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Pollen morphology of Rubiaceae Juss. species occurring in an area of caatinga (dryland) vegetation in Bahia State, Brazil

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RESUMO

(Morfologia polínica de espécies de Rubiaceae ocorrentes em uma área de caatinga no estado da Bahia, Brasil). Rubiaceae foi representada na flora de Brejinho das Ametistas por dezesseis espécies, as quais foram tratadas palinologicamente no presente estudo: *Coccocypselum hirsutum* Bartl. ex DC., *Cordiera rigida* Kuntze, *Coutarea hexandra* K.Schum., *Declieuxia fruticosa* Kuntze, *Diodella apiculata* (Willd. ex Roem. & Schult.) Delprete, *D. radula* (Willd. ex Roem. & Schult.) Delprete, *D. teres* Small., *Emmeorhiza umbellata* K.Schum., *Leptoscela ruellioides* Hook. f., *Mitracarpus baturitensis* Sucre., *Mitracarpus villosus* Cham. & Schltdl., *Palicourea rigida* Kunth, *Psyllocarpus asparagoides* Mart., *Richardia grandiflora* Steud., *Staelia aurea* K. Schum., *Staelia galioides* DC. Para análise sob microscopia de luz e eletrônica de varredura, os grãos de pólen foram acetolisados, mensurados, descritos, foto e eletromicrografados. Os grãos de pólen variaram de pequenos a grandes; suboblatos a subprolatos. Inaperturados em *P. rigida*, colpados, e colporados na maioria das espécies, com número de aberturas variável de três a várias. Exina predominantemente microrreticulada entre as espécies, sendo reticulada (*C. hirsutum, C. rigida, P. rigida*), birreticulada (*D. fruticosa*), microequinada-perfurada (*C. hexandra*), equinado-granulada (*R. grandiflora*), equinado-granulado-perfurada (*D. apiculata* e *D. teres*), e psilada (*P. asparagoides*). Os resultados mostraram que as espécies são palinologicamente distintas.

Palavras-chave: Gentianales, grãos de pólen, Palinologia

ABSTRACT

(Pollen morphology of Rubiaceae species occurring in an area of *caatinga* (dryland) vegetation in Bahia State, Brazil). The palynology of the following 16 species of Rubiaceae, from Brejinho das Amestistas, was investigated: *Coccocypselum hirsutum* Bartl. ex DC., *Cordiera rigida* Kuntze, *Coutarea hexandra* K.Schum., *Declieuxia fruticosa* Kuntze, *Diodella apiculata* (Willd. ex Roem. & Schult.) Delprete, *D. radula* (Willd. ex Roem. & Schult.) Delprete, *D. teres* Small., *Emmeorhiza umbellata* K.Schum., *Leptoscela ruellioides* Hook. f., *Mitracarpus baturitensis* Sucre., *Mitracarpus villosus* Cham. & Schltdl., *Palicourea rigida* Kunth, *Psyllocarpus asparagoides* Mart., *Richardia grandiflora* Steud., *Staelia aurea* K. Schum., and *Staelia galioides* DC. The pollen grains were acetolysed to and their morphological characters were analyzed using light and scanning electron microscopy. They varied in size from small to large; were suboblate to subprolate; inaperturate (*P. rigida*), colpate and colporate in the remaining species, with an aperture number that varied from three to several. The exines were microreticulate in most species, reticulate (*C. hirsutum, C. rigida* and *P. rigida*), bireticulate (*D. fruticosa*), microechinate-perforated (*C. hexandra*), echinate-granulate (*R. grandiflora*), echinate-granulate-perforate (*D. apiculata* and *D. teres*), and psilate (*P. asparagoides*). Based on the results, palynological data can be used to distinguish these species.

Key words: Gentianales, pollen grains, Palynology

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Introduction

The family Rubiaceae, as currently circumscribed, belongs to the order Gentianales and comprises 637 genera and approximately 10,700 species (Robbrecht 1988); approximately 112 genera and 1347 species occur in Brazil (Barbosa *et al.* 2010). The family is widely distributed globally, although its diversity is principally concentrated in tropical regions (Souza & Lorenzi 2008; Judd *et al.* 2009). The individual species of this group have various habits, such as herbs, subshrubs, shrubs, and trees; lianas and epiphytes are less common. Morphologically, the family is characterized by having simple, opposite leaves, interpetiolar stipules, and epipetalous stamens equal in number to the petals (Souza & Lorenzi 2008). Recent phylogenetic studies have suggested support for only three subfamilies: Rubioideae (18 tribes), Cinchonoideae (nine tribes), and Ixoroideae (15 tribes), plus the tribe Coptosapelteae, which cannot currently be placed in any of the subfamilies (Bremer 2009).

Heterostyly is a genetically established floral polymorphism, and Rubiaceae comprises more heterostylous genera than all the other plant families combined (Ganders 1979). According to the same author, this implies both physiological and morphological differences in the reproductive verticils and pollen grains of these plants, and differences in pollen grain sizes in many genera of Rubiaceae can be explained by this polymorphism. Brevistylous (thrum) forms generally have larger pollen grains than longistylous (pin) forms (Ganders 1979; Jung-Mendaçolli & Melhem 1995; Dessein *et al,* 2000), which must be taken into account in palynological studies.

Palynological studies involving species of Rubiaceae in Brazil have been limited to pollinic catalogs or palynological surveys, such as those undertaken by Salgado-Labouriau (1973), Melhem *et al.* (2003), and Silva (2007). The principal palynological studies of the Rubiaceae have characterized the family as eurypollinic (Erdtman 1952; Salgado-Labouriau 1973; Jung-Mendaçolli & Melhem 1995; Dessein *et al.* 2005), and have facilitated the use of pollen morphology in classifying and identifying its taxa. According to Dessein *et al.* (2005), morpho-palynological data in the Rubiaceae can elucidate evolutive relationships among the different taxa, and can be used to reject or corroborate taxonomic decisions.

The following works stand out among the published palynological studies of the family Rubiaceae: Erdtman (1952) palynologically described 230 species belonging to 120 genera; Kirkbride (1979) revised the genus *Psyllocarpus* in Brazil using palynological features; Puff & Robbrecth (1988) investigated the taxonomic position of the Australian genus *Durringtonia*; Roubik & Moreno (1991) palynologically documented the species of Rubiaceae occurring on Barro Colorado Island in Panama; Jung-Mendaçolli & Melhem (1995) analyzed 25 heterostylous species of Rubiaceae and demonstrated differences in pollen size and exine ornamentation among the floral morphs investigated; Pire (1996; 1997) palynologically studied the tribe Spermacoceae; Huysmans *et al.* (2003) documented the pollen morphology of all of the genera of the tribe Rubieae occurring in Europe; Perveen & Qaiser (2007) described the pollen morphology of 50 species of the family occurring in Pakistan; Verellen *et al.* (2007) undertook palynological studies of the tribes Naucleeae and Hymenodictyeae; and Cai *et al.* (2008) studied the pollen grains of Asian species of this family.

