



Comparative pollen morphological analysis in the subgenera *Passiflora* and *Decaloba*

TALIANE L. SOARES¹, ONILDO N. JESUS^{1*}, EVERTON H. SOUZA^{1,2},
MÔNICA L. ROSSI³ and EDER J. OLIVEIRA¹

¹Embrapa Mandioca e Fruticultura, Rua Embrapa, s/n, Chapadinha, C.P. 007, 44380-000 Cruz das Almas, BA, Brazil

²Universidade Federal do Recôncavo da Bahia, Campus de Cruz das Almas, 44380-000 Cruz das Almas, BA, Brazil

³Universidade de São Paulo, CENA/USP, Av. Centenário, 303, São Dimas, 13400-970 Piracicaba, SP, Brazil

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ABSTRACT

The genus *Passiflora* is the most diversified of the Passifloraceae, and its palynology presents wide morphological variability. The objective of the study was to evaluate the pollen morphology of 18 *Passiflora* species in order to identify informative pollinic characteristics to contribute to the taxonomic classification of the genus. The morphology of the pollen grains and the exine structure were investigated using light microscopy and scanning electron microscopy. Differences in the pollen morphology were found in the studied species, mainly in terms of shape, pollen aperture and ornamentation pattern of the exine. Most of the species belonging to the subgenus *Passiflora* presented ornamented 6-syncolpate pollen grains with an oblate-spheroidal shape and an exine with large lumens. In the subgenus *Decaloba* the pollen grains were 6-colporate, 12-colpate and 12-colporate, with subprolate to prolate-spheroidal shape, as well as an exine with smaller lumen size and few ornamented. The pollen morphology of the species of the subgenera *Passiflora* and *Decaloba* has shown palynological characteristics that have specific diagnostic value, thus allowing a better understanding of the taxonomy of the genus *Passiflora*.

Key words: Passifloraceae, pollen grains, scanning electron microscopy, exine, morphological characterization.

INTRODUCTION

The genus *Passiflora* L. is the most representative of the Passifloraceae, with approximately 576 species (Ocampo and D'Eeckenbrugge 2017) from the tropical and temperate regions of South America (Krosnick et al. 2013). Brazil has approximately 150 species, and most of them are endemic in the

country (Bernacci et al. 2015). This genus was initially divided into 23 subgenera according to Killip (1938) and subsequently reduced to only five: *Astrophea* (DC.) Mast., *Deidamioides* (Harms) Killip, *Decaloba* (DC.) Rechb., *Tetrapathea* (DC.) Reichb. and *Passiflora* L. (Feuillet and MacDougal 2003, Krosnick et al. 2009). However, the two largest subgenera are *Passiflora* (~ 250 species) and *Decaloba* (~ 230 species) (Porter-Utley 2014).

Due to the great morphological diversity, pollen grains have been used for taxonomic purposes, assisting in the morphological characterization

Correspondence to: Onildo Nunes de Jesus

E-mail: onildo.nunes@embrapa.br

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and identification of some species to generate phylogenetic information that more accurately expresses the origin and evolution of the organisms (Judd et al. 2007). The pollen characteristics are fundamental for taxonomic classification because the pollen grains present the shape, size, color and ornamentation of the exine defined for each species, genus and family (Mert 2010, Fazal et al. 2013, Silvério and Mariath 2014, Mezzonato-Pires et al. 2015a, Silva et al. 2016).

Passifloraceae is very interesting from the palynological point of view because the pollen grains present great phenotypic variability for several characteristics, some that have still not been explored according to systematic and phylogenetic aspects (Dettke and Santos 2009). Mohl (1834) and Fritzsche (1837) were the first researchers to use the pollen morphology of *Passiflora* species for taxonomic purposes, highlighting the shape and ornamentation as good taxonomic characteristics. Based on these studies, some other research have focused on palynology in the differentiation of *Passiflora* and/or species (Amela Garcia et al. 2002, Milward-de-Azevedo et al. 2010, 2014a, Gabarayeva et al. 2013, Mezzonato-Pires et al. 2015a,b). However, few studies simultaneously analyzed different subgenera of the Passifloraceae (Mezzonato-Pires et al. 2015b). Therefore, the objective of this work was to evaluate the pollen morphology of eighteen *Passiflora* species in order to identify informative pollinic characteristics to contribute to the taxonomic classification of the genus.

MATERIALS AND METHODS

PLANT MATERIAL

Eighteen *Passiflora* species, represented for two subgenera according the classification of Feuillet and MacDougal (2003) were analyzed (Table I; Figure 1): *Passiflora alata* Curtis. (BGP-004, HURB-20749), *P. gibertii* N. E. Br. (BGP-

008, HURB-20753), *P. edulis* Sims f. *flavicarpa* Degener (BGP-038, HURB-20754), *P. edmundoi* Sacco (BGP-046, HURB-20745), *P. tenuifila* Killip (BGP-105, HURB-20750), *P. morifolia* Mast. (BGP-107, HURB-20740), *P. galbana* Mast. (BGP-109, HURB-20743), *P. muchronata* Sessé & Moc. (BGP-114, HURB-20848), *P. rubra* L. (BGP-125, HURB-20752), *P. suberosa* L. (BGP-152, HURB-20742), *P. foetida* L. (BGP-153, HURB-20741), *P. malacophylla* Mast. (BGP-170, HURB-20747), *P. racemosa* Brot. (BGP-172, HURB-20744), *P. setacea* L. (BGP-237, HURB-20755), *P. cincinnata* Mast. (BGP-312, HURB-20746), *P. maliformis* L. (BGP-379, HURB-20756), *P. subrotunda* L. (BGP-394, HURB-20751) and *P. coccinea* Aubl. (BGP-408, HURB-20748). These species belonging to the Active Germplasm Bank of Passion fruit (AGP) from Embrapa Mandioca e Fruticultura located in Cruz das Almas, Bahia Brazil (12° 48' 38" S and 39° 06' 26" O; 220 m). Specimen of each taxon were deposited in the HURB herbarium (Universidade Federal do Recôncavo da Bahia).

In this study, we followed the two taxonomic treatments of genus *Passiflora* proposed by Killip 1938 (with emends by Escobar 1988, 1989, 1994; and MacDougal 1994) by Feuillet and MacDougal (2003) as a point of comparison and discussion of our data. Most of these species were preserved in greenhouses, except for *P. setacea*, *P. maliformis* and *P. alata*, which were preserved under field conditions. Each *Passiflora* species was represented by one accession identified by the BGP code.

MORPHOPOLLINIC CHARACTERIZATION

For the morphopollinic characterization, the pollen grains were fixed in modified Karnovsky's solution (Karnovsky 1965) [glutaraldehyde (2%), paraformaldehyde (2%), CaCl₂ (0.001 M), sodium cacodylate buffer (0.05 M), pH 7.2] for 48 hours and then dehydrated in increasing concentrations of ethanol (35-100%) for 20 minutes each. The

TABLE I
List of 18 *Passiflora* species of the genus *Passiflora* L. used in this study.

