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Laura Benitez Bosco & Cynthia Fernandes Pinto da Luz

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Pollen analysis of Atlantic forest honey from the Vale do Ribeira Region, state of São Paulo, Brazil

LAURA BENITEZ BOSCO & CYNTHIA FERNANDES PINTO DA LUZ 

Núcleo de Pesquisa em Palinologia, Instituto de Botânica, Secretaria do Meio Ambiente de São Paulo, São Paulo, Brazil

Abstract

The pollen contents of 34 *Apis mellifera* honey samples from the Quilombola Community of Porto Velho (Vale do Ribeira, state of São Paulo, Brazil) were studied in order to identify their botanical and geographical origin. A total number of 114 pollen types distributed in 43 families were identified. Fabaceae pollen types were the most diverse (20). Dominant pollen was observed in 27 samples. The dominant type *Piper* was found in 21 samples and *Zanthoxylum*, *Mikania cordifolia*, *Cupania oblongifolia*, *Cecropia*, *Attalea* and *Poaceae* only in one sample. The quantitative analysis revealed a predominance of very-high pollen concentrations in the samples (Category IV, 35.3%). A second frequency of occurrence analysis revealed 67 pollen types from 26 botanical families from nectariferous species. Fabaceae was also the family with the largest number of nectariferous pollen types (15), followed by Sapindaceae, Asteraceae (six pollen types each), and Euphorbiaceae (five types). Dominant pollen occurred in 19 samples. The main nectariferous sources of pollen in the present study were: Arecaceae (*Attalea* and *Euterpe/Syagrus*), Asteraceae (*Mikania cordifolia*), Fabaceae (*Machaerium*), Rutaceae (*Zanthoxylum*), and Sapindaceae (*Cupania oblongifolia*). The honey was classified as predominantly heterofloral (67.6%). Monofloral honey from *Mikania cordifolia*, *Machaerium*, *Zanthoxylum*, *Cupania oblongifolia*, *Euterpe/Syagrus* and *Attalea* were also identified. The geographical origin demonstrates *Apis mellifera* using nectariferous sources available from the Atlantic Rainforest in the south and southeast of Brazil, as well as in the brushwood, secondary forest and field plants. This is one of the first works about melissopalynology in the Vale do Ribeira Region.

Keywords: *Apis mellifera*, melissopalynology, bee flora, native flora, botanical origin, apiculture

Pollen and nectar are vital sources of protein, vitamins, mineral and carbohydrates for bees (Michener 1974; Simpson & Neff 1981; Ramalho et al. 1991a). In order to survive, eusocial bees such as *Apis mellifera* L. need to collect and store constant amounts of these resources and exploit them from many different plant species (Ramalho et al. 1989; Michener 2000; Goodman 2003). Studying the interaction of bees with the flora is of the utmost importance in order to understand their ecology and developing sustainable apiculture (Silveira 1983; Barth 2013; Roubik & Moreno 2013).

Melissopalynological analysis identifies and quantifies pollen grains found on the bodies of bees or their products (Barth 2004). Combined with research on bee flora, the following can be identified: the plant species that produced the nectar used for

the production of honey (determination of its botanical origin through qualitative analysis); specific components of the vegetation from the region where the honey was produced (determination of geographic origin) and the species of apicultural interest around the apiaries (Barth 1989; Barth & Luz 1998; Luz 2001). This information improves the quality of the product and provides data for its certification, enabling the producer to achieve better market values (Luz 2001; von der Ohe et al. 2004; Almeida-Muradian et al. 2005; Barth et al. 2005). It also provides beekeepers with knowledge about the plant species used so that they can conserve the flora in a region and to manage the location of bee colonies (Luz et al. 2007).

Beekeeping is an activity of great commercial interest in Brazil, which is currently the eighth largest

Correspondence: Laura Benitez Bosco, Instituto de Botânica, Secretaria do Meio Ambiente de São Paulo, Núcleo de Pesquisa em Palinologia, Avenida Miguel Stéfano 3687, São Paulo, SP, 04301-902, Brazil. E-mail: laurabenitezbosco@gmail.com

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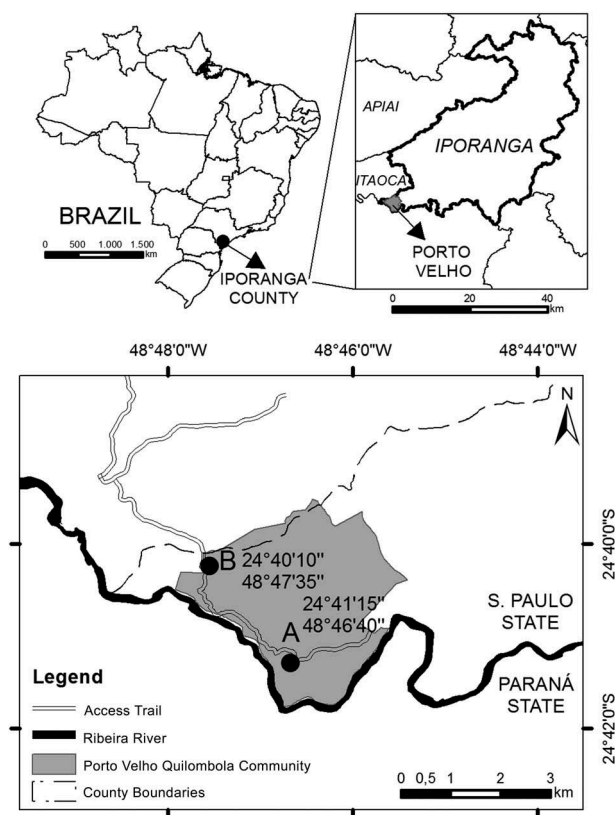


Figure 1. Map of Iporanga City showing the location of Porto Velho Community (grey) with the location of the apiaries investigated.

exporter of honey in the world (Associação Brasileira de Exportadores de Mel 2016). Due to the low initial investment, it is accessible and is especially important for generating income in preserved forest sites where other forms of agriculture are not allowed (Reis 2003; Reis & Filho 2003; Mayana et al. 2006).

The United Nations for Education, Science and Culture (UNESCO) considers the Vale do Ribeira region in São Paulo state a Natural Heritage Site of Humanity (Ribeira 2005; UNESCO 2015). Around 60% of its territory is under legal protection as Conservation Units and there is a rich cultural diversity, including communities descended from slaves called Quilombolas (Resende 2002). Among them is the Porto Velho community (Figure 1) with a population of 70 people, divided into 21 families. In 2013, beekeeping was introduced to the Community as a source of complementary income to agriculture (Quilombolas do Ribeira 2015). In 2015, there were 142 hives, distributed in four apiaries, which together produced around three tons of honey per year (Benitez-Bosco 2015). Thus, beekeeping has become one of the main sources of income for the families of the community (Quilombolas do Ribeira 2015). Despite its great environmental wealth and,

consequently, potential for beekeeping, there have only been two melissopaynological analyses made in the Vale do Ribeira region (Luz et al. 2008; Melo et al. 2009).

Therefore, this study aims to identify the pollen content of *Apis mellifera* honey from the Quilombola Community of Porto Velho, Vale do Ribeira, São Paulo State, Brazil and to identify their botanical and geographical origins.

