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Morphological characterization of pollen grains of Brazilian species of Bombacoideae (Malvaceae *s.l.*)

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ABSTRACT

The pollen grains of 15 species belonging to five genera (*Ceiba* Mill., *Eriotheca* Schott & Endl., *Pachira* Aubl., *Pseudobombax* Dugand, and *Rhodognaphalopsis* A. Robyns) of the subfamily Bombacoideae (Malvaceae *s.l.*) were acetolysed, measured, described and photomicrographed under light microscopy and scanning and transmission electron microscopy, in order to characterize the pollen morphology of species of the subfamily occurring in Brazil. The pollen characters studied were size, shape, exine constitution, and apertural type. Pollen grains were medium to large-sized and isopolar with a (sub)circular and (sub)triangular amb with flat, convex or concave sides; oblate to suboblate; 3(-4)-colp(or)ate or 4-5(-6)-colporate, planaperturate, sinuaperturate; equatorial calottes different or not; exine tectate or semitectate, perforate and microreticulate with or without supratectal spines. The results confirm the eurypalynous nature of the group with variation among the studied genera being mainly in the exine pattern.

Keywords: *Ceiba*, *Pachira*, palynology, *Pseudobombax*, SEM, TEM

Introduction

Bombacoideae is one of nine recognized subfamilies of Malvaceae *s.l.* (Bayer *et al*. 1999). It comprises genera traditionally placed in the family Bombacaceae and, with the subfamily Malvoideae, forms a clade called Malvatheca (Alverson *et al*. 1999). Malvatheca is well-supported by mixed analyses, and includes representatives characterized by modified anthers with two or multiple sporangiate thecae. However, the compositions of Bombacoideae and Malvoideae are not easily determined by morphology, while molecular studies have presented conflicting results (Baum *et al*. 2004; Nyffeler *et al*. 2005). Traditional works (Kunth 1821; Candolle 1824; Cronquist 1981) have always considered the taxa included in Malvoideae and Bombacoideae as being very closely related.

Bombacoideae includes about 17 genera and 160 species with a predominantly Neotropical distribution (Carvalho-Sobrinho *et al*. 2016). According to Stevens (2005), the subfamily is characterized by an often robust trunk with water storage in the parenchyma, fine bark, robust aculeus, usually fasciculate fillets, pollen grains with a triangular amb with more or less flat sides, staminode generally absent, pubescent endocarp and palmately compound leaves, the latter being considered a probable synapomorphy for the group (Alverson *et al*. 1999; Baum *et al*. 2004).

Several representatives of Bombacoideae have numerous uses and are of great economic importance. The light wood of species of *Cavanillesia*, *Ceiba*, *Eriotheca* and *Pseudobombax* is used in the manufacture of packaging, furniture structure, door, linings, toys, and model aircrafts. The kapok is used for filling mattresses, pillows, and furniture upholstery.

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Among other species, representatives of *Bombax*, *Ceiba*, and *Pachira* stand out as ornamental plants used in parks and gardens (Braga 1960; Lorenzi 2002a; b; Maia 2004).

Bombacoideae is considered a eurypalynous subfamily whose pollen grains vary mainly in apertural type and pattern of exine ornamentation. The use of pollen morphology for taxonomic differentiation is of particular importance for eurypalynous groups. Fuchs (1967) emphasized the value of pollen characters for differentiating taxa of Bombacoideae. This is evidenced in the taxonomic study of *Bombax s.l.* (Robyns 1963), which used pollen morphology along with macromorphological characters to delimit infrageneric taxa, and the classification of Cronquist (1981), which used pollen type as one of the characters to separate representatives included in Malvoideae and Bombacoideae.

One of the most significant contributions to the pollen morphology of representatives of Bombacoideae is that of Nilson & Robyns (1986), who analyzed pollen grains of 29 genera of the subfamily using light and electron microscopy. These authors recognized eleven pollen types based mainly on exine ornamentation patterns and apertural types.

Other important works on the pollen morphology of species of the subfamily include Erdtman (1952), who analyzed 90 species of 20 genera, including species of the Brazilian flora. Robyns (1963), as previously mentioned, used pollen characters in the taxonomic study of the genus *Bombax*, including species of *Eriotheca*, *Pachira*, *Pseudobombax*, *Bombacopsis* and *Rhodognaphalopsis*. Using scanning electron microscopy, Robyns (1971) recognized pollen types that differed with regard to apertures, amb and sexine stratification. Tsukada (1964) highlighted the importance of representatives of Bombacoideae in stratigraphic studies when analyzing the pollen morphology of fossil and extant species. Sowunmi (1973), Lozano-García & Hernández (1990) and Palacios-Chávez *et al*. (1991) included morphological descriptions of the pollen of species of Bombacoideae in their works. Moncada & Sotolongo (1994) described four pollen types for the genera that occur in Cuba.

Brazilian works of significance include that of Salgado-Labouriau (1973), who recognized four pollen types for Cerrado species; Melhem & Bissa (1985), who analyzed the pollen morphology of *Pseudobombax grandiflorum*; Bove (1993) who described the pollen grains of *Pseudobombax*, *Bombacopsis*, *Pachira* and *Spirotheca*, which occur in the state of Santa Catarina, grouping them into two pollen types and three pollen subtypes; Carreira *et al*. (1995), who analyzed the pollen grains of species of Bombacoideae growing in the park of the Goeldi Museum and presented a pollen key based on apertural type and exine ornamentation; and Abreu *et al*. (2014), who analyzed eleven species, separating them by a pollen key that considers the presence or absence of callote. It should be noted that most descriptions were made using light microscopy (LM), with scanning electron microscopy (SEM) being used only in the last three cited works.

The present work aimed to describe the pollen morphology of Brazilian species of Bombacoideae with the aim of expanding palynological knowledge of species of this subfamily.

Materials and methods

Pollen grains of 15 species belonging to five genera representative of Brazilian Bombacoideae were examined using light and electron microscopy. Anthers were removed from specimens deposited in the herbaria ALCB, EAC, HST, HUEFS, IPA, PEUFR, SP, SPF and TEPB, all acronyms according to Thiers (2017, continuously updated) (Tab. 1).

For light microscopy (LM), pollen material was acetolyzed (Erdtman 1960), mounted on slides with glycerinated gelatin, sealed with paraffin and analyzed and photomicrographed using a Zeiss Axiostar microscope. The slides were deposited in the pollen collection of Laboratório de Micromorfologia Vegetal (LAMIV) of Universidade Estadual de Feira de Santana. Measurements of the main morphometric parameters (equatorial and polar diameters) were made on 25 pollen grains, whenever possible. Other parameters (diameter of apertures and thickness of exine, sexine and nexine) were measured for 10 randomly-chosen pollen grains. All exine measurements were taken in the mesocolpium region. Quantitative data were submitted statistical analyses adequate for the sample size. The arithmetic mean ($\bar{x}$), standard deviation of the mean ($S\bar{x}$), 95 % confidence interval (CI) and coefficient of variation (CV) were calculated for all of the diameter measurements with a sample size of 25, while only the arithmetic mean was calculated for parameters with a sample size less than 25.

Principal component analysis (PCA) using PC-ORD 5.0 software (McCune & Mefford 2011) was used to identify the palynological variables that best contribute to the distinction of the studied species. This analysis used ten metric variables: polar diameter in equatorial view (P), equatorial diameter in polar view (E), equatorial diameter in equatorial view (Epv), shape (P/E), polar area index (AI), ectoaperture length (EcL), ectoaperture width (EcW), endoaperture height (Eh) and thickness of exine (Ex), nexine (Nex) and sexine (Sex). The results were observed in a biplot graph built from the two first principal components (PC1 and PC2) derived from the PCA.

