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# Pollen analysis of honey and beebread derived from Brazilian mangroves

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**ABSTRACT** – (Pollen analysis of honey and beebread derived from Brazilian mangroves). Pollen analyses were performed on honey and beebread from hives in apiaries located in two distinct mangrove areas dominated by *Laguncularia racemosa* (L.) C.F. Gaernt. One apiary was located at the edge of Guanabara Bay, Rio de Janeiro State, and the other near Maranguá Bay, Bahia State, Brazil. We investigated the contribution of nectar and pollen from mangrove vegetation to *Apis mellifera* L. honey and beebread stocks. Intensive visitation to this plant species by honeybees and the presence of its pollen grains in honey and beebread confirmed the importance of *Laguncularia racemosa* as a polliniferous and nectariferous species.

Key words - apiculture, *Laguncularia racemosa*, melissopalynology

## INTRODUCTION

Countries such as India, the United States, China, and Cuba have long taken advantage of nectariferous and polliniferous mangrove vegetation in the production of honey and beebread by bees of the species *Apis* (Piñeiro 1989, Krishnamurthy 1990, Yao et al. 2006, Singh & Kar 2011). Although mangrove vegetation grows almost continuously along the Brazilian coast from Amapá State in the north to Santa Catarina State in the south (covering a total area of 13,000 km$^2$) (Spalding et al. 2010), its contribution to apicultural activities has not been extensively investigated. Assessing the potential contribution of the mangrove vegetation to apiculture along the Brazilian coast is important as apiculture in native environments is a conservationist activity with many associated benefits. Mangrove apiculture represents a potential alternative economic activity for many populations residing in the coastal regions of Brazil and, if underpinned by competent environmental institutions, could become a promising activity in the conservation of this ecosystem.

Honeybees store flower nectar inside beeswax alveoli (other than those containing beebread). Pollen grains used for beebread are transported by worker bees in pollen pellets/pollen loads attached to pollen baskets (corbicula) on their hindmost pair of legs. Compacting the pollen pellets into the alveoli forms what is known as beebread, which serves as a food source for their broods. Monthly pollen analyses of stored honey and beebread is an indispensable method for identifying the floral sources used by bees, and we sought to obtain data on the representivity of the constituent mangrove plant species in apicultural products. Special attention was given to *Laguncularia racemosa* (L) C.F. Gaertn (white mangrove), which is well represent in the study region.

The white mangrove is a functionally dioecious species, with plants producing only male, or both male and female flowers (Allen 2003). Flowering and fruiting may occur all year long (Jiménez 1985), although flowering is more intensive during rainy periods (Valente et al. 1994, Landry & Rathcke 2007, Menezes et al. 2008). *L. racemosa* has an entomophilous pollination syndrome, and pollination is performed by species of Diptera (Jiménez 1985, Lemus-Jiménez & Ramírez 2003). *Apis mellifera* visits *L. racemosa* flowers but it is not an effective pollinator (Landry & Rathcke 2007) – although *L. racemosa* is a melliferous species, according to CONAFOR (2004).

The pollen morphology of *L. racemosa* has been described in the palynological literature (Barth & Silva 1965) as: monad, isopolar, average sized (25.0-35.4 µm), subprolate, tricolporate, narrow colpus, inconspicuous and lalongate endoapertures, nexine thinner than the sexine, folded microreticulate tectate sexine. The lumina of the reticulum are reduced towards the colpus. Photographs of pollen types of the species using scanning electron and light microscopy were presented by Barth & Luz (1998).

A more complete knowledge of the presence of pollens derived from mangrove, open field, and wooded areas in the food stocks in *Apis mellifera* hives from an apiary located in a coastal region will increase our knowledge of the interactions of bees and nectariferous and polliniferous mangrove floral sources.

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## MATERIAL AND METHODS

### Study site

The Guanabara Bay mangrove swamp covers ca. 60 km$^2$ and is contained within the Guapimirim Environmental Protection Area (EPA). The apiary chosen for this study was located in Barão-do-Iriri (22°40' S and 43°02' W) in the municipality of Magé, Guanabara Bay, Rio de Janeiro State, Brazil. Cattle pastures and mangrove swamps dominate the landscape, but with the presence of cultivated arboreal and shrub fruit species. The climate is hot and humid, with year-round temperatures near 22 °C. There is a rainy season from January to March, and another relatively dry season from July to September (Araújo & Maciel 1979, FEEMA 1979, Amador 1992, Soares et al. 2003).