Material and methods

Study site

The present study was conducted in two small apiaries (averaging ten beehives) (Apiary A at 24° 41' 15.88" S, 48° 46' 40.02" W, Apiary B at 24° 40' 10.25" S, 48° 47' 35.71" W), located at the Quilombola Community of Porto Velho in the city of Iporanga (Vale do Ribeira Region) in the south of the state of São Paulo, close to the border of the state of Paraná (Figure 1). The sites are located in a mosaic of natural vegetation (Tropical Rain Forest and areas of Capoeira Vegetation with secondary vegetation of dense forest), pasture, *Pinus* sp. and *Eucalyptus* sp., silviculture and subsistence farming (Inventário Florestal da Vegetação Natural do Estado de São Paulo 2005; Andrade & Tatto 2013). There is no significant difference in vegetation between the studied apiaries.

The territory of Porto Velho has 9 582 900 m² and is located near the State Park of Alto do Ribeira (PETAR), where around 680 native plant species were identified, 40 of those are classified as endangered species (Ivanauskas et al. 2012). In a flora survey carried out in the territory, 85 species of plants were identified, distributed across 32 botanical families and 69 genera (Benitez-Bosco 2015).

The local economy is mostly based on beekeeping, pig, poultry and cattle farming, kitchen gardens, cassava flour (*Manihot esculenta* Crantz), corn crops (*Zea mays* L.) and beans (*Phaseolus vulgaris* L.), citrus orchards, handcrafts, palmetto plantings (*Euterpe edulis* Mart.) and passionfruit production (*Passiflora edulis* Sims) (Quilombolas do Ribeira 2015). According to the Köppen Climate Classification System (Köppen 1931), the climate in the region is tropical, rainy, having no dry season (Af). It has a mean annual temperature of 24 °C (CEPA-GRI 2015) and mean annual precipitation varies from 1500 to 2000 mm/year (Lepsch et al. 1990).

Sampling description

The present study examined 34 honey samples produced from January 2013 to February 2014

Table I. List of honey samples examined.

Sample	Date of extraction	Apiary	Beehive	Beekeeper
1	January 2013	A	1	Sézar Aparecido dos Santos
2	January 2013	A	2	Sézar Aparecido dos Santos
3	February 2013	A	1	Sézar Aparecido dos Santos
4	Februart 2013	A	2	Sézar Aparecido dos Santos
5	March 2013	A	1	Sézar Aparecido dos Santos
6	March 2013	A	2	Sézar Aparecido dos Santos
7	April 2013	A	1	Sézar Aparecido dos Santos
8	April 2013	A	2	Sézar Aparecido dos Santos
9	May 2013	A	1	Sézar Aparecido dos Santos
10	May 2013	A	2	Sézar Aparecido dos Santos
11	June 2013	A	1	Sézar Aparecido dos Santos
12	June 2013	A	2	Sézar Aparecido dos Santos
13	July 2013	A	1	Sézar Aparecido dos Santos
14	July 2013	A	2	Sézar Aparecido dos Santos
15	July 2013	B	1	Osvaldo dos Santos
16	July 2013	B	2	Osvaldo dos Santos
17	August 2013	A	1	Sézar Aparecido dos Santos
18	August 2013	A	2	Sézar Aparecido dos Santos
19	August 2013	B	1	Osvaldo dos Santos
20	August 2013	B	2	Osvaldo dos Santos
21	13 September 2013	A	1	Sézar Aparecido dos Santos
22	13 September 2013	A	2	Sézar Aparecido dos Santos
23	13 September 2013	B	1	Osvaldo dos Santos
24	October 2013	A	1	Sézar Aparecido dos Santos
25	October 2013	A	2	Sézar Aparecido dos Santos
26	13 November 2013	A	1	Sézar Aparecido dos Santos
27	13 November 2013	A	2	Sézar Aparecido dos Santos
28	December 2013	A	1	Sézar Aparecido dos Santos
29	December 2013	A	2	Sézar Aparecido dos Santos
30	January 2014	A	2	Sézar Aparecido dos Santos
31	January 2014	B	1	Osvaldo dos Santos
32	January 2014	B	2	Osvaldo dos Santos
33	February 2014	A	2	Sézar Aparecido dos Santos
34	February 2014	B	2	Osvaldo dos Santos

(Table I). In each apiary, two Lagstroth®-type beehives of *Apis mellifera* were sampled ($n =$ four hives). One honeycomb from each hive was sampled (completely or partially operculated) every month and aseptically transferred in plastic boxes to the Laboratory of Palynology Research Center (NPP-IBt) at the Botanical Institute, São Paulo, Brazil.

Preparation of the samples

In the laboratory, the honeycombs were squeezed manually, the honey was homogenised and a 50 ml sample from each hive was stored in a -18°C freezer. We followed the method proposed by von der Ohe et al. (2004), a non-acetolytic method, adding the *Lycopodium clavatum* L. foreign marker to perform the pollen concentration calculations. Ten grams of homogenised honey were dissolved in 20 ml of distilled water (20°C). Two tablets of *L. clavatum* were added to the sediment (each tablet contains 12 542 spores) (Buchmann & O'Rourke 1991; Barth & Dutra 2000)

and centrifuged for 5 min at 1800 rpm. The sediment was washed with 10 ml of distilled water and centrifuged again. Then, 5 ml of 1:1 glycerin/distilled water was added to the sediment and allowed to rest for 30 min. It was centrifuged again and finally decanted. The final sediment was mounted on permanent microscope slides (doubled) with glycerine jelly and sealed with paraffin.

Analysis of samples

All optical analysis was performed using an Olympus BX50 microscope at magnifications of $400\times$ and $600\times$. Images were captured using an Olympus U-CMAD-2 camera and the program Olympus Cell-Sens Standard® 1.5. At least 500 pollen grains in each honey sample were counted (250 on each microscope slides) (von der Ohe et al. 2004).

Pollen identification was made by comparison with pollen samples from plants collected from the surrounding areas of the apiaries (Benitez-Bosco

