

Research Article

Pollen Sources for *Melipona capixaba* Moure & Camargo: An Endangered Brazilian Stingless Bee

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Received 20 February 2011; Revised 13 April 2011; Accepted 27 April 2011

Academic Editor: James Charles Nieh

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Pollen samples were collected in three different periods from 11 *Melipona capixaba* Moure & Camargo hives and analyzed with melissopalynological methodology. A total of 33 pollen types were identified, of which 23 genera and 15 families were identified. The following families showed the highest pollen richness: Fabaceae (7), Myrtaceae (3), Solanaceae (3), Arecaceae (2), Asteraceae (2), Euphorbiaceae (2), Melastomataceae/Combretaceae (2), Rubiaceae (2), and Sapindaceae (2). The most frequent pollen types (>45%) were *Eucalyptus*, which generated great similarities between the samples, except one in which the *Tibouchina* was predominant. Although the majority of the pollen types showed low percentage values, the results demonstrated that *M. capixaba* has taken advantage of the polliniferous sources available in the Atlantic Rainforest as well as in the “Capoeira” (brushwood, secondary forest) and “ruderal” (field) plants, probably implying its importance as a pollinator of the native flora and of the exotic species.

1. Introduction

Melipona Illger constitutes the genus of Meliponini tribe with the biggest amount of species. It occurs in the whole neotropical region, which is the most diversified in the Amazon basin [1]. *M. capixaba* (popularly known as uruçu-preta or uruçu-capixaba) is endemic to the Atlantic Rainforest where it is restricted to the mountainous area of Espírito Santo State in the municipalities of Domingos Martins, Conceição do Castelo, Venda Nova do Imigrante, Alfredo Chaves, and Afonso Cláudio embracing Pedra Azul State Park, which is protected by the government. *M. capixaba* was described by Moure and Camargo [2] who referred it to the Meliponini group of the Amazon region and designated by Rocha and Pompolo [3] to the same karyotype group of *M. scutellaris* Latreille. Experiments proved that the lack of anatomical or behavioural isolation mechanisms allowed the crossing of these species when they were brought into the same area. These observations suggest that the two species are capable of forming fertile hybrids [4]. The fact that two

ecologically different species of stingless bees, separated by more than 300 km, could still cross when placed in the same area suggests that there has not been any pressure to develop reproductive isolation [4]. However, *M. capixaba* is mentioned on the list of Endangered Brazilian species (Normative Instructions no. 3, May 27th 2003, Ministry of Environment) because its original habitat has been almost completely fragmented to make room to pastureland, coffee (*Coffea* sp), and *Eucalyptus* sp cultivars. Currently only 10% of the original Atlantic Rainforest remains and has become a “hot spot” for this bee [4, 5].

Despite its endangered status and ecological importance as a pollinator, few studies have examined the ecology and biology of *M. capixaba*. Pollen analysis may be useful to indirectly determine its food sources and help clarify its role as a vegetation pollinator [6]. Knowledge of the several pollen sources that are used by *M. capixaba* in its natural environment helps the beekeepers to manage them. Likewise, complementation of the ecological data,

TABLE 1: Data regarding origin and date of collection of the pollen samples stored in food pots of *Melipona capixaba*.

Samples	Origin (Latitude–Longitude)	Date of collection
FC0	Venda Nova do Imigrante municipality (S20° 18' 57.6"–W41° 07' 55.2")	october 2007
JV1	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	may 2008
JV2	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	may 2008
JV3	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	may 2008
EM4	Domingos Martins municipality (S20° 27' 22.8"–W41° 00' 27.8")	may 2008
JOV5	Alfredo Chaves municipality (S20° 32' 56.4"–W40° 48' 02.8")	may 2008
JOV6	Alfredo Chaves municipality (S20° 32' 56.4"–W40° 48' 02.8")	may 2008
JOV7	Alfredo Chaves municipality (S20° 32' 56.4"–W40° 48' 02.8")	may 2008
FC8	Venda Nova do Imigrante municipality (S20° 18' 57.6"–W41° 07' 55.2")	may 2008
JV9	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	march 2009
JV10	Domingos Martins municipality (S20° 14' 35.3"–W40° 54' 58.1")	march 2009

