-coloured bees (data from Pereboom and Biesmeijer 2003)

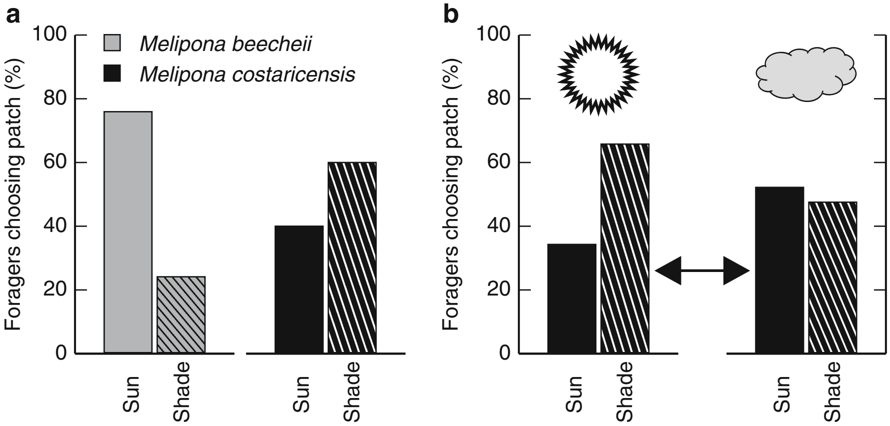

Fig. 13.3 Spatial niche differentiation among stingless bee species differing in body colouration. (**a**) Under clear sky-conditions, foragers of the light-coloured *Melipona beecheii* (*grey bars*) preferentially collect at sunlit patches whereas the dark-coloured *M. costaricensis* (*black bars*) prefer food patches in the shade. (**b**) Under changing weather conditions, foragers of *M. costaricensis* react immediately with respect to their patch preference in response to switches from sunny to cloudy weather or vice versa (data from Biesmeijer et al. 1999a)

large species are capable of attaining ideal flight temperatures even at low ambient temperatures (Heinrich 1993), and can initiate foraging long before the small species warmed up sufficiently. Concerning the onset of flight activity, body colouration might play a decisive role for smaller species, since dark-coloured bees absorb thermal radiation more efficiently (Digby 1955) and, consequently, heat up quicker than light-coloured species (Fig. 13.5).

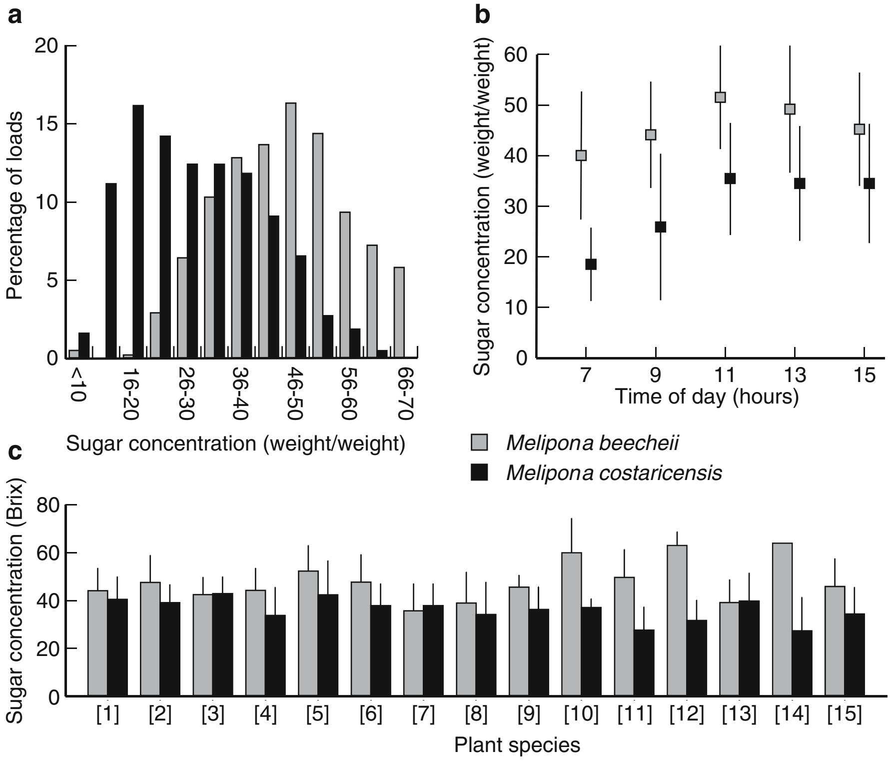

Fig. 13.4 Sugar concentration of nectars collected by stingless bee species differing in body colouration. Light-coloured *Melipona beecheii* (*grey-filled bars* and *squares*) collect nectars of significantly higher sugar concentration than dark-coloured *M. costaricensis* (*black-filled bars* and *squares*). (**a**) Percentage of foragers returning with loads of the respective sugar concentration. (**b**) Variation of sugar concentration (mean ± 1 SD) of nectar collected in the course of a day. (**c**) Sugar concentration (mean + 1 SD) of nectar of different botanic origin obtained from foragers at the nest entrance. [1] *Oyedaea verbesinoides* (Asteraceae); [2] *Vernonia patens* (Asteraceae); [3] *Bidens squarrosa* (Asteraceae); [4] Type 11; [5] cf. *Heliocarpus* (Malvaceae); [6] *Hyptis capitata* (Lamiaceae); [7] *Serjania* sp. (Sapindaceae); [8] *Mikania micrantha* (Asteraceae); [9] *Bravaisia integerrima* (Acanthaceae); [10] *Schlegelia parviflora* (Schlegeliaceae); [11] cf. *Celtis* (Cannabaceae); [12] Type 9; [13] Type 16; [14] Type 42; [15] Type 50 (data from Biesmeijer et al. 1999b). Photos: M. Hrncir

13.5 The Realised Food Niche: Aggression and Dominance at a Feeding Site

Stingless bee colonies are, in essence, sessile. Consequently, both the food sources available in space and time and the presence of potential competitors are determined by the nest's location. In bee assemblages, competition for food putatively is strongest among coexisting colonies of the same species and among species of the same genus, which tend to be similar in body size, colony size, and foraging strategy, and, therefore, tend to have similar fundamental food niches (Biesmeijer and Slaa 2006).

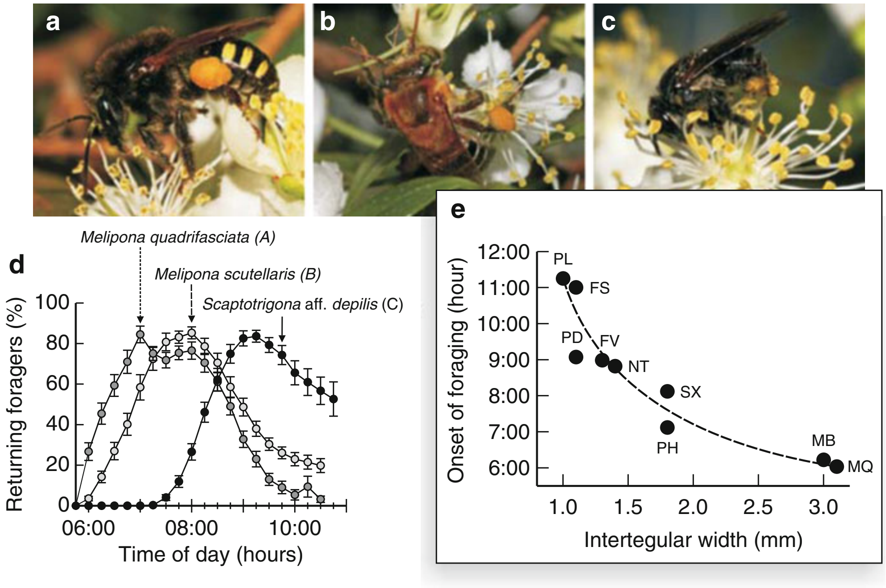

Fig. 13.5 Temporal niche differentiation among stingless bee species differing in body size and colouration. (**a**–**d**) Foraging of big, dark-coloured *Melipona quadrifasicata* (**a**), big, pale-coloured *M. scutellaris* (**b**), and small, dark-coloured *Scaptotrigona* aff. *depilis* (**c**) at mass-flowering *Eugenia uniflora*. (**d**) Onset, maximum, and end of the foraging processes are influenced by body size and colouration of the respective bee species. Data collected in August 2009 at the campus of the University of São Paulo in Ribeirão Preto, Brazil (given are the proportions of bees returning to colonies with pollen loads relative to the maximum number of foragers; data are presented as mean ± 1 SD of 12 observations per species; CMS and MH, unpublished data). (**e**) Onset of foraging in nine stingless bee species differing in body size (given as intertegular width); MQ, *Melipona quadrifasciata*; MB, *Melipona bicolor*, PH, *Partamona helleri*; SX, *Scaptotrigona xanthotricha*; NT, *Nannotrigona testaceicornis*; PD, *Plebeia droryana*; FV, *Frieseomelitta varia*; FS, *Friesella schrottkyi*; PL, *Plebeia lucii*. Note earlier foraging start of dark-coloured PH compared to the similar-sized, light-coloured SX (data from Teixiera and Campos 2005). Photos: M. Hrncir

In these cases, common resources might be shared either through spatio-temporal differences in foraging activity among congeneric species (see above) or through scramble competition.

Consistent with the idea of limiting similarity (MacArthur and Levins 1967), eusocial bee assemblages in the tropics tend to consist largely of species from different genera. Even so, food niches overlap, and there is strong association among several coexisting taxa with respect to food sources used (Biesmeijer and Slaa 2006). Here, differences in foraging strategies and underlying recruitment mechanisms between different genera might be important factors concerning the partitioning of common resources.

In stingless bees, foraging strategies can be described in terms of three basic foraging traits: recruitment ability (solitary or group foraging), individual aggressiveness (present or absent), and local enhancement in heterospecific encounters (attraction or avoidance) (Biesmeijer and Slaa 2004). Among the possible combinations of these traits, a highly successful strategy is aggressive group foraging, as found in several *Trigona* and *Oxytrigona* species (Nagamitsu and Inoue 1997; Johnson 1983; Slaa 2003). These mass-recruiting aggressive species form dense forager groups through local enhancement, and attack everything at or near the exploited food patch. Consequently, these bees "extirpate" less aggressive group foragers or solitary foraging species at the food patch, and, thus, monopolise clumped and rich resources (Johnson and Hubbell 1974, 1975; Johnson 1983; Biesmeijer and Slaa 2004; Lichtenberg et al. 2010). However, due to a low independent scouting activity, aggressive mass-recruiters have a limited capacity of discovering new food sources or even neighbouring food patches independently (Hubbell and Johnson 1978; Biesmeijer and Slaa 2004).

Although aggressiveness can lead to dominance at a food patch, it should not be used as a direct measure for dominance. Rather, dominance should be interpreted as the suppression or exclusion of one species or individual by another (Johnson and Hubbell 1974; Lichtenberg et al. 2010). In solitarily foraging animals, like many vertebrates, larger and stronger species, or individuals within a species, tend to dominate at a feeding site. In social insects, however, the strength often lies in numbers. When a large group of foragers of a single colony arrives at a feeding site, other species are often at a loss due to the sheer fact that they cannot find a free spot to land and feed (Johnson 1983; Biesmeijer et al. 1999a; Hrncir 2009; Lichtenberg et al. 2010). Consequently, non-aggressive mass-recruiters, such as *Scaptotrigona*, *Partamona*, and some *Trigona* species, are able to numerically dominate rich clumped patches, excluding other species even without aggressive interactions[3] (Johnson 1983; Biesmeijer and Slaa 2004; Lichtenberg et al. 2010). Scrambler species that forage individually or in small groups, therefore, would need to move to less disputed, often poorer feeding sites or, alternatively, arrive at rich patches ahead of the mass-recruiting species.

13.6 The Realised Food Niche: First Come First Served

Many medium-sized, unaggressive Meliponini share similar floral resources (Biesmeijer and Slaa 2006) and, therefore, experience scramble competition when foraging. Scramble competition among colonies is highest at rich clumped food sources, such as mass flowering plants (Biesmeijer and Slaa 2006), which produce

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[3] Johnson (1983) described a situation in which two non-aggressive mass-recruiters, *Partamona orizabaensis* (as *Trigona testacea*) and *Scaptotrigona mexicana* (as *Trigona mexicana*), numerically dominated the inflorescences of a *Bactris* palm tree. Although both these scrambler species did not exclude each other from the food patch, insinuators (small, unaggressive, and mostly solitarily foraging bees, such as many *Plebeia* species) did not find space to land at the inflorescences. More surprisingly, even an aggressive group-foraging species, *Trigona silvestriana*, was competitively outnumbered by the scrambling mass of bees and, consequently, left the patch (Johnson 1983).

a large amount of new flowers each day over a short period of time (“big-bang” or “mass-flowering” strategy) (Augspurger 1980; Bawa 1983). Within plant populations, in general, mass-flowering individuals of a species bloom synchronously. Slight differences in the onset of flowering among individuals result in an extended blooming period on the population level (Bawa 1983). Mass-flowering plants, therefore, offer a great opportunity for colonies to hoard large amounts of food within a short period of time, and represent the predominant source of both nectar and pollen for stingless bees, contributing up to 90% of the annual nutritional input into the colonies (Wilms et al. 1996; Wilms and Wiechers 1997; Ramalho 2004).

Fully grown mass-flowering trees are usually too big to be monopolised by a single colony of mass-recruiting bees (aggressive or unaggressive). Individual or group-foraging scramblers, consequently, can exploit such kind of resource virtually undisturbed (Biesmeijer and Slaa 2006). The situation, however, might be different with small mass-flowering trees or shrubs, which can be easily defended by aggressive colonies (Johnson and Hubbell 1975) or numerically dominated by non-aggressive mass-recruiters (Johnson 1983). Here, in order to be able to profit from such foraging bonanzas, non-aggressive scramblers that forage individually or in small groups should get to the food patch prior to others, or as long as the population density of potential competitors is low.

An important trait that allows bees to arrive at a food patch ahead of competitors is their capability to learn both the position of a potential collecting site and the time of resource availability (Johnson 1983; Biesmeijer and Slaa 2004; Schorkopf et al. 2004; Murphy and Breed 2008). Food-patch-experienced foragers, consequently, arrive at familiar feeding sites far quicker than inexperienced bees, which still have to search for it. So far, however, few studies investigated the time–place–memory of stingless bees (Biesmeijer and Slaa 2004). An important topic for future research, therefore, is to investigate whether the capacity to memorise the spatio-temporal characteristics of food sources differs among species with fundamentally different foraging strategies (aggressive mass-recruiters, unaggressive mass-recruiters, group-foraging scramblers, solitary scramblers, insinuators).

For group-foraging bees, a second parameter important for the efficient exploitation of resources is recruitment velocity (Jarau et al. 2003). Here, we have to distinguish, in essence, between mass-recruiting species (aggressive and unaggressive) and species that forage in small groups. The strategy of mass-recruiting species relies on the rapid mobilisation of a huge number of foragers to one particular feeding site. In aggressive mass-recruiters, the overwhelming multitude of recruits extirpates other species at a feeding site and, subsequently, defends this patch against other aggressive colonies (Hubbell and Johnson 1978; Johnson 1983). Through similar fast and goal-oriented recruitment, unaggressive mass-recruiters are able to dominate food patches numerically, thereby diminishing exploitative competition by other scramblers or even keeping off aggressive species (see footnote 3). In contrast to mass-recruiters, the strategy of unaggressive scrambler colonies that forage in small groups, such as *Melipona* or *Nannotrigona* species, relies on a quick mobilisation of all available recruits, yet without indicating the position of a particular food patch. Due to this lack of vector information, the foraging force spreads out over the surroundings to find any patch that carries the odour that has been brought

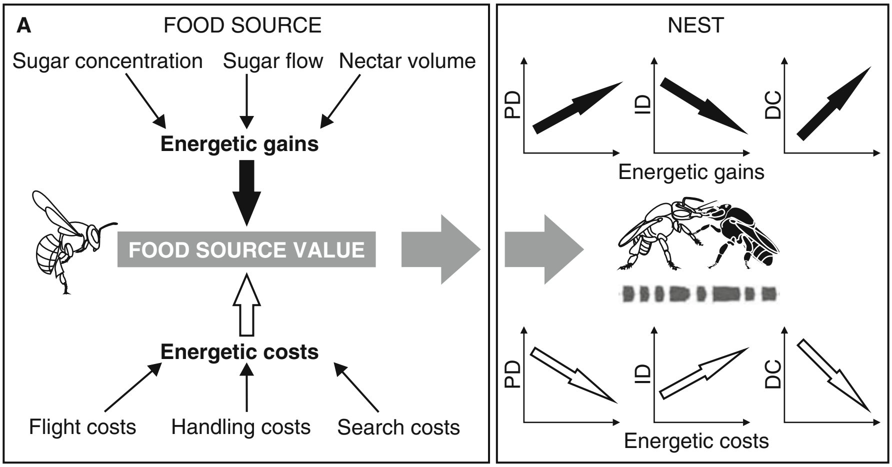

Fig. 13.6 Activation signals of stingless bees. The nest-internal recruitment signals of stingless bees, the thoracic vibrations, are directed at the fast activation of additional foragers. The temporal pattern of the foragers' pulsed vibrations is influenced by the value of the visited food source. Increasing energetic gains at the food patch result in longer pulses (PD), shorter intervals (ID), and, consequently, an increasing duty cycle (DC = PD/[PD + ID]). Increasing energetic costs, by contrast, result in shorter pulses, longer intervals, and a decreasing duty cycle (figure adapted from Hrncir 2009)

back to the colony by successful scouts (Hubbell and Johnson 1978; Jarau et al. 2000; Slaa 2003; Biesmeijer and Slaa 2006; Hrncir 2009). Thus, when excluded from one feeding site by a mass-recruiting species (aggressive or unaggressive), the colonies are still able to profit from a rich food source by switching their foraging focus to less disputed patches (Hubbell and Johnson 1978; Johnson 1983; Biesmeijer and Slaa 2006).

Based on the differences in necessity to guide the foraging force to a specific food patch, recruitment strategies should differ between mass-recruiters and scramblers that forage in small groups with respect to the information about the exact position of a feeding site (important for mass-recruiters, useless for unaggressive scramblers) but not necessarily concerning the velocity of mobilising the foraging force. So far, few meliponine species have been analysed in detail concerning their recruitment strategies. In both mass-recruiters (*Scaptotrigona* aff. *depilis*) and unaggressive scramblers that forage in small groups (*Melipona* spp., *Nannotrigona testaceicornis*), the temporal pattern of thoracic vibrations generated by recruiting scouts within the nest is related to the profitability of a food source (Fig. 13.6). These vibrations, putatively, are an alerting signal, activating the foraging force (Hrncir 2009). Although these nest-internal recruitment signals are similar for mass-recruiters and small-group-scramblers, only the mass-recruiting species have been shown to be able to guide recruits to a specific food patch (aggressive mass-recruiters: *Trigona corvina*, *T. hyalinata*, *T. spinipes*; unaggressive mass-recruiters: *Geotrigona mombuca*, *Scaptotrigona* aff. *depilis*, *S. postica*, *S. mexicana*, *Trigona recursa*). In contrast to honey bees, which indicate the position of a feeding site through their waggle dance (Grüter and Farina 2009), mass-recruiting stingless bees achieve this goal-directed recruitment through species- or even colony-specific pheromone trails or pheromone marks at and near the feeding site (Jarau 2009; Stangler et al. 2009; Jarau et al. 2010; Schorkopf et al. 2011).

13.7 Concluding Remarks

Stingless bee pot-honey is a valuable product with a long tradition of harvest and consumption (Camargo and Posey 1990; Crane 1999). A large diversity of stingless bee species is kept by meliponiculturists all over Latin America to provide this precious gold. The differences found among meliponine honeys with respect to their physiochemical composition, sugar content, and floral origin depend not only on the geographic region where it has been harvested but also on the stingless bee species being used for honey production (Barth 1989; Souza et al. 2006; see related chapters in this book).

Tropical habitats are frequently shared by several dozen meliponine species. Consequently, diet overlap in terms of food sources used is considerable. The selective pressure to maximise food collection led to the evolution of a rich variety of foraging-related traits among the stingless bees. In our chapter, we wanted to give a brief overview of this diversity, discussing the importance of morphological characteristics (tongue length, body colour, and body size) for the separation of fundamental food niches among the Meliponini. In contrast to a species' fundamental niche, which is delimited by the morphological and physiological characteristics of an organism, the food niche realised by a species is determined through the interactions with other organisms that share the same fundamental food niche. Here, differences in foraging strategy among the stingless bees with regard to aggression, recruitment ability, and recruitment precision influence dominance relationships at a feeding site and, thus, are important factors concerning the partitioning of resources.

To be sure, our overview is far from being complete, since our description of the foraging strategies used by stingless bees almost entirely omitted the unaggressive solitary foragers, often very small species that remain competitive through an "insinuation strategy" (Johnson 1983). These insinuators fly off a food patch when threatened by dominant species, yet they quickly return to the same site or nearby flowers and continue feeding as if indifferent to the aggressors (Biesmeijer and Slaa 2006). Several of these insinuator species, like *Tetragonisca angustula* or *Frieseomelitta varia*, are bees important for meliponiculture (Souza et al. 2006). Yet, knowledge about the foraging strategies of the small Meliponini is rather poor, probably because the large bees, like *Melipona* spp., and the aggressive ones, like *Trigona* spp., are more attractive to scientists.

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Part II
Stingless Bees in Culture, Traditions and Environment

Chapter 14
Stingless Bees: A Historical Perspective

Richard Jones

This chapter is dedicated to the memory of Dr. Eva Crane who, in over 50 years of worldwide research, produced the seminal texts on the history of beekeeping and honey hunting. Everyone advancing these studies today owes her a tremendous debt.

14.1 Introduction

Stingless bees are native to all tropical regions although they closely resemble another familiar honey-making bee, *Apis*, which ranges naturally through most tropical and temperate regions of the Old World. The honey bee, *Apis mellifera*, was introduced into many areas, especially in the New World and on islands, by European explorers and settlers in the sixteenth century in the Americas, and as late as the nineteenth century in Indoaustralia. The main distribution of stingless bees in historical times has been described by Kerr and Maule (1964) and summarised by Michener (2007, and in the present book).

It is safe to assume that the connection between bees and man began then when the first honey hunters ripped open nests to release the sweet golden treasure of honey and also perhaps to benefit from the protein provided by the bee brood. Between 15,000 and 10,000 years ago, when people first inhabited the New World, they exploited its tropical honey-making bees. Far before this, in Africa, Asia, and Australia, there were humans taking honey from wild bees and this can be seen in some of the earliest records of human culture (Crane 1999).

Until the introduction to the Americas of the honey bee, *Apis mellifera*, stingless bees were the source of cerumen and honey and therefore played a significant role in native civilisations. Honey bees later provided a much bigger return for the effort of management, but pot-honey is undoubtedly a legacy of stingless bees.

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14.2 Bee Hunting to Beekeeping

Honey hunters were able to harvest the honey stores of bees by tolerating their defensive biting or stinging, using tools to access the native bee nests in tree trunks or in the ground, or even using plants that diminish their aggressiveness, e.g. the Andaman islanders' use of tranquilising plants to harvest nests of giant honey bees (Crane 1990), while the Kayapó Indians of Brazil employ crushed toxic leaves to extract honey from some of the fiercely biting stingless bees (Posey and Camargo 1985). It is but a short evolutionary step from honey hunting to beekeeping. This involves providing a suitable nest site in a location that is easily accessible for exploitation. So a hollow tree becomes a hollow log; the log is cut in such a way that it can be opened and resealed by the owner and thus beekeeping is born. This first step certainly occurred in the area dominated by the Maya Civilisation, between 10° and 23°N in Mesoamerica, but remained comparatively rare in the rest of tropical America.

In 1492 Columbus recorded that there was honey and cerumen in Cuba and Santo Domingo (Schwarz 1949). These must have been the products of stingless bees but it is not known if they were derived from kept or wild bees, although it was probably the latter. Bishop Diego de Landa writing at the time of the Spanish Conquest of Mesoamerica said: “honey was often consumed together with bee maggots” and that the honey was contained in “wax pots as large as doves' eggs” (Kent 1984).

14.3 Commercial and Cultural Importance of Honey and Cerumen

The cerumen was as important as the honey to many early Pre-Columbian societies. No stingless bee builds its nest of pure wax, but uses cerumen mixed with resin, called “cerumen” (as noted in several book chapters herein). These civilisations are famed for their treasures of gold. Indeed the legend of El Dorado—the Golden Man—impelled the Spanish as they exploited the newly discovered lands and people. The cerumen was used to cast exquisite jewellery, usually made from pure gold.

The process known as “lost wax casting” allows quite intricate objects to be sculpted in cerumen or wax. The resultant object is then surrounded by clay hardened by drying in the sun. The ball of clay was then heated so that the cerumen could drain away through vents and molten gold was poured in to take its place and thereby assume the shape of the desired object. This method was mostly used for small objects such as jewellery but artisans doing the work would require a constant and reliable supply of cerumen which would make heavy demands on honey (cerumen) hunters. Such a need might have encouraged more organised beekeeping: a simple example of the economic principle of supply and demand.

The people of South and Central America were expected to pay tribute to their European Conquerors—preferably in gold that was then taken back to the Old

World to reward those who had financed the exploratory expeditions. However, there are several records showing that for the poorer communities some of these tributes were paid in honey and cerumen (Georghiou 1955; Landa 2008).

14.4 Historical Production and Management

One of the first European travellers to report stingless bees in detail was the German Ulrich Schmidel (Crane 1999). Between 1536 and 1545 he traveled extensively in what is now called Northern Argentina. Many years later he wrote:

"An Indian goes into a wood with an axe and the first tree he comes to that has an entrance hole to a bees' nest. By boring other holes he gets five or six jugs of pure honey. These bees are small and have no sting …"

Similarly, Jesuit priest Bernabé Cobo (1892) traveled in Central America and as far as present-day Peru. In "Historia del Nuevo Mundo" (Cobo 1653) he wrote about stingless bees including: "The smallest bee is the size of a fly that breeds in wine; another is somewhat larger … neither can sting, but they burrow in the hair and in the beard".

The first reference in the literature to stingless bees in Australia was made by Dutch explorer, Abel Tasman (1603–1659), in 1648 when he noted that the indigenous people on the island now named after him (Tasmania) cut notches in some trees and used these to help them climb and gain access to individual bees' nests (Wills 1970).

A reference to the importance of cerumen is to be found in Reyne (1962) quoting a 1769 report of two and a half tonnes being exported from what is now Surinam in the year 1745. It seems likely that most of this would have been supplied from stingless bees as it is unlikely that imported *Apis mellifera* would have been established in sufficient numbers to generate this quantity of wax, but we have no certain data on that point.

The records of the amount of honey and cerumen yielded by a single nest vary considerably: one rather dubious 1657 report (Purchas 1657) tells of a nest providing "enough honey to fill a firkin"—an old barrel measurement equating to about 40 L! Goudot (1846) describing the situation in Columbia explains the seasonal cycle and that a nest harvested in April/May or October might yield 3 L of honey and 1 kg of cerumen. Many more reports are available now [see Barceló (Chap. 17) and Ocampo Rosales (Chap. 15) in this book]. Interestingly he also mentions that the honey was often sold in markets using bamboo internodes as containers.

For some tribes brood was an important food source. So the honey and brood were eaten while the wax and propolis (cerumen) that constitute the walls of the storage cells—the honey pots—were chewed and stored in soft balls. The mixture could be heated and used for a multitude of purposes, from fixing feathered flights to arrows (Stearman et al. 2008), to making toys and ceremonial objects.

Mesoamerica was the area directly affected by Mayan culture and this advanced culture certainly embraced beekeeping. The stingless bee of the Maya—*Melipona*

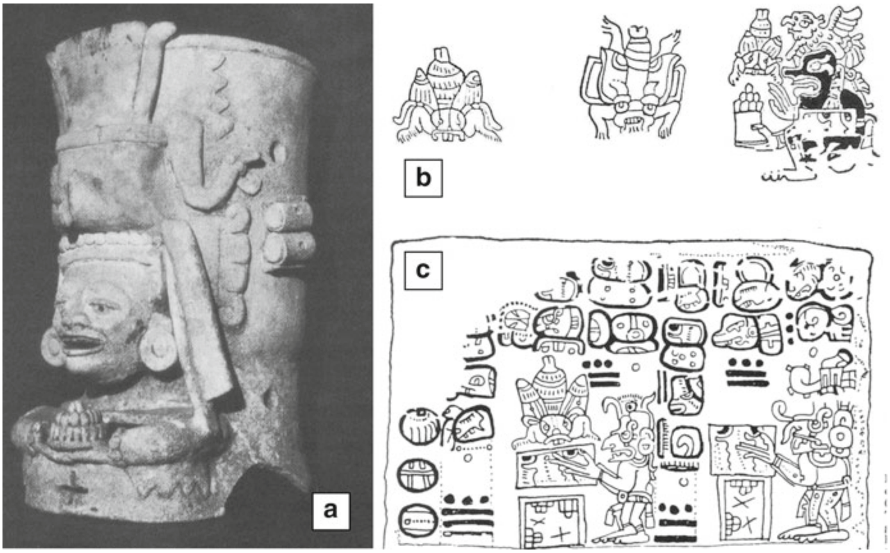

Fig. 14.1 Symbols of *Melipona beecheii* in the Mayan Tro-Cortesian Codex. (**a**) Effigy censer from Cozumel, in the shape of the descending Mayan bee god of honey Ah Mucen Cab, with brood of *M. beecheii*, in the Archaeological Museum of Yucatán, Mérida, Mexico (Darchen and Darchen 1978). (**b**) Bees icons and god-like figure on the right, holding stingless bee brood with the hands, like the Ah Mucen Cab censer. (**c**) Upper portion of page 104, the *lower row* shows two bee gods facing left (Villacorta and Villacorta 1977), each with a hive of *M. beecheii*. Itzamná grandfather god is working in summer close to the bee queen and Chaac (god of rain) fixes a honey supper (Cappas e Sousa 1995) (permission granted by the International Bee Research Association)

beecheii—was known as "colecab" or "xunan cab" (lady bee). *Melipona beecheii* is painted in the Tro-Cortesianus Codex, Museum América, in Madrid. The sacred world of this goddess bee was represented by knowledgeable priests and painters (Fig. 14.1).

Mayan codices are folding books written in Maya hieroglyphic script on paper-sheets obtained from the inner bark of wild-growing fig tree. Tro-Cortesianus is derived from the two fragments Troano, owned by the Spanish palaeographer Don Juan Tro y Ortolano (pp 22–56, 78–112), and Cortesianus (pp 1–21, 57–77), united in the Madrid Codex since 1888, after León de Rosny identified that Troano and Cortesianus were two parts of the same book (FAMSI 2012). The united manuscript is 6.7 m long with 56 leaves, and page dimensions are 12 cm × 24 cm (The University of Arizona Library. Mayan Codex facsimiles. http://www.library.arizona.edu/exhibits/mexcodex/maya.htm).

The cerumen from stingless bees is of lower quality than honey bee wax for candle making, because the resin burns and sputters, emitting some smoke. However, in 1549, 3 tonnes of honey and an amazing 277 tonnes of cerumen, known as "cera de Campeche", were paid in tribute to the conquerors and exported from Yucatan to Spain (Calkins 1974). Such production was only possible because *M. beecheii* were kept on a commercial, almost industrial, scale. This stingless bee is amenable to hive management and gives worthwhile honey yields, but the reason could be more cultural than biological (D. Roubik, personal communication).

The Nicoya peninsula in Costa Rica marks the southern limit of *M. beecheii* and, as it happens, that of Mayan influence. In the 1500s the Spanish referred to traditional hive beekeeping here, so it is likely that the design of the equipment and the necessary accompanying skills had been in existence for centuries. To this day traditional log hives can be seen hanging in the eves of houses (Imperatriz-Fonseca 1989) or, if there are ten or more hives together, sheltered in a specially constructed "A" frame structure near the house.

In Australia cerumen was also used to paint animal and human figures on rock faces. Some of these pictures of the life of the indigenous people have been dated back to 2000 BC. There are no records of any such applications in the Americas although often, similar discoveries, abilities, and cultural mores developed simultaneously, thousands of kilometres apart and without any contact whatsoever between those people concerned.

In Central America there is a musical percussion instrument, the marimba, which in its traditional form uses stingless bee cerumen to adjust the pitch and so control the sound produced from the gourd resonators that are to be found below the wooden keys. While in Australia the mouth piece of the didgeridoo was made of cerumen so as to make an airtight seal with the mouth of the player.

14.5 Recent History and Transitions

Today log hives are used, along with boards fashioned into "rational hives", in the Yucatan peninsula. They have a central flight entrance and closures at each end made from disks of wood or soft stone that can be easily cut to shape. Archaeological digs have revealed many similar stone disks, which shows that this type of hive and its associated beekeeping management techniques existed over a thousand years ago. Many of these finds have been in close proximity indicating that then, as now, some beekeeping was on a grand scale with hundreds of hives in some meliponaries (Calkins 1974). The reader is invited to see the short film "Honey for the Maya" by Buchmann (2011), to appreciate *Melipona beecheii* honey making and meliponiculture. The Maya valued cerumen as they did not use the cerumen for candles but used, instead, reed torches for lighting.

In the latter part of the twentieth century stingless beekeeping has been under threat and suffered some setbacks. Spreading urbanisation and in some regions heavy deforestation have reduced forage and potential nest sites from which the stock for beekeeping activities could be obtained.

Indiscriminate application of pesticides and general pollution have killed many colonies. However, one of the biggest problems is competition for forage. This began with the introduction of *A. mellifera* with the European settlers in the sixteenth century but was greatly exacerbated by the Africanised honey bee from 1956 onwards. Despite early demonstration of competition at flower patches between meliponines and honey bees (Roubik 1978), there is little certainty about what influence Africanised honey bees will ultimately have on native bees; what is certain is that they provide a pollination service which may benefit the native bees (Roubik and Villanueva 2009; Roubik 2000). Arrival of the Africanised honey bee also heavily affected hive bees of European varieties, with reduced yields from 15 to 2–3 L in one Brazilian apiary (Imperatriz-Fonseca 1989).

Traditional hives by definition mean that the designs, and indeed often the actual hives, have been handed down from generation to generation. On the other hand the word rational is used for a hive based on reasoning and thought after a study of the stingless bees' needs (Mariano-Filho 1910). Mariano-Filho (1910) devised a hive consisting of three-tiered boxes. However, Paulo Nogueiro-Neto in São Paulo has undertaken some of the most intuitive and constructive developments in stingless beekeeping over the last 60 years. In 1948 he designed hives for *Trigona* and *Melipona* species, and over the years he has refined the design and, from his own tireless observations, added copious information and instructions for harvesting honey, transferring nests, and dividing colonies. Much of this work has been published on various occasions but it all comes together in one seminal text book "Vida e Criação de Abelhas Indigenas Sem Ferrão" (Nogueira-Neto 1998).

Kempff Mercado (1966) in Bolivia and Nates-Parra (1978) in Colombia, for example, have also promoted rational hives. An interesting modern hive has been developed in Tobago by the University of Utrecht (Sommeijer 1999) which allows harvesting of honey without disturbance of the brood chamber.

Sadly the rapid and almost universal growth of honey bee beekeeping throughout Hispanic America, at both commercial and hobbyist levels, especially over the last 100 years, has been to the detriment of stingless bees. Traditions and the special management skills that are required are being lost almost daily. These bees evolved with the natural ecology and crops of the area and so have a valuable role to play in the pollination of those crops with all the resultant benefits in improved yields and food security. They are valuable bio-indicators of the state of the environment and provide not just honey and cerumen but also, as the nature of these products is being more deeply understood, medicaments that could provide pharmaceutical benefits where so far synthetic substitutes have failed.

Only eusocial bees store honey and pollen as a provision for the brood and for times of dearth. Properly managed and by using rational hives, the honey can be harvested from the stingless bees without damage to the colony. The quantities produced are much smaller than those produced by honey bees. The honey has a higher water content than honey bee honey and is a little more acidic but still very sweet and pleasant. Many stingless bees do not confine their foraging to nectar, pollen, and honeydew—the basis of honey bee honey. However, throughout history to the present time it has been used in its natural state as a pleasurable eating experience or as a sweetener with other food. There is evidence that the Mayan civilisation used considerable stingless bee honey for production of a fermented drink—"balché"—roughly the equivalent of mead (Crane 1975; Ocampo Rosales Chap. 15 in this book).

14.6 Value of Pot-Honey

Some of the stingless bees from Brazil were included in the song of Caetano Veloso "Mel" (http://letras.terra.com.br/caetano-veloso/44746/), honey in English (Souza 2008). In his song about honey (available in the Internet), there are no Africanised bees but the worth of three stingless bees "lambe-olhos" *Leurotrigona muelleri*, "torce cabelos" *Scaptotrigona depilis*, and "vamo-nos embora" *Lestrimelita limao*, is appreciated by the public. In Venezuela, "arica" *Melipona favosa* is present in the poem "Miel de arica" by Guillermo Jiménez Leal (T. Castro, personal communication) and in the novel Doña Bárbara (Gallegos 1976). These are bees of high value since ancient times and expanding legacy of cultural expressions.

Although the quantities produced are small (see Alves Chap. 40 in this book), pot-honey is believed to have healing qualities and plays an important role in folk medicine, particularly in South and Central America. The use in different treatments for coughs and throat infections is well known but it can also be used in fertility treatment and in combination with jungle herbs to treat fever. Preliminary research shows that the honey has many potential benefits in the treatment of ocular cataracts (Vit and Jacob 2008), besides the putative treatment of pterygium with eye drops.

The value of stingless bees is highly prized, but has been somewhat dismissed in pot-honey standards and overshadowed by the commercial honey bees for many years. Now there is a resurgence of interest in these bees and their honey (Main 2012). Efforts are being made to establish controls and standards for the honey produced (Vit et al. 2004; Souza et al. 2006) so that it can take place as a marketable product. This would give a great boost to many areas that would benefit from economic input but above all would be a clear statement of the value of stingless bees and so an important step in ensuring their conservation.

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Chapter 15
Medicinal Uses of *Melipona beecheii* Honey, by the Ancient Maya

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> *La mayor gloria que al secreto oficio de la abeja se da, a la qual los discretos deven imitar, es que todas las cosas por ella tocadas convierte en mejor de lo que son.*
>
> (La Celestina, Fernando de Rojas)
>
> *The greatest glory that is given to the secret craft of the bee, which those that are prudent must imitate, is that all things touched by it are converted into something better than they are.*
>
> (La Celestina, Fernando de Rojas)

15.1 Introduction

In the Yucatan peninsula, the bee *Melipona beecheii* was named "cab" or "kab" in the Mayan language. It was considered of such importance by the Mayan people that, after a long process of appropriation, the bees were deified and named "xunan cab," or "xunan kab." The word "xunan" means principal lady (Barrera Vázquez 1980). With this word, we perceive that the bees were docile, gentle, well born, belonging to the lineage, and, because of this last quality, direct descendants of the Mayan gods. Thus, the deity, "Ah mucen kab," was granted to the native stingless bees, so that he would take care of their nests and hives, due to the delicacy required in all the breeding and collecting activities. "Hobones" is the Mayan name for the traditional nests of bees, built within the hollow trunks of certain tropical trees that the meliponas found in the forest during their reproductive phase, and then colonized.

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Within the family lands, the bees received protection from many natural enemies that did not dare to come close to the hobones to feed on the sweet honey that the bees produced, destroying the hives and killing the larvae and adults. Also benefiting from the closeness of the hives, the Mayan families that had lodged the nests had easy access to the products that the stingless bees manufactured, honey, cerumen, and pollen, and to the benefits they provided.

The melipona honey was an especially coveted product, valued for its medicinal properties and for its ritual importance in the elaboration of beverages used in religious ceremonies. This is documented in the manuscripts carefully preserved throughout the difficult centuries of Colonial domination. The anonymous texts that deal with medical practices, the "Ritual de los Bacabes" (Arzápalo Marín 1987), the "Recetario de Indios en Lengua Maya" (Roys 1976), and the "Libros del Judío" (Barrera and Barrera Vásquez 1983), include a great number of healing incantations and prescriptions for the preparation of remedies based on the honey of the native bee *M. beecheii*, which could be used either alone, as the main ingredient, or as a vehicle for other healing products.

Many years before the Spanish conquest, honey and cerumen were important products exported to other regions of Mesoamerica, Central America, and the Caribbean. We find mention of this trade in the manuscripts known as "Relaciones Histórico-Geográficas de la Gobernación de Yucatán." This translation of quotes was made respecting the style used in the manuscripts:

> In these provinces there are not mines of any type. The profit they give are some cotton sheets and wax and honey, that is the land's trade, and in order to be valuable, they are taken to Mexico, Honduras and other parts. (Garza et al. 1983)

From a thriving industry and trade that survived three centuries of colonial exploitation, meliponiculture is now on the verge of extinction. The activity has suffered from the current economical and social pressures experienced by the Maya people and from the introduction of *Apis mellifera*. Apiculture with that bee has become a very important agroindustry in the Yucatan peninsula. It is probable that due to the medicinal properties and ritual use of the honey and other products of the native bees, meliponiculture continued in practice in a reduced scale in the backyards of Mayan homes and has barely survived.

Studies carried out by bacteriologists have proven that the honey of *Melipona beecheii* has high levels of *Bacillus* that inhibit pathogenic bacterial growth (Quezada Euán 2005; Catzin Ventura et al. 2009). This fact may contribute to its medicinal action, as well as a higher acidity compared with the honey of *Apis mellifera* (Vit et al. 2004). The hydrogen peroxide, an antibiotic found in all honeys, acts as an hypotonic medium that, dehydrates microbes or inhibits their growth (Menezes et al., Chap. 10 in present book).

We will make a brief review of some ideas that the Mayans had about the diseases, the literature where we find notes on how this honey was used as medicine, and will indicate the ways in which the Pre-Hispanic Indians used honey as well as the bee nests and brood. Finally, a description of the maladies cured with honey and its application by the "ah dzaco'ob," general medics of ancient times, will be given.

15.2 Mayan Ideas of Disease

For the Mayan people, a disease was a serious state of physical, mental, and spiritual alteration. The sick person suffered physically in an intense way, and presented mental alterations and emotional or spiritual unsteadiness. A sick man or woman was defenseless and incapable to carry out his or her everyday labors and personal, familiar, and social duties. In the emblematic book of Mayan medicine, the "Ritual de los Bacabes" (Arzápalo Marín 1987), we find that for these people, the diseases were supernatural beings, with origins in a remote mythical time, born to a mother and father in a "temazcal," the traditional steam bath, located in a selected spot of the sacred geography. They also possessed clothes, pieces of gold jewelry, and symbols, which provided them with character.

Human beings fell sick for a number of reasons; most of these had to do with the supernatural world and beings. A man that was negligent, cruel, or naughty with his family or neighbors, or with defenseless people such as youngsters or elders, was prone to anger the gods and to receive their punishment in the form of a sickness.

Dangerous places such as caves, rivers, water springs, lakes, and the forest were abodes of great energy that could affect man in a negative way. The men that dared go into these places were either owners of enough power to arrest the energy that prevailed, or carried out rituals to appease the supernatural beings and forces that prowled there.

If a person was at fault during the rituals that were due to the deities, he or she could also be chastised with a disease. The gods of Mesoamerican religions were capricious creatures that would equally bestow great luck or the worst of fortunes, pain, and maladies on a human being, despite his or her good conduct and respect to his or her obligations to them.

These were the main causes of disease and, as we proceed through the texts to see how the honey of *Melipona beecheii* helped to cure many of them, we will recognize a few of these ideas that persisted in spite of years of cultural repression during the Colonial period. More information regarding these subjects can be found in López Austin (1980) and Ocampo Rosales (2005).

Why was the honey endowed with such power to cure? For the Mayans, the energy was a force called "kinam," whose various meanings are (1) strength, robustness, rigor, and fortitude; (2) virtue, as in the stones, or herbs, etc.; and (3) venom or poison from animals, or pain caused by the poison or the ulcer, and that which is very painful (Ciudad Real 2001).

It is probable that the Maya word "kinam" derives from the word "kin," sun, which might indicate that for these people, a certain kind of power was like that of the sun, or provided from it, thus being especially strong.

The Mayans considered that the sun's power concentrated in the plants' reproductive organ, the flower, in the form of a sweet liquid, the nectar. That strength or energy was transmitted to the bee and from the insect to the honey. That is why "kab," honey, was so powerful that it was even considered as a sacred food, used in rituals.

15.3 Traditional Literature on the Use of *Melipona beecheii* Honey for Medicinal Purposes

In the Mayan literature, written in Latin characters, that has been preserved, there are a few texts that comprise medical aspects of great importance. In some, the health of Mayan populations and their unfortunate contact with the epidemics brought by the Spanish conquerors were recollected, as well as the years when the conditions were most severe. In others, the illnesses were described with their name in Mayan and, occasionally, the translation for this name was provided in Spanish. We also find very complete lists of plants, their medicinal properties, and their use against different maladies.

The main purpose that the Mayan specialists had in writing these texts was the preservation of the part of their culture that dealt with the recovery of health and the prescriptions by means of which the patients were treated. Obviously, in most of these books, the ritual parts, fundamental in the treatment carried out by the "ah dzac," and which had a deep religious background, were scarcely mentioned. To demonstrate this omission, the important collection of prayers and invocations gathered in the "Ritual de los Bacabes" (Arzápalo Marín 1987) is a complete manual of the rituals by means of which the Mayan doctors healed their sick. Characteristic of this manuscript is the use of a language that was only known to the initiated, and the description of complicated ceremonies. Here, the use of honey to heal certain diseases is recorded, but the examples are few. It is in the collection of manuscripts known as "Libro del Judío" (Barrera and Barrera Vásquez 1983) where the Mayan informants wrote widely about the use of honey as a powerful healing agent.

The "Libro del Judío" is a complex, detailed, and long compilation of several manuscripts that was accomplished by an Italian physician, Ricardo Ossado, who lived in the Yucatan Peninsula during the eighteenth century. Probably due to an acute professional curiosity, a considerable knowledge of medicine and the vegetation of the region, the Jew, as Ricardo Ossado was nicknamed, used the "ah dzaco'ob," general doctors, as informants and translators to compile many prescriptions to cure several diseases that were common among the Mayan population. His knowledge of the maladies is clear in this minute register of medical practices of the time. The manuscripts were named after the village where they were recovered and because of their characteristics, we consider that they are copies of prescriptions that were handed down from teacher to disciple since remote times; many exhibit a clear Pre-Hispanic tradition. From this extensive corpus, we extracted those prescriptions in which honey is one of the main ingredients incorporated to act effectively against an illness, particularly, virgin honey, taken directly from the honey pots inside the hives or "hobones," named "hobnil cab," honey of "hobon."

15.4 Preparation of Prescriptions

The honey of the meliponas was used for its effectiveness as a curative product, and for its religious and mythical powers. Due to its properties, honey was used to treat all kinds of diseases, prepared and dosed adequately, but always as a principal ingredient, capable of restoring a patient's lost balance. Honey appears in the prescriptions as the fundamental curative element, added to plants that were macerated, cooked, roasted, or burnt to ashes. Honey was rubbed or anointed alone, or with plants to form a paste or a liquid that was applied over the sick member, skin bruises, ulcers, wounds, on the eyes, inside the ear, or covering the region of the organ to be treated. The "ah dzac" is also advised to use the nests of certain bees or wasps to cure certain illnesses. The way it was done was to burn down the nest of the insect, extract the larvae from it, grind them, and administer all with the ashes in the form of a beverage (Roys 1976).

In the case of burnt skin, honey was applied alone. It is also used in many of the prescriptions as basis of anti-inflammatory liquids or ointments. For "chuchup calil," swollen neck:

> You take the *Malachra palmata* (Malvaceae), mallows and honey. Let them be mashed and let him drink it. Or else let him drink milk and cinnamon mixed to honey, and let a little of it be applied wherever the swelling is. (Roys 1976)

15.5 Diseases Treated with Honey of *Melipona beecheii*

In order to make the copious information of the medicinal properties of the melipona honey more comprehensible, we will use a classification of diseases according to the organs that were affected. In these prescriptions, honey, "kab," was used to cure diseases of respiratory, digestive, circulatory, and immunological systems. It was also used as a remedy for maladies of the sensory organs, such as the skin, eyes, ears, mouth, tongue, gums, and teeth. An important part of the literature is dedicated to a group of diseases that were named fevers which due to their high incidence, importance, and negative effects, were considered as a unit in their particular classification by the Mayans. Another part refers to those illnesses typical of the Mayan worldview, with defined traits and supernatural etiology that are called syndromes of cultural filiation. In these regions characterized by a high biodiversity, another important application of honey was as a remedy against the stings and bites of scorpions, spiders, tarantulas, bugs, ants, and venomous serpents (Barrera and Barrera Vásquez 1983).

15.5.1 "Cold" Diseases

In the Mayan classification of diseases, an important part is dedicated to those considered cold diseases, sent by gods or entities that inhabited the cold, dark, damp portion of the Mayan universe—the underworld. The gods and forces that inhabited this place exhibited traits that reflected their surroundings. They were cold, damp, and dark.

Many of the respiratory maladies were considered cold diseases. To cure the white phlegm, whose symptoms make us suspect tuberculosis, the elements of the prescription included expectorants like pepper (Piperaceae, a recent import from the

Old World), chilli *Capsicum annuum* (Solanaceae), and tobacco *Nicotiana tabacum*, *N. rustica* (Solanaceae). In this particular case, as well as in other prescriptions, it is clear that the ingredients were prepared searching for a balance between the intrinsic qualities of the disease and the properties of the remedies. The phlegm disease was cold and the constituents of the medicine were hot.

Honey was a hot product due to its origin and properties, and this made it especially valuable to treat the problems that women experienced before, during, and after giving birth. In this situation, the parturient was in an extremely cold and dangerous state, because she had come close to death and to the underworld; consequently, she was invaded by the negative forces and spirits that dwelled in this place. Honey was used to expel the placenta, "kal ybin":

> The remedy is honey heated with a little sugar, not much, roasted, powdered and stirred thoroughly into the hot honey. Let it be given to drink to the patient. It will be good to put immediately the blood of a chicken in it, the blood from the leg of the chicken. When for two days the after-birth may be retarded in part, administer the other remedy for the after-birth, grated "chaya" *Cnidoscolus chayamansa*, with horse-dung and honey and chilli *Capsicum annuum*. Let it be drunk warm. (Roys 1976)

In this prescription, we observe the addition of an element that is hot in its very nature, the blood, to counteract the placenta's coldness and promote its detachment. Its second part seems elaborated under the dictations of the "medicine of filth," typical of the knowledge of medieval physicians, medical procedures that had probably been brought to New Spain by the doctors that emigrated from the Spanish territories in Europe and had become popular in America or the American continent. It also involves the use of two plants that originated in this continent, "chaya" *Cnidoscolus chayamansa* (Euphorbiaceae) and chilli *Capsicum annuum* (Solanaceae).

In another prescription, honey was rubbed on the woman's abdomen before birth and was also taken as a beverage. To this day, in the Mexican states of Campeche and Yucatán, women who are attended during labor by traditional midwives also receive this treatment before giving birth. The midwife anoints honey over the woman's stomach to help increase the contractions, to correct the position of the child, and to protect both from the coldness of the labor. This is accompanied by other rituals in which help is summoned from supernatural beings to make the labor short and the delivery successful (González-Acereto et al. 2011).

In the manuscript called "Manuscrito de Chan Cah," recovered from the so-called Maya village, the compiler refers to a problem of the placenta in a few lines, unfortunately incomplete:

> When the unhealthy afterbirth is retained by the woman _____ the afterbirth that is tangled his _____ put honey on them. (Grupo Dzibil 1982)

In the group of diseases that came from the cold places of the universe, a dangerous case of heart failure, "chibal puczik," heart pain, is treated with the integration of three different constituents in the prescription, which are all hot remedies: honey, anise *Pimpinella anisum* (Apiaceae), and wine. They were mixed and placed on a piece of cloth and while still hot applied over the region of the heart. The mixture was probably used as an effort to reanimate this organ in case of heart failure.

We believe that due to the seriousness of this disease, this prescription is one of the longest and most complex.

Three or four different remedies are provided to apply in case of “chibal puczik.” We have to consider, in addition the inclusion of European elements such as anise and wine and their use in Mayan medicine. We only quote the part in which “kab,” honey, is used:

> Or else you burn honey with roasted anise, (mix) with wine and put it on a cloth like a thick cake baked in hot ashes. Then you bind it on the heart, hot… (Roys 1976).

Among the indications given in “El Libro del Judío” to treat heart diseases, we find the following:

> “Chiople” *Eupatorium hemipteropodum* (Asteraceae), “xhóch” *Ricinus communis* (Euphorbiaceae), green tobacco *Nicotiana tabacum* (Solanaceae). An infusion of these three herbs is sweetened [with honey] and you imbibe two spoonfuls, every three hours, and it is very effective to cure heart disease and palpitations of this organ; it is taken for three, six or nine days, continually, and you will be cured. (Barrera and Barrera Vásquez 1983)

15.5.2 Fevers and “Hot” Diseases

In all ancient texts on the subject of medicine, fevers are amply cited. We now know that a fever is an abnormally high body temperature, symptom of infection, autoimmune disease, intoxication, and parasitosis, but even now they are considered as a group, and, in the Mesoamerican world they were known as “hot” diseases. According to the Mayan worldview, these illnesses were sent by gods, beings, or forces that belonged to the hot, dry, luminous part of the universe, the supranatural world, above the terrestrial stratum. These beings possessed a very powerful constitution that could damage humans in a severe way.

In some of the prescriptions to treat these maladies, we do not fully understand the nature of the products that are required. For nocturnal fever, “akab chacuil,” the “ah dzac” recommends administration of “hobnil haa,” “hobon water,” with “kanlecay,” dodder, *Cuscuta americana* (Convolvulaceae) in a tepid bath so that the fever disappears (Roys 1976). At present, it is difficult for us to know exactly what the doctor means by “hobnil haa.” In the hives of *Melipona beecheii*, there are small water reservoirs collected by the bees that are probably utilized, amongst other uses, to regulate the hive temperature (Quezada Euán 2005). It could be that the “hobnil haa” required was, alternatively, waste liquid from the hive, but its quantity is minimal. Perhaps the empty “hobones” or logs were used to collect “virgin” water, that is, the rain gathered in the forest and that had never been touched by human hand. This water was profusely used in rituals and treatments by the Mayan priests. It is also possible that the “ah dzac” referred to the “kab,” honey, in a metaphorical way whose meaning still remains obscure to us.

In these books, certain children’s diseases are mentioned repeatedly: for example, nocturnal fevers, convulsions, and shivers, which bring to our attention the fact that children were more likely to catch maladies and were defenseless against a great many of them.

Honey was used for several diseases that had fevers as symptoms. For example, in the case of a skin eruption accompanied by fever, “u chacuil hobonte kak,” three herbs, lemon juice, and fresh honey were integrated to prepare a beverage for the patient (Roys 1976).

15.5.3 Syndromes of Cultural Origin

The name of syndromes of cultural origin has been given to particular diseases that still exist in indigenous communities, related to their ancient medical traditions by Carlos Zolla and his investigative team (Mellado Campos et al. 1994). The Mesoamerican cultures believed in the existence of a complex collection of diseases that were due to the direct action of the deities or other forces, such as an evil wind. The sick person lost one or several faculties like the ability of speech. They had a sad, anguished heart, “okom puczikal.” They suffered from dizziness or vertigo and consequently were exposed to the danger of falling during a journey; they had pain in the legs, or walker’s tiredness and many others. Some of these patients were treated with a variety of plants integrated with the honey.

Found in sixteenth-century dictionaries, this group of diseases, “tamcaz,” translated as frenzy, madness, could probably be epileptic seizures. Antonio de Ciudad Real, the Franciscan friar who collected thousands of terms to compile the first “calepino” Maya-Spanish dictionary, registers for “tamcaz”: stiffness or numbness, epilepsy or frenzy, that strikes dumb and deaf those who suffer tamcaz (Ciudad Real 2001). The Chan Cah manuscript records a remedy for this illness consisting of a mixture of the root of “kulche” *Cedrela mexicana* (Meliaceae) and the root of “cat” *Parmentiera edulis* (Solanaceae), water, and honey (Grupo Dzibil 1982).

With respect to a malady where the patient fell, we might speculate over its multiple causes. It could be a simple faint or swoon, or a complication of a cardiovascular disease, epilepsy, or a diabetic coma. There are several entries that refer to this disorder, in which the “ah dzac” specified multiple symptoms. In one of the prescriptions, the doctor referred to a blood movement in the bowels, the sick person fell, and spitted or vomited blood. These symptoms remind us of a gastric ulcer. The prescription was integrated with a handful of “xucul”: leaves, stem, and root of purslane (imported from the Old World with the Spanish conquest), *Portulaca oleracea* (Portulacaceae), that were boiled with one-third drachma of honey. It was left to cool, sugar was added, and it was administered to the patient at sunrise, under abstinence, for 3 or 4 days (Roys 1976).

15.5.4 Maladies of the Digestive Tract

Several diseases of the digestive tract were treated with honey. In the first place, diarrhea with severe colic, named “u lom tokil hubnak” with “othcehil,” was treated with the tender tips of the cualote tree *Guazuma polybotrya* (Malvaceae) and green leaves of "taamaay" *Zuelania roussoviae* (Salicaceae), "ixim-che" *Casearia nitida* (Salicaceae), "muloch" *Triumfetta semitriloba* (Malvaceae, Tilioideae), and "buhumkak" *Cordia geraschanthoides* (Boraginaceae) ground and dissolved in a "tumin," Mayan measure, of hot honey. The liquid was left to cool and was given as a drink although it could provoke vomiting or colic (Roys 1976).

A prescription to treat dysentery, "kik choch," bloody diarrhea, appears in the "Ritual de los Bacabes." Honey extracted from the logs was added to the plants required for this medicinal beverage (Arzápalo Marín 1987).

Not only honey had the strength called "kinam" that the Mayans imagined came from the energy that the sun bestowed upon the earth's creatures and plants. For other digestive diseases, an indication was given to the specialist to use bees' or wasps' nests. For yellow stools and spasms, or colic, the nest of a wasp, "kanal," was boiled with some plants. This prescription is an example of sympathetic medicine in which color is fundamental. The malady's signs were yellow, and so were the wasps and their nests. The plants required for the remedy were also yellow, thus having an additional healing power, which could depend on the color division of the Mayan universe (Roys 1976).

Another prescription to treat dysentery required burning a nest of "kan-kub," a bee, taking the larvae, grinding them, and mixing all to administer as a beverage, with honey (Roys 1976). To cure diarrhea, the "ah dzac" could use:

> "Lucal". Residue that is collected in the honey pots or in the hive and dissolved in water cures diarrhea, even chronic ones. (Barrera and Barrera Vásquez 1983)

To eliminate intestinal worms, honey was also applied in an effective enema:

> Take milk and honey and vinegar and apply to the rectum (or lower abdomen). It will draw them out immediately. (Roys 1976)

One of the most important ritual drinks that the Maya manufactured was "balché." This beverage was prepared with water, honey, and the bark of the tree called "balché" *Lonchocarpus longistylus* (Fabaceae, Faboideae), which were mixed and left to ferment for 2 days. The beverage was used by all the members of the Mayan society, according to the ritual that was being enacted. Only children were sometimes exempted from its drinking. Balché was used like a very good purgative, to promote health, strength, and longevity (Garza 1987).

The Catholic priests tried to ban the production and use of this beverage mainly because of its close connection with the idolatrous rituals that the Mayans still had fresh in their memories. To this day, balché is commonly drunk in all the Yucatán peninsula.

15.5.5 Diseases of the Sensory Organs

In the past, honey was used against ear and eye infections and it is still used by the Mayan traditional specialists called "ah men" or "h men" to heal these ailments. In the literature, we find a prescription to use plants like *Hibiscus tubiflorus* (Malvaceae) "tupkin," hibiscus, sorrel, or black mustard *Brassica nigra*, another European import

(Brassicaceae), whose leaves were roasted and introduced in the ear. If it did not get better, the specialist suggested the use of a ripe, red chilli, without seeds. The doctor took a small quantity of honey directly from the "hobon" and added water. Both substances were put into the chilli that was roasted over hot ashes. When the liquid was tepid, it had to be squeezed into the ear (Roys 1976).

When there was pain in the eyes, the medicinal treatment was:

> It is good also to take fresh honey from the hive and the tender tips of the *Carica papaya* (Caricaceae), covered with banana leaves and cooked, add a little salt, then wrap it in cotton–wool and squeeze it into the eye. (Roys 1976)

Some of the most notorious symptoms of a great number of hot diseases are rashes, spots, pustules, and abscesses on the skin. In the documents that support this investigation, a very serious disease called "ek pedz kak," smallpox, is mentioned. The prescription indicates:

> There is also black confluent smallpox "ek pedz kak". This is the remedy, the blossom and the leaf and the outside of the red *Plumeria rubra* (Apocynaceae), frangipani. Let these all be roasted, then you mash them and you add a little honey from the hive, raw honey. Then you heat it to just the right temperature and you give it to drink to anyone who has this eruption, in order that it may put an end to the burning and the throbbing. (Roys 1976)

In another case of infectious rash, "canal kak," the informant registered the months and years when the disease appeared and the symptoms as well as the remedies. The word "kak" means fire and "kak cimil," fire, disease, smallpox in general (Ciudad Real 2001). Contagious skin eruptions were treated with an emetic drink made up with crushed fresh leaves of *Bravaisia tubiflora* (Acanthaceae) "ek-huleb"; the *Croton niveus* (Euphorbiaceae) croton "chuy-che"; the *Zuelania roussoviae* (Salicaceae), "taamaay"; the *Castilla elastica* (Moraceae) rubber tree; the *Alvaradoa amorphoides* (Picramniaceae) "besinikche", Sapindales stub [sic]; and the *Leucaena glauca* (Fabaceae, Mimosoideae) "uaxim", white leadtree and mixed with honey (Roys 1976).

To cure skin burns, "chuhul," the injuries had to be covered with honey: "… let it be anointed with honey fresh from the hive, immediately" (Roys 1976). The prescription is long and complex, but honey was the first product that was used to treat these accidents.

15.6 Conclusions

In Yucatan, the Mayan traditional doctors, "ah dzaco'ob," used honey produced by the stingless bee, *Melipona beecheii*, as a medicinal product of great importance. This fact was rigorously registered in the Colonial chronicles that deal with traditional Mayan medicine. But the complete information on which the practice of these specialists was based gradually disappeared under the pressure of the Spanish culture that was imposed on the native people. The prescriptions lost Pre-Hispanic tradition, and the use of honey was modified from being a curative element of great power or *kinam*, elaborated by deities, to being used only to sweeten the remedies. Reading the prescriptions that were compiled by Ricardo Ossado and comparing them with the invocations of "El Ritual de los Bacabes" (Arzápalo Marín 1987), we realize the loss of medical, ritual, and religious information that the former underwent. It is also clear that many "traditional remedies" in fact included plants introduced by the Europeans to the Mayans.

There are a number of reasons to support the extensive use of this bee's honey to treat a great number of diseases: first, its unequivocal properties, considering its antimicrobial capacity; second, the "kinam" of its origin that makes it a hot product, gift of long forgotten gods; and third, the fact that it is a natural product, with almost null toxicity on the human organism. Much research remains to be done on the medicinal properties of "kab," based on the ancient texts.

The prescriptions reviewed above were quoted as they were written to provide insight into the logical structure of Mayan thought regarding the power of honey as medicine—ideas that led to its extensive use for the many diseases against which human applications of honey were effective. They may also instruct us regarding the Mayan worldview, an issue of great complexity and interest.

Efforts seeking to inform present-day tropical people on the existence and importance of the native stingless bees are very valuable. Let this work be an open invitation to learn more about the native stingless bees of America, their honey stored in pots, the people that have protected them for centuries, and the countries which they inhabit.

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Chapter 16
Staden's First Report in 1557 on the Collection of Stingless Bee Honey by Indians in Brazil

Wolf Engels

Dedicated to my colleague and friend Paulo Nogueira-Neto on the occasion of his 90th birthday, April 18, 2012.

16.1 Introduction

Honey has presumably been much in demand by people since prehistoric times. To procure this unique, delicious food, many modes of honey hunting were developed, of which several are still in use today. To facilitate access to this delicacy, several ancient cultures invented modes of beekeeping, in particular with two species of honey bees, *Apis mellifera* in Europe and Africa, and *Apis cerana* in Asia (Crane 1999). In the Americas, management of stingless bees in artificial hives has only been reported for the culturally advanced Mayans and Aztecs, a tradition of meliponiculture now continued by the indigenous population of the Mexican peninsula, Yucatán (Inoue 1990). As far as we know, the early Brazilians never developed similar techniques, although their methods of honey hunting include sustainable removal without destroying the nest (Posey 2002). Nevertheless, that they knew very well where to find stingless bee colonies was already reported by Hans Staden in the sixteenth century (see also Cobo 1653, in Roubik 2000).

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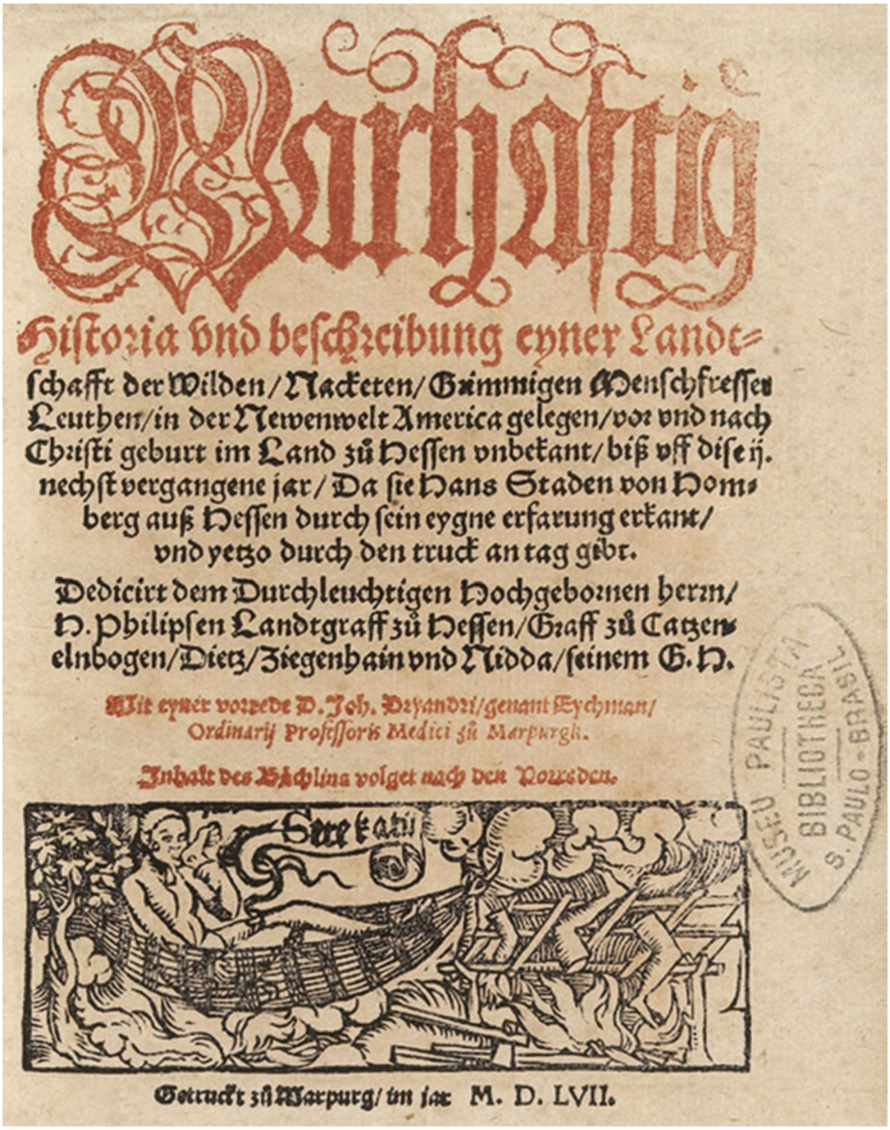
P. Vit et al. (eds.), *Pot-Honey: A legacy of stingless bees*,
DOI 10.1007/978-1-4614-4960-7_16,

16.2 The Oldest Written Report on Brazilian Honey Collection

The first book on Brazil, the “Warhaftig Historia” by Hans Staden (Fig. 16.1), was published in Marburg in 1557. The author was a German adventurer who served as a mercenary on Spanish and French ships exploring the Atlantic coast from the La Plata region north to Cabo Frio near Rio de Janeiro. During two journeys he spent



Warhaftig
Hiſtoria vnd beſchreibung eyner Landt-
ſchafft der Wilden/ Nacketen/ Grimmigen Menſchfreſſer
Leuthen/ in der Newenwelt America gelegen/ vor vnd nach
Chriſti geburt im Land zů Heſſen vnbekant/ biß vff diſe ij.
nechſt vergangene jar/ Da ſie Hans Staden von Hom-
berg auß Heſſen durch ſein eygne erfarung erkant/
vnd yetzo durch den truck an tag gibt.

Dedicirt dem Durchleuchtigen Hochgebornen herrn/
H. Philipſen Landtgraff zů Heſſen/ Graff zů Catzen-
elnbogen/ Dietz/ Ziegenhain vnd Nidda/ ſeinem G. H.

Mit eyner vorrede D. Joh. Dryandri/ genant Eychman/
Ordinarij Profeſſoris Medici zů Marpurgk.

Inhalt des Bůchlins volget nach den Vorreden.

Getruckt zů Marpurg/ im jar M. D. LVII.

Fig. 16.1 Frontispiece of Hans Staden’s book, original edition 1557 about 10 years in the New World, including 10 months as a prisoner of the Tupinambá tribe in the São Paulo region. In his 178-page book he described in New High German language the coastal geography in great detail, based on his experience cruising the coast. In addition, he reported on the life of the indigenous people. Especially because it included description of an anthropophagic cult, the book immediately became a bestseller.

16.3 Hans Staden's Contribution to the Knowledge of Stingless Bees in Brazil

The original publication of Staden's book as well as early illegal editions, and also recent literature on Hans Staden and on stingless bees in Brazil, were consulted. The figures shown here are copies from online facsimiles prepared by the University of São Paulo.

At the very end of his book, in only six pages, some peculiarities of Brazilian nature (Engels and Heinle 2011) were recorded (Fig. 16.2). In the second part of the book, the last chapters discuss nature in Brazil, beginning with Chap. 30, titled "Bericht etlicher Thier im lande" (record on several animals in Figs. 16.1 and 16.2).

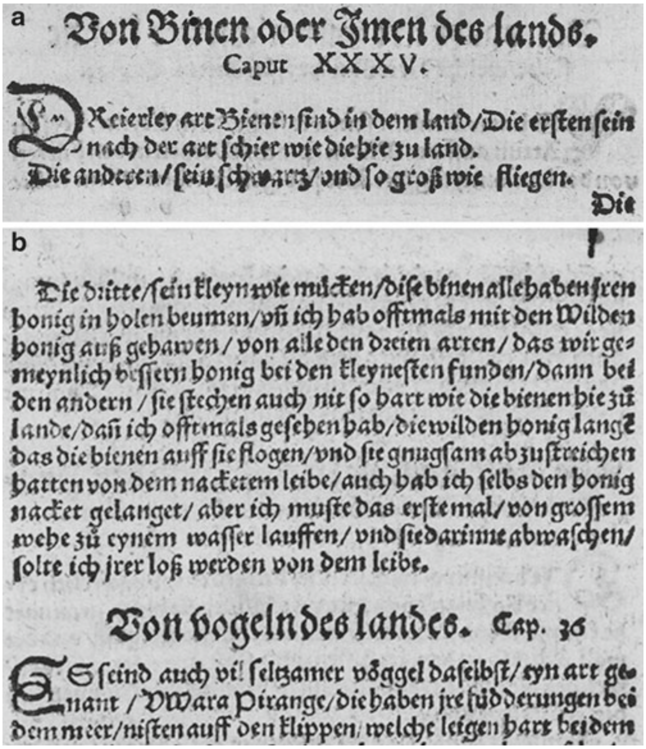
Chapter 35 is entitled "Von Binen oder Imen des lands" (from bees or "ims" of the land), including remarks on stingless bees and the collection of their honey (Fig. 16.3).

With a mere 140 words Hans Staden described stingless bees, mentioned their typical behavior, and noted that nests with honey stores are found in hollow trees. He had observed how the Indians collected the honey and participated in the process, and was attacked vigorously by the non-stinging but biting bees. He wrote [in translation]:

> There are three species of bees in the land. According to their nature, the first are almost like those in our land. The others are black and as large as flies. The third are small like midges. All these bees have honey in hollow trees. Together with the wild men, I frequently collected the honey. Among the three species, we usually found better honey from the smallest bees than from the others. They do not sting so hard as the bees in our country. As I have

Bericht etlicher Thier im lande.
Caput xxx.
ES hat in dem land Rehböck wie hie wild ſchwein/
zweyerley art. Deren art eyne ſein wie hie im land/
Die andern kleyn wie junge Schweinlein/ heyſſen
Teygaſu Dattu/ ſein ſehr vbel zufahen in den fallen/ welche
die wilden brauchen wild zufangen.

Fig. 16.2 Title of Chapter 30 on Brazilian animals



Von Binen oder Imen des lands.

Caput XXXV.

DReierley art Bienen ſind in dem land/Die erſten ſein nach der art ſchier wie die hie zu land. Die anderen/ ſein ſchwartz/ vnd ſo groß wie fliegen.

Die

Die dritte/ſein kleyn wie mücken/diſe binen alle haben jren honig in holen beumen/vñ ich hab offtmals mit den Wilden honig auß gehawen/ von alle den dreien arten/ das wir gemeynlich beſſern honig bei den kleyneſten funden/dann bei den andern / ſie ſtechen auch nit ſo hart wie die bienen hie zů lande/dañ ich offtmals geſehen hab/die wilden honig langē das die bienen auff ſie flogen/vnd ſie gnugſam abzuſtreichen hatten von dem nacketem leibe/auch hab ich ſelbs den honig nacket gelanget/ aber ich muſte das erſtemal/von groſſem wehe zů eynem waſſer lauffen/ vnd ſie darinne abwaſchen/ ſolte ich jrer loß werden von dem leibe.

Von vogeln des landes. Cap. 36

ES ſeind auch vil ſeltzamer vöggel daſelbſt/ eyn art genant / VWara Pirange/die haben jre füdderungen bei dem meer/niſten auff den klippen/ welche leigen hart bei dem

Fig. 16.3 Chapter 35 on Brazilian stingless bees, their behavior, and how the Indians in Brazil collect their honey

> often seen, when the wild people take honey, the bees fly upon them, so that they had much to do in striking them off from their naked bodies. I myself also took honey naked. The first time I had to run with great pain to water and wash them off, merely to get rid of the bees from my body.

16.4 Forward-Thinking Based on the Precise Bee Descriptions of Staden

The original text of this short chapter in German is very precise (Fig. 16.3). I will comment on the above-mentioned sentences. First of all, it was possible for me to deduce the genera and the probable species mentioned by Hans Staden. These are most likely *Melipona quadrifasciata*, *Scaptotrigona postica*, and *Tetragonisca angustula* (Engels 2009), all today still occur in the São Paulo region (Nogueira-Neto 1997; Marcolin 2009).

According to Staden, these stingless bees use hollow trees as nesting sites, a correct observation (Nogueira-Neto 1997). The Indians collected the honey by removing it from the colony after cutting the trunk open. Presumably they only took the honey pots, because it is known from recent studies on apicultural traditions of the North Brazilian Kayapó Indians (Posey and Camargo 1985; Posey 2002) that honey hunting is done by repeated removal of sealed pots from the storage area of stingless bee nests without destroying the colony. In comparing the honey of the three species, Staden favored that from *T. angustula*, and in fact this "jataí" honey also yields the highest price on today's Brazilian market. It is delicious and also is used for medicinal purposes.

The term "stingless bee" was unknown in the sixteenth century; however, Staden mentioned correctly that the Brazilian bees did not sting. In particular, *S. postica* colonies very actively defend their nest. Any enemy is immediately attacked, the workers hang onto hairs and eyelashes, bite into the skin, enter the ears, nostrils, and mouth, and chase the intruder. I experienced this behavior during field work in Brazil, as documented in our film on their nest biology (Engels and Engels 1980). Staden reported that it is not easy to get rid of these defenders, which also recruit many nestmates by releasing an alarm pheromone (Smith and Roubik 1983).

16.5 Conclusions

In summary, Hans Staden's book provided the first published information on stingless bees, unknown then in Europe. He described their nesting habit, non-stinging defense strategy, and in particular, stingless bee honeys of different qualities. This precise record was until recently (Engels 2009; Marcolin 2009) not quoted in the scientific literature on stingless bees (Nogueira Neto 1997; Michener 2007; Moure et al. 2007). The cultural traditions of South American Indians evidently allowed them to harvest honey as a valuable product of the native meliponine bees, similar to various forms of honey hunting developed in Europe, Africa, Asia, and both Americas (Crane 1999). We can assume that detailed knowledge on stingless bee biology was present in the indigenous Brazilian tribes and practiced in the sustainable use of the resources available in the tropical forests. Honey hunting from stingless bees presumably was common long before the Europeans arrived in South America.

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Chapter 17
Melipona Bees in the Scientific World: Western Cultural Views

Raquel Barceló Quintal and David W. Roubik

17.1 Introduction

To study the tiny world of insects, a microscope is a necessary tool. Insects were made large by their deed, in the case of stingless bees, by manufacturing honey from the nectar of flowers—or other sugary resources—in their environment. To follow up on such a novel discovery, many entomologists and natural historians had to use a microscope. Further exploration and taxonomic expertise were required, as illustrated here in examples from the Western World and literature.

17.1.1 Early Studies on the Stingless Bees

For centuries, humans have used honey from bees known as meliponas or the stingless bees (Schwarz 1932, 1948; Lutz 1933; Friese 1903; Ducke 1924), tribe Meliponini, as a natural source of food, as a healing element, and as a product for commercial exchange. It was not, however, until the nineteenth century when European scientific studies on the aforementioned bees began in earnest (e.g., Spinola 1853). This was not the case for *Apis mellifera* because its study was closely linked to the development of optical devices, such as the microscope. According to the Italians, this instrument was invented, in 1610, by Galileo Galilei, but the Dutch attribute it to Zacharias Jansen, in the year 1602. Later, at the workshop of Cornelius Drebbel

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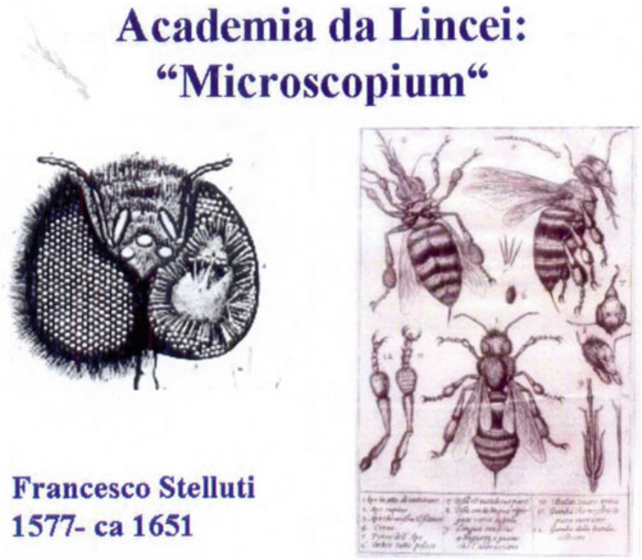

Fig. 17.1 Stelluti's book with compound eye of the honey bee. From Stelluti (1625)

(1572–1633), a similar device was created that was called the microscopium. With this instrument, a new age of biology arose.

It was in Francesco Stellutti's workshop (1577–1651) that the honey bee *Apis mellifera* and its compound eyes were first observed under the microscope (see Fig. 17.1, from Stelluti 1625). Such observations revealed various new characteristics.

Nevertheless, Francesco Redi (1621–1679) may be considered the "father of insect biology," thanks to interesting observations gathered in his work written in 1668 "Esperienze intorno alla generazione degl' insetti," translated in the book "Experiments on the generation of insects" (Redi 1909).

Despite several conceptual mistakes in the seventeenth century, there were a number of direct observations that influenced early treatises on bees and fostered emergence of modern science as a system of approaching reality, whose historical achievements included publication, around 1637, of the work "Discourse on Methods," by René Descartes, who distinguished Physics from Biology. Descartes included, among natural facts, behavioral responses from living beings as events obeying general laws, similar to those that govern inanimate objects. With Descartes' text, science moved ahead, since the old controversy on spontaneous generation of small animals was challenged by the sound experiments of Redi, when for the first time insects were demonstrated not to come from flesh through spontaneous generation.

17.1.2 *Enlightenment and the Study of Insects*

Even though scientific studies in the seventeenth century were devoted to *Apis mellifera*, in the eighteenth century the Western World became interested in stingless bees. In that century, best known as the enlightenment century, there was an optimistic attitude in minority European circles about the possibilities and benefits of reason, education, and science as means of solving mankind's problems. There was important progress from a peculiar constitutive and operational principle, which, in its turn, was conceived as a vital force—ontologically and operationally superior to other cosmic natural forces (mechanics, thermodynamics, electricity, chemistry, and magnetism).

In the eighteenth century, observers of the natural world were concerned about ordering living diversity by means of taxonomy, that is to say a hierarchical system. In 1731, Carl Linnaeus (1758) (1707–1778) invented a biological classification system, presented in its 10th edition in 1758, and considered the origin of modern taxonomy. He developed the modern scheme of binomial nomenclature, first, indicating genus, and second, species. After that, diverse taxonomists added other categories: family, order, class, phylum or division, and kingdom. According to the sociologists Émile Durkheim (1858–1917) and Marcel Mauss (1872–1950), primitive classifications emerged not only from the ability to recognize groups but also as a projection of social organization; they said "man classified things because he was divided into clans" [...]. The first categories were the social ones; the first classes of things were human classes. This was because men were grouped, and they thought about themselves in the form of groups, and in their minds appeared the idea of grouping things [...]. Man was the first genus; clans were the first species (Durkheim and Mauss 1963).

On 22 March 1803, Aimé Bonpland (1793–1858), aboard a Spanish frigate, sailed from Guayaquil (Ecuador) to Acapulco, the most important Pacific Mexican port. He visited and described the places in his diary before leaving for Chilpancingo and Taxco, on 29 March, and then to Mexico City, where he arrived in on 12 April. There, Alexander von Humboldt (1984) (1769–1859) traveled to nearby places. When he published his work "Political Essay on the Kingdom of New Spain," and related Campeche's honey and cerumen production, he wondered if this bee was the same one that Bonpland found on the Eastern slopes of the Venezuelan Cordillera, mentioned in their book "Recueil d'observations de zoologie et anatomie comparée," published in 1811 (Freites 2000).

Baron Alexander von Humboldt knew about melipona bees through the entomologists Johann Karl Wilhelm Illiger (1811) (1775–1813), Pierre André Latreille (1762–1833), and Louis Jurine (1775–1819). In 1806 Illiger was the person who described the characteristics of the genus *Melipona* (Wille 1983), as he mentions in his work "Prodomus systematis mammalium et avium" (1811), which is a treatise on systematics or Linnaean Taxonomy. Another entomologist, Latreille, arranged the entomological collection of the National Museum of Natural History in Paris; in 1814, as a member of the French Academy of Sciences, he studied *Melipona scutellaris*. In 1819, he published his work "Mémoires sur divers sujets de l'histoire naturelle des insectes, de géographie ancienne et de chronologie." He went further, by subdividing the tropical American stingless bees into two genera; *Melipona*, in which the mandibles are not toothed; and *Trigona*, in which mandibles are dentate. The basis of these subdivisions seemed to be supported by the general appearance of the insects (see Schwarz 1932, 1948; Michener 2007).