In spite of these efforts, we are still quite far from completing the palynological documentation of this family because only about 15% its species have been investigated (Dessein *et al.* 2005). Most of the palynological studies of the Rubiaceae have been undertaken on taxa not found in Brazil, which has left a large gap in our knowledge about the pollen of representatives of the Brazilian flora. For this reason, the present study examined regional species of this family in order to contribute to the taxonomic and systematic knowledge of this group.

Materials and methods

We analyzed the pollen of 16 species belonging to 11 genera of Rubiaceae. Floral buds were collected in the field following the methodology recommended by Mori *et al.* (1989). Five specimens of each species were collected, when possible, and deposited in the herbarium at the Universidade do Estado da Bahia (HUNEB - Caetité Collection). Material was also obtained from specimens deposited in the herbaria at the Universidade Estadual de Feira de Santana (HUEFS) and the Universidade Federal da Bahia (ALCB); abbreviations follow *Index Herbariorum* (Thiers, B., continuously updated).

The floral morphologies of the specimens (thrum or pin) were determined using a stereo microscope. Species without annotations regarding their floral morphology are those that do not have published descriptions of heterostyly available in the literature.

Material examined: *Coccocypselum hirsutum*Bartl. ex DC. - **BRAZIL, Bahia**: Caetité, Brejinho das Ametistas. Farm Capão do Plástico, 19/V/2009, *Bonfim, L. G. N. et al.* 42 (HUNEB - CTE).*Cordiera rigida* Kuntze **– BRAZIL, Bahia**: Caetité, Brejinho das Ametistas, road to Pindaí - BA, 06/IX/2008, *Silveira Júnior, C.E.A. et al.* 04 (HUNEB - CTE). *Coutarea hexandra* K.Schum. **– BRAZIL, Bahia**: Caetité, Brejinho das Ametistas, road to Pindaí-BA, 23/I/2009, *Vasconcelos, L.V. et al.* 213 (HUNEB - CTE). *Declieuxia fruticosa* Kuntze - **BRAZIL, Bahia**: Caetité, Brejinho das Ametistas, Passagem da Pedra, 20/I/2009, *Santos, L. S. et al.* 07 (HUNEB - CTE); **BRAZIL, Bahia**: Caetité, Brejinho das Ametistas, *Silveira Jr et al.* 23 (HUNEB - CTE). *Diodella apiculata* (Willd. ex Roem. & Schult.) Delprete - **BRAZIL, Bahia**: Caetité, Brejinho das Ametistas, hill of the Bolívia, 22/I/2009, *Silveira Júnior,*

C.E.A. et al. 29 (HUNEB - CTE). *Diodella radula* (Willd. ex Roem. & Schult.) Delprete - **BRAZIL, Bahia**: Caetité, Brejinho das Ametistas, Passagem da Pedra, 06/IX/2009, *Silveira Júnior, C.E.A.et al.*08 (HUNEB - CTE). *Diodella teres* Small. - **BRAZIL, Bahia**: Caetité, Brejinho das Ametistas, 28/IV/2008, *Guedes, M.L.* 14256 (ALCB). *Emmeorhiza umbellata* K.Schum. - **BRAZIL, Bahia:** Caetité, Brejinho das Ametistas, road to Pindaí-BA, 13/V/2009, *Silva et al.* 20 (HUNEB - CTE). *Leptoscela ruellioides* Hook. f. - **BRASIL, Bahia**: (Brevistylous Caetité, Brejinho das Ametistas, road to Licínio de Almeida-BA, 23/I/2009, *Silveira Júnior, C.E.A. et al.* 38 (HUNEB - CTE); (Longistylous) Caetité, Brejinho das Ametistas, 23/III/2010, *Silveira Júnior, C.E.A. & Cunha, P. N.* 75 (HUNEB).*Mitracarpus baturitensis* Sucre -**BRASIL, Bahia**: Caetité, Brejinho das Ametistas, road Caetité to Brejinho das Ametistas, *Saba, M. D.* 71 (HUNEB - CTE). *Mitracarpus villosus* Cham. & Schltdl. - **BRASIL, Bahia**: Caetité, Brejinho das Ametistas, 28/IV/2008, *Guedes, M.L.* 14360 (ALCB). *Palicourea rigida* Kunth - **BRASIL, Bahia**: Caetité, Brejinho das Ametistas, road Caetité to Brejinho das Ametistas, 20/I/2009, *Silveira Júnior, C. E. A. et al.* 25 (HUNEB - CTE). *Psyllocarpus asparagoides* Mart. - **BRASIL, Bahia**: Caetité, Brejinho das Ametistas, road Caetité to Brejinho das Ametistas, 16/VII/2008, *Saba, M.D. et al.* 43 (HUNEB - CTE). *Richardia grandiflora* Steud. - **BRASIL, Bahia**: Caetité, Brejinho das Ametistas, road to Pindaí-BA, 29/IV/2008, *Silveira Júnior, C.E.A. et al.* 01 (HUNEB - CTE). *Staelia aurea* K. Schum. - **BRASIL, Bahia**: Caetité, Brejinho das Ametistas, Passagem da Pedra, 20/I/2009, *Silveira Júnior, C.E.A. et al.* 17 (HUNEB - CTE). *Staelia galioides* D.C. - **BRASIL, Bahia**: Caetité, Brejinho das Ametistas, 20 km from Caetité to Brejinho das Ametistas, 13/III/2002, *Ribeiro 255* (HUEFS).

Palynological processing –Visible light microscopic analyses (LM) were performed in the Palynological Laboratory at UNEB, *Campus* VI. The pollen grains of the majority of the species were prepared using the classical method of acetolysis (Erdtman 1960), while fragile pollen grains (such as those from *Palicourea rigida*) were treated using the potassa method (Faegri & Iversen 1975). Whenever possible, the anthers of more than one flower from a given specimen were examined to guarantee a more uniform sampling (Salgado-Labouriau 1973). The pollen samples were then mounted on slides with glycerinated gelatin and evaluated qualitatively and quantitatively, and photomicrographs were taken using a Zeiss Axioskop 2 microscope.

The principal morphometric parameters of randomly chosen pollen grains were measured according to the recommendations of Salgado-Labouriau (1973). The polar (PD) and equatorial (ED) diameters of 25 pollen grains were measured whenever possible. The other parameters, such as the polar area, aperture diameter, and exine, sexine, and nexine thicknesses were measured on 10 samples.

The quantitative results were statistically analyzed by calculating the arithmetic average ($\bar{x}$), the standard error of the sample (s), and the average standard error ($S_{\bar{x}}$) for samples sizes of 25; only arithmetic averages were calculated for parameters with sample sizes of 10.

