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Palynological characterization of ornamental plants

Caracterização palinológica de plantas ornamentais

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ABSTRACT
Pollen grains are reproductive structures, which have a very resistant coating (exine outer wall and intine inner wall) and exhibit the main morphological components of palynological characterization. Ornamental plants are recognized for their plastic characteristics (flowers, leaves, perfume, fruits) and are generally grown in gardens. Aiming to carry out the palynological characterization of ornamental species, the following species were analyzed: *Dietes bicolor* (Steud. Klatt ex Sweet, 1866) (Iridaceae), *Neomarica candida* (Hassl. Sprague, 1928) (Iridaceae), *Agapanthus inapertus* (Beauverd, 1912) (Amaryllidaceae), *Calceolaria tripartita* (Ruiz & Pav. 1798) (Calceolariaceae), *Erythrina speciosa* (Andrews, 1806) (Fabaceae), *Begonia radicans* (Vell. 1827) (Begoniaceae), Closed buds of the species were collected, which were conserved in acetic acid. The anthers were removed, macerated and acetolyzed. The pollen grains were mounted on microscopy slides, observed under light and scanning electron microscope, photographed and described in terms of unit, size, scope, polarity, symmetry, openings, shape and ornamentation. For the monocotyledons, the grains were in monads, elliptical in amb, heteropolar, large, with bilateral symmetry, monosulcate, with reticulated exine. For the eudicots, the grains were presented in monads, sub-circular/circular amb, isopolar, small/ medium, with radial symmetry, tri-tetracolporate, prolate/prolate-spheroidal shape, with reticulated /striated exine.
Keywords: Amaryllidaceae; Begoniaceae; Calceolariaceae; Fabaceae; Iridaceae; pollen.

RESUMO
Os grãos de pólen são estruturas reprodutivas que contam com um recobrimento muito resistente (parede externa de exina e interna de intina) e exibem os principais componentes morfológicos de caracterização palinológica. Plantas ornamentais são reconhecidas por suas características plásticas (flores, folhas, perfume, frutos) e geralmente cultivadas em jardins. Objetivando realizar a caracterização palinológica de espécies ornamentais, foram analisadas as seguintes espécies: *Dietes bicolor* (Steud. Klatt ex Sweet, 1866) (Iridaceae), *Neomarica candida* (Hassl. Sprague, 1928) (Iridaceae), *Agapanthus inapertus* (Beauverd, 1912) (Amaryllidaceae), *Calceolaria tripartita* (Ruiz & Pav. 1798) (Calceolariaceae), *Erythrina speciosa* (Andrews, 1806) (Fabaceae) e *Begonia radicans* (Vell. 1827) (Begoniaceae). Coletaram-se botões fechados das espécies, que foram conservados em ácido acético. As anteras foram retiradas, maceradas e acetolisadas. Os grãos de pólen foram montados em lâminas de microscopia, observados em microscópio de luz e eletrônico de varredura, fotografados e descritos em unidade, tamanho, âmbito, polaridade, simetria, aberturas, forma e ornamentação. Para as monocotiledôneas, os grãos se apresentaram em mônade, de âmbito elíptico, heteropolares, grandes, com simetria bilateral, monossulcados, com exina reticulada. Para as eudicotiledôneas, os grãos se apresentaram em mônade, de âmbito subcircular/circular, isopolares, pequenos/ médios, com simetria radial, tri-tetracolporados, forma prolato/ prolato-esferoidal, com exina reticulada/ estriada.
Palavras-chave: Amaryllidaceae; Begoniaceae; Calceolariaceae; Fabaceae; Iridaceae; pólen.

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INTRODUCTION

Ornamental plants are recognized for their plastic characteristics (flowers, perfume, fruits, stem) and are strategically grown, usually for decorative purposes, and used as aesthetic adornments (CASTRO, 2014). Ornamental plants are included in all taxonomic divisions, among which the families Euphorbiaceae, Lamiaceae, Solanaceae, Verbenaceae and Rosaceae stand out (MOUGA *et al*., 2015). Their trade is well developed, making commercial issues often stand out in relation to ecological and environmental problems (TOSCANO *et al*., 2019).

Plants considered ornamental have been cultivated since time immemorial and the use of plants over time, including ornamental ones, can be revealed by archaeopalynology (MONTECCHI & MERCURI, 2018). However, there is a lack of studies on the pollen morphology of ornamental botanical species. In this sense, a palynological study of the following ornamental species was carried out: *Dietes bicolor* (Steud. Klatt ex Sweet, 1866) (Iridaceae), *Neomarica candida* (Hassl. Sprague, 1928) (Iridaceae), *Agapanthus inapertus* (Beauverd, 1912) (Amaryllidaceae), *Erythrina speciosa* (Andrews, 1806) (Fabaceae), *Begonia radicans* (Vell. 1827) (Begoniaceae) and *Calceolaria tripartita* (Ruiz & Pav. 1798) (Calceolariaceae).