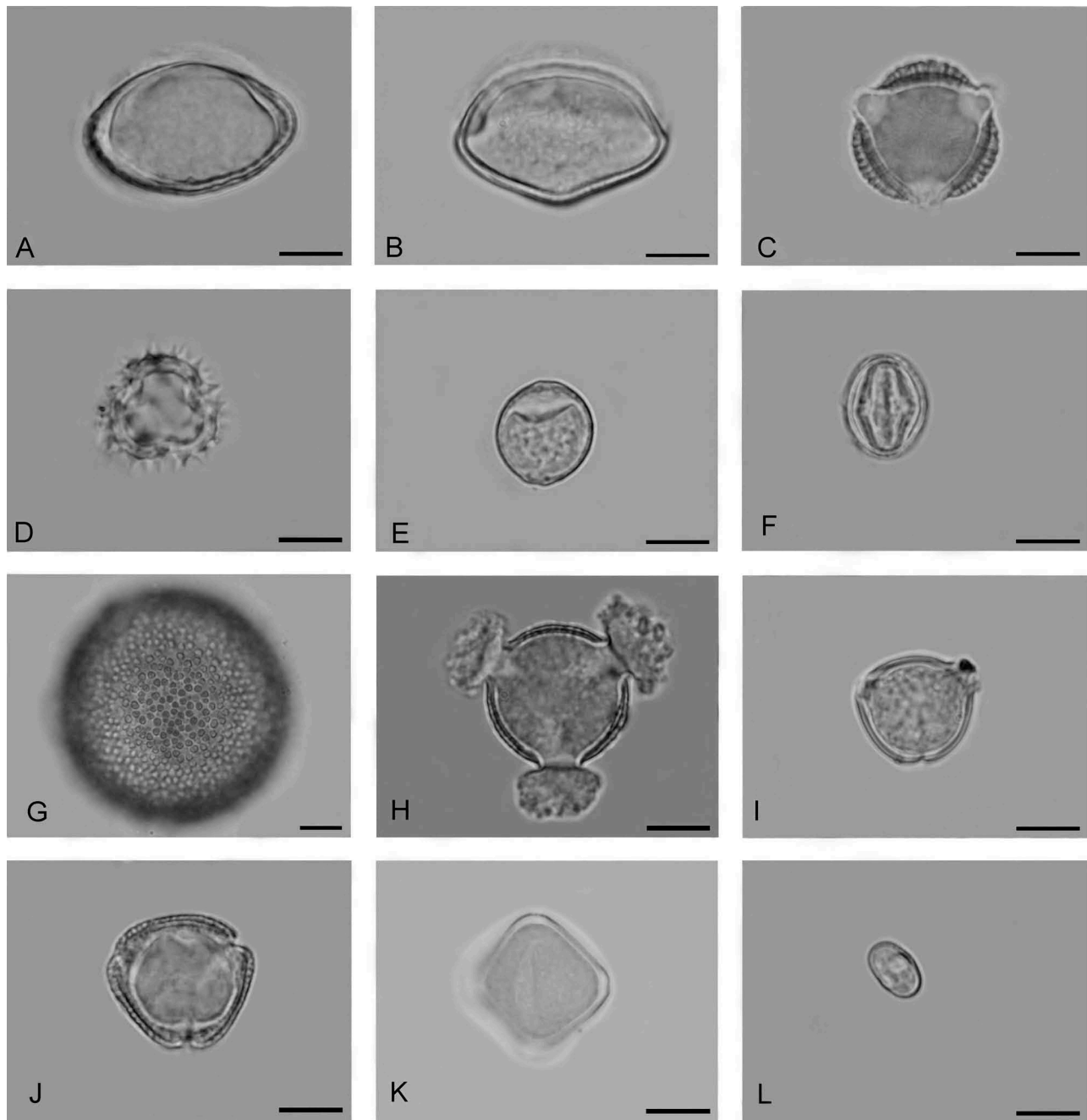


Figure 2. Light microscopy photographs of some pollen types observed in honey samples from *Apis mellifera* from Vale do Ribeira Region, state of São Paulo, Brazil. **A.** Arecaceae: *Attalea*. **B.** Arecaceae: *Euterpe/Syagrus*. **C.** Aquifoliaceae: *Ilex*. **D.** Asteraceae: *Mikania cordifolia*. **E.** Cannabaceae: *Trema micrantha*. **F.** Cunnoniaceae: *Weinmannia*. **G.** Euphorbiaceae: *Croton*. **H.** Fabaceae: *Machaerium*. **I.** Fabaceae: *Tephrosia vogelii*. **J.** Rutaceae: *Zanthoxylum*. **K.** Sapindaceae: *Cupania oblongifolia*. **L.** Urticaceae: *Cecropia*. Scale bars – 20 μ (G), 10 μ (A–F, H–L).

2015), the Palynology Collection of NPP-IBt and relevant published literature (Barth 1970a, 1970b, 1970c, 1970d, 1989, 1998; Melhem et al. 1984; Roubik & Moreno 1991; Cruz-Barros et al. 2006; Moreti et al. 2007). The palynological results were also evaluated based on flora surveys for the region (Costa 2002; Ivanauskas et al. 2012; CRIA 2014;

Lunelli 2014). The microscopy slides are stored in the Palynology Collection of NPP-IBt.

Melissopalynological analysis

A first frequency of occurrence was estimated using the entire pollen spectrum (including anemophilous,

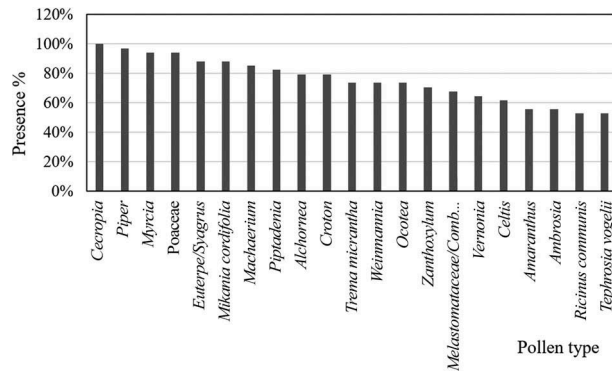


Figure 3. Pollen types found in more than 50% of the samples: *Cecropia* in 100%; *Piper* in 97%; *Myrcia* and *Poaceae* in 94%; *Euterpe/Syagrus* and *Mikania cordifolia* in 88%; *Machaerium* in 85.3%; *Piptadenia* in 82%; *Alchornea* and *Croton* in 79% each; *Trema micrantha*, *Weinmannia* and *Ocotea* in 74% each; *Zanthoxylum* in 71%; *Melastomataceae/Combretaceae* in 68%; *Vernonia* in 65%; *Celtis* in 62%; *Amaranthus* and *Ambrosia* in 56% each; *Ricinus communis* and *Tephrosia vogelii* in 53% each.

polleniferous and nectariferous species) in order to provide an overview of the honeys produced by Porto Velho, and possibly reveal geographical markers for the region and the most valuable plant species used by the bees (Barth 1989; von der Ohe et al. 2004). To determine the botanical origin of the honey, a second frequency of occurrence was estimated using only the relative frequencies of the pollen types of nectariferous species, according to the recommendations of von der Ohe et al. (2004). This shows us the species that actually contributed nectar for the formation of honey, allowing for a more accurate botanical identification of the samples.

First frequency of occurrence. – For the qualitative analysis, the relative frequency of the pollen grains was calculated and presented in five classes: Predominant Pollen (P: >45%), Secondary Pollen (S: 16–45%), Important Minor Pollen (I: 3–15%) and Minor Pollen (m: <3%) (Louveaux et al. 1978). The frequency of occurrence in the samples was based on Jones and Bryant (1996) and used the categories of occurrence: Rare (< 10%), Infrequent (10–20%), Frequent (21–50%) and Very Frequent (> 50%). For the quantitative analysis, the concentration of pollen was estimated from 10 g of honey from each sample and was divided into five categories (von der Ohe et al. 2004): Category I ($\leq 20\,000$ pollen grains), Category II (21 000–100 000), Category III (101 000–500 000), Category IV (501 000–1 000 000) and Category V (> 1 000 000).

