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Pollen morphology of selected species of *Passiflora* L. (Passifloraceae) from the Atlantic Forest

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ABSTRACT

Pollen grains from twelve species of the Passifloraceae family from the Brazilian Atlantic Forest were analyzed: *Passiflora* subg. *Astrophea* (1sp.), *P.* subg. *Decaloba* (1sp.) and *P.* subg. *Passiflora* (10spp.). The current study aims to acquire additional information and feature the pollen morphology of the herein studied species to help setting a more precise taxa delimitation. Acetolyzed pollen grains were measured, described and illustrated using light and scanning electron microscopy. The pollen grains were medium or large sized, oblate spheroidal, suboblate, prolate spheroidal and subprolate; 4-colpate (*P. kermesina*), 6-12-pantocolporate (*P. mediterranea*), 6-colpate (*P. mucronata*), 6-colporate (*P. pentagona*), 12-colporate (*P. misera*) or 6-syncolpate (in most species). The presence of reticulate sexine, pseudopercula, pontopercula and/or opercula was observed. The endoaperture was just found in *P. pentagona* and *P. misera*. It was concluded that pollen morphology is an important source of taxonomic features useful for distinguishing species and characterizing the three subgenera. The current study provides additional information that, along with other previously published studies, will enable a better understanding of phylogenetic relations among these strains.

Keywords: Atlantic Forest, palynotaxonomy, *Passiflora*, Passifloraceae, sandbanks

Introduction

Passifloraceae *s.s.* holds about 17 genera and 630 species, which are widely distributed in tropical regions (Wilde 1974; Holm-Nielsen *et al.* 1988; Brummitt & Powell 1992; Deginani 1999). *Passiflora* is its most representative genus, which presents approximately 400 taxa. The genus was initially divided into 23 subgenera (Killip 1938; Escobar 1989) and subsequently reduced to just four: *Astrophea*, *Deidamioides*, *Decaloba* and *Passiflora*.

Brazil hosts approximately 150 Passifloraceae species, 87 of them are endemic (Bernacci *et al.* 2014). The State of Rio de Janeiro is considered to be one of the greatest fauna and flora endemism centers in the country (Bergallo *et al.* 2000; Rocha *et al.* 2003). According to Bernacci *et al.* (2014), the state is represented by 41 species.

Most of the herein analyzed species (*Passiflora alata*, *P. amethystina*, *P. edulis*, *P. farneyi*, *P. foetida*, *P. kermesina*, *P. mediterranea*, *P. misera*, *P. mucronata*, *P. pentagona*, *P. racemosa* and *P. setacea*) can be found in sandbanks in the State of Rio de Janeiro. These species show wide distribution and are found in almost all Brazilian regions, except for *Passiflora kermesina, P. pentagona* and *P. mucronata,* which are restricted to the Southeast region of the country and *P. farneyi,* which is just found in the State of Rio de Janeiro (Bernacci *et al.* 2014).

The Atlantic Forest is an important "hotspot", i.e., one of the richest and most threatened biological regions in the world; its endemic rate is particularly high (Mittermeier *et al.* 2000). As already highlighted by Candido *et al.* (2013) as well as by preceding authors (Myers *et al.* 2000; Rocha *et al.* 2007), "although *restinga* habitats were once distributed along most all the coastline in the State of Rio de Janeiro, they have been diminishing at alarming rates due to native vegetation removal for human occupation purposes".

The main pollen studies on Passifloraceae *s.s.* species started to be conducted back on 1965, when Presting (1965) analyzed the morphology of pollen grains and suggested an evolutionary theory based on apertural and opercular types. Spirlet (1965) used pollen data to help taxonomic classification. Later studies such as those by García *et al.* (2002), Araújo & Santos (2004), Milward-de-Azevedo *et al.* (2004; 2010), Barrios *et al.* (2005), Dettke & Santos (2009), Evaldt *et al.* (2011), Tangarife *et al.* (2011), Mezzonato-Pires (2013) and Verdasca *et al.* (2013) also helped improving the palynological knowledge on the Passifloraceae *s.s.* family.

The current study aims to feature pollen morphology and identify informative characters that may help increasing knowledge on the genus and subgenus of this plant group. It also aims to study the phylogeny of the group and to gather helpful information to further conservation efforts.

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Material and methods

The current study investigated pollen grains of 12 *Passiflora* species, subgenus *Astrophea* (1 sp.), *P.* subg. *Decaloba* (1 sp.) and *P.* subg. *Passiflora* (10 spp.). The herein used botanical material consisted of flowers in anthesis and /or flower buds picked from specimens deposited in different herbaria in the State of Rio de Janeiro; their acronyms were related according to the Index Herbariorum (Thiers, continuously updated).