Fig. 17.2 Portrait of Captain Frederik William Beechey. From Christian Young (n/d)

17.1.3 The Nineteenth Century and Melittology

During the nineteenth century, there were many formal studies on insects in Mexico. Indeed, nearly a century after Illiger and Latreille established the ground plan of studies on Neotropical stingless bees, a number of publications appeared on regional fauna (Cockerell 1900; von Ihering 1902; Friese 1903; Marianno 1911; von Ihering 1912; Ducke 1924). This century saw the origin of an accredited entomological profession; centers of teaching and research were founded, and museums and collections initiated, together with societies and periodic publications devoted to insects. Meanwhile in Europe, studies on *Apis mellifera* proliferated, among them works on pollen contained in honey, which gave a new impulse to apiculture.

In 1827, Frederik William Beechey (1796–1856; see Fig. 17.2) British naval officer, artist and geographer, went across the Bering Strait with the purpose of meeting John Franklin and William Edward Parry. Although his voyage was unsuccessful, on his return he explored the Pacific Coast, where he discovered several islands and visited the ports, such as San Francisco and Mazatlán, where he arrived in on 3 February 1828, and drew one of the first known maps of the city. He had the good fortune to bring together a variety of rare species from distant localities, some of which had been seldom, if ever, visited by any collector. In 1831, as a result of this travel, Beechey published his work "Narrative of a voyage to the Pacific and Bering Strait to co-operate with the Polar Expeditions, 1825–1828."

Later, in 1831, Edward Turner Bennett (1797–1836), British zoologist, reviewed the notes of captain Beechey[1] on the domestication of the bee that he knew in

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[1] British zoologists studied the notes of captain Beechey and published the book "Zoology of Beechey's Voyage." In 1891 the stingless bee, whose culture in hollow logs was developed by the Mayans, acquired the name *Melipona beecheii* Bennett, named in his honor.

Fig. 17.3 Herbert F. Schwarz. Image reproduced courtesy of J. Ascher and E. Wyman

Mexico. The interconnection between human and stingless bees was typified in the following paragraph:

> In the domestications of the bees of Mexico but little violence is done to their natural habits. In habitants, in their wild state of cavities in trees, a hollow tree is selected to form their hive. A portion of it, of between two and three feet in length; is cut off, and a hole is bored trough the side into the hollow, at about its middle. The ends of the hollow are then stopped to with clay, and the future hive is suspended on a tree, in a horizontal position, with the hole opening the cavity directed also horizontally. Of the hive, this prepared, a swarm of bees speedily take possession, and commence their operations by forming cells for receptions of their larvae, and sacs that contain the superabundant honey collected by them in their excursions (Bennett 1831).

The final decades of the nineteenth century saw several entomologists who describe species of Meliponini from Mexico, among them Ezra Townsend Cresson (1838–1926), Theodore OA Cockerell (1866–1948), and Karl Wilhem von Dalla Torre (1858–1928). Studies in Brazil also produced meliponines new to science (Spinola 1853).

17.1.4 The Meliponas in Twentieth Century Science

Behavior and ecology of stingless bees was beginning to be explored, particularly in regard to the foraging flights and recruitment of individual bees to food sources by others from their colony (Salt 1929; Lutz 1933; see also Lindauer 1961; Wille 1983; Roubik 1989). The foundations of meliponine taxonomy were further extended to other portions of the world, and intensive country-wide surveys continued (Schwarz 1932, 1934, 1937, 1948; Moure and Kerr 1950; Michener 1954; Moure 1961). See Fig. 17.3 with the portrait of HF Schwarz, ca. 1935, from the American Museum of Natural History, New York.

Paleontologists soon joined in stingless bee studies from their external morphology captured in amber, focusing on bees from both Dominican Republic and Mexico (Wille 1983, and see also present book Chap. 9 by Ayala et al.). Regarding this last subject certain specimens that have been found allow observing or inferring relationships. Such is the case of specimens of *Proplebeia dominicana* which became trapped while collecting resin for their nests. The most ancient amber fossil meliponine *Cretotrigona prisca* dating as early as the Upper Cretaceous Period[2] was found in New Jersey, United States, and it is roughly 67 million years old. The first fossils of *Apis* were discovered in Western Germany, and they date back to the Early Miocene Period, from 22 to 25 million years ago (Engel et al. 2009). A bee that looks like *Apis dorsata*, but is smaller, similar to the current size of *Apis mellifera*, was present in the Upper Miocene period, ca. 12 million years ago, in Western North America (Engel et al. 2009). It is thought that *Apis florea* and *Apis dorsata* might have existed as separate species or lineages since the Oligocene period.

With regard to paleontological studies, João María Franco de Camargo (1941–2009), Brazilian entomologist, proposed biogeographical barriers or geological compartments in hierarchies defined by sequences of vicariance and cladogenesis among the fossil and extant stingless bees (Camargo 2008; Vit 2010; Camargo, Chap. 2 in this book).

In the twentieth century, after some paleontological discoveries, several researchers, such as Joachim C. Evenius (1896–1933), Guido Grandi[3] (1886–1970), and Edward Butler (1881–1963) devoted themselves to the study of pollen carried by bees (*Apis* and *Melipona*). Methods of melissopalynology (pollen identification of pollen in honey) were published by Louveaux et al. (1978).

As a result of the discontinuity produced by the Revolution, entomological research in Mexico was disturbed, and it was not until the twentieth century, after 1921, when it regained vitality. During the decades of 1940 and 1950 the proper means for the development of this discipline were established. More recent years were characterized by some important achievements: well-equipped laboratories and proper salaries have allowed entomologists to work on research full-time (Pacheco 1989).

Regarding taxonomy, two major genera were long used for stingless bees. In 1951, Jesús Santiago Moure (1912–2010) and Warwick Estevam Kerr (1922–) proposed 12 genera and 19 subgenera for the Neotropical region (Moure and Kerr 1950). In 1967, Kerr et al. proposed the subgenus *Micheneria*; and Moure, in 1975, changed it to *Michmelia*. Nevertheless, Charles Duncan Michener[4] (1918–) does not consider

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[2] In that time, continents were already separate and had a form similar to now, but they presented distinctive attributes, for example, the inner part of North America contained a sea which divided the continent, known as Cretaceous Seaway.

[3] Italian entomologist, who founded, in 1928, the Institute of Entomology in the University of Bologna.

[4] In 1944, he published a classification system for bees that would be soon adopted by melittologists, and was used until 1995, when he was the co-author of new classifications; again modernized for the world in 2000 and in a revised work, "The bees of the World," in 2007.

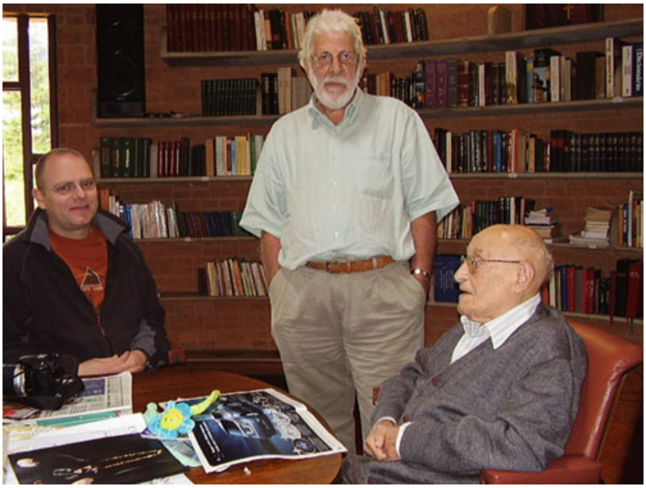

Fig. 17.4 C. Rasmussen, J.M.F. Camargo, and Father J.S. Moure. Three of the twenty-first century entomologists most devoted to stingless bee taxonomic and systematic studies, in the library of the Claretian Home in Batatais, São Paulo, Brazil, 2008. Photo P. Vit

that *Melipona* is heterogeneous enough to be divided into subgenera (Michener 1990). It is important to note that Moure, known as the "Father of bees," was a priest who created a catalogue of Neotropical bees, together with Danuncia Urban, Gabriel AR Melo, and individual authors of large sections, e.g., Camargo and Pedro (2007) Chapter Meliponini Lepeletier, 1836. This catalogue was a product initiated with compilation of Moure's notes about bees, dating back to 1938. In 1975, the catalogue contained over 11,200 typed cards.[5]

During a short stopover in Ribeirão Preto, Brazil, while Dr. Rasmussen was invited for a talk, Professor Camargo suggested a visit to his very appreciated mentor Padre Moure in the Claretian Retirement Home in Batatais, during the local holiday known as "tiradentes" in 2008. Three generations of stingless bee scholars are shown including Padre Moure in Fig. 17.4.

Studies were directed toward discovering Brazilian stingless bee communication by meliponologists Martin Lindauer[6] (1918–2008) and Warwick Estevam Kerr

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[5] Padre Moure's catalogue consisted of handwritten cards; carbon copies can be found at the University of Kansas, where they were deposited by Padre Moure; 11,200 typed cards, which in large part relate to the family Halictidae (around 2,000 cards), were published as a catalogue in 1987 by Moure and Paul David Hurd (1921–1982), for the Smithsonian Institution. Recently, the part containing information about Colletidae (around 750 cards) was published in five articles in the *Magazine of Zoology*, reaching a total of 161 pages. Therefore, most of Padre Moure's catalogue was unpublished until 2007 when the whole catalogue of bees in the Neotropical region was edited by Moure, Urban, and Melo.

[6] German neurobiologist, who was a Zoology professor at Frankfurt University. As a scientist, he discovered communication among bees; their sense of orientation to find their way and live in a society.

(1922–), in Piracicaba, and elsewhere in Brazil. The communication procedure is partly chemical, when the foragers find an important source of nectar, pollen, and presumably resin, they make from six to ten journeys to the hive carrying it as a demonstration of a harvestable resource. Then, bees suddenly change their behavior, they leave the nest, and fly towards the resource, but this time they do not pick it up when returning to the nest; instead they start "marking" the foraging site, leaving signals from place to place. These substrates differ according to bee species; for example, *Trigona spinipes* "irapuã" marks stones, leaves, flowers, or any other objects before entering the nest. The mark that these bees leave consists of tiny drops of the pheromone produced by certain glands in the head. Recent research reveals different combinations of zigzag dances in the nest, or use of marking pheromones, in *Melipona*, *Scaptotrigona*, *Cephalotrigona*, and *Partamona* (Quezada-Euán 2005 and various chapters in the present book).

In the 1970s, in addition to cataloguing native bees, biological studies were extended to the nesting biology, beekeeping, and behavior of stingless bees, for example, by Paulo Nogueira-Neto (1922–)[7] who studied nesting colonies, the fertilization of the queen, and the foraging of worker bees, and published a comprehensive manual on stingless beekeeping (Nogueira-Neto 1970). In addition, the nest architecture and varied biological details of nesting colonies were rendered with detailed drawings and field observations (Camargo 1970).

The 1980s witnessed not only the first detailed ethnography of stingless bee specialists within indigenous American tribes (Posey 1980; Posey and Camargo 1985) but also an integration of literature on tropical bees, highlighting many of the biological features of Meliponini (Roubik 1989).

In the 1990s, study of the Meliponini has been concerned with risk of extinction, crops and their pollination, the impact of pesticides, the devastation of forests, the introduction of non-native species, and reduction of stingless bee abundance. In the same decade there was consideration of stingless bee "re-population" in forests, in such a way that the trees will receive pollination and the latter obtain food and protection (Svensson 1991; Méndez 1999). Other research showed that the stingless bees produce more honey under conditions of ecological balance (Hill and Webster 1995).

Currently, a growing number of studies that consider physicochemical composition of honey from stingless bees are being carried out. Moreover, standards are being devised for their honey quality in different ways, as shown in the present book.

17.1.5 Cultural Studies on the Stingless Bees

Claude Lévi-Strauss (1908–2009) exposed to the eyes of Western scientists the existence of indigenous knowledge about nature and its societies, what he called "sciences of the concrete," that is to say, traditional knowledge, with the aim of

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[7] First Secretary of the Special Secretariat for the Environment, Brazil.

Fig. 17.5 Levi-Strauss in the Brazilian Amazon. From Wilcken (2011)

validating its principles and establishing its cultural rights (Lévi-Strauss 1964). He was one of the social scientists who recovered popular knowledge about stingless bees (Fig. 17.5).

Lévi-Strauss, as an anthropologist, focused on popular knowledge and/or peoples' primitive thought. From 1930 to 1935, he lived in Brazil, where he performed his first ethnographical work, in Mato Grosso, and the Amazon. In 1955, he published his work "Tristes tropiques" (Sad tropics), which is an ethnographical work; and in 1972 "De la miel a la cenizas" (From honey to ashes), second part of his series "Les mythologiques," where he undertakes a structural analysis about diverse myths created over honey by the tribes he visited in Brazil. In brief, through the study of Brazilian indigenous cultures, he understood that despite the differences existing among diverse parts of mankind, human mind is one and the same thing everywhere, with the same abilities (Lévi-Strauss 1972).

The current trend seems to be more holistic, regarding all or a great part of intellectual elements: cognitive, symbolic, economic, cultural, and ecological. In Latin America, in general, and Mexico and certainly several Tropical American countries there is a large cultural diversity that contains a wide knowledge which may be highly correlated to scientific knowledge. Within the fauna of Tropical America, bees have been of great cultural value, since before America's discovery by European explorers, and further conquest. They have been part of religious, festive, and trade customs of several indigenous peoples, the Mayans were the main ethnic group who developed, through *Melipona beecheii* beekeeping and husbandry, the science and art of meliponiculture (see the Ocampo Rosales Chap. 15 in this book). The other meliponas are ever present in the culture of Latin American people.

With regard to research on *Melipona beecheii*, in the culture of the ancient Mayans, Ernst Förstemann (1822–1906), librarian of Dresden, was one of the pioneers in trying to decipher the "calendar of the meliponary" of the Codice Madrid. Later, Alfred Marston Tozzer (1877–1954) spent several seasons in Yucatán to

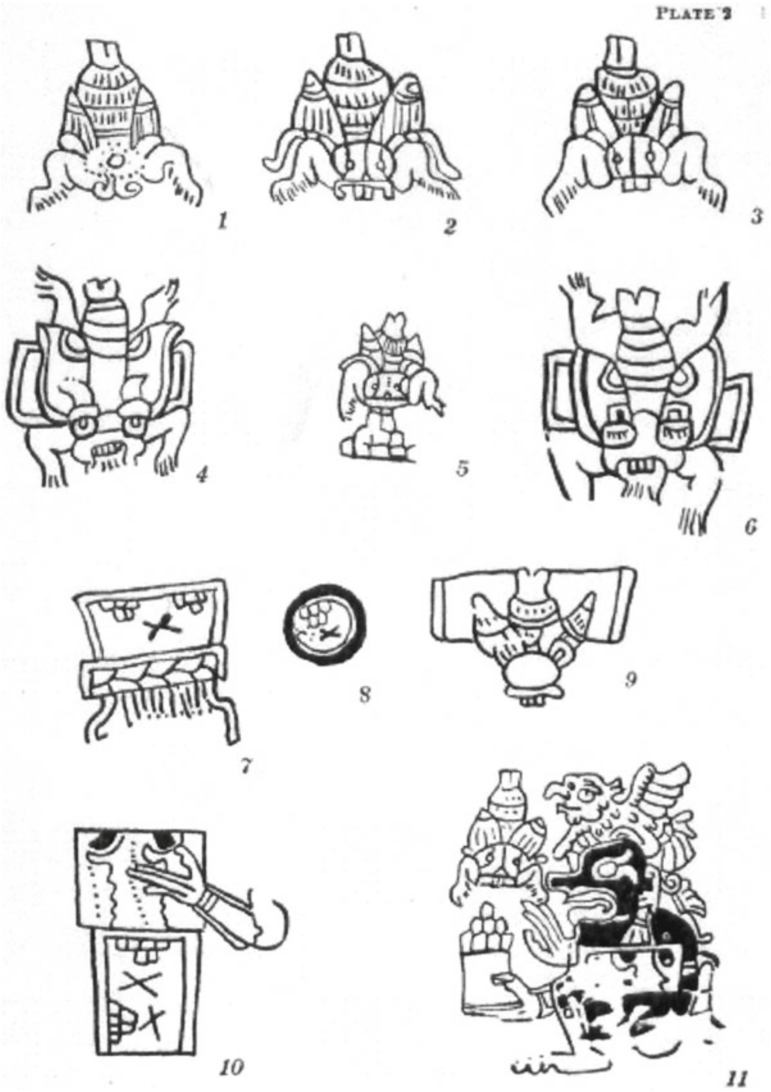

Fig. 17.6 Bees from the Mayan Codex. From Tozzer and Allen (1910)

investigate the Mayan culture. Among the folk stories, he drew attention to those in which the bees were of high value between cultures. In 1910, together with Glover Morrill Allen (1879–1942) he published “Animal Figures in the Mayan Codices” (Tozzer and Allen 1910), see Fig. 17.6. Another pioneering work was that of Édouard Bunge, member of the Société des Américanistes of Paris, published in the Journal of this Society, in 1936, as “Les pages des abeilles du Codex Tro” (Bunge 1936). At the end of the 1950s, Wolfang Cordan (1908–1966) traveled to Mexico where he studied the language and the Mayan writing. In 1966, he studied the rite of harvest of honey in the codice Madrid (Cordan 1966). And finally, among recent works, is that of Mary A. Ciaramella, who interprets the beekeepers in the same codex (Ciaramella 2002).

Studies of the native stingless bees and their relations to humans, because of the complexity of their biological history and cultures, require interdisciplinary research, combining biology, anthropology, cultural ecology, ethnomedicine, ethnozoology, biochemistry, genetics, and combinations thereof. The stingless bees have produced many things, products such as honey, pollen, cerumen, and propolis. They have tangible intellectual and economic value, as well as providing a unique source of food and medicines.

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Chapter 18
Taxonomy as a Tool for Conservation of African Stingless Bees and Their Honey

Connal Eardley and Peter Kwapong

We dedicate this chapter to Professor Charles Duncan Michener who has been a huge inspiration throughout our career. As ecology embodies taxonomy, the background created by Mich will endure and always be treasured.

18.1 Introduction

In Africa stingless bees are most diverse in the equatorial regions. To the north the Sahara Desert abruptly delimits their distribution. Southwards they become progressively less diverse reaching more or less the Tropic of Capricorn in the interior of the Subcontinent. Their distribution extends farther south along the East coast, and to a lesser extent along the west coast (Eardley 2004).

Several species appear confined to the tropical wet forests. Most species, however, occur in both savannah and tropical forests, including the east African coastal forest (Eardley 2004). Two species have been recorded from desert areas, one occurs in the south-western Sahara (*Hypotrigona penna* Eardley) and there is an unpublished record of *Liotrigona* from the Richtersveld, South Africa.

The African stingless bees are smaller than indigenous African honey bees *Apis mellifera* L. and their approximately 30 recognised subspecies (Ruttner 1988;

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Michener 2007). They also do not produce as much honey as the honey bee does, which has a larger flight range than meliponines. The robbing of honey bee nests by indigenous people has been practiced for millennia in Africa, as documented in rock paintings (Crane 1999; Johannsmeier 2001), and they are still being robbed in Africa (Eardley C, personal observations). Currently meliponiculture is practiced in tropical Africa, but for the most part its history has not been documented and its age is unknown. In Ghana, current stingless beekeeping only recently began as an activity complementary to beekeeping with *Apis* (Kwapong et al. 2010). It does not appear to have been practiced in southern Africa. However, stingless bee honey, although less in quantity, is highly sought in all of tropical Africa—primarily for its medicinal uses. It fetches higher prices than honey bee honey, and is culturally important. The current value of stingless bees, as pollinators, to biodiversity conservation and agriculture is unknown, but they do visit flowers of many different plants and crops, as seen in the field and often indicated on museum specimen labels. Being social they can possibly be more easily managed than solitary bees and the expansion of meliponiculture to agriculture should be further investigated (Roubik 1995). Vernacular names for stingless bees in South Africa are “mopani” bees or “mocca” bees. In Ghana several of the species are known by their common names: “anihammoa”, “duro kokoo”, “duro tuntum”, “mimina” and “tifuie”.

18.2 Taxonomy of Stingless Bees

Prior to Eardley (2004) research articles on stingless bees of the Afrotropical Region were relatively few, and by a handful of authors (Ambougo-Atisso 1990; Darchen 1966, 1969a, b, 1970, 1971a, b, 1972a, b, 1973, 1977a, b, 1981, 1985; Darchen and Louis 1961; Darchen and Pain 1966, Fletcher and Crewe 1981a, b; Kajobe 2006, 2007a, b; Kajobe and Echazarreta 2005; Kajobe and Roubik 2006; Lobreau-Callen et al. 1986, 1990, 1994; Michener 1959; Moure 1961; Moritz and Crewe 1988; Portugal-Araújo 1955a, 1955b, 1956, 1958, 1963; Portugal-Araújo and Kerr 1959; Sakagami et al. 1977), excluding those that described new species. Moure (1961) provides keys for the identification of many African stingless bees, but understanding the small differences between species together with intraspecific variation still prevented confident identification of many species. Consequently a taxonomic revision, based on worker bee morphology, was undertaken (Eardley 2004). Since then a lot of interest has been shown in developing meliponiculture in West Africa (Kwapong et al. 2010). Meliponiculture has been practiced for a long time in East Africa but more recently research into foraging and nesting has been undertaken (Kajobe 2006, 2007a, b; Kajobe and Echazarreta 2005), while little interest has been generated in southern Africa. There has also been interest in documenting meliponines as pollinators and the medicinal use of their honey, but to date there are no substantial data for Africa.

Eardley (2004) found that the material available in museums and comparative biological information in the literature were scant, in contrast with the great abundance of these bees in the wild. As now recognised, differentiating many stingless bee species and some genera require microscopic or molecular studies (see Rasmussen and Cameron 2010, Chap. 1, in the present book), and cryptic species have been noted (Camargo and Pedro 2007). It is now widely believed that Eardley (2004) underrepresented the true diversity of the taxon (Macharia J, personal communication). Portugal-Araújo and Kerr (1959) discovered *Hypotrigona araujoi* (Michener) to be a distinct species through observation in a meliponary, and Michener (1959) subsequently discovered subtle differences between it and *Hypotrigona gribodoi* (Magretti). Darchen (1970, 1981) studied stingless bee biology in West Africa that led to the description of three new species; *Meliponula* (*Axestotrigona*) *sawadogoi* (Darchen), *Meliponula* (*Axestotrigona*) *richardsi* (Darchen) and *Meliponula* (*Axestotrigona*) *eburnensis* (Darchen) whose types have not yet been located. Joseph Macharia found differences in the nest architecture in the species that Eardley documented as *Meliponula bocandei* (Spinola) (Macharia J, personal communication) suggesting that this taxon is composite. Katherine Krause found size differences in the species that Eardley documented as *H. gribodoi* (Magretti) which indicate that *H. gribodoi* comprises more than one species. Further, the fact that the majority of species (10 out of a total of 18 species) occur in distinct habitats, such as tropical forest and dry savannah, suggests a potential greater species diversity than recorded by Eardley (2004) (Table 18.1).

The difficulty in separating stingless bees based on morphology necessitates the need for new diagnostic tools. Nest architecture and host plant preferences pose logistical problems in gathering material for taxonomic revisions of genera and would be better suited to studies on differences between identified species rather than being used to recognise different species. The most promising tool for identifying morphologically similar species is evidently DNA barcodes, a method using a short genetic sequence to identify an organism, as suggested by Packer et al. (2009).

18.3 Host Plants and Nests of Stingless Bees

Knowing bee host plant usage is important for understanding pollination as well as the medicinal use of bee honey, as explained among the chapters in the present book, which include studies in Africa, Asia, Australia and the Neotropics. Stingless bees focus their foraging activities on a wide range of food plants. As a group they have been recorded visiting 135 plant genera (Eardley and Urban 2010). A preliminary survey of the data suggests that food plant overlap is greater within bee genera than between the genera. However, the data do not indicate if the bees are collecting pollen or nectar. Until the taxonomy is properly resolved, the degree of host plant specificity will not be understood. In Ghana, stingless bees have been collected from tropical rain forest canopies (Nuttman et al. 2011), crops growing on agricultural landscapes as well as on flowers of vegetables and medicinal plants. The most important native and introduced fruit crops on which stingless bees forage include mango, cashew, avocado, citrus, coconut, oil palm, shea butter tree, passion fruit, pepper and many others.

Table 18.1 African stingless bees and vegetation type in which they occur

Taxon	Ghanaian common names of bees	Tropical forest including east coast forest	Savannah including dry savannah and desert
Cleptotrigona cubiceps (Friese, 1912)		x	x
Dactylurina schmidti (Stadelmann, 1895)		x	x
Dactylurina staudingeri (Gribodo, 1893)	"tifuie"	x	
Hypotrigona araujoi (Michener, 1959)	"mimina" "anihammoa"		x
Hypotrigona gribodoi (Magretti, 1884)	"mimina" "anihammoa"	x	x
Hypotrigona penna Eardley, 2004	"mimina" "anihammoa"		x
Hypotrigona ruspolii (Magretti, 1898)	"mimina" "anihammoa"	x	x
Liotrigona bottegoi (Magretti, 1895)		x	x
Meliponula (*Axestotrigona*) *cameroonensis* (Friese, 1990)		x	
Meliponula (*Axestotrigona*) *ferruginea* (Lepeletier, 1841)	"duro tuntum"	x	x
Meliponula (*Meliplebeia*) *beccarii* (Gribodo, 1879)		x	x
Meliponula (*Meliplebeia*) *griswoldorum* Eardley, 2004		x	
Meliponula (*Meliplebeia*) *lendliana* (Friese, 1900)		x	
Meliponula (*Meliplebeia*) *nebulata* (Smith, 1854)		x	
Meliponula (*Meliplebeia*) *ogouensis* (Vachal, 1903)		x	x
Meliponula (*Meliplebeia*) *roubiki* Eardley, 2004		x	
Meliponula (*Meliponula*) *bocandei* (Spinola, 1853)	"duro kokoo"	x	x
Plebeina hildebrandti (Friese, 1900)		x	x

18.4 Challenges to Stingless Bee Survival

Kwapong et al. (2010) discuss some of the challenges stingless bees face in Ghana in their booklet on their management and utilisation. Conservation of stingless bees is threatened by loss of habitat from logging, bush fires and wild honey hunting, pests and predators. As most stingless bees are arboreal, when trees are cut the colonies are lost. Bush fires which constantly sweep through tropical forest during dry season burn up trees or meliponary rustic hives harbouring stingless bee colonies. Quite a number of rural communities are aware of stingless bee nests. When harvesting honey they often burn the bees and thereby destroy the colonies. The most important obstacles facing domesticated colonies of stingless bees are predators and pests, notably the small hive beetle *Aethina tumida* Murray (Coleoptera: Nitidulidae) whose larvae destroy entire colonies. Hive beetle adults live in close association with both honey bees and stingless bees. If hive beetles get an opportunity to oviposit in a colony the eggs hatch and the larvae quickly destroy the colony or cause the bees to abandon the nest. Other predators such as lizards, ants and spiders also threaten stingless bee colonies.

18.5 Justification for Further Taxonomic Research

The species name is the main tool to access the existing information on biology. If the taxonomy is inadequate, accurate biological information cannot be disseminated. The increasing demands of the human population result in the need for more food. Many foods result from pollination, and therefore pollinators need to be properly studied (Roubik 1995). As agriculture intensifies, pollination management will become more important. Increased agriculture and urban sprawl will most likely also place more pressure on the natural environment, resulting in a greater need to conserve biodiversity and the habitat of these organisms. Social bees have an advantage in pollination management in that many individuals live in a colony and they can be moved more easily than solitary bees, but similarly the loss of a colony results in the loss of many pollinators. The ability to move pollinators also introduces the risk of moving them to areas where they do not naturally occur. Moving honey bees in South Africa has had some disastrous consequences, such as the production of the pseudoclone (Neumann and Hepburn 2002) which is a social parasite of *Apis scutellata* Lepeletier.

18.6 Conclusions

Through personal observations the authors' impressions are that in East Africa the importance of stingless bees in traditional medicine is well appreciated and widely used by traditional healers. Here meliponiculture is practiced, but the detailed uses for the honey appear to be trade secrets. In other parts of Africa stingless bee honey appears to be less widely used for medical purposes, and if meliponiculture exists it is uncommon. Its wide use for food is mainly through nest robbing. Meliponiculture for agriculture is limited and very recent. Consequently, little is published on stingless bees in Africa. Nevertheless it appears from the limited studies that have recently taken place that stingless bees are an invaluable resource in Africa for biodiversity conservation, agriculture and medicine. A number of scientists throughout the continent are showing an interest in studying these bees and in the future their biology and honey should become better documented.

There is clearly a need for an updated taxonomic revision of the African stingless bees, following the recent advance made by Eardley (2004). This need is justified by their apparent importance as pollinators for agriculture and biodiversity conservation.

DNA barcoding could be introduced as a complementary tool for separating stingless bee taxa and facilitate the recognition of those morphological characters that are useful in separating species. A study should be undertaken that systematically surveys the stingless bees of Africa to maximise the likelihood of discovering the entire fauna and to document their biogeography. Where possible, host plants and nest architecture should also be documented, which provides a tool for identifying bees in the field. The data should be stored, using relational database technology, in such a way that they will be useful for research including biogeographic analyses, phylogeny and pollination ecology. Finally, before the honey, its composition and uses can be studied, the taxonomy of all living species needs further consideration.

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Chapter 19
Effects of Human Disturbance and Habitat Fragmentation on Stingless Bees

Virginia Meléndez Ramírez, Laura Meneses Calvillo, and Peter G. Kevan

19.1 Introduction

Nowadays, deforestation and the consequent loss of natural and semi-natural habitats is one of the most important causes for the decline of biodiversity and key species, such as pollinators, in terrestrial ecosystems around the world (Kevan 1999, 2001; Kevan and Imperatriz-Fonseca 2002; Aizen and Feinsinger 2003; Fahrig 2003; Foley et al. 2005; Brown and Paxton 2009). The rate of world deforestation is decreasing, but still continues at an alarmingly high rate in many countries (FAO 2011). Thus, various human activities, like agriculture, cattle ranching, selective logging, timber harvesting, urbanization, and other human activities that cause deforestation, ultimately contribute to habitat fragmentation. With those processes different habitats are reduced or divided into fragments. The degree of disturbance, coupled with the composition and structure of the original and remaining habitat and their physical characteristics are expected to influence the populations and faunal composition of the bee biota in different ways.

One would expect that species restricted to fragmented sites disappear in the short, medium, or long term, depending on the type and extent of disturbance and characteristics of the species. The rate of reduction of population would be affected by dispersal ability and potential for colonization, gene flow (e.g., Allee effect), and changes in the inter-specific interactions (Araújo et al. 2004). At present, insect conservation is based generally on species and specific habitats but ecological data

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are essential to integrating strategies into the larger landscape scale (dynamic and within interconnected habitats) through which bee conservation can be facilitated in the perspective of global environmental change (Murray et al. 2009).

The pollination of plants in tropical regions is mainly carried out by wild bees. Many different species of the social bees called stingless bees comprise ecologically important communities because of their diversity, colony size, and social habits. In fact, social bees are the dominant species in tropical bee communities (Roubik 1992; Nates-Parra et al. 2008) as well as being major pollinators of wild and cultivated plants (Heard 1999; Meléndez et al. 2002; Brosi et al. 2008). The first research on the impact of different perturbations on bees in tropical ecosystems and their fragmentation suggests that stingless bees are affected both in abundance and diversity (Brosi et al. 2007), with some species possibly endangered. For example, in the Yucatán Peninsula, Mexico, stingless bees are evidently suffering decline, as in *Melipona beecheii*, an economically and culturally important species (Cairns et al. 2005). In this chapter, we first explain the effects of human disturbance and fragmentation on the bee communities and their interactions, as now known in particular for stingless bees and then we suggest strategies for conserving these species for their ecology and economic importance.

19.2 Disturbance, Habitat Fragmentation, and Bee Communities

The different human activities like agriculture, livestock management, selective or other timber harvesting, urbanization, and generally all human disturbances that cause deforestation have the ultimate effect of fragmenting habitats. The result is a reduction of continuous habitat into spatially isolated remnants separated from each other by vegetation different from the original. Thus, plant and animal populations are diminished and become spatially isolated. Fragmentation has different effects on various habitat components through time. The total area of fragments may decrease further, the number of fragments may increase as larger tracts become further fragmented, isolation becomes more severe, and fragment shapes become increasingly dominated by straight borders (Bennett and Saunders 2010). Each of those components affects processes within and between resident populations and biotic communities (Fahrig 2003). The effects of human disturbance and fragmentation on bee communities are little studied (Cane 2001; Aizen and Feinsinger 2003; Taki et al. 2007), although it is understood that ecological interactions, such as the mutualisms in pollination, are adversely affected—the occurrence and/or abundance of the mutualistic partners notwithstanding (Bennett and Saunders 2010). Despite current concerns and controversy over the "global pollination crisis" (Kearns et al. 1998; Kevan and Imperatriz-Fonseca 2002; Ghazoul 2005; NASU 2007) there is little information on the responses of bees to land-use change and effects of tropical fragmentation on entire bee communities (Brosi et al. 2008).

Tropical bee communities comprise many species, including stingless bees (Meliponini) and *Apis* spp., which dominate and determine the structure of the communities because of their perennial and large colonies (Roubik 1992; Appanah and Kevan 1995). Bee communities in the Mexican tropics show similar patterns with the composition of species changing between vegetation types and even between cultivated areas (Meléndez et al. 2002; Novelo Rincón et al. 2003). In the on-going current study in a fragmented landscape in this area it was found that bee communities were structurally similar across fragments regardless of size, but species richness and diversity increased with fragment size. It was also found that the greater difference in species composition could be explained by greater degrees of isolation (Meneses et al. 2010).

It is important to understand ecologically that species are embedded in complex webs with mutualistic and antagonistic interactions and nowhere are these webs more complex and diverse than in tropical forest ecosystems. Differences in species interactions between ecosystems and regions reflect the particular sets of species present and the nature of the physical environment (Bennett and Saunders 2010). Extinction cascades are particularly likely to occur in degraded landscapes with reduced native vegetation, low connectivity, and intensive land use, especially if keystone species or entire functional groups of species are lost (Fischer and Lindenmayer 2007). In addition, disrupted inter-specific interactions may have exacerbating effects through other trophic levels in ecology, dispersion is a static feature, and dispersal is a process or action (Bennett and Saunders 2010).

We now know that mutualistic networks, such as pollination and seed dispersal provide well-defined and predictable patterns of interdependence between species and they are highly heterogeneous and nested (Bascompte and Jordano 2007). In such networks, a greater number of links provides greater resilience of the web through buffering between individual species against disruption of any particular interaction (Okuyama and Holland 2008). Because mutualistic webs are highly asymmetric and nested, adding to the robustness of the networks, when invasive species are inserted, web structure can be altered, with consequences for species persistence. Analysis in temperate forests of the southern Andes and oceanic islands revealed that invasive species became integrated into the networks and did not alter the overall connectivity. However, some links were replaced from generalist native species to super-generalist alien species during invasion so that connectivity among native species declined. These alterations in the structure of pollination networks, due to the dominance of alien species, can leave many native species in a new ecological and evolutionary context (Aizen et al. 2008). Until now, the effect of alien mutualists on the architecture of plant–pollinator webs and fragmentation has not been investigated in the tropics. The stingless bees in the mutualistic networks are mostly super-generalist species and could be displaced by alien species, like *Apis mellifera*, at the levels of habitat and floral interactions (Pinkus-Rendon et al. 2005; Meléndez 2006; Roubik and Villanueva-Gutiérrez 2009).

In the context of island biogeography, it is suggested that the number of links of species present in pollination webs increases twice as fast, in comparison to species richness when area increases, as a consequence of decreasing dominance (i.e., increasing evenness) of any particular interaction. This could indicate a faster loss of interaction links than of species as isolated habitats become reduced, and also has implications for conservation (Sabatino et al. 2010). In addition, theoretically one would expect negative effects of fragmentation on wild bee species to arise and cause profound structural and functional changes in plant communities (e.g., Aizen and Feinsinger 1994a; Steffan-Dewenter et al. 2006; Taki et al. 2007). Indeed, recent work has shown that pollination limitation results from the interruption of some plant–pollinator interactions in fragmented areas with high plant diversity, such as in the tropics (Brosi et al. 2008).

19.3 How Can Habitat Fragmentation Affect Stingless Bee Biology?

Stingless bees are the most diverse group (over 500 species worldwide) of all eusocial bees. They found in tropical and southern subtropical areas throughout the world (Roubik 2006; Michener 2007). They have a particular distinctiveness that must be considered to understand how human disturbance and fragmentation could impact them. Stingless bees occur in colonies from a few dozen to one hundred thousand or more workers. They live in permanent colonies, being the only highly eusocial bees together with *Apis* spp. (Michener 2007). Different species have different densities of nests in given landscapes and also differ in their capacities of flight and strategies of foraging, as shown in several book chapters herein.

19.3.1 Stingless Bee Nesting

Most species of stingless bees nest in cavities in live trees, others nest in the ground and some establish within nests of termites or ants (Salmah et al. 1990; Roubik 2006; Michener 2007). Some trees are used by several species, and sometimes several can coexist. Stingless bee nesting in natural forest has been studied in various tropical countries (Kajobe and Roubik 2006). They occur in high numbers in Borneo, Thailand, and Brazil (840, 115, and 1,500, respectively) in small areas of the moist forests there (2.8, 4, and 11.3 ha, respectively). Deforestation and fragmentation cannot but have a negative effect on species richness, abundance, and dispersal. Given that nesting resources are limited the negative effects of deforestation cannot be denied even though there is little numerical evidence to prove the scale, frequency, or severity (Roulston and Goodell 2011).

In Sabah, Malaysia, the nest density of stingless bees in undisturbed and logged-over dipterocarp forests was evaluated (Eltz et al. 2002). It was generally high in the fragments of primary forest (mean 8.4 nests/ha) but extremely low nest densities (0.5–0.7 nests/ha) in newly logged areas reflected direct impact of availability of food. According to Roulston and Goodell (2011), there is strong evidence that food availability regulates bee populations. Moreover, it has been suggested that some species appear not to be affected by disturbances. Batista et al. (2003) find 16 species of stingless bees but *Tetragonisca angustula* is the most abundant, occurring in all habitats (disturbed and undisturbed), with 31% of all nests. The ecological plasticity of this species is associated with aggressive patrolling of potential nests cavities, as documented in numerous studies (Roulston and Goodell 2011) that serve to explain its capacity to withstand perturbations. However, some species of stingless bees are restricted to forests, at least for nesting. There, nests and/or individual bees in deforested habitats may be prone to greater incidences of diseases, parasites, or predation (Brosi et al. 2007).

New research could identify the main factors driving interactions that determine the nesting sites of each species and those could include human activities in the mosaic of tropical environments.

19.3.2 Stingless Bees and Potential Flight Ranges

Another important issue is the potential flight ranges of bees in fragmented areas. When a habitat is fragmented dispersal and potential for colonization is often reduced, especially as fragments become more and more isolated by degraded and highly modified areas between them. The maximum flight ranges in bees, including stingless bees, are a function of body size especially with wing dimensions (Table 19.1). Because they are central place foragers they occupy a maximum effective space proportional to this, thus presenting strong constraint on local populations restricted to forest fragments (Araújo et al. 2004).

From the foregoing, it can be predicted that the risk of extinction is greater for smaller stingless bees than for larger ones. For example, colonies of *Plebeia droryana* (1.35 mm, maximum length of the forewing) could be effectively isolated if inter-fragment distances were greater than 600 m. In contrast, larger species, such as *Melipona compressipes* (3.25 mm) and *Melipona quadrifasciata* (2.90 mm), could be effectively isolated if forest fragments were greater than 2 km apart (Table 19.1). In theory even though larger species have a greater capacity to migrate between forest fragments their doing so but would also depend on other factors (e.g., resources requirements). Additionally, swarming in stingless bees could also act as a limiting factor in nest dispersion because new colonies of stingless bees depend strongly on the parental nest which generally provides the new nest with food and material. Thus, long-distance dispersal by individual reproductive or by swarms is impossible (Michener 2007), unlike the situation for *Apis* spp. However, the effects of fragmentation in this context have not been investigated.

Table 19.1 Bee species and flight distances (according to Araújo et al. 2004)

Bee size	Flight distances
Small bees	Maximal flight distances ranged
Nannotrigona testaceicornis (Lepeletier, 1836)	From 621 to 951 m
Plebeia droryana (Friese, 1900)[a]	540 m
Plebeia poecilochroa Moure and Camargo, 1993	From 621 to 951 m
Scaura latitarsis (Friese, 1900)	
Tetragonisca angustula (Latreille, 1811)	
Trigona sipinipes (Frabricius, 1793)[a]	840 m
Medium-sized species bees	
Cephalotrigona capitata (Smith, 1854)[b]	1,650 m
Frieseomelitta varia (Lepeletier, 1836)	From 1,159 to 1,710 m
Geotrigona inusitata Moure and Camargo, 1992	
Partamona cupira (Smith, 1863)	
Scaptotrigona postica (Latreille, 1807)	
Trigona hypogea Silvestri, 1902	
Trigona recursa Smith, 1863	
Larger bees	
Melipona bicolor Lepeletier, 1836	Greater than 2 km
Melipona compressipes (Fabricius, 1804)[a]	2,470 m
Melipona marginata Lepeletier, 1836[c]	800 m
Melipona quadrifasciata Lepeletier, 1836[a]	2,000 m
Melipona scutellaris Latreille, 1811	Greater than 2 km

With the fitted linear regression, maximum flight distance = 1,383.333 ± 645.185 (generalized wing size) ± error, they estimate the maximum flight distance for 12 species of stingless bees from their generalized wing size. Each estimated value represents a mean expectation of the maximum flight distance for each species with an associated error

[a]Using mark-recapture method: Kerr (1987), [b]Roubik and Aluja (1983), [c]Wille (1983)

19.3.3 Stingless Bee Foraging

Habitat fragmentation could affect foraging by stingless bees, the colonies of which are largely self-organized. Some species (solitary foragers) trust individual forager decision making in the field. Other species belong to the obligate foragers group that relies largely on collective decision making, with foragers following each other and even communicating in the nest. The species-specific balance between individual and collective decision-making determines the foraging niche of each species. The coexistence of multiple species with different foraging strategies indicates that the various strategies are complementary in as to how food is extracted from their ever-changing habitat (Beismeijer and Slaa 2004).

Within a community of stingless bees the species overlap extensively in foraging range (e.g., Eltz et al. 2002; Slaa 2003), and inter-specific encounters are common. Reactions vary widely from avoidance to attack, depending on the species combination. Aggressive species are sometimes attracted to a heterospecific, generally leading to the departure of the latter. However, avoidance seems more common, and is accurately predicted by relative body size of the two species. Thus, unaggressive species avoid aggressive species and smaller species generally avoid larger species (Slaa et al. 2003). The complex interactions between small and large, aggressive and unaggressive species and between species with similar sizes and behaviors suggest that deforestation and fragmentation change the insect–plant and insect–insect interactions with negative results for both species diversity and functional diversity, although some species could be more favored than others.

19.3.4 Stingless Bees, Disturbance and Habitat Fragmentation

In the tropics few studies have investigated factors like deforestation, logging and shifting cultivations, fragmentation, and their relationship with diversity and abundance of stingless bees. Early studies in Sumatra indicate that species diversity and abundance of stingless bees decreased along anthropogenic disturbance gradients in secondary forests and at higher altitudes (Salmah et al. 1990). In Rondônia, species richness in *Melipona* increased with increasing forest cover and proximity to forests and adverse effects of deforestation were detectable, despite the fact that significant areas of tropical forest cover remained (Brown and Albrecht 2001). Samejima et al. (2004) in Sarawak, Malaysia, reported that for stingless bees nest density is positively related to the density of large trees (>50 cm DBH) and that some species were abundant in the primary forests, whereas others in disturbed forests. Nevertheless, in this study, species richness was not affected by human disturbance, but the relative abundance of the species may have been affected both by nest site availability and food resource limitations. Thus, it is suggested that changes in the composition of pollinator community may also affect tree community composition in the long term.

In Costa Rica, Brosi et al. (2007) studied the effects of distance to forest, tree management, and floral resources on bee communities. They found no clear differences in bee diversity or abundance regarding pasture management or floral resources. However, the bee community composition was evidently different at forest edges than in deforested countryside only a few hundred meters away. The sites at the edge of a relatively large forest contained a much higher proportion of social stingless bees and a relatively low proportion of *Apis*, whereas non-edge sites showed the opposite pattern. The eusocial bee fauna of the study area comprised principally stingless bees and honey bees together; they are distinctive in quickly recruiting foragers to high-quality resources. Thus, it is necessary to emphasize the importance of the diverse assemblage of native stingless bees that cover a wide range of body sizes and flower foraging behavior not found in honey bees.

Bee community responses to forest fragment size, shape, isolation, and landscape contexts including pastures adjacent are examined by Brosi et al. (2008) in southern Costa Rica. This study suggests no effects of forest variables on bee diversity and abundance, although strong changes in bee community composition are noteworthy. In particular, tree-nesting stingless bees are associated with larger fragments, smaller edge: area ratios and greater proportions of forest surrounding sample points. Community composition is also markedly different between forests and pastures, despite their spatial proximity. In forests, even in the smallest patches, stingless bees comprise a large proportion of bee communities.

On the other hand, in the Yucatán Peninsula, particularly in Quintana Roo, Mexico, changes in the communities of stingless bees illustrate the effects of human-induced ecosystem disturbance. The community with the greatest anthropogenic disturbance had lower overall species richness of stingless bees and the highest degree of dominance of the Africanized honey bee (*A. mellifera*), while the area with the most ecosystem conserved had the highest diversity of stingless bees, though *A. mellifera* was still the dominant species where in general bee numbers are lowest, richness of stingless bee species and evenness were higher in ecosystem conserved than in the more disturbed sites (Cairns et al. 2005). Similarly, Roubik (2009) found the greater abundance of honey bees in disturbed sites and lower abundance in the forest in neotropical areas.

Although some changes in habitat are directly perceptible after fragmentation (e.g., shifts in habitat pattern, forest structure and composition at edges, changes in population sizes) other changes may emerge only after a long time. For example, genetically related changes on populations and lost or extinction of species often take years to become evident. In stingless bees, as in many organisms, genetic drift is a process frequently exacerbated by the isolation of small, local populations. For example, populations *Melipona* spp. are highly susceptible to the effects of genetic drift. In *M. scutellaris* within a population based on extended breeding from a small number of founder colonies there was a great reduction in the number of alleles even though with low genetic variability the population could be maintained for nearly 10 years (Alves et al. 2011). Thus, in some species of stingless bees, breeding from a small reserve of colonies may have less drastic consequences than previously assumed. Additional studies of genetic variability in other species are urgently needed to support strategies for the conservation of stingless bees.

Recently, an overview of studies in tropical ecosystems on how bees are affected by human disturbances (Winfree et al. 2009) indicated that stingless bees and solitary bees are the most affected (Table 19.2).

19.4 Conservation and Importance of Stingless Bees

Despite the fact that there are few studies in the tropics, all indicate that the local bee communities are negatively affected by human disturbance and fragment size. For stingless bees conservation is essential to identify that the sizes of fragment from medium to large are those in which that maintenance of the greatest number of susceptible species and in this way are adequate to design conservation strategies (Meneses et al. 2010). Also, it is important to consider the establishment of corridors to improve the connectivity between fragments in any conservation strategy for reducing the impacts of fragmentation on wild bee community (Bennett and Saunders

Table 19.2 Effect of anthropogenic disturbance on tropical bees (from data base of Winfree et al. 2009)

BTx	A/R	E[a]	DT	BT	BS	Country	Reference
Apis	A	+	F	Tsdbf	Social	Argentina	Aguilar (2005)
	A	–	F	Tsmbf	Social	Argentina	Chacoff and Aizen (2006)
	A	–	F	Tsmbf	Social	Costa Rica	Ricketts (2004)
	A	+	F	Tsdbf	Social	Argentina	Aizen and Feinsinger (1994b)
	A	–	F	Tsgssh	Social	Australia	Blanche et al. (2006)
Bombus	A	–	F	Tsdbf	Social	Argentina	Aguilar (2005)
Bee[b]	A	–	F	Tsdbf	Solitary	Argentina	Aguilar (2005)
	A	–	F	Tsgssh	Solitary	Australia	Blanche et al. (2006)
	A	–	F	Tsmbf	Solitary	Brazil	Becker et al. (1991)
	A	–	F	Tsmbf	Solitary	Brazil	Powell and Powell (1987)
	R	+	F	Tsmbf	Solitary	Brazil	Becker et al. (1991)
	R	–	F	Tsmbf	Solitary	Indonesia	Klein et al. (2006)
	A	–	F	Tsmbf	Social	Argentina	Chacoff and Aizen (2006)
	A	+	F	Tsmbf	Social	Malaysia	Eltz et al. (2002)
	A	–	F	Tsgssh	Social	Argentina	Blanche et al. (2006)
	A	–	Lg	Tsmbf	Social	Malaysia	Eltz et al. (2002)
	R	+	F	Tsmbf	Social	Malaysia	Eltz et al. (2002)
	R	–	Lg	Tsmbf	Social	Malaysia	Eltz et al. (2002)
Bee[c]	A	–	F	Tsmbf	All	Costa Rica	Ricketts (2004)
	A	+	F	Tsmbf	All	Indonesia	Klein et al. (2003a)
	A	–	F	Tsdbf	All	Argentina	Aizen and Feinsinger (1994b)
	R	–	F	Tsmbf	All	Argentina	Aguilar (2005)
	R	–	F	Tsmbf	All	Costa Rica	Ricketts (2004)
	R	–	F	Tsmbf	All	Indonesia	Klein et al. (2003a)
	R	–	F	Tsdbf	All	Argentina	Aizen and Feinsinger (1994b)
	R	+	F	Tsgssh	All	Australia	Blanche et al. (2006)
	R	+	Ag	Tsmbf	Solitary	Indonesia	Klein et al. (2002)
	R	–	F	Tsmbf	Social	Indonesia	Klein et al. (2002)
	R	–	F	Tsmbf	Social	Brazil	Brown and Albrecht (2001)
Bee[d]	A	–	F	Tsmbf	All	Indonesia	Klein et al. (2003b)
	R	–	F	Tsmbf	All	Indonesia	Klein et al. (2003b)

BTx = bee taxon, A = abundance/R = species richness, E[a] = effect, DT = disturbance type, BT = biome type, BS = bee sociality, Ag = agriculture, F = fragmentation, Lg = logging, Tsdbf = tropical and subtropical dry broadleaf forests, Tsmbf = tropical and subtropical moist broadleaf forests, Tsgssh = tropical and subtropical grasslands, savannahs, and scrublands

[a] The effect was measured by Hedge's unbiased standardized mean difference (Hedge's *d*). Positive values of the effect size (*d*) imply positive effects of anthropogenic disturbance on bee abundance or richness, whereas negative (*d*) values imply negative effects

[b] Non-*Apis* and non-*Bombus*, [c] Non-*Apis*, [d] Non-*Bombus*

2010; Meneses et al. 2010). Although bee populations are known to fluctuate temporally (e.g., Roubik 2001; Roubik and Wolda 2001), they need resources throughout the year.

The strong correlation between body size and flight range in stingless bees could be useful for developing strategies to conserve tropical bee diversity (Araújo et al. 2004).

In addition, the conservation of these bees requires a continual assessing of their genetic diversity where effects of genetic drift (Allee effect) could be operating, as in fragmented areas. A simulation model has been developed to determine the number of colonies needed to maintain a certain number of sex alleles in a population, thereby providing useful guidelines for stingless bee breeding and conservation (Alves et al. 2011).

The decline of stingless bees in the central area Quintana Roo, Mexico, particularly for the once-economically important *M. beecheii* suggests that both habitat change and increased competition with an invasive species (*A. mellifera*) have contributed to this decline. Moreover, selective logging affects several important nesting tree species for stingless bees in general, and other changes in the vegetation mosaic may also have contributed to the decline of *M. beecheii* (Cairns et al. 2005). Thus, habitat management strategies are needed. Conserving stingless bees require maintenance of natural forest because distance between fragments and overall isolation probably explains the presence of a large proportion of rare species restricted to only one fragment (Meneses et al. 2010), but the habitat requirements of wild bees are largely unknown and need investigation.

The potential of native habitat to provide bee pollination services to agriculture is particularly most important in the neotropics, where also stingless bees are the principal pollinators (Kevan and Imperatriz-Fonseca 2002). Although beekeeping is promoted as an agroeconomic activity, the honey bees (*A. mellifera*) used cause the displacement of stingless bees from resources floral (Pinkus-Rendon et al. 2005; Roubik and Villanueva-Gutiérrez 2009). For local agriculture, the synergistic effects of combinations of species of pollinating bees are becoming recognized (Meléndez et al. 2002). Even in a crop as important and well known as coffee wild bee diversity and abundance in association with forest patches have been correlated with larger crops (Ricketts et al. 2004, Klein et al. 2003a). In addition, stingless bees have proven efficient pollinators in crops in greenhouses (e.g., Cauich et al. 2006; Palma et al. 2008a, b) and their domestication has great potential (Meléndez et al. 2004).

Finally, key species, such as stingless bees, in the tropics are required for ecosystem function and ecosystem health, floral resources and nesting sites (i.e., as trees of sufficient thickness) will sustain the bees and their pollination interactions with the wild biodiversity of flowering plants and crops.

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Part III

What Plants Are Used by the Stingless Bees?

Chapter 20
Palynology Serving the Stingless Bees

Ortrud Monika Barth

20.1 Introduction

Like the honey bees, stingless bees collect nectar, pollen grains, and resins from a large group of plant species. Palynological analysis of several bee products, such as honey, bee pollen, bee bread (brood provisions), geopropolis (resin collected by stingless bees), and royal jelly, allows one to identify the associated pollen species, and to understand composition of vegetation used by the bees.

20.2 Bees, Vegetation, and Pollen Grains

20.2.1 The Bees

The stingless bees (Meliponini) and honey bees (Apini) both are pollinators of native and exotic plant species and harvest honey and pollen appreciated by humans—and also by predatory animals. It is of interest to know more about bee food preference and floral choice, and also of economic interest.

Pollen grains obtained directly from bees or taken from nests and colonies indicate foraging activities during a day, a week, a month, or even a year. These data deserve detailed investigation and evaluation in order to assess quality or quantity of bee products and to exploit the bee preferences for flowering plant and crop pollination. Pollen analysis is a refined scientific approach for investigating these subjects.

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Two methods of pollen analysis are normally pursued. Physicochemical methods provide information about honey and pollen grain composition, including the moisture, pH values, acidity, ash, sugars, proteins, and more. On the other hand, palynological methods detect where bees obtain nectar and pollen, and some other materials such as resin.

Stingless bee honey properties depend upon the bee species. The bees consume nitrogen compounds of the plant phloem, while sugars and minerals are maintained in the processed nectar stored as concentrated sugar, in honey. Their honey presents a higher water content and more saccharose and mineral elements than honey of honey bees. On the other hand, the quality of *Apis mellifera* honeys depends upon the plant resources, and the honeys often have lower concentration of water, sucrose, and minerals (Bazlen 2000).

20.2.2 Vegetation to Benefit Bees

Stingless bees occur in several continents, mainly in tropical and subtropical regions, and are adapted to different types of vegetation including forests, savannas, fields, marshes, and mountains. Honey and harvested pollen change in composition depending upon fluctuating plant species flowering.

In South America, Brazil is of continental size and its types of vegetation (Veloso et al. 1991) change across the landscape. The majority of tropical plants depend upon pollination activities of insects, birds, and bats, and the stingless bees play a major role (Roubik 1978, 1980).

Absy and Kerr (1977), using pollen analysis of honey, began the study of stingless bee floral visitation in the Amazon region, which comprises different forest types, savannas and riversides, as well as human-disturbed landscapes and urban areas. Absy and collaborators pursue observations on stingless bees over several years (Oliveira et al. 2009), demonstrating that a great variability of pollen resources, not commonly dominated by a unique plant species, are used by the bee species studied. Furthermore, the trophic niches of stingless bee species frequently overlap (Silva et al. 2004).

Similar investigations in the Brazilian Northeast region concentrate in the state of Bahia, including semiarid localities. A sequence of investigations for 10 years by Bazlen (2000) and Carvalho et al. (2001) analyzed honey samples of Meliponini. Although representing a diversity of flowers, the honey revealed a significant monofloral element. The source of pollen loads (Ramalho et al. 2007) and residual nest pollen (Dórea et al. 2010) is known from different localities during different years and months in this region.

Palynological investigation of pollen loads and honey of stingless bees in the Brazilian Southeast region has a long tradition (Barth 2004), starting with honey analysis by Iwama and Melhem (1979), and more recently with the analysis of forage pollen (Hilario and Imperatriz-Fonseca 2009) and pollen contained in storage pots

(Malagodi-Braga and Kleinert 2009). The last investigation compares the results obtained from corbicular loads on returning foragers to the pollen in storage pots.

Larval food pollen analysis provides additional information about southern Brazil in Santa Catarina (Cortopassi-Laurino et al. 2009). Human activity degrades natural vegetation and is readily revealed by pollen analysis of honey and bee corbicular loads.

Pot-honey and pot-pollen of Meliponini outside Brazil have also been investigated. Recently, Flores and Sánchez (2010) obtained the first results for *Tetragonisca angustula* from Salta, Argentina, showing some monofloral honey. Freitas et al. (2010) compared the food resources of Meliponini in different regions of Brazil and Venezuela using pollen analysis of honey samples.

20.2.3 Pollen Grains

What is the difference between pollen analysis and palynological analysis? When considering pollen grains in honey, pollen loads, bee bread, and propolis/geopropolis, the study involves pollen analysis. On the other hand, when work considers additional structures found among honey, pollen, and nest products, such as bacteria, spores, and fungal hyphae, yeast, oil, wood, plant hairs (trichomes), and other materials, it is a palynological analysis. This enables us to make a better interpretation of the phytogeographic origin, cleanliness, bee storage, and manipulation of these products (Barth 1989).

Research efforts consider in general the pollen grains alone. Distinction between pollen grains of nectariferous, polleniferous, and anemophilous plant species must be made to obtain a valid result and diagnosis. Super- and sub-representation of pollen grains of some plant species, abortive pollen grains (e.g., *Citrus* Rutaceae), and amyloplasts (e.g., *Zea mays* Poaceae), protoplasts (e.g., *Persea* Lauraceae), and gemma (e.g., *Bauhinia* Fabaceae, Caesalpinioideae) inside the palynological preparations must be recognized, and considered for diagnosis.

Pollen morphology overlaps frequently between plant species and genera, so an exact identification cannot be made. For this reason, the usual technical terminology may relate to a pollen type at family, genus, or species level. A detailed knowledge of local plants visited by bees, however, may allow recognition of the pollen and nectar sources.

The knowledge of pollen morphology is most often an accurate instrument with which to analyze bee products. Several publications illustrating pollen and spore morphology and terminology are available. Based upon pollen structure definition in Erdtman (1952) and Faegri and Iversen (1950), ordinary terms of pollen morphology are translated and illustrated in Portuguese by Barth (1965, 1975) and Barth and Melhem (1988). The standard English version today follows Punt et al. (2007).

20.3 Palynological Analysis of Honey

Honey of Meliponini stored in pots, when compared with honey of *A. mellifera* stored in combs, is more liquid, presenting a higher degree of water, sucrose, hydroxymethylfurfural (HMF), and ash, as noted in the literature, considering several phytogeographical regions of honey production. These properties depend mainly on the bee species. Melissopalynological studies of stingless bee honey are rare, limited to *Melipona seminigra merrillae* and *Melipona rufiventris paraensis* (Absy and Kerr 1977), *Melipona compressipes*, *Melipona favosa*, *Melipona trinitatis*, *Frieseomelitta nigra*, *Frieseomelitta* sp. *aff. varia*, *Plebeia* sp., *Scaptotrigona* sp. *aff. depilis*, *Scaura latitarsis* and *T. angustula* (Vit and Ricciardelli D'Albore 1994), *Melipona scutellaris* (Carvalho et al. 2001), *Melipona mandacaia* (Alves et al. 2006), *T. angustula* (Flores and Sánchez 2010), and *M. favosa* (Vit et al. 2012). Specific information about the use of *Eucalyptus* species by the bees was detailed in the thesis of Bazlen (2000).

Stingless bee honey can be divided into two groups. One shows dominance of a unique pollen type (more than 45% of all counted nectariferous pollen grains). Such monofloral (or unifloral) honey maintains similar physicochemical and sensory properties, while heterofloral honey varies in its characteristics (Ferreira et al. 2007, 2009).

Visiting flowers to collect nectar, Meliponini were considered sometimes to be specialists, producing monofloral honeys, and sometimes generalists, producing heterofloral honeys. The observed results depend upon several factors, including the blooming plant species and the available number of flowers, the content of nectar sugars and water, and the weather, as well as the bee species. Bazlen (2000) studied 92 meliponine honey samples, from the Brazilian states of Bahia, São Paulo, and Rio Grande do Sul, and considered physicochemical and palynological characteristics. Seventy-five samples (81.5%) comprised monofloral honeys. The main dominant pollen type was of Myrtaceae (without species or genus identification) in 27 samples (36%), followed by Fabaceae, Mimosoideae in 11 samples (14.5%) which mostly lacks nectar. Altogether 12 plant families were responsible for these monofloral honeys.

Pollen analysis of bimonthly collected honeys of *M. scutellaris* in 15 colonies at Bahia State is presented by Carvalho et al. (2001). *Eucalyptus* was the dominant pollen type in all samples, except one of *Psidium*.

In parallel, pollen analysis of 11 honey samples of *M. mandacaia* (Alves et al. 2006), obtained in a semiarid region at the state of Bahia, reveals that *Piptadenia rigida* (Fabaceae, Mimosoideae) is the dominant nectariferous pollen in six samples, and *Ricinus communis*, an anemophilous plant species, in one sample. *Piptadenia moniliformis* pollen grains were dominant inside one sample (97.6%) from Paraguassu, Bahia (Junior and Santos 2003), and a species of Euphorbiaceae (51%) in *Trigona spinipes* honey at São Cristóvão, Sergipe State (Oliveira et al. 2008).

T. angustula was considered to be a generalist bee in foraging choice, although four honey samples from a total of eight presented dominant pollen types, two of *Mitracarpus* (Rubiaceae), one of *Ziziphus joazeiro* (Rhamnaceae), and one of *Zornia* (Fabaceae, Faboideae), in an arid region of Bahia State (Novais et al. 2006).

Table 20.1 Original and corrected pollen percentages >3%, in Amazonian *Melipona* honey

Samples	Families	Pollen types	Common names	Original %	Corrected %
Amazonas 1	Brassicaceae	*Brassica*	mostarda	–	3.3
	Fabaceae	–	–	3.3	10.0
	Gesneriaceae	–	–	4.9	15.1
	Melastomataceae	–	–	**66.8**	–
	Solanaceae	*Solanum*	lobeira	22.7	**69.8**
Amazonas 2	Lythraceae	*Cuphea*	sete-sangrias	5.8	18.9
	Melastomataceae	–	–	**52.1**	–
	Fabaceae, Mimosoideae	*Mimosa scabrella*	bracatinga	17.3	–
	Solanaceae	*Solanum*	lobeira	24.8	**81.1**
Amazonas 3	Anacardiaceae	–	–	4.1	26.4
	Burseraceae	*Protium*	almecegueira	9.8	**62.6**
	Fabaceae, Caesalpinioideae	*Crudia*	jutairana	–	3.3
	Gesneriaceae	–	–	–	3.3
	Melastomataceae	–	–	**81.2**	–
	Fabaceae, Mimosoideae	*Mimosa scabrella*	bracatinga	3.1	–

– Non-identified pollen types, unknown common names, and frequency below 3%, bold = dominant pollen type, frequency >45%

Three honey samples of *Melipona* obtained in the Brazilian Amazonas region (Table 20.1) were analyzed by Freitas et al. (2010). All of them contained dominant pollen grains. Two samples of *M. compressipes manaosensis* and *M. seminigra*, obtained at the Manacapuru region, were from a nectariferous Solanaceae; this result was based upon a correction of the percentages of counted pollen grains, when those of polleniferous plants (Melastomataceae and *Mimosa scabrella* pollen type) were excluded. The third sample of *M. seminigra*, obtained in Porangaba, was from *Protium* (Burseraceae). A similar result was obtained by Absy et al. (1980) in the Amazon region of Manaus. Two of the four honey samples of *Melipona* obtained at Paraíba State (Freitas et al. 2010) showed a dominant pollen type of *Crotalaria* (Fabaceae, Faboideae) that comprises several species and genera of the Fabaceae presenting the same pollen morphology.

One honey sample of *T. angustula*, obtained at the region of Içara, Santa Catarina State, presents a dominant pollen type of *Hovenia dulcis* (Rhamnaceae), analyzed by Freitas et al. (2010).

20.4 Palynological Analysis of Bee Pollen

Pollen harvested by Meliponini is known from recent work in the Brazilian states of Amazonas and São Paulo. Different methodologies of pollen load collection and of palynological analyses were utilized, and are not readily compared. A standard methodology of pollen load analysis was proposed recently by Barth et al. (2010). In summary, two grams of bee pollen are washed with ethanol, homogenized, and five hundred or more pollen grains of one drop of this well-mixed suspension are considered. It was demonstrated that pollen grain color is not plant species/genus/family specific (Barth et al. 2009).

Papers by Marques-Souza et al. (2002, 2007) analyzed bee pollen in Amazonas State, finding *Stryphnodendron guianense*, *Schefflera morototoni*, *Miconia myriantha*, and *Myrcia amazonica* to be the most frequent. Oliveira et al. (2009) reported no dominance of any pollen type in the same area.

Pollen loads taken off from *M. scutellaris* at several localities of Bahia State (Salvador, Cruz das Almas, Alagoinhas) are investigated and compared with those obtained from *Apis* (Ramalho et al. 2007). Pollen types were recognized at family level only, and no dominance signalized, but an overlapping of resource plants is noted. Chemical composition of pollen loads study was based upon pollen identification, resulting in a predominance of yellow-colored loads of *Mimosa gemmulata* (98.95%) and bright colored loads of a Fabaceae (Silva et al. 2006), also in Bahia. Dórea et al. (2010) investigate pollen residues in nests of *Centris tarsata* at the Canudos Biological Station, a semiarid locality in Bahia State. The caesalpinoid Fabaceae *Chamaecrista ramosa* (46.5%) was the unique dominant taxon among the 31 pollen types identified. Pollen of pot samples investigated by Oliveira et al. (2008) at the locality of São Cristóvão, Sergipe State, shows a dominance of Celastraceae (46.9%) pollen grains.

Pollen resources of *Melipona* are largely known from studies in the Brazilian state of São Paulo, mainly inside the campus of the State University, São Paulo city. Most recently, Malagodi-Braga and Kleinert (2009) present studies in the same locality, and emphasize the importance of *Eucalyptus* pollen nearly throughout the year, and of isolated plant pollen for alternative resources.

Floral origin of pollen harvested by *Plebeia saiqui* inside pots was investigated by Pick and Blochtein (2002) during 1 year in São Francisco de Paula, Rio Grande do Sul state. No dominant plant species could be detected, but species of the Asteraceae were prevalent.

20.5 Palynological Analysis of Geopropolis (Meliponine Propolis)

Plant exudates, resins, waxes, plant tissues, and trichomes, mixed with more or less 5% pollen grains, were the main ingredients of honey bee propolis (Barth 1998; Barth et al. 1999). On the other hand, meliponine geopropolis does not contain trichomes, but in addition these bees mix resins and waxes with earth, and frequently collect mud or clay, small pebbles, seeds, and sometimes sand. Spores and fungal hyphae, soot, and amorphous organic material are commonly present (Fig. 20.1 and Table 20.2). Further, the pollen grain spectrum reflects the vegetation of the phytogeographical regions or localities (Barth 2006; Barth and Luz 2003).

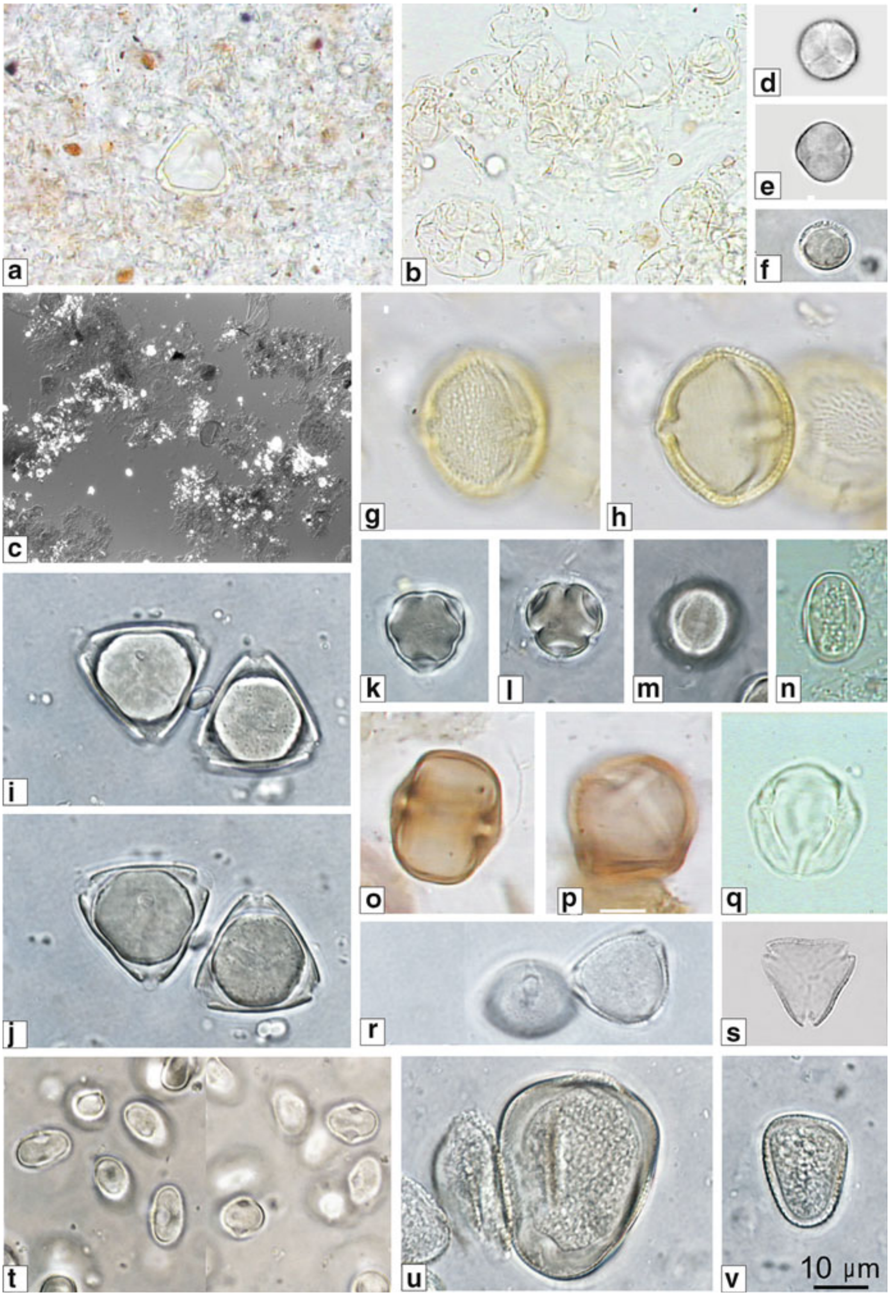

Fig. 20.1 Structured elements of geopropolis sediments. (**a**) *Eucalyptus* (Myrtaceae) pollen grain inside a complex structured sediment of geopropolis (Meliponinae) before acetolysis treatment. (**b**) Glandular trichomes of propolis (*Apis*) before acetolysis treatment (for comparison with a geopropolis sediment). (**c**) Geopropolis sediment after acetolysis treatment, polarized illumination. (**d**–**v**) Pollen grains; (**d** and **e**) tetrads of *M. scabrella* pollen type; (**f**) Piperaceae; (**g** and **h**) *Schinus* (Anacardiaceae); (**i** and **j**) *Eucalyptus*; (**k**–**n**) Melastomataceae; (**o**) *Protium* (Burseraceae); (**p** and **q**) *Solanum* (Solanaceae) pollen type; (**r** and **s**) *Myrcia* (Myrtaceae) pollen type; (**t**) *Cecropia* (Cecropiaceae); (**u** and **v**) Cyperaceae. All figures of 1,000× magnification, except figures 1–3 of nearly half of this magnification. Photos: O.M. Barth

Table 20.2 Evaluation of nest entrance geopropolis of six bee species considering frequency of sediment constituents, except pollen grains, after acetolysis

Bee species	Organic material	Sandy fragments	Spores and hyphae of fungi	Soot (burned organic material)
Lestrimellita cf. limao	+	++ (crystals)	+	+
Trigona recursa	++	+ (crystals)	+	+
Tetragonisca angustula	+	+++ (sandy powder)	++	+++
Melipona quadrifasciata	+++	+ (crystals)	+	+
Nannotrigona testaceicornis	+	–	++	+
Frieseomelitta varia	+	–	+	+

(+++) Very frequent, (++) frequent, (+) few, (–) not detected (Barth 2006)

Dominant pollen types were *Eucalyptus* (Myrtaceae) in samples from São Paulo state (Barth 2006), *Schinus* (Anacardiaceae) in one sample of Minas Gerais, and *Myrcia* (Myrtaceae) in samples of several states (Barth and Luz 2003). Anemophilous and polleniferous pollen, as of *Cecropia* (Urticaceae), *M. scabrella* (Fabaceae, Mimosoideae) pollen type, and *Piper* (Piperaceae), were sometimes well represented in geopropolis samples.

20.6 Conclusions

Summarizing the actual knowledge about pollen analysis of honey, pollen loads, and harvested pollen of the stingless bees, Meliponini, in Brazil, and considering the great size of this undertaking in such a remarkably large tropical country, scant data are available on the plant species offering the bees nectar and pollen. Most investigations recognize only the plant family. Detailed field study, followed by standard laboratory processing of samples and phytogeographic characterization of study sites and regions, will be the most promoting way to provide better resolution of meliponine behavior within the vast Brazilian regions.

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Chapter 21
How to Be a Bee-Botanist Using Pollen Spectra

David W. Roubik and Jorge Enrique Moreno Patiño

21.1 Introduction

To better understand tropical biology, we would like to see the world as a bee does (Fig. 21.1). Two central tasks exist for a foraging bee. The first is to find rewarding resources, and the second to remember the route between the nest or home base and the resource. Once a bee is successful finding food, it can move back and forth until the food is depleted, both within a single day and within the flowering period of that plant. In tropical wild lands, that course of action includes primarily the forest canopy (Roubik et al. 1984). And because so many flowers are not observable, despite the labors of field biologists, we are still woefully ignorant of which flowering plants are most important to the honey-making social bees, especially stingless bees and honey bees (Roubik 1989, 1993; Roubik and Hanson 2004; Roubik et al. 2003; Corlett 2011). Such bees are termed "generalists" because they use many floral species, but this term is qualitative, not quantitative. Substantial research has attempted to give quantitative pollen data and its potentially important role in understanding which plant species are most important to bees (classic studies by Louveaux 1968; Barth 1970a, b; Maurizio 1975; Iwama and Melhem 1979; see also Roubik et al. 1984; Roubik 1989; Villanueva-Gutiérrez and Roubik 2004; Roubik and Moreno 2009). Palynology and its specialized subdisciplines of melittopalynology and melissopalynology (see present book chapters and Roubik 2009)—more simply termed bee-botany and bee-palynology—provide the best approach to connect bees with their food sources, whereby pollen taxonomy is applied to plants—used opportunistically and steadfastly pollinated by bees. Pollen taxonomy, we believe,

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Fig. 21.1 A tropical lowland forest, Barro Colorado Island (9° North latitude) viewed from above the canopy. Photo: Archives at the Smithsonian Tropical Research Institute

is the first step in a process of training persons who study bees to study the plants that bees depend upon and also to extract the most information possible from bee biology field and laboratory studies, using the identification of pollen as a foundation. If we fail to interpret our own data, then we have not been able to graduate as "bee-botanists," which is that to which we must aspire.

We studied the honey of two social bees in tropical American forests and semi-forested areas, and also the actual pollen loads brought in the nest by returning foragers, to demonstrate how melittopalynology (bee–pollen studies) and melissopalynology (honey–pollen studies) can be organized to answer biologically meaningful questions, such as the translation of pollen identification and counts to resource importance for bees, and their ecology.

21.1.1 Pollen and Bee-Botany

Bee resources dominant in pollen counts, both in honey and in pollen provisions in bee nest cells, are often small woody plants, plants thought to be anemophilous, or herbs growing on the forest edge. Their grain number, instead of concentration, weight, or volume in a bee "pollen spectrum" (see Barth 1970a, chapter in present book, and below) indicates they are present, but little else. This presence was determined to be either "isolated" (very rare) or "accessory" (intermediate), when not dominant. We employ a different analytical technique and methods, in an attempt to simplify the categorization of pollen choice, abundance, and importance, especially in honey (nectar) sources. Furthermore, unless careful field studies are made of whether bees or other nectar drinkers, such as flies, butterflies, wasps, or birds, are foraging nectar at a flower, the nature of the floral resource is unknown from its pollen detection in a bee product. Dioecious plants—many palms, for example—and up to 25% of tropical forest tree species in a given natural, mature forest (Henderson 1986), provide only pollen (and scent) at one sex of flower and often only scent at the other. They are deceptive mimics. Variation in the floral resource-pollinator theme (e.g., Latham and Mbuta 2011), without adequate field study, also deceives researchers on bee-flower ecology. Indeed, Iwama and Melhem (1979) summarize the findings of researchers and indicate that, even within a single genus (*Alchornea*, Euphorbiaceae), some species have nectar, but many do not, and this also occurs in *Miconia* (Melastomataceae) and *Acacia* (Fabaceae) (Sornsathapornkul and Owens 1998; Stone et al. 2003; Dos Santos, et al. 2010).

How can we accurately demonstrate the specialization we perceive (Roubik 1992) in the resources a generalist bee uses? Like the foraging bee, the biologist must try to find a way to establish important links. Pollen quantification can be misleading. As already mentioned, pollen does not always indicate a nectar source (and the female flower of dioecious species may often have nectar, but never pollen). Furthermore, because different bee–pollen species have grains ranging 7–300 μm in diameter (Roubik 1989; Roubik and Moreno 1991) there is a difference between individual grains of almost a quarter million (216,000) volumes (see Roubik 1989). In other words, the largest and smallest grains are potentially of identical importance to a bee as harvested food if there is one of one species and 216,000 of the other. When pollen slides are prepared and pollen grains counted along a transect, omission of one of the large grains constitutes a serious loss of biological data. Consider, for example, grains of Cucurbitaceae (e.g., *Cayaponia* spp. 200 μm diameter) versus those of *Miconia* or *Piper* (7 μm), see Roubik and Moreno (1991). Because generalist bees use large numbers of plant species, but not all species are used evenly—either in raw volume as protein or in quality—(Roubik 1988, 1989; Biesmeijer et al. 1992; Roulston et al. 2000) there is certainly a potential and sometimes large degree of specialization for "generalist" foraging bee species.

21.1.2 *Quantitative Methods for Bee-Palynology*

In response to the challenges mentioned above, our first step was to determine pollen volume, either as it comes mixed in honey or as pollen gathered by individual bees as pollen loads. Pollen volumes are quantified, in the case of honey bees, either by counting the pellets trapped from the hind legs and then identified (Roubik et al. 1984), by dry weight, or by computing individual grain volume of the plant species (Villanueva-Gutiérrez and Roubik 2004). Another method, which we use here, is an internal standard from spore tablets of *Lycopodium*, with a known number of spores per tablet batch (Stockmarr 1971). This is a straightforward method, much like weighing individual pollen pellet loads of a single species, to quantify the portion per weight or volume of sample pollen species (Roubik and Moreno 2009).

Three analytical methods are introduced here. First is that of determining pollen concentration, which we call "importance" in a pollen spectrum. The second is to document high importance across sites or apiaries, which we call "consistency." Both methods require corrections from raw pollen counts, based either on number of grains, relative to spore counts, per gram or per cubic centimeter. For corbicular pollen loads, those of *Apis mellifera* at one apiary (from which honey also was collected) were sorted by color. The pollen species, and concentrations of different pollen types per gram, were determined for each designated color variety. In addition to providing a comparison of corbicular pollen to pollen found in honey, this method tests whether color is a valid index of pollen species and whether the density of different pollens in a pellet (pollen load from one bee's leg) is similar across species.

We obtained honey samples from apiaries and meliponaries with collaboration of local beekeepers and also used colonies maintained by the first author in Panama. The two bee species studied were *Tetragonisca angustula* (Latreille 1811) (which may include other cryptic species, Camargo and Pedro 2007) and the Africanized honey bee, Neotropical *Apis mellifera*—close to African *A. mellifera scutellata*, but no longer identified as that subspecies (Francoy et al. 2008). Honey was collected in clean 50 ml plastic vials from a sample of the entire nest honey, or in the case of *Apis* from multiple colonies, at the normal honey harvest time and usually stored under refrigeration until analysis. Pollen pellets of the corbicular load of *Apis* were taken at the hive entrance with an exterior screen commercial pollen trap and collecting pan.

To obtain an estimate of pollen volumes for different species, *Lycopodium* spores were added before carrying out the acetolysis process, with a known weight and volume, to provide an internal standard that allows calculation of relative proportions of the same pollen species in different samples or in multiple slides prepared from a single processed sample (Roubik and Moreno 2009).

Eighteen samples of honey removed from bee hives of *Apis mellifera* at 17 lowland (<500 m elevation) localities in Bolivia, Brazil, Venezuela, Mexico, French Guiana, and Panama and from 11 lowland localities for *Tetragonisca angustula* in Panama, Bolivia, and Peru (Tables 21.1 and 21.2) were used for pollen analysis. For *Apis mellifera*, Step 1 was only applied to nine samples of Venezuelan honeys from seven sites and for two apiary samples from two sites, near Sinnamary, French Guiana. Venezuelan sites varied considerably and were located in agro-ecosystems with some natural vegetation patches available. Both the French Guiana sites were in forest–savanna or along a mangrove areas with coastal forest, with very little human disturbance of vegetation, aside from the roads and seasonal savanna burnings.

Table 21.1 Numbers of plant taxa in pollen spectra, by locality, from honey of *Tetragonisca angustula* in tropical lowlands

	Locality										
	Peru	Bolivia								Panama	
Plant taxa	San Martín	Isozog A Kopere	Isozog B Karapari	Pampas A Beni	Pampas B Beni	Yungas	Ixiamas	Sacramento	Chaco	STRI	Curundu
Families	13	36	21	36	20	29	22	24	18	25	34
Genera	13	43	27	53	22	33	23	28	20	25	47
Species	15	52	31	62	23	41	27	34	22	27	57

Colony samples from: Peru: San Martín; Bolivia: Isozog A—Kopere, Isozog B—Karapari, Beni—Pampas A, Beni—Pampas B, Coroico, Yungas, Ixiamas, Sacramento, Chaco; Panama: Ancon area, Curundu Flats (see Supplemental Data)

Table 21.2 Numbers of plant taxa in pollen spectra, by locality, from honey of Africanized *Apis mellifera* in tropical lowlands

	Locality																	
	Brazil	Bolivia				French Guiana		México	Panama	Venezuela								
Plant taxa	Curitiba	Ixiamas	Isozog	Pampas	Coroico	Bord du Mer	Forêt et Savanne	Chetumal	Curundu Flats	Barinas A	Barinas B	Barinas C	Cojedes	Anzoátegui	Bolivar	Lara	Miranda	Trujillo
Families	14	34	16	28	27	23	17	23	25	22	20	22	27	16	36	34	20	30
Genera	19	43	20	32	34	24	19	29	33	24	21	27	37	18	58	46	24	36
Species	19	52	24	37	37	27	24	36	39	31	23	34	44	26	71	60	30	46

Colony samples from: Brazil, Curitiba; Bolivia, Ixiamas, Isozog, Pampas, Coroico; French Guiana, Sinnamary—bord du mer, Forêt et Savanne; Mexico, Chetumal; Panama, Curundu Flats; Venezuela, Barinas—Altamira de Cáceres A, Barinas—Altamira de Cáceres B, Barinas—Guanare-Barrancas, Cojedes, Anzoátegui, Bolívar, Lara, Trujillo, and Miranda (see Supplemental Data)

The following general methods describe our acetolysis procedure for honey and pollen pellet samples:

1. One *Lycopodium* tablet was added to each sample (a "batch" of the tablets is accompanied by information on its mean spore count per tablet; batch 938934 had a mean = 10.700 spores (*T. angustula* and *A. mellifera*), batch 124961 a mean = 12.500 spores (*A. mellifera* only)).
2. Samples were dissolved in water and sieved with mesh (250 μm).
3. Samples were concentrated at 2,700 rpm/5 min and supernatant discarded.
4. Residues were dried with glacial acetic acid.
5. Samples were concentrated at 2,700 rpm/5 min and supernatant discarded.
6. Solution of Acetolysis was added (nine parts of anhydride acetic acid and one part of sulfuric acid concentrated)/heated 5 min, to destroy all cellulose content and to clean pollen grains.
7. Samples were concentrated at 2,700 rpm/5 min and the solution of Acetolysis discarded.
8. Samples were then washed with distilled water and their residues concentrated.
9. Ethanol was used as dehydratant and samples were concentrated at 2,700 rpm/5 min.
10. The ethanol was discarded and some drops of glycerol were added.
11. Finally, permanent microscope preparations were made using glycerin jelly as mounting media and paraffin as sealant.