Access	Species	Subgenus	Supersection/section or series	Pollinator	2n	Compat.* Systematic
BGP-004	<i>P. alata</i> Curtis	<i>Passiflora</i> L.	<i>Laurifolia</i> (Cervi) Feuillet & MacDougal/ <i>Quadrangulares</i> Feuillet & MacDougal	Bees	18	SI
BGP-008	<i>P. gibertii</i> N. E. Br.	<i>Passiflora</i> L.	<i>Stipulata</i> Feuillet & MacDougal/ <i>Granadillastrum</i> Triana & Planch.	Bees	18	SI
BGP-038	<i>P. edulis</i> Sims f. <i>flavicarpa</i> Deg.	<i>Passiflora</i> L.	<i>Passiflora/Passiflora</i>	Bees	18	SI
BGP-046	<i>P. edmundoi</i> Sacco	<i>Passiflora</i> L.	<i>Stipulata</i> Feuillet & MacDougal / <i>Kermesinae</i> Killip ex Cervi	Hummingbirds	18	SI
BGP-105	<i>P. tenuiflora</i> Killip	<i>Passiflora</i> L.	<i>Passiflora/Passiflora</i>	Bees	18	SC
BGP-107	<i>P. morifolia</i> Mast.	<i>Decaloba</i> D.C Rchb.	<i>Bryonioides</i> (Harms) MacDougal & Feuillet/ <i>Cieca</i> (Medic.) MacDougal & Feuillet	Wasps	12	SC
BGP-109	<i>P. galbana</i> Mast.	<i>Passiflora</i> L.	<i>Passiflora/Simplicifoliae</i> (Harms) Killip	Bats	18	SI
BGP-114	<i>P. muchronata</i> Sessé & Moc.	<i>Passiflora</i> L.	<i>Passiflora/Simplicifoliae</i> (Harms) Killip	Bats	18	SI
BGP-125	<i>P. rubra</i> L.	<i>Decaloba</i> D.C Rchb.	<i>Decaloba</i> (DC.) MacDougal & Feuillet/ <i>Xerogona</i> (Raf.) Killip	Wasps	12	SC
BGP-152	<i>P. suberosa</i> L.	<i>Decaloba</i> D.C Rchb.	<i>Decaloba</i> (DC.) MacDougal & Feuillet/ <i>Cieca</i> (Medic.) MacDougal & Feuillet	Wasps	24	SC
BGP-153	<i>P. foetida</i> L.	<i>Passiflora</i> L.	<i>Stipulata</i> Feuillet & MacDougal/ <i>Dysosmia</i> DC.	Bees	18, 20, 22	SC
BGP-170	<i>P. malacophylla</i> Mast.	<i>Passiflora</i> L.	<i>Passiflora/Serratifoliae</i>	Bees	18	SI
BGP-172	<i>P. racemosa</i> Brot.	<i>Passiflora</i> L.	<i>Stipulata</i> Feuillet & MacDougal/ <i>Calopathanthus</i> Harms	Bees	18	SI
BGP-237	<i>P. setacea</i> L.	<i>Passiflora</i> L.	<i>Passiflora/Setaceae</i> Killip ex Cervi	Bats	18	SI
BGP-312	<i>P. cincinnata</i> Mast.	<i>Passiflora</i> L.	<i>Passiflora/Passiflora</i>	Bees	18	SI
BGP-379	<i>P. maliformis</i> L.	<i>Passiflora</i> L.	<i>Laurifolia</i> (Cervi) Feuillet & MacDougal/ <i>Laurifoliae</i> Killip ex Cervi	Bees	18	SI
BGP-394	<i>P. subrotunda</i> L.	<i>Passiflora</i> L.	<i>Passiflora/Simplicifoliae</i>	Bees	18	SI
BGP-408	<i>P. coccinea</i> Aubl	<i>Passiflora</i> L.	<i>Coccinea</i> Feuillet & MacDougal	Hummingbirds	18	SI

Taxonomic classification, their pollinators, diploid numbers, compatibility systematic, according to Feuillet and MacDougal (2003), Melo et al. (2001), Melo and Guerra (2003), Soares et al. (2015). *SI = self-incompatible, SC = self-compatible.

