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Pollen morphology of Malpighiaceae from Brazilian forest fragments

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Abstract The pollen morphology of 12 Brazilian native species of Malpighiaceae from forest fragments was investigated in search to expand the morphological knowledge of the species analyzed thus contributing to its palynology, taxonomy, and conservation. The pollen grains were acetolysed, measured, photographed using light microscopy and scanning electron microscopy, and described qualitatively. The quantitative data were analyzed by descriptive statistics and multivariate statistics. The pollen grains are monads, apolar or isopolar, small to large, with circular to quadrangular amb, oblate-spheroidal to prolate-spheroidal, tricolporate with long colpi and lalongate endoaperture or 6–(7–8)-porate with or without colpoids and aspides, psilate-rugulate, rugulate or microreticulate exine, sexine thicker than nexine in most species. The results of this study point to differences in the morphology of the pollen grains in the analyzed species, which indicates Malpighiaceae as a eurypalynous family; this fact was also confirmed by quantitative data and the multivariate analysis. These data reinforce the importance of pollen morphology for identification and distinction of genus and species of the family.

Keywords Brazil · Eurypalynous · Palynotaxonomy · Pollen grains

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Introduction

Malpighiaceae, an important family within the eudicots groups, is part of Malpighiales and has approximately 1,300 species in 77 genera (APG III 2009; Davis and Anderson 2010). They occur as trees, shrubs, and climbers that are widespread in tropical, subtropical forests, and savanna occurring in the Old and New World, its current distribution and diversification are probably a result of the breakdown of the Gondwana (Anderson 1990, 1993; Amorim 2002; Davis et al. 2002a). There are about 534 species in 44 genera of Malpighiaceae in Brazil, which are distributed in almost all states (Mamede et al. 2014).

Some Malpighiaceae species were identified as the main representatives of the remnant forest fragments in the northwest of São Paulo state, along with three other prominent families: Apocynaceae, Bignoniaceae, and Sapindaceae (Ranga et al. 2012). These areas initially formed by Atlantic Forest now consist of small forest fragments and large areas of Cerrado, mostly due to forest fragmentation process that usually leads to loss of biodiversity (Kronka et al. 1993; Turner 1996; Myers et al. 2000).

Malpighiaceae has a high morphological variety, especially regarding the types of habits, fruits, and also of pollen grains (Niendenzu 1928; Lobreau 1967, 1968), this probably has happened reflecting the extensive ecological and geographical distribution. An important feature of the family is the presence of unicellular hairs usually attached, so that the trichome has two arms (in T-, V- or Y-shaped), the stalk is often short and the arms straight to twisted (Niendenzu 1928; Robertson 1972; Rao and Sarma 1992). The types of fruits and trichomes are of great systematic importance to the family and also widely used in the characterization of genera (Anderson 1981; Judd et al. 2009)

Recent papers on the molecular biology of Malpighiaceae pointed out new data on the traditional classification of the family, suggesting the importance of more detailed studies (Cameron et al. 2001; Davis et al. 2001, 2002a, b, 2004; Davis and Anderson 2010). The family is currently divided into two subfamilies, Byrsonimoideae W. R. Anderson, with New World genera and Malpighioideae Burnett, with species distributed in the New and Old World (APG III 2009). According to Anderson (2004), the New World Malpighiaceae genera have unicellular branched trichomes, simple and opposite leaves, bilateral flowers with two oil glands on the abaxial surface sepals, 10 stamens and a tricarpellate gynoecium with one ovule per locule. Some of these characteristics have been lost in the Old World genera (Anderson 2004; Davis and Anderson 2010).

The pollen morphology of Malpighiaceae has been described in the studies of Erdtman (1952), Lobreau (1967, 1968), Salgado-Labouriau (1973), Anderson (1982), Lobreau-Callen (1983), Makino (1986), Makino-Watanabe (1988), Roubik and Moreno (1991), Makino-Watanabe et al. (1993a, b, 1998), Amorim (2003), Melhem et al. (2003), Gonçalves-Esteves et al. (2007), Magalhães-e-Silva (2007), Sousa et al. (2010), Dutra et al. (2014), and Sebastiani et al. (2014). These studies report about the palynology of the family, its genera or isolated species, and the differences in the pollen grains analyzed indicate Malpighiaceae as eurypalynous.

Continuing the pollen morphology characterization for forest fragments areas (Souza and Gasparino 2014), the aim of this study is to contribute with morphological data, which will be used in future taxonomic or pollinic studies (such as geopalynology, melissopalynology, etc.) that include Malpighiaceae species and assisting so their conservation.

Materials and methods

We studied the pollen grains of 12 Malpighiaceae native species (Appendix) from the remnant forest fragments of the northwest area São Paulo state. Ranga et al. (2012) describes 14 Malpighiaceae species for the area, however *Janusia intermedia* (A.St.-Hill.) A. Juss. cited in this paper is currently from synonymy with *Byrsonima intermedia* A. Juss., and also *Carolus chlorocarpus* (A. Juss.) W. R. Anderson was not analyzed by lack of pollen material. The pollen materials were obtained from dried herbarium specimens supplied from JABU and SJRP herbaria, of samples taken mainly in the remnant forest fragments of northwest São Paulo State. Pollen grains of 24 specimens were studied by light microscopy (LM) and scanning electron microscopy (SEM), and the pollen grains of *Diplopterys pubipetala*, *Mascagnia cordifolia*, *Peixotoa tomentosa,* and *Stigmaphyllon lalandianum* had not been studied previously. The pollen grains were acetolysed according to the methods of Erdtman (1960) and Melhem et al. (2003) and measured, under LM, within seven days after their preparation (Salgado-Labouriau et al. 1965). For SEM analysis, pollen grains were prepared according to Melhem et al. (2003) to both acetolysed and non-acetolysed pollen grains. Permanent slides of light microscopy are deposited in the pollen reference collection of the Departamento de Biologia aplicada à Agropecuária, Universidade Estadual Paulista, Unesp, Jaboticabal, Brazil.