obtained through melissopalynological analysis, is important for the development of the preservation programs for this bee. This may help in directing the efforts to recover the vegetation in the affected areas utilizing botanic species that guarantee its food supply. There is only one publication in the scientific literature that reports a case of the workers of *M. capixaba* carrying *pollinarium* attached to the *scutellum*, of the orchid subtribe Maxillariinae species, possibly of the genus *Maxillaria sensu lato* or *Xylobium* [7]. However, food storage in the beewax pots of *M. capixaba* has not yet been palynologically analyzed. The aim of this study was to investigate the influence of the local flora on the pollen harvest by *M. capixaba* to characterize the vegetation in which the sources were obtained in order to help in the conservation efforts of this bee.

2. Materials and Methods

Pollen samples were collected from 11 hives of *Melipona capixaba* found inside the tree-trunks and maintained by beekeepers of three different regions. The pollen samples were collected in different periods (October 2007, May 2008, and March 2009) directly from their food storage in the beewax pollen pots. Six samples originated from Domingos Martins municipality were defined as JV1, JV2, JV3, EM4, JV9, and JV10; three from Alfredo Chaves (JOV5, JOV6, and JOV7), and two from Venda Nova do Imigrante (FC0, FC8), all of them from Espírito Santo State, Brazil (Figure 1, Table 1). The meliponary located in Venda Nova do Imigrante municipality (FC) stays near

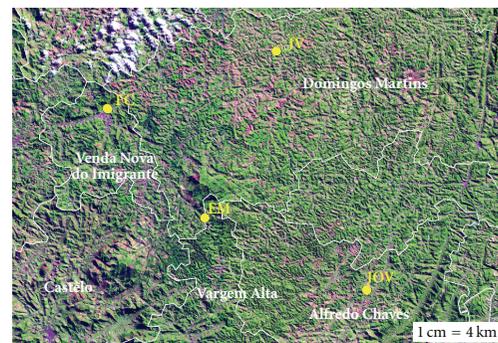
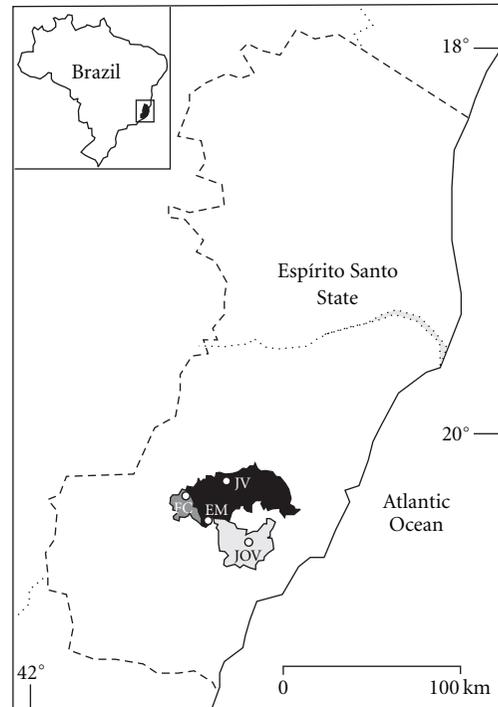


FIGURE 1: Location of the municipalities where the pollen samples of the food pots of *M. capixaba* were collected. Black = Domingos Martins municipality; dark grey = Venda Nova do Imigrante municipality, and light grey = Alfredo Chaves municipality.

