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Palynological characterization of species from the families Cactaceae and Melastomataceae

Caracterização palinológica de espécies das famílias Cactaceae e Melastomataceae

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ABSTRACT
Pollen grains can be classified by the ir morphological characteristics such as the number of openings, ornamentation, size and structure of the exine. Aiming to contribute to the palynological characterization of the Cactaceae and Melastomataceae families, the pollen morphology of two Melastomataceae species was analyzed – *Medinilla speciosa* Blume and *Pleroma candolleanum* (Mart. ex DC.) –, and three Cactaceae species – *Gymnocalycium anisitsii* (K.Schum.), *Mammillaria polythele* Mart. and *Pilosocereus leucocephalus* (Poselg.) Byles & G.D. Rowley. From field trips, unopened buds were collected, whose anthers were removed, macerated and acetolyzed, resulting in slides that were analyzed using light microscopy and SEM (scanning electron microscopy). The Melastomataceae pollen grains studied are in monads, small, tricolporate-tripseudocolpate, subtriangular, suboblate, microverrucate. The pollen grains of Cactaceae are in monads, medium-large, tricolpate (two parasyncolpate), circular, suboblate-oblatospheroidal, perforated granulated and microverrucate.
Keywords: morphology, Palynology, pollen.

RESUMO
Os grãos de pólen podem ser classificados por suas características morfológicas, tais como o número de aberturas, ornamentação, tamanho e estrutura da exina. Visando contribuir com a caracterização palinológica das famílias Cactaceae e Melastomataceae, foi analisada a morfologia polínica de duas espécies de Melastomataceae – *Medinilla speciosa* Blume e *Pleroma candolleanum* (Mart. ex DC.) – e três espécies de Cactaceae – *Gymnocalycium anisitsii* (K.Schum.), *Mammillaria polythele* Mart. e *Pilosocereus leucocephalus* (Poselg.) Byles & G.D.Rowley. Em saídas a campo, foram coletados botões fechados, cujas anteras foram retiradas, maceradas, acetolisadas, resultando em lâminas que foram analisadas em microscopia de luz e MEV (microscopia eletrônica de varredura). Os grãos de pólen de Melastomataceae estudados são em mônade, pequenos, tricolporados-tripseudocolpados, subtriangulares, suboblatos, microverrucados. Os grãos de pólen de Cactaceae são em mônade, médios-grandes, tricolpados (dois parassincolpados), circulares, suboblatos-oblatoesferoidais, granulados perfurados e microverrucados.
Palavras-chave: morfologia, Palinologia, pólen.

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INTRODUCTION

Pollen represents the male gamete of flowers and when it fertilizes the ovules contained in the ovary, it transforms them into seeds, which are necessary for the perpetuation of the species. The

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number of pollen grains is greatly influenced by environmental conditions, so any stress that the plant may suffer may impair their formation (LOPES *et al*., 2017).

Cactaceae is a family represented by a great diversity of succulent plants, popularly known as cacti, which stand out for their evolutionary and adaptive capacities to more arid regions (CAVÉQUIA, 2020). Melastomataceae are characterized by having stamens with pedoconnective (poorly developed or reduced in some genera), appendages when present only ventral and paired, and capsular fruits that contain cochlear seeds with curved embryos (MICHELANGELI *et al*., 2013).

Palynological characterization is of great importance to expand taxonomic and environmental conservation studies, among other research fields. However, there is a lack of pollen morphological studies of species from the Cactaceae and Melastomataceae families. Thus, this study aims to expand the morphological data of pollen grains from the Cactaceae and Melastomataceae families through pollen characterization.

MATERIAL AND METHODS

During field trips, botanical species were collected, including two from the Melastomataceae family, *Medinilla speciosa* Blume and *Pleroma candolleanum* (Mart. ex DC.) Triana, and three species from the Cactaceae family, *Gymnocalucium anisitsii* (K.Schum.) Britton & Rose, *Mammillaria polythele* Mart, and *Pilosocereus leucocephalus* (Poselg.) Byles & G.D.Rowley.

A search was conducted for the existence of pollen studies on the taxa sampled. After classification and identification of the plants, the collected specimens were herborized. Closed buds of the species were removed and preserved in acetic acid (MELHEM, 1978). Just before acetolysis, the buds were opened, the anthers were removed and macerated in a petri dish, and then subjected to the acetolysis process (placed in acid solutions and specific substances and centrifuged) (ERDTMAN, 1960). The acetolyzed pollen grains were mounted in glycerinated gelatin on thin light microscope slides (five slides per species) (BARTH, 1965). The slides were placed in the pollen collection library of the Univille Bee Laboratory (Label).

The slides were observed under a light microscope (400x) with the aid of the “Dino-Eye Capture 2.0” software. The pollen grains on the slides were photographed, with 25 repetitions for each type of view (equatorial and polar), measuring the following parameters in micrometers (µm): equatorial diameter and polar axis, in addition to exine thickness (BARTH & MELHEM, 1988). The grains were classified based on characteristics such as pollen unit, size, scope, symmetry, polarity, shape, openings, ornamentation (PUNT *et al*., 2007). The measurements were tabulated (Microsoft Excel), with the aim of obtaining the size range (Xmin-Xmax), the arithmetic mean and the standard deviation of the arithmetic mean (SALGADO-LABORIAU, 1973). The data were assembled in tables, with the respective measurements of the pollen grains in the polar and equatorial views, measurement of the exine thickness and other characteristics (pollen unit, size, scope, symmetry, polarity, shape, openings, ornamentation) (SALGADO-LABORIAU, 2007).

The pollen grains were photographed using scanning electron microscopy at the Universidade do Estado de Santa Catarina (Udesc), for better analysis and observation of the exine ornamentation.