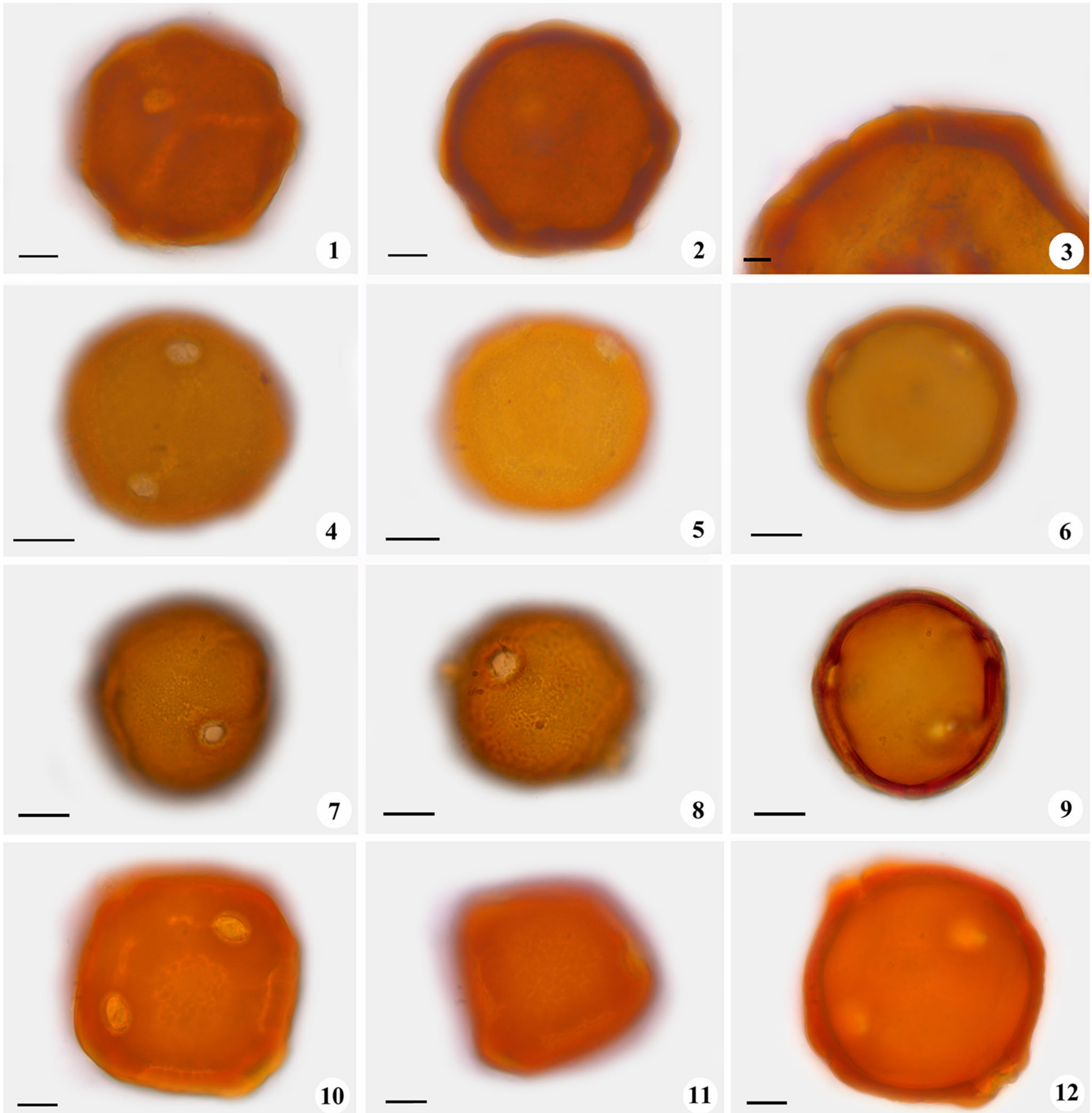
Pollen diameters ($n = 25$) and the other characteristics (of aperture and exine thickness, $n = 10$) were measured in the pollen samples under LM. Statistical analysis was conducted to obtain the means (x), standard deviation (sx), standard error (s), 95 % confidence intervals (CI), coefficient of variability (V), and range (R) following Vieira (1981) and Zar (1996). To compare the values of the pollen grain diameters, we used the diameter I and diameter II (for apolar pollen grains) and diameter in equatorial view (for isopolar pollen grains), and the data represented by graphs of MINITAB program. The mean was calculated for exine thickness, length and width of apertures. To verify whether the pollen data allowed the grouping of species, a principal component analysis (PCA) was performed using the programs FITOPAC (Shepherd 1996) and PC-ORD (McCune and Mefford 1999). For this analysis, we used 11 metric variables: length of colpus (CLEN), width of colpus (CWID), polar diameter in equatorial view or diameter I (DIAI), equatorial diameter in equatorial view or diameter II (DIAII), equatorial diameter in polar view (EDPV), length of endoaperture (ELEN), width of endoaperture (EWID), pore diameter (PDIA), exine (EXIN), nexine (NEXI), and sexine (SEXI).

Photomicrographs were performed with a light microscope Leica IM50 for LM photos, and with a JOEL, JSM5410 scanning electron microscope for the SEM images. Pollen description and terminology follows Barth and Melhem (1988) and Punt et al. (2007) glossaries, Faegri and Iversen (1966) and Gasparino et al. (2013) were adopted for the polar area index and for width index of colpi, respectively.

