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Pollen morphology of some species of Spermacoceae *s.s.* (Rubiaceae) of the Atlantic Forest, Rio de Janeiro, Brazil

Vania Gonçalves-Esteves[1*], Gabrielle Reboredo Menezes Vieira[1], Renata Jacomo Paixão de Carvalho[1], Sonia Regina de Melo Crespo[2] and Claudia Barbieri Ferreira Mendonça[1]

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ABSTRACT

This work aimed to morphologically characterize 22 species of Spermacoceae *s.s* found in the Atlantic Forest in the state of Rio de Janeiro with the purpose of improving palynological knowledge of the studied species. The studied species included six species of *Borreria* and *Denscantia cymosa*, *Emmeorhiza umbellata*, *Hexasepalum apiculatum*, *H. radula*, *H. teres*, *Manettia fimbriata*, *Mitracarpus eichleri*, *M. frigidus*, *M. hirtus*, *M. lhotzkyanus*, *M. megapotamicus*, *Oldenlandia salzmannii*, *Pentodon pentandrus*, *Richardia brasiliensis*, *Richardia scabra* and *Spermacoce rubescens*. Acetolysed pollen grains were analyzed using light (LM) and scanning electron miscroscopy (SEM). The pollen grains varied in size (polar diameter) from medium to large, with the exception of *Hexasepalum teres* which was very large. Pollen grains were oblate spheroidal in most species, suboblate in six species, prolate spheroidal in *Spermacoce rubescens* and subprolate in *Manettia fimbriata*. All pollen grains were colporate, while number of apertures varied (three-19). The endoaperture was lolongate in most species and lalongate in four species. Sexine ornamentation varied among reticulate, reticulate-granulate, microreticulate, granulate with perforation, echinate-perforate and vermiculate. Pollen attributes were found to distinguish the studied species, but do not separate genera, confirming that Spermacoceae is euripolynic.

Keywords: Gentianales, multivariate analysis, palynology, Rubioideae, systematics

Introduction

According to Angiosperm Phylogeny Group (APG IV 2016), Rubiaceae is positioned in the order Gentianales and recent phylogenetic studies have suggested support for only three subfamilies: Cinchonoideae Raf., Ixoroideae Raf. and Rubioideae Verdc. It is a cosmopolitan, predominantly pantropical family (Taylor *et al.* 2007). In Brazil, this family is represented by approximately 126 genera and 1415 species, of which 733 are endemic (Flora do Brasil 2020 2019), with significant representation in the Atlantic Forest and the Amazon Basin (Delprete & Cortes 2006; Delprete & Jardim 2012).

The Spermacoceae *s.s*. is monophyletic tribe belongs the subfamily Rubioideae is one of the most representative tribes in Brazil, about 11 genera and 30 species are registered in the state of Rio de Janeiro (Flora do Brasil 2020 2019).

As already emphasized by Bacigalupo & Cabral (1996); Pire (1996); Dessein *et al.* (2002, 2005); Harwood & Dessein (2005); Cabral & Pire (2006); Souza & Lorenzi (2008), the palynology in Spermacoceae has proven valuable whereas the diversity in the pollinic attributes provides important data for the systematics of the tribe. Variations in the pollen

1 Laboratório de Palinologia Álvaro Xavier Moreira, Departamento de Botânica, Museu Nacional, Universidade Federal do Rio de Janeiro, 20940-040, Rio de Janeiro, RJ, Brazil
2 Universidade Estácio de Sá, 20771-004, Rio de Janeiro, RJ, Brazil

* Corresponding author: esteves.vr@gmail.com

grains of Spermacoceae *s.s.* related, mainly, in number, type of aperture and ornamentation of the sexine were registered by these authors.

The importance of pollen characters in Rubiaceae taxonomy has been highlighted by several authors, such as Campo (1976), Jansen *et al.* (1996a; b) and Dessein *et al.* (2005), among others that prove their use in the systematic of certain groups.

The following work intends the morphological pollinic characterization of 22 species of the Spermacoceae tribe found in Atlantic Forest in the state of Rio de Janeiro, with the purpose of offering a better palynological knowledge of the studied species, as well as provide subsidies for future molecular studies in Rubiaceae.

Materials and methods

Pollen grains of 22 species of the Spermacoceae (Rubiaceae) were examined. The samples were obtained from anthers of flower buds of specimens held in the Brazilian herbaria (GUA, R and RB). Acronyms are according to Thiers (2017 continuously updated).

Whenever possible, four specimens of each species were analyzed and compared in order to obtain accurate results (List S1. in supplementary material). The permanent slides of this study are deposited in Laboratório de Palinologia Álvaro Xavier Moreira, Departmento de Botânica da Universidade Federal do Rio de Janeiro / Museu Nacional.

Pollen grains were prepared by acetolysis (Erdtman 1952), as modified by Melhem *et al.* (2003) for light microscopy (LM). The scanning electron photomicrographs (SEM) were taken by using either a JSM-5310 microscope at the Hertha Meyer Laboratório de Ultraestrutura, Instituto de Biofísica, Universidade Federal do Rio de Janeiro or Jeol 6390 LV at the Centro de Microscopia Eletrônica, Departmento de Invertebrados do Museu Nacional, Universidade Federal do Rio de Janeiro.

One specimen of each species was chosen for statistical treatment and illustrations and is indicated by an asterisk (*) after the collector's name (List S1 in supplementary material). Measurements in equatorial view (PD - polar diameter, ED - equatorial diameter) were taken on 25 pollen grains per sample. Means ($\bar{x}$), and 95 % confidence intervals (CI) were calculated. For measurements of equatorial diameter from polar view (EDPV), apocolpium side (AS), apertures and exine thickness, the arithmetic means of 10 measurements were used. Ten similar measurements of pollen grains were made on additional material, from another collection, to check the stability of the data (treated further as comparison material). Whenever possible, comparison specimens were analyzed for confirmation of the obtained results (Souza *et al.* 2016; Marinho *et al.* 2018; Moreira *et al.* 2018; Sousa *et al.* 2018).

The terminology used for pollen descriptions follows Punt *et al.* (2007), which takes into consideration elements of size, shape, number of apertures, and sexine ornamentation. The descriptions of the polar area and aperture size were made according to the classification system established by Faegri & Iversen (1966) for the polar area index.

Principal component analysis (PCA) and a cluster analysis were performed using PC-ORD 5.0 (McCune & Mefford 2006) software. The metrics for pollen grains were transformed by the power of 0.5 (square root) to standardize analyses.

Principal component analysis (PCA) was conducted to determine whether the pollen features could cluster species. The variance and covariance matrix (var-cov) was obtained from the means of the morphometric data of the palynological analysis, and the coordinates in the biplot graph are based on Euclidian distances and show the first and second principal components. The matrix of characters, the values of the vectors in each axis and the total of accumulative variance are presented in tables. The cluster analysis (AHC Clustering) was conducted with the aim of classifying the analyzed species into groups based on shared pollen variables (similarity). Two factors were considered in forming the groups from the set of analyzed variables: the percentage of information (variables) necessary to arrive at the groups related to the final number of groups formed. A dendrogram was made using Euclidian distances (Caccavari *et al.* 2008) and using Ward's linkage method.

Results

The palynological descriptions are arranged according to the following pollen features: one. Dispersal unit, polarity and size, two. shape and polar area, three. number and type of apertures and four. Stratification of exine and ornamentation.

