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Pollen morphology of Rutaceae from Brazilian forest fragments

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ABSTRACT

This paper presents pollen morphology of 12 native Brazilian species of Rutaceae from forest fragments of São Paulo, Brazil. The aim of this research is to expand the morphological knowledge of the species, thus contributing to taxonomic knowledge and, subsequently, conservation of species and the forest area. The pollen grains were acetolyzed, measured, described qualitatively, and illustrated using light microscopy (LM) and scanning electron microscopy (SEM). The studied species have differences in polarity, size, shape, aperture and exine ornamentation of the pollen grains, confirming the eurypalynous character of Rutaceae pollen. In particular, species of *Zanthoxylum* L. also presented significant differences in the pollen grains, confirming the morphological variation within the genus. The quantitative data and multivariate analysis confirm the morphological description; thus, the results reinforce the importance of pollen morphology in the identification and characterization of species of Rutaceae.

KEYWORDS

Eurypalynous; multivariate analysis; palynotaxonomy; pollen grains

1. Introduction

Currently, global biodiversity is being threatened due to fragmentation and loss of natural environments (Fahrig 2003). In Brazil the primary cause of this fragmentation is the expansion of agricultural frontiers, especially in the Cerrado, where the flora is characterized by high levels of endemism (Myers et al. 2000; Ribeiro & Walter 2008).

Ranga et al. (2012) characterized the floristic structure of the native vegetation remnants from the northwest region of São Paulo, Brazil, searching for data about the occurrence of native species in these areas as well as the presence of endangered species in order to protect them and the conservation of biodiversity in these priority areas.

Rutaceae, with approximately 2040 species in 150–170 genera, is an important family of aromatic species with essential oils, native in the tropics and temperate regions of the Southern Hemisphere, especially South Africa and Australia (Kubitzki et al. 2011; Morton & Telmer 2014). The family is positioned in Sapindales, along with other eight families (APG IV 2016). In Brazil, Rutaceae is represented by 194 species divided into 33 genera, of which 106 species are endemic. These species can be found in all Brazilian biomes. In the Cerrado the family has 41 species and 13 genera (Pirani & Groppo 2016), and its specimens are usually associated with forest fragments, as indicated by Ranga et al. (2012).

The presence of a superior ovary, sieve tubes with S-type plastids, secretory cavities containing aromatic ethereal oils in the pericarp and parenchyma, and exstipulate leaves are morphological features that characterize Rutaceae (Chase et al. 1999). The placement of the family in the order Sapindales is strongly supported by molecular data (Gadek et al. 1996); however, despite several studies also carried out with molecular

data the relationships between the genera of Rutaceae are not yet clear (Chase et al. 1999; Scott et al. 2000; Morton et al. 2003; Poon et al. 2007; Groppo et al. 2008; Bayer et al. 2009; Morton 2009; Razafimandimbison et al. 2010; Appelhans et al. 2011), and further studies are needed to confirm the monophyly of the family (Morton & Telmer 2014).

The recent study of Rutaceae taxonomy by Morton & Telmer (2014) presents a molecular phylogeny for the six previously proposed subfamilies of the family. The authors showed a new classification for Rutaceae, confirming the monophyly of four subfamilies: Aurantioideae, Cneoroideae, Rutoideae and Amyridoideae. The authors also point out that for the establishment of a natural and stable classification of the family, new information about other taxa is needed, combining molecular and morphological data (Morton & Telmer 2014).

The pollen morphology of different species of Rutaceae has been described by Erdtman (1952), Sato (1971), Barth (1980, 1982, 1983, 1985), Arreguin-Sanchez et al. (1986), Roubick & Moreno (1991), Corrêa et al. (1992), Mziray (1992), Breis et al. (1993), Morton & Kallunki (1993), Victor & Van Wyk (1998, 1999a, 1999b, 2000, 2001), Grant et al. (2000), Carreira & Barth (2003), Qaiser & Perveen (2005), Magalhães-e-Silva (2007), Fukuda et al. (2008), Mou & Zhang (2009), Groppo et al. (2010), Cao et al. (2014), Radaeski et al. (2014), Silva et al. (2014) and Al-Anbari et al. (2015). These authors observed the differences in the morphology of the pollen grains of the species studied, especially regarding the aperture, shape and ornamentation of the exine, concluding that the family is eurypalynous.

Pollen studies on native species from remaining forest fragments indicate variations in shape, aperture, amb, ornamentation and size of the pollen grain (Souza & Gasparino 2014; Belonsi & Gasparino 2015), so the aim of this study is to identify

morphological characteristics in pollen grains of the Rutaceae species which may be used for future taxonomic and palynological studies contributing to the conservation of these species in priority conservation areas.

2. Material and methods

We studied the pollen grains of 12 Rutaceae native species (see Appendix 1) from the remnant forest fragments of the northwest state of São Paulo described by Ranga et al. (2012). These species occur throughout the Brazilian territory, mainly concentrated in the Southeast, São Paulo state, in forest fragment areas (Figure 1, Specieslink 2017). We analyzed seven genera (*Balfourodendron* Mello ex Oliv., *Esenbeckia* Kunth., *Galipea* Aubl., *Heli-etta* Tul., *Metrodorea* A.St.-Hil., *Pilocarpus* Vahl and *Zanthoxylum* L.). Each genus is represented by one species, except

Zanthoxylum which has six species. *Zanthoxylum rhoifolium* Lam. is the most widely distributed in the sampled forest fragments. All Rutaceae species listed in Ranga et al. (2012) are new records in the study area. The pollen material was obtained from samples collected mainly in the remnant forest fragments of northwest São Paulo State, deposited as dried herbarium specimens in JABU and SJRP herbaria (acronym by Thiers 2017). When necessary, we used collections from other locations. Pollen grains of 20 different specimens were studied by light microscopy (LM) and scanning electron microscopy (SEM). The pollen grains of *Zanthoxylum monogynum* A.St.-Hil., *Zanthoxylum petiolaris* A. St.-Hill. & Tul. and *Zanthoxylum rigidum* Humb. & Bonpl. ex Willd. had not been studied previously.

For LM analysis, permanent slides were prepared with pollen material acetolyzed according to Erdtman (1960) modified by Melhem et al. (2003). The pollen grains were measured (pollen