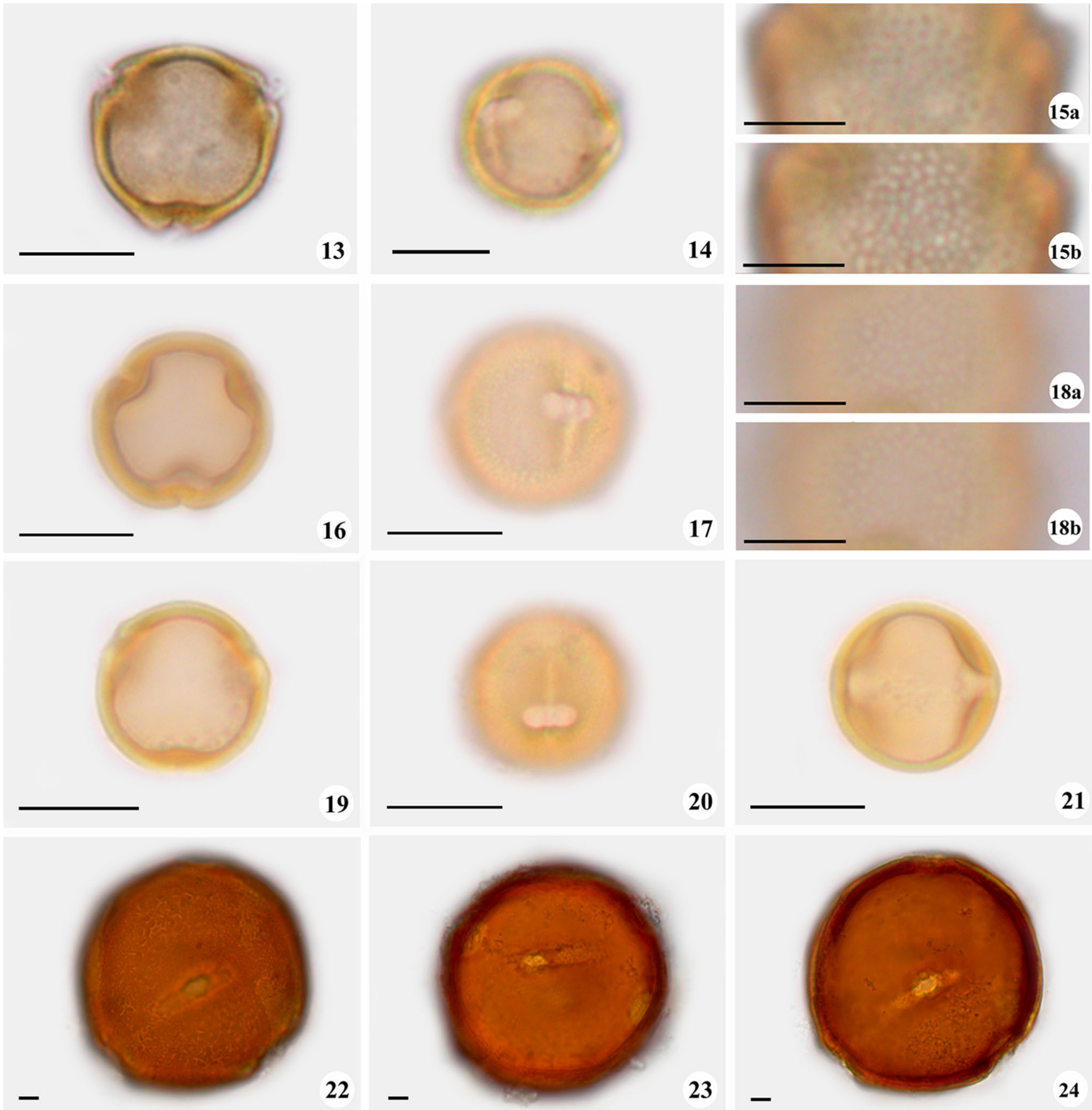
Results

General description

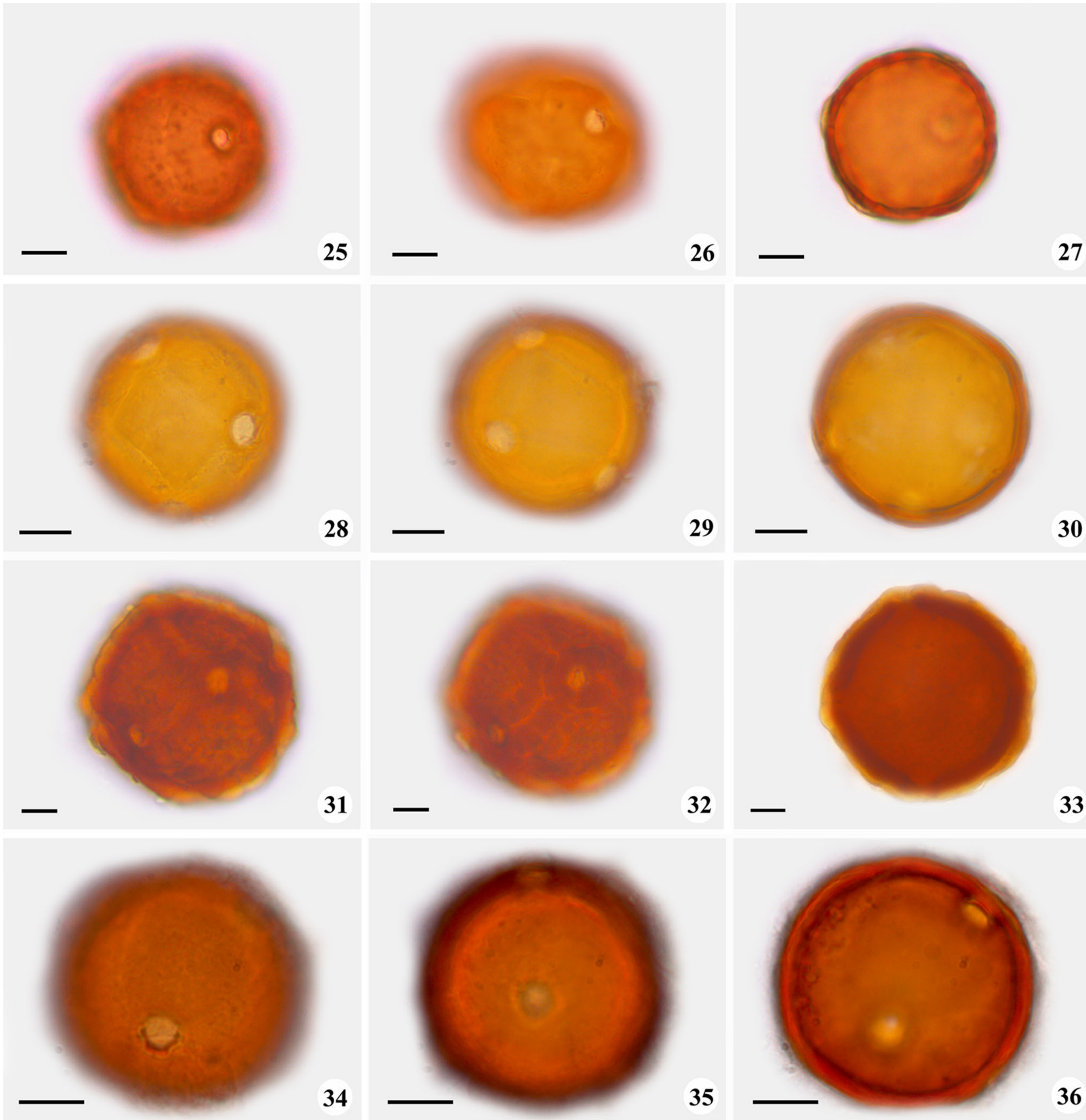
The pollen grains of the Malpighiaceae species studied here (Figs. 1–12, 13–24, 25–36, 37–46) are monads, apolar



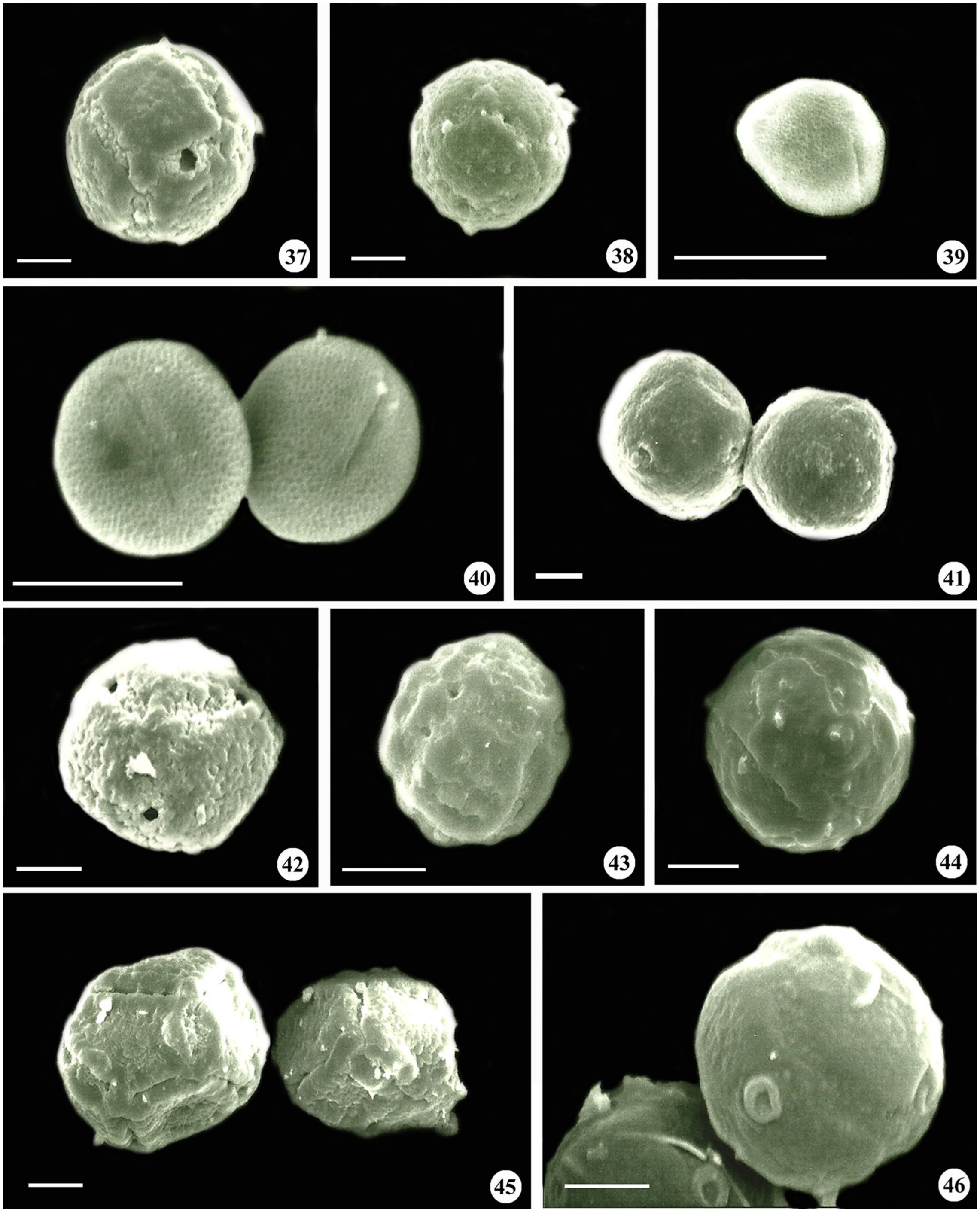
Figs. 1–12 Photomicrographs of the pollen grains of *Banisteriopsis* C.B.Rob. ex Small (Malpighiaceae). **1–3** *B. argyrophylla* (A. Juss.) B. Gattes. **1** General aspect, aperture and ornamentation. **2** General aspect, optical section. **3** Exine, optical section. **4–6** *B. muricata* Cavanilles. **4** General aspect, aperture. **5** General aspect, ornamentation. **6** General aspect, optical section. **7–9** *B. nummifera* (A. Juss.) B.Gates. **7** General aspect, aperture. **8** General aspect, ornamentation. **9** General aspect, optical section. **10–12** *B. oxyclada* (A. Juss.) B. Gattes. **10** General aspect, aperture. **11** General aspect, ornamentation. **12** General aspect, optical section. *Bar* = 5 µm (**3**); 10 µm (**1–2**, **4–12**)