Palynological analyses using a scanning electron microscope (SEM) were undertaken in the Electron Microscope Section of the Department of Biological Sciences at the Universidade Estadual de Feira de Santana. The pollen grains were acetolized, washed, and dehydrated in an ascending ethanol series (50, 70, 90 and 100%, remaining for approximately 10 min. at each step). A small sample of the pollen grains from the absolute alcohol step was placed directly on the specimen holder of the SEM and, after drying, was sputter coated with gold under high vacuum, and viewed and photographed using a LEO 1430 VP scanning electron microscope.

The morpho-palynological characteristics were illustrated by photographs and electron photomicrographs, and the palynological descriptions used the terminology of Punt *et al.* (2007).

Results

The pollen grains of the species analyzed ranged from small to large, with the smallest grains belonging to *Psyllocarpus asparagoides* (ED=13.6 µm) and the largest belonging to *Richardia grandiflora* (PED=92 µm), and were isopolar in most species, with the exception of *Palicourea rigida* (apolar); suboblate to subprolate; and amb circular, sub-circular (*Coccocypselum hirsutum, Declieuxia fruticosa* and *Leptoscela ruellioides*), or triangular to quadrangular (*Cordiera rigida*). In terms of the aperture type, the grains were inaperturate (*P. rigida*), zonoaperturate, colpate (*Diodella apiculata*, *D. teres*, *Mitracarpus baturitensis*, *M. villosus,* and *Richardia grandiflora*), or colporate in the remaining species; the number of apertures varied from 3 to more than 20 (*R. grandiflora*), while the lengths of the ectoapertures varied from short to long, with the smallest being observed in *Diodella teres* (PAI= 1.6) and the largest in *R. grandiflora* (PAI= 0.1). In relation the exine ornamentation, the analysis under light microscopy (LM) revealed that the pollen grains were microreticulate in most species, reticulate (*C. hirsutum, C. rigida* and *P. rigida*), echinate-granulate (*R. grandiflora*), echinate-granulate-perforated (*Diodella apiculata* and *D. teres*), or psilate (*P. asparagoides*). Pollen grains were observed under an SEM that were microreticulate (*L. ruelioides*), bireticulate (*D. fruticosa*), and microechinate-perforated (*Coutarea hexandra*). The sexine was thicker than the nexine in most species, although the sexine of *Coutarea hexandra* was thinner than the nexine; the sexine and nexine were indistinct in *Diodella teres* and *Palicourea rigida*.

The morphological and morphometric data concerning the pollen grains are presented in Tables 1 and 2, respectively.

Coccocypselum hirsutum *Bartl. ex DC. (Fig.1A-C)*

Morpho-floral type longistylous (pin)

Pollen grains large; isopolar; oblate spheroidal; amb subcircular; 3-colporate, **a**ngulaperturate, ectoapertures with tapered extremities, endoaperture lalongate with rounded extremities, fastigiate; reticulate, heterobrocate, **simplicolumellate**; sexine thicker than nexine.

Cordiera rigida *Kuntze (Fig. 1D-F)*

Pollen grains medium; isopolar; suboblate; amb triangular and quadrangular; 3(-4)-colporate, angulaperturate, ectoaperture long with tapered extremities, endoaperture lalongate with inferior and superior extremities parallel; reticulate, heterobrocate, simplicolumellate, undulating semitectum; sexine thicker than nexine, columellae thick and regularly distributed.

Coutarea hexandra *K. Schum. (Fig.1G-I)*

Pollen grains medium; isopolar; subprolate; amb circular; 3-colporate, angulaperturate, apertures difficult to see using light microscopy, more easily observed in the polar view and by an SEM; microechinate-perforated, with coniform microspines with wide base and sharp apex distributed uniformly over the surface of the pollen grains, the perforations surrounded by a thicker region of the sexine; nexine thicker than the sexine.

Declieuxia fruticosa *Kuntze (Fig. 1J-L)*

Morpho-floral types longistylous and brevistylous

Pollen grains medium; isopolar; oblate spheroidal; amb subcircular; 3-colporate, angulaperturate, ectoaperture with tapered extremities, endoaperture lalongate with rounded extremities, fastigium present; bireticulate; sexine thicker than nexine.

The pollen grains of the two morpho-floral types analyzed showed similar characteristics, with only small morphometric variations in the polar diameters and exine thicknesses (Tab.1).

Diodella apiculata *(Willd. ex Roem. & Schult.) Delprete (Fig. 1M-N)*

Pollen grains large; isopolar; oblate spheroidal; amb circular; 13-(-14) (-15)-16-zonocolpate; echinate-granulate-perforated, with coniform spines with large bases and sharp apices, heterogeneous sizes, distributed uniformly over the pollen grain; granules small and distributed uniformly over the pollen grain; columellae distinct; sexine thicker than nexine.

Diodella radula *(Willd. ex Roem. & Schult.) Delprete (Fig.1O-Q)*

Pollen grains medium; isopolar; prolate spheroidal; amb circular; (8)-9(-10)-zonocolporate, ectoaperture long, thin, and narrow, endoaperture lalongate, costa present; microreticulate, with heterogeneous lumens; muri interrupted, simplicolumellate; sexine thicker than nexine.

Diodella teres *Small. (Fig. 17-20) (Fig. 1R-T)*

Pollen grains large; isopolar; prolate spheroidal; with amb circular; (11)(-12)-14(-15)(-16) zonocolpate, colpus narrow and very long; echinate-granulate-perforated, with coniform spines with wide base and sharp apex, heterogeneous in size and distributed uniformly over the pollen grain; granules small and uniformly distributed over the sexine; perforations seem more clear in optical section; sexine and nexine indistinct in optical section.

Emmeorhiza umbellata *Schum. (Fig.2A-C)*

Pollen grains medium; isopolar; prolate spheroidal; amb circular; 7-8(-9)-zonocolporate, ectoaperture long, endoaperture lalongate, costate; microreticulate, homobrocate, simplicolumellate; sexine thicker than nexine.

Leptoscela ruellioides *Hook. f. (Fig.2D-F)*

Morpho-floral type longistylous and brevistylous.

Pollen grains medium; isopolar; suboblate (brevistylous) or prolate spheroidal (longistylous); with amb subcircular; 3-colporate, angulaperturate, ectoaperture with thin extremities, endoaperture lalongate when visible, with rounded extremities, in some grains the endoapertures appear to be longitudinally united, fastigium present; microreticulate, homobrocate, with bifurcated columellae present; sexine thicker than nexine.

Although the pollen grains of both morpho-floral types are medium-sized, the largest diameter grains were observed in the brevistylous form (Tab.1).

Mitracarpus baturitensis *Sucre (Fig.2G-I)*

Pollen grains small; isopolar; prolate spheroidal; amb circular; (5)-6-zonocolpate, colpi short and narrow; microreticulate, homobrocate; sexine thicker than nexine.

Mitracarpus villosus *Cham. & Schltdl. (Fig.2J-L)*

Pollen grains small; isopolar; oblate spheroidal; amb circular; 5(6-)-zonocolpate, colpi long and narrow; microreticulate, with homobrocate; sexine thicker than nexine.

