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Plant anatomy: history and future directions

Pollen morphology in the genus *Dyckia* (Bromeliaceae): endemic species from southern Brazil comprising the proposed infrageneric grouping "*D. selloa* complex"

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Abstract

Dyckia, belonging to the Bromeliaceae, consists of species that share several morphological similarities. Recently, a proposal has grouped 13 species of the genus into a "*D. selloa* complex" based on their limited geographical distribution and seed characteristics. However, further investigation is required to gain a better understanding of this grouping. This study aimed to analyze the pollen grain morphology of the grouped species. The analysis was conducted using scanning electron microscopy (SEM), as well as light microscopy equipped with bright field and fluorescence optics. Various structures were measured. The pollen grains are heteropolar, oblate, and range in size from small to medium. They possess a simple sulcus type and exhibit a sexine (composed of tectum and infratectum columellate) and a nexine 1. Different patterns of ornamentation were identified, including reticulate, microreticulate, reticulate with free granules, reticulate with a partially discontinuous reticulum, and reticulate with a discontinuous reticulum. The aperture margin of some species differs from the described characteristics for the genus (*Puya* type) and is referred to here as the "*Other*" type. The results align with existing data for the genus *Dyckia* and contribute to understanding the pollen morphology within the genus and the studied group. The data obtained in this study, along with the observed variations, could facilitate a better understanding of the "*D. selloa* complex".

Key words: Brazil, Bromeliaceae, *Dyckia*, microscopy, pollen.

Resumo

Dyckia (Bromeliaceae) possui espécies com diversas semelhanças morfológicas. O proposto agrupamento de 13 espécies do gênero está baseado em sua restrita distribuição geográfica e características das sementes como um "complexo *D. selloa*", porém necessita ainda de um melhor entendimento. O presente trabalho possui como objetivo analisar a morfologia dos grãos de pólen das espécies pertencentes ao agrupamento. Foram realizadas análises por meio de Microscopia Eletrônica de Varredura (MEV), Microscopia de Luz em Campo Claro e em Fluorescência, com a medição de algumas estruturas. Os grãos de pólen são heteropolares, oblados, com tamanho pequeno ou médio e sulco do tipo simples. Eles possuem uma sexina (com teto e infrateto columelar) e nexina 1. Diferentes padrões de ornamentação foram identificados: reticulado, microrreticulado, reticulado com grânulos livres, reticulado com um retículo parcialmente descontínuo e reticulado com retículo descontínuo. A margem da abertura de algumas espécies difere do descrito para o gênero (tipo *Puya*) e é aqui indicado como tipo "*Outro*". Os resultados são consistentes com os dados existentes para o gênero *Dyckia* e contribuem para a compreensão da morfologia polínica no gênero e no agrupamento estudado. Os dados obtidos aqui e as variações observadas podem ser úteis para um melhor entendimento do "complexo *D. selloa*".

Palavras-chave: Brasil, Bromeliaceae, *Dyckia*, microscopia, pólen.

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Introduction

Dyckia Schult.f. (Bromeliaceae) belongs to the “xeric clade” of the subfamily Pitcairnioideae and is found in Latin America (Gomes-da-Silva *et al.* 2017, 2019; Bünecker *et al.* 2021). Leaves have been significant in diagnosing species within the genus. Additionally, *Dyckia* species possess peltate scales and CAM-like metabolism, which are important xerophytic traits (Reitz 1983; Strehl 1983; Benzing 2000; Crayn *et al.* 2004). The inflorescences are racemose, with flowers varying in color from yellow to red (Büneker *et al.* 2021). Pollination is achieved by insects or birds, and the fruits are of the capsular type with winged seeds (Smith & Downs 1974; Reitz 1983).

A group of *Dyckia* species endemic to South Brazil demonstrates morphological and habitat similarities at the infrageneric level. These species include *D. agudensis* Irgang & Sobral, *D. alba* S.Winkl., *D. delicata* Larocca & Sobral, *D. domfelicianensis* Strehl, *D. hebdingii* L.B. Smith, *D. maritima* Baker, *D. myriostachia* Baker, *D. nigrospinulata* Strehl, *D. polyclada* L.B. Smith, *D. retroflexa* S.Winkl., *D. rigida* Strehl, *D. selloa* Baker, and *D. tomentosa* Mez (Strehl & Beheregaray 2006; Büneker *et al.* 2015; Büneker *et al.* 2021). These species, distinct from other members of the genus, have elongated seeds with a triangular appearance. The group was initially referred to as the “*maritima* complex” (Strehl & Beheregaray 2006; Büneker *et al.* 2015), but it is currently known as the “*D. selloa* complex” (Büneker *et al.* 2021).