Figs. 13–24 Photomicrographs of the pollen grains of *Byrsonima* Rich. ex Kunth and *Diplopterys* A. Juss. (Malpighiaceae). **13–15** *Byrsonima crassifolia* (L.) Kunth. **13** Polar view, optical section **14** Equatorial view, aperture. **15a**, **b** Ornamentation in high and low focus. **16–18** *Byrsonima intermedia* A. Juss. **16** Polar view, optical section **17** Equatorial view, aperture. **18a**, **b** Ornamentation in high and low focus. **19–21** *Byrsonima verbascifolia* (L.) Rich. **19** Polar view, optical section **20** Equatorial view, aperture. **21** Equatorial view, optical section **22–24** *Diplopterys pubipetala* (A. Juss.) W.R.Anderson & C.Davis. **22** General aspect, aperture. **23** General aspect, ornamentation. **24** General aspect, optical section. *Bar* = 5 µm (**15, 18**); 10 µm (**13–14**, **16–17**, **19–24**)



Figs. 25–36 Photomicrographs of the pollen grains of *Heteropterys* Kunth, *Mascagnia* Bertero, *Peixotoa* A. Juss., and *Stigmaphyllon* A. Juss. (Malpighiaceae). **25–27** *Heteropterys aceroides* Griseb. **25** General aspect, aperture and ornamentation. **26** General aspect, ornamentation. **27** General aspect, optical section **28–30** *Mascagnia cordifolia* (A. Juss.) Griseb. **28** General aspect, aperture. **29** General aspect, ornamentation. **30** General aspect, optical section **31–33** *Peixotoa tomentosa* A. Juss. **31** General aspect, aperture. **32** General aspect, ornamentation. **33** General aspect, optical section **34–36** *Stigmaphyllon lalandianum* A. Juss. **34** General aspect, aperture. **35** General aspect, ornamentation. **36** General aspect, optical section. *Bar* = 10 μm



Figs. 37–46 Electron micrographs of the pollen grains of Malpighiaceae. **37–38** *Banisteriopsis oxyclada* (A. Juss.) B. Gattes. **37** General aspect, apertures. **38** General aspect, ornamentation. **39** *Byrsonima crassifolia* (L.) Kunth, ornamentation. **40** *Byrsonima intermedia* A. Juss., apertures and ornamentation. **41–42** *Diplopterys pubipetala* (A. Juss.) W.R.Anderson & C.Davis. **41** general aspect, ornamentation. **42** General aspect, apertures. **43** *Heteropterys aceroides* Griseb., ornamentation. **44** *Mascagnia cordifolia* (A. Juss.) Griseb., amb and ornamentation. **45** *Peixotoa tomentosa* A. Juss., ornamentation. **46** *Stigmaphyllon lalandianum* A. Juss., apertures and ornamentation. *Bar* = 10 μm

or isopolar, small to large, with circular to quadrangular amb, oblate-spheroidal to prolate-spheroidal, tricolporate with long colpi and lalongate endoaperture or 6–(7–8)-porate with or without colpoids and aspides, psilate-rugulate, rugulate or microreticulate exine, sexine thicker than nexine in most species.

Pollen key to Malpighiaceae species

1. Isopolar and colporate pollen grains, microreticulate exine 2
    2. Endoaperture constricted in the median region, exine ≥2,01 μm .... *Byrsonima intermedia.*
    2′. Endoaperture not constricted in the median region, exine ≤2,00 μm 3
        3. Oblate-spheroidal pollen grains, circular amb, width of endoaperture ≥5,01 μm .... *Byrsonima verbascifolia.*
        3′. Prolate-spheroidal pollen grains, subcircular amb, width of endoaperture ≤5,00 μm. *Byrsonima crassifolia.*

1′. Apolar and porate pollen grains, psilate-rugulate or rugulate exine 4
    4. Pollen grains 7-porate .... *Diplopterys pubipetala.*
    4′. Pollen grains 6– or 8-porate 5
        5. Pollen grains only 8-porate 6
            6. Circular amb, aperture with inconspicuous colpoids, small regular in exile .... *Mascagnia cordifolia.*
            6′. Subcircular amb, aperture with conspicuous colpoids, large rugulae in exine .... *Peixotoa tomentosa.*
        5′. Pollen grains 6-porate or 6(8)-porate 7
            7. Exine ornamentation psilate-rugulate with small rugulae ....*Stigmaphyllon lalandianum.*
            7′. Exine ornamentation rugulate with psilate areas 8
                8. Pollen grains 6(8)- porate .... *Heteropterys aceroides.*
                8′. Pollen grains only 6-porate 9
                    9. Aperture of pollen grains with conspicuous colpoids 10
                        10. Large pollen grains, sexine < nexine .... *Banisteriopsis argyrophylla.*
                        10′. Medium pollen grains, sexine > nexine ....*Banisteriopsis oxyclada.*
                    9′. Aperture of pollen grains with inconspicuous colpoids 11
                        11. Pori with aspides, sexine < nexine ....*Banisteriopsis nummifera.*
                        11′. Pori without aspides, sexine > nexine .... *Banisteriopsis muricata.*

Banisteriopsis C.B.Rob. ex small (Figs. 1–12, 37–38, Tables 1, 3)

Apolar pollen grains, medium to large (*B. argyrophylla*, Table 1), circular to cuboidal (*B. oxyclada*, Figs. 10–11), 6-porate, with colpoids in the pori, conspicuous in *B. argyrophylla* (Fig. 1) and *B. oxyclada* (Figs. 10–11), and inconspicuous in *B. muricata* (Figs. 4–5) and *B. nummifera* (Figs. 7–8). Pori with aspides (*B. nummifera*, Figs. 7–8, *B. oxyclada*, Fig. 12). Exine ornamentation rugulate with psilate areas and granules, sexine thicker than nexine (*B. muricata* and *B. oxyclada*) or nexine thicker than sexine (*B. argyrophylla* and *B. nummifera*, Table 3). In SEM we can observe psilate areas near the colpoids and granules within these (Figs. 37–38).

Studied species: *Banisteriopsis argyrophylla* (A. Juss.) B. Gattes (Figs. 1–3); *Banisteriopsis muricata* Cavanilles (Figs. 4–6); *Banisteriopsis nummifera* (A. Juss.) B. Gates (Figs. 7–9); *Banisteriopsis oxyclada* (A. Juss.) B. Gattes (Figs. 10–12, 37–38).

Byrsonima Rich. ex Kunth (Figs. 13–21, 39–40, Tables 2, 3)

Isopolar and small pollen grains, circular to subcircular amb (*B. crassifolia*, Fig. 13), oblate-spheroidal to prolate-spheroidal, 3-colporate, long and narrow colpi, without margo and rounded ends, lalongate endoapertures sometimes constricted (*B. intermedia*, Fig. 17) and also with rounded ends. Microreticulate exine, homobrocate (Figs. 15, 18). Sexine thicker than nexine (Table 3). Under SEM, it is observed the homobrocate microreticulum and a slightly psilate area near the apertures (Figs. 39–40).

Studied species: *Byrsonima crassifolia* (L.) Kunth (Figs. 13–15, 39); *Byrsonima intermedia* A. Juss. (Figs. 16–18, 40); *Byrsonima verbascifolia* (L.) Rich. (Figs. 19–21).