Second frequency of occurrence. – Nectariferous plants were identified by comparing pollen grains in the

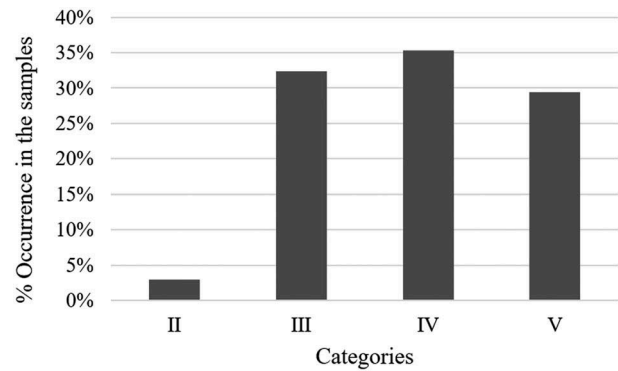


Figure 4. Percentage of honey categories.

samples with pollen grains from plants known to be nectariferous, according to various bibliographical references (see the full table of references in Benitez-Bosco 2015). When the plant related to the pollen type belonged to both polleniferous and nectariferous types, the samples were considered monofloral only when they showed frequency of this pollen type above 90%. For example, of the genera *Eucalyptus* and *Euterpe/Syagrus* (Barth 1989; Barth et al. 2010). Based on this information, the relative frequency (Louveaux et al. 1978) and frequency of occurrence (Jones & Bryant 1996) could be estimated.

Results

First frequency of occurrence, geographical origin of honey

A total of 114 pollen types from 43 families and 24 species was identified (Figure 2, Table II). Four pollen types were identified as belonging to monocotyledons and five could not be identified. All of them had frequencies below 1% in the samples. The mean value of pollen types per honey sample was 28.52. The highest richness was observed in the sample 14 (A2 of July 2013: 56 types) and the lowest in the sample 29 (A2 of December 2013: 11 types) (Table II).

The families with a higher incidence of pollen types were Fabaceae (20 types), Euphorbiaceae (9), Asteraceae (8), Sapindaceae (7), Rubiaceae, Malvaceae and Myrtaceae (5 each). Together these families represent approximately 50% of the pollen types found within the samples.

The Predominant Pollen Category was found in 27 samples (79.4%) (Table I). The *Piper* type had frequencies above 45% (P) in 21 of the 34 samples (61.76%). Another six pollen types were predominant in only one of the samples (*Attalea*, *Mikania cordifolia* (L.f.) Willd., *Poaceae*, *Zanthoxylum*, *Cupania oblongifolia* Mart. and *Cecropia*). Eight types were classified as secondary pollen: *Mikania cordifolia* (four samples), *Myrcia*, *Cecropia*, *Piper* and *Euterpe*

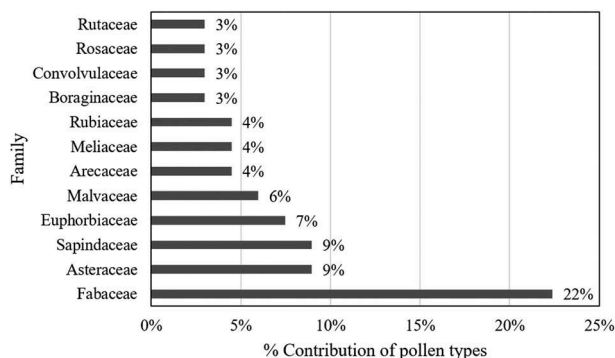


Figure 5. Contribution per family of nectariferous pollen types. Families with more than 3%.

Syagrus (three samples), *Mimosa bimucronata* (DC.) Kuntze (two samples), Poaceae and Melastomataceae/Combretaceae (one sample). The total frequency indicated a rich diversity of pollen types with low frequency (below 1%) in all the samples. Twenty-one types were Very Frequent (> 50%) in the samples and *Cecropia* were present in all samples (Figure 3).

The results of the quantitative analysis (Table II) contained only one sample with medium content of pollen grains (Category II: 2.9%). The other 33 samples all had high (Category III: 32.3%) to very high pollen concentrations (Category IV: 35.3% and Category V: 29.4%). The highest grain concentration in 10 g of honey was observed in the sample 17 (A1 of August 2013: 22 663 394 grains; Category V) and the lowest in 33 (A2 of February 2014: 44 882 grains; Category II) (Figure 4).

Second frequency of occurrence – botanical origin of honey

We found 67 pollen types belonging to 26 families and 15 nectariferous species (Table III). The average number of pollen types was 17. The highest richness of pollen types was observed in sample 14 (A2 of July 2013: 34 types) and the smallest in the sample 29 (A2 of December 2013: 7 types). The families with the greatest representation of nectariferous pollen types were Fabaceae with 15 types (21.7%), Sapindaceae and Asteraceae with six pollen types each (8.7%) and Euphorbiaceae with five types (7.2%) (Figure 5).

Nineteen samples (55.8%) presented Predominant Pollen (> 45%). The types that represent the most important nectariferous sources for the study site were Arecaceae: *Attalea* (one sample) and *Euterpe/Syagrus* (eight samples), Asteraceae: *Mikania cordifolia* (seven samples), Fabaceae: *Machaerium* (one sample), Rutaceae: *Zanthoxylum* (one sample) and Sapindaceae: *Cupania oblongifolia* (one sample).

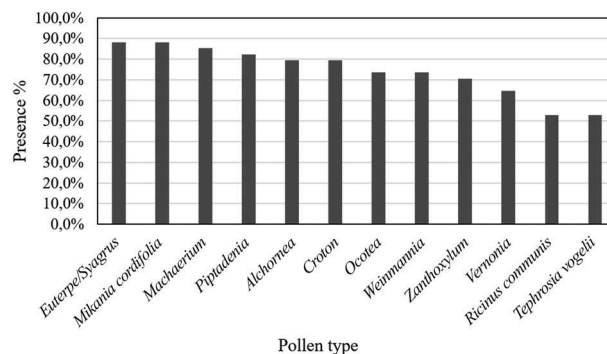


Figure 6. Pollen types of nectariferous plants found in more than 50% of the samples: *Euterpe/Syagrus* and *Mikania cordifolia* (88.2% each); *Machaerium* (85.3%); *Piptadenia* (82.3%); *Alchornea* and *Croton* (79.4% each); *Ocotea* and *Weinmannia* (73.5% each); *Zanthoxylum* (70.5%); *Vernonia* (64.7%); *Ricinus communis* and *Tephrosia vogelii* (52.9% each).

The following types were observed as secondary pollen: *Alchornea*, *Attalea*, *Crotalaria*, *Euterpe/Syagrus*, *Machaerium*, *Matayba*, *Mikania cordifolia*, *Piptadenia*, *Schizolobium parahyba* (Vell.) Blake, *Senecio*, *Vernonia*, *Weinmannia*. A large number of types with low frequency (below 1%) were also observed in the samples. Twelve pollen types were Very Frequent (> 50%) (Figure 6).

The honey from the apiaries of the Quilombola Community of Porto Velho were classified as predominantly heterofloral (67.6% of the samples). Eleven of the samples were classified as monofloral with six being *Mikania cordifolia* and the others from *Machaerium*, *Zanthoxylum*, *Cupania oblongifolia*, *Euterpe/Syagrus* and *Attalea*, respectively (Figure 7).

Discussion

Phytogeographic origin

The richness of pollen types in honey from Porto Velho demonstrates that a wide range of plants are visited by *Apis mellifera*, revealing the great diversity of the flora of the Vale do Ribeira and corroborating the observations made by Kleinert and Giannini (2012) regarding the generalist behaviour of *A. mellifera* when searching for resources.

