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The Botanical Profiles of Dried Bee Pollen Loads Collected by *Apis mellifera* (Linnaeus) in Brazil

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Abstract

A total of 61 dried bee pollen samples collected in four Brazilian macro-regions within 19 municipalities were analyzed aiming to identify the sources used by *Apis mellifera* (L.) for pollen production and to enable a more accurate product certification. Sample preparation followed the standard methodology, including washing the pollen grains with ethanol, then with water and homogenising the sediment in a water/glycerine solution for microscopic observation. Pollen counts included at least 500 pollen grains per sample. Only six samples, presenting a unique species or pollen type comprising more than 90% of the pollen sum, were considered monofloral, including *Ambrosia* sp., *Cecropia* sp., *Eucalyptus* sp., Fabaceae, *Mimosa scabrella* (Benth.) spp. and *Schinus* sp. pollen types. The most frequent pollen types of the heterofloral pollen batches, based on a counting limit of 45%, included *Anadenanthera* sp., Asteraceae, *Brassica* sp., Caesalpiniaceae, *Cocos nucifera* (L.) sp., *Mimosa caesalpiniaefolia* (Benth.) sp., *Mimosa verrucosa* (Benth.) sp., and *Myrcia* sp. pollen types. This result may be related to the great diversity of the Brazilian flora contributing to heterofloral pollen loads and honeys.

Introduction

The marketing for *Apis mellifera* (L). products is widely expanding in Brazil in response to the increased exports in recent years. Bee pollen production has gradually increased in response to the demand for dietary supplements and therapeutic products (Barreto et al. 2005). These products result from the intensive pollination activity of the bees, which support the maintenance of the biodiversity of the pollen-producing flora (Santos 2009). Beekeeping has been widespread in several Brazilian regions as an activity that supplements the income of small farmers while it has a low environmental impact and high importance for the social and the economic aspects of sustainability (Vieira & Resende, 2007).

Brazilian vegetation is geographically diverse and is divided into five macro-regions. The North region includes the largest tropical rainforest in the country such as a huge drainage area through the Amazon basin. The Northeast region occurs in the eastward "bulge" of the country where there is a semiarid interior been largely given over to low-density vegetation. The Southeast region is mainly an upland area with prevalence of high species diversity in the vegetation of the Atlantic forest. The South region is the smallest macro-region, been distinct because of its temperate climate and the presence of Araucaria forest. In the Midwest region there is a dry forest ("cerrado") characterized by drier climate and vegetation resistant to prolonged periods without rain, besides the humid "pantanal" areas (Rizzini 1997).

For many insects, and particularly for bees, pollen is the main source of the proteins and lipids required by larvae. Pollen is essential to the normal growth and development of all individuals in a colony and for the reproduction and maintenance of the colony (Marchini et al. 2006). Pollen grains are collected on the corbicula of the hind legs of the bees during field activity and are stored in hive cells, forming the "bee bread" (Moreti et al. 2002). The increasing variety and availability of apiary derivatives in the Brazilian

market, and international interest in these products encourage research to characterize the botanical origin of different pollen types collected by bees using palynological analysis for quality control (Luz et al. 2007).

The botanical origin of bee pollen loads is of extreme importance to a greater quality control, enabling better commercial value for the manufactured products. Almeida-Muradian et al. (2005) emphasized the importance of this quality control in targeting the commercial production of pollen products, including the flowering plants of each region and a wide range of physicochemical bee pollen analyses.

Melissopalinological analysis is an important tool for increasing knowledge about the botanical origins of bee pollen. Regional data were presented by Barth & Luz (1998) with regard to pollen collection inside mangrove areas, Barth (2004) in a review, Modro et al. (2007), Luz et al. (2007) in a forested area of Rio de Janeiro State, Melo et al. (2009) in São Paulo State, Barth et al. (2009), Luz et al. (2010) in Minas Gerais State, Barth et al. (2010) with regard to the technical processing of pollen loads, Luz et al. (2011) in Espírito Santo State, Boff et al. (2011) in Mato Grosso do Sul State, Novais et al. (2009); Novais et al. (2010); Dórea et al. (2010); Santos (2011) in Bahia State and Barth et al. (2011) in regions of Venezuela.

The present study aimed to investigate the botanical and geographical origins of bee pollen collected in four Brazilian macro-regions. This study aimed to provide also results that will contribute to improve the certification of this bee product and the preservation of the native vegetation.