Diplopterys A. Juss (Figs. 22–24, 41–42, Table 1)

Apolar and medium pollen grains (Table 1), circular, 7-porate with short colpoids in the pori (Figs. 22–24), pori without aspides. Rugulate exine with psilate areas mainly near the colpoids (Fig. 22). Nexine thicker than sexine. The

Table 1 Quantitative data of pollen grains of *Banisteriopsis* C.B. Rob. ex Small, *Diplopterys* (A. Juss.) W.R., *Heteropterys* Kunth, *Mascagnia* Bertero, *Peixotoa* A. Juss., and *Stigmaphyllon* A. Juss. (Malpighiaceae), $n = 25$

Species	(R) $x \pm s_x$	s	CI	V
Diameter I				
B. argyrophylla	(47.50–57.50) 51.50 ± 0.46	2.28	(50.56–52.44)	4.43
B. oxyclada	(32.50–45.00) 39.10 ± 0.61	3.05	(37.84–40.36)	7.80
B. muricata	(27.50–40.00) 35.20 ± 0.72	3.60	(33.72–36.68)	10.23
B. nummifera	(32.50–45.00) 37.80 ± 0.55	2.73	(36.67–38.93)	7.22
D. pubipetala	(40.00–47.50) 44.80 ± 0.48	2.41	(43.81–45.79)	5.38
H. aceroides	(30.00–42.50) 37.20 ± 0.63	3.17	(35.89–38.51)	8.53
M. cordifolia	(25.00–45.00) 34.70 ± 0.82	4.10	(33.01–36.39)	11.82
P. tomentosa	(40.00–60.00) 49.50 ± 0.89	4.45	(47.67–51.33)	8.99
S. lalandianum	(32.50–40.00) 37.40 ± 0.34	1.69	(36.70–38.10)	4.52
Diameter II				
B. argyrophylla	(47.50–57.50) 51.40 ± 0.58	2.89	(50.21–52.59)	5.63
B. oxyclada	(35.00–42.50) 39.00 ± 0.50	2.50	(37.97–40.03)	6.41
B. muricata	(27.50–40.00) 34.70 ± 0.70	3.49	(33.26–36.14)	10.04
B. nummifera	(32.50–45.00) 38.30 ± 0.64	3.20	(36.98–39.62)	8.37
D. pubipetala	(37.50–47.50) 44.80 ± 0.51	2.54	(43.75–45.85)	5.68
H. aceroides	(37.50–40.00) 38.00 ± 0.48	2.39	(37.01–38.99)	6.30
M. cordifolia	(30.00–47.50) 37.50 ± 0.75	3.75	(35.96–39.05)	10.00
P. tomentosa	(40.00–62.50) 50.45 ± 0.98	4.88	(48.44–52.46)	9.67
S. lalandianum	(35.00–41.25) 37.65 ± 0.40	1.99	(36.83–38.47)	5.28

R range, *x* mean (µm), s_x standard deviation (µm), *s* standard error (µm), *CI* confidential interval in 95 % (µm), *V* coefficient of variability (%)

Table 2 Quantitative data of pollen grains of *Byrsonima* Rich. ex Kunth (Malpighiaceae), $n = 25$

Species	(*R*) $x \pm s_x$	s	CI	*V*
Equatorial diameter in polar view				
B. crassifolia	(12.50–17.50) 14.05 ± 0.31	1.54	(13.41–14.69)	10.98
B. intermedia	(12.50–17.50) 14.55 ± 0.28	1.39	(13.98–15.12)	9.57
B. verbascifolia	(12.50–15.00) 13.90 ± 0.25	1.27	(13.38–14.42)	9.11
Polar diameter in equatorial view				
B. crassifolia	(12.50–17.50) 14.40 ± 0.26	1.31	(13.86–14.94)	9.08
B. intermedia	(12.50–17.50) 14.50 ± 0.29	1.44	(13.91–15.09)	9.95
B. verbascifolia	(12.50–15.00) 13.60 ± 0.25	1.27	(13.08–14.12)	9.31
Equatorial diameter in equatorial view				
B. crassifolia	(12.50–15.00) 13.75 ± 0.25	1.25	(13.24–14.27)	9.09
B. intermedia	(12.50–17.50) 14.30 ± 0.27	1.35	(13.74–14.86)	9.47
B. verbascifolia	(12.50–15.00) 13.80 ± 0.25	1.27	(13.27–14.33)	9.24

R range, *x* mean (µm), s_x standard deviation (µm), *s* standard error (µm), *CI* confidential interval in 95 % (µm), *V* coefficient of variability (%)

pollen grains of *Diplopterys pubipetala* present psilate areas in the ornamentation near the apertures, and small granules within the colpoids. (Figs. 41–42).

Studied species: *Diplopterys pubipetala* (A. Juss.) W. R. Anderson & C. Davis (Figs. 22–24, 41–42).

Heteropterys Kunth (Figs. 25–27, 43, Tables 1, 3)

Apolar and medium pollen grains (Table 1), circular (Fig. 27), 6–(8)-porate, with inconspicuous colpoids in the pori (Figs. 25–26), pori without aspides. Rugulate exine with psilate areas irregularly distributed across the pollen grain surface. Sexine thicker than nexine. When observed under SEM, the pollen grains of *Heteropterys aceroides* have large rugula with psilate areas (Fig. 43).

Studied species: *Heteropterys aceroides* Griseb (Figs. 25–27, 43).

Mascagnia Bertero (Figs. 28–30, 44, Tables 1, 3)

Apolar and medium pollen grains (Table 1), circular (Fig. 30), 8-porate, with inconspicuous colpoids in the pori

Table 3 Measures (in µm) of Malpighiaceae pollen grains, apertures and exine. $n = 10$

Species	Pori	Colpus		Endoaperture		Ex	Sex	Nex	WCI
		Length	Width	Length	Width				
Banisteriopsis argyrophylla	7.64	–	–	–	–	6.08	2.94	3.14	–
Banisteriopsis muricata	3.43	–	–	–	–	3.53	2.45	1.08	–
Banisteriopsis nummifera	5.31	–	–	–	–	2.75	1.08	1.67	–
Banisteriopsis oxyclada	4.21	–	–	–	–	3.24	2.06	1.18	–
Byrsonima crassifolia	–	11.50	1.00	2.00	4.00	1.03	0.70	0.33	13.75
Byrsonima intermedia	–	13.04	1.30	1.73	5.20	2.17	1.30	0.87	11.00
Byrsonima verbascifolia	–	12.60	0.48	1.76	9.13	1.66	1.04	0.62	28.75
Diplopterys pubipetala	5.49	–	–	–	–	9.13	2.47	6.66	–
Heteropteris aceroides	3.65	–	–	–	–	3.77	2.30	1.47	–
Mascagnia cordifolia	3.11	–	–	–	–	8.04	3.04	5.00	–
Peixotoa tomentosa	4.41	–	–	–	–	8.04	3.04	5.00	–
Stigmaphyllon lalandianum	4.70	–	–	–	–	3.29	1.67	1.62	–

Pori pori diameter, *Ex* exine thickness, *Sex* sexine thickness, *Nex* nexine thickness, *WCI* width colpus index

(Figs. 28–29), pori without aspides. Regulate exine with small regular distributed across the pollen grain surface. Nexine thicker than sexine (Table 3). Under SEM, we can observe large regulate areas in the ornamentation (Fig. 44).

Studied species: *Mascagnia cordifolia* (A. Juss.) Griseb (Figs. 28–30, 44).

Peixotoa A. Juss (Figs. 31–33, 45, Tables 1, 3)

Apolar, medium to large pollen grains (Table 1), subcircular (Fig. 31), 8-porate with conspicuous colpoids in the pori (Figs. 31–32), pori without aspides. Rugulate exine with thicker and psilate areas near the colpoids (Figs. 31–32). Nexine thicker than sexine (Table 3). Under SEM, we observed large and irregular rugulae distributed throughout the pollen grain ornamentation (Fig. 45).

Studied species: *Peixotoa tomentosa* A. Juss. (Figs. 31–33, 45).

Stigmaphyllon A. Juss. (Figs. 34–36, 46, Tables 1, 3)

Apolar and medium pollen grains (Table 1), circular (Fig. 36), 6-porate with inconspicuous colpoids in the pori (Figs. 34, 36), pori with aspides (Figs. 35, 46). Psilate-rugulate exine with uniform and small rugulae throughout the pollen grain (Fig. 34). Sexine slightly thicker than nexine (Table 3). When analyzed under SEM, we can see small rugulae in the pollen grain surface, granulate areas in colpoids membrane, and a prominently aspis in the pore (Fig. 46).