Pollinic descriptions of the species of *Borreria brachystemonoides* (Fig. 1A-C); *B. capitata* (Fig. 1D-F); *B. latifolia* (Fig. 1G-H); *B. palustris* (Fig. 1I), *B. scabioisoides* (Fig. 1J-L); *B. verticillata* (Fig. 2A-C); *Denscantia cymosa* (Fig. 2D-F); *Emmeorhiza umbellata* (Fig. 2G-I); *Hexasepalum apiculatum* (Fig. 2J-L); *H. radula* (Fig. 3A-C); *H. teres* (Fig. 3D-F); *Manettia fimbriata* (Fig. 3G-I); *Mitracarpus eichleri* (Fig. 3J-L); *M. frigidus* (Fig. 4A-C); *M. hirtus* (Fig. 4D-F); *M. lhotzkianus* (Fig. 4G-I); *M. megapotamicus* (Fig. 4J-L); *Oldenlandia salzmannii* (Fig. 5A-C), *Pentodon pentandrus* (Fig. 5D-F); *Richardia brasiliensis* (Fig. 5G-I); *R. scabra* (Fig. 5J-L); *Spermacoce rubescens* (Fig. 5M-O).

Dispersal unit, polarity and size

All species examined had isopolar and radially symmetrical pollen grains on monads. are generally medium-sized (ranging from 25.5 to 47.9 µm in polar diameter and 25.9-48.7 µm in equatorial diameter, in equatorial view), large in *Hexasepalum apiculatum*, *H. radula*, *Manettia fimbriata*, *Richardia brasiliensis* and *R. scabra* (51.6-91.0 µm in polar diameter and 59.5-82.8 µm in equatorial diameter, in equatorial view) and very large (103.2 µm in polar diameter

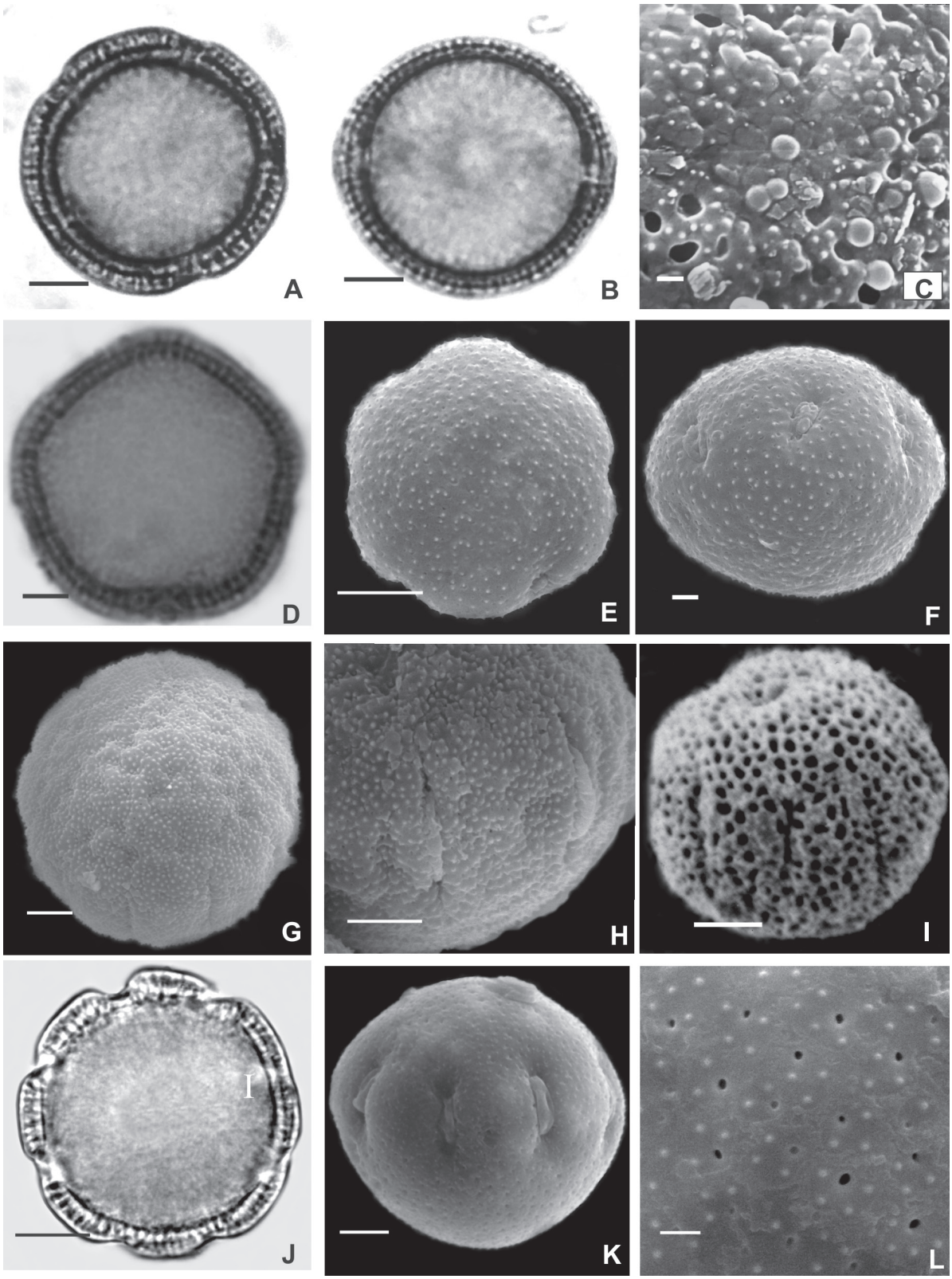

Figure 1. Photomicrographs and eletronphotomicrographs of pollen grains of species Spermacoceae (Rubiaceae) of the Atlantic Forest. *Borreria brachystemonoides* - **A.** polar view: optical section (LM); **B.** equatorial view: aperture (LM); **C.** surface detail (SEM). *B. capitata* - **D.** polar view: optical section (LM); **E.** polar view: general aspect (SEM); **F.** equatorial view: aperture (SEM). *B. latifolia* - **G.** polar view: general aspect (SEM); **H.** equatorial view: aperture detail (SEM). *B. palustris* - **I.** equatorial view: general aspect and aperture. *B. scabioisoides* - **J.** polar view: optical section (LM); **K.** equatorial view: general aspect and aperture (SEM); **L.** surface detail (SEM). Bars: **A**, **B**, **E**, **G**, **K** (= 5 μm); **D**, **H**, **J** (= 10 μm); **F** (=2 μm); **C**, **I**, **L** (= 1 μm).

