Meliponini (Bees)

Gabriela de Oliveira
Colegio Bolivar

Dr. Wojciech S. Waliszewski
Agricultural Class Monograph, Grade 12
Abril 29, 2022

Table of Contents

Introduction

Meliponini is a tribe of the family Apidae and the order Hymenoptera commonly referred to as "stingless bees". Meliponines have stingers, but they are highly reduced and cannot be used for defense. In recent years stingless bees have become very popular for the medicinal benefits of their products like honey, propolis, pollen, and cerumen. Studies have shown that these products have antioxidant, anti-inflammatory, anti-obesity, anticancer, and antimicrobial properties. Furthermore, *Meliponini* are extremely effective pollinators playing a crucial role in tropical ecosystems and showing great promise for the agricultural industry.

This monograph goes into depth about the ecology, biology, propagation and management, and ultimately emerging products and potential markets of *Meliponini* bees. Specifically, we will discuss their distribution, the environmental factors that affect them, their life cycle, hive structure, managment, economic importance of *Meliponini*, their hive products amongst other topics.

Chapter 1: Ecology

1.1 Distributional context

1.1.1 Affinities

Meliponini is a tribe of the family Apidae and the order Hymenoptera commonly referred to as "stingless bees". They are closely related to common honey bees, orchid bees, and bumblebees. Meliponines have stingers, but they are highly reduced and cannot be used for defense, though they have other defensive behaviors. Stingless bees can be found in most tropical regions of the world, such as Australia, Africa, Southeast Asia, and Central and South America. The majority of native bees of Central and South America are stingless bees (Grüter, 2020). They produce low quantity but excellent quality of honey; only a few species produce enough honey to be farmed by humans. In fact, meliponine honey is prized as a medicine in many African and South American communities (Ávila et al., 2018; Grüter, 2020). Stingless bees are important pollinators of annatto, camu-camu, chayote, coconut, cupuaçu, carambola, macadamia, mango, and mapati. They make a contribution to the pollination of about 60 other crop species and have been reported visiting about 20 crop species(Heard, 2001). According to Kerr et al. (1996), bees are responsible for 40 to 90% of the pollination of native plants in a tropical environment. They are often used as pollinators in greenhouses, exceeding honey bees in efficiency and leading to overall larger yields of fruits that were heavier, larger, and contained more seeds (Santos et al., 2009). The Meliponini tribe comprises approximately 600 species in 56 genera that are characterized for being highly eusocial (colonies with adults of two generations, castes and division of labor, cooperative work on brood cells, permanent colonies), but only two genera, *Melipona* and *Trigona*, are commonly domesticated worldwide (Shamsudin et al., 2019). There are 400 known species in Neotropical regions, and it is estimated that more than 100 species are yet to be described (Cortopassi-Laurino 2006). Approximately 244 species occur in Brazil (Pedro 2014), and some of them are under threat of extinction due to the destruction of their natural habitat (Cham et al., 2018). Meliponines have extremely diverse morphology, nest building (nidication) habits, behavior, and ecology. They are small- to medium-sized bees (2–13mm in length, ranging from species smaller than fruit flies to species larger than honey bees) with considerable variation in colony population size (from several hundred up to more than 10,000 workers per colony). They also exhibit a high rate of brood production and require substantial pollen intake (Ramalho etal. 1998)(Cham et al., 2018).

List of unusual stingless bees:

- *Trigona necrophaga* , *Trigona hypogea* , and *Trigona crassipes*: These bees do not collect fresh pollen or nectar from fresh flowers in the way that other species do. Instead, these bees collect dead meat, and also get their sugars from eat rotting fruits or dead flowers
- *Lestrimelitta*: This species raids the food stores of other bees and transfers them to the storage pots in its own nest.
- *Partamona batesi:* build their hives in the nests of other social insects such as termites or ants.
- *Ptilotrigona lurida, P. pereneae, and P. occidentalis:* These bees cultivate yeast associated with pollen.

Taxonomy of Meliponini (*ITIS - Report: Axestotrigona Ferruginea*, n.d.):

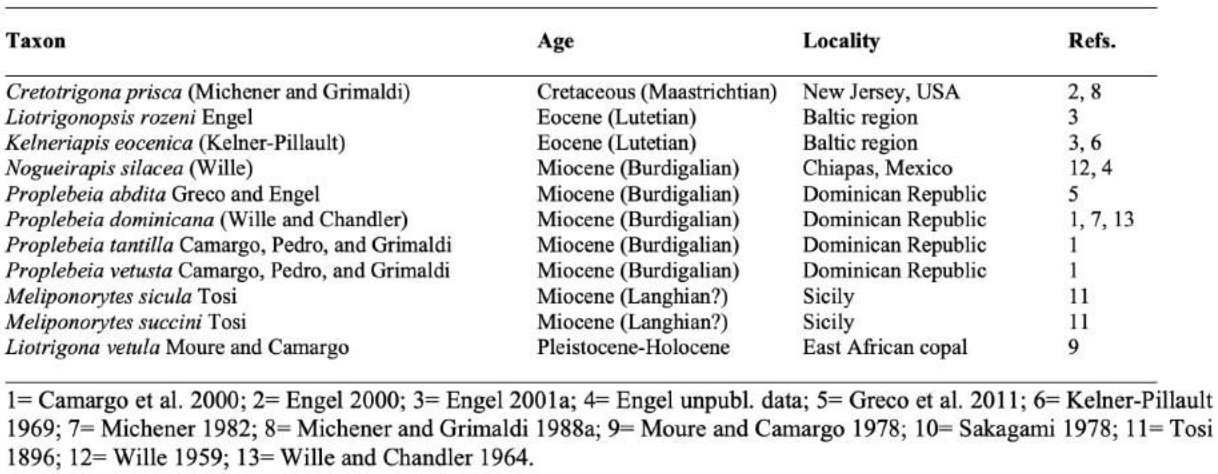
Kingdom:	Animalia
Subkingdom:	Bilateria
Infrakingdom:	Protostomia
Superphylum:	Ecdysozoa
Phylum:	Arthropoda
Subphylum:	Hexapoda
Class:	Insecta
Subclass:	Pterygota – winged insects
Infraclass:	Neoptera – modern, wing-folding insects
Superorder:	Holometabola
Order:	Hymenoptera
Suborder:	Apocrita – ants, bees, wasps
Infraorder:	Aculeata
Superfamily:	Apoidea – bees, sphecoid wasps, apoid wasps
Family:	Apidae – euglossines, honey bees, stingless bees
Subfamily:	Apinae
Tribe:	Meliponini
Genera (selected):	*Lestrimelitta* *Melipona* *Partamona* *Ptilotrigona* *Trigona*

1.1.2 Fossil Record and Origin

Fossils of stingless bees are the most abundant of all fossilized Apoidea. Despite this abundance, most of the fossils represent few species. 11 extinct Meliponini species have been recorded from amber and copal

Figure 1.

Fossilized Meliponini species (adapted from - (Engel & Michener, 2013))



Taxon	Age	Locality	Refs.
Cretotrigona prisca (Michener and Grimaldi)	Cretaceous (Maastrichtian)	New Jersey, USA	2, 8
Liotrigonopsis rozeni Engel	Eocene (Lutetian)	Baltic region	3
Kelneriapis eocenica (Kelner-Pillault)	Eocene (Lutetian)	Baltic region	3, 6
Nogueirapis silacea (Wille)	Miocene (Burdigalian)	Chiapas, Mexico	12, 4
Proplebeia abdita Greco and Engel	Miocene (Burdigalian)	Dominican Republic	5
Proplebeia dominicana (Wille and Chandler)	Miocene (Burdigalian)	Dominican Republic	1, 7, 13
Proplebeia tantilla Camargo, Pedro, and Grimaldi	Miocene (Burdigalian)	Dominican Republic	1
Proplebeia vetusta Camargo, Pedro, and Grimaldi	Miocene (Burdigalian)	Dominican Republic	1
Meliponorytes sicula Tosi	Miocene (Langhian?)	Sicily	11
Meliponorytes succini Tosi	Miocene (Langhian?)	Sicily	11
Liotrigona vetula Moure and Camargo	Pleistocene-Holocene	East African copal	9

1= Camargo et al. 2000; 2= Engel 2000; 3= Engel 2001a; 4= Engel unpubl. data; 5= Greco et al. 2011; 6= Kelner-Pillault 1969; 7= Michener 1982; 8= Michener and Grimaldi 1988a; 9= Moure and Camargo 1978; 10= Sakagami 1978; 11= Tosi 1896; 12= Wille 1959; 13= Wille and Chandler 1964.

The following is a brief review of the fossil record of Meliponini, progressing from oldest to youngest and through the geological periods of the Cretaceous, Paleogene, and Neogene.

Cretaceous (145–65.5 million years ago)

The Cretaceous period had some of the most dramatic episodes of biotic diversification and change, specifically the origin and development of flowering plants and the numerous lineages of animals. These animals arose to take advantage of either the new flora or those creatures associated with it (Engel & Michener, 2013). It was in this context that bees first diverged from the apoid wasps, (at least 125 million years ago) (Engel, 2001a, 2004a, 2011; Ohl and Engel, 2007). After this bee diversification was probably rapid, with the earliest roots of the main families arising during the mid-Cretaceous and subsequently diverging throughout the rest of the Cretaceous period (Engel, 2001a, 2004a; Ohl and Engel, 2007).

The earliest evidence of stingless bees comes from the latest stage of the Cretaceous. From Maastrichtian-aged deposits (the latest age of the Late Cretaceous Epoch), approximately 70 million years old, in New Jersey, amber produced by extinct species of Taxodiaceae was recovered (Michener & Grimaldi, 1988a,1988b; Engel, 2000). *Cretotrigona prisca* (Michener & Grimaldi) (Figure 2b), comprises the whole Mesozoic record of the Meliponini, and is the only true apid from the Cretaceous. The species has remarkable superficial similarity to modern species of Trigona s.str. (Michener & Grimaldi, 1988). *Cretotrigona* demonstrates that stingless bees once occupied what are today higher latitudes in the New World, but were likely dramatically impacted by the globally disruptive events that occurred during the mass extinction event around the K-T boundary (the boundary between the Cretaceous and Tertiary periods), 65.5 million years ago (e.g., Renne et al., 2013; Pälike, 2013). The impact of a large meteor colliding with the earth, off of the Yucatan Peninsula, along with a series of smaller impacts around the globe, set into motion a chain of events which led to a significant reorganization of the flora and fauna. This was particularly dramatic across Central and North America because these were the sites of impact (Schultz & D'Hondt, 1996). Northern Hemisphere effects were perhaps most catastrophic at this time, particularly in the New World, and certainly *C. prisca* and any of its relatives would have been devastated by such an event. In addition to its biogeographic implications, *C. prisca* demonstrates that highly eusocial bees were already well developed and present in the later times of the Mesozoic (Michener & Grimaldi, 1998b). This is not surprising given that many eusocial insect lineages like termites and ants had their origins during the Cretaceous (Grimaldi & Engel, 2005; Engel et al., 2009).

Figure 2.

Fossilized Meliponini species through the geological periods (adapted from - (Engel & Michener, 2013))

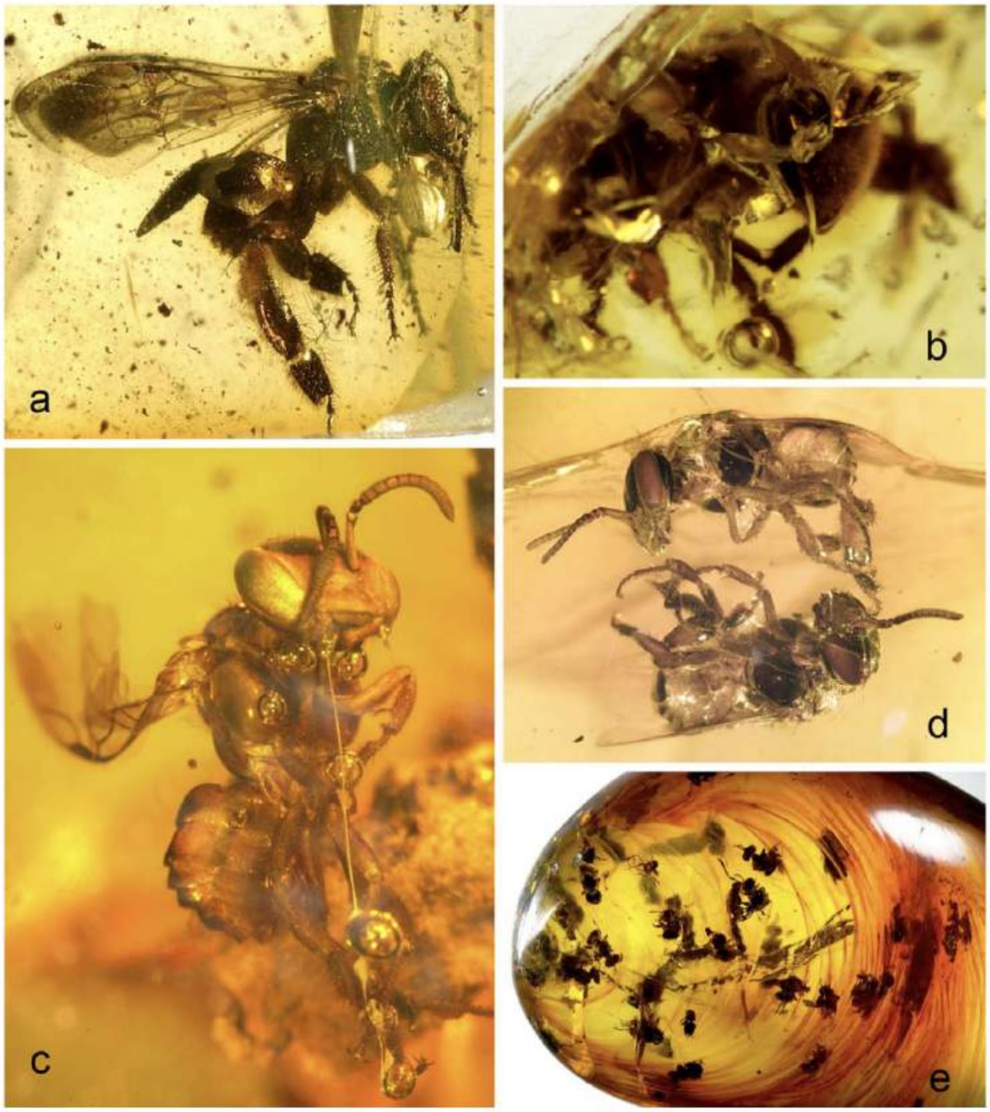


Figure 1. Photomicrographs of amber-entombed workers of melikertine and meliponine bees.
a. *Melissites trigona* Engel (Melikertini) in mid-Eocene Baltic amber. **b.** *Cretotrigona prisca* (Michener and Grimaldi) (Meliponini) in Late Cretaceous New Jersey amber. **c.** *Nogueirapis silacea* (Wille) (Meliponini) in Early Miocene Mexican amber. **d.** Two individuals of *Proplebeia dominicana* (Wille and Chandler) in Early Miocene Dominican amber. **e.** 'Swarm' of *P. dominicana* in Dominican amber. All rights to the images are retained by M.S. Engel, and may not be reproduced without explicit permission.