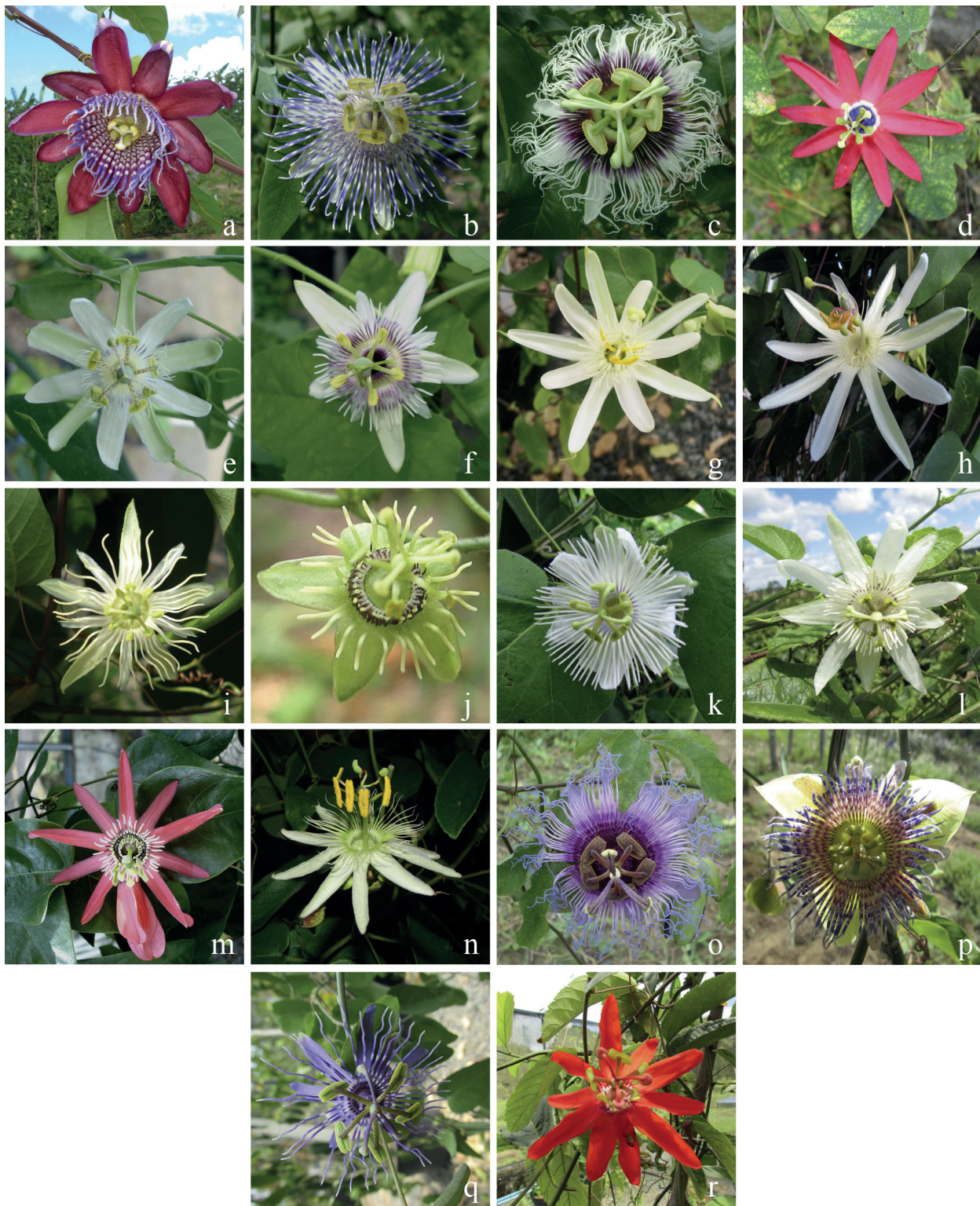


Figure 1 - General view of flowers of 18 *Passiflora* species used in this study: **a)** *P. alata*, **b)** *P. gibertii*, **c)** *P. edulis* f. *flavicarpa*, **d)** *P. edmundoi*, **e)** *P. tenuifila*, **f)** *P. morifolia*, **g)** *P. galbana*, **h)** *P. muchronata*, **i)** *P. rubra*, **j)** *P. suberosa*, **k)** *P. foetida*, **l)** *P. malacophylla*, **m)** *P. racemosa*, **n)** *P. setacea*, **o)** *P. cincinnata*, **p)** *P. maliformis*, **q)** *P. subrotunda* and **r)** *P. coccinea*.

samples were dried out in hexamethyldisilazane, and the dried samples were then mounted on metallic supports (stubs), metallized with gold for 180 s using a sputter coater (MED 010, Balzers Union, Balzers, Liechtenstein). The images were obtained using the LEO 435 VP variable pressure scanning electron microscope (Carl Zeiss, Jena, Germany), at 20 kv.

The pollen grains were submitted to weak lactic acetolysis (ACLAC 40), according to Raynal and Raynal (1979), and the assessments were obtained using the ImageJ 1.46r program (Rasband 1997-2016). Quantitative characters, polar (PD) and equatorial (ED) diameters of 25 pollen grains per species were measured (μm) at random in equatorial view. We also computed the shape (PD/ED ratio), number and aperture type, and exine ornamentation of the pollen grains. The pollen grains were characterized according to the nomenclature described by Punt et al. (2007) and Hesse et al. (2009), and for the general description of the pollen openings, we adopted the definition of Presting (1965).

The pollen grains were photographed digitally using the Olympus microscope (DM1000; Leica Microsystems, Wetzlar, Germany) coupled with a Sony (Sony, Tokyo, Japan) video camera and a microcomputer using Image Pro-plus Software version 3.0 for Windows (Media Cybernetics, Inc., Bethesda, MD, USA).

DATA ANALYSIS

Qualitative (aperture and shape) and quantitative data were submitted to multivariate analysis to determine genetic distance using the Gower distance (1971). The validation of the clusters was determined by the cophenetic correlation (cc) (Sokal and Rohlf 1962). The R (R Development Core Team 2015) program was used to obtain the genetic distance matrix and cophenetic correlation. A dendrogram was generated using the MEGA

5.0 program (Tamura et al. 2011) based on the distance matrix and the clustering method UPGMA (unweighted pair group method with arithmetic means).

RESULTS

Pollen grains of *Passiflora* were characterized by their morphological characteristics (shape, number and aperture type, and exine ornamentation) (Table II and Figs. 2, 3, 4). The multivariate analysis of the 18 *Passiflora* species allowed for the formation of three groups (Fig. 2) using the mean genetic dissimilarity ($D_{dg} = 0.36$) as a cutoff. The cophenetic correlations, which express the relationship between the original distances and graphically distances was 0.88 ($P < 0.0001$, 10,000 permutations). Some authors have affirmed that cophenetic correlation above 0.8 shows the adequacy of the clustering method to represent the genetic diversity among the assessed individuals (Rohlf and Fisher 1968).

The majority of *Passiflora* species were allocated in Group 1 (G1): *P. alata*, *P. gibertii*, *P. edulis*, *P. edmundoi*, *P. muchronata*, *P. racemosa*, *P. setacea*, *P. galbana*, *P. maliformis*, *P. subrotunda*, *P. cincinnata*, *P. tenuifila* and *P. foetida* (Table II and Fig. 2). All species of Group 1 belong to the subgenus *Passiflora* and form a homogeneous palynological complex, mainly in terms of the shape, size and type of the pollen grain openings (Table II and Figs. 2, 3, 4, 5a-i).

The palynological analysis performed using light microscopy on the accessions of Group 1 (G1) showed the presence of large pollen grains ranging from 50.2 μm to 88.9 μm (major axis - polar diameter), oblate spheroidal shape and 6-syncolpate aperture (six colpos fused in pairs in the apocolpus region). The pollen grains of this group were classified as isopolar, of circular scope, with a thick heteroreticulate exine, columellate and simple muri, and with sinuous and apparent

columelles (Table II and Figs. 3a-o, 4a-o, 5a-f). The most notable pollen characteristic that distinguishes the species of this group is the presence of bacula inserted into the lumen, with some variation in the number and height of the bacula. Some species have large lumens with many bacula inserted in their interior (Figs. 3c, 3f, 3i, 4c, 4f, 4i, 4l, 5c, 5f, 5i, 5l, 5r, 5o), and others presented few bacula (Fig. 3l, 3o, 3r, 4o). Although belonging to the subgenus *Passiflora* (supersection *Coccinea*), *P. coccinea* presented an exclusive shape suboblate type and was therefore allocated in Group 2 (G2), which has this characteristic as the main differential (Table II and Fig. 5g-i).

The species (*P. morifolia*, *P. suberosa*, and *P. rubra*) belonging to the subgenus *Decaloba* formed Group 3 (G3) (Table II, Fig. 2). The pollen grains of this subgenus ranged from 47.2 to 72.5 μm , being considered large, with distinct pollen aperture (6-colporate - *P. morifolia*, 6-colpate - *P. suberosa* and 12-colporate - *P. rubra*) (Fig. 5j-r). Regarding the classification of pollen grains based on the PD/ED ratio, these were categorized as subprolate (*P. suberosa* and *P. morifolia*) and spheroidal prolate (*P. rubra*) (Table II).