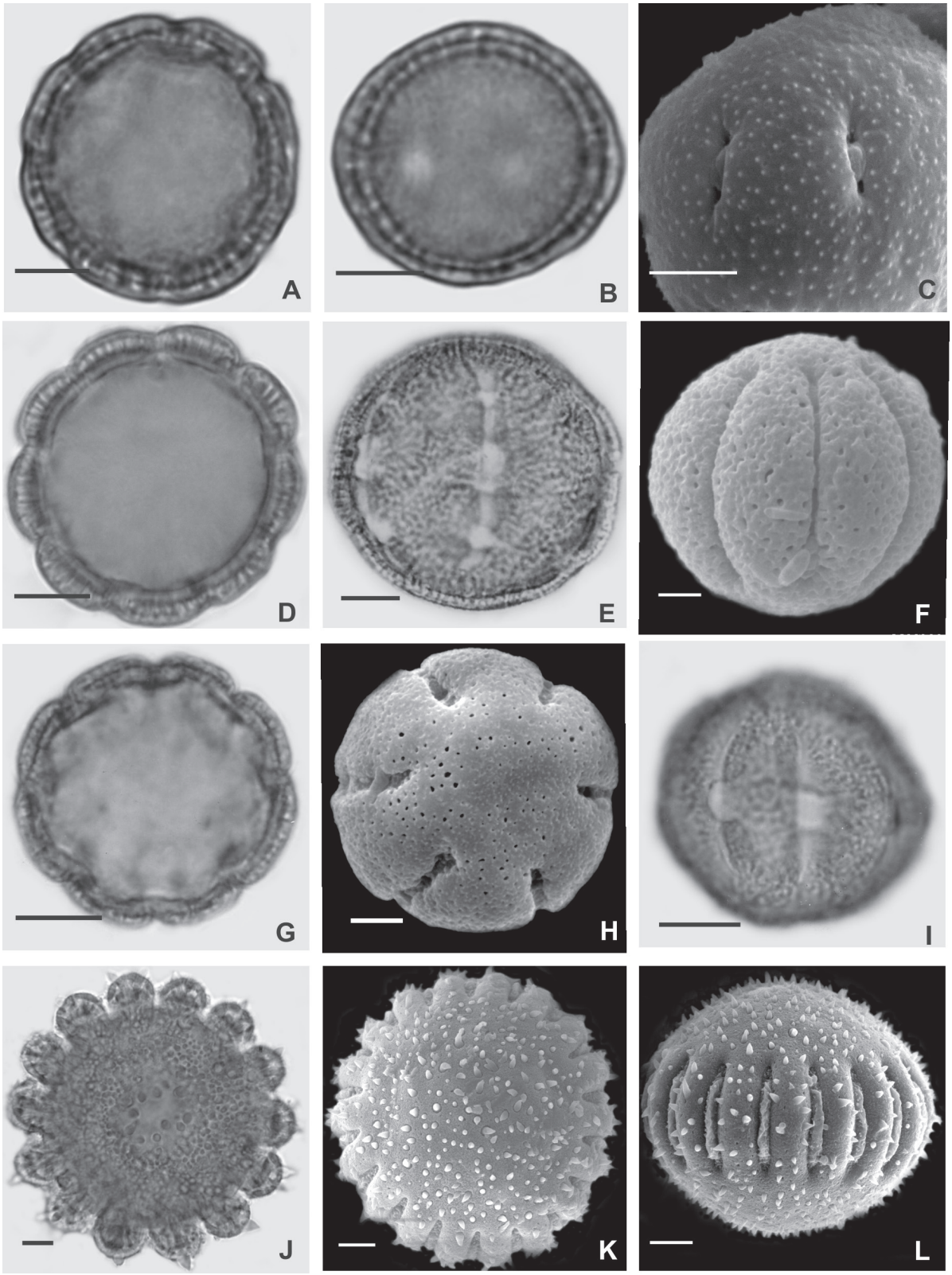

Figure 2. Photomicrographs and eletronphotomicrographs of pollen grains of species Spermacoceae (Rubiaceae) of the Atlantic Forest. *Borreria verticillata* - **A.** polar view: optical section (LM); **B.** equatorial view: optical section and aperture (LM); **C.** equatorial view: aperture detail (SEM). *Denscantia cymosa* - **D.** polar view: optical section (LM); **E.** equatorial view: aperture (LM); **F.** equatorial view: general surface and aperture (SEM). *Emmeorhiza umbellata* - **G.** polar view: optical section (LM); **H.** polar view: general aspect (SEM); **I.** equatorial view: aperture (LM). *Hexasepalum apiculatum* - **J.** polar view: optical section (LM); **K.** polar view: general aspect (SEM); **L.** equatorial view: general aspect and aperture (SEM). Bars: **C**, **F**, **H**, **K**, **L** (= 5 μm); **A**, **B**, **D**, **E**, **G**, **I**, **J** (= 10 μm).

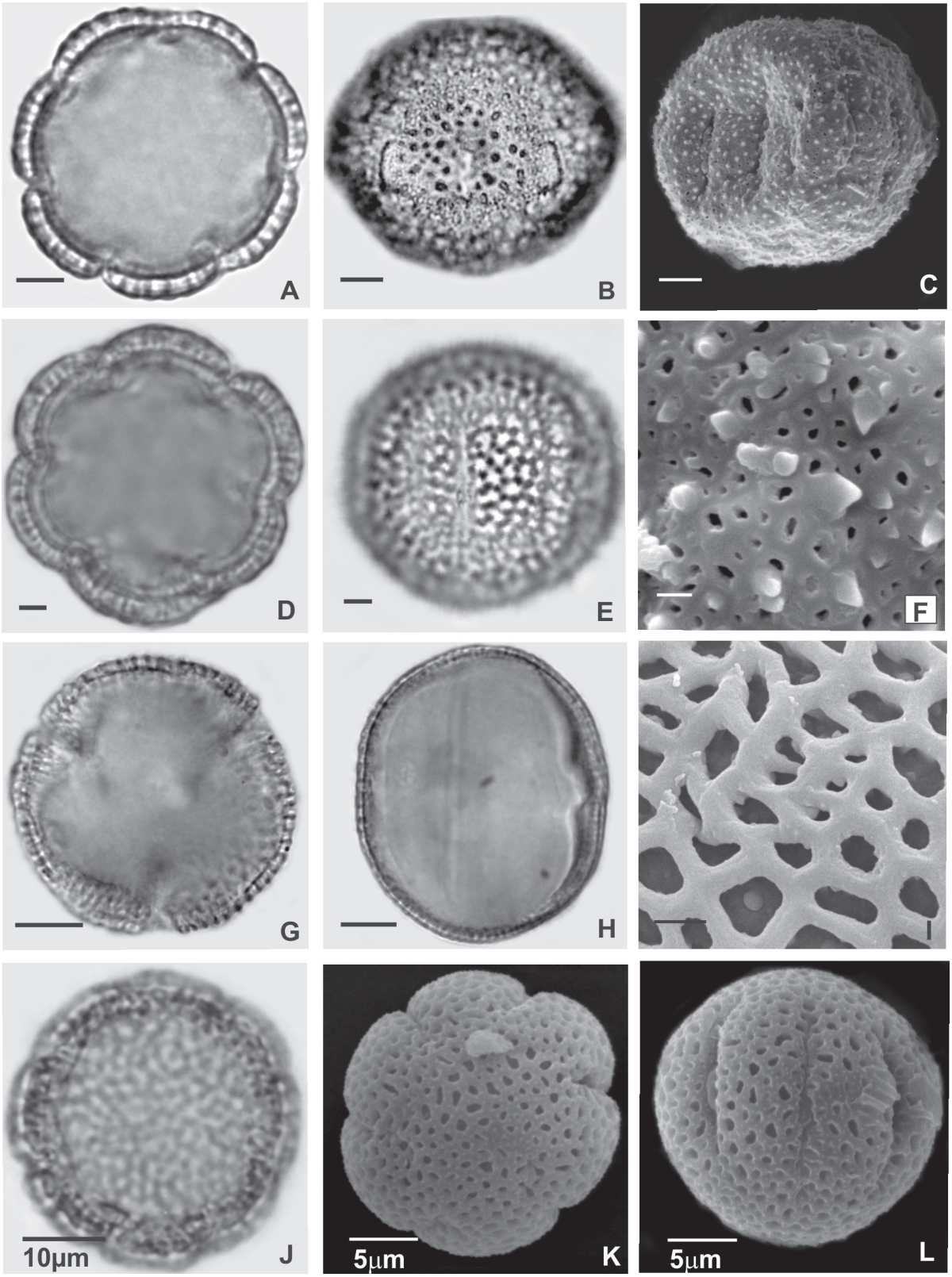

Figure 3. Photomicrographs and eletronphotomicrographs of pollen grains of species Spermacoceae (Rubiaceae) of the Atlantic Forest. *Hexasepalum radula* - **A.** polar view: optical section (LM); **B.** equatorial view: aperture (LM); **C.** equatorial view: general aspect and aperture (SEM). *H. teres* - **D.** polar view: optical section (LM); **E.** equatorial view: aperture (LM); **F.** surface detail (SEM). *Manettia fimbriata* - **G.** polar view: optical section (LM); **H.** equatorial view: general aspect and aperture (LM); **I.** surface detail (SEM). *Mitracarpus eichleri* - **J.** polar view: optical section (LM); **K.** polar view: general aspect (SEM): **L.** equatorial view: general aspect and aperture (SEM). Bars: **C**, **K**, **L** (= 5 µm); **A**, **B**, **D**, **E**, **G**, **H**, **J** (= 10 µm); **F**, **I** (= 1 µm).