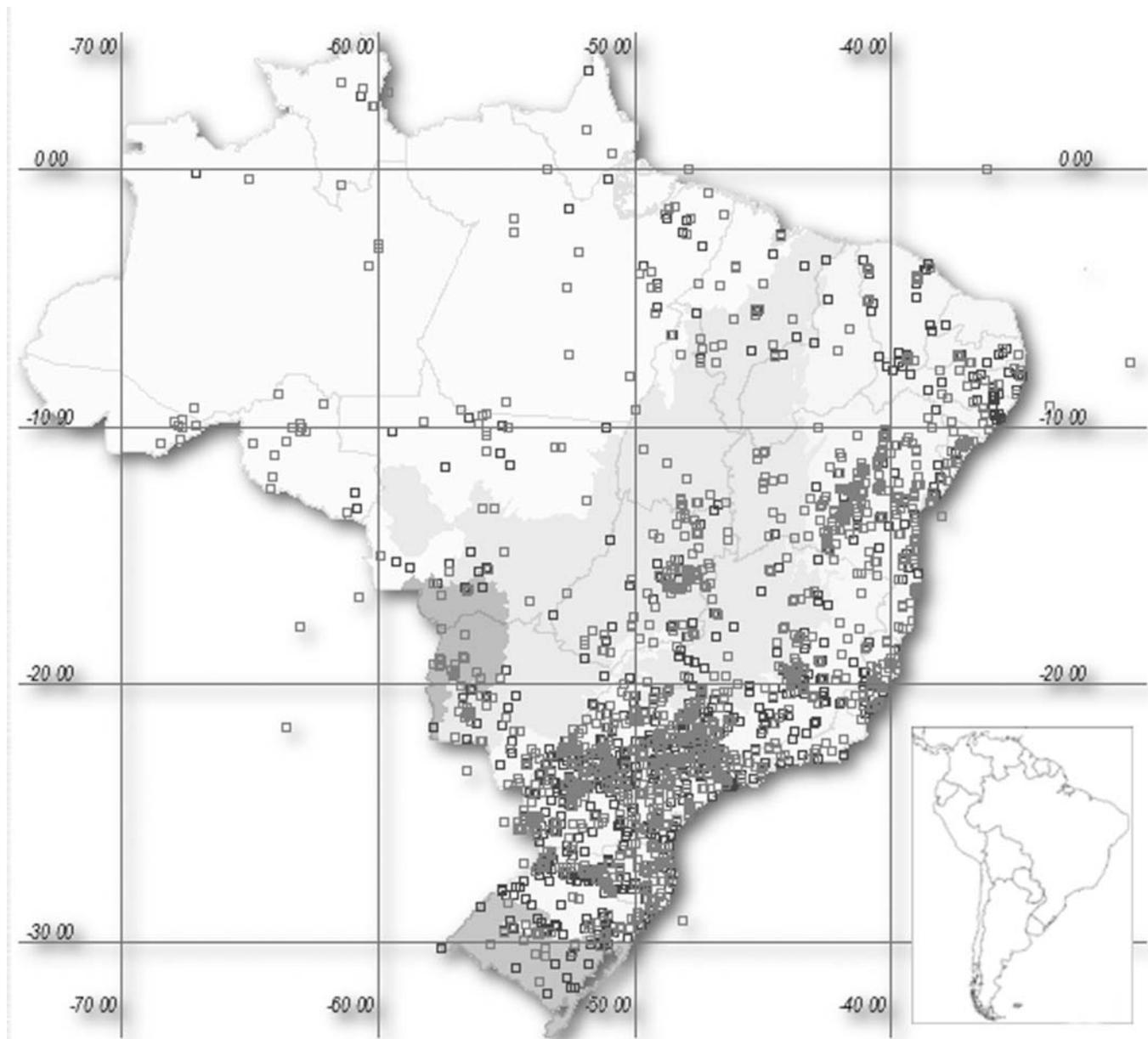


Figure 1. Occurrence map of the analyzed species of Rutaceae in Brazil. The squares correspond to the localities of collections available in the Specieslink database (<http://splink.cria.org.br/>).

diameter, $n = 25$; aperture and exine thickness, $n = 10$) 7 days after preparation as established by Salgado-Labouriau et al. (1965). For SEM we used non-acetolyzed pollen grains, following Melhem et al. (2003). The slides were deposited in the pollen reference collection of the Plant Morphology and Palynology Laboratory, Departamento de Biologia aplicada à Agropecuária, Universidade Estadual Paulista, Unesp, Jaboticabal, Brazil.

A statistical analysis was conducted with the measures taken to obtain the means (\bar{x}), standard deviation (s_x), standard error (s), 95% confidence intervals (CI), coefficient of variability (V) and range (R) according to Vieira (2011) and Zar (1999). For measures of auxin thickness, and length and width of apertures, only the arithmetic mean was calculated. To compare the values of the pollen grain diameters we used the graphs of the MINITAB program which represent the mean and the confidence interval values. A principal component analysis (PCA) was performed using the programs FITOPAC (Shepherd 1996) and PC-ORD (McCune & Mefford 2011). We used eight metric variables for the PCA: length of colpus (CLEN), width of colpus (CWID), polar diameter in equatorial view (PDIA), equatorial diameter in equatorial view (EDIA), length of endoaperture (ELEN), width of endoaperture (EWID), shape (SHAP) and exine (EXIN).

The pollen description and terminology follows Barth & Melhem (1988) and Punt et al. (2007); we adopted the polar area index proposed by Faegri & Iversen (1966), and the colpi width index is according to Gasparino et al. (2013). Photomicrographs were taken with a Bel Photonics light microscope, for LM photos, and with a JEOL JSM5410 scanning electron microscope for the SEM images.

3. Results

3.1. General description

The pollen grains of the Rutaceae species studied here (Plates 1–3) are monads, apolar or isopolar, small to large (Tables 1–3), with circular to subcircular amb, oblate-spheroidal to prolate, inaperturate, 3-colporate or 4-colporate, with long colpi, narrow or wide, with or without margo, rounded or tapered at the polar ends, endoaperture lalongate, microreticulate, reticulate, striate-microreticulate or striate-reticulate exine, sometimes with differences between apocolpium and mesocolpium, sexine thicker than nexine.

3.2. Species description

Balfourodendron riedelianum (Engl.) Engl. (Plate 1 figures 1–5; Tables 1, 3, 4)

Isopolar pollen grains, small to medium ($27.66 \times 21.49 \mu\text{m}$), circular amb, subprolate, 3-colporate, long and narrow colpi rounded at the ends, with margo, lalongate endoapertures, microreticulate homobrochate exine (Plate 1, figure 3).

Esenbeckia febrifuga (A. St.-Hill.) A. Juss ex Mart (Plate 1, figures 6–10; Tables 1, 3, 4)

Isopolar pollen grains, medium ($29.38 \times 25.21 \mu\text{m}$), circular amb, subprolate, 3-colporate, long and narrow colpi, without margo, lalongate endoapertures tapered at the ends, striate-

microreticulate exine (Plate 1, figure 10) with microreticulum homobrochate in mesocolpium (Plate 1, figure 6).

Galipea jasminiflora (A. St.-Hill.) Engl. (Plate 1, figures 11–13; Tables 1, 2, 4)

Apolar pollen grains, large ($72.60 \times 67.80 \mu\text{m}$), circular amb, prolate-spheroidal, inaperturate, reticulate heterobrochate exine, very large lumina, sinuous muri and bacula inside lumina (Plate 1, figures 12–13).

Helietta apiculata Benth. (Plate 1, figures 14–20; Tables 1, 3, 4)

Isopolar pollen grains, medium ($29.80 \times 25.60 \mu\text{m}$), circular amb, subprolate, 3-colporate, long and narrow colpi, with margo, lalongate endoapertures and tapered at the ends, striate-microreticulate exine (Plate 1, figures 19–20) with microreticulum homobrochate in mesocolpium (Plate 1, figure 14).

Metrodorea nigra A. St.-Hill. (Plate 2, figures 1–4; Tables 1, 3, 4)

Isopolar pollen grains, medium ($37.10 \times 29.10 \mu\text{m}$), subcircular amb, subprolate, 3-colporate, long and narrow colpi, with margo, lalongate endoapertures tapered at the ends, striate-microreticulate exine in apocolpium and mesocolpium (Plate 2, figures 1, 4).