Differences in the subgenus *Decaloba* in terms of the colpi sculpture and the number of bacula inserted in the lumen (Fig. 5j-r) were observed from the evaluation of the pollen grains in the electron microscopy. *P. rubra* showed a reticulated exine with sinuous muri, apparent columelles and few bacula inserted in the lumens (Fig. 5p-r). The pollen grains of *P. morifolia* also presented reticulum with sinuous muri, without apparent columelles and with many bacula inserted in the lumen (Fig. 5m-o). However, in *P. suberosa*, the exine of the pollen grains is characterized by presence of reticulum with flat muri and absence of bacula into the lumen (Fig. 5j-l). Another pollen grain characteristic that differentiated these species was the lumen size, which was smaller in *P. suberosa* when compared to *P. morifolia* and *P. rubra*. In addition, the pollen

grains of *P. rubra* and *P. morifolia* presented three mesocolpus, and *P. suberosa* presented six mesocolpus.

DISCUSSION

The pollinic characteristics are useful to differentiate and establish relationships between different species (Fazal et al. 2013, Amorim et al. 2014) because they are genetically determined and very constant and specific to each species (i.e., pollen grain size, number of apertures, aperture type and exine ornamentation) and therefore have a high taxonomic value (Mert 2010, Tangarife et al. 2011).

The pattern of clustering based on the pollinic characteristics allowed to classify the species into two subgenera, *Passiflora* and *Decaloba*, congruent with the classification of Feuillet and MacDougal (2003) and supported by phylogenetic studies using molecular markers and sequencing technique (Yockteng and Nadot 2004, Hansen et al. 2006, Mäder et al. 2010, Porter-Utley 2014, Milward-de-Azevedo et al. 2014b, Muschner et al. 2012, Krosnick et al. 2013, Ocampo and D'Eeckenbrugge 2017). These authors revealed that the *Passiflora* and *Decaloba* subgenera showed monophyletic clades.

In the present study, the subgenus *Passiflora* was represented by 15 species (*P. alata*, *P. gibertii*, *P. edulis*, *P. edmundoi*, *P. tenuifila*, *P. galbana*, *P. muchronata*, *P. foetida*, *P. setacea*, *P. cincinnata*, *P. malacophylla*, *P. racemosa*, *P. maliformis*, *P. subrotunda* and *P. coccinea*). This subgenus is characterized by leaves entire or lobed; glands on petioles, stipules, and margins of leaves; hypanthium tubular or campanulate; operculum tubular or filamentous; membranous lumen, herbaceous vines or woody lianas, free serrate bracts, large colorful flowers, attract as pollinators bees, bats and hummingbirds, are diploid with $2n = 18$ and self-incompatible according to described

TABLE II
Morphopollinic characterization of 18 *Passiflora* species belonging to the subgenera *Passiflora* and *Decaloba*.

Accessions	Species	Polar Diameter (PD)		Equatorial Diameter (ED)		PD/ ED	Shape	Size	Aperture	Group*
		VF	$\bar{x} \pm s_x$	VF	$\bar{x} \pm s_x$					
BGP-004	<i>P. alata</i>	85.1 – 63.1	71.0 ± 8.0	83.5 – 59.7	73.3 ± 9.2	0.97	oblate-spheroidal	Large	6-syncolpate	G1
BGP-008	<i>P. gibertii</i>	80.5 – 64.0	71.7 ± 6.3	83.4 – 61.8	72.1 ± 7.6	0.99	oblate-spheroidal	Large	6-syncolpate	G1
BGP-038	<i>P. edulis</i>	76.4 – 63.4	68.6 ± 3.7	77.5 – 63.7	71.3 ± 5.0	0.98	oblate-spheroidal	Large	6-syncolpate	G1
BGP-046	<i>P. edmundoi</i>	78.6 – 77.6	78.2 ± 0.2	83.6 – 82.4	83.1 ± 0.3	0.94	oblate-spheroidal	Large	6-syncolpate	G1
BGP-105	<i>P. tenuifila</i>	82.1 – 79.5	80.8 ± 1.8	82.0 – 78.9	80.4 ± 2.1	0.94	oblate-spheroidal	Large	6-syncolpate	G1
BGP-107	<i>P. morifolia</i>	72.5 – 58.0	62.9 ± 3.9	58.4 – 52.7	54.6 ± 1.3	1.15	subprolate	Large	6-colporate	G3
BGP-109	<i>P. galbana</i>	88.0 – 87.1	87.6 ± 0.4	89.8 – 89.5	89.7 ± 0.3	0.98	oblate-spheroidal	Large	6-syncolpate	G1
BGP-114	<i>P. muchronata</i>	88.9 – 87.4	88.1 ± 0.3	90.7 – 89.4	90.1 ± 0.3	0.97	oblate-spheroidal	Large	6-syncolpate	G1
BGP-125	<i>P. rubra</i>	62.5 – 47.2	52.9 ± 5.1	63.2 – 46.0	52.4 ± 5.4	1.01	prolato-spheroidal	Large	12-colporate	G3
BGP-152	<i>P. suberosa</i>	69.1 – 58.2	64.1 ± 2.9	66.8 – 51.0	54.9 ± 3.2	1.16	subprolate	Large	12-colpate	G3
BGP-153	<i>P. foetida</i>	73.5 – 64.2	67.3 ± 2.6	76.2 – 66.7	69.7 ± 2.9	0.96	oblate-spheroidal	Large	6-syncolpate	G1
BGP-170	<i>P. mallacophyla</i>	81.7 – 78.4	79.4 ± 0.8	82.0 – 78.2	80.5 ± 0.8	0.99	oblate-spheroidal	Large	6-syncolpate	G1
BGP-172	<i>P. racemosa</i>	83.4 – 79.1	81.6 ± 0.9	84.2 – 80.2	82.8 ± 0.9	0.99	oblate-spheroidal	Large	6-syncolpate	G1
BGP-237	<i>P. setacea</i>	80.3 – 55.8	69.0 ± 5.8	86.2 – 68.1	75.0 ± 5.9	0.92	oblate-spheroidal	Large	6-syncolpate	G1
BGP-312	<i>P. cincinnata</i>	81.6 – 79.8	80.6 ± 0.5	87.8 – 86.4	87.1 ± 0.3	0.92	oblate-spheroidal	Large	6-syncolpate	G1
BGP-379	<i>P. maliformis</i>	72.0 – 58.1	64.9 ± 3.4	72.30 – 57.4	65.7 ± 4.5	0.99	oblate-spheroidal	Large	6-syncolpate	G1
BGP-394	<i>P. subrotunda</i>	78.5 – 58.1	68.0 ± 5.2	80.2 – 63.20	71.1 ± 5.3	0.96	oblate-spheroidal	Large	6-syncolpate	G1
BGP-408	<i>P. coccinea</i>	61.9 – 50.2	55.3 ± 3.4	76.8 – 64.2	69.6 ± 4.6	0.79	suboblate	Large	6-colporate	G2

VF = range of the characteristic, \bar{x} = overall mean, s_x standard deviation of the mean; *groups formed by the clustering analysis.