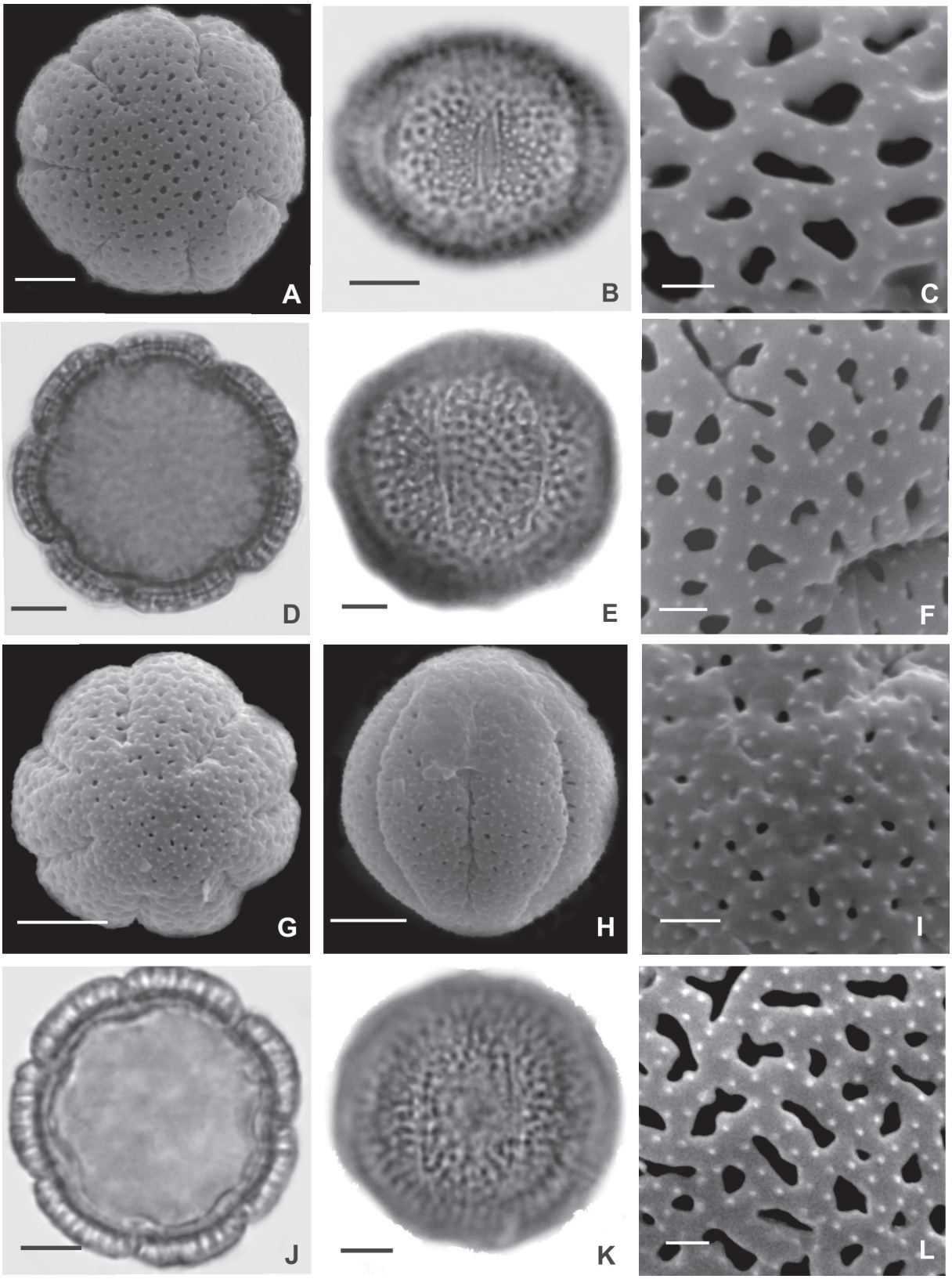

Figure 4. Photomicrographs and eletronphotomicrographs of pollen grains of species Spermacoceae (Rubiaceae) of the Atlantic Forest. *Mitracarpus frigidus* - **A.** polar view: general aspect (SEM); **B.** equatorial view: aperture (LM); **C.** surface detail (SEM). *M. hirtus* - **D.** polar view: optical section (LM); **E.** equatorial view: aperture (LM); **F.** surface detail (SEM). *M. lhotzkianus* - **G.** polar view: general aspect (SEM); **H.** equatorial view: general view and aperture (SEM); **I.** surface detail (SEM). *M. megapotamicus* - **J.** polar view: optical section (LM); **K.** equatorial view: aperture (LM); **L.** surface detail (SEM). Bars: **A**, **G**, **H** (= 5 μm); **B**, **D**, **E**, **J**, **K** (= 10 μm); **C**, **F**, **I**, **L** (= 1 μm).