The apiary chosen in Bahia State was located near Maranguá Bay (13°56' S and 38°55' W) in the municipality of Camamu, which has a mangrove swamp covering ~28 km$^2$ as well as remnant areas of Atlantic Forest, palm oil (*Elaeis guineensis* Jacq) and cashew (*Anacardium occidentale* L) plantations, and extensive areas of secondary forest vegetation (capoeirão). The apiary was installed in the capoeirão region, proximately 350 m from the mangrove. The climate in the region is hot and humid, with average year-round temperatures of ~24 °C, and a rainy season from March to May (Oliveira et al. 1998).

We analyzed samples of honey and beebread stored in the honeycombs of hives in each apiary.

### Sampling Guanabara Bay honey

Samples were collected monthly for one year and were processed the following the European Standard Method, without the application of acetolysis. Ten grams of honey was dissolved in 20 ml of distilled water. The sediment was re-suspended in a 1:1 glycerol:$H_2O$ mixture and subsequently mounted with glycerine-jelly on microscope slides sealed with paraffin. For pollen analyses, 500 pollen grains per sample were counted and the relative frequencies (percentages) of each pollen type were established following Zander (Maurizio & Louveaux 1965). The results were analyzed in terms of pollen type dominance, considering the under- and over-representations in each sample (Barth 1970a, 1970b, 1970c, 1970d, 1989, 1990).

### Sampling Guanabara Bay beebread

Five pollen samples were collected monthly, during one year, from the same hive used for honey analysis. The pollen comb was removed every month and replaced by a new one made of beeswax. Samples were not collected in February and December, due to the lack of significant stocks at those times. The samples were dissolved in 1:1 glycerol:$H_2O$, and the microscope slides sealed with paraffin. For these analyses, 2,000 pollen grains were counted in each sample (making sure to consider the whole slide). The results were analyzed in terms of pollen type dominance, considering the under- and over-representations in each sample.

### Sampling Maranguá Bay honey

Four honey samples were collected during the flowering period of *Laguncularia racemosa*. The methodologies used for sample preparation, counting pollen types, and interpretation of the results followed those previously mentioned for the Guanabara Bay honey sampling. Beebread samples were not collected due to technical problems.

## RESULTS

*Laguncularia racemosa* pollen grains were observed in honey and beebread samples (tables 1 and 2). The main pollen types encountered are shown in figures 1 to 11.