Material used in the study: *Passiflora alata* Curtis: Brazil. Rio de Janeiro: São João da Barra, III/1939, *A. Sampaio 8175 (R); Carapebus, road to Praia da Capivara, 10/29th/1996, M.C. Oliveira 441 (R). *Passiflora amethystina* Mikan: Brazil. Rio de Janeiro: Teresópolis, Serra dos Órgãos National Park, between shelters 3 and 2, 04/29th/1962, *E. Santos 1235 *et al.* (R). *Passiflora edulis* Sims: Brazil. Rio de Janeiro: Guanabara Road, Recreio dos Bandeirantes, Itapeba Restinga, Lagoa das Taxas, 07/06th/1965, Newton Santos 5309 (R); Cabo Frio, Cabo Frio Restinga, 01/20th/1947, D. Sucre 1427 (RB); Maricá, Ponta Negra, 11/26th/1952, *J. Vidal s/n (R106018). *Passiflora farnyei* Pessoa & Cervi: Brazil. Rio de Janeiro: Cabo Frio, 19/11/1967, D. Sucre 1940 (R). *Passiflora foetida* L.: Brazil. Maranhão: Arari, camping area by Lagoa Malhada Grande, without date, J.G. Silva 2021 and J.A.F. Costa 1602 (R). *Passiflora kermesina* Link. & Otto: Brazil. Rio de Janeiro: Carapebus, São Lázaro Farm, 12/07th/1994, Correia 617 *et al* (R). *Passiflora mediterranea* Vell.: Brazil. Rio de Janeiro: Morro Queimados, 01/17th/1932, *Brade 11287 (R). *Passiflora misera* Kunth: Brazil. Santa Catarina: BR 101, Km 348, 3 Km north of Jaguaruna, 01/22nd/2001, *V. J. Pott 4283 & A. Pott (R). *Passiflora mucronata* Lam: Brazil. Rio de Janeiro: Macaé, Cabuinas, Lagomar Resort, Lagoa Jurubatiba, 9.3 Km from NUPEM, Ca. 350m from PNRJ entrance, 02/11th/2009, *M.F. Castilhori 262 and I.E. Santo *et al* (R); Rio de Janeiro, Arpoador, 03/29th/1978, P. Laclette 446 *et al.* (R); Carapebus, 2nd sandy line, after the road to Carapebus, 03/26th/1996, V. Esteves 928 *et al.* (R); Carapebus, close to Lagoa Cabuinas, 09/23rd/1997, M.C. de Oliveira 568 *et al.* (R). *Passiflora pentagona* Mast.: Brazil. Rio de Janeiro: Quissamã, *C. Farney *et al. 3420*, 10/29th/1994 (RB); Espírito Santo, Itaúnas, Itaúnas State Park, Milward, M. & Van der Vem, P.H.L. *35*, 02/01st/2001 (RB). *Passiflora racemosa* Brot.: Brazil. Rio de Janeiro: Maricá, Morro do Macaco, 09/19th/1986, *J.G. Silva 615 *et al.* (R). *Passiflora setacea* DC.: Brazil. Rio de Janeiro: Maricá, Morro do Macaco, 09/19th/1986. *J.G. Silva 600 *et al.* (R).

Standard specimens marked with an asterisk (*) next to the collector's name were chosen for each studied species. Three specimens were chosen for comparison to corroborate the results. The following criteria were adopted to choose the standard material: being preferably collected in the State of Rio de Janeiro and being identified by an expert on the family. The used slides were deposited in the Palynology Collection of Álvaro Xavier Moreira Palynology Laboratory, in the Botany Department at National Museum of Federal University of Rio de Janeiro.

The pollen samples to be analyzed under light microscopy were prepared according to the acetolytic method by Erdtman (1952), which was modified by Melhem *et al.* (2003); and measured after seven preparation days (Salgado-Labouriau 1973).

Twenty-five (25) polar diameter (PD) and equatorial diameter (ED) measurements of pollen grains in equatorial view, 10 equatorial diameter measurements and measurements of the side of the apocolpus – both in polar view (EDPV) - were performed. The grains were distributed among three slides. The statistical analysis was done by calculating the arithmetic mean (x), sample standard deviation (s), mean deviation ($s_{\bar{x}}$), the coefficient of variability (CV%), the confidence interval of 95% and the variation rate. Ten (10) pollen grains were measured to set other features such as apertures and exine layers. Ten (10) similar pollen grain measurements were obtained from additional material deposited in another collection (from this point on referred to as comparison material). Such measures were taken to check the stability of data collected from the reference material (Abreu *et al.* 2012; 2014; Moreira *et al.* 2013).

The terminology herein used meets that by Barth & Melhem (1988), Punt *et al.* (2007). It takes into account the size, shape and ornamental pattern of sexine. The definitions by Presting (1965) and Dettke & Santos (2009) were applied to apertures description.

Non-acetolyzed pollen grains were assembled on stubs with double-sided carbon tape and sputter-coated (for 3 min) with a thin palladium-gold layer. The photomicrographs were taken under scanning electron microscopy using the JSM-5310 microscope (JEOL, Ltd., Tokyo, Japan), in the Electron Microscopy Center of Invertebrates Department at National Museum of Federal University of Rio de Janeiro.

