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Pollen morphology of Myrtaceae visited by social bees

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Abstract

This study aimed to characterize species of Myrtaceae belonging to genera already identified in the pollen spectrum of beehive products of social bees, describing pollen grains morphology and correlating with taxonomic differences the species reported in the literature. For each plant species, measurements were made with 25 pollen grains in equatorial view and the polar diameter (PD) and equatorial diameter (ED) were measured. The exine thickness was obtained by measuring 10 pollen grains in polar view at the medium height of mesocolpium. The description of pollen morphology showed that pollen grains of the species are triangular *amb*, 3-colporates, psilate surface, small to medium size and oblate shape with the P/E ratio ranging from 0.52 to 0.72 μm . The morphological description of Myrtaceae species showed similarity between several characteristics common to pollen grains of this species. The description also allowed separation of nine species studied by size and specific morphological characters with the identification key, which can also be used to study the pollen spectrum of hive products.

Keywords: Pollen grain; palynotaxonomy; plant; bee flora.

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Introduction

The study of bee flora indicates food sources that bees use to collect nectar and pollen, allowing to maximize the use of natural resources, both in the establishment and upkeep of local apicultural flora, in areas of natural or cultivated vegetation (1-2). Several botanical families stand out as nectar and pollen sources for bees and different studies (3-5) have reported Myrtaceae as one of the most important. However, studies on palynotaxonomy of apicultural plants are scarce and our study is the first to correlate pollen morphology of Myrtaceae to bee flora.

Myrtaceae is considered of great ecological relevance, since this family presents characteristics of apicultural plants (pollen and nectar production), besides producing edible fruits, highly appreciated by wild fauna and humans (6-7). Additionally, Myrtaceae is among the most diversified families in Brazilian vegetation formations (8-9).

With roughly 140 genera and more than 3000 species, the Myrtaceae family has its two main dispersion centers in the Americas and Australia. In Brazil, there are approximately 1000 species described and distributed in 23 genera (10-12). The genera of Myrtaceae belonging to the

Leptospermoideae subfamily with greater diversity of species are *Eucalyptus* (500 species) and *Malaleuca* (100 species). For the Myrtoideae subfamily are *Eugenia* (600 species), *Myrcia* (300 species), *Syzygium* (200 species) and *Psidium* (100 species) (13).

Among the exotic species of Myrtaceae in Brazil, the genera *Eucalyptus* (with many timber and essential oil species), *Callistemon* and *Mellaleuca* (as ornamental species) stand out. Other species of relevance are *Pimenta dioica* and *Syzygium aromaticum* (used as spices in culinary), besides the fructiferous genera *Eugenia uniflora*, *S. cumini* and *S. malaccensis* (14-16).

The potential of eucalyptus for honey production is well known and both beekeepers and foresters explore this potential to generate extra income from their eucalyptus crops. *Eucalyptus* flowers are very attractive to bees because of high pollen and nectar concentrations, ideal for good development of hives (17-18). The *Eucalyptus* species have variable pollen production and sometimes are classified as nectariferous or polliniferous (19).

Studies on the botanical identity of pollen in honey or bee pollen from the state of Bahia, Brazil, highlight the Myrtaceae as one of the most important families for the sustainable development of beekeeping and meliponiculture (5,20-21). However, identification of the botanical affinity of pollen types in bee products is not always an easy task. Myrtaceae is considered stenopalynous, with pollen grains peroblates to suboblates, isopolar or heteropolar, radial, 3-colporate, biconvex, convex or concave-convex plane, triangular or sub-triangular *amb* (outline of a pollen grain seen in

polar view), with perforated exine or psilate (22). These complex taxonomic characteristics are important tools to identify pollen types in bee products.

Our study aimed to characterize species of Myrtaceae belonging to genera identified in the pollen spectrum of beehive products of social bees in the Recôncavo of Bahia, Brazil, describing the morphology of pollen grains and correlating with taxonomic differences reported in the literature. The results obtained may be useful in the characterization of hive products. This is the first work that correlates palynotaxonomy of Myrtaceae with bee flora.