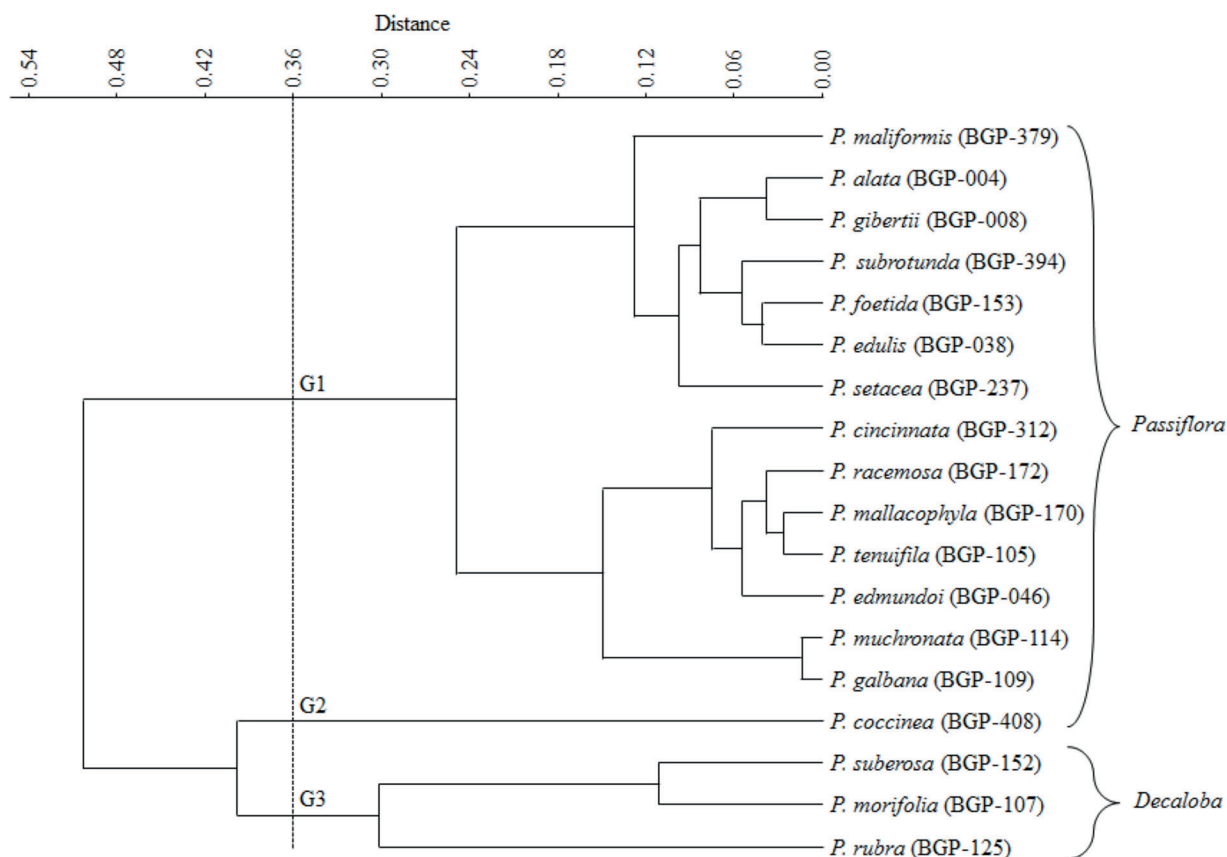


Figure 2 - Dendrogram of the 18 *Passiflora* species, obtained by UPGMA (unweighted pair group method using arithmetic averages) based on the Gower distance, using the qualitative and quantitative morphopollinic characteristics.

by Feuillet and MacDougal 2003, 2004, Melo and Guerra 2003, Santos et al. 2015, Ocampo and D'Eeckenbrugge 2017 (Table I). However, subgenus *Decaloba* is represented by *P. morifolia*, *P. rubra* and *P. suberosa*, characterized by the absence of ocelli on the leaf blades, by the absence of bracts, and by capsular fruits. The species present small flowers (< 4 cm diameter) with one or two series of corolla filaments, a folded operculum, usually white or greenish and small fruits, diploid $2n = 12, 24$, according to described by Ulmer and MacDougal 2004, Milward-de-Azevedo 2014b (Table I).

In general, it was observed that the majority of the *Passiflora* species presented apertural membrane strongly ornamented with a large number

of densified bacula inserted into the lumen. This characteristic may indicate a mechanism against dehydration, mainly during pollination, since the great majority of the analyzed species presented a relatively large apertural area. Among the species of the subgenus *Passiflora*, *P. edulis* was the one that presented pollen grains with large lumens with few bacula inserted inside (Fig. 3r), while in the species of the subgenus *Decaloba*, *P. suberosa* was the only one that did not present bacula inserted into the lumen (Fig. 3l).

The pollinic characteristics based on the presence or absence of bacula are important for the taxonomic delimitation of the subgenus *Decaloba* in the species from southwest Brazil (Milward-de-Azevedo et al. 2004). Indeed, some pollinic

characteristics based on the presence or absence of the bacula have been used as a palynotaxonomic resource of great importance for phylogenetic studies in the Passifloraceae (Presting 1965).

Regarding the appearance of the exine surface of the *Passiflora* pollen grains, lipophilic substances were found between the free reticulum columelles, called pollenkitt (Fig. 3g). This substance has important functions during the pollen grain dispersion because it aids in the adhesion of the pollen grains to the body of the pollinating agent. The pollenkitt can also protect against dehydration and UV radiation damage, as well as with the pollen grain recognition system on the stigmatic surface (Pacini and Hesse 2005). Other authors also observed the presence of pollenkitt in the exine of the pollen grains of *Passiflora* (Souza et al. 2004, Soares et al. 2013, Silvério and Mariath 2014).

The classification of the pollen grain shape according to the PD/ED ratio, it was observed that the majority of the species of *Passiflora* belonging to supersections: *Laurifolia*, *Stipulata* and *Passiflora* (Feuillet and MacDougal 2003) presented a PD/ED ratio above 0.90 and were therefore classified as oblate-spheroidal, with the exception of *P. coccinea* (supersection *Coccinea*), which presented a PD/ED ratio of 0.79 and a suboblate shape. This major difference could lead to the *P. coccinea* species becoming more distant from other species of the subgenus *Passiflora* being allocated in Group 2. Previous studies based on taxonomic progress and phylogenetic analyses using molecular markers particularly over the last 11 years by Muschner et al. (2003), Yockteng and Nadot (2004), and Hansen et al. (2006) did not support the classifications into supersections, sections, and series.