Paleogene (65.5–23 million years ago)

The Paleogene period marked the beginning of the Cenozoic (the Age of Mammals, but just as much a mere fragment in an overarching Age of Insects). Non-avian dinosaurs were gone, along with countless other lineages, and the flora and fauna had to adapt to massive climatic changes, particularly a rapid spike at the Paleocene-Eocene Thermal Maximum (PETM - a time period with a more than 5–8 °C global average temperature rise across the event). Although the PETM was a considerable raise in global temperatures, overall the Cenozoic experienced a trend toward cooling and drying relative to the Cretaceous (particularly after the Eocene and continuing through the Neogene). This shift was partially driven by the formation of the Antarctic Circumpolar Current which also led to the ultimate freezing of Antarctica. It was also during the Paleogene that the 'Grand Coupure' took place, also known as the Eocene-Oligocene extinction event. It was tied to another drop in global temperatures (due to lowered atmospheric carbon dioxide) as well as the beginning of ice sheet formation on Antarctica (Engel & Michener, 2013).

It was at the Eocene Oligocene boundary that there appears to have been a change in the composition of bee faunas. Many primitive forms of bees recognized as extant tribes became extinct. That included the loss of various, highly eusocial, bee lineages, most notably the Melikertini, understood to be the closest relatives of the stingless bees (Engel, 2001a, 2001b). Although the Paleogene record of bees is significantly stronger than that of the Cretaceous (e.g., Engel, 2001a; Michez et al., 2012), there are presently only two definitive stingless bees documented from the Paleogene (Both are preserved in middle Eocene amber of the Baltic region): *Liotrigonopsis rozeni* Engel and *Kelneriapis eocenica* (Kelner-Pillault). Both species are similar to genera today living in sub-Saharan Africa and Southeast Asia (Engel 2001a).

Both of the Baltic amber species highlight a more global distribution of stingless bees. *Cretotrigona, Liotrigonopsis* and *Kelneriapis* push the historical bounds of meliponine distribution well northward, encompassing what are today cool temperate regions of the northern hemisphere. While today stingless bee biogeography is often limited to pantropical distribution, we must recognize that tropical zones have not remained static. Climates have changed considerably, and any consideration of meliponine historical biogeography must take this into account, particularly given that the tribe is of such great antiquity. Prior to the Eocene Oligocene boundary the planet's higher latitudes were subtropical or paratropical, permitting stingless bees to have much broadened ranges; *Liotrigonopsis* and *Kelneriapis* both are indicative of these paratropical conditions within the middle Eocene of Europe (Engel & Michener, 2013).

Neogene (23 million years ago–today)

During the Neogene period the global cooling and drying trend continued, with development of distinct seasonalities, and continental positions largely approximated those of today, particularly with the formation of the Isthmus of Panama and the connection of North and South America. It is from the Neogene that we have the richest sources of fossil stingless bees, though representing relatively few species (e.g., Michez et al., 2012). It is from the Early Miocene (19–17 million years old) amber mines of the Dominican Republic that the greatest wealth of stingless bee fossils has been recovered. Dominican amber is likely an extinct species of *Hymenaea* (Fabaceae). While West Indian stingless bees are today only found in Jamaica, the Lesser Antilles, and putatively adventive in Cuba, they were a diverse and abundant component of the native fauna of ancient Hispaniola. Michener (1982) confirmed that the Dominican amber species was more closely related to *Plebeia* but noted significant differences and therefore established the genus *Proplebeia*. *Proplebeia dominicana* remains the single most common fossil bee anywhere in the world, although subsequent research has added additional species to the genus (Camargo et al. 2000; Greco et al. 2011: Table 1). Multiple individuals of Proplebeia are often found together and these clusters can even be quite numerous (Figures 2d, e). The various species of Proplebeia, as well as many other Dominican amber bees (e.g., Engel, 1996, 1999a, 1999b; Engel et al., 2012), highlight the changes to the composition of the West Indian melittofauna that have taken place during the last 19 million years.

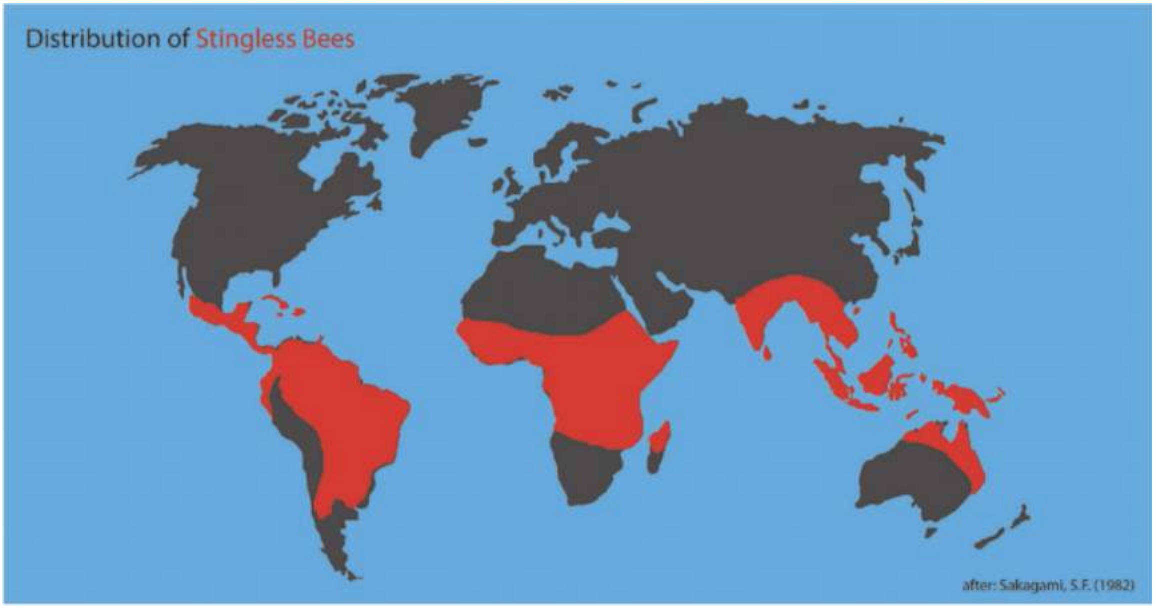
The contemporaneous amber deposits of southern Mexico are another rich source of Early Miocene flora and fauna, and this fossil resin was perhaps also produced by an extinct species of *Hymenaea* much like Dominican amber (Engel, 2004b; SolórzanoKramer, 2007). Unfortunately, unlike the Dominican amber, those fossil resins from Mexico have far fewer bee inclusions, although all presently recorded individuals are stingless bees. The first bees discovered from these deposits were described as an extinct species of *Nogueirapis moure* (Wille, 1959). *Nogueirapis silacea* Wille (Figure 2c) is not as abundant as *P. dominicana*, but is certainly not a rarity in the Mexican amber fauna. This work is ongoing but is already highlighting that there remains much to be discovered about Mexico's fossil meliponines. Pleistocene and younger copals harbor numerous stingless bee individuals and come from a wide diversity of botanical sources. Indeed, some of the young copals from Colombia, Tanzania, Madagascar, the Philippines, and elsewhere are known to contain workers of a variety of living *Meliponine* species, and these can sometimes be quite common or come in large numbers within individual pieces. (Engel & Michener, 2013)

1.1.3 Present distribution

Stingless bees can be found in most tropical regions of the world (Figure 3), such as Australia, Africa, Southeast Asia (India, Malaysia, and Indonesia), and Central and South America.(Camargo and Pedro 2013)

Figure 3.

Map of geographic distribution of Meliponini in the Tropical and Subtropical regions of the world (Sagakami, 1982)



1.2 Environmental Factors

1.2.1 Environmental Factors in Distribution

Most species of stingless bees are geographically restricted to low tropical and subtropical regions which could be a result of their limited ability to warm up their nests at low environmental temperatures, such as those found in Mexico (Figure 4, below). However, few species also dwell at tropical highlands and may show tolerance to cold by means of active mechanism, but this has not been evaluated (Sakagami 1982; Wille1983; Ortiz-Mora et al. 1995; Nogueira-Neto1997).

Consensus distribution models of Melipona species of CAM. Panels a and b correspond to subgenus Melikerria; c to f to subgenus Michmelia; h, i and j to subgenus Melipona, and k to subgenus Eomelipona. Probability estimates of species occurrence is expressed as a continuum; darker colors indicate higher prediction values and lighter colors indicate lower values. The highest prediction interval (darkest shade) was defined using an expert-based threshold (shown as the lower interval limit). Panel l shows the natural protected area (NPA) network within the study region (ProtectedPlanet.net 2015).

Figures 5 and 6 below are an analysis of Meliponini distribution in Northern South America. Figure 5 shows the occurrence of specific species of Meliponini in certain areas while figure 6 shows the suitability of areas focused in Colombia.

Figure 4.

Distributional analysis of Melipona stingless bees (Apidae: Meliponini) in Central America and Mexico

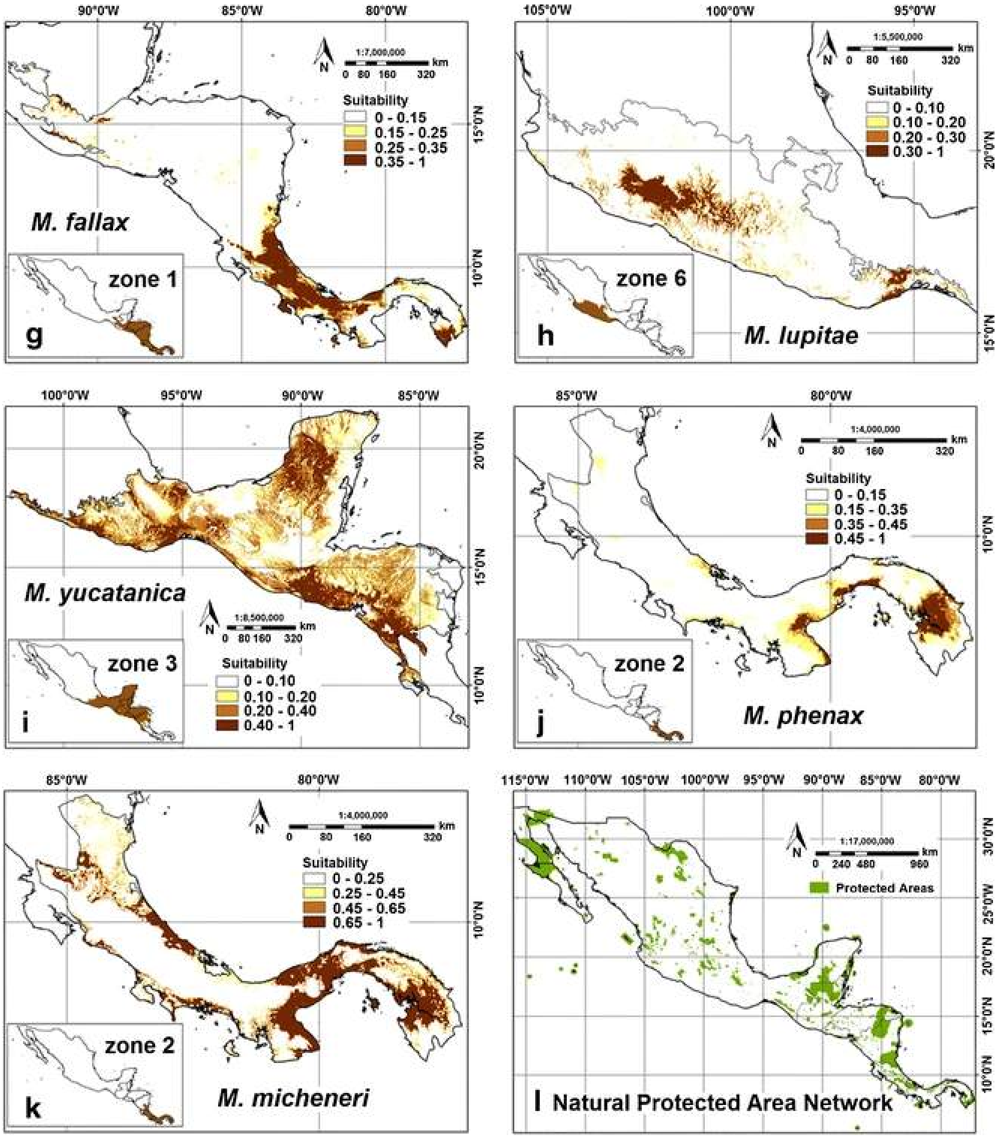


Figure 5.