Results

Pollen grains subordinated to three subgenera were analyzed: *P.* subg. *Astrophea*: *Passiflora pentagona* (Fig. 1A-C), *P.* subg. *Decaloba*: *Passiflora misera* (Fig. 1D-G), and *P.* subg. *Passiflora*: *Passiflora alata* (Fig. 1H), *Passiflora amethystina* (Fig. 1I), *Passiflora edulis* (Fig. 1J-K), *Passiflora farneyi* (Fig. 1L), *Passiflora foetida* (Fig. 2A), *Passiflora kermesina* (Fig. 2B-C), *Passiflora mediterranea* (Fig. 2D-E), *Passiflora mucronata* (Fig. 2F-H), *Passiflora racemosa* (Fig. 2I, J) and *Passiflora setacea* (Fig. 2K-L).

The palynological description was arranged according to the following pollen features (Figs. 1 e 2): size; polarity; dispersal unit; size, shape, and type of apertures; opercula types; exine sculpture. The results were presented in a single description and summarized in Tables 1 to 5.

Polarity, dispersal unit, size and shape

Isopolar pollen grains were found in most of the species and the apolar grains were found in *P. kermesina* and *P. mediterranea* (*P.* subg. *Passiflora*) (Tabs. 2-4, Fig. 2); they were considered to be large sized in most of the species. Medium sized pollen grains were just found in *P. pentagona*. Just five species were analyzed in equatorial view. Their polar and equatorial diameters were measured and shapes established. Thus, the suboblate shape was found in *P. edulis* and *P. mucronata*; oblate spheroidal in *P. alata*, prolate spheroidal in *P. misera* and subprolate in *P. pentagona*. *Passiflora pentagona* presented the smallest polar (48.6 μm) and equatorial diameters (42.6 μm); whereas *P. edulis* showed the largest polar (63.0 μm) and equatorial diameters (67.5 μm), in equatorial view (Tab. 2). *P. pentagona* showed the lowest equatorial diameter, in polar view (44.2 μm) and *P. amethystina* showed the largest one (87.4 μm); *P. farneyi* had the lowest value related to apocolpium side (14.3 μm) and *P. amethystina* showed the largest one (25.0 μm) (Tab. 4). *P. kermesina* species had the lowest values in D1 and D2 (Tab. 3).

Number and type of apertures

The apertures are not easily distinguishable due to the intense ornamentation of most pollen grains. Different types of apertures were found in the studied species. *P. alata, P. amethystina, P. edulis, P. farneyi, P. foetida, P. racemosa* and *P. setacea* had 6-syncolpate apertures; *P. kermesina*, 4-colpate apertures; *P. mediterranea* pollen grains were heteromorphic, with 6-12-pantocolpate apertures; *P. misera*, had 12-colporate pollen grains; *P. mucronata*, 6-colpate apertures; *P. pentagona*, 6-colporate apertures (Tab. 1). *Passiflora misera* featured six slightly lolongate endoapertures (Fig. 1E) and *P. pentagona,* presented three single lalongate endoapertures, in each pair of ectoaperture (Fig. 1B-C, Tab. 5).

Opercula

P. alata, P. amethystina, P. edulis, P. farneyi, P. foetida, P. racemosa and *P. setacea* species showed three pseudopercula (Figs. 1H-J, L, 2A, I, K); *P. kermesina*, four pseudopercula (Fig. 2B); *P. pentagona* and *P. mucronata*, three pontopercula (Figs. 1B, C, 2F, G); *P. mediterranea,* six to twelve pseudopercula (Fig. 2D-E); and *P. misera*, six opercula (Fig. 1D, F). After acetolysis, pseudopercula detached from the murus (Fig. 1H) in syncolpate species (*P. alata, P. amethystina, P. edulis, P. farneyi, P. foetida, P. racemosa* and *P. setacea*).

Exine sculpture

Reticulate exine was found in all species; *P. misera* and *P. pentagona* had the lowest lumina (3.0 e 3.6 μm), the other species had larger lumina (7.3-16.3 μm). *P. alata* had the widest murus (4.8 μm) and *P. misera* presented the narrowest murus (0.9 μm) (Tab. 5).

The scanning electron microscopy showed that muri are supported by conspicuous columellae (except in *P. misera* and *P. pentagona*). The upper part of the muri was psilate, in all studied species.

The species of the *Passiflora* (*P. amethystina*, *P. edulis*, *P. farneyi*, *P. foetida*, *P. mediterranea* and *P. racemosa*) subgenus had muri with greater sinuosity (Figs. 1I, K-L, 2A, D-E J); except for *P. alata*, *P. kermesina*, *P. mucronata* and *P. setacea*, which showed less sinuous muri (Figs. 1H, 2C, H, L). On the other hand, the species in the other two subgenera showed straight muri (Fig. 1B, G). *Passiflora mucronata*, *P. misera* and *P. pentagona* showed perforated muri. The lumina are just ornamented in species of the *Passiflora* subgenus. *P. setacea* showed more dense and attached bacula inside the lumina.

