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TROPHIC RESOURCES COLLECTED BY *Melipona grandis* GUÉRIN, 1844 (APIDAE: MELIPONINA) IN RURAL AREA OF RIO BRANCO, ACRE – BRAZIL

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Abstract: *Melipona grandis* has a wide geographic distribution in Latin America. Moreover, it is a species used in honey production by native meliponicultors of Acre. This study aimed to identify plants visited by *M. grandis* and indicate the degree of specialization of this bee in the collection of trophic resources, being conducted between November 2016 and October 2017. Altogether, 26 samplings were performed. The most common pollen types were: *Solanum* type (Solanaceae); *Cassia mimosoides* (Fabaceae/Caesalpinioideae); *Mimosa caesalpiniifolia*; *Mimosa pudica* (Fabaceae/Mimosoideae) and *Miconia* type (Melastomataceae). The months with greatest wealth of pollen types were: July, May and August. Arboreal and shrubby plants were the main resource suppliers for *M. grandis*, being 48.78 % (arboreal) and 34.15 % (shrubby). The degree of specialization of this bee is clearly associated with the abundance of resources throughout the year, Levins Index (B) for trophic niche amplitude 6.975, Standardized Index (B_{sta}) 0.314, characterizing *M. grandis* as polyletic.

Keywords: Visited plants; pollen; floral resources; stingless bees.

INTRODUCTION

Stingless bees are the most diverse group among social bees (Malerbo-Souza & Halak 2016). 52 genera are currently known, and about 417 species inhabit the Neotropical region (Camargo & Pedro 2013). The records for Brazil are 29 genus and 244 species, of which 100 are present in the Legal Amazon, and 61 species can be found in Acre (Pedro 2014). In tropical forests, species of *Melipona* Illiger, 1806 visit approximately 800 plant species to collect trophic resources used to maintain their colonies (Camargo & Pedro 2013), which demand a large amount of food throughout the year (Ramalho *et al.* 2007, Crowther *et al.* 2014). They are important pollinators in crops and natural ecosystems because of the generalist interactions with Angiosperms (Hilário *et al.* 2000, Slaa *et al.* 2006, Souza *et al.* 2006, Crowther *et al.* 2014, Smith *et al.* 2016).

Melipona grandis (Hymenoptera, Apidae) has wide geographical distribution and can be found in Bolivia (El Beni, La Paz, Pando, Santa Cruz); Brazil (Acre, Amazonas, Mato Grosso, Rondonia); Colombia (Amazonas, Cumdinamarca, Goal); Ecuador (Napo) and Peru (Loreto, Pasco, San Martín) (Camargo & Pedro 2013, Pedro 2014). It is a robust bee, its total length measures approximately 14 mm; the previous wing length is about 9.8 mm and the width about 4.5 mm (Oliveira *et al.* 2013). This species builds perennial and populous nests in tree hollows established in shady areas of floodplain and igapó forests, as well as in anthropized locations (Camargo 1970, Oliveira *et al.* 2013). Moreover, it is a species used in honey production by native meliponicultors of Acre (Magalhães & Venturieri 2010).

However, until now, the trophic niche of this species is little known. The identification of the plants visited by *M. grandis* by pollen analysis is fundamentally important. This method allows to know the preferred food sources and other behavioral aspects of foraging from this group of insects (Marques-Souza *et al.* 2002, Alves *et al.* 2006, Oliveira *et al.* 2009, Vossler 2014). The presente study aimed to identify plants visited by *M. grandis* and indicate the degree of specialization of this bee in the collection of trophic resources.

MATERIAL AND METHODS

The study was conducted between November 2016 to October 2017, in a meliponary set up on a rural property located 2 km from Rio Branco, Acre (9°55'57"S; 67°53'19"W). The property and its surroundings consist of grazing and fragments of native vegetation, being close to urban areas (Figure 1).

To obtain the pollen samples were used three colonies of *M. grandis*, kept in standard boxes, model INPA, at biweekly intervals. Altogether, 26 samplings were performed. Each day sampled, held every two weeks, 10 workers returning from the field were captured randomly using entomological net, always in the morning of the highest pollen collection intensity. The bees were then sacrificed in 70 % alcohol to remove pollen grains trapped in their body.