To identify all pollen grain types, transects of all slides were made at ×40 magnification using an Olympus BH-2 binocular scope. Electronic microphotographs of material were obtained at ×100 magnification using a Pixera Camera System attached to the Olympus scope. The botanical names of families, genera, and species were established by comparisons with pollen atlases (see References). Species names preceded with "cf." Or "prob." indicate the identification based on neotropical pollen collections kept at the Center for Tropical Paleoecology and Archaeology (CTPA) of the Smithsonian Tropical Research Institute (STRI) in Panama require further confirmation, using collections of the local flora at particular sampling sites.

The procedure for analyzing botanical species importance to a bee species was as follows: pollen concentrations or counts were determined for each pollen type. Given that the volume and weight of a subsample from the entire collected pollen were determined and the number of *Lycopodium* spores added to the sample known, each subsample has an exact pollen and spore concentration, revealed by the number of *Lycopodium* counted and its ratio to other pollen types. For example, if 10,000 spores of *Lycopodium* had been added to 1 g or 1 cc of acetolyzed pollen sample, and a transect count had produced six spores with 12, 60, and 300 grains of pollen types A, B, and C, then the total of those grains would be estimated as 20,000, 100,000, and 500,000.

The concentration method, described by Stockmarr (1971)—see also O'Rourke and Buchmann (1991)—was used in one group of honey bee samples from Venezuela, the largest for one region in this study ($N = 9$), pollen of corbicular loads, and in the honey samples of *Tetragonisca*, to calculate the ratio of *Lycopodium* spores to the pollen in question. We provide details here on the methods used to compare the importance of a given botanical resource among sites. The assumption is made that the spores are distributed evenly among all the pollen types on the microscope slide preparation. To compare between sites or different colony samples, the total number of spores counted is also inversely proportional to the total amount of sample grains present. That is, if one sample returned 20 grains of a pollen type and recorded ten spores, and a second sample returned ten grains of the same pollen type with five spores, the proportional representation or concentration of the pollen in the two subsamples was identical.

Our procedure for identification and quantification of pollen indicates three possible categories. The first is the number of grains counted in transects, the second consists of cases in which only one grain was found in transects, and the third category, signifying "presence," occurs when one or more grains were found on the microscope slide preparation, but none within transects used to count the spores and total pollen (see Supplemental Data).

Pollen resource importance is examined first as a histogram, in which species are ranked by their total concentrations or counts, from highest to lowest. This may be done for a single colony or honey sample, or combined samples from several colonies, different areas, or regions. The idea is to see whether certain species, genera, or families stand out as important resources. Pollen resource consistency is next examined, for those resources that registered high importance. This step requires precise taxonomy, so that the same taxa can be registered as present or absent. We also evaluate relative concentration (proportion of the total sample) across sites. Here we chose to make the comparisons using the plant genera scored as important, then expanded that category to include species, as discussed below.

Resource counts and concentration are graphed after ranking in descending order. An overall list of important resources is made on the basis of plants that are both important and consistently used by the bees (see Supplemental Data for individual colonies and honey samples from apiaries of *A. mellifera*). In this way, we try to establish whether in a majority of sites, or samples, the pollen spectrum and relative importance were similar. In addition, also based on the concept of consistency, we examine the plant resources (family or genus) which were used in most sites, but were not among the high-volume resources. As will be made clear in the following sections, the analysis of importance using a histogram is an intuitive approach. A simple curve-fitting procedure was applied to the ranked pollen resource histograms testing logarithmic, exponential, and power functions for goodness of fit, using Microsoft Excel.

21.1.3 Application to Stingless Bees and Honey Bees

After genus and mostly species identifications were made of pollen, resource richness was categorized and analyzed. For the stingless bee *Tetragonisca angustula*,

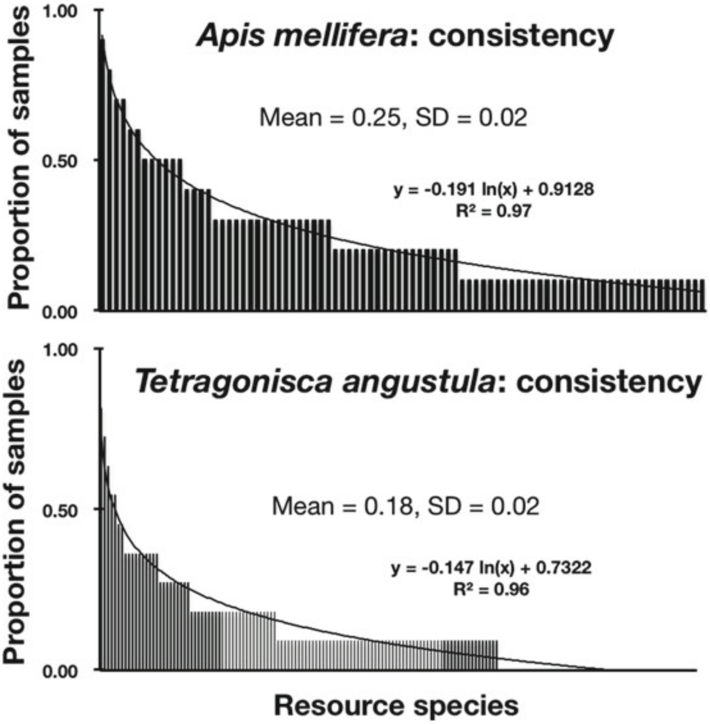

Fig. 21.2 Pollen consistency—representation across sites—for *Tetragonisca angustula* and *Apis mellifera* at 11 and 18 sites, in three and six countries, respectively

and including all pollen identified, the 11 samples produced 175 species and 134 genera in 69 plant families (Table 21.1 and Fig. 21.2). For *Apis mellifera*, the 18 honey samples contained 206 species, 156 genera, and 76 families (Table 21.2). Plant species enumerated from pollen in honey samples from Africanized *Apis mellifera* in largely forested areas of natural vegetation. Honey bee colonies had a mean of 46 species in their honey (range 19–71), while those of the stingless bee averaged 35 species (range 15–62). An index of pollen diversity, the number of botanical species divided by the number of samples, yielded approximately 16 for *T. angustula* and 12 for *A. mellifera*. However, little difference was found between the means of the averages for each region, 33.3 for *T. angustula* and 34.6 for *A. mellifera*. There was an "outlier," with considerably lower pollen richness, in each bee study—that of Curitiba, Brazil for *A. mellifera* and that of San Martín, Peru for *T. angustula*.

Pollen corbicular pellets from Africanized honey bees in French Guiana, sampled during April from one apiary near the coast and one in the interior forest and savanna, included 22 color categories. A total of 1,048 pellets was analyzed, an average of 24 of each color (SD=35). The average pellet weight was 4.39 mg (SD=2.12 mg). The color subsamples of each apiary, to which one *Lycopodium* tablet was added, averaged 134.59 mg (SD=237 mg). Total concentrations of pollen

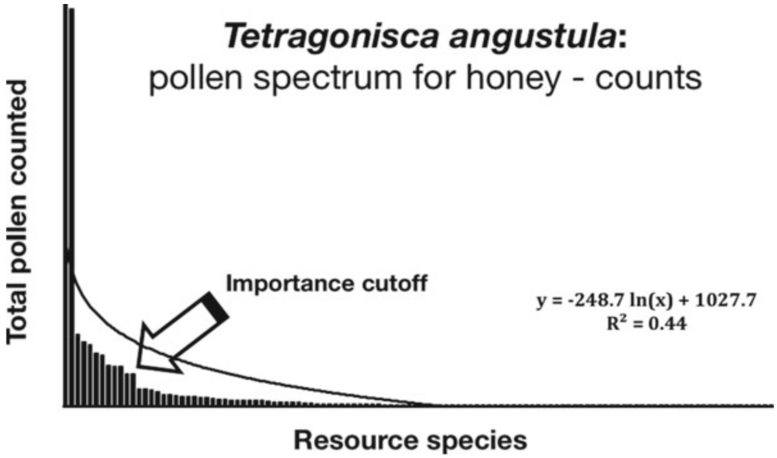

Fig. 21.3 Pollen species as indicated by counted pollen grains of different species in honey of *Tetragonisca angustula* at 11 sites, 3 Neotropical countries

grains per mg in those pellet samples were calculated to range from 4.54×10^6 to 1.573×10^{10}. Thus, individual pollen species grains differed in weight by over 3,000-fold.

There were 37 pollen types in the corbicular pollen samples, and each pellet color contained an average of four (range 1–7) species. A single pollen of the shrub, *Mimosa pudica*, was 89% of total pollen pellets, represented by 1.59×10^9 grains in 5 g. Corbicular pellets strongly dominated by *M. pudica* ranged in color from almost white to light brown to light yellow (see Supplemental Data, pollen loads of *A. mellifera* spreadsheet). Those color differences may be due to the degree to which the pellets had been dried.

Botanical resources of *Tetragonisca angustula* included plants with one or more grains counted in a sample and are ranked in total volume in Fig. 21.3. There is a clear break in the curve after the 11th species, with those below that rank counted as less than 200 grains. The "top 10" species are considered in Table 21.3, further modified for actual pollen volume(Fig. 21.4). The total number of sites and the total volume are given with the plant taxonomy (see also Supplemental Data). From pollen counts alone, summed among the diverse lowland sites, a papilionaceous legume (*Machaerium*) and a genus of Rubiaceae (*Macrocnemum*) were far more common than the next most common families—but Anacardiaceae clearly predominated in consistency and would appear equally important (but not as a source of nectar). The summed pollen concentrations better quantify taxonomic preferences. An Anacardiaceae (*Spondias*) was the most important pollen source (it has no nectar, see Carneiro and Martins 2012), with *Gouania* (Rhamnaceae), *Machaerium*, and *Macrocnemum* following, then the palm *Scheelea* (a pollen-only source), *Anacardium*, *Eugenia*, *Alternanthera*, *Miconia*, and *Calopogonium*. These were the top ten plants, after which the remainder drop well below the predictive distributional

Table 21.3 Pollen counts, consistency, and concentration (volume) of major resources indicated by honey pollen analysis for *Tetragonisca angustula*

Pollen counts and sites present				Pollen concentration		
Plant genus	Plant family	Sum pollen count	Sites	Plant genus	Plant family	Sum % conc.
Machaerium	Fabaceae, Papilionoideae	2,767	6	*Spondias*[a]	Anacardiaceae	136
Macrocnemum	Rubiaceae	1,210	2	*Gouania*	Rhamnaceae	120
Gouania	Rhamnaceae	446	4	*Machaerium*	Fabaceae	97
Eugenia	Myrtaceae	401	3	*Macrocnemum*	Rubiaceae	78
Anacardium	Anacardiaceae	381	8	*Scheelea*[a]	Arecaceae	66
Alternanthera	Amaranthaceae	321	3	*Anacardium*	Anacardiaceae	59
Rubiaceae[b]	Rubiaceae	252	1	*Eugenia*	Myrtaceae	58
Miconia[a]	Melastomataceae	250	3	*Alternanthera*	Amaranthaceae	47
Spondias[a]	Anacardiaceae	248	6	*Miconia*[a]	Melastomataceae	40
Scheelea[a]	Arecaceae	203	4	*Calopogonium*[b]	Fabaceae, Papilionoideae	35
Acacia[a]	Fabaceae, Mimosoideae	200	2	Rubiaceae	Rubiaceae	24
Guazuma[b]	Sterculiaceae[c]	168	2	*Hyptis*[b]	Lamiaceae	19
Poaceae[a]	Poaceae	–	7	*Acacia*[a]	Fabaceae, Mimosoideae	18
Cecropia[a]	Urticaceae	–	6	*Piper*[a]	Piperaceae	17
Asteraceae	Asteraceae	–	7	*Serjania 2*	Sapindaceae	15
Euphorbia	Euphorbiaceae	–	6	*Syzygium*	Myrtaceae	14
Celtis	Cannabaceae	–	11	*Triumfetta*	Tiliaceae[c]	11
				Guazuma	Sterculiaceae[c]	11
				Asteraceae	Asteraceae	11
				Arrabidaea	Bignoniaceae	11

The cutoff range was determined at 200-grain counts (see Fig. 21.4) and at two portions of the pollen spectrum curve—concentration

[a] Nectarless flowers

[b] Concentration rank changed presence and ranking from raw count data

[c] Sterculiaceae and Bombacaceae are now included in Malvaceae sensu APG III

curve (Fig. 21.5). The potentially nectarless *Acacia*, and the solely nectarless and *Piper* were very low in the overall ranking.

Botanical resources of *Apis mellifera* quantified by our ranking methods followed a logarithmic curve, and two relatively minor cutoff points were found for the Venezuelan honey samples (Fig. 21.6). Remarkably, one floral species was the single most important resource for *Apis* in this research. *Apis mellifera* in both Venezuela and French Guiana lowlands used *Mimosa pudica* heavily, a plant with no floral nectar. Identification of corbicular pollen from French Guiana and honey samples of both countries (Table 21.3, Fig. 21.7) ranked this plant species highest, excessively so as a seasonal pollen source. In Fig. 21.6 this species is depicted far

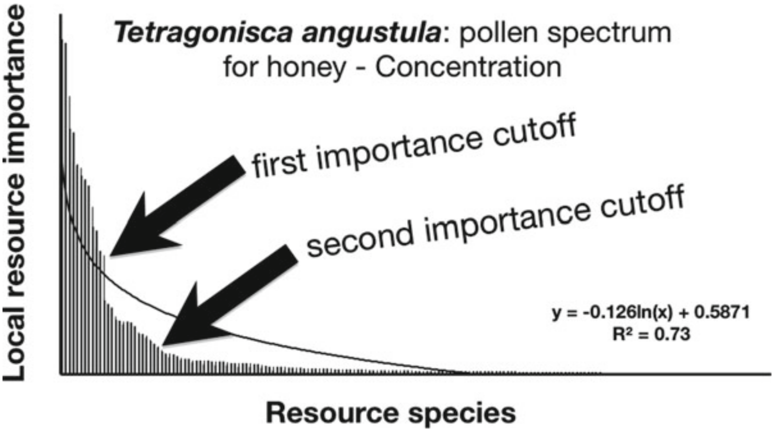

Fig. 21.4 Pollen concentrations provided by internal calibration marker spores of *Lycopodium* in Neotropical honey of *Tetragonisca angustula* (see Fig. 21.3). The "cutoffs" are points where the importance declines greatly between sequential ranked species

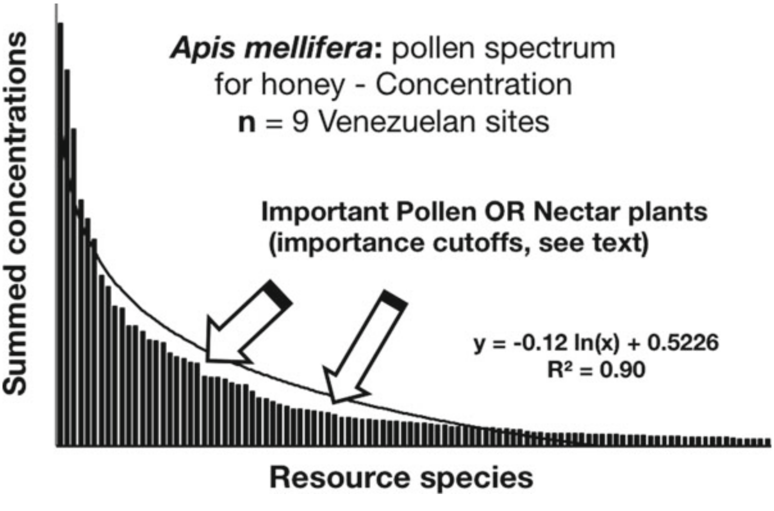

Fig. 21.5 Pollen concentrations and cutoff points for floral resource importance in Neotropical honey of Africanized *Apis mellifera*

above the predictive curve ranking pollen found in honey. The simple pollen counts without concentration marker *Lycopodium* also registered relatively abundant *Mimosa* in four of the additional six regions—southern Brazil, Mexico, Panama, and Bolivia. In Suriname, in contrast (Biesmeijer et al. 1992; Kerkvliet and Beerlink 1991) there was a predominance of *Cecropia* among pollen species, which indicates recently disturbed habitats, like forest edges that have been cleared or burned

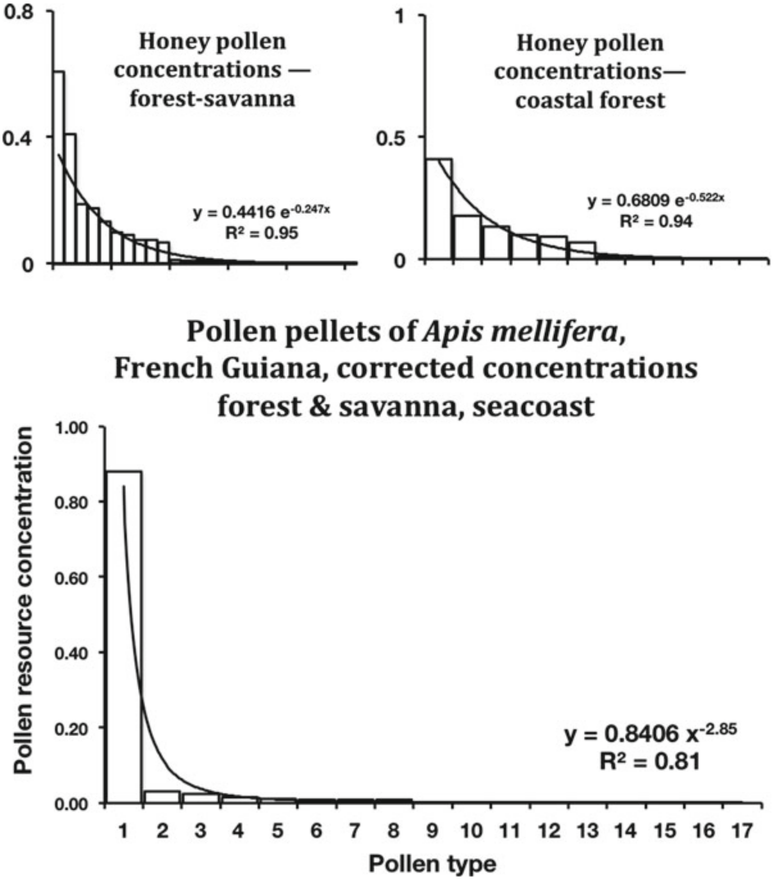

Fig. 21.6 French Guiana corbicular pollen data and honey data for *Apis mellifera* data at two natural sites, with the combined sites shown for honey pollen species concentrations

(Roubik 2009), and little in common, aside from various palms in the sample, with the forest, coastal, and savanna samples taken in French Guiana.

The consistency of pollen genera across sites averaged 0.25, SD 0.02, so that we would expect each recorded genus to occur in one of four lowland sites. The most consistently scored genera and the summed pollen concentrations (the concentrations summed across sites) indicated that eight floral resources were outstanding in their importance to *Apis mellifera*; half of them do not produce nectar (Table 21.4). The potentially most important nectar sources, those not obviously pollen-only flowers, were *Psidium* (Myrtaceae), *Alchornea* (Euphorbiaceae), *Hyptis* (Lamiaceae), and *Roystonea* (Arecaceae). Many palms have no nectar in their flowers (Henderson 1986). The *Alchornea* are dioecious, and nectar of female flowers therefore leaves no trace of pollen (but flowers of both sexes, at least of some species, have nectar; Latham and Mbuta 2011).

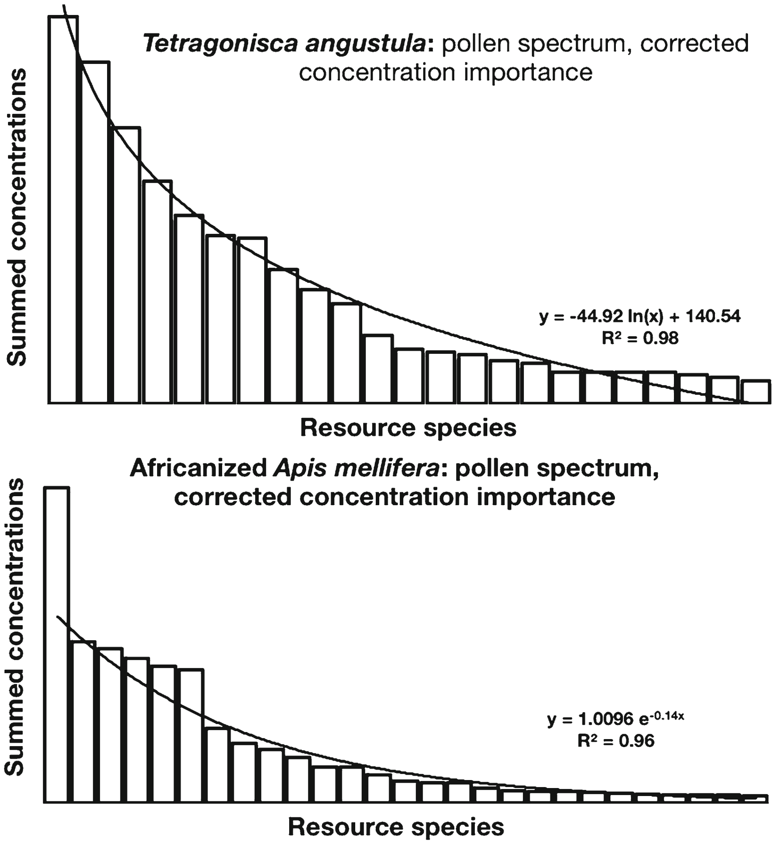

Fig. 21.7 Corrected (summed) pollen concentrations combining all Neotropical sites sampled for *Tetragonisca angustula* and *Apis mellifera*, shown for honey pollen species concentrations

Pollen pellets had similar pollen types of major importance, in proximal habitats of French Guiana—each with some natural savanna and extensive forest, and were combined (Fig. 21.6). These showed a power function ranking, with only one type far more abundant than the more uniformly abundant remainder. In their honey pollen types, the forest–savanna area had ten abundant pollen types, while that of the coastal forest contained only five.

The following significant forage species, indicated in Fig. 21.7 as decreasing gradually in importance, contained scattered pollen-only flowers in the ranking—*Cecropia*, *Doliocarpus*, Poaceae, *Mimosa*, and *Anthurium*. This leaves, as likely important nectar sources, two Myrtaceae, one Sapindaceae, one Bignoniaceae, two Asteraceae, one Euphorbiaceae, one Rutaceae, one Ulmaceae, two papilionaceous legumes, one Anacardiaceae, one Melastomataceae, and one Elaeocarpaceae.

Corrections for consistency and volume of pollen types across sites provide distributions plotted in Fig. 21.7, which returned a power function for *Apis* and a logarithmic one for *Tetragonisca*, each highly significant ($R^2 = 0.96$–0.98). There is

Table 21.4 Pollen concentration indicating rank in importance of floral nectar and pollen sources, and consistency (no. sites present) for honey of *Apis mellifera* from seven Venezuelan sites (see Table 21.2)

Plant	Family	Sum pollen concentration	No. sites
Mimosa pudica[a]	Fabaceae, Mimosoideae	667	6
Psidium	Myrtaceae	500	3
Piper[a]	Piperaceae	269	4
Alchornea[a]	Euphorbiaceae	251	2
Hyptis	Lamiaceae	217	5
Roystonea	Arecaceae	170	2
Cecropia[a]	Urticaceae	166	2
Doliocarpus[a]	Dilleniaceae	162	4
Poaceae 1[a]	Poaceae	131	1
Eugenia	Myrtaceae	130	1
Serjania	Sapindaceae	105	1
Poaceae 2[a]	Poaceae	96	2
Asteraceae	Asteraceae	88	1
Bignoniaceae	Bignoniaceae	70	1
Asteraceae	Asteraceae	69	1
Mimosa casta[a]	Fabaceae, Mimosoideae	62	2
Croton	Euphorbiaceae	60	1
Zanthoxylum	Rutaceae	57	1
Celtis	Cannabaceae	57	1
Syzygium	Myrtaceae	54	1
Fabaceae, Papilionoideae	Fabacaeae, Papilionoideae	52	1
Desmodium	Fabaceae, Papilionoideae	48	1
Mangifera	Anacardiaceae	42	1
Spondias[a]	Anacardiaceae	40	1
Anthurium[a]	Araceae	40	1
Miconia[a]	Melastomataceae	37	1
Sloanea	Elaeocarpaceae	36	1

[a] Nectarless flowers

a sudden drop in importance for *Apis* after the first and sixth-ranked species. *Tetragonisca* displays a smooth curve with no sudden decline in rank, except after the tenth species in the ranking. The rankings and consistency overviews, including raw counts, are given for *T. angustula* in Table 21.3, and the consistently major pollen, based on volume (concentration), for *Apis mellifera* is given in Table 21.4. Some of our photomicrographs of the most important pollen types are given for comparison in Fig. 21.8.

21.1.4 Pitfalls of Pollen Analysis and Need for Field Observation

We frequently observe plants flowering over many weeks, or even all year, and this may explain their abundance in seasonal or yearly samples. Moreover, those pollen

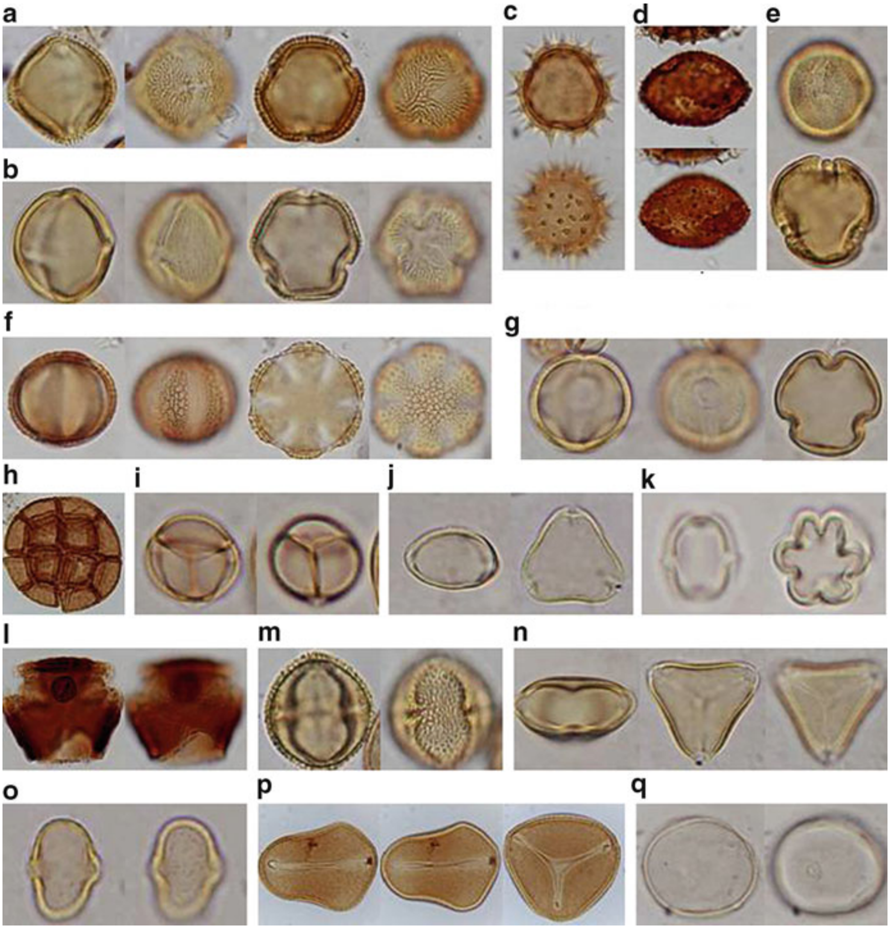

Fig. 21.8 Most important pollen resources. *Dicotyledoneae*: Anacardiaceae: (**a**) *Anacardium* sp., (**b**) *Spondias* sp. Asteraceae: (**c**) Undetermined. Boraginacae: (**d**) *Cordia* sp. Euphorbiaceae: (**e**) *Alchornea* sp. Lamiaceae: (**f**) *Hyptis* sp. Fabaceae-Faboideae: (**g**) *Machaerium* sp. Fabaceae-Mimosoideae: (**h**) *Acacia* sp., (**i**) *Mimosa* sp. Myrtaceae: (**j**) *Eugenia* sp. Melastomataceae: (**k**) *Miconia* sp. Rhamnaceae: (**l**) *Gouania* sp. Rutaceae: (**m**) *Zanthoxyllum* sp. Sapindaceae: (**n**) *Serjania* sp. Urticaceae: (**o**) *Cecropia* sp. *Monocotyledoneae*: Arecaceae: (**p**) *Scheelea* sp. Poaceae: (**q**) Undetermined (×100) (photos not in same scale). Photos: J.E. Moreno Patiño

grains are often relatively small, smaller than 10 μm in diameter, and may occur in high density in a honey sample. The same kind of small and numerous pollen is sometimes incorrectly associated with a nectar source. Although the pollen is found in honey in a hive comb or food pot, the plant does not have nectar and cannot be a honey source (e.g., *Piper*, in Kerkvliet and Beerlink 1991, or *Cecropia*, in Villanueva-Gutiérrez and Roubik (2004), or *Spondias* (Carneiro and Martins 2012), or *Acacia* (apparently, from a detailed study of a hybrid, Sornsathapornkul and Owens 1998)); numerous small grains do not signify importance (Biesmeijer et al. 1992). Further, we believe there is nectar in *Alchornea*, *Trema*, *Theobroma*, and many palms, but more study is needed.

We found the Africanized honey bees and *Tetragonisca angustula* tend to use diverse but distinctive groups of floral resources (typically three or four dozen families, genera, and species) in lowland Neotropical areas, and they specialize heavily among them—shown by pollen ranking using power, logarithmic, and exponential functions. Dominant pollen is often no indication of a nectar source, if flowers are nectarless, thus the less abundant pollen types in honey may serve to indicate some important nectar plants. The consistencies with which resources were utilized across a range of sites were predictable for both bees by a logarithmic curve, but for pollen pellets (pollen analysis alone) a power function was superior, and *A. mellifera* showed high consistency. Simple pollen counts for *T. angustula* were difficult to fit with a regression model as to rank ($R^2=0.44$). Pollen counts corrected for density in the sample—concentration and total volume—produced different species ranks and even introduced or removed species from consideration in the top 20 floral species. We do not yet have a comprehensive picture for annual pollen use and floral visitation for an entire year at any site. However, the combination of sites, during wet season, dry season, and primarily, the time in which most honey is harvested, or peak "honeyflow" (see Villanueva-Gutiérrez et al. 2009) give us some confidence that the data are representative of floral importance.

The pollen types of honey from the nest, and from pollen loads, were dominated by a nectarless "roadside weed," *Mimosa pudica*, which provides pollen to diverse bees, primarily *Melipona* in vast forest regions, in early morning, but is often monopolized by Africanized *A. mellifera* near de-forested areas (Roubik 1996). Nine of the most widespread honey bee sources were nectarless or dioecious. This was not true for the stingless bee, although it had nectarless flowers of *Spondias* as one of its major, consistent resources. In addition, the honey bee used many grasses and, although *T. angustula* also uses nectarless grasses, sedges, and palms (see Chap. 23 by Obregón et al. in this book) these were not among its main resources. The vast majority of both bee resource spectra were trees (in the semi-forested and forested habitats, see Supplemental Data).

Pollen content, presented as a list of species (see Supplemental Data, pollen pellets of *A. mellifera*), provides basic information on flowering plants used by bees, but often, particularly in botanically rich environments, does not lead to any particular insight or prediction. Honey bees and solitary bees both are generalists (Roubik and Villanueva 2009) but this is not a guide toward understanding either ecology or management. Counts of grains as indices of resource importance, with no further quantification, are likely to be inadequate or misleading. The pollen concentration in honey and nectar varies greatly (Bryant and Jones 2001). As mentioned in the pollen and bee-botany section, grain volumes vary widely, and pollen importance (concentration) in one sample may not be comparable to that in another. We believe the *Lycopodium* density marker can be used to make adequate corrections for the different grain sizes found in melittopalynological samples. As for simply examining pollen taken from the field or the bees—with no chemical treatment to remove the interior protoplasm and expose exine characteristics—in the tropics, where there are roughly 1,000 species in a given habitat, and 20% have the same gross pollen characteristics (Roubik and Moreno 1991), correct identification, even at the family level, is very often impossible. On the other hand, some playnologists, if they have an adequate reference collection, discern tropical genera or families with only pollen grains taken from bees and slide-mounted in water (M. Burgett, personal communication).

Our organization of the pollen obtained from honey has made clear, for both a stingless bee and the Africanized *Apis* of the Neotropics, the difference between an abundant pollen source and the source of nectar. This kind of information is seldom regarded in melissopalynology, but now there is easy access to literature with Internet search engines, but there is still, above all, the need for direct observation, in the field, of bees visiting flowers. If they are using the tongue to extract nectar, it should be noted.

Apis uses large amounts of pollen to support its brood production and swarming, while the stingless bees do not often swarm and should use less pollen (Roubik 2006). Therefore, the honey bee leaves much more pollen from nectarless sources within its nest, and that pollen finds its way into the honey. For honey, honey bee samples contained large quantities of pollen that may be called "contaminants" in nectar (Iwama and Melhem 1979) of other species which provide the liquid source of honey. The flowers of *Mimosa*, *Acacia*, *Piper*, *Senna*, *Cassia*, *Cecropia*, *Spondias*, *Doliocarpus*, all grasses (Poaceae), most Solanaceae, most Melastomaceae, and many palms have no nectar foraged by bees, and some dioecious flowers may be exploited solely for pollen. Yet pollen of this botanical origin, at species or higher level, is common in honey among *Apis* (Kiew 1997; Roubik 1989, 2005; Adekanmbi and Ogundipe 2009; and chapters of the present book).

In lowlands from sea level to several hundred meters altitude, tropical flowers first open near 6 a.m. or sunrise, and flowers that were open during the night also present their surplus nectar and pollen (Roubik 1989, see Corlett 2011). Our lowland samples reflected these trends, but it remains to be seen whether similar findings would apply to tropical highlands. Pollen usually is depleted at flowers in the morning and before nectar, because it is not continuously secreted. With most foraging in early morning for pollen, loose pollen is distributed throughout the bee nest. That pollen can easily be carried all day, on the bodies of active nest bees and foragers, into areas of nectar storage. Why is so much pollen from non-nectar species contained in honey of certain tropical bees? The timing and intensity of foraging are likely the key. Pollen is present in large quantities in the early morning, for example, from *Mimosa pudica*, *Piper*, or grasses (Roubik 1996). For a seasonal pollen in Venezuela, *Apis* had 89% of its pollen volume or mass one nectarless type—also a major food for *Melipona*—among 37 species identified. It was *Mimosa pudica* (Roubik 1996). Barth (1970a) also found much *Mimosa* pollen in the honey of *Apis mellifera* in Brazil, as did Iwama and Melhem (1979) in the honey of *Tetragonisca angustula* there. The value of the present comparative study is this: the stingless bee used fewer major pollens but used them more evenly than did *Apis*. We suggest a generally more even distribution of resource types for stingless bees, and predominance of non-nectariferous pollen in honey of Africanized honey bees contrasted to stingless bees, is due to their extensive swarming and greater demand for pollen.

The stingless bees take a long time to reproduce, whereas *Apis* does so freely, and apparently at least once a year (Roubik 1989, 2006). However, both groups take advantage of pollen and also nectar, that is ostensibly to feed large nocturnal animals that visit large flowers, dioecious species or those with no nectar, and dense inflorescences. This still appears to be the general situation for tropical honey-making bees, when importance and not only species-lists are considered (Roubik 1989).

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Chapter 22
Important Bee Plants for African and Other Stingless Bees

Robert Kajobe

22.1 Introduction

Stingless bees are distributed throughout the tropical parts of the world (Michener 1979, 2000; Camargo and Pedro 1992; Roubik 1992). Their highest abundance may be in the neotropical region. There are hundreds of stingless bee species existing in the world and these vary in colony size, body size, body color, and biology (Roubik 1989, 1992; Michener 2000; Eardley 2004). The fauna of Afrotropical meliponines is smaller than that in neotropical or Indo-Malayan/Australasian faunas (Rasmussen and Cameron 2006). The Afrotropical meliponines have relatively fewer species (Eardley 2004) and genera (Moure 1961). The African meliponine has also a relatively low abundance in most parts of Africa (Darchen 1972; Kajobe and Roubik 2006). Stingless bee colonies have a single queen (Sakagami 1982; Velthuis et al. 2001). The founding of a new colony occurs by colony fission and swarming. Stingless bees, like solitary bees, produce brood, with an egg placed on top of a food mass in a sealed cell (Sakagami 1982). Unlike *Apis*, meliponines have no sting, mate only once, and do not use pure wax to build their nests or use water to cool the nests. Meliponines cannot freely swarm to reproduce and the males feed at flowers, while the gravid queens cannot fly (Roubik 2006). Stingless bees are dependent on flowering plants because plants offer bees food in the form of nectar and pollen. The colonies of stingless bees make less honey, as compared to honey bees.

In the tropical regions, there are a variety of families and species of trees, shrubs, and agricultural crops that provide pollen and nectar to the bees. Most of the plants in this chapter were obtained from what the various authors considered to be important nectar and pollen source for the bees, and offering shelter or nesting tree cavities.

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22.2 Plants Used by Stingless Bees for Food

Pollen and nectar are a primary reward to insect pollinators in general and to the bees in particular (Roubik 1989). As honey bees require large quantities of pollen and nectar at specific times, they utilize particular plant species for a limited period of time. During the flowering period, there is a significant movement by bees between plants of the same species. This in turn favors the successful cross-pollination of plants (Faegri and der Pijl 1979; Free 1970). The utilization of plant resources as food (pollen and nectar) by tropical bees has been extensively studied. Studies have been made in areas with different types of vegetation such as natural forests, grasslands, agricultural land, pasture land, or urban areas. Most of the methods for obtaining information about bee plants in an area are based on direct field observation of foraging bees on flowers. The analysis of bee plant pollen loads and palynological analysis of honey samples can provide the accurate depiction of the bee flora of an area (Johnson and Hubbell 1974; Hubbell and Johnson 1977; Sommeijer et al. 1983; Kleinert-Giovannini and Imperatriz-Fonseca 1987; Roubik 1989, 1992; Ramalho et al. 1990; Ramalho et al. 1994; Eltz et al. 2001; Kajobe 2008; Hilario and Imperatriz-Fonseca 2009). Many of such stingless bee foraging behavior studies are based on analysis of pollen and nectar diets, and bee foraging behavior.

Our review provided the list of selected important bee plants (Table 22.1). The genera of stingless bees (*Meliponula*, *Melipona* and *Trigona*) and *Apis melifera* were used for this analysis. The important pollen plant species include Mimosaceae, Caesalpiniaceae, Myrtaceae, Asteraceae (sometimes called Compositae), and Moraceae. The other important families include Anacardiaceae, Euphorbiaceae, and Solanaceae. The most important plant species used were trees followed by shrubs, herbs, climbers, and runners in order of importance.

22.2.1 Pollen and Nectar Plant Sources

Pollen is extensively used by many species of insects, and by bees for brood rearing. Many studies consider chemical composition and nutritive value of pollen, effect on brood rearing growth, and longevity of bees or colonies. Pollen ordinarily provides bees with their only natural source of protein, which is needed for larval development and also satisfies other dietary needs for lipids, sterols, vitamins, and minerals (Roubik 1989; Herbert 1992). The protein content of the pollen is a direct measure of pollen quality in the diet of the bee (Pernal and Currie 2001). Foraged pollen loads are good indicators of the surrounding flowering plant species that provide pollen for the bees. They also indicate availability of dominant food resources for the different pollinators in an ecosystem. Stingless bees collect nectar from flowering plants and transport it to the nest, to be used in feeding larvae and for preparing honey. Kajobe (2006b) found that nectar concentration of plant species differs considerably in the amount and concentration of sugar they produce. He found that certain plant species produce large quantities of nectar to attract more pollinators, or

Table 22.1 Some of the important bee plants, their life form, nectar (N) and pollen (P) sources

			Genera of stingles bees					
Bee plants	Plant form	Reference	*Meliponula*	*Melipona*	Trigona	*A. mellifera*	N	P
Acanthaceae								
Acanthaceae Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Avicennia		Vit and Ricciardelli D'Albore (1994b)		x				x
Amaranthaceae								
Alternanthera		Dórea et al. (2010)				x		x
Amaryllidaceae								
Allium cepa	Crop	Adjare (1990)				x	x	x
Anacardiaceae								
Anacardium occidentale	Tree	Katende et al. (1995)				x	x	x
Astronium fraxinifolium		D'Apolito et al. (2010)				x		x
Lannea barteri	Tree	Katende et al. (1995)				x	x	x
Mangifera indica	Tree	Mouga (1984)			x	x	x	x
Spondias mombim	Tree	Sommeijer et al. (1983) and Dórea et al. (2010)		x	x	x		x
Spondias radlkoferri	Tree	Dórea et al. (2010)			x			x
Tapirira guianensis	Tree	Absy et al. (1984) and Dórea et al. (2010)		x	x	x		x
Apocynaceae								
Adenium obesum	Shrub	Crane et al. (1984)				x	x	x
Couma utilis		Rech and Absy (2011)			x			x
Rauvolfia caffra	Tree	Crane et al. (1984)				x	x	x
Arecaceae								
Attalea maripa		Rech and Absy (2011)			x			x
Bactris gasipaes		Rech and Absy (2011)			x			x
Cocos nucifera	Tree	Adjare (1990)				x	x	x
Elaeis guineensis	Tree	Dórea et al. (2010)				x	x	x

(continued)

Table 22.1 (continued)

Bee plants	Plant form	Reference	Genera of stingles bees			A. mellifera	N	P
			Meliponula	*Melipona*	Trigona			
Euterpe precatoria		Rech and Absy (2011)			x			x
Leopoldinia pulchra		Rech and Absy (2011)			x			x
Phoenix reclinata	Tree	Crane et al. (1984)				x	x	x
Asparagaceae								
Agave sisalana	Herb				x	x	x	x
Asteraceae								
Baccharis sp.		Orth (1983)			x			x
Baccharis erioclada		Orth (1983)		x	x			x
Baccharis semiserrata		Orth (1983)		x	x			x
Bidens pilosa	Herb	Kajobe and Roubik (2006)	x					x
Elephantopus		Dórea et al. (2010)				x		x
Eupatorium		Dórea et al. (2010)				x		x
Helianthus annuus	Crop	Horn (2004)				x	x	x
Mikania		Dórea et al. (2010)				x		x
Vernonia amygdalina	Shrub	Kajobe and Roubik (2006)	x					x
Vernonia auriculifera	Shrub	Kajobe and Roubik (2006)	x					x
Vernonia pauciflora	Shrub	Vit and Ricciardelli D'Albore (1994a)		x			x	x
Wedelia trilobata		Leonhardt et al. (2007)			x		x	
Balsaminaceae								
Impatients balsamina	Tree	Ramalho et al. (1985)			x			x
Impatients sultanii	Tree	Ramalho et al. (1985)			x		x	x
Impatiens walleriana	Tree	Ramalho et al. (1985)			x		x	x
Bignoniaceae								
Jacaranda mimosifolia	Tree	Katende et al. (1995)				x	x	x
Markhamia lutea	Tree	Kajobe (2006a)	x				x	x

Boraginaceae								
Cordia africana	Tree	Crane et al. (1984)			x	x	x	x
Cordia millenii	Tree	Crane et al. (1984) and Katende et al. (1995)			x	x	x	x
Cordia monoica	Tree	Crane et al. (1984) and Katende et al. (1995)			x	x	x	x
Cordia sinensis	Tree	Crane et al. (1984) and Katende et al. (1995)			x	x	x	x
Burseraceae								
Protium Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Cannaceae								
Canna indica	Herb	Orth (1983)			x			x
Caricaceae								
Carica papaya	Tree	Orth (1983)			x	x	x	x
Combretaceae								
Combretaceae Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Combretum collinum	Tree	Horn (2004)				x	x	x
Combretum molle	Tree	Horn (2004)				x	x	x
Combretum	Shrub	Kajobe (2006b)	x				x	
Combretum	Tree	Vit and Ricciardelli D'Albore (1994a)		x			x	x
Commelinaceae								
Commelina africana	Climber	Katende et al. (1995)				x	x	x
Cucurbitaceae								
Citrullus lanatus	Vine-like	Katende et al. (1995)				x	x	x
Cucumis sativus	Vine	Katende et al. (1995)				x	x	x
Cucurbita pepo	Vine	Katende et al. (1995)				x	x	x
Cunoniaceae								
Weinmannia		Vit and Ricciardelli D'Albore (1994b)		x				x
Ebenaceae								
Diospyros		Leonhardt et al. (2007)			x		x	
Diospyros mespiliformis	Tree	Katende et al. (1995)				x	x	x

(continued)

Table 22.1 (continued)

Bee plants	Plant form	Reference	Genera of stingles bees					
			Meliponula	*Melipona*	Trigona	*A. mellifera*	N	P
Euphorbiaceae								
Acalypha		Vit and Ricciardelli D'Albore (1994b)		x				x
Alchornea discolor		Absy et al. (1984) and Rech and Absy (2011)		x	x		x	x
Alchornea sidifolia		Kleinert-Giovannini (1989)		x	x		x	x
Aparisthmium cordatum		Rech and Absy (2011)			x			x
Croton macrostachyus	Tree	Kajobe (2006b)	x				x	x
Euphorbia splendens				x	x		x	x
Mallotus		Leonhardt et al. (2007)			x		x	
Ricinus		Vit and Ricciardelli D'Albore (1994b)		x				x
Fabaceae, Caesalpinioideae								
Acrocarpus fraxinifolius	Tree	Kajobe (2006a)	x				x	x
Afzelia africana	Tree	Kajobe (2006a)	x			x		x
Caesalpinia decapetala	Shrub	Kajobe (2006a)	x					x
Cassia		Vit and Ricciardelli D'Albore (1994b)		x				x
Cassia fistula		Leonhardt et al. (2007)			x		x	
Cassia undulata		Rech and Absy (2011)			x			x
Cynometra alexandri	Tree	Kajobe (2006a)	x			x	x	x
Julbernardia		Vit and Ricciardelli D'Albore (1994b)		x				x
Parkinsonia aculeata	Tree	Crane et al. (1984)	x					x
Peltophorum pterocarpum		Leonhardt et al. (2007)			x		x	
Tamarindus indica	Tree	Kajobe (2006b)	x			x	x	x
Fabaceae, Faboideae								
Cajanus cajan	Shrub	Adjare (1990)				x	x	x
Crotalaria		Vit and Ricciardelli D'Albore (1994b)		x				x
Crotalaria Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Gliricidia sepium	Tree	Adjare (1990)					x	x
Machaerium Type		Vit and Ricciardelli D'Albore (1994b)		x				x

Machaerium	Shrub	Vit and Ricciardelli D'Albore (1994a)		x			x	x
Vicia		Vit and Ricciardelli D'Albore (1994b)		x				x
Fabaceae, Mimosoideae								
Acacia sp.	Tree	Katende et al. (1995) and Dórea et al. (2010)	x			x	x	x
Albizia coriaria	Tree	Katende et al. (1995)				x	x	x
Albizia gummifera	Tree	Katende et al. (1995)	x				x	x
Archidendron jiringa		Leonhardt et al. (2007)			x		x	
Calliandra calothyrsus	Shrub	Kajobe (2006b)	x			x	x	
Faidherbia albida	Tree	Adjare (1990)					x	x
Leucaena leucocephala	Tree	Adjare (1990)				x	x	x
Mimosa bimucronata	Tree	Vit and Ricciardelli D'Albore (1994a)		x				x
Mimosa caesalpiniaefolia		Vit and Ricciardelli D'Albore (1994b)		x				x
Mimosa pudica	Herb	Vit and Ricciardelli D'Albore (1994a, b)		x				x
Mimosa scabrella	Tree	Vit and Ricciardelli D'Albore (1994a)		x				x
Mimosa scabrella	Tree	Adjare (1990)	x				x	x
Schrankia		Vit and Ricciardelli D'Albore (1994b)		x				x
Lamiaceae								
Gmelina arborea	Tree	Adjare (1990)				x	x	x
Vitex doniana	Tree	Adjare (1990)				x	x	x
Lythraceae		Vit and Ricciardelli D'Albore (1994b)		x				x
Malvaceae								
Grewia		Vit and Ricciardelli D'Albore (1994b)		x				x
Grewia bicolor	Tree	Adjare (1990)				x	x	x
Triumfetta		Vit and Ricciardelli D'Albore (1994b)		x				x
Meliaceae								
Azadirachta indica	Tree	Adjare (1990)				x	x	x
Carapa guianensis		Rech and Absy (2011)			x			x
Ekebergia capensis	Tree	Adjare (1990)				x	x	x
Melia azedarach	Tree	Adjare (1990)				x	x	x

(continued)

Table 22.1 (continued)

Bee plants	Plant form	Reference	Genera of stingles bees			*A. mellifera*	N	P
			Meliponula	*Melipona*	Trigona			
Moraceae								
Artocarpus heterophyllus		D'Apolito et al. (2010)				x		x
Moraceae Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Morus alba	Tree	Adjare (1990)				x	x	x
Moringaceae								
Moringa oleifera	Tree	Adjare (1990)				x	x	x
Musaceae								
Musa sp.	Herb	Kajobe and Roubik (2006)	x				x	
Myrtaceae		Vit and Ricciardelli D'Albore (1994b)		x				x
Eucalyptus	Tree	Kajobe and Roubik (2006) and D'Apolito et al. (2010)	x			x	x	x
Syzygium		Kajobe (2006b)	x					x
Oleaceae								
Olea capensis	Tree	Adjare (1990)				x	x	x
Passifloraceae								
Passiflora	Vine	Kajobe and Roubik (2006)	x				x	x
Piperaceae								
Piper	Tree	Vit and Ricciardelli D'Albore (1994a)		x				x
Piperaceae Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Phytolaccaceae								
Phytolacca dodecandra	Climber	Adjare (1990)				x	x	x
Poaceae								
Pariana		Rech and Absy (2011)			x			x
Zea mays	Crop	Kajobe and Roubik (2006)	x			x		x
Polygonaceae		Vit and Ricciardelli D'Albore (1994b)		x				x
Antigonon		Vit and Ricciardelli D'Albore (1994b)		x				x

Proteaceae								
Faurea saligna	Tree	Adjare (1990)				x	x	x
Rhamnaceae								
Rhamnaceae Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Ziziphus abyssinica	Shrub	Adjare (1990)				x	x	x
Rosaceae		Vit and Ricciardelli D'Albore (1994b)		x				x
Eriobotrya japonica	Tree	Adjare (1990)				x	x	x
Prunus africana	Tree	Adjare (1990)				x	x	x
Rubiaceae								
Coffea		Kajobe (2006a)				x	x	
Ixora javanica		Leonhardt et al. (2007)	x		x		x	
Richardia brasiliensis		D'Apolito et al. (2010)				x		x
Rutaceae								
Calodendrum capense	Tree					x	x	x
Citrus	Tree	D'Apolito et al. (2010)				x	x	x
Citrus		Vit and Ricciardelli D'Albore (1994b)		x				x
Zanthoxylum		Vit and Ricciardelli D'Albore (1994b)		x				x
Salicaceae								
Dovyalis abyssinica	Shrub	Adjare (1990)				x	x	x
Flacourtia indica	Shrub or tree	Adjare (1990)				x	x	x
Sapindaceae								
Allophylus rubifolius		Leonhardt et al. (2007)			x		x	
Dodonaea angustifolia	Tree	Adjare (1990)				x	x	x
Sapotaceae								
Butyrospermum paradoxum	Tree	Adjare (1990)				x		x
Scrophulariaceae		Vit and Ricciardelli D'Albore (1994b)		x				x
Solanaceae								
Datura suaveolens	Shrub	Adjare (1990)				x	x	x

(continued)

Table 22.1 (continued)

Bee plants	Plant form	Reference	Genera of stingles bees			*A. mellifera*	N	P
			Meliponula	*Melipona*	Trigona			
Solanaceae Type		Vit and Ricciardelli D'Albore (1994b)		x				x
Solanum		Vit and Ricciardelli D'Albore (1994b)		x				x
Stilbaceae								
Nuxia congesta	Tree	Kajobe (2006a)	x				x	x
Urticaceae								
Cecropia		Vit and Ricciardelli D'Albore (1994b)		x				x
Verbenaceae								
Aloysia triphylla		D'Apolito et al. (2010)				x		x

large pollinators such as birds or mammals, while others produce little. Nectar secretion among plant species differs, depending on the time of day and environmental factors. The frequencies of visitors for nectar gathering have been strongly correlated with the quantity of sugar and chemical constituents of the nectar (Biesmeijer 1997).

The characterization and quantification of pollen is an important indicator of the geographical and botanical origin of bee-collected forage. The pollen spectrum of honey depends on the plants (agricultural and forest) where it is collected. In tropical regions there are some important palynological studies (Eltz et al. 2001; Villanueva and Roubik 2004; Hilario and Imperatriz-Fonseca 2009). For the African countries, some palynological studies have been undertaken. There are also lists of bee plants made from field observations at flowers (Kajobe 2008; Gikungu 2006; Munyuli 2009). Results from the palynological studies show that stingless bees and honey bees collect pollen from a wide range of plant species. This may not be surprising because these eusocial bees have perennial colonies which make them forage for food throughout the year. The bees cannot therefore specialize on one or a few food plant resources. Rather, they are generalists, using a wide range of food plant resources (Michener 1974; Roubik 1989; Biesmeijer 1997; Hilario and Imperatriz-Fonseca 2009). In a comparative palynological study among stingless bees and honey bees in Uganda, Kajobe (2006a) finds a considerable overlap in pollen resources for three bee species (*Apis mellifera*, *Meliponula bocandei*, and *Meliponula nebulata*), and concludes that the overlap represented the bees' attraction to few sources or lack of attractive alternatives for other pollen sources. Such overlap may also be a partial adjustment according to local conditions, including kinds and abundance of competitors and distance to pollen sources (Sommeijer et al. 1983; Roubik et al. 1986; Kleinert-Giovannini and Imperatriz-Fonseca 1989; Roubik 1989; Biesmeijer 1997; Slaa 2003; Roubik and Villanueva-Gutiérrez 2009).

22.3 Plants Used for Stingless Bee Nests

22.3.1 Nesting Sites in Trees

Nests are notable points of bee activity which exhibit spectacular examples of animal architecture. Nesting biology is therefore a highly visible aspect of stingless bee behavior (Michener 1974; Roubik 2006). Stingless bees nest in tree cavities (Figs. 22.1 and 22.2), house wall crevices, and underground, with trees containing more nests as compared to the other nesting substrate (Michener 1974; Hubbell and Johnson 1977; Roubik 1989, 2006; Eltz et al. 2003; Slaa 2003; Martins et al. 2004; Kajobe 2007). Many *Melipona* (such as *M. quadrifasciata* and *M. marginata*), *Scaptotrigona* (such as *S. postica* and *S. xanthotricha*), and *Plebeia* build their nests inside living trees or branch hollows. Other meliponine nests, such as those built by *Frieseomelitta*, *Friesella schrottkyi*, and *Tetragonisca angustula*, can be found in available hollows in dead trees, fences, and walls. *Geotrigona*, such as *Geotrigona mombucae*, *Schwarziana*

Fig. 22.1 Two nest entrances of *Meliponula ferruginea* in Bwindi Impenetrable National Park (BINP), Uganda. Photo: R. Kajobe

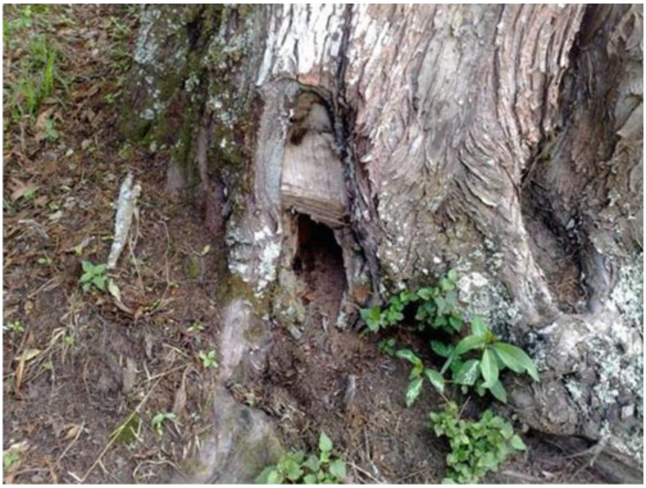

Fig. 22.2 Nest of *Meliponula bocandei* in a live tree predated upon by Batwa Pygmies in Bwindi Impenetrable National Park (BINP), Uganda. Photo: R. Kajobe

quadripunctata, *Melipona quinquefasciata*, and some *Paratrigona*, nest in underground existing cavities, often abandoned ant and termite nests. *Melipona bicolor* chooses to nest in damp places with fresh air, including tree trunks near soil. Some *Partamona* and *Scaura latitarsis* build their nests inside living termite nests. Further, nests of *Trigona spinipes*, *Trigona truculenta*, and other *Trigona* species are built in exposed areas, supported by tree branches, walls, or similar places (Kerr et al. 1967; Michener 1974; Sakagami 1982).

A broad range of trees (194 trees of 57 species) may be used as nesting sites. The predominant plant families include Anacardiaceae, Euphorbiaceae, Fabaceae, Dipterocarpaceae, and Lauraceae (Eltz et al. 2003; Slaa 2003; Martins et al. 2004; Kajobe 2007). Generally, nest tree diversity is high and many tree species are represented by a single individual (Table 22.2). Stingless bees are known to be generalists in relation to nest site selection (Hubbell and Johnson 1977; Roubik 1989). Relatively few bee species have a clear preference to nest in a particular tree species. The non-specificity normally results in high overlap in the use of nest tree species (Hubbell and Johnson 1977). In few cases where there appears to be some nest site specificity, the preference is for the location or the structure of that particular tree and not for the species of the tree (Kajobe 2007). Most stingless bee nests are located in or under large to very large canopy trees. For example, Eltz et al. (2003) found that a total of 86.1% of nest trees were larger than 60 cm dbh and 73.0% were between 60 and 120 cm dbh. Kajobe (2007) found that over 79% of nests of all the tree cavity nesting species were situated in large trees of dbh above 60 cm. These authors concluded that tree species probably differed in their tendency to form suitable cavities due to differences in wood and growth characteristics. These stingless bees are of greatly different worker and colony sizes but build nests in tree cavities of roughly the same dimensions. Roubik (1989) explained that this may probably be because cavity-nesting meliponines can significantly reduce the size of a tree cavity by blocking part of it.

Nests in large trunks are in most cases well insulated. In most cases stingless bees nest on either living trees or dead wood randomly. However, Eltz et al. (2003) found that at least 8.5% of the nest trees were dead while 91.5% were living trees. Stingless bees nest in any type of tree species suggesting that they are opportunistic in selection of a nest site and use whatever tree species that presents a cavity of the correct dimensions and purpose. In the forest, unoccupied tree cavities are fairly common (Johnson and Hubbell 1986). However, the size of the tree hole leading to the nest cavity markedly influences acceptability to bees (Roubik 1983) and whether resident colonies saturate their environment with bees, regardless of nest abundance, is an open question. In some nest trees, there are cases of multiple nests in one tree. In most cases the mature nest tree of such species is characteristically big, and has cavities left after its core of living tree is rotten from rainwater entering through the scars left by its fallen branches (Roubik 1989). The availability of such cavities can account for the clumping of the stingless bee nests. Eltz et al. (2003) reported that over 40% of nest trees contained more than one (maximum: 8) stingless bee nest in an undisturbed forest in Malaysia.

22.3.2 Tree Nest Height Partitioning

Kajobe and Roubik (2006) found some degree of height partitioning with regard to the larger stingless bee species. In general, the mean height given for a species building nests in tree cavities was biased towards lower heights, since nests are mostly

Table 22.2 Trees used for nesting by stingless bee species in the Afrotropical, Indo-Malayan, and Neotropical regions (reference: Eltz et al. 2001 = Indo-Malayan; Martins et al. 2004 = Neotropical; Kajobe 2007 = Afro-tropical)

Nest tree species	Stingless bee species				Afrotropical	Indo-Malayan	Neotropical
Achariaceae							
Hydnocarpus sp.				Not named		x	
Alangiaceae							
Alangium chinense	Mb	Mn	Mf		x		
Anacardiaceae							
Gluta oba				Not named		x	
Gluta sabahana				Not named		x	
Gluta				Not named		x	
Myracrodruon urundeuva			Fv				x
Schinopsis brasiliensis			Fv				x
Spondias tuberosa	Ms	Fd	Fv				x
Apocynaceae							
Aspidosperma pyrifolium	Ms						x
Araliaceae							
Polyscias fulva		Mn			x		
Schefflera barteri			Mf		x		
Bignoniaceae							
Tabebuia caraiba		Fd					x
Burseraceae							
Commiphora leptophloeos	Ms		Fv				x
Calophyllaceae							
Calophyllum sp.				Not named		x	
Celastraceae							
Lophopetalum sp.				Not named		x	
Maytenus acuminata		Mn	Mf		x		
Chrysobalanaceae							
Licania rigida	Ms						x

Parinari excelsa	Mb	Mn	Mf		x		
Cupressaceae							
Cupressus lusitanica	Mb	Mn	Mf		x		
Dipterocarpaceae							
Dipterocarpus grandiflorus				Not named		x	
Dipterocarpus				Not named		x	
Shorea sp.				Not named		x	
Ericaceae							
Agauria salicifolia		Mn	Mf		x		
Euphorbiaceae							
Chaetocarpus castanocarpus				Not named		x	
Cnidoscolus phyllacanthus	Ms						
Trigonopleura malayana				Not named		x	
Fabaceae, Caesalpiniodeae							
Caesalpinia pyramidalis	Ms	Fd	Fv				x
Intsia palembanica				Not named		x	
Sympetalandra borneensis				Not named		x	
Fabaceae, Mimosoideae							
Albizia gummifera	Mb		Mf		x		
Anadenanthera colubrina	Ms						x
Dialium				Not named		x	
Mimosa acutistipula		Fd					x
Piptadenia communis	Ms						x
Lamiaceae							
Premna angolensis		Mn			x		
Lauraceae							
Dehaasia				Not named		x	
Eusideroxylon zwageri				Not named		x	
Litsea caulocarpa				Not named		x	

(continued)

Table 22.2 (continued)

Nest tree species	Stingless bee species				Afrotropical	Indo-Malayan	Neotropical
Litsea				Not named		x	
Phoebe macrophylla				Not named		x	
Malvaceae							
Glyphaea brevis	Mb				x		
Scaphium affine				Not named		x	
Triumfetta macrophylla	Mb				x		
Melastomataceae							
Dichaetanthera corymbosa			Mf		x		
Meliaceae							
Carapa grandiflora	Mb		Mf		x		
Ekebergia capensis		Mn	Mf		x		
Entandrophragma cylindricum		Mn			x		
Entandrophragma excelsum	Mb				x		
Melianthaceae							
Bersama abyssinica		Mn			x		
Monimiaceae							
Xymalos monospora		Mn			x		
Moraceae							
Ficus natalensis					x		
Ficus	Mb	Mn	Mf		x		
Ficus				Not named		x	
Myricaceae							
Myrica salicifolia		Mn	Mf		x		
Myrtaceae							
Eucalyptus			Mf		x		
Syzygium guineense	Mb	Mn	Mf		x		
Syzigium				Not named		x	
Olacaceae							

Scorodocarpus borneensis				Not named		x
Strombosia scheffleri	Mb	Mn	Mf		x	
Penaeaceae						
Olinia usamberensis		Mn	Mf		x	
Podocarpaceae						
Podocarpus milanjianus		Mn			x	
Primulaceae						
Maesa lanceolata	Mb		Mf		x	
Proteaceae						
Faurea saligna		Mn	Mf		x	
Putranjivaceae						
Drypetes gerrardii	Mb		Mf		x	
Rosaceae						
Hagenia abyssinica		Mn			x	
Prunus africana	Mb	Mn	Mf		x	
Rutaceae						
Zanthoxylum gilletii		Mn	Mf		x	
Zanthoxylum macrophyllum			Mf		x	
Sapotaceae						
Chrysophyllum albidum	Mb	Mn			x	
Chrysophyllum gorungosanum		Mn	Mf		x	
Theaceae						
Ficalhoa laurifolia	Mb	Mn	Mf		x	
Thymelaeaceae						
Wikstroemia				Not named		x

Key: MB = *Meliponula bocandei*, Mn = *Meliponula nebulata*, Mf = *Meliponula ferruginea*, Ms = *Melipona subnitida*, Ma = *Melipona asilvai*, Fd = *Frieseomelitta doederleini*, Fv = *Frieseomelitta varia* found near ground level. Roubik (1979, 1983) found that eusocial bee species do not often make their nest entrances level with the ground or in the tallest branches of forest trees but a height of 30 m seems to be their normal limit. The differences in height are explained by the fact that different species are most often active at different strata above the ground.

22.4 Conclusions

The importance of plants to stingless bees was discussed based on available literature in tropical areas of the world. In this chapter emphasis was made on resources needed by stingless bees to survive, mainly food (pollen and nectar) and shelter (tree nest) availability. The data showed that stingless bees collect pollen and nectar from a wide range of plant species. Also, a broad range of tree species were used as nesting sites for stingless bees.

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Chapter 23
Botanical Origin of Pot-Honey from *Tetragonisca angustula* Latreille in Colombia

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23.1 Introduction

Tetragonisca angustula, known in Colombia as "angelita", is the stingless bee most widely distributed in the country, found in all natural regions below 1,800 m elevation (Nates-Parra 2001). *Tetragonisca angustula* is widely kept and recognized for medicinal value attributed to its honey, commercialized in various local markets (Cepeda et al. 2009). However, so far there has been no complete characterization of the honey's botanical origin.

In studies conducted in other countries, *T. angustula* shows a broad pollen spectrum, classifying it as a bee with a generalist foraging habit (Cortopassi-Laurino 1982), but with some plant families represented by many species, such as Euphorbiaceae, Asteraceae, and Myrtaceae (Carvalho and Marchini 1999; Braga et al. 2009; Flores and Sánchez 2010). In this context, our investigation aims to provide knowledge about the plants that are nectar sources for this bee and help to identify the honey in different regions.

23.2 Honey Collection and Pollen Frequency Classes

The study was conducted between 2008 and 2010, in different regions and agroecosystems. Seventy-six honey samples were collected in the Andean region, in the following states: Tolima (1), Antioquia (6), Cauca (1), Cundinamarca (12), and Santander (29), and in the Caribbean region: Cesar (1), Magdalena (24), and Sucre (2).

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Pollen in honey was prepared using the acetolysis method (Erdtman 1952) and mounted on a slide. To calculate the frequency of pollen types, 250 and 400 grains were counted per sample (depending upon the pollen content and sample volume).

Identification of pollen species was carried out by comparison to pollen collections in the Bee Research Laboratory LABUN, and the Palynology and Paleoecology Laboratory of Instituto de Ciencias Naturales, both located at Universidad Nacional de Colombia, and also by using pollen atlases: Moreno and Devia (1982); Roubik and Moreno (1991); Colinvaux et al. (1999); Bush and Weng (2007). Frequency classes (predominant pollen "D" (> 45%); secondary pollen, "S" (16–45%); important minor pollen, "M" (3–15%); minor pollen, "m" (between >1 and <3%)) (Louveaux et al. 1970) were estimated for the pollen types to determine the types of honeys. Honey was characterized as "monofloral" when a species was predominant, "bifloral" when two pollen types had secondary percentages, and "multifloral" for other honeys. Pollen belonging to plants without nectar production was excluded from the counts of the honey because it is considered irrelevant to the botanical origin of nectar sources (von der Ohe et al. 2004; Barth 2005). The botanical names of pollen types were based on "The plant list."

The samples were classified in groups according to similarity by a cluster analysis based on Euclidean distances and correspondence analysis using the statistical package Past (Hammer et al. 2001).

23.3 Botanical and Geographic Origin of Pot-Honey

A total of 306 pollen types, belonging to 49 families, were identified. Twenty-five pollen types were not included in the analysis because they belong to plants that do not produce nectar, such as *Piper aduncum*, *Cecropia*, *Acalypha*, *Trema micrantha*, *Myrsine*, *Theobroma cacao*, *Alchornea*, *Fraxinus uhdei*, *Ricinus communis*, Amaranthaceae, Moraceae, Poaceae, Arecaceae, and Cyperaceae types. Frequency classes in 76 honey samples of *Tetragonisca angustula* are presented in Table 23.1.

The families with a higher representation in the number of pollen types were Asteraceae (47), Fabaceae (39), Malvaceae (11), Rubiaceae (11), Melastomataceae (11), and Euphorbiaceae (11). The samples had an average of 13 ± 5.30 pollen types. The most frequent pollen types were *Heliocarpus americanus* occurring in 46% of the samples, followed by *Coffea arabica* 35.50%, *Citrus* 32.89%, and *Myrcia* Type 30.26%. The most important pollen types are illustrated in Fig. 23.1.

The general palynological spectrum used by *T. angustula*, according to the analyzed samples, is large. At the plant family level, pollen types are similar to those found in other studies (Iwama and Melhem 1979; Cortopassi-Laurino 1982; Imperatriz-Fonseca et al. 1984; Knoll 1990; Carvalho and Marchini 1999; Vossler 2007; Flores and Sánchez 2010), but generic and specific levels differ greatly, probably due to the plant composition of the ecosystems in which samples were taken. This has also been observed in other studies when comparing different locations and seasons, where

Table 23.1 List of pollen types with frequency of occurrence percentage >10% and with the respective frequency classes in 76 honey samples of *Tetragonisca angustula*

Family	Pollen type	FO	D	S	IM	m
Acanthaceae	*Justicia*	10.5			1.3	9.2
Apiaceae	Apiaceae Type	18.4			9.2	9.2
Boraginaceae	*Cordia alliodora*	14.5		2.6	2.6	9.2
Caryophyllaceae	*Stellaria* Type	30.3		1.3	13.2	15.8
Cleomaceae	*Cleome* Type	19.7		7.9	9.2	2.6
Asteraceae	Asteraceae Type	10.5			3.9	6.6
	Austroeupatorium inulifolium	17.1		2.6	5.3	9.2
	Critonia aff. *morifolia*	10.5			1.3	9.2
	Hypochaeris radicata	10.5			1.3	9.2
Euphorbiaceae	*Croton sp.*	26.3			9.2	17.1
	Euphorbia cotinifolia	11.8	1.3	1.3	1.3	7.9
	Euphorbia hirta	15.8	2.6	5.3	5.3	2.6
	Euphorbiaceae Type	15.8	1.3	1.3	3.9	9.2
Lamiaceae	*Hyptis brachiata*	10.5		1.3	2.6	6.6
	Hyptis	14.5			1.3	13.2
Fabaceae, Caesalpinioideae	Fabaceae Type 1	15.8		1.3	3.9	10.5
	Fabaceae Type 2	13.2	1.3	1.3	2.6	7.9
Fabaceae, Mimosoideae	*Mimosa*	30.3		1.3	6.6	22.4
Fabaceae, Faboideae	Fabaceae Type 3	17.1		3.9	6.6	6.6
Loranthaceae	*Oryctanthus* sp.	17.1	1.3	1.3	5.3	9.2
Lythraceae	*Adenaria floribunda*	15.8		2.6	7.9	5.3
Malpighiaceae	*Tetrapteris*	11.8		1.3	3.9	6.6
Malvaceae/Grewioideae	*Heliocarpus americanus*	46.1	5.3	5.3	21.1	14.5
Muntingiaceae	*Muntingia calabura*	25.0	1.3	3.9	5.3	14.5
Myrtaceae	*Myrcia* Type	30.3	1.3	3.9	15.8	9.2
	Myrtaceae Type	25.0		1.3	9.2	14.5
	Syzygium jambos	15.8		2.6	5.3	7.9
Rhamnaceae	*Gouania polygama*	21.1	11.8		2.6	6.6
Rubiaceae	*Coffea arabica*	35.5	11.8	1.3	9.2	13.2
Rutaceae	*Citrus*	32.9	1.3	2.6	11.8	17.1
Verbenaceae	*Lantana* aff. *fucata*	11.8		3.9	6.6	1.3
Vitaceae	*Vitis tiliifolia*	15.8		1.3	3.9	10.5

FO frequency of occurrence percentage. Frequency classes: Value indicating the number of samples in which different pollen types appear in the following percentages: *D* dominant pollen (>45%), *S* Secondary pollen (16–45%), *IM* Important minor pollen (3–15%), *m* minor pollen (<3%)

T. angustula has to take advantage of all food sources it has within its reach (Landaverde et al. 2004). Analyzing the individual samples, each of which corresponds to a certain locality and specific sampling date, we can observe that they each contain only a few pollen types. This may indicate that the worker bees of the same colony have a tendency to be constant in their visits to flowers of the same species, and that they have some favorite sources for nectar, especially those with massive blooms.

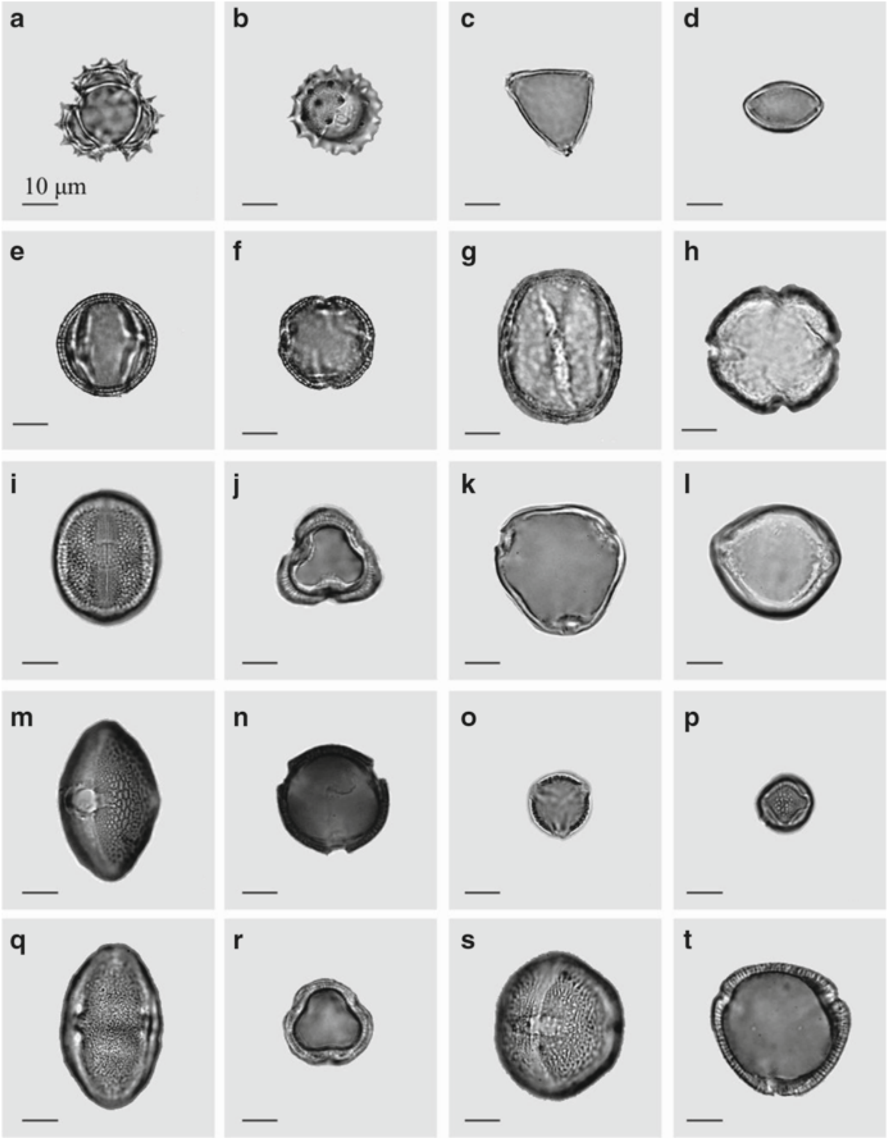

Fig. 23.1 Some important pollen types found in honey samples of *Tetragonisca angustula*: (**a**, **b**) *Austroeupatorium inulifolium*, (**c**, **d**) *Calycolpus moritzianus*, (**e**, **f**) *Citrus* Type, (**g**, **h**) *Coffea arabica*, (**i**, **j**) *Euphorbia hirta*, (**k**, **l**) *Gouania polygamya*, (**m**, **n**) *Heliocarpus americanus*, (**o**, **p**) *Muntingia calabura*, (**q**, **r**) *Spananthe paniculata*, (**s**, **t**) *Toxicodendron striatum.* Scale: 10 μm. Photos: LABUN (Laboratorio de Investigaciones en Abejas) Archives at Universidad Nacional de Colombia

According to the frequency classes, 40 monofloral, 13 bifloral, and 23 multifloral honeys (Table 23.2) were detected from different regions of the country (Fig. 23.2). Based on results of the multivariate analyses of similarity and correspondence, some groupings of samples were found that allow us to classify groups by botanical and geographical origin, as follows:

- Honey from coffee agroecosystems: Forty-four samples from coffee agroecosystems: nine monofloral *Coffea arabica* honeys, nine monofloral *Gouania polygama* honeys, four monofloral *Heliocarpus americanus* honeys, 16 honeys from Sierra Nevada de Santa Marta group (Magdalena state), and six mixed honeys similar in composition but with different geographical origin.
- Honey from dry ecosystems: Twelve samples from a specific locality in Santander called Giron group.
- Undifferentiated honey: Nineteen samples from different places, which were not clearly differentiated by the analysis.

Among the honey samples, palynological composition categorized honey from coffee regions as a typical Colombian agroecosystem, located between 1,000 and 2,000 m elevation (García and Vallejo 2002). Some samples show a clear botanical origin of typical regional plants and can be classified as monofloral honey of *Coffea arabica, Gouania polygama*, or *Heliocarpus amercianus*. There is also one group with a clear geographic origin from the region called Sierra Nevada de Santa Marta, which also belongs to the coffee area. The remaining samples were not clearly grouped (mixed honeys). There were nine monofloral samples of *Coffea arabica*, with an average relative abundance of 76.26 ± 17.70%, coming from different locations in the states of Antioquia and Magdalena (Sierra Nevada de Santa Marta). Within these honeys, 27 complementary pollen types occur in low proportion, the most frequent being *Heliocarpus americanus*, *Stellaria* type, and *Cleome* Type. In addition, there were nine monofloral samples of *Gouania polygama*, with an average relative abundance of 72.34 ± 14.35% coming from different locations in the states of Santander (Charalá, Floridablanca and Socorro) and Magdalena (Sierra Nevada de Santa Marta). In these honeys there were 20 complementary pollen types in low proportion, the most frequent being *Heliocarpus americanus*, *Muntingia calabura*, *Coffea arabica*, and *Myrcia* Type. Finally, there were four monofloral samples of *Heliocarpus americanus*, with an average relative abundance of 69.4 ± 21% in the states of Santander (Charalá) and Antioquia (Medellín). In these honeys were 25 complementary pollen types in low proportion, the most frequent being *Myrcia* Type, *Eucalyptus*, and *Oryctanthus*. The floral preference of *T. angustula* for *Heliocarpus* has been reported elsewhere (Landaverde et al. 2004; Martínez-Hernández et al. 1994). *H. americanus* is a pioneer species, common in secondary forests, and blooms during several months of the year (Cole et al. 2010; Riaño 2005).

Sierra Nevada de Santa Marta is a mountainous region located in the state of Magdalena, where all samples in this group originate. This includes 16 samples, some of monofloral origin: *Astronium* (1), Asteraceae Type 1 (1), Fabaceae/Caesalpinoideae Type (1), and Euphorbiaceae Type 1 (1). The rest include a wide spectrum with 113 pollen types, within which the most frequent were *Cleome* (11),

Table 23.2 Honey types according to botanical origin and geographical distribution

Honey type	Pollen types	Number of samples	States in Colombia
Monofloral (40 samples)	Asteraceae Type 1	1	Santander
	Asteraceae Type 2	1	Magdalena
	Astronium graveolens	1	Magdalena
	Austroeupatorium inulifolium	2	Santander
	Calycolpus moritzianus	1	Santander
	Citrus	1	Santander
	Coffea arabica	3	Antioquia
		6	Magdalena
	Euphorbia cotinifolia	1	Cundinamarca
	Euphorbia hirta	1	Santander
	Euphorbia	1	Sucre
	Euphorbia thymifolia	1	Santander
	Euphorbiaceae Type	1	Magdalena
	Gouania polygama	2	Magdalena
		7	Santander
	Heliocarpus americanus	1	Antioquia
		3	Santander
	Undetermined Type 1	1	Santander
	Fabaceae, Caesalpinioideae Type	1	Magdalena
	Muntingia calabura	1	Santander
	Oryctanthus	1	Santander
	Rosaceae Type	1	Cundinamarca
	Spananthe paniculata	1	Cundinamarca
Bifloral (13 samples)	*Cleome* Type—Fabaceae, Caesalpinioideae Type	1	Magdalena
	Coffea arabica—Asteraceae Type	1	Antioquia
	Euphorbia hirta—Citrus	2	Santander
	Euphorbiaceae type—*Vitis tilifolia*	1	Magdalena
	Heliocarpus americanus—Lantana aff. *fucata*	2	Cundinamarca
	Hyptis brachiata—Cordia spinescens	1	Magdalena
	Lantana aff. *fucata—Adenaria floribunda*	1	Cundinamarca
	Melastomataceae type—Undeter mined Type 2	1	Sucre
	Myrcia—Cuphea racemosa	1	Cundinamarca
	Rosaceae Type—Asteraceae Type	1	Magdalena
	Fabaceae, Caesalpinioideae Type 1—Solanaceae Type	1	Santander
Multifloral (23 samples)	12 pollen types	1	Antioquia
	11 pollen types	1	Cauca
	13 pollen types	1	Cesar
	16 ± 1.4 pollen types	5	Cundinamarca
	15.7 ± 5.7 pollen types	8	Magdalena
	15.3 ± 1.4 pollen types	6	Santander
	14 pollen types	1	Tolima

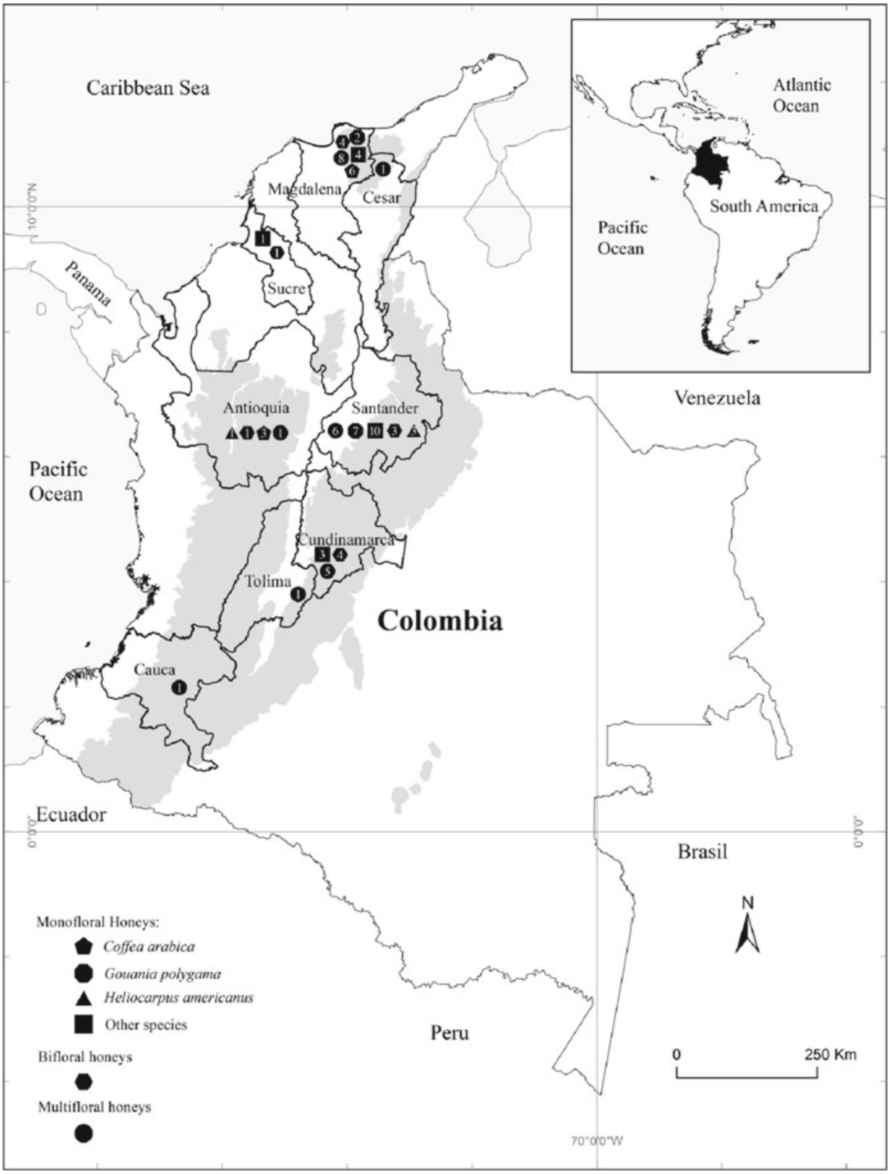

Fig. 23.2 Honey types of *Tetragonisca angustula* found in the study area. The number inside each symbol indicates the number of samples in each state

Coffea arabica (8), Euphorbiaceae Type 1 (8), and *Toxicodendron striatum* (6). As reflected in the samples and the characterization of vegetation (Carbonó and Lozano-Contreras, 1997; Rangel and Garzón, 1995), this is a region with great diversity and many endemic flora species, allowing the production of unique and varied honey.