Exine thickness widely varied among species. The thickest exine was found in *P. amethystina* (exine: ca. 9.9 μm, sexine: ca. 5.9 μm and nexine: ca. 4.0 μm). The thinnest exine was seen in *P. misera* (exine: ca. 2.4 μm, sexine: ca.1.7 μm, nexine: ca. 0.7 μm).

*Pollen key to pollen grain separation in the studied **Passiflora** species:*

1. Apolar pollen grains
    2. 4-colporate pollen grains .... *P. kermesina* (subg. *Passiflora*)
    2. 6-12-colporate pollen grains .... *P. mediterranea* (subg. *Passiflora*)
1. Isopolar pollen grains
    3. Colporate pollen grains
        4. Large pollen grains, 6 apertures .... *P. misera* (subg. *Decaloba*)
        4. Medium pollen grains, 12 apertures .... *P. pentagona* (subg. *Astrophea*)
    3. Colpate pollen grains
        5. Non-syncolpate apertures .... *P. mucronata* (subg. *Passiflora*)
        5. Syncolpate apertures
            6. Small polar area (ca. 0.29-0.33 μm)
            7. Granula densely together in lumen, exine thickness ca. 5.2 μm .... *P. setacea* (subg. *Passiflora*)
            7. Granula not densely together in lumen, exine thickness ≥ 6.3 μm
                8. Exine thickness ca. 9.9 μm .... *P. amethystina* (subg. *Passiflora*)
                8. Exine thickness ca. 6.3 μm .... *P. edulis* (subg. *Passiflora*)

6. Very small polar area (ca. 0.21-0.25 µm)
  9. Little sinuous reticulum muri, wide (ca. 4.8 µm); exine ca. 4.9 µm .... *P. alata* (subg. *Passiflora*)
  9. Very sinuous reticulum muri, narrow (ca. 1.3-1.9 µm); exine ≥ 6.8 µm
  10. Exine ca. 6.8 µm, colpus width ca. 5.0 µm...*P. racemosa* (subg. *Passiflora*)
  10. Exine ≥ 7.6 µm, colpus width ca. 1.0-1.5µm
    11. Exine ca. 8.1µm, Confidence Interval 95% of equatorial diameter in polar view (EDPV) = 60.9-63.0 µm .... *P. farneyi* (subg. *Passiflora*)
    11. Exine ca. 7.6 µm, Confidence Interval 95% of equatorial diameter in polar view (EDPV) = 79.0-84.5 µm .... *P. foetida* (subg. *Passiflora*)

