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Pollen morphology of Convolvulaceae from Serra dos Carajás, southeastern Amazonia

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Abstract: Canga vegetation has high ecological importance in the Serra dos Carajás, southeastern Amazonia, but it coexists with the potential threat of large-scale iron ore mining. Considering Convolvulaceae, this canga environment has only 17 species, and 15 of these species had their pollen grains described in detail in this present work, aiming to contribute to the taxonomic knowledge of critically endangered plants, including *Ipomoea cavalcantei*. Pollen grains were examined by light and scanning electron microscopy, and the morphological parameters obtained were statistically analyzed using principal component analysis. Therefore, *Aniseia cernua* was described for the first time in the literature. In general, the echinae morphology were the main differentiating character among the *Ipomoea* species and was the basis for the proposed pollen key. The family Convolvulaceae in Carajás is eurypalynous, and the genus *Ipomoea* may be classified as stenopalynous. The set of morphological characters statically treated and correlated was effective for the separation of *Ipomoea*.

Keywords: Palynology; taxonomy; pollen morphology; Convolvulaceae; Carajás

Introduction

Convolvulaceae Juss. comprises approximately 1,900 species distributed in 60 genera in the tropical and subtropical regions of the planet (Cheek & Simão-Bianchini 2013; Buril et al. 2015; Yadav et al. 2018). Its representatives are mainly herbs, vines and shrubs (Smith et al. 2004; Saensouk 2007; Ashfaq et al. 2017). In Brazil, 22 genera and approximately 400 species are recognized, occurring in various vegetation formations (Souza & Lorenzi 2005; BFG 2015) and widely cultivated as ornamentals (Rajurkar et al. 2011). *Ipomoea* and *Jacquemontia* are the genera with the most endemic species in Brazil (Bianchinni & Ferreira 2013; Buril et al. 2015).

Serra dos Carajás, located in southeastern Amazonia, harbors approximately 30 Convolvulaceae species distributed in 9 genera, with 17 of these species and 8 genera occurring exclusively in canga vegetation areas (Simão-Bianchini et al. 2016). Serra dos Carajás is a target of mineral exploitation because it is the largest iron reserve in the world, which poses many challenges for the conservation of the vegetation that grows in these areas (Duddu 2014; Gibson et al. 2017; Zappi et al. 2017). Convolvulaceae is highly represented in Carajás, and the species are also prominent due to the endemism observed in *Ipomoea cavalcantei* D.F. Austin, known as *flor de Carajás* [flower of Carajás] (Austin & Cavalcante 1982; Mota et al. 2015). *I. cavalcantei* is considered to be at risk of extinction according to the criteria of the International Union for Conservation of Nature (IUCN), and for this reason, it has been the subject of many studies (Martinelli & Moraes 2013; Babiychuk et al. 2019). Another species of great concern is *I. maurandioides* Meisn (*Ipomoea carajasensis* D.F. Austin), which is considered vulnerable (Martinelli & Moraes 2013). Therefore, the compilation of databases on these environments is a priority, with a view to environmental impact assessments and to guide conservation strategies and mitigation measures of the impacts caused by mining in the Carajás region (Nunes et al. 2015).

The pollen morphology of the Convolvulaceae has been analyzed by several researchers as an important taxonomic tool for the genera (Hallier 1893; Erdtman 1952; Sengupta 1972; Ferguson et al. 1977; Pedraza 1983 and Tellería and Daners 2003). The family is classified as eurypalynous because it has a wide diversity of characters (Erdtman 1969). Medium to very large pollen grains are observed, with exine ornamentation from psilate to echinate, with different numbers of colpate to porate apertures (Tellería & Daners 2003; Leite et al. 2005). Regarding the *Ipomoea* L., Tellería & Daners (2003) described the pollen grains as pantoporate, reticulate and with echinae supported by edges formed by columellae. In *Cuscuta* L., the grains in most species are tricolpate, although the number of apertures is not constant in this genus (Liao et al. 2005). In *Jacquemontia* Choisy and *Evolvulus* L., the apertures may be tricolpate and pantocolpate, respectively (Vital et al. 2008). In *Merremia*, the 3-colpate apertural type with a long colpus is predominant (Vasconcelos et al. 2015).

However, to continue studies with these different types of plants, complete data, including pollen morphology, are necessary (Yadav et al. 2018). The importance of pollen morphology in the taxonomy of Convolvulaceae has been investigated in many studies (Rajurkar et al. 2011). Researching pollen morphological characters is important to avoid increasing taxonomic disagreements and nomenclatural instability, as these characters are taxonomically important in the identification and demarcation of species (Ullah et al. 2018). Indeed, Wood & Scotland (2017) observed several names of *Ipomoea* L. species with distribution in South America that are poorly identified in herbaria and in the literature.

Palynological studies in South America are scarce and restricted to descriptions of a few species belonging to the regional flora (Laguardia 1961; Heusser 1971). Although Convolvulaceae is considered to be eurypalynous, Buril et al. (2013) highlighted that palynological studies on the family are generally defined by a single character, causing uncertainty in the taxonomic classification. In addition, Telleria & Daners (2003) emphasize that the diversity of pollen morphology is not reflected in the studies, especially *Ipomoea* species. Therefore, this study aims to present a detailed description of the pollen morphology based on optical and scanning electron microscopy of the representatives of Convolvulaceae occurring in the canga vegetation areas of the Serra dos Carajás to contribute to the discussion of the taxonomy of the family.

Materials and methods

Study area

Serra dos Carajás comprises the largest mineral province in Brazil and one of the largest in the world, located in the southeastern Amazonia. There are two vegetation types in Serra dos Carajás, dense and open ombrophylous forest (IBGE 2012), which occur on the slopes of the plateau, interrupted by savanna-like vegetation (canga), which colonizes the lateritic crusts under edaphic conditions (Guimarães et al. 2014; Nunes et al. 2015). The cangas are areas with high species richness and unique floristic composition, including several endemic species that make the Carajás region an important area for the conservation of Amazonian flora (Mota et al. 2018).

The climate of the study area, according to the Köppen climate classification, is tropical (Group A) subtype Am, defined based on the lowest temperature (Chen and Chen 2013). The average annual temperature is 26 °C, and the total rainfall in the region is above 2,000 millimeters per year, with approximately 140 rainy days annually (Alvares et al. 2013; Viana 2016).

Samples collection and slide preparation for morphological descriptions

Flower buds from three specimens belonging to Aniseia cernua Moric., Cuscuta insquamata Yunck., Evolvulus filipes Mart., Ipomoea asplundii O'Donell, I. cavalcantei D. F. Austin, I. decora Meisn., I. goyazensis Gardner, I. hederifolia L., I. marabaensis D. F Austin & Secco, I. maurandioides

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Meisn, I. procumbens Mart. Ex Choisy, I. setifera Poir., Jacquemontia tamnifolia (L.) Griseb., Merremia macrocalyx (Ruiz & Pav.) O'Donell and Operculina hamiltonii (G.Don) D. F. Austin & Staple (Appendix B; Figure 1) were collected from exsiccata duly identified by experts of the family Convolvulaceae deposited in the herbaria MG (Museu Paraense Emílio Goeldi) and HCJS (BioParque Vale Amazônia). All slides were deposited in the Palinoteca of the Instituto Tecnológico Vale (PALIITV).