Further research is needed to better understand the intraspecific relationships within the group, both conceptually and in terms of evolutionary implications, to enhance its cohesive delimitation. Exploring various fields of study, such as morphology, anatomy, ecology, physiology, and palynology, is crucial for this purpose. This research focuses specifically on palynology.

Previous studies have described *Dyckia* pollen grains as medium-sized, oblate, with a simple sulcus type, heteropolar, and exhibiting reticulate, microreticulate, or perforate ornamentation, with a *Puya* type aperture margin (Erdtman 1952; Salgado-Laboriau 1973; Wanderley & Melhem 1991; Halbritter 1992; Pire & Schinini 1992; Smith & Till 1998; PalDat 2000; Souza *et al.* 2004; Hesse *et al.* 2009; Silva *et al.* 2016). However, these studies did not compare the pollen grains among *Dyckia* species. Examining specific differences through a detailed analysis can aid in understanding these structures and contribute to the genus’s circumscription. Hence, this study aims to analyze the exine, intine, and other structures of the pollen grains of species belonging to the “*D. selloa* complex”, aiming to provide data for a better understanding of the group’s species.

Material and Methods

Thirteen species of *Dyckia* belonging to the “*D. selloa* complex” were analyzed. All samples were obtained from cultivated living material in the Plant Anatomy Laboratory (LAVeg) at the Federal University of Rio Grande do Sul (Brazil) (Fig. 1). The complete species names, LAVeg registration numbers, and herbarium voucher numbers (ICN) are listed in Table 1.

The nomenclature used to characterize the pollen structures follows Halbritter & Hesse (1993), Punt *et al.* (2007), Hesse *et al.* (2009), and Halbritter *et al.* (2018).

Scanning electron microscopy (SEM)

Mature anthers were extracted from flower buds and dehydrated immediately in 2.2 Dimethoxypropane (DMP) (Halbritter 1998; Hesse *et al.* 2009). The dehydrated anthers were then dried using a BAL-TEC CPD 030 Critical Point Dryer (Gersterberger & Leins 1978), dissected, and the released pollen grains were attached to double-layer cylindrical aluminum sample holders. Finally, the samples were coated with a 25 nm layer of gold using a BAL-TEC SPD 050 metallizer. Sample analysis and image capture were conducted using a JEOL JSM6060 scanning electron microscope at 10 kV.

Brightfield and fluorescence light microscopy

Mature anthers were extracted from flower buds and fixed in a solution of 1% glutaraldehyde and 4% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.2 (McDowell & Trump 1976). The samples were washed and dehydrated following the protocol of Russin & Trivett (2001), using a PELCO bioWave® Pro MW microwave equipment. The fixed anthers were washed in the same buffer and dehydrated in an increasing ethanol series (10, 30, 50, 70, 92, 100, and 100% v/v). Subsequently, the samples were embedded in synthetic resin 2-hydroxyethyl methacrylate (Gerrits & Smith 1983), after an intermediate stage

