

# Plant species used by *Melipona eburnea* bees in seasonally-flooded agroforestry systems in the Peruvian Amazon

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## Abstract

From September 2018 to December 2019 and between July and November 2021 we conducted a study on the association of *Melipona eburnea* bees with fruit-producing trees in seasonally flooded small agroforestry systems in the Peruvian Amazon. We found 40 plant species that were used by *Melipona eburnea*: 22 flowering species that were visited by the bees as a source for food, 15 species that were used as nest habitat, and 8 species that were used by the bees for their resin.

The plant species most visited as a food source were *Eugenia stipitata* with 45 (16.9% of total) individuals, *Myrciara dubia* with 37 individuals (13.9% of total) and *Inga edulis* with 32 individuals (12% of total). The species most used as nesting habitat were *Calycophyllum spruceanum* with 31 individuals (32% of total) *Maquira coriacea* with 15 individuals (15.5% of total), together representing 47.5% of the total number of individuals. We recorded eight plant species that are used as a resin source: *Croton lechleri*, *Protium apiculatum*, *Copaifera officinalis*, *Mangifera indica*, *Anacardium occidentale*, *Garcinia macrophylla* and two species of *Vismia*. *M. eburnea* is an important bee species that depends on a large diversity of plants for its survival. An effective conservation strategy will have to take into account all of the different uses of plants by the bees.

**Keywords:** feeding, floodplains, meliponini, nesting, resin

## Introduction

Globally, there are more than 500 species of stingless bees in the tribe Meliponini (Michener 2013), with the great majority (391 species) described from the Neotropics (Camargo and Pedro 2008, Freitas et al 2009). In Peru, more than 175 different species of bees are known, not including many undescribed species (Rasmussen, personal communication); a recent study reported 69 species of stingless bees for the Department of Loreto, in the Amazonian lowlands of Peru (Rasmussen and Delgado 2019).

### *Melipona eburnea*

Is distributed in the Amazonian region in Bolivia, Brazil, Colombia, Ecuador, Peru and Venezuela (Camargo and Pedro 2008, Obregon and Nates-Parra 2014). In Colombia, the species is reported from 50 to 2100 masl (Nates-Parra 2001), and in Peru up to 950 masl (Rasmussen and Gonzales 2009). The species is locally known as *ronsapilla*, *pacucha* or *boca de sapo* (toad mouth), and it is the most favored species for indigenous communities and other rural people in the region for the quality and quantity of honey that it produces (Rasmussen and Castillo 2003, Rasmussen and Delgado 2019, Delgado et al 2020).

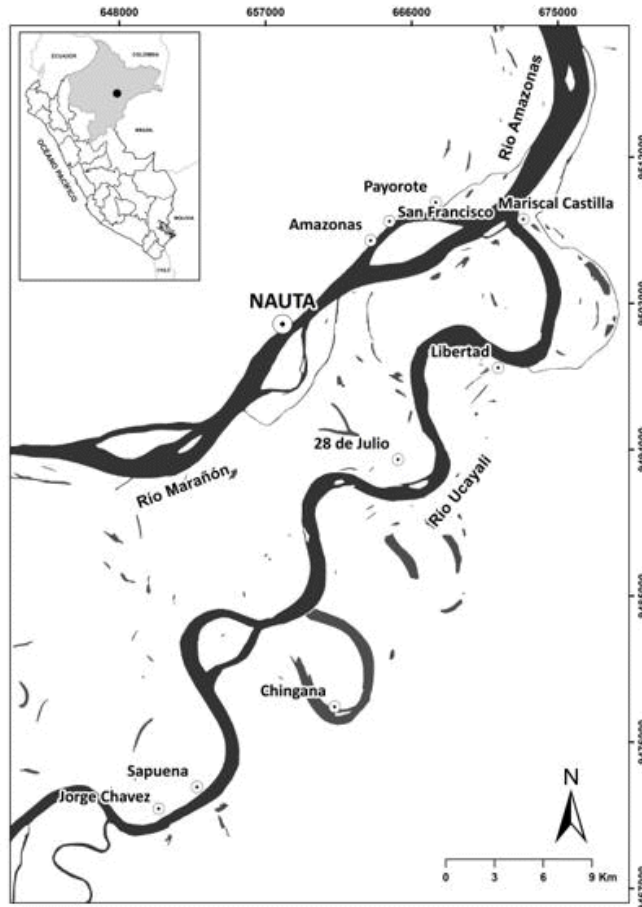
The stingless bees from the tribe Meliponini maintain a strict association with a wide variety of plant species, because plants provide food, nesting sites that are appropriate for some but not all kinds of bees (Roubik 1989, Barquero-Elizondo et al 2019) and resins for nest construction or use as defenses against predators and pathogens (Shanahan and Spivak 2021); and at the same time the bees play an important role in pollination, leading to fruit and seed production of many plants (Batista et al 2016, Frankie et al 2018).