Material and methods

Sampling and site study

We selected species *Eucalyptus alba* Reinw. ex Blume, *Eucalyptus* sp.I, *Eucalyptus* sp.II, *Eucalyptus torelliana* F. Muell., *Eugenia uniflora* L., *Psidium araca* Raddi, *Psidium guajava* L., *Syzygium cumini* (L.) Skeels and *Syzygium malaccensis* L. The species were selected based on studies that report the importance of Myrtaceae species as a trophic resource used by social bees (Table 1). The vegetal material was collected and herborized to identify the taxons. The samples were collected in the municipality of Cruz das Almas, located in the Recôncavo of Bahia (12° 40' 12" S; 39° 06' 07" W and altitude 220m) (Brazil) in the Atlantic Forest biome.

Sample preparation

Flower buds were collected from each species to prepare the slides for microscopy with the respective polliniferous materials, using the

Table 1. Species of Myrtaceae identified as trophic resources for social bees (Hymenoptera: Apidae).

Species / Pollen Type	Beehive products / flora visited by bees	Bee species	Source
<i>Eucalyptus</i> sp.	honey; stored pollen; flora visited	<i>Apis mellifera</i> L.; <i>Melipona scutellaris</i> Latreille.	Nascimento <i>et al.</i> (4-5); Oliveira <i>et al.</i> (20)
<i>Eucalyptus torelliana</i> F. Muell.	flora visited	<i>Apis mellifera</i> L.	Nascimento <i>et al.</i> (5)
<i>Eugenia</i> sp.	honey	<i>Melipona mandacaia</i> Smith; <i>Melipona scutellaris</i> Latreille.	Santana <i>et al.</i> (3)
<i>Eugenia uniflora</i> L.	honey	<i>Melipona asilvai</i> Moure; <i>Apis mellifera</i> L.	Santana <i>et al.</i> (3); Nascimento <i>et al.</i> (4)
<i>Eugenia uvalha</i> L.	honey	<i>Melipona quadrifasciata</i> Lepeletier.	Santana <i>et al.</i> (3)
<i>Psidium araca</i> Raddi.	flora visited	<i>Apis mellifera</i> L.	Nascimento <i>et al.</i> (5)
<i>Psidium guajava</i> L.	honey	<i>Scaptotrigona tubiba</i> Smith.	Santana <i>et al.</i> (3)
<i>Psidium guajava</i> L.	honey; flora visited	<i>Apis mellifera</i> L.	Nascimento <i>et al.</i> (4-5)
<i>Psidium</i> sp.	honey; stored pollen	<i>Tetragonisca angustula</i> Latreille; <i>Melipona scutellaris</i> Latreille.	Santana <i>et al.</i> (3); Oliveira <i>et al.</i> (20)
<i>Syzygium cumini</i> (L.) Skeels	flora visited	<i>Apis mellifera</i> L.	Nascimento <i>et al.</i> (5)
<i>Syzygium malaccensis</i> L.	flora visited	<i>Apis mellifera</i> L.	Nascimento <i>et al.</i> (5)
<i>Syzygium</i> sp.	honey	<i>Apis mellifera</i> L.	Nascimento <i>et al.</i> (4)

standard method of acetolysis (23). After assembly of the pollen material on slides for microscopy images were captured, photomicrographs of pollen grains in equatorial and polar views of each species, within a maximum of seven days. We use a microscope Olympus (CH30) with a digital camera (Moticam-2300) coupled. All photomicrographs were captured with the 100x objective.

Pollen morphology

For each plant species, measurements were made with 25 grains of pollen in equatorial view, measuring the polar diameter (PD) and equatorial diameter (ED). The exine thickness was obtained by measuring 10 pollen grains in polar view at the medium height of mesocolpium. Morphological descriptions were based on specialized literature (24-26). Morphometric measurements were performed using Motic Imagens Plus 2.0ML software.

Statistical analysis

We performed the descriptive statistical analysis, calculating the arithmetic mean (\bar{x}), the standard deviation of the mean ($SD\bar{x}$) and the confidence interval (95%) of the respective diameters, as well as the P/E ratio. In addition, Principal Component Analysis (PCA) was used to identify the similarity of species of Myrtaceae. The linear correlation coefficient was also calculated by the Euclidean Distance Matrix (EDM), using PAST 3.x software version 3.20, April 2018 (27).

