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Stingless Bees of Mexico

The Biology, Management and Conservation of an Ancient Heritage

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Preface

Stingless beekeeping (meliponiculture) in the Yucatan is an ancient activity. Nowhere else in the world meliponiculture reached the level of technical skills and productivity attained by the Maya in Mesoamerica with the stingless bee *Melipona beecheii*. However, changes in the economic system, together with severe deterioration of the habitat, caused a steady decline of this activity, almost to the verge of extinction. Fortunately, a series of events coincided in the mid-1980s that helped raising concern and renewed interest with important initiatives to rescue meliponiculture in the Maya region. In my opinion, 1986 was a critical year due to the arrival of the Africanized bee. The Africanization of European apiaries, drastically changed beekeeping in the whole of Mexico, but especially in the Yucatan Peninsula. In this area, with one of the largest densities of managed honey bee colonies in the Americas (17–21/km^2), hives were traditionally kept in the backyard of rural homes. To avoid stinging accidents, apiaries had to be relocated in the forests, further away from human settlements, leaving a vacant niche in rural homes. At around the same time Jorge González Acereto, a tireless promoter of stingless bees and meliponiculture in Mexico, started the characterization of nests of species from the Yucatán Península, with the objective of designing modern hives to keep them. Other students and I were involved in that project. Then, like most people, I was totally unaware of the existence of these insects and their ancient cultivation. We travelled the Yucatan, searching for colonies and species and got to know several stingless beekeepers from whom I learnt the importance and cultural value of this Mayan heritage. We visited remote rural villages in the forests of the Yucatan where stingless beekeeping had survived thanks to isolation from modern cultural influences. New species, like *M. yucatanica*, were discovered during our travel. With advice of various recognized researchers, as Professor Paulo Nogueira and Virgilio de Portugal Araujo, wooden hives of suitable size were produced for our different stingless bee species. This started the modernization of stingless beekeeping in the Yucatan, allowing better management and improvement on the production and quality of bee products. I obtained my bachelor's degree with a thesis investigating the development of colonies of *M. beecheii* in various types of hive and concluded that, colonies did not adapt to hives with excess volume because of thermoregulation problems. The

development of modern husbandry, together with the promotion of stingless beekeeping as an alternative activity for rural villages, triggered a rebirth of the activity. With support from the government, several groups of farmers, especially women, got involved in meliponiculture, and started to take over the empty niche left by honey bees. Initiatives like the Seminario Mesoamericano sobre Abejas Nativas, devised by Margarita Medina and other stingless bee enthusiasts, helped to gather scientists and beekeepers around similar objectives.

Nowadays, stingless bees have become a phenomenon all over the Yucatan Península and other regions of Mexico. Indigenous groups and agencies are promoting *M. beecheii* and other species like never before. However, here lays a paradox: the recent popularity of stingless beekeeping is also becoming a major hazard. Uncontrolled selling of colonies because of high demand, and the negative impact of inexperienced instructors are threatening the activity, perhaps at a larger scale than ever. An avalanche of written and visual information on stingless bees can be found today in social networks, especially for Latin America. Unfortunately, much information found on these platforms lacks support and can be seriously mistaken, but accepted to the letter by inexperienced hobbyists, in detriment of colonies.

A great deal of scientific information has accumulated on stingless bees. However, it is generally dispersed and not easily available to the Spanish speaking public. One purpose of this book is to compile information on different aspects of the biology and management of stingless bees and (hopefully) make it accessible to students, academics, instructors, and the general public. The original work in Spanish, is aimed at readers in México, where information on these insects is limited. I dared to translate this work to English, intending that the information produced in Mexico (and Latin America), could be better known in other regions. I take full responsibility for the mistakes.

I would like to acknowledge many colleagues, with whom I have shared constructive scientific discussion, and pleasant times for several years. I particularly thank friends, who kindly reviewed and commented on earlier versions of the different chapters: Rodolfo Jaffé, Claus Rasmussen, Rob Paxton, and Adam Hart. My sincere thanks to all of you. My profound gratefulness to the agencies that have supported my research during all this time: the International Foundation for Science in Sweden, Fundación Produce Yucatán 2001–2007 (Manejo tecnificado de abejas sin aguijón y su uso en la polinización de cultivos), SISIERRA (Rescate de melponicultura en la Península de Yucatán), CONACyT Projects 2002–1556 (Rescate,conservación y mejoramiento genético de los recursos apícolas de México), 103341 (Conservación de las abejas sin aguijón de México), B237532 (Climate change and pollinators), and SAGARPA-CONACYT 291333 (Manejo sustentable de polinizadores).

I would also like to acknowledge my colleagues in the Department of Tropical Apiculture and my institution the Universidad Autónoma de Yucatán. Teresita Solis and Humberto Moo-Valle contributed with photographs and information in Chaps. 6 and 8, thank you for your help. Finally, I thank the many students and stingless beekeepers for their help and enthusiasm; you have made my work truly enjoyable. I dedicate this book to my beloved parents, Evangelina and José Guadalupe,

my sisters Elia and Rosa, my dear friend and companion Alvaro Pat, my aunt Catalina, and my niece and nephew Mariana and Bernardo. Thank you all for your support and understanding.

Mérida, Yucatán, Mexico
February 2018

José Javier G. Quezada Euán

Contents

Chapter 1
Taxonomy and Diversity of the Stingless Bees

The two species of *Melipona* present in the Yucatan Peninsula. Left *M. (Melikerria) beecheii* and right *M. (Melipona) yucatanica.*

The bees are among the largest and most diverse group of insects. A worldwide estimation of 20,000 bee species is presently acknowledged (Roubik 1989). Mexico's bee fauna is considered mega-diverse encompassing an estimated 1800 species, representing approximately 10% of the world richness (Ayala 2006).

Together with their close relatives, the ants and wasps, the bees constitute the order Hymenoptera or membrane-winged insects (Michener 1974) (Fig.1.1). As a taxonomic group, the bees can be separated from their wasp relatives mainly by their feeding habits, being predominantly vegetarian (with a few exceptions). They feed upon nectar and pollen during their larval development and adulthood. The bees have evolved especialized structures for the collection of resources from plants, which are also useful in their taxonomic characterization (Michener 2000).

The bees or Apiformes are classified into seven families within the superfamily Apoidea, which also includes the sphecoid wasps (Michener 2000). One of these families of bees, the Apidae, is the most diverse and includes a small group in which the pollen-transporting apparatus of the females is the corbicula or pollen basket (Michener 1999; Hedtke et al. 2013; Fig. 1.2). The other Apidae as well as members

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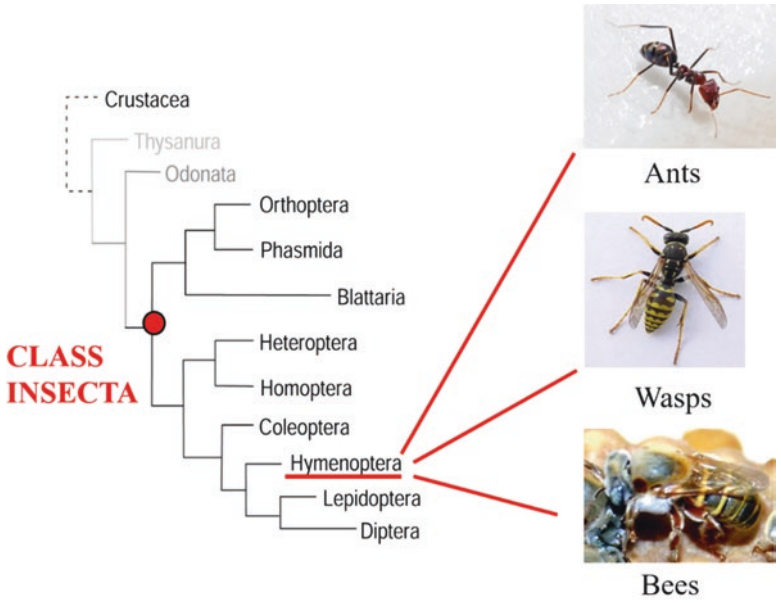