Although some studies have documented the visits of stingless bees to flowers (Roubik 1989, Heard 1999, Gaona et al 2019, Ribeiro de Souza et al 2019, López-Roblero 2021), very few studies have described all three forms of association, including nesting and resin use of plant species by bees. Obregon and Nates-Parra (2014) in the Colombian Andes reported 92 kinds of pollen in the honey and pollen analysis of *M. eburnea*, with the largest amounts belonging to the Myrtaceae family (>94%). Bardales and Bardales (2021) in a study in Loreto, Peru found 82 kinds of pollen in the corbiculae of *M. eburnea*, with Melastomataceae, Fabaceae, and Myrtaceae (21.1%), representing the most common families.

Delgado et al (2020) reported *M. eburnea* as the most common stingless bee species pollinating the flowers of the *camu-camu* bush, *Myrciara dubia* (Myrtaceae). The objective of this study is to document the association of *Melipona eburnea* with the species of plants that are used as food sources, resin sources, and nest habitat in the agroforestry systems found in the seasonally-flooded forests of the Peruvian Amazon, with the aim of establishing a baseline to support strategies for the conservation of this important honey-producing bee.

## Materials and methods

The study was conducted between September 2018 and December 2019 and between July and November 2021, in 9 communities from the Department of Loreto. These were located in two main areas: first, in the mouth of the Marañón River, in the district of Nauta, province of Loreto; second, on the Ucayali River, in the district of Bagazán, province of Requena (Figure 1)



**Figure 1.** Location map of the communities studied, in the basins of the Marañón and Ucayali rivers

All of these sites are found on nutrient-rich soils and with a flat topography that is inundated for three or four months each year with the rise of the river due to Andean snowmelt (Photo 1, 2). The climate is tropical humid with two well-defined seasons: The lower (or dry) season of the river, which coincides with the time of lowest precipitation (May to October) and the growing season (or winter season) which coincides with the greatest rainfall (November to April).



**Photo 1.** Floodable agroforestry system 15 years old in the community of Chingana, with soils 51 days under water (Photo: César Delgado)



**Photo 2.** Four-year-old floodable agroforestry system in the community of Sapuena, with soils above water (Photo: César Delgado)

The average annual temperature is  $25.9^{\circ}\text{C} \pm 2.0$ , relative humidity is 90% and annual rainfall is 2750 mm (Kvist and Nebel 2001). The inhabitants have managed and adapted their crops for hundreds of years to this environment. These agroforestry systems include a large diversity of plant species, including plants for food, medicinal use, timber, firewood, etc., and together with the surrounding natural vegetation, constitute an abundant and diverse source of floral resources for the bees throughout the year.

The evaluation of plant species used by *M. eburnea* for food sources was carried out in 21 agroforestry plots each of which averaged 1 ha in size. Bees were collected only on fruiting trees at anthesis, from 5.30 to 10 am (peak hours of bee flying activity). For the collection, we used a 5m long (40 cm diameter) telescopic entomological net. The collection was standardized by taking two sweeps with the net on each fruit tree. Once identified, the collected bees were released to the natural environment. Fruit species were identified in-situ with the help of a community member. Some specimens were transported to the laboratory of the Research Institute of the Peruvian Amazon, for confirmation by botanists.