Results and Discussion

The description of the pollen morphology of the species Myrtaceae evaluated in our study with the respective averages of diameters and thicknesses of exine are presented in Table 2 and 3. The pollen grains of Myrtaceae presented triangular *amb*, 3-colporate, regularly parasyncolpate aperture type, psilate surface of exine, oblate shape and size of small to medium.

Myrtaceae pollen is trizonocporate, isopolar and radiosimetric. Triangular in polar view, with apertures at the angles and elliptic in equatorial view. Small to medium size (28-30). According to Silva *et al.* (31), pollen grains of this family are peroblates to suboblates, isopolar or hetero-polar, radial and with exine perforated or psilate. These characteristics were also observed for the species analyzed in our study (Table 2-3 and Fig. 1).

Erdtman (32) considered the Myrtaceae family relatively stenopalynous in a study of 45 species of 30 genera. Silva *et al.* (22,31) also reported this characteristic. Stenopalynous taxa present great similarity in pollen morphology within the group, hindering separation of individuals (33).

Barth (34) found a like result for *E. uniflora*, *P. araca* and *P. guajava* for *Myrcia rostrata* D.C.,

which, according to this author, presents small pollen grains, 3-colporate, oblate spheroids, measuring 13.5 x 17.5 μm , long and strait colpus, rarely syncolpates, circular endo-apertures (4.5 μm), triangular *amb*, with a smooth surface exine. This author (34) also reports that pollen grains of *Myrcia* type were found as accessory pollen in two honey samples from the state of Bahia, indicating the importance of Myrtaceae species for the collection of trophic resources by social bees.

There was variation between the measurements for the Myrtaceae species within the same genus and different genera (Table 3). In addition, we performed the Principal Component Analysis (PCA), which revealed the greatest similarity between *Eucalyptus alba* and *Eucalyptus* sp.II, influenced by measurements of the polar diameter (PD). *Eucalyptus* sp.I and *Eucalyptus torelliana* presented measurements that were more similar with greater contribution of the equatorial diameter (ED) (Fig. 2). The first two main components accounted for 99.85% of the total variability of the results analyzed. The Euclidean Distance Matrix (EDM) confirmed the PCA results (Table 4) and *E. alba* x *Eucalyptus* sp.I were the nearest (d=2.64); *Eucalyptus* sp.II x *E. alba* (d=2.06) and *E. torelliana* x *Eucalyptus* sp.II (d=1.96).

A study carried out with three species of *Eucalyptus* showed that the species presented size varying between small and medium, triangular *amb*, 3-colporate, syncolpates, exine thickness \pm 2.1 μm and psilate surface (35), similar to our results (Table 2-3).

Takeda *et al.* (36) described the pollen morphology of *Eugenia involucrata* DC. as pollen grains of small size, tricolporate, angulaperturate, peroblate, with the P/E ratio of 0.38 μm . We found a similar result for *E. uniflora* in our study, with variation in the P/E ratio of 0.64 μm ; however, the pollen grain was also classified as small size.

For *Psidium guineense*, Matos *et al.* (37) observed small pollen grain; prolate; tricolporate/tetracolporate, psilate and micoreticulate, the P/E ratio of 1.38 μm . Comparing the results of our study for species of the same genus (*P. araca* and *P. guajava*) shows that some morphological characteristics, such as size and shape, are evident for separation of these species (Table 3). The pollen grain morphology for *Psidium* species is useful to distinguish similarity between species of this group, and the exine ornamentation, the polar diameter in equatorial view and shape of the pollen grains are the most relevant morphological features (38).

The *Eucalyptus* species presented higher exine thickness (Table 3), while the lowest thickness was recorded for *Syzygium* species. Comparing the two species of genus *Syzygium*, we observed that *S. malaccensis* L. presented the lowest P/E ratio (0.52 μm). The *Eucalyptus* species stood out, because all presented pollen grains of

Table 2. Description of the pollen morphology of Myrtaceae species visited by social bees (Apidae) in Cruz das Almas, Recôncavo region of Bahia, Brazil.