Fig. 1.1 Simplified phylogeny of the orders within the class Insecta. Underlined is the order Hymenoptera, which includes the wasps, ants, and bees

Fig. 1.2 Pollen-collecting apparatus of females of different bee groups. Most bees have hair brushes or scopas on the abdomen (Megachilid) or legs (Anthophorid). The corbiculate bees are characterized by the pollen basket or corbicula, a naked concavity of the tibia of their hind legs (examples, *Euglossa* and *Melipona*)

of the other six families (but not the parasitic ones) possess scopas (hair brushes) on the legs or abdomen, which are used for similar purpose (Fig. 1.2). The evolution of the pollen basket in the form of a naked concavity on the outer side of the hind tibia, allowed the corbiculate bees the transport of sticky materials and rapid dislodging of their load (Roubik 1989). The first corbiculate bees probably diverged 72–95 mya from New World Centridini ancestors preceding the evolution of eusociality (Martins et al. 2014).

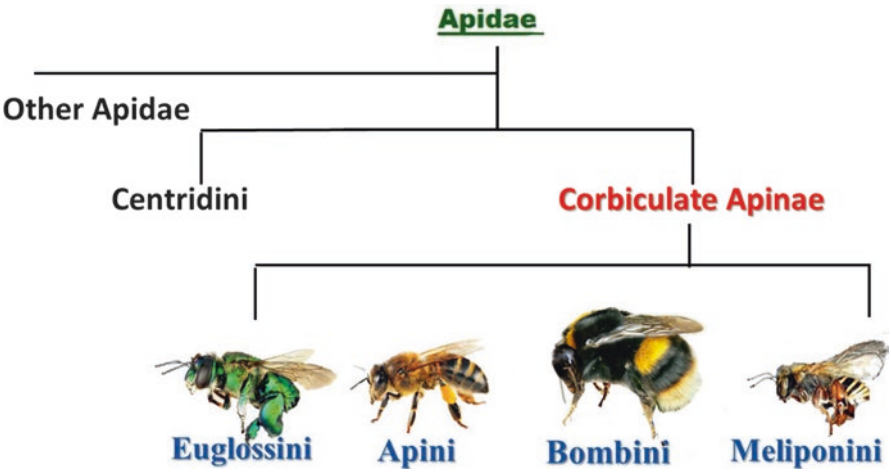


Fig. 1.3 A simplified classification of the four tribes encompassing the corbiculate bees, a subset within the Apinae (after Hedtke et al. 2013)

The corbiculate bees are placed within the subfamily Apinae and comprise four tribes: Apini (honeybees), Bombini (bumble bees), Euglossini (orchid bees), and Meliponini (stingless bees) (Michener 2000) (Fig. 1.3).

Most bee species worldwide are solitary, but a small group of species have evolved different social lifestyles, from primitive to highly social behavior or eusociality (Michener 1974). At the highest level of eusociality two groups of bees are found: the honeybees and the stingless bees. In accordance to Michener (1974), the highly eusocial bees are distinguished by the following traits: morphological and physiological differentiation among females (true reproductive castes), division of labor with cooperative work, colonies in which individuals of different generations overlap, and reproduction by swarming.

1.1 General Features of the Meliponini

The Meliponini or stingless bees are by far the most diverse group within the corbiculate bees, not only for the current number of species (ca. 500), but also for the contrasting differences in morphology, nesting habits, and behavior between species (Roubik 2006). Nonetheless, it is possible to separate the stingless bees from the other corbiculate bees by some common features Wille (1979):

1. A vestigial sting.
2. The presence of a penicillium, a comb of rigid bristles on the internal margin of the corbicula. This structure is weaker or absent in the cleptobiotic genera *Lestrimelitta* and *Cleptotrigona*.

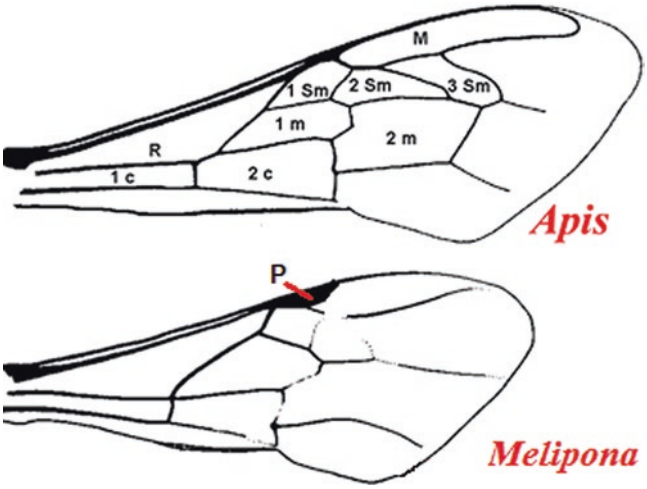


Fig. 1.4 Comparative forewing venation and cells of *Apis* and *Melipona*. A reduction of veins and cells, in particular the marginal cell (M) and the three submarginal cells (Sm), is observed in the latter. The P indicates the pterostigma

3. Weak and reduced forewing venation with a marginal cell relatively short, and submarginal cells weak or absent (Fig. 1.4).
4. A pterostigma of the forewing of moderate (*Melipona* species), to large size (non-*Melipona* species) (Fig. 1.3).
5. A reduced number of hamuli (hooks) on the hind wings (8 to 16 in *Melipona* and 4 to 10 in non-*Melipona*), compared with *A. mellifera* (ca. 20), and *Bombus* (14 to 28).
6. Absence of a pollen press (auricle) between the corbicula and the basitarsus. The basitarsus is small and slender compared with *Apis.*
7. Maxillary palps absent.
8. Wax-producing glands on the dorsal part of the metasoma.
9. Tibial spurs absent.
10. Female claws simple, bifurcate in the males (Fig.1.5).

1.2 Biogeography and Phylogeny

The stingless bees are exclusively found in the tropical and subtropical regions of the planet. In accordance to Moure et al. (2007), 60 genera are currently recognized. However, this figure may vary in accordance to the classification system used, of which two are presently accepted (Michener 2000; Camargo and Pedro 2007; Moure et al. 2007; Rasmussen and Cameron 2010).