Materials and Methods

A total of 61 dried pollen load samples of *A. mellifera* collected by traps in four Brazilian macro-regions, were analyzed using pollen analysis (Figure 1). The procedure followed the methodology proposed by Barth et al. (2010) using two grams of each sample of dried bee pollen homogenate stirring in 70% ethanol. Centrifuge tubes were filled to 13 mL, and the material remained at rest for 30 min or overnight and was then submitted to sonication for five minutes to dissociate the pollen grains.

After centrifugation, the samples containing a large amount of oil were submitted twice to ethanol extraction. The obtained sediment was diluted in a 1:1 mixture of water/glycerine for 30 min. One drop of this well-homogenised pollen grain suspension was applied to a microscope slide and covered with a 24x24 mm cover glass. The stock pollen suspension was kept in glycerine at room temperature.

Two microscope slides, sealed with nail-lack, were prepared and more than 500 pollen grains were counted from each sample considering the two slides. Samples were observed using light and polarized light microscopy. Pollen

Figure.1 Map of the five Brazilian macro-region displaying the studied area with the municipalities collection. 1. Rio Grande do Norte; 2. Sergipe; 3. Bahia; 4. Espírito Santo; 5. São Paulo; 6. Santa Catarina; 7. Rio Grande do Sul; 8. Distrito Federal; 9. Mato Grosso.

classes usually followed those established by Zander (Louveaux et al. 1978) meaning honey analysis PP (predominant pollen > 45%), AP (accessory pollen 15-45%), IP (important pollen 3-15%). These classes were used for qualitative and quantitative analyses of bee pollen loads in the present paper.

Samples were classified as monofloral or heterofloral batches according to their pollen grain percentages. Samples were considered monofloral when presenting more than 90% of a unique pollen type. Barth (1989), Roubik & Moreno (1991) and Moreti et al. (2002) were used to aid in pollen identification. Pollen morphology in the Asteraceae family is not genus specific and was presented at the family ranking Asteraceae.

Results

The results of the palynological analysis showed a wide variety of pollen types found in each Brazilian macro-region (Tables 1, 2, 3). Six of the 61 samples were monofloral. The monofloral samples were found in two samples from the Southeast, two from the South and two from the Midwest (Tables 2 and 3).

Northeast region (Table 1) – Pollen from *Cocos nucifera* was present in all 19 samples analyzed, with a concentration above 3%. This pollen type was present as a dominant pollen in seven samples (>45%), as an accessory pollen in ten samples and as an isolate pollen in two samples throughout the three states of the Northeast region. Pollen types of *Mimosa caesalpiniaefolia*. (sample Sergipe-7), *M. scabrella* (samples Bahia-2, -5), *M. verrucosa* (samples Sergipe-5, -6, -8, -10, -11), and *Myrcia* sp. (samples Sergipe-2, -3, -11) also exhibited a dominant pollen frequency.

Southeast region (Table 2) – The unique dried bee pollen sample from Espírito Santo State was a monofloral sample of an unidentified plant of the Fabaceae family was only found in that one sample. Nine samples from Pariquera Açu in São Paulo State all came from *Cocos nucifera* and were very similar.

The Ribeirão Preto municipality presented dominant pollen grain percentages of *M. caesalpiniaefolia* in six samples (São Paulo-10, -11, -12, -13, -14, -15). *Schinus* sp. was the main pollen type in two samples (São Paulo-16, -18) and *Anadenanthera* sp. in one sample only (São Paulo-19). The other two samples (São Paulo-17, -20) exhibited no dominant pollen types but had in common the pollen types of *Anadenanthera* sp., *Eucalyptus* sp., *Myrcia* sp., and *Cecropia* sp. Pollen grains of the *Persea* sp. type (avocado tree) were detected in only one sample (São Paulo-17).

Taubaté municipality exhibited the *M. caesalpiniaefolia* (samples São Paulo-21, -23) and *M. scabrella* (São Paulo-22, -24) pollen types as dominant in two samples each, with the latter including a monofloral sample (São Paulo-24).

South region (Table 3) – All samples analyzed from Fraiburgo municipality (Santa Catarina-1, -2, -3, -4, -5, -6) were very similar, presenting a high percentage of Asteraceae pollen grains as well as *Amaranthus*/Chenopodiaceae, *Brassica* sp. and Euphorbiaceae pollen grains in several samples. A Caesalpiniaceae pollen type was dominant in Campos Novos (Santa Catarina-7), and *M. caesalpiniaefolia* was dominant in Serra Catarinense (Santa Catarina-8).