Pilocarpus spicatus A. St.-Hill. (Plate 2, figures 5–9; Tables 1, 3, 4)

Isopolar pollen grains, small to medium ($30.00 \times 16.50 \mu\text{m}$), circular amb, prolate, 3-colporate, long and narrow colpi, without margo, lalongate endoapertures (Plate 2, figures 5–7), striate-reticulate exine with reticula heterobrochate in mesocolpium (Plate 2, figures 8–9) and apocolpium striate-reticulate.

Zanthoxylum caribaeum Lam. (Plate 2, figures 10–14; Tables 1, 3, 4)

Isopolar pollen grains, small to medium ($26.43 \times 18.51 \mu\text{m}$), subcircular amb, prolate, 3-colporate, long and wide colpi (Plate 2, figures 11–12), with margo, lalongate endoapertures tapered at the ends, striate-reticulate exine in apocolpium and mesocolpium (Plate 2, figures 13–14).

Zanthoxylum monogynum A.St.-Hil. (Plate 2, figures 15–19; Tables 1, 3, 4)

Isopolar pollen grains, small ($17.38 \times 15.53 \mu\text{m}$), subcircular amb, prolate-spheroidal, 3-colporate, long and wide colpi, without margo, lalongate endoapertures (not evident), striate-reticulate exine with reticula homobrochate in mesocolpium (Plate 2, figures 16, 18) and apocolpium striate-reticulate.

Zanthoxylum petiolari A. St.-Hill. & Tul. (Plate 2, figure 20, Plate 3, figures 1–4; Tables 1, 3, 4)

Isopolar pollen grains, small ($20.57 \times 18.92 \mu\text{m}$), circular amb, oblate-spheroidal, 3-colporate, long and narrow colpi, without margo, lalongate endoapertures tapered at the ends, striate-reticulate exine with reticula homobrochate in mesocolpium (Plate 3, figure 3) and apocolpium striate-reticulate (Plate 2, figure 20).

Zanthoxylum rhoifolium Lam. (Plate 3, figures 5–9; Tables 1, 3, 4)

Isopolar pollen grains, small to medium ($23.10 \times 24.60 \mu\text{m}$), circular amb, oblate-spheroidal, 4-colporate (Plate 3, figure 5), long and narrow colpi, without margo, lalongate endoapertures tapered at the ends, striate-reticulate exine with reticula heterobrochate in apocolpium (Plate 3, figure 5) and mesocolpium striate-reticulate (Plate 3, figures 8–9).

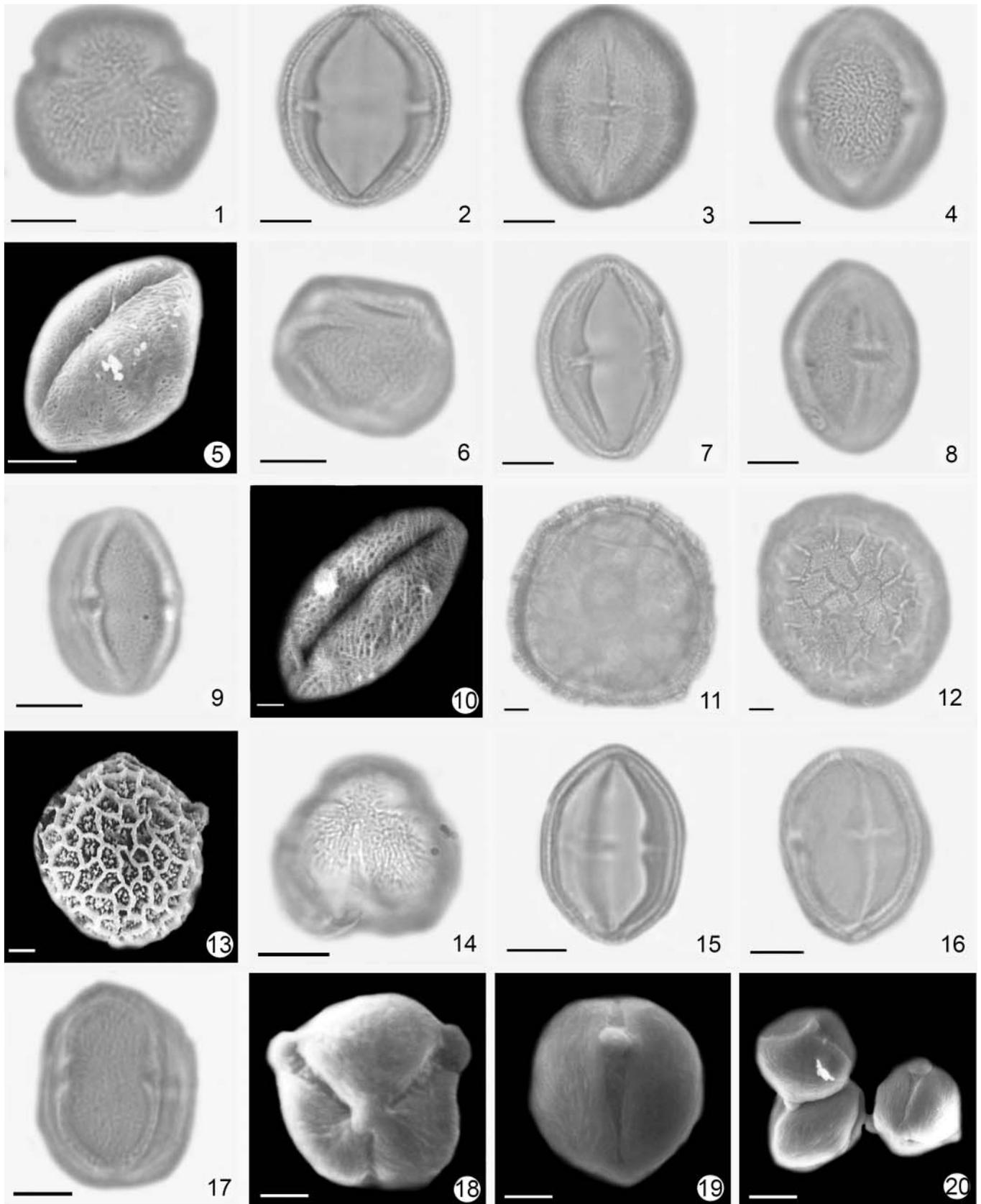


Plate 1. Photomicrographs and scanning electron micrographs (SEM) of the pollen grains of Rutaceae A. Juss. Figures 1–5. *Balfourodendron riedelianum* (Engl.) Engl. 1. General aspect, polar view. 2. Polar view, optical section. 3. Equatorial view, aperture. 4. Equatorial view, ornamentation. 5. Equatorial view, in SEM. Figures 6–10. *Esenbeckia febrifuga* (A. St.-Hill.) A. Juss ex Mart. 6. General aspect, polar view. 7. Equatorial view, optical section. 8. Equatorial view, aperture. 9. Equatorial view, ornamentation. 10. Equatorial view, in SEM. Figures 11–13. *Galipea jasminiflora* (A. St.-Hill.) Engl. 11. General aspect, optical section. 12. General aspect, ornamentation in SEM. 13. General aspect, ornamentation in SEM. Figures 14–20. *Heliotta apiculata* Benth. 14. General aspect, polar view. 15. Equatorial view, optical section. 16. Equatorial view, aperture. 17. Equatorial view, ornamentation. 18. Polar view, in SEM. 19. Equatorial view, in SEM. 20. General aspect, in SEM. Scale bars: 1–9, 11–17 = 10 μm ; 10, 18–20 = 5 μm .