MATERIAL AND METHODS

The six species in focus (figure 1) were selected from field trips in the district of Pirabeiraba, in the municipality of Joinville, State of Santa Catarina, Brazil. After identifying the selected species, closed buds were collected, which were preserved in acetic acid until processing. The buds were opened, the anthers removed and macerated, placed in solutions and subjected to acetolysis (ERDTMAN, 1952). Afterwards, the pollen grains were mounted in glycerinated gelatin on microscopy slides, five slides per species (ERDTMAN, 1960). The slides were placed in the palinotheque of the Bee Laboratory (Label) at Univille-University of the Region of Joinville and, subsequently, observed and photographed under a light microscope (ML) (400x), using the Dino-Eye capture 2.0 software. The samples were also analyzed by scanning electron microscopy (SEM) at the State University of Santa Catarina (Udesc) in Joinville. For this procedure, the pollen grains that were stored in acetic acid were removed from the anthers, fixed on coverslips, and for image processing, the grains were metallized with palladium-gold, subjected to analysis and photographed (SALGADO-LABOURIAU, 2007).

The pollen grains were measured in micrometers, the measures for monocots being the largest equatorial diameter, the smallest equatorial diameter, the polar diameter and the exine thickness (figure 2) and for eudicots, polar axis, equatorial diameter and exine thickness (BARTH & MELHEM, 1988). 25 repetitions of each measurement were performed, per species. The measures were tabulated (Microsoft Excel) and generated arithmetic mean data (X), size range (Xmin-Xmax) and standard deviation of the mean. The grains were described on characteristics such as pollen unit, size, scope, symmetry, polarity, shape, openings and ornamentation. The study took place from June 2022 to July 2023.