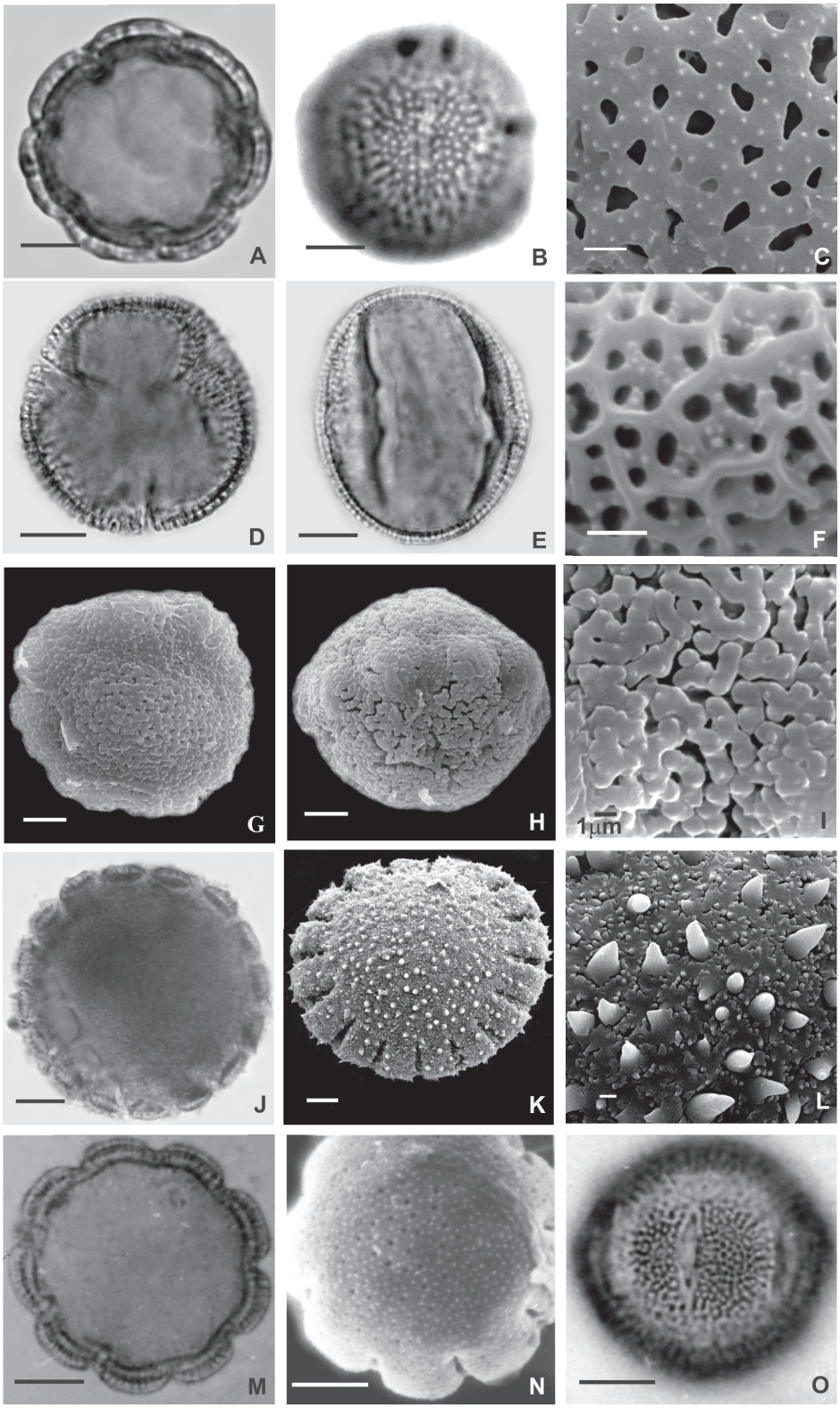

Figure 5. Photomicrographs and eletronphotomicrographs of pollen grains of species Spermacoceae (Rubiaceae) of the Atlantic Forest. *Oldenlandia salzmannii*. **A.** polar view: optical section (LM); **B.** equatorial view: aperture (LM); **C.** surface detail (SEM). *Pentodon pentandrus* - **D.** polar view: optical section (LM); **E.** equatorial view: aperture (LM); **F.** surface detail (SEM). *Richardia brasiliensis* - **G.** polar view: general aspect (SEM); **H.** equatorial view: general aspect and aperture (SEM); **I.** surface detail (SEM). *R. scabra* - **J.** polar view: optical section (LM); **K.** polar view: general aspect and aperture (SEM); **L.** surface detail (SEM). *Spermacoce rubescens* - **M.** polar view: optical section (LM); **N.** polar view: surface detail (SEM); **O.** equatorial view: aperture (LM). Bars: **G**, **H**, **N** (= 5 µm); **A**, **B**, **D**, **E**, **J**, **K**, **M**, **O** (= 10 µm); C, F, I, L (= 1 µm).

and 114.9 µm in equatorial diameter) only in *Hexasepalum teres* (Tabs. 1-3).

Shape and polar area

Most species have oblate spheroidal shape (PD/ED 0.90-0.97), suboblate in *Borreria palustris*, *Hexasepalum apiculatum, H. radula*, *Pentodon pentandrus*, *Richardia brasiliensis*, *R. scabra* (PD/ED 0.77-0.87), prolate spheroidal in *Spermacoce rubescens* (PD/ED 1.02) or subprolate in *Manettia fimbriata* (PD/ED 1.32). Polar area was large in most species, very small in *Borreria latifolia*, *B. palustris, Emmeorhiza umbellata*, *Hexasepalum radula*, *H. teres*, small in *Borreria brachystemonoides*, *Manettia fimbriata* and *Mitracarpus hirtus* or very large in *Borreria capitata*, *Denscantia cymosa*, *Mitracarpus megapotamicus* and *Richardia brasiliensis* (Tabs. 1-3).

Apertures

Pollen grains are colporate with the variable number of aperture (three-19) (Tab. 1). Long ectoaperture in most species, very long in *Denscantia cymosa* and *Richardia brasiliensis*, short in most species, very long in n *Borreria latifolia*, *B. palustris*, *Emmeorhiza umbellate*, *Hexasepalum radula*, *H. teres*, long in *Borreria brachystemonoides*, *Manettia fimbriata* and *Mitracarpus hirtus* and very small in *Borreria capitata*, *Denscantia cymosa*, *Mitracarpus megapotamicus* and *Richardia brasiliensis* (Tab. 4). The ectocolpus membrane is psilated. The ectocolpus is wide (4.0-4.6 µm) in *Borreria latifolia*, *B. palustris*, *Emmeorhiza umbellata*, narrow (1.0-3.0 µm) in most species and narrowest (0.9 µm) only in *H. radula* (Tab. 4). The endoaperture is lolongate in most species and lalongate in *Borreria brachystemonoides* (Fig. 1B), *Denscantia cymosa*, *Manettia fimbriata* (Fig. 3H) and *Pentodon pentandrus*. *Denscantia cymosa* was the only species to present three endoapertures in each ectocolpus (Fig. 2E).

Stratification of exine and ornamentation

Sexine reticulate was found in *Denscantia cymosa* (Fig. 2F), *Manettia fimbriata* (Fig. 3I), reticulate-granulate with gemmae in *Borreria brachystemonoides* (Fig. 1C), reticulate-granulate in *Borreria latifolia* (Fig. 1H), *B. palustris* (Fig. 1I), in the species of the genus *Mitracarpus* (Fig. 4C, F, I, L), in *Oldenlandia salzmannii* (Fig. 5C) and *Pentodon pentandrus* (Fig. 5F). Sexine microreticulate was found only in *Emmeorhiza umbellata*. Sexine granulate with perforation was recorded in *B. capitata* (Fig. 1E, F), *B. scabiosoides* (Fig. 1L), *B. verticillata* (Fig. 2C), *Spermacoce rubescens* (Fig. 5O). In *E. umbellata* (Fig. 3H) and *M. lhotzkianus* (Fig. 4G, I), the lumens of the reticulum diminish towards the poles and are considered perforations. Only in *Manettia fimbriata* are observed granules inside the lumens (Fig. 3I). In the genera *Hexasepalum* the sexine is echinate, being in *H. apiculatum*, *H. radula*, *H. teres* and *Richardia scabra*. Only in *Richardia brasiliensis* was observed vermiculate sexine (Fig. 5I).

Hierarchical cluster analysis (HCA)

The cluster analysis of species of *Spermacoceae* produced a dendrogram with a linkage value of 31.03 for explaining the data. Taking into account the percentage of information

Table 1. Morphological characters of the species of Spermacoceae tribe (Rubiaceae) of the Atlantic Forest.

Species	Size	Shape	Aperture Nº	Sexine ornamentation
Borreria brachystemonoides	medium	oblate spheroidal	5-6	reticulate-granulate
B. capitata	medium	oblate spheroidal	5-6	granulate-perforate
B. latifolia	medium	oblate spheroidal	11-12	reticulate-granulate
B. palustris	medium	suboblate	9-11	reticulate-granulate
B. scabioisoides	medium	oblate spheroidal	8-9-10	granulate-perforate
B. verticillata	small	oblate spheroidal	6-7-8	granulate-perforate
Denscantia cymosa	medium	prolate spheroidal	6-7	reticulate
Emmeorhiza umbellata	medium	oblate spheroidal	8-9	microreticulate
Hexasepalum apiculatum	large	suboblate	13-19	echinate-perforate
H. radula	large	suboblate	9-10-11-12	echinate-perforate
H. teres	very large	oblate spheroidal	13-19	echinate-perforate
Manettia fimbriata	large	subprolate	3	reticulate
Mitracarpus eichleri	medium	oblate spheroidal	7-8	reticulate-granulate
M. frigidus	medium	oblate spheroidal	7-8-9	reticulate-granulate
M. hirtus	medium	oblate spheroidal	7-8	reticulate-granulate
M. lhotzkianus	medium	oblate spheroidal	6-7-8	reticulate-granulate
M. megapotamicus	medium	oblate spheroidal	7-8	reticulate-granulate
Oldenlandia salzmannii	medium	oblate spheroidal	7-8	reticulate-granulate
Pentodon pentandrus	medium	suboblate	3	reticulate-granulate
Richardia brasiliensis	large	suboblate	18-19	vermiculate
R. scabra	large	suboblate	14-15-16	echinate-perforate
Spermacoce rubescens	medium	prolate spheroidal	8-9	granulate-perforate

Table 2. Measurements (in μm) pollen grains in equatorial view. of species of Spermacoceae tribe (Rubiaceae) of the Atlantic Forest. (n=25; *n=10) arithmetic mean ($\bar{x}$); standard deviation ($s_{\bar{x}}$); confidence interval (CI 95 %); PD/ED = relationship between the polar and equatorial diameters.