The highest abundance and diversity of stingless bees are found in the neotropics with ca. 391 species and approximately 32 genera (Camargo and Pedro 2007; Fig. 1.6). Other regions of the world seem to have less diversity, although it is noted

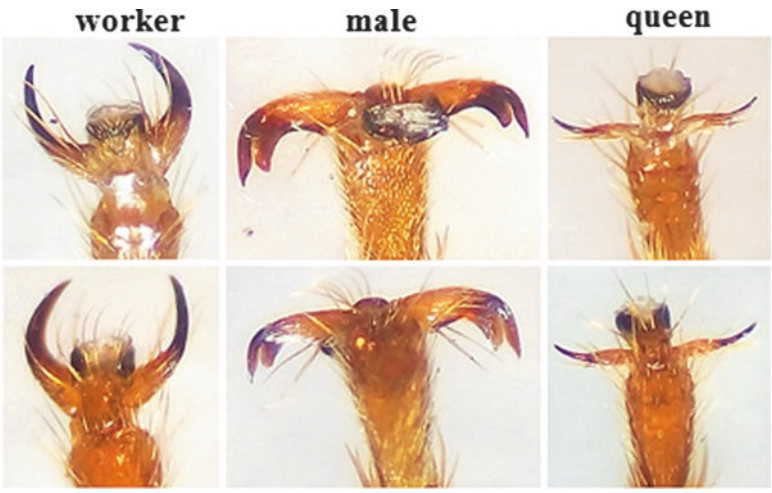


Fig. 1.5 Distal portion of the hind leg of *M. beecheii*: ventral (above) and dorsal view (below). The claws are simple in the worker and queen and bifurcate in the male

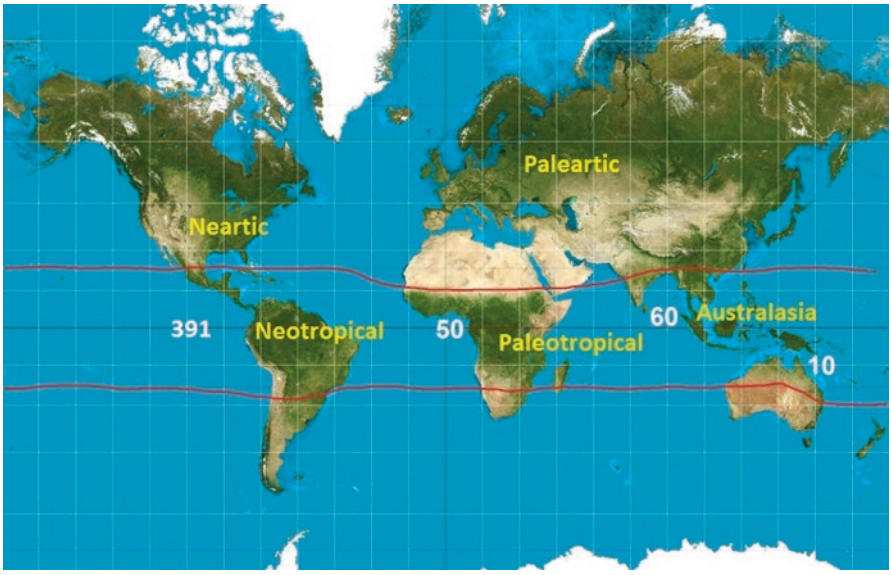


Fig. 1.6 Present geographic distribution of the stingless bees. The red lines represent the limits of their distribution confined to the tropics and subtropics. In white is the estimated number of estimated species per biogeographic region

that they have been less intensely studied. In Africa, for instance, recent revisions have reported some 50 species, and 70 species for Australasia and Indo-Malaysia (Eardley 2004; Rasmussen and Cameron 2007). The advent of accessible molecular methods has increased the number of studies on species complexes and the number

of new species being identified (Halcroft et al. 2016). In addition, detailed revisions have also recognized new genera in museum collections (Melo 2016).

The stingless bees are an ancient group. The fossil evidence suggests that the time of emergence of Meliponini may be 70–90 million years ago; this is long before the evolution of *A. mellifera* (Rasmussen and Cameron 2010). Thus, it seems that the stingless bees are Gondwanian (Camargo and Vit 2013), but experienced a posteriori diversification, mainly in South America. It is thought that vicariance events (geographic separation of populations within a species) have contributed to the high diversity of stingless bees in the neotropical region (Camargo and Vit 2013).

Four main hypotheses have been proposed to explain the biogeography of the stingless bees, constructed upon information on the extant and fossil distribution and their morphology. A first hypothesis, proposed by Kerr and Maule (1964), suggested that stingless bees first appeared in South America and from here their expansion occurred during the Eocene (50–60 mya). They moved to the Palearctic region through the Bering Strait and from there they colonized the tropical regions of the Old World in the late Oligocene (20–30 mya). The main argument for Kerr and Maule's (1964) hypothesis is that the higher diversity in genera and species is presently found in South America. Their hypothesis assumes the existence of a land bridge between South America and Central America during the Paleocene-Eocene. However, it is now accepted that both subcontinents were separated for more than 100 mya, and that a connection with North America was established via the Panama Canal only 3–4 mya (perhaps only with patchy connections before).

Wille (1979), considering that the species with the more primitive or ancestral characteristics are presently found in Africa, suggested that stingless bees originated in this continent during the late Cretaceous (80–100 mya). From Africa stingless bees supposedly moved to the southern parts of Europe during the Eocene, when some land bridges connected both continents. From here, stingless bees eventually dispersed to the New World.

Later on, Camargo and Wittmann (1989) proposed a hypothesis suggesting the appearance of meliponines in Gondwana during the early Cretaceous (100–120 mya), and subsequent movement through land connections that existed between South America, the Antarctic, and Australia. This hypothesis attempted to explain the disjunct distribution of modern species in the genus *Plebeia* across all three subcontinents.

Finally, Michener (1990) proposed that stingless bees originated in North America, when tropical conditions extended to septentrional parts of the New World. However, this hypothesis does not explain a route of dispersion, and does not recognize that Africa and South America share taxa. Michener (1990) also proposed *Melipona* as a sister group to all the other stingless bees, and that *Trigona* sensu *lato (s.l.)* should include species of the neotropical and Indo-Malayan regions, but considered African species a separate clade.

In a further document, Camargo and Pedro (1992) elaborated on the original hypothesis by Camargo and Wittmann (1989), and suggested two main routes of dispersion of the meliponines from West-Gondwana via the Holartic and Panaustral regions. Camargo and Pedro (1992) proposed that *Trigona* from the neotropical, Indo-Malayan, and Australasian regions should form one group. However, these authors considered the Afrotropical species a sister group of all other stingless bees.

A first attempt at constructing a molecular phylogeny of the stingless bees, was undertaken by Costa et al. (2003). The authors built phylogenies based on the fragment 16S of the rRNA. However, due to the low number of species, and the use of only one fragment, not enough resolution for 34 species of stingless bees was obtained.

A larger phylogenetic analysis was conducted by Rasmussen and Cameron (2007, 2010) based on 9 genes for the basal framework, and including 79 taxa from the Old and New World (Fig. 1.7). The results revealed three groups clearly differentiated: Neotropical, Afrotropical, and Indo-Malayan-Australasian. The Neotropical group diverged early from the others. An important finding was that the species of *Trigona* from the New and Old World formed two separate clades implying different origin. The authors argue that since the first descriptions of *Trigona* were made for Neotropical specimens, the genus should be used for American species exclusively. In the analysis by Rasmussen and Cameron (2010) *Melipona* was more parsimoniously placed within the neotropical clade, and not as a sister group of all stingless bees as proposed by Michener (1990). In addition, the Neotropical clade appeared composed by three groups (Fig. 1.7): one including the species of *Melipona*, another for the minute species (e.g., *Trigonisca s.l.*), and a third one with the other New World taxa (Rasmussen and Cameron 2010).

The findings of Rasmussen and Cameron (2010) are useful to test hypotheses on the biogeography of stingless bees. The Gondwanian origin of the stingless bees seems accurate. Rasmussen and Cameron (2010) also estimated the time of divergence between Old and New World stingless bees at around 71–73 mya, the separation of the Afrotropical clade in 61 mya, and the clade Indo-Malayan-Australasia in 49 mya (Fig. 1.7).

Similar to *Trigona*, Old and New World *Plebeia* are not closely related, which proves that the hypothesis for the dispersion of this genus is incorrect (Camargo and Wittmann 1989). In the case of Kerr and Maule's (1964) hypothesis, it does not seem congruent with recent geological findings. Nonetheless, the other hypotheses, in the opinion of Rasmussen and Cameron (2010), cannot be ruled out due to a lack of evidence from fossils for a better phylogenetic picture.