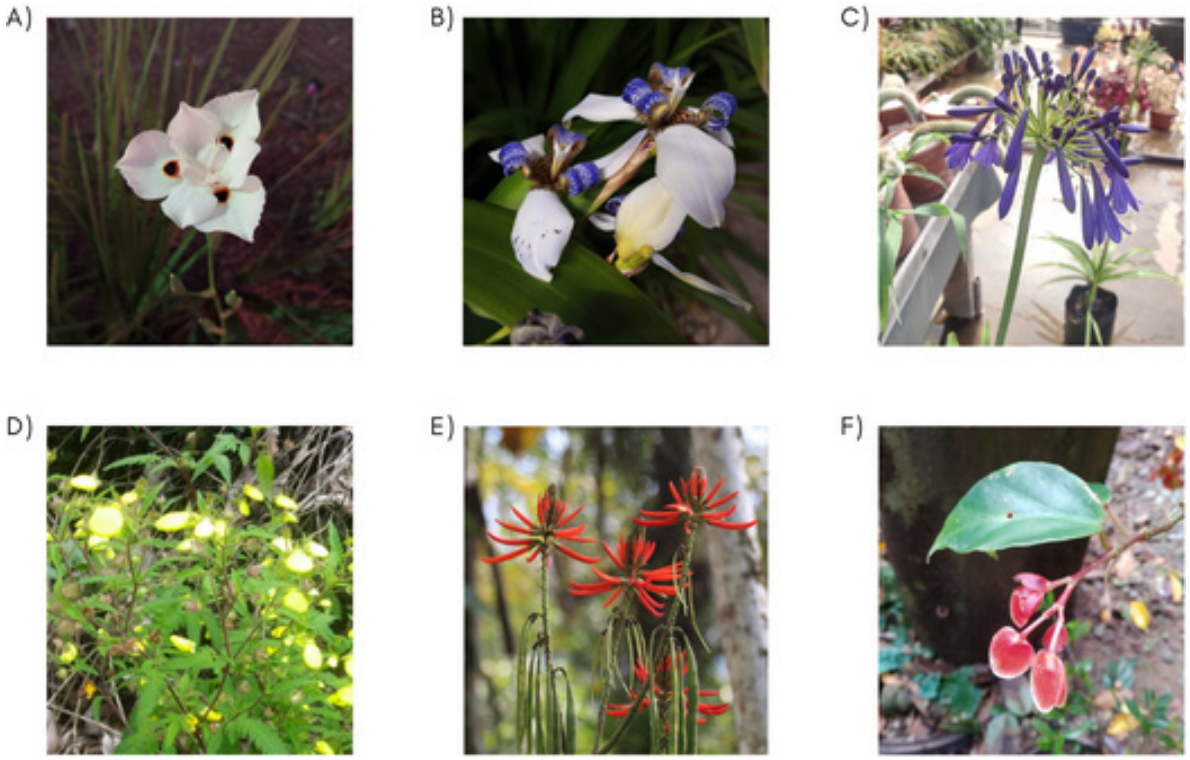

Figure 1 – Images of the studied species: A) Iridaceae – *Dietes bicolor*; B) Iridaceae – *Neomarica candida*; C) Amaryllidaceae – *Agapanthus inapertus*; D) Calceolariaceae – *Calceolaria tripartita*; E) Fabaceae – *Erythrina speciosa*; F) Begoniaceae – *Begonia radicans*. Source: primary.

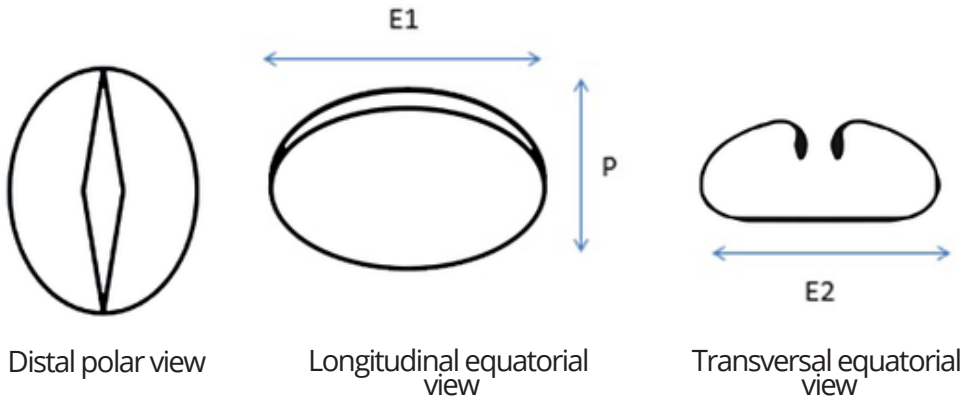

Figure 2 – Illustration of the measures taken for the pollen grains of monocots (larger equatorial diameter, smaller equatorial diameter, polar diameter). Source: primary.

RESULTS

Data refering to the pollen grains of monocots are in table 1 and figures 3 and 4; data refering to the pollen grains of eudicots are in table 2 and figures 5 and 6. All species were in monads.

Dietes bicolor (figure 3 – 1a, b; figure 4 – 1a, b)

Description: bilateral simmetry, heteropolar, elliptic amb, size large.
Apertures: monosulcate.
Exine: average thickness 3,3 (minimum 2,135, maximum 4,353, standard deviation 0,43), ornamentation reticulate.
Measures: average polar diameter 48,935 (minimum 37,36, maximum 56,824, standard deviation 4,479); average smaller equatorial diameter 75,632 (minimum 66,489, maximum 87,46, standard deviation 4,544); average larger equatorial diameter 66,182 (minimum 56,867, maximum 73,935, standard deviation 4,696).

Neomarica candida (figure 3 – 2a, b; figure 4 – 2 a, b)

Description: bilateral simmetry, heteropolar, elliptic amb, size large.
Apertures: monosulcate.
Exine: average thickness 1,721 (minimum 1,153, maximum 2,335, standard deviation 0,304), ornamentation reticulate.
Measures: average polar diameter polar 47,41 (minimum 44,272, maximum 51,865, standard deviation 1,964); average smaller equatorial diameter 71,076 (minimum 63,725, maximum 79,672, standard deviation 4,105); average larger equatorial diameter 57,41 (minimum 44,272, maximum 60,919, standard deviation 2,642).

Agapanthus inapertus (figure 3 – 3a, b; figure 4 – 3a, b)

Description: bilateral simmetry, heteropolar, elliptic amb, size large.
Apertures: monosulcate.
Exinea: average thickness 2,204 (minimum 1,41, maximum 2,843, standard deviation 0,342), ornamentation reticulate.
Measures: average polar diameter 35,343 (minimum 29,72, maximum 48,14, standard deviation 4,664); average smaller equatorial diameter 68,091 (minimum 63,459, maximum 73,755, standard deviation 2,828); average larger equatorial diameter 35,733 (minimum 29,133, maximum 42,024, standard deviation 3,548).