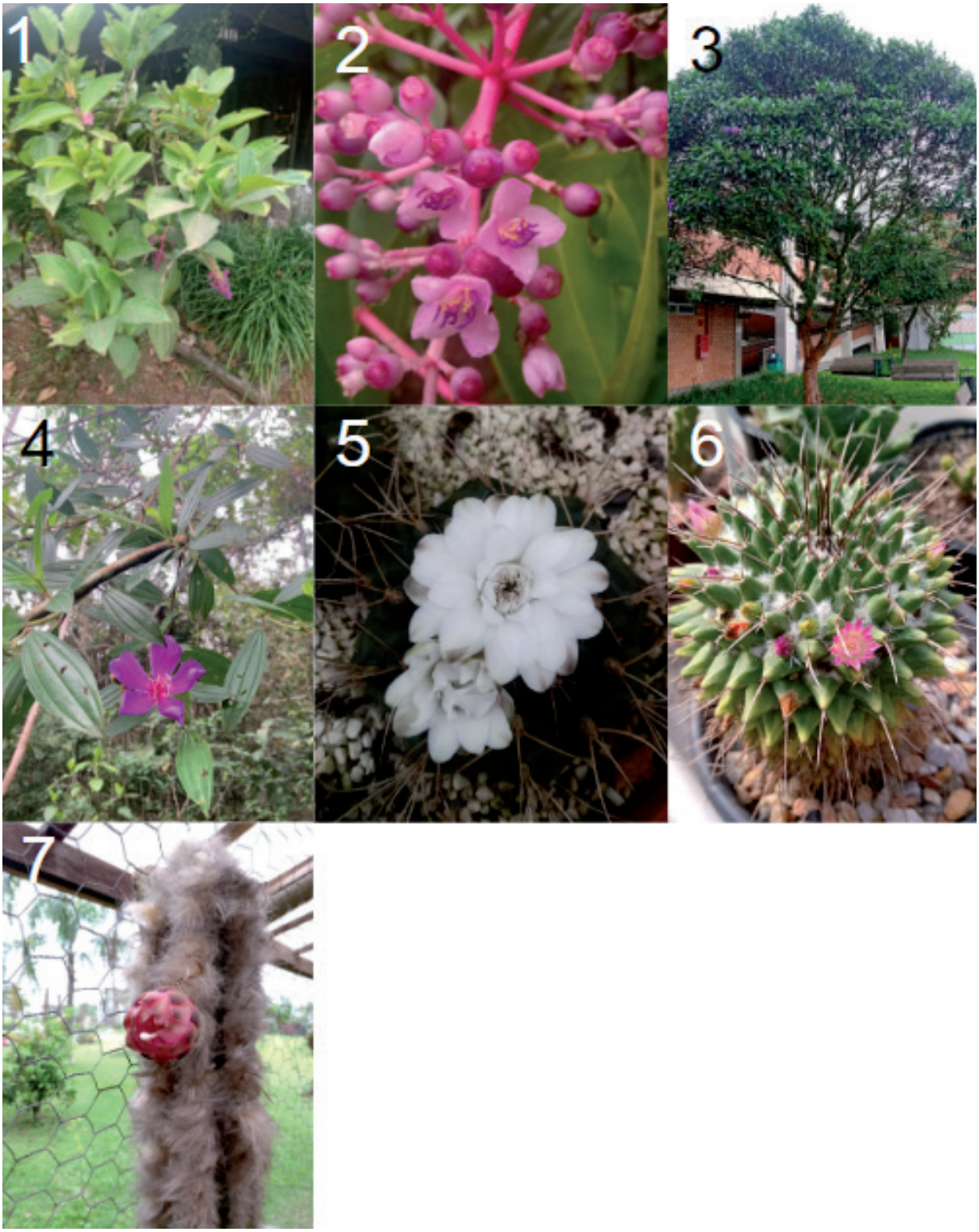

Figure 1 – Images of the studied species: 1) *Medinilla speciosa*, whole plant; 2) *Medinilla speciosa*, flower; 3) *Pleroma candolleanum*, whole plant; 4) *Pleroma candolleanum*, flower; 5) *Gymnocalycium anisitsii*, flower; 6) *Mammillaria polythele*, whole plant with flower; 7) *Pilosocereus leucocephalus*, plant and flower. Source: primary.

RESULTS

The results are presented in tables 1 and 2. Figure 2 shows general views, in images 1, 2, 3, 4 and 5. All pollen grains are isopolar monads with radial symmetry.

The grains of Melastomataceae species are small (*Medinilla speciosa* and *Pleroma candolleanum*), with both species being 3-colporate and pseudocolpate with subtriangular scope. The shape and thickness of the exine of these species are subprolate (*M. speciosa* [P= 14.311 µm; E=17.672 µm; ex. 2.15 µm], *P. candolleanum* [P= 18.465 µm; E=22.429 µm; ex.= 1.95 µm]). For Cactaceae species (*Gymnocalycium anisitsii, Mammillaria polythele* and *Pilosocereus leucocephalus*), the grain size varies between medium and large. The species present pollen grains with three openings, with a circular scope. The shape and thickness of the exine vary between oblate-spheroidal and suboblate (*G. anisitsii* [P= 28.198 µm; E=34.647 µm; ex. 2.27 µm], *M. polythele* [P=37.822 µm; E=42.108 µm; ex. 2.52 µm], *P. leucocephalus* [P=61.664 µm; E=73.302 µm; ex. 3.26 µm]).

Table 1 – Morphometric data of the pollen grain of the analyzed species. Abbreviations: P/E = polar axis/ equatorial diameter.