Five samples were analyzed from Rio Grande do Sul State. Three of these samples showed *Eucalyptus* sp. pollen dominance in the municipalities of Arvorezinha (Rio Grande do Sul-4), Santana do Livramento (Rio Grande do Sul-2) and São Gabriel (Rio Grande do Sul-5). The *Ambrosia* sp. pollen type occurred in a sample from Santana do Livramento (Rio Grande do Sul-1), and the *Brassica* sp. pollen type was found in a sample from Cruz Alta (Rio Grande do Sul-3), both of which were classified as monofloral.

Midwest region (Table 3) - Midwestern Brazil was represented by four samples. The Distrito Federal sample showed a high percentage of the *Schinus* sp. pollen type, which was considered monofloral. Three samples from Mato Grosso showed *Cecropia* sp. pollen dominance, one of which (Mato Grosso-2) was considered monofloral.

The most frequent or characteristic pollen types in the Brazilian macro-regions of the present study can be summarised as follows:

Cocos nucifera (n = 19/19) is a typical pollen product in the Brazilian Northeast region (n = 19) and, pollen types of *M. caesalpiniaefolia* (n = 3), *M. scabrella* (n = 8) and *M. verrucosa* (n = 7) were common. Pollen grains of Asteraceae species were poorly represented in the samples of this macro-region.

The main pollen types of the Southeast region (n = 25) were Asteraceae and *Myrcia* sp. (n = 11/25 of each), such as *M. caesalpiniaefolia* (n = 12/25). The characteristic pollen types of the South macro-region (n = 13) were Asteraceae (n = 10/13) and *Brassica* sp. (n = 8/13). In the few samples of the Midwest macro-region (n = 4) *Cecropia* sp. (n = 4) pollen was detected in all samples, including in one monofloral sample primarily of *Schinus* sp. pollen (n = 1).

Discussion

Northeast region – Coconut trees grow frequently on seashores along the northeastern Brazilian coastline (Pires et al. 2004) and are naturally spread throughout the beaches. These trees are considered an important pollen source in Neotropical regions (Biesmeijer et al. 1992). *Cocos nucifera* pollen grains were well represented in all samples of the three Northeast states (Rio Grande do Norte, Sergipe and Bahia). The pollen of this species was associated with *M. verrucosa* ("espinheiro preto"), a typical plant throughout the Brazilian Northeast (Sodré et al. 2007), and mainly in samples of the state of Sergipe. In addition, *M. caesalpiniaefolia* ("sabiá")

was found in association with *Cocos nucifera*. Novais et al. (2009) and Dórea et al. (2010) highlight other Mimosacea and Arecaceae species for the Bahia State.

M. caesalpiniaefolia has a natural occurrence in the semi-arid Brazilian region (Carvalho 2007, Queiroz, 2009) and has been introduced in more humid regions (Ribaski et al. 2003) and is widely used in the reforestation of degraded areas (Lorenzi 1998). Caesalpiniaceae has been reported by several authors (Absy & Kerr, 1977; Absy et al. 1980; Kerr et al. 1986/1987; Imperatriz-Fonseca 1989; Ramalho 1990) as an important food resource for native bees in different Brazilian states. Monofloral pollen loads of *M. caesalpiniaefolia* have also been collected in the state of Rio de Janeiro (Luz et al. 2007). Therefore it may be considered that the pollen of *Cocos* and *Mimosa* is very important in the Northeast Brazilian region.

Southeast region - The Fabaceae family is often visited by bees in search of nectar and pollen for the maintenance of the hive (Moreti et al. 2007). A monofloral pollen batch of a Fabaceae species came from Espírito Santo State. This species may be wild or a crop species, and the pollen morphology of several related species similar (Carreira et al. 1996; Moreti et al. 2007). The Mimosaceae family comprises a large number of species of the Brazilian flora that are of extreme significance for pollen and honey production.

One batch with predominant pollen of the *M. scabrella* pollen type was obtained in the State of São Paulo. The pollen grain morphology of this specie is not characteristic of a unique plant species but rather, groups of species (Barth 1989). Based on a survey of Neotropical region, Ramalho et al. (1990) reported that the *M. scabrella* pollen type was commonly collected by *A. mellifera*.