Analysis of Meliponini Distribution in Northern South America (Gonzalez et al., 2021)

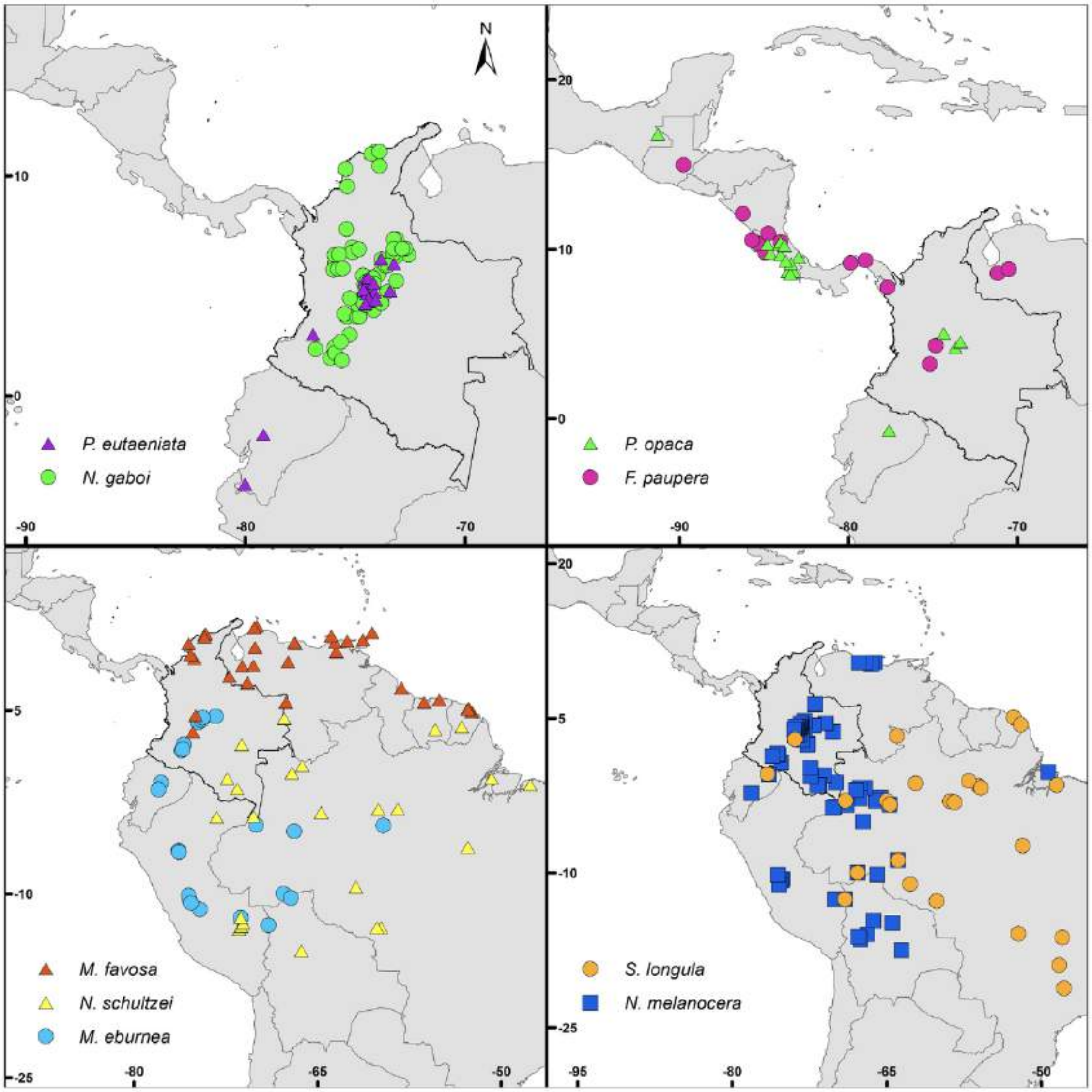


Fig. 1. Occurrence records used to create ecological niche models for the studied stingless bee species. Genus names: *F* = *Frieseomelitta*, *M* = *Melipona*, *N* = *Nannotrigona*, *P* = *Paratrigona*, *S* = *Scaura*.

Figure 6.

Analysis of Meliponini Area Suitability in Colombia (Gonzalez et al., 2021)

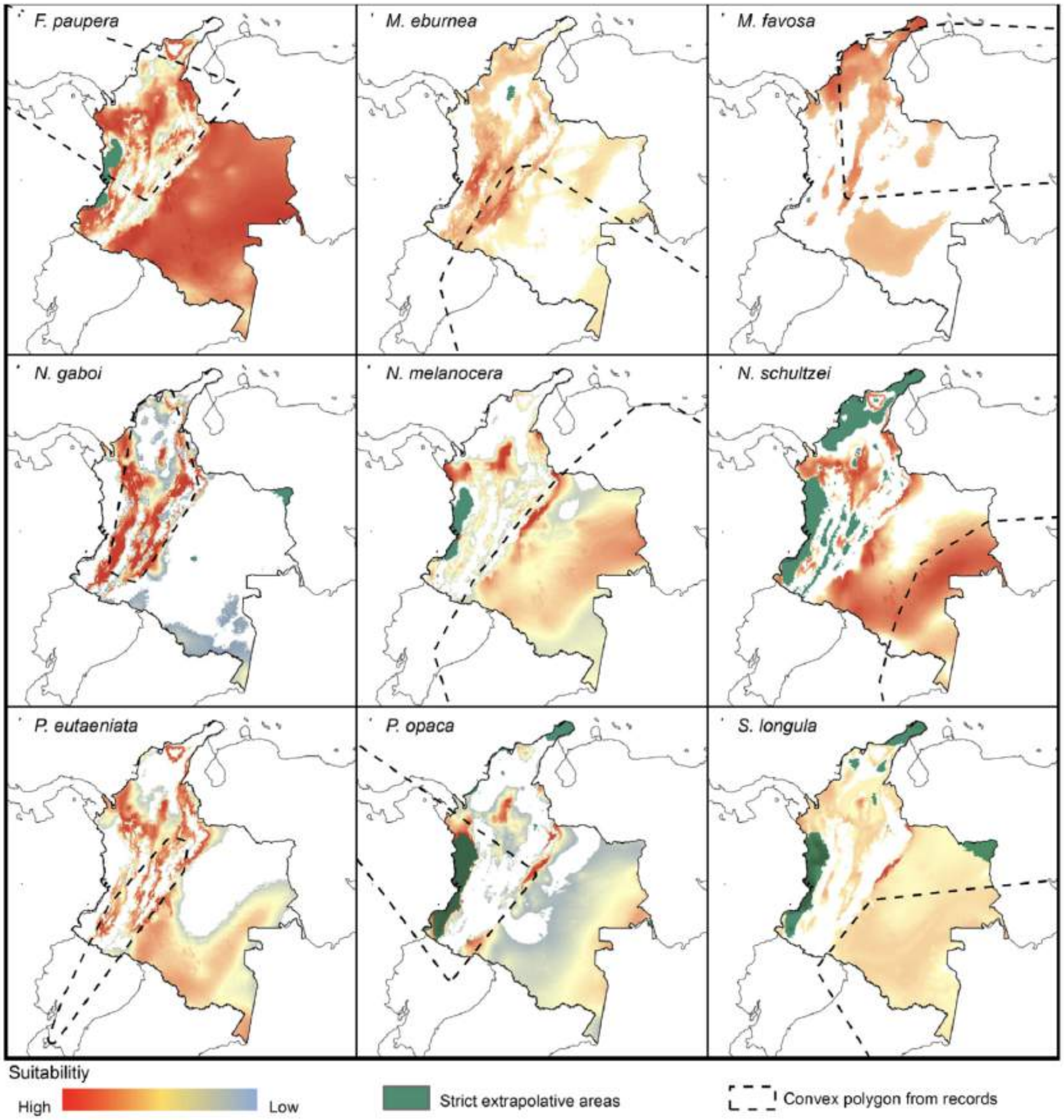


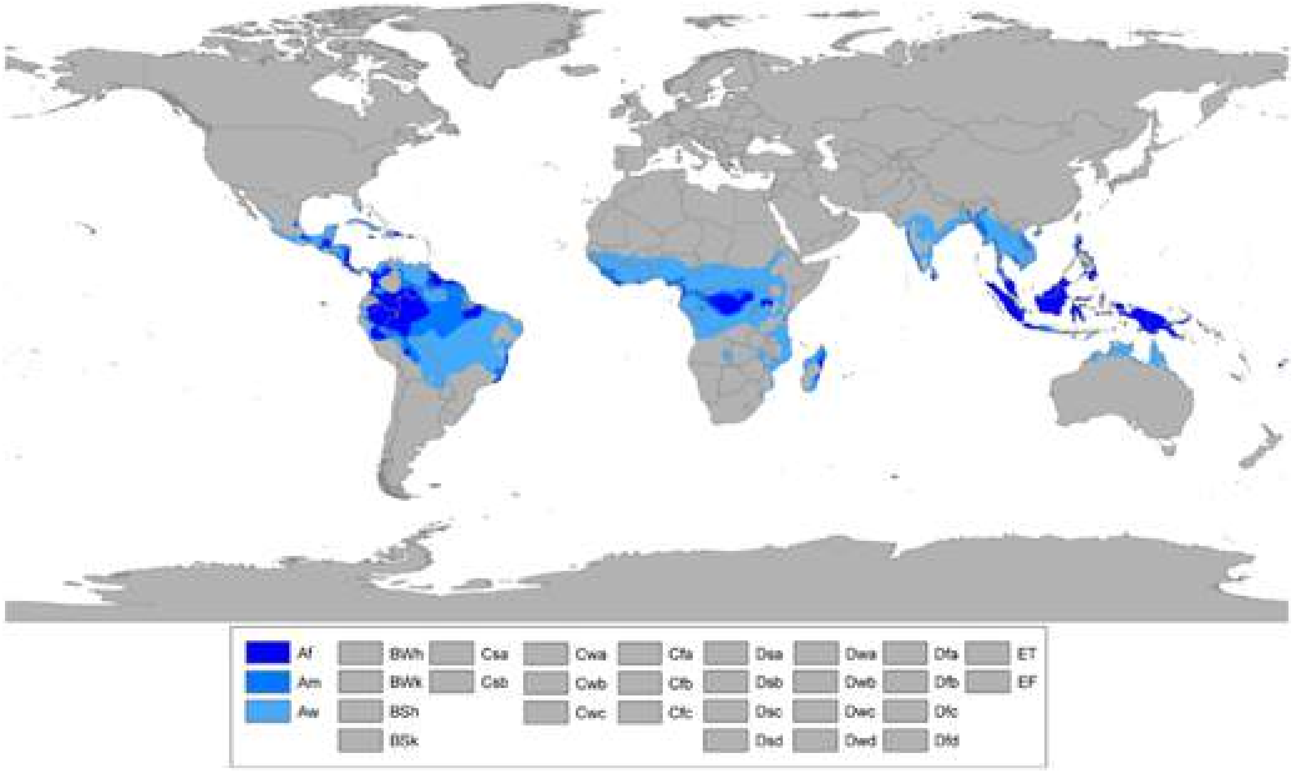
Fig. 2. Current suitable areas for the studied species in Colombia. Broken lines enclose areas with occurrence data. Genus names: *F* = *Frieseomelitta*, *M* = *Melipona*, *N* = *Nannotrigona*, *P* = *Paratrigona*, *S* = *Scaura*.

1.2.1 Climate & Elevation

Stingless bees are mainly associated with tropical dry and humid forests, in low and warm lands although some species can be found in cloud forest and pine-oak forests in highlands. The tropics are warm all year, averaging 25 to 28 degrees Celsius (77 to 82 degrees Fahrenheit). This is because the tropics get more exposure to the sun, sunlight is intense. The tropical seasons are broken up into the wet season and the dry season. The amount of rain can vary greatly from one area of the tropics to another. Some areas, like parts of the Amazon in South America, get almost 3 meters (9 feet) of rain per year. Other areas in the tropics have a drier climate like the Sahara Desert in northern Africa only gets 2-10 centimeters (.793.9 inches) of rain per year (National Geographic, 2011). The map below shows the areas within the tropics which have all 12 months of the year with temperatures above 18 °C. The three types of tropical climate are classified as Tropical Rainforest or Equatorial (Af), Tropical Monsoon (Am) and Tropical Wet and Dry or Savannah (Aw). The general pattern of the tropical climate is warm temperatures. Depending on the type of tropical climate, humidity is variable with Equatorial climates experiencing large quantities of precipitation all year round and Tropical Wet and Dry and Tropical Monsoon climates experiencing seasonal shifts in rain patterns. (The Climate of Tropical Regions - The British Geographer, n.d.).

Figure 6.

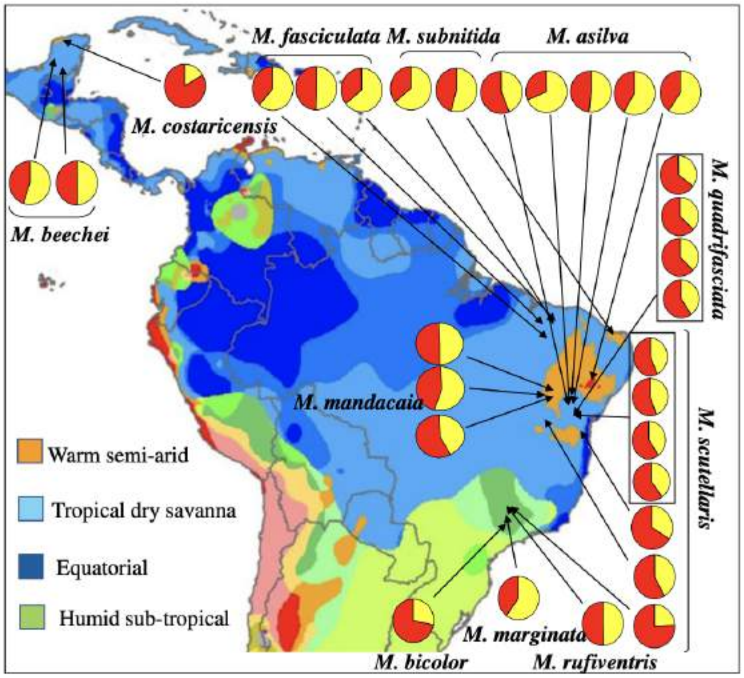
Tropical Climate Distribution (The Climate of Tropical Regions - The British Geographer, n.d.)



Isomeric Alkenes were used to recognize species of Neo -Tropical Stingless Bees. Figure 7 below summarizes the findings (Martin et al., 2017). We can clearly see the proportion of alkanes (yellow, which indicates the presence of bees) and olefins (red) for eleven Melipona species of stingless bees that have been sampled in four different climatic zones across South America.

Figure 7.

Location of Neo -Tropical Stingless Bees (Martin et al., 2017)



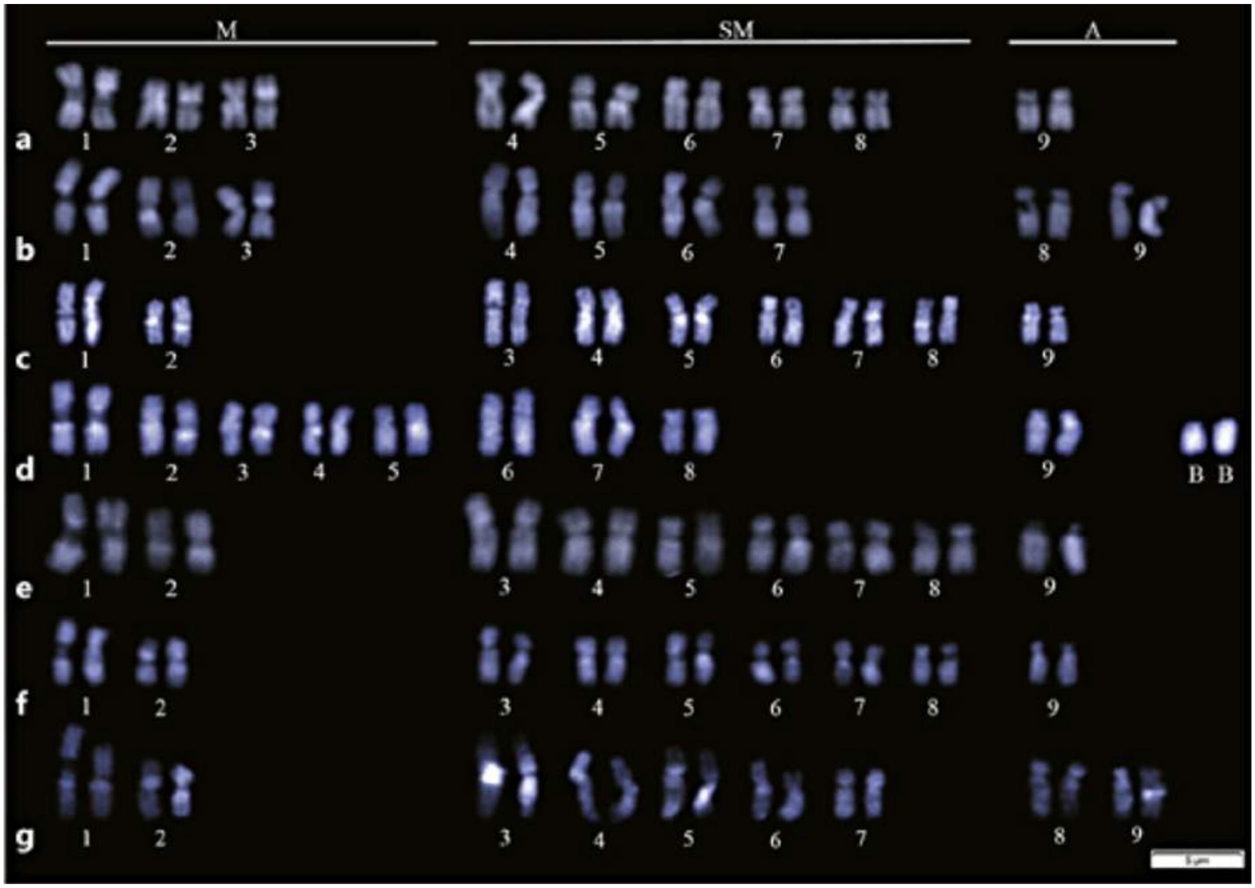
Chapter 2: Biology

2.1 Chromosome Complement

Within the Meliponini tribe there are multiple genera with distinct characteristics. Cytogenetically, Melipona is the most studied genus of the tribe which, in general, have 2n = 18 chromosomes (Travenzoli et al., 2019). Below is an image of karyotypes of multiple Melipona species.