Palicourea rigida *Kunth (Fig. 3A-B)*

Morpho-floral type brevistylous

Pollen grains large; spheroidal; inaperturate; reticulate, heterobrocate, semitectate, simplicolumellate; sexine and nexine indistinct.

Psyllocarpus asparagoides *Mart. (Fig.3C-D)*

Pollen grains small; subprolate; amb circular; 5(6)-zonocolporate, aperture located in invaginations in the wall; ectoaperture thin with tapered extremities; endoaperture lalongate with upper and lower extremities parallel; psilate; sexine and nexine of same thickness.

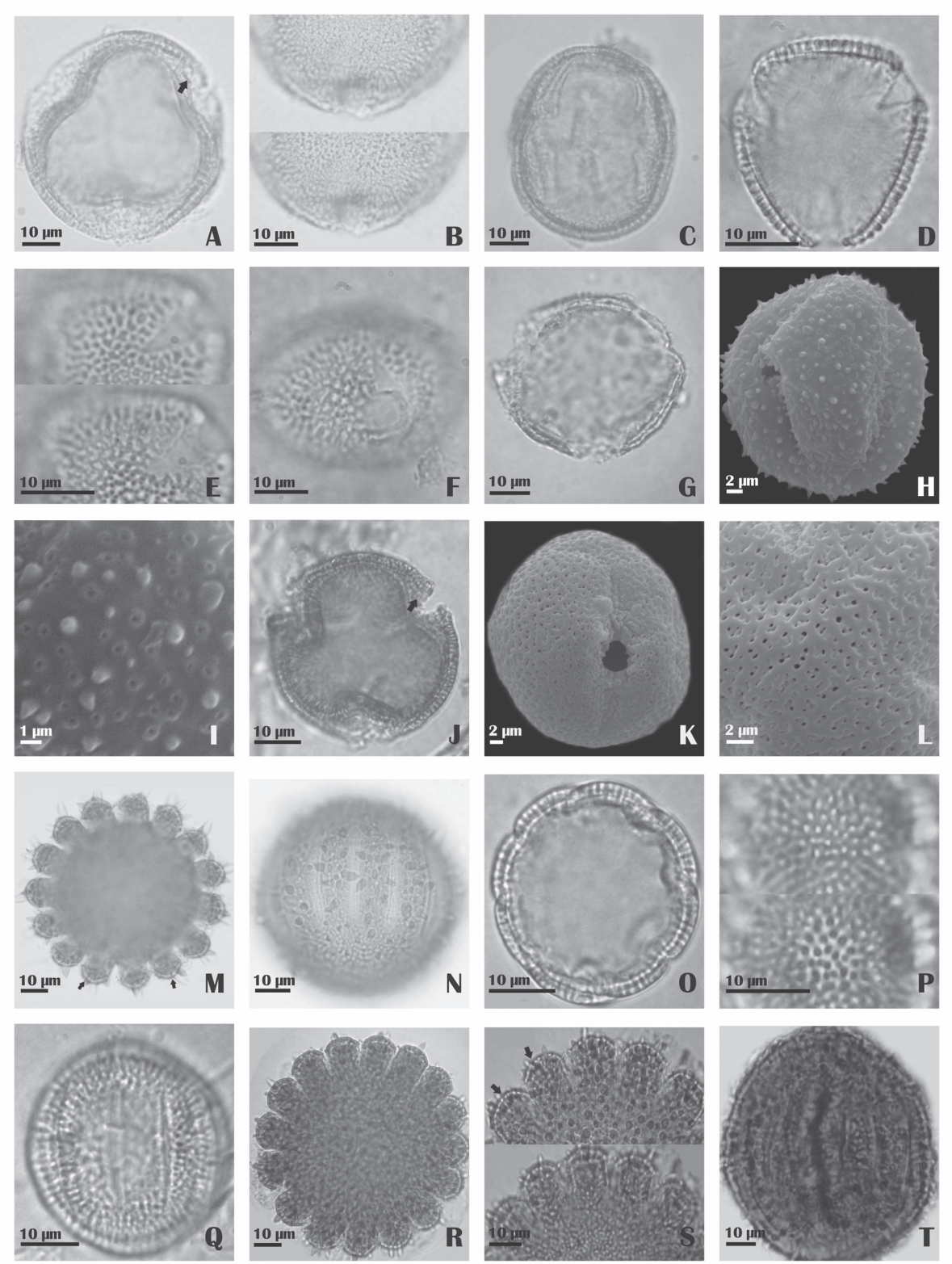

Figure 1. A-T. Photomicrographs and electron photomicrographs of pollen grains of the family Rubiaceae. A-C *Coccocypselum hirsutum* Bartl. ex DC.: A- Polar view (optical section), emphasizing the fastigium (arrow); B-LO analysis; C- Equatorial view. D-F *Cordiera rigida* Kuntze: D- Polar view (optical section); E- LO analysis; F- Equatorial view. G-I *Coutarea hexandra* K.Schum.: G- Polar view (optical section); H- Equatorial view (SEM); I- Surface detail (SEM). J-L *Declieuxia fruticosa* Kuntze (longistylous form): J- Polar view (optical section), emphasizing the fastigium (arrow); *D. fruticosa* Kuntze (brevistylous form): K- Equatorial view (SEM); L-surface detail (SEM). M-N *Diodella apiculata* (Willd. ex Roem. & Schult.) Delprete: M- Polar view (optical section), emphasizing the perforations (arrows); N- Equatorial view. O-Q *D. radula* (Willd. ex Roem. & Schult.) Delprete: O- Polar view (optical section); P- LO analysis; Q- Equatorial view. R-T *Diodella teres* Small.: R- Polar view (optical section); S-LO analysis, showing perforations (arrows); T- Equatorial view.