The numerous monofloral batches of the *Cocos nucifera* pollen type obtained in the state of São Paulo were probably derived from the extensive coconut plantations in the region. Surprisingly, these pollen grains dominated all samples from the munipality of Pariquera Açu, São Paulo State, indicating that coconut plantations are significant for beekeeper activities.

The attractive flowering of the wild trees or shrubs of *Anadenanthera* sp. is extremely important for beekeepers as because, in addition to pollen and honey harvesting, these flowering plants draw hungry bees away from crop plantations, as of *Passiflora* sp. such as those pollinated by bumblebees, where bees are not welcome (Monika Barth, personal information).

South region - The *Ambrosia* sp. pollen type was characteristic of the state of Rio Grande do Sul (RS). The anemophilous pollen of this genus, dispersed by the wind, is considered allergenic in this region and is suspected to have induced hay fever in several patients (Vergamini et al. 2006). According to Lorenzi (2008), species of the *Ambrosia* genus have been found in agricultural areas of southern Brazil and are considered a weed species. This pollen type is visited by a variety of insects.

A monofloral pollen batch of *Eucalyptus* sp. was also obtained in Rio Grande do Sul state, but this genus is common throughout the Brazilian regions where it is cultivated. Pollen analyses of honey have certified the presence of *Eucalyptus* sp. (Barth et al. 2005; Bastos et al. 2004; Luz et al. 2007). The pollen morphology of *Brassica* sp. which is similar in several species may be derived from crops (Brassica napus L.) in the south of Brazil (Rosa et al. 2011) or from wild species occurring in field vegetation (Moreti et al. 2002).

Midwest region - Another monofloral batch of wind-dispersed pollen was *Cecropia* sp. from the region of Mato Grosso do Sul. *Cecropia* is a widespread small tree in Brazil and produces small pollen that is very attractive to bees (Luz et al. 2007). This genus was also observed by Boff et al. (2011) as an accessory pollen type in analyzed pollen loads from southern Pantanal in the state of Mato Grosso do Sul (MS).

Bees have a "preference" for *Schinus* sp. pollen. The main species, *S. terebinthifolius* (L.), has a worldwide distribution in tropical regions. The present monofloral sample was obtained in the Brazilian Federal District. The pollen of this genus occurs as an accessory percentage of the total pollen samples, including in honey samples (Carreira & Jardim, 1994). This genus is characteristic of drier vegetation (Smith et al. 2004) and is widely sought by bees, especially when other species are not in the flower (Baggio 1988).

All dominant pollen types were present as accessory pollen in numerous samples. In addition, six non-dominant pollen types were identified as accessory pollen in the present study including pollen types of *Anadenanthera* sp., *Brassica* sp., Caesalpiniaceae, *Cocos nucifera*, *M. caesalpiniaefolia* and *Myrcia* sp.

Several plant species and genera showed very similar pollen morphology to that of *Myrcia* sp. (Barth 1972). This pollen type occurs in pollen batches of nearly all Brazilian states and was also highlighted by Barth (1989) as an accessory pollen type in honey samples from Bahia.

From the data presented here, the great floristic diversity in Brazil is apparent, as shown through the main pollen types identified in bee pollen loads. Thus, there is a need for a comprehensive and detailed botanical certification program to increase the commercial value of pollen products.

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Table 1. The pollen types identified in the pollen load samples of *A. mellifera* collected in Northeast macro-region of Brazil and their main botanical origins, considering a frequency of more than 3%. (Samples were considered monofloral when presenting more than 90% of a unique pollen type).