Figure 8.

Karyotypes of Melipona species. aM. asilvai. bM. bicolor. cM. puncticollis. dM. quinquefasciata. eM. mandacaia. fM. quadrifasciata. gM. Subnitida. (Travenzoli et al., 2019)

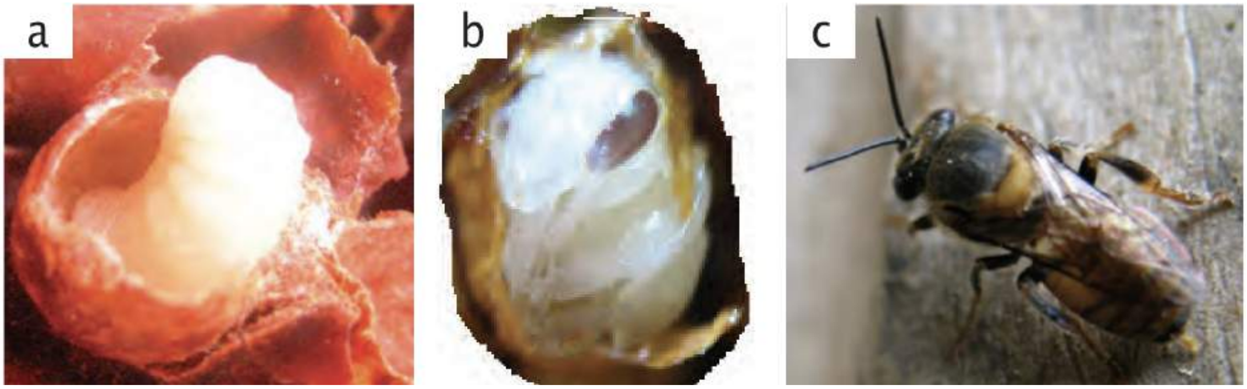


2.2 Life Cycle

The life cycle of Meliponini varies according to their role. It all begins with the queen that mates in flight with a single drone from a different colony and stores all the sperms she needs for egg fertilization during her life. Then she returns to the hive and begins to lay two types of eggs: Fertilized eggs that develop into worker or queen larvae, and unfertilised eggs that develop into drone larvae. What determines whether a fertilized egg becomes a worker bee or a queen is the diet. The soon to bee queen Larvae is exclusively fed royal jelly while worker bee Larvae are fed a combination of royal jelly and worker jelly (Kofi et al., 2010). The development stages of stingless bees include: a larva known as maggot, a pupa, and an adult individual (imago).

Figure 9.

Developmental stages of stingless bees (a. larva, b. pupa and c.adult) (Kofi et al., 2010)

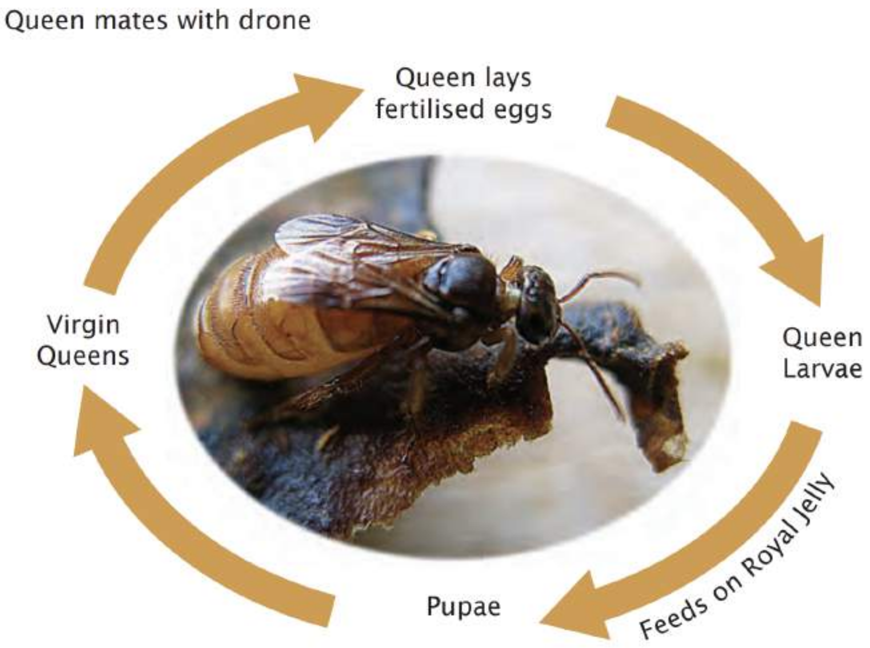


2.2.1 The Queen

The queen is the supreme authority in the hive as she controls the operation of the bees through the emission of pheromones. Depending on the pheromones she releases from her body, members of the colony interpret and act accordingly. For example by releasing a specific pheromone she is able to suppress the laying ability of all workers of the nest. Below is a figure that generally describes the life cycle of the queen (Kofi et al., 2010). The queen usually lives 1 to 2 years.

Figure 10.

Life Cycle of Stingless Bee Queen (Kofi et al., 2010)

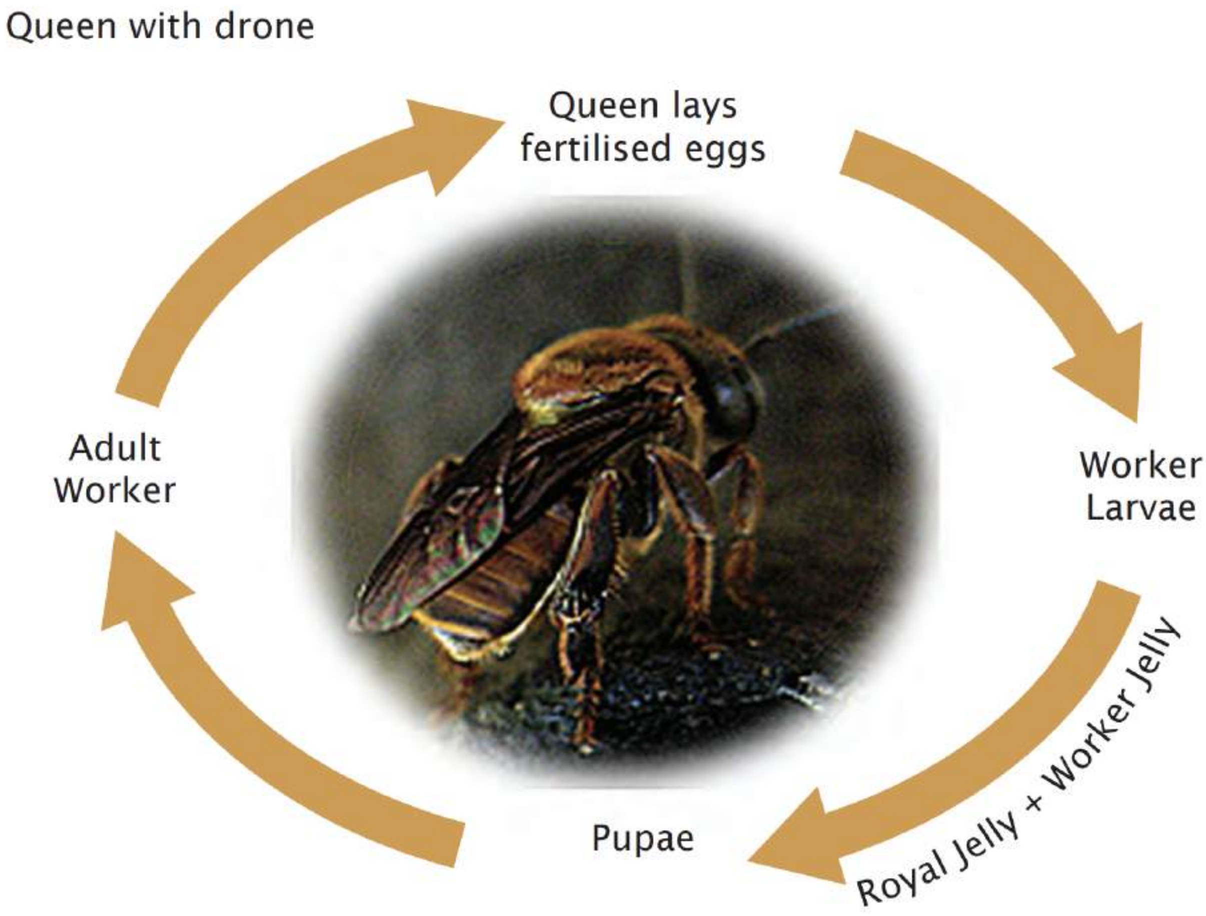


2.2.2 Worker Bees

Worker bees are females that develop from fertilised eggs. The eggs develop into larvae that are initially fed with royal jelly and then given worker jelly until the pupal stage when the cells are sealed. Adults emerge as workers and are responsible for most of the activities in the hive such as foraging, cleaning, defence and larval feeding (Kofi et al., 2010). The division of labour associated with the advancing age of colony members is known as age polyethism (Wilson, 1971; Michener, 1974; Hartfelder et al., 2006), in which worker bees move from low-risk, innest tasks to high-risk, defence and foraging tasks as they age. This results in low mortality rates during the early stages of life with increasing mortality rates later in life. Individuals live longer when high-risk tasks are postponed (O'Donnell and Jeanne, 1995). Once workers move to foraging tasks their physical and metabolic activity increases, thus increasing molecular damage and morphological decline (Cartar, 1992; Brys et al., 2007). Basically, foragers 'wear out' more quickly than house bees (Halcroft et al., 2013). figure 11 below generally describes the life cycle of worker bees (Kofi et al., 2010). They live for approximately 160 days.

Figure 11.

Life Cycle of Stingless Worker Bees (Kofi et al., 2010)

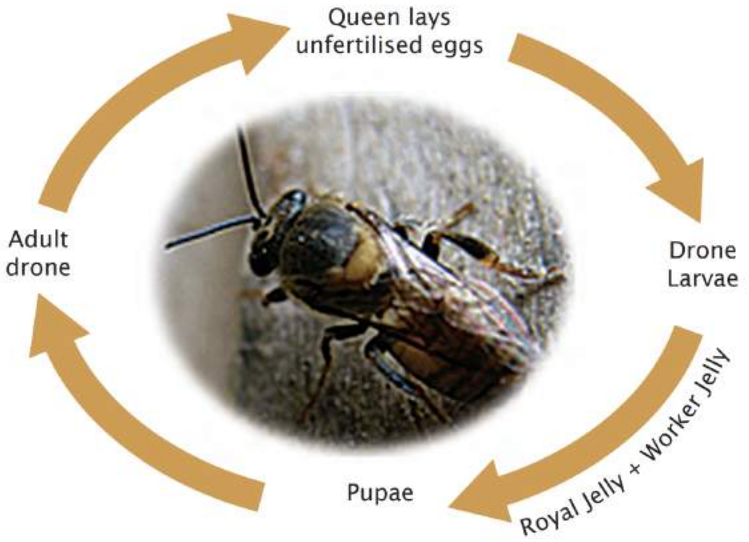


2.2.3 Drones

Drones are the male component of the colony and are crucial for colony reproduction. This is achieved by mating with virgin queens from other colonies. They are developed from unfertilised eggs and are fed with larval food just like workers. They have a similar life span to worker bees. Figure 12 below describes the life cycle of drones (Kofi et al., 2010).

Figure 12.

Life Cycle of Drones (Kofi et al., 2010)



2.2.4 Hive

Colonies of stingless bees can live perennially and reproduce by swarming. The life cycle begins when scout bees, probably aroused by high density in the old nest, begin to find a locality for a new nest. When the new locality is chosen, the workers seal all openings around the fracture in the ground and construct a nest entrance. At first, the building material is taken from the old nest. The workers collect beeswax in baskets located on the third pair of legs, and carry honey and pollen as fluid suspension in their goitres. At the same time, groups of males gather around the new nest. The new queen raised by the workers lives in the old nest for some time with the mother queen. Next, the new queen flies to the new nest alone or surrounded by a group of bees; soon, it performs the mating dance, which, for example, in Melipona quadrifasciata lasts for about 4 to 5 minutes. in Melipononi, there is a strong bond between the home nest and the offspring nest, and the formation of a new family is gradual. The new queen of stingless bees migrates to a new nest, already organized by the workers (Bąk-Badowska et al., 2019).

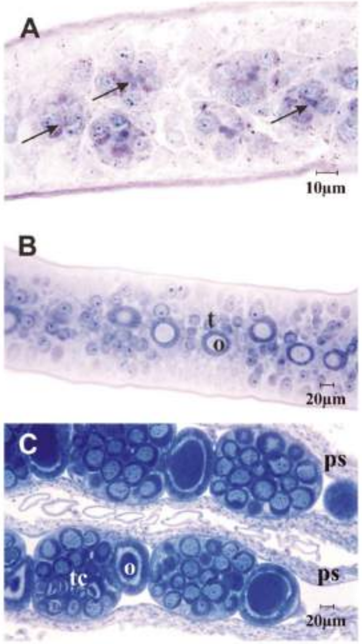
2.3 Reproductive Biology

2.3.1 Female

Most stingless bees, queens and workers, have four ovarioles in each ovary (Cruz-Landim, 2000). Opposed to honey bees Meliponini workers at a certain age are active egg layers aswell. In queens, these ovarioles are more elongated than in workers. As they become active egg layers, the ovarioles extend enormously in length under progressive oogenesis activity, causing the abdomen to swell and the queen to become physogastric. There is a clear separation between the germarial region and the region of follicular growth. In the germarium, all of the early stages of oogenesis are identifiable, starting with the formation of cystocyte clusters leading to initial follicle formation in the region where the germarium widens and forms a transition zone to the region of previtellogenic follicles (Tanaka et al., 2009).