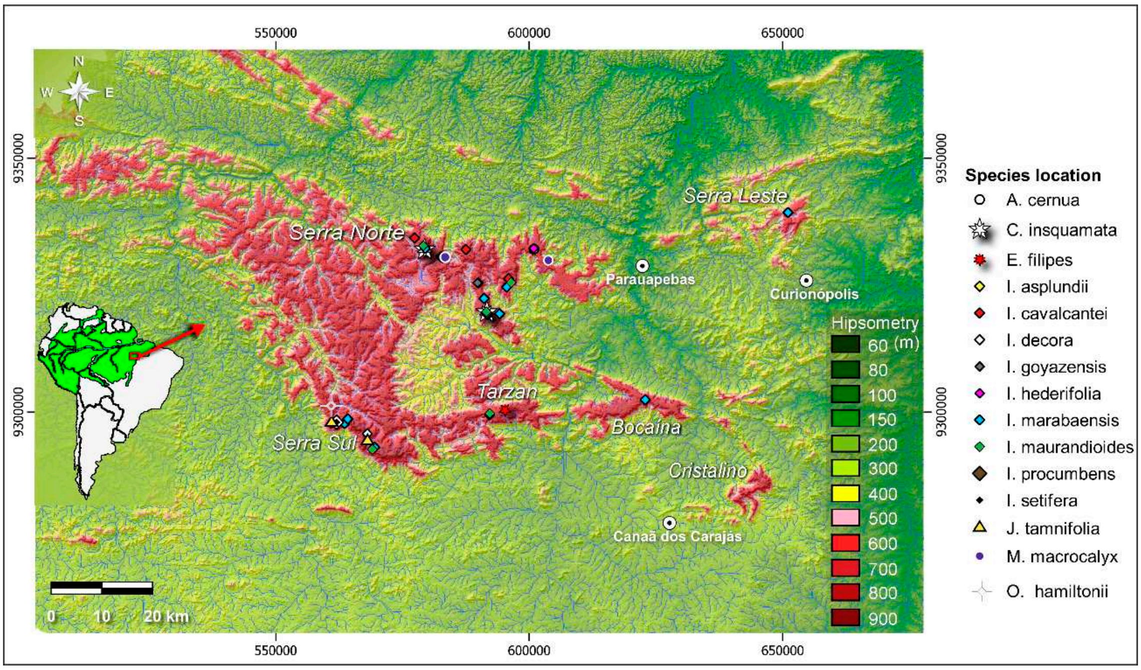

Figure 1. A Digital Elevation Model (SRTM, 30 m resolution) of the study site with the location of the Convolvulaceae species described in this work. The list of each species with geographic coordinates can be observed in Appendix A.

The flower buds were treated according to the acetolysis method proposed by Erdtman (1952), and morphologically analyzed by Light (LM; Zeiss Axio Imager.M2) and Scanning Electron (SEM; Zeiss SIGMAVP) Microscopy. The following were measured: grain diameter (GDL and GDW), excluding echinae in *Ipomoea*; for the pores, the largest and smallest diameters (Pores_length and Pores_width) and distance between them (C_pores) were calculated. For the echinae, the base (Width_base_echinae) and height (Height_echinae), as well as the distance between them (DE), were measured. Measurement of exine stratification, composed of nexine (Nexine_thickness) and sexine (Sexine), was obtained at the interechinae region. The estimation of the number of echinae and pores per pollen grain (X_echinae and X_porus) was based on Hanks & Fryxell (1979). These variables were examined in 20 grains per sample (Ceter et al. 2013).

For the description of pollen morphology, the terminology used followed Punt et al. (2007). In the morphometric description, the data were organized in the following sequence: minimum value (standard deviation) and maximum value. For echinae, the classification was adapted from Vasconcelos et al. (2015): conical; bulbous with apiculate apex (type 1); bulbous with rounded apex (type 2); and bulbous with bulbous apex (type 3).

Statistical analysis

Principal component analysis (PCA) was performed using 17 variables to assess whether the morphometric pollen characteristics allow the separation or clustering of species. The variables used for the analysis were as follows: largest grain diameter (GDL); smallest grain diameter (GDW); GDL/GDW ratio; largest porus diameter (Pores_length); smallest pores diameter (Pores_width); Pores_length/Pores_width ratio (Pl.Pw); C_pores/GDL ratio (C.GDL); C_pores/GDW ratio (C.GDW); distance between pores (C_pores); echinae base (Width_base_echinae); echinae height

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(Height_echinae); distance between echinae (DE); number of pores (X_pores); number of echinae (X_echinae); sexine (Sexine); nexine (Nexine_thickness) and exine.

The first three principal components with eigenvalues greater than 1 were considered. The results were presented in a biplot along the PC1 and PC2 axes. All analyses were performed using the statistical software R version 4.0.1 (R Core Team 2021), and graphs were generated using 'factoextra' (Sebastien et al. 2008) and 'corrplot' (Wei & Simko, 2021).

Results

Pollen morphology

A pollen key was prepared for the studied species (Appendix A), with new data for *Aniseia cernua*. In addition, the pollen morphologies of 10 species in this study are described for the first time from Serra dos Carajás (Table 1).

Table 1. Pollen morphology of Convolvulaceae species. M = medium, L = large; VL = very large. *The size and shape classes follow Erdtman (1952).

Species	Size	Shape	Aperture		Exine ornamentation	Spine type
			Type	No.		
Aniseia cernua Moric.	L	circular	colpus	-	microreticulate and microverrucate	-
Cuscuta insquamata Yunck.	M	subprolate	colpus	3	microreticulate and microechinate	-
Evolvulus filipes Mart.	M	circular	colpus	12	microreticulate and microechinate	-
Ipomoea asplundii O'Donell	L	circular	porus	~163	echinate and microreticulate with granules	Conical
I. cavalcantei DF Austin	L	circular	porus	~93	echinate and microreticulate with granules	bulbous type 3
I. decora Meisn.	L	circular	porus	~160	echinate and microreticulate with granules	bulbous type 2
I. goyazensis Gardner	VL	circular	porus	~199	echinate and microreticulate with granules	bulbous type 1
I. hederifolia L.	VL	circular	porus	~217	echinate and microreticulate with granules	bulbous type 2
I. marabaensis D.F. Austin & Secco	L/VL	circular	porus	~182	echinate and microreticulate with granules	bulbous type 2
I. maurandioides Meisn	L	circular	porus	~183	echinate and microreticulate with granules	conical
I. procumbens Mart. ex Choisy	L/VL	circular	porus	~144	echinate and microreticulate with granules	conical
I. setifera Poir.	L/VL	circular	porus	~163	echinate and microreticulate with granules	conical
Jacquemontia tamnifolia (L.) Griseb.	F/M	circular	colpus/porus	5(4)	microreticulate and microechinate	-
Merremia macrocalyx (Ruiz & Pav.) O'Donell	L	subprolate	colpus	3	microreticulate and microgranulate	-
Operculina hamiltonii (G.Don) D.F. Austin & Staple	L	prolate spheroidal	colpus	3	microreticulate and microgranulate	-

Aniseia Choisy

Aniseia cernua Moric.
Figure 2A–D

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Pollen grains monad, large, apolar, radially symmetrical, circular; pantocolpate; tectate, sexine is much thicker than nexine in mesocolpus and decreases in thickness as it approaches the apertural region (Table 2). Columellate, ornamentation of the exine is microreticulate, heterobrochate. On SEM, exine ornamentation microreticulate-microverrucate, microverrucae irregular in size and distribution.