Municipality Brazilian Macro-region = Northeast	Pollen analysis and pollen types	Predominant botanical origin (representative pollen types)
Rio Grande do Norte-1 (Natal)	**PP**: *Cocos nucifera* (60.2%) **AP**: *Mimosa verrucosa* (36.0%)	Heterofloral sample with primary contributions from *Cocos nucifera* and *Mimosa verrucosa*
Rio Grande do Norte-2 (Grupo Santa Luzia)	**PP**: *Cocos nucifera* (47.2%) **AP**: *Eucalyptus* (25.0%) **IP**: *Mimosa caesalpiniaefolia* (6.4%), *Myrcia* (5.2%), *Mimosa scabrella* (3.7%), Asteraceae (3.4%), *Richardia* (3.2%)	Heterofloral sample with primary contributions from *Cocos nucifera* and *Eucalyptus*
Sergipe-1 (Povoado Brejão)	**AP**: *Mimosa scabrella* (42.1%), *Cocos nucifera* (35.4%), *Myrcia* (17.5%)	Heterofloral sample with primary contributions from *Mimosa scabrella*, *Cocos nucifera* and *Myrcia*
Sergipe-2 (Povoado Brejão)	**PP**: *Myrcia* (67.0%) **IP**: *Cocos nucifera* (3.5%)	Heterofloral sample with a primary contribution from *Myrcia*
Sergipe-3 (Povoado Brejão)	**PP**: *Myrcia* (55.5%) **AP**: *Mimosa scabrella* (25.3%), *Cocos nucifera* (17.2%)	Heterofloral sample with primary contributions from *Myrcia*, *Mimosa scabrella* and *Cocos nucifera*
Sergipe-4 (Povoado Brejão)	**PP**: *Cocos nucifera* (47.5%) **AP**: *Myrcia* (23.2%), *Mimosa scabrella* (23.2%)	Heterofloral sample with primary contributions from *Cocos nucifera, Myrcia* and *Mimosa scabrella*
Sergipe-5	**PP**: *Mimosa verrucosa* (50.7%) **AP**: *Cocos nucifera* (41.1%) **IP**: Poaceae (8.0%)	Heterofloral sample with primary contributions from *Mimosa verrucosa* and *Cocos nucifera*
Sergipe-6	**AP**: *Mimosa verrucosa* (42.8%), *Cocos nucifera* (35.4%), *Myrcia* (16.3%) **IP**: *Tapirira* (5.4%)	Heterofloral sample with primary contributions from *Mimosa verrucosa*, *Cocos nucifera* and *Myrcia*
Sergipe-7	**PP**: *Mimosa caesalpiniaefolia* (50.1%) **AP**: *Cocos nucifera* (41.3%) **IP**: *Myrcia* (5.7%)	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia* and *Cocos nucifera*
Sergipe-8	**AP**: *Mimosa verrucosa* (39.7%), *Cocos nucifera* (36.8%), *Mimosa caesalpiniaefolia* (15.7%) **IP**: *Myrcia* (7.8%)	Heterofloral sample with primary contributions from *Mimosa verrucosa*, *Cocos nucifera* and *Mimosa caesalpiniaefolia*
Sergipe-9	**AP**: *Cocos nucifera* (35.1%), *Brassica* (20.3%), *Myrcia* (18.6%), *Mimosa verrucosa* (16.5%) **IP**: *Tapirira* (9.4%)	Heterofloral sample with primary contributions from *Cocos nucifera*, *Brassica*, *Myrcia* and *Mimosa verrucosa*
Sergipe-10	**PP**: *Mimosa verrucosa* (56.0%) **AP**: *Cocos nucifera* (29.3%) **IP**: *Brassica* (14.8%)	Heterofloral sample with primary contributions from *Mimosa verrucosa* and *Cocos nucifera*
Sergipe-11	**PP:** *Cocos nucifera* (53.3%); **AP:** *Mimosa verrucosa* (39.9%) **IP:** *Myrcia* (3.8%)	Heterofloral sample with primary contributions from *Cocos nucifera* and *Mimosa verrucosa*
Bahia-1 (Canavieiras)	**PP**: *Cocos nucifera* (67.6%) **AP**: *Eucalyptus* (31.3%)	Heterofloral sample with primary contributions from *Cocos nucifera* and *Eucalyptus*
Bahia-2 (Canavieiras)	**PP:***Mimosa scabrella* (59.0%) **AP**: *Cocos nucifera* (41.0%)	Heterofloral sample with primary contributions from *Mimosa scabrella* and *Cocos nucifera*
Bahia-3 (Canavieiras)	**PP**: *Cocos nucifera* (76.3%) **AP**: *Mimosa scabrella* (18.4%) **IP**: *Eucalyptus* (5.3%)	Heterofloral sample with primary contributions from *Cocos nucifera* and *Mimosa scabrella*
Bahia-4 (Canavieiras)	**PP**: *Cocos nucifera* (85.5%) **IP**: *Vernonia* (6.9%)	Heterofloral sample with a primary contribution from *Cocos nucifera*
Bahia-5 (Ilhéus)	**PP**: *Mimosa scabrella* (58.3%) **AP**: *Cocos nucifera* (32.7%) **IP**: Asteraceae (6.7%)	Heterofloral sample with primary contributions from *Mimosa scabrella* and *Cocos nucifera*
Bahia-6 (Ilhéus)	**PP**: Asteraceae (81.5%) **IP**: *Cecropia* (6.4%), *Mimosa scabrella* (5.3%), *Cocos nucifera* (4.5%)	Heterofloral sample with a primary contribution from Asteraceae

PP = predominant pollen (> 45%); AP = accessory pollen (15-45%); IP = isolate pollen (3-15%).