Species/Myrtaceae	<i>amb</i> (outline)	Size	Surface	Aperture type	Aperture number	Fig. 1
<i>Eucalyptus alba</i> Reinw. ex Blume	triangular	medium	psilate	parasyncolpate	3-colporates	A-B
<i>Eucalyptus</i> sp. I	triangular	medium	psilate	parasyncolpate	3-colporates	C-D
<i>Eucalyptus</i> sp. II	triangular	medium	psilate	parasyncolpate	3-colporates	E-F
<i>Eucalyptus torelliana</i> F. Muell.	triangular	medium	psilate	parasyncolpate	3-colporates	G-H
<i>Eugenia uniflora</i> L.	triangular	small	psilate	parasyncolpate	3-colporates	I-J
<i>Psidium araca</i> Raddi.	triangular	small	psilate	parasyncolpate	3-(4)-colporates	K-M
<i>Psidium guajava</i> L.	triangular	small	psilate	parasyncolpate	3-colporates	N-O
<i>Syzygium cumini</i> (L.) Skeels	triangular	small	psilate	parasyncolpate	3-colporates	P-Q
<i>Syzygium malaccensis</i> L.	triangular	small	psilate	parasyncolpate	3-colporates	R-T

Table 3. Dimensions of pollen grains of Myrtaceae species visited by social bees (Apidae) in Cruz das Almas, Recôncavo region of Bahia, Brazil.

Species/ Myrtaceae (n=9)	PD (n=25)		ED (n=25)		P/E (μ m)	Exine thickness (n=10)		Form
	Mean \pm SD (μ m)	CI to 5% (μ m)	Mean \pm SD (μ m)	CI to 5% (μ m)		Mean \pm SD (μ m)	CI to 5% (μ m)	
	<i>Eucalyptus alba</i>	17.72 \pm 0.98	17.33 - 18.10	26.35 \pm 1.05		25.94 - 26.76	0.67	
<i>Eucalyptus</i> sp. I	15.39 \pm 0.69	15.12 - 15.70	25.74 \pm 0.85	25.41 - 26.08	0.60	1.69 \pm 0.27	1.53 - 1.86	Oblate
<i>Eucalyptus</i> sp. II	17.38 \pm 0.91	17.02 - 17.70	26.60 \pm 0.89	26.25 - 26.95	0.65	2.40 \pm 0.50	2.09 - 2.70	Oblate
<i>Eucalyptus torelliana</i>	15.95 \pm 1.46	15.38 - 16.50	26.76 \pm 1.31	26.24 - 27.27	0.60	1.53 \pm 0.15	1.44 - 1.63	Oblate
<i>Eugenia uniflora</i>	10.98 \pm 0.78	10.68 - 11.30	17.08 \pm 1.25	16.59 - 17.57	0.64	1.17 \pm 0.12	1.09 - 1.24	Oblate
<i>Psidium araca</i>	16.73 \pm 0.83	16.40 - 17.10	23.33 \pm 1.05	22.91 - 23.74	0.72	1.59 \pm 0.16	1.49 - 1.69	Oblate
<i>Psidium guajava</i>	10.39 \pm 0.77	10.09 - 10.70	16.69 \pm 0.55	16.47 - 16.91	0.62	1.26 \pm 0.13	1.18 - 1.35	Oblate
<i>Syzygium cumini</i>	9.68 \pm 0.91	9.33 - 10.00	16.21 \pm 0.68	15.94 - 16.47	0.60	1.17 \pm 0.23	1.03 - 1.31	Oblate
<i>Syzygium malaccensis</i>	8.76 \pm 0.76	8.32 - 9.20	17.00 \pm 0.87	16.48 - 17.52	0.52	1.12 \pm 0.12	1.04 - 1.19	Oblate

CI= confidence interval, ED= equatorial diameter, PD= polar diameter and SD= standard deviation

medium size. A study of 140 Myrtaceae species from southern Brazil showed that all have in common the general palynological characteristics of this family, such as pollen grain of medium to small size, oblate to peroblate, 3-colporate of triangular *amb*, surface granulated in mesocolpium and apocolpium, smoother around the apertures (24).