For the evaluation of plant resources used by bees as nesting habitat and as a source of resins, we conducted brief interviews with beekeepers and honey extractors from the community, then we took guided visits with community members to visit the places where the population cares for the beehives and extracts honey from the forest. To identify the sources of resins, a spatula was used to extract small amounts of resins present in the entrance and in the internal part of the hive. With the help of the beekeeper, the resin sample was identified to plant species according to the texture, smell and taste of the resin. As combinations of resins were collected on several occasions making identification difficult, we also made some direct observations of the bees visiting plants to extract resin on known species reported by the community members.

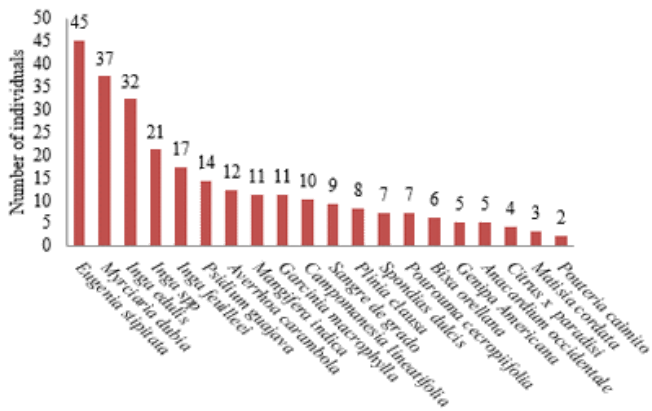
## **Results and discussion**

In our study we found 40 plant species from 18 different families that were used by *Melipona eburnea*: 22 plant species were used as a food source, 15 as nesting habitat and eight to extract resin. Four species were used both for food and to obtain resin, one as both a food source and nesting habitat, and no species was recorded used for all three purposes (Table 1).

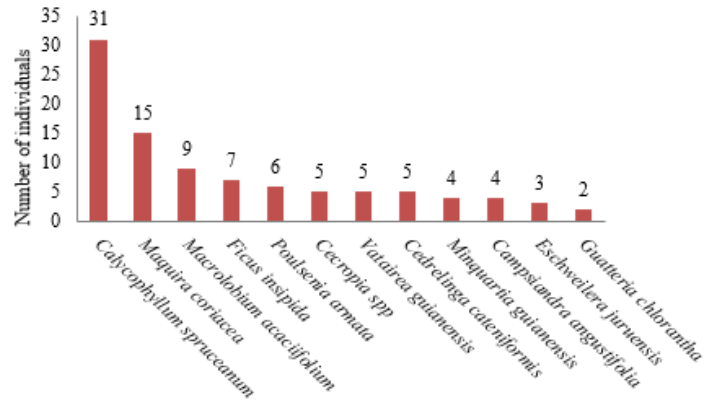
**Table 1.** Diversity of plant species used by stingless bees as a source of food, resins and nesting habitat in nine communities located in floodplains in the lower basin of the Marañón and Ucayali rivers