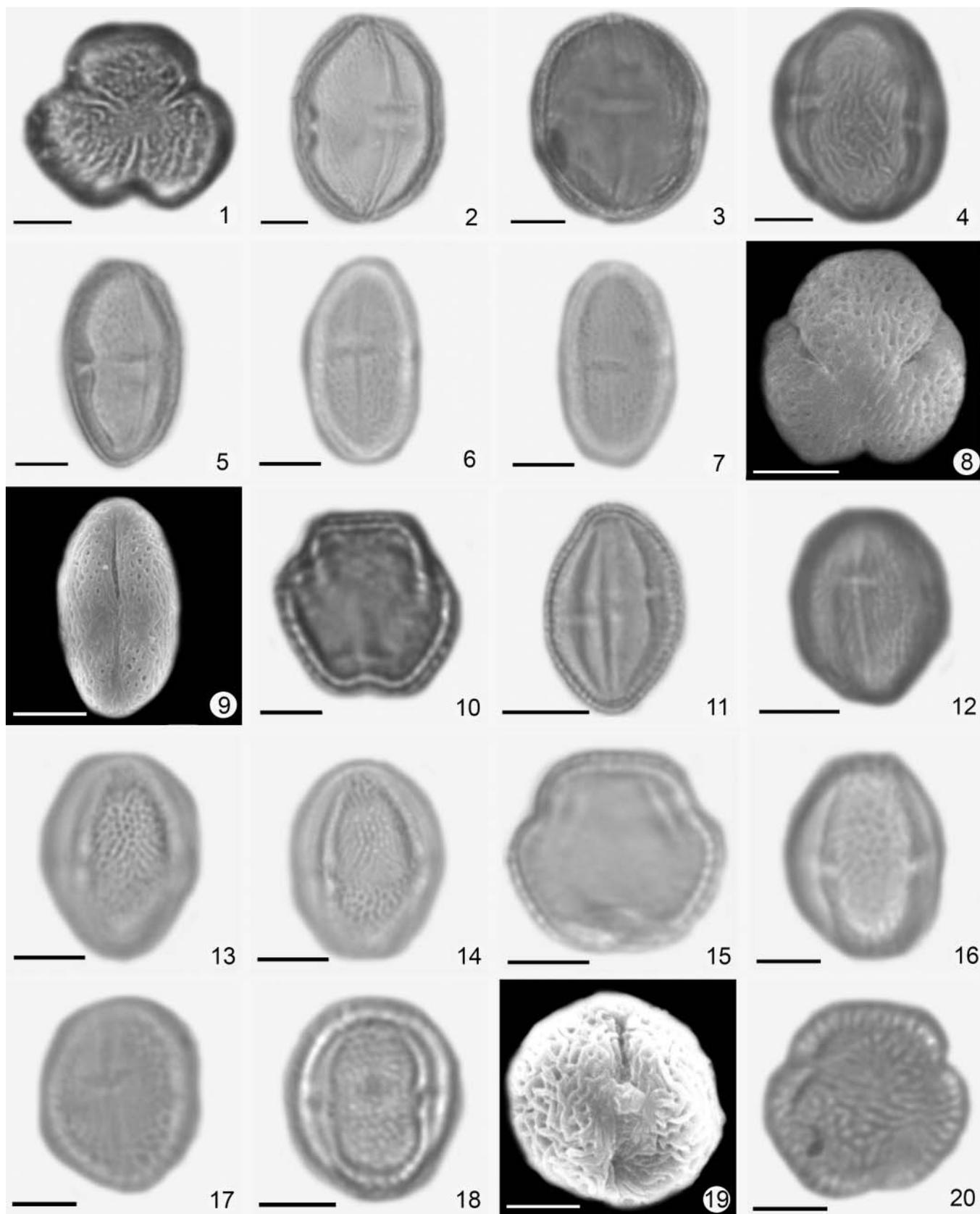


Plate 2. Photomicrographs and scanning electron micrographs (SEM) of the pollen grains of Rutaceae A. Juss. Figures 1–4. *Metrodorea nigra* A. St.-Hill. 1. General aspect, polar view. 2. Equatorial view, optical section. 3. Equatorial view, aperture. 4. Equatorial view, ornamentation. Figures 5–9. *Pilocarpus spicatus* A. St.-Hill. 5. General aspect, equatorial view. 6. Equatorial view, aperture. 7. Equatorial view, ornamentation. 8. Polar view, in SEM. 9. Equatorial view, in SEM. Figures 10–14. *Zanthoxylum caribaeum* (A. St.-Hill. & Tul.) Reynel. 10. General aspect, polar view. 11. General aspect, equatorial view. 12. Equatorial view, aperture. 13. Equatorial view, ornamentation in high focus. 14. Equatorial view, ornamentation in low focus. Figures 15–19. *Zanthoxylum monogynum* A. St.-Hill. 15. General aspect, polar view. 16. Equatorial view, optical section. 17. Equatorial view, aperture. 18. Equatorial view, ornamentation. 19. Equatorial view, in SEM. Figure 20. *Zanthoxylum petiolaris* A. St.-Hill. & Tul. Polar view, ornamentation. Scale bars = 10 μm .