Studied species: *Stigmaphyllon lalandianum* A. Juss. (Figs. 34–36, 46).

Analysis of quantitative data

When we analyzed the mean and confidence interval of the pollen grain diameters (Fig. 47a, b), we clearly observe the distinction of three separate groups considering the pollen size. The first group is represented by the *Byrsonima* species, which have smaller pollen grains, being *Byrsonima intermedia* with larger pollen grains. These species are not separated from each other by the values of diameters in this group.

The larger diameters of the pollen grains are observed for species *Peixotoa tomentosa* and *Banisteriopsis argyrophylla*, which stand out from the other species concerning these values.

Pollen grains with intermediary diameters values were observed in the other *Banisteriopsis* species along with *Mascagnia cordifolia*, *Heteropterys aceroides*, and *Stigmaphyllon lalandianum*. These species form a continuous group and cannot be separated from each other. An outstanding position is given to *Diplopterys pubipetala* which appears among the group of species with intermediate sizes and the two largest species observed (Fig. 47a, b).

The quantitative data of the pollen grains were exploited by PCA in order to identify the link between the species studied (Fig. 48). This analysis summarized in their two axes 98.09 % of total data variability.

The first axis of the principal component analysis was the most significant for the species ordination and summarized 92.91 % of the total variability of the analyzed data. This component explained the variance based mainly on the values of the diameters in polar view (EDPV), length of colpus (CLEN), and width of endoaperture (ELAR—Table 4), i.e. these variables influenced the

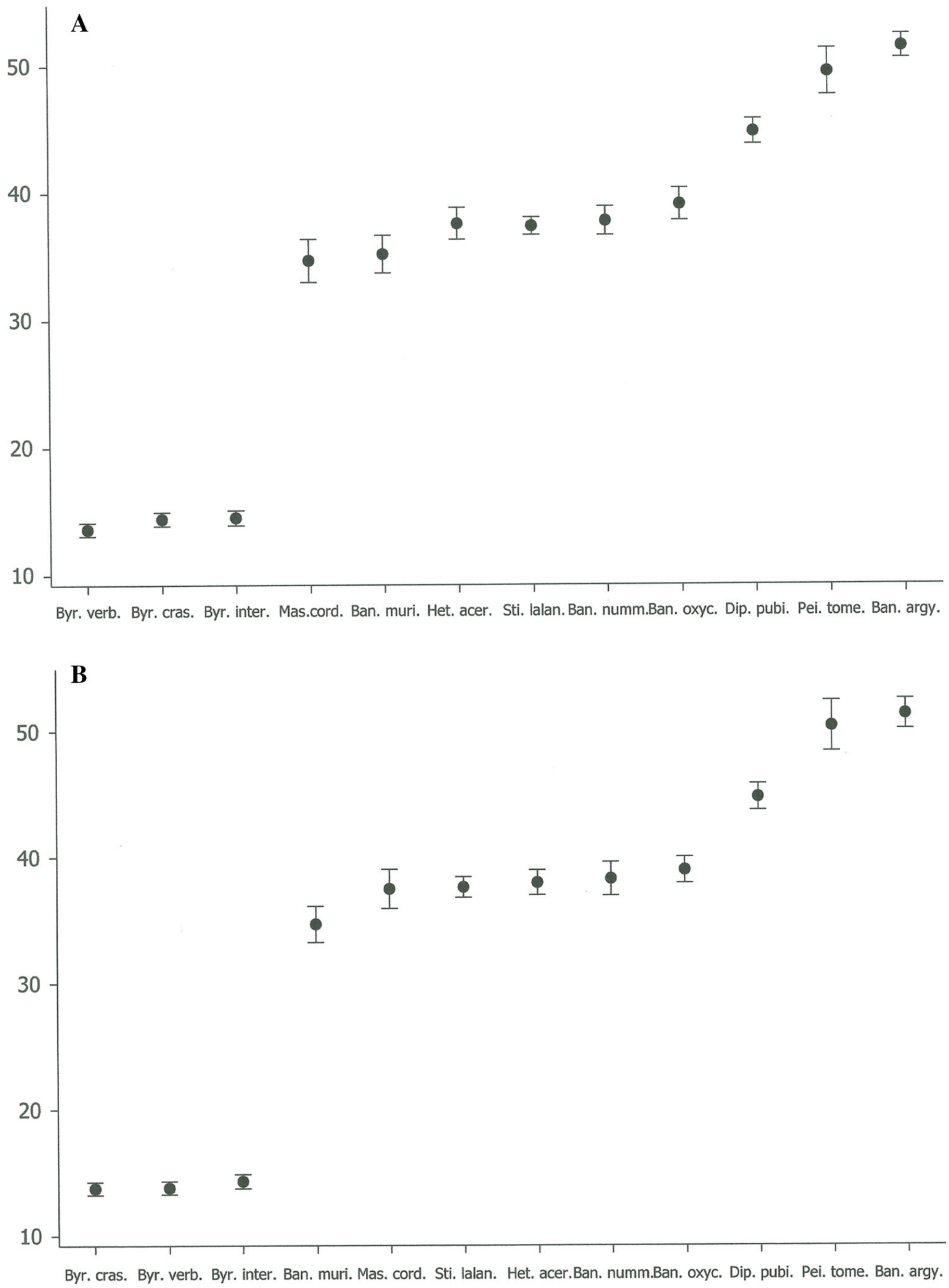


Fig. 47 Representation of confidence interval of mean in 95 % of the pollen grains of Malpighiaceae. **a** Diameter I or polar diameter in equatorial view. **b** Diameter II or equatorial diameter in equatorial view. The *higher* and *lower boundaries* showing the confidence interval; the *average circle* showing the arithmetic mean. Ban. argy. = *Banisteriopsis argyrophylla*, Ban. muri. = *Banisteriopsis muricata*, Ban. numm. = *Banisteriopsis nummifera*, Ban. oxyc. = *Banisteriopsis oxyclada*, Byr.cras. = *Byrsonima crassifoliai* Byr. inter. = *Byrsonima intermedia*, Byr. verb. = *Byrsonima verbascifolia*, Dip. pubi. = *Diplopterys pubipetala*, Het. acer. = *Heteropterys aceroides*, Mas. cord. = *Mascagnia cordifolia*, Pei. tome. = *Peixotoa tomentosa*, and Sti. lalan. = *Stigmaphyllon lalandianum*. The values are in μm

placement of the *Byrsonima* species on the positive side (on the right) of the axis 1, thereby separating the apolar and polar pollen grains by metric values of these variables and also width of colpus and length of endoaperture (Fig. 48).

On the negative side of axis 1, it is possible to observe the species with higher values of diameters of pollen grains and pore diameter; they are divided into two distinct groups, separated by the variables related to the axis 2 of the PCA (characteristics of exine thickness).