Species / Family	Nectar/ pollen	Resin	Nesting habitat
<i>Anacardium occidentale</i> L. <b>Anacardiaceae</b>	X	X	
<i>Mangifera indica</i> L. <b>Anacardiaceae</b>	X	X	
<i>Spondias dulcis</i> Parkinson <b>Anacardiaceae</b>	X		
<i>Guatteria chlorantha</i> Diels <b>Annonaceae</b>			X
<i>Bixa orellana</i> L. <b>Bixaceae</b>	X		
<i>Protium apiculatum</i> Swart <b>Burceraceae</b>		X	
<i>Matisia cordata</i> Bonpl. <b>Bombacaceae</b>	X		
<i>Garcinia macrophylla</i> Mart. <b>Clusiaceae</b>	X	X	
<i>Rheedia floribunda</i> Planch. <b>Clusiaceae</b>	X		
<i>Croton lechleri</i> Muell-Arg <b>Euphorbiaceae</b>	X	X	
<i>Inga edulis</i> Mart <b>Fabaceae</b>	X		
<i>Inga feuillei</i> Mart <b>Fabaceae</b>	X		
<i>Inga</i> spp <b>Fabaceae</b>	X		
<i>Copaifera officinalis</i> (Jacq.) <b>Fabaceae</b>		X	
<i>Macrobium acaciifolium</i> (Benth.) <b>Fabaceae</b>			X
<i>Schizolobium</i> spp <b>Fabaceae</b>			X
<i>Vatairea guianensis</i> Aubl <b>Fabaceae</b>			X
<i>Cedrelinga cateniformis</i> Ducke <b>Fabaceae</b>			X
<i>Campsiandra angustifolia</i> Spruce Ex Benth <b>Fabaceae</b>			X
<i>Vismia gracilis</i> Hieron. <b>Hypericaceae</b>		X	
<i>Vismia</i> spp <b>Hypericaceae</b>		X	
<i>Eschweilera juruensis</i> Knuth <b>Lecythidaceae</b>			X
<i>Ficus insipida</i> Willd. <b>Moraceae</b>			X
<i>Maquira coriacea</i> (Karst.) <b>Moraceae</b>			X
<i>Poulsenia armata</i> (Miq.) <b>Moraceae</b>			X
<i>Artocarpus altilis</i> Fosberg <b>Moraceae</b>			X
<i>Campomanesia lineatifolia</i> R. et P. <b>Myrtaceae</b>	X		
<i>Eugenia stipitata</i> Mc Vaugh <b>Myrtaceae</b>	X		
<i>Myrciaria Dubia</i> (H.B.K.) <b>Myrtaceae</b>	X		
<i>Plinia clausa</i> Mc Vaugh <b>Myrtaceae</b>	X		
<i>Psidium guajava</i> L. <b>Myrtaceae</b>	X		
<i>Minquartia guianensis</i> Aubl. <b>Oleaceae</b>			X
<i>Averrhoa carambola</i> L. <b>Oxalidaceae</b>	X		
<i>Calycophyllum spruceanum</i> (Benth.) <b>Rubiaceae</b>			X
<i>Genipa Americana</i> L. <b>Rubiaceae</b>	X		
<i>Citrus x paradisi</i> Macfad <b>Rutaceae</b>	X		
<i>Citrus sinensis</i> Osbeck <b>Rutaceae</b>	X		
<i>Pouteria caimito</i> (Ruiz et Pavon) <b>Sapotaceae</b>	X		X
<i>Pourouma cecropiifolia</i> Mart. <b>Urticaceae</b>	X		
<i>Cecropia</i> spp <b>Urticaceae</b>			X

In our agroforestry plots, we collected 266 individuals of *M. eburnea* from the flowers of 22 species of fruit trees, from 12 different families. The Myrtaceae family represents the greatest diversity with 5 species. The families that represent the greatest abundance of individuals are the Myrtaceae with 114 individuals (43.1% of total) and the Fabaceae with 70 individuals (26.3%), these two families together represent 69.3% of the total individuals collected. The species with the highest abundance of bees were *Eugenia stipitata* with 45 (16.9% of the total) individuals, *Myrciaria dubia* with 37 (13.9% of the total) and *Inga edulis* with 32 (12% of the total). Other important species were *Matisia cordata*, *Pouteria caimito*, *Citrus x paradisi*, *Anacardium occidentale* and *Genipa americana* (Figure 2, Photo3).



**Figure 2.** Diversity and abundance of plant species used as a food resource by *M. eburnea*.



**Figure 3.** Diversity and abundance of plant species used as nesting habitat by *M. eburnea*.



**Photo 3.** Plant species most used by *M. eburnea* in agroforestry systems: as a source of pollen and nectar, a) *Eugenia stipitata*, b) *Myrciaria dubia*, c) *Inga edulis* and as a nesting habitat, d) *Calycophyllum spruceanum* (Photo: Gladis Atías and César Delgado)

The low diversity of plant species recorded for *M. eburnea* as a food source in our study, compared to the higher diversity of species reported from the Colombian Andes by Obregon and Nates-Parra (2014), and in Loreto - Peru by Bardales and Bardales (2021), must be taken into account with the different methodology used in the three different studies. Our study captured bees in the flowers of fruit trees in a field study, while the previous cited studies cited were carried out using techniques identifying pollen from honey and corbicular pollen load; These techniques make it possible to identify (almost) all the plant species that are used by bees in their environments. However, even with these large differences in methodology, similar to the previous studies we found that the families Myrtaceae and Fabaceae were the most preferred for *M. eburnea*. This high diversity of plant species and preferences for the botanical families Myrtaceae and Fabaceae, were also observed in other regions for species of the genus *Melipona* (Antonini et al 2006, Ramalho et al 1989).