Cuscuta L.

Cuscuta insquamata Yunck.

Figure 2E–H

Pollen grains monad, medium, isopolar, radially symmetrical, subtriangulate, subprolate (Polar/Equatorial; P/E= 1.30); 3-colpate. Tectate, columellate, with microreticulate surface. On SEM, exine ornamentation microreticulate and microechinate, with granules inside the colpi.

Evolvulus L.

Evolvulus filipes Mart.

Figure 2I–M

Pollen grains monad, medium, apolar, with radial symmetry, circular; pantocolpate with 12 apertures. Columellate, exine ornamentation microreticulate. On SEM, exine ornamentation microechinate.

Ipomoea L.

Figure 2N–U; Figure 3A–U; Figure 4A–H

Pollen grains monad, ranging from large to very large, with radial symmetry, apolar, circular and pantoporate. The number of pores varies greatly among species, and they are circular to elliptical. Columellate, sometimes supporting the tectal elements, nexine sometimes thinner than sexine, as in *Ipomoea goyazensis*. In all species, the macro-ornamentation is echinate, varying according to the type of echinae (Table 1). The micro-ornamentation is microreticulate in the interechinae area, with the presence of granules (Figure 2Q, Figure 3D, Figure 3U). In *I. hederifolia*, echinae are evenly distributed around the pores, forming rosettes (Figure 3I–M).

Jacquemontia Choisy

Jacquemontia tamnifolia (L.) Griseb.

Figure 4I–M

Pollen grains monad, medium to large, isopolar, radially symmetrical, circular; 5-(4) colporate. Tectate, sexine is thicker than nexine in mesocolpus (Table 2), and decreases in thickness as it approaches the apertural region. Columellate, exine ornamentation microreticulate and microechinate; these elements are more easily visualized under SEM.

Merremia Dennst. ex Endl.

Merremia macrocalyx (Ruiz & Pav.) O'Donell

Figure 4N–Q

Pollen grains monad, large, isopolar, radially symmetrical, subtriangulate, subprolate (P/E= 1.32); 3-colpate. Sexine twice the thickness of nexine in the mesocolpus (Table 2), and decreases in thickness near the apertural region. Exine ornamentation microgranulate, microreticulate on SEM. Granules vary in size and shape and colpi are also granulated.

Operculina Silva Manso

Operculina hamiltonii (G.Don) D.F. Austin & Staples

Figure 4R–U

Pollen grains monad, large, isopolar, radially symmetrical, subtriangulate, prolate spheroidal (P/E= 1.13); 3-colpate. Sexine twice the thickness of nexine in the mesocolpus (Table 2), and decreases in thickness near the colpus. Exine ornamentation granulate, baculate and easily visualized by SEM.

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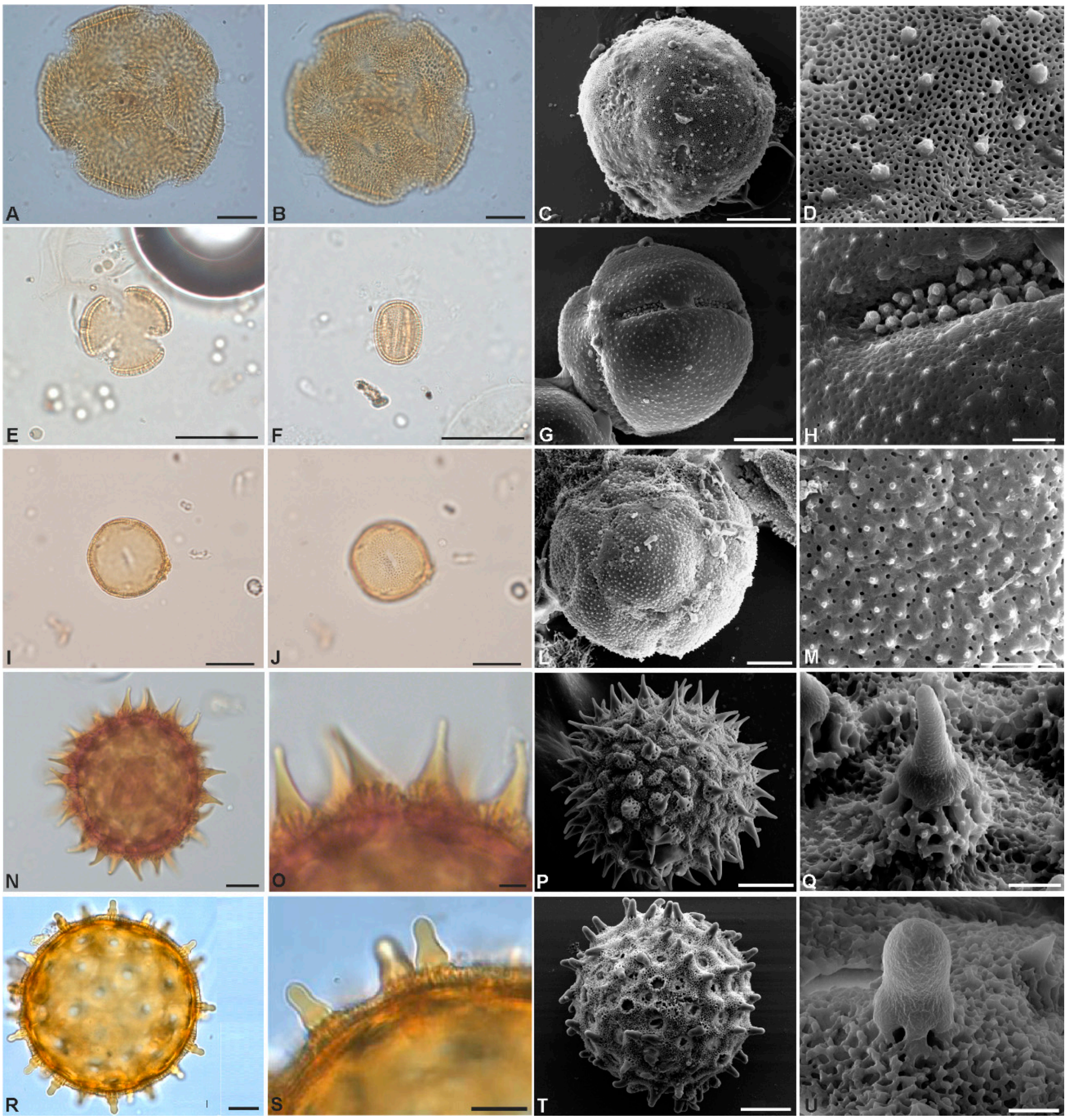

Figure 2. A–U. Pollen grains of Convolvulaceae from Serra dos Carajás, Pará, Brazil. *Aniseia cernua* (A–D): A—optical section; B—ornamentation; C—surface (SEM); D—detail of the exine ornamentation; *Cuscuta insquamata* (E–H): E—optical section of the polar view; F—optical section of the equatorial view; G—surface (SEM); H—ornamentation detail of the exine and colpus; *Evolvulus filipes* (I–M): I—optical section; J—pantocolpate aperture; L—surface (SEM); M—detail of the exine ornamentation; *Ipomoea asplundii* (N–Q): N—optical section; O—conical echinae; P—surface SEM; Q—echinus and detail of the columellae; *I. cavalcantei* (R–U): R—optical section; S—bulbous echinae type 3; T—Surface (SEM); U—detail of the echinae (SEM). (A,B,C,E,F,G,I,J,L,N,P,R,T)—20-µm scale; (O,S)—5-µm scale; (D,H,M,Q,U)—3-µm scale).