For scanning electron microscopy (SEM), acetolyzed pollen grains were washed in distilled water, dehydrated in an ascending hydroethanolic series (50, 70, 80, 90 and 100 %), mounted on a SEM specimen holder, metallized with gold, and electron micrographed using a LEO 1430 VP Electron Microscope of the Departamento de Ciências Biológicas of the Universidade Estadual de Feira de Santana.

Ceiba erianthos (Cav.) K. Schum. was selected, because of the availability of material, for analysis using transmission electron microscopy (TEM). Closed anthers were fixed

Table 1. Species used in the morphological analysis of pollen grains of Bombacoideae. *Material used in photomicrographs and electromicrographs.

Species	Voucher	Herbarium
Ceiba erianthos K. Schum	Andrade-Lima 8145, Harley & Giulietti 54045, Guedes & Eudes 4839	IPA*, HUEFS, ALCB
C. glaziovii (Kuntze) K. Schum.	Queiroz *et al*. 997, Oliveira 258, Lyra-Lemos *et al*. 7030	HUEFS, HUEFS, HUEFS*
Eriotheca candolleana (K. Schum.) A. Robyns	Monteiro 23510, Pirani *et al*. 4356	PEUFR*, SPF
E. gracilipes (K. Schum.) A. Robyns	Andrade-Lima 5491	IPA*
E. macrophylla (K. Schum.) A. Robyns	Lima 12632, Ducke & Lima 65	HST*, IPA
E. obcordata A. Robyns	Silva *et al*. 107	HUEFS
Pachira aquatica Aubl.	Miranda 72, Fróes 26.629, Alunos de Taxonomia da FUFPI s.n.	HUEFS, SP*, TEPB 450
P. nervosa (Uitt.) A. Robyns	Esteves *et al*. 02	SP*
P. retusa (Mart. & Zucc.) Fern Alonso	Freire-Fierro *et al*. s.n., Queiroz *et al*. 9634, Junqueira *et al*. 142	SPF 106500*, HUEFS, HUEFS
P. tocantina (Ducke) Fern. Alonso	Fróes 23378	SP*
Pseudobombax calcicola Carv.-Sobr. & L.P.Queiroz	Carvalho-Sobrinho & Queiroz 573	HUEFS
Ps. marginatum (A. St.-Hil., A. Juss. & Cambess.) A Robyns	Castro s.n., Figueiredo *et al*. 175, Du Bocage 267	EAC 32129, EAC, IPA*
Ps. minimum Carv.-Sobr. & L.P.Queiroz	Forzza *et al*. 1591	HUEFS
Ps. simplicifolium A. Robyns	Queiroz & Nascimento 4628, Queiroz *et al*. 7361, Du Bocage 274	HUEFS, HUEFS*, IPA
Rhodognaphalopsis faroensis (Ducke) A. Robyns	Ribeiro & Pereira 1704, Esteves & Assunção 07	SP*, SP

in glutaraldehyde (2.5 %) with 0.1 M sodium phosphate solution at pH 7.4, post-fixed in 1 % osmium tetroxide (OsO_4), dehydrated in an ascending acetone series (30, 50, 70, 90 and three baths at 100 % for 30 min each bath) and included in EPON resin. Sections made using an Ultracut E ultramicrotome equipped with a diamond razor were contrasted with 7 % aqueous uranyl acetate and lead citrate for observation using a Zeiss M 109 microscope of the Electron Microscopy Laboratory of the Gonçalo Moniz Research Center - Oswaldo Cruz Foundation.

The terminology adopted is in accordance with Punt *et al*. (2007). The denomination 'equatorial calotte' is in agreement with the definition of Robyns (1963).

Results

Pollen grains of the studied species of Bombacoideae are characterized as medium to large in size, isopolar, oblate to suboblate with a (sub)circular and (sub)triangular amb (planaperturate) and a small to large polar area; 3-4-5(-6)-zonoaperturate, colpate, colporate, equatorial calottes different or not, exine tectate-perforate to microreticulate, reticulate with or without supratectal processes and sexine thicker than nexine.

Table 2 summarizes the quantitative pollen characters of the investigated specimens. Figures 1-5 illustrate morphopalynous characteristics.

Ceiba erianthos (Cav.) K. Schum. (Fig. 1A-E); *C. glaziovii* (Kuntze) K. Schum. (Fig. 1F-K)

Pollen grains large; oblate to suboblate; isopolar; amb circular; 4-5(-6)-zonocolporate. Ectoapertures long with sharp ends; endoapertures lalongate, circular or less frequent lalongate; membrane psilate-aperturate. Exine semitectate, reticulate, heterobrochate. Muri high, sinuous, simple to dupli-columellate. Sexine thicker than nexine.

In addition to the 4-5-aperturate pollen grains, 6-aperturate pollen grains (Fig. 1A) were recorded in the specimen Andrade-Lima 8145 of *C. erianthos* (ca. 6 %) and in the specimen Oliveira 258 of *C. glaziovii* (ca. 11 %). The endoaperture is difficult to visualize in *C. erianthos*; it was lolongate in most specimens analyzed but specimens with circular (Fig. 1H) or lalongate endapertures could also be found.

The lumina of the reticulum are irregular, gradually decreasing in size from the apocolpium to the mesocolpium; free bacula and granules could be identified using SEM (Fig. 1D; 1J). Branched muri of the reticulum supported by oblique columellae could also be observed using SEM (Fig. 1D, 1I, 1J).

The TEM analysis (Fig. 1E) of *C. erianthos*, which is the first for the species, revealed stratification of the exine consisting of a semitectum, which is as thick as the nexine (referring to the basal layer plus endexine, layers which were not discernible by the methodology used), supported by short columellae, and the intine, which is thicker and stratified under the apertures.

Eriotheca candolleana (K. Schum.) A. Robyns (Fig. 2A-E); *E. macrophylla* (K. Schum.) A. Robyns (Fig. 2F-H); *E. gracilipes* (K. Schum.) A. Robyns (Fig. 2I-J); *E. obcordata* A. Robyns (Fig 2K-L)

Pollen grains medium to large in size; oblate; isopolar; amb (sub)triangular with flat to convex sides; equatorial calottes undifferentiated; 3(-4)-colporate; planaperturate. Ectoapertures short and narrow with sharp ends; endoapertures lolongate and lalongate; membrane psilate-aperturate. Exine semitectate, reticulate, heterobrochate,

Table 2. Pollen morphometric characteristics and measurements (μm) for species of Bombacoideae (Malvaceae *s.l.*): polar axis (P), equatorial axis (E), equatorial axis in polar view (Epv), polar axis/equatorial axis (P/E), range (R), apocolpium index (AI), ectoaperture length x ectoaperture width (Ec), endoaberture height (Eh), sexine (Sex), nexine (Nex), * n < 25.