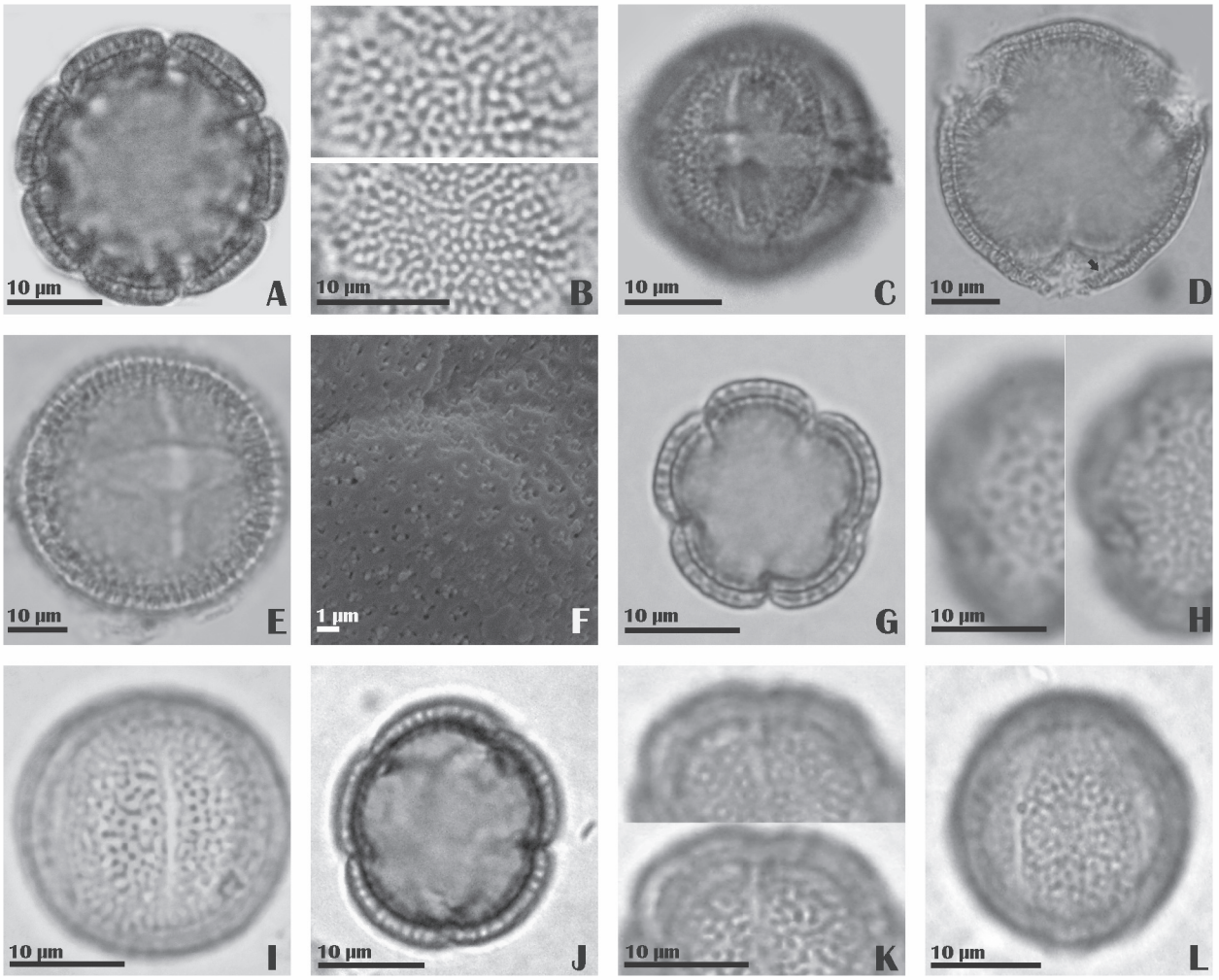

Figure 2. A-L. Photomicrographs and electron photomicrographs of pollen grains of the family Rubiaceae. A-C *Emmeorhiza umbellata* K.Schum.: A- Polar view (optical section); B- LO analysis; C- Equatorial view. D-F *Leptoscela ruelioides* Hook. f.: D- Polar view (optical section), emphasizing the fastigium (arrow); E- Equatorial view; F- Surface detail (SEM); G-I *Mitracarpus baturitensis* Sucre: G- Polar view (optical section); H- LO analysis; I- Equatorial view. J-L *Mitracarpus villosus* Cham. & Schltdl.: J- Polar view (optical section); K- Analysis de LO; L- Equatorial view.

Richardia grandiflora *Steud. (Fig.3E-G)*

Pollen grains large; isopolar; suboblate; amb circular; 13-23-zonocolpate, colpi with rounded extremities with irregular margins, surrounded by spines; echinate-granulate, with sharp spines of heterogeneous sizes, granules distributed homogeneously over the surface of the grain; sexine thinner than nexine.

Staelia aurea K. *Schum. (Fig.3H-J)*

Pollen grains medium, isopolar; oblate spheroidal; amb circular; (-7)(-8)(-9)10(-11)-zonocolporate, with thin ectoapertures with tapered extremities, endoapertures lalongate, costate; microreticulate with homogeneous lumens, columellae distinct and short; sexine thicker than nexine.

Staelia galioides *DC. (Fig.3K-L)*

Pollen grains medium; isopolar; prolate spheroidal; amb circular; (7)-8(-9) zonocolporate, ectoaperture thin with tapered extremities; endoaperture lalongate with extremities parallel; microreticulate with heterogeneous lumens and thin and long columellae; sexine thicker than nexine.

Discussion

Our observations of the pollen grains of *Coccocypselum hirsutum* were different from those encountered in the published literature. Medium-sized pollen grains have been reported for this species by a number of authors (Colinvaux *et al.* 1999; Jung-Mendaçolli & Melhem 1995; Piesschaert *et al.* 2000; Melhem *et al.* 2003); amb triangular to sub-triangular has also been described (Jung-Mendaçolli & Melhem 1995; Melhem *et al.* 2003). Pollen grains with a different type of aperture, 3-porate, have only been reported by Delprete & Cortés (2006), while Colinvaux *et al.* (1999) noted the occurrence of annuli in the ectoapertures. In terms of the

Table 1. Morphological characters of pollen grains of Rubiaceae species occurring in an area of caatinga vegetation of Bahia, Brazil.

Species/Morpho-floral	Size	Shape	Apertural Type	Apertual Number	Exine
Coccocypselum hirsutum					
longistylous	large	oblate spheroidal	colporus	3	Reticulate
Cordiera rigida					
	medium	suboblate	colporus	3	Reticulate
Coutarea hexandra					
	medium	subprolate	colporus	3	Microechinate-perforate
Declieuxia fruticosa					
brevistylous and longistylous	medium	suboblate	colporus	3	Bireticulate
Diodella apiculata					
	large	oblate spheroidal	colpus	13-(-14) (-15)-16	Echinate-granulate-perforate
Diodella radula					
	medium	prolate spheroidal	colporus	(8)-9-(10)	Microreticulate
Diodella teres					
	medium	prolate spheroidal	colpus	(-11)14(-15)	Echinate-granulate-perforate
Emmeorhiza umbellata					
	medium	prolate spheroidal	colporus	7-8-(-9)	Microreticulate
Leptoscela ruelioides					
brevistylous	medium	suboblate	colporus	3	Microrreticulate
longistylous	medium	prolate spheroidal	colporus	3	Microrreticulate
Mitracarpus baturitensis					
	short	prolate spheroidal	colpus	(5)-6	Microreticulate
Mitracarpus villosus					
	short	prolate spheroidal	colpus	5-(6)	Microreticulate
Palicourea rigida					
brevistylous	large	spheroidal	inaperturate	—	Reticulate
Psyllocarpus asparagoides					
	very short	subprolate	colporus	(5)-6	Psilate
Richardia grandiflora					
	large	subprolate	colpus	(20)21(-22)	Echinate-granulate
Staelia aurea					
	medium	oblate spheroidal	colporus	(-7)(-8)(-9)-10(11)	Microreticulate
Staelia galioides					
	medium	prolate spheroidal	colporus	8 (9-7)	Microreticulate

ornamentation of the exine, Jung-Mendaçolli & Melhem (1995) reported a pilate to pilate-rugulate exine. This latter ornamentation pattern was described by Piesschaert *et al.* (2000), using an SEM, as being a "complex reticulum" formed by a suprareticulum psilate with an echinate infrareticulum, with fusion of the muri of both of the reticula. Delprete & Cortés (2006) reported the occurrence of bireticulate pollen grains for the genus.