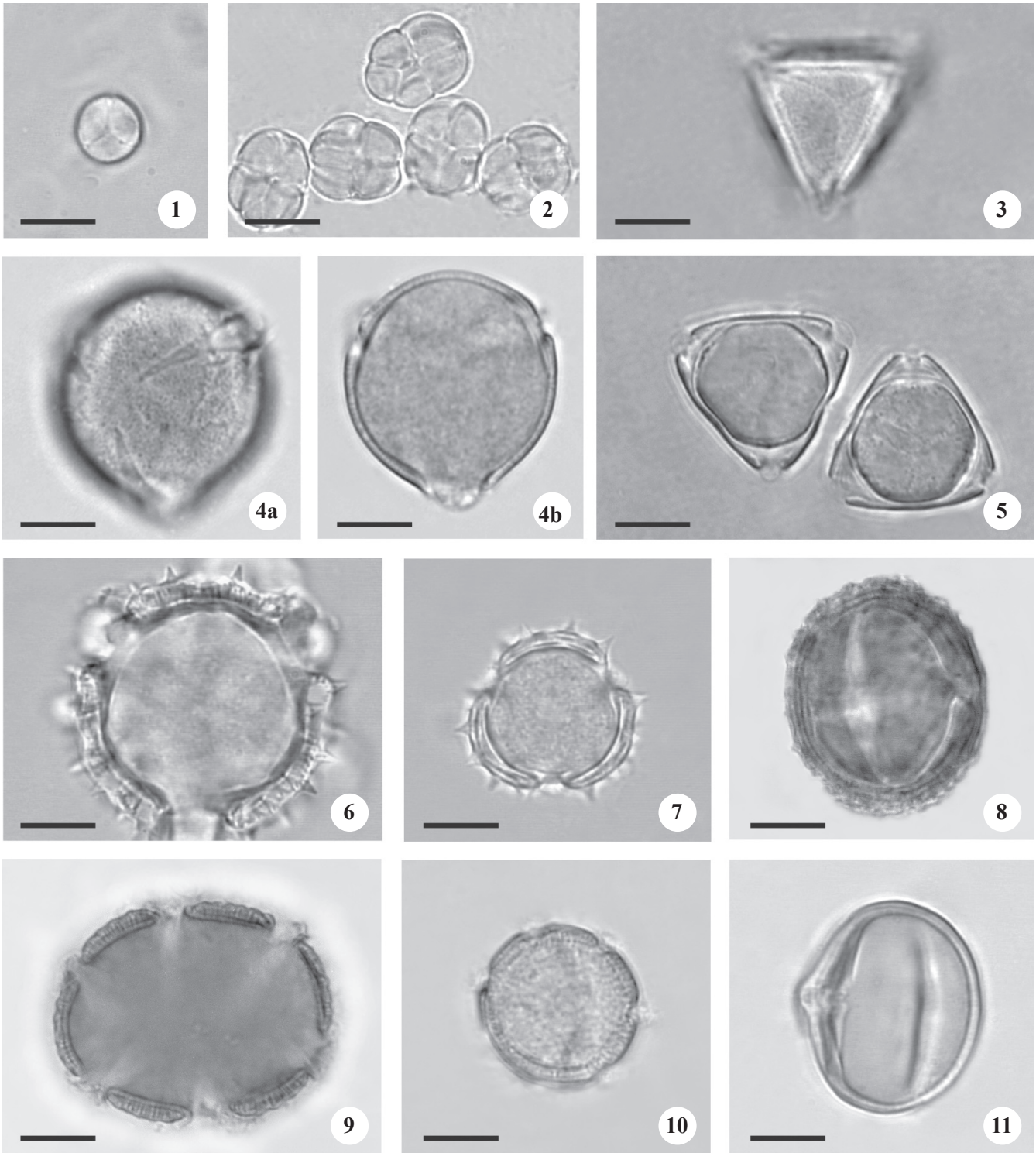
Table 1. Occurrences of pollen class percentages in honey and beebread samples from Guanabara Bay, Rio de Janeiro, Brazil. (D = dominant pollen (> 45%); A = accessory pollen (15%-45%); I = isolated pollen (3%-15%); O = occasional pollen (< 3%); H = honey samples; P = beebread samples).

| Habit | Pollen types | Jan | | Feb | Apr | | May | | Jun | | Jul | | Aug | | Sep | | Oct | | Nov | | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H | P | H | H | P | H | P | H | P | H | P | H | P | H | P | H | P | H | P | H |
| Herbs | ASTERACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Bidens* | | | | | | | | | | | | I | | | | | | | | |
| | *Eupatorium* | | | O | D | A | | | O | I | D | D | D | | O | A | I | | I | | I |
| | *Montanoa* | | | | I | | O | | | | | | O | | I | | I | O | O | | |
| | *Tithonia* | | | | | | | | O | | | | | | | | | | O | | |
| | CONVOLVULACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Merremia* | | | | | | | | O | | | | | | | | | | | | |
| | CYPERACEAE | | | | | | | | | | I | | O | | O | | | | O | O | |
| | POACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Panicum* | O | A | I | O | I | I | I | I | I | I | | I | | I | | O | | I | I | I |