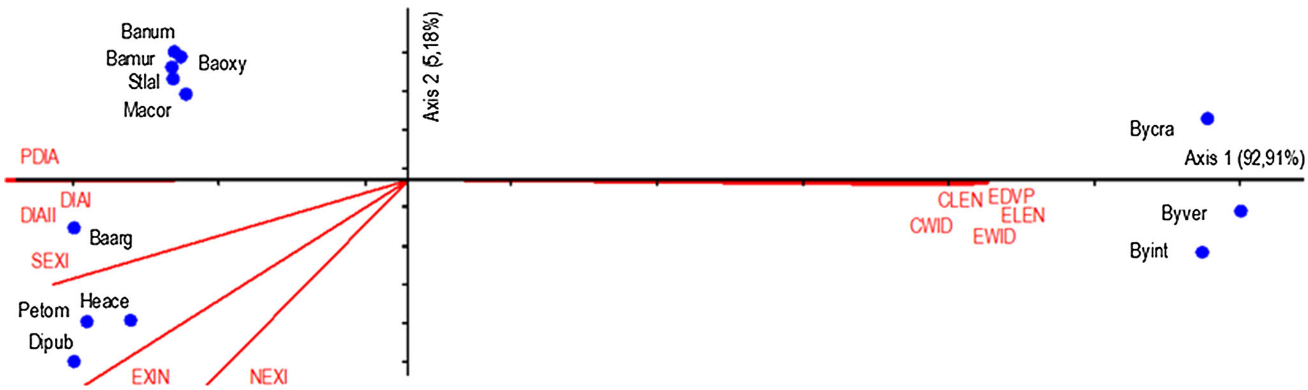


Fig. 48 Principal component analysis performed with the pollen metrical variables from Malpighiaceae species. Baarg = *Banisteriopsis argyrophylla*, Bamur = *Banisteriopsis muricata*, Banum = *Banisteriopsis nummifera*, Baoxy = *Banisteriopsis oxyclada*, Bycra = *Byrsonima crassifoliai* Byint = *Byrsonima intermedia*, Byver = *Byrsonima verbascifolia*, Dipub = *Diplopterys pubipetala*, Heace = -*Heteropterys aceroides*, Macor = *Mascagnia cordifolia*, Petom = *Peixotoa tomentosa*, and Stlal = *Stigmaphyllon lalandianum*

Table 4 Pearson and Kendall correlation coefficients for pollen grains metric variables of the first and the second axis of PCA ordination in Malpighiaceae species

Variables	Principal components	
	Axis 1	Axis 2
EDVP	0.5295	−0.1621
DIAI	−0.2062	−0.0191
DIAII	−0.2093	−0.0376
PDIA	−0.3368	0.0534
CLEN	0.5050	−0.1641
CWID	0.1238	−0.0417
ELEN	0.2024	−0.0510
EWID	0.3744	−0.1483
EXIN	−0.1719	−0.5802
SEXI	−0.1004	−0.2303
NEXI	−0.1697	−0.7254

The second component explained only 5.18 % of the variability of the species studied, the values of nexine (NEXI) and exine (EXIN) were important for the ordering of species that appear on the negative side of the axis (*Diplopterys pubipetala*, *Peixotoa tomentosa*, *Heteropterys aceroides,* and *Banisteriopsis argyrophylla*). As we could see in the graph (Fig. 48), the axis 2 contributed little to the ordering of the species analyzed, nevertheless, the values of the exine of pollen grains of Malpighiaceae were important in the grouping of species along this axis.

Discussion

Pollen data about the Malpighiaceae genera analyzed were previously characterized in the studies of Salgado-Labouriau (1973), Anderson (1982), Makino (1986), Roubik and Moreno (1991), Makino-Watanabe et al. (1993a), Melhem et al. (2003), Magalhães-e-Silva (2007), Gonçalves-Esteves et al. (2007) e Sousa et al. (2010) Table 5.

To *Banisteriopsis campestris* (A. Juss.) Little, Salgado-Labouriau (1973) described spherical pollen grains, 6-porate, with colpoids, and obscure ornamentation. The author also highlights the similarity between the pollen grain of *Banisteriopsis campestris* and *Peixotoa reticulata*. The data of *Banisteriopsis* in our study support the description of Salgado-Labouriau (1973) for *Banisteriopsis campestris*, however, the ornamentation was here defined as rugulate, and we observed a difference in the number of apertures when comparing the pollen grains of this genus with *Peixotoa*.

Makino (1986) showed variations in the number of apertures ((4–5) 6-porate) and ornamentation (granulate, microreticulate, and rugulate) when she studied the pollen grains of *Banisteriopsis*, the same is true for *B. cornifolia* (HBK) C.B.Robinson ex Small, described by Roubik and Moreno (1991) as 6–8-porate and psilate. Though we have analyzed other species, the pollen data found generally agree with the data of these authors, but we did not observe the variations of aperture and ornamentation, describing here 6-porate and rugulate pollen grains.

The pollen grains of a considerable number of *Banisteriopsis* species were analyzed by Makino-Watanabe et al. (1993a), the authors described pollen grains with a variable number of apertures (between 3 and 10 pori), with colpoids or not, psilate, microreticulate, reticulate, or rugulate. In this paper, Makino-Watanabe et al. (1993a) analyzed the four species studied by us, reporting the values of pollen grain diameter and pore diameters superior to those assessed herein (except for the pore diameters of *B. muricata* and *B. nummifera*). The authors also indicated the rugulate ornamentation with psilate areas only for *B.*

Table 5 Important pollen studies in Malpighiaceae which include the analyzed genera in this study

	Banisteriopsis C.B.Rob. ex Small	*Byrsonima* Rich. ex Kunth	*Diplopterys* A. Juss.	*Heteropterys* Kunth	*Mascagnia* Bertero	*Peixotoa* A. Juss.	*Stigmaphyllon* A. Juss.
Salgado-Labouriau (1973)	1	2	–	–	–	1	–
Anderson (1982)	–	–	–	–	–	2	–
Makino (1986)	4	2	–	3	–	–	–
Roubik and Moreno (1991)	1	1	–	1	2	–	4
Makino-Watanabe et al. (1993a)	41	–	–	–	–	–	–
Melhem et al. (2003)	–	1	–	–	–	–	–
Magalhães-e-Silva (2007)	2	1	–	1	–	1	–
Gonçalves-Esteves et al. (2007)	1	1	–	6	–	1	4
Sousa et al. (2010)	–	–	–	1	–	–	–
This study	4	3	1	1	1	1	1

– absent genus in the study. The numbers present the number of species

argyrophylla, for other species Makino-Watanabe et al. (1993a) reported pollen grains psilate (*B. oxyclada*), reticulate-rugulate (in *B. nummifera*) or with obscure exine (for *B. muricata*).

When analyzing the pollen grains of *Banisteriopsis muricata*, Magalhães-e-Silva (2007) also indicated variations in the number of apertures and exine ((4–5)6(7)-porate, scabrate) as previously mentioned in other studies. In our study, we observed that the quantitative data described for this species are similar to those reported by Magalhães-e-Silva (2007) however 6-porate and rugulate pollen grains were found. We believe that these differences in aperture and exine ornamentation described in the studies are mainly due to the difficulty of observing the pori and colpoids and by the irregularity of the ornamentation on the pollen grain surface.

Gonçalves-Esteves et al. (2007) studied the pollen morphology of *Banisteriopsis sellowiana*, and have portrayed apolar, medium and cuboidal pollen grains, 6-porate, with colpoids and rugulate with psilate areas in the sexine. Here we found similar pollen data for *Banisteriopsis sellowiana*, however, there are differences related to the size and shape of the pollen grains when compared with the data of those authors.

The pollen grains of *Byrsonima* species were described as isopolar, small, 3-colporate with lalongate endoaperture, constricted or not, and reticulate or microreticulate exine by Salgado-Labouriau (1973), Makino (1986), Roubik and Moreno (1991), Melhem et al. (2003), Magalhães-e-Silva (2007), and Gonçalves-Esteves et al. (2007). Our data for *Byrsonima crassifolia*, *B. intermedia*, and *B. verbascifolia* confirm the previously published data pollen.

Makino (1986) analyzed the pollen grains of *Byrsonima intermedia* and observed the endoaperture constriction as seen here. This detail also appears for other species in the genus, e.g. Salgado-Labouriau (1973), Melhem et al. (2003), Gonçalves-Esteves et al. (2007). It is also worth emphasizing that even indicating reticulate ornamentation for *Byrsonima* species, both Salgado-Labouriau (1973) and Roubik and Moreno (1991) report that the lumen of the reticulum has less 1 µm, what characterizes microreticulate pollen grains.