In the products of the hive Myrtaceae taxa are often identified (3-5). In a study conducted by Nascimento et al. (5) the pollen types *E. uniflora* and *P. guajava* occurred among samples of *Apis mellifera* Linnaeus, 1758 honey as predominant pollen and very frequent. Oliveira et al. (20) analyzed honey samples of *Melipona scutellaris* Latreille, 1811 verified the presence of pollen type *Eucalyptus* sp. classified as secondary pollen between the samples. In addition, these authors observed that only *Melipona quadrifasciata anthidioides* Lepeletier, 1836 collected trophic resources in *E. uniflora* in the studied area. These results indicate the relevance of this plant group as

a source of trophic resources for social bees. In this way, a key to identify pollen of the species commonly represented in the pollen spectrum of bee products is presented below.

Identification key of pollen grains of Myrtaceae species visited by social bees (Hymenoptera: Apidae) (based on Table 3):

1. Pollen grains with a polar diameter smaller than 12.00 μ m.....2
- 1'. Pollen grains with a polar diameter greater than 12.00 μ m5
2. Pollen grains with a polar diameter smaller than or equal to 10.00 μ m3
- 2'. Pollen grains with a polar diameter greater than 10.00 μ m4
3. Pollen grains with a polar diameter smaller than 9.20 μ m and equatorial diameter between 16.48 and 17.52