Species / specimens	P		E		Epv		P/E	AI	Ec	Eh	Sex	Nex
	$\bar{X}\pm S_{\bar{X}}$	R	$\bar{X}\pm S_{\bar{X}}$	R	$\bar{X}\pm S_{\bar{X}}$	R						
Ceiba erianthos												
Guedes & Eudes 4839 (ALCB)	46.1*	43.5-52.5	55.5*	46.5-63.0	57.6±1.1	46.5-67.5	0.83	0.44	23.6x1.0	—	2.1	1.7
Harley & Giulietti 54045 (HUEFS)	42.1*	39.0-45.0	60.5*	55.5-64.5	57.8*	52.5-64.5	0.70	0.46	21.9x4.1	7.8	2.0	1.2
Andrade-Lima 8145 (IPA)	38.6*	36.0-43.5	52.9*	51.0-54.0	64.4*	52.5-70.5	0.73	0.42	15.5x4.8	—	1.7	0.7
C. glaziovii												
Queiroz *et al.* 997 (HUEFS)	47.8*	42.5-51.3	58.8*	53.8-63.8	56.0*	50.0-66.3	0.81	0.47	14.9x2.7	5.0	2.7	0.8
Lyra-Lemos *et al.* 7030 (HUEFS)	44.8*	42.0-49.5	57.8*	54.0-63.8	56.9*	52.5-61.5	0.77	0.53	16.9x4.2	5.3	2.5	0.9
Oliveira 258 (HUEFS)	46.5*	43.5-48.0	57.5*	55.5-60.0	58.6±0.5	52.5-63.0	0.81	0.47	16.7x3.6	7.1	3.2	1.0
Eriotheca candolleana												
Monteiro 23510 (PEUFR)	28.8±0.4	25.0-32.5	43.7±0.6	40.0-50.0	41.5±0.6	37.5-47.5	0.66	0.65	10.9x2.3	4.0	1.7	0.6
Pirani *et al* .4356 (SPF)	24.0*	20.0-27.5	34.5*	32.5-36.2	33.1±0.5	32.1-34.0	0.69	0.59	7.2x1.0	2.5	1.7	0.7
E. gracilipes												
Andrade-Lima 5491 (IPA)	27.8±0.3	25.5-30.0	38.0±0.4	31.5-42.0	36.7±0.4	31.5-40.5	0.73	0.55	12.3x1.5	4.7	1.6	0.9
E. macrophylla												
Lima 12632 (HST) (3-apert)	22.0±1.0	18.0-27.0	41.3±1.8	39.0-46.5	42.7±0.5	39.0-49.5	0.53	0.57	9.8x2.4	4.3	1.5	0.6
Lima 12632(HST) (4-apert)	—	—	—	—	42.1±0.4	39.0-45.0	—	0.55	—	—	1.4	0.6
Ducke & Lima 65 (IPA)	28.6*	25.0-33.8	41.5*	33.8-45.0	36.8±1.6	33.8-45.0	0.69	0.64	9.0x2.4	4.5	1.1	0.6
E. obcordata												
Silva *et al.* 107 (HUEFS)	30.1±0.5	27.0-33.0	44.2±0.6	40.5-52.5	42.7±0.6	37.5-48.0	0.68	0.60	11.9x2.0	5.4	1.4	0.7
Pachira aquatica												
Fróes 26629 (SP)	56.3±0.4	52.5-60.0	69.1±0.5	63.0-73.5	66.0±0.8	57.0-70.5	0.81	0.62	17.9x3.5	7.9	1.8	1.2
Alunos Taxonomia da FUFPI s.n. (TEPB450)	—	—	—	—	61.2*	58.5-63.0	—	0.61	14.2x3.3	6.2	1.4	1.0
Miranda 72 (HUEFS)	49.0*	40.5-55.5	60.6*	52.5-67.5	59.1±0.7	52.5-67.5	0.81	0.65	20.9x5.4	10.5	1.2	1.1
P. nervosa												
Esteves *et al.* 02 (SP)	38.2*	33.0-43.5	62.2*	60.0-66.0	57.8±0.5	52.5-63.0	0.61	0.54	13.9x3.1	5.3	1.8	0.6
P. retusa												
Freire-Fierro *et al.* s.n. (SPF106500)	46.6*	42.0-51.0	64.0*	60.0-70.5	58.2*	48.0-63.0	0.73	0.56	14.7x4.1	7.8	1.5	0.6
Junqueira *et al.* 142 (HUEFS)	48.3*	45.0-52.5	66.3*	60.0-72.0	61.4±1.0	49.5-75.0	0.73	0.56	13.2x3.4	—	1.1	0.6
Queiroz *et al.* 9634 (HUEFS)	46.0*	45.0-46.5	64.5*	63.0-67.5	60.2*	54.0-64.5	0.71	0.60	17.4x2.4	—	1.1	0.6
P. tocantina												
Fróes 23378 (SP)	45.3*	39.0-52.5	64.6*	57.0-70.5	60.7±0.8	49.5-69.0	0.70	0.56	12.6x2.2	—	2.3	0.6
Pseudobombax calcicola												
Carvalho-Sobrinho & Queiroz 573 (HUEFS)	52.9±0.9	45.0-65.0	90.8±0.7	82.5-100.0	79.9±0.8	70.0-87.5	0.58	0.51	23.3x4.7	—	1.7	1.2
Ps. marginatum												
Castro s.n. (EAC32129)	—	—	—	—	62.4*	58.5-66.0	—	0.57	17.8x2.0	7.0	1.7	0.8
Du Bocage 267 (IPA)	41.0*	37.5-45.0	64.0*	63.0-64.5	62.5*	55.5-70.5	0.64	0.54	24.0x6.0	15.6	1.6	0.9
Figueiredo *et al.* 175 (EAC)	39.0*	37.5-40.5	73.5*	67.5-79.5	63.4*	54.0-76.5	0.53	0.53	18.5x3.4	—	1.6	0.7
Ps. minimum												
Forzza *et al.* 1591 (HUEFS)	42.4±0.8	32.5-50.0	61.3±0.5	57.5-67.5	57.2±0.7	52.5-67.5	0.69	0.53	20.1x3.1	—	1.5	0.7
Ps. simplicifolium												
Queiroz & Nascimento 4628 (HUEFS)	38.8*	34.5-45.0	62.5*	58.5-67.5	61.2±1.1	48.0-69.0	0.62	0.42	20.6x5.4	—	1.9	0.6
Du Bocage 274 (IPA)	42.1*	30.0-50.0	65.4*	56.3-78.8	61.8±1.1	50.0-71.3	0.64	0.40	22.4x2.4	—	1.9	0.6
Queiroz et al. *7361 (HUEFS)*	39.0±0.5	33.0-43.5	61.0±0.9	49.5-70.5	57.0±0.8	48.0-63.0	0.64	0.47	22.1x5.3	—	1.7	0.6
Rhodognaphalopsis faroensis												
Ribeiro & Pereira 1704 (SP)	42.1±0.4	39.0-45.0	58.9±0.6	48.0-63.0	53.8±0.6	46.5-60.0	0.71	0.62	13.0x2.2	4.7	1.3	0.9
Esteves & Assunção 07 (SP)	—	—	—	—	38.6±0.8	33.0-48.0	—	0.60	—	—	1.4	0.6

lumina polyhedral with free granules in the interior; muri high, smooth and simplicolumellate. Sexine thicker than nexine.

The pollen grains of species of *Eriotheca* are uniform in size (Tab. 2). The largest pollen grains (52.5 μm) were observed for *E. obcordata*; however, the average size class was predominant among the analyzed specimens. The amb varied between subtriangular and triangular, with the latter predominating. The 4-aperturate pollen grains (Fig. 2F) had a subcircular amb. 4-aperturate pollen grains were recorded in one specimen of *E. candolleana* (Monteiro 23510) and for