In contrast, in the subgenus *Decaloba* represented by *P. morifolia*, *P. rubra* and *P. suberosa* belonging to two supersections (*Bryonioides* and *Decaloba*) according to classification proposed by Feuillet and MacDougal (2003) presented a subprolate pollen grain shape was observed for *P.*

morifolia and *P. suberosa*, while a prolate-spheroidal shape of the pollen grain was observed in *P. rubra*, similar to the results described by Milward-de-Azevedo et al. (2010). Some authors have affirmed that the oblate-spheroidal, prolate-spheroidal and spheroidal shapes confer the Passifloraceae with one of their main palynological characteristics and may help in the taxonomic classification of this group (Mezzonato-Pires et al. 2015b).

Other descriptors of high value diagnostic for studies taxonomic in *Passiflora* are those related to the pollen grain morphology and the pollen aperture (Dettke et al. 2009, Mezzonato-Pires 2015a,b). The wide morphological diversity of the Passifloraceae has enabled the creation of different types of pollen. Presting (1965) identified typical pollen grains for the species of the Passifloraceae using the reticulum pattern (lumen diameter and presence or absence of bacula) and exine thickness as criteria. On the other hand, Araújo and Santos (2004) chose pollen types based on the operculum variations (elliptic and pontoperculum operculum) and sizes of the reticles lumens. In the present study, the pollen types were typified based on the apertural pattern, shape of the pollen grains and the muri characteristics with the presence or absence of bacula in the lumen.

However, previous studies have shown contradictory results due to the complexity of the pollen grains of the Passifloraceae species, especially in relation to the pollen grain size and the type and number of pollen grains. The pollen aperture in *P. alata* was described in the literature as being 6-colpate (Dettke and Santos 2009) or 6-syncolpate (Araújo and Santos 2004, Evaldt et al. 2011). For *P. edulis*, Tangarife et al. (2011) classified the pollen aperture as 6-colporoid, while Dettke and Santos (2009) described it as 6-colpate. Recently, Mezzonato-Pires et al. (2015b) classified the pollen aperture of *P. alata*, *P. racemosa*, *P. edulis*, *P. foetida* and *P. setacea* as 6-syncolpate and that of *P. mucronata* as 6-colpate. The results

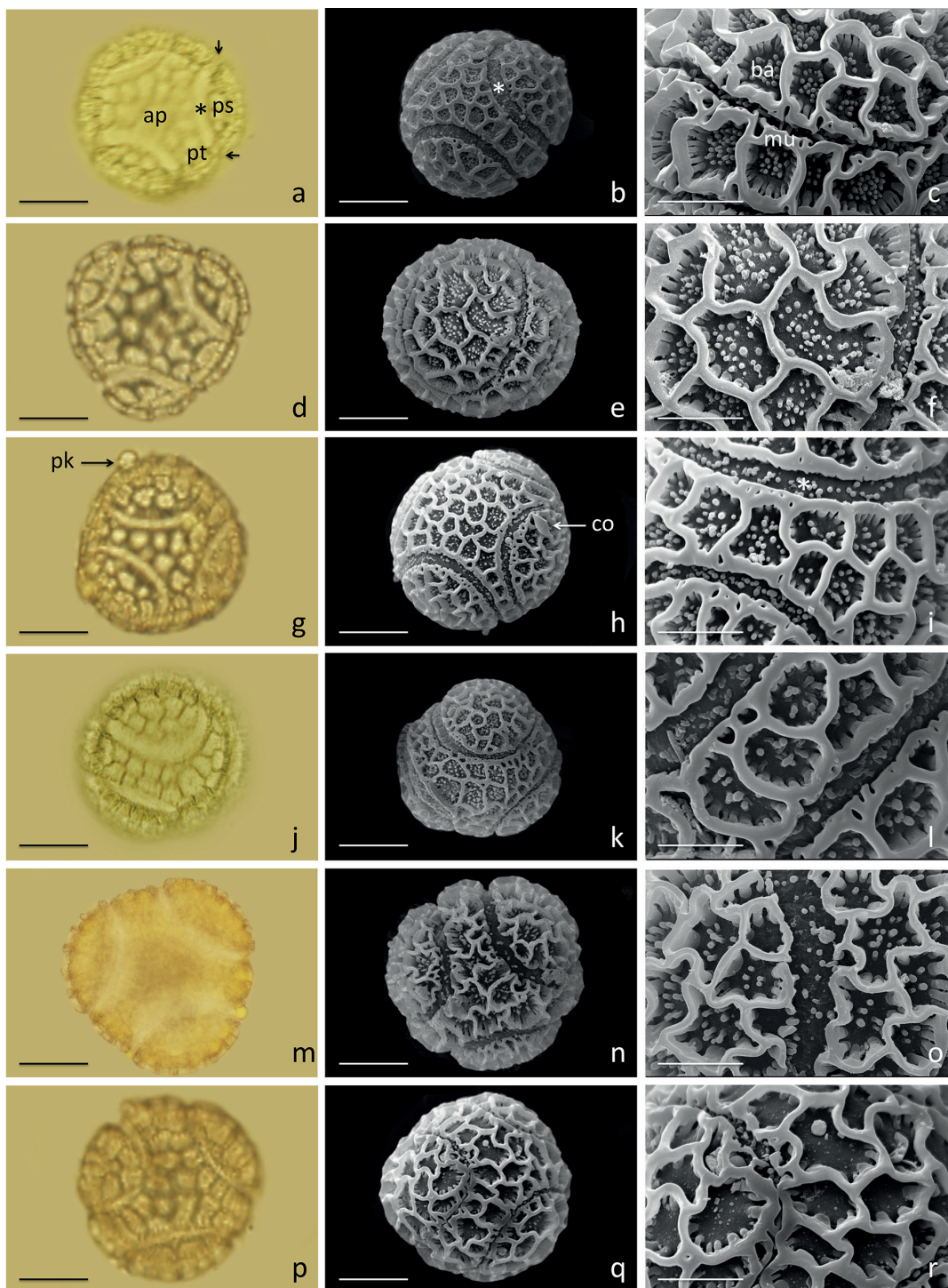


Figure 3 - Pollen grains of *Passiflora* using a light microscope (LM) and a scanning electron microscope (SEM). **a-c)** *P. maliformis* (BGP-379); **d-f)** *P. alata* (BGP-004); **g-i)** *P. gibertii* (BGP-008); **j-l)** *P. subrotunda* (BGP-394); **m-o)** *P. foetida* (BGP-153); **p-r)** *P. edulis* (BGP-038); a, d, g, j, m, p) LM overview; b, e, h, k, n, q) SEM overview; c, f, i, l, o, r) details of the exine and colpi using an SEM. ap: apocolpus; ba: bacula; co: calcium oxalate crystals; mu: muri; pk: pollenkitt; ps: pseudopericulum; pt: pontopericulum; arrow: apertures; *fusion of the apertures within the apocolpus. Bars: a, b, d, e, g, h, j, k, m, n, p, q = 20 μ m; c, f, i, l, o, r = 10 μ m.