	P/E	Shape	Exine (µm)	Apertures
Medinilla speciosa	0,80	Suboblate	2,15	3-colporate 3-pseudocolpate
Pleroma candolleanum	0,82	Suboblate	1,95	3-colporate 3-pseudocolpate
Gymnocalycium anisitsii	0,81	Suboblate	2,27	3-colpate
Mammillaria polythele	0,89	Oblate-spheroidal	2,52	3-colpate parassincolpate
Pilosocereus leucocephalus	0,84	Suboblate	3,26	3-colpate parassincolpate

Table 2 – Morphometric data of the pollen grain of the analyzed species.

	Ornamentation	Âmb	Polar axis mean (µm)	Equatorial diameter mean (µm)	Size
Medinilla speciosa	Microverrucate	Subtriangular	14,311	17,672	Small
Pleroma candolleanum	Microverrucate	Subtriangular	18,465	22,429	Small
Gymnocalycium anisitsii	Granulate	Circular	28,198	34,647	Medium
Mammillaria polythele	Microverrucate perfurated	Circular	37,822	42,108	Medium
Pilosocereus leucocephalus	Granulate perfurated	Circular	61,664	73,302	Great

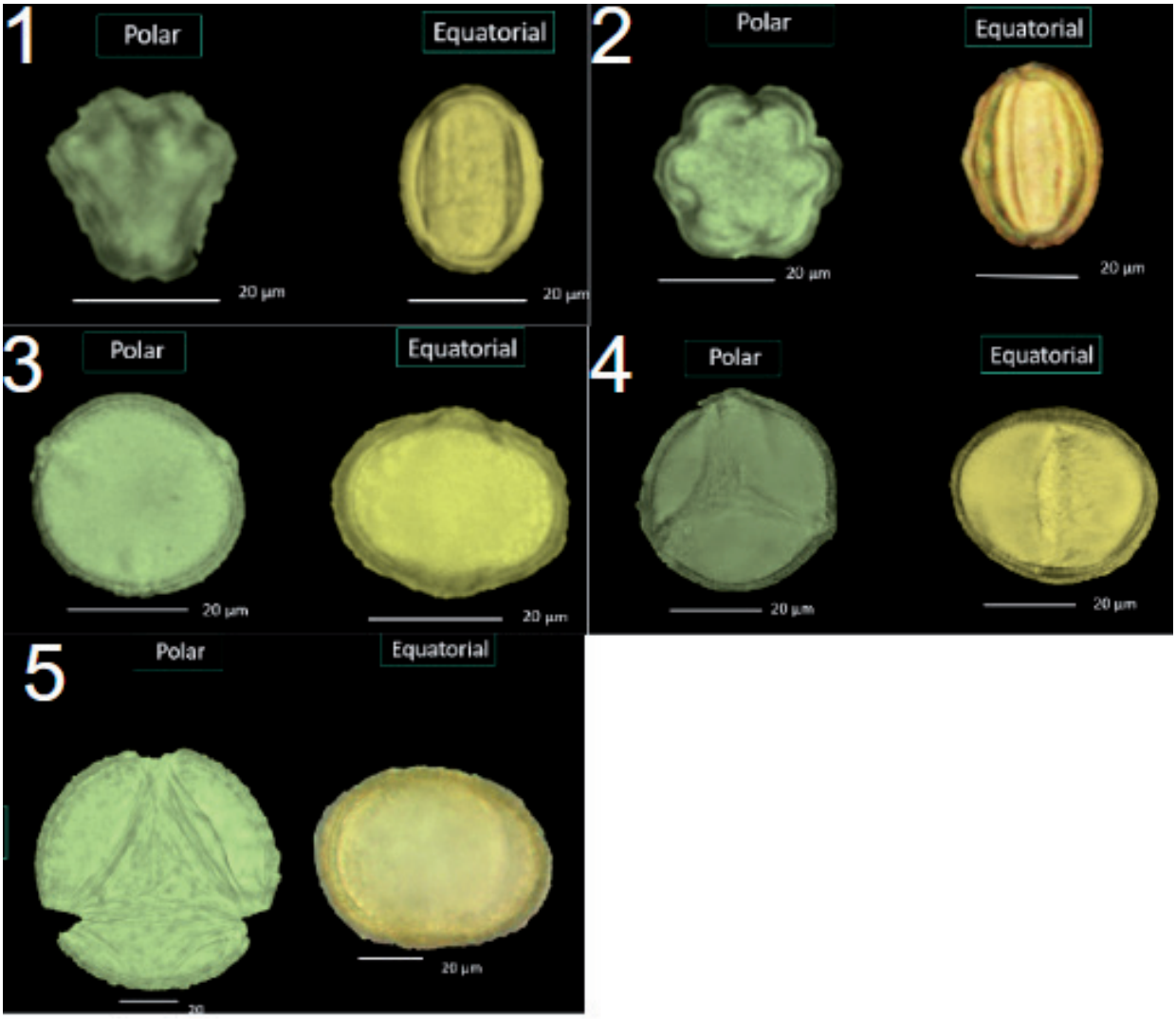

Figure 2 – Images of the pollen grains of the analyzed species: 1) *M. speciosa*; 2) *P. candolleanum*; 3) *G.anisitsii*; 4) *M. polythele*; 5) *P. leucocephalus.* Source: primary.

The images 1A, 2A, 3A, 4A of the figure 3 show the apertures and the ornamentation of the exine of the species of the family Melastomataceae (*Medinilla speciosa* and *Pleroma candolleanum*) on stained grains.