Stingless bees are presently classified within a single tribe, the Meliponini (Michener 2000). However, contrasting differences within the Meliponini have long been recognized between the species of *Melipona* and the other genera (Wille 1983; Table 1.1; Fig. 1.8).

It is interesting to note that *Melipona* and *Trigona* derive from the Greek, meaning in the first case working incessantly for the honey (*meli* (honey) + *ponein*

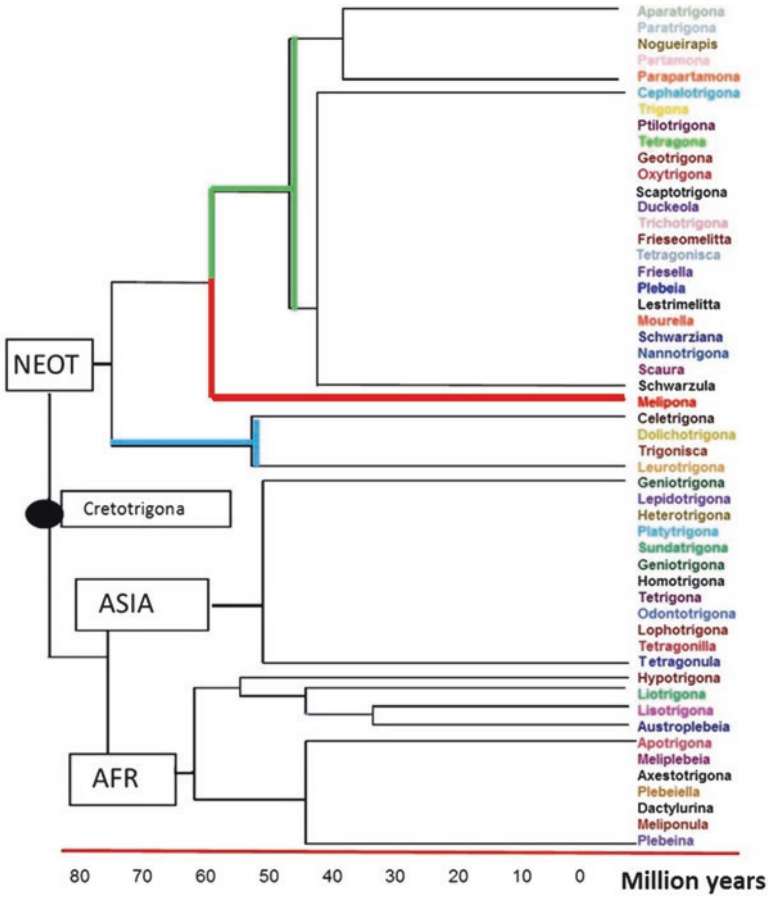


Fig. 1.7 Phylogenetic relationships of the stingless bees in accordance to the molecular study by Rasmussen and Cameron (2010). Three main clades are identified, NEOT = neotropical, ASIA = Indo-Malaya, AFR = Africa. Within the NEOT clade, three groups are found: the minute stingless bees (blue root), *Melipona* (red root), and the other taxa of New World (green root). *Cretotrigona*, the oldest fossil for the group, was used to provide an estimation of the time of emergence of each branch

(to toil)). *Trigona* makes reference to the triangular shape of the abdomen of these bees (*tri* (three) + *gono* (angle)) (Schwarz 1932).

In the neotropics, some 33 genera (Camargo and Vit 2013; Melo 2016), and approximately 418 species of stingless bees are recognized (Pedro 2014). The genus *Melipona* (Illiger, 1806) is exclusively Neotropical, and it encompasses an estimate of 74 species found between Mexico and Argentina (Camargo and Pedro 1992; Ramírez et al. 2010; Pedro 2014).

Melipona includes some of the most representative, economically and culturally, species in the Americas. A first comprehensive phylogeny of the genus *Melipona*

Table 1.1 Characteristics of the species in the genus *Melipona* and the other genera of the stingless bees

	Meliponini	
Characteristic	*Melipona*	Non-*Melipona*
Nest entrance	Made of mud, with a radiated shape	Made of cerumen or resin. Some species are distinctive for their tube or trumpet-like entrances
Worker body size	Robust, with a total length >1 cm	Wasplike, slender, total length <1 cm
Body pilosity	Abundant, the integument is furry, which gives an overall opaque appearance	Scarce—the integument is mostly glabrous, which gives a shiny appearance
Forewing	Total length not beyond the tip of the abdomen Pterostigma of moderate size	Total length well beyond the tip of the abdomen Pterostigma comparatively large in relation to wing size
Hind wing	8–16 Hamuli	Generally <8 hamuli
Dorsal vessel	Type II (see Chap. 3)	Type I
Digestive tract	Long	Short
Royal cells	There are no royal cells—cells where queens are reared are of the same size as workers and males	Royal cells are larger than those of males and workers
Distribution of queen cells	The cells where queens are reared are intermingled with those of males and workers	Queen cells are normally found in the periphery of the combs or the outer parts of clustered cells
Gyne size	Gynes are generally smaller than workers and males—thorax width notably smaller	Normally gynes are larger than males and workers
Ovaries of gynes at emergence	Partially developed	Well developed
Gyne rate production	Gynes are frequently produced	Gynes are produced at a lower rate
Caste determination	Tropho-genetic	Trophic

Fig. 1.8 Workers of *M. beecheii* and *Cph. zexmeniae* to show some contrasting features between *Melipona* and non-*Melipona* stingless bees. Body pilosity is more abundant in *M. beecheii*, and the forewing is comparatively larger in *Chp. zexmeniae* (Table 1.1)

was constructed using morphological characters by (Moure 1992), who recognized four subgenera.

The four subgenera recognized by Moure (1992) within *Melipona* are:

Melipona (*Melipona s. str.*) Illiger, 1806
Melipona (*Michmelia*) Moure, 1975
Melipona (*Melikerria*) Moure 1992
Melipona (*Eomelipona*) Moure 1992

Molecular studies have confirmed that *Melipona* is a modern clade within the stingless bees, of post-Gondwanian origin, which probably rose approximately 24 mya in the upper Eocene (Rasmussen and Cameron 2010). However, an extensive study of 35 *Melipona* species using mitochondrial, ribosomal, and nuclear DNA markers, suggests even a more recent origin (14–17 mya) of this genus (Ramírez et al. 2010). Interestingly, the phylogeny of *Melipona* by Ramírez et al. (2010), is largely in agreement with the phylogeny proposed by Moure (1992), and more recently by Camargo and Pedro (2007), both using morphological characters.

It is important to mention that in his most recent classification, Michener (2000) did not recognize the subgenera proposed by Moure (1992) for *Melipona,* due to the apparently subtle morphological differences between them. Michener (2000) neither recognized many of the genera used by Camargo and Pedro (2007) and Moure et al. (2007), for other stingless bees.

The *Melipona* clade seems largely monophyletic, with a common ancestor (Fernandes-Salomão et al. 2005; Ramírez et al. 2010; Rasmussen and Cameron 2010) (Fig. 1.9). Nevertheless, in the most recent study by Ramírez et al. (2010), it was found that three of the subgenera seem monophyletic (*Melikerria*, *Melipona s. str*., and *Michmelia*), but in the case of *Eomelipona*, it seemed polyphyletic (more than one possible ancestor, Fig. 1.9). Moreover, there was a group of species, which were not assigned to any of the four proposed subgenera (*Incertae sedis*). These non-classified *Melipona* species, are considered a sister group to all others, and apparently form a monophyletic clade with *Michmelia* (Ramírez et al. 2010).