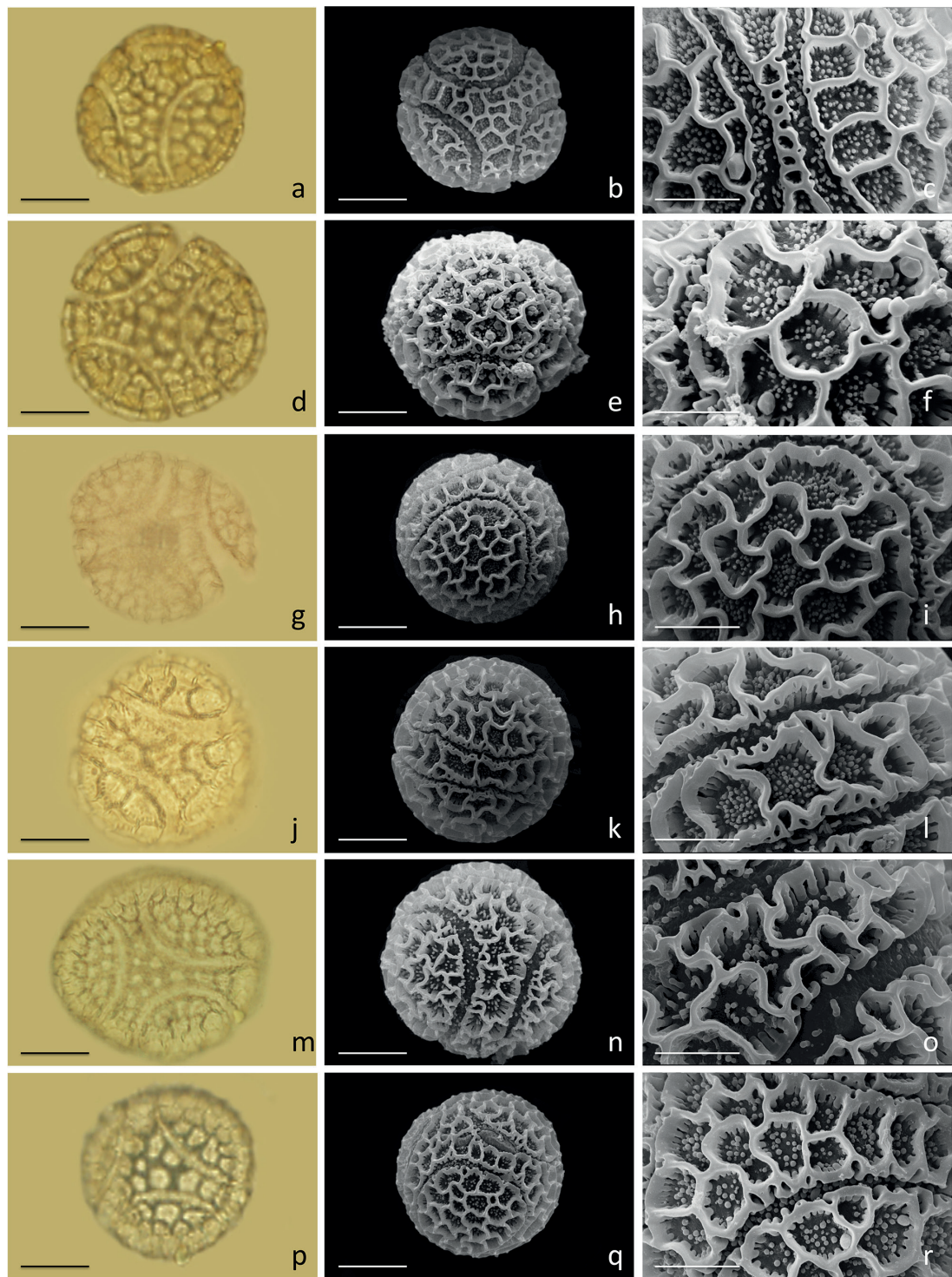


Figure 4 - Pollen grains of *Passiflora* using a light microscope (LM) and a scanning electron microscope (SEM); **a-c**) *P. setacea* (BGP-237); **d-f**) *P. cincinnata* (BGP-312); **g-i**) *P. racemosa* (BGP-172); **j-l**) *P. mallacophyla* (BGP-170); **m-o**) *P. tenuifila* (BGP-105) **p-r**); *P. edmundoi* (BGP-046); a, d, g, j, m, p) LM overview; b, e, h, k, n, q) SEM overview; c, f, i, l, o, r) details of the exine and colpi using an SEM. Bars: a, b, d, e, g, h, j, k, m, n, p, q = 20 μ m; c, f, i, l, o, r = 10 μ m.