Variations in the number of aperture and the exine ornamentation were also observed for the pollen grains of *Heteropterys* species (Makino 1986; Magalhães-e-Silva 2007; Gonçalves-Esteves et al. 2007). Makino (1986) described the genus pollen grains 3-porate to poliporate (until 12 pores) with psilate, microreticulate and reticulate-rugulate exine. Magalhães-e-Silva (2007) observed in *Heteropterys catingarum* A. Juss. pollen grains 4–6–(7)-porate, scabrate, and Gonçalves-Esteves et al. (2007) pollen 6–8-porate with rugulate, psilate, or scabrate sexine in the six species studied by them. However, the studies of Roubik and Moreno (1991) and Sousa et al. (2010) indicate pollen grains 6-porate with psilate or rugulate exine, with no evidence to large variations.

The pollen grains of *Heteropterys aceroides* examined by us had 6–(8)-pori with conspicuous colpoids and psilate-rugulate exine, characteristics also described by Makino (1986) for this specie. Therefore, due to the morphological variation presented in the literature about *Heteropterys* pollen, we suggest that it belongs to an euripalynous genus.

Roubik and Moreno (1991) analyzed the pollen of two *Mascagnia* species and described their pollen grains as 6-porate, with inconspicuous colpoids if present, and exine ranging from verrucate (in *M. hippocrateoides* (Tr & Planch.) Niedenzu) to psilate (in *M. nervosa* Niedenzu). For *Mascagnia cordifolia* here analyzed, we observed 8-porate pollen grains, with inconspicuous colpoids and rugulate exine, differing from the results of Roubik and Moreno (1991) about the number of apertures and the exine ornamentation. By comparing these data, we can conclude that there is possibly a variation in pollen morphology to the genus *Mascagnia*.

Three species of *Peixotoa* had their pollen grains previously described, *P. reticulata* Griseb. with spherical pollen grains, 6-porate, with irregular colpoids and obscure exine was observed by Salgado-Labouriau (1973). Anderson (1982) describes for *P. goiana* C. E. Anderson and *P. parviflora* A. Juss. 6-porate and cuboid pollen grains, which he believes to be typical for most of the genera in tribe *Banisteriae*, we also observe in this paper ornamented colpoids and rugulate exine. There is a variation in the number of apertures in *P. tomentosa* analyzed here (8-porate) and the species described above, however, the presence of colpoids and ornamentation of exine (rugulate) appears to be a common character for the genus.

Roubik and Moreno (1991) observed variations in pollen morphology for *Stigmaphyllon*, its pollen grains are medium to large, stephanocolporate (6-colporate) and periporate (6-porate or >7-porate), sexine psilate to verrucate, scabrate, or psilate. This variation was also indicated by Gonçalves-Esteves et al. (2007) when observed medium to large pollen grains, apolar, 6–8–10-porate with colpoids and rugulate or rugulate-pilate sexine. The specie analyzed by us showed medium pollen grains, 6-porate with inconspicuous colpoids, pori with aspides and psilate-rugulate exine, supporting the pollen data presented by Roubik and Moreno (1991) and Gonçalves-Esteves et al. (2007), confirming the variation in *Stigmaphyllon* pollen.

For *Diplopterys* no pollen morphology data were found in literature, and therefore this analysis in *Diplopterys pubipetala* is the first palynological study.

The data obtained confirm the euripalynous character of the Malpighiaceae, as mentioned before in the papers involving species of the family and emphasize the importance of pollen study in the analyzed genera. We observed pollen morphology particularly similar among the *Byrsonima* species, which could suggest a stenopalynous genus. In the *Banisteriopsis* species we found differences about the amb (circular to cuboidal), details of apertures (with or without aspides), and exine (sexine sometimes thicker, sometimes thinner than nexine), that also show a pollen morphology variation in the genus. The quantitative data helped in the separation of species, as evidenced by PCA, and by comparison to other studies with *Heteropterys*, *Mascagnia*, *Peixotoa*, and *Stigmaphyllon*, we can conclude that these genera also exhibit significant variations in the pollen morphology which could assist in the identification of its species.

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Appendix: Specimens examined

Banisteriopsis argyrophylla (A. Juss.) B. Gattes: BRASIL. São Paulo: Novo Horizonte, 25 Mar 2010, *N.T. Ranga* et al. *F03* (SJRP); BRASIL. São Paulo: Sales, 24 May 2008, *N.T. Ranga* et al. *19* (SJRP); BRASIL. São Paulo: Votuporanga, 15 Mar 2008, *N.T. Ranga* et al. *26* (SJRP).

Banisteriopsis muricata Cavanilles: BRASIL. São Paulo: Barretos, 05 Mar 2009, *N.T. Ranga* et al. *21* (SJRP); BRASIL. São Paulo: Taquaritinga, 12 Mar 2009, *N.T. Ranga* et al. *02* (SJRP).

Banisteriopsis nummifera (A. Juss.) B.Gates: BRASIL. São Paulo: Jaboticabal, 14 Aug 1991, *E.H.A. Rodrigues 141* (JABU).

Banisteriopsis oxyclada (A. Juss.) B. Gattes: BRASIL. São Paulo: Ida Iolanda, 30 Jun 2006, *N.T. Ranga* et al. *45* (SJRP); BRASIL. São Paulo: Macaubal, 05 Apr 2008, *N.T. Ranga* et al. *01* (SJRP); BRASIL. São Paulo: Onda Verde, 01 Jan 2007, *N.T. Ranga* et al. *14* (SJRP).

Byrsonima crassifolia (L.) Kunth: BRASIL. Minas Gerais: São Roque de Minas, Serra da Canastra, 14 Jun 2009, *B.F. Bueno 005* (JABU).

Byrsonima intermedia A. Juss.: BRASIL. Goiás: Rio Verde, 11 May 2009, *A.C. Lofego* s/n. (SJRP 30024); BRASIL. Mato Grosso: Itiquira, 03 Jun 2003, *R.D. Daud & P.R. Demite 37* (SJRP); BRASIL. São Paulo: Barretos, 21 Aug 2008, *N.T. Ranga* et al. *24* (SJRP); BRASIL. São Paulo: Bebedouro, 19 Feb 2009, *N.T. Ranga* et al. *28* (SJRP).

Byrsonima verbascifolia (L.) Rich.: BRASIL. Goiás: Edealina, 11 May 2009, *A.C. Lofego* s/n. (SJRP 30017); BRASIL. Mato Grosso: Itiquira, 03 Jun 2003, *R.D. Daud & P.R. Demite 50* (SJRP).

Diplopterys pubipetala (A. Juss.) W. R. Anderson & C. C. Davis: BRASIL. São Paulo: Birigui, Fazenda Água Branca, 11 Aug 1999, *Montilha* et al. *31* (SJRP); BRASIL. São Paulo: Sales, Fazenda Água Clara, 24 Aug 1995, *M.D.N. Grecco* et al. *105* (SJRP).

Heteropterys aceroides Griseb.: BRASIL. São Paulo: Paulo de Faria, Estação Ecológica de Paulo de Faria, 23 Jun 1993, *V. Stranghette 234* (SJRP); BRASIL. São Paulo: Paulo de Faria, Estação Ecológica de Paulo de Faria, 12 Jan 1994, *V. Stranghetti 251* (SJRP).

Mascagnia cordifolia (A. Juss.) Griseb.: BRASIL. São Paulo: Campinas, Distrito de Souzas, 22 Jun 1996, *K. Santos* s/n. (SJRP 30104); BRASIL. São Paulo: José Bonifácio, antiga Fazenda Jacaré, 07 Apr 1995, *M.R. Pietrobom da Silva 2270* (SJRP); BRASIL. São Paulo: São José do Rio Preto, Mata dos Macacos, 1 May 1999, *F. Tomasetto* s/n. (SJRP 29374).

Peixotoa tomentosa A. Juss.: BRASIL. São Paulo: Itirapina, cerrado de Itirapina, 07 May 1962, *Homero do Amaral s/n* (JABU514).

Stigmaphyllon lalandianum A. Juss.: BRASIL. São Paulo: Jaboticabal, 09 Mar 1991, *E.H.A. Rodrigues 129* (JABU).

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