Figure 13.

"Histological sections of ovarioles of Melipona. quadrifasciata virgin queens kept in an incubator with a small group of accompanying workers. (A) upper germarial region of 7 day-old queen showing a sequence of cystocyte rosettes (arrows); (B) lower portion of germarium of 5 day-old queen showing a series of oocytes, each surrounded by trophocytes; (C) two ovarioles of 7 day-old queen sectioned in the region of early vitellogenic growth of the follicles. o, oocyte; ps, peritoneal sheath; t, trophocyte; tc, trophic chamber." (Tanaka et al., 2009)

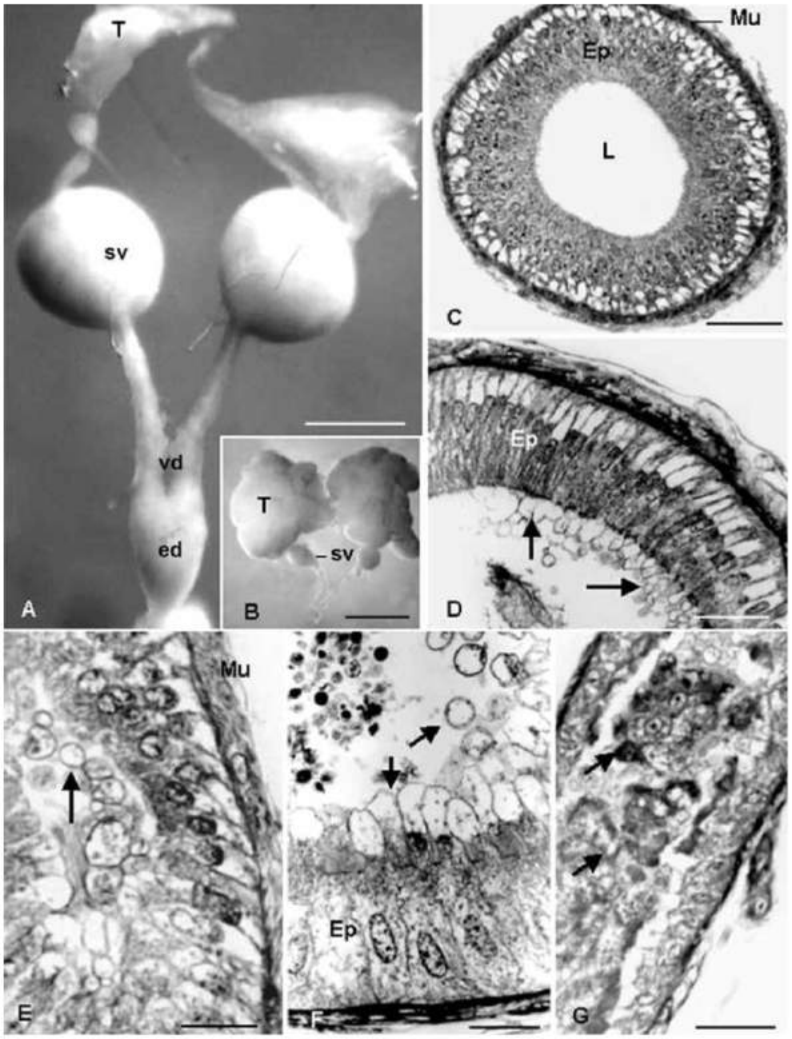


2.3.2 Male

The male reproductive organs are a pair of testes, each with four follicles, pair of vasa deferentia with enlarged region forming the seminal vesicle that opens in an ejaculatory duct. In the male reproductive system of these bees there is no structure that can be recognized as accessory glands as seen in insects in general. From brown-eyed pupae to newly-emerged adults, the epithelia of the seminal vesicles and post-vesicular vasa deferentia have prismatic cells that release secretion to the organs lumen. In 5-days old adults the testes undergo degeneration, the seminal vesicles filled with secretion and spermatozoa, and the epithelium has cubic cells rich in inclusions in the basal region suggesting that this is the age in which males reach the sexual maturation (Araujo et al., 2020).

Figure 14.

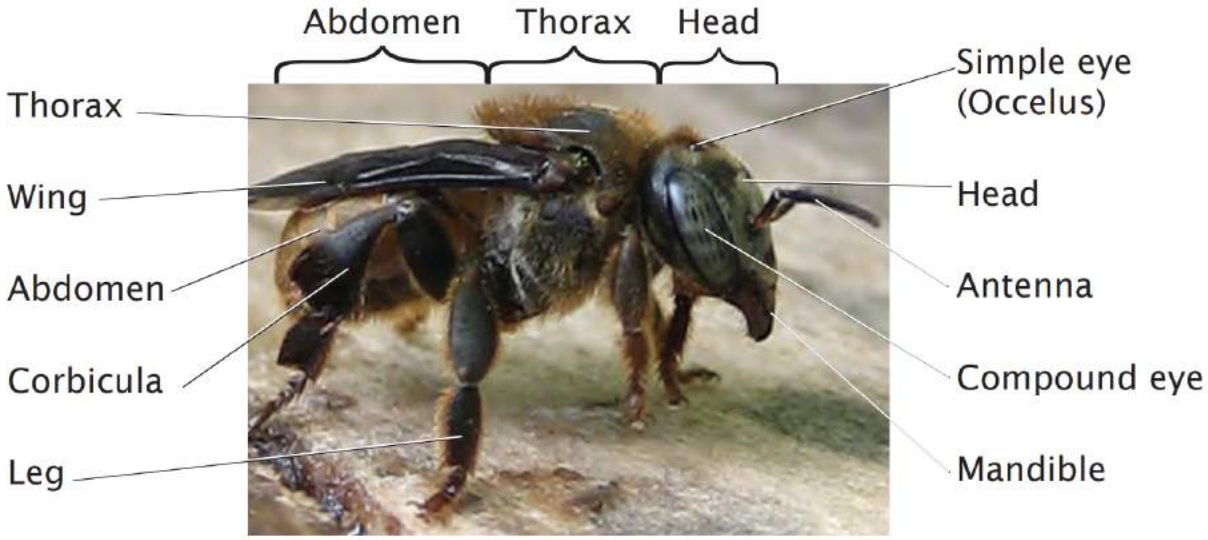
"The male reproductive tract of Scaptotrigna xanthotricha. A. Sexually mature male 10-day-old showing degenerated testes (T), enlarged seminal vesicles (sv), vasa deferentia (vd) and the ejaculatory duct (ed). B. Black-eyed pupae showing well-developed testes (T) and dilated seminal vesicles (sv). C-D. Cross section of a seminal vesicle in pink-eyed pupae (C) and brown-eyed pupae (D) showing empty lumen (L), simple prismatic epithelium (Ep), vesicles (arrow), and a muscle layer (Mu). E-F. Cross section of vasa deferentia (E) and seminal vesicles (F) in black-eyed and non-pigmented body pupae showing the secretion in the lumen (arrows). G. Longitudinal section of a vas deferens in black-eyed and pigmented body pupae, showing the different secretions in the lumen (arrows). Bars: A-B =0,7mm; C-G=50um;" (Araujo et al., 2020)



2.4 Physiology

Figure 15.

Morphology of Stingless Bees (Kofi et al., 2010)



The size of these insects varies from 16 mm (in a worker of the genus Trigona) to about 2 cm in the species of Melipona. They are black to golden in colour; some species have shiny bodies, whereas others are covered with hair. The body of bees from the tribe Meliponini comprises three parts – the head (cephalon), the trunk (thorax) and the abdomen. The head contains a pair of antennae, a pair of compound eyes, simple eyes and chewing-sucking mouthparts. The thorax bears two pairs of membranous wings and three pairs of legs. Bees as the only hymenopterids have the ability to collect pollen. The pollen apparatus in the Meliponini is a basket located on the hind legs. The pollen basket is built of long, thick hairs, surrounding the external, non-hairy, concave and smooth surface of the hind legs. The hairs hold a pollen lump, stuck to the hind legs and forming the pollen trap. Bees feed on plant pollen and nectar. Depending on the species, they may fly as far as 2 km from their nests. Some representatives of the Meliponini collect resin from tree trunks and branches. A completely different feeding strategy occurs in three species of the Melipolini from the Trigona hypogea group. Workers of these bees feed their larvae with partly digested tissues of dead animals [Kwapong et al., 2010]. (Bąk-Badowska et al., 2019)

Chapter 3: Propagation and Management

3.1 Hive design and construction

The keeping of stingless bees (Tribe Meliponini), also known as meliponiculture, is an ancient practice in the Americas, carried out principally by traditional communities that keep the stingless bees in a rudimentary way. Yet major advancements to enhance general beekeeping techniques and practices have been developed. Many hive models in the meliponiculture are directly linked to the vast diversity of stingless bee species present in these regions (Venturieri et al., 2012), and also based on personal or cultural preferences, as many beekeepers tend to create new hive models on their own even when the more known models (INPA, Embrapa, PNN, UTOB) are available (Leão et al., 2016).

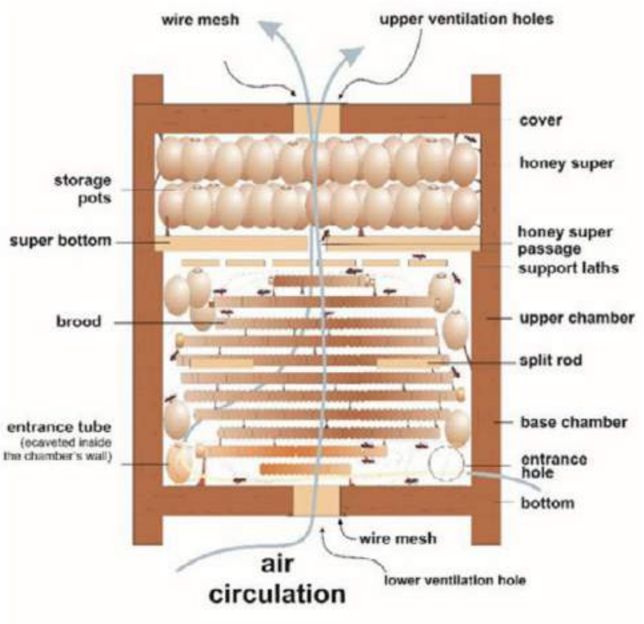
Proper hive design and construction are crucial to guarantee the success of a colony. This entails a strategic definition of materials, volume, orientation, and position of nest entrance. Regarding the materials, depending on the purpose of the hive (harvesting products or reproduction or recreational) and the species, one determines the materials and design of the artificial hive. Materials may include wood (sawn timber and planks), clay pots, baboo, hollow logs, plastic bottles etc. In terms of the volume this should be determined according to the volume of the natural nest considering the hive should have sufficient space to enable food storage and reproduction. The orientation of the hive should be designed in a way that enables stingless bees to arrange the nest either vertically or horizontally depending on the species (Kofi et al., 2010). Horizontal hives are usually completely hollow, without any internal division, or more elaborate, with internal divisions to separate the nest from the honey storage space (Nogueira-Neto, 1997; Sommeijer, 1999). “The vertical (Baiano or Nordestino, Uberlândia, PNN, the UTOB and the INPA) hive models follow the natural brood comb pattern in tree trunks, divided into two main modules, the base chamber (to shelter the nest) and the upper chamber (to store honey and pollen; Portugal-Araújo, 1955). The creation of a specific honey storage compartment enables a faster and cleaner harvest, with minimum nest damage, the great advantage of this hive model (Venturieri et al., 2003; Venturieri, 2008a). The number of beekeepers using vertical hives is growing despite the horizontal models still being more widely used (Venturieri, 2008a). However, actual comparisons on the advantages of each type of hive in regards to biological parameters, such as hive occupation and thermoregulation, as instance, are scarce, but in general point to that the type of hive that different species are bred result in clear differences in the health and development of the colonies (Quezada-Euán & Gonzalez-Acereto, 1994).” (Leão et al., 2016). Figure 16 below shows various examples of hives built with different materials, shapes, and sizes. Figure 17 is a detailed diagram of the commonly used vertical hive design in meliponiculture.

Figure 16.

Examples of different types of hives (Fig 3. Indigenous Stingless Bee Husbandry in Mesoamerica Involves The..., n.d.; “Stingless Bee Keeping (Meliponiculture),” n.d.; “Trap Hives for Stingless Bees,” 2018)

Figure 17.

Vertical hive design (Leão et al., 2016).



3.2 Identification and collection of wild nests

Wild nests are the natural nests of stingless bee colonies found in their habitats. Various stingless bee species construct nests that are unique with a particular architecture that allows us to easily identify them. First of all, search for stingless bee species on flowers in agricultural fields, orchards, forests and woodlands. If you recognize the Dactylurina species, concentrate your search on branches of trees for the brown or black ball-shaped nest. If you see Hypotrigona, look for the transparent entrance tubes on walls, bamboo stems or logs. Finally if you see Meliponula, search for entrance holes on trunks of trees and fallen logs. These are just a few examples of the most common stingless bee species and how to identify their nests. Figure 18 below shows the different types of nest entrances of distinct species as well as where you can find them.

Figure 18.

Different types of nest entrances of distinct stingless bee species (Grüter, 2020)

Figure 19.

Some nest entrances of stingless bees (Fig. 3. Some Nest Entrances of Stingless Bees. (A) Plebeia Minima..., n.d.)

(A) Plebeia minima Gribodo, 1983 (Hymenoptera: Apidae). (B) Partamona helleri Friese, 1900 (Hymenoptera: Apidae). (C) Nannotrigona sp. (D) Melipona quadrifasciata. (E) Nannotrigona testaceicornis. (F) Scaptotrigona bipunctata Lepeletier, 1836 (Hymenoptera: Apidae). (G) Paratrigona sp.

Schwarz, 1938 (Hymenoptera: Apidae). (H) Melipona flavolineata Friese, 1900 (Hymenopetera: Apidae). (I) Paratrigona sp. (J) Leurotrigona muelleri Friese, 1900 (Hymenoptera: Apidae). (K) Partamona helleri. (L) Schwarziana quadripunctata Lepeletier, 1836 (Hymenoptera: Apidae). (M) Trigona pallens Fabricius, 1798 (Hymenoptera: Apidae). (N) Tetragonisca angustula. All Pictures: Cristiano Menezes.