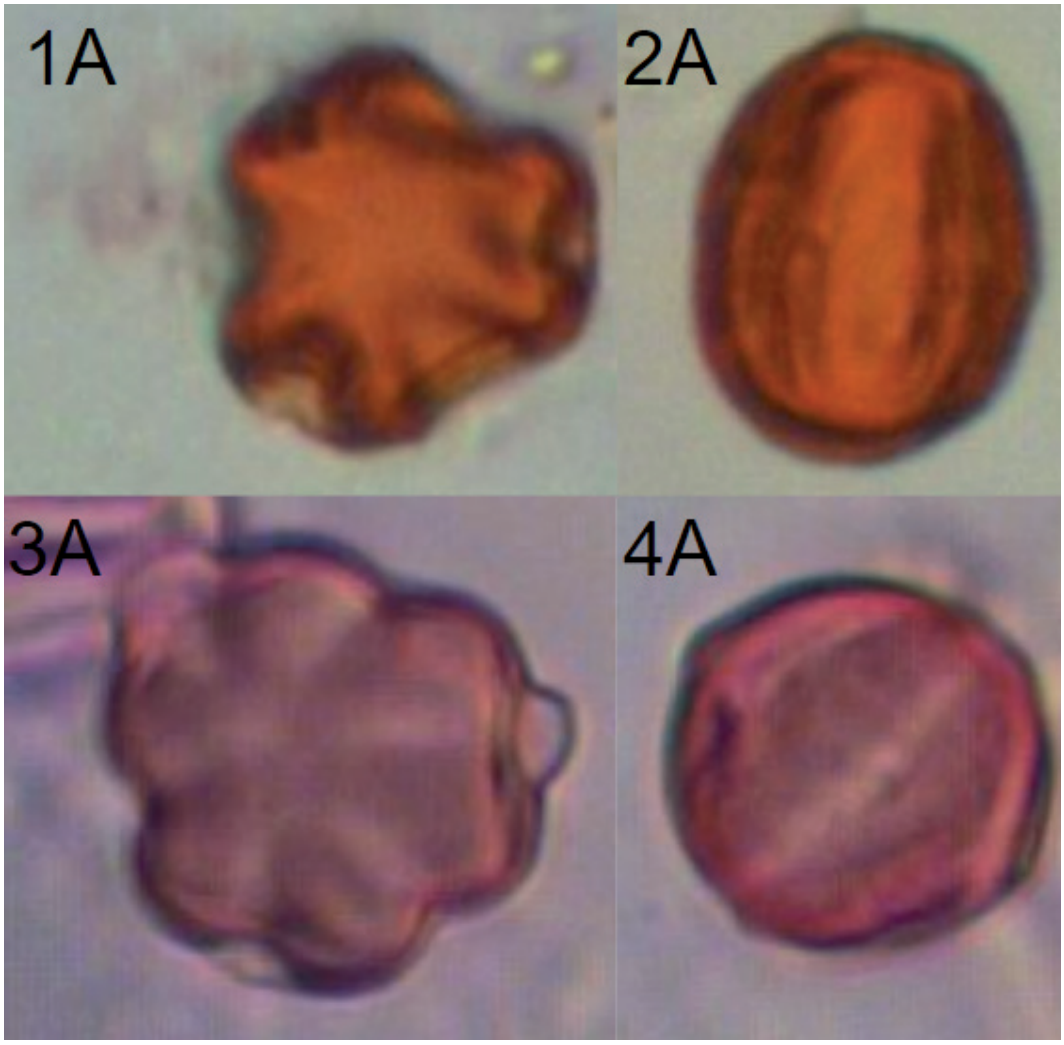

Figure 3 – Stained pollen grains of the species of Family Melastomataceae: 1A and 2A) *M. speciosa*; 3A and 4A) *P. candolleanum*. Source: primary.

The images 1B, 2B, 3B, 4B, 5B e 6B of figure 4 show pollen grains of species of Cactaceae (*M. polyhele*, *G. anisitsii*, and *P. leucocephalus*) stained, evidencing the ornamentation of the exine.