For each sample a set of three microscopy slides was assembled. The pollen grains were fixed in glacial acetic acid and treated by Erdtman's (1960) acetolysis method, observed and photographed using a Leica DM750 optical microscope coupled with a 3.3-megapixel EC4 high definition color camera. The images were captured at 1,000x magnifications and used for

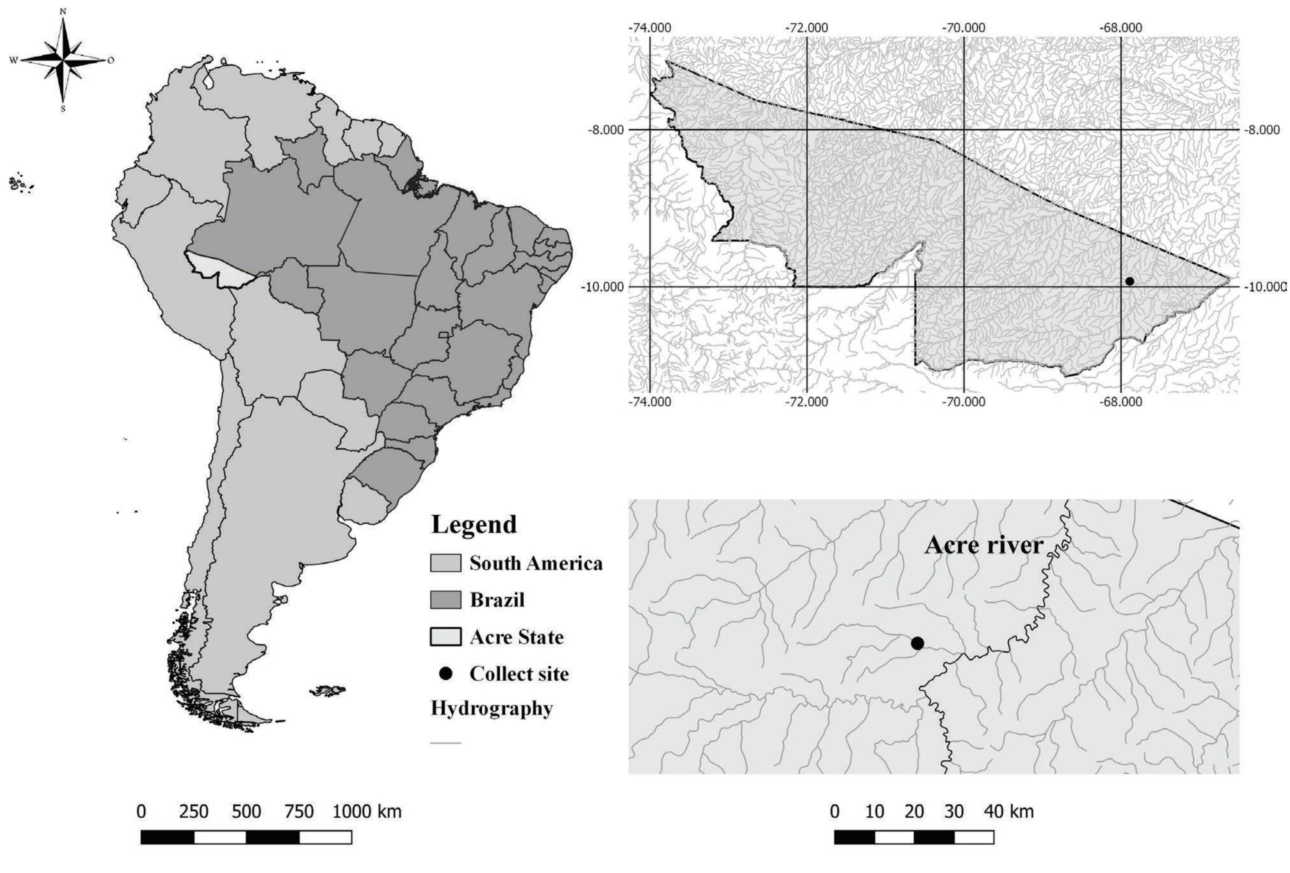

Figure 1. Aerial view indicating the location of the meliponary where the study was developed and its surroundings (Google Earth ™).

characterization of the grains in family, genus or species, according to the “type” methodology described by De Klerk & Joosten (2007). For identification we used a reference palinoteca, built with pollen grains collected from flowering plants, in the study place and its surroundings. After the identifications, it was also verified as to the habit (Arboreal, herbaceous, shrub, creeper or subshrub) of the resource supplying plants.

The frequency of occurrence (FO) of pollen types in the sample set followed the proposed model by Jones & Bryant (1996): rare (< 10 %); uncommon (from 10 to 20 %); frequent (from 21 to 50 %) and very frequent (> 50 %), based on the percentage of occurrence of each pollen type. The trophic niche amplitude (diet amplitude) was estimated using the Ecological Methodology software, version 7.3 (Krebs 2016) according to the Levin’s Standardized Index (B) (Krebs 1999):

$$B = 1/\Sigma pi^2$$

, where, B is the amplitude of the trophic niche of the species; *i* is the category of used resource; *p* is the proportion of category *i* used by the species.