A group of six samples from different localities (4 Santander, 2 Antioquia) were similar in composition and characterized by typical pollen types present in coffee-growing areas, including *Coffea arabica*, *Myrcia* Type, *Heliocarpus americanus*, and *Gouania polygama*, but occurring in low proportion. However, this group also contains two monofloral samples from *Myrcia* Type and from *Oryctanthus*.

The Santander-Girón region is between 150 and 1,200 m elevation, and the climate is dry with a tendency toward desertification (UIS 2009). The vegetation is of low stature and much of the area is degraded, reflected in the palynological spectrum. This group includes 12 samples. The pollen types with the highest average relative abundance were *Euphorbia hirta* 19 ± 20% and *Muntingia calabura* 12 ± 20% (typical plants of disturbed ecosystems). The most frequent pollen types were *Stellaria* Type (11), *Euphorbia hirta* (8), *Muntingia calabura* (8), Euphorbiaceae type (8), and *Citrus* (6). Within the group there were monofloral honeys of *Citrus* (1), Euphorbiaceae Type (1), Undetermined (1), *Euphorbia hirta* (1), and *Muntingia calabura* (1).

We named undifferentiated honey, 19 samples from different localities that were not clearly separated by multivariate analysis or geographic or botanical origin. Cundinamarca (Fusagasugá) had 11 samples and they were taken in this location. The most frequent pollen types were *Myrcia* Type 90%, *Heliocarpus americanus* 90%, *Eucalyptus* 90%, *Citrus* 90%, Fabaceae, Faboideae 1 80%, *Lantana fucata* 80%, and *Adenaria floribunda* 80%. Santander (Oiba) had three samples and they were taken in this location. The most frequent pollen types were Asteraceae Type 15.33%, *Mimosa* Type 1 33%, *Stellaria* Type 33%, and *Spermacoce* 33%. Two samples were taken from Sucre (Colosó, Sincelejo) where pollen types with the highest average relative abundance were *Euphorbia* Type 1 28.8 ± 40% and *Austroeupatorium inulifolium* 12.3 ± 17%. Tolima (Dolores), with a single location and sample, contained pollen of *Adenaria floribunda* 16%, *Croton* 13.50%, *Syzygium jambos* 10%, and *Dalechampia* 10%. Similarly, with a single sample Cauca (Popayán) contained Asteraceae Type 1, 36.7%; *Myrcia* Type, 20%; and Bignoniaceae Type 2, 16%. With one sample, Cesar (Pueblo Bello) had *Syzygium jambos*, 41.5%; Asteraceae Type 13, 21.1%; and undetermined, 17%.

23.4 Conclusions

Multivariate analysis of palynological composition helped to identify the geographical origin *T. angustula* honey. We distinguished honey from coffee agroecosystems and from other localities. The honey from coffee areas contains *Coffea arabica*, *Gouania polygama*, *Heliocarpus americanus, Muntingia calabura*, and *Myrcia* Type, which are useful as pollen indicators because they have a high frequency in the samples and they are characteristic components of those areas. Honey from Santander, specifically from a dry region called Girón, can be also recognized by pollen analysis due to the occurrence of *Euphorbia hirta* and *Muntingia calabura*.

The detection of monofloral and bifloral honeys from specific pollen types such as *Coffea arabica* or *Heliocarpus americanus* allowed characterizing the botanical origin. This information is useful for stingless bee-keepers because it helps to characterize the products of this species and to recognize the plants that provide nectar and contribute to the maintenance of colonies. This information contributes to recognition of floral preferences of *T. angustula* in areas where it is mostly kept in Colombia. It is desirable to expand sampling from different regions of the country to continue the characterization of honey from *T. angustula* by botanical and geographical origin.

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Part IV
Sensory Attributes and Composition of Pot-Honey

Chapter 24
Sensory Evaluation of Stingless Bee Pot-Honey

Rosires Deliza and Patricia Vit

To Michel Gonnet, for the first imprinting with the sensory message of a lavanda honey served in a crystal gobblet in Monfavet, France

24.1 Introduction

The sensory characteristics of honey play an important role in producing quality standards, as they determine consumer acceptance. The sensory attributes in terms of appearance, aroma, flavor, and texture vary from product to product, revealing the need for investigating every honey in order to better understand their characteristics. When one evaluates honey sensory quality, several perspectives are taken into account, and among them is the consumer perception that leads to different honey evaluations. Consumers are more and more concerned about health and wellness and, consequently, they are more interested in the benefits from food and beverage (Sloan 2011). Honey is a health product (Amtmann 2010), and therefore, a thorough investigation of honey sensory properties is desirable.

Sensory analysis as a discipline uses the five human senses (sight, smell, taste, touch, and hearing) to analyze food, beverages, and other products. By using human panels to sample the products, with an adequate experimental design and statistical

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Table 24.1 Main characteristics of qualitative and quantitative sensory methods for investigating honey

Sensory issues in qualitative studies
How do you perceive this honey? What did you specifically like and dislike about it?
Please explain what do think about this honey color compared to the other one
Please describe the consistency of this honey
Please tell me more about why the color of this honey is unacceptable to you
Why in your opinion honey 371 is nicer than product 775?
Why your family preferred this honey instead of the others?
Characteristics of sensory quantitative studies
The honey consumer is invited to participate:
A relatively large group of consumers participates (depending on the statistical power required)
Careful honey preparation for a large number of participants
Written questionnaire with attributes and scales to score consumer response. Sensory questions may include overall liking, liking and perceived intensity of attributes, and preference
The selection of attributes in the questionnaire is critical
Data are statistically analyzed

Adapted from Deliza and Glória (2009)

analysis, it is possible to evaluate products in terms of appearance, aroma, flavor, texture, and aftertaste (Meilgaard et al. 1999). Assessment can derive from a panel selected according to specific criteria and trained to evaluate product sensory attributes, or from a consumer panel, i.e., any person who consumes the product under investigation or matches predefined recruitment criteria, normally based on demographics (e.g., gender, age, education, product consumption).

Several quantitative sensory methods are available and well defined in terms of application procedures (Stone and Sidel 2004). The choice regarding panel selection (trained people or consumers) will depend on the study objective. Three distinct methods are applied, as follows: the descriptive method (used when the aim is to have a sensory characterization of samples), discriminative tools (useful to investigate whether there are sensory differences between products), and affective tests, which investigate how much a product is liked/accepted by consumers. Consumer studies can be carried out through qualitative and quantitative studies. Qualitative research often has an exceptional value, since the consumer can be queried to obtain information not easily obtainable in quantitative studies. Qualitative information can provide the most important data and cannot be easily measured through a written questionnaire. The qualitative studies do not replace quantitative ones, but complement them (Muñoz 1998). Quantitative studies, on the other hand, are geared to collect data that can be summarized and analyzed statistically. The main characteristics quantitative and qualitative sensory studies are indicated in Table 24.1.

Consumers may have subjective impressions regarding product quality, and several aspects contribute to their product evaluation. Among them are psychological processes. Such processes are influenced by many factors, including the level of previous knowledge and cognitive competencies of each consumer (Deliza and MacFie 1996; McBride and MacFie 1990). Thus, from a consumer perspective, quality refers to the perceived quality and not to quality in an objective sense (Deliza and Glória 2009; Steenkamp 1990).

We illustrate a number of distinctive sensory characteristics of pot-honey. Comparisons between honey produced by *Apis* (in combs) or meliponines (in pots) are presented and discussed, as well as the sensory evaluation of fermented honey. The latter is, objectively, fairly common for honey in different stingless bee species. Sensory implications based on the extractive techniques are also included considering the new odor–aroma families needed to describe such a product. Preliminary data on acceptance of pot-honey produced by different species are given. A Free-choice profile described is a useful method to group honeys according to their entomological origin, by untrained panels.

24.2 Sensory Characteristics of Pot-Honey

Honey consumers in the cities can find honey from *A. mellifera* on supermarket shelves. In tropical villages where many of the stingless bees are appreciated, as well as the several species of tropical *Apis* produced in combs, there is also a great variety of honey produced in pots. Familiarity with local species of meliponines is also reflected in the cultural uses of honey by stingless bee honey hunters and stingless bee keepers. Their honeys were widely relished in tropical America before Columbus (Schwarz 1948). Honey is as varied as the different species that produce it and the different seasons and habitats in which it is harvested. Therefore, when we taste honey it is like a communication between man and the habits of bees through the human senses.

Honey produced in pots by Meliponini shares compositional properties with *A. mellifera* honey produced in combs, but differs in others such as higher water content and free acidity (Vit et al.; Souza et al. 2006). Therefore, their sensory attributes vary accordingly. For example, a higher acidity increases the sour taste perceived in pot-honey, as observed since Gonnet et al. (1964). The higher water content causes a lower visual viscosity, and has different implications in the perception of odors and aromas, caused by a flavor dilution factor. A wide range of applications derives from the perception of a paradoxical honey, so far the most ancient honey in the planet (Camargo, personal communication) but a new product in the honey market, with few recent sensory studies (Ferreira et al. 2009; Vit et al. 2011a, d).

Classical work on sensory characteristics and defects of honey from *A. mellifera* (Gonnet and Vache 1984) were expanded towards perception evaluation by human consumers. Persano Oddo et al. (1995) characterized honey by visual, olfactory, and flavor attributes, later organized in complete sheets of 20 European honey types (Persano Oddo and Piro 2004). Anupama et al. (2003) developed a specific lexicon for Indian honey by quantitative descriptive analysis (QDA). They applied Principal Component Analysis (PCA) to appearance, aroma, mouthfeel, and flavor descriptors and physicochemical variables. Galán-Soldevilla et al. (2005) developed a sensory lexicon for floral honey with 15 descriptors, in categories of odor, flavor, texture, and trigeminal sensations, i.e., more associated with the sense of touch, perceived through the action of specific compounds on the trigeminal receptors (e.g., the tingling effect of citric acid, cooling sensation from menthol, fizzy feeling of carbonated beverages, astringency caused by unripe persimmons and bananas, or the hotness perceived after eating chilli). Additionally, postharvest conservation methods (see Menezes et al. chapter in this book) cause variable sensations according to the stingless bee species, which leads to the human reaction and distinctive sensory perception, that needs to be considered.

A number of distinctive sensory characteristics of honey derive from extractive techniques. As we will discuss, some new odor–aroma families are needed to describe this product. The sensory interpretation of fermented honey, preliminary data on acceptance of pot-honey produced by different species, and the free-choice profile as a useful method to group honey according to their entomological origin are explored by untrained panels.

24.3 New Odor–Aroma Families for Pot-Honey

The system used to describe the honey of *A. mellifera* has identified and arranged seven families of sensory attributes in the odor–aroma wheel (Piana et al. 2004). This was adapted to eight odor–aroma families for pot-honey produced by stingless bees (Table 24.2), as follows: (1) Floral-fruity, (2) Vegetable, (3) Fermented, (4) Wood, (5) Bee hive, (6) Mellow, (7) Primitive, and (8) Industrial chemicals (Vit et al. 2007a, b). For the public the family bee hive makes sense, but for scholars bee nest would be a better expression.

24.4 Pot-Honey Extraction by Pressure or By Suction?

Compression of mature honey pots is the traditional method of extraction. Compared to modern honey extraction by suction after piercing sealed pots, more pollen is added to the honey by squeezing the storage pots, which may include adjacent pollen pots. The extractive technique has implications related to the fermented pollen (see Menezes et al., chapter this book) added to the honey.

Using descriptors of Table 24.2, eight assessors tasted pressed pot-honeys of *Melipona aff. fuscopilosa* [= *Melipona* (*Michmelia*) sp. 1, see Table in Pedro and Camargo chapter, this book, until the revision of *Melipona* is done] and *Tetragona clavipes* from the Venezuelan Amazon (Vit et al. 2007a, b). The intense fermented odor and aroma reduced the relative frequencies of descriptors from the other seven sensory families. Fermented odor was perceived more frequently than fermented aroma, somehow associated to volatile components of fermentation.

For honey of *A. mellifera*, fermentation is considered an off-odor, something that is not normal (Gonnet and Vache 1984). It represents not only a sensory defect,

Table 24.2 Organized odor–aroma descriptors for pot-honey

Family	Subfamily	Sensory descriptors
1. Floral-fruity	Floral	Orange blossom, jasmine, rose, violet
	Citrus fruit	Citrus zesty, lemon, mandarine, orange, grapefruit
	Fresh fruit	plum, coconut, apricot, berries, apple, melon, passion fruit, watermelon, pear, pineapple, rose apple, fig, peach, grape
	Processed fruit	Candied fruit, dehydrated fruit, syrup fruit, fruit jam
2. Vegetable	Fresh	Sugar cane, raw beans, fresh leaves, sweet corn, sweet parsnip, bitter plants, vegetation
	Dry	Dry hay, malted, chamomile, straw, tea
	Aromatic	Lemongrass, eucalyptus, bay leaves, peppermint, oregano, rue, lime, liquorice
3. Fermented	Acetic	Vinegar, meliponine pollen pots
	Alcoholic	Aguardiente, fermented fruit, yeast, liqueur, must, sake, vinasse, white wine, red wine
	Lactic	*Miso*, cheese, yogurt
4. Wood	Woody	Sawdust, cork, wood flakes
	Resinous	Cedar, incense, pine resin
	Spicy	Anise, cocoa, coffee, cinnamon, clove, nutmeg, tobacco, vanilla
	Seeds	Sesame, almond, marzipan, chestnut, hazelnut
5. Bee hive	Stingless bee	Bee, batumen, cerumen, pot-honey
	Apis mellifera	Beeswax, bee excrement, honey, bee pollen, propolis, moth
6. Mellow	Sugary	White sugar, brown sugar, syrup, tablets, chocolate
	Caramelized	*Arequipe*, burned sugar, candy, caramel, maple, molasses, jaggery, toffee, malt
	Pastry	Pudding, butter
7. Primitive	Animal	Formic acid, pet food, leather, stable, manure, fat, eggs, cat urine, sweat
	Smoke	Smoked food, burned straw
	Wet	Floor mop, after the rain, humus, moldy
	Sulfate	Artichoke, cabbage
	Mineral	Water, clay, ice, water
	Marine	Nori seaweed, fish
	Oily	Oil, rancid
8. Industrial Chemical	Petrochemical	Engine oil, book glue, rubber, paint, plastic, photographic film, solvent
	Medicinal	Ascorbic acid, soap, quinine, soap, vitamin B1

Vit et al. (2007)

but is considered to result from harvesting unripe honey which has a higher water content which causes fermentation. Meliponini process honey differently. Fermentation is accomplished by associated microorganisms inside the storage pots and also after harvest. Therefore, fermentation of pot-honey is not a defect but an aspect of honey maturation by meliponines and a human sensory attribute that needs further consideration. The consumer's preferences are related to cultural backgrounds, and tropical cultures value sour tastes, possibly because tropical fruits are sour-sweet. A group of 20 Venezuelan assessors tasted compressed pot-honey and honey extracted by suction. Despite the very small number of participants in this preliminary study, the results demonstrated that the acceptance was higher for the compressed honey than for the honey extracted by suction. Honey compressed with surrounding sour pollen pots contains fermented pollen, and was perceived with a more intense "lemon-like" flavor (unpublished data) i.e., the honey was perceived as having a citrusy note similar to lemon. This result suggests that such characteristic ("lemon-like" flavor) might have contributed to increase the compressed honey acceptance by consumers, compared to the honey extracted by suction.

The sensory evaluation and interpretation of fermented pot-honey is a challenge for those who work in the field. A transition from defect to value could be based on a direct preference for a more fruity-sour characteristics, a complex perception of fermentation patterns, and also an indicator of medicinal properties derived from the fermentive process.

Stingless bees have associations with microorganisms that transform and help to preserve honey and pollen (see Menezes et al. and Rosa et al. chapters in this book). Different microorganisms have a characteristic fermentation pathway. The presence of lactic acid was confirmed in honey of Meliponini (Vit et al. 2011c). Honey of *Tetragonisca angustula* was studied during a 30-day-postharvest experiment. The gradual increase of ethanol enhanced the antioxidant activity in fermented honey stored at 30ºC (Pérez-Pérez et al. 2007).

24.5 Acceptance of Pot-Honeys from Different Species of Meliponini

Considering that food acceptance depends on several consumers' and individual cultural background, the stingless bee honey's acceptance has been evaluated in different populations. In separate studies, participants from Spain, Venezuela, Mexico, and Australia rated how much they liked the honeys on 10-cm unstructured line scales anchored with the expressions "dislike it a lot" and "like it a lot", in the left (1 cm) and right ends (9 cm), respectively. The acceptance scores were measured and the data were analyzed, with ANOVA, followed by a Tukey test to check differences between means. The results are presented in Tables 24.3, 24.4, 24.5, 24.6 and 24.7.

Spanish consumers tasted pot-honey from Australia, Bolivia, Brazil, Mexico, and Venezuela (Vit et al 2010b). The results in Table 24.3 reveal that on average Spanish consumers did not like the pot-honeys, as the higher acceptance mean was 6.2, which is situated slightly above of the neutral score 5 (neither like nor dislike). Stratified sampling is suggested to see if any type of consumer emerges and we can identify people who most like the products.

Little is known about the perception of pot-honey from the forest by native communities of stingless bee-hunters and stingless bee-keepers. For this reason, the acceptance of honey was evaluated in a Huottuja group in Paria Grande, Amazonas

Table 24.3 Average honey acceptance evaluated by Spanish consumers

Common name of the bee	Country of origin	Bee species	Acceptance[1] (Mean ± SD)
"negrita"	Mexico	*Scaptotrigona mexicana*	4.3 ± 2.5^{a}
"suro negro"	Bolivia	*Scaptotrigona polysticta*	4.9 ± 2.1^{a}
"carby"	Australia	*Tetragonula carbonaria*	5.1 ± 2.3^{a}
"uruçú"	Brazil	*Melipona scutellaris*	5.6 ± 2.4^{a}
"erica"	Venezuela	*Melipona favosa*	6.2 ± 2.2^{a}

[1] Evaluated in 10-cm unstructured line scales varying from "dislike it a lot" (1) and "like it a lot" (9). Significant differences between honeys ($P < 0.05$, ANOVA) are indicated by different superscripts

Table 24.4 Average acceptance of honey by Huottuja consumers in Amazonas State, Venezuela

Common name of the bee	Bee species	Acceptance[1] (Mean ± SD)
honey bee	*Apis mellifera*	5.4 ± 3.3^{a}
"angelita" artificial	–	6.5 ± 3.1^{a}
"isabitto"	*Melipona aff. fuscopilosa*[2]	6.9 ± 3.6^{a}
"ajavitte"	*Tetragona clavipes*	7.9 ± 2.2^{a}
"angelita" artificial	–	8.4 ± 1.5^{a}

[1] Evaluated in 10-cm unstructured line scales varying from "dislike it a lot" (1) and "like it a lot" (9). Significant differences between honeys ($P<0.05$, ANOVA) are indicated by different superscripts

[2] *Melipona aff. fuscopilosa* [= *Melipona* (*Michmelia*) sp. 1, see Table in Pedro chapter, this book]

Table 24.5 Average acceptance of "tiúba" *M. fasciculata* honey from different locations

Location	Acceptance[1] (Mean ± SD)
Todos os Santos	3.5 ± 2.9^{a}
Limoeiro	$4.4 \pm 0.8^{a,b}$
Tabocas	$4.8 \pm 1.4^{a,b}$
Moura	5.1 ± 1.1^{b}
Preazinho	6.5 ± 2.6^{c}

[1] Evaluated in 10-cm unstructured line scales varying from "dislike it a lot" (1) and "like it a lot" (9). Significant differences between honeys ($P < 0.05$, ANOVA) are indicated by different superscripts

State, Venezuela (Vit et al. 2010a). Two artificial honeys sold as "angelita" *Tetragonisca angustula* in the indigenous market from Puerto Ayacucho, one honey bee and two genuine stingless bee honeys of "isabitto" *Melipona aff. fuscopilosa* and "ajavitte" from *Tetragona clavipes*, were evaluated. The acceptance results are given in Table 24.4.

Another study was carried out with commercial pot-honey produced by "tiúba" *Melipona fasciculata* in five different places: Limoeiro, Moura, Preazinho, Tabocas, and Todos os Santos, all located in Maranhão state, Brazil. In that honey, natural fermentation was completed, as the postharvest processing aiming at stabilizing the

Table 24.6 Average Mexican acceptance scores for pot-honey from different stingless bees

Common name of the bee	Bee species	Year of harvest	Acceptance[1] (Mean ± SD)
"ala blanca"	*Frieseomelitta nigra*	2011	4.7 ± 2.4^{a}
"uruçú"	*Melipona scutellaris*	2011	4.8 ± 2.5^{a}
"criolla"	*Melipona solani*	2011	5.2 ± 3.3^{a,b}
"colmena real"	*Melipona fasciata*	2010	5.3 ± 2.2^{a,b}
"abeja bermeja"	*Scaptotrigona hellwegeri*	2010	5.5 ± 1.9^{a,b}
"mijui"	*Scaptotrigona polysticta*	2011	5.7 ± 2.3^{a,b}
"pisilnekmej"	*Scaptotrigona mexicana*	2009	6.5 ± 2.1^{a,b}
"abeja bermeja"	*Scaptotrigona hellwegeri*	2009	6.6 ± 2.0^{a,b}
"abeja real"	*Melipona beecheii*	2011	6.8 ± 2.3^{a,b}
"pisilnekmej"	*Scaptotrigona mexicana*	2010	6.8 ± 1.9^{a,b}
"pisilnekmej"	*Scaptotrigona mexicana*	2011	7.3 ± 2.2^{b}

[1] Evaluated in 10-cm unstructured line scales varying from "dislike it a lot" (1) and "like it a lot" (9). Significant differences between honeys ($P < 0.05$, ANOVA) are indicated by different superscripts

Table 24.7 Average Australian acceptance scores of pot-honey from different stingless bee species and unifloral *A. mellifera* honeys

	Acceptance[1] (Mean ± SD)
Stingless bee species	
Melipona fasciata	3.7 ± 2.6^{a}
Scaptotrigona mexicana	4.0 ± 3.0^{a}
Tetragonula carbonaria	4.1 ± 2.6^{a}
Frieseomelitta nigra	4.1 ± 2.8^{a}
Melipona beecheii	4.7 ± 3.2^{a}
Unifloral honey	
Passion fruit	4.1 ± 2.7^{a}
Lychee	5.1 ± 2.5^{a}
Leatherwood	5.5 ± 2.6^{a,b}
Manuka	6.0 ± 2.5^{a,b}
Avocado	7.3 ± 0.2^{b}

[1] Evaluated in 10-cm unstructured line scales varying from "dislike it a lot" (1) and "like it a lot" (9). Significant differences between honeys ($P < 0.05$, ANOVA) are indicated by different superscripts

honey prior to packaging. The word "natmel" was created for naming this type of honey. Honey was collected during the X IberoLatinamerican Congress of Apiculture held in Natal, Brazil 2010. The honey was taken to Venezuela to be tasted by Venezuelan honey consumers. Table 24.5 presents the acceptance results (Vit et al. 2011b).

During the VII Mesoamerican Seminar on Native Bees held in Cuetzalán, Puebla, Mexico, May 2011, the Municipality of Cuetzalán was declared Sanctuary of *S. mexicana* "pisilnekmej" (from the Nahuatl "pisil" small, "nektsin" bee).

Pot-honeys from eight species of stingless bees were tasted by a panel of Mexican creole, Mayan, and Nahuatls. Two species had honeys harvested in different years. Higher acceptance mean scores were observed for recently harvested *S. mexicana* (2011) (Table 24.6).

Another study investigated the acceptance of pot-honeys produced by five species of stingless bees (*M. beecheii*, *M. fasciata guerreroensis*, *S. mexicana*, *T. carbonaria*, and *T. nigra*) and five unifloral honeys: avocado *Persea americana* (Lauraceae), lychee *Litchi chinensis* (Sapindaceae), passion fruit *Passiflora edulis* (Passifloraceae), leatherwood *Eucriphia lucida* (Cunoniaceae), and manuka *Leptospermum scoparium* (Myrtaceae) of *A. mellifera* from Kuranda forest, Queensland, Australia. Table 24.7 shows the average acceptance results achieved in the study.

24.6 Descriptive Sensory Studies of Pot-Honey

Descriptive studies were also carried out with pot-honey, to investigate the relationship between sensory attributes and the bee origin of the honey produced in pots by Vit et al. (2011a and 2011d). Samples were analyzed by free-choice profiling (FCP) (Deliza et al. 2005), a quick and inexpensive method in which participants are asked to both identify attributes in the sample, and score their intensities on appropriate scales. They should be provided with adequate instruction on how to perform this test, and possibly given product categories to describe them in terms of appearance, aroma, flavor, texture, etc. Each participant will generate his/her own set of attributes, and consumers should be recruited as product users, age/gender/education level. It is important to note that consumers may use terms in different ways. Researchers may be able to separate consumers into groups, aiming at better identifying which characteristics are most important for that consumer segment. Generalized Procrustes Analysis (GPA) is a common statistical tool for analysis of FCP data. Figures 24.1 and 24.2 present the results of the studies conducted with the Huottuja (Piaroa) community and Spanish consumers, respectively.

24.7 Final Considerations

Perception is a multifactorial process that needs to be considered to explain any sensory response, in our case the pot-honey results. Orthonasal (breathing, nasal mucosal tissues, nasal metabolism) and retronasal (physicochemical release, salivation, oral metabolism, oral and pharyngeal) peripheral factors, besides chewing and swallowing patterns, and tongue movements affect the tasting process (Buettner and Beauchamp 2010). Odor, aroma, and taste are released from the honey matrix according to chemical and physical features. Although we are interested in comparing honeys—not assessors, we cannot forget the individual

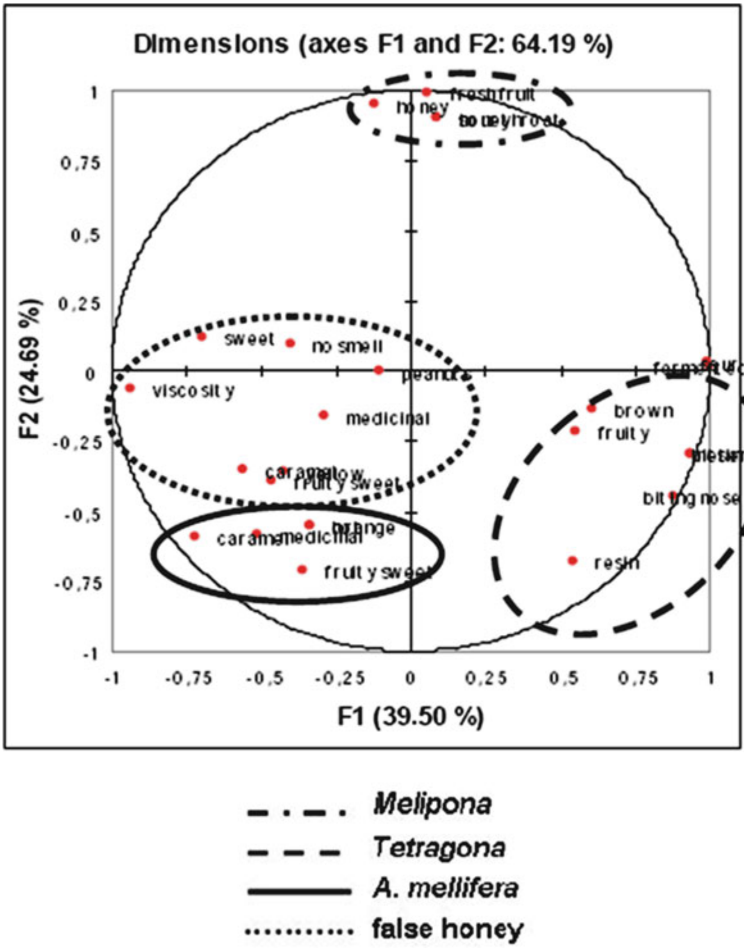

Fig. 24.1 Honey descriptive sensory evaluation by Huottuja community (from Vit et al. 2011a). Used by permission of Sociedade Brasilera de Farmacognosia

differences of participants regarding honey perception with a strong cultural imprinting since their childhood (Barthomeuf et al. 2009). In addition, due to today's market competitiveness, it is necessary to understand the factors influencing consumers at the emotional level. Identifying the emotional elements that consumers experience and expect in a product can help providing a complete perspective on consumer affective behaviors, and contributing to the identification of the products most liked by consumers. In this context, scales for measuring different emotions associated to food product have been developed to test food by consumers (King and Meiselman 2010), and may be a useful tool to help better understand consumer's honey perception.

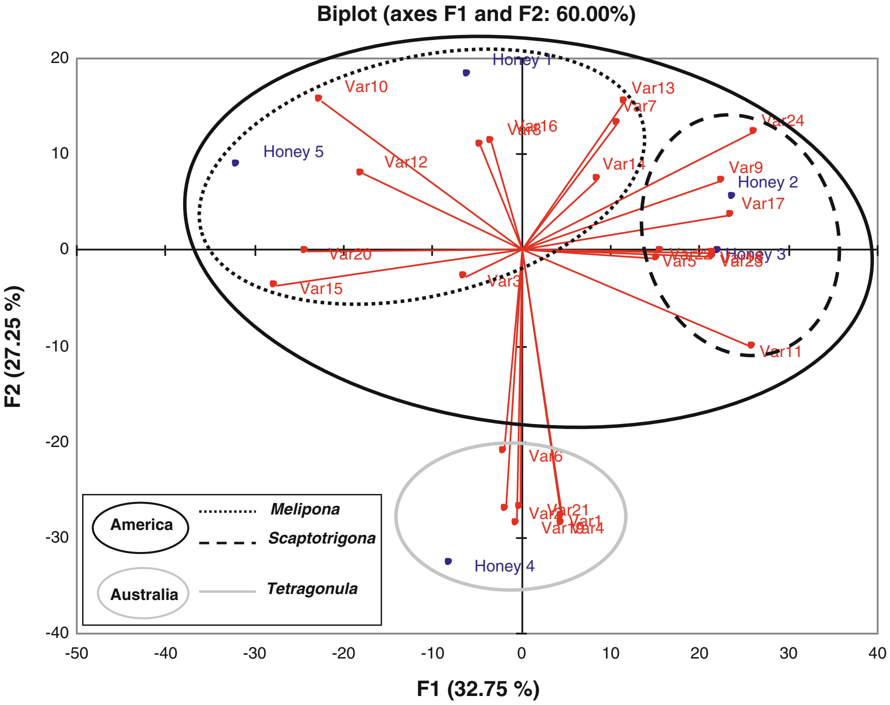

Fig. 24.2 Pot-honey descriptive sensory evaluation by Spanish consumer (from Vit et al. 2011d). Permission granted by the International Bee Research Association

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Chapter 25
Melipona favosa Pot-Honey from Venezuela

Patricia Vit

To the memory of Father Santiago López Palacios of Universidad de Los Andes, Venezuela, for his inspiration to investigate bee botany and honey.

To the retired Dr. Livia Persano Oddo and Dr. Stefan Bogdanov for their truthful interest and timely scientific collaboration to study this unknown honey in Europe.

To the memory of Professor João MF Camargo for identifying stingless bee species to name pot-honey beyond expectations.

25.1 Introduction

During his visit to Venezuela in 2008, Prof. JMF Camargo could not observe the *Melipona favosa* (Fabricius 1798) that he kindly identified, in their cactus wild nests (see Fig. 25.1). However, he informed us that this was the first species of Meliponini accurately described, probably with a specimen from French Guiana. Prof. Camargo also authored and anchored the idea of pot-honey as the first honey on planet Earth, dating back to the late Cretaceous, before comb honey was produced by *Apis mellifera*. He had studied the oldest bee fossil, *Cretotrigona prisca*, preserved in amber from New Jersey (Michener and Grimaldi 1988a, b), and knew that dinosaurs and stingless bees shared landscapes 97–74 million years before present. This bee from the Paraguaná Peninsula (Falcón state, Venezuela) was undisturbed by *Apis mellifera*, until honey bee swarms were seen after the floods caused by el Niño at the end of 1999. But the Africanized honey bee colonized Venezuela since 1975 in southern Amazon state, and 1976 in Santa Elena de Uairén, Bolívar state (Gómez Rodríguez 1986).

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Fig. 25.1 Entrance of *Melipona favosa* nest in a columnar cactus "cardón" *Stenocereus griseus*, Paraguaná Peninsula, Falcón state, Venezuela Photo: P. Vit

M. favosa is mostly known as "erica" but is also named "maba" in a few places. It is a smaller bee than other Venezuelan *Melipona* such as *M. compressipes* and *M. trinitatis*, named "guanota." However, it is bigger than stingless bees from other genera different from *Melipona*, like *Tetragonisca* and *Scaptotrigona*. The honey pots also have an intermediate size. This honey is reported in the classic novel "Doña Bárbara" (Gallegos 1973), the Venezuelan book on creole bees (Rivero Oramas 1972), and the chapter on Meliponini in the Catalogue of Bees in the Neotropical Region (Camargo and Pedro 2007), but is not considered in Venezuelan honey standards (Vit 2008a). It was available during field work in the plains and coastal regions of Venezuela. The honey harvest is traditionally made by removing sealed pots from the storage area in the hive, on a dish. The honey pots are compressed with forks or hands, and honey is decanted, and bottled, as learned from Venezuelan stingless bee-keepers, also known as "meliponicultors" (Vit 1994a, b).

Our analytical pot-honey harvests were done by extraction with rubber tube adapted to a syringe, after piercing sealed honey pots, to avoid contamination from pollen pots. However, in a preliminary sensory trial in the Food Science Department at Universidad de Los Andes held in 2007, the additional sour taste, derived from fermented pollen in honey extracted by compression (by hand, with honey and pollen pot contents admixed), was highly appreciated (Vit et al. 2010b). Currently, suction pumps are used for meliponine honey extraction in Brazil (see Alves chapter in this book), while piercing and decantation are used in Australia (TA Heard and M Halcroft, personal communication).

Comb honey from *A. mellifera* is different from pot-honey of *Melipona*. However, both honey types have practical applications as sweeteners, and prototypical medicinal uses conferred by the high osmotic pressure, and the action of minor components of botanical (see Tomás-Barberán chapter in this book) and bee origin. The enormous biodiversity of Meliponini, and their associated microorganisms (see chapters by Menezes et al., and Morais et al., this book), may add further functional properties to pot-honey, unknown for comb honey. Here I analyze the *M. favosa* honey composition in a collection of five samples from the Paraguaná Peninsula and review a database of 40 *M. favosa* pot-honeys from Venezuela, including their bioactive and sensory properties.

25.2 A Peculiar Honey, with Similarities to and Differences from *Apis mellifera*

Since 1985, the collection of *M. favosa* honey has steadily increased. Only recently, a false *M. favosa* honey invaded the Venezuelan market (Vit et al. 2011). This fact should be of interest for Venezuelan sanitary authorities, and not ignored, as is often the case for fraudulent or adulterated honey of *A. mellifera*. It remains the responsibility of the consumer to determine the authenticity of honey, when needed for medicinal use. Venezuelan norms for honey created in 1984 (Comisión Venezolana de Normas Industriales 1984a, b) have not been revised, in contrast to the recent assessment of Colombian norms, in which honey produced by native bees was included for the first time in a honey regulation (ICONTEC 2007). This is a promising example for other countries to join the quest of setting standards for the honey produced by Meliponini, instead of searching for a new word such as "divine elixir" (Vit et al. 1998b). The word honey is not a trademark for that made by bees in combs and can be used for both the honey produced in pots and in combs (Vit 2010a).

A number of collaborators were attracted by this honey processed in pots, and from that of other stingless bee species (their ability of transporting and storing the energy of the sun—as watery sugars—in flexible pots built up with cerumen, able to expand and reduce volumes during fermentive process) (see Fig. 25.2).

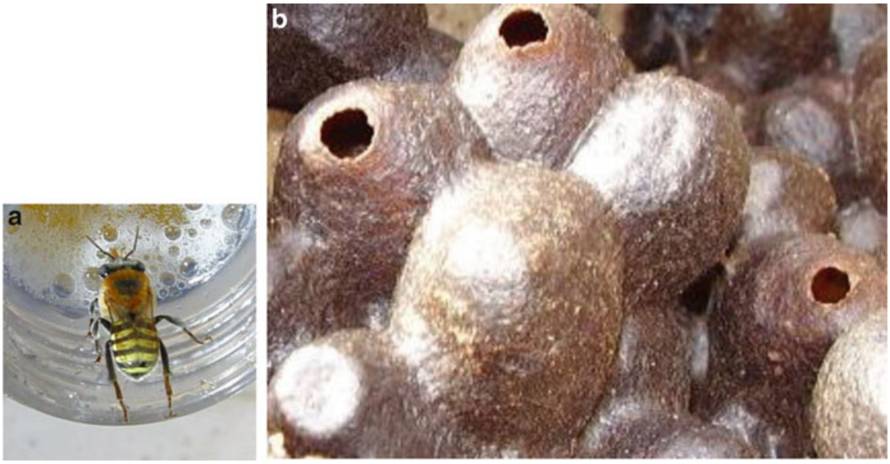

Fig. 25.2 *Melipona favosa* (**a**) on a bottle of fermenting honey harvested from sealed honey pots and (**b**) her storage pots in the nest Photo: P. Vit

This sour–sweet honey, with its own sugar spectra (Bogdanov et al. 1996; Vit et al. 1998a), has very low diastase activity, higher moisture and higher free acidity than that of *A. mellifera*, but similar ash, sucrose, and nitrogen content (Vit et al. 1994, 1998b). Possibly, it is a honey finished and stored with lower processing of nectar, causing nose perceptions resembling those of the foraged flowers, from a more diluted sugar matrix less transformed than *A. mellifera* comb honey, which is permitted by resin, which kills the bacteria, and by mutualistic microbes in the gut—just like *Apis*—which kill pathogenic microbes that would otherwise ruin both the honey and pollen (DW Roubik, personal communication).

25.3 Composition of *Melipona favosa* Honey Collected in Rational Hives

Honey pots of *M. favosa* from Paraguaná Peninsula, Falcón state, Venezuela, were pierced to collect the honey by syringe extraction. Honey samples were harvested from five *M. favosa* hives, the same day. Physicochemical parameters were analyzed in duplicate according to the methods recommended by the Venezuelan regulations (COVENIN 1984a). Parameters measured included ash (gravimetric method), water content (refractometric method), reducing sugars and sucrose (titrimetric method), pH, free acidity (titrimetric method). Color was measured by optical comparison (instrumental method). Nitrogen was determined by a standard micro Kjeldahl method (AOAC 1984). The analytical results on chemical composition of the five samples of *M. favosa* honey are shown in Table 25.1.

The honey produced by *M. favosa* is light in color. In the five samples analyzed here, the color varied between 20 and 27 mm Pfund. The moisture content varied between 29.7 and 30.2 g water/100 g honey, which is higher than the honey standard for *A. mellifera*, and typical for the values in meliponine honeys reported since Gonnet et al. (1964). The ash content varied between 0.07 and 0.14 g ash/100 g honey, falling below the maximum 0.5 g/100 g *A. mellifera* honey standard. The pH

Table 25.1 Composition of *Melipona favosa* pot-honey from the Paraguaná Peninsula of Venezuela, $n=5$

Physicochemical parameters	Mean ± SD	Min	Max
Color (mm Pfund)	23.2 ± 2.7	20	27
Moisture (g/100 g honey)	30.0 ± 0.2	29.7	30.2
Ash (g/100 g honey)	0.10 ± 0.02	0.07	0.14
pH	3.7 ± 0.2	3.5	3.9
Free acidity (milliequivalents/kg honey)	50.6 ± 18.3	34.2	85.2
Nitrogen (mg/100 g honey)	41.7 ± 8.1	30.0	53.4
Sugars (g/100 g honey)			
Reducing sugars	64.6 ± 2.3	61.4	69.0
Apparent sucrose	1.3 ± 0.5	0.7	2.0

values are in the same range of *A. mellifera* honey, whereas the average free acidity (50.6 meq/kg honey) is higher than the maximum 40 meq/kg *A. mellifera* honey standard (COVENIN 1984b). This indicates the presence of higher amounts of weak acids, such as organic acids with low ionization.

The nitrogen content varied between 30.0 and 53.4 mg N/100 g honey with an average of 41.7, similar to 40.66 mg N/100 g reported for *M. favosa* honey in a previous work, and slightly lower than 57.1 mgN/100 g found in *A. mellifera* honey from Venezuela (Vit et al. 1994). The average concentration of reducing sugars is into the limit of the minimum 65 g/100 g and of the maximum 5 g/100 g prescribed by the *A. mellifera* honey standards (COVENIN, 1984b). This means that some *M. favosa* honey samples do not fulfill this parameter due to a slightly lower concentration of reducing sugars, which is consistent with previous results (Vit et al. 1998b).

25.4 Sensory Attributes of *Melipona favosa* Honey

A honey tasting sensory assay was initiated with the system used for *A. mellifera*. Sensations in the nose are called "odor," whereas the multimodal sensations in the mouth—differing from taste and trigeminal sensations, are called "aroma." Seven families of sensory attributes in the odor-aroma wheel (Piana et al., 2004) were adapted to eight sensory odor-aroma families in a table for stingless bees: (1) Floral-fruity. (2) Vegetable. (3) Fermented. (4) Wood. (5) Bee hive. (6) Mellow. (7) Primitive. (8) Industrial chemicals (Vit et al. 2007). This is a cognitive construct to facilitate the perception of pot-honey in this system. A histogram of odor-aroma families perceived in one sample of *M. favosa* honey by eight assessors is shown in Fig. 25.3. The highest count for odor was halved with a dotted line, and for aromas with a straight line. Bars above the lines are considered primary odors and aromas, respectively, and below the lines are considered secondary odors and aromas.

The family floral-fruity described both primary odor and aroma. The peculiar smell of the *M. favosa* nest is a primary attribute more frequent than woody, mellow, and primitive odors. Fermented, vegetable, and primitive secondary aromas are more frequent than woody, nest, and mellow. Overall, this *M. favosa* is a floral-fruity and fermented honey with the bouquet of the hive (given by the bees, collected substances and products). Other secondary odors and aromas were less frequent.

The fermenting honey, noted as a sensory attribute, is interpreted as an indication that Meliponini process their food with microorganisms, possibly as evolutionary ability. The sensory concept, that fermented meliponine honey is not spoiled, was recently assessed during the 8th Pangborn Sensory Science Symposium (Vit et al. 2009b). In fact, meliponine honey is not to be considered a spoiled honey, even if it may ferment in the storage pots inside the hive and after harvest, due to the high water content and associated microorganisms. On the contrary, fermentation contributes to the typical sensory profile of this honey and also increased the antioxidant activity of *T. angustula* honey (Pérez-Pérez et al. 2007).

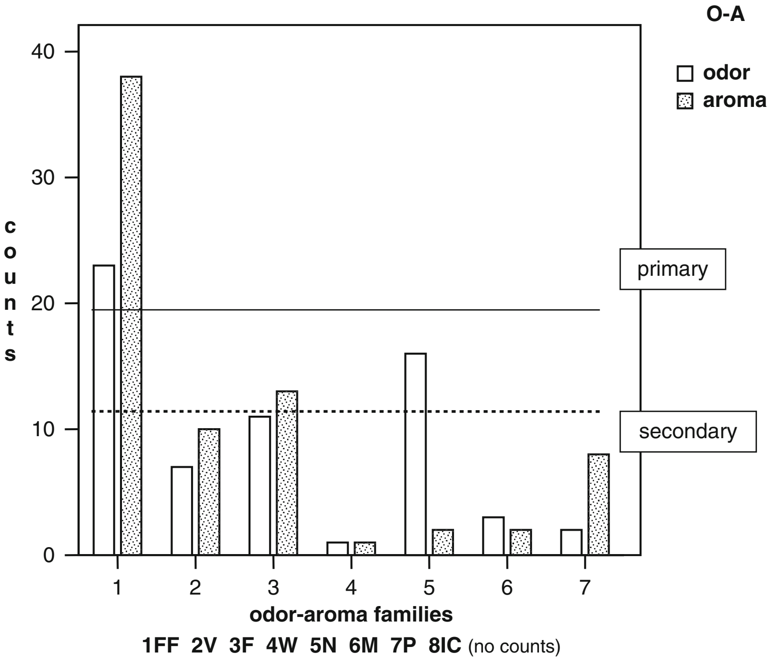

Fig. 25.3 Sensory profile of *Melipona favosa* honey with a trained panel. *FF* floral-fruity, *V* vegetable, *F* fermented, *W* woody, *N* bee hive, *M* mellow, *P* primitive. The highest count for odor was halved with a *dotted line*, and for aromas with a *straight line*. *Bars* above the lines are considered primary odors and aromas, respectively, and below the lines are considered secondary odors and aromas. No counts for the industrial chemical family 8IC Modified from: Vit (2008b). Permission granted by Revista de la Facultad de Farmacia

25.5 Database of *Melipona favosa* Honey from Venezuela

Settings of honey standards were suggested for the most studied stingless bees, four species of *Melipona* (*M. asilvai*, *M. compressipes*, *M. favosa*, *M. mandacaia*) and *Tetragonisca angustula*. The averages values found for 20 samples of *M. favosa* honey in a previous review were free acidity 49.9 meq/kg, 0.22 g ash/100 g, 55.8 mg nitrogen/100 g, 71.2 g reducing sugars/100 g, 1.7 g apparent sucrose/100 g, and 24.8 g water/100 g (Souza et al. 2006).

The seven physicochemical standards in the Venezuelan norm COVENIN 2191–84 are set for *A. mellifera* but not for Meliponini pot-honey: (1) Moisture (Max 20%), (2) Reducing sugars (min 65%), (3) Sucrose (max. 5%), (4) Free acidity (max 40 meq/100 g), (5) Ash (max 0.5%), (6) Hydroxymethylfurfural HMF (negative), (7) Diastase activity (positive). These last two parameters are qualitative and refer

Table 25.2 Composition of *Melipona favosa* pot-honey from Venezuela highlighted values are different from *Apis mellifera* honey standards

Physicochemical parameter	*N*	Mean ± SD	Min	Max
Moisture (g/100 g honey)	40	28.0 ± 2.7	22.1	32.0
Ash (g/100 g honey)	40	0.14 ± 0.13	0.01	0.61
Diastase (DN)[a,b]	6	2.86 ± 0.36	2.64	3.50
Free acidity (milliequivalents/kg honey)	40	51.7 ± 25.2	12.7	97.1
Invertase (IU)[c]	6	90.08 ± 48.03	31.80	150.70
Nitrogen (mg/100 g honey)	39	45.7 ± 18.3	10.5	102.0
HMF (mg/kg honey)	21	17.7 ± 8.5	5.04	24.69
Sugars (g/100 g honey)				
Reducing sugars	40	67.3 ± 4.1	60.9	78.6
Apparent sucrose	40	2.1 ± 1.3	0.5	5.1

[a] The Diastase Number (DN) indicates g starch hydrolyzed/100 g honey/h, at pH 5.2 and 40°C

[b] Semiquantitative data not included

[c] An Invertase Unit (IU) indicates μmoles p-nitrophenyl glucopyranoside hydrolyzed/kg honey/min, at pH 6.0 and 40°C

to the heating and aging of the honey. Findings in previous works indicated the low diastase activity of *M. favosa* honey, as well as an HMF content similar to that of *A. mellifera* honey (Vit et al. 1994, 1998b). The natural low diastase activity values found in previous qualitative (Vit 1992) and quantitative (Vit et al. 1994, 1998b) measurements suggest this is not a quality indicator for *M. favosa* honey. For this reason, diastase activity was measured in half of the samples. The average composition and variations of 40 samples of *M. favosa* honey studied from samples taken over 20 years are indicated in Table 25.2.

Free acidity, ash, reducing sugars, sucrose, and water content of honey are useful quality indicators for *M. favosa,* as they are for *A. mellifera*, although standards may differ. Flavonoid and polyphenol contents, antioxidant and antibacterial activities, and sensory analysis are biochemical, biological, and consumer analyses which also contributed to *M. favosa* honey characterization.

25.6 Suggested Standards for *Melipona favosa* Honey Compared to *Apis mellifera*

Compared to Venezuelan honey standards for *A. mellifera* (COVENIN 1984b), the following changes in reference values may be adopted for *M. favosa* honey (see Table 25.3): (1) No variation for HMF values, (2) Increased maximum values for water content (up to a maximum of 35%), apparent sucrose (up to a maximum of 6%), free acidity (up to a maximum of 100 meq/100 g), and ash (up to a maximum of 1.0%), (3) Decreased minimum for reducing sugars (down to a minimum of

Table 25.3 Suggested standards for *Melipona favosa* honey, compared to *A. mellifera*

Quality factor	*Melipona favosa* suggested standard	Relation	*Apis mellifera* standard
Moisture (g/100 g)	Max 35.0	>	Max 20.0
Ash (g/100 g)	Max 0.5	=	Max 0.5
Free acidity (meq/100 g)	Max 100.0	>	Max 40.0
Nitrogen (mg/100 g)	10.0–105.0	New	–
Reducing sugars (g/100 g)	Min 60.0	<	Min 65.0
Apparent Sucrose (g/100 g)	Max 6.0	>	Max 5.0
HMF (mg/kg)	Max 40.0	=	Max 40.0

60%), (4) The nitrogen content is not included in the standards for *A. mellifera* honey, but a range 10–100 mg N/100 g honey would be useful for protection against adulteration and falsification, (5) Diastase activity is not included because the activity of this enzyme is very low in *M. favosa* honey; therefore, it is not a practical quality factor to measure freshness or heating.

25.7 The Inclusion of Biological Activity Descriptors

In addition to compositional quality factors, the biological activity of honey could also become a useful descriptor for medicinal use. However, there are no simple descriptors for that purpose. For instance, the variable contents of flavonoids and polyphenols in *A. mellifera* unifloral honeys (Frankel et al. 1998) did not correlate with antioxidant capacity. The flavonoid content is lower than the polyphenols, as generally observed in the honey produced by other species of stingless bees, such as *T. carbonaria* from Australia (Persano Oddo et al. 2008), *M. beecheii* and *M. solani* from Guatemala (Gutiérrez et al. 2008), *M. crinita*, *M. eburnea*, *M. grandis*, *M. illota*, *Nannotrigona melanocera*, *Partamona epiphytophyla*, *Ptilotrigona lurida*, *Scaptotrigona polysticta*, *Scaura latitarsis*, and *Tetragonisca angustula* from Peru (Rodríguez-Malaver et al. 2009), *Tetragonisca fiebrigi* from Argentina and Paraguay (Vit et al. 2009a), and also in *M. favosa* from Venezuela (Vit et al. 2012). This means that other polyphenol types in pot-honey may explain their antioxidant activities. Seminal findings on greater contents of flavonoid glycosides than aglycones in *M. favosa* honey strongly differentiate them from *A. mellifera* honey. Pot-honey of *M. favosa* has more aglycones, from hydrolyzed *O*-glycosides in the nectar and propolis (Truchado et al. 2011). Values of 45.9–227.92 μmole Trolox equivalents/100 g honey, positioned *M. favosa* honeys in the categories low (0–100) and high (200–300) reported for unifloral *A. mellifera* Czech honeys (Vit et al. 2008a).

Considering antibacterial activity, a successful marker of antibacterial activity is the unique manuka factor (UMF) described by Prof. Peter Molan from Waikato University in New Zealand (Molan 2005). However, this is a useful marker for honey of a botanical origin including only Myrtaceae, genus *Leptospermum*. More conservative are the tests to measure inhibition of bacterial growth under controlled condition. The Gram positive *S. aureus* is more resistant to these honeys than the Gram negative *E. coli*, because lower MICs of honey were needed to kill *E. coli* than *S. aureus*. This was also observed in Venezuelan honeys of *A. mellifera* (Vit et al. 2008b) and *M. favosa* (Vit et al. 2012), other stingless bee species from Argentina (Vit et al. 2009a), and *Geotrigona acapulconis* from Guatemala (Dardón and Enríquez 2008). Although *E. coli* and *S. aureus* MICs were similar to those found in other *Melipona* species, *E. coli* was more resistant than *S. aureus* to *Tetragonisca angustula* honey from Guatemala.

The anticancer activity of two *M. favosa* honeys (IC_{50} 3.39–16.50 mg/mL) was measured in vitro using a model based on ovarian cancer (see Vit et al. chapter 35, this book). Considering that both samples were collected in the same meliponary but in different months, the effect of the botanical origin (see Obregón et al. chapter 23 in this book) becomes relevant to the bioactive properties of pot-honey.

Melissoplaynology will be useful in the future, for understanding the contribution of botanical origin to the composition, sensory and biological properties of *M. favosa* honey. Denomination of unifloral honeys of each stingless bee species is not envisaged, but some exceptions may be valid, as well as for the geographical origin.

25.8 Contemporary Interactions to Value *Melipona favosa* Honey

Expert scientists, technicians, and keepers of traditional meliponiculture can benefit consumers in search of information. Emotion, cognition, and communication are relevant components to spread the tradition and to foster technological progress. Observing a living stingless bee hive is the ultimate learning experience concerning pot-honey and the meliponines. *M. favosa* is a gentle bee that could be easily kept in schools, where young people can observe them. However, this bee thrives in the plains and coastal regions, and other species will be needed in different locations of Venezuela. The *M. favosa* bee can be kept by women, children, and the elderly.

The entomological origin of honey should be on the label (common and scientific name of the bee). Consumers and stingless bee-keepers should be protected from producers of false meliponine honeys without stingless bee apiaries (meliponaries) to back up their honey production. Labels of organic certified honey may help to safeguard the reputation of pot-honey and be useful to promote this industry, but they demand great organization to be reliable.

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Chapter 26

Tetragonisca angustula Pot-Honey Compared to *Apis mellifera* Honey from Brazil

Ligia Bicudo de Almeida-Muradian

26.1 Introduction

According to Brazilian legislation (Brasil 2000), honey is considered a food product produced by bees from floral nectar, plant secretions and sap-feeding insects, collected from living plants that bees transform, combine with specific substances, and store. Commercial honey is usually produced from floral nectar. Honeydew is produced from exudates of some insects and is called in Portuguese "mel de melado" (Campos et al. 2003).

In Brazil there are two types of beekeeping: (1) the commercial kind with *Apis mellifera*, and (2) meliponiculture, which uses stingless bees. Honey from stingless bees is more expensive than commercial honey. However, it is sold without proper regulation. There are no identity and quality parameters, or regulation, for this type of honey which is popularly known by its beneficial properties to human health (Vit et al. 2004; Sousa 2008).

The characteristics of beekeeping products have specific laws for quality control of honey (Brasil 2000), pollen, propolis and royal jelly (Brasil 2001). Meliponiculture is the art of dealing with indigenous stingless bees, obtaining honey as the primary product (Nogueira-Neto 1997). As cited by Kerr et al. (2005), stingless bees were the only species producing commercial honey in Brazil, until 1838. Because they are traditionally kept by indigenous people, they can be also referred to as indigenous bees. *Tetragonisca angustula* (Latreille 1811), a small bee known as "jataí", is the most abundant stingless bee in the southeast and southern regions of Brazil

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(Villas Bôas and Malaspina 2005) and produces an excellent honey with distinct characteristics from *A. mellifera*.

It is difficult to establish a single standard for all of Brazil, a country rich in stingless bee species and characterized by great environmental diversity (Almeida-Muradian 2009). Honey samples produced by *A. mellifera* and *T. angustula* in the same region of Brazil are compared here.

26.2 Beekeeping and Meliponiculture in Brazil

Beekeeping with honey bees is practiced in Brazil since the immigration of Europeans, mostly Italians and Germans, who brought the bees in the middle of the nineteenth century, introducing them in Rio Grande do Sul, Santa Catarina and Paraná states (Kerr et al. 2005); but stingless beekeeping potentially originated 15,000 years ago (David W Roubik, personal communication). Meliponiculture can be a sustainable activity and economically viable, since honey produced by native bees has a guaranteed market (Oliveira 2002). There are about 400 species of meliponines in Brazil, and many others are found in the tropical world, with more than 70% occurring in the Americas (Carvalho et al. 2003) and Camargo, Michener, in this book.

Although the amount of honey obtained from a colony of stingless bees is not much, compared with the Africanized honey bee, stingless bees have advantages such as: (1) they are more suitable for the pollination of trees from Brazilian forest and cultures and (2) their honey has the best price in the market, as a special organic product, with particularities of taste and aroma, which depend on flora and bee species (Venturieri 2003). According to Nogueira-Neto (1997), the different food collection habits of native bees, compared to the Africanized bees, varies the composition of their honey.

26.3 Honey Composition of Brazilian *Tetragonisca angustula*

The stingless bee jataí has a characteristic nest and a cerumen entrance tube. This is one of the meliponine species most adaptable in nesting. They live in cities and towns, virgin forests, and secondary vegetation, under the ground, in trees, and in the hollows between rocks (Nogueira-Neto 1970).

Jataí honey is collected by piercing the honey pots. Honey is removed with a large syringe, or a suction pump, and later filtered. As a sanitary precaution, the honey is removed from closed pots, considered "mature honey", to prevent absorption of moisture and consequently deterioration. Although they produce honey in lesser amount, the meliponines supply a varied product compared to common honey from *A. mellifera*, because of their special flavours (Carvalho et al. 2005). *T. angustula* produces a honey well appreciated by the consumers. Jataí honey is used also for therapeutic treatments including ophthalmic and pulmonary uses (Iwama 1977).

26.4 Legislation for *Apis mellifera* Honey and Quality Parameters

Honey is considered food that provides energy, being elaborated from the dehydration and transformation of floral nectar. For human consumption, honey needs to comply with the minimum requirements of identity and quality demanded by proper regulation (Sousa 2008).

For *A. mellifera*, Brazilian honey standardization employs Normative Instruction 11 October 2000 (Brasil 2000). This regulation was based on European legislation and honey from *A. mellifera,* not the honey from native bees, which presents differences in some physicochemical parameters (Azeredo et al. 2000; Sousa 2008). However, some works had suggested quality standards for the regulation of the stingless bee honeys.

Some researchers suggested maximum and minimum values for each quality parameter for stingless bee honey. Vit et al. (2004) proposed quality standards for stingless bee honey from Venezuela, divided in three groups: *Melipona*, *Scaptotrigona* and *Trigona*. Villas-Bôas and Malaspina (2005) suggested parameters for Brazilian stingless bee honey. The values defined for Brazilian legislation of quality control concerning *A. mellifera* and values suggested for stingless bee honey, by Villas-Bôas and Malaspina (2005), are shown in Table 26.1.

The Brazilian Legislation standardizes the quality of the honeys evaluating the parameters indicating physicochemical characteristics of maturity (reducing sugars, apparent sucrose and moisture) of authenticity (insoluble solids in water and minerals) and of deterioration (acidity, diastase activity and hydroxymethylfurfural). The recommended method for sugar analyses in honey, using Lane and Eynon method which consists of the reduction of copper ions in alkaline Fehling solution (Brasil 2000).

Beyond the physicochemical traditional methods, other methods more selective have been studied that can be applied like high performance liquid chromatography

Table 26.1 Legislation standards for quality control of *Apis mellifera,* suggested values for honey of Meliponini and *Tetragonisca angustula* in Brazil

Chemical parameters	*Apis mellifera* honey (Brasil 2000)	Meliponini honey (Villas-Bôas and Malaspina 2005)
Reducing Sugars (%)	Min. 65.0	Min. 50.0
Moisture (%)	Max. 20.0	Max. 35.0
Apparent Sucrose (%)	Max. 6.0	Max. 6.0
Insoluble Solids (%)	Max. 0,1	Max. 0.4
Minerals (%)	Max. 0.6	Max. 0.6
Acidity (meq/Kg)	Max. 50.0	Max. 85.0
Diastase activity (DN)	Min. 8.0	Min. 3.0
Hydroxydometylfurfural (mg/kg)	Max. 60.0	Max. 40.0

(HPLC), which is capable of identifying a high variety of carbohydrates in the sample. This method is more sensible, reducing the time of the analysis (Cano et al. 2006).

Another important parameter for the determination of honey quality is the amount of moisture (water content), responsible for conservation of the honey. Water content of honey presents a great variation (14–25%), with the ideal values between 17 and 18%, when it is not prone to fermentation (Louveaux 1968).

Moreover, sugars and water correspond to a major part of honey composition, but there are also small amounts of enzymes, whose presence vary compared with the substances producing sweetness. The main enzymes presented in honey are invertase, diastase and glucose oxidase (White 1975).

Invertase originates from the hypopharyngeal glands of the bees. It is the main factor responsible for the chemical transformation of the nectar in honey (Maurizio 1959). It is added to the nectar and its activity can continue in the product after extraction. Invertase hydrolyzes sucrose into glucose and fructose; other more complex sugars are also transformed under the action of this enzyme (Iwama 1977). The reduction of this enzyme can be caused by the processing, heat and shelf life (Huidobro et al. 1995). The activity of the enzyme diastase is used as a quality parameter for authenticity of honey from *A. mellifera*. This quantification indicates the intensity of heating and natural degradation of the product.

Another indicative characteristic from adulteration of the honey is the quantity of hydroxymethylfurfural (HMF). It is a cyclic aldehyde ($C_6H_6O_3$) formed by decomposition of fructose in the presence of acid (pH 3.8–3.9). The process of dehydration from fructose indicate ageing and heating of the honey (González 2002; White 1975; Gonnet 1963). The identification of this compound is used to verify honey adulteration with commercial sugar (beetroot or maize), inadequate storage and overheating (Vilhena and Almeida-Muradian 1999). Another quality parameter is the free acidity of honey. The acid found in honey is responsible for its stability against microorganisms (White 1975). The pH of honey varies from 3.2 to 4.2 (average of 3.9) being influenced by the mineral percentage. Generally the honeys rich in ash present high values of pH (White 1975). The quantification of insoluble solids is another quality parameter demanded by legislation used to verify the pureness from honey and the efficiency in the extraction process (Leite and Santos 2001). The maximum allowed by Brazilian legislation for insoluble solids in honey is of 0.1%, except the pressed honey that tolerates 0.5% (Brasil 2000).

Brazilian honey possesses a large variety of colours, which can influence the preference of the consumers. Honey colour can be correlated with its floral origin, processing storage, climatic factors and the temperature which the honey ripens in the beehive (Seemann 1988).

Dark honeys have largest amounts of minerals compared with light ones. The percentage of mineral (total ash) varies from 0.02 to 0.6%. Ashes constitute mainly of salts from calcium, sodium, potassium, magnesium, iron, chlorine, phosphorus, sulphur and iodine (Sepúlveda Gil 1980).

26.5 Physicochemical Properties of *T. angustula* and *A. mellifera* Honey in Brazil

Samples from *T. angustula* ($n=6$) and *A. mellifera* ($n=6$) honey were collected in six cities from São Paulo state, Brazil (Amparo, Itaberaba, Lins, Marília, Pedreira, Santo Antonio de Posse). The honey was kept frozen until analysis.

Moisture was measured with an Abbe refractometer, and refraction index was converted into humidity using the Chataway table at 20°C (Brasil 2000; AOAC 1990; Almeida-Muradian and Bera 2008). Reducing sugar content and apparent sucrose were determined by titration using Fehling reagent (CAC 1989; Bogdanov et al. 1997). Insoluble solids were analyzed by gravimetry according to Brazilian regulation (Brasil 2000) and the Codex Alimentarius Commission (CAC 1989). Minerals (ash) were determined by gravimetric methods (oven at 550°C) (CAC 1989; Brasil 2000). Free acidity was measured by potentiometric titration (AOAC 1990; Brasil 2000; Bogdanov et al. 1997). Diastase employed the spectrophotometric method—wavelength 660 nm (Brasil 2000; CAC 1989). Hydroxymethylfurfural (HMF) content was measured by spectrophotometry at 284 nm, subtracting the back absorbance at 336 nm according to AOAC (1990) and Brazilian regulation (Brasil 2000). All measurements were made in triplicate.

The composition of the two types of honeys (*A. mellifera* and *T. angustula*) from Brazil obtained by Sousa (2008) as well as the values used for honey quality control required by the Brazilian regulation for *A. mellifera* Brasil 2000) can be seen in Table 26.2.

Lower moisture offers some security against fermentation, because below 18%, this process does not occur (Crane 1975; Rodrigues et al. 2005). Campos et al. (2003), analyzing floral and honeydew samples from *A. mellifer*a, obtained variation between 15 and 20.8% moisture content. Azeredo and Azeredo (1999), working with honeys from São Fidelis (RJ) found levels between 18.96 and 19.6%. In Bahia State (Brazil), Sodré (2000) obtained moisture values between 18 and 21.9% for coastal region honey. Brazilian regulation for *A. mellifera* (Brasil, 2000) establishes a maximum of 20% moisture. Sousa (2008) gave honey of *T. angustula* values varying from 23.40 to 25.60% for São Paulo state (in Lins, Amparo, Pedreira, Itaberaba, Marília and Santo Antônio de Posse) which are adequate if we use the suggested values for stingless bee honey of Villas-Bôas and Malaspina (2005) (<35% moisture). Similar values were found by Souza et al. (2006), between 26.10 and 26.62, and by Denadai et al. (2002), 23.70%. However, Iwama (1977) found wide variation (22.70–35.4%). In Table 1, Sousa (2008) jataí honey presents values not meeting standards for honey of *A. mellifera*: moisture (23.40–25.60%), acidity (21.65–63.85 meq/kg) and reducing sugars (44.78–67.54%). However, they are in accordance with the values suggested for stingless bee honey (Villas-Bôas and Malaspina 2005).

Regarding the honey free acidity values obtained for *T. angustula* (Table 26.2), they were similar to Cortopassi-Laurino and Gelli (1991) (acidity between 30.0 and 90.0 meq/kg) for different species of stingless bees. Reducing sugars were similar to data from Almeida—Anacleto (2007) (48.66–57.94%) and Rodrigues et al. (1998) 58.19% average

Table 26.2 Composition of *T. angustula* and *Apis mellifera* honey, compared to the Brazilian legislation parameters

	Tetragonisca angustula honey[a] *n* = 6	*Apis mellifera* honey[a] *n* = 6	Brazilian regulation for *A. mellifera* honey[b]
Parameters	Mean ± SD (Min–Max)	Mean ± SD (Min–Max)	Standard
Reducing sugars (g/100 g honey)	57.09 ± 7.83 (44.78–67.54)	71.50 ±10.45 (52.98–84.24)	Min. 65.0
Moisture (g/100 g honey)	24.37 ± 0.77 (23.4–25.6)	17.29 ± 1.23 (15.40–19.00)	Max. 20.0
Apparent sucrose (g/100 g honey)	2.14 ± 1.80 (0.43–4.46)	2.99 ± 2.60 (0.56–7.64)	Max. 6.0
Insoluble solids (g/100 g honey)	0.06 ± 0.03 (0.02–0.10)	0.04 ± 0.03 (0.01–0.08)	Max. 0.1
Minerals/Ash (g/100 g honey)	0.28 ± 0.11 (0.17–0.42)	0.20 ± 0.06 (0.11–0.26)	Max. 0.6
Free acidity (meq/kg)	37.34 ± 16.74 (21.65–63.85)	25.48 ± 5.66 (16.82–32.47)	Max. 50.0
Diastase activity (DN)	16.93 ± 3.94 (11.01–22.45)	7.32 ± 3.50 (2.20–11.49)	Min. 8.0
hydroxymethylfurfural (mg/kg) (HMF)	0.65 ± 0.25 (0.30–0.93)	11.37 ± 7.78 (2.0–21.0)	Max. 60.0

[a] Sousa (2008)
[b] Brasil (2000)

for *T. angustula*; apparent sucrose values were similar to those obtained by Souza et al. (2006) who analyzed 152 samples of different stingless bee honey (1.1–4.8%).

HMF values for honey of *T. angustula* were similar to "uruçú" *M. scutellaris* (mean = 0.38 mg/kg) (Marchini et al., 1998). Diastase values (Table 26.2) were in accordance with the values obtained by Vit et al. (1998) for stingless bees (excluding *Melipona*) from Venezuela (2.60–36.60). Insoluble solids obtained were low, indicating there are no impurities in samples, similar to values obtained from *M. fasciculata* known as "uruçú cinzenta" (Silva 2006), with a mean value of 0.02%. Ash content was in accordance with that presented by Carvalho et al. (2005), varying between 0.04 and 0.50% for different stingless bees.

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Chapter 27
Honey of Colombian Stingless Bees: Nutritional Characteristics and Physicochemical Quality Indicators

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27.1 Introduction

The geographic location of Colombia and its mega-biodiversity have been identified as advantages for beekeeping and for meliponiculture. Beekeeping is a potentially sustainable activity and presents an interesting opportunity to identify new products—mostly yet-to-be-discovered—with unique features related to their natural origin and functional characteristics. There are certainly more than an estimated 900 native bee species in Colombia (Freitas et al. 2009).

As among other Latin American countries, pre-Hispanic cultures that lived in different territories now located in Colombia practiced meliponiculture (especially of the genera *Melipona* and several others), for the extraction and processing of honey and the use of cerumen in metalwork. The European colonization of Central and South America minimized the practice of meliponiculture, introduced beekeeping with hives of *Apis mellifera*, and largely ended meliponiculture in Colombia. More recently, the trends of increased consumption of natural foods and health products have played an important role in the renewed interest in bee products, particularly honey from stingless bee species, and the recovery of traditional knowledge.

Because of this, meliponiculture in Colombia has recently developed. Products such as honey produced by *T. angustula*, called “angelita” (“little angel” in English), is available in traditional markets and commands a significantly higher price relative to *A. mellifera* honey (e.g., because of its scarcity and because it is commonly thought to have medicinal features, the price of *T. angustula* honey can reach over ten times the price of honey from *A. mellifera*) (Rosso and Nates-Parra 2005). Although data on the marketing of pot-honeys in Colombia is not available, this product, known in Spanish as “miel de pote,” is mainly sold in natural foods stores.

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Beekeepers generally maintain relatively few nests, without financial gain, and often express a desire to make them a source of income, but they often lack knowledge about breeding techniques and maintenance.

Technological and environmental issues, such as complex ecosystem interactions, the susceptibility of some species to human practices and relatively low honey production yields of individual nests, must be studied and overcome in order to make meliponiculture feasible in Colombia. Sustainable meliponiculture must be based on the generation of knowledge about native bee biology, their environment and characteristics of their products; therefore, the assessment of physical and chemical features of various honeys of Colombian stingless bees is of great interest. This chapter summarizes existing information regarding the physicochemical properties, nutritional information and quality of Colombian pot-honey.

27.2 Physicochemical Characteristics of Colombian Pot-Honey

Only very recently have data been obtained on composition and physicochemical properties of Colombian pot-honeys. In fact, the Colombian technical standard for *A. mellifera* honey was extended from the stingless bee data published by Souza et al. (2006) and lacks information regarding Colombian stingless bee honeys (ICONTEC 2007). This general lack of knowledge has had several consequences. For example, there are difficulties in regulating the adulteration and falsification of stingless bee honey.

The objective differentiation between authentic pot-honey and adulterated honey is especially interesting. Adulteration is often achieved by mixing pot-honey with common *A. mellifera* honey, and even by using adulterated honey of that species, containing added molasses and fructose syrup. Therefore, physicochemical characteristics are useful for regulating adulteration, and that knowledge will allow the development of regulatory standards.

Previous physicochemical characterization of Colombian pot-honey (Zuluaga et al. 2009) has focused on *T. angustula* or non-compositional analysis (Torres et al. 2004, 2007), or remained unpublished. Information provided in this chapter derives from studies performed in the Institute of Food Science and Technology (ICTA), Universidad Nacional de Colombia, since 2008. The data are compared to those of Zuluaga (2010).

Among the hundreds of Colombian stingless bee species (belonging to more than 13 genera; Nates-Parra 2001; Nates-Parra et al. 2006), the chemical composition of honey from seven genera has been explored. The species for which honey has been analyzed, as well as the number of samples and location for each are shown in Table 27.1. Several samples identified only to taxonomic group, e.g. genus. Often, the small amount of pot-honey that can be harvested at one time limits parameters assessed for a sample; therefore, some physicochemical characteristics are evaluated for few species or samples. In most cases, the analytical methods for

Table 27.1 Physicochemical composition of pot-honey from Colombia (general information about the samples)

Taxon	Geopolitical regions	Number of pot-honey samples
Frieseomelitta sp.[a]	Magdalena, Santander, Caldas	6
Melipona compressipes	Santander, Caldas	12
Melipona favosa	Sucre, Magdalena, Cundinamarca	7
Melipona eburnea	Cundinamarca	7
Melipona sp.	Santander	14
Nannotrigona testaceicornis	Santander, Cundinamarca	3
Nannotrigona sp.	Cundinamarca, Boyacá, Sucre, Santander	4
Paratrigona opaca	Santander	4
Partamona peckolti	Santander	1
Partamona sp.	Santander	1
Plebeia spp.	Santander	1
Scaptotrigona limae	Sucre, Santander	2
Scaptotrigona sp.	Cundinamarca, Caldas, Magdalena, Santander	4
Tetragona sp.[a]	Santander	21
Tetragonisca angustula[a]	Magdalena, Santander, Cundinamarca, Sucre, Caldas, Tolima, Huila	45

[a]Previously denominated as a subgenus of *Trigona* (Rasmussen and Cameron 2010)

pot-honey are the same as for *A. mellifera* honey (AOAC 1998). The number of analyzed honey samples varies according to the genus and the species; the largest number of samples corresponds to the genera *Melipona* and *Tetragonisca* because there is ample breeding of those species (see Souza et al. 2006).

27.2.1 Main Composition (Water and Sugars)

The honey from stingless bees, like *A. mellifera* honey, is composed primarily of simple reducing sugars (mainly fructose and glucose), and non-reducing sugars (mainly sucrose and maltose), water and ash. These quality parameters depend on many factors, even for the same species, such as the maturity achieved in the bee nest or hive during the harvesting season, climatic and geographic factors, and other elements affecting floral abundance.

The concentration of sugars and water for Colombian varieties of pot-honey are given in Table 27.2. Mean moisture content values ranged from 24.3 g/100 g for *T. angustula* to 42.7 g/100 g for *Partamona pecktolti*. The high water concentration in the former species is consistent with the relatively low total sugar content (°Brix) reported by Souza et al. (2006); such large moisture values had only been reported for *Melipona quadrifasciata* (Gonnet et al. 1964; Pamplona 1989) and *Plebeia* (Bijlsma et al. 2006; Carvalho et al. 2005). Most honey moisture content values

Table 27.2 Water and sugar contents of stingless bee honey from Colombia

Taxon	Moisture[a] (g/100 g)	Fructose[b] (g/100 g)	Glucose[b] (g/100 g)	Fructose + Glucose (g/100 g)	Disaccharides[b, c] (g/100 g)
Frieseomelitta sp.[d]	33.1 ± 3.3 (6)	17.1 ± 6.6 (5)	12.6 ± 7.5 (5)	29.7 ± 7.5 (5)	3.1 ± 2.7 (5)
Melipona compressipes	25.8 ± 2.0 (12)	36.9 ± 3.7 (11)	34.2 ± 4.4 (11)	71.1 ± 8.1 (11)	3.4 ± 2.2 (11)
Melipona favosa	24.8 ± 1.8 (3)	39.3 ± 7.0 (7)	33.5 ± 3.1 (3)	72.2 ± 7.4 (3)	3.1 ± 1.8 (3)
Melipona eburnea	27.6 ± 2.1 (7)	39.3 ± 7.0 (7)	38.5 ± 7.5 (7)	72.2 ± 7.4 (7)	3.6 ± 1.5 (7)
Melipona sp.	26.2 ± 1.8 (14)	36.7 ± 3.5 (14)	30.9 ± 4.0 (14)	67.6 ± 7.5 (14)	6.0 ± 2.3 (14)
Nannotrigona testaceicornis	27.5 ± 4.2 (3)	40.1 ± 18.1 (2)	25.7 ± 17.0 (2)	65.8 ± 35.1 (2)	7.9 ± 4.3 (2)
Nannotrigona sp.	25.7 ± 1.8 (4)	33.1 ± 4.1 (4)	17.7 ± 3.7 (4)	50.8 ± 7.4 (4)	9.7 ± 4.3 (4)
Paratrigona opaca	26.6 ± 1.2 (4)	30.9 ± 2.4 (4)	27.2 ± 10.7 (4)	58.1 ± 12.4 (4)	3.9 ± 2.8 (4)
Partamona peckolti	42.7 (1)	26.6 (1)	14.0 (1)	40.6 (1)	6.1 (1)
Partamona sp.	28.9 (1)	29.0 (1)	9.3 (1)	38.3 (1)	13.1 (1)
Plebeia spp.	28.6 (1)	17.4 (1)	19.3 (1)	36.7 (1)	0.9 (1)
Scaptotrigona limae	25.8 ± 2.2 (2)	39.0 ± 0.7 (2)	28.7 ± 3.4 (2)	67.7 ± 4.1 (2)	6.6 ± 4.6 (2)
Scaptotrigona sp.	26.9 ± 2.9 (4)	31.8 ± 2.9 (4)	23.9 ± 3.1 (4)	55.7 ± 5.0 (4)	12.1 ± 7.4 (4)
Tetragona sp.[d]	25.8 ± 3.6 (21)	31.8 ± 3.9 (19)	29.0 ± 6.8 (19)	60.8 ± 10.7 (19)	4.4 ± 5.6 (19)
Tetragonisca angustula[d]	24.3 ± 2.3 (44)	30.1 ± 5.4 (41)	23.5 ± 6.4 (41)	53.6 ± 11.8 (41)	4.2 ± 2.4 (41)

Mean values, ± standard deviation and (number of samples) are presented

[a] Measured by refractometry according to the AOAC 969.38B standard methodology (AOAC 1998)

[b] Assessed using an HPLC method based on the AOAC 979.23 and 983.22 standard methodologies (AOAC 1998)

[c] Sucrose plus maltose

[d] Previously denominated as a subgenus of *Trigona* (Rasmussen and Cameron 2010)

ranged between 24 and 27 g/100 g; this parameter maybe a promising distinctive criterion for this kind of honey. It is important to mention that this assessment is performed via the indirect refractometric methodology (AOAC 1998), and thus, equations originally developed for *A. mellifera* honey are used as an approximation; the accuracy of this methodology should be scrutinized for each honey. To obtain more reliable data on this important feature, methods such as vacuum drying (an official and a low cost procedure), the Karl-Fischer method, and similar techniques are recommended.

Because of their floral origin, the main sugars present in stingless bee honey are glucose, fructose, maltose and sucrose; other disaccharides and oligosaccharides occur in lower proportion and in trace quantities. The sugar composition shown in Table 27.2 includes the most important sugars, all of which were evaluated using an HPLC (high pressure liquid chromatography) method, which does not differentiate sucrose and maltose. Therefore, the sum of these sugars is presented as disaccharides. Mean glucose content varied between 9.3 g/100 g (*Partamona* sp.) and 38.5 g/100 g (*Melipona eburnea*), mean fructose content between 17.1 g/100 g *Frieseomelitta*, and 40.1 g/100 g (*Nannotrigona testaceicornis*). The disaccharides varied between 0.9 g/100 g (*Plebeia*) and 13.1 g/100 g (*Partamona*). Honey from all *Melipona* had mean glucose content >30 g/100 g and mean fructose content >36 g/100 g. The mean fructose–glucose ratio for all species is >1 with an exception of one sample of *Plebeia*. An exceptionally high fructose/glucose value was found for *Partamona*, accompanied by the lowest total reducing sugars value and a relatively low value of total sugars. The fructose–glucose ratio for this species had not been previously reported as an unusually high value, although the low total sugar content has an antecedent in the study by Roubik (1983) (cited by Souza et al. 2006) in which honey of *P. pecktolti* had the lowest values of total sugars (°Brix) from among more than 25 types of stingless bee honey from Panama. Torres et al. (2004) reported values of fructose (36.1–37.6 g/100 g) and glucose (29.8–31.8 g/100 g) for honey of *T. angustula* from Colombia that are at the higher end of the range shown in Table 27.2.

Unusually low glucose content occurred in honey of *Frieseomelitta* (12.6 ± 7.5 g/100 g) and *Nannotrigona* (17.7 ± 3.7 g/100 g), whereas *M. eburnea* had the highest mean glucose content (38.5 ± 7.5 g/100 g). Moreover, high disaccharide content was found for *Scaptotrigona* (12.1 g/100 g) and *Partamona* (13.1 g/100 g). These values differ from those reported by Santiesteban-Hernández et al. (2003) in Mexico for the former genus (1.1 g/100 g). Such divergent values have high variability and probably too few samples analyzed, and thus, further characterization must be performed to better establish sugar concentration value as an origin denomination criterion, and to set regulatory quality standards.

27.2.2 Ash and Minerals

The ash and mineral contents depend strongly not only on botanical and geographical origin, but also on the species (Vit et al. 1994, 2004, 2005; Vit 2005; Souza et al.

2006). The concentration of ash and some minerals (Na, K, Ca, Mg, Fe, Cu, and Zn) for Colombian pot-honey from four genera is shown in Table 27.3. For Colombian honey known thus far, obvious differences exist between species or genera.