3.3 Collection of Wild Nests

There are two principle methods of wild hive collection; trap nests which are temporary collecting containers that can be used to bait and trap swarms of bee colonies, and whole nest collection when the whole colony is collected in their natural hive and relocated into meliponiaries (artificial hives which were discussed earlier in the chapter). More detail regarding the transfer of the captured hive is provided in the next subsection of the chapter.

3.3.1 Trap nests

Traps can be made with various materials like wooden boxes, cavities in logs or bamboo, calabashes, pots and plastic bottles etc. Once the material of the trap is determined it is baited with stingless bee wax and propolis tincture that attracts bee swarms. Traps can take long for colonies to occupy since stingless bees take longer times to swarm. In order to transfer to the trap, worker bees spend weeks gradually constructing a new nest inside the trap near the original nest. When the new nest (inside the trap) is nearly finished, a young, newly mated queen moves in with some worker bees to complete the new nest and start a new colony. Therefore it’s necessary to do monthly inspections of trap nests to check their state and provide some care until they are colonized. Once nests are colonized, they can be relocated into the permanent artificial hive previously built. (Kofi et al., 2010)

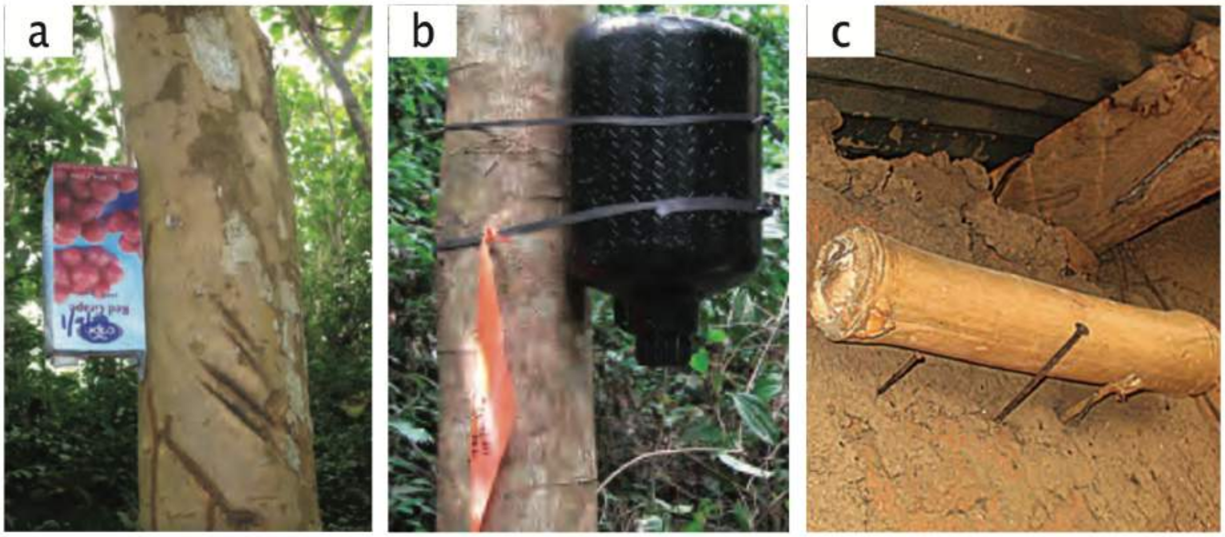
Steps (Kofi et al., 2010):

1. Obtain a container of the material of your choice from the previously mentioned (wooden boxes, cavities in logs or bamboo, calabashes, pots and plastic bottles etc.)
2. Spray with dark paint on the outside to avoid light inside the container.
3. Drill a hole to permit access to the targeted bee species
4. Coat the inside of the container with melted wax and a tincture of propolis and alcohol to serve as baits for bee swarms.
5. Using binding wire, tie the traps to trees strategically located near wild nests.

6. Monthly inspection is recommended to check on the state of the traps and give the necessary attention.
7. Remove the trap when colonized at night when foragers would have all returned by closing the entrance.
8. Relocate the new colony safely and carefully.
9. Help bees to settle as quickly and comfortably as possible. Make sure predators do not have access to them.
10. Provide ready food and water.

Figure 20.

Trap nests for stingless bees attached to trees in the forest (a) empty fruit juice container (b) plastic gallon (c) bamboo internode (Kofi et al., 2010)



3.3.2 Whole Wild Nest Collection

This method consists of withdrawing the whole nest from their natural location whether it is in a tree or the ground and transferring it directly to an artificial hive.

Steps (Kofi et al., 2010):

1. Locate the nest and start the procedure in the late afternoon when most forager bees are back in the colony. If the nest is in the ground, dig around it and extract it, placing it in a plastic bag or container and making sure most bees are safely secured inside the bag for transportation. If the

bees are in a tree carefully cut the branch or the part of the tree where the nest is and again place it in a plastic bag/container.

2. Carry the nest wrapped in the bag to the new location before untying it. It is very important to keep the orientation of the nest as before. This will prevent eggs from drowning within cells resulting in casualties and colony absconding.
3. Carefully and gently place the nest into the artificial hive with the workers and the queen in the same orientation as found in the original nest.

3.4 Transfer into hive and nest placement

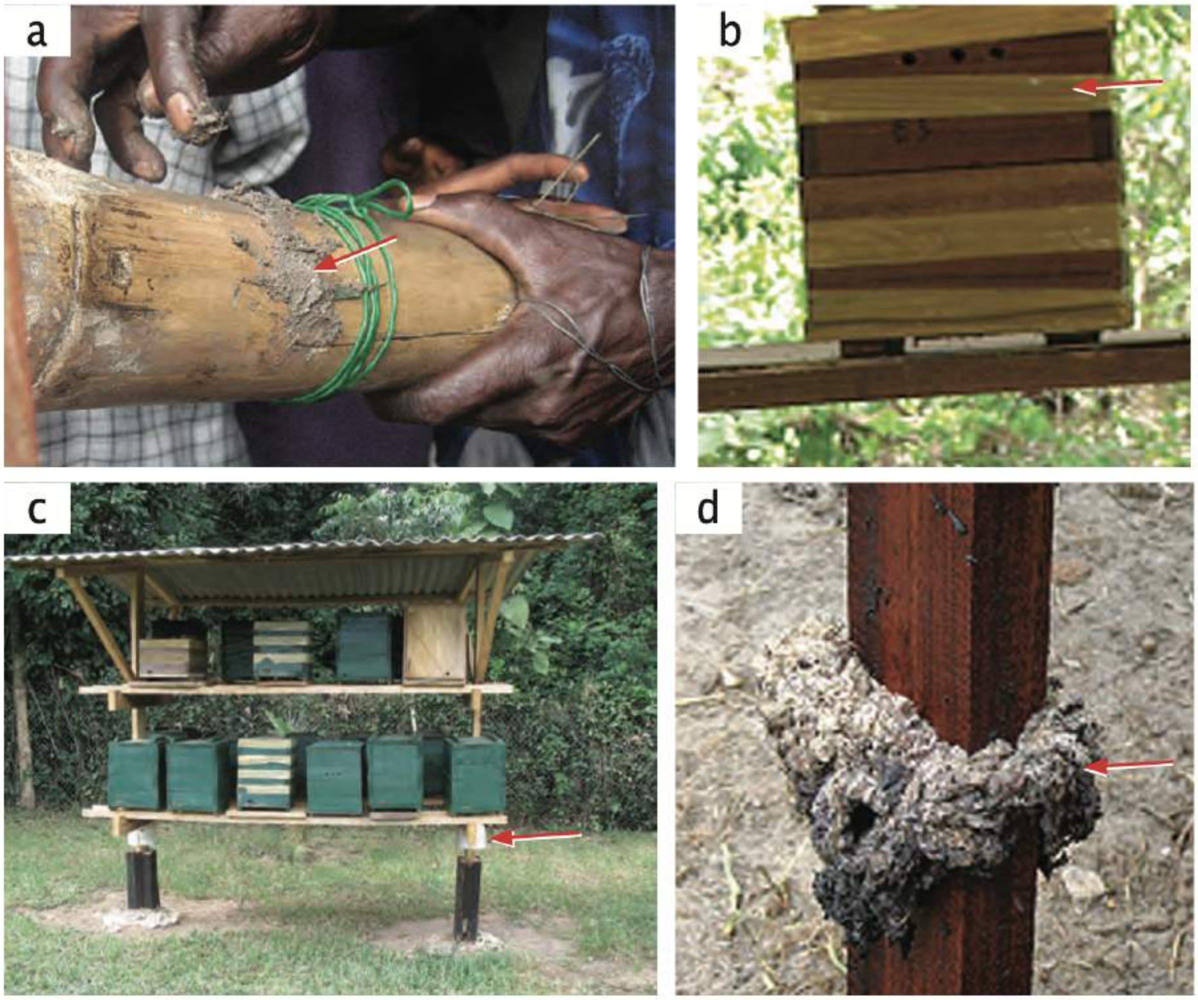
It's crucial to re-establish a stingless bee colony into a well constructed beehive. A well designed and constructed beehive enables the colony to develop successfully and store abundant volumes of honey and pollen. These stored foods in addition to other hive products can be harvested by the beekeeper without much difficulty. Colonies can also be managed more easily in well designed bee hives rather than in their natural logs. (Kofi et al., 2010)

3.4.1 Precautions for transfer

Timing, nest security, and invasion of pests during and after the transfer are serious precautions that must be taken into account before realizing the transfer operation. Regarding timing "colonies should be transferred during the early parts of the beekeeping season (beginning of the dry season). This is the time when food and other resources are abundant in the environment to help colonies establish and grow quickly."(Kofi et al., 2010). In terms of nest security, some intruders such as flies and beetles take advantage of exposed nests and devastate colonies. Therefore constructed artificial bee hives should be built in a way that all openings and gaps are closed. This enables colonies to re-establish quickly and grow . In order to avoid the invasion of pests the operation must be done quickly and when the transfer operation is over it's fundamental to make sure that the site of the new hive is free of pests, especially ants. Hive stands and hanging ropes should be effectively protected from pests and crawling insects. This can be done by placing dirty engine oil in cans and grease on ropes. (Kofi et al., 2010)

Figure 21.

Various means of protecting hives (a. clay, b. sellotape, c. plastic lizard gard and dirty oil as well as d. foam soaked with dirty oil) (Kofi et al., 2010)



3.4.2 Transfer Operation

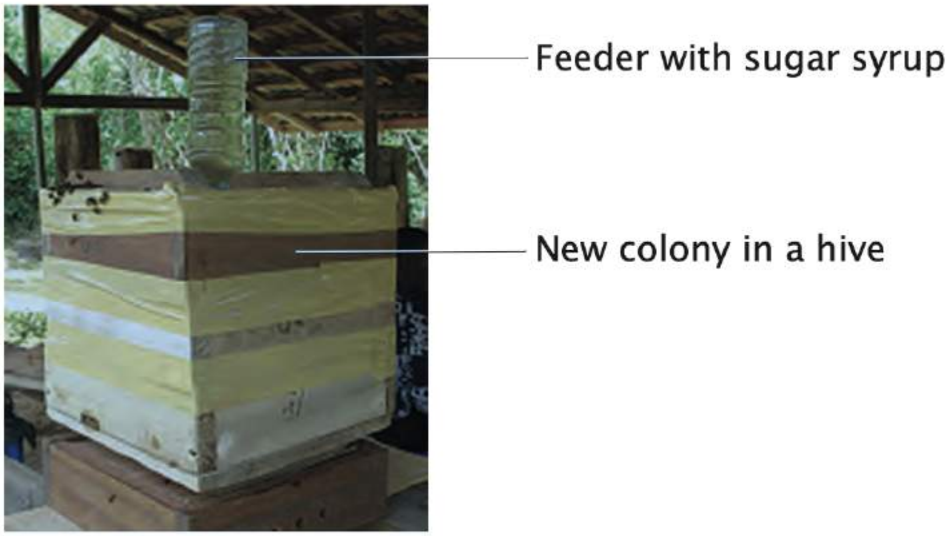
Steps (Kofi et al., 2010):

1. Remove the whole brood section of the nest and place this inside the new beehive close to the entrance hole.
2. Close the new hive and secure it well. Use appropriate material such as clay, or cellotape to seal all openings except the entrance.
3. The food (pollen and honey pots) content of the original nest must not be added to the new hive to prevent contamination and possible pest infections in the new hive. Eggs and adult hive pests, especially beetles may be present in the pollen and honey storage pots of the natural nest.

4. Sugar syrup (sugar:water = 1:1) can be prepared for feeding the new colony while it recovers its strength. Figure 22 below shows artificial feeding.
5. Remove the old empty nest away from the site to avoid attracting hive pests.
6. Monitor the re-establishment of the new hive externally and check the nest for ants and other pests.

Figure 22.

Artificial Feeding colonies within hives (Supplementary feeding of a baby colony with sugar syrup) (Kofi et al., 2010).



3.4.3 Nest Placement

A proper site for the placement of stingless bee nests should protect the colonies from strong weather conditions such as rain, and intense sunlight as well as pests. Additionally it’s important that the site has abundant forage resources (flowering plants), a water source, that it’s adequately shaded with good air circulation, and on top of relatively dry land (avoiding damp and water-logged areas). Stingless bee colonies are no threat to humans so the hives can be placed near homes (backyard, on corridors or verandas) and in farms. (Kofi et al., 2010)

3.5 Management

Good management practices are vital to ensure the success of Meliponine colonies and to derive maximum benefits from the bees. A well managed colony has good records for improved management, grows constantly, produces lots of hive products, has reduced pests and disease problems as well as reduced stress from external factors. To guarantee a successful colony it's important to protect them from extreme weather conditions, pests, diseases, weeds, bushfires, chemical pesticides, pollution from charcoal burning, and lack of forage and water. This can be done by placing hives under sheltered areas that prevent access of pests such as ants, lizards, and spiders, and keeping it clean of weeds. Furthermore, colonies are best kept at places where activities like bush and charcoal burning are limited considering intense heat and smoke is a direct threat to colonies. Additionally colonies are greatly affected and debilitated by seasons where food and water is scarce. In these cases supplementary feeding is needed which can be done by growing plants that stingless bees feed on near the meliponiary or providing artificial feeding. Ultimately the principal threat to Meliponie colonies is pesticides. When foragers visit crops or flowers that have been sprayed with pesticides, "they either get killed or collect contaminated forage which may poison other hive members and thereby destroy the colonies. If possible colonies should be sited at places where pesticide contamination is minimal. On the other hand, if colonies are placed near agro-ecosystems, where regular spraying is carried out, frequent monitoring should be required so as to reach an agreement with potential sprayers. For example, hives could be locked-up before spraying." (Kofi et al., 2010).

Figure 23.

Designs on hive stands that prevent ants, lizards and other intruders access to bee hives (Kofi et al., 2010).