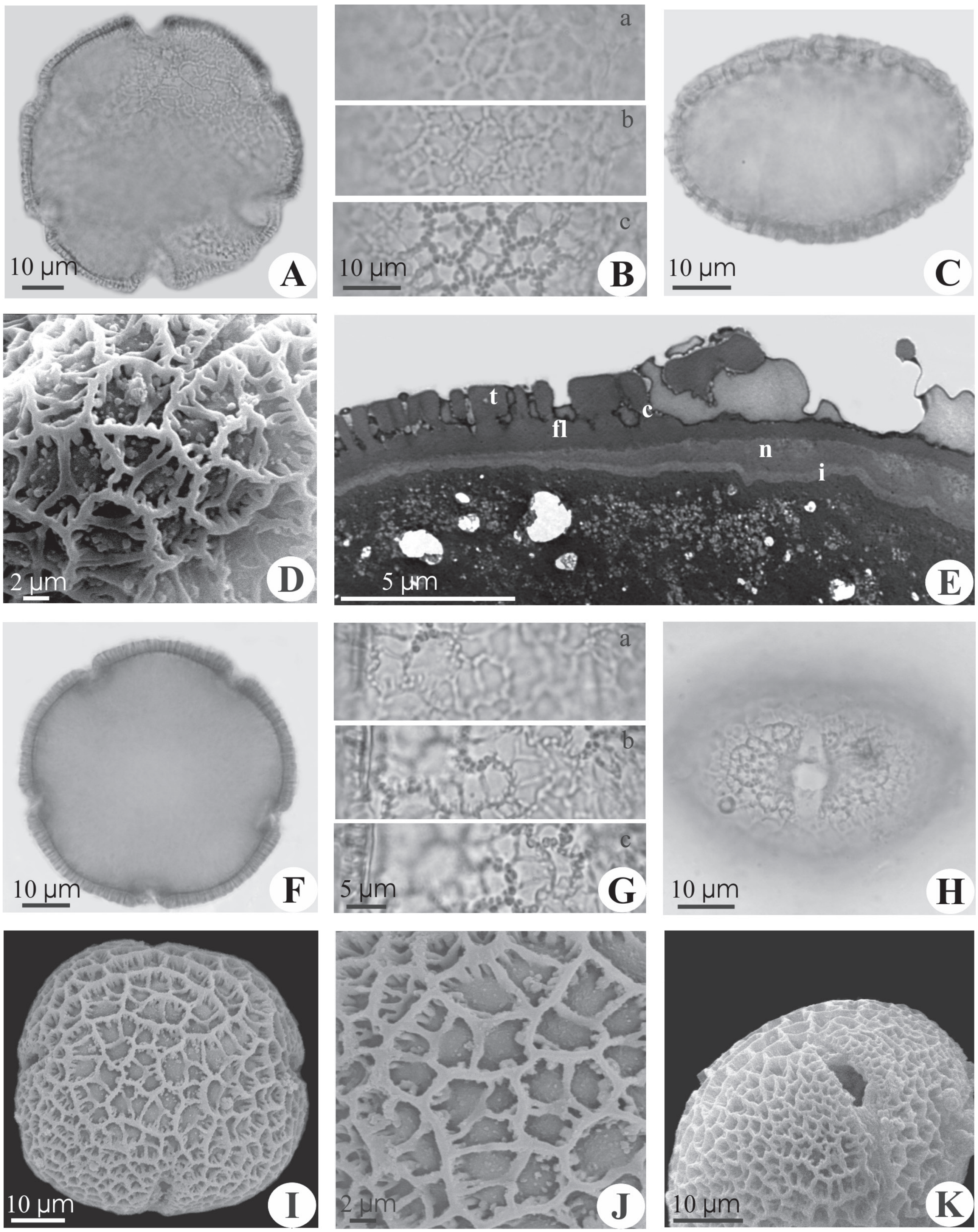

Figure 1. Pollen grains of species of *Ceiba* Mill. **A-E.** *C. erianthos* K. Schum. **A.** Optical section in polar view. **B.** LO-analysis. **C.** Optical section in equatorial view. **D.** Surface detail (SEM). **E.** Detail of murus structure in cross-section (SEM). **F-L.** *C. glaziovii* (Kuntze) K. Schum. **F.** Optical section in polar view. **G.** LO-analysis. **H.** Detail of aperture and surface. **I.** Polar view (SEM). **J.** Surface detail (SEM). **K.** Aperture detail (SEM). (t = tectum, c = columella, b = basal layer, i = intine)

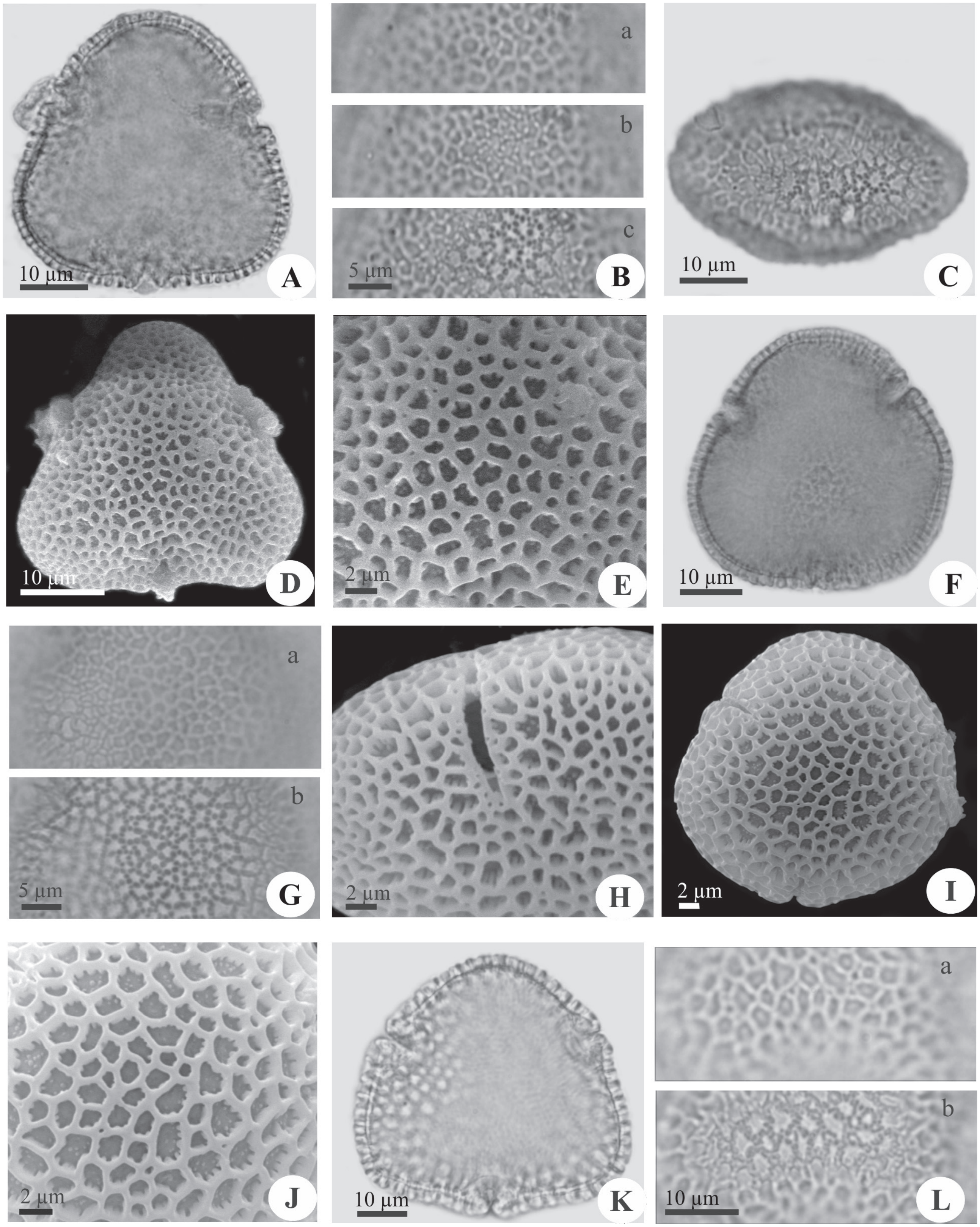

Figure 2. Pollen grains of species of *Eriotheca* Schott & Endl. **A-E.** *E. candolleana* (K. Schum.) A. Robyns- **A.** Optical section in polar view. **B.** LO-analysis. **C.** Aperture and surface in equatorial view. **D.** Polar view (SEM). **E.** Surface detail (SEM). **F-H.** *E. macrophylla* (K. Schum.) A. Robyns- **F.** Optical section in polar view. **G.** LO-analysis. **H.** Detail of aperture (SEM). **I-J.** *E. gracilipes* (K. Schum.) A. Robyns- **I.** Polar view (SEM). **J.** Surface detail (SEM). **K-L.** *E. obcordata* A. Robyns. **K.** Optical section in polar view. **L.** LO-analysis.

specimens of *E. macrophylla*; however, the specimen Lima 12632 had the most significant percentage of 4-aperturate pollen grains with 12.7 %, while other specimens had 2 % or less. All specimens analyzed of *E. gracilipes* had 3-aperturate pollen. The ectoapertures were short and narrow, sometimes hindering visualization of endoapertures under LM, which was characterized as lolongate in *E. candolleana*, *E. gracilipes* and *E. macrophylla*, and lalongate with straight to concave upper and inferior margins in *E. obcordata*. Using SEM, it was possible to detect the presence of heterogeneously-sized granules in the reticulum lumina of the specimens analyzed (Fig. 2J). The smooth surface of muri was also observed with SEM.