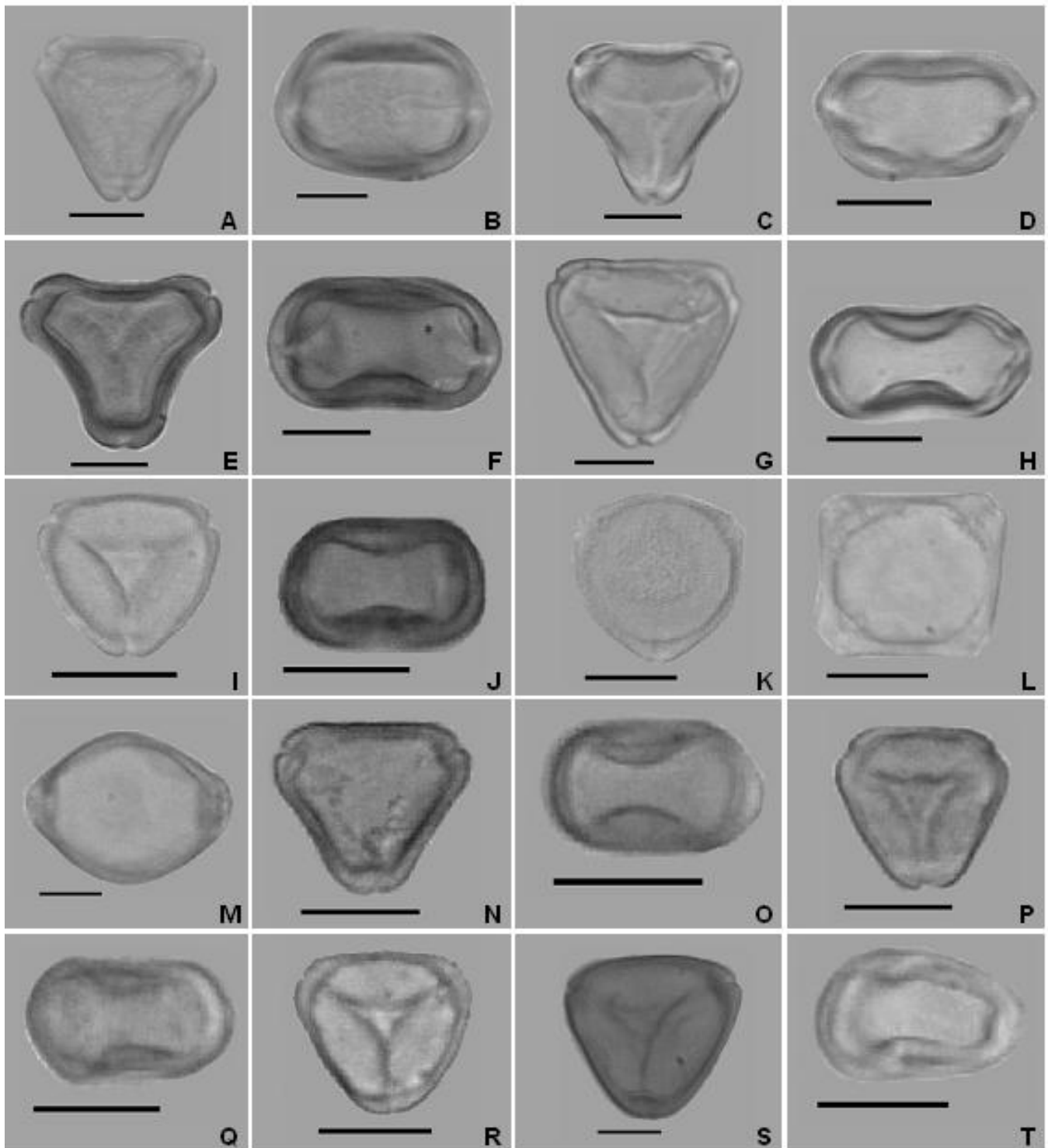


Fig. 1: Photomicrograph of pollen grains (1000x) of Myrtaceae species visited by social bees (Apidae) in Cruz das Almas, Recôncavo region of Bahia, Brazil, in equatorial view (EV) and polar view (PV) where: *Eucalyptus alba* Reinw A (PV) - B (EV); *Eucalyptus* sp.I C (PV) - D (EV); *Eucalyptus* sp.II E (PV) - F (EV); *Eucalyptus torelliana* F. Muell. G (PV) - H (EV); *Eugenia uniflora* L. I (PV) - J (EV); *Psidium araca* Raddi K-L (PV) - M (EV); *Psidium guajava* L. N (PV) - O (EV); *Syzygium cumini* (L.) Skeels P (PV) - Q (EV); *Syzygium malaccensis* L. R-S (PV) - T (EV). (Scale: 10 μ m).

- μ m; exine thickness between 1.04 and 1.19 μ m *Syzygium malaccensis* L. (Fig. 1 R-S).
- 3'. Pollen grains with a polar diameter between 9.30 and 10.00 μ m and equatorial diameter between 15.94 and 16.47 μ m; exine thickness between 1.03 and 1.31 μ m *Syzygium cumini* (L.) Skeels (Fig. 1 P-Q).
4. Pollen grain with triangular *amb* with the sides of the convex *amb*; exine thickness between 1.09 and 1.24 μ m; polar diameter between 10.68 and 11.30 μ m; equatorial diameter between 16.59 and 17.57 μ m *Eugenia uniflora* L. (Fig. 1 I-J).
- 4'. Pollen grain with characteristic triangular *amb* or with the sides of the concave *amb*; exine thickness between 1.18 and 1.35 μ m; Polar diameter less than 10.70 μ m; equatorial diameter between 16.47 and 16.91 μ m *Psidium guajava* L. (Fig. 1 N-O).