To learn about which plant trees were being used as nesting habitat, we interviewed 19 people dedicated to bee breeding and honey extraction from the forest. The informants identified 16 plant species from nine different families. The most diverse families were Fabaceae with 5 (31.2% of total) species and Moraceae with 4 (25% of total), which together represent 56.2% of the plant species used by *M. eburnea* as nesting habitat. The most used species were *Calycophyllum spruceanum* (Rubiaceae) with 31 individuals (32% of total) *Maquira coriacea* (Moraceae) with 15 individuals (15.5% of total), both representing 47.5% of the total number of individuals (Figure 3, Photo 3). Other minor species were *Artocarpus altilis*, *Guatteria chlorantha*, which represented 3% of the total individuals.

Regarding the use of resins, we identified resin from eight plant species from six different families. The number of identified species must be considered a large underestimate, because bees probably collect resins from many different plant species on the same day. Therefore, in any given nest there will be combinations of resins from various species of trees, not all of which could be identified by the informants. Despite the low number of confirmed species, this work contributes to the general knowledge of plant species that are important for bees in the construction and protection of their nests. In addition, for the people living in these communities, these plant species represent an important contribution of their local economies, due to their medicinal use, as is the case for sangre de grado (*Croton lechleri*), copal (*Protium apiculatum*), cobaiba (*Copaifera officinalis*), among others. The use of copal resins collected by bees has been recorded by other authors (Roubik 1989). We observed *M. eburnea* collecting copal resin secreted by wounds made on the tree by the beetle *Papista* sp (Coleoptera: Curculionidae).

In agroforestry systems in Indonesia, the stingless bee *Tetragonula laeviceps* was reported using nectar and pollen from 22 plant species, with the Fabaceae family being the most preferred (Agussalim et al 2020), and three species as a source of resin (propolis) (Erwan et al 2021), of which two species are reported in our study (Table 2). Agroforestry systems in Indonesia and Peru share many cultivated plant species (Table 2). However, when we compare these data with our study, we observe a low similarity of plant species visited for nectar and pollen between the two geographic regions. Regarding the use of resins, unlike Indonesian stingless bees, it is very common to find *M. eburnea* using *A. occidentale* and *M. indica*, but it was not found in *Musa* sp. despite the fact that this plant species is very abundant in the plots studied.

**Table 2.** Diversity of plant species used by stingless bees as a source of food (X) and resins (O) in Agroforestry systems of Peru and Indonesia (Agussalim et al 2020 and (Erwan et al 2021).