Calceolaria tripartita (figure 5 – 4a, b, c, d; figure 6 – 4a, b, c, d, e, f, g, h)

Description: radial simmetry, isopolar, subcircular amb, size small, shape prolate-spheroidal.
Apertures: 3-4 colpate, ocasionally syncolpate, ocasional *pseudocolpi* (*sensu* Iversen & Trolls-Smith, 1950).
Exine: average thickness 1,659 (minimum 1,286, maximum 2,073, standard deviation 2,4), ornamentation reticulate.
Measures: average polar axis 22,279 (minimum 19,529, maximum 24,764, standard deviation 1,559); average equatorial diameter 21,22 (minimum 18,168, maximum 23,6, standard deviation 1,676). The measures of tetracolpate grains were similar to those of tricolpate.

Erythrina speciosa (figure 5 – 5a, b; figure 6 – 5a, b)

Description: radial simmetry, isopolar, subcircular amb, average size, shape prolate-spheroidal.
Apertures: tricolporate, brevicolporate.
Exine: average thickness 2,494 (minimum 2,116, maximum 3,002, standard deviation 0,231), ornamentation reticulate.
Measures: average polar axis 39,373 (minimum 33,194, maximum 42,688, standart deviation 2,331); average equatorial diameter 36,366 (minimum 29,036, maximum 39,03, standard deviation 2,330).

Begonia radicans (figure 5 - 6a, b; figure 6 – 6a, b)

Description: radial simmetry, isopolar, circular amb, size small, shape prolate.
Apertures: tricolporate, margo.
Exine: average thickness 1,331 (minimum 0,788, maximum 1,673, standard deviation 0,190), ornamentation striate-perforate.
Measures: average polar axis 19,539 (minimum 17,604, maximum 22,865, standard deviation 1,27); average equatorial diameter 13,071 (minimum 12,061, maximum 13,938, atandard deviation 0,46).

Table 1 – Morphological data of the pollen grains of the monocots species analysed. Presentation of data: maximum, average, minimum and standard deviation, respectively.

N	Species	Polar diameter (P)	Smaller equatorial diameter (E1)	Larger equatorial diameter (E2)	Exine thickness	Ornamentation of the exine
1	*Dietes bicolor*	56,824 48,935 37,369 4,479	87,46 75,632 66,489 4,544	73,935 66,182 56,867 4,696	4,353 3,3 2,135 0,43	reticulate
2	*Neomarica candida*	51,865 47,41 44,272 1,964	79,672 71,076 63,725 4,105	60,919 57,41 51,905 2,642	2,335 1,721 1,153 0,304	reticulate
3	*Agapanthus inapertus*	48,14 35,343 29,72 4,664	73,755 68,091 63,459 2,828	42,024 35,733 29,133 3,548	2,843 2,204 1,41 0,342	reticulate

Table 2 – Morphological data of the pollen grains of the species of eudicots analysed. Presentation of data: maximum, average, minimum and standard deviation, respectively.

N	Species	Polar axis (P)	Equatorial diameter (E)	Exine thickness	Ornamentation of the exine
4	*Calceolaria tripartita*	24,764 22,279 19,529 1,559	23,6 21,22 18,168 1,676	2,073 1,659 1,286 2,4	reticulate
5	*Erythrina speciosa*	42,688 39,373 33,194 2,331	39,03 36,366 29,036 2,330	3,002 2,494 2,116 0,231	reticulate
6	*Begonia radicans*	22,865 19,539 17,604 1,27	13,938 13,071 12,061 0,46	1,673 1,331 0,788 0,190	striate perforate