Chapter 4: Emerging Products & Potential Markets

4.1 Economic importance of Stingless Bees

Stingless bees have substantial economic relevance due to the importance of pollination for plant reproduction as well as the multiple hive products like medicinal honey, pollen, propolis, cerumen, and the colonies themselves. Furthermore, Meliponines have been sought after for research purposes and ecotourism.

4.1.1 Pollination

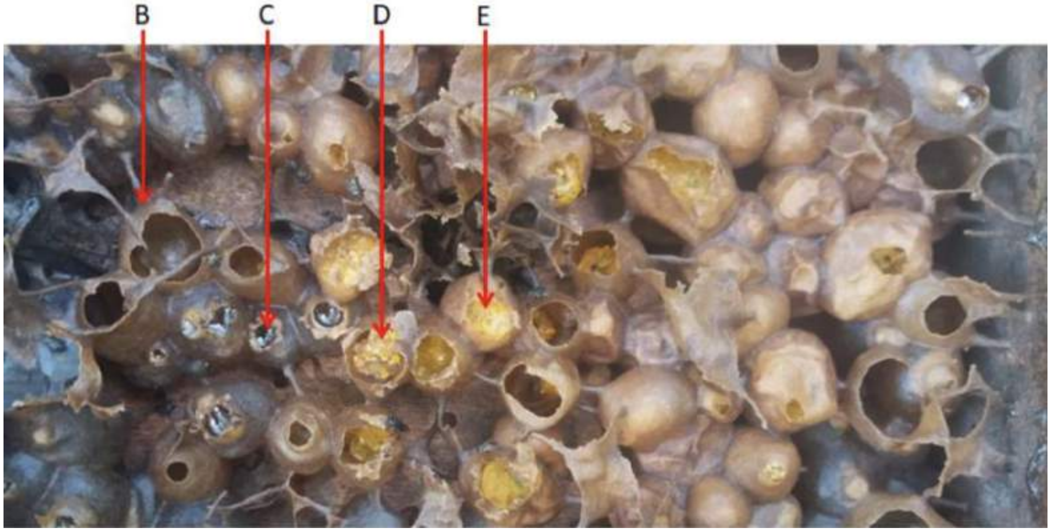
The characteristic small size of stingless bees, the great diversity of species, and their vibration makes stingless bees the most effective and efficient pollinators particularly of forest trees. By visiting multiple plants collecting nectar, pollen, wax, resins, oils and other plant substances they transfer pollen to the stigma fertilizing the plants. This leads to both a high quality and quantity of yields of fruits and seeds. Stingless bee pollination can increase crop production by up to 40% (Mustafa et al., 2018). This demonstrates the crucial role of Meliponini not only in sustaining ecosystems but their potential for agricultural use.

4.1.2 Hive Products

Meliponines have a great number of products with unique medicinal properties. Some of these products are honey, pollen, beebread, propolis, and cerumen. Figure. 24 below shows these different products within the hive.

Figure. 24

Hive box containing colony of stingless bees (Heterotrigona itama). A, cerumen; B, empty propolis pot; C, honey; D, bee pollen; E, fermented pollen (beebread) (Al-Hatamleh et al., 2020).



Honey produced by Meliponines has a greater value than regular *Apis mellifera* honey due to the antioxidant, anti-inflammatory, anti-obesity, anticancer and antimicrobial properties. “The major composition of stingless bee honey includes sugars (fructose and glucose) with nearly zero hydroxymethylfurfural (HMF). It also contains small amounts of other compounds, such as organic acids, phenolic compounds (eg., phenolic acids and flavonoids), proteins, amino acids (eg., phenylalanine, alanine, tyrosine, valine, acetate and trigonelline), enzymes, vitamins and minerals. The polyphenolic content is nearly tenfold higher compared to other types of honey. The honey has great potential for prevention of chronic diseases, such as cancer, stroke, hypertension and diabetes, as measured by its ability to manipulate signaling pathways of disease development. It was recentley showed that supplementation with honey from the stingless bee Heterotrigona itama led to enhancement in memory and learning in mice and increased anti-obesity parameters in high fat diet-induced obese rats, indicating the potential role of honey in controlling obesity-associated problems.” (Mustafa et al., 2018) Although research regarding the medicinal benefits of stingless bee honey is still premature figure. 25 below is a compilation of some of the most important discoveries regarding Meliponine honey health benefits.

Figure. 25

List of studies on the potential medicinal properties of stingless bee honey (Al-Hatamleh et al., 2020).

Study ID	***Country of Origin***	***Species***	***Reported Properties***
Arshad 2020 [24]	*Malaysia*	*Trigona*	*Improve memory* *Reduces anxiety*
Biluca 2020 [25]	*Brazil*	*Melipona marginata* *Tetragona clavipes* *Scaptotrigona bicunctata* *Melipona quadriasciata* *Tetragonisca angustula* *Trigona hypogea*	*Antioxidant* *Anti-inflammatory*
Mustafa 2019 [26]	*Malaysia*	*Heterotrigona itama*	*Improves memory and learning*
Abdul Malik 2019 [27]	*Malaysia*	*Heterotrigona itama*	*Antiaging*
Hazirah 2019 [28]	*Malaysia*	*Trigona*	*Antioxidant*
Al Kafaween 2019 [29]	*Malaysia*	*Trigona*	*Antimicrobial*
Avila 2019 [30]	*Brazil*	*Melipona bicolor* *Melipona quadrifasciata* *Melipona marginata*	*Antimicrobial* *Antioxidant*

Study ID	***Country of Origin***	***Species***	***Reported Properties***
Arshad 2020 [24]	*Malaysia*	*Trigona*	*Improve memory* *Reduces anxiety*
		Scaptotrigona bipuncata	
Selvaraju 2019 [31]	*Malaysia*	*Heterotrigona itama* *Geniotrigona thoracica*	*Antioxidant*
Ranneh 2019 [32]	*Malaysia*	*Trigona*	*Anti-inflammatory* *Antioxidant*
Ahmad 2019 [33]	*Malaysia*	*Heterotrigona itama*	*Anticancer*
Mohd Rafie 2018 [34]	*Malaysia*	*Heterotrigona itama*	*Anti-obesity*
Mohamad 2018 [35]	*Malaysia*	*Trigona itama*	*Antiproliferative*
Nordin 2018 [36]	*Malaysia*	*Trigona itama* *Trigona thorasica*	*Improve wound healing*
Ng 2017 [37]	*Malaysia*	*Trigona*	*Antibacterial*
Aziz 2017 [38]	*Malaysia*	*Geniotrigona thoracica*	*Anti-obesity* *Antidiabetic* *Antioxidant*

Study ID	***Country of Origin***	***Species***	***Reported Properties***
Arshad 2020 [24]	*Malaysia*	*Trigona*	*Improve memory* *Reduces anxiety*
Budin 2017 [39]	*Malaysia*	*Trigona*	*Antioxidant* *Improves fertility*
Zamora 2017 [40]	*Costa Rica*	*Tetragonisca angustula* *Melipona beecheii*	*Antimicrobial*
Saiful Yazan 2016 [41]	*Malaysia*	*Trigona*	*Anticancer*
Syam 2016 [42]	*Indonesia*	*Trigona*	*Anti-inflammatory*
Massora 2014 [43]	*Australia*	*Tetragonula carbonaria*	*Antimicrobial*
Borsato 2014 [44]	*Brazil*	*Melipona marginata*	*Anti-inflammatory* *Antioxidant*
Ilechie 2012 [45]	*Ghana*	*Meliponula*	*Antimicrobial* *Anti-inflammatory*
Boorn 2010 [46]	*Australia*	*Trigona carbonaria*	*Antimicrobial*
Garedew 2004 [47]	*Ethiopia*	*Trigona*	*Antimicrobial*

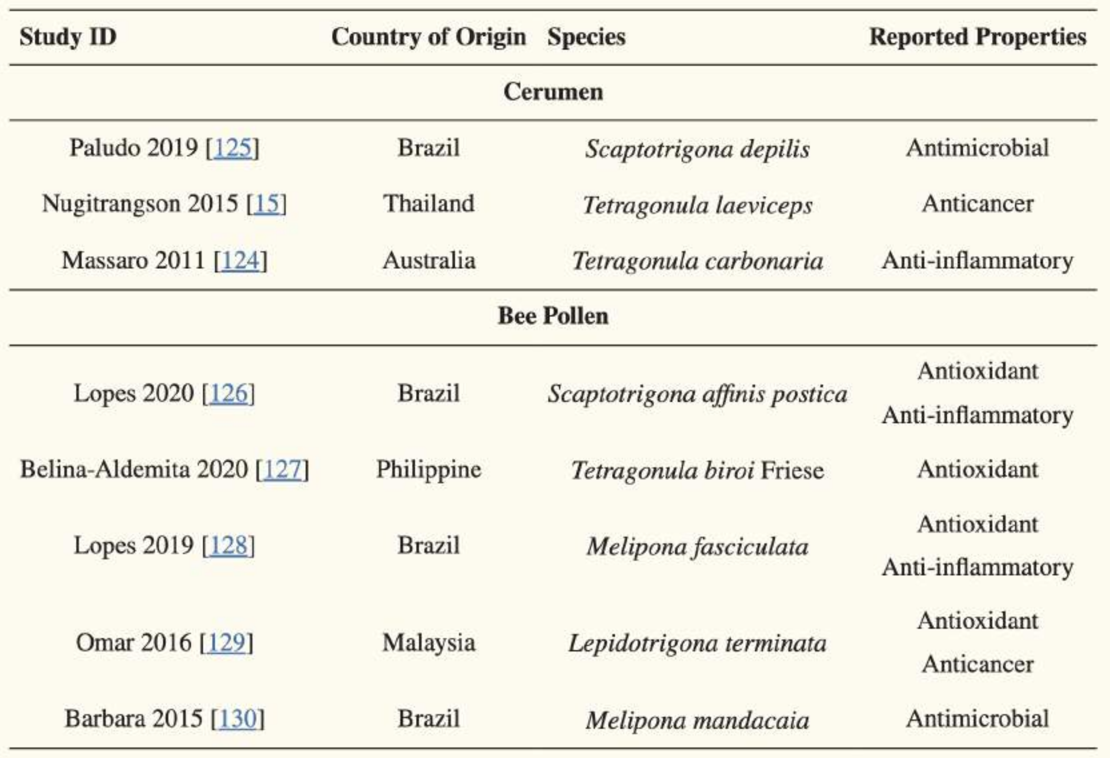
Study ID	***Country of Origin***	***Species***	***Reported Properties***
Arshad 2020 [24]	*Malaysia*	*Trigona*	*Improve memory* *Reduces anxiety*
Torres 2004 [48]	*Colombia*	*Tetragonisca angustula*	*Antimicrobial*
Patricia 2002 [49]	*Venezuela*	*Melipona favosa*	*Anti-inflammatory*

Meliponini also produce propolis, a resin-like substance that bees produce by mixing salivary secretions, beeswax, pollen and other resins harvested from botanical sources to seal holes and crevices, build the entrance to the hive and protect the community against microbial infections. Additionally, specific species of Meliponini (for example, Melipona fasciculata and Melipona quadrifasciata anthidioides) make geopropolis which is the mixture of propolis with soil or clay. Stingless bee propolis is well known for its main medicinal properties: antioxidant, anti-inflammatory, anticancer and antimicrobial activities. Moreover, propolis has shown "promising regenerative capacity, also making it a potential novel therapy for wound healing" (Al-Hatamleh et al., 2020).

Furthermore, stingless bees collect pollen which is composed of microscopic particles produced by the male flower. Pollen is the principal source of protein for bees; it's composed of carotenoids, chlorophyll, numerous vitamins, phenolic compounds, and has essential amino acids that provide a high nutritional value. After being deposited in pots within the nest, pollen is enriched with bee honey and digestive enzymes contained in the salivary glands of bees. Pollen can ferment during its storage creating what is commonly known as beebread. Bees also make cerumen which is a "mixture of propolis with the wax secreted by stingless bees before they use it for nest construction…It has been reported that stingless bee cerumen extracts have anti-inflammatory potential as they are capable of inhibiting enzymes responsible for catalyzing the activity of pro-inflammatory mediators" (Al-Hatamleh et al., 2020). Very few studies have been conducted regarding stingless bee cerumen and pollen yet in figure. 26 below you can see some of the few discoveries that have been made.

Figure. 26

List of studies on the potential medicinal properties of cerumen and bee pollen from stingless bees. (Al-Hatamleh et al., 2020)



Study ID	Country of Origin	Species	Reported Properties
Cerumen			
Paludo 2019 [125]	Brazil	*Scaptotrigona depilis*	Antimicrobial
Nugitrangson 2015 [15]	Thailand	*Tetragonula laeviceps*	Anticancer
Massaro 2011 [124]	Australia	*Tetragonula carbonaria*	Anti-inflammatory
Bee Pollen			
Lopes 2020 [126]	Brazil	*Scaptotrigona affinis postica*	Antioxidant Anti-inflammatory
Belina-Aldemita 2020 [127]	Philippine	*Tetragonula biroi* Friese	Antioxidant
Lopes 2019 [128]	Brazil	*Melipona fasciculata*	Antioxidant Anti-inflammatory
Omar 2016 [129]	Malaysia	*Lepidotrigona terminata*	Antioxidant Anticancer
Barbara 2015 [130]	Brazil	*Melipona mandacaia*	Antimicrobial

4.2 Potential Markets

The stingless bee product industry is considerably premature and underdeveloped for multiple reasons. Stingless bees produce much less quantity of honey (an average of 4 kg honey per colony each year) compared to *Apis Mellifera* (between 13 kg- 17kg of honey per colony per year), they are very delicate and fragile requiring special attention and conditions, their presence is limited to tropical and subtropical environments, and meliponiculture is still in its early stages. Although the industry is small, the potential is astounding. For instance, recent discoveries regarding the health benefits of Meliponini honey, propolis, and pollen have made them very desirable and expensive products. Beyond its medicinal value, honey, pollen and propolis can be used in cosmetics, pharmaceuticals and other health products. On the other hand, according to Tim Heard, former research scientist at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia, "Among the greatest commercial potential of stingless bees is as crop pollinators." Interestingly stingless bees have short flight ranges of aproximatley 500 meters, keeping them among crops for pollination while apis meliferas have a flight range of 2km that is harder to control for agricultural purposes. Furthermore, the empowerment of today's stingless bee industry would have a direct impact on the production of high quality honey, while increasing pollination and therefore maintaining biodiversity. Moreover, reinventing the Meliponini industry in tropical countries could be a new medium for targeting socio-economics and ecology. Finally in places like Brazil stingless bees have transcended into a highly profitable pet industry with many secondary benefits like those shown in figure. 27 below.

Figure 27.