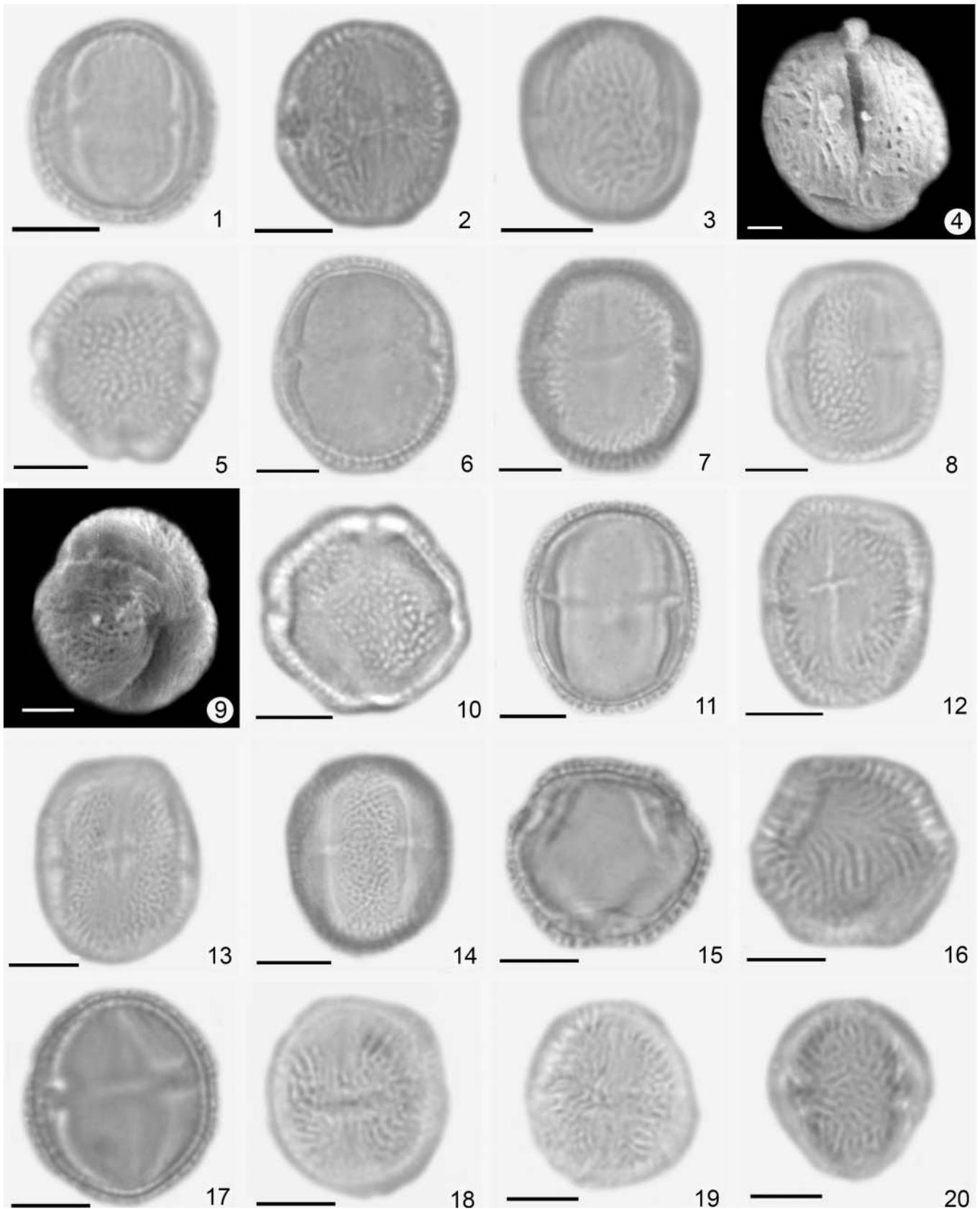


Plate 3. Photomicrographs and scanning electron micrographs (SEM) of the pollen grains of Rutaceae. Figures 1–4. *Zanthoxylum petiolari* A. St.-Hill. & Tul. 1. Equatorial view, optical section. 2. Equatorial view, aperture. 3. Equatorial view, ornamentation. 4. Equatorial view, in SEM. Figures 5–9. *Zanthoxylum rhoifolium* Lam. 5. Polar view, ornamentation. 6. Equatorial view, optical section. 7. Equatorial view, aperture. 8. Equatorial view, ornamentation. 9. Polar view, in SEM. Figures 10–14. *Zanthoxylum riedelianum* Engl. 10. General aspect, optical section. 11. Equatorial view, aperture. 12. Equatorial view, colpus and ornamentation. 13. Equatorial view, ornamentation. Figures 15–20. *Zanthoxylum rigidum* Humb. & Bonpl. ex Willd. 15. Polar view, optical section. 16. Polar view, ornamentation. 17. Equatorial view, optical section. 18. Equatorial view, aperture. 19. Equatorial view, ornamentation in high focus. 20. Equatorial view, ornamentation in low focus. Scale bars = 1–3, 5–8, 10–20 = 10 μm ; 4, 9 = 5 μm .

Table 1. Morphological characterization of Rutaceae pollen grains (P/E = ratio between polar and equatorial diameter; AN = aperture number; EO = exine ornamentation; S = small; M = medium; L = large; OS = oblate spheroidal; PR = prolate; PS = prolate spheroidal; SP = subprolate; MR = microreticulate; SM = striate-microreticulate; SR = striate-reticulate; RT = reticulate; ST = striate).

Species	Size	P/E	Shape	AN	EO
<i>Balfourodendron riedelianum</i>	S–M	1.29	SP	3	MR
<i>Esenbeckia febrifuga</i>	M	1.17	SP	3	SM
<i>Galipea jasminiflora</i>	L	1.07	PS	0	RT
<i>Helietta apiculata</i>	M	1.16	SP	3	SM
<i>Metrodorea nigra</i>	M	1.27	SP	3	SM
<i>Pilocarpus spicatus</i>	S–M	1.82	PR	3	SR
<i>Zanthoxylum caribaeum</i>	M	1.43	PR	3	SR
<i>Zanthoxylum monogynum</i>	S	1.12	PS	3	SR
<i>Zanthoxylum petiolari</i>	S	0.92	OS	3	SR
<i>Zanthoxylum rhoifolium</i>	M	0.94	OS	4	SR
<i>Zanthoxylum riedelianum</i>	M	1.18	SP	4	SM
<i>Zanthoxylum rigidum</i>	S–M	1.09	PS	3	SR

Table 2. Quantitative data of inaperturate pollen grains of Rutaceae, $n = 25$.

Species	(R) $x \pm s_x$	s	CI	V
<i>Galipea jasminiflora</i>				
Diameter I	(62.50–87.50) 72.60 \pm 0.97	4.87	(70.59–74.61)	6.70
Diameter II	(62.50–75.00) 67.80 \pm 0.86	4.29	(66.03–69.57)	6.33

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

Zanthoxylum riedelianum Engl. (Plate 3, figures 10–14; Tables 1, 3, 4)

Isopolar pollen grains, small to medium (27.15 \times 22.93 μm), circular amb, subprolate, 4-colporate (Plate 3, figure 10), long and narrow colpi, without margo, lalongate endoapertures tapered at the ends, striate-microreticulate exine with

Table 3. Quantitative data of aperturate pollen grains of Rutaceae, $n = 25$.

Species	(R) $x \pm s_x$	s	CI	V
Polar diameter in equatorial view				
<i>B. riedelianum</i>	(23.14–30.85) 27.66 \pm 0.43	2.14	(26.78–28.54)	07.72
<i>E. febrifuga</i>	(25.00–32.50) 29.38 \pm 0.01	2.17	(29.36–29.39)	07.37
<i>H. apiculata</i>	(25.00–37.50) 29.80 \pm 0.72	3.60	(28.32–31.28)	12.09
<i>M. nigra</i>	(32.50–42.50) 37.10 \pm 0.57	2.86	(35.92–38.28)	07.70
<i>P. spicatus</i>	(25.00–37.50) 30.00 \pm 0.78	3.89	(28.40–31.60)	12.95
<i>Z. caribaeum</i>	(23.14–33.42) 26.43 \pm 0.58	2.92	(25.23–27.63)	11.06
<i>Z. monogynum</i>	(15.43–20.57) 17.38 \pm 0.37	1.86	(16.61–18.15)	10.70
<i>Z. petiolari</i>	(15.43–23.14) 20.57 \pm 0.42	2.10	(19.70–21.43)	10.21
<i>Z. rhoifolium</i>	(18.75–27.50) 23.10 \pm 0.47	2.34	(22.14–24.06)	10.14
<i>Z. riedelianum</i>	(23.14–30.85) 27.15 \pm 0.45	2.24	(26.23–28.07)	08.24
<i>Z. rigidum</i>	(22.50–30.00) 26.00 \pm 0.32	1.61	(25.34–26.66)	06.21
Equatorial diameter in equatorial view				
<i>B. riedelianum</i>	(18.00–25.71) 21.49 \pm 0.43	1.95	(20.69–22.30)	09.06
<i>E. febrifuga</i>	(22.50–30.00) 25.21 \pm 0.57	1.98	(24.03–26.39)	07.86
<i>H. apiculata</i>	(20.00–30.00) 25.60 \pm 0.42	2.08	(24.74–26.46)	08.11
<i>M. nigra</i>	(25.00–35.00) 29.10 \pm 0.58	2.88	(27.92–30.28)	09.88
<i>P. spicatus</i>	(12.50–20.00) 16.50 \pm 0.46	2.28	(15.56–17.44)	13.83
<i>Z. caribaeum</i>	(12.86–25.71) 18.51 \pm 0.65	3.24	(17.18–19.84)	17.48
<i>Z. monogynum</i>	(12.86–18.00) 15.53 \pm 0.35	1.74	(14.81–16.24)	11.19
<i>Z. petiolari</i>	(15.43–23.14) 18.92 \pm 0.39	1.95	(18.12–19.72)	10.29
<i>Z. rhoifolium</i>	(22.50–27.50) 24.60 \pm 0.31	1.56	(23.96–25.24)	06.35
<i>Z. riedelianum</i>	(20.57–25.71) 22.93 \pm 0.39	1.95	(22.13–23.74)	08.51
<i>Z. rigidum</i>	(20.00–25.00) 23.80 \pm 0.36	1.79	(23.06–24.54)	07.50