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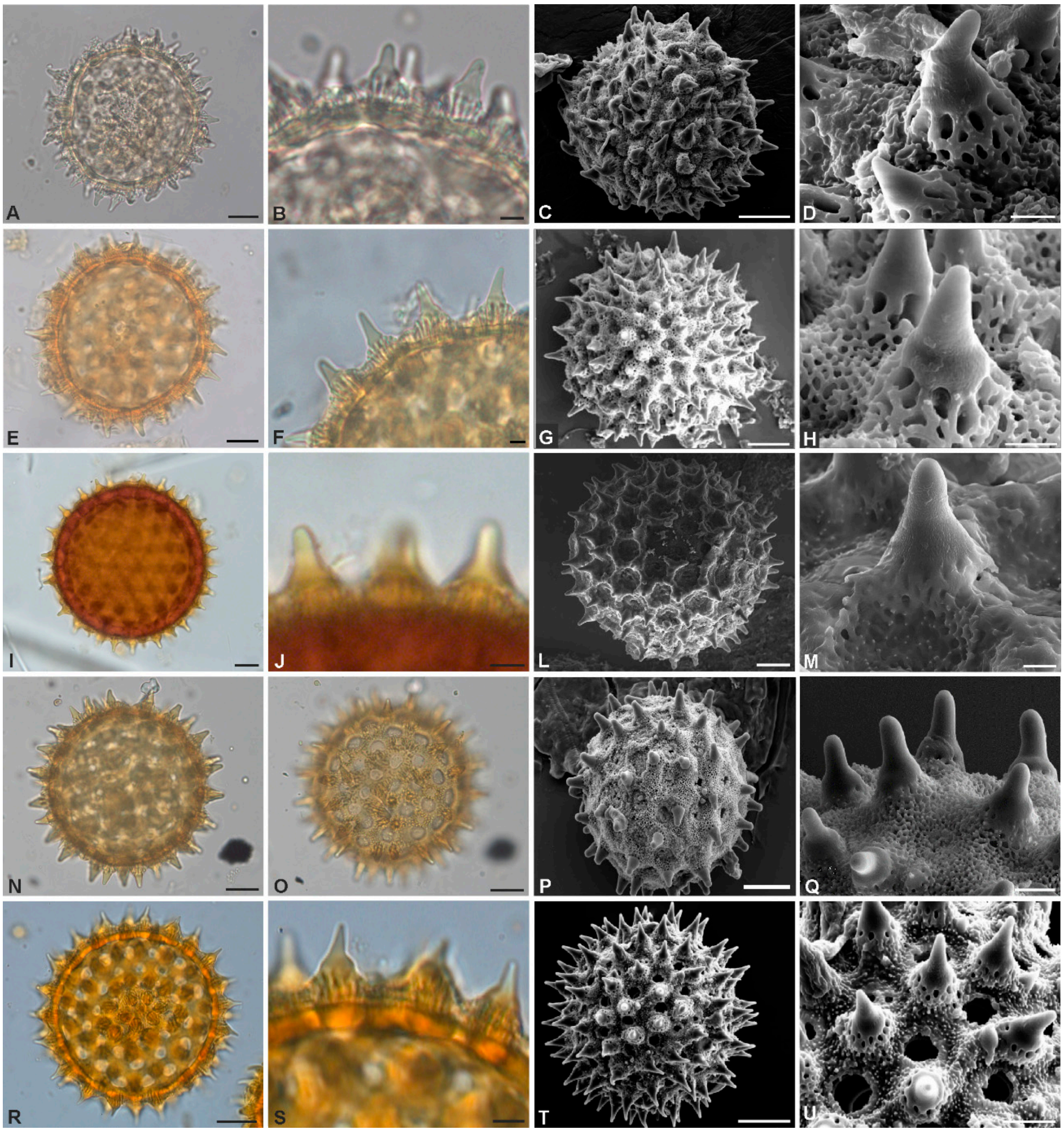

Figure 3. A–U. Pollen grains of Convolvulaceae from Serra dos Carajás, Pará, Brazil. *Ipomoea decora* (A–D) A—optical section; B—type 2 bulbous echinae; C—surface on SEM; D—echinae and detail of the columellae. *I. goyazensis* (E–H): E—optical section; F—type 1 bulbous echinae; G—surface on SEM; H—echinae and detail of columellae. *I. hederifolia* (I–M): I—optical section; J—type 2 bulbous echinae; L—surface on SEM; M—echinae and detail of the columellae. *I. marabaensis* (N–Q): N—optical section; N—type 2 bulbous echinae; P—surface on SEM; Q—echinae and detail of the columellae. *I. maurandioides* (R–U): R—optical section; S—conical echinae; T—surface on SEM; U—echinae and apertures. (A,C,E, G, I, L, N, P, R, T)—20-µm scale; (B, F,J,O,S)—5-µm scale; (D,G,M,Q,U)—3-µm scale).