The value of B varies from 1 to *n*, where *n* represents total number of registered resources. The standardization is expressed by the formula:

$$B_{sta} = B\text{-}1/n\text{-}1$$

Niche values close to 1 indicate that the species collects resources in a wide range of plant species. Values close to zero indicate preference for few plant species and zero, the use of a single species.

RESULTS

The comparison between pollen grains collected from the bee body and the reference palinoteca, it was possible to identify 41 pollen types distributed in 20 families and 34 genera. Of this quantity, 16 were identified at species level (Figures 2, 3, 4 and 5).

Fabaceae was the family with the highest frequency of occurrence (FO) in the sample (34.15 %), and the genus *Mimosa* presented FO = 12.20 %, being the most representative. All the other taxa had FO < 10 % (Figure 6). Therefore, there were no very frequent (21 to 50 %) or frequent (> 50 %) classifications for pollen types.

Particularly, the most common pollen types were: *Solanum* type in 10 of the 12 months sampled, followed by *Cassia mimosoides*; *Mimosa caesalpiniifolia* and *Miconia* type in nine and *Mimosa pudica* in eight. In addition, the months with the highest pollen types were: July with 17 types, May with 16 and August with 13 (Figure 7).

When the habit of the identified plants was evaluated, it was found that plants of arboreal and shrub size were the most visited by workers of *M. grandis*, being 48.78 % (arboreal) and 34.15 % (shrub) (Figure 8).

Regarding the body pollen profile of *M. grandis* workers, it was found that the degree of specialization of this bee in the use of floral resources is clearly associated with the abundance of pollen sources offered throughout the year. The Levins Index (B) for trophic niche amplitude of the species was 6.975, while the standardized index (B_{sta}) was 0.314, indicating inequality in the use of some sources of floral resources.

DISCUSSION

Among the 20 botanical families identified in the sample set, Fabaceae was the most visited by workers of *M. grandis* (FO = 34.15 %). This family is among the most important sources of nectar and pollen for stingless bees in the Amazon (Oliveira *et al.* 2009, Absy *et al.* 2018, Souza *et al.* 2018). In surveys conducted by Marques-Souza *et al.* (2007) with pollen basket samples of *Scaptotrigona fulvicutis* (Hymenoptera, Apidae) identified 97 pollen types belonging to 36 families, with Fabaceae being the most frequent with 45.1 %; Ferreira (2014) analyzed the trophic niche of the species *M. seminigra* (Hymenoptera, Apidae) and *M. interrupta* (Hymenoptera, Apidae) identifying 67 pollen types distributed in 27 botanical families and Fabaceae was the second most abundant with 33.2 % of the total taxa sampled; Correia (2016) analyzing the pollen niche of the species *M. eburnea* in the same area evaluated in this study, observed that Fabaceae represented 67.2 % of all pollen collected by this bee. These works demonstrate the importance of this family as a supplier of floral resources