Pachira aquatica Aubl. (Fig. 3A-D); *P. nervosa* (Uitt.) Fernández-Alonso (Fig. 3E-F); *P. retusa* (Mart. &Zucc.) Fernández-Alonso (Fig. 3G-J); *P. tocantina* (Ducke) Fernández-Alonso (Fig. 3K-L)

Pollen grains large; oblate to suboblate; isopolar; amb triangular, less frequently quadrangular, with flat to slightly convex sides; equatorial calottes separate, protrusions with rounded angles; planaperturate; 3-colp(or)ate, sometimes 4-colporate; costate and with differentiated margin. Ectoapertures short with sharp ends; endoapertures lolongate, sometimes difficult to visualize. Exine reticulate and heterobrochate in the polar and apertural regions, psilate-perforate, microreticulate in the equatorial calottes. Muri thin to wide, simplicolumellate to duplicolumellate, with simple and digitate columellae, anastomosed, with supratectal protrusions. Sexine thicker than nexine.

Morphologically, the pollen grains of the studied species of *Pachira* are similar. There was a predominance of 3-aperturate pollen grains; 4-colporate (7 %) pollen grains were found only in the examined specimen of *P. nervosa*. The aperture was well defined in *P. aquatica*, with a margin devoid of ornamentation (Fig. 3D); the aperture was difficult to visualize in the other species using LM, with continuous ornamentation up to the apertural region, without a differentiated margin. The endoapertures were lolongate with an irregular outline, and were difficult to visualize in some of the analyzed specimens. It was not possible to measure the endoaperture of all of the analyzed specimens of *P. retusa*, since most pollen grains were kneaded making it difficult to visualize the endoaperture (Fig. 3H). The surface of pollen grains had reticulate ornamentation in polar and apertural regions, with irregular lumina and ornate muri, but differing in the equatorial calottes where it was psilate-perforate (SEM). There were concentrated perforations towards the reticulate regions (Fig. 3C-D) in *P. aquatica*, and microreticulate in the other species (Fig. 3E).

The SEM examination revealed longitudinally-elongated supratectate protrusions, with predominantly rounded apices, starting from the point of intersection of the reticulate muri in *P. aquatica* (Fig. 3C, 3D), characterizing a "cristate reticulum" *sensu* Hesse *et al*. (2009). In the other species, the branched columellae (Fig. 3J, L) fuse distally, forming supratectate structures of varied shapes (Fig. 3F, I). Granules were observed by SEM inside the lumina of *P. nervosa* (Fig. 3F). The sexine was thicker than the nexine in all specimens (Tab. 2), with the largest difference being for a specimen of *P. tocantina*, for which the sexine measured 2.3 μm and the nexine measured 0.6 μm.

Pseudobombax calcicola Carv.-Sobr. & Queiroz LP (Fig. 4A-E); *Ps. marginatum* (A. St.-Hil., A. Juss. & Cambess.) A. Robyns (Fig. 4F-I); *Ps. minimum* Carv.-Sobr. & LP Queiroz (Fig. 4J-N); *Ps. simplicifolium* A. Robyns (Fig. 4O-Q)

Pollen grains large, oblate, isopolar; amb triangular with flat to slightly concave sides; equatorial calottes slightly to very evident, with rounded angles; 3-colp(or)ate; planaperturate; costate. Ectoapertures short and long with rounded ends; endoapertures lolongate when present. Exine reticulate, heterobrochate, with circular to elongated lumina in the polar region and around the apertures with granules in the interior, psilate, granulate and microreticulate in the mesocolpium region and colpus margin. Muri wide, smooth and perforated, single-, dupli- or less frequent pluricolumellate, with distinct columellae using optical (LM), and short and wide columellae using SEM. Sexine thicker than nexine.

The pollen grains of most of the analyzed species were homogeneous in shape, size and amb, with the exception of *Ps. calcicola*, which reached a size of up to 100 μm (Tab. 2).

As for apertures, colporate pollen grains were observed in *Ps. marginatum*, *Ps. simplicifolium* and *Ps. minimum*, but it was not possible to measure the endoapertures in any of the analyzed specimens of the latter two species. The endoaperture was characterized as circular or lolongate in most of the analyzed specimens, but difficult to visualize using light microscopy (Fig. 4H, 4O). A differentiated margin was noted using SEM (Fig. 4I). The pollen grains of specimen Figueiredo *et al*. 175 of *Ps. marginatum* and the analyzed specimen of *Ps. calcicola* were characterized as colpate.

The ornamentation of the exine is reticulate with circular to elongated lumina in the polar region and around the apertures. Using SEM, the exine was observed to be psilate in the equatorial calotte in *Ps. Calcicola* and *Ps. Simplicifolium*, finely granulate and irregularly perforate in *Ps. Marginatum*, and microreticulate in *Ps. Minimum*. The reticulum of *Ps. minimum* had comparatively smaller lumina, uniform up to the apertural region, without a differentiated margin (Fig. 4L).

With SEM it was possible to observe few and spaced perforations in the reticulum muri (Fig. 4L, M, Q). Figures 4E and 4N show how the tectum is supported by large and short columellae that form single rows, double rows or more rarely several rows (Fig. 4G) under the muri.

The sexine was much thicker than nexine in all the studied species. Using SEM, short columellae and the