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

Table 4. Measurements (in μm) of Rutaceae pollen grains, apertures and exine ($n = 10$).

Species	Colpus		Endoaperture		Ex	WCI
	Length	Width	Length	Width		
<i>Balfourodendron riedelianum</i>	19.28	2.31	1.62	9.00	1.30	9.30
<i>Esenbeckia febrifuga</i>	24.42	2.70	2.57	12.60	2.57	9.33
<i>Galipea jasminiflora</i>	–	–	–	–	4.37	–
<i>Helietta apiculata</i>	20.17	2.57	2.57	13.63	2.57	9.96
<i>Metrodorea nigra</i>	25.10	2.60	2.70	13.70	2.70	11.19
<i>Pilocarpus spicatus</i>	25.30	1.00	1.90	7.70	1.30	16.50
<i>Zanthoxylum caribaeum</i>	17.74	2.83	2.57	7.46	2.57	6.54
<i>Zanthoxylum monogynum</i>	25.70	2.83	2.55	16.71	3.00	5.48
<i>Zanthoxylum petiolari</i>	14.14	2.57	2.57	9.38	2.70	7.36
<i>Zanthoxylum rhoifolium</i>	20.56	2.57	2.44	10.80	2.57	9.57
<i>Zanthoxylum riedelianum</i>	16.71	1.89	2.16	10.28	1.77	11.97
<i>Zanthoxylum rigidum</i>	14.39	2.57	2.57	5.91	2.57	9.26

Ex exine thickness, WCI width colpus index.

microreticulum homobrochate in mesocolpium (Plate 3, figure 14) and apocolpium striate-microreticulate (Plate 3, figure 10).

Zanthoxylum rigidum Humb. & Bonpl. ex Willd. (Plate 3, figures 15–20; Tables 1, 3, 4)

Isopolar pollen grains, small to medium (26.00 \times 23.80 μm), subcircular amb, prolate-spheroidal, 3-colporate, long and narrow colpi, without margo, lalongate endoapertures tapered at the ends, striate-reticulate exine in apocolpium and mesocolpium (Plate 3, figures 16, 20).

3.3. Pollen key to Rutaceae species

1. Apolar and inaperturate pollen grains
Galipea jasminiflora
- 1'. Isopolar and aperturate pollen grains 2
2. Pollen grains 4-colporate 3
3. Oblate-spheroidal pollen grains with reticulate heterobrochate apocolpium
Zanthoxylum rhoifolium
- 3'. Subprolate pollen grains with striate-microreticulate apocolpium
Zanthoxylum riedelianum
- 2'. Pollen grains 3-colporate 4
4. Exine microreticulate ... *Balfourodendron riedelianum*
- 4'. Exine striate-reticulate or striate-microreticulate 5
5. Ornamentation striate-microreticulate 6
6. Apocolpium and mesocolpium striate-microreticulate *Metrodorea nigra*
- 6'. Mesocolpium microreticulate 7
7. Colpi without margo *Esenbeckia febrifuga*
- 7'. Colpi with margo *Helietta apiculata*
- 5'. Ornamentation striate-reticulate 8
8. Apocolpium and mesocolpium striate-reticulate ... 9
9. Prolate pollen grains, wide colpi with margo
Zanthoxylum caribaeum
- 9'. Prolate-spheroidal pollen grains, narrow colpi without margo *Zanthoxylum rigidum*
- 8'. Apocolpium and mesocolpium reticulate 10
10. Heterobrochate reticula in mesocolpium
Pilocarpus spicatus

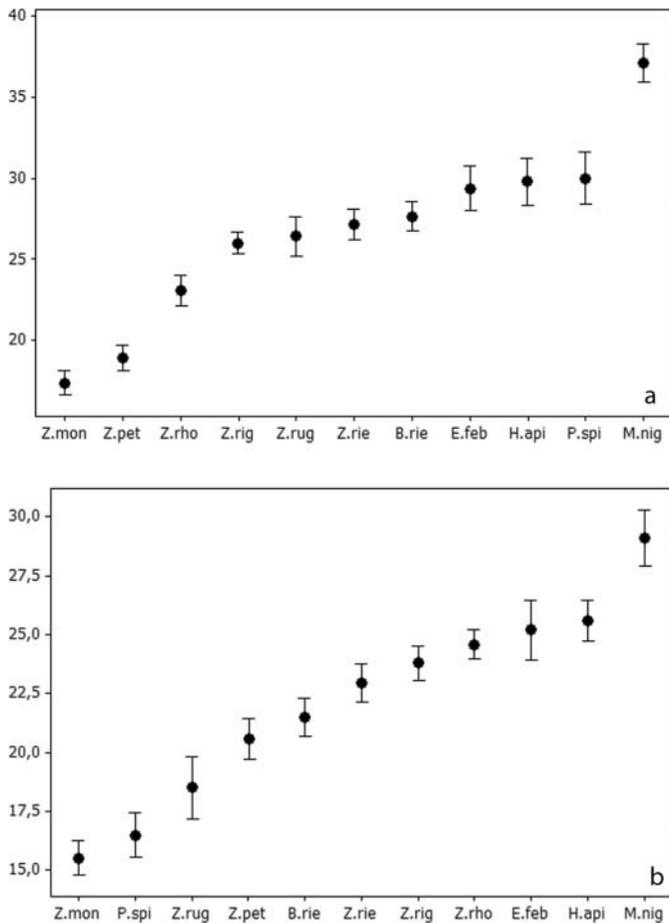


Figure 2. Representation of confidence interval of mean in 95% of the pollen grains of Rutaceae. (a) Polar diameter in equatorial view. (b) Equatorial diameter in equatorial view. The higher and lower boundaries show the confidence interval; the average circle shows the arithmetic mean. B.rie = *Balfourodendron riedelianum*; E.feb = *Esenbeckia febrifuga*; H.api = *Helietta apiculata*; M.nig = *Metrodorea nigra*; P.spi = *Pilocarpus spicatus*; Z.car = *Zanthoxylum caribaeum*; Z.mon = *Zanthoxylum monogynum*; Z.pet = *Zanthoxylum petiolaris*; Z.rho = *Zanthoxylum rhoifolium*; Z.rie = *Zanthoxylum riedelianum*; Z.rig = *Zanthoxylum rigidum*. The values are in μm .