Species	Polar diameter (PD)			Equatorial diameter (ED)			(PD/ED)
	Range	$\bar{x}$+$s_{\bar{x}}$	CI 95 %	Range	$\bar{x}$+$s_{\bar{x}}$	CI 95 %	
Borreria brachystemonoides	22.5-25.5	24.1±0.2	23.7-24.5	22.5-27.5	25.7±0.1	25.5-25.9	0.94
B. capitata	22.5-27.5	26.3±0.2	25.7-26.9	20.0-30.0	27.6±0.4	26.7-28.4	0.95
B. latifolia	42.5-46.2	43.6+0.3	43.0-44.2	47.5-52.0	48.7±0.2	48.3-49.1	0.90
B. palustris	39.5-42.2	40.8+0.2	40.4-41.2	46.2-48.2	47.2+0,1	47.0-47.4	0.86
B. scabioisoides	22.5-31.5	29.4±2.3	24.6-34.2	27.0-36.0	30.1±3.9	22.7-38.2	0.97
B. verticillata	22.5-25.0	23.9+0.2	23.5-27.5	23.8-27.5	25.9+0.2	25.5-26.3	0.92
Denscantia cymosa	45.5-50.0	46.9+0.4	46.0-47.8	42.5-47.5	44.3+0.4	43.5-45.1	1.05
Emmeorhiza umbellata	28.8-31.5	30.1+0.2	29.7-30.5	32.5-35.0	33.6+0.2	33.2-34.0	0.90
Hexasepalum apiculatum	82.5-95.0	91.0±0.9	89.2-92.8	82.5-90.0	83.6±0.4	82.8-84.4	0.78
H. radula	55.0-62.5	57.8±0.5	56.8-58.8	67.5-75.0	70.7±0.5	69.7-71.6	0.81
H. teres	100.0-105.0	103.2*	-	112.5-117.5	114.9*	-	0.90
Manettia fimbriata	45.0-57.5	51.6+0.7	50.2-53.1	37.5-42.5	38.8+0.3	38.2-39.6	1.32
Mitracarpus eichleri	24.3-26.9	25.6+0.1	25.4-25.8	26.2-28.1	27.0+0.1	26.8-27.2	0.95
M. frigidus	25.0-30.0	27.8±1.6	24.5-31.1	27.5-32.5	30.0±1.4	27.2-32.9	0.92
M. hirtus	23.8-27.4	25.5+0.2	25.1-25.9	26.2-28.6	27.8±0.1	27.6-28.0	0.92
M. lhotzkianus	22.5-25.0	23.9+0.2	23.5-24.3	23.8-27.5	26.3+0.2	25.9-26.7	0.91
M. megapotamicus	27.0-28.5	27.6+0.1	27.4-27.8	28.5-30.0	29.4+0.1	29.2-29.6	0.94
Oldenlandia salzmannii	25.0-30.0	27.5+0.3	26.9-28.2	27.5-32.5	29.9+0.2	29.4-30.4	0.93
Pentodon pentandrus	27.5-35.0	29.5±0.35	28.8-30.2	30.0-37.5	33.7±0.41	32.8-34.5	0.87
Richardia brasiliensis	46.2-48.7	47.9+0.2	47.6-48.2	57.5-61.2	59.5+0.1	59.1-59.8	0.80
R. scabra	50.0-70.0	58.5+1.5	55.5-61.5	65.0-85.0	75.3+1.4	72.4-78.3	0.77
Spermacoce rubescens	27.0-36.0	30.1±3.9	22.7-38.2	22.5-31.5	29.4±0.3	24.6-34.2	1.02

Table 3. Measurements (in μm) pollen grains in polar view: equatorial diameter (EDPV); apocolpus side (AS); polar area index (PAI) and the exine layers of species of Spermacoceae tribe (Rubiaceae) of the Atlantic Forest (n=10). Arithmetic mean ($\bar{x}$).

Species	Equatorial diameter in polar view (EDPV)		AS		PAI
	Range	$\bar{x}$	Range	$\bar{x}$	
Borreria brachystemonoides	23.8-26.2	24.9	9.5-12.2	10.3	0.41
B. capitata	27.5-30.0	28.5	20.0-25.0	21.8	0.76
B. latifolia	48.8-52.5	49.9	17.5-22.5	10.2	0.20
B. palustris	42.5-45.0	43.7	9.5-10.8	10.1	0.23
B. scabioisoides	31.5-41.0	36.2	20.2-29.8	25.0	0.69
B. verticillata	27.5-35.0	30.3	15.0-22.5	18.2	0.60
Denscantia cymosa	35.0-42.5	39.5	31.2-40.0	34.4	0.87
Emmeorhiza umbellata	30.0-32.5	31.6	5.8-7.0	6.2	0.20
Hexasepalum apiculatum	87.5-110.0	97.2	60.0-82.5	68.2	0.70
H. radula	58.8-61.2	60.2	11.2-12.8	12.3	0.20
H. teres	110.0-125.5	115.8	12.5-15.0	14.2	0.12
Manettia fimbriata	37.5-40.0	38.8	7.5-17.5	11.3	0.29
Mitracarpus eichleri	27.5-30.0	29.5	17.5-20.0	19.5	0.66
M. frigidus	26.7-31.6	29.1	16.8-21.7	19.2	0.66
M. hirtus	26.2-28.6	27.0	15.5-14.5	13.2	0.49
M. lhotzkianus	32.5-35.0	33.9	12.5-22.5	17.4	0.51
M. megapotamicus	30.0-35.0	33.1	22.5-30.0	25.0	0.76
Oldenlandia salzmannii	25.0-32.5	29.5	17.5-20.0	19.5	0.66
Pentodon pentandrus	27.5-32.5	30.5	17.5-27.5	20.5	0.67
Richardia brasiliensis	47.5-52.5	48.8	37.8-40.2	39.3	0.80
R. scabra	70.0-87.5	78.8	35.0-45.0	40.3	0.51
Spermacoce rubescens	31.5-41.0	36.2	20.2-29.8	25.0	0.69

Table 4. Measurements mean (μm) of the aperture and exine layers of species of Spermacoceae (Rubiaceae) of the Atlantic Forest. (n=10).

Species	Ectocolpus		Endoaperture		Exine	
	length	width	length	width	sexine	nexine
Borreria brachystemonoides	16.0	2.5	3.2	6.0	1.6	0.9
B. capitata	15.0	1.0	4.0	3.5	1.6	0.9
B. latifolia	17.8	4.0	6.2	2.9	2.9	1.0
B. palustris	17.5	1.5	7.0	2.0	3.4	1.5
B. scabioisoides	19.0	2.9	4.5	3.0	2.6	2.0
B. verticillata	10.2	2.6	5.0	2.0	2.1	1.3
Denscantia cymosa	16.0	1.5	3.0	3.5	2.5	1.9
Emmeorhiza umbellata	26.2	4.6	9.1	5.0	1.1	1.0
Hexasepalum apiculatum	27.5	1.6	6.0	3.0	1.6	1.0
H. radula	15.0	0.9	7.0	4.6	2.6	1.8
H. teres	43.8	2.2	3.0	1.5	1.8	1.0
Manettia fimbriata	45.6	4.0	10.4	21.8	1.2	1.5
Mitracarpus eichleri	18.9	1.6	2.5	2.0	2.0	1.1
M. frigidus	18.7	1.5	2.5	1,7	2.0	1.1
M. hirtus	22.0	1.4	2.7	2.0	2.2	1.2
M. lhotzkianus	18.1	1.0	2.0	1.0	1.9	1.6
M. megapotamicus	19.3	1.4	2.5	1.0	2.3	1.9
Oldenlandia salzmannii	18.5	1.2	3.0	1,3	1.6	1.1
Pentodon pentandrus	19.8	2.5	6.0	11.0	1.2	1.0
Richardia brasiliensis	20.0	3.0	3.0	2.7	2.0	1.0
R. scabra	25.3	1.3	3.5	2.5	2.7	2.1
Spermacoce rubescens	19.0	1.3	4.5	3.0	2.6	2.0

(variable) and the final number of groups, three groups were recognized when 75 % of the information was analyzed.