According to mean ash content value, most analyzed honey meets the standard for Codex Alimentarius proposed by Vit et al. (2004), which is a maximum of 0.5 g/100 g (for honey from *A. mellifera*, *Melipona*, *Scaptotrigona* and *Tetragonisca* (formerly labeled a subgenus of *Trigona*), with the exception of honey from *Tetragona*) which had a mean content of 0.495 g/100 g and a standard deviation of ±0.077 g/100 g. This difference implies that some samples would not meet the suggested standard, in spite of authenticity, unless only one decimal place was used. In this case, the value could be approximated as 0.5 g/100 g. Some 40% of the *Tetragona* samples that were characterized exceeded 0.5 g of ash/100 g. Therefore, this suggestion needs to be clarified, at least for pot-honey from this species.

For all types of honey, the most concentrated mineral element yet quantified is potassium (188.3–1,669.4 ppm), and the least concentrated element is copper (0.8–1.2 ppm). Other minerals, in increasing order of concentration, are iron (3.3–49.6 ppm), zinc (6.7–19.6 ppm), magnesium (4.7–85.5 ppm), sodium (63.6–178.3 ppm), and calcium (51.0–267.8 ppm). This order of concentration is the same found for Colombian *A. mellifera* honey (Zuluaga 2010). In general, the honey that exhibits higher ash concentration has higher concentration of each mineral element, as may be expected. High variability indicates that this parameter can be used as a differentiation criterion, since it has been widely used for *A. mellifera* honey, and other apicultural products.

27.2.3 Other Physicochemical Quality Parameters

Physicochemical analyses are important for establishing the identity of each variety of pot-honey, according to bee species and geographical origin, and to provide regulatory organizations with objective tools for preventing honey falsification in commerce. The quality parameters of honey produced by *A. mellifera* are not directly related to nutritive value (i.e., water, sugar and mineral content), but to authenticity, denomination of origin, and safety (pH, acidity, content of hydroxymethylfurfural, diastase activity, specific rotation, conductivity and color). They have been widely assessed for several types of this product, throughout the world. Such characterization, together with the need to avoid adulteration and falsification, have led food regulation agencies to set standards, which are generally very accurate for *A. mellifera* honey but regularly exclude the honey of other species from the legal definition of honey. This situation occurs in several countries, including Colombia. To set accurate quality standards for Colombian stingless bee honey, an extensive knowledge base regarding the behavior of these variables for each species must be gathered in the same manner currently used by other countries such as Venezuela,

Table 27.3 Ash and mineral contents of Colombian stingless pot-honey

Taxon	Ash[a] (g/100 g)	Sodium[b] (mg/kg)	Potassium[b] (mg/kg)	Calcium[b] (mg/kg)	Magnesium[b] (mg/kg)	Iron[b] (mg/kg)	Copper[b] (mg/kg)	Zinc[b] (mg/kg)
Melipona compressipes	0.09 (1)	63.6 (1)	299.8 (1)	55.0 (1)	20.0 (1)	4.8 (1)	1.2 (1)	10.8 (1)
Melipona favosa	0.01 ± 0.01 (2)	–	–	–	–	–	–	–
Melipona sp.	0.20 ± 0.00 (2)	67.7 ± 33.6 (2)	545.7 ± 138.2 (2)	150.3 ± 0.9 (2)	32.5 ± 1.8 (2)	3.3 ± 0.3 (2)	0.8 ± 0.4 (2)	6.7 ± 2.5 (1)
Nannotrigona sp.	0.33 (1)	154.5 (1)	961.2 (1)	117.0 (1)	4.7 (1)	49.6 (1)	1.9 (1)	14.9 (1)
Scaptotrigona limae	0.04 (1)	–	–	–	–	–	–	–
Scaptotrigona sp.	0.06 (1)	–	188.3 (1)	51.5 (1)	37.4 (1)	15.1 (1)	0.6 (1)	19 (1)
Tetragona sp.[c]	0.50 ± 0.08 (5)	178.3 ± 29.5 (5)	1669.4 ± 388.8 (5)	267.8 ± 113.3 (5)	85.5 ± 7.1 (5)	6.2 ± 0.8 (5)	1.2 ± 0.7 (5)	18.1 ± 3.1 (5)
Tetragonisca angustula[c]	0.21 ± 0.70 (12)	155.0 ± 65.1 (9)	576.6 ± 177.6 (9)	199.6 ± 63.4 (9)	56.0 ± 27.5 (9)	5.8 ± 2.3 (9)	0.9 ± 0.3 (9)	19.6 ± 8.3 (9)

Mean values, ± standard deviation and (number of honey samples) are presented

[a] Ash content was determined according to the AOAC 920.181 standard methodology (AOAC 1998)

[b] Mineral elements (Na, K, Ca, Mg, Fe, Cu, and Zn) were quantified by atomic absorption spectrometry according to the AOAC 979.23 standard methodology (AOAC 1998)

[c] Previously denominated as a subgenus of *Trigona* (Rasmussen and Cameron 2010)

Mexico, Guatemala, and Brazil (Vit et al. 2004; Souza et al. 2006). In Colombia, little knowledge on these quality parameters is published (Torres et al. 2004; Quicazán et al. 2009). However, such studies signal differences between honey from a stingless bee species in different countries (see Chap. 21). In addition, although our results agree with other reports in most cases, some values fell outside the suggested ranges. Table 27.4 presents the existing information regarding color, pH, acidity, diastase activity, HMF, conductivity and specific rotation of honeys of Colombian stingless bees.

Color was assessed using the Pfund scale, which is the most common color scale for *A. mellifera* honey, using a colorimeter (HI C221 Hanna Instruments). For *Melipona* honey, color is highly variable and may correspond to the particular species. Among the *Melipona*, some lacking current taxonomic certainty have the darkest honey, which can be considered light amber to amber according to the USDA color standard designation, whereas most honey of other *Melipona* ranges from very white to very light amber. *Nannotrigona* honey is considered to be light amber, and *Paratrigona* and *Scaptotrigona* honeys vary from white to light amber (high variability is found for these genera). For the former genus *Trigona* (here considered among the three genera *Tetragona*, *Tetragonisca* and *Frieseomelitta*) the lighter honeys appear to be those of *T. angustula*, even though they range from very white to light amber, and the darker honey is that of *Frieseomelitta*. The free acidity in honey of Meliponini is usually significantly higher than that of *A. mellifera*, reflected in pH, and in the flavor (Vit et al. 1994, 2004, 2005, 2006; Souza et al. 2004, 2006; Sosa López et al. 2004; Zuluaga 2010). This is likely associated with a higher tendency to spontaneously ferment due to a higher water content; fermentation is not necessarily an undesirable process, even though is typically not controlled (Vit et al. 1994, 2004). All of the analyzed Colombian honey meets the standards proposed by Vit et al. (2004) for pot-honey varieties from Venezuela, Guatemala, and Mexico. An unusual value of acidity was found for *M. compressipes*. Such low acidity has only been reported in honey from *Melipona beecheii* and *Melipona scutellaris* (Souza et al. 2006); therefore, because of the low number of samples, further assessment needs to establish whether this is normal in Colombia or only among analyzed samples.

Currently, the diastase activity of Colombian meliponine honey is known for only a few species. *Melipona* and *Scaptotrigona* pot-honey presented lower values than *Frieseomelitta*, *Tetragona*, *and Tetragonisca* for diastase activity, which is consistent with previously reported information (Vit et al. 1994, 2004). Unlike the activities of *A. mellifera* and *Tetragonisca*, these results indicate a lack of high enzyme activity, not due to possible heating of the product. It is important to note that the diastase activity for *Melipona* and *Scaptotrigona* honey was less than 3.0 DN, which is the lower detection limit of the Schade method (Bogdanov et al. 1997) used in this assessment; therefore, the diastase activity is not a standard to be considered for the quality of pot-honey.

The hydroxymethylfurfural (HMF) contents for Colombian pot-honey were much lower than the maximum accepted content for *A. mellifera* (40 mg/kg)

Table 27.4 Physicochemical quality parameters of Colombian stingless bee honey

Taxon	Color[a] (mm Pfund)	pH[b]	Free acidity[b] (meq/kg)	Diastase activity[c] (DN)	HMF[d] (mg/kg)	Conductivity[e] (μS/cm)	Specific rotation[e]
Frieseomelitta sp.[f]	82 ± 7 (3)	–	–	–	–	–	–
Melipona compressipes	42 ± 19 (10)	–	7.0 (1)	n.d. (2)	3.0 (1)	1049 ± 56 (2)	−12.6 ± 2.6 (2)
Melipona favosa	36 ± 4 (3)	–	–	n.d. (1)	–	–	–
Melipona eburnea	34.4 ± 8 (7)	–	–	–	–	–	–
Melipona sp.	45.2 ± 27.8 (13)	–	–	–	–	–	–
Nannotrigona sp.	65 ± 4 (2)	–	–	–	–	–	–
Paratrigona opaca	36 ± 15 (4)	4.1 (1)	31.7 (1)	–	–	–	–
Plebeia spp.	62 (1)	–	–	–	–	–	–
Scaptotrigona sp.	54 ± 27 (4)	4.5 (1)	57.83 (1)	2.4 (1)	6.0 (1)	392 (1)	–
Tetragona sp.[f]	70 ± 15 (18)	4.2 ± 0.3 (4)	44.3 ± 21.8 (4)	17.8 ± 5.5 (2)	1.0 ± 1.1 (2)	1183 ± 122 (3)	−1.1 (1)
Tetragonisca angustula[f]	49 ± 19 (23)	4.2 ± 0.3 (12)	39.2 ± 22.9 (12)	16.7 ± 9.2 (8)	1.3 ± 2.1 (6)	658 ± 57 (2)	2.6 ± 1.3 (3)

Mean values, ± standard deviation and (number of honey samples) are presented

[a] Estimated photometrically on the Pfund scale using a C-221 colorimeter (Hanna Instruments, USA)

[b] pH was measured at 20 °C (10 g of honey/75 ml water); free acidity was assessed by neutralization according to the AOAC 962.19 standard methodology (AOAC 1998)

[c] Diastase activity assessed by the method of Schade (Bogdanov et al. 1997); DN: diastase number

[d] Hydroxymethilfurfural (HMF) evaluated spectrophotometrically according to the White method as described by Bogdanov et al. (1997)

[e] Electrical conductivity and specific rotation evaluated according to methods described by Bogdanov et al. (1997)

[f] Previously denominated as a subgenus of *Trigona* (Rasmussen and Cameron 2010)

(Table 27.4). It is interesting to note changes of this variable during long-term storage, considering that meliponine honey should be kept refrigerated, and the high moisture content could eventually enhance product appearance. Electrical conductivity has not been commonly assessed for stingless bee honey. In the case of *T. angustula*, conductivity (0.66 ± 0.06 mS/cm) was different from values reported by Vit et al. (1994) for Venezuelan honey (7.32 mS/cm), but similar to the value reported by Santiesteban-Hernández et al. (2003) for Mexican honey of this species (0.78 mS/cm), although there may be several species involved (see Chap. 21). The singular honey of *Scaptotrigona*. for which conductivity has been assessed had a particularly low value (0.39 mS/cm), which to the best of our knowledge is the lowest reported value for any stingless bee honey; a conductivity of 0.49 mS/cm for *Scpatotrigona mexicana* (reported erroneously as *S. luteipennis*) in Mexico was apparently the previous minimum reported value (Santiesteban-Hernández et al. 2003). The specific rotation is also a property that is not widely explored for stingless bee honey. The data presented in Table 27.4 indicate that specific rotation is a potential criterion for differentiating honeys because values for each species seem to stay within a consistent range. This property is related to the concentration of levorotary (as fructose) and dextrorotary (as glucose) compounds. However, the correlation is not known for pot-honey that has been evaluated and may be due to the presence of other sugars that have not been quantified, and other compounds with rotation capacity.

27.3 Conclusions

Even though most of the Colombian pot-honey display physicochemical properties within the range of values previously reported for diverse stingless bee species, the values show that physicochemical data can potentially be used as criteria to differentiate the honey from adulterated products, *A. mellifera* honey, other stingless bees honey, and even honey of the same species from different regions. Nevertheless, it is necessary to continue the characterization process that leads to a better knowledge of this valuable product, and the establishment of laws that regulate falsification and adulteration. The result will enable or stimulate sustainable meliponiculture across Colombia. In the Zuluaga-Domínguez et al. chapter of the present book, we tackle a further classification and differentiation of stingless bee honey with multivariate statistical analysis of physicochemical properties and the novel analytical methodology known as an “electronic nose.”

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Chapter 28
The Pot-Honey of Guatemalan Bees

María José Dardón, Carlos Maldonado-Aguilera, and Eunice Enríquez

28.1 Introduction

In Guatemala there are at least 32 species of native stingless bees that produce honey. Guatemalan beekeepers have developed, since Pre-Columbian times, skills for bee breeding and nowadays refer to about 15 species by their common name. However, the species with superior realized breeding potential and honey production are *Melipona beecheii* Bennett, 1831, *Tetragonisca angustula* (Latreille, 1811), *Scaptotrigona pectoralis* (Dalla Torre, 1896), and *Scaptotrigona mexicana* (Guérin-Méneville, 1844). *Geotrigona acapulconis* (Strand, 1919) is also greatly appreciated for its honey, which is believed to have medicinal properties. However, the bee nests underground and is not kept in hives easily thus no traditional breeding apparently exists (Yurrita et al. 2004; Enríquez et al. 2001, 2004, 2005).

In some regions stingless bee breeding and artificial feeding, in the rainy season, are practiced. This is an economic alternative currently promoted by nongovernmental organizations, to benefit families in the rural area. However, there are still regions of Guatemala where stingless bee colonies are kept in traditional log hives, and beekeeping practical knowledge is transmitted orally, from generation to generation (Yurrita et al. 2004; Enríquez et al. 2001, 2004, 2005). Honey is the hive's most coveted product; there are few reports on the use of wax (i.e., cerumen—a mixture of wax with resin), pollen and no reports on the use of propolis (i.e., pure resin). Most of the beekeepers use the honey only for their own consumption, either as medicine and food, because of the scarcity of the product. Only those who have many hives sell the honey, but

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always locally. The honey of stingless bees is priced three times higher than that of *Apis mellifera* L., as in other countries of the region (Yurrita et al. 2004; Enríquez et al. 2001, 2004, 2005).

Popularly, the honey of stingless bees is claimed to have a great number of medicinal properties that together with cultural, historic, and biologic components, gives an added value to it (Enríquez et al. 2001, 2004, 2005 and chapters in this book). However, the exact composition of the honey is unknown, which represents a challenge that has to be overcome to encourage the conservation of these species and their honey. There are many characteristics to study in honey, for example physicochemical, pollen composition, nutrition and taste or sensorial evaluation. Also the sanitary quality of the product and popular beliefs regarding properties and uses require validation, before marketing can be pursued. Part of this work has already begun, and the results are discussed below.

28.2 Physicochemical Characteristics of Guatemalan Pot-Honeys

Honey presents a great variety of physicochemical characteristics that have been used to determine its quality. In *A. mellifera* some useful parameters are acidity, ash, sucrose, reducing sugars, moisture content, diastase and hydroxymethylfurfural. These parameters may also be used to establish quality control and to avoid adulteration of stingless bee honey. However, the composition of honey should be known, throughout the regions from which it comes, to define normal values for such parameters and lead to its commercialization. Composition has been studied, preliminarily, in 18 samples of honey from *Melipona beecheii, M. solani* Cockerell, 1912, *M. yucatanica* Camargo, Moure and Roubik 1988, *Tetragonisca angustula*, *Plebeia sp.*, *Nannotrigona perilampoides* Cresson 1878, *Scaptotrigona mexicana* and *Geotrigona acapulconis* (Dardón and Enríquez 2008).

28.2.1 Reducing Sugars

The principal reducing sugars found in honey, generally in almost equal proportions, are glucose and fructose (Alves et al. 2005). The reducing sugars in the honey of Guatemalan stingless bees (Table 28.1) are of higher content than the minimum proposed by Vit et al. (2004) (50 g/100 g) and Souza et al. (2006) (58.0–75.7 g/100 g), as honeys show values between 57.22 and 75.97 g/100 g. Average values of reducing sugars are not very different among honeys of different stingless bee genera. The honey of the genera *Melipona* and *Trigona* present a higher quantity of reducing sugars, while honey of *Scaptotrigona* has about 20% less reducing sugars, compared to honey of *Melipona*, so their honey is usually less sweet.

Table 28.1 Sugars content of stingless bees honey from Guatemala

Bee species	Honey samples n	Reducing sugars (g/100 g)	Apparent sucrose (g/100 g)	Total sugars (g/100 g)
Melipona beecheii	7	68.77 ± 3.82	3.50 ± 4.14	72.45 ± 6.10
Melipona solani	1	75.97	1.7	76.19
Scaptotrigona mexicana	1	57.22	0.06	57.28
Tetragonisca angustula	1	65.78	4.83	70.86

After Dardón and Enríquez (2008)

28.2.2 Sucrose

Sucrose represents about 2–3% of the carbohydrates in honey of *A. mellifera* (Swallow and Low 1990); high values of this disaccharide are related with premature honey harvest, where sucrose has not been converted into glucose and fructose by the action of invertase (Alves et al. 2005). The sucrose in honey of Guatemalan stingless bees (Table 28.1) is in the allowed parameters for the Codex alimentarius (maximum of 5 g/100 g) and the values coincide with those reported by Souza et al. (2006) for stingless bees (1.1–4.8 g/100 g). Values for sucrose in the honey of *Scaptotrigona* are lower than those of *Melipona* and *Geotrigona*, as suggested by Vit et al. (2004) and Dardón and Enríquez (2008).

28.2.3 pH

The pH values in honey refer to the hydrogen ions present in solution that participate in formation of other components (e.g., hydroxymethylfurfural) (Carvalho et al. 2005). According to Alves et al. (2005), pH is determined by nectar, the cephalic secretions of the bees while they carry the nectar to the hive, by the origin of the honey and the concentration of different ions like calcium, potassium, and sodium. Most (Table 28.2) are found in the ranges reported by Souza et al. (2006), with values between 3.71 and 5.18, with the highest pH in the honey of *Geotrigona* (Dardón and Enríquez 2008).

28.2.4 Free Acidity

Honey contains acids that contribute to its stability and retard development of microorganisms; gluconic acid is the most common (Mato et al. 1997). This acid is formed by the action of glucose-oxidase on glucose, this enzyme is produced in the hypopharyngeal glands of bees, acting even after the honey is stored (Alves et al. 2005). Acids found in smaller quantities include acetic, benzoic, butyric, citric, phenylacetic, formic, isovaleric, lactic, maleic, oxalic, propionic, pyroglutamic, succinic, and valeric acids (Carvalho et al. 2005). The values were less than 20 meq/100 g

Table 28.2 Physicochemical parameters of stingless bees honey from Guatemala

		Physicochemical parameters					
Bee species	Honey samples n	pH	Free acidity (meq/100 kg)	Moisture content (g/100 g)	Ash content (g/100 g)	Diastase activity (DN)	HMF (mg/kg)
Mb	7	3.67 ± 0.12	23.2 ± 30.0	17.3 ± 2.6	0.07 ± 0.05	21.3 ± 32.8	n.d.
Ta	4	5.18 ± 1.35	17.4 ± 10.4	17.5 ± 2.8	0.35 ± 0.26	12.3 ± 10.3	n.d.
Sm	2	4.04 ± 0.4	12.7 ± 3.0	18.7 ± 0.2	0.10 ± 0.04	18.6 ± 12.7	n.d.
Ms	1	3.81	4.95	19.66	0.06	8.3	n.d.
Ga	1	3.06	85.53	32.09	0.09	2.6	n.d.
Pl	1	3.8	15.31	30.26	1.25	7.6	n.d.
My	1	3.79	10.59	20.37	0.06	10.0	n.d.
Np	1	3.8	9.93	16.54	0.33	6.8	n.d.

Mb = *Melipona beecheii*, Ms = *Melipona solani,* My = *Melipona aff. yucatanica,* Ta = *Tetragonisca angustula,* Pl = *Plebeia* sp., Np = *Nannotrigona perilampoides,* Sm = *Scaptotrigona mexicana,* Ga = *Geotrigona acapulconis*

After Dardón and Enríquez (2008)

in our study, although in *G. acapulconis* the value is four times higher and tends to reach values above 80 meq/100 g (Table 28.2) (Dardón and Enríquez 2008). Vit et al. (2004) proposed maximum values between 70 and 85 meq/100 g for the genera *Melipona*, *Scaptotrigona* and *Trigona*. The free acidity range was (5.9–109.0), with averages between 36.6 and 49.7 in the most studied species (Souza et al. 2006).

28.2.5 Moisture Content

The moisture content, besides water, has a relation with the viscosity, specific weight, maturity, crystallization and taste of honey. The honey of Guatemalan stingless bees (Table 28.2) is, on average, below 20 g/100 g, which is an acceptable value for commercial *A. mellifera* honey. There is also an exception for *Geotrigona acapulconis* and *Plebeia* sp., which acquire moisture values above 30 g/100 g and give honey the lowest viscosity. Souza et al. (2006) point out that, in honey of these species, the most common range is 19.9–41.9 g/100 g. However, Vit et al. (2004) proposed a maximum of 30 g/100 g for *Melipona*, *Scaptotrigona* and *Trigona*. According to observations on honey of *Plebeia* and *Geotrigona* with higher moisture values, an extension of the parameter should be considered.

28.2.6 Ash Content

The amount of ash found in honey is a quality criterion influenced by botanical origin. This parameter is correlated with the color of the honey; darker honeys have more ash and, consequently, more minerals (González-Miret et al. 2005). Our honey

(Table 28.2) contains, an average of 0.23 g/100 g of ash content. However, the high quantity of ash in the honey of *Plebeia* sp. stands out, acquiring values above 1.25 g/100 g. Vit et al. (2004) propose a maximum of 0.5 g/100 g for ash of stingless bee honey, while Souza et al. (2006) list the common values of stingless bee honey at 0.01–1.18 g/100 g.

28.2.7 Diastase (a-Amylase)

Enzymes present in honey are formed by bee hypopharyngeal glands in the head and are found in small proportions in collected pollen (Moritz and Crailsheim 1987). Diastase is a heat-sensitive enzyme, so it is recommended for testing honey quality. The diastase activity is calculated as diastase number (DN = units of diastase activity). One unit is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end point in that 40°C under the condition of the test (Vorlová and P idal, 2002). The stingless bee honey in Guatemala is highly variable in diastase number. This is reflected in the values of standard deviations presented in Table 28.2, particularly in *M. beecheii* honey. Vit et al. (1998) reported diastase values 2.9–23.0 DN for *Melipona favosa* honey, somewhat similar to values found in some Guatemalan stingless bees, 2.6–21.3 DN (Table 28.2), in agreement with the minimum of 3 DN for *Melipona* honey, initially proposed by Vit et al. (2004).

28.2.8 Hydroxymethylfurfural (HMF)

HMF is a degradation compound formed by the reaction of certain sugars with acids, principally by the decomposition of fructose (Spano et al. 2006). Its presence is an indicator of honey quality because it is found in small quantities in recently collected honey, and also because the quantity increases with time and overheating. HMF was not detected in honey of Guatemalan stingless bees (Table 28.2). Vit et al. (2004) proposed a maximum of 40 mg/kg. For Souza et al. (2006) the averages for the stingless bee honey most studied varied between 2.4 and 16.0 mg/kg, although the highest HMF value known so far is 78.5 mg/kg from an abstract meeting (Grajales et al. 2001).

28.3 Nutritional Characteristics

The honey of *A. mellifera* is recognized as a high-energy and nutritive food, and for being a sugar substitute of wide use in the food industry. The honey is principally composed by carbohydrates, which are about the 95–99% of the solids, and of those, 85–95% corresponds to reducing sugars that give honey its sweet taste and energy.

Table 28.3 Nutritional characteristics of stingless bees honey from Guatemala

Bee species	Honey samples n	Carbohydrates (g/100 g)	Proteins (g/100 g)	Calories kcal/100 g
Scaptotrigona pectoralis	2	70.22	0.41	283
Melipona beecheii	3	75.08	0.07	300
Tetragonisca angustula	3	70.22	1.19	286
Scaptotrigona mexicana	1	71.73	0.47	289

After Rodas et al. (2008)

The protein content of honey, in *A. mellifera,* presents a maximum of 0.1% and 7 proteins have been identified, five from the bees and two from plants. Of these proteins, enzymes are the most important for their role in the conservation of honey. Proline is the most abundant protein amino acid in honey (Carvalho et al. 2005). Honey also contains most of the essential chemical elements for the organism, such as K, Na, Ca, Mg, Mn, Ti, Co, Mo, Fe, Cu, Li, Ni, Pb, Sn, Zn, Os, Ba, Ga, Bi, Ag, Au, Ge, Sr, Be, and Ba (Freitas et al. 2006). Other compounds are found in smaller quantities, like organic acids, vitamins and aromatic substances, which play an important role in nutrition.

Preliminary studies of the honey of four Guatemalan stingless bees (Table 28.3) demonstrate an energy value of 280–300 kcal/100 g, 70–75% carbohydrate, each lower values than honey of *A. mellifera*. The percentage of protein in the honey of stingless bees varies between 0.073 and 1.19, for *M. beecheii* and *T. angustula* with the lowest and highest protein contents, respectively.

28.4 Antibacterial Properties of Guatemalan Pot-Honey

Honey has been used since ancient times in efforts to cure many diseases. It has been utilized by Chinese, Egyptian, Hebrew, Greek, Hindu, Persian, Roman, and Mayan cultures (see the Ocampo Rosales chapter in this book). The scientific mechanism known for the antibacterial activity in honey is hydrogen peroxide (H_2O_2), slowly released by the action of glucosidase and ingredients including antioxidant activity, vitamins, osmotic pressure, and polyphenol content, etc., which are of botanical origin (Aguilera et al. 2006). The study of antibacterial activity of honey validates its therapeutic use and has shown activity against some pathogenic bacteria. There should be valid reasons for medicinal use of this hive product, and its derivatives, in the treatment of infectious disease (Aguilera et al. 2006). After evaluating the antibacterial activity (Table 28.4) it was found that honey of eight among nine species shows antibacterial activity, against eight pathogen microorganisms, at concentrations of 2.5–10%. The honey of *M. solani*, however, had no such activity. The least susceptible microorganisms to the honey were *Candida albicans* and *Salmonella tiphy*. However, in dilutions of 2.5%, the honey of *S. pectoralis* was effective (Table 28.4). The stingless bee honey inhibited growth of *Staphylococcus aureus*, in

Table 28.4 Antimicrobial activity of stingless bees honey from Guatemala

Stingless bee species[a]	Mb	Ms	My	Ta	Pl	Np	Sm	Sp	Ga
Sample size	12	3	1	5	1	6	1	1	1
Bacterias and yeasts	Dilutions with microbial growth								
Staphylococcus aureus	5	–	5	10	5	5	5	2.5	10
Salmonella typhi	5	–	10	10	5	5	5	2.5	10
Mycobacterium smegmatis	5	–	5	5	5	2.5	5	2.5	5
Bacillus subtilis	5	–	5	5	5	2.5	5	2.5	5
Pseudomonas aeuroginosa	5	–	5	10	5	5	5	2.5	5
Escherichia coli	5	–	5	5	5	5	5	5	5
Candida albicans[b]	10	–	5	10	10	5	5	5	–
Criptococcus neoformans[b]	5	–	5	5	5	2.5	5	2.5	5

[a]Stingless bee species are indicated in the Table 28.2

[b]Yeast

After Dardón and Enríquez (2008)

dilutions ranging from 2.5 to 10%, with exception of *S. pectoralis*, which inhibited at 2.5%, and *M. solani,* which had no activity. *Mycobaterium smegmatis* was inhibited by honey of eight species, at an average dilution of 5% (Table 28.4).

28.5 Sensory Characteristics of Guatemalan Pot-Honey

Sensory characteristics are those perceived through the sense organs (eyes, nose, tongue, skin, or ears) to evaluate the color, size, shape, smell, aroma, taste, texture, malleability, and sound of consumables. Honey has a wide range of qualities that are very useful for detecting or describing its attributes (Vit 2007; Vit et al. 2008). The honey of five Guatemalan stingless bees was analyzed in color, smell, taste, and viscosity. Color allowed recognition of four descriptors ranging from transparent white (honey of *M. solani*) to orange (honey of *T. angustula*) (Table 28.5). Generally, the honey of *Melipona* is characterized for color ranging from pale yellow to white, or "white honey". In addition, refrigerated honey, stored for 10 years, changes color, giving rise to many colors of the same origin but different age.

For the taste of honey, of Guatemalan stingless bees, 10 descriptors were identified: strong acetic acid, sugar, sugarcane, sweet, slightly sweet, floral, formaldehyde, fruity, slightly acetic acid, and "nance" (the sour, edible fruit from a tree, *Byrsonima crassifolia*, Malpighiaceae). For the smell, 11 descriptors were recognized: accentuated acetic acid, sugar, "panela" (jaggery), fermented, floral, slightly formaldehyde, slightly fat, slightly acetic acid, slightly alcoholic, slightly fruity, and hive (Table 28.5). Both the smell and taste varied between the samples analyzed, influenced possibly by their location of origin. According to these results we can say that the pot-honey of Guatemalan stingless bees present sweet smell and taste, but the smell is also slightly acetic acid because of the relatively high water content, which triggers the fermentation processes.

Table 28.5 Sensory characteristics of stingless bees honey from Guatemala

Bee species	Honey samples n	Color	Odor/aroma	Taste	Viscosity
Melipona beecheii	5	Pale yellow	Slightly fat, floral, hive, slightly acetic acid, slightly frutal	Slightly sweet	78.8
Scaptotrigona mexicana	3	Pale yellow	Slightly ethanolic, floral	Slightly sweet	72
Melipona solani	3	Transparent white and pale yellow	Slightly acetic acid, slightly formaldehyde	Sweet	76
Trigona angustula	2	Yellow and orange	Fermented, jaggery, strong acetic acid	Sweet, slightly acid	81
Geotrigona acapulconis	1	Yellow	Strong acetic acid	Sweet, strong acid	64

After Rodas et al. (2008)

28.6 Pollen Composition of Guatemalan Pot-Honey

Melissopalynology considers pollen types found in honey and information on botanical origin, sometimes used for honey classification as unifloral or multifloral (Louveaux et al. 1970). A unifloral honey is the one that presents at least 45% of a single species, while a multifloral honey presents a high number of pollen resources or, at least, three different species in similar proportion. Honey characteristics are strongly influenced by botanical origin due to bee-plant interaction (i.e., bee foraging preferences), and it is useful to apply palynology for understanding bee flora. Our 53 honey samples of 9 different species revealed 20 botanical families (Table 28.6). The families Asteraceae, Fagaceae, Melastomataceae, and Tiliaceae were found in the honey of at least five different species and were the most commonly visited families. The honey of *T. angustula* presented a higher richness of families (18), while the honey of *S. mexicana* and *G. acapulconis* were the poorest (3). *Melipona* honey in Guatemala did not exceed eight plant families in pollen content.

Table 28.6 Floral resources of stingless bee honey from Guatemala

Bee species[a]	Mb	Ms	M	Ta	P	Np	Sm	Sp	Ga
Sample size	13	6	1	21	1	4	4	2	1
Botanical Family	Pollen types								
Acanthaceae				X	X				
Amaranthaceae				X					
Asclepiadaceae				X					
Asteraceae	X	X	X	X	X	X			X
Begoniaceae	X			X					
Bignoniaceae				X					
Cochlospermaceae[b]				X					
Convolvulaceae				X	X				
Fabaceae	X	X		X	X		X		
Fagaceae[b]	X	X		X			X	X	X
Lamiaceae				X					
Malvaceae						X			
Melastomataceae	X	X	X	X	X		X	X	
Myrsinaceae							X		
Myrtaceae	X	X		X		X			
Onagraceae				X					
Piperaceae[b]		X		X					
Rutaceae				X					
Solanaceae[b]	X	X		X		X			
Malvaceae (Tilioideae)	X	X		X		X		X	X
Total	8	8	2	18	5	5	4	3	3

[a] Bee species are indicated in Table 28.2

[b] Pollen is not indicator of nectar origin

28.7 Sanitary Quality of the Honey of Guatemalan Stingless Bees

The sanitary quality control of a product insures a safe product by detecting the presence of components that may negatively affect human health. Honey of stingless bees has been studied to detect presence or absence of insecticides. During flight and foraging, as well as in search of water, nectar, and/or honey, a bee may have contact with agricultural pesticides and other artificial chemical sources. This is why they are considered excellent bioindicators of the distribution of pesticides (Kevan 1999). One type of the most common pesticides is the organophosphates, which have been detected in low levels in the honeys of *A. mellifera*. The presence of pesticides represents a major risk to public health and maximum values allowed in honey have not been established, although some acaricide residues are regulated (Blasco et al. 2004).

In Guatemala, organochlorides, organophosphates, pyrethroids, bipiridils, glyphosate, and atrazines are used around apiaries and meliponaries (Rodas et al. 2008). Therefore, there may be pesticide contamination of honey from agricultural areas. Four Guatemalan stingless bees studied by gas chromatography/mass spectrophotometry revealed no contaminants (Rodas et al. 2008). Detectable levels of pesticides were not found in six samples of honey from *M. beecheii*, 3 *T. angustula*, 2 *G. acapulconis*, and 1 of *S. pectoralis.* There is no detectable risk, at present, of pesticide in the honey, despite the fact that these compounds are used in the immediate environment.

28.8 Honey Attributes of the Four Most Appreciated Stingless Bee Species in Guatemala

28.8.1 Melipona beecheii

This species is popularly known in Guatemala as the creole bee “abeja criolla,” large beehive “colmena grande,” “bichi,” and, in Mayan language, “sak’q qaw.” This species has been used extensively since PreColumbian times. Its pot-honey, denominated “white honey,” is very prized in Guatemala and is used against various maladies, such as stomach, respiratory and ocular disease or sickness, bumps, sores, and skin wounds.

Due to its physicochemical components, the honey of *M. beecheii* presents a high degree of acidity, 23.2 meq/kg honey, in comparison with the other species studied (excluding *G. acapulconis*). The ash content is relatively low, possibly the reason for the pale yellowish color, also reflected in low protein content (in comparison with *T. angustula*). The floral-fruity, fermented and woody odors and aromas make this honey very pleasant to the consumer. The price of *M. beecheii* honey ranges from Q75.00 to Q300.00 (US$ 10–40), per L, which is a price two to eight times higher than the local honey of *A. mellifera*. When evaluated against various microbial pathogens, *M. beecheii* honey inhibited their growth at dilutions of 5–10% and was least effective against the yeast *C. albicans* (Table 28.6).

28.8.2 Geotrigona acapulconis

This species is commonly called "talnete". It produces a considerable amount of honey that is popularly used to treat broken bones, internal injuries, eye diseases, cleaning the kidneys, and as a purgative. Due to the biology of this bee and its strict nesting habits, captive breeding is not practiced. Honey is obtained by digging up underground nests. The free acidity, 85.5 meq/kg honey of one sample, was at least four times higher than other Guatemalan stingless bees. Accordingly, the smell of the honey has relatively high acetic acid content and its flavor is described as sweet and strong acetic acid. The moisture content is high, making it a very liquid honey, and ash values are similar than those found in the genus *Melipona*. It has low diversity in pollen content, with only three plant families recorded. These families are often visited by stingless bees kept in our country. The honey, of yellow color when extracted, is not very well known and its sale is by a prior agreement. It is strictly a product of "honey hunting," not rational beekeeping. It is also a highly prized honey, and it is conducive to fraud and adulteration. It has been observed that some people offer a honey prepared with panela and lemon, as "talnete" honey.

28.8.3 Scaptotrigona mexicana

The breeding of this bee, commonly named "magua negro" or "congo negro," has advanced because it produces a considerable amount of honey. The honey has a pale yellow color and its smell is alcoholic and slightly floral. All the sensory families described by Vit (2007) for the aroma and smell of stingless bee honeys were found here floral-fruity, fermented, woody, mellow, primitive, industrial chemicals, hive, and vegetable.

The honey of *S. mexicana*, as in *S. pectoralis*, presents a higher percentage of protein, more than honey of *M. beecheii*, although it shows lower values for carbohydrates and this is reflected in its kilocalorie content. The study of four honey samples of this species allowed identification of four plant families in its pollen composition. With respect to the biotic activity of honey, *S. mexicana* was effective against all the evaluated microorganisms, in a dilution of 5%, being therefore one of the most active pot-honey (Table 28.6). Curiously, beekeepers report little medicinal use, even though the honey shows a potential for therapy.

28.8.4 *Tetragonisca angustula*

T. angustula is a very small and normally docile bee, commonly known as "chumelo," "doncella," "doncellita," in Mayan language it is known as "an us" and "qán us." It can form big colonies, but due to the small size of the honey pots, the quantities of honey produced are considerably less than those obtained in species like *M. beecheii*, with larger honey pots. This honey is very popular for the treatment of eye diseases (cataract and pterygium) but is also used for stomach illness, wounds and ulcers, and sometimes as an energy food or drink. The honey of *T. angustula* has yellow to orange color, with the aroma and smell families: floral-fruity, fermented, woody, mellow, primitive, industrial chemicals, hive, and vegetable. Its honey contains 19 families identified in Guatemala, reflected in color variation and high values of ash and protein.

Its physicochemical composition stands out from the other stingless bees, having the highest pH (>5) and the highest sucrose content (4.8 g/100 g). Antibacterial activity occurs at 5–10% honey dilution and was least effective of all evaluated honey. The microorganisms *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Candida albicans* were the most resistant (Table 28.6). Popularly, this honey is considered useful for the treatment of eye diseases, so it has to be evaluated to confirm this putative medicinal property.

28.9 Conclusions

The honey of stingless bees is a patrimony for tropical regions, especially for Latin America, where most of these bees exist. The honey of each varies among species and also within the same species, depending on the region where they are found and the plant resources they utilize. Determining the composition of this greatly varied honey, and knowing its attributes, is a difficult task. However, the challenge has been taken by research from Argentina, Bolivia, Brazil, Colombia, Costa Rica, Perú, Venezuela, and us, in Guatemala. We have 33 species of stingless bees, 32 produce honey and of these, only 9 species have been studied: all of them in manners considering antibacterial activity and pollen composition, 8 in physicochemical properties, 5 in sensory attributes and 4 for its sanitary quality. There are still 23 species that have not been studied, this corresponding to 60% of the entomological diversity of honey in the country. Efforts for understanding more about the pot-honey of stingless bees have begun, and for the moment, boosted stingless bee keeping. However, it is necessary to continue, to get to know all the diversity of honey, and promote its commercialization, and to validate potential therapeutic use.

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Chapter 29
Pot-Honey of Six Meliponines from Amboró National Park, Bolivia

Urbelinda Ferrufino and Patricia Vit

> *Our forests wait to be discovered, shaped by David W. Roubik's interpretations of foraging bees and seminal work of Francisco Tomás-Barberán and Federico Ferreres on flavonoids of tropical honey*

29.1 Introduction

A project on sustainable meliponiculture promoted by the Ecological Association of the East (ASEO, "Asociación Ecológica de Oriente") initiated the Association of Native Honey Producers (APROMIN, "Asociación de Productores de Miel Nativa") in Amboró National Park, S17°43′–17°53′ W60°30′–0°04′, 637,600 ha, located in the eastern lowlands of Bolivia, near San Carlos. Forty families became stingless bee-keepers to improve their economy with a new product from the forest. Each associate started with one hive and added up to 40. The web site "Amazonia Boliviana" advertises stingless bee honey on the web at prices ranging from 30 to 300 USD/l. The highest value in the Amboró community corresponds to "señorita" honey, produced by the widespread *Tetragonisca fiebrigi*, used to treat ocular diseases. Stingless bee honey yield is about 1–15 kg/year, and the fact that the honey is highly appreciated for potential medicinal use increases the price up to 10–25 times that of *Apis mellifera* honey. Packaging of pot-honey for commercial distribution

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includes a creative approach, based on a traditional spheroidal ceramic jar called "puño," which simulates darkness inside the hive.

In the Carmen Surutú community, Amboró National Park, six species of stingless bees have been selected by stingless bee-keepers (meliponicultors) to be kept in hives: *Melipona brachychaeta*, *M. grandis*, *Scaptotrigona depilis*, *Scaptotrigona polysticta*, *S.* near *xanthotricha*, and *T. fiebrigi*. A general comparison of honey, pollen and propolis production is given for each species. The chemical composition (moisture, ash, pH, free acidity, reducing sugars, sucrose), minerals (Ca, Cu, Fe, K, Mg, P, Zn), and microbiological counts (mesophilic bacteria, molds, yeasts) are compared here.

29.2 Species of Stingless Bees Producing Pot-Honey in Amboró National Park

Bolivian stingless bees were collected and sent to Dr. Silvia RM Pedro at the Biology Department, Universidade de São Paulo, Ribeirão Preto, Brazil, for identification. Additional data including location, behavior and images were also submitted to the Camargo Collection RPSP (São Paulo, Ribeirão Preto), as stingless bee-keeper information.

From ten species of stingless bees identified in a brief and incomplete survey of Amboró National Park (see Table 29.1), only six are kept by stingless bee-keeper.

Pot-honey was extracted by syringe and collected in PET recycled bottles, from *M. brachychaeta*, *M. grandis*, *S. depilis*, *S. polysticta*, *S.* sp. *aff. xanthotricha*, *T. fiebrigi* of stingless bees kept in Amboró National Park. Nest entrances are shown in Fig. 29.1.

Each species of stingless bee produces honey, pollen, and propolis in different ratios. In Table 29.2 an annual yield for stingless bee products in Amboró National Park is characterized, and relative stingless bee species abundance. *S. polysticta* "suro negro" is the most abundant, and *T. fiebrigi* "señorita" also is abundant, but is the lowest producer because this is as small bee with small storage pots. The *Melipona* "erereú barcina" and "erereú choca" are less abundant. *S. depilis* "obobosí"

Table 29.1 Scientific and common names of Bolivian stingless bees

Scientific names of Bolivian stingless bees	Honey	Common names
Melipona brachychaeta Moure, 1950	1	"erereú choca"[a]
Melipona grandis Guérin, 1834	2	"erereú barcina"[a]
Melipona aff. crinita Moure and Kerr, 1950		"unknown"
Plebeia droryana (Friese, 1920)		"lambeojitos"
Plebeia kerri Moure, 1950		"boca de vieja"
Scaptotrigona depilis (Moure, 1942)	3	"obobosí"[a]
Scaptotrigona polysticta Moure, 1950	4	"suro negro"[a]
Scaptotrigona aff. xanthotricha Moure, 1950	5	"suro choco"[a]
Tetragonisca fiebrigi (Schwarz, 1938)	6	"señorita"[a]
Trigona chanchamayoensis Schwarz, 1948		"sicae amarilla"

[a] Pot-honey studied here

Fig. 29.1 Nest entrances of Bolivian stingless bees in hives in the Carmen Surutú community, Amboró National Park, Bolivia. (**a**) *Melipona brachychaeta*, (**b**) *Melipona grandis*, (**c**) *Scaptotrigona depilis*, (**d**) *Scaptotrigona polysticta*, (**e**) *Scaptotrigona aff. xanthotricha*, (**f**) *Tetragonisca fiebrigi*, not shown to scale Photos: P. Vit

Table 29.2 Relative annual yield of stingless bee products

Common name of the bees	Average honey (l/year)	Average pollen (kg/year)	Average propolis (kg/year)	Abundance in the park
Erereú barcina	1	0.5	0.5	Very low
Erereú choca	1	0.5	0.5	Very low
Obobosí	3	2	1	Abundant
Suro negro	2	1	3	Abundant
Suro choco	3	1.5	4	Medium
Señorita	0.5	0.5	0.25	Very high

produces 2 kg pollen/year and 3 kg honey/year, like "suro negro," but this is a rare species. *S.* near *xanthotricha* "suro choco" is a remarkable propolis producer with 4 kg/year and also yields an average of near 3 kg honey/year. Local common names of the bees, familiar to consumers, are used for marketing purposes.

29.3 Chemical and Microbial Composition of Bolivian Pot-Honey

The chemical composition (quality factors and mineral contents) and the microbiological analysis were performed with a sample of 300 g pot-honey, for each meliponine species, by Quality Control Laboratory, Food and Natural Products Centre, Faculty of Science and Technology, Universidad Mayor de San Simón, in Cochabamba, Bolivia (report number CAPN M197/08-3/6).

Physicochemical parameters were analyzed in duplicate: ash (gravimetric method), water content (refractometric method), reducing sugars and sucrose (titrimetric method), pH, and free acidity (titrimetric method) (AOAC 1984). The minerals were measured by spectrophotometry (Cu, Mg, Zn) (Perkin Elmer 1996), flame (Ca, K) (Vogel 1978), and colorimetry (Fe, P) (AOAC 1984) methods.

Microbiological spectra of mesophilic bacteria, molds, and yeasts were measured in colony-forming units (cfu)/g, using plate count agar. The analytical results of pot-honey produced by six species are shown in Table 29.3.

In Table 29.3, three sets of data are given for quality factors, mineral contents, and microbe content. Compared to *A. mellifera* honey standards (Codex Standard for Honey 1981), those of the meliponines were often different, including: (1) moisture (24.1–26.5 g water/100 g) for *M. grandis* and *S. polysticta*, all values higher than the honey standard <20%, (2) ash content (0.01–0.33 g ash/100 g) for *M. brachychaeta* and *T. fiebrigi*, complies with the honey standard of not more than 0.5%, (3) pH (3.4–4.5) for *S. depilis* and *T. fiebrigi*, as reference values not included in the honey standards, (4) free acidity (10.4–49.4 meq./kg) for *M. brachychaeta* and *S. depilis*, included in the maximum of 50 meq./kg for honey standards, (5) reducing sugar content (58.6–73.4 g reducing sugars/100 g) for *T. fiebrigi* and *M. brachychaeta*; standards are >60% glucose and fructose, and (6) sucrose content (0.0–1.5 g sucrose/100 g) for *S. aff. xanthotricha*, and *M. brachychaeta*, like the standards, <5%.

Honey mineral content was measured, for the first time, for Bolivian meliponines. The Ca, Cu, Fe, K, Mg, P, and Zn content was lower in honey produced by *M. grandis* and higher in the honey of *T. fiebrigi*, in agreement with ash content (0.01–0.33 g ash/100 g).

Microbe composition information is useful for sanitary quality control and is a routine analysis in the Brazilian Norm for honey (Brasil 1997). The counts for total aerobic mesophilic bacteria varied between 9.6×10^2 and 3.2×10^5 cfu/g for *T. fiebrigi* and *M. brachychaeta*, respectively. These values cover a wider range than the 1.0×10^3 and 5.0×10^3 cfu/g for Nigerian *A. mellifera*, where mold and yeasts were not detected in the honey (Omafuvbe and Akanbi 2009). Yeasts are usually present in honey, while other fungi were found only in the honey produced by three species: *S. depilis*, *S.* near *xanthotricha* and *T. fiebrigi*, in concentrations of 1.0×10 to 1.6×10^2 cfu/g. Souza (2008) also reported molds and yeasts in *S. xanthotricha* (2.5×10 to 4.6×10^2) and *T. angustula* (3.5×10 to 4.4×10^3) Brazilian pot-honeys. Molds were absent in the *Melipona* and *S. polysticta* honey. Yeast concentration varied between 3.0×10 and 4.1×10^3 cfu/g for *S. depilis* and *M. grandis*. A similar range, 1.3×10 to 1.6×10^3 cfu/g, was found in two samples of *M. mandacaia* from Brazil. *Melipona* such as *M. asilvai*, *M. quadrifasciata anthidioides*, and *M. scutellaris* were also within that range (Souza 2008). This author also observes increasing mold and yeast counts in pot-honey of *M. asilvai*, *M. quadrifasciata anthidioides*, *T. angustula*, and *M. scutellaris*, respectively. Therefore, molds and yeasts are fairly common in pot-honey. Association of microorganisms with Meliponini is discussed elsewhere in this book (see Chaps. 10 and 11).

The identification of yeasts, molds, and bacteria associated with the six bees is needed, in order to explain their function for the bees and for human health. The fact that meliponines cannot migrate (Roubik 2006) may lead to eventual fermentation and regulation of this factor within stingless bee nests. Flexible cerumen pots are ideal containers to do that, in contrast with the more rigid besswax combs, with

Table 29.3 Average values in chemical composition and antibacterial activity for six species of Bolivian meliponine pot-honeys from Amboró National Park

Chemical parameters	Stingless bees[a]					
	1 erereú choca	2 erereú barcina	3 obobosí	4 suro negro	5 suro choco	6 señorita
Quality factors						
Moisture (g/100 g honey)	24.9	24.1	26.0	26.5	24.9	25.1
Ash (g/100 g honey)	0.01	0.02	0.03	0.06	0.09	0.33
pH	3.8	3.6	3.4	3.5	3.8	4.5
Free acidity (milliequivalents/kg honey)	10.4	16.0	49.4	49.1	34.5	43.8
Sugars (g/100 g honey)						
Reducing sugars	73.4	72.5	67.7	67.8	67.0	58.6
Sucrose	1.5	0.9	1.0	1.0	0.0	1.8
Minerals (mg/100 g honey)						
Calcium	2.10	2.47	2.97	2.97	2.98	10.99
Cupper	n.d.	0.04	0.01	0.01	0.08	0.11
Iron	0.02	0.06	0.09	0.13	0.27	0.40
Magnesium	0.36	0.71	1.58	1.48	2.77	4.97
Phosphorus	0.97	1.32	3.00	5.38	7.01	16.85
Potassium	9.63	12.52	14.75	29.1	43.58	144.92
Zinc	0.02	0.02	0.01	0.68	0.15	0.63
Microbe composition (cfu/g)						
Mesophilic bacteria	3.2×10^5	2.3×10^4	$4,6\times10^3$	1.4×10^4	1.5×10^3	9.6×10^2
Molds	Absent	Absent	2.0×10	Absent	1.0×10	1.6×10^2
Yeasts	2.8×10^2	4.1×10^3	3.0×10	$3.1\text{x}10^2$	6.4×10^2	4.7×10^2

[a] See Table 29.1 for species

potentially thicker walls and aggregated arrangements to store higher quantities of honey. Honey microbes may be used to set sanitary standards for meliponines. For organic honey (Sereia et al. 2010), it has been suggested that microbe counts are of primary importance, but we believe this is still undetermined for meliponine honey. One example of the possible relationship between a yeast and health is the protective role of *S. cerevisiae*, acting as a probiotic able to colonize and survive in the mice enteron, and the immune modulation exerted against *Salmonella* infection (Martins et al. 2007).

MICs of *T. fiebrigi* honey from Argentina and Paraguay are lower for the Gram negative *E. coli* than the Gram positive *S. aureus* (Vit et al. 2009). For Bolivian pot-honey, only mesophilic bacteria, molds, and yeast concentrations were measured here. The measurement of antibacterial activity and probiotic action will be useful analyses to include with prospective medicinal value in these honeys.

29.4 Sensory Approaches to Evaluate Pot-Honey from Bolivia

The sensory evaluation for consumer acceptance included a Spanish panel of students and staff at the University of Burgos, Spain, who had never tasted meliponine honey before (Vit et al. 2010). The panel consisted of honey users with adequate physiological conditions. The six honey samples were evaluated at the same time, in an individual booth of the sensory room, under natural daylight. Water and toast were provided to clean the palate between samples. Instructions suggested trying all honeys first from left to right, and then to rank each one in a free order, and describe a short reason for this choice. Participants rated how much they liked each honey, manually, on a 10-cm line anchored with the words "dislike it a lot" and "like it a lot," in the left and right ends. This procedure provided a baseline rating the following averages of acceptance ± SD: "suro negro" *S. polysticta* 5.6 ± 2.2, "obobosí" 5.5 ± 2.5, "ereureú choca" *M. brachychaeta* 5.0 ± 2.5, "suro choco" 4.9 ± 2.2, "señorita" *T. fiebrigi* 4.8 ± 2.4, and "erereú barcina" *M. grandis* 3.7 ± 2.1. Although *M. grandis* honey was very light amber color, similar to acacia honey, it was the honey with the lowest score, due to a bitter taste, and animal notes. This average acceptance could be improved by a better knowledge of the honey and would be very interesting to compare with acceptance by consumers from urban and rural Bolivia.

Another sensory approach compared one pot-honey of *S. polysticta* from Bolivia with that from four species in Australia, Brazil, Mexico, and Venezuela, using the free-choice profile method. In this method there is no need of a trained panel, because sensory descriptors of honey are elicited from the assessors, and then quantified. The *S. polysticta* honey in this international set was characterized by fresh fruit aroma, sour taste and an astringent trigeminal sensation, and was grouped with another species of *Scaptotrigona*, *S. mexicana* (Vit et al. 2011).

29.5 Need for Networking to Market Bolivian Pot-Honey

The main honey importers in the world are Germany, the USA, UK, Japan, and France, and commercial interest is growing for organic and special honey (Hernández 2010). However, producing pot-honey and achieving a market niche are two different aspects of the business. Most projects, assisted or not, attain successful production and community interest. The chain of marketing needs to fulfill a system and a philosophy, valid in all the steps of the process, from raw materials in the environment to packaged honey as a commodity for the consumer and the "cradle to grave" perspective to attract consumers of organic honey (Hilmi, n.d.). Small to medium-sized enterprises (SMEs) like meliponiculture are not focused on conventional marketing. The additional lack of marketing resources makes alternative marketing approaches necessary, which benefit from a variety of networking processes (Gilmore 2001).

The Bolivian effort of 11 years with this meliponiculture project in Santa Cruz de la Sierra Department, Ichilo Province and three counties (Buena Vista, San Carlos and Yapacaní) was successfully coordinated by ASEO (Aguilera Peralta and Ferrufino Arnéz 2004; Ferrufino Arnéz and Aguilera Peralta 2006). Seven communities with 40 associates evidence the cooperative organization of APROMIN.

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Chapter 30
An Electronic Nose and Physicochemical Analysis to Differentiate Colombian Stingless Bee Pot-Honey

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30.1 Introduction

Honey derived from *Apis mellifera* (Linnaeus, 1758) are well known by consumers worldwide. Honey has been valued since ancient times and has been used as a nutritional and therapeutic supplement in many cultures (Vit et al. 1994). Previous studies have focused research on defining distinctive characteristics of honey from *A. mellifera* to obtain quality and authenticity labels (Acquarone et al. 2007; Kaškonienė et al. 2008; Baroni et al. 2009; Cajka et al. 2009; Truchado et al. 2009; Castro-Vázquez et al. 2010; Kaškonienė et al. 2010; Kropf et al. 2010; Stanimirova et al. 2010; Wang and Li 2011).

Geographical differentiation and the establishment of quality standards give added value to bee products such as honey and facilitate their commercial exploitation. Protected Geographical Status (PGS) is a legal framework defined in the European Union law to protect the names of regional foods, which ensures that only products genuinely originating in that region are allowed to be identified as such in commerce (EC 2008).

The purpose of this law is to protect the reputation of regional foods, to promote rural and agricultural activity, to help producers obtain a premium price (or fair price) for their authentic products, and to eliminate unfair competition and the deception of consumers by false or adulterated products, which may be of inferior quality. According to these laws, the quality of bee products—especially honey—can be defined by providing additional information about floral and geographic origin. As of 2011, more than 24 different kinds of European honey have been registered with PGS (EC 2011).

In addition to that of *A. mellifera*, honey from stingless bees (Meliponini, or meliponines) is found in Latin America. Meliponini live in tropical and subtropical areas,

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often acting as pollinators. Stingless bees have been on the continent far longer than *A. mellifera*; the latter was introduced during the Hipsanic period of conquest, mostly in the 1500s. Historical reports show that honey from the stingless bees was considered to be a treasure of great value for the indigenous population and that it was used as a trading instrument (see Chap. 14).

In Latin American culture, honey has traditionally been conferred with different therapeutic effects in addition to its nutritional properties. Stingless bee honey from Mexico, Guatemala, Venezuela, Brazil, Ecuador, Colombia, and Bolivia (Vit et al. 2004; Souza et al. 2006; Almeida-Muradian et al. 2007; Guerrini et al. 2009; see Chaps. 7, 28, 29) have physical and chemical properties of interest. On the other hand, due to reduction in forests where stingless bees thrive, there is a severe decrease in stingless bee populations, even to the point where they may be in danger of extinction. Different government entities have made efforts to increase the population of the bees by encouraging beekeepers to breed them and to commercialize their honey (Imperatriz-Fonseca and Peixoto 2006).

The distribution of stingless bee honey in the market is limited, compared with the honey from *A. mellifera*, as a consequence of limited production, shorter shelf life and lack of an institutional quality standard, due to the scant knowledge about the products. The main objective of this research was to establish quality attributes of stingless bee honey based on its physicochemical properties and the application of an “electronic nose” to monitor the volatile components of honey. This preliminary research determines if an ‘electronic nose’ is a valuable device for determining the quality and authenticity of stingless bee pot-honey. An electronic nose analysis has been conducted for *A. mellifera* honey (Benedetti et al. 2004; Lammertyn et al. 2004; Zuluaga et al. 2011). In this chapter we report for the first time, an electronic nose multivariate approach to pot-honey from Colombia.

30.2 Physicochemical and Electronic Nose Analysis of Honey

Fifty-five honey samples were collected from *Melipona* sp. (10 samples), *Tetragona* sp. (21 samples), *Melipona compressipes* (10 samples), *Melipona favosa* (7 samples), and *Melipona eburnea* (7 samples). The samples were immediately stored at 4°C in airtight containers in the dark to prevent degradation prior to analysis. To make a comparison of analyzed properties, 15 honey samples were collected from *A. mellifera* and processed in the same manner.

30.2.1 Physicochemical Analysis

The water content was determined by measuring the refraction index according to AOAC 969.38B (AOAC 2005) using a table refractometer ABBE (Euromex, The Netherlands) at 20°C. The water content (g/100 g) was obtained by correlation with a Chataway table (Chataway 1932).

Sugars analysis included the quantification of disaccharide (maltose–sucrose) and monosaccharide (glucose and fructose) content. This procedure was performed according to AOAC 979.23 and 983.22 (AOAC 2005) by high performance liquid chromatography (JASCO CO-2065, Japan) with a refraction index detector (JASCO RI-2031, Japan) and a calcium cationic exchange resin column Metacarb Ca Plus (VARIAN A5205, USA). In the mobile phase, distilled, degassed, and deionized water was used, with a flow of 0.5 mL/min; column temperature was kept at 80°C, and the detector at 45°C. Sugars results are expressed as g/100 g.

30.2.2 The Electronic Nose Analysis

The electronic nose consists of an array of weakly specific or broad-spectrum chemical sensors that mimic human olfaction and convert sensor signals into data that can be analyzed with appropriate statistical software. Such characteristics greatly facilitate monitoring volatile components of food, providing real-time information about the various characteristics of food under study (Schaller et al. 1999).

A number of potential applications of an electronic nose in the food industry have been reported, such as quality parameters for *A. mellifera* honey (Benedetti et al. 2004; Lammertyn et al. 2004) and quality assessment of meat (García et al. 2005; García et al. 2006), fruit and vegetables (Lebrun et al. 2008; Pani et al. 2008), wines (Aleixandre et al. 2008; Berna et al. 2008; Lozano et al. 2008), and dairy products (Pillonel et al. 2003; Brudzewski et al. 2004; Benedetti et al. 2005; Labreche et al. 2005).

Analyses were performed with an Airsense PEN 3 electronic nose (Germany) that consisted of three parts: a sampling apparatus, a detector unit containing the sensor array, and software for pattern recognition. Samples were introduced to the sampling apparatus randomly and after an adequate sensor flush time to avoid undesirable effects caused by sensor drift on readings.

The sensor array was composed of ten Taguchi type sensors (metal oxide semiconductors—MOS). Sensors were kept at 400–500°C during all of the process phases. The MOS sensors are the most suitable for food headspace analysis as they work at high temperatures and thus are not sensitive to humidity (Benedetti et al. 2004). The sensors used in this work are: W1C (aromatic compounds), W5S (wide range of compounds, especially nitrogen), W3C (aromatic compounds), W6S (mainly hydrogen), W5C (aromatic and aliphatic compounds), W1S (short chain hydrocarbons), W1W (sulphur compounds), W2S (alcohols), W2W (sulphur–chlorine compounds), and W3S (short chain aliphatic compounds).

The operative procedure was standardized and optimized as reported by Zuluaga et al. (2011). Three grams of each sample were placed in 40 mL Pyrex® vials with silicone caps and then introduced to the sampling unit of the electronic nose. Preliminary trials indicated that using larger sample volumes did not significantly increase signal intensities and reproducibility. After an equilibration time of 20 min at 40°C, the measurement sequence began (Zuluaga et al. 2011).

The measurement procedure consisted of pumping reference air over the sensors (the air in the room filtered through active carbon) at a constant flow rate (1 cm^3/s) for 10 s to set a stable baseline. Then the honey gas headspace sampled with a syringe was pumped over the sensor surfaces for 150 s. The sensors were then exposed to the reference air to recover the baseline. The total cycle time for each measurement was 7.5 min. Sensor drift was not experienced during the measurement period.

30.2.3 Data Analysis

The data obtained from the sensor array and physicochemical analyses for all of the honey samples were analyzed by partial least squares-discriminant analysis (PLS-DA) performed with MATLAB (v. 7.0 The Mathworks, Natick, MA, USA).

PLS-DA is a combination of the PLS technique, and regression to correlate an experimental response with a calculated response from a model, and DA analysis, which discriminates the experimental response among classes. The dimensions (components) extracted are composed such that they exhibit the maximum correlation with Y (class membership, e.g., origin and species) (van Ruth et al. 2010). This technique is a "supervised method," thus validated to obtain a reliable classification model. Some indicators were used to evaluate the robustness and prediction capacity of this model: non-error rate, specificity, sensibility, and precision.

For a better understanding of PLS-DA techniques, see Beebe et al. (1998), Wold et al. (2001), Gemperline (2006), Bereton (2007), and Aguilera et al. (2010).

30.3 Aromatic Profile and Physicochemical Results for the Genus *Melipona*

The physicochemical results for stingless bee and *A. mellifera* honey are presented in Table 30.1.

To create the classification models, data were organized in two matrices, analyzed separately with PLS-DA. The first data matrix grouped stingless bee honey from *M. compressipes*, *M. favosa*, and *M. eburnea*. The second data matrix grouped stingless bee honey from *Melipona* and *Tetragona* and *A. mellifera*.

The PLS-DA results are shown in Fig. 30.1 for the sample plot and the loading plot, respectively. *Melipona* are well classified in three defined classes. Samples from *M. compressipes* have high sugar values and an appreciable response from the sensors identified as W1W, W2W, and W3S. The same analysis for *M. eburnea* shows higher glucose content and a specific response for the sensors identified as W1C, W3C, and W5C. *M. favosa* has the highest moisture content.

The validation model shows adequate results for non-error rate and error rate for both the fitting and the cross-validation stages (Table 30.2), which indicates

Table 30.1 Physicochemical results for analyzed honeys from Colombia

Genus/species	Moisture (g/100 g)	Glucose (G) (g/100 g)	Fructose (F) (g/100 g)	Disaccharides (D)[a] (g/100 g)	Sugars (G+F+D) (g/100 g)
Melipona					
Melipona compressipes	25.8±2.0	34.2±4.4	36.9±3.7	3.4±2.2	75.2±8.0
Melipona eburnea	27.6±2.1	38.5±7.5	39.3±7.0	3.6±1.5	73.0±3.4
Melipona favosa	24.8±1.8	33.5±3.1	38.7±4.3	3.1±1.8	75.3±6.2
Melipona sp.	26.8±5.3	30.5±5.6	36.9±5.7	6.5±3.2	73.5±8.0
Tetragona					
Tetragona sp.	25.8±3.6	29.0±6.8	31.8±3.9	4.4±5.6	69.1±4.3
Apis					
Apis mellifera	18.6±1.5	32.6±4.4	40.1±3.9	6.8±2.1	82.6±9.3

Mean values±standard deviation
[a] Sucrose plus maltose

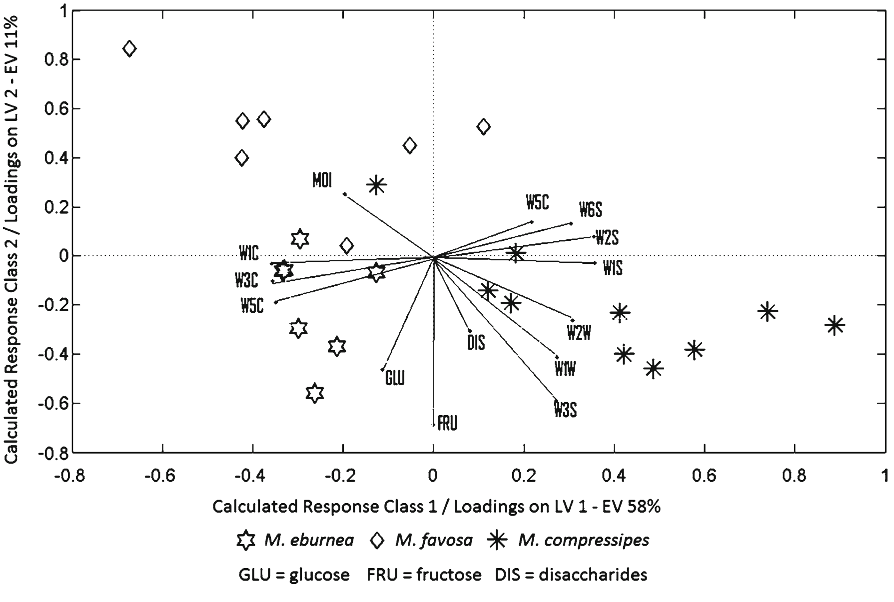

Fig. 30.1 PLS-DA result for *Melipona* pot-honey

that the model has a good capacity for recognizing classes and should be tested for prediction in future.

The other parameters, such as *specificity*, *sensibility*, and *precision*, established that the capacity of prediction is very accurate for *M. compressipes* and *M. eburnea*. However, for *M. favosa* the model has a fair capacity to differentiate samples from this class, but a low capacity to predict new, unknown samples.

Table 30.2 PLS-DA model fitting and validation results for species *Melipona*

Fitting Error rate: 0.08 Non-error rate: 0.83				Cross-validation Error rate: 0.17 Non-error rate: 0.75			
Class	Specificity	Sensibility	Precision	Class	Specificity	Sensibility	Precision
M. compressipes	1.00	0.80	1.00	*M. compressipes*	0.93	0.80	0.89
M. favosa	0.94	0.71	0.83	*M. favosa*	0.88	0.43	0.60
M. eburnea	0.94	1.00	0.88	*M. eburnea*	0.94	1.00	0.88

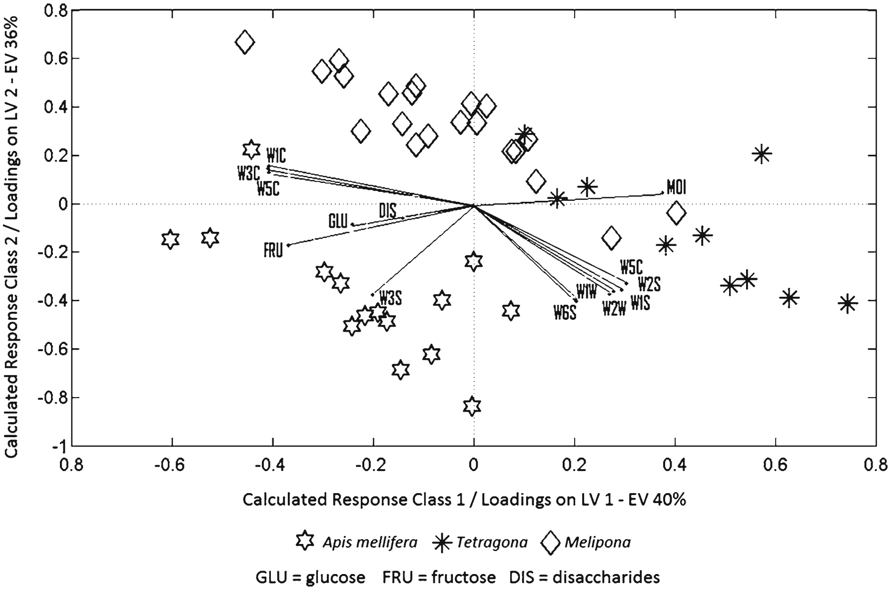

Fig. 30.2 PLS-DA results for *Melipona*, *Tetragona*, and *Apis mellifera* honey

30.4 Aromatic Profile and Physicochemical Results for the Species *Melipona*, *Tetragona* and *A*. *mellifera*

A. mellifera is included to establish differences from *Melipona* and *Tetragona*. The results from PLS-DA (Fig. 30.2) show differentiation from *A. mellifera* and separation between *Melipona* and *Tetragona*. *A. mellifera* is distinguished by high levels of fructose and low moisture content, also by responses of the sensor identified by the manufacturer as W3S. These results corroborate with those reported in the chapter of Deliza and Vit in this book, using assessors to evaluate pot-honey. *Tetragona* is characterized by the response of sensors W1C, W3C, and W5C; the same analysis concluded that the *Melipona* was characterized by W1S, W2S, W5S, W6S, W1W, and W2W.

The model evaluation (Table 30.3) shows a well-adjusted classification and a robust prediction capacity, especially for the *Tetragona* and the *A. mellifera* species. In the case of the *Melipona*, the model is adequate in differentiating samples of this species, but according to the results from cross-validation, the model has a low prediction of new unknown samples for this class.

30.5 Classification Model

Honey classification was made possible with sensor responses and data from simple chemical analysis. Both results showed that it is possible to create a model that facilitates the differentiation and classification of honey according to

Table 30.3 PLS-DA model fitting and validation results for species *Melipona*, *Tetragona*, and *Apis mellifera*

Fitting				Cross-validation			
Error rate: 0.09				Error rate: 0.20			
Non-error rate: 0.80				Non-error rate: 0.76			
Class	Specificity	Sensibility	Precision	Class	Specificity	Sensibility	Precision
Melipona	0.92	0.70	0.70	*Melipona*	0.86	0.60	0.55
Tetragona	0.96	0.81	0.94	*Tetragona*	0.84	0.76	0.80
Apis mellifera	1.00	0.87	1.00	*Apis mellifera*	1.00	0.87	1.00

bee species—in this case, from Colombian stingless bees. The PLS-DA model can be implemented as a useful tool for classification to guarantee the quality and the authenticity of honey. Data from the electronic nose analysis confirmed that volatile and semi-volatile organic compounds present in the headspace contributed significantly to the honey aroma and to the aroma variation in relation to the bee species. Aroma is a very important parameter for defining the quality of apicultural products (Ampuero et al. 2004; Benedetti et al. 2004).

Pot-honey has different flavors depending on various factors, one of which is the bee species (Vit et al. 2011a, b). However, in Colombia, there have been no studies aimed at characterizing and differentiating honeys from an objective point of view. It is clear that other types of analyses exist that facilitate the discrimination of honey according to species (e.g., gas chromatography), but using an electronic nose has shown that the proposed methodology is simple, rapid and does not require isolation of the volatile components. This makes the technique particularly useful for online quality control because any alteration that causes changes in the volatile fraction can be detected, which is of great importance to control adulteration and counterfeiting (very common activities in stingless bee honey sales).

Despite the fact that PLS-DA model classification parameters for *M. favosa* and *Melipona* could not achieve 100 % prediction, the results confirm the influence of the variables analyzed here for creating new models. It is advisable to increase the number of samples to enhance the fitting and predictive capacity of the statistical method to ensure reliability of results.

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Chapter 31
Nuclear Magnetic Resonance as a Method to Predict the Geographical and Entomological Origin of Pot-Honey

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31.1 Introduction

Nuclear Magnetic Resonance (NMR) is a powerful spectroscopic method, traditionally used as a very important tool in chemistry for structure verification, elucidation and purity analysis. However, driven by the needs of multidisciplinary topics such as biochemistry, medicine, pharmaceutical sciences, food chemistry, and others, NMR has rapidly expanded its applications to many other fields, and recent examples are the analysis of complex mixtures and screening applications (Lindon et al. 2000; Spraul et al. 2009).

NMR is an especially suited *detector* in the analysis of fluids of biological origin, food materials or drinks. It combines truly quantitative and structural information with high throughput (a 1D spectrum can be measured in a few minutes) and excellent reproducibility, which depends mostly on the minimal sample preparation required and the absence of any derivatization step.

For these reasons, it can be used to detect small molecules to generate global metabolite profiles in metabolomic studies, which aim to categorize or classify samples and to understand the basic underlying principles that contribute to the differences among them (Kang et al. 2008). Pattern recognition is followed by related multivariate statistical approaches to analyze the latent structures in the multivariate data.

Principal Component Analysis (PCA) and Partial Least-Squares Discriminant Analysis (PLS-DA) have often been used to identify sample groups and to relate specific biochemical compounds to the group separation.

^{1}H NMR-based metabolomic studies have been applied also to food science (Cevallos-Cevallos et al. 2009), including for example assessments of green tea

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(Tarachiwin et al. 2007), rosemary (Xiao et al. 2008), honey (Schievano et al. 2012), and grape wine (Son et al. 2008).

In this chapter, a study of the honey matrix is illustrated, performed using an NMR-based metabolomic approach combined with multivariate analysis.

31.2 Nuclear Magnetic Resonance

NMR is a branch of spectroscopy which uses radio waves, with a frequency between 20 MHz and 1 GHz on sensitive nuclei. The most common NMR experiments are performed on ^1H nuclei, but spectra on many other nuclei (^{13}C, ^{31}P, ^{19}F, and ^{15}N are the most common ones) are frequently acquired. The principles of NMR spectroscopy are well known nowadays; they are available in many textbooks (Günther 1995; Claridge 1999) and they are not discussed in detail here. Some basic principles will be quickly illustrated, to enhance the comprehension of this work. Nuclei with an intrinsic magnetic moment may be oriented by a strong magnetic field; two orientations are possible for ^1H nuclei. A consequence is the tendency to absorb and emit energy at a specific resonance frequency. Based on this phenomenon, a very large number of different NMR experiments have been developed, which explore different properties of the material under study. Samples can be analyzed in the solid (CP-MAS NMR), semisolid (HR-MAS NMR), and solution state (HR-NMR): the last one has been utilized in this work.

The ^1H 1D spectrum is the simplest NMR experiment: a radio frequency pulse inverts the orientation of some of the ^1H nuclei in the magnet; then, relaxation toward the original situation results is an electric signal (free induction decay: FID), which can be processed with a Fourier Transform to give a resonance peak.

Samples must be completely dissolved in a solvent. In the solvents for NMR analyses, protons are normally replaced with deuterium atoms to avoid saturation of the NMR receiver with the solvent protons, which would otherwise hide the signals of the protons of the solute. Each peak in the spectrum is the signal of a particular kind of proton in the mixture and its resonance position, the *chemical shift*, is measured in ppm units on the *x*-axis of the spectrum. The *y*-axis is an intensity scale, relative to the amount of protons. *Integration* of a peak area is directly proportional to the number of protons resonating at that same frequency.

31.3 Metabolomic Analysis

Metabolomics is the study of the global metabolic profile in a system (cell, tissue, or organism) under a given set of conditions. Metabolic profiling first appeared in the literature in the 1950s, and developed throughout the following decades (Rochfort 2005). The metabolome is formally defined as a collection of small molecules, including a range of endogenous and exogenous chemical entities such as peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins, polyphenols, alkaloids, minerals, and just about any other chemical that can be used, synthesized, or ingested by a given cell or organism. Over the past few years, two schools of thought have emerged for processing and interpreting metabolomic data: the chemometric and the quantitative metabolomics (or targeted profiling) approaches (Wishart 2008).

The chemometric approach (untargeted metabolomics) includes the analysis of multiple samples (for example by NMR) and statistical comparison of the results, without identifying the chemical compounds, but only using the recorded spectral pattern to recognize the relevant spectral features that distinguish sample classes. This method involves unsupervised clustering (PCA) or supervised classification (e.g., PLS-DA). After discovering significant differences, the most informative peaks in the spectra are identified and these molecules can then be used as markers.

In the quantitative metabolomics approach, most compounds in the sample are first identified and quantified, and this information is then used to perform multivariate statistical analyses and to find the most important markers and informative metabolic pathways.

From the perspective of a metabolomics researcher, most foods can essentially be viewed as complex chemical mixtures consisting of various metabolites and chemical additives in a solid, semisolid, or liquid mixture. In food science, metabolomics has become a tool to assess the quality, the processing history, and the safety of raw materials and final products. Recent applications involve geographical or botanical origin, or authenticity, of several foods.

In this work, a chemometric approach to differentiate the geographical and entomological origin of stingless bee honey has been used: ^{1}H NMR spectra provided signals, which were integrated and used as inputs for PCA and PLS-DA studies. Formally, PCA is a clustering technique that reduces the dimensions of a complex data matrix to orthogonal linear combinations (Principal Components visualized as principal axes) which describe variation in the data. These components can be displayed graphically as a score plot, where the separation of the observations is visualized in the space between the two axes.

Unsupervised PCA was initially used to explore variation in the NMR spectra dataset while PLS-DA was subsequently applied to maximize the separation among the samples.

The score plot can be visualized also in 3D corresponding to three principal components. In the loading plot, the most influential variables are highlighted: the farther they are from the center of the graph, the more they influence cluster separation. The Hotelling's T2 region, shown as an ellipse in score plots of the models, defines the 95% confidence interval of the modeled variation. The quality of the models is described by R^2x and Q^2 values. R^2x is defined as the proportion of variance in the data explained by the models and indicates goodness of the fit. Q^2 is defined as the proportion of variance in the data predictable by the model and indicates predictability (Eriksson et al. 2006). Thus, PCA is most commonly used to identify how one sample is different from another, and which variables contribute most to this difference. PLS-DA is based on the same basic principles as PCA, but it uses the labeled set of class identities, enhancing the separation between groups of observations.

31.4 NMR-Based Metabolomics Applied to Pot-Honey

Because of the complexity and diversity of the metabolites present in a complex food matrix, it is unlikely that one single analytical method would generate information about all the metabolites present and it would probably be necessary to perform a wide range of chemical analyses, which should be both rapid and reproducible. ^{1}H NMR has the potential to detect and identify a large number of compounds; as such, it is emerging as a leading technique in the area of metabolomic studies. An important advantage of the use of NMR spectroscopy in metabolomic studies is that the sample requires hardly any physical or chemical treatment prior to analysis. MS studies usually require separation of the metabolites, and for GC-MS it may be necessary to modify the metabolites to render them volatile. On the other hand, separation via HPLC requires conveniently detectable chromophores or functional groups.

NMR methodologies overcome these problems, and the range of compounds that can be analyzed is not limited by their volatility, presence of chromophores, or polarity, or other properties. Although the detection limit of NMR is still higher than that of other techniques, new pulse sequences have been introduced that lower the detection limit to about 10 μM in the sample solution (Rastrelli et al. 2009). Moreover, NMR spectroscopy simultaneously gives definitive structural information on many different compounds in the sample, maximizing the chance to identify important but unexpected or previously unknown metabolites (Teresa and Fan 1996).

^{1}H NMR has been successfully used, for example, in the area of toxicology, clinical diagnostics, and in the field of plant metabolites; it is frequently applied to food samples that can be directly examined as liquids (Belton et al. 1996), but very simple extraction or sample preparation procedures may also be used (Schievano et al. 2008). In the last decades, specific chemical and physical properties of honey have been used to determine its *botanical* origin (Anklam 1998; Bogodanov et al. 2004; Arvanitoyannis et al. 2005), and new analytical techniques have been proposed to this aim. An improvement in determination of botanical origin can certainly be achieved by a multivariate analytical approach. Recently, NMR techniques have been proposed also to identify and classify honey of different floral sources (Beretta et al. 2008; Lolli et al. 2008; Schievano et al. 2010) or geographical origin (Donarski et al. 2008; Consonni and Cagliani 2008).

The composition and properties of a particular honey sample depend strongly on the type of bee, on the type of flowers visited by the bees, as well as on the climatic conditions in which the plants grow and on contributions of the beekeeper (Al et al. 2009; Azeredo et al. 2003). In fact, Schievano et al. (2012) have shown that ^{1}H NMR spectra of organic extracts of honey can be used as a fingerprint to differentiate the botanical origin, when coupled with chemometric analysis.

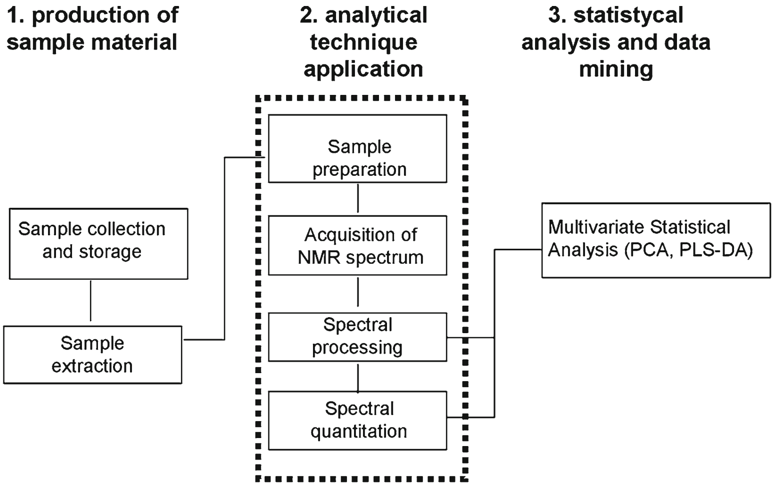

Fig. 31.1 The work flow of the NMR-based metabolomic approach applied to honey

The extraction method is simple and reproducible: a water/chloroform mixture was used as extracting solvent, with the advantage to eliminate the compounds most present in the honey mixture, i.e., the carbohydrates, with the water layer. The aroma compounds and those hydrophobic substances that differ the most in honeys of various sources are retained in the organic solution. Also, the extraction procedure yields a concentrated solution amenable to rapid NMR analysis. In more detail, portions of honey samples (6 g) were weighted in a centrifuge tube and dissolved with 15 ml of deionized water. 15 ml of $CHCl_3$ were added and the mixture was mechanically stirred for 10 min. The biphasic mixture was then centrifuged at 10,000 rpm for 15 min at 4 °C. The lower chloroform phase was collected and the solvent was evaporated under a gentle stream of nitrogen. The solid residue was dissolved in 600 μl of $CDCl_3$ and put in an NMR tube. The scheme of this NMR-based metabolomic approach is shown in Fig. 31.1.

The ^1H spectrum provides a fingerprint for each honey type showing many characteristic peaks in all spectral regions. Figure 31.2 shows a representative NMR spectrum from a *Melipona fuscopilosa* honey sample from the Amazon. Generally, the strongest signals in a honey spectrum are in the aliphatic region (0.0–2.5 ppm) while signals of comparable intensities rise in the other regions. All the regions appear very crowded. Specifically, many peaks are present in the 3.0–3.5 ppm region ($–C\boldsymbol{H}_2OH$ resonances), in the 4.0–4.5 ppm ($–C\boldsymbol{H}_2O–CO–$ signals), in the olefinic proton region (4.5–5.5 ppm), and in the aromatic region (6.5–8.5 ppm); also aldehydic and acidic proton signals are present (9.0–13.0 ppm).

The 1D spectra were acquired at 298 K, with a 600 MHz NMR instrument, using a modified double pulsed field gradient spin echoes (DPFGSE) sequence (Rastrelli et al. 2009). The introduction of a π pulse in the DPFGSE sequence allows the removal of the strongest signals present in the 0–2 ppm region, and this results in improved digitization of the weaker peaks, lower integration errors, and eventually,

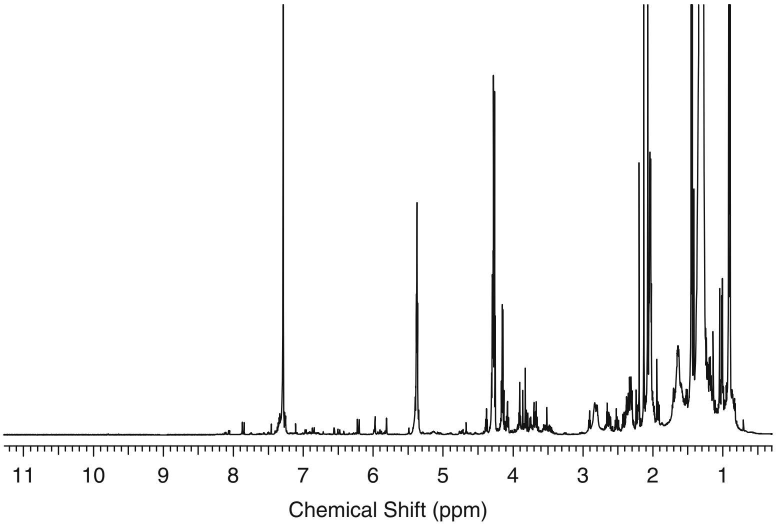

Fig. 31.2 Representative ^{1}H NMR spectrum of a *M. fuscopilosa* honey sample from the Amazon. The extract was dissolved in deuterochloroform and acquired with a 600 MHz NMR instrument

better quantification of the number of resonant spins. The spectra collection, processing, and analysis require 30 min.