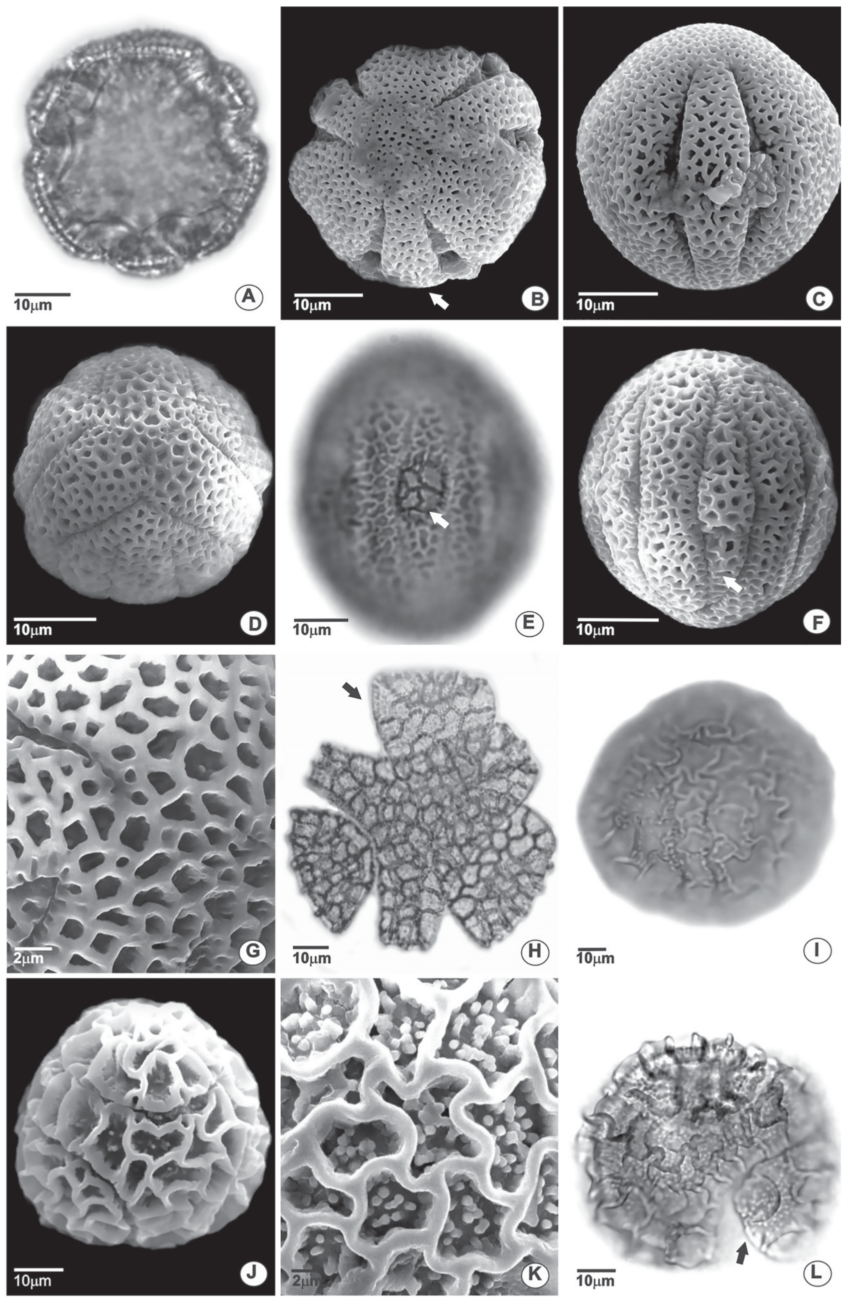

Figure 1. Photomicrographs and electron micrographs of *Passiflora* species pollen grains. A-C. *P. pentagona* – A. optical cut in polar view (LM), B. general aspect in polar view (SEM), C. general aspect in equatorial view (SEM); D-G. *P. misera* – D. general aspect in polar view (SEM), E. endoaperture detail (LM), F. general aspect in equatorial view (SEM), G. ornamentation detail (SEM); H. *Passiflora alata* Curtis – general aspect in polar view, detaching pseudopercula (LM); I. *P. amethystina* Mikan – general aspect in polar view (LM); J-K. *P. edulis* Sims – J. general aspect in polar view (SEM), K. ornamentation detail (SEM); L. *P. farneyi* – general aspect in polar view (LM). Arrows: B = pontopercula, E = endoaperture, F = opercula, H-L = pseudopercula.