Table 2. The pollen types identified in the pollen load samples of *A. mellifera* collected in Southeast macro-region of Brazil and their main botanical origins, considering a frequency of more than 3%. (Samples were considered monofloral when presenting more than 90% of a unique pollen type.)

Municipality Brazilian macro-region = Southeast	Pollen analysis and pollen types	Predominant botanical origin (representative pollen types)
Espírito Santo	**PP**: Fabaceae- Faboideae (90.6%) **IP**: *Myrcia* (4.7%), *Eucalyptus* (3.1%)	**Monofloral sample** of a Fabaceae- Faboideae species
São Paulo-1 (Pariquera Açu)	**PP**: *Cocos nucifera* (62.5%) **AP**: Asteraceae (15.6%) **IP**: Poaceae (12.5%), Melastomataceae (9.4%)	Heterofloral sample with primary contributions from *Cocos nucifera*, and Asteraceae
São Paulo-2 (Pariquera Açu)	**PP**: *Cocos nucifera* (50.0%) **AP**: *Myrcia* (22.2%), *Mimosa caesalpiniaefolia* (19,4%) **IP**: *Mimosa scabrella* (8.3%)	Heterofloral sample with primary contributions from *Cocos nucifera*, *Myrcia* and *Mimosa caesalpiniaefolia*
São Paulo-3 (Pariquera Açu)	**PP**: *Cocos nucifera* (55.3%) **AP**: *Mimosa caesalpiniaefolia* (27.8%), *Myrcia* (16.9%)	Heterofloral sample with primary contributions from *Cocos nucifera Mimosa caesalpiniaefolia* and *Myrcia*
São Paulo-4 (Pariquera Açu)	**PP**: *Cocos nucifera* (47.5%) **AP**: *Myrcia* (17.0%), *Cecropia* (16.4%) **IP**: *Mimosa caesalpiniaefolia* (13.8%)	Heterofloral sample with primary contributions from *Cocos nucifera, Myrcia*, and *Cecropia*
São Paulo-5 (Pariquera Açu)	**PP**: *Cocos nucifera* (81.2%) **IP**: *Ilex* (6.2%), *Sebastiania* (6.2%), Asteraceae (5.3%), *Myrcia* (4.2%),	Heterofloral sample with a primary contribution of *Cocos nucifera*
São Paulo-6 (Pariquera Açu)	**PP**: *Cocos nucifera* (78.3%) **IP**: *Ilex* (11.6%), Asteraceae (5.8%), *Myrcia* (4.3%)	Heterofloral sample with a primary contribution from *Cocos nucifera*
São Paulo/SP 7 (Pariquera Açu)	**PP**: *Cocos nucifera* (57.7%) **IP**: *Cecropia* (12.1%), *Ilex* (9.1%), *Eucalyptus* (4.8%), Asteraceae (7.0%), *Myrcia* (5.8%), unidentified (3.0%)	Heterofloral sample with a primary contribution from *Cocos nucifera*
São Paulo-8 (Pariquera Açu)	**PP**: *Cocos nucifera* (68.3%) **IP**: *Eucalyptus* (7.6%), *Myrcia* (3.8%)	Heterofloral sample with a primary contribution from *Cocos nucifera*
São Paulo-9 (Pariquera Açu)	**PP**: *Cocos nucifera* (58.2%) **AP**: *Cecropia* (23.9%), *Mimosa caesalpiniaefolia* (17.9%) **IP**: *Myrcia* (3.8%)	Heterofloral sample with primary contributions from *Cocos nucifera*, *Cecropia* and *Mimosa caesalpiniaefolia*
São Paulo-10 (Ribeirão Preto)	**PP**: *Mimosa caesalpiniaefolia* (57.9%) **AP**: Asteraceae (26.8%) **IP**: Poaceae (14.6%)	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia* and Asteraceae
São Paulo-11 (Ribeirão Preto)	**PP**: *Mimosa caesalpiniaefolia* (60.3%) **AP**: Asteraceae (17.6%), Poaceae (16.6%), **IP**: *Myrcia* (3.8%)	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia*, Asteraceae and Poaceae