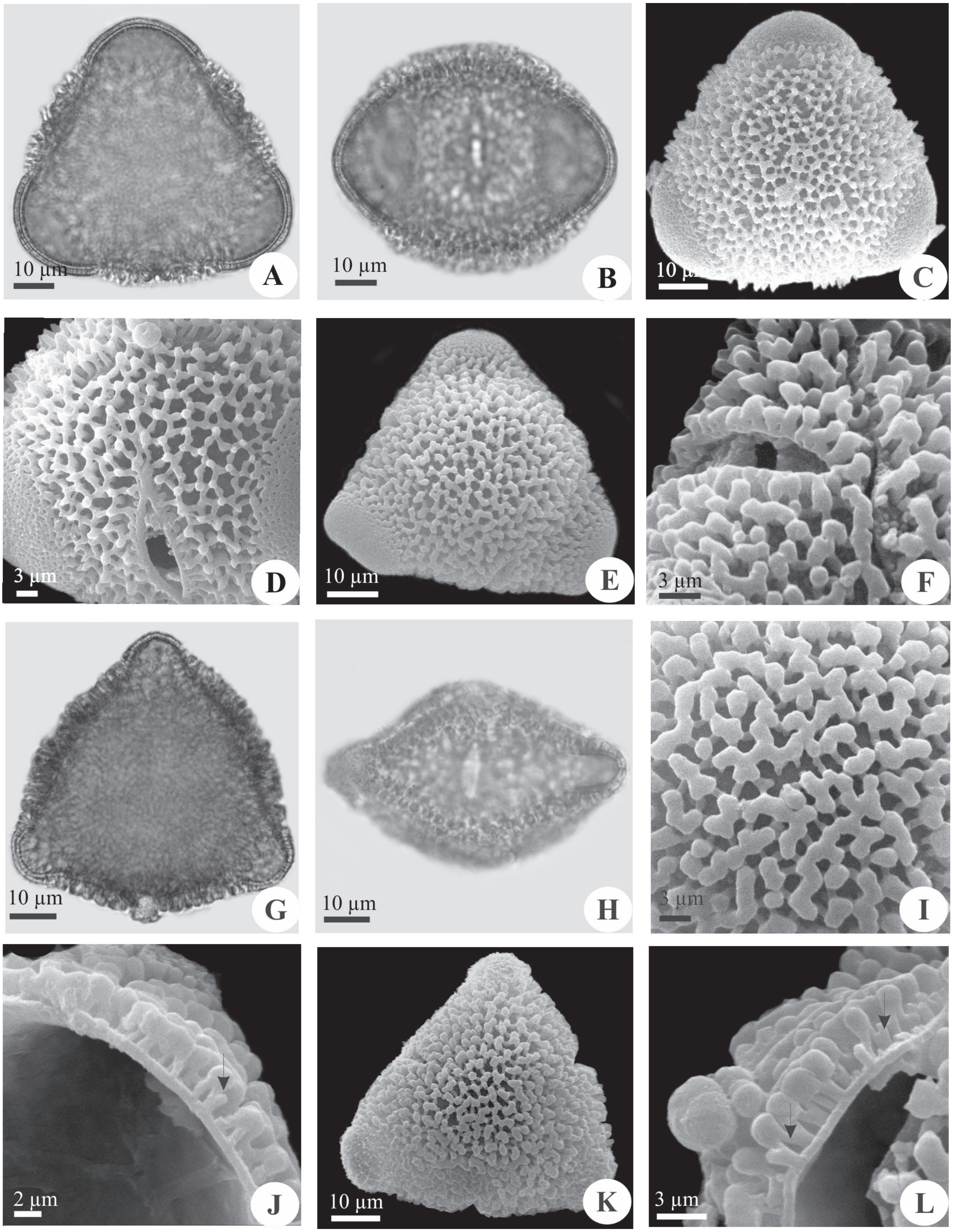

Figure 3. Pollen grains of *Pachira* Aubl. **A-D.** *P. aquatica* Aubl.- **A.** Optical section in polar view. **B.** Optical section in equatorial view. **C.** Polar view (SEM). **D.** Detail of surface and aperture (SEM). **E-F.** *P. nervosa* (Uitt.) Fernández-Alonso- **E.** Polar view (SEM). **F.** Detail of aperture (SEM). **G-J.** *P. retusa* (Mart & Zucc.) Fernández-Alonso- **G.** Optical section in polar view. **H.** Optical section in equatorial view. **I.** Surface detail (SEM). **J.** Structure of exine with forked columella (arrow) (SEM). **K-L.** *P. tocantina* (Ducke) Fernández-Alonso- **K.** Polar view (SEM). **L.** Structure of exine with forked columellae (arrows) (SEM).

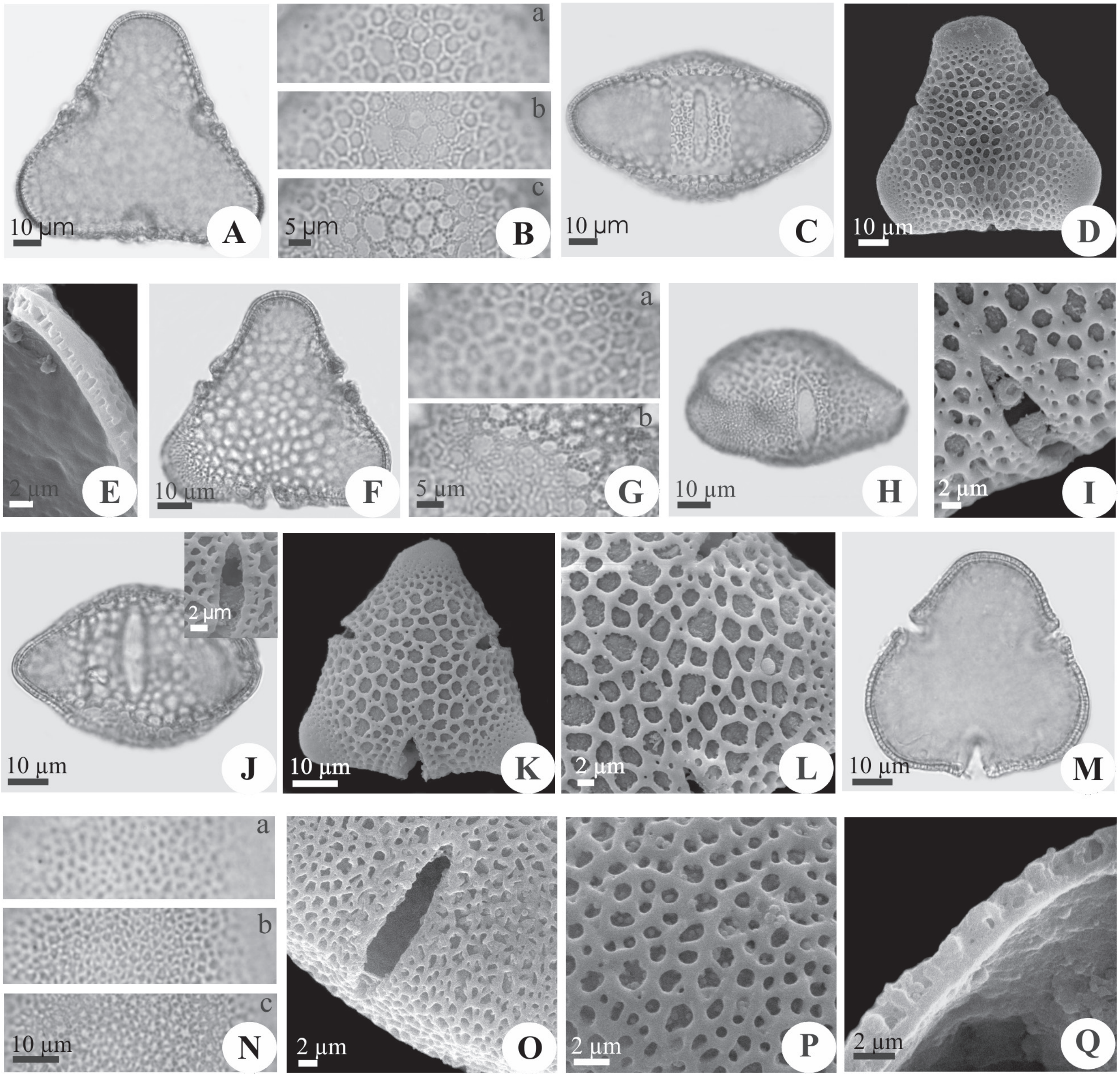

Figure 4. Pollen grains of species of *Pseudobombax* Dugand. **A-E.** *Ps. calcicola* Carv.-Sobr. & L.P. Queiroz - **A.** Optical section in polar view. **B.** LO-analysis **C.** Optical section in equatorial view. **D.** Polar view (SEM). **E.** Structure of exine (SEM). **F-I.** *Ps. marginatum* (A. St.-Hil., A. Juss. & Cambess.) A. Robyns- **F.** Optical section in polar view. **G.** Analysis of L.O. **H.** Aperture and surface in equatorial view. **I.** Detail of aperture (SEM). **J-N.** *Ps. minimum* Carv.-Sobr. & L.P. Queiroz - **J.** Optical section in polar view. **K.** LO-analysis **L.** Detail of aperture (SEM). **M.** Surface detail (SEM). **N.** Structure of exine (SEM). **O-Q.** *Ps. simplicifolium* A. Robyns- **O.** Optical section in equatorial view, detail of the aperture in the upper right corner (SEM). **P.** Polar view (SEM). **Q.** Surface detail (SEM).

inner surface of the rough nexine could be observed for *Ps. minimum* (Fig. 4N) and an undulate nexine for *Ps. calcicolate* (Fig. 4E).