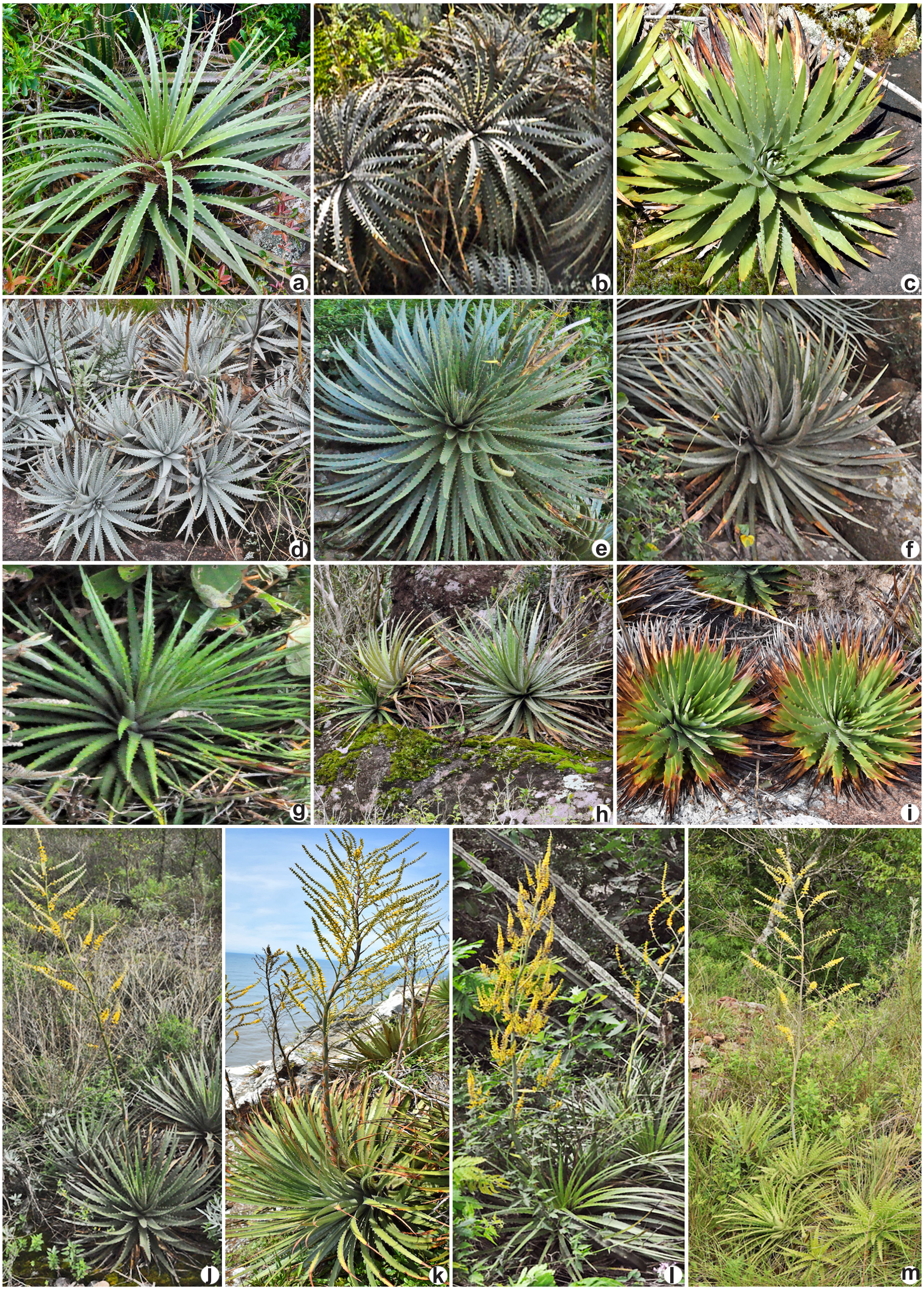

Figure 1 – a-m. *Dyckia* species belonging to the "*D. selloa* complex" in their natural habitat – a. *D. agudensis*; b. *D. delicata*; c. *D. domfelicianensis*; d. *D. hebdingii*; e. *D. myriostachya*; f. *D. nigrospinulata*; g. *D. retroflexa*; h. *D. rigida*; i. *D. tomentosa*; j. *D. alba*; k. *D. maritima*; l. *D. selloa*; m. *D. polyclada*. Images kindly provided by Henrique Mallmann Büneker.

Table 1 – Information for the studied species with registration in LAVeg collection (CV) and Voucher information.

Species[1]	**CV**[2]	**Voucher**[3]
Dyckia agudensis Irgang & Sobral	1374	ICN 188075
Dyckia alba S.Winkl.	1326	ICN 188076
Dyckia delicata Larocca & Sobral	1322	ICN 188077
Dyckia domfelicianensis Strehl	1370	ICN 188078
Dyckia hebdingii L.B.Sm.	1338	ICN 188079
Dyckia maritima Baker	1332	ICN 188080
Dyckia myriostachya Baker	1323	ICN 188081
Dyckia nigrospinulata Strehl	1316	ICN 188082
Dyckia polyclada L.B.Sm.	1375	ICN 188083
Dyckia retroflexa S.Winkl.	1318	ICN 188087
Dyckia rigida Strehl	1334	ICN 188084
Dyckia selloa (K.Koch) Baker	1372	ICN 188085
Dyckia tomentosa Mez	1319	ICN 188086

[1] = Species according to Büneker *et al.* (2021); [2] = Collection registration number (LAVeg); [3] = Herbarium ICN of Universidade Federal do Rio Grande do Sul, Brazil.

of pre-inclusion in absolute ethanol and resin (1:1). Sections of 2 µm were obtained using a Leica RM2265 rotary microtome.