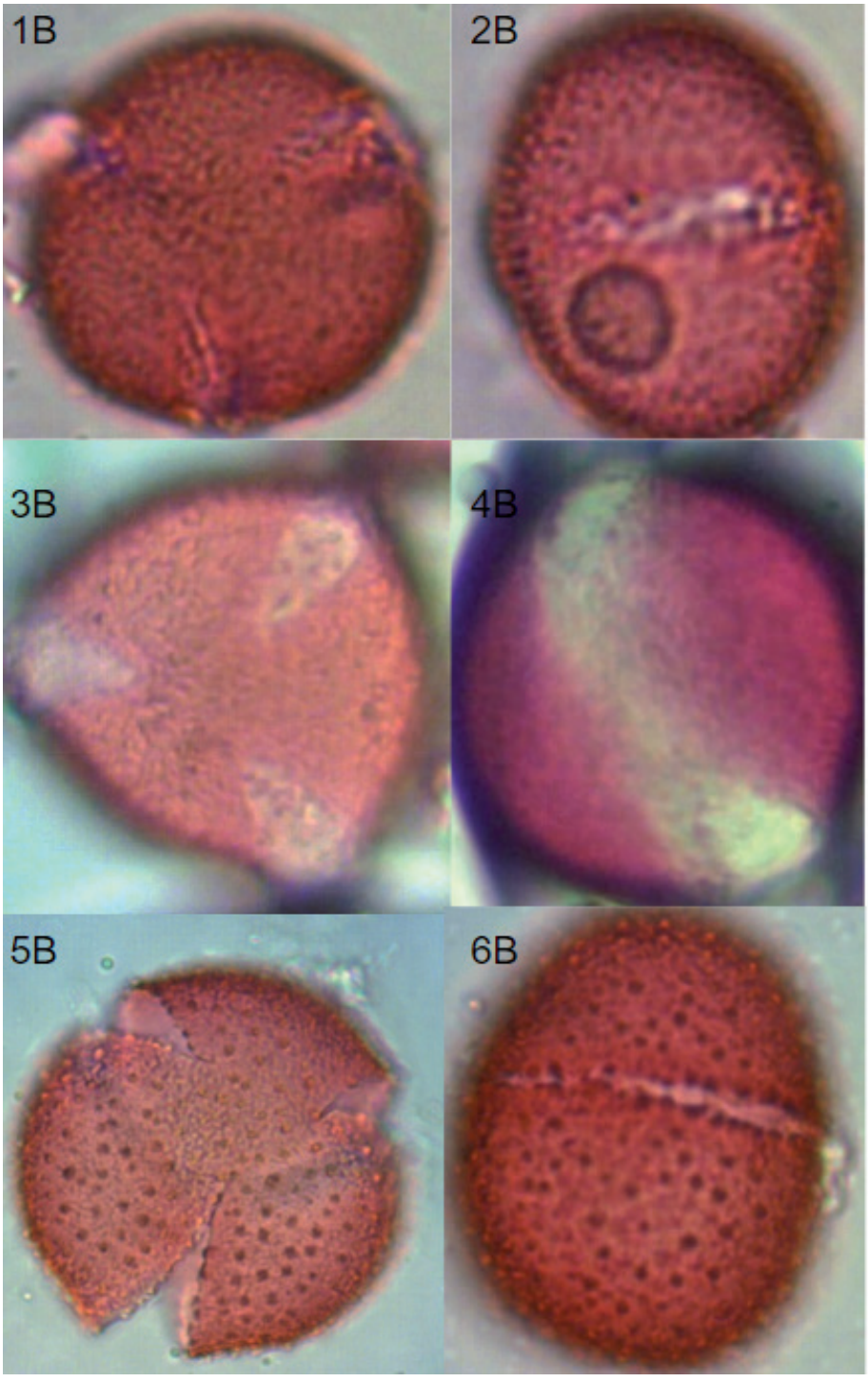

Figure 4 – Stained pollen grains: 1B and 2B) *G. anisitsii*; 3B and 4B) *M. polythele*; 5B and 6B) *P. leucocephalus.* Source: primary.

The grains of the mentioned species were subjected to scanning electron microscopy at the State University of Santa Catarina (Udesc). The images 1C, 2C, 3C and 4C correspond to the species *Medinilla speciosa* (figure 5), the images 1D, 2D, 3D and 4D correspond to the species *Pleroma candolleanum* (figure 6), the images 1E, 2E, 3E and 4E represent the species *Gimnocalycium anisitsii* (figure 7), the images 1F, 2F, 3F and 4F correspond to the species *Mammillaria polythele* (figure 8) and the images 1G, 2G and 3G refer to the species *Pilosocereus leucocephalus* (figure 9).

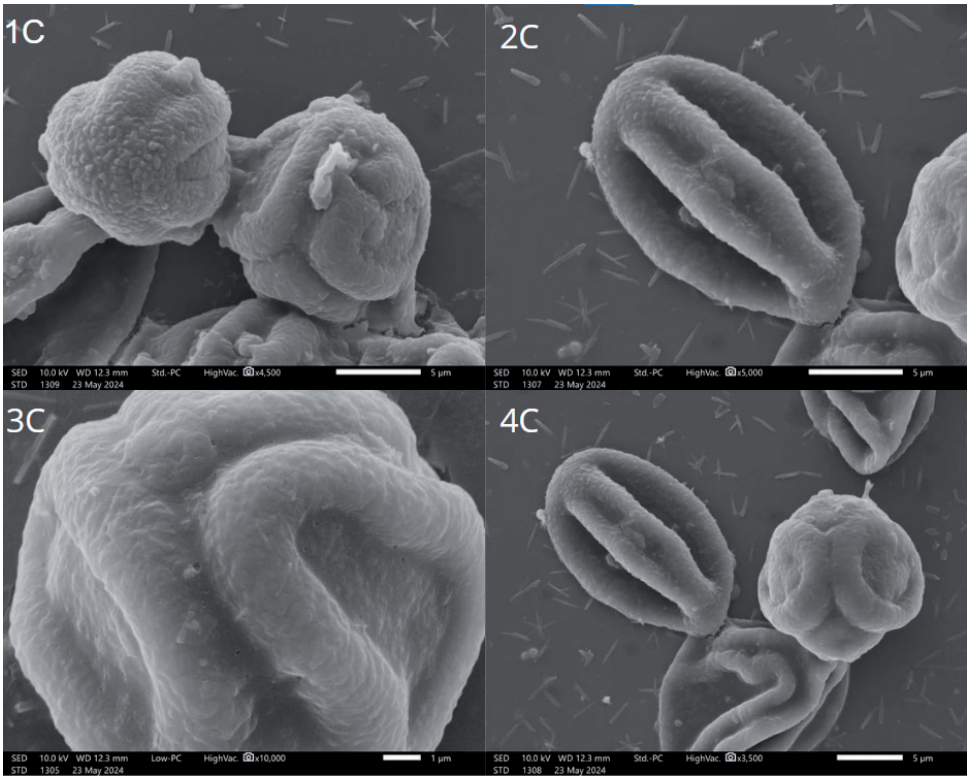

Figure 5 – Pollen grains of the species *M. speciosa* seen under SEM: 1C) polar axis; 2C) equatorial diameter; 3C) polar axis (exine); 4C) pollen grains in polar axis and equatorial diameter. Source: primary.