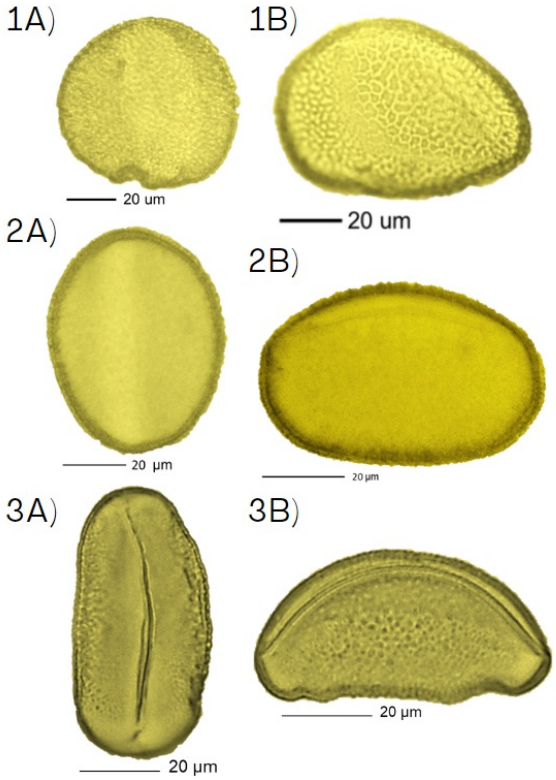

Figure 3 – Images in light microscopy of the pollen grains of the monocots species studied: 1 – (*Dietes bicolor*) a) pollen grain distal polar view; b) pollen grain equatorial longitudinal view; 2 – (*Neomarica candida*) a) pollen grain distal polar view; b) pollen grain equatorial longitudinal view; 3 – (*Agapanthus inapertus*) a) pollen grain distal polar view; b) pollen grain equatorial longitudinal view. Source: primary.

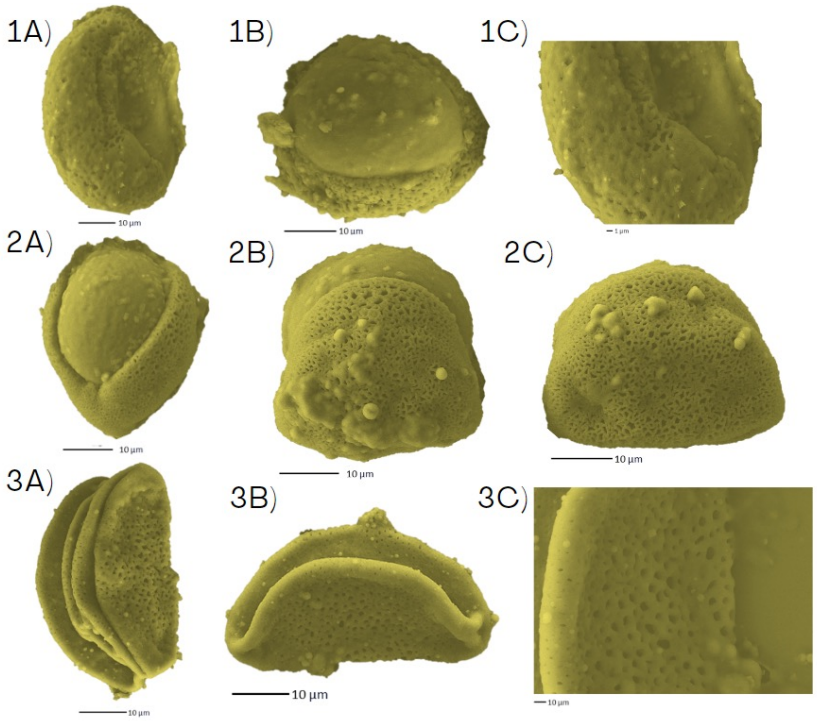

Figure 4 – Images in scanning electronic microscopy (SEM) of the pollen grains of the monocots species studied: 1 – (*Dietes bicolor*) a) pollen grain distal polar view; b) pollen grain longitudinal equatorial view; c) pollen grain focus on the exine; 2 – (*Neomarica candida*) a) pollen grain distal polar view; b) pollen grain longitudinal equatorial view; c) pollen grain focus on the exine; 3 – (*Agapanthus inapertus*) a) pollen grain distal polar view; b) pollen grain longitudinal equatorial view; c) pollen grain focus on the exine. Source: primary.