Different sections underwent the following treatments for brightfield microscopy: 0.1% Toluidine Blue O, pH 4.4 (O'Brien & McCully 1981); Ruthenium Red at 1000 ppm (Johansen 1940), in a drop of distilled water, to identify pectins; IKI (0.5% iodine solution with 1% potassium iodide (Johansen 1940) to identify starch; 2.5% Basic Fuchsin in alcoholic solution (Faegri & Iversen 1950) to differentiate endexine from ektexine (Punt *et al.* 2007); 0.01% Calcofluor White M2R aqueous solution (Hugher & McCully 1975) to identify cellulose; and 0.01% Auramine O in 0.05 M tris-HCL buffer (Evans *et al.* 1992; Nepi & Franchi 2000) for sporopollenin.

Fixed anthers were also dissected to release pollen grains into a drop of pure glycerol that had been placed on a histological slide. The slide was then covered with a coverslip.

Sample analysis and photomicrographs were conducted using a Leica DMR HC microscope, which was equipped with a Zeiss Axiocam HCr digital camera and a brightfield and fluorescence system. For samples treated with Calcofluor White, a Leica DM A filter (PN513804; BP 340-380 nm, DM 400 nm, LP 425 nm) was used. Samples treated with Auramine O were examined using a Leica DM I3 filter (PN513808; BP 450-490 nm, DM 510 nm, LP 515 nm).

Measurements

Measurements were conducted by observing the entire pollen grain under brightfield light microscopy. Major (E) and minor (e) equatorial diameters were measured with the pollen grain in distal polar view, while the polar axis (P) was measured with the pollen in equatorial view. The thickness of different sporoderm layers was measured on pollen grain sections under brightfield light microscopy, after treatment with Toluidine Blue O (exine (H), nexine (N), sexine (S), infratectum (I), tectum (T), major measurement of intine in the region of aperture (IN), and intine along the wall opposite the aperture (in)). Measurements were obtained from 25 randomly chosen pollen grains of each species, taken from anthers of the same floral bud.

To objectively distinguish between reticulate or microreticulate ornamentations in SEM, the maximum extension of the largest lumina of the reticulum in the proximal polar region (L) was measured (Punt *et al.* 2007, pp. 44 and 62). The measurement was conducted without any pre-fixed orientation, with n = 25. The proximal polar region was chosen because it showed less variation in size among lumina. These measurements, along with qualitative observations of the exine, were used to determine the type of ornamentation for each pollen grain.

The following simple descriptive statistics were calculated for quantitative characters: mean, standard deviation, coefficient of variation, standard error, and median.

Results and Discussion

Pollen morphology: general characterization

The sampled species exhibited several common characteristics in their pollen grains. The general characterization of the pollen is presented in Figure 2. All analyzed pollen grains are monad, heteropolar, oblate, or oblate-spheroidal (*D. rigida*) and elliptical, ranging in size from small (10–25 µm) to medium (26–50 µm) (Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>).

Regarding the aperture type, all species have a monosulcate aperture (Fig. 2a,b,g-i) and belong to the simple type, characterized by the absence of exine deposition on the surface (Fig. 2g-i).

However, it is important to note that the works of Salgado-Labouriau (1973), Wanderley & Melhem (1991), Souza *et al.* (2004), and Silva *et al.* (2016) adopt a different methodology from the one used in this study for similar purposes and consider the aperture of *Dyckia* pollen grains as a "colpus". The difference in nomenclature is likely due to the general use of the term "colpus" to describe an elongated aperture, regardless of its location, which is also accepted by Punt *et al.* (2007).