The pollen type described for *Cordiera rigida* (3-colporate with reticulate exine) is shared by *Coccocypselum hirsutum*, but the pollen of these species differs in size and shape. The characteristics of the pollen grains of *C. rigida* were also reported by Delprete & Cortés (2006) for other species of the genus.

Roubik & Moreno (1991) and Huysmans *et. al.* (1999) described the pollen grains of *Coutarea hexandra* using

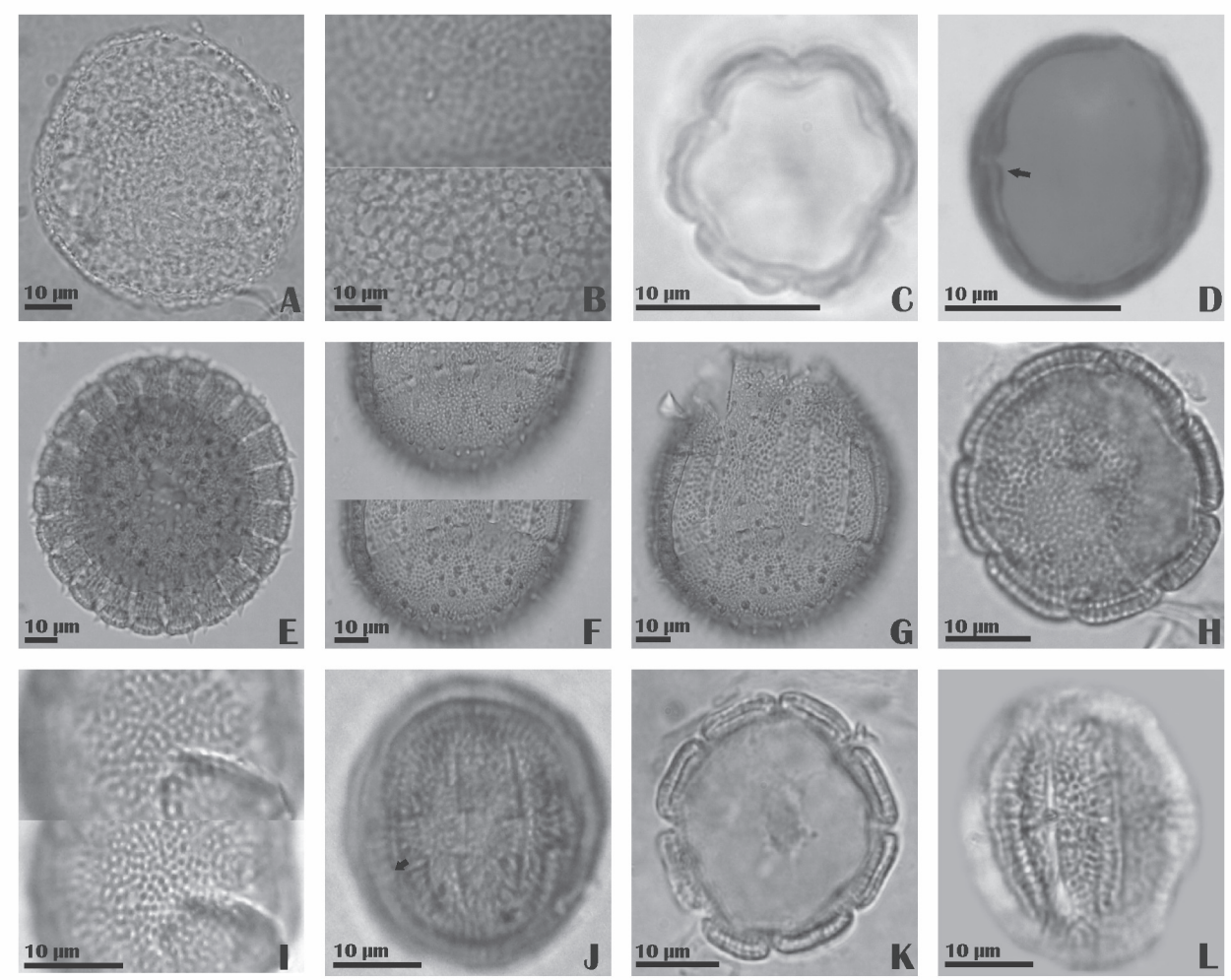

Figure 3. A-L. Photomicrographs of pollen grains of the family Rubiaceae. A-B *Palicourea rigida* Kunth: A- General view (optical section); B-LO analysis. C-D *Psyllocarpus asparagoides* Mart. ex Mart. & Zucc.: C-Polar view (optical section); D- Equatorial view, detail of costa (arrow); E-G *Richardia grandiflora* Britton: E- Polar view (optical section); F- LO analysis; G- Equatorial view; H-J *Staelia aurea* K. Schum.: H- Polar view (optical section); I- LO analysis; J- Equatorial view; K-L *Staelia galioides* D.C.: K- Polar view (optical section); L- Equatorial view.

LM and an SEM, respectively, and, according to these authors, this species has pollen grains that are medium-sized, spheroidal to subprolate, amb circular, tricolpate, with a microechinate exine. The aperture type described by these authors was not observed in the present work. In terms of exine ornamentation, Roubik & Moreno (1991) did not observe the perforations seen in the tectum under light microscopy that are described in the present study.

The pollen morphology of *Declieuxia fruticosa* was described by Piesschaert *et al.* (2000) using an SEM, and these authors reported similar results to those of the present study; they also observed a "complex reticulum" in this species. This type of ornamentation is very similar to the bireticulate pattern described by Punt *et al.* (2007) as a reticulum composed of two layers (a suprareticulum sustained by a microreticulate tectum). As such, the ornamentation types encountered in the specimens in the present study differed from the patterns described by Piesschaert *et al.* (2000) in that micro-spines were not observed in the infrareticulum. Pollen grains with pilate-rugulate exines may also occur in this genus, which was observed by Jung-Mendaçolli & Melhem (1995) and Melhem *et al.* (2003).