In Mexico, the most important species of *Melipona, M. beecheii*, belongs in the subgenus *Melikerria* (Roubik and Camargo 2012). Two derived (apomorphic) traits are characteristic of the subgenus *Melikerria* (Moure 1992):

1. The inner half of the apex of the mandibles is subdivided by strong emargination into two distinct contiguous teeth (the median tooth with sinuosity).
2. The anterolateral angles of the mesonotum show a rust-red patch of hair.

The males have a scythe-shaped gonostyle (Roubik and Camargo 2012).

In addition to the Mesoamerican *M. beecheii*, eight other species are included in the subgenus *Melikerria*, most of South American distribution. The following species with the present geographic distribution are recognized in *Melikerria* (Camargo and Pedro 2007; Roubik and Camargo 2012):

M. beecheii from Mexico to Costa Rica
M. triplaridis from Eastern Panama
M. salti, from the north of Colombia

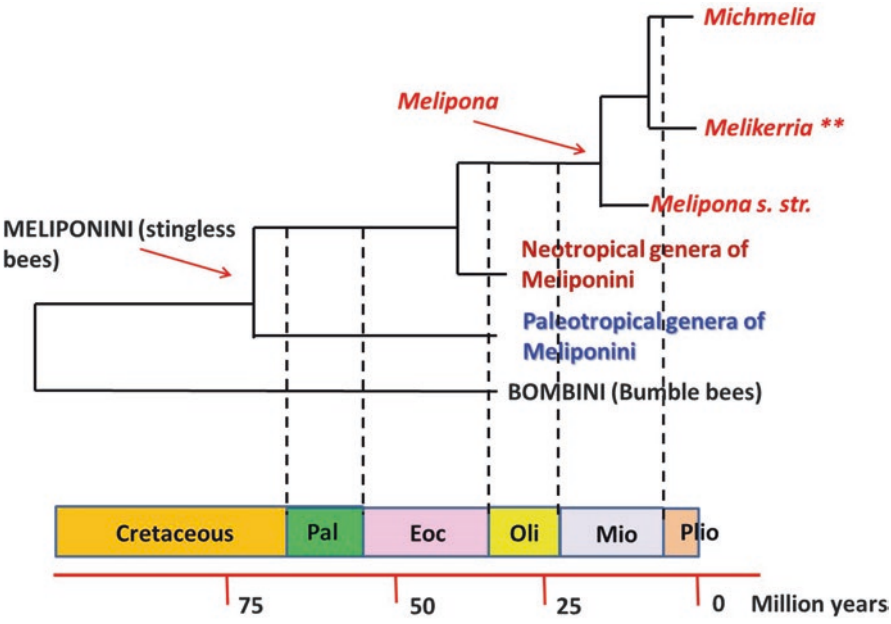


Fig. 1.9 Simplified phylogeny of the genus *Melipona* in accordance to Ramírez et al. (2010). The tribes are written in capital letters. The estimated time of emergence of each clade is presented, Pal (Paleocene), Eoc (Eocene), Oli (Oligocene), Mio (Miocene), Plio (Pliocene). The subgenus *Eomelipona* has a likely polyphyletic origin, and is not included in the figure. The subgenus *Melikerria* where *M. beecheii* is included is marked with a double asterisk

M. compressipes from Venezuela, Guyana, Suriname, French Guiana, and the north of Brazil and Colombia
M. interrupta from Guyana, Suriname, French Guiana, and the north of Brazil
M. grandis from South Western Amazonia
M. fasciculata from oriental Amazonia
M. quinquefasciata from North East Brazil and Argentina
M. insularis from the island of Coiba, province of Veraguas, Panama
M. ambigua from North East Colombia

The recent discovery of two new *Melikerria* species, namely *M. insularis* and *M. ambigua*, from Panama and Colombia, agrees with the idea that a land bridge united North and South America around 20 mya, long before the generally accepted estimation of 3 mya (Roubik and Camargo 2012). Thus, *M. beecheii* may have recently derived from the most northern species of *Melikerria*.

1.3 Diversity of 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 Pleistocene. Presently, 46 recognized species of stingless bees exist in Mexico (Ayala 1999). However, the

advent of molecular tools and analyses of chemical profiles have contributed to discover a large genetic diversity within several species complexes, some of which could represent cryptic species.

Compared to other bees, stingless bee populations frequently show genetic differentiation (structure) across regions. This may derive firstly, because swarms of stingless bees exhibit limited dispersal (Rasmussen and Cameron 2010). Colonies of stingless bees maintain long-time bonds with daughter swarms and these are established near by (see Chap. 6). This system of reproduction, called philopatry, reduces the spreading of colonies (and female reproductives) over long distances, causing genetic differentiation over time (Quezada-Euán et al. 2012). Interestingly, managed species tend to show weaker genetic structure than feral ones (Jaffé et al. 2016; Landaverde-González et al. 2017); this is possibly due to man-mediated colony movement across locations.

It should also be considered that meliponine species may be phenotypically similar, yet exhibiting substantial differentiation, in genetic and nonvisual traits, i.e., chemical profiles (Bickford et al. 2006). Such species are known as cryptic and seem frequent in stingless bees (Michener 2000). On the other hand, large phenotypic variation may lack significant genetic differentiation (Quezada-Euán et al. 2012; Hurtado-Burillo et al. 2017). Thus, accurate definition of true species in stingless bees should involve the use of multiple criteria (Halcroft et al. 2016).

In Mexico, the genus *Melipona* is one of the most important. It is represented by seven species, which comprise 15% of the Mexican stingless bee fauna (Ayala et al. 2013; Table 1.2). Among the Mexican species of *Melipona*, two are found in highland forests: *M. colimana* and *M. fasciata*, both in the subgenus *Michmelia.* The workers of these species have the largest body size of them all. There are two other species of the subgenus *Michmelia* in Mexico; both are found in lowland tropics, *M. solani* and *M. belizae*. The workers of both species are clearly distinguished by the orange pilosity. The smallest Mexican *Melipona* are both endemic, namely *M. yucatanica* and *M. lupitae.* These species are phenotypically similar and are close phylogenetically (Ayala 1999; Table 1.2).

The species with the most extensive geographic distribution is *M. beecheii*, and also the most relevant due to its economic and cultural importance (Ayala 1999) (Fig. 1.9a). It is also the only species of the subgenus *Melikerria* out of South America (Roubik and Camargo 2012).

Molecular comparisons have shown that *M. beecheii* has probably experienced earlier divergence from the other *Melikerria* (Roubik and Camargo 2012).