The aforementioned characteristics align with existing descriptions in the literature for the genus (Wanderley & Melhem 1991; Halbritter 1992; Souza *et al.* 2004; Halbritter 2016a, b, h, j, k, m, n). They are also consistent with descriptions in other genera of the subfamily Pitcairnioideae. Similar pollen grains can be found in the genus *Encholirium* Mart. *ex* Schult & Schult.f. (Halbritter 2017a) and *Fosterella* L.B.Sm. (Halbritter 2016c), which are also part of the "xeric clade" of the subfamily. The pollen grains of *Deuterocohnia* Mez shares the same characteristics except for the shape, which is considered spheroidal (Halbritter 2017b). *Pitcairnia* L'Hér presents significant differences compared to the sampled species, including larger pollen grains and a sulcus that is not of the simple type, although other discussed characteristics are observed in it as well (Halbritter 2016d; Silva *et al.* 2016). Additionally, the descriptions provided in this study align with other genera in the family Bromeliaceae, such as *Brocchinia* Schult.f., Catopsis Griseb., *Cottendorfia* Schult.f., *Hechtia* Klotzsch, and *Puya* Molina (Givnish *et al.* 2011; Halbritter 1992; Halbritter 2015; Halbritter 2016e, f, g).

Another shared characteristic among all studied species is the presence of exine strata (Fig. 3). The sampled species exhibit clearly distinguishable exine stratification, consisting of a columellate sexine (tectum and infratectum) and nexine (Nepi & Franchi 2000; Punt *et al.* 2007; Hesse *et al.* 2009) (Fig. 3d-f). The sexine (S) is thicker than the nexine (N) (Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>; Fig. 3e). These results differ from those reported by Salgado-Labouriau (1973) and Wanderley & Melhem (1991), who observed similar thickness for these layers in acetolyzed pollen grains. However, the findings align with the research of Souza *et al.* (2004), which also found a greater thickness of the sexine.

A columellate tectum and infratectum were observed in the sexine (Fig. 3d-f; Tab. S2, available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>), while no supratectal elements were detected. The nexine shows a very uniform layer (Fig. 3e), identified as a nexine 1 since there is no differentiation in color compared to the rest of the exine when stained with basic Fuchsin (Fig. 3f) (Punt *et al.* 2007). In this study, endexine was not identified using the employed method.

Lastly, it is worth noting that starch grains were not detected in the sampled pollen grains (Fig. 3a-b). Microscopic examination using Toluidine Blue O revealed the presence of polysaccharides, but no starch grains were identified through reaction with IKI. Thus, the studied pollen grains can be characterized as "starchless", which is consistent with other research findings (Baker & Baker 1979; Oliveira *et al.* 2015).

Distinctive characters

Aperture margin

As shown in Table S1 (available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>), the qualitative observation of the aperture margin was classified as either *Puya* type or *Other* type. The term "*Other*" is used when it is not possible to unambiguously characterize the aperture margin as a Puya type or closely resembling the Catopsis type (Halbritter 1992).

Table S1 (available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>) demonstrates that the following species have an aperture margin easily characterized as the *Puya* type, as shown in Figures 4b and 4d: D. agudensis, *D. domfelicianensis*, *D. hebdingii*, *D. maritima*, *D. myriostachya*, *D. polyclada*, *D. retroflexa*, and *D. rigida*. On the other hand, the following species had an aperture margin that was difficult to characterize, as demonstrated in Figures 4a and 4c: *D. alba*, *D. delicata*, *D. nigrospinulata*, *D. selloa*, and *D. tomentosa*. The difference in characterization was not based on the criterion of similarity but rather on the careful application of the conceptual typology proposed by Halbritter (1992). Figure 4c shows an image of a pollen grain of *D. delicata*, which was characterized as having an *Other* type (2) aperture margin. Notice the unclear decreasing deposition of exine in the aperture region and the relatively abrupt transition from the region with exine deposition to the aperture region.