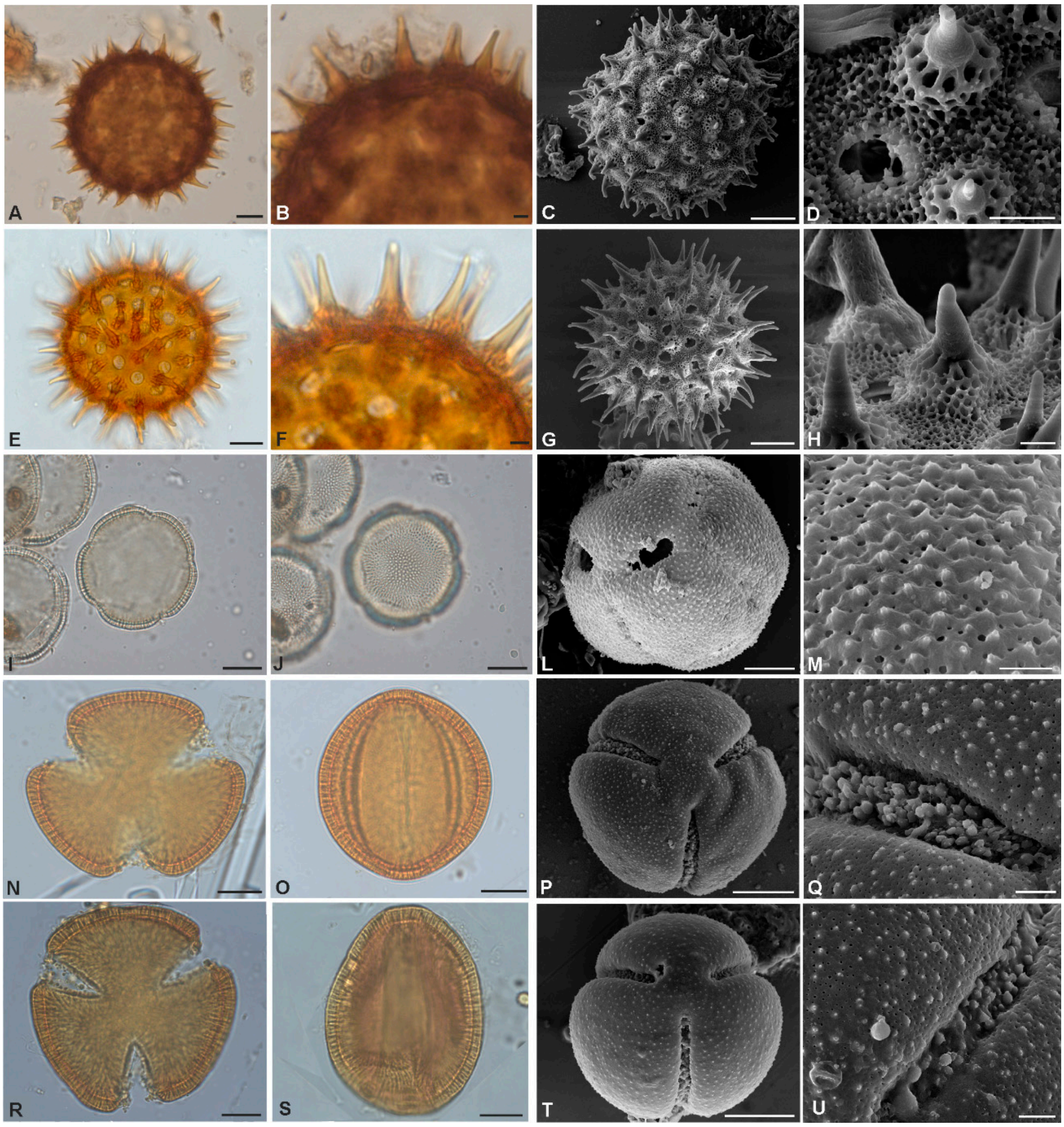

Figure 4. A–U. Pollen grains of Convolvulaceae from Serra dos Carajás, Pará, Brazil. *Ipomoea procumbens* (A–D) A—optical section; B—conical echinae; B—surface on SEM; D—echinae and detail of columellae and aperture. *Ipomoea goyazensis* (E–H): E—optical section; F—conical echinae; G—surface on SEM; H—echinae and detail of columellae. *Jacquemontia tamnifolia* (I–M): I—optical section; J—exine ornamentation on OM; L—surface on SEM; M- detail of the exine on SEM. *Merremia macrocalyx* (N–Q): N—optical section of polar view; O—optical section of equatorial view; P—surface on SEM; Q—detail of the exine ornamentation and colpus. *Operculina hamiltonii* (R–U): R—optical section of polar view; S—optical section of equatorial view; T—surface on SEM; U—detail of the exine ornamentation and colpus. (A,C,E,G,I,J,L,N,O,P,R,S,T)—20-µm scale; (B,F)—5-µm scale; (D,H,M,R,U)—3-µm scale).

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Table 2. Morphometry (μm) of pollen grains of Convolvulaceae species. PD = polar diameter (μm); ED = equatorial diameter (μm); LD = largest diameter of apolar grains; SD = smallest diameter of apolar grains; Di = interechinae distance (μm).

Species	PD/ED	ED/SD	Sexine	Nexine	Exine	Spine			
						Base	Height	No.	Di
Aniseia cernua	82.7 (5.8) 108.2	76.5 (6.4) 99.9	4.3 (0.6) 6.4	1.2 (0.3) 2.4	6.0 (0.7) 11.6	-	-	-	-
Cuscuta insquamata	25.9 (6.4) 48.4	18.3 (4.3) 39.6	0.4 (0.1) 1.0	0.5 (0.1) 1.0	1.0 (0.5) 3.2	-	-	-	-
Evolvulus filipes	28.2 (2.6) 36.0	25.7 (2.6) 33.9	0.6 (0.1) 1.0	0.8 (0.1) 1.1	1.4 (0.2) 1.9	-	-	-	-
Ipomoea asplundii	81.2 (5.5) 101.8	79.2 (5.0) 97.9	1.6 (0.4) 3.8	2.2 (0.4) 3.9	4.5 (0.6) 7.4	6.2	11.7	~185	13.0
I. cavalcantei	92.7 (6.8) 125	85.2 (6.9) 125	2.9 (0.2) 3.5	2.3 (0.3) 3.2	5.4 (0.5) 6.8	6	8.9	~126	17.2
I. decora	98.6 (5.0) 116.3	87.4 (5.6) 112.2	2.7 (0.7) 5.6	2.6 (0.5) 4.7	6.4 (1.1) 10.0	6.2	8.7	~190	14.2
I. goyazensis	93.6 (12.2) 155.7	91.7 (8.5) 120.9	2.8 (0.6) 4.7	2.3 (0.5) 4.3	5.6 (0.9) 8.9	6.1	10.7	~197	14.9
I. hederifolia	131.2 (3.5) 144	126.7 (3.4) 140.7	4.0 (1.3) 9.2	4.6 (1.4) 10.9	11.8 (1.2) 16.6	6.7	9.0	~200	16.6
I. marabaensis	98.4 (8.8) 128	96.5 (6.5) 122	2.4 (0.5) 4.2	2.9 (0.3) 4.1	5.8 (0.6) 7.9	6.3	9.9	~185	15.2
I. maurandioides	83.6 (5.3) 111	82.2 (11.2) 101.2	2.8 (0.6) 6.1	2.8 (0.4) 4.8	6.4 (0.9) 9.8	5.1	8.7	~184	12.2
I. procumbens	109.7 (4.2) 122.9	107.3 (2.9) 119.6	1.2 (0.8) 4.8	2.2 (0.6) 4.5	4.5 (1.2) 9.2	6.9	15.7	~175	15.8
I. setifera	99.1 (2.2) 100.5	87.5 (2.6) 99.1	1.4 (0.6) 3.5	2.5 (0.5)4.2	3.9 (0.8) 7.5	5.1	15.2	~147	13.9
Jacquemontia tamnifolia	42.9 (7.9) 69.1	41.6 (8.0) 65.7	1.8 (0.3) 3.3	0.6 (0.3) 1.6	2.5 (0.5) 4.8	-	-	-	-
Merremia macrocalyx	69.0 (4.4). 86.6	83 (7.8) 72.2	2.9 (0.4) 4.7	0.8 (0.2) 1.8	3.9 (0.5) 6.0	-	-	-	-
Operculina hamiltonii	84.8 (9.7) 122	66.7 (9.8) 98.3	2.9 (0.7) 5.3	0.1 (0.4) 2.6	3.9 (0.7) 6.9	-	-	-	-

Statistical analysis

PCA, an exploratory analysis of quantitative data, was used to analyze the pollen grains of the *Ipomoea* species, a stenopalynous genus that therefore requires a greater number of analyzed traits for its separation (Figure 5). This analysis was performed with 17 morphometric variables measured in the pollen grains studied. The first three axes of the analysis, with eigenvalues greater than 1, summarized 65% of the total data variance (Table 3).