Roubik and Camargo (2012) suggested that *M. beecheii* probably derives from the lineage of *M. insularis*, the most northern of all South American *Melikerria*. Its lineage may have gone extinct on mainland, but relict populations survived on islands of the Panama microplate, which eventually colonized the northern parts of Central America and the tropical regions of Mexico. This scenario may also explain the larger

Table 1.2 Description of the seven species of the genus *Melipona* found in Mexico (based on Ayala 1999)

Species		Distribution	Main features
Melipona beecheii Subgenus: *Melikerria*		The Yucatán Península, Gulf Coast (Tabasco, Veracruz, Tamaulipas) and Pacific Coast (Chiapas, Jalisco, Nayarit, Oaxaca). Also found in San Luis Potosí and Zacatecas	Integument black with variable yellow, red, or black marks on the legs. Body length 9.7–10.7 mm. Front of the antennal scape yellow. Scutum of the thorax with orange/yellow hair. Lateral tufts of dense orange-red hairs in strong contrast with the rest of the scutum. Black abdomen with well-defined yellow bands
Melipona solani Subgenus*:* *Michmelia*		Tropical forests of Chiapas and possibly Tabasco. More common in Central America	Orange and black integument with orange pilosity. Body length 8.0 mm. Hair on face orange-brown with some black hair. Pilosity red-orange with black hairs. Abdominal segments orange generally with no lines
Melipona belizeae Subgenus: *Michmelia*		Belize, Southern areas of Campeche and Quintana Roo	Integument black and orange. Body length 8.0 mm. Face without marks with grey-brown hair around the ocelli. Scutum with ocher and some black hairs. Sides of thorax with ocher or red hair only. Abdomen brown with yellow bands in last segments
Melipona fasciata Subgenus: *Michmelia*		One of the two highland *Melipona* species in México between 500 and 3000 m. From Guerrero and Michoacán, also Orizaba and Córdoba on the Gulf coast of Mexico.	Integument of head and thorax black, but orange in the abdomen with variable yellow lines. Pilosity clear yellow and orange. Body length 9.0 mm. Yellow marks below the eye (as in *M. colimana*). Sides of the thorax with orange tufts

(continued)

Table 1.2 (continued)

Species		Distribution	Main features
Melipona colimana Subgenus: *Michmelia*		Endemic to the highland forests on the west Coast in Jalisco above 1000 m (sierra de Manantlán, Volcán and Nevado de Colima and sierra del Tigre)	Black integument and yellow marks with orange pilosity no black hair. Body length 9.5 mm. Yellow marks below the eye. Side portions of the thorax with orange hairs and a tuft of dark orange hairs. Abdominal segments black with well-defined yellow apical bands
Melipona lupitae Subgenus: *Melipona*		Endemic to Mexico, only found in low tropical deciduous forests on the west side of the River Balsas Basin, in the state of Michoacán	Black integument with white and yellow marks. Light brown-orange fur with some whitish or black hairs. Body length 7.9–8.5 mm. Black scutellum. Tibia dark brown with black hairs. Abdominal segments with yellow stripes well defined with some black hair.
Melipona yucatanica Subgenus: *Melipona*		Endemic to the Southern forests of the Yucatan Península and the Tehuantepec Isthmus. Also present in Guatemala but the population is genetically different to the Mexican ones	Morphologically similar to *M. lupitae.* Black integument with yellow marks. White to orange hair on the face. Body length 8.2–8.5 mm. Scutum with orange hairs. Lateral portion of the thorax with a yellow thin line. Brown-red tibiae with yellow hairs. Corbicula with a black mark on its distal portion. Abdominal segments almost hairless and intensely colored yellow lines

Photographs kindly provided by Dr. Ricardo Ayala (UNAM), Dr. Terry Griswold, and Chelsey Ritner (USDA ARS—Utah State University)

genetic diversity found in *M. beecheii* from Central America compared to Mexico (May-Itzá et al. 2009), in agreement with a more recent colonization of the latter.

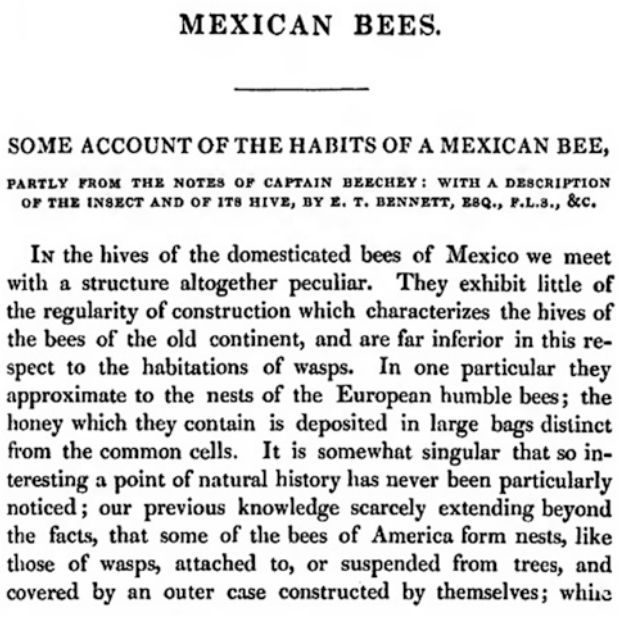
Presently, the distribution of *M. beecheii* in Mexico includes the low tropical and subtropical forests of the Pacific and Gulf coasts, the Isthmus of Tehuantepec, and the Yucatan Peninsula. Outside Mexico, it can be found in Guatemala, Belize, Honduras, Salvador, and Nicaragua. The southernmost distribution is the Guanacaste Peninsula of Costa Rica (Ayala 1999; Fig. 1.10A). This species is also one of the only two *Melipona* found in the Antilles, on the islands of Cuba and Jamaica (Genaro 2008). In the Yucatan Peninsula and some regions of Central America,

Fig. 1.10 (**A**) Geographic distribution (in orange) of *M. beecheii* in accordance with Ayala (1999) and Moure et al. (2007). (**B**) The two species of *Melipona* present in the Yucatan Peninsula: (**a**) *M. (Melikerria) beecheii* and (**b**) *M. (Melipona) yucatanica*

M. beecheii is sympatric with *M. yucatanica* (*ts'ets* in Maya), both recognized by the Maya as different species (González-Acereto 1984). Although externally both species may look similar, especially in the color of the integument (Fig. 1.10B), they are not related (Camargo et al. 1988). Indeed, in accordance with Moure et al. (2007), *M. yucatanica* belongs to the subgenus *Melipona s. str.* while *M. beecheii* is included in *Melikerria.*

Across its wide geographic distribution, *M. beecheii* is known with a diversity of names: abeja-real, *Yilkil-kab*, *Colel-kab*, *Xunan kab* (*Xunaan-kaab*), *Pool-kab*, colmena-kab, abeja-alazana, *pipioli*, *mimialcuatl*, *tsaspena*, gato, abeja-aluva, blanco-aluva, criolla, jicote-gato, jicote-estrella, abeja de la tierra, *Ajau-chab*, and *Suk-ajatié*, the latter two among the Maya, Chol, Chontal, and Tzeltal from Tabasco and Chiapas, in Mexico (González-Acereto 2008; Moure et al. 2007). The diversity fo names may be an indication of the extensive use of this species.

The first scientific description of *M. beecheii* was made by captain F.W. Beechey in his manuscript narrating a 3-year voyage to the Pacific coasts of Mexico: *Narrative of a voyage to the Pacific and Beering's strait, to cooperate with the polar expeditions: performed in his Majesty's Ship Blossom, under the command of Captain F. W. Beechey.* Vol. 2. (1831) London: H. Colburn & R. Bentley. In this volume's Appendix, from pages 357 to 365, the navigator includes a section called "*Mexican bees*. *Some account of the habits of a Mexican bee*" (Fig. 1.11).



MEXICAN BEES.

SOME ACCOUNT OF THE HABITS OF A MEXICAN BEE,

PARTLY FROM THE NOTES OF CAPTAIN BEECHEY: WITH A DESCRIPTION OF THE INSECT AND OF ITS HIVE, BY E. T. BENNETT, ESQ., F.L.S., &C.