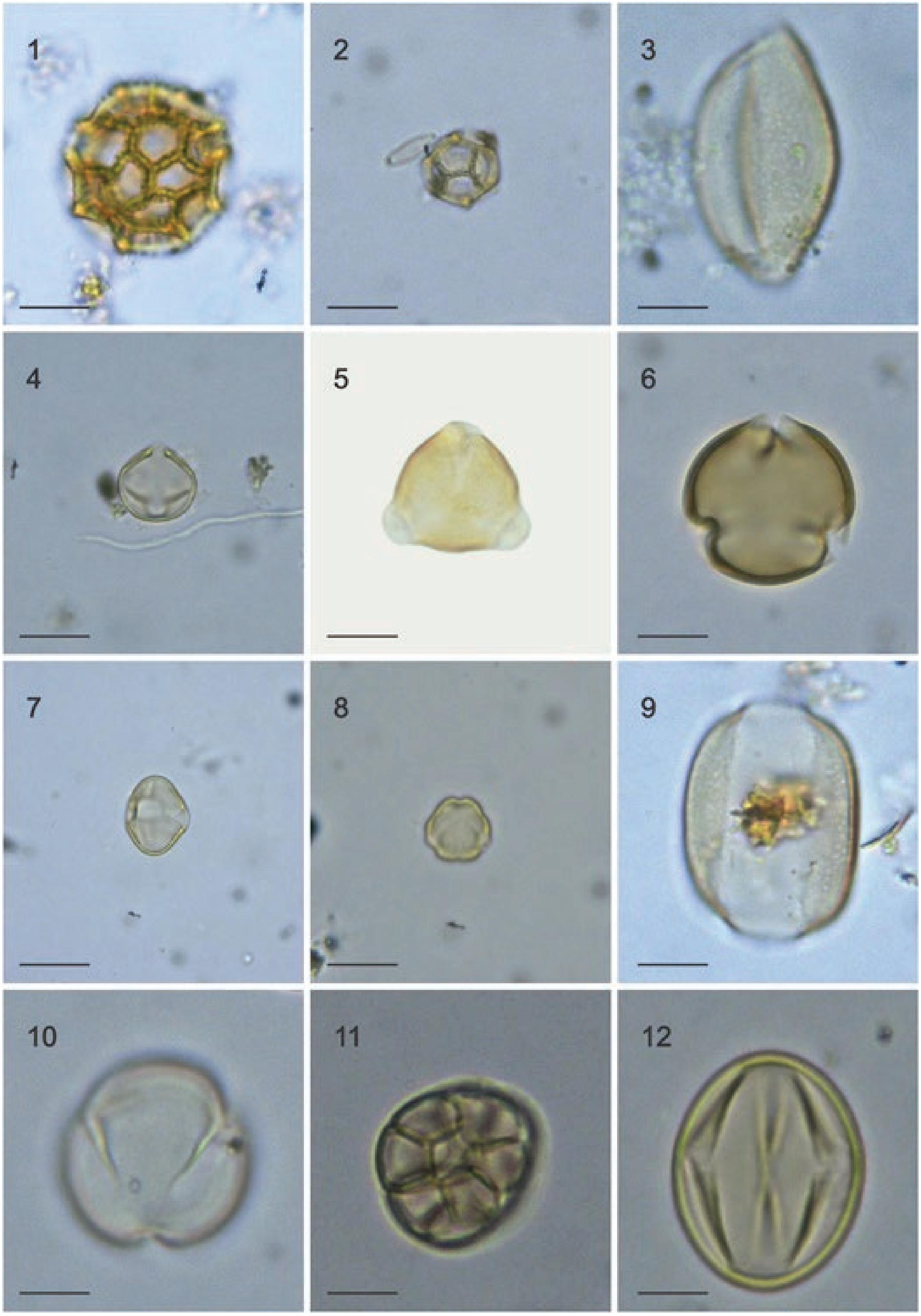

Figure 2. Optical photomicrographs of pollen types collected by *Melipona grandis*: Amaranthaceae –*Gomphrena* (1), *Alternanthera* (2); Amaryllidaceae – *Crinum* (3); Anacardiaceae – *Tapirira guianensis* (4); Arecaceae – *Astrocaryum aculeatum* (5); Boraginaceae – *Cordia* (6); Burseraceae – *Protium* (7); Combretaceae – *Combretum* (8); Cyperaceae – *Scleria* (9); Dilleniaceae – *Doliocarpus* (10); Fabaceae – *Cassia mimosoides* (11), *Cassia* (12). (bars = 20 μm).

Figure 3. Optical photomicrographs of pollen types collected by *Melipona grandis*: Fabaceae – *Cynometra* (13); *Diplotropis* (14); *Hymenaea* (15); *Leucaena* (16); *Machaerium* (17); *Mimosa* (18); *Mimosa caesalpiniifolia* (19); *Mimosa guilandinae* (20); *Mimosa pudica* (21); *Mimosa pigra* (22); *Schizolobium* (23); *Swartzia* (24). (bars = 20 μm).