to the urban area, being a small farm where they raise chicken and cultivate orchids, besides raising stingless bees. The bees collect floral resources in small forest fragments nearby, and there are some *Eucalyptus* cultivars a few meters far from the meliponary. The meliponary from Alfredo Chaves municipality (JOV) is located within a Particular Reserve of the Natural Patrimony (a private land), an area with secondary forest that has been acquired by the land owner for ecological tourism. There are a lot of coffee and *Eucalyptus* cultivars in the region. The meliponary located north Domingos Martins municipality (JV) is inside a small farm near small forest fragments that are permanent forest reserves inside private properties in the neighborhood. There are fruit, vegetable, and *Eucalyptus* cultivars. The meliponary south Domingos Martins municipality (EM) is inside a small farm near great areas of primary or secondary native forests in high stages of succession, within a permanent forest reserve in “Hotel Monte Verde”, occupying an area of about

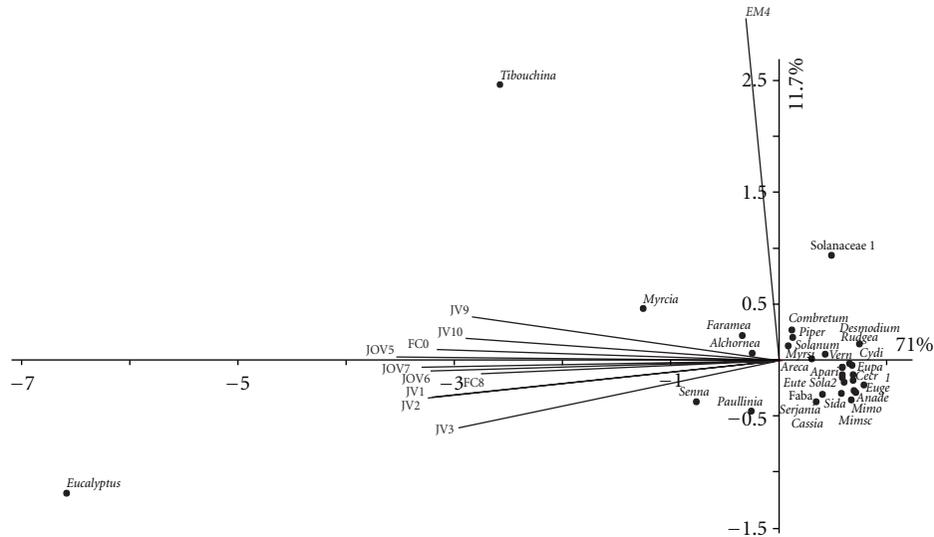


FIGURE 2: Analysis of the main components (PCA) of pollen samples, using the absolute value variables per sample. Pollen samples = FC0, JV1, JV2, JV3, EM4, JOV5, JOV6, JOV7, FC8, JV9, JV10. • = Pollen types.

3,000 hectares, and that uses the place for ecological tourism. Besides that, this meliponary is close to two State Parks, State Park of Forno Grande and State Park of Pedra Azul. There are many family farms in the region that cultivate flowers, fruits, and vegetables, besides agro-ecotourism, without *Eucalyptus* cultivars.

All the sediments of one pollen pot were analyzed as a single samples of one hive. The samples were acetolysed [8]. Microscope slides were prepared using jelly glycerine and sealed with paraffin. All samples were observed under traditional light microscopy. Pollen identification was done using literature data [9, 10] and the reference pollen slide collection of the Palynology Research Center, Instituto de Botânica, from the Environment Department of São Paulo State. A total of 500 pollen grains of each sample were counted for the frequency calculations, as shown in Table 2. Percentage values above 2% of the total count are specified. Interpretation of the data takes in account all the pollen grains of plant taxa. The term “pollen type” means a single plant species or a group of species, or higher taxa, presenting similar pollen morphology. “Monofloral” means originating mostly from a unique plant species (pollen type with >90% of the total) [11].

The multivariate analysis was performed through Principal Component Analysis (PCA) in order to verify the pollen grain occurrence in the samples. The matrix comprised the absolute value of all taxa found in each sample. The absolute numerical variables were transformed into natural logarithms $[\log(x+1)]$ using the FITOPAC program [12] and the ordination was done through covariance matrix using PC-ORD 4.0 [13].