In the hives of the domesticated bees of Mexico we meet with a structure altogether peculiar. They exhibit little of the regularity of construction which characterizes the hives of the bees of the old continent, and are far inferior in this respect to the habitations of wasps. In one particular they approximate to the nests of the European humble bees; the honey which they contain is deposited in large bags distinct from the common cells. It is somewhat singular that so interesting a point of natural history has never been particularly noticed; our previous knowledge scarcely extending beyond the facts, that some of the bees of America form nests, like those of wasps, attached to, or suspended from trees, and covered by an outer case constructed by themselves; while

Fig. 1.11 A section of the first page of the Appendix of the narrative of the journey by captain Beechey (1831) along the Pacific coast of Mexico, where he described *M. beecheii* for the first time

Because captain Beechey was the person who first gave an account on this bee and its colonies, Bennett in 1831 used the captain's name for its species nomenclature, and assigned it to the genus *Melipona*, described by Illiger in 1806.

Beechey (1831: on the page 444 from the same Appendix) indicates the localities visited along the Mexican Pacific coast: San Blás and Tepic in Nayarit, as well as Mazatlán in Sinaloa and Acapulco in Guerrero. However, gives no precise locality where he collected his specimens. Paradoxically, the economic and cultural value of *M. beecheii* for the indigenous inhabitants of the west coast of Mexico is much less representative compared with the Yucatan Peninsula (Quezada-Euán et al. 2001; Fig. 1.10). It is believed that the description made by Bennett of *M. beecheii* was based on a single specimen whose present location is unknown (Moure et al. 2007).

In his revision of the stingless bees of Mexico, Ayala (1999) describes *M. beecheii* as a medium-sized bee, measuring between 9.7 and 10.7 mm, with a forewing span between 7.7 and 7.9 mm. The integument is black with yellow, brown, or black marks on the legs, depending on the geographic origin (Fig. 1.12). The metasomal tergi are black, with well-defined yellow bands. Noteworthy, there is a wide variation in size and coloration among specimens from different regions, particularly on the maculae on the clypeus and the dark marks on the hind legs (Ayala 1999).

Ayala (1999) assigned *M. beecheii* in what he called the *fasciata* group. The *fasciata* group of *Melipona*, have the lower part of the antennal scape yellow; the head vertex on frontal view slightly concave with no elevation at the level of the ocelli; the frontal-lateral sides of the scutum are covered by tufts of dense red hair, in contrast with the rest of the pubescence, which is clear and less dense.

There has long been controversy on the taxonomy of *M. beecheii* as a single species. This due to contrasting variation of color, body size, and occurrence of intergrades in specimens from different geographic regions (Schwarz 1932; Wille 1976; Quezada-Euán et al. 2001, 2007). Schwarz (1932) proposed the existence of two subspecies of *M. beecheii*. One such subspecies (*M. b. beecheii*), may involve populations from southern Mexico, Guatemala, Nicaragua, and Costa Rica, and the other (*M. b. fulvipes*), may include specimens from Cuba, Jamaica, Belize, and the Yucatan Peninsula in Mexico. Camargo et al. (1988) also acknowledged the possible existence of these two subspecies in *M. beecheii*. However, to date, *M. b. beecheii* and *M. b. fulvipes* are not accepted as true subspecies (Moure et al. 2007).

A first quantitative approach to analyze the morphological variation in *M. beecheii* was done by Carrillo et al. (2001). Their study included specimens from the Yucatan, Costa Rica, and Chiapas and compared body size and degree of maculation of the clypeus (the section on the head below the antennae and above the mandibles) and the malar region (the area beside the clypeus) (Fig. 1.12). The results showed that the bees from the Yucatan Peninsula were smaller and had significantly more yellow marks on the face than the bees from the other two regions.

In a following study Quezada-Euán et al. (2007) showed that morphological differentiation between bees from the Yucatan Peninsula and Costa Rica was genetically supported (*FST* value = 0.280). In spite of clear genetic differences, the sequencing of the mitochondrial *cox*1 revealed a differentiation between both populations of only 1.2%. Typically, sequence divergence of *cox*1 between insect species

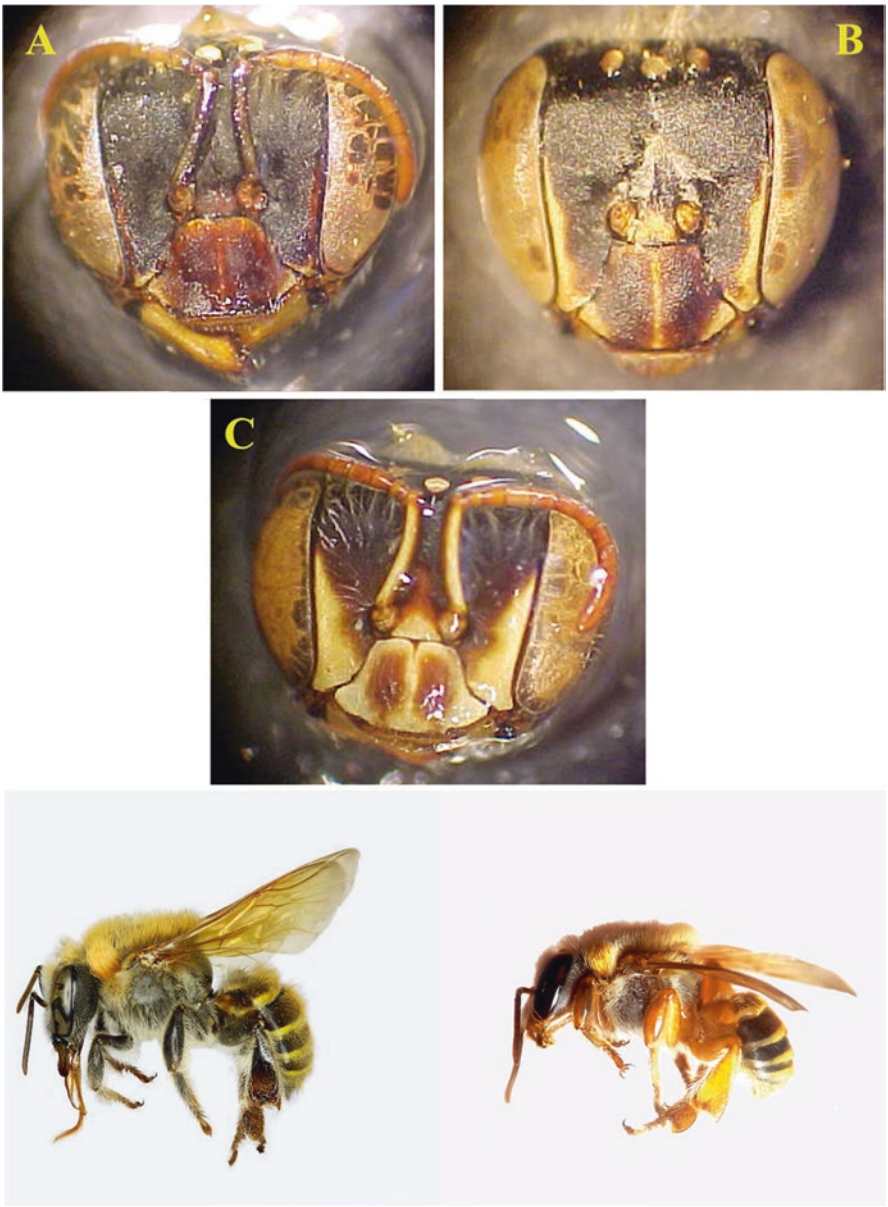


Fig. 1.12 Intraspecific diversity in the pattern of coloration (maculation) of the face and body of *M. beecheii* workers from (**a**) Costa Rica, (**b**) Chiapas, Mexico, and (**c**) Yucatan, Mexico. There is a clinal distribution in the degree of face and leg maculation decreasing from North to South. The lower image shows workers from Chiapas (left) and Yucatan (right). The specimens from Chiapas are larger and darker than the specimens from the Yucatan

is in a range between 3 and 10% (Hebert et al. 2003), though sibling species may show far lower sequence divergence (Hebert et al. 2004). Although the observed divergence of 1.2% between populations of *M. beecheii* representing different species is not yet clear, distinct genotypes can be found in both regions (Quezada-Euán et al. 2007).