- 10'. Homobrochate reticula in mesocolpium 11
- 11. Prolate-spheroidal pollen grains, wide colpi *Zanthoxylum monogynum*
- 11'. Oblate-spheroidal pollen grains, narrow colpi *Zanthoxylum petiolaris*

3.4. Analysis of quantitative data

For quantitative analysis of the pollen grain size we used the polar and equatorial diameters in equatorial view for the aper-turate species (Figure 2a, b). *Galipea jasminiflora*, with inaperturate pollen grains, was excluded from this comparison. When we observe the mean and confidence interval of the pollen grain diameters, we find that *Zanthoxylum monogynum* and *Zanthoxylum petiolaris* are separated from the other species by lower polar diameters. The same applies to the *Metrodorea nigra* pollen grains; however, this species has higher values for the polar diameter. The other species are in a continuous group of intermediate diameter values.

When we consider the equatorial diameter, *Metrodorea nigra* has a larger diameter than all the other species. We can also observe a continuous group with all other species in which the *Zanthoxylum* species have the lowest values for this diameter (Figure 2a, b).

The PCA is an exploratory analysis of quantitative data val-ued for Rutaceae pollen grains. This analysis summarizes in its two axes (Figure 3) 94.42% of total data variability. The first axis of the PCA explained 89.88% of the total variability according to the metric variables. On this axis we note a separation in the ordination of the species, so that *Galipea jasminiflora*, the only species with large and inaperturate pollen grains, appears alone on the positive side of the first axis, in contrast to the others that are very close together on the negative side of this axis (except *Zanthoxylum rigidum*). The most relevant variables for the species grouping in the first axis were the length of the col-pus (CLEN) and width of the endoaperture (EWID) (Table 5).

Although the contributions of the second PCA axis were much smaller than those of the first axis (only 4.54%), we can

Table 5. Pearson and Kendall correlation coefficients for pollen grain metric variables of the first and the second axis of principal component analysis (PCA) ordination in Rutaceae species.

Variables	Principal components	
	Axis 1	Axis 2
EDIA	0.2381	-0.2882
PDIA	0.2171	0.1993
CLEN	-0.6682	0.3400
CWID	-0.2640	-0.4772
ELEN	-0.2661	-0.2141
EWID	-0.5501	-0.2356
EXIN	0.0941	-0.5893
SHAP	-0.0122	0.2921

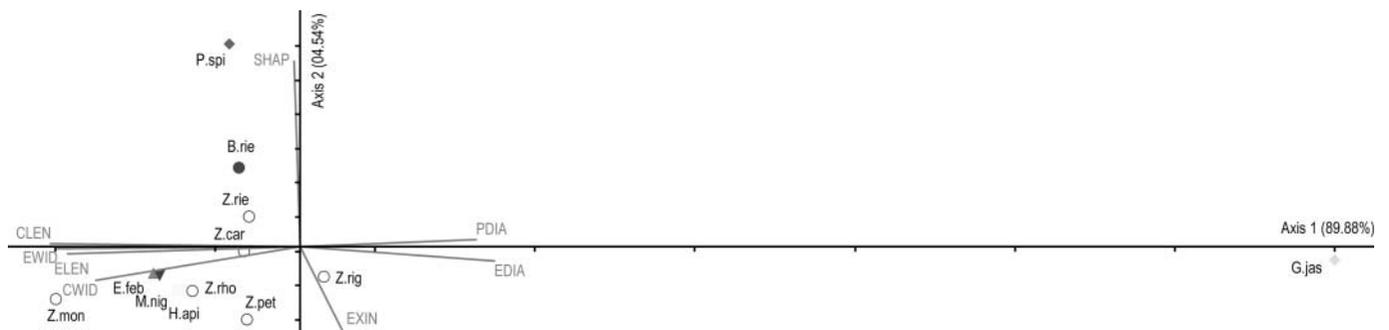


Figure 3. Principal component analysis performed with the pollen metrical variables from Rutaceae species. B.rie = *Balfourodendron riedelianum*; E.feb = *Esenbeckia febrifuga*; G.jas = *Galipea jasminiflora*; H.api = *Helietta apiculata*; M.nig = *Metrodorea nigra*; P.spi = *Pilocarpus spicatus*; Z.car = *Zanthoxylum caribaeum*; Z.mon = *Zanthoxylum monogynum*; Z.pet = *Zanthoxylum petiolaris*; Z.rho = *Zanthoxylum rhoifolium*; Z.rie = *Zanthoxylum riedelianum*; Z.rig = *Zanthoxylum rigidum*.

check the distance of *Pilocarpus spicatus* (on the positive side) from the other analyzed species. The values of exine (EXIN) and width of colpus (CWID) were the most important variables for the placement of the species along the second axis (Table 5).

The PCA of the Rutaceae species here analyzed confirms the qualitative data described for the pollen grains since only *Galipea jasminiflora* (with large and inaperturate pollen grains) is well separated from all other species. The high values in shape also contributed to the distance of *Pilocarpus spicatus* pollen grains from other species. Although important, the measurements obtained of pollen grains without the qualitative data contribute little to separate the genera and species.

4. Discussion

The pollen grains of the Rutaceae generally present variations in shape, aperture number and type, and exine ornamentation, as portrayed in previous studies (Morton & Kallunki 1993; Grant et al. 2000; Fukuda et al. 2008). They are usually colpulate (rarely porate as in *Spiranthera* A.St.-Hil. or pantoaperturate as in *Almeida* A.St.-Hil.), with three to seven apertures and microreticulate, reticulate, striate, striate-reticulate or, rarely, psilate exine, such as in *Hortia* Vand. species (Morton & Kallunki 1993; Groppo et al. 2010). The Rutaceae genera here analyzed were previously reported by Barth (1980, 1982, 1985), Corrêa et al. (1992), Roubick & Moreno (1991), Carreira & Barth (2003), Magalhães-e-Silva (2007), Cao et al. (2014) and Radaeski et al. (2014).

We found in this study, for *Balfourodendron riedelianum*, small to medium pollen grains, with circular amb, subprolate, 3-colpulate with microreticulate exine. The pollen data are similar to those of Magalhães-e-Silva (2007) for *B. molle*. In other species of this genus, however, this author classified the exine as striate, as did Barth (1982), which may indicate an ornamentation variation of *Balfourodendron*.

Corrêa et al. (1992) described *Esenbeckia febrifuga* as having medium, prolate, 3-colpulate and psilate pollen grains. The results in our study for this species corroborate the previous data; however, we observed subprolate shape and striate-microreticulate ornamentation.