3. Results

Thirty-three pollen types were identified with regards to family, genus, and species taxa (Table 2). The families that

showed the highest richness of pollen types were Fabaceae (7), Myrtaceae (3), Solanaceae (3), Arecaceae (2), Asteraceae (2), Euphorbiaceae (2), Melastomataceae/Combretaceae (2), Rubiaceae (2), and Sapindaceae (2). The variability between the samples of pollen comprised 82.7% on the two first axis in the Principal Component Analysis (PCA) (Figure 2). Axis 1 alone comprised 71.0%. Regarding correlation among the pollen types, the pollen samples showed great similarity related to the high occurrence of the *Eucalyptus* (Figure 3(e)), except for the EM4 sample, from Domingos Martins, in which it was absent. Further *Tibouchina* (Figure 3(d)) was common in the samples, absent only in the JV3 sample, but predominant in the EM4 sample (Table 2). *Myrcia* (Figure 3(f)), even though is also a main characteristic component, occurred in small percentages in all samples. *Paullinia* (Figure 3(h)) was observed in most samples, however in small quantities. *Senna* (Figure 3(b)) occurred in 5 samples with varying percentages. Other pollen types that were expressive, although present in few samples, were *Alchornea* (Figure 3(a)), *Combretum* (Figure 3(c)), *Euterpe/Syagrus*, *Faramaea*, *Piper* (Figure 3(g)), and *Solanaceae* type 2 (Figure 3(i)) (Table 2).

4. Discussion

The species found in the State Park of Pedra Azul forest [14] include *Alchornea triplinervia*, *Andira* sp, *Annona* sp, *Astronium graveolens*, *Cariniana estrellensis*, *Carpotroche brasiliensis*, *Cedrela* sp, *Didymopanax morototoni*, *Erythroxylum subsessilis*, *Euterpe edulis*, *Faramaea* sp, *Fuchsia regia*, *Geonoma schottiana*, *Guapira opposita*, *Melanoxylon* sp, *Miconia inaequidens*, *M. latecrenata*, *Myrsine coriacea*, *M. parvifolia*, *M. umbellata*, *Nectandra* sp, *Ocotea* sp, *Rollinia* sp, *Senna* sp, *Serjania* sp, *Schizolobium* sp, *Solanum* sp, *Solanum capsicoides*, *Sorocea ilicifolia*, *Tabebuia* sp, *Tibouchina* sp, and several species of the Myrtaceae family (mainly *Myrcia*

TABLE 2: Pollen types observed in the pollen sediment storage in the beewax pollen pots of *Melipona capixaba*.