São Paulo-12 (Ribeirão Preto)	**PP**: *Mimosa caesalpiniaefolia* (70.2%) **AP**: Poaceae (18.2%) **IP**: Asteraceae (11.6%)	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia* and Poaceae
São Paulo-13 (Ribeirão Preto)	**PP**: *Mimosa caesalpiniaefolia* (51.3%) **AP**: *Mimosa scabrella* (15.0%) **IP**: Poaceae (12.6%), Euphorbiaceae (6.6%), Fabaceae-Faboideae (4.2%),	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia* and *Mimosa scabrella*
São Paulo-14 (Ribeirão Preto)	**PP**: *Mimosa caesalpiniaefolia* (72.1%) **IP**: Poaceae (12.5%), *Mimosa scabrella*, (6.3%), Asteraceae (4.7%), Euphorbiaceae (4.4%)	Heterofloral sample with a primary contribution from *Mimosa caesalpiniaefolia*
São Paulo-15 (Ribeirão Preto)	**PP**: *Mimosa caesalpiniaefolia* (64.5%) **AP**: Poaceae (19.3%) **IP**: Asteraceae (12.9%), Euphorbiaceae (3.2%)	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia* and Poaceae
São Paulo-16 (Ribeirão Preto)	**PP**: *Schinus* (58.6%) **AP**: *Alternanthera* (34.6%), Asteraceae (3.0%)	Heterofloral sample with primary contributions from *Schinus* and *Alternanthera*
São Paulo-17 (Ribeirão Preto)	**AP**: *Persea* (25.9%), *Myrcia* (20.3%), *Anadenanthera* (18.6%), *Eucalyptus* (18.4%) **IP**: *Cecropia* (6.9%), *Tapirira* (4.7%), Asteraceae (3.5%)	Heterofloral sample with primary contributions from *Persea*, *Myrcia*, *Anadenanthera* and *Eucalyptus*
São Paulo-18 (Ribeirão Preto)	**PP**: *Schinus* (82.3%) **AP**: *Vernonia* (15.3%)	Heterofloral sample with primary contributions from *Schinus* and *Vernonia*
São Paulo-19 (Ribeirão Preto)	**PP**: *Anadenanthera* (82.5%) **IP**: *Eucalyptus* (10.0%), *Cecropia* (5.7%)	Heterofloral sample with a primary contribution from *Anadenanthera*
São Paulo-20 (Ribeirão Preto)	**AP**: *Cecropia* (43.2%) *Anadenanthera* (33.9%) **IP**: *Eucalyptus* (12.3%), *Myrcia* (9.3%)	Heterofloral sample with primary contributions from *Cecropia* and *Anadenanthera*
São Paulo-21 (Taubaté)	**PP**: *Mimosa caesalpiniaefolia* (46.1%) **AP**: *Mimosa scabrella* (37.2%) **IP**: *Syagrus* (10.2%)	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia* and *Mimosa scabrella*
São Paulo-22 (Taubaté)	**PD**: *Mimosa scabrella* (80.0%)**IP**: Malpighiaceae (5.9%)	Heterofloral sample with a primary contribution from *Mimosa scabrella*
São Paulo-23 (Taubaté)	**AP**: *Mimosa caesalpiniaefolia* (43.1%), *Antigonon leptopus* (23.2%), *Eucalyptus* (15.4%) **PI**: Poaceae (11.0%), *Cecropia* (7.3%)	Heterofloral sample with primary contributions from *Mimosa caesalpiniaefolia*, *Antigonon leptopus* and *Eucalyptus*
São Paulo-24 (Taubaté)	**PP**: *Mimosa scabrella* (92.3%) **IP**: *Antigonon leptopus* (4.9%)	**Monofloral sample** of *Mimosa scabrella*

PP = predominant pollen (> 45%); AP = accessory pollen (15-45%); IP = isolate pollen (3-15%)

Table 3. The pollen types identified in the pollen load samples of *A. mellifera* collected in South and Midwest macro-region of Brazil and their main botanical origins, considering a frequency of more than 3%. (Samples were considered monofloral when presenting more than 90% of a unique pollen type).