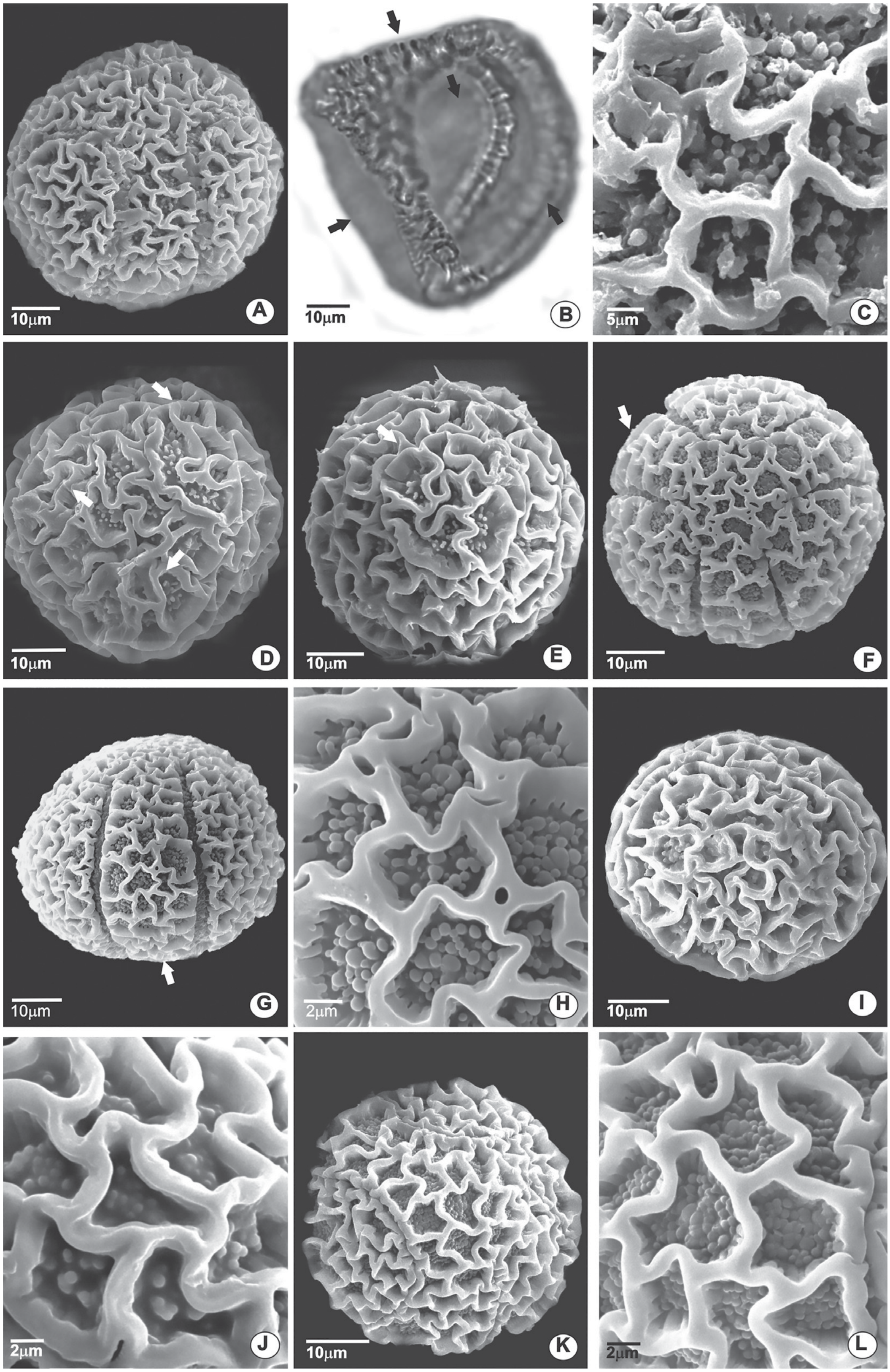

Figure 2. Photomicrographs and electron micrographs of *Passiflora* species pollen grains.
A. *P. foetida* – general aspect in polar view (SEM); B-C. *P. kermesina* – region of the four pseudopercula (LM), B. general aspect in polar view (SEM), C. ornamentation detail (SEM); D-E. *P. mediterranea* – general aspect (SEM); F-H. *P. mucronata* – F. general aspect in polar view (SEM), G. general aspect in equatorial view (SEM), H. ornamentation detail (SEM); I-J. *P. racemosa* – I. general aspect in equatorial view (SEM), J. ornamentation detail (SEM); K-L. *P. setacea* – K. general aspect in polar view (SEM), L. ornamentation detail (SEM). Arrows: B = apertures, D, E = pseudopercula, F, G = pontopercula.

Table 1. Morphological characterization of the analyzed *Passiflora* species pollen grains.

Subgenus	Taxa	Size	Aperture	Endoaperture
Astrophea				
	P. pentagona	medium	6-colporate	lalongate
Decaloba				
	P. misera	large	12-colporate	slightly lolongate
Passiflora				
	P. alata	large	6-sincolpate	absent
	P. amethystina	large	6-sincolpate	absent
	P. edulis	large	6-sincolpate	absent
	P. farneyi	large	6-sincolpate	absent
	P. foetida	large	6-sincolpate	absent
	P. kermesina	large	4-colpate	absent
	P. mediterranea	large	6-12-pantocolpate	absent
	P. mucronata	large	6-colpate	absent
	P. racemosa	large	6-sincolpate	absent
	P. setacea	large	6-sincolpate	absent

Table 2. Measures (in µm) of pollen grains in equatorial view: polar diameter (PD); equatorial diameter (ED), *Passiflora* species (n=25). $\bar{x}\pm s_{\bar{x}}$ – arithmetic mean ± standard deviation; 95% CI – 95% confidence interval; PD/ED – polar:equatorial diameter ratio.

Species	Polar Diameter			Equatorial Diameter			PD/ED	Shape
	Range	$\bar{x}\pm s_{\bar{x}}$	CI 95%	Range	$\bar{x}\pm s_{\bar{x}}$	CI 95%		
P. alata	61.5-67.5	62.6±0.5	61.5-63.7	65.0-75.0	68.9±0.6	67.5-70.2	0.90	oblate spheroidal
P. edulis	67.5-72.5	63.0±1.0	60.9-65.1	67.5-75.0	72.1±0.7	57.7-86.5	0.87	suboblate
P. misera	50.0-62.5	56.9±0.7	55.5-58.3	47.5-57.5	51.4±0.5	50.3-52.5	1.11	prolate spheroidal
P. mucronata	50.0-65.0	57.0±1.0	55.0-59.0	65.0-74.5	68.8±0.6	67.5-69.9	0.83	suboblate
P. pentagona	41.2-52.5	48.6±0.5	47.6-49.6	37.5-47.5	42.6±0.6	41.4-43.8	1.14	subprolate

Table 3. Measures (in µm) of diameter 1 and 2 of apolar pollen grains from *Passiflora* species (n = 25). $\bar{x}\pm s_{\bar{x}}$ – arithmetic mean ± standard deviation; 95% CI – 95% confidence interval; D1/D2 – relation between diameter 1 and diameter 2.

Species	D1			D2		
	Range	$\bar{x}\pm s_{\bar{x}}$	CI 95%	Range	$\bar{x}\pm s_{\bar{x}}$	CI 95%
P. kermesina	60.0-75.0	64.5±0.6	63.3-65.7	60.0-75.0	65.3±0.6	64.1-66.5
P. mediterranea	55.0-87.5	65.5±0.1	61.9-69.0	50.0-80.0	80.8±0.1	78.3-83.2

Table 4. Measures (in µm) of pollen grains in polar view: equatorial diameter in polar view (EDPV), side apocolpium (SA), polar area index (PAI) and exine from *Passiflora* species (n=10, *n=25, for pollen grains which fall preferably in polar view). $\bar{x}\pm s_{\bar{x}}$ – arithmetic mean ± standard deviation; 95% CI – 95% confidence interval.