The choice of chloroform as a solvent offers great advantages compared to other solvents previously used in NMR studies of honey. The residual chloroform signal is very sharp, and obscures a very small region at 7.26 ppm, which does not affect the analysis. On the other hand, solvents such as DMSO and MeOH are less suitable since they exhibit large signals in very important areas (around 3.4 ppm for MeOH and around 2.5 ppm for DMSO).

Data were processed using the ACD software (ACD/Specmanager 7.00 software, Advanced Chemistry Development Inc., 90 Adelaide Street West, Toronto, Ont., Canada M5H 3V9). Principal component analysis (PCA) and PLS-DA were conducted using the software SIMCA-P11 (Umetrics, Umea Sweden).

31.5 Geographical and Entomological Differentiation of Pot-Honey by NMR

The present study was performed on a total of 67 honey samples: 63 were obtained from stingless bees (see Table 31.1), one pot-honey was bought at the Indigenous market of Puerto Ayacucho, Amazonas state, Venezuela, as “erica” honey, one additional pot-honey sample was obtained after sugar feeding *M. quadrifasciata* bees, São Paulo state, Brazil, and two commercial honeys from *Apis mellifera* (one from Venezuela and one from Italy).

Table 31.1 Table of stingless bee pot-honey samples tested in this study

Common name	Stingless bee species	Geographical origin	Honey samples
"carby"	*Tetragonula carbonaria*	Australia, Brisbane	1–10
"uruçú"	*Melipona scutellaris*	Brazil, João Pessoa	11
"mijui"	*Scaptotrigona polysticta*	Brazil, Xingú	12
	Melipona sp.	Brazil	13
"tiúba"	*Melipona fasciculata*	Brazil, Maranhão	14–18
"jandaíra"	*Melipona subnitida*	Brazil, Rio Grande du Norte	19–22
"erica"	*Melipona favosa*	Venezuela, Falcón	23–30
"isabitto"	*Melipona aff. fuscopilosa*[a]	Venezuela, Amazon	31–33
"ajavitte"	*Tetragona clavipes*	Venezuela, Amazon	34–37
"pisilnekmej"	*Scaptotrigona mexicana*	Mexico	38–40
"colmena real"	*Melipona fasciata guerreroensis*	Mexico	41
"abeja real roja"	*Melipona fasciata guerreroensis*	Mexico	42
"criolla"	*Melipona solani*	Mexico	43
"abeja bermeja"	*Scaptotrigona hellwegeri*	Mexico	44–46
"ala blanca"	*Frieseomelitta nigra*	Mexico	47
"abeja real"	*Melipona beecheii*	Mexico	48–50
"erereú barcina"	*Melipona grandis*	Bolivia, Amboró National Park	51
"erereú choca"	*Melipona brachychaeta*	Bolivia, Amboró National Park	52
"obobosí"	*Scaptotrigona depilis*	Bolivia, Amboró National Park	53–54
	Melipona sp.	Bolivia, Amboró National Park	55
"suro choco"	*Scaptotrigona sp. aff. xanthotricha*	Bolivia, Amboró National Park	56, 57
"suro negro"	*Scaptotrigona polysticta*	Bolivia, Amboró National Park	58, 59
"señorita"	*Tetragonisca fiebrigi*	Bolivia, Amboró National Park	60, 61
"obobosí"	*Scaptotrigona depilis*	Bolivia, Amboró National Park	62
"abejita"	*Plebeia* sp.	Bolivia, Amboró National Park	63

[a] *Melipona aff. fuscopilosa* (= *Melipona* (*Michmelia*) sp. 1, see table in Pedro chapter 4, this book)

The pot-honey samples in Table 31.1 are from different entomological and geographical origins: 10 came from Australia, 12 from Brazil, 15 from Venezuela, 13 from Mexico, and 13 from Bolivia. The principal bees are *Melipona*, *Scaptotrigona*, *Tetragonisca*, *Tetragonula*, and *Frieseomelitta nigra.* In the sample data set, the differences due to geographical and entomological origin are not easily separable. In fact, the different species of bees generally live in different ecosystems. This consideration suggests that it might be difficult to discriminate the effects of different geographical origin from bee identify.

From the geographical point of view, our data set is composed of five main classes of honeys from five different regions. Furthermore, samples from Venezuela and Brazil can be divided in two other classes of samples collected in different regions of the same nation. Usually, projection methods for classification, such as PLS-DA, are able to produce efficient classification models for not more than four classes of samples.

For this reason, we did not consider the entomological origin of our honey samples at first, and PLS-DA models were obtained from groups of honey samples of different geographical origin, compared three at a time. Figure 31.3 shows the PLS-DA score plots (in 3D, corresponding to PC1/PC2/PC3) derived from the NMR spectra of the honey extracts, and they visualize good separations among these extracts (R^2x and Q^2 value of 0.70 and 0.80 for the "a" plot, 0.63 and 0.56 for the "b" plot, 0.91 and 0.80 for the "c" plot).

As a prediction test, we randomly selected two test samples from each region and built the PLS-DA prediction models without them. The approach yielded similar statistical characteristics to those previously obtained using the entire data set and correctly predicted the origins of the ten test samples. These results show that our method could be applicable to discriminate other unknown honey samples on the basis of their geographical origin.

If we apply the same PLS-DA calculations to the classification of the different entomological origins, there can be some ambiguity because some bees are found only in a specific geographical zone (e.g., the *Tetragonula carbonaria* and the *Melipona favosa* honeys sampled here are found only in Australia and in the Amazon, respectively). When we considered restricted regions, we were able to achieve good discrimination based on the entomological origin. The best results were obtained with the honeys collected in Venezuela (n° 23–37 of Table 31.1). Within these samples, we have honey of the same geographical origin, but of different entomological origin. A PLS-DA model (Fig. 31.4) is able to discriminate *T. clavipes* (four samples), *M. aff. fuscopilosa* (three samples), and *M. favosa* (eight samples). Specifically, samples from the same ecosystem (the Amazon) are very clearly separated in two groups (R^2x of 0.88, Q^2 of 0.97) corresponding to honey produced by two different bees (*M. aff. fuscopilosa* and *T. clavipes*). The honey sample bought at the local indigenous market in Puerto Ayacucho (State Amazonas) as "erica" *M. favosa* honey was used to test the predictive capability of our model. In Fig. 31.4, PLS-DA assigns it to the *Tetragona* group, not to *M. favosa* as claimed.

PCA of Mexican honeys (Fig. 31.5a) readily separated the groups of the most numerous samples from *Melipona* and *Scaptotrigona*. The remaining samples, produced by different bees, are in different regions of the plot. Pot-honey N° 48 is known to be produced by *M. beecheii*; however, it is found in a different area, and the most probable reason for that is the presence of a high content of hydroxymeth-

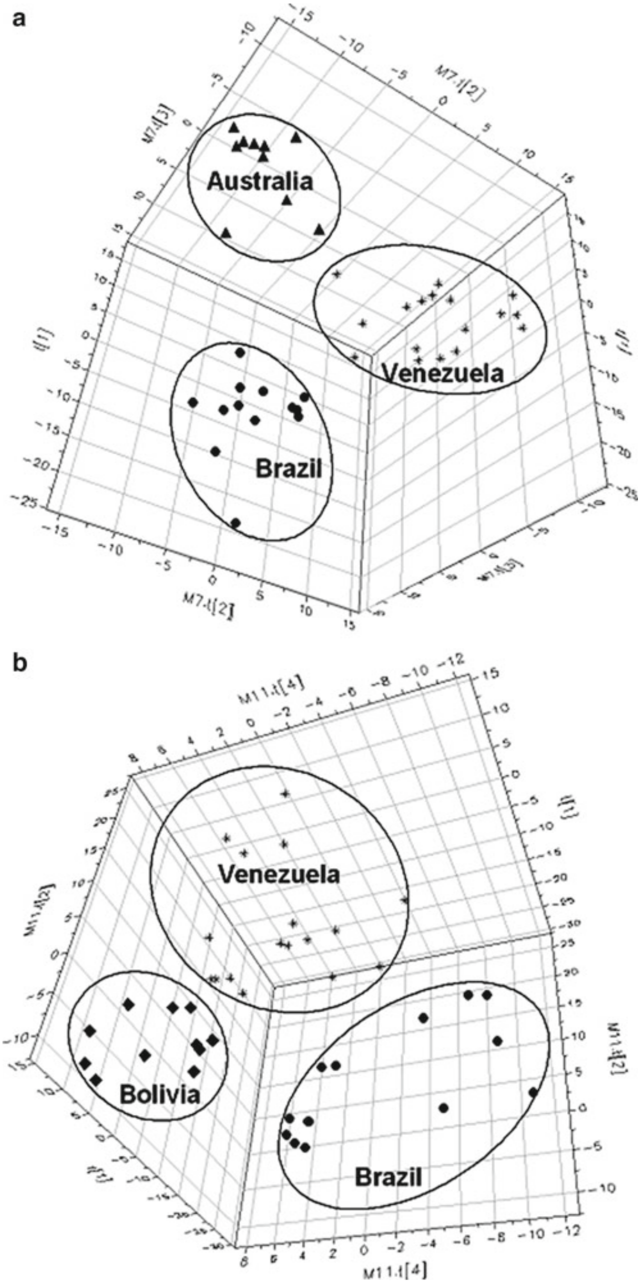

Fig. 31.3 PLS-DA score plots derived from 600 MHz ^{1}H NMR spectra of chloroform honey extracts. (**a**) PLS-DA on samples from Australia, Brazil, and Venezuela. (**b**) PLS-DA on samples from Bolivia, Brazil, and Venezuela. (**c**) PLS-DA on samples from Australia, Brazil, and Mexico. (*Filled triangle*) Australia, (*filled circle*) Brazil, (*asterisk*) Venezuela, (*filled diamonds*) Bolivia, (*open diamonds*) Mexico

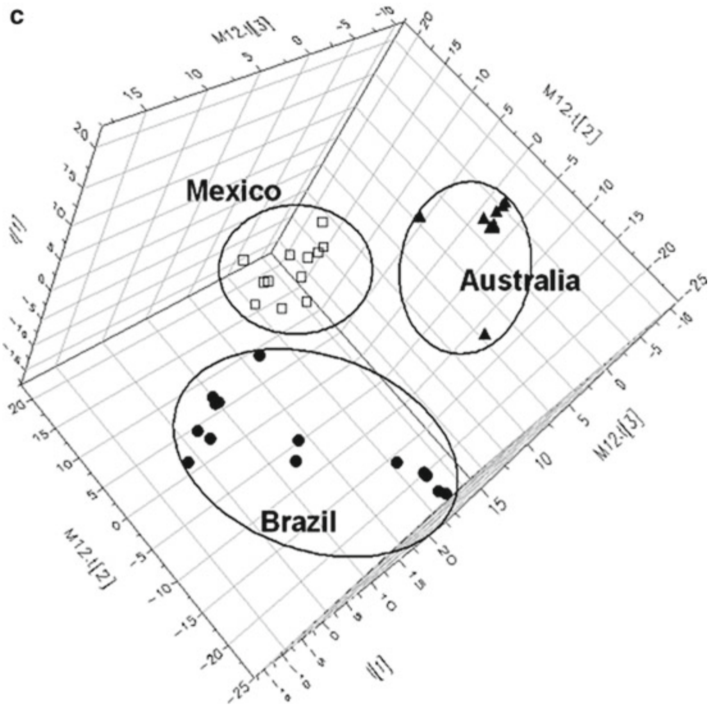

Fig 31.3 (continued)

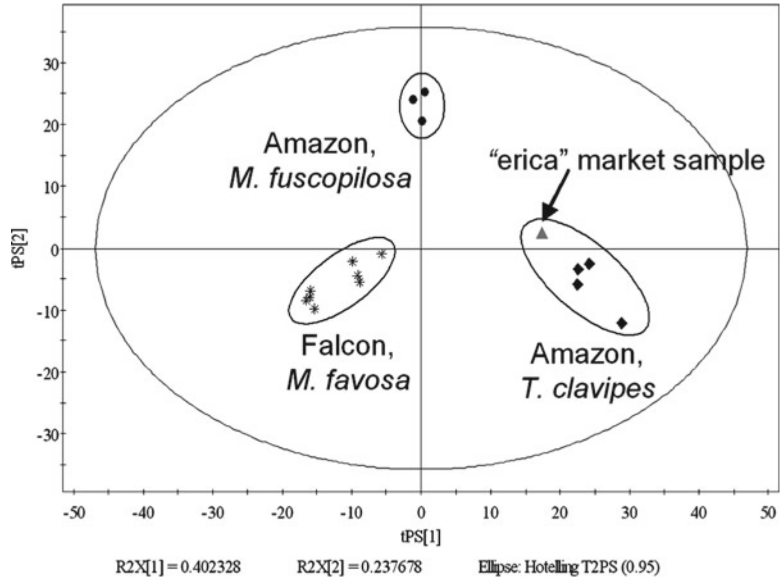

Fig. 31.4 PLS-DA on Venezuelan pot-honey samples. *M. fuscopilosa* (= *Melipona aff. fuscopilosa* = *Melipona* (*Michmelia*) sp. 1, see table in Pedro chapter 4, this book)

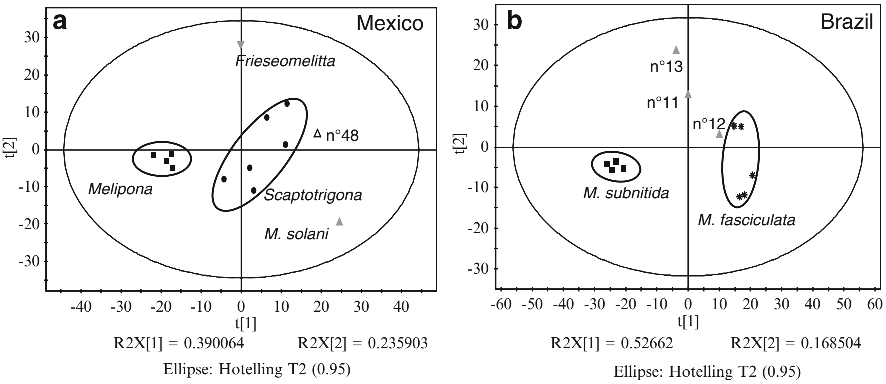

Fig. 31.5 PC1 and PC2 scores on pot-honey from (**a**) Mexico (**b**) Brazil. See honey numbers in Table 31.1

ylfurfural (HMF), which indicates lack of freshness or bad storage conditions and substances from fermentation. The sample produced by *M. solani* is different from the other *Melipona* honeys.

When a PCA on Brazil samples (n° 11–22 of Table 31.1) was performed (Fig. 31.5b), a clear differentiation, by the first PCA component, was seen between *M. fasciculata* and *M. subnitida*. The three samples outside the ellipses originated from different species. Again, samples produced by different bees are in different regions of the plot.

Our NMR-based metabolomic approach, even if applied to a limited number of samples, confirmed the validity of the multivariate statistical analysis in discrimination. We developed an efficient tool to differentiate the honeys by their geographical origin; additionally, to highlight the entomological origin, we understood that the field of investigation must be restricted to a smaller geographical region.

The following step was the identification of chemical shift resonances indicating specific marker molecules, responsible for the separation of origins. This was achieved by analyzing the loading plots of PCA, which explain the influence of the selected variables on the PCA model. As an example, we show here the assignment of a chemical compound in the Brazilian honeys. The loading plot is shown in Fig. 31.6a (the corresponding score plot is reported in Fig. 31.5b).

M. subnitida honeys from Maranhão are characterized by the following NMR resonances: 5.79, 5.96, 5.89, 6.16, 6.29, 6.44, and 2.28 ppm (see continuous line spectra in Fig. 31.6c, in comparison with the dotted line from Rio Grande du Norte honey). Characteristic peaks for *M. fasciculata* honey resonate at 5.32, 5.36, 4.24, and 4.12 ppm (see dotted line spectra in Fig. 31.6c).

In the case of *M. subnitida* honeys, the resonances were assigned and attributed to the *cis* and *trans* isomers of abscisic acid, which is present in large amounts (in comparison with the other compounds) in these samples. Unequivocal structural identification of this marker compound was obtained using homo- and hetero-nuclear

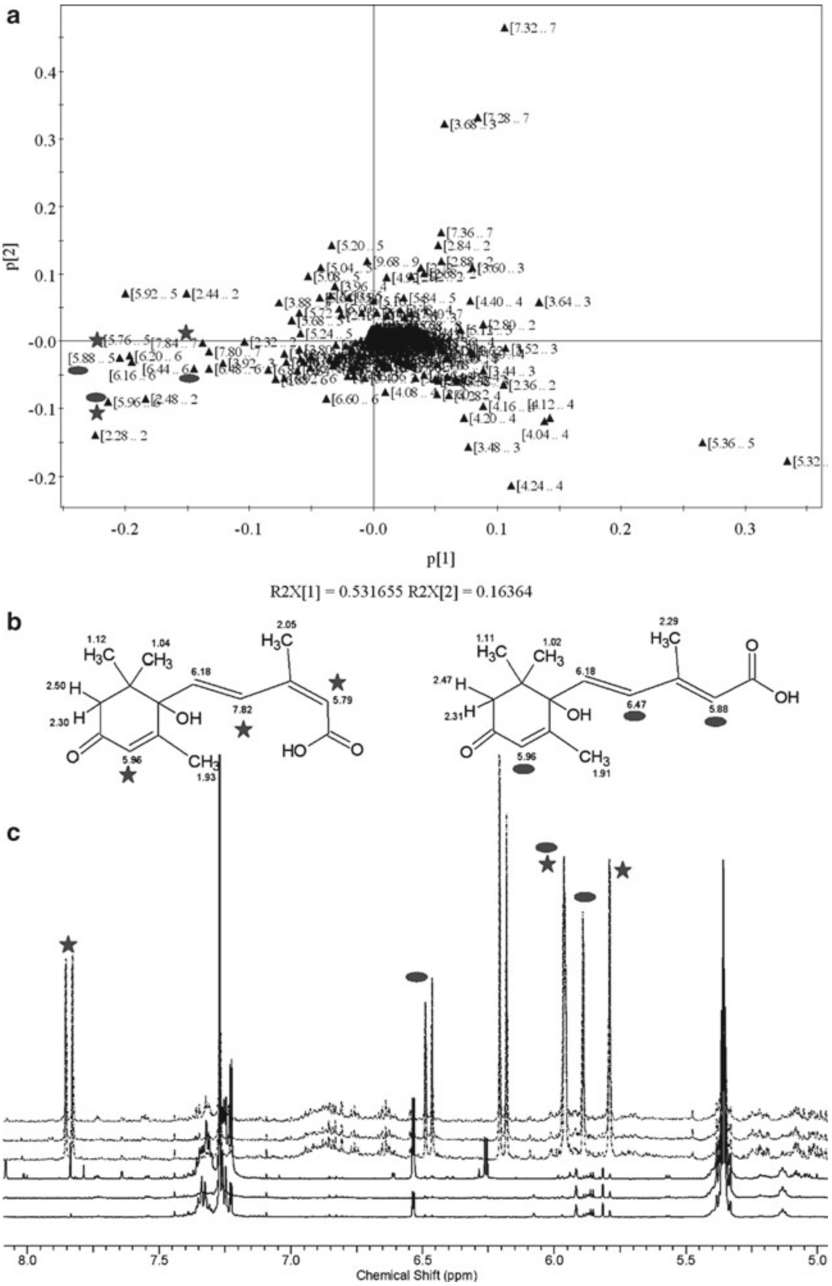

Fig. 31.6 Assignment of abscisic acid. (**a**) Loading plot of PCA on Brazilian honey samples (the corresponding score plot is shown in Fig. 31.5b on the right). (**b**) Chemical structures of *trans* and *cis* abscisic acid. *Asterisks* and *ellipsoids* indicate protons and the corresponding resonances. (**c**) Expanded region of ^{1}H spectra of three samples of *M. subnitida* (*dotted line*) and of three samples of *M. fasciculata* (*continuous line*) where the same resonances of abscisic acid are found

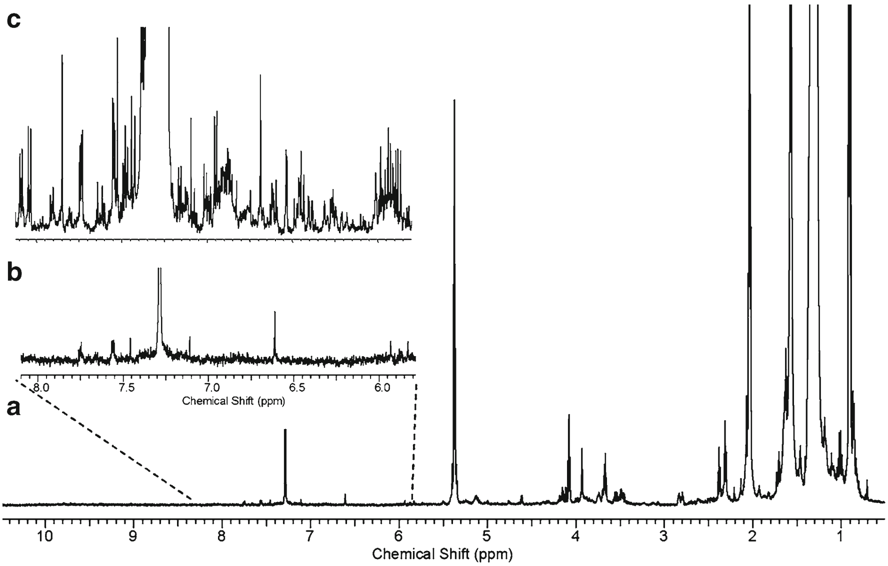

Fig. 31.7 Comparison of ^{1}H NMR spectra of chloroform extract of bees fed either sucrose syrup or floral resources (**a**) "sucrose honey" from *M. quadrifasciata*. (**b**) Expanded aromatic region of the "sucrose honey" extract. (**c**) Expanded aromatic region of a floral *Scaptotrigona mexicana* honey extract

correlation 2D-NMR experiments, and MS analysis. In Fig. 31.6b, the molecular structure of abscisic acid is reported, with its resonance assignment.

Concerning the Amazon honey samples, PCA led to a clear discrimination of the different bees present in the same ecosystem (*M. aff. fuscopilosa* and *T. clavipes*, as evident also in the PLS-DA of Fig. 31.4). According to the loading plot analysis, the discriminating region of the ^{1}H NMR spectrum is between 2.3 and 5.4 ppm. Assuming that in the same territory bees visit the same kind of plants and flowers, these data suggest that signals in this region of the spectrum come from organic compounds secreted by the specific bees.

To find the contribution of the bees and cerumen pots on the honey composition, a blank-trial probe was prepared, in which *M. quadrifasciata* were fed a sucrose syrup, to obtain a "sucrose honey". The ^{1}H NMR spectrum was acquired (see Fig. 31.7a,b) and compared with a typical pot-honey spectrum (as *Scaptotrigona mexicana* in Fig. 31.7c). The expanded aromatic region (6–8 ppm) of the sucrose honey (Fig. 31.7b) is poor of signals, in contrast to floral honey (Fig. 31.7c). However, most of the peaks in the aliphatic region (0–5.5 ppm), at frequencies typical of the fatty acid protons, are present in both sugar and floral honeys. It is evident that these aliphatic compounds must be part of the endogenous metabolism of the bee, rather than of the floral, exogenous resources. Therefore, discriminating signals that differentiate bee species are expected in the aliphatic region, whereas the foraging variation was observed in the aromatic region of the spectrum.

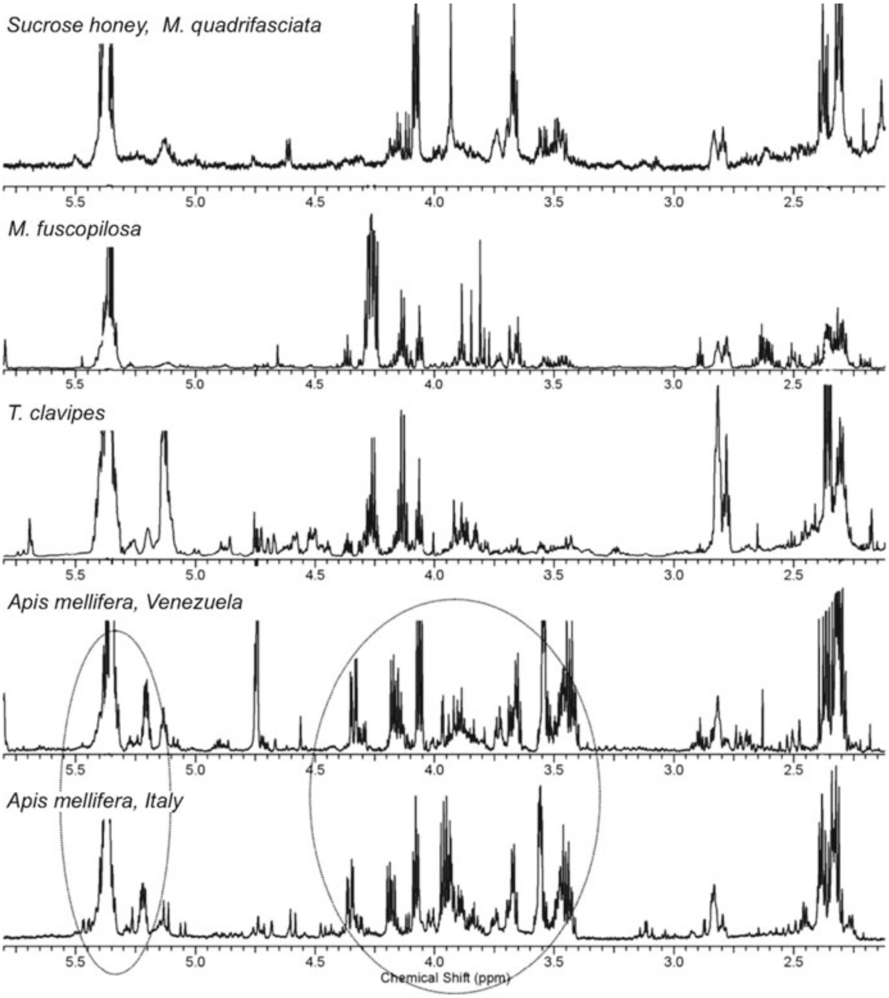

Fig. 31.8 ^{1}H NMR spectra (region 2–5.5 ppm) of the chloroform extract of honey produced by *A. mellifera*, and stingless bees. *M. fuscopilosa* (=*Melipona aff. fuscopilosa*= *Melipona* (*Michmelia*) sp. 1, see table in Pedro chapter 4, this book)

To substantiate this point, a comparison between the 2 – 5.5 ppm regions of the ^{1}H NMR spectra of different species of meliponine honeys was conducted and is shown in Fig. 31.8. This region of the spectra shows the typical resonances of the free or bound fatty acids and many other signals of the glycerol esters. In particular, the very high similarity between *A. mellifera* from Venezuela and from Italy confirms that these resonances are not geographical but entomological markers, clearly characterizing honeys produced by *A. mellifera*.

31.6 Conclusions

In this work, 65 honey samples from Meliponini (63 in Table 31.1, one from the local market, one obtained from a sugar fed colony) and two from *A. mellifera*, were analyzed by our NMR-based metabolomic approach. The ^{1}H NMR spectrum of the chloroform honey extract represents a mixture profile containing both endogenous bee metabolites and exogenous compounds coming from plants and flowers visited by the bees. For this reason, the data set is particularly suitable for a multivariate statistical analysis to distinguish both geographical and entomological origin. Moreover, as a preliminary work, the number of the analyzed samples was sufficient a higher number would allow us to have a test set to perform a prediction analysis to confirm these first results. Using an NMR-based metabolomic approach, we showed that:

1. Considering the entire data set samples, the stingless bee pot-honeys were well differentiated by their geographical origin.
2. The structural identification of abscisic acid, as an example of geographical marker compound for the Brazilian honeys, was achieved using 1D and 2D NMR spectroscopy.
3. If the analysis is restricted to a smaller region, it is possible to group honeys according to their entomological origin, because the entomological discriminant character becomes stronger than the geographical differences.
4. The application of NMR to authenticate the entomological origin of pot-honey (i.e., the market honey sold as “erica” was not a honey produced by *M. favosa*, but by *T. clavipes*), is demonstrated for the first time.
5. Sugar-fed *M. quadrifasciata* produced a honey with an unusual NMR profile, very poor in signals in the aromatic region (6–8 ppm), compared to natural floral honey. Therefore, the specific region in the NMR spectrum responsible for entomological separation seems to be the 0–5 ppm aliphatic region, where the protons from endogenous fatty chains resonate.
6. Discriminating signals to differentiate stingless bee species are expected in the aliphatic region of the NMR spectrum of honey.

Acknowledgments See the chapter on anticancer activity by Vit et al. in this book for the pot-honey samples received for this study. Prof. Paulo Nogueira-Neto provided the sucrose pot-honey of *M. quadrifasciata*, from São Paulo, Brazil. The *Apis mellifera* honeys were provided by Rigoni S.p.A. within the Veneto Region, Italy, UNIMIELE project 2008, and Miel La Encantada, Venezuela. We acknowledge Prof. Vit’s proposal to initiate this research in our lab.

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Chapter 32
Nonaromatic Organic Acids of Honeys

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32.1 Introduction

The composition of stingless bee (Meliponini) honey, also called pot-honey, has been researched since the 1960s (Gonnet et al., 1964 *apud* Souza et al. 2006). Despite having particular organoleptic properties and being highly appreciated in tropical areas, stingless bee honeys are not commonly available for purchase by consumers in most parts of the world.

Stingless bees have been widely studied by several researchers (Wille 1979; Kerr 1987; Camargo and Menezes Pedro 1992, 2007; Roubik 1995; Heard 1999; Michener 2000). As food commodities, some pot-honeys have been described as delicate and with delicious flavors (Kent 1984; van Veen et al. 1990), as well as honeys with sweet and sour flavors (Vit et al. 2010).

Many researchers have studied the physical and chemical properties of stingless bee honeys, as reviewed by Souza et al. 2006. With regard to acidity, scientists have reported that in general, pH of these honeys ranges from 2.0 to 4.7, whereas the values of free acid may be close to 200 meq/kg (Souza et al. 2006; Persano Oddo et al. 2008; Sgariglia et al. 2010). Although high values of free acid have been sometimes related to honey fermentation, the high acidity shown by stingless bee honeys has not been characteristically associated with spoilage of this food, and therefore, a high free acid could be a normal parameter of pot-honeys. In fact, several researchers have pointed out that an organic acids profile could be a better parameter than free acidity to determine *Apis mellifera* honey spoilage (Mato et al. 2006a).

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Stingless bee honeys are included neither in the revised codex standard for honey (CODEX 2001) nor in the European council directive 2001/110/EC relating to honey (OJEC 2002). Current studies on this food are needed because these standards would provide the consumers with a guarantee of food safety and food control by responsible laboratories.

This chapter reviews the importance and methods of analysis of nonaromatic organic acids of honey, based mainly on data obtained for *Apis mellifera*, compared to *Tetragonula carbonaria* and *Melipona favosa*, as well as its relationship to other parameters of this food.

32.2 Importance of Nonaromatic Organic Acids in Honey

More than 30 different nonaromatic organic acids have been identified in honey (Mato et al. 2003), most of them added by bees (Echigo and Takenaka 1974). Along with the concentration of sugars and hydrogen peroxide, nonaromatic organic acids are responsible for the excellent resistance of honey against microbial spoilage (White 1979a). Gluconic acid is the predominant nonaromatic organic acid in honey (Stinson et al. 1960), instead of malic or citric acids as previously thought (Nelson and Mottern 1931). Gluconic acid in equilibrium with gluconolactone is present in all honeys, in concentrations much higher than others (White 1978). Besides gluconic acid, other nonaromatic organic acids commonly present in honey are malic, citric, lactic, succinic, fumaric, maleic, formic, acetic, oxalic, and pyruvic, among others (Mato et al. 2003). Malic acid was one of the first acids identified in honey (Hilger 1904) and has been usually considered the second in importance after gluconic acid (Cherchi et al. 1994). Citric acid is a tricarboxylic acid, and the relationship between the acid forms and salt depends on honey pH, total citric acid content, and citric acid dissociation constants (Mato et al. 2000). The content of citric acid has been considered potentially useful to differentiate between nectar and honeydew honeys (Talpay 1988).

Honey gluconic acid comes mainly from the action of bee glucose-oxidase on nectar or honeydew glucose. Part of this acid is also produced by *Gluconobacter* spp., bacteria that are common in a bee's gut and stay throughout the ripening of honey. In aerobic environments with high glucose concentrations, *Gluconobacter* spp. microorganisms produce large amounts of gluconic acid (Ruiz-Argüeso and Rodríguez-Navarro 1973). The variation in the amounts of gluconic acid depends on the time required to completely transform the nectar or honeydew into honey; the longer it is, the greater the addition of glucose oxidase by the bee, and the greater therefore the amount of gluconic acid. Other factors that also influence the process are the strength of the colony and the quality and quantity of nectar coming into the hive (White 1979b). The origin of the other nonaromatic organic acids in honey is not fully known. They may come directly from nectar or honeydew, and some of them are produced from nectar and honeydew sugars by the action of enzymes secreted by worker bees and added to honey at ripening (Echigo and Takenaka 1974). Many honey nonaromatic organic acids are intermediates of such enzymatic pathways as Krebs cycle and others, being oxidized throughout the mentioned pathways (Echigo and Takenaka 1974; White 1979b; FAO 1990).

Honey organic acids have been proposed as potentially useful to characterize the botanical and geographical origin of honeys (Steeg and Montag 1988; Talpay 1989; Cherchi et al. 1994; Anklam 1998; Del Nozal et al. 1998; Mato 2004; Kaskoniene and Venskutonis 2010). 2-Methoxybutanedioic and 4-hydroxy-3-methyl-*trans*-2-pentenedioic acids were described as possible markers of *Knightia excelsa* (Proteaceae) honeys (Wilkins et al. 1995). In *Erica* sp. (Ericaceae) honeys, *cis*,*trans*-abscisic acid and *trans*,*trans*-abscisic acid (Ferreres et al. 1996), as well as high concentrations of quinic acid (Del Nozal et al. 1998), were found as possible markers, being the concentrations of *cis*,*trans*-abscisic acid about ten times higher than those found in honeys of other botanical origins (Gheldof et al. 2002). Low concentrations of pyruvic acid and high quantities of both malic and succinic acid were typical of *Quercus* sp. (Fagaceae) honeys, whereas high citric acid concentrations were described as a possible marker of *Thymus* sp. (Lamiaceae) honeys (Del Nozal et al. 1998). In *Castanea sativa* (Fagaceae) honey, high levels of formic acid were found, contrary to the low levels of formic acid described in *Eucalyptus* spp. (Myrtaceae) honey (Suárez-Luque et al. 2006).

Acetic acid has been proposed as possible indicator of honey fermentation, when its levels are excessively high (Mato et al. 2003). Such osmophilic yeasts as *Saccharomyces* spp., *Zygosaccharomyces* spp., *Torula* spp. and others, produce alcohols and eventually organic acids from honey sugars (Gonnet 1982). These yeasts come from flowers, soil, air, or the equipment used for honey extraction and processing, and are very sensitive to heat, so many companies pasteurize their honeys in order to prevent fermentation (Piana et al. 1989). For unpasteurized honeys, the possible usefulness of nonaromatic organic acid profile as a fermentation indicator should be researched (Mato et al. 2003).

Among other parameters such as phenolics, peptides, aminoacids, Maillard reaction products and enzymes, and nonaromatic organic acids, also contribute to antioxidant capacity observed in honeys (Gheldof et al. 2002). Such honey organic acids as citric, malic, and others act as metal ion chelators, and are considered as synergists of primary antioxidants enhancing antioxidant activity (Gheldof et al. 2002; Wanasundara and Shahidi 2005).

There is evidence that some acidic components of honey show antibacterial activity (Russel et al. 1988; Wahdan 1998). Acidic substances identified to date as antibacterial in honeys are mainly aromatic organic acids; such as ferulic and caffeic acids (Wahdan 1998), benzoic acid derivatives (Russel et al. 1988; Weston et al. 1999), and acids of royal jelly (Isidorov et al. 2011). Possible relationships between honey acidity and antibacterial activity have been studied, as well as between honey pH and antibacterial activity (Yatsunami and Echigo 1984; Bogdanov 1997). Honey antibacterial activity was significantly correlated with free acid and total acidity, showing the acidic fraction of several honeys with the greatest non-peroxide antibacterial activity (Bogdanov 1997; Kirnpaul-Kaur et al. 2011). In an acidic medium, honeys show better antibacterial activity (Bogdanov 2011).

Stingless bee honeys have been used in traditional and Mesoamerican aboriginal medicine (Vit and Tomás-Barberán 2004; Vit et al. 2004; Sgariglia et al. 2010).

Pot-honeys show high free acid values, and antibacterial activity is found in them by many scientists (DeMera and Angert 2004; Dardon and Enríquez 2008; Irish et al. 2008; de Almeida et al. 2009; Rodríguez-Malaver et al. 2009; Vit et al. 2009a; Boorn et al. 2010; Sgariglia et al. 2010). Therefore, it would be very interesting to study antibacterial activity of stingless bee honeys in relation with their levels of organic acids.

32.3 Honey Components and Parameters Related to Nonaromatic Organic Acids

Honey contains less than 0.5% of organic acids. Nevertheless, they are a group of constituents that contribute to several properties of this food, such as its color, aroma, taste, pH, acidity, and, to a lesser extent, electrical conductivity.

Color is an optical property of honey, described as the result of different degrees of absorption of light at different wavelengths by honey compounds (FAO 1990). The color of honey varies widely, from nearly colorless to almost black. This variability depends heavily on its origin and thus on its composition. Dark honeys tend to have higher acidity and higher organic acids contents (White 1979b; Crane 1990) than light honeys.

Aroma and flavor of honey are mainly due to a complex mixture of substances that are highly dependent on the botanical origin, but also influence the processing and storage conditions of this food (Anklam 1998). Among these substances organic acids are important, in particular for the taste of honey (Louveaux 1985; Crane 1990; Bogdanov 2009).

Honey acidity depends mainly on the presence of organic acids (White 1979b). Lactones are internal esters of organic acids and do not contribute to honeys' active acidity (Bogdanov 2009). Lactones hydrolyze over time, therefore increasing honey free acid. Total acidity is the sum of free acid and lactones. Honey pH depends on the amount of ionized acids, as well as the content in such minerals as potassium, sodium and calcium (White 1979b). Small oscillations in the range of pH in relation to the large swings in the free acid values were attributed to the buffer properties of honey, due to such mineral salts as phosphates, carbonates and others (Bogdanov 2009).

Electrical conductivity is a physical property of honey mainly related to the content of mineral salts, and to a lesser extent to the content of organic acids, proteins, sugars, and polyols (Crane 1990). It was found that the electrical conductivity was directly proportional to ash content and acidity of honey (Vorwohl 1964).

32.4 Methods of Analysis of Nonaromatic Organic Acids in Honey

The most important and frequently employed methods to determine honeys' nonaromatic organic acids are enzymatic assays, chromatographic techniques, and electrophoretic procedures (Mato et al. 2006b). Enzymatic assays are based on spectrophotometric measurements, usually at 340 nm, of the increase or decrease in absorbance of the reduced form's coenzymes nicotinamide adenine dicucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH), after the reaction of organic acids with specific enzymes. Enzymatic methods are precise and accurate. In addition, their specificity is excellent, allowing quantification of the D/L isomers of several organic acids. Furthermore, enzymatic procedures require very simple equipment, normally available in every quality control laboratory. Unfortunately, the stability of the enzymatic kits is not very long, and enzymatic procedures are tedious and time-consuming, allowing the determination of only one organic acid each time. Enzymatic analyses were commonly used to determine nonaromatic organic acids in *Apis mellifera* honeys (Tourn et al. 1980; Stoya et al. 1986, 1987; Hansen and Guldborg 1988; Talpay 1988, 1989; Sabatini et al. 1994; Mato et al. 1997, 1998a, b; Mutinelli et al. 1997; Cossu and Alamanni 1999; Alamanni et al. 2000; Bogdanov et al. 2002; Gheldof et al. 2002; Pulcini et al. 2004; and Vit et al. 2009a, b, among others). In respect of honeys produced by stingless bees, total D-gluconic, citric, and L-malic acids were quantified enzymatically in honeys from Australian *Tetragonula carbonaria* (Persano Oddo et al. 2008) and Venezuelan *Melipona favosa*.

Organic acids of honeys have been widely determined by chromatographic techniques. At first, these compounds were analyzed by paper and on-column ion exchange chromatography (Stinson et al. 1960). Gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionization detector (GC-FID) were applied to analyze honey nonaromatic organic acids with a previous derivatization process, due to the fact that most of these acids are not volatile (Echigo and Takenaka 1974; Wilkins et al. 1995; Horváth and Molnár-Perl 1998; Pilz-Güther and Speer 2004; Sanz et al. 2005), albeit recently, 29 organic acids were analyzed by GC-MS in honeys and other food commodities, using a procedure based on continuous solid-phase extraction without prior derivatization (Jurado-Sánchez et al. 2011).

Many researchers analyzed honey nonaromatic organic acids by high-performance liquid chromatography with ultraviolet detection (Cherchi et al. 1994, 1995; del Nozal et al. 1998, 2003a, b; Alamanni et al. 2000; Suárez-Luque et al. 2002a, b; Serra-Bonvehí et al. 2004; Hrobonová et al. 2007), although ionic chromatography with conductivity detection was also used to determine some nonaromatic organic acids in honeys (Pérez-Cerrada et al. 1989; Defilippi et al. 1995; del Nozal et al. 2000), as well as anionic exchange chromatography with UV detection (del Nozal et al. 1998) or constant voltage amperometric detection (Casella and Gatta 2001). Liquid chromatographic methods allow the simultaneous determination of several organic acids, showing a good versatility, reproducibility, and sensitivity. However, there are many interferences that must be removed by pretreatment of honey samples, or by using several columns in series, thus liquid chromatographic methods to determine honey nonaromatic organic acids are tedious and time-consuming.

Capillary electrophoresis with ultraviolet detection is another method that was successfully employed to quantify nonaromatic organic acids in honeys (Boden et al. 2000; Navarrete et al. 2005; Mato et al. 2006a; Suárez-Luque et al. 2006). Capillary electrophoresis is a rapid and low cost procedure that allows the simultaneous determination of several nonaromatic organic acids with a very simple preparation of the honey sample. The drawbacks of this method, if compared with other procedures, are its lower reproducibility and sensitivity. Nevertheless, capillary electrophoresis is a very promising technique that should be intensively studied for future analysis of honey compounds. Its application to analyze nonaromatic organic acids of pot-honeys could contribute to their characterization, which would be very interesting to promote and improve the commercialization of stingless bee honeys.

32.5 Nonaromatic Organic Acids in Pot-Honey

The content of D-gluconic, L-malic, and total citric acids was analyzed in eight samples of pot-honey produced by *Tetragonula carbonaria*, (Persano Oddo et al. 2008, as *Trigona carbonaria*, but see Rasmussen and Cameron 2007), and seven samples of *Melipona favosa* from Venezuela (Fig. 32.1). In all these pot-honeys, the quantities of L-malic and total citric acids were in general similar to those of *Apis mellifera* honeys described in the literature. As usual, D-gluconic acid values were one thousand times higher than L-malic and total citric acid concentrations. The quantities of D-gluconic acid in *Trigona carbonaria* honeys were in the same range of levels of D-gluconic acid of *Castanea* sp., *Thymus* sp., *Arbutus* sp. and honeydew honeys from *Apis mellifera* (Pulcini et al. 2004). The values of D-gluconic acid were about ten times higher in *Melipona favosa* samples (Fig. 32.1a), which might be indicative of a very high glucose oxidase activity at ripening (Persano Oddo et al. 2008), and could contribute to characterize *Melipona favosa* pot-honeys. Conversely, the concentrations of both L-malic and total citric acid were about ten times lower in honeys from *Melipona favosa* than in samples from *Trigona carbonaria* (Fig. 32.1b, c). It is interesting to highlight the fact that the *Melipona favosa* honey (sample 2) with the highest quantities of both L-malic and citric acid was the sample with the lowest concentration of D-gluconic acid. In contrast, the *Melipona favosa* sample with the lowest value of citric acid was the sample with the highest quantity of D-gluconic acid. In pot-honey from *Trigona carbonaria* it was observed that, in general, samples with the highest contents of D-gluconic acid contained the lowest quantities of total citric acid and vice versa. Most studies of pot-honey characterized the honey produced by different bee species of stingless bees (Vit et al. 1994; Souza et al. 2006; Persano Oddo et al. 2008; Sgariglia et al. 2010). It should be very interesting to research the nonaromatic organic acid profiles of these honeys, of particular interest the possible identification of the acid(s) responsible for the high free acid of pot-honey.

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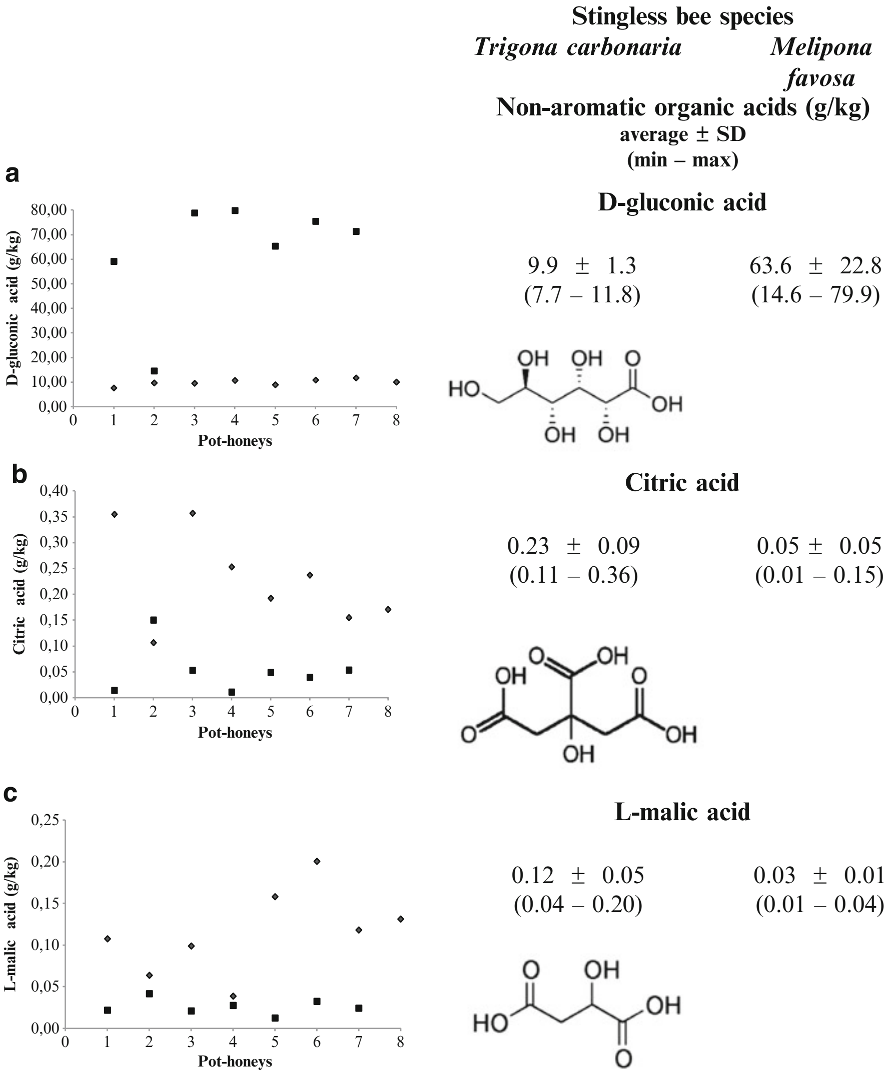

Fig. 32.1 Content of nonaromatic organic acids in pot-honey. (**a**) D-Gluconic acid, (**b**) citric acid, and (**c**) L-malic acid contents in pot-honey of *T. carbonaria* (*filled diamond*) from Australia and *M. favosa* (*filled square*) from Venezuela

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Part V
Biological Properties

Chapter 33
Flavonoids in Stingless-Bee and Honey-Bee Honeys

Francisco A. Tomás-Barberán, Pilar Truchado, and Federico Ferreres

33.1 Introduction

Honey produced in cerumen pots by stingless bees is a tropical ingredient for medicinal preparations since the Mayans (see Ocampo Rosales Chap. 15 in this book), widely relished before Columbus (Schwarz 1948). The Neotropical diversity of stingless bees, some 400 species reported by Camargo and Pedro (2007), is a challenge for any phytochemical investigation considering bee–plant interaction.

The sugar and water acidic matrix of honey has a set of minor components used as quality indicators, such as hydroxymethylfurfural and diastase activity (Bogdanov 1999). All the natural products and minerals of nectar and plant exudates used for honey-making are concentrated in honey as such or transformed by the bees and associated microflora.

Flavonoids are plant secondary metabolites that are associated with different physiological and ecological functions, such as protection of plant epithelial cells from ultraviolet rays, defense against biotic and abiotic stress, plant pigmentation, and signaling for interaction with animals, including bees, microbes, and other plants (Harborne 1982).

Flavonoids from floral nectar, pollen (Tomás-Barberán et al. 1989), and different plant exudates (Tomás-Barberán et al. 1993a) are incorporated into honey by the bees, and the metabolites present in plants can be modified during the honey elaboration process, mainly by the action of bee enzymes, bee microbiota metabolism, and chemical transformations during honey maturation.

Honey flavonoid profiles help to determine botanical (Ferreres et al. 1992, 1993, 1994, 1996b; Soler et al. 1995; Martos et al. 2000) and geographical

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(Tomás-Barberán et al. 1993b) origins of honey. It seems clear that honey contains complex phenolic and flavonoid profiles that could be associated with floral and geographical origin, although it is rather difficult to establish valid floral origin biomarkers, specific for a given plant. The study of potential phenolic fingerprints would be a very appropriate methodology for this purpose (Tomás-Barberán et al. 2001). Changes occur in the flavonoid profile with honey maturation in the bee nest and provide a method for evaluation of the degree of honey ripening (Truchado et al. 2010).

The flavonoid content in *Apis mellifera* honey has been extensively studied (Frankel et al. 1998; Ferreres et al. 1996a; Martos et al. 1997). The content in stingless-bee honey, however, has only been recently reported for samples from Australia (Persano Oddo et al. 2008) and Venezuela (Truchado et al. 2011), although previous qualitative studies exist (Vit et al. 1997; Vit and Tomás-Barberán 1998).

Recent research correlates flavonoid content (measured by a spectrophotometric method) to the antioxidant activity of honey produced by several species of stingless bees (Rodríguez-Malaver et al. 2007, 2009; Persano Oddo et al. 2008; Duarte et al. 2012). In this chapter the flavonoids of stingless-bee honey are reviewed, including new data presented here, from several countries.

33.2 Methods of Extraction and Analysis of Flavonoids in Honey

In the analysis of flavonoids from honey, a major problem is the extraction of these minor compounds from a matrix very rich in polar compounds (sugars). This problem is successfully solved by filtration of the diluted honey in acidified water, through nonionic polymeric resins such as Amberlite XAD (Ferreres et al. 1991). This methodology is combined with a final liquid–liquid extraction in which the flavonoids are extracted from water with dyethyl ether. The extraction renders flavonoid extracts that contain most flavonoid aglycones present in *Apis mellifera* honey—the main flavonoids present. Recent studies reveal that some unifloral honey, e.g., *Robinia pseudoacacia* (Fabaceae, Papilionoideae), contains mainly flavonoid glycosides, considered an uncommon honey trait (Truchado et al. 2008). For its analysis, extraction using solid phase extraction cartridges, in combination with HPLC-MS analyses, is considered very useful. In fact, in a more recent paper, the widespread occurrence of flavonoid glycosides in *A. mellifera* honey from different floral origins is demonstrated (Truchado et al. 2009b) although in most cases, flavonoid aglycones are the main metabolites. For stingless-bee honey, since this type of honey contains glycosides in a higher proportion than aglycones (Vit et al. 1997), the same extraction methodology was applied to a number of samples collected in South America and Australia.

The methodology used was the following. Flavonoid compounds from honey samples (5 g) were isolated with a Sep-Pak solid phase extraction cartridge (reversed phase C18 cartridge). The samples were diluted with ultrapure water and centrifuged at 9,000 × *g* for 10 min. The supernatants were filtered through a cartridge previously activated with methanol (10 mL) followed by water (10 mL). Following this, the phytochemicals that remained adsorbed in the cartridge were eluted with 1 mL methanol. The methanol fractions were filtered through a 0.45 μm membrane filter and stored at −20°C until further analyzed by HPLC-DAD-MS^n/ESI (Truchado et al. 2011).

33.3 Analysis of Honey Flavonoids Using Advanced HPLC-MS Methods

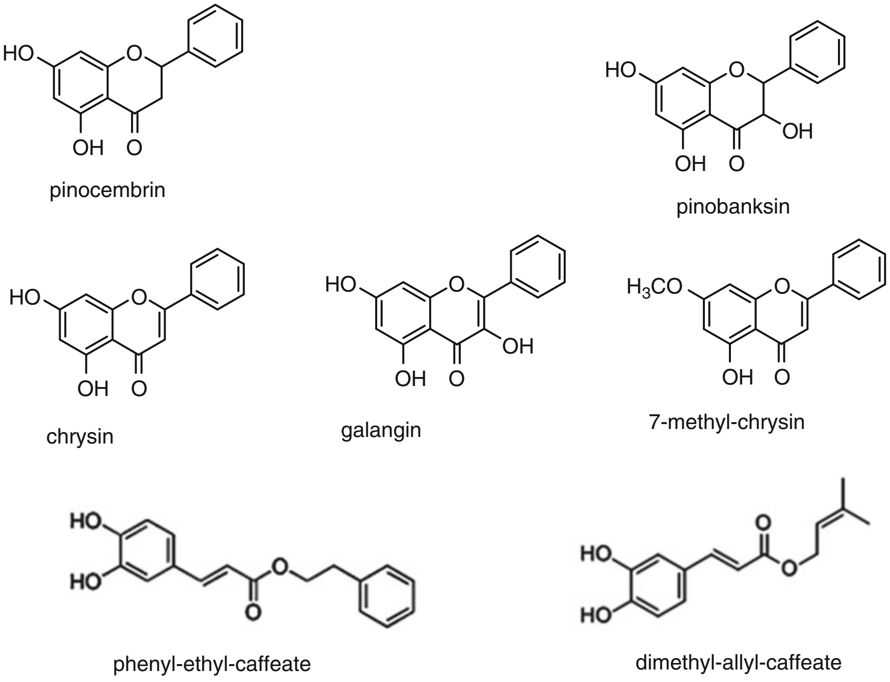
Analysis of honey flavonoid glycosides and aglycones was carried out in an Agilent HPLC 1100 series equipped with a diode array detector and mass detector in series (Agilent Technologies, Waldbronn, Germany). The HPLC consisted of a binary pump (model G1312A), an autosampler (model G1313A), a degasser (model G1322A), and a photodiode array detector (model G1315B). The HPLC system was controlled by ChemStation software (Agilent, v. 08.03). The mass detector was an ion trap spectrometer (model G2445A) equipped with an electrospray ionization interface, controlled by LCMSD software (Agilent, v. 4.1). The ionization conditions were adjusted to 350°C and 4 kV for capillary temperature and voltage, respectively. The nebulizer pressure and flow rate of nitrogen were 65.0 psi and 11 L/min, respectively. The full scan mass covered the range from *m*/*z* 100 up to *m*/*z* 2,000. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, with voltage camping cycles from 0.3 to 2.0 V. Mass spectrometry data were acquired in the negative ionization mode. MSn was carried out in the automatic mode on the more abundant fragment ion in MS($^{n-1}$). Chromatographic analyses were carried out on a LiChroCART column (250mm ×4 mm, RP-18, 5 μm particle size, LiChrospher®100 stationary phase, Merck, Darmstadt, Germany) protected with a LiChroCART guard column (4 mm × 4 mm, RP-18, 5 μm particle size, Merck, Darmstadt, Germany). The mobile phase consisted of two solvents: water–formic acid (1%) (A) and methanol (B) (99.9%, HPLC grade; Merck, Darmstadt, Germany), starting with 10% B and using a linear gradient to obtain 30% at 20 min, 60% at 40 min, 70% at 45 min, and 90% at 60 min. The flow rate was 1 mL/min, and the injection volume 20 μL. Spectral data from all peaks were accumulated in the range of 240–600 nm, and chromatograms were recorded at 280, 320, 330, 360, or 520 nm. The phenolic compounds were identified according to their UV spectra, molecular weights, retention times, and their MS–MS fragments, and whenever possible, with commercially available standards.

33.4 Flavonoids Observed in Honey from Combs and Pots

33.4.1 Apis mellifera Comb Honey

This type of honey contains flavonoid aglycones and other lipophylic compounds as the main plant secondary metabolites. Some honey samples of specific floral origin contain metabolites that may be considered biomarkers of the particular plant, as is the case of the flavanone hesperetin for citrus honey (Ferreres et al. 1993) and the alkaloid kinurenic acid for chestnut honey (Truchado et al. 2009a). Other honey samples contain specific compounds that are common to a number of different plant species, as in the case of the flavone tricetin and the flavonol myricetin in eucalyptus honey (Martos et al. 2000) and ellagic acid and abscisic acid in heather honey (Ferreres et al. 1996a).

Some *A. mellifera* honey contains relatively high amounts of flavonoid aglycones from propolis (poplar bud exudates collected by bees) (Fig. 33.1) including the flavones chrysin, galangin and techtochrysin, the flavanones pinocembrin and pinobaknsin and the caffeic acid derivatives dimethyl-allyl-caffeate and phenyl-ethyl-caffeate. Some of these compounds have also been reported in beeswax and in freshly secreted wax scales. It is suggested that bees may ingest propolis to incorporate these flavonoid metabolites in the secreted wax (Tomás-Barberán et al. 1993c).



pinocembrin

pinobanksin

chrysin

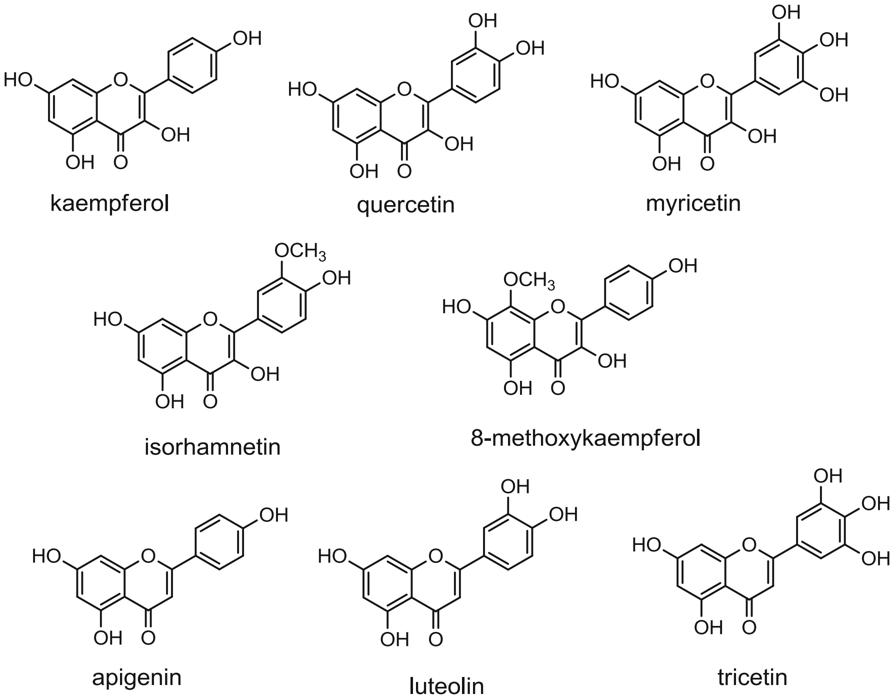
galangin

7-methyl-chrysin

phenyl-ethyl-caffeate

dimethyl-allyl-caffeate

Fig. 33.1 Propolis-derived flavonoids and other phenolic compounds from *Apis mellifera* honey



kaempferol

quercetin

myricetin

isorhamnetin

8-methoxykaempferol

apigenin

luteolin

tricetin

Fig. 33.2 Nectar and pollen derived flavonoid aglycones in honey and pot-honey

In addition, *A. mellifera* honey contains a large number of flavonoid aglycones derived from the naturally occurring flavonol-glycosides present in nectar, and probably pollen, from hydrolysis caused by bee saliva enzymes. These flavonoid aglycones include mainly polyhydroxylated flavones, but also their mono methyl ethers (i.e., isorhamnetin and 8-methoxykaempferol) and flavanones like hesperetin (Fig. 33.2).

A good example to illustrate hydrolytic activity of bee saliva is found in eucalyptus nectar and honey which clearly shows the presence of flavonol glucosides and diglucosides in nectar, and the transformation of these polar metabolites into the corresponding aglycones in mature honey (Fig. 33.3) (Truchado et al. 2009b).

When flavonoid rhamnosides or rhamnosyl-glucosides are present in nectar, those glycosides are not hydrolyzed by bee enzymes, as the bee does not have rhamnosidases in its saliva, and therefore the natural plant nectar glycosides are found in mature honey (Fig. 33.4). This occurs with *Robinia pseudacacia* honey, reported to contain mainly nectar flavonoid glycosides that bees cannot hydrolyze (Truchado et al. 2008).

When the transformation of nectar flavonoid glycosides is followed during the maturation of nectar in the comb to produce mature honey, the original flavonoid glycosides that are present in freshly deposited nectar are hydrolyzed sequentially, This process releases the aglycones found in mature honey, as demonstrated in *Diplotaxis tenuifolia* (Brassicaceae) honey (Truchado et al. 2010) (Fig. 33.5).

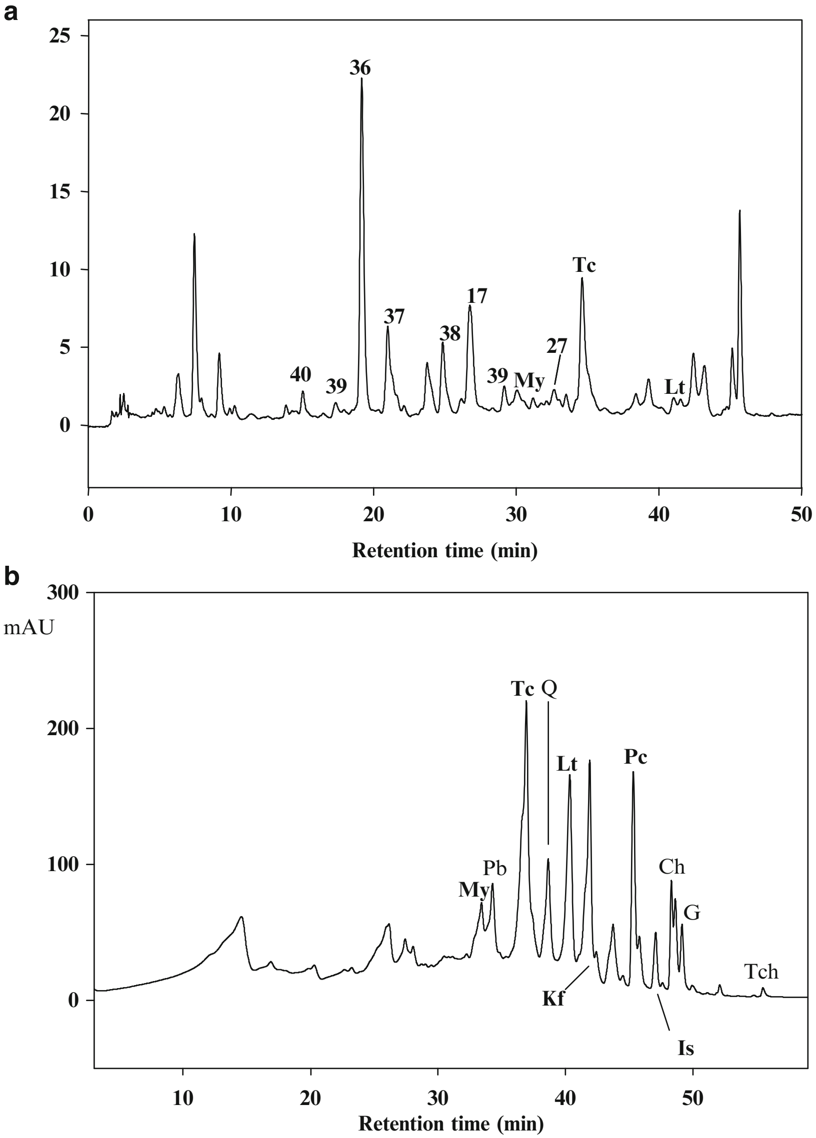
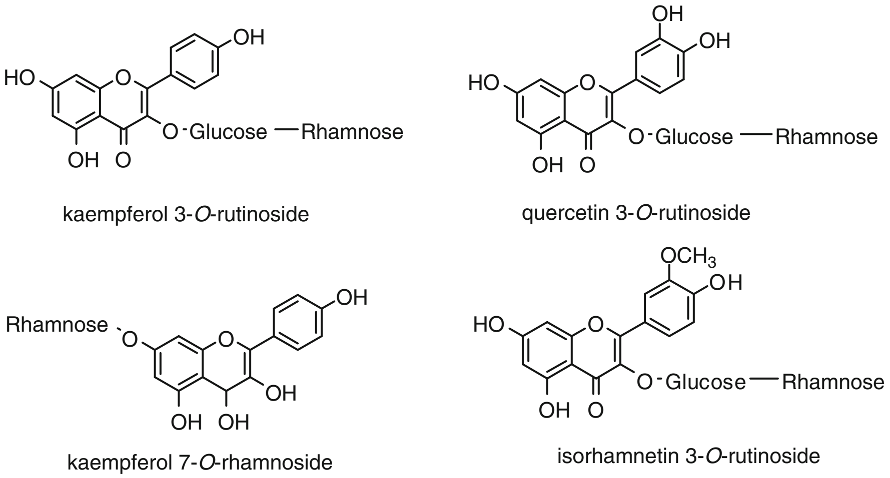


Fig. 33.3 Nectar (**a**) and honey (**b**) flavonoid profiles of *Apis mellifera* Eucalyptus honey. For flavonoid identification see Table 33.1

It can be concluded that, as a general rule, mature *A. mellifera* honey contains a larger amount of flavonoid aglycones than glycosides, although some specific honeys maintain large fractions of the original flavonoid glycosides, particularly when rhamnosides are present.



kaempferol 3-*O*-rutinoside

quercetin 3-*O*-rutinoside

kaempferol 7-*O*-rhamnoside

isorhamnetin 3-*O*-rutinoside

Fig. 33.4 *Apis mellifera* honey representative flavonoid glycosides

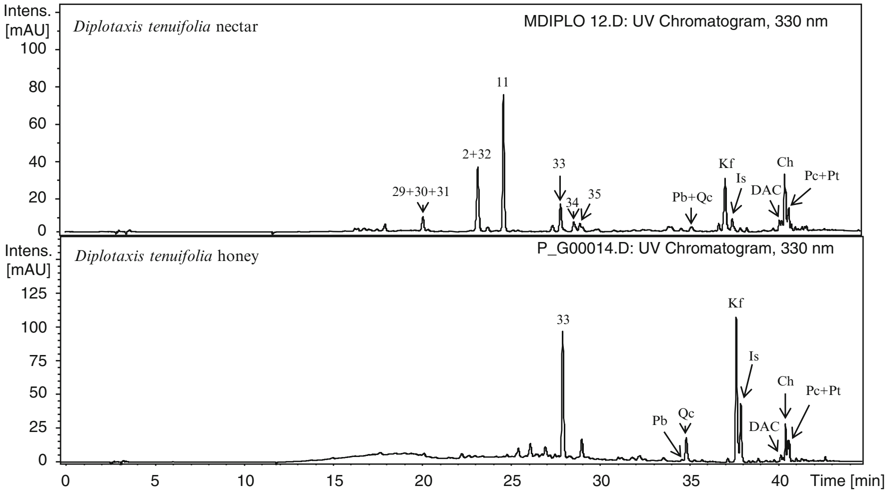
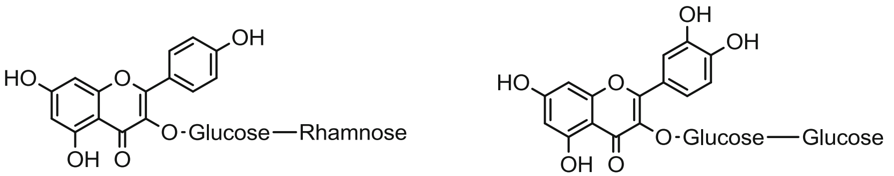


Fig. 33.5 HPLC/DAD (330 nm) phenolic profile of *Diplotaxis tenuifolia* honey from Argentina. The chromatogram from nectar is immature honey. For compound identification see Table 33.1

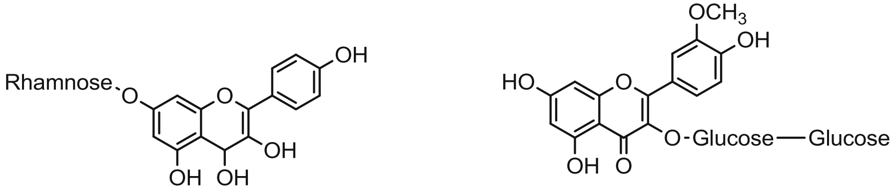
33.4.2 Stingless-Bee Pot-Honey

Pot-honey is generally characterized by a higher content of flavonoid glycosides than *A. mellifera* honey. This characteristic difference might be explained by the very low diastase activity of stingless bees compared to *Apis* (Persano Oddo et al. 2008). Recent studies report the occurrence of flavone di-*C*-glycosides and flavonoid *O*-glycosides in stingless-bee honey (Truchado et al. 2011) (Fig. 33.6).



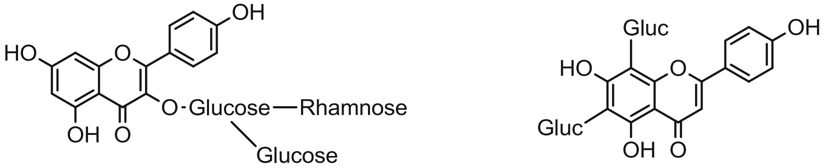
kaempferol 3-*O*-rutinoside

quercetin 3-*O*-dihexoside



kaempferol 7-*O*-rhamnoside

isorhamnetin 3-*O*-dihexoside



kaempferol 3-*O*-glucosyl-rutinoside

apigenin-6,8,-di-*C*-glucoside

Fig. 33.6 Stingless-bee honey representative flavonoid glycosides

A collection of eight *Tetragonula carbonaria* honey samples collected from nests in various locations around Brisbane (Queensland, Australia), in suburban areas where the flora was composed mainly of ornamental shrubs and flowering trees (Persano Oddo et al. 2008) was studied to evaluate the content of flavonoid compounds. This screening showed a similar chromatographic profile for all samples (Fig. 33.7a), in which flavonoid aglycones [tricetin (*Tc*), pinobanksin (*Pb*), luteolin (*Lt*), kaempferol (*Kf*), apigenin (*Ap*), isorhamnetin (*Is*), and pinocembrin (*Pc*)], were identified together with large number of flavonoid glycosides derived from quercetin, kaempferol, and isorhamnetin and a possible tetrahydroxydihydroflavone (*H*). Six flavonoid triglycosides, namely, one flavonoid trihexoside (**1**), two compounds with a –3-*O*-(2-hexosyl, 6-rhamnosyl)hexoside substitution (**3**, **9**), another two with a –3-*O*-(2,6-di-rhamnosyl)hexoside substitution (**5**, **14**), and another compound isomeric of **3** and **9** with a tentative –3-*O*-(2-hexosyl, 3-rhamnosyl)hexoside substitution (**7**), were detected. In the same way several flavonoid diglycosides derived from the triglycosides mentioned above and with –3-*O*-(2-hexosyl)hexoside (**2**, **4**, **11**), –3-*O*-(2-rhamnosyl)hexoside (**6**, **15**, **16**) (Fig. 33.7a), and –3-*O*-(6-rhamnosyl)hexoside (**17**) substitutions were, as well as two –3-*O*-(2-pentosyl)hexosides (**10**, **13**) and one tentative –3-*O*-(3-pentosyl)hexoside (**18**), detected (Table 33.1).

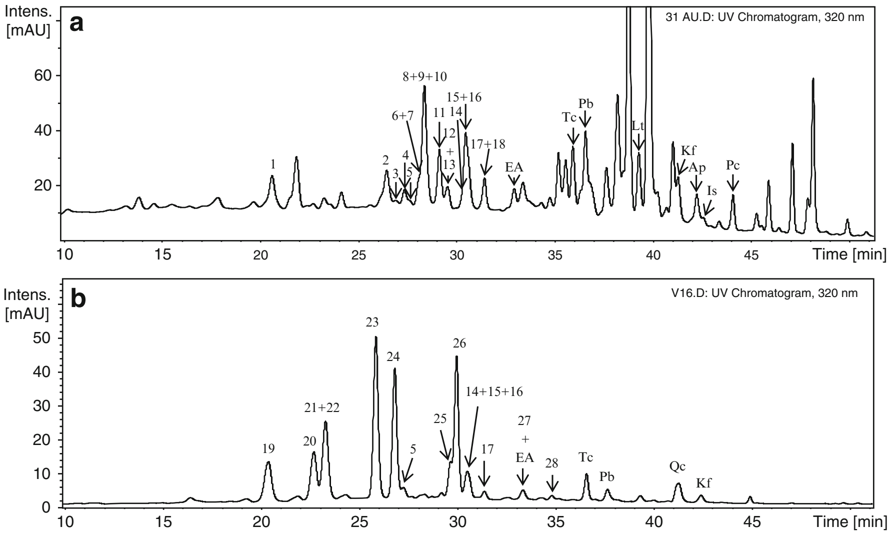


Fig. 33.7 HPLC/DAD (320 nm) phenolic profile of stingless-bee honeys (**a**) *Tetragonula carbonaria* honey from Australia, (**b**) *Melipona favosa* honey from Venezuela. For compound identification see Table 33.1

In the same way, 12 stingless-bee (*Melipona favosa*) honey samples from Venezuela collected in the arid climate area of Moruy were analyzed. The vegetation of this area was rich in Cactaceae and Mimosaceae species (Truchado et al. 2011) and all of them showed a similar chromatographic profile (Fig. 33.7b). The samples were characterized by the occurrence of five flavonoid di-*C*-glycosides: three apigenin 6,8-di-*C*-hexoside isomers (**19**, **20**, **21**), apigenin 6-*C*-pentoside-8-*C*-hexoside (**23**), and apigenin 6-*C*-hexoside-8-*C*-pentoside. Compounds with this *C*-glycosylation type had not been reported in honey (Truchado et al. 2011). In addition, these honey samples contained flavonol 3-*O*-glycosides, similar or identical to those reported from Australian stingless-bee honey described above. Compounds **5** and **14** and kaempferol 3-*O*-(2,6-di-rhamnosyl)hexoside (**26**) with a similar glycosylation to that of compound **5**, the diglycosides **15** and **16,** and the 3-*O*-(6-rhamnosyl)hexoside derivatives **17**, **27,** and **28**, in which only the aglycone was different, were detected and quantified. In addition, some propolis-derived aglycones, ellagic acid (*EA*), a flavonoid tetraglycoside [kaempferol 3-*O*-(2-hexosyl) rhamnosyl, 6-rhamnosyl)hexoside] (**25**), and a pentahydroxy-dihydroflavone, most likely dihydroquercetin (**22**), were detected (Fig. 33.7b).

Several stingless-bee honeys from Bolivia were also studied [“erereú choca” *Melipona brachychaeta* Moure, 1950; “erereú barcina” *Melipona grandis* Guérin, 1834; “obobosí” *Scaptotrigona depilis* (Moure, 1942); “suro negro” *Scaptotrigona polysticta* Moure, 1950; “suro choco” *Scaptotrigona* sp., *aff. xanthotricha* Moure, 1950; “señorita” *Tetragonisca fiebrigi* (Schwarz, 1938)] from Parque Nacional Amboró at different geographical areas with different vegetation. Only one honey

Table 33.1 Flavonoids from nectar and honey samples from *Tetragonula carbonaria* (T), *Melipona favosa* (M), *Apis mellifera* (*Diplotaxis tenuifolium*) (D) and *Apis mellifera* (*Eucalyptus globulus*) (E)

No.	Compound	T	M	D	E
1	Quercetin-*O*-trihexoside[a]	×			
2	Quercetin-3-*O*-sophoroside[a]	×		×	
3	Isorhamnetin-3-*O*-(2-hexosyl, 6-rhamnosyl)hexoside[a]	×			
4	Isorhamnetin-3-*O*-sophoroside[a]	×			
5	Quercetin-3-*O*-(2,6-di-rhamnosyl)hexoside[a]	×	×		
6	Quercetin-3-*O*-(2-rhamnosyl)hexoside[a]	×			
7	Isorhamnetin-3-*O*-(2-hexosyl, 3-rhamnosyl)hexoside[a]	×			
8	Tetrahydroxydihydroflavone[b]	×			
9	Kaempferol-3-*O*-(2-hexosyl, 6-rhamnosyl)hexoside[a]	×			
10	Quercetin-3-*O*-(2-pentosyl)hexoside[a]	×			
11	Kaempferol-3-*O*-sophoroside[a]	×		×	
12	Isorhamnetin-3-*O*-(hexosyl)hexosideisomer[a]	×			
13	Kaempferol-3-*O*-(2-pentosyl)hexoside[a]	×			
14	Isorhamnetin-3-*O*-(2,6-di-rhamnosyl)hexoside[a]	×	×		
15	Kaempferol-3-*O*-(2-rhamnosyl)hexoside[a]	×	×		
16	Isorhamnetin-3-*O*-(2-rhamnosyl)hexoside[a]	×	×		
17	Quercetin-3-*O*-(6-rhamnosyl)hexoside[a]	×	×		×
18	Quercetin-3-*O*-hexoside[a]	×			
19	Apigenin-6,8-di-*C*-hexoside[c]		×		
20	Apigenin-6,8-di-*C*-hexoside isomer[c]		×		
21	Apigenin-6,8-di-*C*-hexoside isomer[c]		×		
22	Dihydroquercetin[b]		×		
23	Apigenin-6-*C*-pentoside-8-*C*-hexoside[c]		×		
24	Apigenin-6-*C*-hexoside-8-*C*-pentoside[c]		×		
25	Kaempferol-3-*O*-(2-hexosyl)rhamnosyl, 6-rhamnosyl)hexoside[a]		×		
26	Kaempferol 3-*O*-(2,6-di-rhamnosyl)hexoside[a]		×		
27	Kaempferol-3-*O*-(6-rhamnosyl)hexoside[a]		×		×
28	Isorhamnetin-3-*O*-(6-rhamnosyl)hexoside[a]		×		
29	Quercetin-3,3′,4′-*O*-triglucoside[a]			×	
30	Isorhamnetin-3-*O*-glucoside-4′-*O*-gentiobioside[a]			×	
31	Quercetin-3,4′-*O*-diglucoside[a]			×	
32	Kaempferol-3-*O*-diglucoside isomer[a]			×	
33	Isorhamnetin 4′-*O*-gentiobioside[a]			×	
34	Isorhamnetin 4′-*O*-glucoside[a]			×	
35	Kaempferol-4′-*O*-glucoside[a]			×	
36	Tricetin 7-*O*-sophoroside (diglucoside)[a]				×
37	Tricetin 7,4′-di-*O*-glucoside[a]				×
38	Quercetin 3-*O*-glucuronide[a]				×
39	Myricetin 3,7-di-*O*-glucoside[a]				×
40	Myricetin 3-*O*-sophoroside (diglucoside)[a]				×
EA	Ellagic acid[d]		×		
DAC	Dimethylallylcaffeate[d]			×	
My	Myricetin[b]				×
Qc	Quercetin[b]		×	×	×

(continued)

Table 33.1 (continued)

No.	Compound	T	M	D	E
Lt	Luteolin[b]	×			×
Kf	Kaempferol[b]	×	×	×	×
Ap	Apigenin[b]	×			
Is	Isorhamnetin[b]	×		×	×
G	Galangin[b]				×
Ch	Chrysin[b]			×	
Tch	Techtochrysin[b]				×
Tc	Tricetin[b]	×	×		×
Pb	Pinobanksin[b]	×	×	×	
Pc	Pinocembrin[b]	×		×	×
Pt	Pinostrobin[b]			×	×

[a] *O*-glycoside
[b] Aglycone
[c] *C*-glycoside
[d] Phenolic acid derivative

sample from each stingless-bee species was available for analysis and suggests strong limitations of this study. The flavonoid profile observed was not as consistent as observed in the pot-honey from Venezuela and Australia. Nevertheless, the flavonoid glycosides detected which were also derivatives of quercetin, kaempferol, and isorhamnetin showed a glycosidic combination similar to those reported above for other stingless-bee honeys: normally hexosyl-hexosides although the second sugar could also be rhamnose or a pentose. Flavonoid triglycosides were also detected and in this case the additional sugar was often rhamnose. Several of these glycosides are common to all the analyzed samples, and in some cases flavonoid aglycones were also observed.

Several stingless-bee samples from Brazil were also analyzed: seven from “tiúba” *Melipona fasciculata*, four from “uruçú” *M. scutellaris*, and three from “jandaíra” *M. subnitida*, two from “mandaçaia” *M. quadrifasciata* and one from “uruçú amarela” *M. rufiventris*. All of them are characterized by having a very limited number of flavonoids, and in a very low quantity. These samples do not show a similar or common flavonoid profile, even for the same bee species, although this could be explained by different localities and therefore different floral origin. Some of them, and particularly the three samples from *M. subnitida*, have an abundant content of *tt* and *ct*-abscisic acid. In other samples they contained very small amounts of di-*C*-glycosyl flavonoids. Among the flavonoid *O*-glycosides, isorhamnetin and kaempferol derivatives, with a similar structure to those reported above, were detected, as well as other derivatives with glycosylations in the 3 and 7 positions. The aglycones pinobanksin and kaempferol were also detected.

A recent study reports the flavonoid glycoside content of stingless-bee honey (2.7 mg/100 g honey) is considerably higher than the content of aglycones (0.3 mg/100 g) (Truchado et al. 2011), and this differs from previous studies on *A. mellifera*, with much higher aglycone content and smaller flavonoid-glycoside content.

33.5 Conclusions and Further Research

Although the flavonoid content of *A. mellifera* honey has been extensively studied for potential use in determining botanical and geographical origin and also considering potential health benefit, the composition of stingless-bee honey is still largely unknown. An appealing topic of research is thus available due to the large number of bee species and the many and diverse plant sources used for honey production. The transformation of nectar flavonoids by bee enzymes is less relevant for the Meliponini, and therefore honey may better preserve the natural plant compounds. This observation deserves exploration in more detail. The fact that pot-honey is processed in storage pots containing resins may cause a transfer from the food container to the stored food which has never been measured, but certainly would add to its phytochemical spectra and bioactivity.

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Chapter 34
Antioxidant Activity of Pot-Honey

Antonio Jesús Rodríguez-Malaver

34.1 Introduction

Stingless bee honey has been used in traditional medicine for centuries. In countries including Peru, Guatemala, Mexico, and Venezuela, this honey is used widely and sold at local markets, often as a sweetener, but more often as an ingredient of folk medicine (Vit et al. 2004). This honey is a complex mixture that contains different botanical and entomological compounds. Such compounds contribute to honey's bioactive properties and are important in apitherapy.