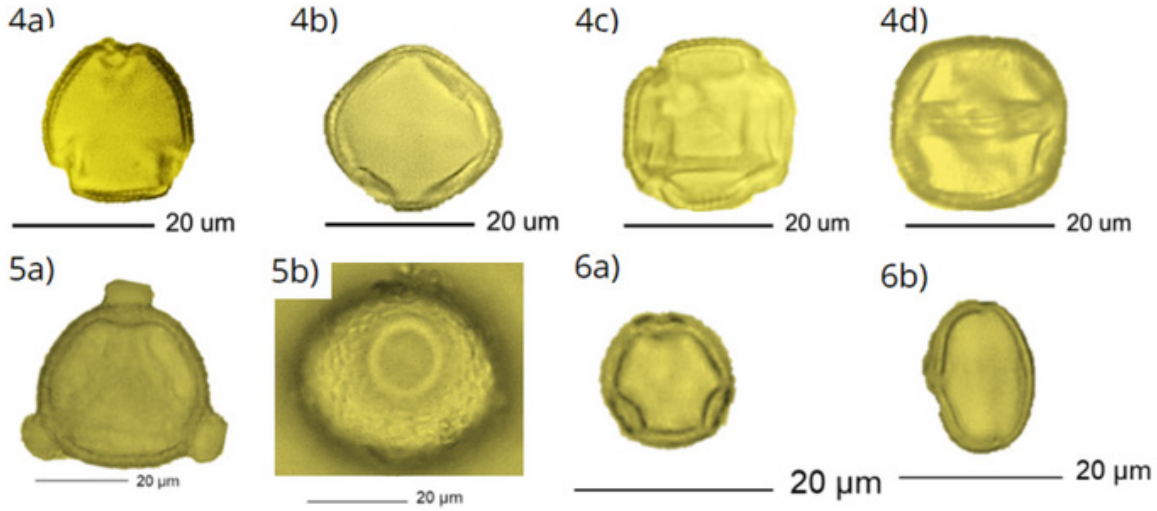

Figure 5 – Images in light microscopy of the pollen grains of the eudicots species studied: 4 – (*Calceolaria tripartita*) a) pollen grain polar view, tricolpate grain; b) pollen grain equatorial view, tricolpate grain; c) pollen grain polar view, tetracolpate grain; d) pollen grain equatorial view, tetracolpate grain; 5 – (*Erythrina speciosa*) a) pollen grain polar view; b) pollen grain equatorial view; 6 – (*Begonia radicans*) a) pollen grain polar view; b) pollen grain equatorial view. Source: primary.

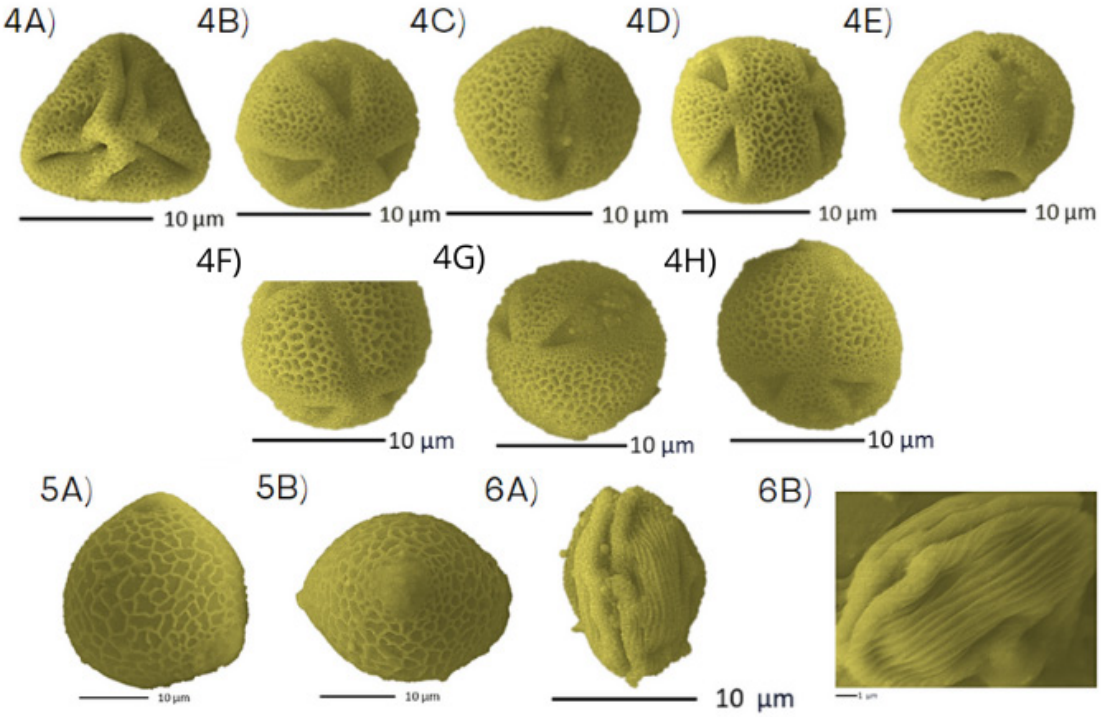

Figure 6 – Images in scanning electronic microscopy (SEM) of the pollen grains of the eudicots species studied: 4 – (*Calceolaria tripartita*) a) dry pollen grain polar view, tricolporate grain; b) pollen grain polar view, tricolporate grain; c) pollen grain equatorial view, tricolporate grain; d) pollen grain polar view, tetracolporate grain; e) pollen grain equatorial view, tetracolporate grain; f) syncolpate grain with sulcus in H shape; g) tetracolpate grain with symmetric regions between the sulcus; h) tetracolpate grain with asymmetric regions between the sulcus; 5 – (*Erythrina speciosa*) a) pollen grain polar view; b) pollen grain equatorial view; 6 – (*Begonia radicans*) a) pollen grain equatorial view; b) pollen grain focus on the exine. Source: primary.