Spill-over impacts of the stingless bee industry. (Mustafa et al., 2018)

Works Cited

Ávila, S., Beux, M. R., Ribani, R. H., & Zambiazi, R. C. (2018). Stingless bee honey: Quality parameters, bioactive compounds, health-promotion properties and modification detection strategies. *Trends in Food Science & Technology*, *81*, 37–50. https://doi.org/10.1016/j.tifs.2018.09.002

Bąk-Badowska, J., Żeber-Dzikowska, I., Gworek, B., Kacprzyk, W., & Chmielewski, J. (2019). The role and significance of stingless bees (Hymenoptera: Apiformes: Meliponini) in the natural environment. *Environmental Protection and Natural Resources*, *30*(2), 1–5. https://doi.org/10.2478/oszn-2019-0005

Brutscher, L. M., & Flenniken, M. L. (2015). RNAi and Antiviral Defense in the Honey Bee. *Journal of Immunology Research*, *2015*, 1–10. https://doi.org/10.1155/2015/941897

Camargo, J. M. F. de, Grimaldi, D. A., & Pedro, S. R. M. (2000). *The extinct fauna of stingless bees (Hymenoptera, Apidae, Meliponini) in Dominican amber: Two new species and redescription of the male of Problebeia dominicana (Wille and Chandler). American Museum novitates ; no. 3293*. https://digitallibrary.amnh.org/handle/2246/2984

Cham, K., Nocelli, R., Borges, L., Viana-Silva, F., Tonelli, C., Malaspina, O., Menezes, C., Rosa-Fontana, A., Blochtein, B., Freitas, B., Pires, C., Oliveira, F., Contrera, F., Torezani, K., Ribeiro, M., Siqueira, M., & Lima, M. (2018). Pesticide Exposure Assessment Paradigm for Stingless Bees. *Environmental Entomology*, *48*. https://doi.org/10.1093/ee/nvy137

Choudhari, M. K., Haghniaz, R., Rajwade, J. M., & Paknikar, K. M. (2013). Anticancer Activity of Indian Stingless Bee Propolis: An *In Vitro* Study. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 1–10. https://doi.org/10.1155/2013/928280

Cortopassi-Laurino, M., Lucia Imperatriz-Fonseca, V., David Ward Roubik, Dollin, A., Heard, T., Aguilar, I., C. Venturieri, G., Eardley, C., & Nogueira-Neto, P. (2006). Global meliponiculture: Challenges and opportunities. *Apidologie*, *37*(2), 275–292.

ENGEL, M. (2009). A New Interpretation of the Oldest Fossil Bee (Hymenoptera: Apidae). *American Museum Novitates*, *3296*, 1–11. https://doi.org/10.1206/0003-0082(2000)3296<0001:ANIOTO>2.0.CO;2

Engel, M. S. (1999a). *Megachile glaesaria, the first megachilid bee fossil from amber (Hymenoptera, Megachilidae). American Museum novitates ; no. 3276*. https://digitallibrary.amnh.org/handle/2246/3014

Engel, M. S. (1999b). *The first fossil Euglossa and phylogeny of orchid bees (Hymenoptera, Apidae, Euglossini). American Museum novitates ; no. 3272*. https://digitallibrary.amnh.org/handle/2246/3046

Engel, M. S. (2001a). *A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). Bulletin of the AMNH ; no. 259*. https://digitallibrary.amnh.org/handle/2246/1437

Engel, M. S. (2001b). Monophyly and extensive extinction of advanced eusocial bees: Insights from an unexpected Eocene diversity. *Proceedings of the National Academy of Sciences*, *98*(4), 1661–1664. https://doi.org/10.1073/pnas.98.4.1661

Engel, M. S. (2011). Systematic melittology: Where to from here? *Systematic Entomology*, *36*(1), 2–15. https://doi.org/10.1111/j.1365-3113.2010.00544.x

Engel, M. S., Grimaldi, D. A., González, V. H., Hinojosa-Díaz, I. A., & Michener, C. D. (Charles D. (2012). *An exomalopsine bee in early Miocene amber from the Dominican Republic (Hymenoptera, Apidae). (American Museum novitates, no. 3758)*. https://digitallibrary.amnh.org/handle/2246/6371

Engel, M. S., & Michener, C. D. (2013). *Geological History of the Stingless Bees (Apidae: Meliponini)*. http://www.saber.ula.ve/handle/123456789/37108

Farias, J. H. C. de, Reis, A. S., Araújo, M. A. R., Araújo, M. J. A. M., Assunção, A. K. M., Farias, J. C. de, Fialho, E. M. S., Silva, L. A., Costa, G. C., Guerra, R. N. M., Ribeiro, M. N. S., & Nascimento, F. R. F. do. (2014). Effects of Stingless Bee Propolis on Experimental Asthma. *Evidence-Based Complementary and Alternative Medicine*, *2014*, 1–8. https://doi.org/10.1155/2014/951478

Gonzalez, V. H., Cobos, M. E., Jaramillo, J., & Ospina, R. (2021). Climate change will reduce the potential distribution ranges of Colombia's most valuable pollinators. *Perspectives in Ecology and Conservation*, *19*(2), 195–206. https://doi.org/10.1016/j.pecon.2021.02.010

Greco, M. K., Welz, P. M., Siegrist, M., Ferguson, S. J., Gallmann, P., Roubik, D. W., & Engel, M. S. (2011). Description of an ancient social bee trapped in amber using diagnostic radioentomology. *Insectes Sociaux*, *58*(4), 487–494. https://doi.org/10.1007/S00040-011-0168-8

Grimaldi, D. A., & Engel, M. S. (2005). *Evolution of the insects*. Cambridge University Press.

Grüter, C. (2020). *Stingless Bees* (1st ed.). Springer.

Heard, T. (2001). Stingless bees and crop pollination. *Annual Review of Entomology*, *82*, 110–112.

Hennebert, M. (2014). The Cretaceous-Paleogene boundary and its 405-kyr eccentricity cycle phase: A new constraint on radiometric dating and astrochronology. *Carnets de Geologie - Notebooks on Geology*. http://paleopolis.rediris.es/cg/14/09/

Hrncir, M., Jarau, S., & Barth, F. (2016). Stingless bees (Meliponini): Senses and behavior. *Journal of Comparative Physiology A*, *202*. https://doi.org/10.1007/s00359-016-1117-9

ITIS - Report: Axestotrigona ferruginea. (n.d.). Retrieved October 26, 2021, from https://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=763722#null

Lyons, J. R., & Ahrens, T. J. (2003). Terrestrial Acidification at the K/T Boundary. In L. Davison, Y. Horie, & T. Sekine (Eds.), *High-Pressure Shock Compression of Solids V: Shock Chemistry with Applications to Meteorite Impacts* (pp. 181–197). Springer. https://doi.org/10.1007/978-1-4613-0011-3_8

Martin, S. J., Shemilt, S., da S. Lima, C. B., & de Carvalho, C. A. L. (2017). Are Isomeric Alkenes Used in Species Recognition among Neo-Tropical Stingless Bees (Melipona Spp). *Journal of Chemical Ecology*, *43*(11), 1066–1072. https://doi.org/10.1007/s10886-017-0901-5

Michener, C. (1982). A New Interpretation of Fossil Social Bees from the Dominican Republic. *Sociobiology*, *7*(1), 37–45.

Michener, C. D. (Charles D., & Grimaldi, D. A. (1988). *A Trigona from late Cretaceous amber of New Jersey (Hymenoptera, Apidae, Meliponinae). American Museum novitates ; ; no. 2917*. https://digitallibrary.amnh.org/handle/2246/5163

Michener, C. D., & Grimaldi, D. A. (1988). The oldest fossil bee: Apoid history, evolutionary stasis, and antiquity of social behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(17), 6424–6426.

Michener, C. D., & Poinar, G. (1996). The Known Bee Fauna of the Dominican Amber. *Journal of the Kansas Entomological Society*, *69*(4), 353–361.

Michez, D., Vanderplanck, M., & Engel, M. S. (2011). *Fossil bees and their plant associates*. https://doi.org/10.1017/CBO9781139014113.006

Morayma Solórzano Kraemer, M. (2007). Systematic, palaeoecology, and palaeobiogeography of the insect fauna from Mexican amber. *Palaeontographica Abteilung A*, 1–133. https://doi.org/10.1127/pala/282/2007/1

National Geographic. (2011, January 21). *Tropics*. National Geographic Society. http://www.nationalgeographic.org/encyclopedia/tropics/

Patiny, S. (2011). *Evolution of Plant-Pollinator Relationships*. Cambridge University Press.

Pedro, S. R. M. (2014). The Stingless Bee Fauna In Brazil (Hymenoptera: Apidae). *Sociobiology*, *61*(4), 348–354. https://doi.org/10.13102/sociobiology.v61i4.348-354

RAMALHO, M., IMPERATRIZ-FONSECA, V. L., & Giannini, T. C. (1998). Within-colony size variation of foragers and pollen load capacity in the stingless bee Melipona quadrifasciata anthidioides Lepeletier (Apidae, Hymenoptera). *Apidologie*, *29*(3), 221–228.

Santos, S., Roselino, A., Hrncir, M., & Bego, L. R. (2009). Pollination of tomatoes by the stingless bee Melipona quadrifasciata and the honey bee Apis mellifera (Hymenoptera, Apidae). *Genetics and Molecular Research : GMR*, *8*, 751–757. https://doi.org/10.4238/vol8-2kerr015

Shamsudin, S., Selamat, J., Sanny, M., Abd. Razak, S.-B., Jambari, N. N., Mian, Z., & Khatib, A. (2019). Influence of origins and bee species on physicochemical, antioxidant properties and botanical discrimination of stingless bee honey. *International Journal of Food Properties*, *22*(1), 239–264. https://doi.org/10.1080/10942912.2019.1576730

Vit, P., R. M., S., & Roubik, D. (Eds.). (2013). *Pot-Honey* (1st ed.). Springer, New York, NY.

Wille, A. (1959). A New Fossil Stingless Bee (Meliponini) from the Amber of Chiapas, Mexico. *Journal of Paleontology*, *33*(5), 849–852.

Araujo, V. A., Serrão, J. E., Antonini, Y., Dias, L. G., & Neto, J. L. (2020). Structural Changes in the Male Reproductive Tract of the Stingless Bee Scaptotrigona xanthotricha Moure 1950 (Meliponini, Apidae) During Sexual Maturation. *Sociobiology*, *67*(4), 526–534. https://doi.org/10.13102/sociobiology.v67i4.5513

Bąk-Badowska, J., Żeber-Dzikowska, I., Gworek, B., Kacprzyk, W., & Chmielewski, J. (2019). The role and significance of stingless bees (Hymenoptera: Apiformes: Meliponini) in the natural environment. *Environmental Protection and Natural Resources*, *30*(2), 1–5. https://doi.org/10.2478/oszn-2019-0005

Halcroft, M., Haigh, A. M., & Spooner-Hart, R. (2013). Ontogenic time and worker longevity in the Australian stingless bee, Austroplebeia australis. *Insectes Sociaux*, *60*(2), 259–264. https://doi.org/10.1007/s00040-013-0291-9

Kofi, K., K, A., Combey, R., & Karikari, A. (2010). *Stingless Bees: Importance, Management and Utilisation: A Training Manual for Stingless Bee Keeping.*

Tanaka, É. D., Santana, W. C., & Hartfelder, K. (2009). Ovariole structure and oogenesis in queens and workers of the stingless bee Melipona quadrifasciata (Hymenoptera: Apidae, Meliponini) kept under different social conditions. *Apidologie*, *40*(2), 163–177. https://doi.org/10.1051/apido/2008071

Travenzoli, N. M., Lima, B. A., Cardoso, D. C., Dergam, J. A., Fernandes-Salomão, T. M., & Lopes, D. M. (2019). Cytogenetic Analysis and Chromosomal Mapping of Repetitive DNA in Melipona Species (Hymenoptera, Meliponini). *Cytogenetic and Genome Research*, *158*(4), 213–224. https://doi.org/10.1159/000501754

Bradley, K. (2013, June 7). A peek inside a stingless beehive... *Milkwood: Permaculture Courses, Skills + Stories*. https://www.milkwood.net/2013/06/08/inside-a-stingless-beehive/

Fig 3. Indigenous stingless bee husbandry in Mesoamerica involves the... (n.d.). ResearchGate. Retrieved March 21, 2022, from https://www.researchgate.net/figure/Indigenous-stingless-bee-husbandry-in-Mesoamerica-involves-the-use-of-specially-made_fig3_328520925

Fig. 3. Some nest entrances of stingless bees. (A) Plebeia minima... (n.d.). ResearchGate. Retrieved March 21, 2022, from https://www.researchgate.net/figure/Some-nest-entrances-of-stingless-bees-A-Plebeia-minima-Gribodo-1983-Hymenoptera_fig3_330005753

Grüter, C. (2020). Nesting Biology. In C. Grüter (Ed.), *Stingless Bees: Their Behaviour, Ecology and Evolution* (pp. 87–130). Springer International Publishing. https://doi.org/10.1007/978-3-030-60090-7_3

Kofi, K., K, A., Combey, R., & Karikari, A. (2010a). *Stingless Bees: Importance, Management and Utilisation: A Training Manual for Stingless Bee Keeping.*

Kofi, K., K, A., Combey, R., & Karikari, A. (2010b). *Stingless Bees: Importance, Management and Utilisation: A Training Manual for Stingless Bee Keeping.*

Leão, K., Queiroz, A. C. M. D., Veiga, J., Contrera, F., & Venturieri, G. (2016). *COLONY DEVELOPMENT AND MANAGEMENT OF THE STINGLESS BEE SCAPTOTRIGONA AFF. POSTICA (APIDAE, MELIPONINI) IN DIFFERENT HIVE MODELS*. https://doi.org/10.13102/SOCIOBIOLOGY.V63I4.1041

Stingless Bee Keeping (meliponiculture). (n.d.). *MADHUSREE HONEY*. Retrieved March 21, 2022, from https://madhusreehoney.com/stingless-bee-keeping-meliponiculture/

Trap hives for stingless bees. (2018, August 26). *Australian Native Bee*. https://www.australiannativebee.com/2018/08/26/trap-hives-for-stingless-bees/

Al-Hatamleh, M. A. I., Boer, J. C., Wilson, K. L., Plebanski, M., Mohamud, R., & Mustafa, M. Z. (2020). Antioxidant-Based Medicinal Properties of Stingless Bee Products: Recent Progress and Future Directions. *Biomolecules*, *10*(6), 923. https://doi.org/10.3390/biom10060923

Kofi, K., K, A., Combey, R., & Karikari, A. (2010). *Stingless Bees: Importance, Management and Utilisation: A Training Manual for Stingless Bee Keeping.*

Mustafa, M. Z., Yaacob, N. S., & Sulaiman, S. A. (2018). Reinventing the Honey Industry: Opportunities of the Stingless Bee. *The Malaysian Journal of Medical Sciences : MJMS*, *25*(4), 1–5. https://doi.org/10.21315/mjms2018.25.4.1

The emerging market of propolis of stingless bees in tropical countries. (2019). *MOJ Food Processing & Technology*, *Volume 7*(Issue 2). https://doi.org/10.15406/mojfpt.2019.07.00215