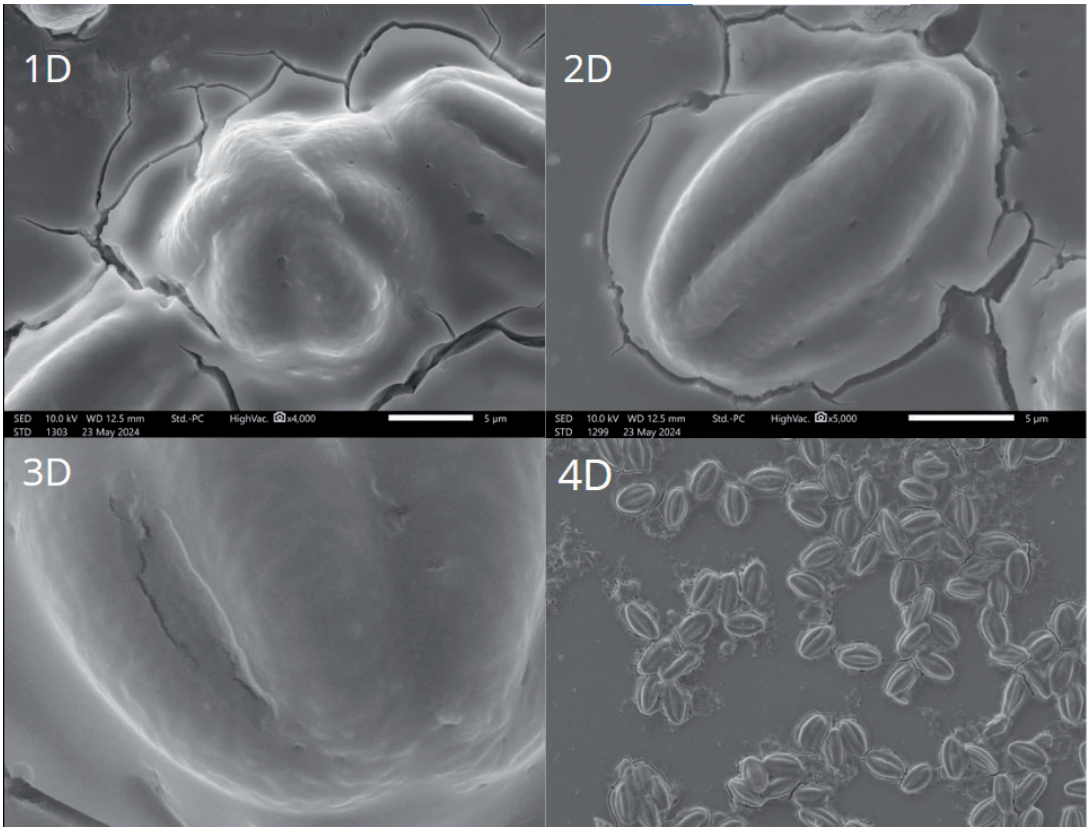

Figure 6 – Pollen grains of the species *P. candolleanum* seen under SEM: 1D) polar axis; 2D) equatorial diameter; 3D) exine; 4D) pollen grains. Source: primary.

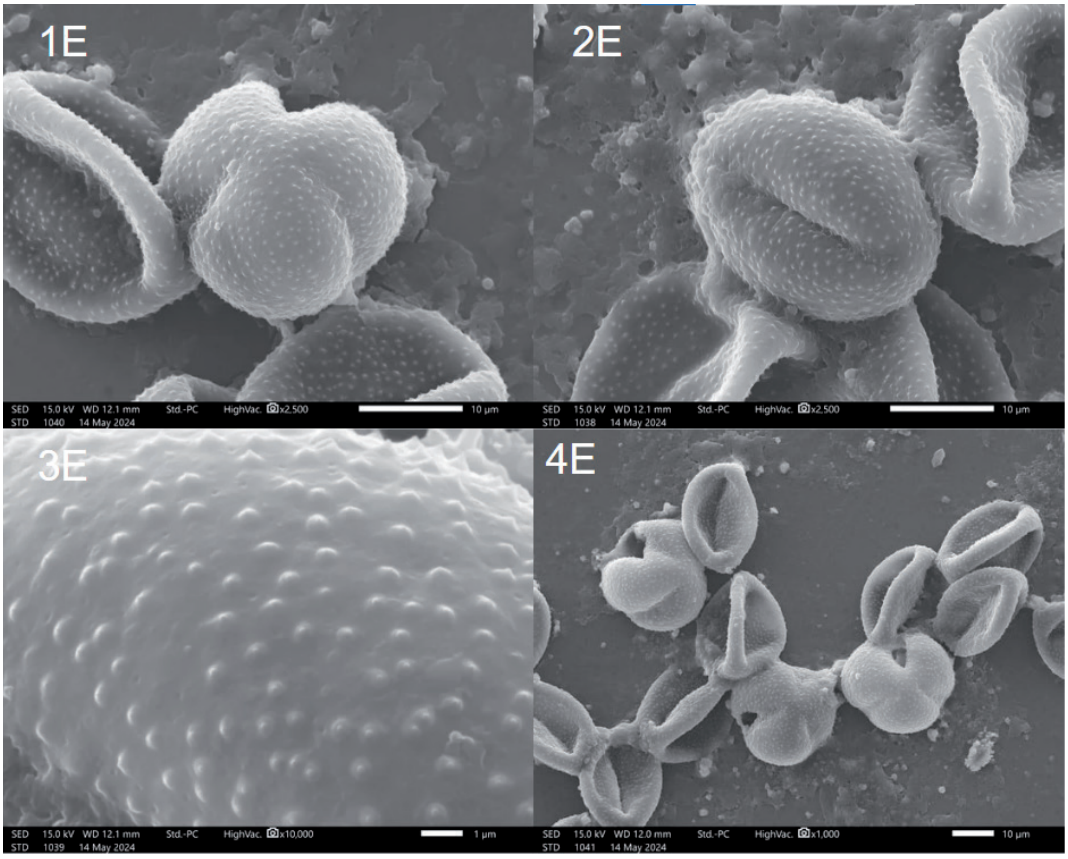

Figure 7 – Pollen grains of the species *G. anisitsii* seen under SEM: 1E) polar axis; 2E) equatorial diameter; 3E) exine; 4E) pollen grains. Source: primary.