The pollen spectrum of the honey in Iporanga provided information that allowed for its geographical characterization. It includes native species from the Atlantic Forest of the southeast and south of Brazil, as well as ruderal and secondary plants that occur in areas with some level of environmental disturbance. In the latter group (ruderal and secondary plants occurring in areas with some degree of environmental disturbance), we found the types *Piper*, which was Predominant Pollen in 61.76% samples, and also *Cecropia* and Poaceae, which had the highest

Table II. Pollen types and their frequency-class distribution in the 34 honeys studied.

Pollen types	P	S	I	m	FO	CO
Amaranthaceae						
<i>Amaranthus</i>	0	0	3	16	56	VF
Anacardiaceae						
<i>Schinus terebintifolius</i>	0	0	1	11	35	F
Aquifoliaceae						
<i>Ilex</i>	0	0	1	4	15	I
Arecaceae						
<i>Arecaceae</i>	0	0	0	5	15	I
<i>Attalea</i>	1	0	1	12	41	F
<i>Astrocaryum</i>	0	0	0	2	6	R
<i>Euterpe/Syagrus</i>	0	4	10	16	88	VF
Asteraceae						
<i>Trixis</i>	0	0	0	3	9	R
<i>Ambrosia</i>	0	0	3	16	56	VF
<i>Baccharis</i>	0	0	0	5	15	I
<i>Bidens</i>	0	0	1	15	47	F
<i>Vernonia</i>	0	0	3	19	65	VF
<i>Mikania cordifolia</i>	1	4	4	21	88	VF
<i>Montanoa</i>	0	0	0	4	12	I
<i>Senecio</i>	0	0	1	0	3	R
Bignoniaceae						
<i>Jacaranda micrantha</i>	0	0	0	3	9	R
Boraginaceae						
<i>Cordia</i>	0	0	0	11	32	F
<i>Echium</i>	0	0	0	3	9	R
Burseraceae						
<i>Protium</i>	0	0	0	13	38	F
Cannabaceae						
<i>Celtis</i>	0	0	1	20	62	VF
<i>Trema micrantha</i>	0	0	0	25	74	VF
Caryophyllaceae						
<i>Drymaria</i>	0	0	0	2	6	R
Casuarinaceae						
<i>Casuarina</i>	0	0	0	2	6	R
Celastraceae						
<i>Maytenus</i>	0	0	0	3	9	R
Convolvulaceae						
<i>Jacquemontia blanchetii</i>	0	0	0	1	3	R
<i>Merremia</i>	0	0	0	3	9	R
Cunnoniaceae						
<i>Weinmania</i>	0	0	5	20	74	VF
Cyperaceae						
<i>Cyperaceae</i>	0	0	0	4	12	I
<i>Rhynchospora</i>	0	0	0	1	3	R
Euphorbiaceae						
<i>Acalypha gracilis</i>	0	0	0	1	3	R
<i>Alchornea</i>	0	0	1	26	79	VF
<i>Euphorbia</i>	0	0	0	1	3	R
<i>Croton</i>	0	0	0	27	79	VF
<i>Dalechampia</i>	0	0	0	2	6	R
Euphorbiaceae						
<i>Euphorbiaceae 2</i>	0	0	0	3	9	R
<i>Ricinus communis</i>	0	0	0	18	53	VF
<i>Sebastiania</i>	0	0	1	1	6	R
Fabaceae						
<i>Schizolobium parahyba</i>	0	0	3	10	38	F
<i>Acacia</i>	0	0	0	3	9	R
<i>Anadenanthera</i>	0	0	0	4	12	I
<i>Caesalpinia</i>	0	0	0	2	6	R
<i>Centrosema</i>	0	0	0	3	9	R

(Continued)

Table II. (Continued).

Pollen types	P	S	I	m	FO	CO
<i>Copaifera</i>	0	0	0	2	6	R
<i>Crotalaria</i>	0	0	3	8	32	F
<i>Desmodium</i>	0	0	0	4	12	I
Fabaceae	0	0	0	5	15	I
<i>Galactia</i>	0	0	0	2	6	R
<i>Holocalyx balansae</i>	0	0	0	1	3	R
<i>Indigofera</i>	0	0	0	1	3	R
<i>Inga</i>	0	0	0	12	35	F
<i>Machaerium</i>	0	0	1	28	85	VF
<i>Mimosa bimucronata</i>	0	2	1	13	47	F
<i>Mimosa scabrella</i>	0	0	0	8	24	F
<i>Mimosa verrucosa</i>	0	0	0	1	3	R
<i>Piptadenia</i>	0	0	2	26	82	VF
<i>Tephrosia vogelii</i>	0	0	0	18	53	VF
<i>Vigna</i>	0	0	0	1	3	R
Haloragaceae						
<i>Haloragaceae</i>	0	0	0	1	3	R
Lamiaceae						
<i>Leonurus sibiricus</i>	0	0	0	9	26	F
Lauraceae						
<i>Ocotea</i>	0	0	0	25	74	VF
Loranthaceae						
<i>Loranthaceae</i>	0	0	0	2	6	R
Malpighiaceae						
<i>Byrsonima</i>	0	0	0	1	3	R
Malpighiaceae						
<i>Stigmaphyllon tomentosum</i>	0	0	0	1	3	R
Malvaceae						
<i>Sida rhombifolia</i>	0	0	0	4	12	I
<i>Sida</i>	0	0	0	1	3	R
<i>Bombacopsis</i>	0	0	0	3	9	R
<i>Calianthe rufinerva</i>	0	0	0	3	9	R
<i>Triumfetta</i>	0	0	0	1	3	R
Melastomataceae/ Combretaceae	0	1	1	21	68	VF
Meliaceae						
<i>Cedrela fissilis</i>	0	0	0	1	3	R
<i>Guarea</i>	0	0	0	3	9	R
<i>Trichilia</i>	0	0	0	2	6	R
Moraceae						
<i>Moraceae</i>	0	0	0	4	12	I
<i>Moraceae 2</i>	0	0	1	9	29	F
<i>Morus</i>	0	0	0	9	26	F
<i>Sorocea bonplandii</i>	0	0	0	6	18	I
Myrtaceae						
<i>Eucalyptus</i>	0	0	0	14	41	F
<i>Eugenia</i>	0	0	1	5	18	I
<i>Myrcia 2</i>	0	0	0	2	6	R
<i>Myrcia</i>	0	3	15	14	94	VF
Myrtaceae						
<i>Myrtaceae</i>	0	0	1	1	6	R
Nyctaginaceae						
<i>Guapira</i>	0	0	0	4	12	I
Passifloraceae						
<i>Passiflora</i>	0	0	0	3	9	R
Phyllanthaceae						
<i>Hyeronima alchorneoides</i>	0	0	0	1	3	R
<i>Phyllanthus</i>	0	0	0	1	3	R
Piperaceae						
<i>Piper</i>	21	3	3	6	97	VF
Plantaginaceae						
<i>Mecardonia</i>	0	0	0	1	3	R

(Continued)

Table II. (Continued).