Table 3. Loadings of *Ipomoea* pollen morphological characters for three principal components. The first three PCs with eigenvalues greater than one are represented here. Each percentage in parentheses indicates the amount of variation explained by each PC. Largest grain diameter (GDL); smallest grain diameter (GDW); GDL/GDW ratio; largest pores diameter (Pores_length); smallest pores diameter (Pores_width); Pores_length/Pores_width ratio (Pl.Pw); C_pores/GDL ratio (C.GDL); C_pores/GDW ratio (C.GDW); distance between pores (C_pores); echinae base (Width_base_echinae); echinae height (Height_echinae); distance between echinae (DE); number of pores (X_pores); number of echinae (X_echinae); sexine (Sexine); nexine (Nexine_thickness) and exine.

Characters	PC1 (30.6%)	PC2 (24.17%)	PC3 (10.25%)
GDL	-0.0968558487	-0.4448954923	-0.2094374965
GDW	-0.1111947746	-0.4466275440	-0.1449467515
GDL.GDW	0.0422884533	-0.0570466784	-0.2967254499
Pores_length	-0.3257246993	0.0875787758	-0.2761904120
Pores_width	-0.3215687248	0.0680506747	-0.2752009761
Pl.Pw	0.0446138575	0.0255071817	0.0460258691
C_pores	-0.3429523932	-0.2791425832	0.0095841515
C.GDL	-0.3469113525	0.0158739705	0.2663134002
C.GDW	-0.3578827122	0.0074901452	0.2482642165
Width_base_echinae	-0.1234947500	-0.2173683353	-0.3158851823
Height_echinae	0.0009557029	0.0921775804	-0.4765036952
DE	-0.3106893994	-0.2284124864	0.0206751589
X_echinae	0.2658107702	-0.1293933233	-0.2076926665
X_pores	0.3675813652	-0.0432977352	-0.2368559242
Nexine_thickness	0.1765245315	-0.3882438747	0.0903857449
Sexine	0.1251662916	-0.2628771862	0.2887846217
EXINE	0.1858772727	-0.4022241713	0.2054888818

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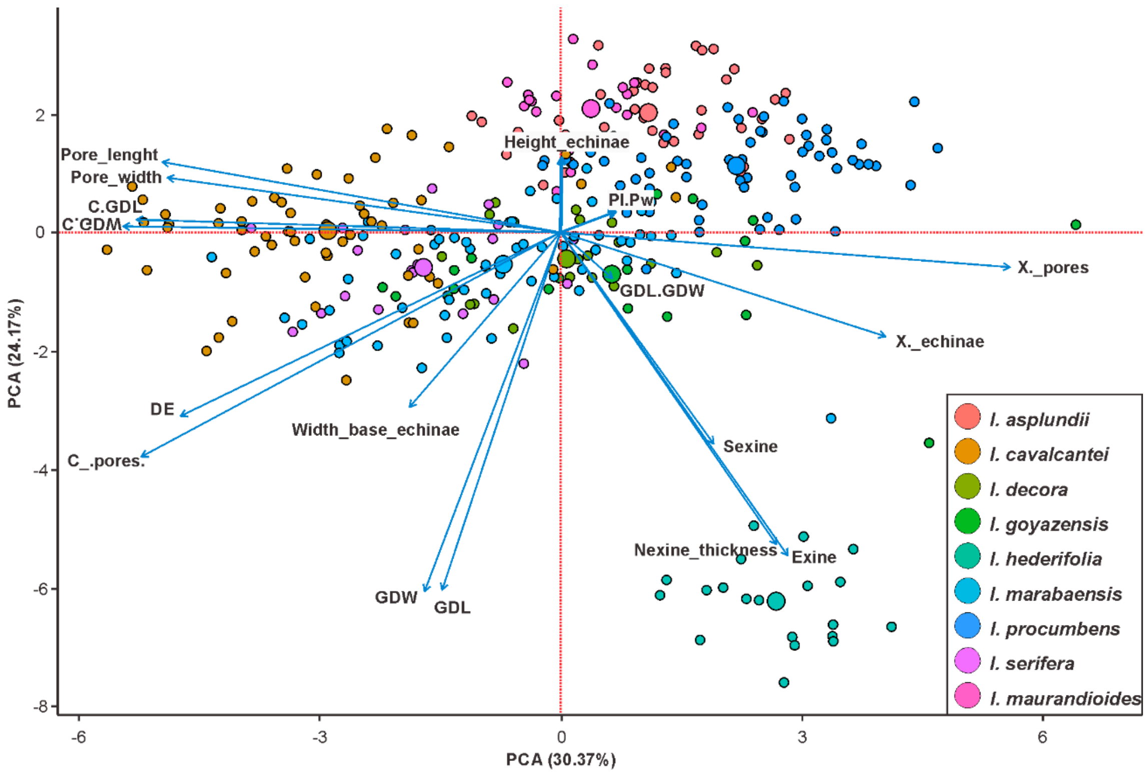

Figure 5. Principal component analysis scatterplot with the morphometric pollen variables of *Ipomoea*. The first two principal components were plotted, and each color represents a different species. Largest grain diameter (GDL); smallest grain diameter (GDW); GDL/GDW ratio; largest pores diameter (Pores_length); smallest pores diameter (Pores_width); Pores_length/Pores_width ratio (Pl.Pw); C_pores/GDL ratio (C.GDL); C_pores/GDW ratio (C.GDW); distance between pores (C_pores); echinae base (Width_base_echinae); echinae height (Height_echinae); distance between echinae (DE); number of pores (X_pores); number of echinae (X_echinae); sexine (Sexine); nexine (Nexine_thickness) and exine.

The first major axis (PC1) was the most significant for species ordination (Figure 5), which explained 30.66% of the variation based mainly on the estimate of the number of pores (X._pores) and number of echinae (X._echinae), followed by the ratios of the distance between the pori and the largest grain size and smallest grain size (C.GDL and C.GDW) and the distance between pores (C. pores). PC2 was responsible for 24.17% of the data variability, mainly related to the grain size (GDL and GDW), exine thickness and echinae height (Height_echinae). However, echinae height and width contributed negatively to the construction of the third axis (PC3), explaining 10.25% of the data variability.