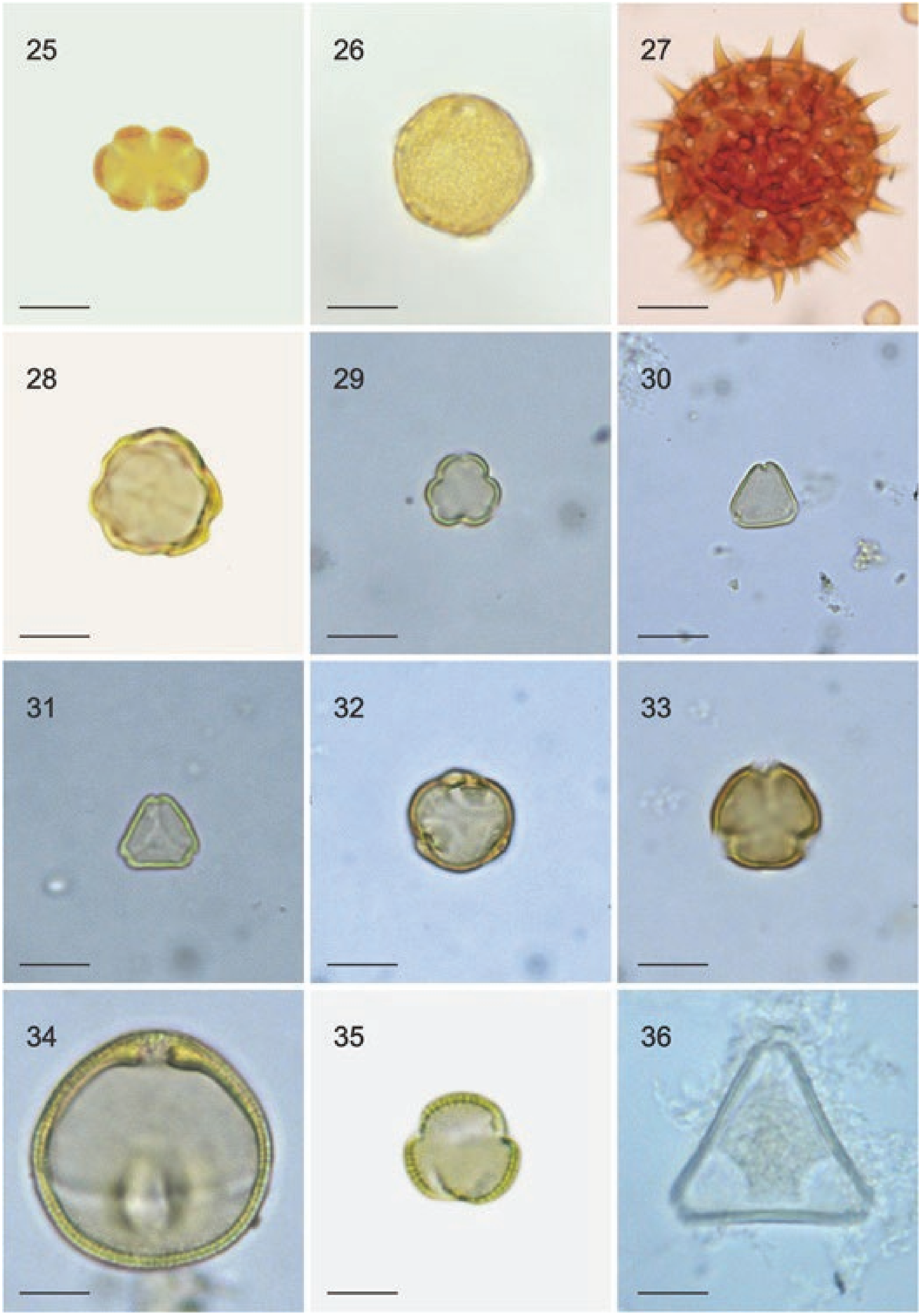

Figure 4. Optical photomicrographs of pollen types collected by *Melipona grandis*: Lamiaceae – *Hyptis* (25); Malpighiaceae – *Malpighia glabra* (26); Malvaceae – *Hybiscus* (27); Melastomataceae – *Bellucia imperialis* (28), *Miconia* (29); Myrtaceae – *Eugenia stipitata* (30), *Syzygium malaccense* (31); Polygonaceae – *Antigonon leptopus* (32), *Triplaris* (33); Rubiaceae – *Isertia* (34); *Warszewiczia coccinea* (35); Sapindaceae – *Paullinia cupana* (36). (bars = 20 μm).