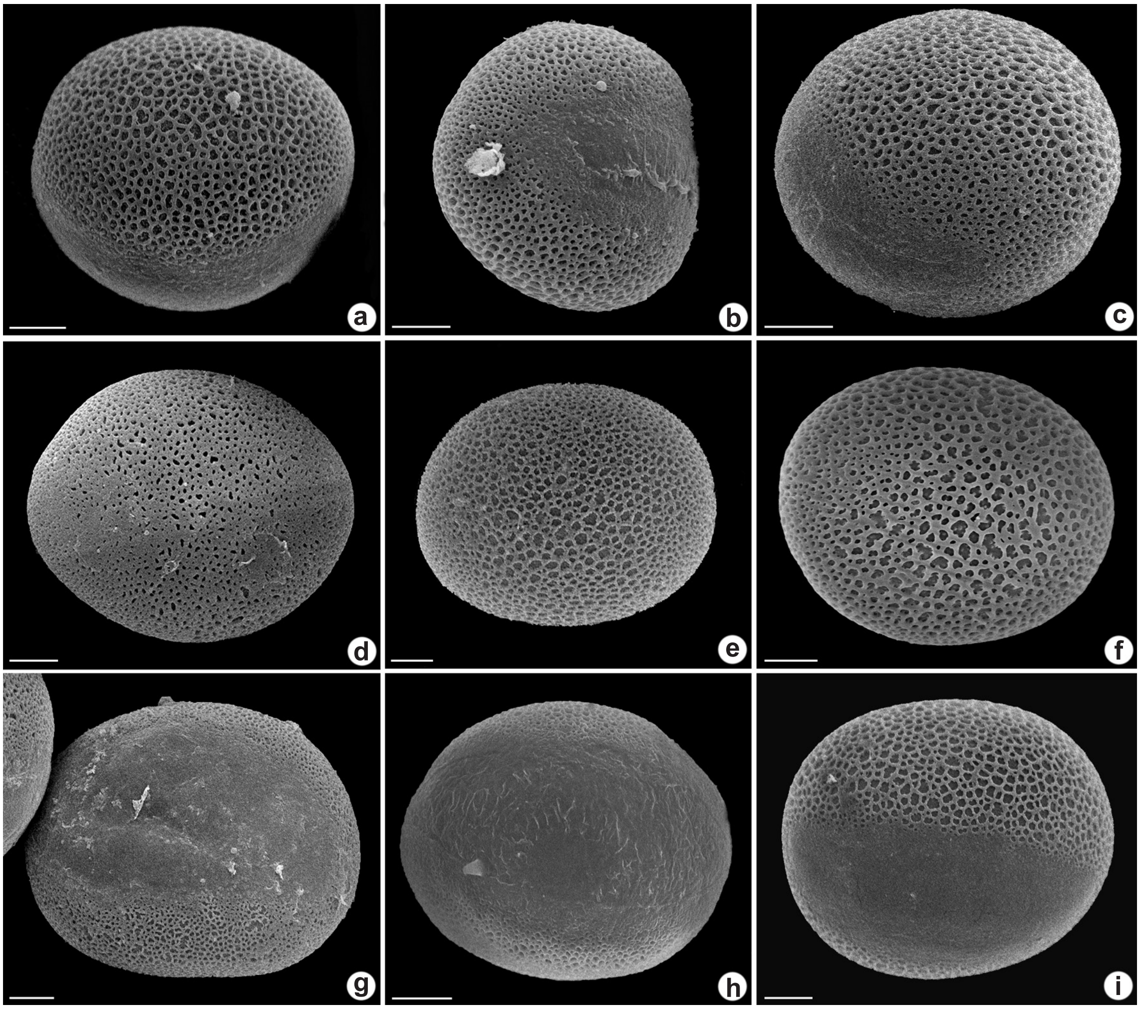

Figure 2 – a-i. General aspect of the sampled pollen grains, obtained under scanning electron microscope (SEM) – a-c. equatorial view – a. *D. alba*; b. *D. rigida*; c. *D. hebdingii*; d-f. proximal polar view – d. *D. agudensis*; e. *D. domfelicianensis*; f. *D. selloa*; g-i. distal polar view, showing a simple sulcus type – g. *D. myriostachya*; h. *D. rigida*; i. *D. alba*. Scale bars = 5 μm.

This aperture margin is very similar to that of *Puya floccosa* É.Morren (Halbritter 2016g) and *Catopsis floribunda* (Brongn.) L.B.Sm. (Halbritter 2016e).

Furthermore, Figure 4d depicts a pollen grain of *D. domfelicianensis*, characterized as having a *Puya* type aperture margin. The margin in Figure 4d is similar to that in Figure 4c. However, upon closer observation of Figure 4d, the decreasing deposition of exine in the aperture region is much clearer, with no indication of an abrupt interruption of its deposition. These observations reinforce the earlier discussion about