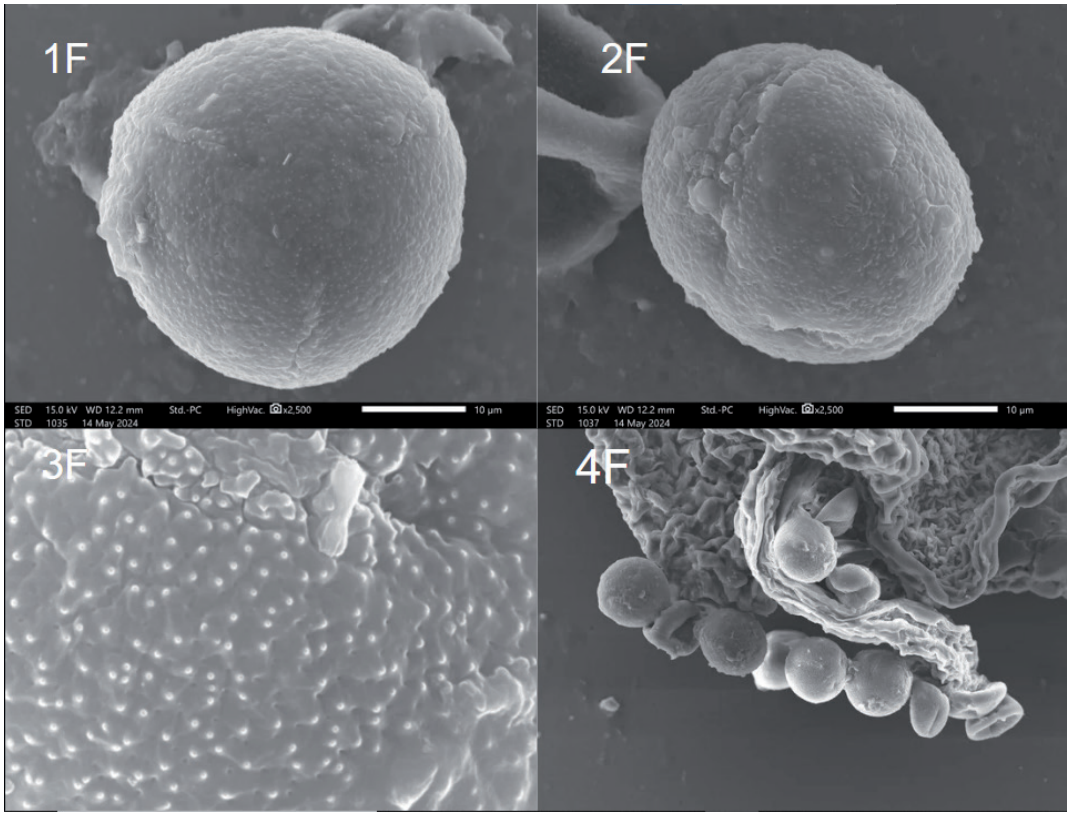

Figure 8 – Pollen grains of the species *M. polythele* seen under SEM: 1F) polar axis; 2F) equatorial diameter; 3F) exine; 4F) pollen grains. Source: primary.