The cluster one grouped six genera and 13 species: *Borreria brachystemonoides*, *B. capitata*, *B. scabiosoides, B. verticillata, Emmeorhiza umbellata, Mitracarpus eichleri, M. frigidus, M. hirtus, M. lhotzkianus, M. megapotamicus, Oldenlandia salzmannii, Pentodon pentandrus* and *Spermacoce rubescens*, but did not group all species of *Borreria*. These species present sexine reticulate-granulate, granulate-perforate in *S. rubescens*, granulate-perforate in *B. capitaita*, reticulate-granulate with gemmae in *B. brachystemonoides*, granulate-perforate in *B. scabiosoides* and *B. verticillata*. The shape is oblate spheroidal with exception of *P. pentandrus* (suboblate) and *S. rubescens* (prolate spheroidal). All species have medium pollen size except for *B. verticillata* which has small pollen grains. *Oldenlandia sazlmannii, M. eichleri* and *M. frigidus* were not separated, as were *B. scabiosoides* (=*Spermacoce* scabiosoides) and *S. rubescens*. The cluster two grouped two species of *Borreria* (*B. latifolia* and *B. palustres*), *D. cymosa, H. radula, M. fimbriata, R. brasiliensis* and *R. scabra*. The two species of *Borreria* and *Manettia fimbriata* present reticulate-granulate sexine or with granules in the lumen. The genus *Richardia* and the species *H. radula* and *Manettia fimbriata* present large-sized pollen. We can conclude that the qualitative data do not corroborate with the quantitative data in the two cluster above. In cluster three only two species of the genus *Hexasepalum* (*H. apiculatum* and *H. teres*) were grouped by the quantitative data and were confirmed by the qualitative data such as number of apertures (13-19), large-size pollen grain and echine-perforated sexine (Fig. 6).

Principal component analysis (PCA)

The first two axes of the PCA explain 94.78 % of the total variance, with first and second axes explaining 85.92 % and 8.89 % of the variance, respectively. The most significant variables of the first principal component are: polar diameter/PD, equatorial diameter/ED, EDPV. The most significant variables of the second axis is index of polar area/AS.

The negative side of axis one concentrated most species of different genera: *Borreria* (five species), *Emmeorhyza*, *Mitracarpus* (five species), *Oldenlandia*, *Pentodon* e *Spermacoce*. The positive side of axis one and two concentrated four species: genus *Borreria latifolia*, *Hexasepalum* (two species), *Manettia* and the negative side of axis one and two concentrated species of the genera *Borreria* (three species), and *Mitracarpus* (three species), *Oldenlandia* and *Spermacoce*. *Hexasepalum teres* and *H. apiculatum* were noted to be vertically opposite at the ends of axis one and two to the right, completely distant from all other species, *H. teres* on the positive side and *H. apiculatum* on the negative side. *Borreria scabiosoides, Mitracarpus megapotamicus* and *Spermacoce rubescens* were very similar. *Borreria brachystemonoides* was completely separated from the other species of the genus on the positive side of axis one and negative axis two, *Borreria latifolia* and *B. palustris,* although they were together, also distanced themselves from the other species of the genus being on the positive side of axis one and two, *Descanttia* and *Richardia* are close from eachother at the positive side of axis one and negative axis two (Fig. 7).

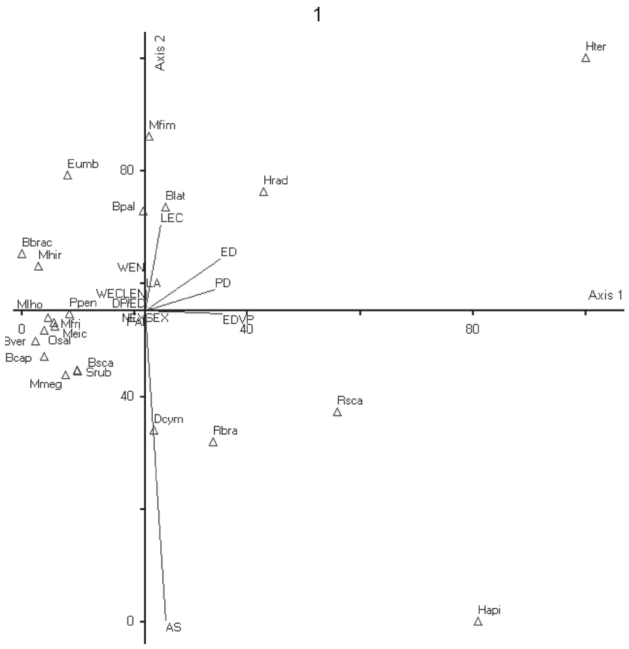

Figure 6. Principal component analysis performed with the mesured variables of pollen from species of Spermacoceae. Abbreviations: PD- polar diameter, ED- equatorial diameter, PD/PE - shape , LEC- ectoaperture length, WEC – ectoaperture largure, LEN – endoaperture length, WEN - endoaperture largure, SEX- sexine, NEX -nexine, EDPV- equatorial diameter from polar view, AS- apocolpium side.

Discussion and conclusion

The species *Manettia fimbriata* and *Pentodon pentandrus*, presented three-colporate pollen grains while the most species, had many apertures ranging from five to 19 colpore. With regard to endoaperture, the majority of the species presented lolongated endoaperture. As for the shape, the pollen grains ranged from suboblate, oblate spheroidal, subprolate to prolate spheroidal. Regarding the sexine ornamentation, the following types were recorded: a) reticulate-granulate with few gemmae (*B. brachysatemonoides*), b) reticulate with granules in lumen (*Manettia fimbriata*), reticulate-granulate (all species of *Mitracarpus*, *Oldenlandia salzmannii* and *Pentodon pentandrus*), c) microreticulate (*Emmeorhyza umbellata*), d) vermiculate *Richardia brasiliensis*), e) perforate with granules (*Borreria scabiosoides*), f) echinate-perforate (all species of *Hexasepalum*, *Richardia scabra*), g) granulate-perforate (*Borreria capitata*, *B. latifolia*, *B. verticillata*, *Spermacoce rubescens*), h) reticulate (*Descantia cymosa*).

The genus *Borreria* was palynologically described in several studies, among those is the study by Salgado-Labouriau (1973) that, through the species *B. capitata*, established the *Borreria* type as zonoaperturate, with six-eight pores and sexine retipilate; Carreira (1976) described the pollen grains as small, six-seven-pantoporate, retipilate sexine. Cabral (1985) considered pollen grains