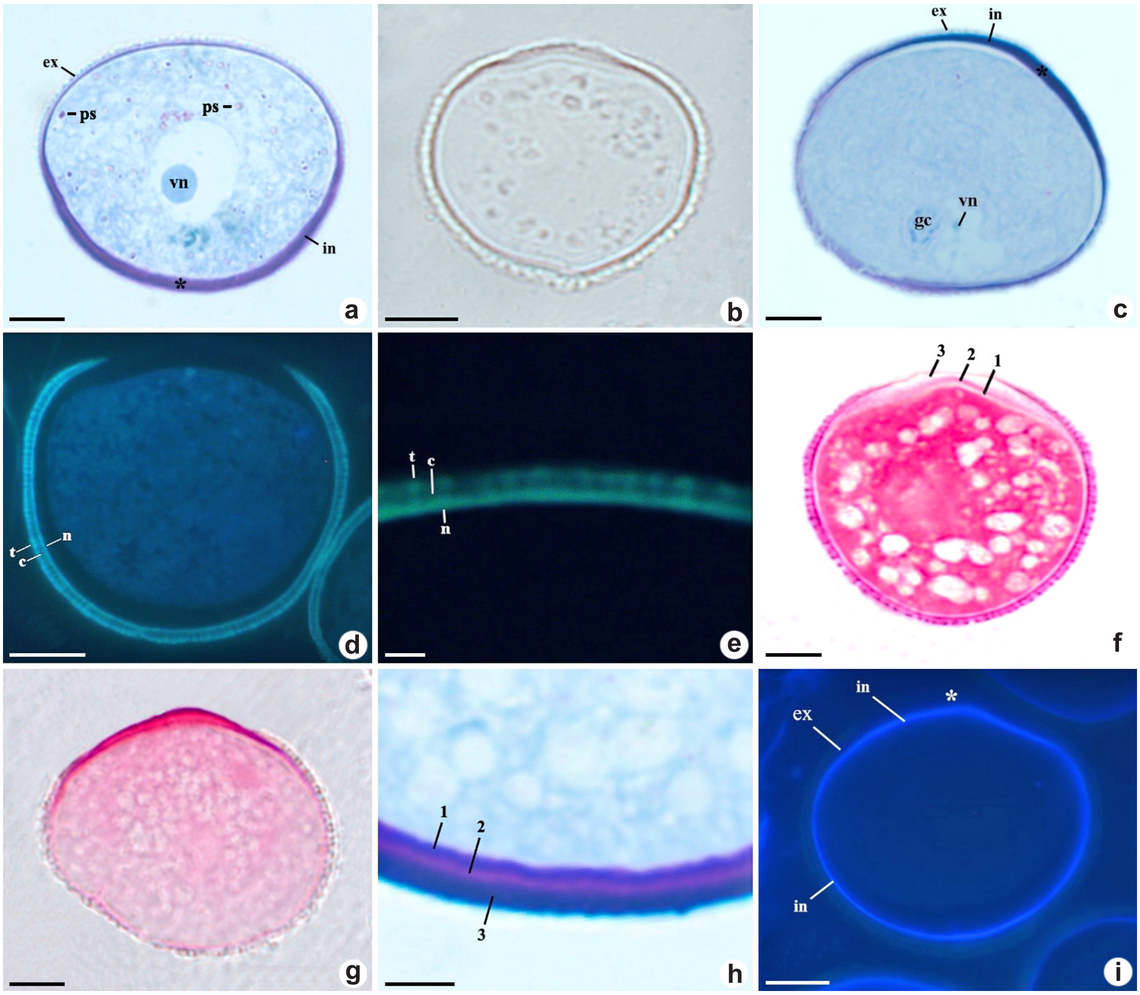

Figure 3 – a-i. General appearance of pollen grains in section – a. evidence of polysaccharides, coloring with Toluidine Blue O. *D. nigrospinulada*; b. treatment with IKI, no starch grain detection. *D. domfelicianensis*; c. general appearance of pollen grain in section. *D. maritima*; d-f. exine strata, showing sexine (tectum and infratectum columellate) and nexine 1 – d. treatment with Auramine O. *D. agudensis*; e. in detail, treatment with Auramine O. *D. rigida*; f. coloring with Basic Fuchsin (note the uniformity of color between the different layers of the exine, evidencing the presence of a nexine 1). *D. tomentosa*; f-i. intine observation – f. coloring with Basic Fuchsin (note the stratification of the intine in the aperture region with three distinguishable layers). *D. tomentosa*; g. coloring with Ruthenium Red (note the greater deposition of intine in aperture region; also note the stratification of the intine in the aperture region, with more intense staining in the outermost stratum, evidencing the presence of pectins). *D. polyclada*; h. in detail, coloring with Toluidine Blue O (stratification of the intine in the aperture region has three distinguishable layers). *D. tomentosa*; i. treatment with Calcofluor White (note the uniformity of the reaction with cellulose even in the aperture region). *D. retroflexa*. Abbreviations: ps = polysaccharides; ex = exine; in = intine; t = tectum; c = columellae; n = nexine; vn = vegetative nucleus; gc = generative cell; * = aperture region. Scale bar: e, h = 1 µm; a-d; f; g; i = 5 µm.

the aperture margin and highlight the need for studies aimed at improving the existing typologies proposed by Halbritter (1992).