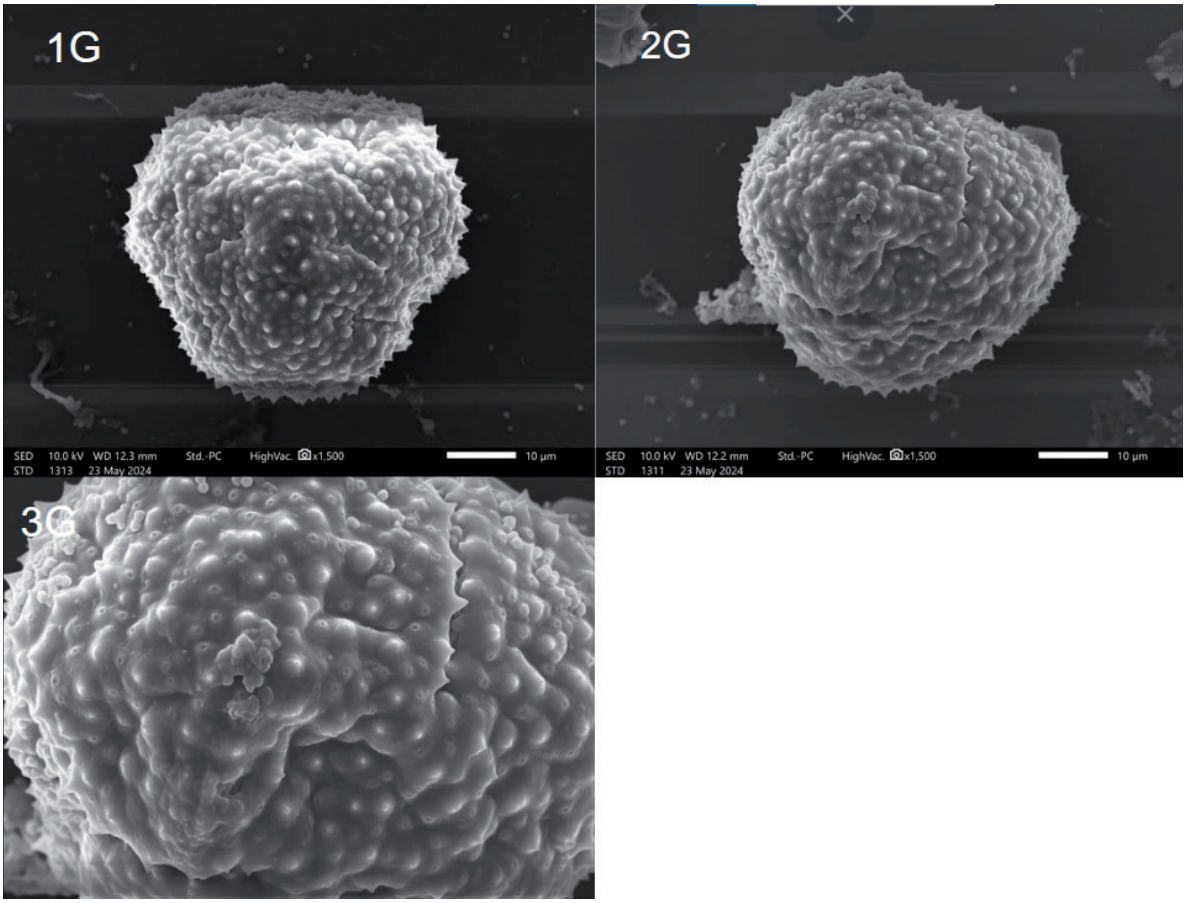

Figure 9 – Pollen grains of the species *P. leucocephalus* seen under SEM: 1G and 2G) polar axis; 3G) exine. Source: primary.

DISCUSSION

MELASTOMATACEAE

The studied species of the Melastomataceae family present uniformity, with the pollen grains having practically the same arrangement, being small, suboblate, 3-colporate, 3-pseudocolpate and, furthermore, both species being microverrucate. According to Barth & Barbosa (1975), with regard to the palynology of the Melastomataceae family, the pollen grains present a pattern, being generally small/medium, prolate, mostly perprolate (P/E > 2.00), 3-colporate, 3-pseudocolpate, with a psilate or wavy surface, rarely striated, with or without the existence of an *operculum* in the pseudocolpi, thus being able to be assimilated to stenopollinic grains. Also according to the mentioned authors, the *operculum* is a thickening of the apertural membrane, of variable dimensions and clearly defined; for Melastomataceae in particular, it is a portion of sexine, similar to that of the regions between the colpi, which covers the pseudocolpi, clearly detached from the margins of these by means of a narrow zone devoid of sexine; thus, the operculum resembles a bottle cap (*sic*); in the case of Melastomataceae, the nexines beneath the *opercula* remain with constant thickness. No opercula were observed in the two species studied here. Uribe & Fonnegra (1979) give importance and recognition to the palynology of this family.

For *Medinilla*, Halbritter (2017) cites *Medinilla scortechinii* having a pollen grain in a monad, small, 3-colporate, isopolar, spheroidal in shape, with a circular, prolate, heteroaperturate scope, with pseudocolpi. In other words, it presents a difference in terms of shape and scope in relation to the species studied here (*Medinilla speciosa*), which has a suboblate and subtriangular grain, similar

in relation to the pollen unit (both monad) and the openings (three colporate openings and also the presence of pseudocolpi).

The genus *Pleroma* was previously included in *Tibouchina*, having been separated from it for taxonomic reasons, with *Tibouchina* being the homotypic synonym of *Pleroma*. Stebler (2023) cites the species *Tibouchina urvilleana* as having a triangular, spheroidal to optically slightly flattened pollen grain (P/E approximately 0.87), isopolar, without visible sculptures under the light microscope, that is, psilate, with six openings, alternating colpate-colporate and with finely ornamented opening membranes. The species *Pleroma candolleanum* studied here differs in its microverrucate ornamentation, in the fact that it is tricolporate-tripseudocolpate, subtriangular, but it is similar in that the grain is suboblate and isopolar.

CACTACEAE

Regarding the species studied here from the Cactaceae family, a similarity can be seen between the species: tricolpate grains, circular scope, with small variations (medium and large size, oblate-spheroidal and suboblate shape, exine ornamentation (granulated, microverrucate).

As for the morphology of Cactaceae pollen grains, Kurtz Jr. (1963) identified relevant variation in grain size, differences in the number of grooves (openings) in several genera and useful variation in the pollen sculpture, in the length of the spicules and in the exine perforation in several genera. According to Santos *et al.* (1997), the pollen group in which the genus *Pilosocereus* is included is characterized by 3-colpate, perforated, spiculate to spiny pollen grains. The species studied here, *Pilosocereus leucocephalus,* resembles the description given above since the grains have three colpi and perforated ornamentation. and sharply granulated.

For Wittmann & Schlindwein (1995), the species *Gymnocalycium denudatum* has medium-sized pollen grains, uniformly 3-zonocolpate, radiosymmetric, isopolar, suboblate (P/E 0.83), circular to subtriangular in polar view, elliptical in equatorial view, scabrate ornamentation (in ML), with *pollenkitt* (coating of pollen grains consisting of adhesive substances, mainly lipids), grains frequently swollen in the colpi and inconspicuous colpi in unstained glycerinated gelatin. For the species studied here (*Gymnocalycium anisitsii*), the grain can be described as having several similarities with the species mentioned, as being isopolar, medium, suboblate (presenting P/E of 0.81), circular, and the grains being 3-colpate.

Mouga *et al.* (2019) cite *Mammillaria* species as presenting a conservative pattern, with few or small variations, mainly in size (medium and large), ambit (circular, subcircular and quadrangular) and shape (suboblate, oblate-spheroidal), with an exine ornamentation that is also very uniform (rugulate or microechinate/microperforate). The species studied here, *Mammillaria polythele*, presents an average size, oblate-spheroidal shape, circular ambit and perforated microverrucate exine, therefore, in agreement. It is worth noting that, for Miesen *et al*. (2015), however, the pollen grains of the Cactaceae family have a large diameter (40-100 µm), being subspheroidal, prolate or oblate.

CONCLUSION

The pollen grains of the taxa studied here show similarities when compared with literature, with small differences in certain aspects. These nuances, although relatively slight, reveal the individuality of the pollen characteristics, contributing to a more comprehensive understanding of the diversity among the species studied.

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