Pollen types	P	S	I	m	FO	CO
Poaceae						
Poaceae	1	1	2	28	94	VF
Proteaceae						
<i>Roupala</i>	0	0	0	1	26	F
Rosaceae						
<i>Rubus</i>	0	0	1	13	41	F
<i>Rubus</i> 2	0	0	0	1	3	R
Rubiaceae						
<i>Diodella teres</i>	0	0	0	1	3	R
<i>Borreria</i>	0	0	0	1	3	R
<i>Ixora</i>	0	0	0	2	6	R
Rubiaceae	0	0	0	1	3	R
<i>Rudgea</i>	0	0	0	8	24	F
Rutaceae						
<i>Citrus</i>	0	0	0	6	18	I
<i>Zanthoxylum</i>	1	0	0	23	71	VF
Salicaceae						
<i>Casearia</i>	0	0	0	5	15	I
Sapindaceae						
<i>Cupania oblongifolia</i>	1	0	0	1	6	R
<i>Cupania vernalis</i>	0	0	0	1	3	R
<i>Cupania</i>	0	0	0	6	18	I
<i>Matayba</i>	0	0	0	3	9	R
<i>Paullinia/Allophylus</i>	0	0	0	5	15	I
<i>Paullinia</i>	0	0	0	1	3	R
<i>Serjania</i>	0	0	0	8	24	F
Solanaceae						
<i>Acnistus</i>	0	0	0	3	9	R
<i>Cestrum</i>	0	0	0	10	29	F
<i>Solanum</i>	0	0	0	6	18	I
Tiliaceae						
<i>Tiliaceae</i>	0	0	0	1	3	R
Urticaceae						
<i>Cecropia</i>	1	3	18	12	100	VF
Verbenaceae						
<i>Aloysia virgata</i>	0	0	0	3	9	R
Indeterminate						
Indetermined 1	0	0	0	8	24	F
Indetermined 2	0	0	0	1	3	R
Indetermined 3	0	0	0	1	3	R
Indetermined 4	0	0	0	3	9	R
Indetermined 5	0	0	0	1	3	R
Monocotyledon 1	0	0	0	5	15	F
Monocotyledon 2	0	0	0	2	6	R
Monocotyledon 3	0	0	0	1	3	R
Monocotyledon 4	0	0	0	2	6	R

Note: P: Predominant Pollen (> 45%); S: Secondary Pollen (16–45%); I: Important Minor Pollen (3–15%); m: Minor Pollen (< 3%); FO: frequency of occurrence; CO: categories of occurrence (R, Rare [< 10%]; I, Infrequent [10–20%]; F, Frequent [21–50%]; VF, Very Frequent [> 50%]).

frequencies of occurrence in the samples. These are very polleniferous species that apparently do not produce nectar as a resource for the bees (Barth 1989; Figueiredo & Sazima 2000; Vit et al. 2013) and are all pioneer species that occur in riparian vegetation, forest borders and clearings. They are also indicators of open or very anthropic areas (Lorenzi 1992, 2008). *Piper* has many species and five

species of the genus *Piper* are found in the bee flora survey conducted in the study site (*Piper aduncum* L., *P. arboreum* Aubl., *P. cernuum* Vell., *P. dilatatum* Rich. and *Piper* sp.) (Benitez-Bosco 2015). The *Cecropia* type was present in every sample studied (although at low frequencies as Minor Pollen or Important Minor Pollen and Predominant Pollen only in sample 27) and it is considered common in the honey produced in the southeast region of Brazil (Barth 1989). Two *Cecropia* species (*C. pachystachya* Trécul and *C. glaziovii* Sneathl) were identified in the surrounding areas of the apiaries (Benitez-Bosco 2015).

Several other pollen types from Porto Velho can be used for their geographical characterization. According to Ramalho et al. (1991b) and Barth (1989), the following types characterise the south region of the country: *Senecio*, *Mimosa scabrella*, Benth. *Matayba*, *Casearia*, *Allophylus* and *Vernonia*. Meanwhile, the types *Ilex*, *Anadenanthera*, *Piptadenia*, *Trixis*, *Triumfetta* and *Zanthoxylum* are characteristic from the southeast region of Brazil (Barth 1989). *Syagrus* has also been cited as one of the most important pollen types for the São Paulo region (Ramalho et al. 1991b), while *Croton*, *Vernonia*, *Passifloraceae* and *Trema* were found in the honey and in the bee pollen from different municipalities of the Vale do Ribeira (Luz et al. 2008; Melo et al. 2009).

The Predominant Pollen types *Mikania cordifolia* (Asteraceae) and *Cupania oblongifolia* (Sapindaceae) correspond to native species that are very nectariferous, highly attractive to the bees and are important for beekeeping in the southeast region of Brazil (Brandão et al. 1984; Lorenzi 2008; Somner et al. 2009, 2015; Ritter et al. 2015). *Mikania cordifolia* blooms abundantly from February to November and corresponds to the period in which this pollen type was found in the Porto Velho honey (Lorenzi 2008). The *Euterpe/Syagrus* type was very frequent between the samples (88%) and can correspond to *Euterpe edulis*. This species is native to the Atlantic Rainforest and Cerrado and indicates undisturbed areas, since it is threatened with extinction (Centro Nacional de Conservação da Flora 2012).

The richest families in pollen types in the honey samples from Porto Velho (Fabaceae, Euphorbiaceae, Asteraceae, Sapindaceae, Malvaceae, Myrtaceae and Rubiaceae) were also the ones with the highest number of species in the flora survey conducted by Luz et al. (2008) and Ivanauskas et al. (2012) in honeys from Vale do Ribeira. Furthermore, our results are in agreement with the data published by Alves (2008) and Borsato et al. (2014) in which Fabaceae, Euphorbiaceae, Rubiaceae and Asteraceae are families significantly represented in honey samples from *Apis mellifera* in the

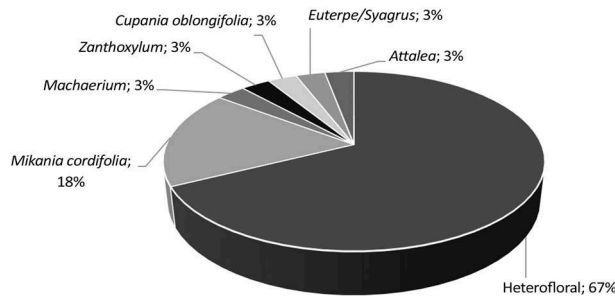


Figure 7. Percentage distribution of honey types: 23 honeys were heterofloral (67%) and 11 were monofloral (33%).

state of Paraná. The families Euphorbiaceae, Asteraceae and Fabaceae have been identified in honey samples from the states of São Paulo and Paraná (Marchini et al. 2001; Sereia et al. 2011).

The quantitative analysis of the samples also reflected the richness of pollen grains and abundance of polleniferous species. The calculated concentrations of pollen (von der Ohe et al. 2004) showed 33 samples with high (Category III: 32.3%) to very high pollen concentrations (Category IV: 35.3% and Category V: 29.4%). Category III had Predominant Pollen, in general from species that produce large quantities, corroborating the description of this category as including monofloral honey with pollen from over-represented species. Category V includes squeezed honey with large amounts of pollen.