Species	EDPV			SA		Polar area		Exine		
	Range	$\bar{x}\pm s_{\bar{x}}$	CI 95%	Range	$\bar{x}$	PAI		total	sexine	nexine
P. alata	62.5-72.5	68.5	-	16.2-18.7	17.3	0.25	very small	4.9	2.2	2.7
*P. amethystina**	77.5-90.0	87.4±0.6	86.2-88.6	22.5-27.5	25.0	0.29	small	9.9	5.9	4.0
P. edulis	71.2-80.0	73.6	-	17.5-25.0	22.5	0.31	small	6.3	5.3	1.0
*P. farneyi**	57.5-67.5	62.0±0.5	60.9-63.0	13.0-15.0	14.3	0.23	very small	8.1	4.1	4.0
*P. foetida**	55.0-87.5	81.8±1.3	79.0-84.5	19.0-23.0	19.9	0.24	very small	7.6	4.7	2.9
P. misera	45.0-62.5	50.9	-	18.0-25.0	20.9	0.41	small	2.4	1.7	0.7
P. mucronata	42.5-50.0	45.5	-	20.0-28.0	23.1	0.51	large	8.8	5.8	3.0
P. pentagona	37.5-50.0	44.2	-	22.5-30.0	24.8	0.56	large	2.8	1.7	1.1
*P. racemosa**	62.5-80.0	73.9±0.7	72.3-75.4	12.0-20.0	15.7	0.21	very small	6.8	4.4	2.4
*P. setacea**	62.5-72.5	69.3±0.6	68.0-70.6	17.5-25.0	23.1	0.33	small	5.2	4.1	1.1

Table 5. Measures (in μm) of the apertures, murus width and lumen length of *Passiflora* species pollen grains (n=10).

Species	Ectoaperture/Colpus		Endoaperture		Muri	Lumen
	length	width	length	width	width	diameter
P. alata	---	5.1	---	---	4.8	12.6
P. amethystina	---	4.0	---	---	1.9	14.3
P. edulis	---	1.7	---	---	2.5	16.3
P. farneyi	---	1.5	---	---	1.9	10.3
P. foetida	---	1.0	---	---	1.3	12.8
P. kermesina	---	5.0	---	---	1.6	10.3
P. mediterranea	---	1.7	---	---	2.0	11.9
P. misera	41.0	1.3	9.9	8.5	0.9	3.6
P. mucronata	---	2.0	---	---	1.5	7.3
P. pentagona	36.5	3.8	8.5	21.0	2.5	3.0
P. racemosa	---	5.0	---	---	1.8	11.5
P. setacea	---	5.0	---	---	1.0	10.0

Table 6. Comparison of the main papers already published on *Passiflora* species, focusing on the apertural type.
CPO = colporoidate; CP = colporate; C = colpate ; SC = syncolpate; GC = geminicolpate; PP = pantoporate; PC = pantocolpate.

Species studied	Presting (1965)	Spirlet (1965)	Roubik & Moreno (1991)	García *et al*.. (2002)	Mellhem *et al.* (2003)	Araujo & Santos (2004)	Milward-de Azevedo (2004; 2010)	Dettke & Santos (2009)	Evaldt *et al.* (2011)	Tangarife *et al.* (2011)	Mezzonato-Pires (2013)	Verdasca *et al.* (2013)	Present study
P. alata	-	-	-	-	6C	6SC	-	6C	6SC	-	-	6SC	6SC
P. amethystina	6CPO	6GC	-	-	-	-	-	6C	6SC	-	-	-	6SC
P. edulis	6CPO	6GC	-	-	-	-	-	6C	6SC	6CPO	-	6SC, 8-10 PP	6SC
P. farneyi	-	-					-			-	-	-	6SC
P. foetida	6CP	-	6C	6C	-	6SC	-	-	6SC	-	-	-	6SC
P. kermesina	8C	-	-	-	-	-	-	-	-	-	-	-	4C
P. mediterranea	12C	-	-	-	-		-	-	-	-	-	66SC, 8(10-12) PC	6-12C
P. misera	6CP	-	-	12C	-	6C	12CP	6CP	6CP	-	-	-	12CP
P. mucronata	6CPO	-	-	-	-	-	-	-	-	-	-	-	6C
P. pentagona	-	-	-	-	-	-	-	-	-	-	6CP	-	6CP
P. racemosa	-	6GC	-	-	-	-	-	-	-	-	-	-	6SC
P. setacea	8C	-	-	-	-	6SC	-	-	-	-	-	-	6SC

CPO = colporoidate; CP = colporate; C = colpate ; SC = sincolpate; GC = geminicolpate; PP = pantoporate; PC = pantocolpate

Discussion

The 12 species subordinated to the three palynologically studied subgenera could be initially separated into two major groups according to apertural type (colpate and colporate). The group with colpate aperture can be syncolpate and it coveres most of the species: apolar colpate (*P. kermesina* and *P. mediterranea*) or 6-colpate (*P. mucronata*); all species with colpate aperture belong to the *Passiflora* subgenus. The other group consists of two species, *P. misera* and *P. pentagona*, which belong to the *Decaloba* and *Astrophea* subgenera, respectively. *P. misera* pollen grains are 12-colporate with 6 endoapertures, whereas *P. pentagona* pollen grains are 6-colporate with 3 endoapertures.