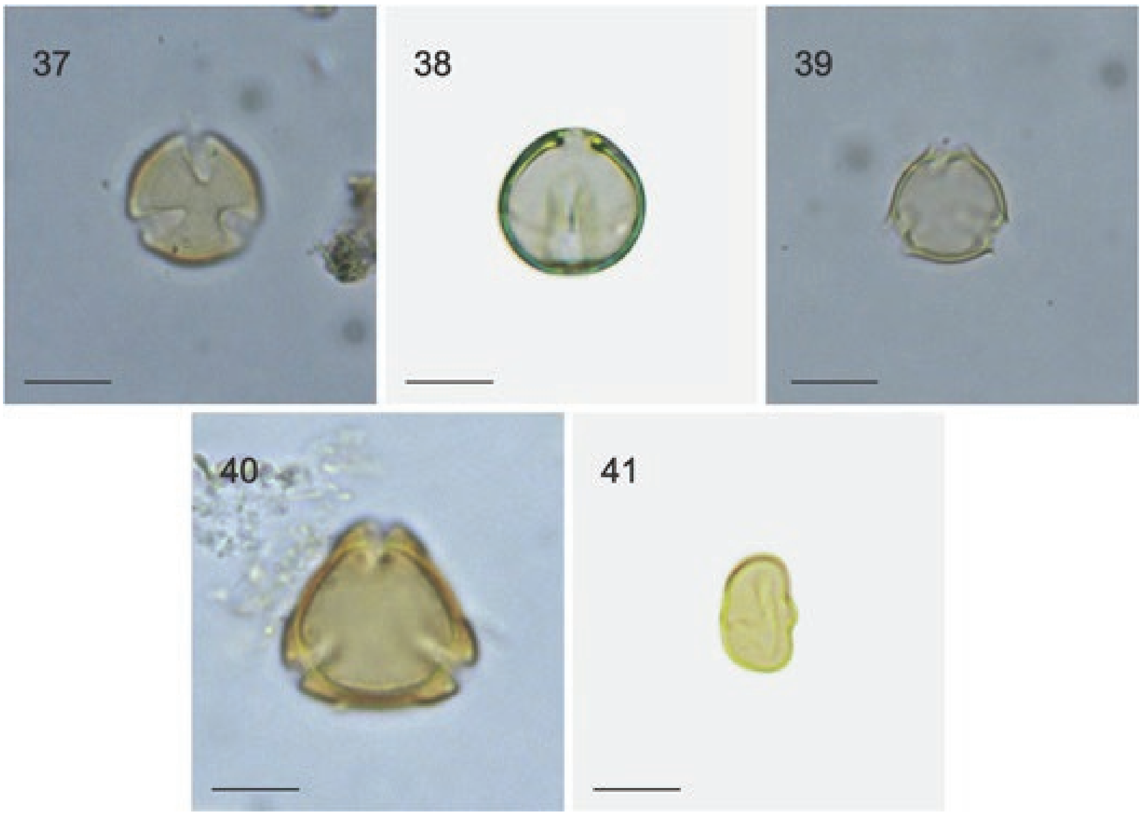

Figure 5. Optical photomicrographs of pollen types collected by *Melipona grandis*: Sapindaceae – *Talisia* (37); Solanaceae – *Solanum stramonifolium* (38), *Solanum rugosum* (39), *Solanum* (40); Urticaceae – *Cecropia* (41). (bars = 20 µm).

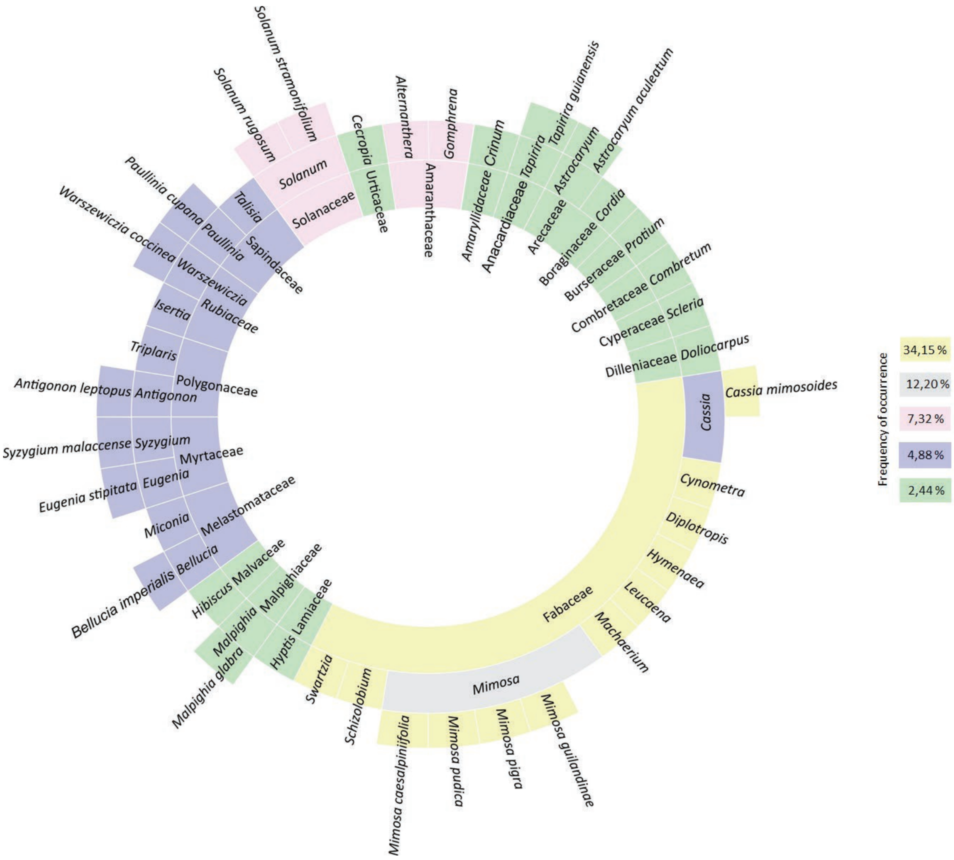

Figure 6. Pollen types present in *Melipona grandis* samples and their respective percentages in the sample set.