*continue*

*continuation*

| Habit | Pollen types | Jan | | Feb | Apr | | May | | Jun | | Jul | | Aug | | Sep | | Oct | | Nov | | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H | P | H | H | P | H | P | H | P | H | P | H | P | H | P | H | P | H | P | H |
| | LAMIACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Hyptis* | O | | | | | O | | | | | | O | | | | | | | | |
| | MIMOSACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Mimosa pudica* | D | A | D | | | A | | D | | | | I | | I | | | | | | D |
| | FABACEAE | | | O | | | | | | | | | | | | | | | O | | O |
| | *Chamaecrista* | I | | | | | | | | | | | | | | | | | | | |
| | *Crotalaria pallida* | O | O | | | | | | | | | | | | | | | | | | |
| | LYTHRACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Cuphea* | O | | O | | | | | | | | | I | O | | | | | | | |
| | MALVACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Sida* | | | | O | | O | O | | | I | O | O | | | | | | O | | |
| | ONAGRACEAE | | | | | | | | O | | I | I | O | | O | O | | | | | |
| | RUBIACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Borreria* | | | | O | | O | A | A | | | | I | O | O | | O | | | | |
| | TILIACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Triumfetta* | | | | | | O | A | A | | | | O | O | I | O | O | | | | |
| Trees, shrubs and vines | ANACARDIACEAE | | | | | | | | | | | | | | I | | | | | | |
| | *Spondias* | | | | A | A | I | | | | | | | | | | | | | | |
| | BOMBACACEAE | | | | | | | | | | | | | | | | O | O | | | |
| | COMBRETACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Laguncularia racemosa* | | | | O | I | | | | | | | | | | | O | | | | |
| | *Terminalia* | | | O | | | | | | | | | | | O | | I | | | | |
| | ASTERACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Gochnatia* | I | A | A | | | O | | I | | | | D | | | | | | | | A |
| | *Vernonia* | | | | O | I | | | I | | I | A | O | O | I | I | A | A | O | O | |
| | EUPHORBIACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Croton* | O | O | O | | | O | | O | | | | O | | O | | O | | A | O | O |
| | *Ricinus communis* | | | | | | | | | | | | I | | | | D | | | | |
| | CAESALPINIACEAE | | | | | | O | | | | | | | | | | O | | | | |
| | MIMOSACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Albizzia lebbeck* | O | | O | | | | | | | | | | | | | I | I | | | O |
| | *Mimosa bimucronata* | | | | O | | A | | A | | | | | | A | | | | | | |
| | *Piptadenia* | | | | | | | | | | | | | | | | I | O | O | I | |
| | *Schrankia* | | | | O | | O | | | | | | O | | O | | | | | | |
| | MORACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Cecropia* | | | | O | | A | | I | | | | I | | A | A | I | I | I | I | |
| | MYRTACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Eucalyptus* | | | O | I | | I | I | O | | O | | O | I | A | I | I | | O | | |
| | *Eugenia* | | | | O | | | | | | | | I | | | | | | | | |
| | *Jambosa* | | | | I | | | | | | | | | | | | | | | | |
| | *Myrcia* | | | | | | | | O | | | | O | | I | | I | | | | O |
| | ARECACEAE | | | | | | | | | | | | | | | | | | | | |
| | Psilate type | | | | O | | I | | I | | O | O | I | O | | | O | I | O | | O |
| | Verrucate type | | | | | | | | | | | | O | O | I | | I | O | | | |
| | PASSIFLORACEAE | O | | O | | | | | | | | | O | | | | | | | | O |
| | POLYGONACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Coccoloba* | | | | O | | | | | | | | | | | | | | | | |
| | SAPINDACEAE | | | | O | | | | I | | | | | | I | | | | D | D | O |
| | STERCULIACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Dombeya* | | | | | | | | | | | | | | | | | | I | | |
| | ULMACEAE | | | | | | | | | | | | | | | | | | | | |
| | *Trema micranta* | | | | O | | | | | | | | | | | | | | | | I |
| | Not identified | | | | I | | O | A | I | | | | I | I | I | I | A | O | I | | |
| | Total of pollen types | 8 | 7 | 11 | 14 | 10 | 17 | 6 | 8 | 12 | 7 | 6 | 18 | 15 | 14 | 13 | 15 | 14 | 12 | 11 | 12 |




Figures 1-11. Light photomicrographs of dominant or characteristic pollen types from honey and beebread samples collected in mangrove apiaries. 1. *Mimosa pudica* (Fabaceae-Faboideae), tetrad. 2. *Mimosa bimucronata* (Fabaceae-Faboideae), ditetrads. 3. *Cupania* (Sapindaceae), polar view, optical section. 4. *Ricinus communis* (Euphorbiaceae): 4a. nearly polar view, surface and apertures; 4b. nearly polar view, optical section. 5. *Eucalyptus* (Myrtaceae), polar view, optical section. 6. *Vernonia* (Asteraceae), polar view, optical section. 7. *Eupatorium* (Asteraceae), polar view, optical section. 8. *Gochnatia* (Asteraceae), equatorial view, optical section. 9. *Hyptis* (Lamiaceae), polar view, optical section. 10. *Borreria verticillata* (Rubiaceae), polar view, optical section. 11. *Laguncularia racemosa* (Combretaceae), equatorial view, optical section. Bar = 10 µm.