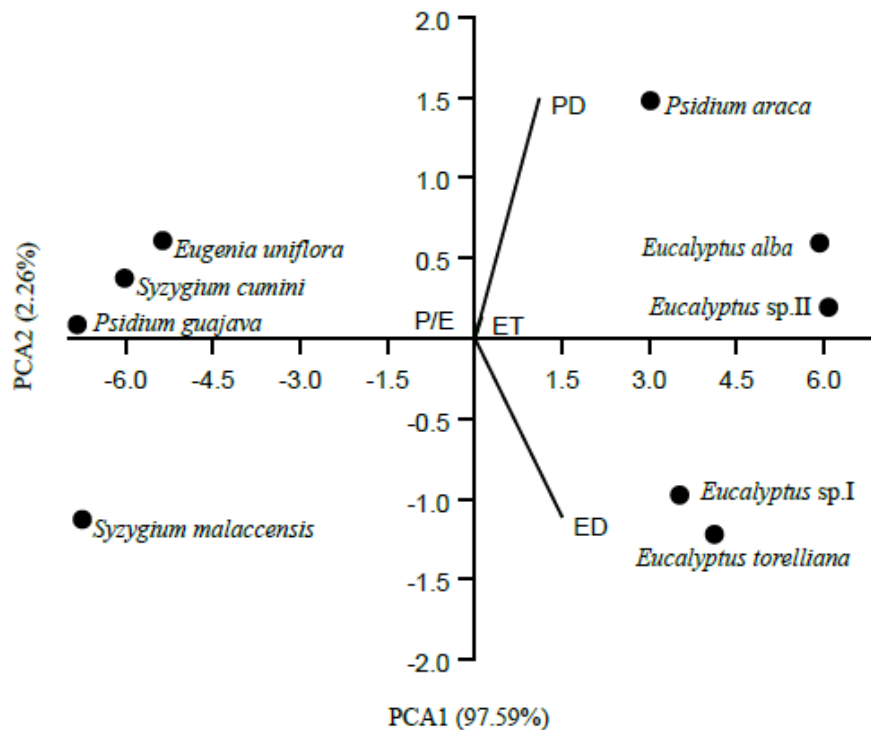


Fig. 2. Principal Component Analysis (PCA) of pollen grains of Myrtaceae species visited by social bees (Apidae) in Cruz das Almas, Recôncavo region of Bahia, Brazil. ET= exine thickness; PD= polar diameter; ED= equatorial diameter and P/E= P/E ratio.

Table 4. Euclidean distance matrix (EDM) of pollen grains of Myrtaceae species visited by social bees (Apidae) in Cruz das Almas, Recôncavo region of Bahia, Brazil.

	<i>Eucalyptus alba</i>	<i>Eucalyptus sp.I</i>	<i>Eucalyptus sp.II</i>	<i>Eucalyptus torelliana</i>	<i>Eugenia uniflora</i>	<i>Psidium araca</i>	<i>Psidium guajava</i>	<i>Syzygium cumini</i>
<i>Eucalyptus sp.I</i>	2.64							
<i>Eucalyptus sp.II</i>	2.06	2.49						
<i>Eucalyptus torelliana</i>	3.56	2.32	1.96					
<i>Eugenia uniflora</i>	12.18	10.18	11.71	10.93				
<i>Psidium araca</i>	5.95	4.86	4.56	4.05	8.56			
<i>Psidium guajava</i>	13.56	11.49	12.82	11.89	2.12	9.24		
<i>Syzygium cumini</i>	14.74	12.64	13.92	12.91	3.38	10.23	1.32	
<i>Syzygium malaccensis</i>	15.25	13.03	14.29	13.12	4.58	10.62	2.60	1.57

- 5. Pollen grains with an equatorial diameter greater than 24.00 μm 6
- 5'. Pollen grains with an equatorial diameter smaller than 24.00 μm 9
- 6. Exine thickness greater than 2.00 μm 7
- 6'. Thickness of exine smaller than 2.00 μm 8
- 7. Pollen grains with exine thickness smaller than 2.15 μm ; polar diameter between 17.33 and 18.10 μm ; equatorial diameter between 25.94 and 26.76 μm ; the P/E ratio equals to 0.67 μm *Eucalyptus alba* Reinw (Fig. 1 A-B).
- 7'. Pollen grains with exine thickness greater than 2.15 μm ; polar diameter between 17.02 and 17.70 μm ; equatorial diameter between 26.25 and 26.95 μm ; the P/E ratio equals to 0.65 μm ... *Eucalyptus sp.II* (Fig. 1 E-F).
- 8. Pollen grains with exine thickness equals to 1.69 μm ; polar diameter between 15.12 and 15.70 μm ; equatorial diameter between 25.41 and 26.08 μm *Eucalyptus sp.I* (Fig. 1 C-D).
- 8'. Pollen grains with exine thickness equals 1.53 μm ; polar diameter between 15.38 and 16.50 μm ; equatorial diameter between 26.24 and 27.27 μm *Eucalyptus torelliana* F. Muell (Fig. 1 G-H).

9. Pollen grains with polar diameter between 16.40 and 17.10 μm ; P/E = 0.72; exine thickness equals to 1.59 μm *Psidium araca* Raddi. (Fig. 1 K-L).

Conclusion

The description of pollen morphology of the Myrtaceae species reveals similarity between the several characteristics common to pollen grains of species of this family. This description allows separation of nine species studied by size and specific morphological features with the identification key, which can be used to study the pollen spectrum of beehive products.

Competing Interests

The authors declare no conflict of interests.

Author's contributions

ASN (ORCID: <https://orcid.org/0000-0001-5236-0460>) carried out fieldwork and compiled the data. ASN and CALC (ORCID: <https://orcid.org/0000-0002-3306-3003>) conceptualized the work plan and structured the manuscript.

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