Although *Esenbeckia* and *Metrodorea* are phylogenetically very close (Groppo et al. 2008), the revision of Barth (1982) indicates that these have a distinct pollen morphology, with pollen grains 3-colpulate and striate in *Metrodorea*. We found similar data in *Metrodorea nigra* in this study, with variation only in the ornamentation, described here as microreticulate.

The *Galipea jasminiflora* pollen grains were described by Erdtman (1952) as subprolate, 3-colpulate and reticulate. Barth (1982) confirmed the results of Erdtman (1952) and also defined the ornamentation of this species as reticulate with numerous bacula inside each lumen. The pollen data introduced by Erdtman (1952) differ from ours in shape (here prolate spheroidal) and aperture (inaperturate). Through the analysis of SEM, we observed bacula inside the lumina of the reticulum as reported by Barth (1982).

The differences in the results previously reported on *Galipea jasminiflora* and the morphological variations found between the pollen grains of several *Galipea* species described by Barth (1982), such as *G. pentagina* A.St.-Hil. with 4-zonocolpulate

pollen grains and *G. bracteata* (Nees & Mart.) Schult. with brevicolpulate pollen grains, indicate a eurypalynous genus.

Helietta was cited in the review of Barth (1982) as stenopalynous with prolate, 3-colpulate and reticulate pollen grains, with discrete variation in the ornamentation, just as for *Zanthoxylum*, *Pilocarpus* and *Esenbeckia*. Radaeski et al. (2014) described *Helietta apiculata* pollen as subtriangular, subprolate, 3-colpulate, lalongate and with a striate-microreticulate exine. Our results corroborate Radaeski et al. (2014), and we observed an ornamentation variation of the apocolpium (striate-microreticulate) and mesocolpium (microreticulate) for these pollen grains, as mentioned by Barth (1982).

Pollen grains of *Pilocarpus* species were described in Barth's studies (1982, 1985); in general, the author pointed out prolate, 3-colpulate and reticulate pollen, similar to those described by Carreira & Barth (2003) for *P. microphyllus* Stapf. ex Wardlew. *Pilocarpus spicatus* here analyzed has prolate and 3-colpulate pollen grains as described above; however, we observe a striate-reticulate ornamentation, with heterobrochate reticula very evident in the mesocolpium. These details of the pollen surface can be used in species distinctions within *Pilocarpus*, as noted by Barth (1982) for other genera of Rutaceae.

Barth (1980) classified *Zanthoxylum rhoifolium* pollen grains as medium, prolate, 3-colpulate with exine partially striate, while Corrêa et al. (1992) described them as small, subprolate, 3-colpulate and striate-reticulate. Our results are similar to those of Corrêa et al. (1992) but differing in shape, here oblate spheroidal and aperture numbers (4-colpulate).

For *Zanthoxylum riedelianum* and *Z. caribaeum* (still under the name *Z. rugosum*), Barth (1980) found prolate, 3-colpulate and reticulate pollen grains, differing from our results in terms of aperture (4-colpulate to *Z. riedelianum*) and ornamentation (striate-reticulate or striate-microreticulate). The pollen grains of *Zanthoxylum* species have variations in the margo of the apertures, and differences in ornamentation between apocolpium and mesocolpium.

Roubick & Moreno (1991) classified the pollen grains of *Zanthoxylum* as circular, subprolate to oblate spheroidal, 3-colpulate, reticulate or striate-reticulate. Similar results were reported by Cao et al. (2014), with small to medium pollen grains, spheroidal to prolate, 3-colpulate, macroreticulate, striate-rugulate or striate. In our study we observed in the *Zanthoxylum* species some differences in shape, aperture number and pollen grain ornamentation. We did not identify reticulate pollen grains (as previously mentioned by Roubick & Moreno 1991), nor macroreticulate or striate-rugulate (as cited in Cao et al. 2014); likewise, previous studies did not observe 4-colpulate apertures in pollen grains for the genus, so these palynological data are important to the species identification.

The pollen morphological variation found in the analyzed species and genera of Rutaceae reinforce the importance of palynological studies in Rutaceae. We verified that the quantitative data in some cases could help distinguish the species, as in *Galipea jasminiflora* (with large pollen grains) and *Metrodorea nigra* (among colpulate species that also have larger pollen grains). For Rutaceae, as well as in other families also analyzed in forest fragments (Souza & Gasparino 2014; Belonsi & Gasparino 2015), qualitative data are the main tools in pollen species distinction.

5. Conclusions

- (1) The aperture number of Rutaceae pollen grains allows the distinction of inaperturate (*Galipea jasminiflora*, with apolar pollen grains), 4-colporate (*Zanthoxylum rhoifolium* and *Z. riedelianum*) and 3-colporate species (the remaining species).
- (2) We find different types of pollen ornamentation: microreticulate (*Balfourodendron riedelianum*), reticulate (*Galipea jasminiflora*), striate-microreticulate (*Esenbeckia febrifuga*, *Heliectia apiculata*, *Metrodorea nigra* and *Zanthoxylum riedelianum*) and striate-reticulate (*Pilocarpus spicatus* and all other species of *Zanthoxylum*).
- (3) The species of *Esenbeckia*, *Heliectia*, *Pilocarpus* and *Zanthoxylum* show differences in ornamentation between the apocolpium and mesocolpium of the pollen grains, which helps in the identification of the taxa.
- (4) The results obtained from this study demonstrate the pollen diversity of Rutaceae. It is regarded as a eurypalynous family. As mentioned, details of the presence or absence, number of apertures, and ornamentation (and its variations) have contributed to species recognition in the Rutaceae examined.

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Disclosure statement

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Appendix 1. Specimens examined

Balfourodendron riedelianum (Engl.) Engl.: Brasil. Paraná: Tabagi, 27 Oct 1995, F. Chagas e Silva & L.H. Soares-Silva 1859 (SJRP); Brasil. São Paulo: Jaboticabal, 19 Dec 1994, E.A. Rodrigues 254 (JABU).

Esenbeckia febrifuga (A. St.-Hill.) A. Juss ex Mart: Brasil. Paraná: Londrina, 02 Apr 1990, M. F. Gouvea s/n (SJRP); Brasil. Paraná: Rolândia, 25 Nov 1997, V.F. Kinupp 188 (SJRP).

Galipea jasminiflora (A. St.-Hill.) Engl.: Brasil. São Paulo: Matão, 24 Mar 2008, N.T. Ranga 88 (SJRP); Brasil. São Paulo: Matão, 15 Feb 2008, N.T. Ranga s/n (SJRP).

Helietta apiculata Benth.: Brasil. São Paulo: General Salgado, 14 Nov 1999, E. Montilha et al. 59 (SJRP); Brasil. Paraná: Sapopema, 06 Dec 1997, C. Medri & E. M. Francisco 544 (SJRP).

Metrodorea nigra A. St.-Hill.: Brasil. Paraná: Lobato, 25 Oct 1986, G. Hatschbach 50660 (SJRP).

Pilocarpus spicatus A. St.-Hill.: Brasil. Minas Gerais: Monte Azul, 18 Apr 1996, G. Hatschbach et al. 64970 (SJRP); Brasil. Bahia: Caetitê, 21 Apr 1996, G. Hatschbach et al. 65173 (SJRP).

Zanthoxylum caribaeum Lam.: Brasil. São Paulo: Sertãoópolis, 29 Sep 1995, A.T. Dias 41 (SJRP).

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