The species of *Diodella* Small studied here were similar in terms of the large numbers of apertures seen in the equatorial region. *Diodella apiculata* and *D. teres* appeared to be palynologically very similar because they were the same size, had the same aperture type and exine ornamentation, and were distinct only in terms of the shape of the grains. The palynological studies of *D. apiculata* (= *Diodia rigida* Cham. & Schltdl.) undertaken by Erdtman (1952) differed from the results of the present study, because this author reported very large pollen grains (ca. 100 µm) that were 16-18-colporate (brevicolpate), punctate and echinate. Very large pollen grains were also encountered by Dessein *et al.* (2005) in *D. teres* (133 µm). Delprete & Cortés (2006) reported that the pollen of species of this same genus were multicolporate and commonly had foveolate-perforate exines. Of the species studied here, *D. radula* was the most

Table 2. Morphometric characters of the pollen grains of Rubiaceae species occurring in an area of caatinga vegetation of Bahia, Brazil.

Species/Specimen	PD		ED		EDp		P/E	Ecto	Endo	PAI	Sex	Nex	Exi
	x̄±S x̄	Fv	x̄±S x̄	Fv	x̄±S x̄	Fv							
Coccocypselum hirsutum													
Bonfim, L.G.N. *et al.* 42 (HUNEB) (L)	50,0*±2,4	40,0-55,0	52,9*±1,1	50,0-57,5	55,5±0,4	50,0-72,5	0,94	__	__	0,6	2,3	1,5	3,8
Cordiera rigida													
Silveira Jr *et al.* 04 (HUNEB)	27,3±0,2	25,0-32,5	31,7±0,2	25,0-37,5	31,0±0,2	25,0-37,5	0,86	19,3x4,3	7,5	0.4	2,0	1,1	3,1
Coutarea hexandra													
Vasconcelos *et al.* 213 (HUNEB)	41,6±0,3	35,0-52,5	33,4±0,2	27,5-40,0	42,5±0,3	35,0-52,5	1,24	__	__	__	1,2	1,3	2,5
Declieuxia fruticosa													
Silveira Jr *et al.* 23 (HUNEB) (B)	42,9±0,7	27,5-47,5	46,7±0,3	32,5-52,5	47,6±0,2	40,0-52,5	0,91	29,8x6,8	5,0	0,4	2,6	1,5	4,1
Santos, L. S. 07 (HUNEB) (L)	39,6±0,2	37,5-42,5	44,5±0,3	35,0-50,0	45,2±0,3	35,0-52,5	0,88	__	6,0	0,3	2,3	1,0	3,3
Diodella apiculata													
Silveira Jr. *et al.* 29 (HUNEB)	65,1*±4,1	50,0-80,0	69,6*±3,2	60,0-92,5	72,8±5,2	55,0-92,5	0,93	25,6x2,5	__	0,1	5,0	2,5	7,2
Diodella radula													
Silveira Jr *et al.* 08 (HUNEB)	32,5±0,2	27,0-40,0	31,1±0,2	30,6-31,5	31,8±0,2	25,0-40,0	1,04	22,5x1,2	5,3	0.2	2,0	1,7	3,7
Diodella teres													
Guedes, M.L. 14256, (ALCB)	73,3*±3,7	62,5-80,0	72,5*±3,6	62,5-80,0	83,8*±4,4	60,0-97,5	1,01	17,0x1,0	__	1.6	__	__	2,0
Emmeorhiza umbellata													
Silva *et al. 20* (HUNEB)	29,8±0,1	27,5-32,5	29,4±0,1	25,0-32,5	29,6*±0,8	25,0-35,0	1,01	19,4x2	4,4	0.2	1,7	1,2	2,9
Leptoscela ruelioides													
Silveira Jr *et. al.* 38 (HUNEB) (B)	37,1±0,1	35,0-40,0	42,7±0,2	37,5-47,5	43,3±0,3	35,0-50,0	0,86	__	__	0,4	1,8	1,0	2,8
Silveira Jr. & Cunha 75 (HUNEB) (L)	37,4±0,2	30,0-42,0	36,6±0,1	32,5-40,0	39,4±02	32,5-45,0	1,02	26,5x2,0	4,3	0,6	2,0	1,0	3,0
Mitracarpus baturitensis													
Saba, M. D. 71 (HUNEB)	20,2±0,2	17,5-27,5	19,2±0,1	15,0-25,0	20,6±0,2	15,0-25,0	1,05	14,2x1,0	__	0,6	2,0	1,0	3,0
Mitracarpus villosus													
Guedes *et al.* 14360 (ALCB)	20,0±0,1	17,5-22,5	19,1±0,6	16,2-20,0	18,9±0,7	17,5-22,5	0,92	14,0x1,0	__	0,3	1,2	1,0	2,2
Palicourea rigida													
Silveira Jr *et al.* 25 (HUNEB) (B)	56,6*±4,5	32,5-72,5	56,6*±4,5	32,5-72,5	___	___	1,00	___	__	__	__	__	1,0
Psyllocarpus asparagoides													
Saba, M.D. *et. al.* 43 (HUNEB)	16,5±0,2	12,5-20,0	13,6±0,2	10,0-20,0	13,7*±0,2	12,5-15,5	1,21	12,7x1,3	__	0,4	1,0	1,0	2,0
Richardia grandiflora													
Silveira Jr *et al.* 01 (HUNEB)	80,3*±2,3	72,5-90,0	91,2*±4,7	82,5-120	92,0*±4,4	80,0-120	0,88	27,8x3,5	__	0,1	2,3	3,0	5,3
Staelia aurea													
Silveira Jr *et al.* 17 (HUNEB)	30,1±0,3	28,0-32,0	32,7±0,3	30,6-34,0	31,5±0,2	30,0-35,0	0,62	18,5x1,1	5,4	0,3	2,1	1,0	3,1
Staelia galioides													
Ribeiro 255 (HUEFS)	30,6±1,2	25,0-35,0	27,5±0,8	20,0-30,0	27,9±0,7	25,0-30,0	1,11	18,3x1,2	__	0,3	1,8	1,3	3,1

PD = polar diameter; ED = equatorial diameter; EDp = equatorial diameter in polar view; PAI = polar area index; Ecto = length x width of the ectoaperture; Endo = height of the endoaperture; Sex= sexine; Nex= nexine; Exi= exine; * n < 25; measurements in µm and indices in absolute numbers.

similar to descriptions supplied by these authors because it had grains that were (8)-9(-10)-colporate, although none of the species studied here had foveolate-perforate exine ornamentations. The descriptions encountered in the literature demonstrate a significant degree of heterogeneity within the genus in terms of palynological characteristics when compared to those of the present study.

Melhem *et al.* (2003) described the pollen grains of *Emmeorhiza umbelllata* using visible light microscopy, and differences were restricted to the reticulate ornamentation pattern and to the apparent lack of costae on the ectoapertures (as observed in the present study). In terms of the genus, Delprete & Cortés (2006) pointed out the occurrence of multicolporate pollen grains as well as an echinate-perforate exine.

Leptoscela ruellioides is endemic to Brazil and was invest here for the first time. This species differed palynologically from the others by having a microreticulate exine and a bifurcated columellae. This species did, however, have pollen grains with characteristics common to other taxa, such as: pollen grains with amb subcircular, 3-colporate with a fastigium (which occurs in both *Coccocypselum hirsutum* and *Declieuxia fruticosa*).