Samples	Total of pollen types	Main pollen types	Pollen types with minor importance (<5%)	Classification of the sample
FC0	11	<i>Eucalyptus</i> (85.3%) and <i>Tibouchina</i> (9.4%)	Arecaceae type 1, <i>Faramea</i> , <i>Myrcia</i> , <i>Myrsine</i> , <i>Senna</i> , <i>Serjania</i> , <i>Solanum</i> , Solanaceae type 1 and Solanaceae type 2	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Tibouchina</i>
JV1	6	<i>Eucalyptus</i> (96.1%)	Fabaceae type 1, <i>Faramea</i> , <i>Tibouchina</i> , <i>Myrcia</i> and <i>Paullinia</i>	Monofloral
JV2	9	<i>Eucalyptus</i> (95.7%)	<i>Alchornea</i> , <i>Faramea</i> , <i>Tibouchina</i> , <i>Mimosa</i> , <i>Myrcia</i> , <i>Myrsine</i> <i>Paullinia</i> and <i>Sida</i>	Monofloral
JV3	6	<i>Eucalyptus</i> (96.6%)	<i>Faramea</i> , <i>Mimosa scabrella</i> , <i>Myrcia</i> , <i>Paullinia</i> and not identified type 1	Monofloral
EM4	8	<i>Tibouchina</i> (87.8%) and Solanaceae type 2 (8.4%)	<i>Eupatorium</i> , <i>Faramea</i> , <i>Myrcia</i> , <i>Paullinia</i> , <i>Desmodium</i> and <i>Vernonia</i>	Heterofloral with great contribution of <i>Tibouchina</i> and Solanaceae
JOV5	5	<i>Eucalyptus</i> (87.9%) and <i>Tibouchina</i> (10.3%)	Arecaceae type 1, Fabaceae type 1 and <i>Myrcia</i>	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Tibouchina</i>
JOV6	8	<i>Eucalyptus</i> (87.2%) and <i>Senna</i> (8.9%)	<i>Alchornea</i> , <i>Crotalaria</i> , <i>Tibouchina</i> , <i>Myrcia</i> , <i>Paullinia</i> and <i>Vernonia</i>	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Senna</i>
JOV7	8	<i>Eucalyptus</i> (91.4%)	<i>Alchornea</i> , <i>Cassia</i> , <i>Tibouchina</i> , <i>Paullinia</i> , <i>Myrcia</i> , Fabaceae type 1, and not identified type 2	Monofloral
FC8	13	<i>Eucalyptus</i> (75.4%), <i>Senna</i> (11.9%) and <i>Myrcia</i> (6.6%)	<i>Euterpe/Syagrus</i> (2.4%), <i>Eupatorium</i> , <i>Paullinia</i> , <i>Tibouchina</i> , <i>Combretum</i> , <i>Anadenanthera</i> , <i>Alchornea</i> , <i>Solanum</i> , <i>Piper</i> and not identified type 3	Heterofloral with great contribution of <i>Eucalyptus</i> , <i>Senna</i> and <i>Myrcia</i>
JV9	16	<i>Eucalyptus</i> (53.4%) and <i>Tibouchina</i> (28.6%)	<i>Piper</i> (4.7%), <i>Alchornea</i> (3.1%), <i>Myrcia</i> (2.8%), <i>Paullinia</i> , <i>Aparisthmium</i> , <i>Faramea</i> , <i>Rudgea jasminoides</i> , <i>Sida</i> , <i>Myrsine</i> , <i>Senna</i> , <i>Cecropia</i> , <i>Solanum</i> , <i>Combretum</i> and <i>Cydista heterophylla</i>	Heterofloral with great contribution of <i>Eucalyptus</i> and <i>Tibouchina</i>
JV10	14	<i>Eucalyptus</i> (59.5%), <i>Tibouchina</i> (13.9%), <i>Combretum</i> (8.2%) and <i>Myrcia</i> (6.5%)	<i>Senna</i> (3.0%), <i>Alchornea</i> (2.1%), <i>Faramea</i> (2.1%), <i>Aparisthmium</i> , <i>Piper</i> , <i>Cydista heterophylla</i> , <i>Euterpe/Syagrus</i> , <i>Solanum</i> , <i>Myrsine</i> and <i>Rudgea jasminoides</i>	Heterofloral with great contribution of <i>Eucalyptus</i> , <i>Tibouchina</i> , <i>Combretum</i> and <i>Myrcia</i>

and *Eugenia* genera), among others. In the forest outskirts one can frequently observe *Guapira opposita*, *M. latecrenata*, *Erythroxylum ovalifolium*, *Psychotria hancornifolia*, and *M. coriacea*. The areas of rocky outcrops present a singular herbaceous-shrubby stratum with *Aechmea* sp, *Alcantarea imperialis*, *Baccharis* sp, *Epidendrum denticulatum*, *Fuchsia regia*, *Leandra* sp, *Melinis minutiflora*, *M. inaequidens*, *M. latecrenata*, *Poliavana* sp, *Polypodium* sp, *Pseudolaelia vellozicola*, *Tibouchina* sp, *Vellozia* sp, *Vernonia* sp, and *Vriesea carinata*. The glades are characterized by *Cecropia* sp, *Mimosa* sp, *Solanum* sp, and *Solanum capsicoides*, with significant populations of the pteridophyta *Pteridium aquilinum* and *Melinis minutiflora* grass. In the urban gardens are found bromeliads and orchards (with guava, lime, orange, etc.). Pasture lands are dominated by *Baccharis dracunculifolia*, *Bidens pilosa*, *Borreria verticilata*, *Chamaesyce prostrata*, *Chamaecrista* sp, *Crotalaria claussemi*, *Emilia sonchifolia*, *Mellines minutiflora*, *Panicum maximum*, and *Sida* sp, among others. Based on this, it is confirmed that *M. capixaba* harvested the polliniferous resources from natives trees,