Rhodognaphalopsis faroensis (Ducke) A. Robyns (Fig. 5A-G)

Pollen grains large; oblate; isopolar; amb triangular with concave sides; 3-colporate; costate. Ectoapertures short with rounded ends; endoapertures lalongate with concave upper and lower margins. Exine microreticulate-echinate using LM, tectate-perforate with supratectate protrusions with cylindrical and conical shapes and heterogeneous sizes and diameters using SEM. Sexine thicker than nexine.

The ectoapertures were observed to be short, narrow, with a regular outline and rounded ends, and without a differentiated margin using LM and SEM (Fig. 5C, G). The endoaperture was lalongate with concave upper and lower margins while the extremeties were difficult to view. Figure 5D shows the costae of the pollen grains of the studied species.

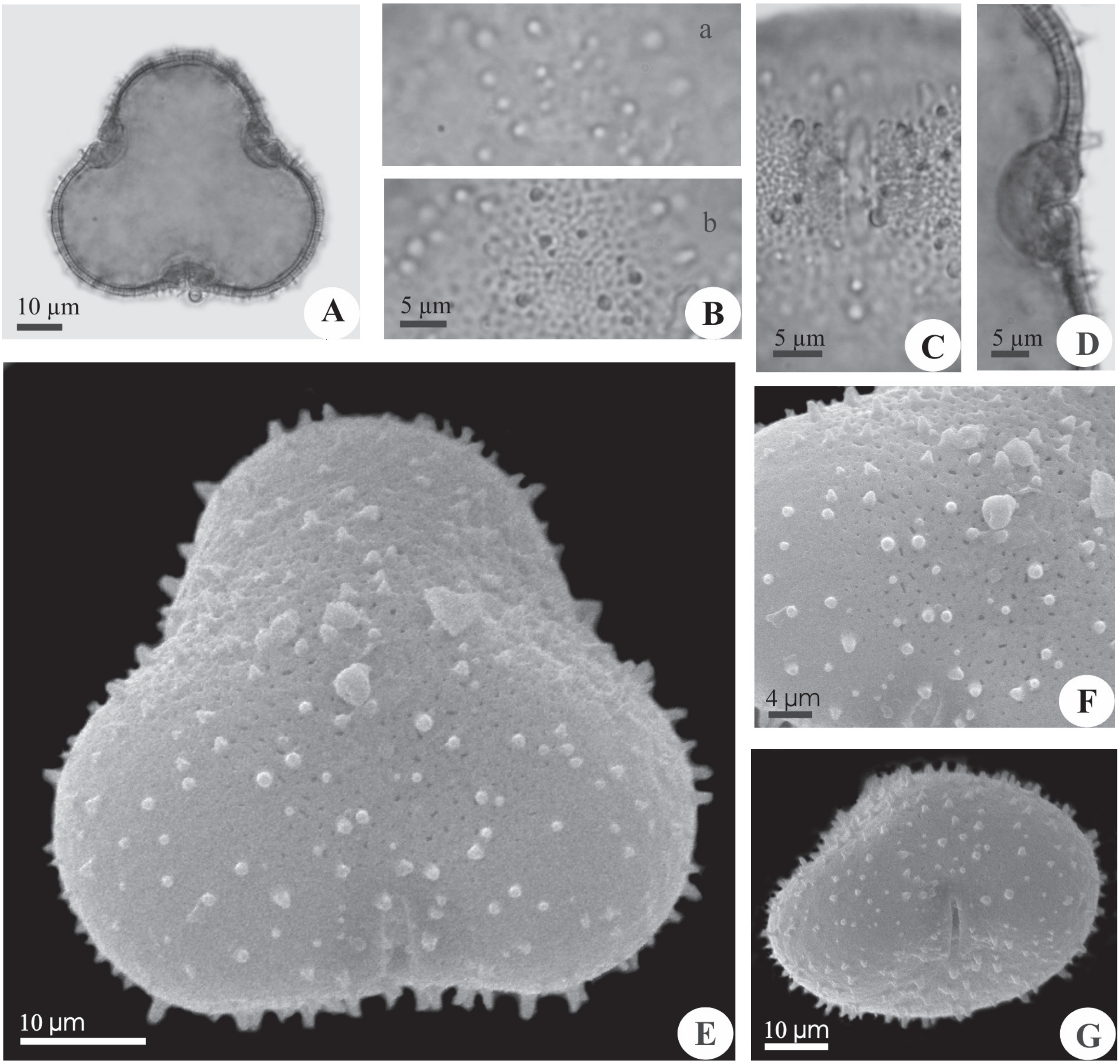

Figure 5. A-G. Pollen grains of *Rhodognaphalopsis faroensis* (Ducke) A. Robyns- **A.** Optical section in polar view. **B.** LO-analysis. **C.** Detail of aperture. **D.** Optical section of aperture. **E.** Polar view (SEM). **F.** Surface detail (SEM). **G.** Equatorial View (SEM).

LO-analysis (Fig. 5B) revealed a tectum with a pattern similar to that of a microreticulum. Higher amplifications using SEM, however, revealed the presence of perforations of varied diameter and shape (spherical and elongated) concentrated in the polar region. The supratectate protrusions also varied in shape and diameter, with cylindrical protrusions with rounded ends and conical protrusions with a sharp end (Fig. 5E-G).

Principal component analysis

The first two axes of the PCA explained 85.43 % of the total variance (Fig. 6). The first axis (PC1) explained 75.66 % of the total variance with ectoaperture length (EcL) and width (EcW) being the most significant variables, followed equatorial diameter in polar view (E) (Tab. 3). The species of *Pseudobombax* (*Ps. calc*, *Ps. simp*, *Ps. marg*, *Ps. mini*) and *Paquira aquatica* (*Pa. aqua*) were grouped having the highest values for these variables of the first axis. In contrast, species of *Eriotheca* (*Er. macr*, *Er. obco*, *Er. cand*, *Er. grac.*) were grouped on the opposite side with lower values of E, EcL and EcW. The second axis (PC2) explained 9.77 % of the total variance, with sexine (Sex) and exine (Ex) being the most significant variables (Tab. 3). The variables polar area index (AI) and nexine (Nex) were correlated with the second axis. The taxa in the bottom left quadrant have lower values for AI (Fig. 6).

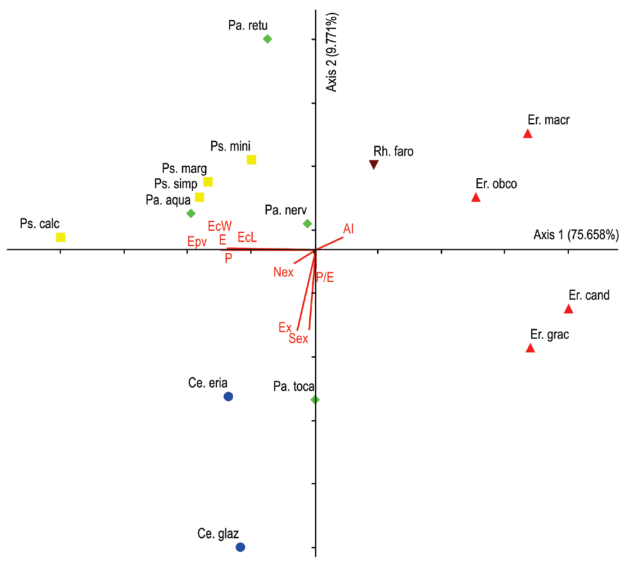

Figure 6. Principal component analysis performed with the pollen metrical variables of Brazilian species of Bombacoideae. Ce. eria = *Ceiba erianthos*, Ce glaz = *C. glaziovii*, Er. cand = *Eriotheca candolleana*, Er. grac = *E. gracilipes*, Er. macr = *E. macrophylla*, Er. obco = *E. obcordata*, Pa. aqua = *Pachira aquatica*, Pa. nerv = *P. nervosa*, Pa. retu = *P. retusa*, Pa. toca = *P. tocantina*, Ps. calc = *Pseudobombax calcicola*, Ps. marg = *Ps. marginatum*, Ps. mini = *Ps. minimum*, Ps. simp = *Ps. simplicifolium*, Rh. faro = *Rhodognaphalopsis faroensis*.