Although there is a vast Neotropical biodiversity of 391 stingless bee species (Camargo and Pedro 2007), only the honey produced by a few species has been studied. In general, the main differences between stingless bee honey and *Apis mellifera* (honey bee) honey are a higher water content and acidity, lower diastase, and a different sugar content in the stingless bee honey compared to *Apis mellifera* honey (Vit et al. 2004; Souza et al. 2006).

It has been demonstrated that fermentation increased the antioxidant bioactivity of *Tetragonisca angustula* honey. This observation, signaling the importance of antioxidants, could partly explain the reputed medicinal properties of stingless bee honey (Pérez-Pérez et al. 2007).

Rodríguez-Malaver et al. (2007) measured the antioxidant capacity of *Apis*, *Melipona*, and *Trigona* honey from Venezuela with three oxidative systems, to test the effectiveness of honey at scavenging (i.e., removing) superoxide anions, hydroxyl radicals, and benzoate degradation. All the honey samples showed higher antioxidant capacity indicators than those of artificial honey and lipoic acid. The authors suggested that the antioxidant capacity could serve as a test to detect and then control adulterated honey on the commercial market.

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In this chapter, the antioxidant capacity of pot-honey is reviewed, and further scrutinized using information available for stingless bee pollen and propolis.

34.2 Bioactivity of Stingless Bee Products (Honey, Propolis, Pollen)

Among natural products, honey bee-derived apicultural products such as pollen and propolis have been applied for centuries in traditional medicine, as well as in food diets and supplementary nutrition (Kroyer and Hegedus 2002). Propolis has been used as a folk medicine and has been reported to possess therapeutic or preventive effects against inflammation, heart disease, diabetes mellitus, microbes hepatotoxity, and cancer (Burdock 1998).

Kujumgiev et al. (1999) report no differences in the antibacterial, antifungal, and antiviral activities of propolis from different geographic origins, including four samples from Brazilian *A. mellifera* and two stingless bees. The flavonoids in propolis (mainly pinocembrin) are considered responsible for its inhibitory effect on bacteria and fungi, but only traces of these compounds have been found in propolis of South American origin (Tomás-Barberán et al. 1993); thus, propolis from that region may possess other active compounds.

Farnesi et al. (2009) demonstrated that the antibacterial activity of green propolis from honey bee nests against *Micrococcus luteus* and *Staphylococcus aureus* was superior to that taken from nests of stingless bee, *Melipona quadrifasciata* and *Scaptotrigona*, propolis. Two samples of propolis (green propolis and *Scaptotrigona* propolis) were effective against *Escherichia coli*. *Melipona quadrifasciata* propolis was more active than green propolis and *Scaptotrigona* propolis against *Pseudomonas aeruginosa*, suggesting a potential importance for human and veterinary medicine.

It was found that Fenton reagent causes a decrease in salivary total antioxidant activity (TAA) and *Apis mellifera* propolis protects and even increases salivary TAA. On the other hand, *Melipona favosa* propolis only protects salivary TAA against oxidative stress (Sánchez et al. 2010).

Silva et al. (2009) show that the extracts of pollen from *Melipona rufiventris* are good scavengers of active oxygen species. Those authors suggest this property of pollen is important in prevention of diseases such as cancer, cardiovascular disease, and diabetes, among others.

34.3 Comparison of Pot-Honey and *Apis mellifera* Honey

Pot-honey shows differences in antioxidant activity, in comparison to *Apis mellifera* honey. In a study on Peruvian stingless bee honey from ten species, the Trolox equivalent antioxidant capacity (TEAC) ranged from 93.84 to 569.65 μmol Trolox equivalents (TE)/100 g (Rodríguez-Malaver et al. 2009). Some species (*Nannotrigona melanocera*) showed higher TEAC than both Czech *A. mellifera* honey (from 43.55 to 290.35 μmol TE/100 g) (Vit et al. 2008) and Venezuelan *A. mellifera* (from 34.90 to 203.21 μmol TE/100 g) (Vit et al. 2009a). In this work, flavonoid and polyphenol contents of stingless bee honey were measured; they ranged from 2.6 to 31.0 mg quercetine equivalents (QE)/100 g, and 99.7–464.9 mg gallic acid equivalents (GAE)/100 g, respectively. Those values were higher than Czech *A. mellifera* honey (from 1.90 to 15.74 mg QE/100 g and from 47.39 to 265.49 mg GAE/100 g) and Venezuelan *A. mellifera* honey (from 2.32 to 14.41 mg QE/100 g and 38.15 and 182.10 mg GAE/100 g).

The antioxidant activity, flavonoid and polyphenol contents are compared in pot-honey produced by several stingless bee genera. The highest values are found in *Nannotrigona* honey, followed by *Scaura* and *Ptilotrigona*. The lowest values are found in *Melipona* and *Partamona*, followed by *Tetragonisca* and *Scaptotrigona*. However, such comparisons are only preliminary, because more honey samples are needed. Only one honey was available for most of the genera, whereas 28 *Melipona* honeys and 18 *Tetragonisca* honeys were analyzed (Gutiérrez 2008).

34.4 Factors that Explain the Antioxidant Capacity and Possible Role for Authentication

Persano Oddo et al. (2008) report that the TEAC of *Tetragonula carbonaria* (formerly named *Trigona carbonaria*) honey from Australia is higher (233.96 ± 50.95 μmol/100 g) than that reported for Czech floral honey of *Apis mellifera*, while the radical scavenging activity (RSA) (48.03 ± 12.58% ascorbic acid equivalents) is similar to that of floral and honeydew blends of Spanish honey (Pérez et al. 2007). The flavonoid content of *T. carbonaria* honey (10.02 ± 1.59 mg QE/100 g) is higher than those of Czech floral and honeydew honey (6.59 and 7.25 mg QE/100 g, respectively). In contrast, the polyphenol content is higher in the floral (115.03 mg GAE/100 g) and honeydew (129.03 mg GAE/100 g) Czech honeys than in *T. carbonaria* honey (55.74 ± 6.11 mg GAE/100 g) (Vit et al. 2008). The authors suggest that organic acids might explain its high antioxidant activity. The antioxidant capacity of *T. carbonaria* and other stingless bee honey represents an important added value, to encourage further research on medicinal attributes with both nutritional and pharmaceutical application. In a recent study, a high level of antibiotic activity was found in honey from *T. carbonaria* (Irish et al. 2008).

In another study with pot-honey from Guatemala, *M. beecheii* "abeja criolla" and *M. solani* "chac chow" were compared. The antioxidant activity, flavonoid and polyphenol contents are given in Table 34.1. The TEAC values, flavonoid and polyphenol contents were significantly higher in *M. beecheii* than in *M. solani* honey (Gutiérrez et al. 2008). Such a difference could be explained by the floral species visited. Asteraceae and Melastomataceae were the most abundant plant families in the *Melipona* honey pollen spectrum in Guatemala (Dardón and Enríquez 2008).

Table 34.1 Bioactivity of *Melipona* honey from Guatemala (permission granted by Revista de la Facultad de Farmacia)

	Stingless bee species	
Bioactive parameter	*M. beecheii*, $N=4$	*M. solani*, $N=2$
Flavonoids* (mg QE/100 ghoney)	3.60 ± 0.61	1.88 ± 1.64
Polyphenols* (mg GAE/100 g honey)	107.35 ± 17.79	68.66 ± 15.11
TEAC* (μmol TE/100 ghoney)	87.38 ± 12.92	39.07 ± 10.52

Averages ± SD values

*Significant differences between *M. beecheii* and *M. solani* ($P<0.05$), *t*-test

Tetragonisca fiebrigi Schwarz, 1938 is a stingless bee named "yateí" in Argentina and Paraguay. Vit et al. (2009b) compared a honey sample from both countries and found that TEAC was higher in honey from Argentina (160.15 ± 60.50 μmol TE/100 g) compared to Paraguay (120.91 ± 38.67 μmol TE/100 g). However, they did not find a difference in flavonoid (14.37 ± 11.11 and 12.66 ± 4.82 mg QE/100 g) and polyphenol (240.74 ± 94.05 and 148.29 ± 17.75 GAE/100 g) content.

High nitrite content was found in Peruvian pot-honey (Rodríguez-Malaver et al. 2009). It was hypothesized that nitric oxide and/or nitrite might be responsible, in part, for the biological and therapeutic effects of honey (Al-Waili 2003). In addition, this metabolite could be used for authentication of honey. Also in this research, there were positive Pearson correlations ($P<0.01$) between flavonoids-TEAC (0.879), polyphenols-TEAC (0.942), proteins-TEAC (0.911), color-TEAC (0.771), and nitrites-TEAC (0.422). Those correlations indicated compounds that could be involved in the antioxidant action of stingless bee honey. Similar results have been reported for polyphenols, flavonoids, and color in *A. mellifera* honey (Bertonceij et al. 2007; Frankel et al. 1998; Taormina et al. 2001; Vela et al. 2007, 2008). It has also been reported that the antioxidant activity of stingless bee honey increases with free acidity ($r^2=0.97$, $P<0.01$) (Vit et al. 2006). Due to a controversy about which compounds signify honey antioxidant activity, Gheldof et al. (2002) suggested that total antioxidant content of honey may be better explained by interactions of a wide range of compounds, including phenolics, peptides, organic acids, enzymes, and Maillard reaction products.

34.5 Conclusions

Diversity of stingless bees in America is very high. Thus, bioactivities of stingless bee products are diverse because they depend on bee species, their habits, and also on external factors such as geography, climate, season, harvesting method, etc. Comparisons of bioactivities from bee products of native stingless bee species has been widely studied and reported. It was found that both internal and external factors affect classes, types, and contents of active compounds and their derivatives, which mainly belong to phenolic compounds and flavonoids.

The correlation between chemical compounds such as water, sugars and free acidity and the bioactivities has been widely studied. Standard control of stingless bee products in traditional medicine would require identifying new bioactive agents of interest in order to demonstrate their bee origin, and to avoid or reduce the side-effects of using present modern medicine.

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Chapter 35
Use of Honey in Cancer Prevention and Therapy

Patricia Vit, Jun Qing Yu, and Fazlul Huq

This chapter is dedicated to cancer sufferers and survivors, and researchers engaged in its prevention and therapy

35.1 Introduction

The typical composition of honey (Codex Alimentarius Commission 2001) provides a generalization that misses variability in composition of an apparently homogeneous sugary product. Therefore, it was referred to as enigmatic honey in a book on melissopalynology (Vit 2005) meaning honey not being a standard syrup. Commonality and variability in properties of honey is considered to be useful in making informed health-care choices (Gethin 2008). Honey composition and other factors may readily explain this variability, as shown in several chapters in this book.

Variability in either composition of honey and characteristics of cancer raise a question: what type of honey for what cancer, at what stage of the disease, and in what dosage and timing? Further questions arise on the usefulness of honey intake alone or as an ingredient of natural remedies, or used in combination with conventional chemotherapy. Honey alone showed moderate murine antitumor activity and pronounced antimetastatic effects, but combined with anticancer drugs, 5-fluorouracil and cyclophosphamide, resulted in antitumor activity (Gribel and Pashinkii 1990). The use of honey with *Aloe arborescens* has been associated with tumor regression and survival time in patients

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treated simultaneously with oncologic chemotherapy (Zago 2004; Lissoni et al. 2009). In a review of 131 studies, *Aloe vera* and honey prevented or reduced mucositis, varying with the type of cancer and treatment (Worthington et al. 2010). *Aloe vera* and honey were hepatoprotective, reduced cell proliferation, and increased apoptosis in murine tumors (Tomasin and Gomes-Marcondes 2011).

Two recent reviews covered the ethnopharmacological uses of honey in northeastern Brazil, with a number of stingless bee species (*Melipona scutellaris*, *Melipona subnitida*, *Partamona seridoensis*, *Scaptotrigona* sp., and *Tetragonisca angustula*) (Oliveira et al. 2010; Souto et al. 2011). However, the term cancer was not included as a disease descriptor. Possibly cancer as such cannot be diagnosed in traditional medicine, but can only be related to inflammations and swellings.

Cancer, the most dreaded disease of our time, is curable if detected in its early stages (Cantor 2008). The use of honey in cancer prevention and therapy has been tested both in vitro and in vivo, but the data do not cover the range of honey types or cancer symptoms known to exist. A number of cellular pathways in diverse cancer cell lines that are being investigated may eventually lead to a unified concept applying to the plethora of diseases termed cancer. The apoptotic ability (anti-proliferative potential, arresting cell growth at the subpopulation sub-G_1, activation of the caspase cascade) of honey varies according to the cell type, e.g., in colon cancer cells (Jaganathan and Mandal 2009b), and involves nonprotein thiols, mitochondrial dysfunction, reactive oxygen species, and protein p53 (Jaganathan and Mandal 2010). The group of Nada Oršolić at the University of Zagreb in Croatia demonstrated growth inhibition of certain tumor types, reduction of metastases and prolonged survival in mice, after treatment with honey alone (Oršolić 2009), or propolis combined with chemotherapeutic agents (Benkovic et al. 2007).

The ability of health scientists to measure the activity of honey in cancer is related to factors within a matrix of diverse botanical, entomological and geographical origin (major sugar components, water, polyphenols and other secondary plant metabolites, acids, enzymes, minerals, etc.), cancer type (adenoma, carcinoma, myeloma), organ site, cancer stage (initiation, metastasis, double tumor), cancer care (mucositis, radiation burns), patient age, and presence of other diseases. Cascades of molecular markers as indicators of cancer onset and anticancer action are actively investigated. Whether honey is useful to treat cancer is a question to be answered in relief of oncologic suffering and death.

This study aims to provide an overview in the usefulness of honey in cancer prevention and therapy. Our data on the antiproliferative action of pot-honey from *Frieseomelitta*, *Melipona*, *Scaptotrigona*, and *Tetragonula* in three human ovarian cancer cell lines are described and evaluated here.

35.2 Cancer

The name “cancer” originated with Hippocrates and the Greek word ‘carcinos’ “καρκίνος” to indicate tumors with the shape of a crab. All cancer cells in a patient originate from a unique cell starter among the 10^{14} cells in the human body (Pecorino

2008) as the primordium of this progressive disease. One initial mutation accumulates in a single cell, causes unregulated cell growth, invasion of surrounding tissues, and eventually spreads. The disease is therefore clonal, and may evolve more than 10 years before clinical detection. The multistep process leading to the development of cancer is known as carcinogenesis. Proto-oncogenes are activated, while tumor suppressor and genomic stability genes are inactivated. A colon cancer model gave seminal evidence for cancer genetic and histological multistage progression (Volgestein et al. 1988). Age is the biggest risk factor for cancer (Tovey et al. 2007).

The following six cell-markers differentiate cancer cell behavior from normal cells: (1) Evasion of apoptosis. (2) Growth signal autonomy, (3) Evasion of growth inhibitory signals, (4) Angiogenesis, (5) Unlimited replicative potential, and (6) Invasion and metastasis (Hanahan and Weinberg 2000). Molecular pathways and signaling used in cell function are considered to understand how a normal cell transforms into a cancer cell, and also how cancer cells alter tissue, organ and body functions. Any group of cells out of place is considered cancer in medical imaging. A new growth of cells is called a "neoplasm". Oncology is the medical discipline specialized in cancer, and is also originated from a Greek word "onkos" "ογκος", which means bulky mass.

Carcinomas are the most common tumors and occur in epithelial cells (e.g., brain, colon, kidney, lung, skin, stomach); sarcomas develop in mesoderm cells (e.g., bone, muscle), and adenocarcinomas develop in glandular tissue (e.g., breast, prostate, pancreas). The situation becomes more complex when examining molecular mechanisms, target tissues and cell types, patterns of metastasis, and causes. Besides the ability of cancer to invade other organs during final stages, secondary effects of cancer treatment also cause pain. Cancer patients tend to have wounds that fail to heal (Mc Nees and Dow Meneses 2007), causing suffering and death. Radiation-induced oral mucositis, stomatitis, malignant ulcers, infected lesions, and an infected oral cavity in head and neck cancer are common (Bardy et al. 2008). The feeling of helplessness is often the main cause of increasing pain in cancer (Toon 2008).

Official labeling of a cancer drug contains approved information for the product. It covers a number of categories for precise use in terms of type and subtype of cancer, dose, association, schedule and route of administration, and duration of treatment according to the course of the disease. In medical practice, use outside this frame is considered "off-label" prescription (Levêque 2008) but does not apply to traditional use of phytochemicals, including honey.

35.3 Multidrug Resistance Caused by Chemotherapy

Cells repeatedly exposed to anticancer drugs may develop drug resistance due to intrinsic or extrinsic factors of diverse nature. Tumor cells exposed to toxic agents increase their tolerance to drugs by adaptive response. Several molecular mechanisms that cause multidrug resistance have been described. First, there may be a reduced drug uptake and increased drug efflux at the membrane level. Second, enhanced drug detoxification in cytoplasmic thiol systems, through glutathione S-transferases may occur. Third, there may be increased DNA repair by enzymes. Additionally, decreased apoptosis has three metabolic pathways; (1) overexpression of anti-apoptotic proteins, (2) underexpression of pro-apoptotic proteins, and (3) altered subcellular distribution of wild type p53 protein, called the "guardian of the genome". Studies on sequenced combination of cisplatin and other platinum compounds with phytochemicals are being carried out in the cancer research laboratory at the Discipline of the Biomedical Science at The University of Sydney (F. Huq 2011, personal communication) with the aim of surmounting cisplatin resistance in ovarian cancer.

35.4 Honey and Cancer

Because honey may be viewed as a medicinal dietary substance, scientific evidence on the benefits of honey have been growing since the ancient claims about health and longevity, e.g., by Hippocrates (Skiadas and Lascaratos 2001). Markers of human health suggest that honey consumption reduces the risk of diseases causing death (Cooper et al. 2010). The immunological activity mediated by cytokines is an important functional property modulated by honey (Tonks et al. 2001, 2003, 2007). Healing properties of bee products are related to the antioxidant, anti-inflammatory, antimicrobial, and anticancer activities of flavonoids. However, other substances such as amino acids, vitamins and organic acids can also contribute to the healing power of honey (Frankel et al. 1998) and its useful inclusion in the diet to complement other polyphenols (Blasa et al. 2006). One study indicated the presence of a tumor-promoting factor in honey (Upadhyay et al. 1980), but in current research honey is found to be healing. The antitumor activity of honey may occur through the activation of macrophages, T- and B-cells (Attia et al. 2008). The antiproliferative effect of honey in colon cancer cells is found to vary depending on honey's botanical and geographical origin (Jaganathan and Mandal 2009b). Although Indian honey has been applied in culture media (Jaganathan et al. 2010), most studies use phenolic extracts of honey. Methanol extracts of Malaysian honey showed a higher phenolic content, whereas an ethyl acetate extract was more active to reverse the toxicity caused by tumor necrosis factor (Kassim et al. 2010).

In research with human cancer cell lines, antiproliferative action of honey was observed by apoptosis with IC_{50} values (the concentration at which cell proliferation is inhibited by 50%) of 4, 10, and 14% after 24, 48, and 72 h, respectively, in a prostate PC-3 cell line (Samarghandian et al. 2010), and with an IC_{50} of 1.7 and 2.1 μg/mL after 48 and 72 h in renal cell carcinoma (Samarghandian et al. 2011). Therefore, the apoptotic nature of honey has potential for the treatment of prostate and kidney cancer. Honey of the giant honey bee *Apis dorsata*, reportedly from nesting in the large forest tree "Tualang" (*Koompassia excelsa*, Fabaceae) in Malaysia was found to induce apoptosis in human oral squamous cell carcinomas, osteosarcoma (Ghashm et al. 2010), and breast and cervical cancer cell lines by depolarization of the mitochondrial membrane (Fauzi et al.).

Evidence of medicinal uses of honey in oncological care is found in reviews in the Journal of Clinical Nursing (Bardy et al. 2008; Gethin 2008). Nurses are directly involved in healthcare intervention, and have extensive contact with patients. They have often encountered secondary effects caused by conventional treatments of neoplasias. Honey is used to prevent neutropenia (Zidan et al. 2006), in pediatric hematology–oncology wound care (Wiszniewsky et al. 2006), for radiation induced skin toxicity (Moolenaar et al. 2006), mucositis (Motallebnejad et al. 2008), and as a potent antibacterial agent in cancer patients (Majtan et al. 2011).

35.4.1 The Botanical Diversity of Honey

Plants visited by bees have been of great interest to diverse disciplines, and melissopalynology provides a tool to study the pollen residues of honey as a “fingerprint” potentially indicating botanical origin of nectar (but see Chap. 21, Roubik and Moreno in this book). Honey with more than 45% pollen counts of one taxon is considered unifloral (Louveaux et al. 1978). The honey of chestnut (*Castanea sativa*) has been studied for aroma composition (Castro-Vázquez et al. 2010), and manuka (*Leptospermum*) honey for its medicinal properties (Molan 2001; Tonks et al. 2007). Different plants may well confer different properties to honey. Sensory and physicochemical patterns described for 13 unifloral European honeys produced by *Apis mellifera* (Persano Oddo and Piro 2004) were further investigated for their aroma composition and medicinal properties. As an example, the antimutagenic activity of honey from seven different floral sources: acacia (*Robinia pseudoacacia*), buckwheat (*Fagopyrum esculentum*), clover (*Melilotus*), fireweed (*Epilobium angustifolium*), soybean (*Glycine max*), tupelo (*Nyssa*), and Christmas berry (*Schinus terebinthifolius*), and the sugars glucose, fructose, maltose, and sucrose, was measured against nonpolar heterocyclic amine Trp-p-1 (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole) and tested via Ames assay (Wang et al. 2002). Sucrose was not active, but fructose and glucose were more antimutagenic than honey and the weak maltose, against Trp-p-1. Buckwheat honey, which is extremely high in phenolics caused the greatest inhibition (52.1%) at 1 mg/mL, indicating its potential for use in anticancer therapy.

35.4.2 How Many Kinds of Bees Produce Honey?

There are approximately 750 bee species that make honey, about 250 of which are in the genus *Bombus*, and not considered here (Michener 2007). Hymenoptera are one of the largest and most biologically diverse orders of phytophagous insects with various social grades, and a range of parasitic species (La Salle and Gauld 1993). Phylogenetic relationships of the hymenopteran superfamily, to which all types of bees belong, were initially resolved by sequenced mytochondrial genomes as a single analytical approach (Dowton et al. 2009). However, mtDNA is not conservative enough to have any resolution power earlier than the Pliocene, needed to study bee phylogenies, as reviewed by Roubik 2012.

In nature, honey is derived from water–sugar resources available in the environment, processed and accumulated for energy needs of the bee colony. Honey bees (*Apis* spp., Apini) store their honey in beeswax combs, while stingless bees (Meliponini) use cerumen pots of different sizes, shapes, and colors. Apini has 11 or 12 species in the single genus *Apis*, but Meliponini has more than 500 species in approximateley 61 genera (Rasmussen and Cameron 2010; Roubik 2012). The great biodiversity of Meliponini is treated in the contributions by Camargo and by Michener (Chaps. 1 and 2), in this book. Honey produced by Meliponini clustered naturally according to entomological origin, using compositional data (Vit et al. 1998). Therefore, the entomological origin of honey adds an important descriptor to any medicinal application of honey.

35.4.3 Flavonoids as Anticancer Components of Honey

Cancer chemoprevention is an important issue concerning dietary components such as polyphenols, and their epigenetic role as modulating agents of gene expression (Jaganathan and Mandal 2009a; Link et al. 2010; Szic et al. 2010). Thus, flavonoids in honey have been studied for their chemopreventive action. Chemopreventive properties of dietary polyphenols (catechin, chrysin, epicatechin, epigallocatechin-3-gallate, quercetin, rutin, myricetin, resveratrol, and xanthohumol) are associated with multiple molecular mechanisms of action against colorectal cancer cell lines (Araújo et al. 2011). Phytochemicals are also studied as agents that may help to counter multidrug resistance in combined treatments (Yunos et al. 2010). An hypothesis on the genotoxic role of honey flavonoids targeting cancer cells has been proposed (Jaganathan 2011).

Flavonoids are a group of small molecules (C6-C3-C6, MW ~ 300) widely known to contribute to the colors of flowers and fruits. Five subclasses of dietary flavonoids were considered in selected food: flavones, flavonols, flavanones, flavan-3-ols, and anthocyanidins (USDA 2007). In this database there is an entry for a content of reference flavonoids in 100 g honey: 0.05 mg apigenin, 0.63 mg luteolin (flavones) and 0.17 mg isorhamnetin, 0.11 mg kaempferol, 1.03 mg myricetin, 0.51 g quercetin (flavonols). Over the past few years, a number of studies have used flavonoid profiles of honey to find botanical and other markers, such as bee species (Vit and Tomás-Barberán 1998), and locations of origin (Tomás-Barberán et al. 2001).

The removal of free radicals—named scavenging, is one of the outstanding medicinal attributes of flavonoids (Havsteen 2002). Phosphorylation and dephosphorylation reactions that regulate the Na^+/K^+ ion pump are sensitive to flavonoids. Quercetin removes the phosphate ester from the phenol group tyrosine and restores the pH value in cancer cells (Spector et al. 1980). Apigenin and luteolin are potent inhibitors in human thyroid carcinoma cell lines (Yin et al. 1999). Polyphenols studied to characterize and differentiate bee products are a valuable background for predictions on what honey types may have anticancer value.

The antiproliferative effects of honey are mainly explained by the presence of the flavonoid chrysin (5,7-dihydroxyflavone). Flow cytometry analysis indicated that cytotoxicity induced by honey or chrysin was mediated by G(0)/G(1) cell cycle arrest. Chrysin was therefore considered a potential candidate for both cancer prevention and treatment (Pichichero et al. 2010). Chrysin has been widely studied by several authors for its effect in suppressing inflammation caused by NF-κB and JNK activations (Ha et al. 2010), to trigger the unfolded endoplasmic reticulum resident protein GRP78 response (Sun et al. 2010), to enhance the apoptosis induced by a ligand (Li et al. 2011), p38 and Bax activation (Pichichero et al. 2011). However, in another study, chrysin inhibited the apoptosis induced by the antitumor-drug topotecan by inhibiting ATP-binding cassette (ABC) transporters (Schumacher et al. 2010).

35.5 Is Pot-Honey Cytotoxic to Human Ovarian Cancer Cells?

Substances such as antioxidants that can be chemopreventive to normal cells can also be cytotoxic to cancer cells. Often, these opposing properties are manifested in different cell receptors. It is possible that honey can play both chemopreventive and cytotoxic roles, perhaps due to a variety of antioxidants. To answer this question, the survival of human ovarian cancer cells was measured in the presence of 200 mg honey/mL and three lower serial dilutions up to 1.6 mg honey/mL. The MTT reduction assay (Mosmann 1983) was carried out to determine cell kill due to 16 pot-honey samples produced by 13 species of stingless bees (eight *Melipona* species, three *Scaptotrigona* species, *Tetragonula carbonaria*, and *Frieseomelitta nigra* obtained from Australia, Brazil, Mexico, or Venezuela).

The IC_{50} values of honey samples against three human ovarian cancer cell lines (i.e., concentrations of honey required for 50% cell kill) are given in Table 35.1. The results show that honey samples vary widely in their ability to cause cell kill. The most active honey sample against parent A2780 cell line is *Melipona solani* (2.74 mg/mL) and the least active one is *Melipona scutellaris* (24.37 mg/mL). The next two more active honey samples are *Melipona favosa* (3.39 mg/mL) and *Scaptotrigona polysticta* (3.60 mg/mL), followed by *Scaptotrigona hellwegeri* (4.19 mg/mL), *Melipona beecheii* (4.24 mg/mL), and *Frieseomelitta nigra* (4.58 mg/mL). The activity of cisplatin is found to be much lower in the resistant $A2780^{cisR}$ (3.88 μM) and $A2780^{ZD0473R}$ (3.44 μM) cell lines, as compared to that in the parent A2780 cell line (0.88 μM). Unlike that of cisplatin, generally the activity of the honey samples in the resistant cell lines is found to be comparable to that in the parent cell line or greater except in the case of *Melipona subnitida* (as applied to $A2780^{ZD0473R}$) where the activity is some 50% lower in the resistant cell lines. Greater activities of some honey samples, especially *Melipona solani* (1.66 and 0.79 mg/mL) and *Scaptotrigona polysticta* (1.54 and 1.36 mg/mL) in the resistant $A2780^{cisR}$ and $A2780^{ZD0473R}$ cell

Table 35.1 IC$_{50}$ values of pot-honeys in the human ovarian cancer cell lines

		Ovarian cancer cell lines				
		A2780	A2780$^{\text{CisR}}$		A2780$^{\text{ZD0473R}}$	
		IC$_{50}$	IC$_{50}$	RF	IC$_{50}$	RF
Geographical origin, city, country	Cisplatin (control)	0.88	3.88	4.42	3.44	3.91
	Pot-honey bee species					
Chetumal, Mexico	*Melipona beecheii*	4.24	3.35	0.79	4.14	0.98
El Reventón, Mexico	*Melipona fasciata*	6.17	4.72	0.77	4.28	0.69
Moura, Brazil	*Melipona fasciculata*	6.18	5.83	0.94	5.89	0.95
Tabocas, Brazil	*Melipona fasciculata*	8.00	3.97	0.50	5.15	0.64
Preazinho, Brazil	*Melipona fasciculata*	13.56	6.69	0.49	7.69	0.57
Moruy, Venezuela	*Melipona favosa*	16.50	4.21	**0.26**	12.81	0.78
Moruy, Venezuela	*Melipona favosa*	3.39	3.68	1.08	3.65	1.08
Belém, Brazil	*Melipona rufiventris*	5.10	4.68	0.92	3.80	0.74
João Pessoa, Brazil	*Melipona scutellaris*	**24.37**	**25.72**	1.06	**27.64**	1.31
Chiapas, Mexico	*Melipona solani*	**2.74**	1.66	0.61	**0.79**	**0.29**
Natal, Brazil	*Melipona subnitida*	17.54	27.60	**1.57**	34.36	**1.96**
El Reventón, Mexico	*Scaptotrigona hellwegeri*	4.19	4.59	1.10	4.10	0.98
Cuetzalan, Mexico	*Scaptotrigona mexicana*	7.71	4.43	0.57	5.62	0.73
Xingú, Brazil	*Scaptotrigona polysticta*	3.60	**1.54**	0.43	1.36	0.38
Brisbane, Australia	*Tetragonula carbonaria*	8.96	4.76	0.53	4.54	0.51
Guerrero, Mexico	*Frieseomelitta nigra*	4.58	4.72	1.03	4.19	0.92

IC_{50} honey (mg/mL), cisplatin (μM), *RF* resistance factor as the ratio IC$_{50}$ resistant cell line/IC$_{50}$ parent cell line

lines, respectively, than in the parent A2780 cell line, indicate that the pot-honey samples have been able to overcome (at least partially) cisplatin resistance operating in the cell lines. The lowest resistance factor in this set of experiments was achieved by honeys of *Melipona favosa* against A2780$^{\text{cisR}}$ (0.26) and *Melipona solani* against A2780$^{\text{ZD0473R}}$ (0.29). Further studies would be required to obtain information about the mechanisms of cell killing effect by the pot-honeys, and what active components confer their antiproliferative activity.

The second honey of *Melipona favosa* (V12 in APIBA honey collection), was 4.5× richer in flavone *C*-glycosides than V9, and half in flavonol *O*-glycosides (Truchado et al. 2011). More precisely, enzymatic hydrolysis of flavone *C*-glycosides could produce cytotoxic metabolites, or a *C*-glycoside fit in a signaling molecular pocket to explain the observed higher cell kill.

Much needed experiments should compare honey of the same species of bee fed from different kinds of flowers, and of different species of bees fed on the same species of flower. With bee colonies in greenhouses, so that the flowers available to them would be clearly known, such experiments would be possible. With such experiments, the sources of anticancer compounds, whether from flowers or bees or both, could be determined. The very different numbers sometimes shown in Table 35.1 for the same species of bees may suggest the great influence of the floral resources.

35.6 Adaptive Response of Cancer and Normal Cells to Honey

This review to approach the anticancer action of honey involved studies of a variety of mechanisms. We have highlighted three main issues. First, the complexity of the problem from both sides of honey and cancer biodiversity is discussed. Second, the role of honey in chemoprevention is shown. The action of some active components such as flavonoids and the well-known nature of high sugar concentration are discussed. Third, the therapy after cancer onset, with combined treatments using conventional chemotherapy and alternative medicine, is considered. Finally, the effect of pot-honey in a model based on human ovarian cancer cell lines was compared between the stingless bee genera *Frieseomelitta*, *Melipona*, *Scaptotrigona*, and *Tetragonula*.

The adaptive response of cancer and normal cells to honey is a mosaic under construction, and we hope that it will lead to a model for a better understanding of flavonoid interactions with cells, as a chemopreventive and genotoxic tool. Generations of anticancer agents with reduced toxicity in cancer patients may have honey as an ingredient of preparations with other natural products such as *Aloes*, or combined with targeted therapy.

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Chapter 36
Bioactivity of Honey and Propolis of *Tetragonula laeviceps* in Thailand

Chanpen Chanchao

36.1 Introduction

Stingless bee products are used in traditional medicine in Thailand. The “chan-narong” *Tetragonula laeviceps* is of primary interest because of its wide distribution and management. Honey, propolis, bee pollen, royal jelly, and cerumen are among the many natural bee products that are applied for medicinal purposes (Riches 2000). For example, patients with hay fever and pollen-induced asthma purportedly alleviate their symptoms if they eat local honey. Litwin et al. (1997) suggest symptoms of ragwort hay fever are controlled by eating ragwort pollen present in honey.

Natural medicines are a primary focus of one hospital in Thailand, Chao Phya Abhaibhubejhr Hospital, whose efforts are directed toward discovering information about the safety and efficacy of chemical raw materials which then can be applied and developed into traditional Thai medicines. Clinical uses of bee products have continued to increase in recent years. For example, Aburahma et al. (2010) surveyed 176 children who were patients at the pediatric neurology clinic of King Abdullah University Hospital in North Jordan during March to July of 2008. It was found that 29% of the children who used complementary and alternative medicine consumed honey products. It has been reported that honey can treat coughs better than the commercial drugs dextromethorphan and diphenhydramine (Shadkam et al. 2010; Paul et al. 2007). In Thailand, at Bangkok’s Ramathibodi Hospital, honey is successfully used to treat a wound after a radical operation for vulvar carcinoma, and efficiently works in a povidone-iodine solution to heal an abdominal wound disruption (Phuapradit and Saropala 1992; Phuapradit 2002).

Stingless bee products are as economically important as honey bee products in Thailand. Interestingly, stingless bees can produce a large amount of propolis, which

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is used as a sealant in the nest. It appears that propolis is the bee product most widely used for medical purposes (Butnariu and Giuchici 2011; Guney et al. 2011; Saxena et al. 2011). Here I address chemical properties and application of *T. laeviceps* honey and propolis extracts for medicinal purposes. Honey and propolis yields of this bee are approximately 300 g hive/year. The antiproliferative activity of propolis extracts was tested against cancer cell lines, and compared to normal cells. Besides the beneficial aspects of bee products, hygienic concern is also discussed, medical-grade honey is therefore suggested.

36.2 Composition of Honey and Propolis of *Tetragonula laeviceps*

Honey pots, dark resin collected in the entrance, and propolis in the *T. laeviceps* hive are shown in Fig. 36.1.

Although honey contains many monosaccharides and disaccharides that account for its sweet taste, it is very acidic. For example, the pH of honey from *Apis dorsata* is 3.81, that of *Apis cerana* is 3.87, *Apis florea*, 3.76, and *Apis mellifera* ranges 3.41–3.95, depending on foraged food sources (Chanchao et al. 2006),

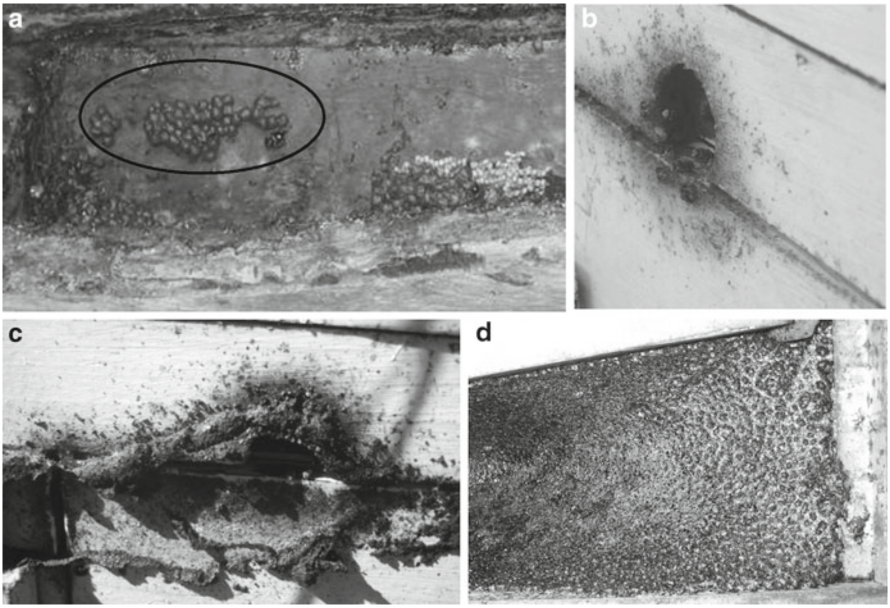

Fig. 36.1 Honey pots and propolis of *T. laeviceps*. (**a**) Honey pots. (**b**) Resins collected in the entrance. (**c**) Propolis outside the hive. (**d**) Propolis inside the hive. Photos: C. Chanchao

Table 36.1 Chemical components of WEP[a] and EEP[b] from *T. laeviceps*

Propolis extracts	Total sugar (µg/ml)	Reducing sugar (µg/ml)	Total polyphenols (µg/ml)	Flavonoids (µg/ml)	Total protein (µg/µl)
WEP	1.41	42.35	0.57	0.04	11.3
EEP	0.23	0.00	16.88	0.26	25.0

After Boonsai (2009) and Kaewwongwattana (2009)

[a] WEP water extract of propolis

[b] EEP ethanol extract of propolis

while *T. laeviceps* is 3.37 (Chanchao 2009). The honey of stingless bees tastes sour and bitter, and also smells different from honey of *Apis*; it is not as often consumed as honey bee honey. It has become widely used in traditional medicine instead. More bioactivities are obtained from the honey of stingless bees than from the honey of honey bees, since the honey pots of stingless bees are made from resin combined with beeswax, known as cerumen. In contrast, the honey cells of honey bees are made entirely of wax. Thus, the active chemical compounds in honey pots, many derived from terpenoids in natural resin (Langenheim 2003), may work together with those from honey. Honey of *T. laeviceps* has 1.72 mg proline/kg, 0.28 protein g/100 g (44.8 mg N/100 g), and 15.2 g invert sugars/100 g (Chanchao 2009).

The chemical components of water extracts of propolis (WEP) and ethanol extracts of propolis (EEP) from *T. laeviceps* are shown in Table 36.1 (Boonsai 2009; Kaewwongwattana 2009). The data support the idea that propolis could provide bioactivity, based on plant-derived polyphenol and flavonoid contents, and other factors such as the extraction methods, collecting seasons, collecting sites, and other external factors (Gülçin et al. 2010; Li et al. 2010; Miguel et al. 2011).

36.3 Bioactivity of *Tetragonula laeviceps* Products

36.3.1 Antimicrobial Activity

Antimicrobial activity of *T. laeviceps* pot-honey was assayed against *Staphylococcus aureus* (a Gram-positive bacteria), *Escherichia coli* (a gram-negative bacterium), *Candida albicans* (yeast), and *Aspergillus niger* (fungus) (Wongchum 2007). In Fig. 36.2 the antibacterial, antiyeast, and antifungal activities of serial dilutions of *T. laeviceps* honey (0, 25, 50, 75, 100%) is determined by the diameter of a clear zone (no-growth area) in the agar-well diffusion method. It is obvious that the inhibition zone increased with higher doses of honey.

Neat honey possesses the most effective antimicrobial activity. Using honey at a concentration of 50% (v/v) or higher, *S. aureus* was the most sensitive microorganism, followed by *E. coli*, *C. albicans*, and *A. niger*, respectively (Fig. 36.3). The yeast

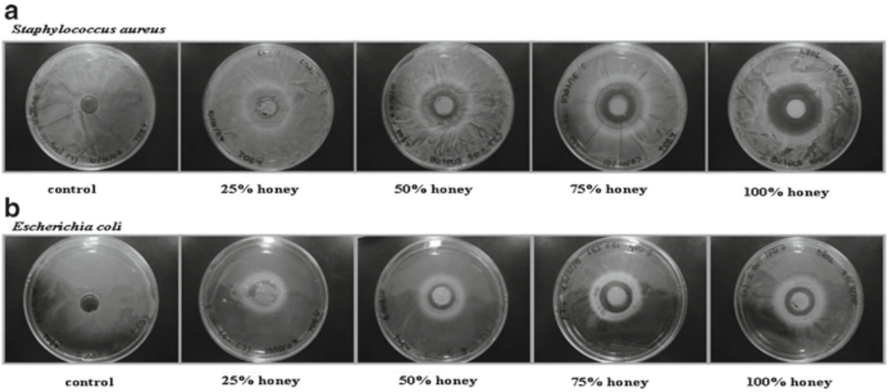

Fig. 36.2 Antimicrobial activity of *Trigona laeviceps* honey, by agar-well diffusion method, against (**a**) *Staphylococcus aureus* and (**b**) *Escherichia coli*. Photos: C. Chanchao

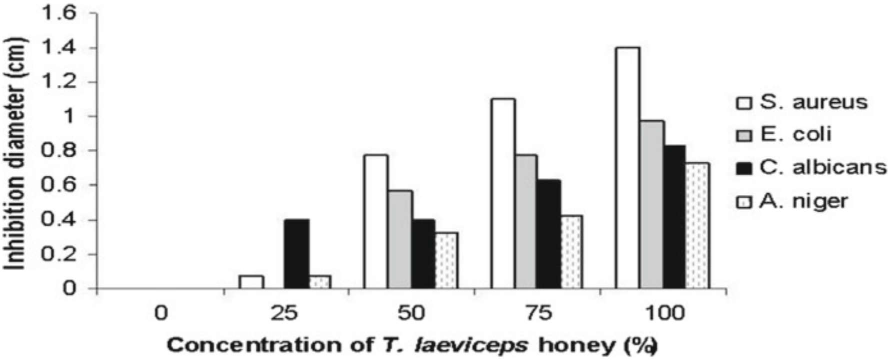

Fig. 36.3 Antimicrobial activity of 0–100% honey from *T. laeviceps* (Modified from Wongchum 2007)

C. albicans was more sensitive to the diluted honey at 25% (v/v) than the bacteria and the fungus.

Honey extracts were tested for antimicrobial activity against *Micrococcus luteus* and *Pseudomonas aeruginosa*. Raw honey was partitioned with organic solvents of different polarities (nonpolar hexane, slightly polar dichloromethane, polar methanol). Considering the minimum inhibitory concentration (MIC in mg/ml) and the minimum bactericidal concentration (MBC in mg/ml), results showed active compounds of low polarity, since efficient antimicrobial activity was found in dichloromethane extract of honey (DEH) and hexane extract of honey (HEH), but not in the methanol extract of honey (MEH) (Fig. 36.4). The most efficient antimicrobial activity against *M. luteus* and *P. aeruginosa* was demonstrated by DEH at MIC of 10 mg/ml (Chartthai 2010).

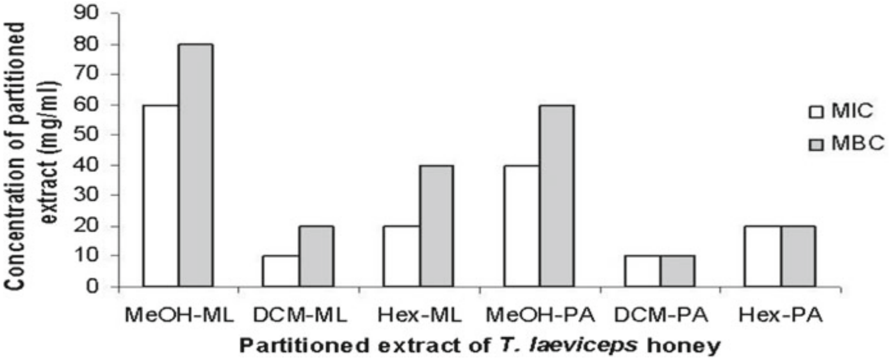

Fig. 36.4 Antimicrobial activity of partitioned extracts of honey. MIC and MBC of methanol (MeOH), dichloromethane (DCM), and hexane (HEX) honey extracts determined by *Micrococcus luteus* (ML) and *Pseudomonas aeruginosa* (PA) (Modified from Chartthai 2010)

Table 36.2 Diameter of inhibition zones (cm) from ethanol extract of honey (EEH), showing antimicrobial activity against *S. aureus* and four isolates of MRSA

Concentration (mg/ml)	Isolates				
	S. aureus	MRSA 20645	MRSA 20646	MRSA 20651	MRSA 20652
0	0.00	0.00	0.00	0.00	0.00
64.5	1.60±0.20	1.60±0.05	1.48±0.10	1.58±0.13	1.70±0.26
129	2.17±0.21	2.20±0.00	2.13±0.32	2.17±0.15	2.43±0.13
193.5	2.33±0.25	2.60±0.10	2.53±0.25	2.33±0.15	2.63±0.15
258	2.62±0.24	2.83±0.06	2.70±0.20	2.60±0.00	2.80±0.20

After Jirakanwisal (2010)

Not only pathogenic bacteria are susceptible to honey extract, methicillin-resistant *S. aureus* (MRSA) is also susceptible (Jirakanwisal 2010). This indicates that honey may contain a promising new antibiotic. As shown in Table 36.2, the efficiency of an ethanol extract of honey (EEH) against *S. aureus* and MRSA increases with higher concentration.

In addition to honey, the crude extract of propolis has presented antimicrobial activity. In 2009, Umthong et al. reported that both a water extract of propolis (WEP) and a methanol extract of propolis (MEP) from *T. laeviceps* inhibited the growth of *A. niger*, *B. cereus*, *C. albicans*, *E. coli*, and *S. aureus*. The *T. laeviceps* water extract of propolis was more active than the methanol extract, showing a remarkable anti-*B. cereus*, anti-*Herpes simplex* virus type 1, and anti-*Mycobacterium tuberculosis* activities inhibiting 25–33% of growth with a MIC of 50 μg/ml. WEP was no cytotoxic to Vero cells. Unlike WEP, EEP demonstrated antimalaria (*Plasmodium falciparum*, K1 strain) activity at an IC_{50} of 4.48 μg/ml (Kaewmuangmoon et al. 2012).

36.3.2 Antiproliferative Activity

Nowadays, cancer is one of the leading causes of death in the Thai population. From statistical records of the Thai Ministry of Public Health during 2005–2009, 13.57% of overall deaths were from cancer. A propensity for cancer is not only inherited, but it can also be triggered by environmental factors such as ultraviolet rays, carcinogens, etc. Research and development of treatments for this disease has been ongoing not only in Thailand but worldwide. Other than surgery, radiation, and chemotherapy—which are the most effective therapies at present—the search for a novel anticancer agent from natural products offers a promising alternative.

In 2010, Tasaniyananda reported that honey of *T. laeviceps* could provide antiproliferative activity against breast tissue (BT474) cancers (Fig. 36.5).

It was also found that this activity depended mainly on the type of organic solvent; a water extract of honey (WEH) provided better antiproliferation than an ethanol extract (EEH). Unlike EEH, EEP (IC_{50} of 25.54 μg/ml) demonstrated better anticancer activity against small-cell lung cancer (NCI-H187) than WEP, for which the percentage of inhibition was <50%. Moreover, EEP showed cytotoxicity against a human leukemia cell line (HL-60) at an IC_{50} of 29.29 μg/ml (Kaewmuangmoon et al. 2012).

The antiproliferative action of *T. laeviceps* WEP and MEP on a colon cancer cell line (SW620) showed IC_{50} values of 60 and 80 mg/ml, respectively (Umthong et al. 2009). Not only could this be assayed by the percentage of cell viability, but DNA fragmentation and a change in morphology in SW620 cells were also observed. Later, purification was performed by partition and chromatography. The hexane extract of EEP, which showed the best antiproliferative activity against cancer cell lines from breast (BT474), lung (Chago), colon (SW620), hepatic (Hep-G2), and stomach (Kato-III), was further purified by column chromatography and size-exclusion

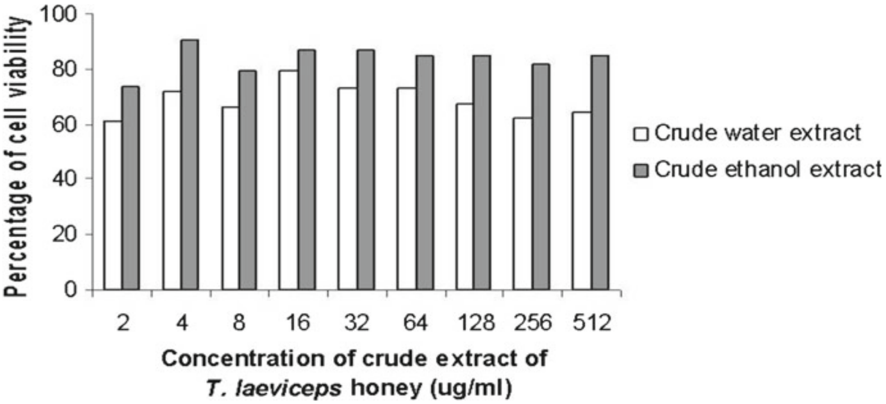

Fig. 36.5 Percentage of cell viability of breast cancer cell lines (BT474) after being treated with water or ethanol extracts of *T. laeviceps* honey (From Tasaniyananda 2010)

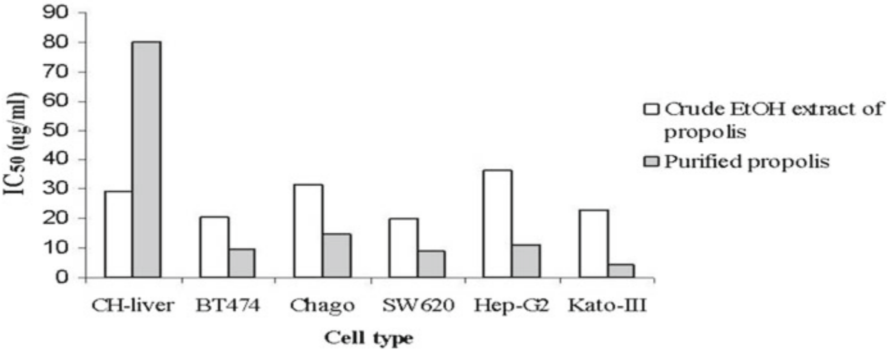

Fig. 36.6 Antiproliferative activity of crude extract and purified fractions of *T. laeviceps* propolis. The activity was tested against breast (BT474), lung (Chago), colon (SW620), hepatic (Hep-G2), and stomach (Kato-III) tissue cancers; liver (CH-liver) cells were used as a control (Modified from Umthong et al. 2011)

chromatography. As shown in Fig. 36.6, IC_{50} values were lower for purified *T. laeviceps* propolis than the ethanol extract in all cancer cells except CH-liver. In addition, much lower cytotoxicity to normal cells (CH-liver) was found when using purified propolis at the IC_{50} value of 80.15 µg/ml, compared to EEP (IC_{50} value of 29.14 µg/ml) (Umthong et al. 2011).

36.4 Antimicrobial Peptides of Honey

Antimicrobial peptides are ubiquitous gene-encoded peptide antibiotics (20–40 amino acids) with a folded size similar to the thickness of cellular membrane (Huang 2000). Honey also contains an antimicrobial peptide (AMP) (Kwakman and Zaat 2011; Kwakman et al. 2011a, b). Thus, its direct target is the microbial membrane, because the cationic domain of AMP specifically interacts with the negatively charged outer membrane. Later, a hydrophobic domain will act to disrupt the membrane and translocate into the cells (Epand and Vogel 1999).

Several antimicrobial peptides have been reported for *Apis*. For example, Casteels-Josson et al. (1993) found the apidaecin in *A. mellifera* body. Later, in 2009, Viljakainen et al. (2009) reported the amino acid sequences of hymenoptaecin in *A. mellifera* body. Moreover, Yoshiyama and Kimura (2010) reported the amino acid sequences of defensin (GenBank: AB540997.1) and abaecin (GenBank: AB90717.1) from *Apis cerana japonica*. In Thailand, Wannakul (2007) reported epinecidin-1, which was another AMP, in honey of the giant honey bee (*A. dorsata*). These antimicrobial peptides could explain the antimicrobial action of honey and propolis.

In 2011a, Kwakman et al. developed medical-grade honey containing 75 µM of the synthetic peptide known as bactericidal peptide 2 (BP2). It was able to rapidly inhibit the growth of many antibiotic-resistant strains of bacteria, including MRSA and extended-spectrum beta-lactamase-producing *E. coli*. Given the choices of medical-grade honey, BP2 alone, or honey alone, medical-grade honey clearly provided the best antimicrobial activity.

Antibacterial peptides in stingless bees have not yet been reported, but considering the important function they have, it seems worthwhile to study them, in addition to known active compounds such as flavonoids (Tomás Barberán et al. 1993).

36.5 Awareness of Using Stingless Bee Products

The main problem of using hive products concerns dosage and safety. This is especially true for honey, because it is usually consumed raw, and thus can easily be contaminated with plant pollen or spores of pathogens (Boukraa and Sulaiman 2009; Antúnez et al. 2004; Piccini et al. 2002). Interestingly, although honey is supersaturated, it does contain abundant water in which microorganisms can grow—including lactic acid bacteria of the genera *Lactobacillus* and *Bifidobacterium* (Olofsson and Vásquez 2008). Toxic or "mad honey" is also a possible concern. Grayanotoxin is a toxin known to be found in *Rhododendron* species and other Ericaceae, and can contaminate honey local to that area (Koca and Koca 2007). It can cause symptoms of bradycardia, atrioventricular block, and hypotension (Cagli et al. 2009; Dubey et al. 2009; Okuyan et al. 2010).

Besides certain honeys, bee pollen should also be used with caution. For example, Akiyasu et al. (2010) reported that ingestion of bee pollen in nutritional supplements could cause renal failure. Moreover, it has been reported that propolis ointment could cause a dermatological problem: an enlarged, fluid-filled pruritic lesion on a minor trauma (Ting and Silver 2004).

Thus, it is necessary to process honey. It can then safely be used to heal a wound or for other medical purposes. Good examples of processed honey are medical-grade honey or "manuka" honey, which are produced under standardized conditions in a greenhouse. Also, the honey is sterilized by gamma irradiation which can kill bacterial spores efficiently without affecting the honey's bioactivity (Postmes et al. 1995). More antimicrobial peptides can also be added to medical-grade honey for even more rapid bactericidal activity (Kwakman et al. 2011a, b).

In addition, since the chemical compositions and bioactivities of bee hive products depend on seasonal variation and other external factors, it is very important to establish standards for types and amounts of active chemical compounds before selling them commercially (Salomão et al. 2008; Teixeira et al. 2010).

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Chapter 37
Costa Rican Pot-Honey: Its Medicinal Use and Antibacterial Effect

Gabriel Zamora, María Laura Arias, Ingrid Aguilar, and Eduardo Umaña

37.1 Introduction

Honey is the natural sweet substance produced by honey bees from the nectar of flowers or extrafloral nectaries, or from excretions of plant sucking insects, which the bees collect and transform by adding specific substances of their own, dehydrate, and store in the honey comb to ripen and mature (Codex Alimentarius Commission 2001). Many studies have shown the honey of *Apis mellifera* possesses antimicrobial properties and also favors the healing of wounds and burns (Molan 1992; Bowler et al. 2001; Fournier et al. 2006; Aguilera et al. 2009). Nevertheless, stingless bee honey is locally considered to have stronger healing effects than the honey from *A. mellifera* of the same regions (de Jong 1999; Sommeijer 1999; Gonçalves et al. 2005; Boorn et al. 2009).

The Mesoamerican region is the natural habitat for native stingless bees (Meliponini), acknowledged as indispensable pollinators with a key role in tropical forest conservation (Roubik et al. 1982; Roubik and Aluja 1983; Paxton 1995; Michener 2000; Slaa et al. 2006). Among them, the most commonly domesticated species are *Melipona beecheii* and *Tetragonisca angustula*. The Mayan and Aztec cultures started the keeping of these bees and used their honey for medicinal purposes (de Jong 1999; Vit et al. 2004). At present, treatment of infected wounds, digestive disorders, respiratory tract infection and eye problems like cataracts and

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conjunctivitis with the honey of stingless bees is widespread (Grajales et al. 2004; Vit et al. 2004, 2009). However, there are no studies that evaluate the medicinal properties of honey from stingless bees in Costa Rica.

Due to the growing problem of antimicrobial resistance, it is of vital importance to discover innovative topical treatments for infected burns and wounds. This chapter provides updates on antibacterial activity of the pot-honey produced by several of our stingless bee species, and new data on *M. beecheii* and *T. angustula*, compared to *A. mellifera*.

37.2 Traditional Medicinal Use of Pot-Honey in Costa Rica

The traditional use of honey collected by stingless bees as a medicine is deeply embedded in Costa Rican ethnopharmacology. This natural product remains a traditional medicine, since pre-Columbian times. At present, is still highly regarded as a burn and wound dressing and a topical treatment for cataracts and conjunctivitis (Kent 1984; de Jong 1999; Sommeijer 1999).

Pot-honey collected by the stingless bee species *T. angustula* and *M. beecheii* have received the most commercial interest in Costa Rica. It is common to find stingless bee honey bottled in small dropper containers in natural medicine stores, sold at a substantially higher price than *A. mellifera* honey (Sommeijer 1996; Cortopassi-Laurino et al. 2006). Stingless bee honey in Costa Rica have the folk medicine reputation of having better medicinal properties as a burn and wound dressing than *A. mellifera* honey (DeMera and Angert 2004; Bijlsma et al. 2006).

The ideal antimicrobial topical agent contains active constituents of a burn and wound dressing—inhibitory activity against common agents of infection, among other qualities (Bryskier 2005). In order to determine if the traditional value given to stingless bee honey over *A. mellifera* honey is valid, an evaluation over the antimicrobial activity of honey samples of *T. angustula*, *M. beecheii*, and *A. mellifera* was performed.

37.3 Comparative Study of *Apis mellifera, Tetragonisca angustula*, and *Melipona beecheii* Honey

37.3.1 Honey Collection

A total of 56 honey samples (500 g to 1 kg) collected from *A. mellifera* ($n=34$), *T. angustula* ($n=14$), and *M. beecheii* ($n=8$) were obtained from producers. The honey under study belonged to several geographical locations were meliponiculture is practiced (see Table 37.1). All samples were kept in storage at 23°C, in a cool and dry place, away from light.

Table 37.1 Geographical origin of 56 Costa Rican honey samples

	Bee species		
Region	*A. mellifera*	*T. angustula*	*M. beecheii*
Central Valley	8	7	1
Mountain South	12	–	–
Central Pacific	2	–	–
North Pacific	12	3	7
South Pacific	–	4	–
Total honey samples	34	14	8

37.3.2 Evaluation of Antibacterial Activity

Pot-honey solutions with final concentrations of 75, 50, 25, and 12.5% (w/v) were prepared in sterile peptone water 0.1%, pH 7.2. These solutions and pure honey were subjected to an antibacterial activity test following a Mueller-Hinton agar-well diffusion assay as described by Mitscher et al. (1972). A test solution was qualitatively considered antimicrobial if a clear zone without microbial growth was present surrounding the well after incubation. The analysis was conducted three times for all honey samples against the following American Type Culture Collection (ATCC) strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076), *Listeria monocytogenes* (ATCC 19166), and *Pseudomonas aeruginosa* (ATCC 9027). In addition, a clinical isolate of *Staphylococcus epidermidis* (UCR 2902) was included in the present trial. The results of antimicrobial activity evaluation are presented in Table 37.2. All descriptive and inferential statistics used InfoStat Software (InfoStat Group, Universidad Nacional de Córdoba, Argentina).

A previous study performed by DeMera and Angert (2004) compared antimicrobial activity of honey produced by *T. angustula* and *A. mellifera* from Costa Rica. In their evaluation, *S. aureus* showed no susceptibility to any of the samples analyzed. In contrast, Estrada et al. (2005) reported 80% of *A. mellifera* honeys were active against *S. aureus*. By means of the same method, in our trial, all *T. angustula*, *M. beecheii* and 82% of *A. mellifera* honey exerted antibacterial activity against *S. aureus*. The present study shows no statistical difference ($p > 0.05$) from results presented by Estrada et al. (2005) for the inhibitory activity against *S. aureus* by *A. mellifera* honey.

At a honey concentration of 25%, the differences observed in inhibition of *S. aureus* are statistically significant between *A. mellifera* and *T. angustula* ($p < 0.05$) and highly significant comparing *A. mellifera* to *M. beecheii* ($p < 0.001$). Hence, at lower concentration, stingless bee honey was more active against *S. aureus*. Moreover, at the lowest concentration tested, *M. beecheii* honey were the most active ($p < 0.001$).

The results obtained for *A. mellifera*, *T. angustula* and *M. beecheii* honey, inhibitory against *S. epidermidis* and *L. monocytogenes* at a concentration of 50%, show

Table 37.2 Antibacterial activity of honey and pot-honey from Costa Rica

	Honey concentrations grouped by bee species[a]														
	100%			75%			50%			25%			12.5%		
Bacterial strains	Am	Ta	Mb	Am	Ta	Mb	Am	Ta	Mb	Am	Ta	Mb	Am	Ta	Mb
Staphylococcus aureus	82	100	100	79	100	100	71	100	100	21	64	100	0	7	78
Staphylococcus epidermidis	85	100	100	76	100	100	38	93	100	6	21	78	0	0	0
Escherichia coli	97	100	100	85	86	89	74	7	67	3	0	0	0	0	0
Salmonella enteritidis	94	100	100	88	100	100	85	7	56	18	0	0	0	0	0
Listeria monocytogenes	79	100	100	47	100	89	9	50	67	3	0	22	0	0	0
Pseudomonas aeruginosa	9	93	100	0	86	100	0	21	78	0	0	33	0	0	0

Results are expressed as percentages of honey successful to inhibit bacterial growth

[a] *Am Apis mellifera*, *Ta Tetragonisca angustula*, *Mb Melipona beecheii*

significant differences ($p < 0.05$, $p < 0.001$ respectively). With 50% honey solutions, *E. coli* and *S. enteritidis* were the only cases in which *A. mellifera* was more active than *T. angustula* ($p < 0.001$). Nevertheless, there was no statistical difference between *A. mellifera* and *M. beecheii* ($p > 0.05$).

Finally, the inhibitory effect on *P. aeruginosa* revealed a statistically significant difference in the results. The samples collected from both stingless bee species were more active than those of *A. mellifera* ($p < 0.001$, for 100 and 75% solutions).

37.4 Pot-Honey as Alternative Antibiotic

The antibacterial effects presented herein invite further study of the nature of medicinal activity exerted by Costa Rican pot-honey. In general, these results exemplify the broad-spectrum antimicrobial activity of pot-honey from Costa Rica. Antibacterial activity towards *S. aureus* and *P. aeruginosa* was higher in *T. angustula* and *M. beecheii* pot-honey than in *A. mellifera* comb honey. The actual medical panorama reflects an increasing number of antibiotic resistant microorganisms that cause resilient disease (Bowler et al. 2001; Howell-Jones et al. 2005; Salyers and Whitt 2005). Under this turn of events, innovative therapies towards wound healing are urgent (Bryskier 2005) and pot-honey is an alternative treatment.

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Chapter 38
Immunological Properties of Bee Products

José Angel Cova

38.1 Introduction

Since ancient times, bee products have been used in medicine. Several reports have attributed anti-inflammatory, antitumoral and antioxidant properties to honey bee products (Majtán 2009; Attia et al. 2008; Bariliak et al. 1996; Rekka et al. 1990). Their mechanism of action often involves participation of the immune system, and it is important to know the impact of such substances in immune system defense, suppression and immunoregulation functions. In this chapter, we focus on the principal characteristics of the immune system and the impact of bee products on animal and human immune response.

38.2 Honey Bee Products and Innate Immune Response

The immune system has been designed to protect animals from invader pathogenic microorganisms. Immunity—its main and unique function—has evolved until differentiating into two complementary forms: innate and adaptive.

Innate immunity is considered the first line of defense against pathogenic microorganism such as bacteria, viruses, parasites and the cellular and humoral components of immunity are mainly located and distributed in the external surface of the body. Most of these components are present long before pathogen invasion or the infection's settlement. Their molecular mechanisms are nonspecific and of short duration. They also cannot discriminate among different antigens, either nonself or

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self in origin. They have no memory and their response remains unalterable even with the occurrence of repeated organism substance encounters (Abbas and Litchman 2005b; Goldsby et al. 2007).

Innate immunity includes physical and anatomic barriers such as skin, the epithelial layer that covers intestinal, respiratory and urogenital tracts and some humoral and cellular components of the immune system. The humoral elements include complement system (CS) components, acute-phase proteins (APP) and Interferon, especially α and β interferon. The cells that participate in innate immunity are neutrophils, eosinophils, macrophages, natural killer cells (NK), dendritic cells (DC), mast cells, and endothelial cells, among others (Abbas and Lichtman 2005a).

The complement system (CS) is a group of proteins, most of them synthesized in the liver, that circulate in an inactive state in sera and other body fluids. Several complement proteins are proteases that are self-activated by proteolytic cleavage (Janeway 2005; Trevani and Geffner 2005). The CS is activated through three different pathways: (1) the classical pathway (CP) which is activated through interaction between one of two isotypes of G or M immunoglobulin (IgG or IgM), bound to C1 complement component onto the microbial surface. As a cascade, activated C1 cleaves and activates C4, which activates C2 and subsequently activates C3. (2) The alternative pathway (AP) does not require antibody presence and is initiated in most cases by foreign cell-surface constituents: AP by means of factor D, factor B and properdin activates C3 to C5. (3) The mannan-binding-lectin (MBL) pathway is another route for complement activation. The MBL binds to mannose residues on glycoproteins or carbohydrates of the microbe surface and initiates complement activation in a similar manner than to C1, which resembles its structure. All these three pathways activate from C5 to C9 components of the system sequentially and form the membrane-attack-complex (MAC) which damages the membrane of pathogenic organisms. Thus, complement activation facilitates the clearance of bacteria through phagocytosis by macrophages and neutrophils. One of the most important complement activation components is C3 because of its role as a connector between the different pathways. The pharmacological intervention on C3 could switch all the system from an anti-inflammatory state to a pro-inflammatory state or vice versa (Trevani and Geffner 2005; Janeway 2005; Volanakis 1998).

Acute-phase proteins (APP) are a family of proteins that include C-reactive protein, serum amyloid A protein, α-antiquimiotripsin, fibrinogen, and MBL and are produced by hepatocytes and macrophages in an inflammatory response. APP increase phagocytosis of opsonized bacteria, induce complement's activation and inhibit bacterial proteases which help to eliminate dangerous microorganisms from the body (Goldsby et al. 2000).

Interferon comprises a family of proteins produced by virus-infected cells. Interferon has many functions, especially one that enables its binding to nearby cells to induce a generalized antiviral state that prevents the spread of infection to other cells and organs. Other proteins involved in the humoral response belong to the cytokine and chemokins families such as tumor necrosis factor (TNF), interleukin 1β (IL-1), transforming growth factor-β (TGF-β), interleukin-12 (IL-12), interleukin-8 (IL-8), and others. These cytokines participate in the innate immune response and their inhibition or increase by action of the honey bee products might modify the immune response. There is a growing interest to find molecules that induce the production of TGF-β by T regulatory cells in order to control several hypersensitivity reactions as arthritis and inflammatory bowel disease, among others.

The function of cells that participate in the innate immunity is to recognize the pathogens when they invade the body. Cells have many different mechanisms to identify foreign invaders and most of these are based in the interaction between pathogens-associated-molecules-patterns (PAMP) present in the surface of microorganisms (viruses, bacteria, mycobacteria and parasites) and PAMP-recognize-receptors (PRR) also expressed in DC and macrophages surfaces. PAMP includes lypopolisaccharide (LPS), teicoic acid, non-methylated DNA, dsRNA, a class of molecules unique to microbes and are never found in multicellular organisms. Besides, PRR is placed in a different class of receptors that can activate a phagocyte cell after binding to PAMP as do the toll-like receptors (TLR) (Trevani and Geffner 2005, Akira et al. 2006). TLR4, as an example, recognizes the LPS of Gram-negative bacteria and initiates the activation of macrophages via MyD88-NFκB, which induces phagocytosis and secretion of proinflammatory cytokines (IL-1, IL-6, etc). Promising results of propolis usage to enhance TLR expression in cells have appeared as a new and exciting research area for natural medicine (Orsatti et al. 2010). Another mechanism to eliminate pathogens involves recognition of virus-infected cells and intracellular bacteria by the activation receptor (AR) expressed in natural killer (NK) cells. The AR includes NKp receptor group: NKR-P1, CD2, NKp30, and NKp44. Their binding to a specific ligand on target cells initiates a cytotoxic lysis. Whether the honey bee products can modify the expression of these receptors and enhance the lysis of cancer cells or virus by NK cells is a subject that requires further study.

The results of studies about the effect of bee products from the honey bee in the immune system have been obtained under different conditions. These include varied botanical origin of compounds, extraction solvent (ethanol extraction vs. aqueous extraction), variable concentration of compounds, different times of incubation and different drug administration routes (peritoneal, subcutaneous, etc.). For this reason, interpretation of the cited evidence deserves to be analyzed very carefully.

38.2.1 Honey

In humans, honey inhibits the basophil degranulation at high and low concentration levels of anti-IgE antibody used to stimulate them (Poitevin et al. 1988). With regard to this result, honey might be used as a homeopathic medicine in human allergic disease after controlled in vitro and in vivo assays.

As a complex process, inflammation is studied using indicators of anti-inflammatory activity, such as the lipoxigenase (LOX) essay. Salomón et al. (2011) studied the LOX inhibition by pot-honey of *Tetragonisca fiebrigi*, *Scaptotrigona jujuyensis* and *Plebeia molesta* from Northern Argentina (Chaco, Formosa, Misiones and Tucumán). The *S. jujuyensis* honey showed the most anti-inflammatory action, and positive correlations between radical scavenging activity and LOX inhibition (MI Isla, personal communication).

38.2.2 Propolis

Honey bee products have been demonstrated to induce alteration in intracellular space and the cellular membrane. At the intracellular level, propolis decreases DNA synthesis in peripheral blood mononuclear cells (PBMC) including macrophages. In this report, propolis and its studied constituents were capable of suppressing DNA synthesis in dose-dependent phytohemagglutinin (PHA)-induced cells as well as in T cells. The production of cytokines (IL-1β, IL-2, IL-4, and IL-12) was also suppressed in these cells (Ansorge et al. 2003). However, when the macrophages are in the peritoneal compartment, propolis stimulates pro-inflammatory cytokine production, such as IL-1β and TNF-α in mice, after stimulation at a dose of 0.2–1 mg/ml (Moriyasu et al. 1994). These results show differences that could be explained based on the compartment in which cells are located. The immunosupressor effect of cyclophosphamide can be reversed at a dose of 50 mg/kg of propolis and could be possible via nonspecific immunity modulation through activation of macrophages (Dimov et al. 1991).

In the complement system, propolis modulates the production of C1 complement component in macrophages after incubation at a dose of 0.150 mg/g (Dimov et al. 1992). It inhibits the classical and alternative pathways of the complement at higher doses (Ivanoska et al. 1995). Possibly, propolis causes inactivation or suppression of the one or more components of the complement and in this way diminishes the activity of these pathways. Georgieva et al. (1997) found compounds like flavonoids and phenolic substances with anticomplementary activities through inactivation of C3.

Reactive-oxygen intermediate (ROI) and nitric oxide (NO) produce macrophages and activate neutrophils that help eliminate bacteria. Propolis increases generation of H_2O_2 in macrophages after incubation at doses of 5, 10 and 20 μg/ml. Otherwise, neutrophils obtained from rabbit decrease the superoxide anion ($O_2^{\bullet-}$) production at different dose of propolis (range 2–25 μg/ml). In general, the production of NO is inhibited in macrophages treated with propolis (Krol et al. 1996). Also, in human neutrophils, propolis enhances the secretion of cytokines, both spontaneous and induced cytokine release, but plasma levels do not change (Orsi et al. 2000; Simoes et al. 2004).

Commercial laying hens fed a diet supplemented with propolis show lower counts of heterophil cells (macrophage-like cells) than a control group. Likewise, this experiment demonstrates that supplementation with propolis improves performance and egg mass for commercial production (Galal et al. 2008).

Table 38.1 Biological activity of pot-honey

Effect	Action mechanism	Reference
Antiinflammatory	Propolis and honey inhibits the production of nitric oxide by peritoneal macrophages	Orsi et al. (2000); Kassim et al. (2010)
	Honey inhibits the oxidative burst in phagocytes cells	Mesaik et al. (2008)
	Propolis and its component suppress prostaglandins and leukotriene production in murine peritoneal macrophages	Mirzoeva and Calder (1996)
Antibacterial	Propolis improves the bactericidal activity against *Salmonella typhimurium* on macrophages	Orsi et al. (2005)
	Propolis increases the bactericidal activity against *Paracoccidioidis brasiliensis* on macrophages	Murad et al. (2002)
	Honey has antibacterial activity against *Staphylococcus aureus*	Miorin et al. (2003)
	Honey inhibits the *H. pylori* grow	Ali et al. (1991)
Antitumoral	Propolis increases the NK cytotoxic activity against tumor	Sforcin et al. (2002)
	Honey bee reduces tumor cells proliferation	Attia et al. (2008)
Adjuvant	Propolis increases the specific antibodies production after vaccination with inactivated SuHV-1 vaccine preparation	Fischer et al. (2007)

Bullfrogs fed with propolis at 0.2, 0.5 and 1.0% of concentration in their diet significantly increase monocytes density in peripheral blood. However, other cell-basophils, neutrophils and eosinophils do not produce a statistical difference between groups (Romero et al. 2006).

38.2.3 Royal Jelly

The antiinflammatory effects and immunomodulatory properties of glandular products secreted by worker honey bees (royal jelly) could ameliorate immunological disorders (ID) and act as an immunomodulatory agent. In fact, royal jelly treatment in lymphocytes from patients with Graves' disease shifted the T helper cell Th1/Th2 cytokine ratio to the side of Th1 cytokine (Erem et al. 2006). Therefore, royal jelly may control tissue damage in the thyroid gland and induce remission in this disease.

In conclusion, the major pharmacological activities of the products from bees have been focused on anti-inflammatory properties that induce immunosuppression. This effect modifies the innate immune response making it useful for treatment of hyper immune responses. Other biological activities attributed to bee-products are summarized in Table 38.1.

38.3 Action Mechanisms of Bee Products in the Adaptive Immune Response

The adaptive immunity is a branch of the immune system developed to recognize and selectively eliminate foreign microorganisms (e.g., bacteria or viruses) and molecules. Unlike the innate immune response, adaptive immune responses are not the same in all members of a given species, but are reactions to specific antigenic challenges. Adaptive immunity displays four characteristic attributes: antigenic specificity, diversity, immunologic memory and self/nonself recognition (Goldsby et al. 2007).

B-lymphocytes and T-lymphocytes are the cellular elements of the adaptive immune response, and they express specific receptors for antigens on the membrane: B-cell receptor (BCR) and T-cell receptor (TCR) in B-lymphocytes and T-lymphocytes, respectively. Each B cell or T cell clone will recognize only one antigenic structure (antigenic specificity). After the interaction between antigen and a specific cell receptor the cell is activated. After activation, the B or T lymphocyte will undergo a clonal expansion and produce millions daughter cells with identical antigenic specificity. In the case of B cells, the progeny undergo differentiation into memory B cells and effector B cells called plasma cells (Liu and Banchereau 1997). Memory B cells have a longer life span and circulate in the body until a reencounter with the antigen, followed by clonal expansion. Plasma cells live for a short time and produce enormous amounts of antibodies or immunoglobulins, secreted for binding to the antigen prior to their clearance by phagocytosis, and activation of complement and/or antibody-dependent cell-mediated cytotoxicity (ADCC).

T-lymphocytes are divided into two well-defined populations known as T-helper (Th) or T/CD4$^+$ and T-cytotoxic (Tc) or T/CD8$^+$ cells. Following the stimulation, the Th cell can differentiate into Th1 cells (producing IL-2 and IFN-γ), Th2 cells (producing IL-4 and IL-5), T regulatory cells (producing IL-10 and TGF-β) and Th17 cells (producing IL-17 and IL-6) (Mosmann and Coffman 1989; Murphy and Reiner 2002; Sakaguchi 2000; Harrington et al. 2005). Each subtype of Th cells and their pattern of secreted cytokines results in different types of immune response. For example, IFN-γ—produced by Th1 cells—activates macrophages and helping to activation and expansion of naive CD8$^+$ T cell transforms it into an effector cell called cytotoxic T lymphocyte (CTL). The CTL and macrophages play an important role in the defense against intracellular bacterial infection, virus-infected cells, tumor cells and cells of a foreign tissue graft (Abbas and Litchman 2005c). The Th2 cell and IL-4 secreted by them induces activation and differentiation of the B-lymphocyte into a plasma cell that secretes antibody into the extracellular space. This type of immune response is important in the control of helminth parasites, along with extracellular bacterial and some viral infections (Liu and Banchereau 1997).

The immune response must terminate when the pathogen or parasite is eliminated or controlled if the antigen persists. The T regulatory cells (Treg) participate in regulating the immune response by at least two types of interaction. First, Treg produces immunosuppressive cytokines like IL-10 and TGF-β. Second, Treg interacts with T effector cells through cell-cell contact and delivery inhibitory signal into activated Th cells (Thornton and Shevac 1998; Sakaguchi 2000).

From this knowledge, new pharmacological applications for honey, propolis and royal jelly could be investigated through in vitro and in vivo studies. The influence of these social bee products after incubation of different duration and concentration with the immune cells can be measured using diverse techniques: expression of new molecules in the membrane of cells by flow cytometry, detection of RNA by molecular biology methods, and studies of protein by proteomics, among others. The compounds found in the honey may be useful to treat maladies in which immune system dysfunction is responsible for the disease.

38.3.1 Royal Jelly and Propolis Modify the Adaptive Immune Response

In lymphocytes using proliferation assay, Del Valle-Pérez et al. (2001) do not observe changes in lymphocyte proliferation after incubation of cells with royal jelly. Instead, propolis diminishes DNA synthesis and is able to suppress IL-2 (pattern-Th1 cytokines) and IL-4 (pattern-Th2 cytokines) in T-lymphocytes, revealing an antinflammatory action. Moreover, TGF-β, an immunosuppressor cytokine, is enhanced after propolis incubation, indicating T regulatory cell activation (Ansorge et al. 2003). This could be the explanation for antinflammatory properties, inhibition of NO production and respiratory cell burst observed after incubation with propolis. The effect of stingless bee products in other Th cells (Th17, Th9, and Th22) has not been studied yet. On the other hand, Ivanoska et al (1995) observed a proliferative tendency in splenocytes incubated with propolis. Further propolis inhibited proliferation in Con A-stimulated cells compared to a control group in experiments with mice.

Treatment with honey or propolis administered to Newcastle disease virus NDV-infected chicken produces an increase in the amount of antibodies as well as higher percentage of macrophages, both in sera. Likewise, the mortality rate is reduced in groups infected with virulent NDV and subsequently treated either with propolis or honey, if compared with the infected group only (Hegazi et al. 1995, 1996).

With B lymphocytes, the evidence suggests an increase in antibody (Ab) production by cells after incubation with bee-products. Propolis increases Ab production in mice immunized with sheep red blood cell in different amounts (Scheller et al. 1998). Similarly, the administration of propolis at 10% concentration to rats significantly increases antibody titres, even after 15 days of immunization (Sforcin et al. 2005; Hegazi et al. 1997).

Propolis and other bee products seem to have adjuvant activity in the adaptive immune response. The production of antibodies might be induced after the action of honey on macrophages which activate, directly or by soluble signal, B-cells that transform into antibody producing-cells. Further research will give insight to understanding the participation of honey bee-products in immune response.

38.4 Future Perspectives to Use Honey Bee Products in Treatment of Immune Diseases

The main immunological diseases comprise allergy, autoimmune disease and immunodeficiency. Both allergic and autoimmune diseases are mediated through a hypersensitivity mechanism and inflammation plays a critical role in pathogenesis. On the other hand, immunodeficiency occurs by defects in the elements or organs of the immune system.

The inhibition on cyclooxygenase-2 (COX) induced by honey and propolis may improve the inflammatory process in autoimmune diseases (Viuda-Martos et al. 2008).

Other targets to treat autoimmune diseases have been discovered, such as cytokines, cellular receptors, intracellular signals and pro-apoptotic molecules. One of them is IL-17secreted by Th17 cells that participate in chronic inflammation observed in the autoimmune diseases. IL-17 induces the production of inflammatory cytokines by synovial cells, recruitment of leukocytes into inflamed joints, upregulation of matrix metalloproteinase, and nitric oxide causing destruction of tissue and bone in rheumatoid arthritis (RA). The effect of propolis, royal jelly and honey in IL-17 secretion and Th17 cell proliferation need to be studied. For example, the expression of cellular markers and IL-17 secretion in T-lymphocyte culture from patients with RA using flow cytometry techniques and ELISA assays may provide insight for the treatment of RA.

The production of antibodies against self-proteins is involved in pathogenesis of autoimmune diseases due the breakdown of tolerance mechanisms. Briefly, an autoreactive B-cell is activated following interaction of self-protein-MHC II complex and costimulatory signal (B7 and CD40) instead the inhibitory signal (BTLA). It induces transformation of B-cells into plasma cells that produce immunoglobulin which reacts against self-protein. Given the action of bee products over B-cells, new therapeutic approaches using these products seem highly promising.

Allergic disease is a worldwide health problem and is increasing in many countries. The hypersensitivity reaction is initiated by antigen-presenting cells that internalize, process, and present allergic protein (allergen) to specific T-lymphocytes, inducing activation of those cells. By action of IL-4, cells proliferate and differentiate into Th2 cells secreting cytokines for the stimulation of B-cells. Following this, B-cells undergo immunoglobulin gene class switching, leading to their terminal differentiation into plasma cells that produce antigen-specific IgE antibodies. Once released by plasma cells, antigen specific IgE binds to the high-affinity IgE receptor in mast cells and basophils, leading to sensitization of these cell types. When mast cells and basophils with such IgE on their surface come in contact with native protein antigen, they are induced to degranulate, releasing histamine, tryptase, proteoglycans, serotonin, and other compounds. All of these are responsible for allergic symptoms.

In allergic diseases, the influence of honey in inhibition of basophils and mast cells, lower expression of CD63, and lower levels of IgE, among others, will clearly be essential to demonstrate and understand, in order to design effective treatment and sound experiments.

38.5 Conclusions

Bee products, known primarily from the honey bee but now starting to be investigated with stingless bees (as revealed in many chapters of the present book) contain various active compounds responsible for many positive effects in both normal and altered immune systems.

A new avenue of experimental studies should be designed in order to evaluate the immunological effects of pot-honey in the different forms of the immune response, recently initiated in Argentina by evaluating the anti-inflamatory action on one key enzyme. Likewise, testing the effects of bee products on immunological disorders through clinical studies might provide us a new class of drugs to be employed in allergy and autoimmune disease treatment.

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Chapter 39
Chemical Properties of Propolis Collected by Stingless Bees

Ömür Gençay Çelemli

39.1 Introduction

Propolis is known in folk medicine since ancient times. Egyptians benefited from anti-putrefactive properties of propolis in order to embalm their dead. It was used as an antiseptic and healing agent by Greek and Roman physicians. Incas employed propolis as an anti-pyretic agent, and the London Pharmacopeia of the seventeenth century listed propolis as an official drug (Ghisalberti 1979). Studies on composition and biological properties of propolis reveal the interest of researchers on this bee product and its potential for the development of new drugs as well (Sforcin and Bankova 2011).