shrubs, and vines (*Alchornea*, Arecaceae, *Cassia*, *Combretum*, *Eugenia*, *Euterpe/Syagrus*, *Faramea*, *Myrcia*, *Myrsine*, *Paullinia*, *Senna*, *Serjania*, several Solanaceae, and *Tibouchina*), as well as pollen types from native trees not present in the referred list (*Anadenanthera*, *Aparisthmium*, and *Cydista heterophylla*). The altered vegetation “Capoeira” (brushwood, secondary forest) and ruderal (field) plants were represented by the pollen types *Cecropia*, *Combretum*, *Crotalaria*, *Desmodium*, *Eupatorium*, *Mimosa scabrella*, *Piper*, *Sida*, several Solanaceae, *Tibouchina* and *Vernonia*, apart from the exotic species of *Eucalyptus*, and *Rudgea jasminoides*. In spite of the high richness of pollen types, the results demonstrated a similarity between the hives regarding the preferences of pollen of *Eucalyptus*, a widely cultivated tree in the region, and, with less intensity, pollen of *Tibouchina*, a common plant in the native forest. The EM4 sample from the meliponary south Domingos Martins was the only one that did not show *Eucalyptus* pollen grains, because it is located in one of the most well-preserved areas of the region, without cultivars of this plant in the surroundings. The other areas