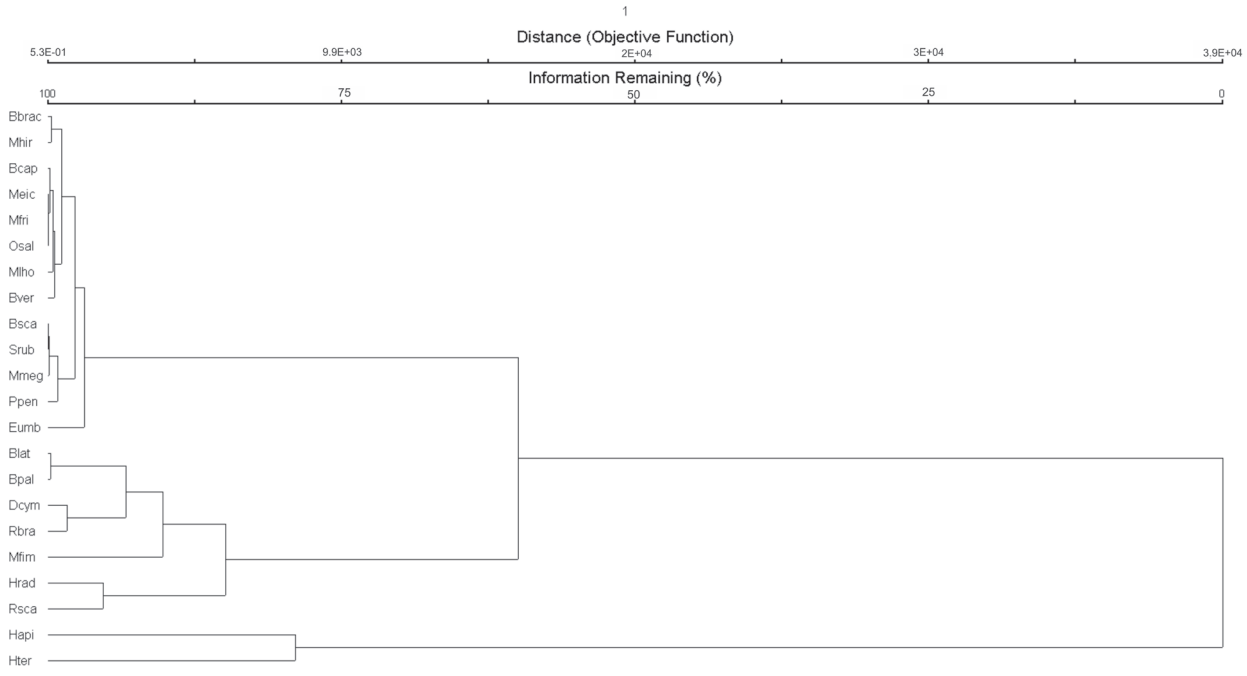

Figure 7. Cluster analysis performed with the measured variables of pollen from Spermacoceae. Abbreviations: Bbrac = *Borreria brachystemonoides*, Bcap = *Borreria capitata*, Blat = *Borreria latifolia*, Bpal = *Borreria palustres*, Bsca = *Borreria escarabeoideo*, Bver =*Borreria verticillata*, Dcym = *Denscantia cymosa*, Eumb = *Emmeorhiza umbellata*, Hapi = *Hexasepalum apiculatum*, Hrad = *Hexasepalum radula*, Hter = *Hexasepalum teres*, Mfim = *Manettia fimbriata*, Meic = *Mitracarpus eichleri*, Mfri = *Mitracarpus frigidus*, Mhir = *Mitracarpus hirtus*, Mlho = *Mitracarpus lhotzkyanus*, Mmeg = *Mitracarpus megapotamicus*, Osal = *Oldenlandia salzmannii*, Ppen = *Pentodon pentandrus*, Rbra = *Richardia brasiliensis*, Rsca = *Richardia scabra*, Srub= *Spermacoce rubescens*.

of *B. capitata* medium, with five-six pores and granulate ornamentation.

Authors such as Chávez *et al.* (1991), Arreguín-Sánchez *et al.* (1995) and Dessein *et al.* (2002) analyzed the pollen grains of *B. verticillata* and, in general, the results found here are similar to the last author. The results differ from Chávez *et al.* (1991) and Arreguín-Sánchez *et al.* (1995) regarding type of aperture and ornamentation of the sexine that Chávez *et al.* (1991) described as six-colpate and sexine microreticulate. Arreguín-Sánchez *et al.* (1995) characterized as seven or more colpi and per-reticulate surface, whereas, in the present work, we found grains of pollen (six)-seven-(eight) colporate and perforate-granulate sexine.

Melhem *et al.* (2003) analyzed 20 species of Rubiaceae for Campos do Jordão, São Paulo, Brazil. In this study the authors analyzed three species of *Borreria* of which *B. capitata* and *B. verticillata*. The results are different from those obtained when the type of aperture (pores in *B. capitata*) and the ornamentation of *B. verticillata* (perforated). These species were here considered to have colpori and sexine granulated-perforated.

Denscantia cymosa was the only species that presented three endoapertures by ectoaperture. This species was analyzed by Cabral (1985), Pire & Cabral (1992) and Silveira-Junior *et al.* (2012). The results here obtained (sexine reticulate) differed from the previous analyzes by regarding the surface da sexine (granulate-perforate).

The pollen grains of *Hexasepalum* were characterized by Silveira-Junior. *et al.* (2012) who also analyzed the species and the results found here were similar regarding ornamentation of sexine and aperture number. *Hexasepalum radula* and *H. teres* were analyzed by Dessein *et al.* (2005) who considered the pollen grains of *H. teres* to be very large. Our results agree with those of the author and differ from the descriptions made by Delprete & Cortés (2006) that considered pollen grains *H. radula* with ornamentation foveolate-perforate sexine. The pollen data of *H. teres* presented in this study also differ from that described by Silveira-Junior *et al.* (2012) regarding the number of zonocolpate apertures (11) (-12)-four-(-15) (-16) and the echinate-granulate-perforate surface. It is believed that the differences in the ornamentation of the sexine between the present study and the previous ones are justified by the type of microscopy used.

The species *Emmeorhiza umbellata* has been described herein as having medium size, oblate spheroidal, eight-(nine) colporate pollen grains and the microreticulate surface. Pire & Cabral (1992), Melhem *et al.* (2003), Delprete & Cortes (2006) and Silveira-Junior *et al.* (2012) studied the *E. umbellata* pollen grains and the results found here differ from most of the authors cited above for surface (equinate-perforate). The results are similar only with those of Silveira-Junior *et al.* (2012) regarding surface (microreticulate).

Melhem *et al.* (2003) described the pollen grains of the *Mitracarpus* species as medium, oblate spheroidal three-four-six-seven-nine-colpate, granulate exine. The results found here were similar differing in the type of aperture (colporate) and in ornamentation of sexine, described here as reticulate-granulate. Melhem *et al.* (2003) considered it as granulate and Souza and Lorenzi (2008) reported the presence of five-10-colporate pollen grains. The data of Delprete & Cortés (2006) are different regarding type of aperture (colporate) and ornamentation of the exine.

Pentodon pentandrus was described in the present work as medium, suboblate, three-colporate pollen grains and reticulate-granulate surface, by the bibliographic survey, shows that the species was analyzed for the first time in the present study.

Authors such as Erdtman (1952), Salgado-Labouriau (1973), Delprete & Cortés (2006) and Silveira-Junior *et al.* (2012) studied species of the genera *Richardia*, and the results found were different when compared to the size, shape, type and number of aperture and ornamentation of sexine.

Based on the results obtained here, it can be concluded that the pollen attributes such as size, shape, number of apertures and ornamentation of the sexine were important to define species but did not characterize the genera. However, in relation to ornamentation, some species of a genus presented the same type, for example: *Hexasepalum* species, echinate-perforate; *Mitracarpus* species reticulate-granulate confirming that the group is euripolynic.

Borreria, *Mitracarpus*, *Spermacoce* and *Oldenlandia* were grouped togheter in the PCA and HCA analysis, showing that the pollinic characteristics didn't resolve those genera. *Borreria palustre* and *B. latifolia* are exceptions, forming a cluster different from the other species of the genera.

Hexasepalum are separeted in the PCA, due to apocolpium side and lenght ectolpus measures, but the species *H. apiculatum* and *H. teres* are agrouped in a cluster, from the HCA perspective.

From the multivariate analysis used here it can be concluded that the quantitative traits are not enough to completely separate the species of Spermacoceae.

It can be affirmed that the pollinic characteristics can be useful in taxonomic studies of the Spermococeae. The application of pollen knowledge is an additional tool for describing and identifying taxa to increase understanding of tribe systematics, presently established infrageneral categories arrangements for reassessment of circumscriptions, and a better understanding of the phylogenetic lineages of Spermacoceae.

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