Botanical origin

Despite the importance of pollen grains of species without nectar for bee feeding and geographic characterization of their products, the nectariferous species are the ones that effectively contribute to the production of honey. The second frequency of occurrence showed that 58.7% of the pollen types present in honey produced in Porto Velho are from nectariferous plants, which suggests the area has a great potential for the production of honey and a large pasture for bees.

The nectariferous families with greatest richness of pollen types in the honey (Fabaceae, Sapindaceae, Asteraceae and Euphorbiaceae) have also been identified as important in research on beekeeping flora in the same area (Benitez-Bosco 2015). Ramalho et al. (1990) demonstrated that families that have a high number of species in an area also tend to be the main sources of nectar and/or pollen for *Apis mellifera*.

The pollen types *Croton*, *Machaerium*, *Mikania cordifolia*, *Piptadenia*, *Matayba*, *Cupania oblongifolia*, *Vernonia* and *Alchornea* were very frequent in the samples and are related to very nectariferous species abundantly present in the study area and

are important for apiculture. The pollen types *Attalea*, *Euterpe/Syagrus* and *Weinmannia*, frequently found in the samples, are of species that other studies indicate as a source of nectar for bees (Benitez-Bosco 2015). Although these species were identified as sources of pollen and nectar, the proportion of these resources is still unknown, except for the *Euterpe/Syagrus* type, which produces both resources (Dorneles et al. 2013).

The honeys from Quilombola Community of the Porto Velho apiaries were classified as predominantly heterofloral (67.6% of the samples), corroborating the potential of the states of São Paulo and Paraná, and the Vale do Ribeira, for the production of this kind of honey (Barth 2004). Monofloral honeys were mainly from *Mikania cordifolia*. This species is very nectariferous and of great importance for beekeeping. It has abundant flowering from February to November, which corresponds to the period in which this pollen type was predominant in the honey of Porto Velho (Pirani & Cortopassi-Laurino 1993; Marchini et al. 2001; Mendonça et al. 2008). Another monofloral honey was *Zanthoxylum* (Rutaceae), which was also observed in honey samples in the state of Paraná by Sekine (2011) as Very Frequent. *Zanthoxylum* includes nectariferous tree species native to the Atlantic Forest in primary forest clearings or secondary forests (Embrapa 2011). Various species of this genus are found in the Ribeira Valley, some of them are: *Zanthoxylum chiloperone*, Mart. ex Engl. *Z. fagara*, (L.) Sarg. *Z. riedelianum*, (Engl.) *Z. rhoifolium* Lam. (Coffani-Nunes & Weissenberg 2010). *Zanthoxylum rhoifolium* is found in the municipality of Iporanga (CRIA 2014) and its flowering is concentrated in September–December (Embrapa 2011), during which period it was observed in honey samples from Porto Velho (August).

The number of pollen types in the samples combined with the knowledge of the regional flora provided us with a relevant dataset. The richness of nectariferous pollen types observed in sample 14 (A2 of July 2013: 34 types) and the low diversity in sample 29 (A2 of December 2013: seven types) are contrary to the periods of higher and lower flowerings of the species in the study area. Benitez-Bosco (2015) observed that between September and December (possibly extending until the end of January) is the period of the highest blooming of the species and between May to July is the period of the lowest blooming and production of honey in the region. According to many authors, in periods of high abundance of flowers, *Apis mellifera* can show high foraging activity in few floral sources (Cortopassi-Laurino & Ramalho 1988; Ramalho et al. 1989, 1990). Meanwhile, in seasons with a reduced availability of floral resources, the bees have to search

Table III. Summarised results of second frequency of occurrence (only nectariferous pollen types)

Sample	Frequency-class distribution			Number of pollen types
	P	S	I	
1	—	<i>Euterpe/Syagrus</i> (26%), <i>Crotalaria</i> (17.1%), <i>Machaerium</i> (17.1%), <i>Piptadenia</i> (17.1%)	<i>Bidens</i> (7.5%), <i>Alchornea</i> (4.8%), <i>Croton</i> (4.8%), <i>Weinmannia</i> (3.4%)	9
2	—	<i>Mikania cordifolia</i> (32.3%), <i>Machaerium</i> (29.6%)	<i>Bidens</i> (10%), <i>Groton</i> (5%), <i>S. paralytba</i> (4.4%), <i>Euterpe/Syagrus</i> (4.4%), <i>Vernonia</i> (3.4%)	22
3	<i>Mikania cordifolia</i> (71.3%)	—	<i>Piptadenia</i> (12.6%), <i>Machaerium</i> (4.6%), <i>Euterpe/Syagrus</i> (3.4%)	13
4	<i>Mikania cordifolia</i> (47.6%)	—	<i>Euterpe/Syagrus</i> (11.9%), <i>Ocotea</i> (11.9%), <i>Croton</i> (9.5%), <i>Machaerium</i> (4.8%)	11
5	—	<i>Attalea</i> (32.3%)	<i>Piptadenia</i> (12.3%), <i>Mikania cordifolia</i> (9.2%), <i>Croton</i> (7.7%), <i>Alchornea</i> (6.1%), <i>Echium</i> (4.6%), <i>Protium</i> (3%), <i>Inga</i> (3%), <i>Machaerium</i> (3%)	14
6	—	<i>Machaerium</i> (26.7%)	<i>Euterpe/Syagrus</i> (15.7%), <i>Mikania cordifolia</i> (12.2%), <i>Weinmannia</i> (11.9%), <i>Casearia</i> (9.4%), <i>Piptadenia</i> (5%), <i>Alchornea</i> (3.7%)	21
7	<i>Machaerium</i> (50%)	—	<i>Alchornea</i> (10.9%), <i>Mikania cordifolia</i> (10.8%), <i>Crotalaria</i> (8.7%), <i>Attalea</i> , <i>Croton</i> , <i>Piptadenia</i> (4.3%)	10
8	<i>Euterpe/Syagrus</i> (45.2%)	—	<i>Mikania cordifolia</i> (11.5%), <i>Machaerium</i> (10.5%), <i>Weinmannia</i> (7.3%), <i>Alchornea</i> (6.3%), <i>Schinus terebinifolius</i> (5.3%), <i>Piptadenia</i> (4.2%), <i>Croton</i> (3.2%), <i>Ocotea</i> (3.2%)	12
9	—	<i>Alchornea</i> (40.5%), <i>Weinmannia</i> (24.1%)	<i>Euterpe/Syagrus</i> (14.1%), <i>Machaerium</i> (9.2%)	23
10	—	<i>Mikania cordifolia</i> (22.3%), <i>Euterpe/Syagrus</i> (21.4%)	<i>Weinmannia</i> (14.1%), <i>Machaerium</i> (7.6%), <i>Croton</i> (7%), <i>Cupania</i> (6.8%), <i>Alchornea</i> (5%), <i>Ocotea</i> (3.4%)	20
11	—	<i>Weinmannia</i> (39.1%)	<i>Euterpe/Syagrus</i> (15.7%), <i>Machaerium</i> (12.4%), <i>Cupania</i> (6.5%), <i>Piptadenia</i> (4.5%), <i>Croton</i> (3.8%)	25
12	—	<i>Machaerium</i> (21.6%), <i>Matayba</i> (19.8%),	<i>Weinmannia</i> (15.1%), <i>Ixora</i> (15.4%), <i>Euterpe/Syagrus</i> (13.1%), <i>Croton</i> (11%)	22
13	—	<i>Weinmannia</i> (22.3%), <i>Mikania cordifolia</i> (18.2%)	<i>Euterpe/Syagrus</i> (15.6%), <i>Ixora</i> (10%), <i>Machaerium</i> (7.8%), <i>Tephrosia vogelii</i> (5.2%), <i>Croton</i> (3.3%), <i>Piptadenia</i> (3.3%), <i>Euterpe/Syagrus</i> (3.8%)	23
14	—	<i>Mikania cordifolia</i> (31.2%)	<i>Schinus terebinifolius</i> (8%), <i>Alchornea</i> (6.5%), <i>Vernonia</i> (6%), <i>Zanthoxylum</i> (5%), <i>Rubus</i> 1 (5%), <i>Ricinus communis</i> (5%), <i>Ocotea</i> (4.4%), <i>Tephrosia vogelii</i> (3.9%), <i>Bidens</i> (3.8%), <i>Triumfetta</i> (3.8%)	34
15	<i>Mikania cordifolia</i> (59.6%)	<i>Crotalaria</i> (20.3%)	<i>Tephrosia vogelii</i> (4.8%), <i>Vernonia</i> (4.7%)	18
16	<i>Euterpe/Syagrus</i> (55.95%)	—	<i>Croton</i> (7.1%), <i>Anadenanthera</i> (7.1%), <i>Piptadenia</i> (7.1%), <i>Tephrosia vogelii</i> (5.9%), <i>Zanthoxylum</i> (5.9%), <i>Vernonia</i> (3.5%), <i>Weinmannia</i> (3.5%)	10
17	<i>Zanthoxylum</i> (74.2%)	—	<i>Sebastiania</i> (7%), <i>Baccharis</i> (6.4%), <i>Crotalaria</i> (4.8%), <i>Ricinus communis</i> (3.4%)	14
18	<i>Mikania cordifolia</i> (61.3%)	—	<i>Machaerium</i> (6.6%), <i>Bidens</i> (5.8%), <i>Serjania</i> (4.4%), <i>Vernonia</i> (3.6%)	15
19	<i>Mikania cordifolia</i> (59.52%)	—	<i>Euterpe/Syagrus</i> (15.7%), <i>Rubus</i> (5.9%)	17
20	<i>Mikania cordifolia</i> (48.37%)	<i>Vernonia</i> (20%)	<i>Euterpe/Syagrus</i> (12.6%), <i>Citrus</i> (5.5%)	19