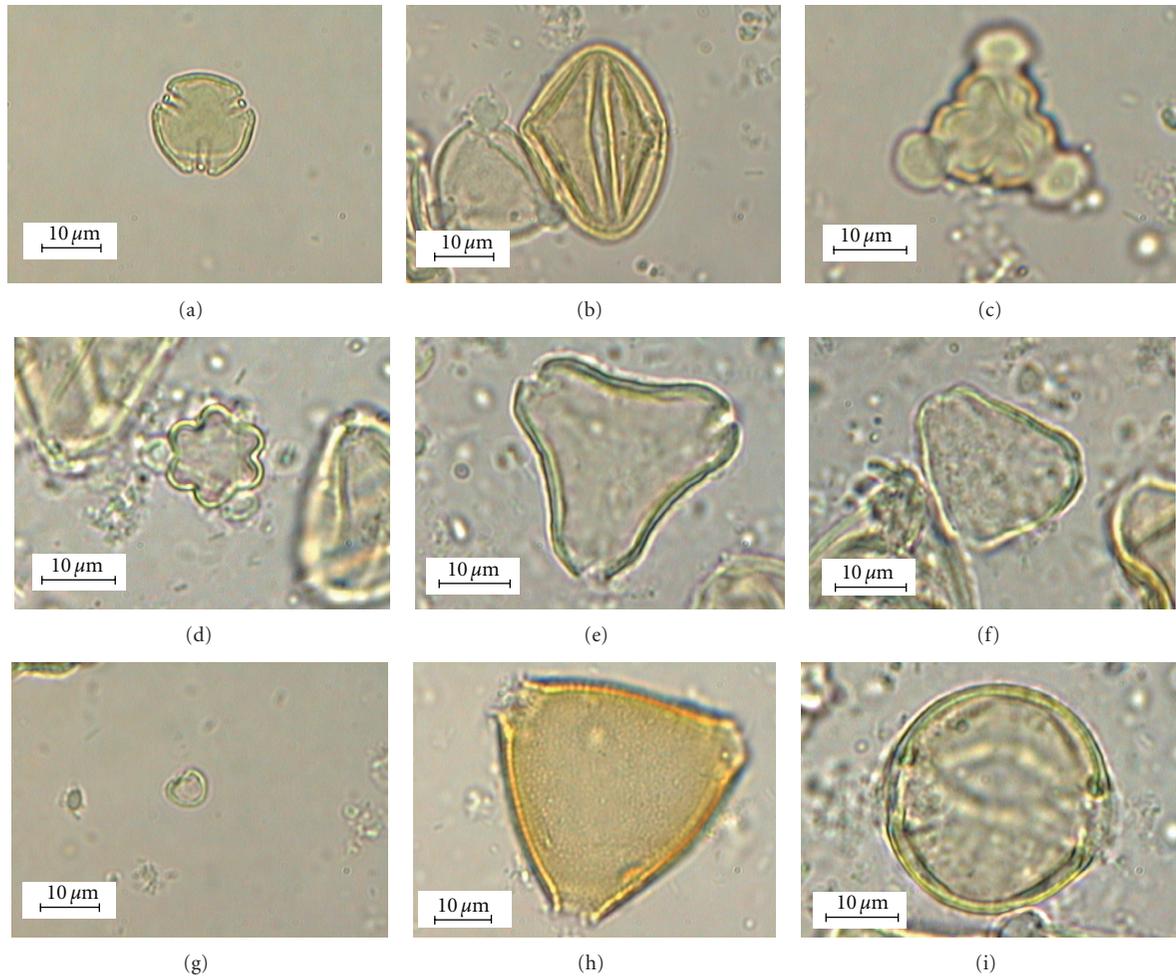


FIGURE 3: Most frequent pollen types observed in pollen samples from *Melipona capixaba* food pots. (a) Euphorbiaceae, *Alchornea*, polar view; (b) Fabaceae, *Senna*, equatorial view; (c) Melastomataceae, *Combretum*, polar view; (d) Melastomataceae, *Tibouchina*, polar view; (e) Myrtaceae, *Eucalyptus*, polar view, optical section; (f) Myrtaceae, *Myrcia*, polar view, optical section; (g) Piperaceae, *Piper*; (h) Sapindaceae, *Paullinia*, polar view, surface; (i) Solanaceae, type 2, equatorial view, optical section; Bar = 10 μ .

of the meliponaries (JOV, JV, and FC) are more deforested and replaced by pastures, and are near *Eucalyptus* cultivars. The palynological results from this sample showed that in the presence of native floral resources *M. capixaba* efficiently visits and harvest those resources, indicating their original pollen sources.

Studies based on the visitation of the pollinators of the flowers of the Atlantic Rainforest concluded that stingless bees are not specialized visitors in certain plant species. Only 7% of the plants in the Atlantic Rainforest are intensely visited by native bees; 77% of the plants are visited with less frequency, and 16% are not visited at all [15]. Analysis of palynological results in Brazil point to a great variety of trophic resources and generalized habits of pollen harvesting by the Meliponini that commonly visit a larger number of plant species [16]. However, regional studies show that the Meliponini frequently concentrate the pollen harvest to a few floral sources [17, 18]. Several studies under natural conditions in the Tropical Atlantic Domain concluded that colonies of different species of *Melipona* frequently searched floral sources of the trees of families Melastomataceae,

Mimosaceae, Myrtaceae, and Solanaceae, apart from the genus *Cassia* of family Caesalpiniaceae [17, 19–21]. The present study corroborates in great part this tendency as the families Melastomataceae and Myrtaceae were the most procured families by *M. capixaba* for pollen harvest while families Mimosaceae, Solanaceae, and plants of the genus *Cassia* (or *Senna*), although utilized, were visited to a significantly lesser extent.