In the first axis (Figure 5), *Ipomoea cavalcantei* obtained, in general, larger distance between echinae (DE) values and, therefore, a lower number of echinae and pores (X._echinae and X._pores). *I. procumbens* also had larger pores (Pl and Pw), distance between pores, and echinae base. The species *I. maurandioides*, *I. asplundii* and *I. setifera* were grouped on the positive side of axes one and two because they had higher echinae heights (Height_echinae), smaller exines and echinae bases (Width_echinae), and smaller pollen grains (GDL and GDW). In addition, in the second axis, *I. hederifolia* was grouped separately from the other species, with the main characteristic being the largest grains among species (GDL and GDW), with smaller pores (P and Pw) and higher exine, nexine and sexine values. In addition, *I. hederifolia* had a higher number of echinae and pori calculated by the formula of Hanks and Fryxell (1979).

The pollen of some species, namely, *I. procumbens*, *I. setifera*, *I. asplundii*, *I. maurandioides*, *I. goyazensis*, *I. decora* and *I. marabaensis*, were clustered due to similar values of the Pl.Pw ratio and Height_echinae metrics (Figure 5). In general, the species were concentrated mainly on the positive side of axes one and two and on the negative side of axes one and two.

Discussion

Among the studied species, *Cuscuta insquamata* and *Evolvulus felipes* presented the smallest pollen grains. According to Vij & Sachdeva (1974), grain size is related to flower size. Within the family Convolvulaceae, species with smaller flowers usually also have smaller pollen grains. This morphological information may bring species closer together and assist in taxonomic delimitation, since until the present study, little was known about the pollen of *Cuscuta* species (Noshad et al. 2020).

Evolvulus filipes was described as having pantocolpate pollen grains with microechinate exine ornamentation on SEM, which corroborates the descriptions by Tellería & Daners (2003) and Ketjarun et al. (2016).

Considering the grouping of different pollen morphology characters, Segunpta (1972) divided the family Convolvulaceae into four groups, namely, Group 1: 5(-6)-colpate, Group 2: 3-colpate, Group 3: dodecacolpate and Group 4: pantoporate. According to this classification, *Cuscuta insquamata* falls into Group 2, characterized by medium-sized colpate pollen grains and the presence of granules inside the colpi. The *Ipomoea* species in Group 4, due to the type of aperture and its arrangement in the grains, are pantoporate.

The nine *Ipomoea* species analyzed exhibit pollen grains with semitectate to tectate, pantoporate and echinate exine, which corroborates the descriptions in several studies (Erdtman 1952; Laguardia 1961; Sengupta 1972; Andrade & Miranda 1986; Machado & Melhem 1987; Araújo et al. 2000).; Tellería & Daners 2003; Vital et al. 2008; Rajukar et al. 2011; Vasconcelos et al. 2015; Sudhama et al. 2019). Echinae of *Ipomoea* are supported by thick columellae, which increase in height in the aperture-echinae direction, similar to the description by Andrade & Miranda (1986) and Tellería & Daners (2003).

The morphological characters of echinae are more relevant for the separation of *Ipomoea* species due to the observed variations in echinae: a) conical (*I. asplundii*, *I. maurandioides*, *I. procumbens* and *I. setifera*); b) bulbous type 1, with bulbar base and apiculate apex (*I. goyazensis*); c) type 2 bulbous, with bulbar base and rounded apex (*I. decora*, *I. hederifolia* and *I. marabaensis*); d) bulbous type 3, with bulbous apex (*I. cavalcantei*). Echinae were also highlighted as the main differentiating character of this genus, with the species separated according to the base of the echinae, which ranges from straight to bulbar (Vital et al., 2008). For *Ipomoea*, the sexine was thicker than the nexine, with the exception of *I. hederifolia*. In addition, this species presented the largest pollen grains with the largest diameter, ranging from 131–144 µm, classified as very large, which corresponds to the data found by Vij & Sachdeva (1974). However, the data obtained by Machado & Melhem (1987) for *I. hederifolia* do not agree with the data presented here, as they described the echinae as conical and located on edges, with bulbous echinae type 2.

Intraspecific variations are observed, hindering the standardization of the morphological description, and are better detected in some species, such as *I. cavalcantei* and *I. marabaensis*. Likewise, it is difficult to establish the number of pores due to the density of echinae and thick exine. The large number of apertures is possibly associated with derived taxa and with greater reproductive efficiency due to increased opportunities for pollen tube germination (Furnes & Rudall 2004).

Few diagnostic characters are known for the identification of *Jacquemontia*, resulting in identifications that are, in many cases, inaccurate. This is reflected in several botanical collections where the genus is erroneously identified as *Evolvulus* L. or *Convolvulus* L. (Robertson 1971; Moreira 2014). However, the studied pollen grains determined characters that have taxonomic potential for delimitation between these genera, namely, the number of apertures and their distribution in the grains, which helped in the differentiation of the studied species (*Jacquemontia tamnifolia* and *Evolvulus filipes)*.

This is different from what was observed by Carreira & Barth (2003), as they described *Merremia macrocalyx* as having psilate ornamentation. In the SEM analysis, granulate and microreticulate exines were observed, as described by Melhem & Corrêa (1987), who reported elements deposited above the tectum, which Vasconcelos et al. (2015) called granules. The main differences between *Merremia macrocalyx* and *Operculina hamiltonii* are the size and shape, where *M. macrocalyx* has large and subprolate pollen grains and *O. hamiltonii* very large pollen grains with a spheroidal prolate shape.

These findings corroborate Lewis & Oliver (1965) and Vasconcelos (2015) but differ from those reported by Leite et al. (2005) for *M. macrocalyx* (prolate grains). According to Laguardia (1961), 4-colpate pollen grains are found in *M. macrocalyx*, a characteristic that was not observed in the studied grains.

This study indicated that the qualitative characteristics of echinae type, and in some cases, grain size (character measured to be subsequently classified) and aperture arrangement, are important characteristics to describe the genera of Convolvulaceae, thus establishing the classification of the pollen types for the analyzed species. In addition, quantitative data (morphometry) confirm, in some cases, the attributes used to define pollen types. Statistical methods such as principal component analysis (PCA) have been frequently used to evaluate the systematic utility of pollen data (Akhila & Beevy 2015; de Abreu et al. 2015; da Luz et al. 2015; Mezzonato-Pires et al. 2015; Mezzonato-Pires et al. 2015; Ok & Hong 2015; Bellonzi et al. 2020; Gonçalves-Esteves et al. 2019; Moreira et al. 2019).

Species such as *I. goyazensis* and *I. decora* presented conflicts in their taxonomic delimitations, and currently, studies such as that by Wood and Scotland (2017), based on identifications by O'Donell, argue that these are conspecific species, referring to them by name *I. goyazensis* only. Therefore, even though this is something to be discussed, based on the PCA performed in the present study, we observed that the two species were grouped together (Figure 5), which may serve as evidence of their similarity.

The studied specimens of the genus *Ipomoea* were collected in different areas, which suggests that the geographical boundaries were not sufficient to result in a significant difference in pollen morphology, with the exception of *I. hederifolia*, which was grouped separately from the others. According to Wang and Dobritsa (2018), closely related species generally produce similar pollen grains.

Some specimens, represented by same-color points on the PCA plot (Figure 5) are dispersed in the cluster, differing in the number of apertures in their pollen grains, such as *I. maurandioides.* This trend is often due to hybridization processes and is linked to the level of ploidy in individuals (García et al. 2015; Albert et al. 2018; Reeder et al. 2016).