Natural products are a promising source for the discovery of new pharmaceuticals. In the last decades, propolis has received regard for its potential in medicine and cosmetics, even if it is known primarily only in folk medicine and ancient times. The antimicrobial properties of propolis have been widely investigated, confirming its antibacterial, antiviral, and antifungal activities (Sforcin et al. 2000). Stingless bee propolis is used in folk medicine for the healing properties on digestive and respiratory systems, female fertility, skin and visual disorders. Pollen of stingless bees has also therapeutical uses, and the larvae of *Melipona* and other stingless bee genera are consumed in local diets (Freitas et al. 2008). Stingless bee honeys attract attention of researchers for their importance as foodstuffs and traditional remedies in folk medicines (Vit et al. 2004). For example, honey from *Tetragonisca angustula* bee is highly appreciated for its pleasant flavor and is used for the treatment of respiratory, eye infections, and anti-cataract properties (Torres et al. 2004).

The aim of this study is to determine the chemical composition of ethanol extracts of propolis collected by the stingless bees *Melipona favosa* from Venezuela,

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Melipona grandis, *Scaptotrigona depilis*, and *Scaptotrigona polysticta* from Bolivia, and *Tetragonula biroi* from Philippines.

The chemical composition of the propolis of stingless bees is still not clear. Therefore our results will be a step toward the identification of the chemical profile of stingless bee propolis, needed for further applications. Also with this study we can compare the chemical profile of propolis collected by five different stingless bee species using gas chromatography–mass spectrometry (GC–MS). Using GC–MS analysis we can easily observe the volatile profile of terpenes to consider whether there is a variation in chemical composition of propolis samples among bee species.

39.2 Why Are Resins Collected by Honey Bees and Stingless Bees?

Resin, a sticky plant substance, is produced by various plant families and is secreted in response to an injury or infection of plant parts. However, resin secretion can also occur spontaneously, as has been shown for the tropical legume *Hymenaea* (Fabaceae, Caesalpinioideae) (Langenheim et al. 1978). Resins of different botanical origin serve as a deterrent against herbivorous insects, such as lepidopteran larvae (*Hymenaea* resin), as well as against ants (*Pinus* Pinaceae resin), termites ("guayule" *Parthenium argentatum* Asteraceae, gray resin), bacteria (*floral Clusia* Clusiaceae resin), and fungi (*Dipterocarpus* Dipterocarpaceae; guayule pine resin). This deterrent function is most likely due to the presence of terpenes, especially mono- and sesquiterpenes (Leonhardt and Blüthgen 2009). Some plant species use resin as an attractant for pollinators and seed dispersers (birds, mammals, reptiles, ants, bees) (Wallace and Trueman 1995). They secrete resin both to defend themselves against herbivores and to attract bees. In Borneo (Malaysia), bees use resin and resin-derived compounds not only to build and defend their nests but also to enrich their cuticular/chemical profiles (Leonhardt et al. 2011). Cuticular lipids are thought to preserve insects from desiccation, cuticle abrasion, and infection. In several insect taxa, cuticular lipids have become further involved in the communication system by enabling them to reliably differentiate between friend and foe or find a mate based on differences in the chemical composition of cuticular profiles (Leonhardt et al. 2009). The prominence of resin-derived compounds on the bees' body is unique to stingless bees and has not been described in any other social insect (Leonhardt et al. 2011).

Propolis is any resinous mixture or pure resin substance collected by bees and stored within the nest for construction and defense purposes (D. Roubik, personal communication). These natural resinous products collected by foraging bees, from various plant sources, are used to build, strengthen, isolate, and disinfect their nests to fill holes and to embalm dead predators inside the hives (Simone and Spivak 2010). Cerumen is a mixture of wax, and plant resins, potentially enriched with stingless bee secretions. There is suggestive evidence that stingless bees add cephalic gland secretions during cerumen production (Massaro et al. 2011). Cerumen storage pots can expand and contract without breaking during fermentive processes (P. Vit personal communication).

In some literature meliponine propolis is called geopropolis (Barth 2004). While propolis is prepared by Meliponini using resins of plants mixed with waxes and sometimes mud, honey bees (*Apis mellifera*) do not use soil material when preparing propolis (Barth and Luz 2003), and most stingless bees do not add wax or clay and earth. The presence of silica and clay and absence of trichomes was used, besides pollen grains, to differentiate propolis of Meliponini from propolis of *A. mellifera* (Barth 2004). However, *Trigona* workers (Meliponini) collect *Maxillaria rufescens* trichomes (Singer et al. 2004). On the contrary Barth and Luz (2003) investigated ten geopropolis samples collected from three meliponine species in Brazil. They observed pollen grains, hyphae and spores of fungi, organic material and burned plant fragments in most of the samples. Sandy or earth materials were present in all geopropolis sediments. Only one sample contained plant trichomes and was considered a propolis sample of *A. mellifera*.

Propolis is mainly used by honey bees and stingless bees to protect the nest against infection and also as a multipurpose cement and varnish. Social immunity, which describes how individual behaviors of group members effectively reduce disease and parasite transmission at the colony level, is an emerging field in social insect biology (Finstrom and Spivak 2010). “Hygienic behavior” first described for honey bees is now a classical example of a social defense, whereby workers identify and remove infected larvae from among the healthy brood. Other defenses enabled by sociality include the construction of nests from antimicrobial materials, the raising of offspring in sterile nurseries, social “fever” in response to disease, transference of immune traits, and heightened risk-taking by infected individuals. Like most eukaryotes, colony members also possess individual defenses, including immune responses toward disease agents (Evans et al. 2006).

39.3 How Do Bees Collect Resins to Produce Propolis and Cerumen?

The sticky resin known as propolis or bee glue is a material collected from plants by foraging workers. Bees break off pieces of the resinous exudates from the plants, using their mouth parts. The pieces are moistened with the tongue and formed into pellets by the mandibles with the help of the legs. The bees bring the pellets from the mouth along the hair of the tibia and into the corbiculae. While packing one piece of propolis into the corbicula, the bee is simultaneously collecting more. The collection of propolis could take a long time and might be interrupted by visits to the nest for feeding. When the corbicula has been filled, the bees deliver the propolis to the nest (Ghisalberti 1979). Resin foragers have shown a preference for young leaves and vegetative buds over older leaves. The process of obtaining a full corbicular load of resin has been noted to take about 7 min, but can extend up to 1 h depending on the weather. Unloading the resin from corbiculae in the nest is a process that typically takes approximately 15 min, but can extend to 7 h or even overnight (Finstrom and Spivak 2010).

Stingless bees are the major visitors of many flowering plants in the tropics (Heard 1999). Some stingless bees may also incorporate mud, fecal material, chewed plant matter, and artificial products such as tar into their nest (Wallace and Trueman 1995; Roubik 2006). Plant resins are an essential resource for nest building and defense. In contrast with pollen and nectar flows, resin resources are generally unpredictable and short-lived and are aggressively defended by some species (Wallace and Lee 2010).

To reveal factors that influence bee decisions about where and when to collect resin, resin collection was studied in ten stingless bee species by Leonhardt and Blüthgen (2009) in Southeast Asia. Bees prefer resins of particular trees and neglect resins of others. Most trees offering resins to be collected by bees belonged to the Dipterocarp family. Dipterocarps are highly resinous, and their resin is known to inhibit the growth of pollen-associated fungi (Leonhardt and Blüthgen 2009). Stingless bees appear to use the same mechanism and compounds to locate and recognize resin sources as honey bees do (Leonhardt et al. 2010). Stingless bees also important for seed dispersal of three plant species as of a rain forest eucalypt *Corymbia torelliana* (see Chap. 3).

39.4 Botanical Origin of Propolis

Single or compound hairs from plants (trichomes), especially leaves, are commonly an additional component of propolis. Some of these indicate the plant species from which they were collected by their morphological characteristics (Ricciardelli D'Albore 1979). When bees prepare propolis from plant exudates pollen grains already are present, and with contact from bees and their nest, more are introduced. These pollen grains come from the flowers visited by bees for nectar and pollen and also from wind pollinated plants. Because of this, identification of the plant species, whose pollen occurs in propolis samples, allows a characterization of the surrounding vegetation, and frequently the geographical region from which the resin was collected (Warakomska and Maciejewicz 1992). Pollen analysis, besides chemical analysis, is a method used to characterize regionally different propolis samples (presenting different characteristics of hardness, elasticity, smell and colour). It is a good tool for defining the phytogeographical origin of resins and quality of the propolis (Barth and Luz 2003). Meliponini collect not only resin but also clay and, in separate loads, the latex of fruits of *Coussopoa* Moraceae (formerly thought to be *Vismia* Clusiaceae), for propolis confection (Barth 2004).

It is possible to characterize the environmental conditions and the vegetation around the apiary using pollen from propolis, as well as the trophic preferences for some of the bees (Barth 2006). Barth and Luz (2003) investigated ten samples of Brazilian propolis from three species *Melipona quadrifasciata*, *Melipona orbygnii* (*sic*, = *orbignyi*), and *T. angustula*, and two of the samples did not contain pollen grains. With one exception, propolis samples had no trichomes. Different sized grains of sand and/or small particles of soil were detected in all samples examined; these are of earth material utilized by the Meliponini in preparing propolis. With the exception of two samples, they observed pollen grains in all samples. About 64 pollen types could be identified, 22 occuring at a frequency of more than 3%. Pollen grains of *Eucalyptus* (Myrtaceae) and *Schinus* (Anacardiaceae) were dominant in several propolis samples. Barth (2006) analyzed six samples of propolis that showed different physicochemical properties. Only 21 pollen types occured with a frequency higher than 3% and only four with more than 25% of the pollen sum. According to their results, forest taxa were represented by Anacardiaceae, *Anadenanthera* (Fabaceae, Mimosoideae) and Aceraceae and open-land vegetation by species of Asteraceae, Poaceae, *Alternanthera* (Amaranthaceae), Scrophulariaceae, and *Typha* (Typhaceae).

The chemical composition of propolis depends on the phytogeographic characteristics of the site of collection, because the bees choose different plants as sources of resins in different habitats. Thus, the complex standardization of propolis should relate biological properties to a detailed investigation of chemical composition and botanical sources (Bankova 2005; Sforcin and Bankova 2011).

39.5 Chemical Composition and Biological Properties of Propolis

Most components of bud exudate are incorporated into propolis without alteration, although it is possible that some glucosides are subjected to enzymic hydrolysis by the bees either during collection of the bud exudate or during its addition to beeswax to make propolis (Greenaway et al. 1987).

The compound groups identifed from propolis are: amino acids (researchers suggest that the traces of amino acid present in propolis come from the bees), aliphatic acids and their esters, alcohols (of these the α- and β-glycerophosphate probably derive from bee metabolism, the glycerol from wax and other components from bud exudate), aldehydes, chalcones (the chalcones are related to the flavanones and may be formed from them during propolis manufacture and during preparation and analysis of samples), dihydrochalcones, flavanones (these compounds, together with flavones, are often mentioned as having antimicrobial properties), flavones, hydrocarbons (the C 25 and C 27 hydrocarbons are common in poplar bud exudates), but it is likely that in propolis these compounds are derived both from bee metabolism and from bud exudates. It is believed that other hydrocarbons arise primarily from bee metabolism, ketones, terpenoids (the volatile C 10 terpenoids have strong odours and this group of compounds may be responsible for much of the odour of propolis), and sugars (such as glucose, fructose, and sucrose) are frequently present in propolis.

It is suspected that these are due to contamination by honey (Greenaway et al. 1990). Therefore, some compounds of propolis originate from bee metabolism (e.g., alcohols, hydrocarbons) and others from plant exudates (e.g., terpenes, flavonoids).

Researchers find it puzzling that European and Ecuadorian propolis are very different. Ecuadorian propolis contains neither the aromatic acids and esters nor the flavones and flavanones, indicated as the active antimicrobial principles of European propolis. Greenaway et al. (1990) compared propolis from colonies of native stingless bees (*Melipona*, *Nannotrigona tristella*, *Scaptotrigona* and *Tetragonisca*) and from *A. mellifera*. The unique phenolic compound in propolis of *N. tristella* and *Melipona* is 3,5-dihydroxybenzoic acid. They speculate exudates incorporated by Ecuadorian stingless bees probably come from flowers of *Dalechampia* and *Clusia*—although seldom visited by *Nannotrigona* and *Melipona*, which have evolved within their flowers special structures secreting a resin which is attractive to bees.

There is another medical aspect of propolis: it may cause allergic reactions in susceptible persons. Prenyl caffeate (1,1-dimethylallyl caffeic acid ester) has been particularly identified as a contact allergen. This compound occurs in poplar bud exudates in varying amounts (Burdock 1998).

Propolis from the honey bee *A. mellifera* is used in folk medicine in the countries of Eastern Europe as an antiseptic and anti-inflammatory agent for healing wounds and burns. There are limited indications that propolis from Meliponini can be used in the same way (Velikova et al. 2000).

As a natural product of the bee colony, propolis possesses several biological activities such as anti-inflammatory, immunostimulatory, and antibacterial activity especially against Gram-positive bacteria. This activity is reported to be due to flavonoids, aromatic acids, and esters present in the resin (Marcucci et al. 2001). Ethanol extracts of propolis (EEP) are rich in various flavonoid aglycones, phenolic compounds, sesquiterpenes, steroids, amino acids, and inorganics—including trace—elements (Krol et al. 1993).

Pereira et al. (2003) compared the propolis collected by *A. mellifera* and *T. angustula*, in southeastern Brazil. They found a total of 64 compounds. Both propolis samples were almost entirely comprised of pentacyclic triterpenes, mainly lupeol and lupeol acetate. On the other hand, polar compounds differed in propolis collected by *A. mellifera* and *T. angustula*. They identified seven amino acids (alanine, glicine, valine, isoleucine, leucine, proline, and threonine) from only propolis of *A. mellifera*. The main differences between the two propolis samples were the concentrations of an aldotetrol, characterized as erythritol (1.8% *A. mellifera*, *T. angustula* 4.0%).

Analysis of propolis from *Friesomellita varia*, *M. favosa*, *Melipona compressipes*, *Scaptotrigona depilis*, and *Paratrigona anduzei* in tropical Venezuela revealed a phenolic profile characterized by polyprenylated benzophenones. In the chemical investigation of propolis of *M. compressipes*, *M. quadrifasciata anthidioides*, and *Tetragona clavipes* by GC–MS analysis, diterpenic acids were found in all samples, and their amounts were significant in *M. quadrifasciata anthidioides* and *T. clavipes*. On the other hand, the pentacyclic triterpene β-amyrin was identified as the main component in *T. clavipes*, the flavonoid pinobanksin in *M. compressipes* and aromatic aldehydes in *Melipona quadrifasciata anthidioides*, respectively. Suprisingly, the prenylated benzophenones characteristic of propolis from Venezuela were absent in propolis from Brazil, including the one from *M. compressipes* that was analyzed in both tropical areas (Freitas et al. 2008).

Farnesi et al. (2009) examined the antibacterial activities of several types of propolis, including Africanized honey bee green propolis and propolis produced by meliponine bees. They concluded that these resins have the potential for human and veterinary medicine. Massaro et al. (2011) contrasted the extensive research on therapeutic properties of honey bee propolis with the largely unknown biological and medicinal properties of stingless bee propolis. These authors investigated the chemical and biological properties of polar extracts of cerumen from *Tetragonula carbonaria* in South East Queensland, Australia using GC–MS analyses. Distinct GC–MS fingerprints of a mixed diterpenic profile typical of native bee cerumen were obtained with pimaric acid (6.31 ± 0.97%, w/w), isopimaric acid (12.23 ± 3.03%, w/w), and gallic acid (5.79 ± 0.81%, w/w) tentatively identified as useful chemical markers. Characteristic flavonoids and prenylated phenolics found in honey bee propolis were absent in cerumen of *T. carbonaria*.

39.6 Chemical Composition of Stingless Bee Propolis from Bolivia, Philippines, and Venezuela

39.6.1 Propolis Samples

Geographical origin and time of propolis collection are listed in Table 39.1. Eight propolis samples (Fig. 39.1) were investigated to determine their chemical composition by GC–MS. Three propolis samples were from Venezuela (*M. favosa*), one from Philippines (*T. biroi*), four from Bolivia (*M. grandis*, *S. depilis* and *S. polysticta*).

Table 39.1 Stingless bee species and geographical origin of the propolis samples

Sample no.	Common name	Stingless bee species	Propolis type	Country
1	"erica"	*Melipona favosa*	Hive	Venezuela
2	"erica"	*Melipona favosa*	Hive	Venezuela
3	"erica"	*Melipona favosa*	Hive	Venezuela
4	"kiwot"	*Tetragonula biroi*	Hive	Philippines
5	"erereú barcino"	*Melipona grandis*	Hive	Bolivia
6	"obobosí"	*Scaptotrigona depilis*	Hive	Bolivia
7	"obobosí"	*Scaptotrigona depilis*	Hive	Bolivia
8	"suro negro"	*Scaptotrigona polysticta*	Entrance tube	Bolivia

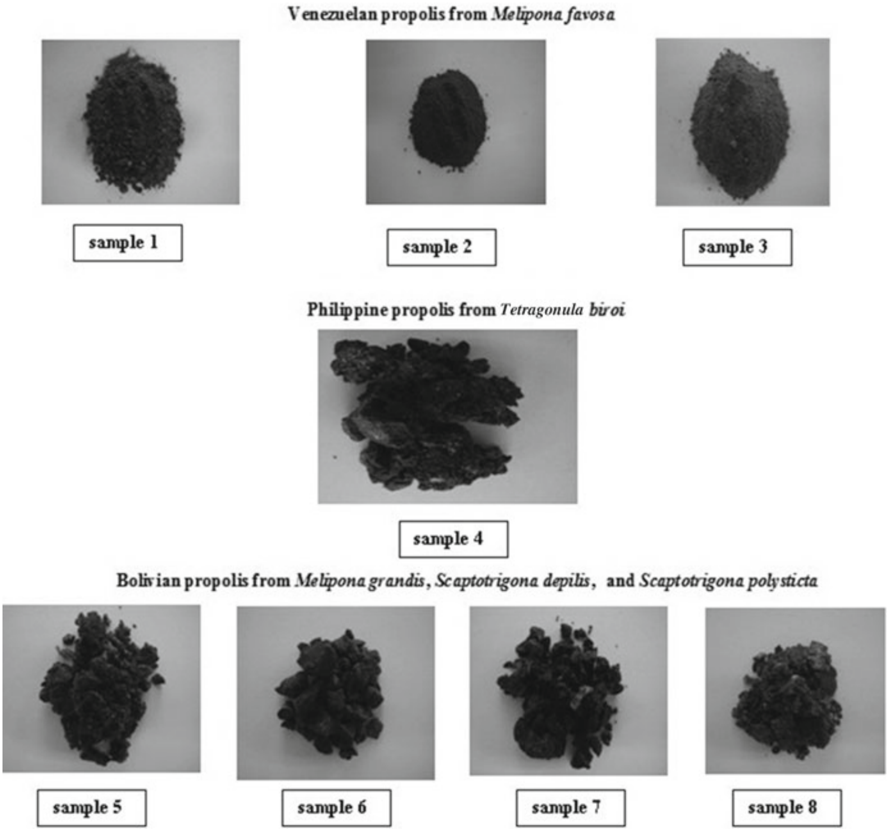

Fig. 39.1 Propolis samples of stingless bees from Venezuela, Philippines, and Bolivia. See Table 39.1 for propolis sample numbers (Photos Omur Gençay Çelemli)

39.6.2 Propolis Extraction and Preparation

Frozen propolis was pulverized and dissolved in 96% ethanol. This mixture was kept in the incubator at 30°C for 2 weeks, in a bottle closed tightly. After incubation, supernatant was filtered twice through Whatman No. 4 and No. 1 filter paper. The final filtered concentrated solution (1:10, w/v), ethanol extracts of propolis (EEP), was evaporated until dry. About 5 mg of residue was mixed with 75 μl of dry pyridine and 50 μl bis (trimethylsilyl) trifluoroacetamide (BSTFA), heated at 80°C for 20 min, then the final supernatant was analyzed by GC–MS.

39.6.3 GC–MS Analysis

A GC 6890N from Agilent (Palo Alto, CA, USA) coupled with mass detector (MS5973, Agilent) was used for the analysis of EEP samples. Experimental conditions of the GC–MS system were as follows: DB 5MS column (30 mm × 0.25 mm and 0.25 μm of film thickness), flow rate of mobile phase (He) set at 0.7 ml/min. For gas chromatography, temperature was kept for 1 min at 50°C and then increased to 150°C with a 10°C/min heating ramp. After this period, temperature was kept at 150°C for 2 min. Finally, temperature was increased to 280°C, with a 20°C/min heating ramp, then kept at 280°C for 30 min.

Organic compounds in samples were identified using standard Wiley and Nist Libraries, available in the data acquisition system of GC–MS, if the comparison scores were higher than 95%, or our own library. For ethanol extracts, instead of internal or external standards, percentage sample compounds were used. This standard was primarily used to identify organic compounds in propolis samples; the error could not be higher than 5% (Gençay and Salih 2009).

39.6.4 Chemical Components of Stingless Bee Propolis

Compounds of aliphatic acids and their esters, alcohols, aromatic acids and their esters, hydrocarbons, and terpenes were identified. In Venezuela, where *Populus* are not native plants, stingless bees and honey bees visit *Clusia* species in order to collect a resin excreted in a ring at the bases of their flower stamens. As a consequence, the chemical composition of both tropical propolis and stingless bees' propolis is particularly characterized by the presence of polyprenylated benzophenones, in accordance with the chemical constituents identified from *Clusia* flowers (Tomás-Barberán et al. 1993; Freitas et al. 2008). But in our Venezuelan samples from *M. favosa* too few compounds were observed. Particularly in two samples we could not find any compound. In the third sample, 6,6,10-trimethyl-1-phenylthiospiro (3.6) dec-1-ene, a hydrocarbon, was the only compound identified. Due to these results we can say that these samples can be only clay, earth or soil, and did not include resin.

The Philippine propolis ethanolic extract of *T. biroi* (sample 4, Fig. 39.1) contained aliphatic acids and their esters, alcohols, carboxylic acids and their esters. Terpenes also were observed. From aliphatic acids and their esters group; ethyl oleate, octadecanoic acid, ethyl ester, hexadecadien-1-ol acetate, linoleic acid ethyl ester, and ethyl tridecanoate compounds were identified. From these compounds, ethyl oleate showed the highest ratio of 4.51%. The *T. biroi* propolis (sample 4, Fig. 39.1) had lower terpene content than the propolis from Bolivia (samples 5–8, Fig. 39.1) but higher than the propolis of *M. favosa* (samples 1–3, Fig. 39.1) without terpenes. According to Table 39.2, we can say higher aliphatic acids and their esters group ratio could be a marker for Philippine propolis. However, there is a preliminary observation for Philippine propolis because we could investigate only one sample.

Half of the propolis samples (samples 5–8) were collected by different bee species (Table 39.1) from Bolivia (Fig. 39.1). In these propolis we observed aliphatic acids, alcohols, carboxylic acids and their esters, hydrocarbons, and terpenes. A common trait of the Bolivian propolis was the presence of terpenes in high ratios

Table 39.2 Chemical compound groups determined in the Meliponini propolis types

	Propolis types[a]							
Compound groups	1	2	3	4	5	6	7	8
Aliphatic acids and their esters	–	–	–	5.48	2.35	3.62	0.34	0.06
Alcohols	–	–	–	0.45	5.64	0.06	4.89	22.22
Carboxylic acids and their esters	–	–	–	1.69	–	2.09	0.03	0.08
Hydrocarbons	–	–	10.17	–	–	3.47	0.34	0.35
Terpenes	–	–	–	3.72	20.91	45.37	39.99	6.92

[a] See Table 39.1 for the stingless bee species that collected the propolis

(6.92–45.31%). The highest terpene ratio was observed in sample 6, from *S. depilis* (45.37%) and sample 7, from *S. depilis*, collected inside the hive (39.99%).

Aliphatic acids and their esters, as we found in our study (see Table 39.2), are known for stingless bees (Velikova et al. 2000). From this group we found octadecanoic acid, as Pereira et al. (2003) found in propolis of *T. angustula*. Similiar to our results, in previous studies flavonoids were not observed in propolis of stingless bees (Massaro et al. 2011). These authors compared cerumen of *T. carbonaria* and propolis of *A. mellifera* and suggest that gallic acid and diterpenic acids of the pimaric and abietic type are chemical markers of *T. carbonaria*. Cinnamic acid, monosaccharide, gluconic acid, fructose, β-glucose, p-coumaric acid, and monosaccharide were present in both types of propolis. However, other propolis constituents such as p-hydroxybenzoic acid, hydroxybenzoic ester, monosaccharide, ferulic acid, caffeic acid, pentenyl ester iso-ferulic acid, pentenyl ester caffeic acid, pentenyl ester caffeic acid isomer, pinostrobin, pinocembrin, sterol, cinnamic acid ester, dihydroxy-2-methyl-anthroquinone, and galangin were not found in *T. carbonaria* cerumen. The researchers found mainly pimaric acid, isopimaric acid, and gallic acid in the cerumen of *T. carbonaria*. We did not find any of these compounds.

We found the terpene delta-cadinene in two Bolivian samples from *Scaptotrigona* (samples 6 and 7). Patricio et al. (2002) observed this compound in the tibia of *F. silvestrii* and *F. varia* (Patricio et al. 2002). Another terpene observed in our results was germacrene D. This compound was found in the tibia of *F. varia* in previous studies (Patricio et al. 2002). We also observed mostly terpenic compounds. From this chemical group, similiar to our results, gamma-Terpinene, α-Amyrin, α-Caryophyllene, β-Amyrin, α-Gurjunene, α-Copaene were identified in previous studies (Freitas et al. 2008; Patricio et al. 2002). From terpenes, β-Amyrin was mostly observed in propolis of stingless bees in previous studies. Patricio et al. (2002) isolated β-Amyrin from the tibia of *F. varia*. Furthermore, Freitas et al. (2008) identified β-Amyrin as the main component of geopropolis of *Tetragona clavipes*, and Massaro et al. (2011) found this compound in cerumen of *T. carbonaria*. We observed β-Amyrin only in *S. depilis* (sample 7) but with a higher ratio (11.75%). With regard to our GC–MS results, we can say that the Bolivian ethanol extracts of *Scaptotrigona* have a richer and more concentrated chemical spectra than the *M. favosa* from Venezuela and the *T. biroi* from the Philippines. Therefore, the thesis that different bee species use different plant sources to collect propolis is potentially correct, but needs much further study and corroboration.

We also analyzed a commercial tincture of Bolivian stingless bee propolis. It presented major chemical differences when compared to the four Bolivian propolis samples as mentioned in Table 39.2 (samples 5–8). It presented fewer chemical compounds and the presence of sugars. We identified compounds from aliphatic acids and their esters with a minor amount (0.89%), hydrocarbons (1.42%), terpenes (6.39%), and sugars with quite higher amount (11.38%). From sugars only the compound Ethyl.alpha.-D-glucopyranoside was observed. Also we did not identify any alcohol compound in the commercial ticture like the other samples (samples 4–8). Most probably it is an alcohol-free product. We can argue that the dissimilarity of the propolis tincture from the Bolivian propolis analyzed here can be traced from its being mixed with other ingredients by its producers. They possibly added a sugar compound to make it sweeter. Also, the sugar content of the tincture can be attributed to contamination of propolis by honey. Besides these some producers put some plant extracts in these kind of products.

According to previous work, propolis of *A. mellifera* shows a wide spectrum of chemical compounds. Generally, alcohols, acids, aldehydes, fatty acids, hydrocarbons, flavonoids, and terpenes form the chemical composition of propolis from *A. mellifera*. Flavonoids are found in high ratios in propolis. The high amount of flavonoids provides antioxidant activity (Gençay and Salih 2009). However, in propolis samples that we investigated, we could not find flavonoid components.

39.7 Conclusions

The Kaur-16-ene (8.beta.13.beta.), Olean-12-ene (β-Amyrene), 3-KETO-URS-12-ENE found in our samples are new constituents for stingless bee propolis. Kaur-16-ene (8.beta.13.beta.) compound is found in *S. depilis* (0.24%), Olean-12-ene in *M. grandis* (4.07%), and 3-KETO-URS-12-ENE in *S. depilis* (21.66%). All new constituents belong to the Bolivian propolis.

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Part VI
Marketing and Standards of Pot-Honey

Chapter 40
Production and Marketing of Pot-Honey

Rogério Marcos de Oliveira Alves

40.1 Introduction

A light yellow liquid, translucent, sour and slightly sweet. That description characterizes the honey of indigenous stingless bees or native honey-making bees in tropical America (Nunes 2009). The honey has a flavor different from that of Africanized bees used for beekeeping today, and was noticed by the early settlers of America when savoring honey that the native people used as a natural sweetener, medicine, and in religious rituals. Honey in the Neotropics came from stingless bees, before introduction of the Western honey bee, *Apis mellifera* (Kerr et al. 2005). This delicacy, found in different parts of Brazil and elsewhere, is still not well known to urban connoisseurs. Stingless bee honey carries a universe of components that go well beyond the traditional product of beekeeping. Another wealth is revealed when the honey is put in the mouth: an impressive array of flavors. Acidity, floral aromas and earthy notes are provided in honey from bees such as "jataí" and "tiúba," among other stingless bees (Marques 2010).

Although hundreds of bee species are known to make honey in the Americas, the entire consumption of honey in America is focused on exotic *A. mellifera*, considered the most productive per colony. However, this perception is changing, the market is becoming more selective, and now wants information on products it consumes. This means flavor, aroma, bouquet, and composition from bees such as "jataís," "uruçús," "tiúbas," "canudos," and "mandaçaia." Stingless bee honey occupies a niche market with diverse value, added from natural sources of honey production.

A major concern of the world market is the total elimination of waste and toxic antibiotics in honey, and organic farming is the most promising strategy to market

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such honey. The marketing of honey labeled “socially fair” is also known as “fair-trade,” with prices paid above the average market value to help poor communities that have a role in conservation (Paula Neto and Almeida Neto 2005). Honey of native species has the right profile for this segment because its production is developed in regions free of pesticide residues and is also free of antibiotics added by the beekeeper. The labor employed is from poor communities with low environmental impact. The product is stored in pots made with propolis and wax (meliponine cerumen). There are species of high productivity well-suited to fill a niche that includes only 3% of world trade (IBCE 2010).

40.2 Stingless Bee Species and Production of Pot-Honey

Among hundreds of native bee species, some produce honey to satisfy the nutritional needs of the colony, others produce an excess available for humans. Only a few of them are excellent honey producers, like *Melipona*, with species of great potential and widely kept in Tropical America.

The best known pot-honeys are produced by “mandaçaia” (*Melipona quadrifasciata*), “jataí” (*Tetragonisca angustula*), “jandaíra” (*Melipona subnitida*), “papaterra” (*Melipona asilvai*), “canudo” (*Scaptotrigona* sp.), “tiúba” (*Melipona compressipes*), “uruçú verdadeira” (*Melipona scutellaris*), “uruçú amarela” (*Melipona rufiventris*), “xunan cab” (*M. beecheii*). Some, like “jataí,” are widely distributed. Others—“papaterra” and “jandaíra,” are more restricted to some habitats, and live in savannas known as “caatinga” (Lopes et al. 2005).

Few stingless bee species have been explored in all their technical potential, needed to increase pot-honey production. Stingless bee keeping should be optimized by bee management, genetic control and promotion of bee plant cultivars. The evaluation of meliponine honey production (Table 40.1) is difficult due to traditional practice differences. The colonies are mostly kept in logs or boxes (literally “tenements”), without management and inspection, insufficient forage, and rudimentary techniques of honey harvest. Therefore, it is easy to envisage greater honey production with adequate management. Estimated pot-honey annual yields were collected personally visiting meliponaries, and by searching the literature.

Villar (2002) estimates that 4,000 tons of stingless bee honey is produced in Brazil every year, a volume ten times lower than the national production of 42,000 tons of total honey. About 1 ton of pot-honey is “Paulista” (produced in São Paulo), with the remarkable contribution of “jataí.” However, during visits to producers, the author estimates that harvests of native bee honey reach 100 tons per year.

Research conducted by Londono (2011) using the internet, with 35 meliponiculturists, found that 40% of the producers were devoted to honey for sale, but only one was a full-time stingless bee keeper. This activity can be promoted by professional efforts. Well-supported stingless bee keeping may lead to high pot-honey yields, reduction of costs and greater benefits for the producer. The practice of migratory meliponiculture apparently increases honey production some 300%.

Table 40.1 Country of origin and estimated honey production by native stingless bees

Country	Species	Pot-honey annual yield
Australia	*Trigona carbonaria* (*s.l.*) = *Tetragonula carbonaria* *Austroplebeia australis*	1 kg (Wikipedia 2011)
Brazil	*Melipona asilvai*	1 l (Carvalho et al. 2003)
Brazil	*Melipona fasciculata*	3–4 l (Magalhães and Venturieri 2010)
Brazil	*Melipona flavolineata*	2–3 l (Magalhães and Venturieri 2010)
Brazil	*Melipona mandacaia*	2.0 l (Carvalho et al. 2003)
Brazil	*Melipona quadrifasciata anthidioides*	2.0 l (Carvalho et al. 2003)
	Melipona quadrifasciata quadrifasciata	2.0 l (Carvalho et al. 2003)
Brazil	*Melipona rufiventris*	3.0 l (Carvalho et al. 2003)
Brasil	*Melipona scutellaris*	2–15 kg (Alves, personal observation)
Brazil	*Melipona subnitida*	2.5 kg (Bezerra 2002)
Brazil	*Scaptotrigona*	3.0 l (Carvalho et al. 2003)
Brazil	*Tetragonisca angustula*	1.0 l (Carvalho et al. 2003)
Costa Rica	*Melipona* "*fasciata*" = *M. costaricensis*	2.5 kg (Cortopassi-Laurino et al. 2006)
Costa Rica	*Melipona beecheii*	2.5 kg (Cortopassi-Laurino et al. 2006)
Indonesia	"*Trigona*" (*s.l.*)	1 kg (Soekartiko 2011)
Mexico	*Melipona beecheii*	2.5 kg (Cortopassi-Laurino et al. 2006)
Paraguay	*Scaptotrigona*	3.0 l (Carvalho et al. 2003)

40.3 Marketing of Meliponine Honey

The world production of honey has increased 4.6%, honey export increased 35.6%, and honey import 38.8%, during 2002–2003. This fact means that lucrative external markets caused a decrease of internal honey consumption in several honey producing countries. Brazil doubled the value of honey exports in 2008, with a record of US$ 43.57 million, increasing by 42% the revenue of 2007, US$ 21.2 million. In 2010 Brazil increased its export by 54%. The exported honey volume also increased, from 12,900 to 18,270 tons in 2010 (IEA 2005).

The world market of meliponine honey is still in its infancy and restricted to particular initiatives in Brazil, Mexico, Costa Rica, and Australia, with regional impact. According to the IBCE (2010), current tendencies in developed countries consider consumer attitude and preference toward organic honey and special honey. This interest to consume organic products is caused by consumer awareness of environmental protection, causing an increase in organic honey demand.

Therefore meliponine honey is becoming better known while production increases. Pot-honey is becoming more familiar and consumed for its singular features and is widely appreciated, considered as an artisan bee product with organic origin, produced in natural environments of tropical nature. In Fig. 40.1 some pot-honey packaging in sachets, bottles, and ceramic.

Fig. 40.1 Commercial presentation of pot-honey. (**a**) Sachet presentation of Brazilian honey produced by *M. compressipes*, known as "tiúba" in Maranhão, (**b**) bottled *Scaptotrigona* honey also from Brazil, (**c**) bottled Australian honey produced by "sugarbag" *Tetragonula carbonaria*, (**d**) jar of "urucú," bottle with artisanal cover of "tiúba" from Brazil, jar of "pisilnekmej" *Scaptotrigona mexicana*, from Mexico, and ceramic "puño" to bottle Bolivian honey produced by "suro negro" *Scaptotrigona polysticta*. Photos: (**a-b**) R.M.O. Alves, (**c**) T.A. Heard, (**d**) P. Vit

40.3.1 America

Consumption of pot-honey in Mexico is as old as food, but mainly as medicine (see Chap. 15) and for use in religious rituals. However, due to low productivity of the colonies, the production is consumed mainly by the stingless bee keeper and the local community (Maganã 1998). The growth of beekeeping has caused a disincentive among farmers to raise native bees, but there is a movement now to preserve traditional stingless bee keeping. The bees considered best for honey are *M. beecheii* and *Scaptotrigona* (Quezada-Euán 2005). In Central America there are *M. beecheii* and *M. fasciata* (currently known as *M. costaricensis*, *M. panamica*, *M. melanopleura*, and others, Roubik D, personal communication) primarily in Costa Rica (Wikipedia 2011).

In South America, economic growth led to increased purchasing power and providing better education, which also increased the consumption of honey, no longer an unusual product in daily diet. Currently the market for bee products experiences tremendous growth, fueled mainly by exports and improvement in the internal market (Koshiyama et al. 2011). In Brazil, the consumption of honey from stingless bees is still small mainly due to availability constraints rather than ignorance about the product. In the north and northeast, despite low availability the honey is very popular and consumed often. However, the increase in consumption is subject to quality improvement and increased honey production. Meliponiculture is less developed in the south and southeast of the country; initiatives there are aimed at conserving colonies, except for the State of Paraná with the keeping of "jataí" (*T. angustula*) and "mandaçaia" (*M. quadrifasciata*) whose objective is the production of pot-honey (Laginsky 2011).

40.3.2 Africa, Asia and Australia

No quantitative information is available for most of Asia. However, like Asia, Australia has no large bee like *Melipona*. In Australia, *T. carbonaria* and *Austroplebeia australis* are the main native honey sources (see chapter in present book). According to Klumpp (2007) a hive of Australian stingless bees produces <1 kg per year. The product is sold in jars of 50 ml at a price of AU$ 30.00 for consumers in urban centers, where it is appreciated for its taste and strong acidity.

There is certainly a growing interest in meliponiculture in Africa (Kwapong et al. 2010). As Kajobe indicates (see chapter in present book) there is information being gathered on the biology of stingless bees, and the management techniques, many of them gleaned from work in the Neotorpics; there will soon be much more data on practices and commercial preference in pot-honey consumption.

40.3.3 Production and Consumption of Pot-Honey

The market for native honey experienced an increase in recent years, accompanying the increase in consumption and insufficient production of honey of *A. mellifera*, especially in the greatest traditional Brazilian beekeeping areas (North–Northeastern Brazil).

Traditionally known as the greatest producer of native honey, the Northeast serves as the development center, with the largest pot-honey producers located in the states of Maranhão, Bahia, Rio Grande do Norte, Pernambuco, and Piauí. This large region has highlighted the technical aspects of meliponiculture specialists who obtain the highest productivity per colony. Meanwhile the Northern Brazil states of Amazonas and Pará have outstanding potential, both in number of species and

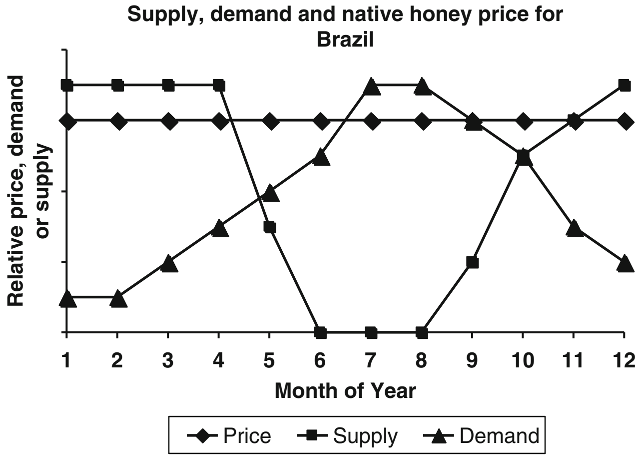

Fig. 40.2 Market behavior, variation in supply, demand, and price of pot-honey

production. The investments made by government and the private sector foster studies and projects that expand the number of species and honey production.

Honey is marketed regionally and considered a seasonal, handcrafted or "artesinal" product. The statistics on colony productivity are estimates, mainly due to lack of product regulation and mode of regional marketing, without official records. Data collected on trips to interview beekeepers allows one to sketch a graph of market behavior of honey from stingless bees in the main producing regions of Brazil (Fig. 40.2).

The consumption of honey from native bees is expanding, driven by the appeal health, social "fairness" and product appearance. With the range of color from white to light amber, honey from native species has a very strong appeal in consumer preference. Other factors that enhance value in production are a pollution-free environment and income, observing principles of sustainable environment.

Oliveira et al. (2005), based on data analysis and research conducted in the State of Pernambuco (Brazil) observed that 86% of respondents said they had consumed honey. Of those, about 70% claim to eat honey from bees of the genus *Apis* while 30% consumed honey from native bees. In Bogotá (Colombia), a recent survey revealed that almost 70% of consumers would not buy pot-honey produced by *Tetragonisca angustula* because it is unknown. Indeed, consumers of "angelita" honey in this capital city are some 80% middle class of socioeconomic strata 3 and 4 (Rodríguez Reyes 2007).

Frequency of native bee honey consumption showed around 8% on a monthly basis and 92% annually. When questioned as to use, the predominant response was that this was primarily used as a sweetener and folk remedy (Oliveira et al. 2005). Seasonality and unfamiliarity of the consumer with product characteristics constitute obstacles to increasing the consumption of pot-honey, as visualized in Table 40.2.

Table 40.2 Periods and causes guiding consumption according to pot-honey supply in north and northeast Brazil

Month	Supply	Causes
January to March	Production and high supply	Hottest time of year Habit of low traditional use Economic factors, school and other costs, fairs, festivals Lack of information on benefits of honey
April to June	Little supply	Cool and rainy weather High honey consumption (folk remedy use) Honey crystalization, difficult to consume
July to September	Lack of supply	Very cool and rainy weather High folk remedy consumption
October to December	Little supply	Beginning of the hot season Reduced consumption related to heat

From: Alves, personal observations during 30 years

It is important that price remain stable throughout the year. The large difference between supply and demand causes disincentive to the farmer, who needs guidance on how to reduce the effects of factors that create this relationship.

40.3.4 Cultural Aspects of Pot-Honey Consumption

Vilckas et al. (2001) reports that the frequency of honey consumption decreases in lower social strata. In the case of low-frequency, they argue that it is lack of custom, it is fattening, or they do not care for it, while some believe it increases their blood cholesterol, is too expensive a product, or is superfluous. Individuals in higher strata can be more knowledgeable and look for special honey types, such as unifloral and organic honey. However, pot-honey is better understood, from direct experience, in rural and forest villages.

Native honey is well known to consumers in rural areas, usually people with lower incomes and little education, but for whom cultural factors are of great importance. Families generally maintain colonies in the yard for use in remedies as needed.

In Bolivia, the honey of stingless bees, in addition to food characteristics, is widely used in traditional medicine to treat eye diseases, and respiratory and digestive maladies (IBCE 2010). Honey is characterized as a remedy, is consumed only in times of onset of colds and respiratory infections, yet in these times the production is lower.

Attempts at honey processing using dehumidification caused an unusual situation. Accustomed to native bee honey as very fluid (less viscous) and sour, processed honey was initially refused because of similarities with the honey of *A. mellifera* (dense and sweet). Whereas honey of *Apis* might be eaten with a spoon, that of stingless bees is often drunk as though a liquor.

Another important feature that restricts consumption is the crystallization of some honey that leads consumers to not accept the product, claiming it has been adulterated by addition of sugar. However, in southern Brazil the crystallized honey of *T. angustula* and *M. rufiventris* is usually consumed.

40.3.5 Low Production and Seasonality of Pot-Honey

The low productivity of colonies is a significant consideration in the marketing of honey from stingless bees. Due to lack of technology to get the most of the colonies the producer realizes an average of 1 l per hive per year in species that have potential for 10 l per hive per year, as in *M. scutellaris*. In Manaus, *M. fasciculata* productivity reaches 3 kg per hive per year. In the state of Rio Grande do Norte meliponines produce about 2.5 l (Vollet Neto et al. 2011). A group of 30 native bee hives produces on average (depending on the flower) 5 pounds of honey per hive, totaling 150 pounds of honey from native bees in a year (CESMAG/COIMP 2007). The lack of product is a factor discouraging both the grower and the consumer and causes disruption in the consumption process.

The major producing regions and greatest number of stingless bee species are in the humid forest biome with a dry season during the months at the end and beginning of the year. In the savanna biome (arid) and in the cerrado, production is greater in mid year, when the rains are reduced. Production of pot-honey is fundamentally different in its timing in the two regions. However, when human discomfort and virus-incuded colds are most frequent—leading to a consistently high demand for pot-honey as a remedy for sore throats and colds, tends to be seasonal and often associated with the beginning or end of the rainy season. Thus, the cerrado and the moist forest somewhat complement each other in the timing of honey production or demand.

40.3.6 Quality of Pot-Honey

Methods of destroying the pots when turning the hive or box supers over to allow drainage into a sieve produce honey of lower quality, constituting a barrier to consumption in larger population centers. The honey harvest is done by piercing or squeezing the pots of honey and pollen, without the need for personal hygiene preparation, now required for the SIF (Federal Inspection Seal), i.e., requirement that harvest is done in a sterile place that is clean and has well ventilated facilities, and includes a sink (Melo 2010).

Use of modern technology for honey extraction—such as a suction device—is frequent in Brazil. This allows improvement of product quality, as regulatory governmental bodies have been aiming to increase honey consumption. A major obstacle to honey of native bees in world trade is that the Codex Alimentarius Commission

Table 40.3 Price of honey according to the bee species and location of production

Bee species	Price (USD/kg)	Price (BR$/L)	Locality
Melipona asilvai	7.00–10.00	20–30	Bahia
Melipona compressipes	10.00–18.00	30.00–50.00	Maranhão
Melipona fasciculata	7.00–9.00	20.00–25.00	Pará
	7.00	20.00	Manaus
Melipona flavolineata	10.00–13.00	30.00–40.00	Piauí
	9.00–12.00	25.00–35.00	Amazonas and Pará
Melipona mandacaia	7.00–12.00	20.00–35.00	São Paulo
Melipona mondury	10.00–18.00	30.00–50.00	Bahia
Melipona quadrifasciata	9.00–21.00	25.00–60.00	Bahia
Melipona scutellaris	10.00–18.00	30.00–50.00	Bahia
	25.00	70.00	Alagoas
	35.00	100.00	Pernambuco
Melipona subnitida	7.00–10.00	20.00–30.00	Bahia
	12.00–18.00	35.00–50.00	Rio Grande do Norte
Tetragonisca angustula	21.00–32.00	60–90	Bahia/Paraná
Scaptotrigona sp.	7.00–10.00	20–30	Bahia/Pará

From: Alves et al. 2005 (updated 2011)

only recognizes honey produced by *Apis* (Vit et al. 2004; Quezada-Euán 2005; Souza et al. 2006), and pot-honey chapters in the present book (Vit, Almeida-Muradian, Fuenmayor et al., Dardón et al., Ferrufino and Vit).

40.3.7 Cost-Value-Price

The value of honey in the market is a function of quality, presentation, and more recently, certification as organic produce, which adds value and may raise the price by 50%. The price of honey varies according to the site and producing species (Table 40.3).

The price of honey produced by stingless bees can reach a value up to 1,100% higher than the common honey, ranging between BR$40 and BR$100 a pound, against BR$3 a pound of honey from the traditional *A. mellifera* (Villar 2002).

When asked about the value of a liter of honey, consumers shopping at “Garanhuns” in the State of Pernambuco indicated an average of BR$ 15.00 per kg as the ideal price for genuine honey of *A. mellifera* (Oliveira et al. 2005). The short supply of native honey places the product on the market with values that may exceed BR$ 30.00 per kg. In Manaus, 1 kg of honey costs BR$ 20.00 and production is 3 kg per hive per year (Portal Extraction 2011). In Maranhão prices range from BR$ 6.00 to BR$ 18.00 for packages of 200 g (INVESTENE 2011). On the west coast of the Yucatán Peninsula the price of honey from *M. beecheii* ranges from USD 2.00 to USD 15.00 per liter (Maganã 1998).

Alves et al. (2005) reports that the difficulty in calculating the price of honey stems from the lack of standardization of protocols, which prevents establishment of basic pricing. Although stingless bee keepers sustain no losses by not marketing their product, the expansion of production could force down the price. As costs for maintaining the bees are low, the activity allows the production of a relatively inexpensive food with a strong commercial appeal (CESMAG/COIMP 2007).

40.3.8 Vending Locations of Pot-Honey

The low yield associated with lack of regulation affects consumption of the product. Currently the production is sold "directly from the hive" or as on-site production, usually in the home, place or establishment of the producer, leading to a special relationship where trust in the product is more important than the amount paid. Another factor is the lack of registration by the government, which otherwise would allow honey to be sold at all pharmacies and supermarkets, considered by Magalhães et al. (2007) to be places of greater access to the product by buyers.

40.3.9 Packaging

In producing regions honey is sold in glass or plastic containers with a capacity of 700–1,000 ml. Glass jars are the best and most suitable, but plastic predominates because it costs less. Modern beekeepers use narrow or wide mouth jars with a capacity of 50, 150, 200 or 500 ml, allowing higher consumption, better product presentation and higher added value. In Maranhão, Vilckas et al. (2001) found honey of "tiúba" (*M. compressipes*) sold in glass containers with a capacity of 205, 315 and 460 g.

40.3.10 Legislation

The lack of regulation of native bee honey makes it impossible to trade efficiently, hindering consumer access to the product and discouraging activity. In Brazil there are initiatives for the characterization of honey in order to provide benefits for its regulation. In the state of Bahia, Brazil the legislation for pot-honey marketing is already in the planning design stage. To my knowledge, as informed by Vit P (see her chapter on *M. favosa* honey in this book), the information provided by Nates-Parra G during the regulation process for honey in Colombia included pot-honey produced by native bees in the annex (ICONTEC 2007) after the publication of their suggested standards (Souza et al. 2006).

40.4 Strategies to Increase the Production and the Consumption of Pot-Honey

40.4.1 Production

The production process in animal husbandry obeys the equality of the variables feeding-management-genetics. This implies that nourishment is provided in times of shortage of flowers, using deployment and improvement of “bee pastures,” performing management operations periodically, and performing selection of the best queens so that the producer is able to get the maximum possible production, and with lower costs.

The deployment of meliponicultural “grazing” contributes to increased production and also to improving honey quality. The supply of trophic resources (nectar, pollen and resin) comes from existing plants, and maintenance of native species should encourage the beekeeper to introduce flowering plants recognized for increasing nectar production potential of the pasture. Unlike the honey bees, stingless bees have not such an extensive foraging area (Roubik 1989) thus improvement of available floral resources is a possibility that can be pursued profitably by an individual beekeeper. Otherwise, spreading plants that are profitable has a minimal impact on bee forage within foraging range.

The utilization of efficient, timely and low-cost, hive inspection, calendars of beekeeping activities, control of enemies, colony division, equipment for harvesting and processing of honey allow for increased production through organization of time and less waste of product. The choice of the best queens allows better development of the colonies for more efficient storage of honey.

The supplementation of nectar and pollen through the use of artificial food allows maintenance of colonies to be standardized, reducing losses and allowing more efficient management of colonies.

40.4.2 Consumption

The current trend in developed countries, especially in the European Union includes consumers seeking organic and other special honey. For such consumers honey is a natural product, pure and healthy. It is a natural product that has several properties which improve health and has always been valued for its therapeutic qualities (IBCE 2010).

There are several strategies to expand markets for honey of stingless bees, such as apitherapy, to stimulate consumption in various areas, greater media coverage, and reducing the price to the consumer (Paula Neto and Almeida Neto 2005). In addition, the use of standardized packaging—with labels and information—participation in fairs, exhibitions, and publicity in schools all are excellent tools for effective marketing and merchandising.

40.4.3 *Cooperative Marketing of Pot-Honey*

Established in regions distant from the consumer centers, perishable and seasonal native bee honey production forces the producer to seek other markets through trade shows and exhibitions. Marketing honey in smaller packages can increase income and encourages consumption with greater frequency.

In the state of Rio Grande do Norte, a stingless bee keeper sells about 300 gallons of honey within the state only in packages of 200 ml (Lopes et al. 2005). In a survey conducted by the author, the honey sold in packs of 1 l is consumed within a year by family of three. The pack split into small sachets of honey is the best method for stimulating consumption and reaching mainly children—future consumers of honey.

A strategy to increase consumption is to form associations or cooperatives of producers, making it easier and less costly to disseminate information and increase consumer confidence in the product. For an individual to produce and market a product is extremely difficult, even if they are a great producer. It may be that small producers, associating with each other, will accomplish the task. It may be an association or a group that shares the same interest (Melo 2010).

40.4.4 *Processing and Storage of Pot-Honey*

The use of effective preservation methods provides honey quality insurance and allows longer shelf life. Currently used methods are refrigeration, maturation, pasteurization, and dehumidification (see Chap. 10), which conserve physicochemical and organoleptic properties (Alves et al. 2007). This activity could facilitate regulation by government agencies.

40.5 Major Initiatives of Pot-Honey Production in Brazilian States

Maranhão—Commonly found in the State of Maranhão, the culture of tiúba proved viable commercial and socially. Each year, a colony can produce up to 300 kg of honey. In each community there are about ten families of “meliponicultors” as stingless bee keepers are called (INVESTENE 2011).

Generating income, promoting social inclusion and preserving native species, meliponiculture with the tiúba bee (*Melipona compressipes*) has changed the lives of 18 communities in the semiarid region of Maranhão. The project was called “Native Bees,” developed by Maranhão for natural conservation and the Federal University of Maranhão since 2001 (INVESTENE 2011).

The commercial manager of the Cooperative Agroecological Meliponary “Baixada Maranhaense,” Luis Pedro, reports that since 2005 a project was implemented aimed at increasing production and quality of honey from *M. compressipes*.

In 2011 there were 12,000 colonies in honey production. They produce 15 tons annually, sold in the regional market and in part in fairs, exhibitions and events across the country.

Amazon—Honey production is quite impressive, though very large distances and logistic difficulties hinder the flow of the product and marketing. The number of colonies is about 80 colonies per individual stingless bee keeper, with a production of 2 kg annually per colony. Projects involving government agencies and associations have the objective of significantly increasing production and selling abroad as well as helping residents to find sustainable income. A liter of pot-honey produced by the community has a value of BR$ 40, but can reach BR$ 80. In each village 150 hives are maintained and each produces an average of 3 l of honey, which is little. This is because, apart from being marketed, it is also consumed by the indigenous people (INOVABRASIL 2011).

Bahia—In the State of Bahia honey production is sourced from producers possessing few colonies, focused on productivity and honey quality. The largest producers are in the central, north, and northeast part of the state, with a mean of 30 colonies per producer and 2–5 l per hive per year. The bee species used are *M. mandacaia*, *M. quadrifasciata*, *M. scutellaris*, and *M. mondury*.

Paraná—In 2007 the Breeders Association of Native Bee Conservation Area Guaraqueçaba (Acriapa) was created. We are already in the third honey harvest. The first two occurred in the summer of 2007–2008 and in late 2008 they were very modest, 30 and 40 pounds, respectively. The last harvest, in February, was 130 pounds, considered very good. The product is currently being sold in bottles of 65 g, priced at BR$ 7.00. According to our calculations, it is estimated that the stingless bee-keeper with the highest production should earn BR$ 1,200 annually (Laginsky 2011).

Rio Grande do Norte—Paulo Menezes is one of the largest suppliers of pot-honey to supermarkets and retail chains in the region. The stingless bee product is sold for up to BR$ 60.00 a gallon, compared to an average of BR$ 5.00 for *A. mellifera* honey. In 2004, Menezes produced 300 l of honey, an average of 1 l per hive. The entire product was sold to supermarkets in Rio Grande do Norte and Fortaleza, and to buyers from Brasilia and Rio de Janeiro. The sales yielded no less than BR$ 18,000 a year. “If you divide by 12 months, it was an income of BR$ 1,500 per month,” says the producer, satisfied with the result (SEBRAE 2005).

Rio Grande do Sul—Stingless bee keeping for pot-honey production is already a reality for family farmers in the Sun Valley Center for the Support of Small Farmers. The bee leading the way is *Tetragonisca angustula*, which is popularly called “jataí.” Beekeeping there allows diversification and can be integrated into forest plantations, fruit and food crops, also contributing to the increase of agricultural production. Recently 1.5 pounds of honey has been harvested per colony (Mezziga 2011).

The stingless bee keeper João Batista Ferreira, in the municipality of Belterra, Pará, is testimony to traditional knowledge and the conservation of Meliponini. Currently, “Mr. John” manages 23 species of stingless bees with an average production, among them, ranging from 0.5 to 5 kg per hive per year. The main producing species are *M. fasciculata* and *Scaptotrigona*. Beekeeping contributes a significant part of family income (Ferreira and Rebello 2005; Lopes et al. 2005).

Sergipe—Bee keeping is encouraged in communities throughout the state. Courses and meliponary community building are the means used to organize and improve food quality, and generate income for residents in rural areas. The honey produced is totally consumed by the regional community and marketed in the same establishment.

40.6 Concluding Remarks

Meliponiculture is a fundamental activity that maintains communities by revenues and improvement in quality of local products. Joint public and private strategies are needed for channel marketing to get pot-honey into the customer's hands. Increased pot-honey demand will benefit meliponiculture. Integrating modern technology with traditional methods, and merchandising of native bee honey, promotes native species use and socially fair policies with a consumer product.

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Appendix A
Taxonomic Index of Bees*

*The names of species (or subspecies) were organized by the specific (or subspecific) epithet (e.g., *Apis mellifera* must be searched as *mellifera*, *Apis* and *Apis mellifera scutellata* as *scutellata*, *Apis mellifera*). Species referred as sp. or spp. are indicated only by the generic name (e.g., *Anthophora* sp. appears only as *Anthophora*). Also, species named approximately to another one, mentioned as *affinis*, near, sp. gr., etc., can be found in the name of the affined species (e.g., *Scaptotrigona aff. depilis* appears as *depili*s, *Scaptotrigona*). Names in bold are junior synonyms (senior synonyms are indicated in brackets).

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Appendix B
List of Bee Taxa

In this entry of bee taxa by genus, after the taxonomic index of bees, countries mentioned in this book are given. Broad distribitutions of the taxa are not included in this list, e.g., Table 5.1 in the French Guiana chapter. Names in bold are junior synonyms (senior synonyms are indicated in brackets or square brackets).

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Appendix C
Common Names of Stingless Bees

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Appendix D
Taxonomic Index of Plant Families

Plant uses or mutualisms, thought to include but not restricted to: Mayan medicinal use (M), nectar (N) excluding extrafloral nectar, pollen (P), pollen only (PO) certain species or genera nectarless, trichomes (T) or resin source (R), used to make honey (H), build nests (B), or visited for nectar and/or pollen by stingless bees (S), and *Apis mellifera* (A). In parenthesis total number of genera and species per family.

(691 morphospecies distributed into: Families = 125, Genera = 437, Species = 611, Varieties = 1)

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Mimosa PO, S, A, 306, 308, 310, 312, 339, 344
Mimosa acutistipula PO, B, S, 329
Mimosa bimucronata PO, S, 321
Mimosa caesalpineifolia PO, S, 589
Mimosa casta PO, A, 309
Mimosa gemmulata PO, S,290
Mimosa invisa PO, S, 589
Mimosa pigra PO, S, A, 589
Mimosa pudica PO, S, 304, 305, 309, 311, 312, 321
Mimosa pulcherrima PO, S, 589
Mimosa scabrella PO, S, 289, 291, 292, 321
Pentaclethra macroloba B, S, 121
Piptadenia communis B, S, 329
Piptadenia moniliformis N, P, S, 288
Piptadenia rigida N, P, S, 288
Pithecellobium S, A, 589
Pithecellobium dinizii A, 589
Pseudosamanea guachapele B, S, 121
Schrankia PO, S, 321
Stryphnodendron guianense N, P, S, 290

Fagaceae PO, H, S (2–2), 105, 403, 449
Castanea sativa H, A, 449, 485
Quercus H, A, 449

Humiriaceae (1–1), 590
Humiriastrum S, A, 590

Hydrangeaceae (1–1), 590
Hydrangea A, 590

Hypericaceae (1–1), 590
Vismia R, S, 528

Juglandaceae (1–1), 590
Juglans australis S, A, 590

Lamiaceae N, P, H, S (5–9), 105, 207, 305, 307, 309, 310, 321, 329, 339, 403, 449
Gmelina arborea N, P, A, 321
Hyptis N, S, A, 207, 305, 307, 309, 310, 339
Hyptis brachiata N, S, 339, 342
Hyptis capitata N, S, 207
Hyptis mutabilis N, S, 590
Premna angolensis B, S, 329
Thymus H, A, 449, 452
Vitex doniana N, P, A, 321
Vitex orinocensis N, S, 590

Lauraceae (6–8), 57, 146, 287, 327, 329, 357
Dehaasia B, S, 329
Eusideroxylon zwageri B, S, 329
Litsea B, S, 329, 330
Litsea caulocarpa B, S, 329
Ocotea veraguensis B, S, 121
Persea N, P, S, 287
Persea americana B, L, S, 57, 121, 146, 357
Phoebe macrophylla B, S, 330

Loranthaceae N, S, A (5–6), 339
Aetanthus S, 590
Gaiadendron S, A, 590
Oryctanthus N, S, 339, 341, 342, 344
Struthanthus S, 590
Struthanthus subtilis N, S, 590
Tristerix S, 590

Lythraceae P, S (3–4), 105, 289, 321, 339
Adenaria floribunda N, S, 339, 342, 344
Cuphea N, S, 289, 342
Cuphea racemosa N, S, 342
Lagerstroemia S, 590

Malpighiaceae P, S, A (6–7), 105, 106, 339, 401
Bunchosia S, 590
Byrsonima crassifolia P, S, 106, 401
Hiraea S, A, 590
Mascagnia hippocrateoides S, 590
Stigmaphyllon A, 590
Stigmaphyllon hypargyreum S, 590
Tetrapteris N, S, 339

Malvaceae M, N, P, H, S, A (27–39), 105, 204, 207, 233, 236, 237, 305, 321, 330, 338, 339, 403

(Bombacoideae), 590
Bombacopsis A, 121

Bombacopsis quinata B, S, 121
Cavanillesia platanifolia A, 590
Ceiba aesculifolia S, 590
Ceiba pentandra N, B, S, 590
Ochroma A, 590
Ochroma pyramidale P, B, S, 590
Pachira aquatica A, 590
Pseudobombax septenatum N, S, A, 590
Scleronema A, 590

(Byttnerioideae), 590
Guazuma polybotra M, 236
Guazuma ulmifolia N, S, 590
Theobroma cacao N, P, S, 338
Waltheria glomerata A, 590
Waltheria rotundifolia N, S, 204

(Grewioideae), 339
Apeiba S, A, 590
Corchorus S, 590
Corchorus orinocensis N, S, 590
Glyphaea brevis B, S, 330
Grewia P, S, 321
Grewia bicolor N, P, A, 321
Heliocarpus N, S, A, 207, 341
Heliocarpus americanus N, S, 338, 339–342, 344
Luehea A, 590
Luehea seemannii B, S, 121
Trichospermum A, 590
Triumfetta P, S, 305, 321
Triumfetta macrophylla B, S, 330
Triumfetta semitriloba M, 237

(Malvoideae), 590
Abutilon S, A, 590
Hampea A, 590
Hampea trilobata A, 590
Hibiscus tubiflorus M, 237
Malachra palmata M, 233
Malvastrum A , 590
Pav*onia* N, S, 591
Sida N, S, A, 591

(Sterculioideae), 591
Scaphium affine B, S, 330
Sterculia apetala N, S, 591

Marcgraviaceae (1–1), 591
Souroubea S, 591

Melastomataceae N, P, H, S (3–4), 105, 289, 291, 297, 305, 308–310, 330, 338, 342, 403, 477
Dichaetanthera corymbosa B, S, 330
Miconia N, PO, S, A, 297, 304, 305, 309, 310
Miconia myriantha P, S, 591
Tibouchina N, S, 591

Meliaceae M, N, P, S, A (8–12), 236, 321, 330
Azadirachta indica N, P, A, 321
Carapa grandiflora B, S, 330
Carapa guianensis P, S, 321
Cedrela S, 591
Cedrela mexicana M, 236
Cedrela odorata B, S, 121
Ekebergia capensis N, P, B, A, 321, 330
Entandrophragma cylindricum B, S, 330
Entandrophragma excelsum B, S, 330
Guarea S, 591
Melia azedarach N, P, A, 321
Trichilia S, A, 591

Melianthaceae (1–1), 330
Bersama abyssinica B, S, 330

Menispermaceae (2–2), 591
Abuta A, 591
Cissampelos S, 591

Monimiaceae (1–1), 330
Xymalos monospora B, S, 330

Moraceae M, N, P, S, A (6–10), 238, 316, 322, 330, 338
Artocarpus heterophyllus P, A, 322
Brosimum S, 591
Brosimum alicastrum B, S, 121
Castilla elastica MM, 238
Clarisia biflora B, S, 121

Ficus B, S, 121, 330
Ficus goldmanii B, S, 121
Ficus natalensis B, S, 330
Ficus trachelosyce B, S, 121
Morus alba N, P, A, 322

Moringaceae N, P, A (1–1), 322
Moringa oleífera N, P, A, 322

Muntingiaceae (1–1), 339
Muntingia calabura N, S, 339–342, 344
Myricaceae (1–1), 330
Myrica salicifolia B, S, 330

Myrtaceae N, P, H, S (8–14), 43, 62, 105, 288, 291, 292, 305, 307–310, 316, 322, 330, 337, 339, 357, 371, 403, 449, 529
Callistemon N, S, 591
Calycolpus moritzianus N, S, 340, 342
Corymbia torelliana (Australian native) L, S, 62, 528
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Appendix E
List of Plant Taxa Used by Bees

In this book, 691 plant taxa are referred to and are listed below, at species, genus, and/or family level. Nomenclature was checked and updated following the Missouri Botanical Garden database: Tropicos.org. Missouri Botanical Garden http://www.tropicos.org

Major changes of family names (the currently preferred names appear in upper case letters) include:

Agavaceae = ASPARAGACEAE, Asclepiadaceae = APOCYNACEAE, Bombacaceae = MALVACEAE, Cecropiaceae = URTICACEAE, Chenopodiaceae = AMARANTHACEAE, Compositae = ASTERACEAE, Flacourtiaceae = SALICACEAE, Gramineae = POACEAE, LEGUMINOSAE (Caesalpinioideae, Mimosoideae, Papilionoideae/Faboideae) = FABACEAE, Maesaceae = PRIMULACEAE, Myrsinaceae = PRIMULACEAE, Oliniaceae = PENDEACEAE, Papilionoideae = FABOIDEAE, Sterculiaceae = MALVACEAE, Tiliaceae = MALVACEAE, Umbelliferae = APIACEAE.

In addition, transfer changes of some genera into different families include:

Agave (Agavaceae) = ASPARAGACEAE, *Alangium* (Alangiaceae) = CORNACEAE, *Alvaradoa* (Simaroubaceae) = PICRAMNIACEAE, *Avicennia* (Avicenniaceae/Verbenaceae) = ACANTHACEAE, *Banara* (Flacourtiaceae) = SALICACEAE, *Calophyllum* Guttiferae/Clusiaceae) = CALOPHYLLACEAE, *Casearia* (Flacourtiaceae) = SALICACEAE, *Cecropia* (Cecropiaceae) = URTICACEAE, *Celtis* (Ulmaceae) = CANNABACEAE, *Chenopodium* (Chenopodiaceae) = AMARANTHACEAE, *Cleome* (Capparaceae) = CLEOMACEAE, *Cochlospermum* (Cochlospermaceae) = BIXACEAE, *Drypetes* (Euphorbiaceae) = PUTRANJIVACEAE, *Ficalhoa* (Malvaceae) = SLADENIACEAE

Glyphaea (Tiliaceae) = MALVACEAE, *Gmelina* (Verbenaceae) = LAMIACEAE, *Heliocarpus* (Tiliaceae) = MALVACEAE, *Hydnocarpus* (Flacourtiaceae) = ACHARIACEAE, *Hydrangea* (Saxifragaceae) = HYDRANGEACEAE, *Hydrocotyle* (Apiaceae) = ARALIACEAE

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Hyeronima (Euphorbiaceae) = PHYLLANTHACEAE, *Maesa* (Maesaceae) = PRIMULACEAE

Myrsine (Myrsinaceae) = PRIMULACEAE, *Nuxia* (Loganiaceae) = STILBACEAE, *Phyllanthus* (Euphorbiaceae) = PHYLLANTHACEAE, *Picramnia* (Simaroubaceae) = PICRAMNIACEAE

Sambucus (Caprifoliaceae) = ADOXACEAE, *Strombosia* (Oleaceae) = OLACACEAE, *Ternstroemia* (Theaceae) = PENTAPHYLACEAE, *Trema* (Ulmaceae) = CANNABACEAE

Triumfetta (Tiliaceae) = MALVACEAE, *Viburnum* (Caprifoliaceae) = ADOXACEAE, *Vismia* (Guttiferae/Clusiaceae) = HYPERICACEAE, *Vitex* (Verbenaceae) = LAMIACEAE

Plants with Mayan medicinal use (M), nectar (N), pollen (P), trichomes (T), or resin source (R) used to make honey (H), build nests (B), or pollinated (L) by stingless bees (S), and *Apis mellifera* (A)

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Acalypha discolor (Euphorbiaceae) N, P, S, A, 596
Acalypha diversifolia (Euphorbiaceae) P, S, 596
Acalypha macrostachya (Euphorbiaceae) P, S, 596
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Appendix F
Common Names of Plants Used for Nesting by Stingless Bees

“espavel, rabito” *Anacardium excelsum* (Anacardiaceae) Costa Rica, 121
“mangle blanco” *Bravaisia integerrima* (Acanthaceae) Costa Rica, 121
“jiñocuabe” *Bursera simaruba* (Burseraceae) Costa Rica, 121
“laurel” *Cordia alliodora* (Boraginaceae) Costa Rica, 121
“guachipelín” *Diphysa americana* (Fabaceae, Papilionoideae) Costa Rica, 121
“higuerón” *Ficus* (Moraceae) Costa Rica, 121
“higuerón” *Ficus trachelosyce* (Moraceae) Costa Rica, 121
“madero negro” *Gliricidia sepium* (Fabaceae, Papilionoideae) Costa Rica, 121
“siete cueros” *Lonchocarpus lasiotropis* (costaricensis) (Fabaceae, Faboideae), Costa Rica, 121
“guayaba, guayabo” *Psidium guajava* (Myrtaceae) Costa Rica, 121
“cortez amarillo” *Tabebuia ochracea* (Bignoniaceae) Costa Rica, 121
“gavilán” *Pentaclethra macroloba* (Fabaceae, Mimosoideae) Costa Rica, 121
“manú” *Minquartia guianensis* (Olacaceae) Costa Rica, 121
“ojoche” *Brosimum alicastrum* (Moraceae) Costa Rica, 121
“pochote” *Bombacopsis quinata* (Malvaceae, Bombacoideae) Costa Rica, 121
“cítricos” *Citrus* (Rutaceae) Costa Rica, 121
“aguacate” *Persea americana* (Lauraceae) Costa Rica, 121
“guanacaste” *Enterolobium cyclocarpum* (Fabaceae, Mimosoideae) Costa Rica, 121
“almendro de montaña” *Andira inermis* (Fabaceae, Faboideae) Costa Rica, 121
“cedro amargo” *Cedrela odorata* (Meliaceae) Costa Rica, 121
“papaturro blanco” *Coccoloba caracasana* (Polygonaceae) Costa Rica, 121
“guaba” *Inga sapindoides* (Fabaceae, Mimosoideae) Costa Rica, 121
“aguacatillo” *Ocotea veraguensis* (Lauraceae) Costa Rica, 121
“jobo” *Spondias mombin* (Sapindaceae) Costa Rica, 73, 121
“roble de sabana” *Tabebuia rosea* (Bignoniaceae) Costa Rica, 121

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Appendix G
Common Names of Medicinal Plants Used with Honey by Mayas

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Appendix H
Microorganisms Associated to Stingless Bees or Used to Test Antimicrobial Activity

Legend:

H – Honey origin
N – Nectar origin
P – Pollen origin
PI – Propolis origin
L – Larval origin
E – Adult bee origin
G – Gut origin
T – Nest/hive origin
C – Brood comb origin
R – Hive floor origin
D – Bee bread origin
GP – Garbage pellet origin
SP – Spores stored in lieu of pollen
B – Bacteria
F – Mold
O – Other Fungi
LA – Cause lactic fermentation
Y – Yeast
S – Stingless bee
A – *Apis mellifera*
I – Solitary bees
PA – Propolis antibacterial activity
HA – Honey antibacterial activity
HF – Honey antifungal activity
HY- Honey antiyeast activity

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List of microorganisms:

Actinobacteria B, A, G, 175
Alphaproteobacteria B, A, G, 175
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Aspergillus flavus F, A, D, 179
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Aspergillus terreus F, S, 179
Aspergillus versicolor F, A, D, 179
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Bacillus megaterium B, S, T, 178
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Bacillus mycoides B, I, 175
Bacillus pumilis nad B, S, 178
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Bacillus subtilis B, S, HA, 178, 401
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Candida batistae, Y, I, T, 176, 177
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Candida bombicola (*Starmerella bombicola*) Y, I, N, P, 176, 177, 180
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Appendix I

Summary of Meliponine and *Apis* Honey Composition

[Number of Honey Samples Analyzed]

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				Physicochemical parameters[a]										
Bee species	Chapter	Country	Number of honey samples analyzed (N)	pH	Free Acidity (meq/Kg honey)	Ash (g/100 g honey)	Diastase activity (DN)[b]	Insoluble solids (g/100 g honey)	HMF (mg/kg honey)	Invertase activity (IU)[c]	Nitrogen (mg/100 g honey)	Reducing sugars[d] (g/100 g honey)	Apparent sucrose[e] (g/100 g honey)	Water (g/100 g honey)
Frieseomelitta sp.	27	Colombia	5–6	–	–	–	–	–	–	–	–	29.7 ± 14.1 [5]	3.1 ± 2.7 [5]	33.1 ± 3.3 [6]
Geotrigona acapulconis	28	Guatemala	1	3.06	85.53	0.09	2.6	–	n.d.	–	–	–	–	32.09
Melipona beecheii	28	Guatemala	7	3.7±0.1	23.2 ± 30.0	0.07±0.05	21.3±32.8	–	n.d.	–	–	68.8 ± 3.8	3.5 ± 4.1	17.3±2.6
Melipona brachychaeta	29	Bolivia	1	3.8	10.4	0.01	–	–	–	–	–	73.4	1.5	24.9
Melipona compressipes	27, 30	Colombia	1–12	–	7.0 [1]	0.09 [1]	n.d. [2]	–	3.0 [1]	–	–	71.1 ± 8.1 [11]	3.4 ± 2.2 [11]	25.8 ± 2.0 [12]
Melipona eburnea	27, 30	Colombia	7	–	–	–	–	–	–	–	–	77.8 ± 14.5	3.6 ± 1.5	27.6 ± 2.1
Melipona favosa	25	Venezuela	6–40	–	51.7 ± 25.2 (12.7–97.1) [40]	0.14 ± 0.13 (0.01–0.61) [40]	2.86±0.36 (2.65–3.50) [6]	–	17.7 ± 8.5 (5.04-24.69) [21]	90.08 ± 48.03 (31.80-150.70) [6]	45.7 ± 18.3 (10.5–102.00) [39]	67.3 ± 4.1 (60.9–78.6) [40]	2.1 ± 1.3 (0.5–5.1) [40]	28.0 ± 2.7 (22.1–32.0) [40]
Melipona favosa	27, 30	Colombia	1–7	–	–	0.01 ± 0.01 [2]	–	–	n.d. [1]	–	–	72.2 ± 7.4 [7]	3.1 ± 1.8 [7]	24.8 ± 1.8 [7]
Melipona grandis	29	Bolivia	1	3.6	16.0	0.02	–	–	–	–	–	72.5	0.9	24.1
Melipona solani	28	Guatemala	1	3.8	4.95	0.06	8.3	–	n.d.	–	–	76.0	1.7	19.66
Melipona aff. yucatanica	28	Guatemala	1	3.8	10.59	0.06	10.0	–	n.d.	–	–	–	–	20.37

Melipona sp.	27	Colombia	2–18	–	–	0.20 ± 0.00 [2]	–	–	–	–	–	67.6 ± 7.5 [18]	6.0 ± 2.3 [18]	26.15 ± 1.8 [18]
Melipona sp.	30	Colombia	10	–	–	–	–	–	–	–	–	67.4	6.5 ± 3.2	26.8 ± 5.3
Nannotrigona perilampoides	28	Guatemala	1	3.8	9.93	0.33	6.8	–	n.d.	–	–	–	–	16.5
Nannotrigona testaceicornis	27	Colombia	2–3	–	–	–	–	–	–	–	–	65.8 ± 35.1 [2]	7.9 ± 4.3 [2]	27.5 ± 4.2 [3]
Nannotrigona sp.	27	Colombia	1–4	–	–	0.33 [1]	–	–	–	–	–	50.8 ± 7.4 [4]	9.7 ± 4.3 [4]	25.7 ± 1.8 [4]
Paratrigona sp.	27	Colombia	1–4	4.1 [1]	31.7 [1]	–	–	–	–	–	–	58.1 ± 12.4 4]	3.9 ± 2.8 [4]	26.6 ± 1.2 [4]
Partamona peckolti	27	Colombia	1	–	–	–	–	–	–	–	–	40.6	6.1	42.7
Partamona sp.	27	Colombia	1	–	–	–	–	–	–	–	–	38.3	13.1	28.9
Plebeia sp.	27	Colombia	1	–	–	–	–	–	–	–	–	36.7	0.9	28.6
Plebeia sp.	28	Guatemala	1	3.8	15.31	1.25	7.6	–	n.d.	–	–	–	–	30.3
Scaptotrigona depilis	29	Bolivia	1	3.4	49.4	0.03	–	–	–	–	–	67.7	1.0	26.0
Scaptotrigona limae	27	Colombia	2	–	–	–	–	–	–	–	–	67.7 ± 4.1	6.6 ± 4.6	25.8 ± 2.2
Scaptotrigona mexicana	28	Guatemala	1–2	4.0 ± 0.4 [2]	12.7 ± 3.0 [2]	0.10 ± 0.04 [2]	18.6 ± 12.7 [2]	–	n.d.	–	–	57.2 [1]	0.1 [1]	18.7 ± 0.2 [2]
Scaptotrigona polysticta	29	Bolivia	1	3.5	49.1	0.06	–	–	–	–	–	67.8	1.0	26.5
Scaptotrigona near *xanthotricha*	29	Bolivia	1	3.8	34.5	0.09	–	–	–	–	–	67.0	0.0	24.9

(continued)

				Physicochemical parameters[a]										
Bee species	Chapter	Country	Number of honey samples analyzed (N)	pH	Free Acidity (meq/Kg honey)	Ash (g/100 g honey)	Diastase activity (DN)[b]	Insoluble solids (g/100 g honey)	HMF (mg/kg honey)	Invertase activity (IU)[c]	Nitrogen (mg/100 g honey)	Reducing sugars[d] (g/100 g honey)	Apparent sucrose[e] (g/100 g honey)	Water (g/100 g honey)
Scaptotrigona sp.	27	Colombia	1–4	4.5 [1]	57.83 [1]	0.06 [1]	2.4 [1]	–	6.0 [1]	–	–	55.7 ± 5 [4]	12.1 ± 7.4 [4]	26.9 ± 2.9 [4]
Tetragona sp.	27, 30	Colombia	2–21	4.2 ± 0.3 [4]	44.3 ± 21.8 [4]	0.50 ± 0.08 [5]	17.8 ± 5.5 [2]	–	1.0 ± 1.1 [2]	–	–	60.8 ± 10.7 [19]	4.4 ± 5.6 [19]	25.8 ± 3.6 [21]
Tetragonisca angustula	26	Brazil	6	–	37.3 ± 16.7 (21.7–63.9)	0.28 ± 0.11 (0.17– 0.42)	16.9 ± 3.9 (11.0– 22.5)	0.06 ± 0.03 (0.02– 0.10)	0.65 ± 0.25 (0.30– 0.93)	–	–	57.1 ± 7.8 (44.8–67.5)	2.1 ± 1.8 (0.4–4.5)	24.4 ± 0.8 (23.4–25.6)
Tetragonisca angustula	27	Colombia	6–44	4.2 ± 0.3 [12]	39.2 ± 22.9 [12]	0.21 ± 0.70 [12]	16.7 ± 9.2 [8]	1.3 ± 2.1 [6]	–	–	–	53.6 ± 11.8 [44]	4.2 ± 2.4 [44]	24.3 ± 2.3 [44]
Tetragonisca angustula	28	Guatemala	1–4	5.9±1.6 [4]	17.4±10.4 [4]	0.35±0.26 [4]	12.3±10.3 [4]	–	n.d.	–	–	65.78 [1]	4.83 [1]	17.5 ± 2.8 [4]
Tetragonisca fiebrigi	29	Bolivia	1	4.5	43.8	0.33	–	–	–	–	–	58.6	1.8	25.1
Tetragonula laeviceps	36	Thailand	–	3.37	–	–	–	–	–	–	44.8	–	15.2	–

[a] Mean values ± SD (minimum and maximum), and [number of honey samples tested, only where N varies] are presented

[b] The Diastase Number (DN) indicate g /100 g honey/h, at pH 5.2 and 40 °C

[c] An Invertase Unit (IU) indicates μmoles p-nitrophenyl glucopyranoside hydrolyzed/kg honey/min, at pH 6.0 and 40 °C

[d] Chapters 27 and 30 measures of glucose + fructose are considered reducing sugars in this table

[e] Chapters 27 and 30 measures of dissacharides (sucrose + maltose) are considered reducing sugars in this table

Appendix J
Information of Collected Stingless Bees

Studies of pot-honey need a backup of identified stingless bees by competent entomologists. Regulations to exchange insects for academic purposes between different countries should be met. If it helps, besides the entomological sample, it is useful to send available information such as that organized in the table below, including few images:

No.	013-2008	Country	VENEZUELA
Nest			
Location		La Vega del Corozo, Aricagua, Estado Mérida	
Geographic Coordinates (if possible with GPS)		N 08° 14.581' W 071° 08.336'	
Height (feet)		3259	
Type of hive (feral o meliponary)		Adobe wall	
Type of meliponario (modern o crafted)		–	
Origin of the nest (location of nest collection transported to the hive, if possible with GPS)		–	
Substrate description (tree cavity, underground, termite nest, ant nest, bee nest, exposed on tree branches, exposed on walls, inside walls, etc.)		Dark mass	
Entrance (tubular shape, etc.) and material (resin, cerumen, vegetal particles, clay, seeds, etc.)		Flat trumpet, resin, aprox. 1 m height	

(continued)

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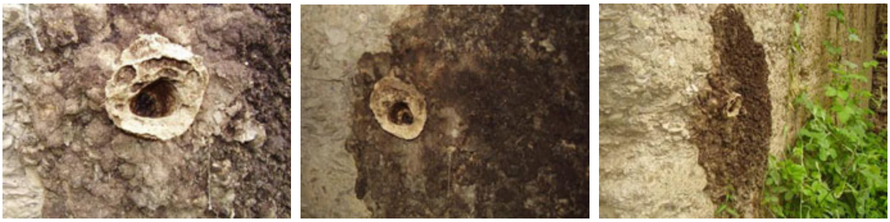
Stingless bee	
Common name	pegona negra
Identification jmafcama@ffclrp.usp.br	*Partamona peckolti* (Friese, 1901)
Collector	
Name	Patricia Vit, Marilin Pérez, Anacely Rivas, Llenis Toro
Date	18.05.08
Address	Departamento Ciencia de los Alimentos, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida 5101, Venezuela
e-mail	vit@ula.ve
Meliponicultor	
Name	–
Address	–
e-mail	–
Phone	–
Mobile	0426-7772466 (Marilin), 0274-5116918 (Berta)
Observations	
Flora	
Flight	
Behavior	Chases, is disturbed by flash, and bites the head



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