The species of *Mitracarpus* Zucc. studied here were very similar palynologically: being the same size and having the same aperture type, exine ornamentation and shape. Published descriptions of this genus indicate, however, that the size, exine ornamentation, and aperture type of its pollen grains can vary. Melhem *et al.* (2003) observed that the pollen grains of *M. hirtus* were medium sized, (3,4,6)-7(-9)-colpate and the ornamentation of the exine was granulate-perforate, while Delprete & Cortés (2006) reported 6-7-colporate pollen grains with an echinate-perforate exine. However, this data does demonstrate that it is possible to separate the species of this genus by analyzing their pollen morphologies.

Salgado-Labouriau (1973) studied the pollen morphology of *Palicourea rigida* and encountered results similar to those of the present work, although this author did not refer to the morpho-floral types examined. This author described the sexine as being much thicker than the nexine, while the exine layers observed in the present work were indistinct. Additionally, this author observed bi- or tri-columellate muri, while in the present study the walls were described as simplicolumellate. Other authors who studied the pollen of species of this genus reported that these taxa had very homogeneous palynological characteristics, with generally large pollen grains that are spheroidal and inaperturate (Erdtman 1952; Jung-Mendaçolli & Melhem 1995; Dessein *et al.* 2005). Jung-Mendaçolli & Melhem (1995) described the genus as stenopollinic and included it the inaperturate pollen type. These authors noted, however, that different ornamentation patterns were encountered in this genus and that this characteristic not only permitted distinctions to be made between species, but also allowed the separation of the pollen grains of the longistylous and brevistylous morpho-floral types (because they had distinct exine ornamentation in at least two of the three species they analyzed). In addition to the reticulate ornamentation found by this author, *Palicourea* Aubl. can also have retipilate, pilate, pilate-rugulate, and pilate-reticulate pollen grains.

Kirkbride (1979) analyzed the pollen grains (using SEM) of species of the genus *Psyllocarpus* Mart. that occur in the Amazon and Pantanal regions of Brazil, and divided them into two sections: 1) an Amazon section characterized by oblate spheroidal pollen grains, 6 to 8-colporate, perforated, with micro-spines distributed over the entire pollen grain; and 2) the section *Psyllocarpus*, characterized by prolate spheroidal pollen grains, 5 to 7-colporate, exine psilate, with micro-spines surrounding the colpus. This author did not refer to the sizes of the pollen grains of the species studied. The micro-spines observed by this author for this species were not seen in the light microscopic examinations in the present study.

The pollen grains from three species of the genus *Richardia* L. were studied by Salgado-Labouriau (1973), including *R. grandiflora,* and were found to be medium to large, oblate spheroid to peroblate, and multicolporate with an exine ornamentation that was microechinate or pilate. Erdtman (1952) analyzed the same taxa as the previous author although in contrast, described the species analyzed here. This author did not specify the aperture type, describing it as polycolp(or?)ate, with a peroblate to oblate shape and an echinate exine. The present study corroborated the results of these authors in terms of the size of the pollen grains and exine orientation, although the specimen analyzed in the present study had colpate pollen grains that were suboblate. This same genus can have rugulate, echinate-perforate, or echinate-reticulate pollen grains (Delprete & Cortés 2006).

The two species of *Staelia* Cham. & Schltdl. had very similar pollen, differing only by their shape and the presence of costa in *S. aurea*. According to Delprete & Cortés (2006), 7 to multiaperturate pollen, and finely reticulated exines occur in the genus. Salas and Cabral (2010) also described the pollen of species of *Staelia* from Paraguay and, like the latter authors, reported that the grains were multi-colporate. These same authors provided complete descriptions of the morpho-palynological characters: medium pollen grains, isopolar, prolate spheroidal, 7-10-colporate, with long ectoapertures and lolongate endoapertures, exine perforated and scabrate, with circular perforations and scabrae distributed over the entire surface of the grain. Our data generally corroborates these observations because the two species had many apertures and similar ornamentation patterns (microreticulate), although it was not possible to observe the lolongate endoapertures or the scabrate exine using light microscopy.

The results encountered in the present work demonstrated variations in almost all of the morpho-palynological characters investigated, especially in the number and type of aperture and exine ornamentation – characters usually

utilized in taxonomic delimitations (Miranda *et al.* 1993, Pire 1996, 1997; Dessein *et al.* 2005). Considering the variability observed here, the present study is in consonance with the authors who consider this group eurypollinic (Erdtman 1952; Salgado-Labouriau 1973; Jung-Mendaçolli & Melhem 1995; Dessein *et al*. 2005).

Among the heterostylous species studied, *Declieuxia fruticosa* and *Leptoscela ruelioides* had pollen grains of the two morpho-floral types analyzed, and the data presented here corroborates information in the literature concerning heterostyly in the family; the pollen grains from the brevistylous (thrum) morpho-floral type had larger diameters (Ganders 1979; Jung-Mendaçolli & Melhem 1995; Dessein *et al.* 2000; Piesschaert *et al.* 2000; Melhem *et al*. 2003). There were no differences between the specimens investigated in terms of aperture types or exine ornamentation.

The present study contributes to our palynological knowledge of the tribe Spermacoceae *sensu lato* (*s.l.*), because most of the genera examined were members of this group (*Diodella*, *Emmeorhiza, Leptoscela, Mitracarpus*, *Psyllocarpus*, *Richardia* and *Staelia*). This tribe is well delimited phylogenetically, although there are still some doubts concerning some of its generic and specific relationships, for example, between the genera *Borreria* G. Mey., *Diodella* L., *Diodia* L., and *Galianthe* Griseb. (Groeninckx *et al.* 2009). Additional phylogenetic studies are needed that focus on this group and include larger numbers of taxa and characters (Groeninckx *et al.* 2009), and pollen morphology will have an important role in these investigations.

The newly described palynological characteristics of the monospecific genus *Leptoscela*, presented here, will contribute to future studies of this taxon because this species had not been securely placed in one of the subfamilies of Rubiaceae. *Leptoscela ruelioides* was assigned to the expanded tribe Spermacoceae *s.l.* that includes the traditional tribes Spermacoceae *sensu stricto* (*s.s.*), Manettieae, and the Hedyotis–Oldenlandia group, to which this genus belongs (Bremer & Manen 2000). However, several monospecific genera of the tribe have various peculiar characteristics that make them difficult to relate to other members of the same tribe. In addition, a morphological investigation would be very important to improve the phylogenetic studies of this group (Groeninckx *et al.* 2009).

Based on the present work, it can be concluded that the species studied were palynologically distinct. However, additional studies are necessary (principally employing SEM) to provide more detailed observations of some structures (such as the apertures and the exine ornamentation), because pollen morphology has the potential to greatly aid in the understanding of the taxonomy and phylogeny of this group.

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