Conclusions

These results reinforce the importance of studying pollen morphology to identify and distinguish genera and species and reaffirm the eurypalynous character of the family Convolvulaceae. Based on the data obtained, it is concluded that palynotaxonomy is considered an important and effective tool in species identification for taxonomic studies. This study of the Convolvulaceae taxa contributes to the knowledge on the Brazilian and worldwide pollen flora and may contribute to taxonomic circumscription and thus improve the understanding of the phylogenetic relationships of Convolvulaceae. In general, the set of morphological characters was effective for separating Convolvulaceae genera and species occurring in Serra dos Carajás.

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Appendix A. Convolvulaceeae species described in this study.

Genera	Species	Ocorrence	Geographic coordinate	Herbarium Nº	Colector
Aniseia	*Aniseia cernua* Moric.	N5	6°02′30′′ S, 50°05′16′′ W	MG222501	Meirelles,J.968
Cuscuta	*Cuscuta insquamata* Yunck	N1	6º02′35′′ S, 50º16′57′′ W	MG 215432	Viana, P.L. 5594
		N7	6º09′16′′ S, 50º10′18′′ W	MG 215060	Mota, N.F.O. 3434
Evolvulus	*Evolvulus filipes* Mart	Serra do Tarzan	6°19′44′′ S, 50°08′20′′ W	MG 214460	Mota, N.F.O. 3003
Ipomoea	*Ipomoea asplundii* O'Donell	N7	6°09′29′′ S, 50°10′10′′ W	MG 226331	Vasconcelos, L.V. 1102
		N7	6°09′29′′ S, 50°10′14′′ W	MG 222398	Harley, R.M. 57379
		N1	6°01′20′′ S, 50°18′02′′ W	MG 223584	Falcão, B.F. 182

	Ipomoea cavalcantei D.F.Austin	N2	6º03′21′′ S, 50º15′13′′ W	MG 215469	Viana, P.L. 5631
		N3	6º02′34′′ S, 50º12′33′′ W	MG 214408	Mota, N.F.O. 2951
		N4	6º06′08′′ S, 50º11′13′′ W	MG 223179	Harley, R.M. 57491
		N5	6º05′40′′ S, 50º07′59′′ W	IAN 173120	L.V. Vasconcelos
	Ipomoea decora Meisn.	Estrada S11D A S11A	6º22′17′′ S, 50°23′04′′ W	MG 213191	Lobato, L.C.B. 4406
	Ipomoea goyazensis Gardner	N4	-	MG 227195	L.V. Vasconcelos
	Ipomoea hederifolia L.	N5	6º02′26′′ S, 50º05′18′′ W	HCJS 1224	Silva, D. F. & Tyski, 773
	Ipomoea marabaensis D.F.Austin & Secco	N1	6º02′27′′ S, 50º16′54′′ W	-	-
		N2	6º03′25′′ S, 50º15′04′′ W	-	-
		N5	6º06′35′′ S, 50º08′11′′ W	MG 223537	Falcão, B.F. 135
		N6	6º07′48′′ S, 50º10′36′′ W	MG 223639	Falcão, B.F. 237
		N7	6º09′25′′ S, 50º10′19′′ W	BHCB 162046	Arruda, A. J. 1366
		N8	6º09′26′′ S, 50º08′56′′ W	BHCB 162045	Arruda, A. J. 1365
		S11A	6°21′11′′ S, 50°25′30′′ W	BHCB 140215	Costa, F. M. 100
		S11B	6°20′42′′ S, 50°25′10′′ W	MG 223488	Falcão, B.F. 86
		S11D	6°23′44′′ S, 50°22′17′′ W	MG 213954	Carreira, L.M.M. 3337
		Serra da Bocaina	6°18′36′′ S, 49°53′20′′ W	MG 223758	Falcão, B.F. 356
		Serra Leste	5°58′32′′ S, 49°38′07′′ W	HCJS 5000	-
		Serra do Tarzan	6°20′15′′ S, 50°10′00′′ W	BHCB 130853	Giorni, V. T. 144
	Ipomoea maurandioides Meisn	N1	6°02′10′′ S, 50°17′06′′ W	MG 224469	Pastore, M. 343
		N5	6º06′05′′ S, 50º07′42′′ W	MG 214372	Mota, N.F.O. 2915
		N7	6º09′13′′ S, 50º10′21′′ W	BHCB 157742	Arruda, A. J. 858
		S11D	6°23′58′′ S, 50°22′31′′ W	MG 214004	Carreira, L.M.M. 3387
		S11A	6°21′09′′ S, 50°26′54′′ W	MG 214059	Carreira, L.M.M. 3442
		Serra do Tarzan	6°20′06′′ S, 50°09′58′′ W	MG 223299	Vasconcelos, L.V. 845
	Ipomoea procumbens Mart. ex Choisy	S11B	6°21′04′′ S, 50°26′22′′ W	MG 222344	Carreira, L.M.M. 3527
	Ipomoea setifera Poir.	Parque	6°03′39′′ S, 50°03′43′′ W	-	Rodrigues, T.
Jacquemontia	*Jacquemontia tamnifolia* (L.) Griseb.	S11C	6°23′06′′ S, 50°23′03′′ W	MG 223119	Harley, R.M. 57430
		S11D	6°21′09′′ S, 50°26′54′′ W	MG 214054	Carreira, L.M.M. 3437
Merremia	*Merremia macrocalyx* (Ruiz & Pav.) O'Donell	N2	6º03′23′ S, 50º14′46′′ W	MG 215937	Lobato, L.C.B. 4447
		Parque	6º03′42′′ S, 50º03′44′′ W	-	Rodrigues, T.
Operculina	*Operculina hamiltonii* (G.Don) D.F.Austin & Staples	Estrada para Serra Sul	6º19′15′′ S, 50º26′58′′ W	HCJS 2045	Tyski, L. 198

Appendix B. Pollen Key

1. Pollen grains colpate

1.1. 3-colpate

1.1.1. Microreticulate ornamentation

Cuscuta insquamata

1.1.2. granulate ornamentação

1.1.2.1. subprolate

Merremia macrocalyx
Operculina hamiltonii

1.1.2.2. prolate spheroidal

Jacquemontia tamnifolia

1.2. 4(-5)-colpate

1.3. Pantocolpate

1.3.1. microreticulate-microverrucate ornamentation

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Aniseia cernua

1.3.2. microechinate ornamentation

Evolvulus filipes

2. Porate pollen

2.1. Echinate, Pantoporate

2.1.1. bulbous echinae

2.1.1.1. apiculate apices (Tipo 1)

goyazensis

2.1.1.2. rounded apices (Tipo 2)

2.1.1.2.1. uniform distribution

Decora

marabaensis

2.1.1.2.2. with metareticulum

hederifolia

2.1.1.2. bulbous apices (Tipo 3)

cavalcantei

2.1.2. Coniform echinae

2.1.2.1. Large pollen grains

asplundii

maurandioides

setifera

2.1.2.2. Very large pollen grains

procumbens

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