Table 2. Occurrence of pollen class percentages in honey samples from Maranguá Bay, Bahia State, Brazil. (D = dominant pollen (> 45%); A = accessory pollen (15%-45%); I = isolated pollen (3%-15%); O = occasional pollen (< 3%); H = honey samples).

| Habit | Pollen types | Sep H | Oct H | Nov H | Dec H |
|---|---|---|---|---|---|
| Herbs | AMARANTHACEAE | | | | |
| | *Althernanthera* | | | O | |
| | ASTERACEAE | | | | |
| | *Eupatorium* | O | O | O | I |
| | *Elephantopus* | | | I | |
| | *Montanoa* | | O | O | |
| | CYPERACEAE | O | O | O | O |
| | POACEAE | | | | |
| | *Panicum* | | O | O | |
| | LAMIACEAE | | | | |
| | *Hyptis* | | I | A | O |
| | MIMOSACEAE | | | | |
| | *Mimosa pudica* | D | A | A | D |
| | LYTHRACEAE | | | | |
| | *Cuphea* | | | O | O |
| | RUBIACEAE | | | | |
| | *Borreria* | | I | A | O |
| | *Richardia* | | | O | |
| | VERBENACEAE | O | | | |
| Trees, shrubs and vines | ANACARDIACEAE | | | | |
| | *Anacardium* | | O | O | O |
| | BORAGINACEAE | | | | |
| | *Cordia* | | | O | |
| | BURSERACEAE | | | | |
| | *Protium* | O | | | |
| | COMBRETACEAE | | | | |
| | *Laguncularia racemosa* | | | O | O |
| | *Terminalia* | O | O | O | O |
| | ASTERACEAE | | | | |
| | *Vernonia* | I | I | I | O |
| | EUPHORBIACEAE | | | | |
| | *Ricinus communis* | O | | | |
| | MIMOSACEAE | | | | |
| | *Acacia* | I | I | I | I |
| | *Mimosa caesalpinaefolia* | | O | I | |
| | *Piptadenia* | | I | I | |
| | *Schrankia* | | I | I | |
| | MORACEAE | | | | |
| | *Cecropia* | O | O | O | O |
| | MYRTACEAE | | | | |
| | *Myrcia* | O | O | | |
| | RUTACEAE | | | | |
| | *Zanthoxylum* | O | O | O | O |
| | ARECACEAE | O | O | O | O |
| | SAPINDACEAE | O | O | O | |
| | Not identified | O | O | O | O |
| | Total of pollen types | 15 | 20 | 25 | 15 |

**Guanabara Bay honey samples**

Thirty four pollen types were identified representing 31 genera and 24 families (table 1). The most frequent pollen type belonged to the families Mimosaceae and Asteraceae. The dominant nectar in August was from *Gochnatia velutina* (cambará), *Eupatorium maximilianii* (salseira) in April and July, Sapindaceae in November, and *Mimosa pudica* (malícia-roxa) from December to February and again in June. Heavy rains jeopardized the handling and extraction of honey from the hive during March. In April, the honey was a nectar combination of *Eupatorium maximilianii* and *Spondias* sp. (cajá) nectars, with only a small contribution from *Laguncularia racemosa*. From May to June, the predominant nectar sources were *Mimosa pudica* and *M. bimucronata* (maricá). In September, the honey was largely composed of nectar from *Eucalyptus* sp. and *M. bimucronata*, in October mainly from *Vernonia* sp. (assa-peixe), and in November the honey was predominantly from *Dombeya* sp. (astrapéia-rósea), *Croton* sp. (morrão-de-candeia), and Sapindaceae.

**Guanabara Bay beebread samples**

Thirty two pollen types were identified, representing 27 genera and 22 families (table 1). The most predominant pollen types were from the families Asteraceae and Mimosaceae, followed by Euphorbiaceae, Lythraceae, Moraceae, Poaceae, Rubiaceae, Sapindaceae, and Tiliaceae. The lowest pollen type richnesses were observed in January, June, and July. *Laguncularia racemosa* contributed to pollen stocks only in April and October, and only low frequencies.