Table 3. Pearson and Kendall correlation coefficients for pollen metrical variables of the first and the second axis of PCA ordination of species of Bombacoideae.

	Principal components	
Variables	Axis 1	Axis 2
P	-0.4097	-0.0667
E	-0.4249	0.1661
Epv	-0.4106	0.0164
P/E	0.0032	-0.0982
AI	0.0400	0.0757
EcL	-0.4951	0.0199
EcW	-0.4555	0.1916
Ex	-0.1122	-0.6455
Sex	-0.0712	-0.6666
Nex	-0.1054	-0.2328

Discussion

Data reported in the literature for other species of *Ceiba* have revealed that, in addition to having a reticulum and gems and piles, sculptural elements such as the exine may also be present (Erdtman 1952; Palacios-Chavez *et al*. 1991). Using SEM, Nilson & Robyns (1986) described for this genus the presence of clava or bacula-like processes in the reticulum lumina, in addition to ramified muri supported by oblique columellae, as was corroborated by the present study. Abreu *et al*. (2014) also recorded elements of ornamentation in the lumina of *C. erianthos*.

The results obtained in this research regarding aperture type are in agreement with data found in the literature for this genus, except for the number of apertures. The 3-aperture condition cited by Erdtman (1952), Tsukada (1964), Sowunmi 1973), Nilson & Robyns (1986), Palacios-Chavez *et al*. (1991), Moncada & Sotolongo (1994), Carreira *et al*. (1995), and Abreu *et al*. (2014) for other species of the genus, was not observed in any of the specimens analyzed here.

There are no data in the literature on the ultrastructure of pollen grains of other species of Bombacoideae. However, some of the data presented here for *Ceiba erianthos* (*e.g.*, short columellae and thicker intine at the apertural region) are in agreement with those reported for other species of Malvaceae *s.l.* (Saba & Santos 2015; Silveira-Júnior *et al*. 2015; 2017).

Robyns (1963) and Salgado-Labouriau (1973) described the pollen grains of the species of *Eriotheca* studied here and reported similar pollen characteristics. However, these authors referred to duplicolumellate muri, rarely pluricolumellate, for *E. candolleana* (Robyns 1963) and *E. gracilipes* (Robyns 1963; Salgado-Labouriau 1973). Furthermore, the colpate pollen grains described by Robyns (1963) for *E. macrophylla* (*crenulaticalyx*) are not in accordance with the data presented here. Visualization of the endoaperture is very difficult by LM, but the SEM analysis of the present work was able to confirm its presence.

For Nilson & Robyns (1986), species of *Eriotheca*, including the species studied here, along with species of the genera *Aguiaria* , *Pachira* (*Bombacopsis*), *Pseudobombax* and *Spirotheca*, comprise the *Bombax* pollen type. The data presented here are in agreement with those presented by these authors with the exception of the pattern of rugulate ornamentation of the exine, which was not observed in any of the species analyzed here. Abreu *et al*. (2014) recorded perforations in the reticulum muri of *E. pentaphylla*, which were not observed in the present study. The other characteristics reported by Abreu *et al*. (2014) are consistent with the data presented here.

Robyns (1963) and Salgado-Labouriau (1973) described the pollen grains of *Pachira retusa* and *P. tocantina* as colpate and colporate, respectively. These data differ from those presented here, which may be explained by the lolongate type of endoapertures, which was difficult to visualize in the analyzed species, and by the kneaded condition of the majority of pollen grains after acetolysis. The authors did not mention the presence of costae, as evidenced here for *P. nervosa*.

Robyns (1963) and Moncada & Sotolongo (1994) using LM, and Carreira *et al*. (1995) using SEM, reported the presence of spines in equatorial calottes. Such elements were not observed in any of the specimens analyzed here. The equatorial calottes of the analyzed specimens of *P. tocantina* were less prominent than those of the other studied species.

Specialized literature includes references of pollen grains with spinous processes for the species studied here (Robyns 1963; Tsukada 1964; Nilson & Robyns 1986; Moncada & Sotolongo 1994) as well as for other species of *Pachira* (Salgado-Labouriau 1973; Nilson & Robyns 1986; Bove 1993). Supratectate protrusions were observed for the species studied here, but they were not considered spinous processes. Carreira *et al*. (1995) did not refer to such structures.

The species of *Pachira* studied by Nilson & Robyns (1986) were included in the *Bombax* pollen type, which is characterized as 3(-4)-colp(or)ate, rarely porate, reticulate, microreticulate to rarely tectate-perforated, equinate, and rarely rugulate. Most of the pollen traits presented by these authors were corroborated by the present study, with the exception of the presence of equinate and rugulate pollen grains, which was not observed in the specimens analyzed here.

The occurrence of colpate and colporate pollen grains in the same species of *Pseudobombax* was reported in the literature by Robyns (1963) and Salgado-Labouriau (1973) using LM. Considering the difficulty of visualizing the endoaperture using LM, identifications of apertural type in some of the descriptions presented for species of this genus may be mistaken.

According to Nilson & Robyns (1986), in addition to the presence of a reticulum and microreticulum, rugulae can also be present as sculptural elements of pollen grains of species of *Pseudobombax*. Pollen data in the literature for *Ps. simplicifolium* and *Ps. marginatum* (Robyns 1963; Salgado-Labouriau 1973; Nilson & Robyns 1986; Abreu *et al*. 2014), and for other species of the genus (Tsukada 1964; Melhem & Bissa 1985; Bove 1993; Moncada & Sotolongo 1994), were corroborated by the present study.

Data reported in the literature (Robyns 1963; Nilson & Robyns 1986) on the pollen morphology of *Rhodognaphalopsis* are consistent those of presented here for most morphopalynous characters. However, the 4-aperturate and colpate type of aperture described by Nilson & Robyns (1986), and the type of endoaperture observed by Robyns (1963) and Nilson & Robyns (1986), were not observed in any of the specimens analyzed here.

The data obtained in the present study confirmed the heterogeneous nature of the pollen of representatives of Bombacoideae. Ornamentation type was found to be an important character for recognizing the studied species of this subfamily. *Rhodognaphalopsis* differs by having perforate to microreticulate pollen grains with cylindrical and conical supratectate protrusions, while other genera have a foveolate sexine. *Pseudobombax* and *Pachira* both have pollen grains with differentiated equatorial calottes, as well as a triangular amb and a planaperture-type of aperture, however, the reticulum of the analyzed species of these genera differs. The ordering of species by PCA confirmed the separation of the studied species of Bombacoideae using only quantitative variables of the pollen grains. Some attributes show potential usefulness for characterizing species, such as equatorial diameter and sexine thickness. In conclusion, morphological and morphometric pollen data provided useful information for distinguishing the studied species, and can contribute substantially to understanding the diversity and relationships among genera and species of the subfamily Bombacoideae.

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