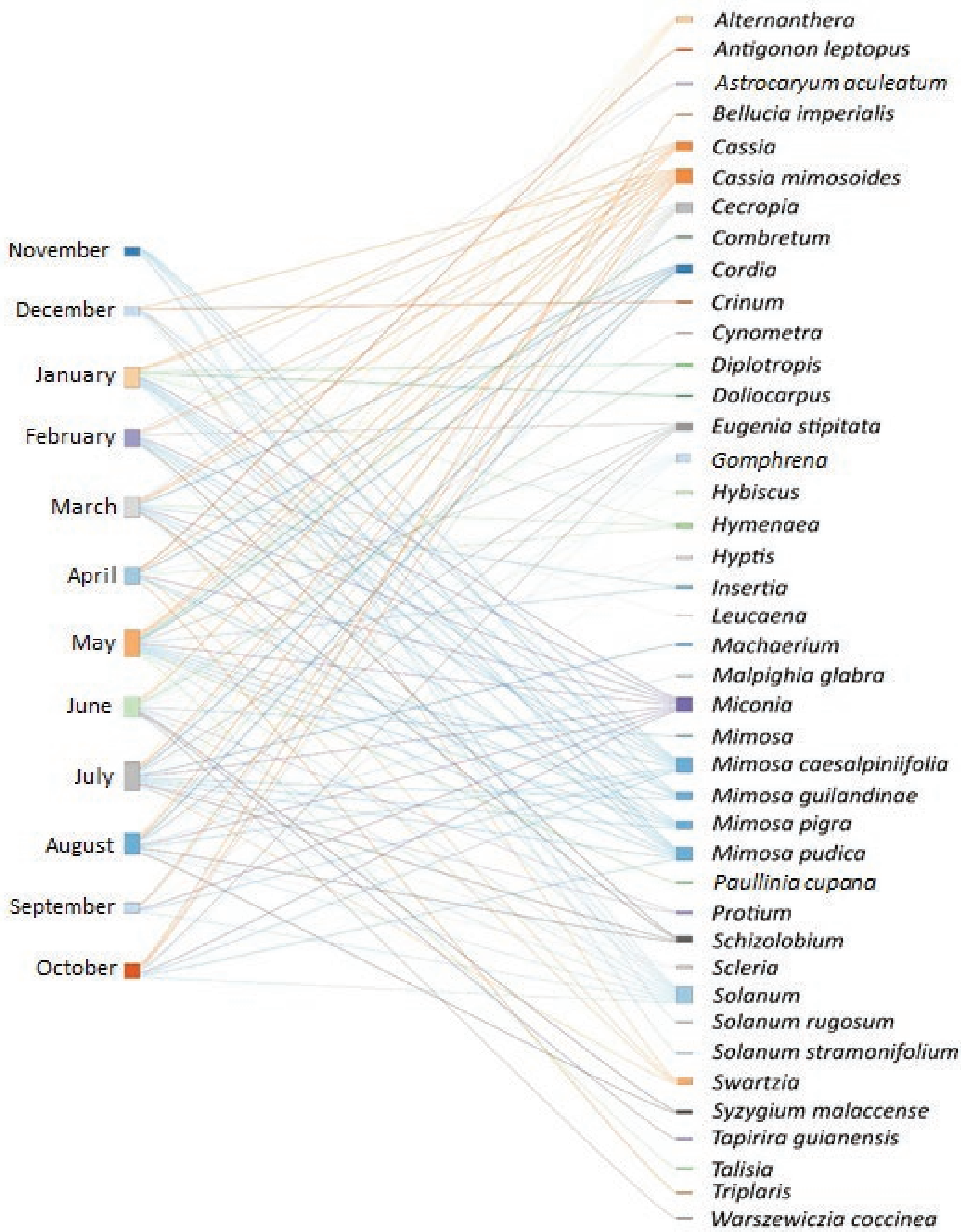

Figure 7. Pollen types found in *Melipona grandis* for each month sample, from November 2016 to October 2017.

for stingless bees, as well as in the formation of meliponícola pastures.