Municipality Brazilian macro-region = South	Pollen analysis and pollen types	Predominant botanical origin (representative pollen types)
Santa Catarina-1 (Fraiburgo)	**PP**: Asteraceae (54.2%); **AP**: *Brassica* (36.4%); **IP**: A*maranthus*/Chenopodiaceae (3.3%); Euphorbiaceae (3.6%)	Heterofloral sample with primary contributions from Asteraceae and *Brassica*
Santa Catarina-2 (Fraiburgo)	**PP**: Asteraceae (76.2%) **AP**: *Brassica* (20.0%)	Heterofloral sample with primary contributions from Asteraceae and *Brassica*
Santa Catarina-3 (Fraiburgo)	**PP**: Asteraceae (56.3%) **AP**: *Brassica* (35.6%) **IP**: Euphorbiaceae (6.1%)	Heterofloral sample with primary contributions from Asteraceae and *Brassica*
Santa Catarina-4 (Fraiburgo)	**PP**: Asteraceae (71.8%) **AP**: *Brassica* (19.7%) **IP**: Euphorbiaceae (7.0%)	Heterofloral sample with primary contributions from Asteraceae and *Brassica*
Santa Catarina-5 (Fraiburgo)	**PP**: Asteraceae (56.7%) **AP**: *Brassica* (23.3%) **IP**: Euphorbiacae (10.0%), *Amaranthus*/ Chenopodiaceae (6.7%), Poaceae (3.3%)	Heterofloral sample with primary contributions from Asteraceae and *Brassica*
Santa Catarina-6 (Fraiburgo)	**PP**: Asteraceae (50.0%) **AP**: Euphorbiaceae (26.6%), *Brassica* (18.7%) **IP**: A*maranthus*/Chenopodiaceae (4.7%)	Heterofloral sample with primary contributions from Asteraceae, *Brassica* and Euphorbiaceae
Santa Catarina-7 (Campos Novos)	**PP**: Caesalpiniaceae (63.1%) **IP**: Asteraceae (13.6%), *Sebastiania* (9.5%), Apiaceae (8.5%), *Myrcia* (3.8%)	Heterofloral sample with a primary contribution from Caesalpiniaceae
Santa Catarina-8 (Serra Catarinense)	**PP**: *Mimosa caesalpiniaefolia* (85.2%) **IP**: Asteraceae (11.8%)	Heterofloral sample with a primary contribution from *Mimosa caesalpiniaefolia*
Rio Grande do Sul-1 (Santana do Livramento)	**PP**: *Ambrosia* (99.4%)	**Monofloral sample** of *Ambrosia*
Rio Grande do Sul-2 (Santana do Livramento)	**PP**: *Eucalyptus* (93.0%) **IP**: Asteraceae (7.0%);	**Monofloral sample** of *Eucalyptus*
Rio Grande do Sul-3 (Cruz Alta)	**PP**: *Brassica* (57.4%) **AP**: *Eucalyptus* (42.6%)	Heterofloral sample with primary contributions from *Brassica* and *Eucalyptus*
Rio Grande do Sul-4 (Arvorezinha)	**PP**: *Eucalyptus* (49.9%) **AP**: *Eupatorium* (24.5%) **IP**: Onagraceae (10.1%), *Ilex* (6.5%), Asteraceae (4.6%), *Myrcia* (4.1%)	Heterofloral sample with primary contributions from *Eucalyptus* and *Eupatorium*
Rio Grande do Sul-5 (São Gabriel)	**PP**: *Eucalyptus* (64.6%) **AP**: *Brassica napus* (35.3%)	Heterofloral sample with primary contributions from *Eucalyptus* and *Brassica napus*
Distrito Federal	**PP**: *Schinus* (92.1%) **IP**: *Eucalyptus* (4.8%), *Cecropia* (3.2%)	**Monofloral sample** of *Schinus*
Mato Grosso-1 (Sinop)	**PP**: *Cecropia* (66.6%) **AP**: Euphorbiaceae (26.7%); **IP**: Poaceae (6.7%)	Heterofloral sample with primary contributions from *Cecropia* and Euphorbiaceae
Mato Grosso-2 (Sinop)	**PP**: *Cecropia* (97.1%)	**Monofloral sample** of *Cecropia*
Mato Grosso-3 (Sinop)	**PP**: *Cecropia* (51.3%) **AP**: Poaceae (44.3%)	Heterofloral sample with primary contributions from *Cecropia* and Poaceae

PP = predominant pollen (> 45%); AP = accessory pollen (15-45%); IP = isolate pollen (3-15%).

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