The presence of sporopollenin orbicular granules associated with the pollen grains was observed in the scanning microscopy images of the studied monocotyledonous species of the genera *Dietes* and *Neomarica.* To assess their presence, the method of Pacini & Hesse (2005) was used to identify.

DISCUSSION

DIETES

For *Dietes bicolor*, of the Iridaceae family, there is no morphological description of the pollen grain in the literature. The Iridaceae family is a well-defined set of approximately 1800 species, belonging to the Liliidae (the monocotyledons), and has two large generic assemblages, Ixioideae and Iridaceae, respectively (GOLDBLATT, 2000). *Dietes* is a small genus of the Iridaceae, closely related to the African genus *Moraea* and the genus *Iris*, the latter widely spread in the Northern Hemisphere (GOLDBLATT, 1981). Six species of *Dietes* are currently recognised, five of which are African and one is restricted to Lord Lowe Island in the Tasman Sea between Australia and New Zealand, and, despite the extraordinary disjunction in distribution, there seems to be no doubt that *Dietes* is a natural genus, all species sharing unique vegetative and floral structures and a basic number = 10 of chromosomes (GOLDBLATT, 1981).

Goldblatt & LeThomas (1992) examined *Dietes grandiflora, D. flavida* and *D. iridoides* pollen grains, and reported that the cited species have monosulcate grains, reticulated, as in the present work, and may be zonasulculate in *Dietes iridioides* and, occasionally, in *D. grandiflora*, which was not verified in the present work.

NEOMARICA

For *Neomarica candida*, from the Iridaceae family, there is no morphological description of the pollen grain in the literature. *Neomarica* Sprague (Trimezieae, Iridoideae, Iridaceae) is a South American genus with about 30 recognized species and, in addition to a Mexican and Central American species, the genus is distributed mainly in the coastal states of Brazil (HENRICH & GOLDBLATT, 1987; GIL *et al.*, 2019; LOVO, 2009).

Golblatt & LeThomas (1992) described *Neomarica coerulea* and *N. northiana*, stated that most genera with monosulcate grains have undifferentiated exine around the aperture, and highlighted that when there is a thickened aperture margin, it is generally narrow and diffuse, citing *Neomarica.* They also stated that *Neomarica* has a very wide *reticulum*, that the bases of the *lumina* can be verrucate, granular or baculate and that relatively straight *muri* surround polygonal l*umina*. There is another species of the genus, palynologically described, *Neomarica gracilis*, by Stebler (2019), which cites a circular to elliptical grain, sulcate, with an unornamented membrane, reticulate, and a pattern of larger reticules near the edge of the *colpus*, which does not occur in *N. candida*, where ornamentation is uniform over the entire exine.

AGAPANTHUS

For *Agapanthus inapertus,* of the Amaryllidaceae family, there is no morphological description of the pollen grain in the literature. Amaryllidaceae is a family of monocotyledonous flowering plants comprising three subfamilies, with approximately 71 genera and 588 species, in addition to unidentified species (XU & CHANG, 2017; Li *et al.*, 2019). The three subfamilies are Agapanthoideae, Allioideae and Amaryllidoideae (CHASE *et al.*, 2009). *Agapanthus* is a unique genus in the subfamily Agapanthoideae (ZHANG *et al.*, 2010; YOUNIS *et al.*, 2022).

There are other species of the family described palynologically: *Agapanthus africanus* (STEBLER, 2016), *Agapanthus praecox* (STEBLER, 2019a) and *Agapanthus caulescens* (STEBLER, 2021). The species studied here (*A. inapertus*) differs from these three species by having margo without reticulate and ornamentation of the exine with coarse reticulate (in this last aspect, it resembles *A. praecox*).

CALCEOLARIA

For *Calceolaria tripartita*, from the Calceolariaceae family, there is no morphological description of the pollen grain in the literature. *Calceolaria* Linnaeus (1770) is an American genus of about 250 species that extends from central Mexico to Chile and southern Argentina, which has always been regarded as a distinct genus, although the relationships between species were unclear until recently (PUPPO, 2014). *Calceolaria,* together with two minor genera, *Porodittia* Don (1838: 608) and *Jovellana* Ruiz & Pavón (1798: 12), were considered to form the tribe Calceolariceae, of Scrophulariaceae, and its closest relations to Gesneriaceae (*op. cit*.). In the last revision for *Calceolaria* Neotropical, Molu (1988) subdivided the genus into three subgenera and 24 sections (mainly based on habit, leaf shape, corolla and stamen morphology). The genus *Calceolaria* includes *C. tripartita* Ruiz & Pavón (1798: 14), the most widespread species in the genus (PUPPO, 2014).