21	—	<i>Euterpe/Syagrus</i> (33.3%)	<i>Weinmania</i> (15.9%), <i>Piptadenia</i> (11%), <i>Alchornea</i> (9.1%), <i>Machaerium</i> (8.2%), <i>Mikania cordifolia</i> (4%)	29
22	—	<i>Vernonia</i> (35.4%), <i>Schizolobium parahyba</i> (24.4%)	<i>Bidens</i> (11%), <i>Galactia</i> (6.2%), <i>Mikania cordifolia</i> (5.3%), <i>Rubus</i> 1 (4.3%)	16
23	<i>Mikania cordifolia</i> (41.32%)	<i>Euterpe/Syagrus</i> (18.2%)	<i>Attalea</i> (6.9%), <i>Schinus terebinthifolius</i> (6.7%), <i>Rubus</i> (5.3%), <i>Bidens</i> (3.6%), <i>Ocotea</i> (3.4%)	30
24	<i>Cupania</i> <i>oblongifolia</i> (94%)	—	<i>Schizolobium parahyba</i> (3.8%)	10
25	—	<i>Euterpe/Syagrus</i> (20.2%), <i>Senecio</i> (19.2%)	<i>Ilex</i> (13.4%), <i>Weinmania</i> (12.5%), <i>Schizolobium parahyba</i> (12.5%), <i>Vernonia</i> (7.7%), <i>Mikania cordifolia</i> (6.7%), <i>Maytenus</i> (3.8%)	12
26	—	<i>Weinmania</i> (37.6%), <i>Euterpe/Syagrus</i> (16.2%), <i>Alchornea</i> (16.2%)	<i>Croton</i> (6.4%), <i>Inga</i> (6.2%)	20
27	<i>Euterpe/Syagrus</i> (88%)	—	<i>Desmodium</i> (3.7%)	14
28	<i>Euterpe/Syagrus</i> (64.8%)	—	<i>Piptadenia</i> (13.7%), <i>Weinmania</i> (8.3%), <i>Croton</i> (4.2%)	12
29	<i>Euterpe/Syagrus</i> (94%)	—	<i>Zanthoxylum</i> (3.3%)	7
30	<i>Euterpe/Syagrus</i> (38.9%)	<i>Mikania cordifolia</i> (16.8%)	<i>Croton</i> (9.2%), <i>Machaerium</i> (6.9%), <i>Weinmania</i> (5.3%), <i>Inga</i> (5.3%), <i>Piptadenia</i> (4.6%), <i>Casearia</i> (3%)	16
31	<i>Euterpe/Syagrus</i> (64.24%)	<i>Piptadenia</i> (17.4%)	<i>Machaerium</i> (4.1%), <i>Zanthoxylum</i> (3.8%), <i>Attalea</i> (3.5%), <i>Croton</i> (3.5%),	11
32	<i>Euterpe/Syagrus</i> (70.75%)	—	<i>Mikania cordifolia</i> (10.1%), <i>Ocotea</i> (5%)	19
33	—	<i>Euterpe/Syagrus</i> (35.8%), <i>Crotalaria</i> (21%)	<i>Alchornea</i> (9%), <i>Mikania cordifolia</i> (7.5%), <i>Baccharis</i> (6%), <i>Weinmania</i> (6%)	15
34	<i>Attalea</i> (97.4%)	—	—	14

Note: P: Predominant Pollen (> 45%); S: Secondary Pollen (16–45%); I: Important Minor Pollen (3–15%).

for resources in a greater number of plant species, which in turn would be reflected in the abundance of pollen types in the resulting honey (Gary 1992). Thus, sample 14 was classified as heterofloral while sample 29 as monofloral of *Attalea*.

Conclusion

The melissopalynological analysis for the present study enabled us to determine the botanical and geographical origin of honey from Porto Velho, expanding the knowledge about the relation between the bees and the plant species in the Vale do Ribeira. The presence of pollen types related to species from the Atlantic Rainforest and the ruderal and secondary species confirms the viability of using apiculture as a source of income in areas with native forests. The samples analysed can be classified according to their geographical origin as belonging to the states of the south and southeast of Brazil and coming from areas of Atlantic Rainforest with some degree of disturbance. The most important nectariferous sources for the beekeepers from the Community of Porto Velho are *Attalea*, *Euterpe/Syagrus*, *Mikania cordifolia*, *Machaerium*, *Zanthoxylum* and *Cupania oblongifolia*. Recognition of native plants used by bees can help persuade beekeepers to preserve them around apiaries. Determining the composition of the honeys could help in obtaining a quality seal for the product, without which it would not be possible to market, and increase the value of the product.

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No potential conflict of interest was reported by the authors.

ORCID

Cynthia Fernandes Pinto da Luz  <http://orcid.org/0000-0001-7908-155X>

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