Selectivity or floral preferences is a behaviour that is frequently observed among *M. scutellaris* [22]. Field research in the northeast Brazil confirmed that it prefers trees of the Atlantic Rainforest, as well as “Capoeira” vegetation, to herbs, and that it is rather selective with reference to the food source [23]. Palynological studies on pollen pellets of *M. scutellaris* in forest areas showed that the floral source preferences depended on the plant species, demonstrating harvesting selectivity as follows, with decreasing order of importance: Myrtaceae, Mimosaceae, Anacardiaceae, Sapindaceae, Caesalpiniaceae, and Fabaceae [6]. In our analyses, the selectivity also occurred in relation to the *M. capixaba* with the Myrtaceae and the Melastomataceae families as the

most popular pollen suppliers. Four pollen samples were monofloral and seven samples were heterofloral with a major percentage contribution of pollen types from these families. During the analyzed periods (March, May, and October) there was a preference of *Eucalyptus* and *Tibouchina*, in spite of other pollen sources in the region. *Combretum* (in one sample), *Myrcia* (two samples), *Senna* (two samples), and Solanaceae (one sample), also contributed with high pollen percentages. Other plants, including herbs, were sporadically visited by *M. capixaba*.

The search for *M. capixaba* in *Eucalyptus* is not a new fact for the Meliponini as the occurrence of its nectar and its pollen has been reported dominant or important in the pollen spectrum of honey and pollen pellets of other species of *Melipona* [18, 19, 21, 24, 25]. However, the almost exclusive usage of only one floral resource by *M. capixaba* can put this species in danger on the absence of this resource, besides the fact that it cannot offer all the nutritional necessities needed by the bees. Other research demonstrated that the pollen nutritional composition harvest by *Apis mellifera* did not show a correlation with the pollen type diversity, but showed a correlation with the predominance of specific pollen types [26, 27]. These authors pointed as well that the different floristic compositions have influenced on pollen pellet quality, and the harvest in different food sources by the bees is important for obtaining a well-balanced diet. The fact that the predominant polliniferous source for *M. capixaba* was *Eucalyptus* demonstrates as well an important economic issue. Nowadays there is some interest in local *Eucalyptus* cultivars, but this is not constant, because it depends on market interest. If in the future *Eucalyptus* cultivars would be replaced by any other reason (environmental or economic importance) and enough native pollen sources to feed the colonies would be absent it would have a significant prejudice for the bees, because *M. capixaba* is restricted to this montane region within the Espírito Santo's Atlantic Forest. Its restriction to this small occurrence area can be evolutionary related to the local biological characteristics, as local native flora. The palynological analysis showed the main pollen sources of native plants for *M. capixaba* and thus provided important information for future researches on the pollination biology, allowing decision making on the creation of sustainable pastures to these bee.

In conclusion, *M. capixaba* took advantage of the polliniferous sources in the forest, especially *Combretum*, *Euterpe/Syagrus*, *Faramea*, *Myrcia*, *Senna*, and several species of Solanaceae and *Tibouchina*, indicating its importance as a pollinator of the native flora, as well as in ruderal plants ("Capoeira"), even though the characteristic pollen types of these environments occurred in low percentages. The main pollen harvest was in *Eucalyptus* cultivars. The mechanisms that increased the frequency of utilization of *Eucalyptus*, even in natural forest areas, still have to be better understood in order to clarify the influence of the nutritional quality of this pollen on its diet. Physiochemical analysis of the pollen from the Myrtaceae family showed a positive and highly significant relation of crude proteins in *Eucalyptus* and, consequently, a complementary negative relation for total carbohydrates [26, 27]. Studies should be undertaken to verify the influence

that a diet almost exclusively consisting of *Eucalyptus* has on *M. capixaba*.

In order to guarantee a varied supply of *M. capixaba* pollen, it is recommended that the beekeepers and others interested in its preservation recover affected areas, reforesting with polliniferous native plants that now are known. The foraging behaviour of the *M. capixaba* explains important ecological consequences in terms of persistence capacity of the species in the environment, as in cases of local extinction of their preferential native floral sources.

Acknowledgments

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship of "Produtividade em Pesquisa" (Process number 301220/2009-3) to the first author and to FAPEMIG for financial support. They are grateful to the beekeepers Ernesto Mazzoco, Fábio Calimã, José Valim, and Jovane R. Carvalho for providing the samples. Thanks are due to Erik Bernhard Lidgren for the translation.

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