There are other species of the family described palynologically: *Calceolaria crenatiflora* (STEBLER, 2017a), *C. calynopsis* (STEBLER, 2020), *C. biflora* (HEIGL, 2021a) and *C. cypripedifolia* (HEILG, 2021b). One of the similarities observed between the mentioned species and the species studied here is the opening membrane which is not ornamented. Furthermore, *C. tripartita* coincided in small size with the species *C. biflora* and *C. cypripedifolia.*

Calceolaria and *Jovellana* are two genera that formerly belonged to the tribe Calceolarieae, in Scrophulariaceae, a tribe that was elevated to a family (ANDERSSON, 2006), with the two genera mentioned and approximately 270 species distributed in America, from Mexico to the south of Argentina, and some species of *Jovellana* that grow in New Zealand (SOUZA & LORENZI, 2008). The species of both genera, analyzed palynologically, were included in a single pollen type, due to their morphological uniformity, in agreement with what was observed by Erdtman (1966), Varghese (1968) and Minkin & Eshbaugh (1989). The palynomorphological homogeneity found, already highlighted by Molau (1988), reinforces the hypothesis of the monophyletic origin of the family Calceolariaceae.

The species *C. calinopsis*, like *C. tripartita*, also occasionally has tetracolpate and, eventually, syncolpate grains. Johnson (1989) reports the observation of tetracolpate grains, in species where there are normally tricolpate grains, these tetracolpate grains showing non-parallel colps, unorganized colps, colps at right angles to other colps, and abnormally syncolpated grains with shaped grooves in H. According to Faegri & Iversen (1964), tetracolpate grains are not common in eudicotyledonous plants but frequent in virus-infected plants. Besides that, among the causative agents of these anomalies in pollen grains, hybridization (WELLS, 1971), a genetic control that reflects cytological races (KING & ROBINSON, 1967) and apomixis (JOHNSON, 1989) were also mentioned.

ERYTHRINA

For *Erythrina speciosa*, from the Fabaceae family, there is no morphological description of the pollen in the literature. Fabaceae Lindl. has approximately 751 genera and more than 19,000 species, being the richest botanical family in Brazil (BELLO *et al*., 2009) and one of the most representative in the world (BRUNEAU *et al.*, 2013). It has six subfamilies: Cercidoideae, Detarioideae. Duparquetioideae. Dialioideae, Caesalpinioideae and Faboideae (STEVENS, 2017). *Erythrina* is one of the main genera of the subfamily Faboideae, with approximately 120 species distributed in tropical and subtropical regions of the world and occurs in all Brazilian biomes (DA SILVA *et al.*, 2013).

Hemsley & Ferguson (1985) studied 99 species of *Erythrina*, trying to find a correlation between the pattern of the pollen grains and the pollinating birds of the plant species, and mention *E. speciosa* as having grain sizes between small and medium, triangular to rounded scope, with small *lumina* and medium *muri* and few or no sexine granules. There are other species of the genus palynologically described: *E. fusca* (HALLBRITTER, 2016d), *E. humeana* (STEBLER, 2017b) and *Erythrina crista-galli* (HEIGL, 2021c) which are reticulate and brevicolpate/colporate. Like the species studied here (*E. speciosa*), *E. fusca, humeana* and *christa-galli* species show a ring (“costa” *sensu* Punt *et al.*, 1997) around the brevicolpus/colporus.

BEGONIA

For *Begonia radicans*, of the Begoniaceae family, there is no morphological description of the pollen grain in the literature. The 2,089 species currently accepted in *Begonia* are divided into 70 sections (ARDI *et al*., 2022). *Begonia radicans* is native to the Atlantic Forest and endemic to Brazil, occurring in the North, Northeast and South regions (REFLORA, 2023).

There are other species of the family described palynologically. Stebler (2016) studied 20 species of *Begonia* (https://pollen.tstebler.ch/MediaWiki/index.php?title=Artenliste) and reports small grains, tricolporate, striated, with an ornate aperture membrane. Hallbritter (2016a, b, c) and Hallbritter & Buchner (2016a, b, c) in turn studied the species *Begonia convovulacea, B. heracleifolia*, *B. humilis*, *B. metallica*, *B. nelumbifolia*, *B. tomentosa* and also reports similar grains, some with margo. The species studied here corroborates these data and presented margo.

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