Species / Family	In the present study	In Indonesia
<i>Anacardium occidentale</i> L. <b>Anacardiaceae</b>	X,O	O
<i>Mangifera indica</i> L. <b>Anacardiaceae</b>	X,O	X,O
<i>Spondias dulcis</i> Parkinson <b>Anacardiaceae</b>	X	
<i>Stelechocarpus burahol</i> (Blume) <b>Annonaceae</b>		X
<i>Cichorium intybus</i> L. <b>Asteraceae</b>		X
<i>Helianthus annuus</i> L. <b>Asteraceae</b>		X
<i>Bixa orellana</i> L. <b>Bixaceae</b>	X	
<i>Canarium</i> sp <b>Burseraceae</b>		X
<i>Protium apiculatum</i> Swart <b>Burseraceae</b>	O	
<i>Matisia cordata</i> Bonpl. <b>Bombacaceae</b>	X	
<i>Garcinia macrophylla</i> Mart. <b>Clusiaceae</b>	X,O	
<i>Rheedea floribunda</i> Planch. <b>Clusiaceae</b>	X	
<i>Terminalia catappa</i> L. <b>Combretaceae</b>		X
<i>Croton lechleri</i> Muell-Arg <b>Euphorbiaceae</b>	X,O	
<i>Gliricidia sepium</i> (Jacq.) <b>Fabaceae</b>		X
<i>Inga edulis</i> Mart. <b>Fabaceae</b>	X	
<i>Inga feuilleei</i> Mart. <b>Fabaceae</b>	X	
<i>Inga</i> spp <b>Fabaceae</b>	X	
<i>Indigofera tinctoria</i> L. <b>Fabaceae</b>		X
<i>Copaifera officinalis</i> (Jacq.) <b>Fabaceae</b>	O	
<i>Calliandra</i> sp. <b>Fabaceae</b>		X
<i>Medicago sativa</i> L. <b>Fabaceae</b>		X
<i>Tamarindus indica</i> L. <b>Fabaceae</b>		X
<i>Vismia gracilis</i> Hieron. <b>Hypericaceae</b>	O	
<i>Vismia</i> spp <b>Hypericaceae</b>	O	
<i>Ceiba pentandra</i> (L.) <b>Malvaceae</b>		X
<i>Campomanesia lineatifolia</i> R. et P. <b>Myrtaceae</b>	X	
<i>Mussa</i> sp. <b>Musaceae</b>		X,O
<i>Eugenia stipitata</i> Mc Vaugh <b>Myrtaceae</b>	X	
<i>Myrciaria dubia</i> (H.B.K.) <b>Myrtaceae</b>	X	
<i>Plinia clausa</i> Mc Vaugh <b>Myrtaceae</b>	X	
<i>Psidium guajava</i> L. <b>Myrtaceae</b>	X	X
<i>Syzygium samarangense</i> (Blume) <b>Myrtaceae</b>		X
<i>Averrhoa carambola</i> L. <b>Oxalidaceae</b>	X	X
<i>Averrhoa bilimbi</i> L. <b>Oxalidaceae</b>		X
<i>Genipa Americana</i> L. <b>Rubiaceae</b>	X	
<i>Citrus limon</i> L. <b>Rutaceae</b>		X
<i>Citrus x paradisi</i> Macfad <b>Rutaceae</b>	X	
<i>Citrus sinensis</i> Osbeck <b>Rutaceae</b>	X	
<i>Nephelium lappaceum</i> L. <b>Sapindaceae</b>		X
<i>Pometia pinnata</i> <b>Sapindaceae</b>		X
<i>Pouteria caimito</i> (Ruiz et Pavon) <b>Sapotaceae</b>	X	X
<i>Capsicum frutescens</i> L. <b>Solanaceae</b>		X
<i>Pourouma cecropiifolia</i> Mart. <b>Urticaceae</b>	X	

Several plant species used by *M. eburnea* are rapidly declining due to selective logging for commercial extraction purposes including construction, firewood, charcoal, honey extraction, as well as general deforestation for monoculture agriculture. A notable case is *Calycophyllum spruceanum* which has been heavily logged since the rubber era (1882-1912), due to the quality of the wood, which is considered the best charcoal. In the rubber boom it was used to run steamships; today, it is harvested in large quantities for brick factories, bakeries, restaurants and chicken roasters in the largest cities of the Peruvian Amazon. Another factor observed and commented on by the local population, are the increasingly frequent floods in the past decade, which have caused changes in the phenology and death of individuals of some important tree species, which do not have sufficient tolerance for prolonged flooding.

## Conclusions

- *Melipona eburnea* is one of most economically-important stingless bee species, and it uses a large diversity of plants as a source of food resources, obtaining resins and as nesting habitat.
- The Myrtaceae and Fabaceae families represent the plant species with the greatest diversity and abundance for food sources, while the Fabaceae and Moraceae families are most important for nesting habitat.
- Conservation strategies for *M. eburnea*, in addition to the important food source and nesting habitat plants should also include prioritize the protection of the trees *Croton lechleri*, *Protium apiculatum* and *Copaifera officinalis*, due to the biological activities of their resins.

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