In subsequent studies, samples from Guatemala, El Salvador, and Nicaragua, together with samples from Mexico and Costa Rica were compared using the segments *ITS*2 and *ITS*1 of the ribosomal DNA (De La Rúa et al. 2007; May-Itzá et al. 2009). An interesting finding was that the *ITS*2 segment of *M. beecheii* is one of the longest found in insects, with 1788 bp (De la Rúa et al. 2007). After digestion, three different patterns were found for both *ITS*2 and *ITS*1, which are in general congruent with the geographic origin of the samples (De la Rúa et al. 2007; May-Itzá et al. 2009). A first pattern of *ITS*1 and *ITS*2 was obtained in specimens from Mexico (Yucatan and Chiapas) and northern de Guatemala, around the Petén region (Fig. 1.13). A second *ITS*1 pattern (B), was found in populations from southern Guatemala and El Salvador, and a third *ITS*1 pattern (C), was found in the bees from San Marcos, in the southwestern part of Guatemala (Fig. 1.13). In the case of *ITS*2, a characteristic pattern was found in specimens from southern Guatemala, Salvador, and Costa Rica, and third pattern was only found in the bees from San Marcos (De la Rúa et al. 2007). The largest genetic diversity of *M. beecheii* was found in Guatemala, where all the different patterns of *ITS*1 and *ITS*2 found, are present (Fig. 1.13).

The evidence from molecular and morphometric analyses, suggests that *M. beecheii* is a monophyletic species with two clearly differentiated groups (May-Itzá et al. 2012). Both groups are largely in agreement with the original subspecies proposed by Schwarz (1932). The distribution of the two main genetic lineages in *M. beecheii* seems defined by the Sierra Madre del Sur, which starts in Chiapas and extends down to Central America (Fig. 1.14). This large mountain chain may have

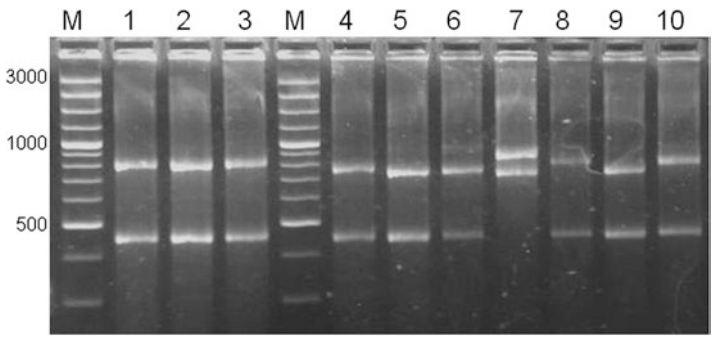


Fig. 1.13 The three different patterns found after digestion of the ribosomal segment *ITS*1 of *M. beecheii*. *ITS*1-A is obtained in specimens from Mexico (Yucatan Peninsula and Chiapas, lanes 1, 2, and 3, respectively) and the north of Guatemala (Petén, lanes 8 and 10). The *ITS*1-B pattern is found in bees from El Salvador (lane 4), and southern Guatemala (lanes 5, 6, and 9, respectively). Pattern *ITS*1-C (lane 7), has only been detected in specimens from San Marcos, in southwestern Guatemala

acted as a barrier for genetic flow between populations to the north and south. Speciation seems to frequently occur in stingless bees as a result of vicariance due to geographic isolation (Quezada-Euán et al. 2012; Jaffé et al. 2016).

There is also evidence for the existence of a cline in body size within the lineages of *M. beecheii* (Fig. 1.14). The bees from central Guatemala have the largest body size and this gradually decreases in the specimens from Salvador, Nicaragua and Costa Rica. Interestingly, a similar pattern occurs to the north, with the bees from the Yucatan Peninsula having the smallest body size of all populations studied (Fig. 1.14).

The mobilization of colonies is a potential threat for the genetic identity of the different lineages of *M. beecheii*, as this species is extensively used in Mexico and

Fig. 1.14 Phenotypic and genetic diversity in *M. beecheii.* There is a cline in body size represented by the mean length of the forewing (mm) across the different regions. Two clear *ITS* patterns are also evident (black and red dots), geographically separated by the Sierra Madre del Sur: (1) Yucatan Peninsula-northern Guatemala-Chiapas, and (2) Central America. There are also *ITS* differences within the Central American clade, suggesting one more genetic lineage in it (May-Itzá et al. 2009; May-Itzá et al. 2012)

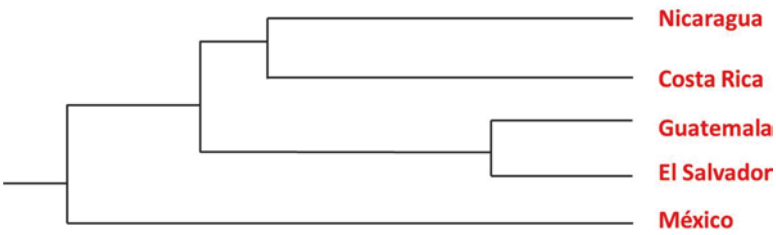


Fig. 1.15 UPGMA tree based on the geometric morphometrics of wing shape in *M. beecheii* specimens from different geographic regions (Francoy et al. 2011). The clades are in agreement with the molecular results of *ITS*1

Central America for honey production, and colonies can be exchanged among regions (May-Itzá et al. 2012). In order to find quick and reliable methods that allow geographic traceability of populations and lineages in *M. beecheii*, Francoy et al. (2011) analyzed the geometric morphometrics of wings of the samples used in the studies by May-Itzá et al. (2009) and May-Itzá et al. (2012). Their approach correctly assigned 87.1% of the colonies to their geographic region, and 92.4% to their haplotype (Fig. 1.15). Thus, geometric morphometrics can be a cheap and reliable method to accurately trace the geographic origin of specimens of *M. beecheii*, and serve as a tool to control the movement of specimens and colonies across geographic regions (Francoy et al. 2011).

Although a clearer picture is now evident for the biogeography of *M. beecheii* across the southern part of its distribution, nothing is yet known for populations along the Gulf Coast of Mexico (Veracruz, San Luis Potosí), and the Pacific coast, between Chiapas and Sinaloa. Stingless beekeeping is increasing in these areas, and it is important to develop studies on the genetic makeup of such populations in order to preserve them.

M. beecheii is one of the two species of *Melipona* found in Cuba (Fonte 2007; Genaro 2008), and the only one in the Greater Antilles. Given the limited ability for colonies to move across large bodies of water, a plausible explanation for its presence on these islands seems human introduction (Michener 1982; Genaro 2008).

Because of their similarity in size and color, Schwarz (1932) and Camargo et al. (1988), considered the bees from Cuba as *M. fulvipes* or a subspecies (*M. beecheii fulvipes*), and placed together with the bees from the Yucatan Peninsula. Recently, Lóriga-Peña and Quezada-Euán (2009) compared the body size and the maculation of the head and legs of *M. beecheii* samples from Cuba with bees from the Yucatan Peninsula and Costa Rica. Although the bees from Cuba and the Yucatan differed in individual characters, a multivariate comparison showed extensive overlapping in their overall body size. The bees from Costa Rica were significantly larger than those from Yucatan and Cuba (Fig. 1.16).

DNA microsatellite markers confirmed that the samples from Cuba were genetically more similar to those from the Yucatan Peninsula too (Lóriga-Peña et al. 2011;