The genus *Mimosa* (Fabaceae) with significant representation in present study has been mentioned as an important supplier of trophic resources for bees (Carvalho *et al.* 2006, Nascimento *et al.* 2009, Ferreira & Absy 2015, Correia 2016), including in Amazon region (Oliveira *et al.* 2009). According to Ramalho *et al.* (1990) and Martins *et al.* (2011), the dominance of the genus *Mimosa* in pollen samples is related to its ability to develop abundantly in various regions, to have mass flowering and flowering periods in succession, and to well adapted in disturbed environments. Here we work with frequency of occurrence and maybe that's why Fabaceae was highlighted with several species, placing it as the most important in this study. Thus, the richness of pollen types of

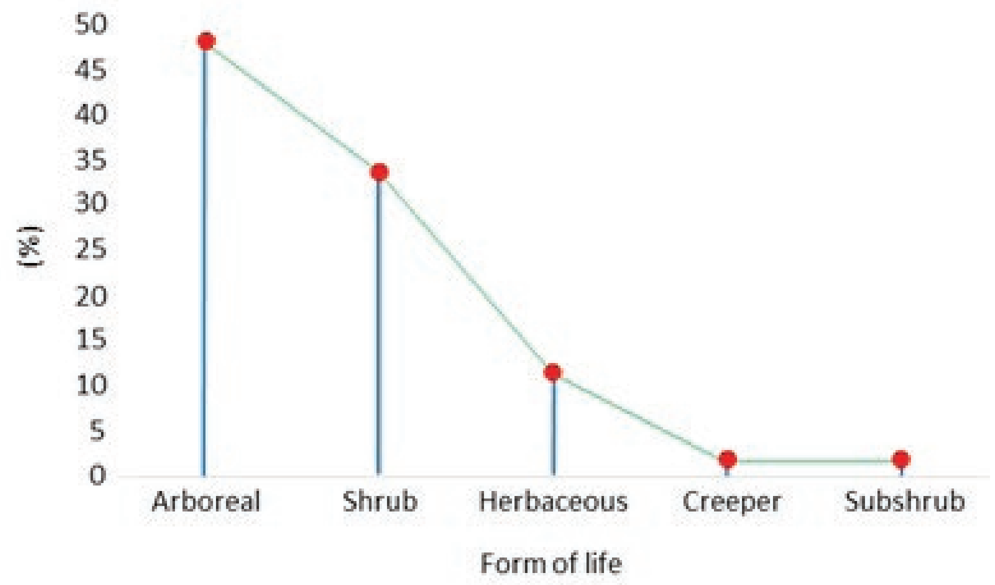

Figure 8. Visitation frequency from pollen identified in *Melipona grandis* body based on the life-form of visited plants.

the Fabaceae family found in the body profile was determinant.

Melipona bees tend to have selectivity regarding the visitation of plants to collect food resources, concentrating on a few botanical species such as a way to maximize foraging efficiency (Absy *et al.* 1984, Antonini *et al.* 2006, Kleinert *et al.* 2009, Fidalgo & Kleinert 2010, Ferreira & Absy 2015, Chidi & Odo 2017) On the other hand a greater variety of plant visitation may be related to factors outside the colony, the main one being rainfall (Correia *et al.* 2018). This statement corroborates the observations made in present work, given that the months with the greatest variety of pollen types coincided with the decrease of the rainfall in the Amazon, which generally varies from May to September (Fisch *et al.* 1998). Similar results were also described by Absy *et al.* (1984), Marques-Souza (1996), Correia *et al.* (2018).

Although the results show that *M. grandis* prefers some plant species to collect floral resources, Ramalho *et al.* (1985), Velthus (1992), Modro *et al.* (2011) report that pollen types with (FO) below < 10 % can be considered secondary resources, being used as a food supplement when there are fewer species in flowering. This way *M. grandis* would increase or decrease its trophic niche according to the flowering intensity.

As for *M. grandis* preference in collecting tree and shrub resources, it happens because bees tend to forage on plants that offer large amounts of flowers, allowing workers to visit as many anthers on the same trip (Vossler 2014, Rech & Absy 2011 Absy *et al.*, 2018). Also, a tree with massive flowering increase the attractiveness for bees (Brito et al. 2015). Moreover, the close relationship between stingless bees and mass flowering plants seems to be related to the abundance of trees in neotropical areas (Ramalho 2004).

The observation of the broad floral spectrum visited by *M. grandis* characterizes this species as polyletic, although with a strong preference for plants grouped in Fabaceae. However, palynological studies by Biesmeijer & Slaa (2006), Hilgert-Moreira *et al.* (2013), Vossler (2014) demonstrate that the habit of foraging in a large number of plants concentrating on some is characteristic of several species of stingless bees, and results from the association of several factors, among them the quality resources offered, high energy expenditure for long-distance travel, floral constancy and information exchange among workers about the location of food sources (Roubik 1989, Ramalho *et al.* 2007).

Although *M. grandis* showed significant diversity in the trophic resources collection throughout the year, the methodology employed in the study did not make it possible to determine supplier plants and pollen and / or nectar. On the other hand, it was possible to demonstrate potential food sources for this bee species.

Studies like this have contributed significantly to the development of meliponiculture in Brazil. However, in Acre, there are few palynological studies focused on the knowledge of meliponicola pastures for species of stingless bees of the genus Melipona created in the region.

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