**Maranguá Bay honey samples**

Twenty eight pollen types were identified in the four samples analyzed, representing 23 genera and 19 families (table 2). The honey was monofloral for *Mimosa pudica* in September and December, while from October to November the honey was polyfloral. The pollen of *Laguncularia racemosa* was observed in honey only at low frequencies from November to December.

## DISCUSSION

The pollen spectra of the honey and beebread samples from Guanabara Bay showed good representation from hygrophytes, several ruderal species, as well as introduced and cultivated species near the study area. Native trees were little represented in the samples. The most significant pollen types were from *Mimosa bimucronata* (a native

hygrophyte), *Gochnatia polymorpha* (ruderal), *Mimosa pudica* (ruderal), *Eupatorium maximilianii* (ruderal), *Vernonia* (ruderal), *Croton* (ruderal), *Eucalyptus* (an exotic tree species), *Myrcia* (several fruit tree species introduced into the apiary), Arecaceae (a tree species introduced into the region), *Spondias* (a fruit tree species introduced to the apiary), and the family Combretaceae (native hygrophytes, including *Laguncularia racemosa*). The honey samples from Guanabara Bay revealed the occurrence of episodes of monofloral honey from *Mimosa pudica* (January, February, June, and December), *Eupatorium* (April and July), *Gochnatia* (August), and a Sapindaceae (November). The pollen of *Eupatorium* (July and August) and *Ricinus communis* (castor-oil plant, mamoneiro, in October) were usually dominant, indicating intense visitation by the honeybees in this region.

The intense collection by *Apis mellifera* of pollen types considered anemophilous and of low nutritional value (Poaceae, Cyperaceae and *Cecropia*) revealed the lack of better floral sources during a good part of the year. The search for food obliged them to range widely during periods of scarcity – as could be seen by the presence of *Eucalyptus* pollen in the samples derived from plantations located 3 km from the apiary. The pollen spectra of the samples characterize this coastal region as having experienced intense anthropogenic intervention.

The most representative pollen types at Maranguá Bay were *Mimosa pudica* (ruderal), *Vernonia* (ruderal), *Acacia* (a native tree), *Borreria latifolia* (ruderal), *Hyptis* (ruderal), *Eupatorium* (ruderal), Arecaceae (several native tree species), *Myrcia* (several native fruit species), *Zanthoxylum* (a native tree species), *Anacardium* (the cashew tree), Combretaceae (hygrophyte), Sapindaceae (vines and native tree species), *Schrankia* (a native tree species), and *Piptadenia* (a native tree species). The honey samples from Maranguá Bay showed wide pollen spectra composed of plants occurring in the woodlands and capoeirão of the surrounding area – which indicated better preservation conditions than seen in Guanabara Bay. Monofloral honey was observed in September and December from *Mimosa pudica*, while the honey collected in October and November was polyfloral.

Contributions of *Laguncularia racemosa* nectar to *Apis mellifera* honey were observed during the month of April in the Guanabara Bay region, and its pollen was found in beebread during the months of April and October. At Maranguá Bay, on the other hand, the nectar of *Laguncularia racemosa* contributed to honey produced during the months of November and December. *Laguncularia racemosa* can be considered a principally nectariferous species as only low quantities of pollen grains were found inside the honey samples examined, but large nectar contributions.

Our analyses demonstrated that the highly-scented inflorescences of *Laguncularia racemosa* readily attract *Apis mellifera* in both degraded and preserved areas. The use of *Laguncularia racemosa* nectar was intense in both the mangrove apiary (Guanabara Bay – Rio de Janeiro state) and in the apiary located farther away from the mangrove swamp (Maranguá Bay – Bahia state) (personal communication from the beekeepers). *Laguncularia racemosa* can be considered an important apicultural tree species as it flowers for long periods of time in these regions and occurs at high densities even though its pollen is poorly represented in the honey. During the six months when *Laguncularia racemosa* was in flower, 500 liters of honey were obtained from 26 hives installed in the apiary in the Guanabara Bay (personal communication from the beekeepers) – which is considered an excellent yield. These harvests represent a major economic stimulus for the development of apiculture in mangrove regions in Brazil and should bring important benefits to the conservation of this threatened ecosystem. Relevant recommendations include installing apiaries in drier areas of the landscape (and not inside the mangrove swamp itself) as well as choosing areas near fresh water sources.

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