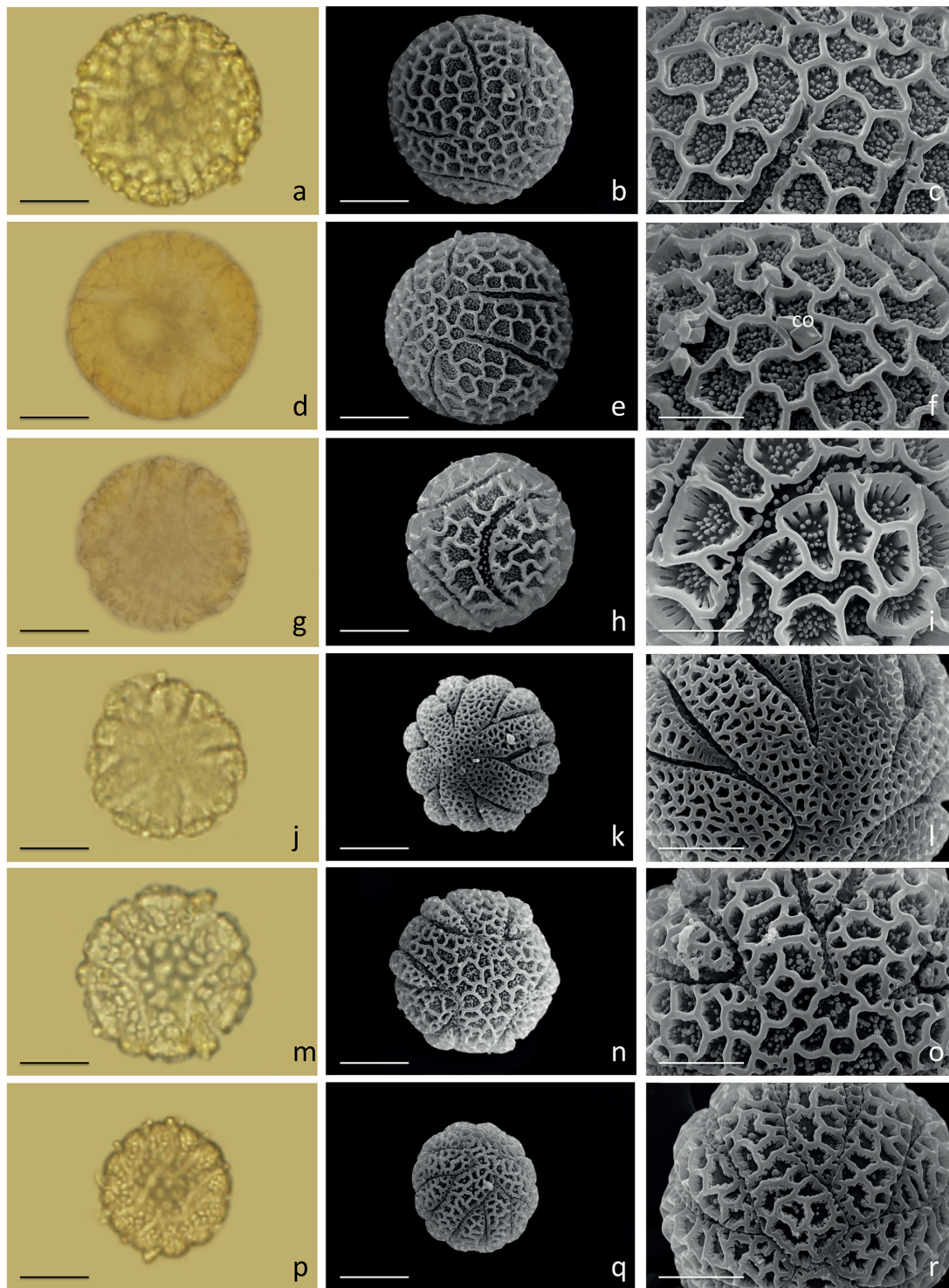


Figure 5 - Pollen grains of *Passiflora* using a light microscope (LM) and a scanning electron microscope (SEM); **a-c**) *P. muchronata* (BGP-114); **d-f**) *P. galbana* (BGP-109) **g-i**); *P. coccinea* (BGP-408); **j-l**) *P. suberosa* (BGP-152); **m-o**) *P. morifolia* (BGP-107); **p-r**) *P. rubra* (BGP-125); a, d, g, j, m, p) LM overview; b, e, h, k, n, q) SEM overview; c, f, i, l, o, r) details of the exine and colpi using an SEM. co: calcium oxalate crystals. Bars: a, b, d, e, g, h, j, k, m, n, p, q = 20 μ m; c, f, i, l, o, r = 10 μ m.

related to the type of ornamentation and pollen aperture found by the authors were partially similar to those obtained in the present study, except for *P. mucronata*, with 6-syncolpate aperture. Studies carried out by other authors have shown that there is some divergence in the pollen morphology in the majority of *Passiflora* species, with differences in the pollen grain number, type of pollen aperture and nomenclatures adopted for the pollen grain structures. This divergence likely occurs because the studies of pollen characterization of *Passiflora* deal with descriptions based mainly on the external aspect of the exine, which contributes to several interpretations (Dettke 2009, Evaldt et al. 2011).

For the characteristics shape, number and aperture type of the pollen grains of the subgenus *Decaloba*, there is a consensus in the pollen descriptions for *P. rubra* (Milward-de-Azevedo et al. 2010, 2014a, Amorim et al. 2014). However, this was not observed for the other two species of the subgenus *Decaloba*, *P. morifolia* and *P. suberosa*, which had pollen grains classified as 6-colporate (Dettke and Santos 2009), 12-colpate (Amela García et al. 2002, Milward-de-Azevedo et al. 2004), oblate (Silvério and Mariath 2014), prolate-spheroidal (Milward-de-Azevedo et al. 2004) and subprolate (Milward-de-Azevedo et al. 2010). The results reported by these authors were similar to those observed in the present work for *P. suberosa* and *P. morifolia* in terms of the pollen grains subprolate format and the 12-colporate pollen opening.

However, our results demonstrate relative congruence with most of the taxonomic affinities related to the subgenera *Passiflora* studied and show that the classification of the *Passiflora* species can be carried out based on morphological characteristics of the pollen grains, thus identifying morphopollinic patterns (i.e., pollen grain shape, pollen apertures and ornamentation of the exine), which can contribute to the taxonomy of this genus.

Most of the species studied belong to the

subgenus *Passiflora* ($2n = 18$) area presents self-incompatible, while those of the subgenus *Decaloba* are self-compatible ($2n = 6$) (Melo et al. 2016). Although there is no chromosome homology between these subgenera, many interspecific hybrids of *Passiflora* have been successfully obtained, because in many cases the barriers of interspecific incompatibility are relatively fragile (Meletti et al. 2005, Soares et al. 2015). Thus, the *Passiflora* species used in the present work have great potential in interspecific hybridization programs with the purpose of introgression of resistance genes in yellow passion fruit (*P. edulis*), which is the main commercial species in Brazil. Among the interesting characteristics present in some evaluated species are: resistance to foliar diseases (Santos et al. 2015, Jesus et al. 2016, Freitas et al. 2016a) and soil (Freitas et al. 2016b) and the presence of self-compatibility which can increase productivity and decrease variation in fruit size among plants in commercial plantations. In addition, interspecific hybrids among the wild species studied have shown ornamental potential (Bugallo et al. 2011, Santos et al. 2012, 2014, Ocampo et al. 2016, Melo et al. 2016, Coelho et al. 2016).

It is interesting to note that although the pollen morphology analysis may be useful in taxonomic studies of the *Passiflora* genus, it should only be considered an additional tool for species classification to increase our understanding of the systematics of the genus, for re-evaluating the circumscriptions and arrangements of the infrageneric categories currently established, and for better understanding the phylogenetic lineages of the *Passiflora*. Further studies should be carried out under the light transmission electron microscopy and ontogeny of the structures that compose the pollen grain sporoderm of the *Passiflora*, enabling better understanding of the exine ornamentation and probably highlighting other traits that may be useful to the classification of the taxa.

CONCLUSIONS

The morphology of Passifloraceae pollen grains can be used as a morphological descriptor to establish the taxonomy and phylogenetic relationships of the species of this family. However, future studies involving transmission electron microscopy will be necessary to enable better understanding of the exine ornamentation, probably highlighting other palynological characteristics that can be used in the delimitation of the taxa.

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