The exine in most of the species presented large and very ornamented lumina. *P. setacea* was different, because it had a bigger amount of densely distributed bacula in the lumina in comparison to other species. *Passiflora mucronata* stood out for the presence of perforations; a feature not found in other species from the *Passiflora* subgenus. The ornamentation in *P. misera* and *P. pentagona* species is similar because these species show reticula with smaller and little ornamented lumina and perforated muri.

The herein elaborated pollen key allowed separating the species among subgenera, although some attributes were found in more than one subgenus. *Passiflora* subg. *Passiflora* showed apolar and isopolar pollen grains, besides syncolpate (one species) or non-syncolpate apertures. *Passiflora* subg. *Astrophea* and *Passiflora* subg. *Decaloba* were close in the key. However, they were separated from *Passiflora* subg. *Passiflora,* due to these species colpori-type aperture. Other important features that allowed separating the species regard details of lumen ornamentation, muri sinuosity, polar area and apertures size as well as exine thickness.

Previous studies showed great controversy in describing the types and number of apertures in genus *Passiflora* (see Tab. 6). Such difficulty concerned terminology (colpus, colporus, operculum types) and the level of observation (light microscope x scanning electron microscope). Some of these differences could be especially seen in older articles.

Passiflora alata was already described as having 6-colpate pollen grains (Melhem *et al*. 2003; Dettke & Santos 2009) or as being 6-syncolpate (Araújo & Santos 2004; Evaldt *et al*. 2011), similarly to the pollen grain described in the current paper.

Presting (1965) considered *P. amethystina* to be 6-colporoidate; Spirlet (1965), found it to be 6-geminicolpate; Dettke & Santos (2009), classified it as 6-colpate; and Evaldt *et al*. (2011), as 6-syncolpate; as it was described in the present paper.

According to Presting (1965) and Tangarife *et al.* (2011), *P. edulis* is 6 colporoid; Spirlet (1965) classify it as 6 geminicolpi; the description by Dettke & Santos (2009) find it to be 6-colpi; and, according to Evaldt *et al*. (2011) and the current results, it is 6-syncolpi.

Presting (1965) found 6 colpori in *P. foetida*; Roubik & Moreno (1991) and García *et al.* (2002), 6-colpi; and Araújo & Santos (2004), Evaldt *et al*. (2011) and the current study, classified it as 6-syncolpi.

P. kermesina and *P. mucronata* pollen grains were analyzed by Presting (1965) and the current study. Presting considered *P. kermesina* aperture as 8-colpate and *P. mucronata,* as 6-colporoidate. Results in the current study do not meet those found by Presting (1965), since he considered the presence of 4-colpi in the first species and colpus aperture type in the second specie. Presting (1965) described *P. mediterranea* aperture as 12-colpate; and Verdasca *et al.* (2013), as 6-syncolpate or 8 (10-12) pantocolporate, whereas the current study considered the aperture as 6-12-colporate.

Passiflora misera was considered by Araújo & Santos (2004) as having 6-colpi; it was 6- colpori, according to Presting (1965), Dettke & Santos (2009) and Evaldt *et al.* (2011); 12 colpi, according to García *et al*. (2002); and 12-colpori, according to Milward-de-Azevedo *et al.* (2004; 2010). The current study corroborates the results found by the last authors.

The 6-colporate aperture type found in *P. pentagona* by Mezzonato-Pires (2013) was the same one found by the current study.

Passiflora racemosa pollen grains were described by Spirlet (1965) as 6-geminicolpate. The current study described it as 6-syncolpate.

Passiflora setacea was described by Presting (1965) as having 8-colpi. According to Araújo & Santos (2004) and to the current study it had 6-syncolpi.

P. farneyi is an endemic species in the State of Rio de Janeiro (Bernacci *et al*. 2014), and it is not easily found in the field. Its first pollen grain analysis was done in the current study.

As it was already emphasized by other authors, it is worth analyzing pollen morphology under transmission electron microscope as well as studying its ontogeny to confirm whether or not and when aperture fusion takes place in pollen grains from this family.

As it was evidenced in previous studies, pollen morphology in the Passifloraceae family is very important for the taxonomy of the group. The family is eurypalynous and in-depth knowledge of its features will enable assessing the constituencies and the organization of its infrageneric categories, since such attributes have been completely recorded. The current study is another source of information that, along with other previously published studies, will enable better understanding the already known phylogenetic strains.

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