Aperture margin was initially proposed as an important character for analyzing pollen grains of Bromeliaceae by Halbritter (1992). Several studies have considered this character when examining different groups within the family (Halbritter & Hesse 1993; Barfuss *et al.* 2016; Leme *et al.* 2017). Two types of margins in simple sulcus have been proposed: the *Puya* type, characterized by decreasing deposition of exine in the aperture margin region, and the *Catopsis* type, characterized by an abrupt interruption of exine deposition in the aperture margin region. Halbritter (1992) also suggests that the *Puya* type margin is found only in specimens of the subfamily Pitcairnioideae, while the *Catopsis* type margin is restricted to the genus *Catopsis* (subfamily Tillandsioideae).

However, characterizing the proposed typology for the aperture margin in the pollen grains studied here proved to be challenging. This led us to examine other pollen grains in the literature, both within and outside of *Dyckia*, to gather more information for analysis. Contrary to expectations, this procedure confirmed the difficulty in pattern characterization.

For instance, comparing *D. pernambucana* L.B.Sm. (Halbritter 2016a) with *D. fosteriana* L.B.Sm. (Halbritter 2016h) revealed that they do not have similar margins. The expected decreasing deposition of exine in the margin of *D. fosteriana* is not clear, and characterizing the species as having a Catopsis type aperture margin would be inappropriate. Similarly, comparing *Catopsis floribunda* (Halbritrer 2016f) with *Catopsis juncifolia* Mez & Wercklé (Halbritter 2016i) also leads to uncertainties. The latter exhibits a well-defined abrupt interruption of exine deposition in the margin, which is very different from the former and could be characterized as having a *Puya* type margin. Even *Puya floccosa* (Halbritter 2016g) can be used as an example, with the aperture margin better characterized as having an abrupt interruption of exine deposition, thus closer to the Catopsis type. It is important to note that these examples highlight an inversion of the traditional conceptual characterization: a species of the genus *Dyckia* or the genus *Puya* characterized as having a Catopsis type margin, and a species of the genus *Catopsis* characterized as having a *Puya* type margin. The species mentioned here serve as examples, but the problem extends to several other species when the character is analyzed diligently and attentively.

The purpose of this discussion is not to question the character itself but to emphasize its relative application. In the mentioned cases, it would be possible to characterize species in an unprecedented manner by utilizing different types of aperture margins in the simple sulcus of the family, deviating from Halbritter's (1992) proposition and yielding different results. Another reasonable alternative is proposing new types of aperture margins or even establishing intermediate types for specimens with imprecise characterization.

Ornamentation

The following ornamentations were observed (Fig. 4; Tab. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>): microreticulate [*D. agudensis* (Figs. 2d; 4h) and *D. maritima*], reticulate [*D. delicata* (Fig. 4c), *D. domfelicianensis* (Figs. 2e; 4d-e), *D. hebdingii* (Fig. 2c), *D. nigrospinulata*, *D. polyclada* (Fig. 4f), *D. retroflexa*, *D. rigida* (Fig. 2b), and *D. selloa* (Fig. 2f)], reticulate with free granules or bireticulate (*D. alba*; Fig. 4g), reticulate with a partially discontinuous reticulum (*D. myriostachya*; Fig. 4k), and reticulate with a discontinuous reticulum (*D. tomentosa*; Fig. 4i-j).

The reticulum pattern (Punt *et al.* 2007) is defined as the deposition of exine in a mesh-like structure with lumina larger than 1 μm, surrounded by exine elements that are narrower than the lumen and irregularly shaped. In this case, the reticulum is also heterobrochate. The ornamentation is illustrated in Figures 4e and 4f, and the measurements of the lumina are provided in Table S1 (available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>). Reticulate ornamentation was the most commonly observed among the studied species, which aligns with previous studies on the genus (Salgado-Labouriau 1973; Wanderley & Melhem 1991; Halbritter 1992; Pire & Schinini 1992; Paldat 2000). However, there is a significant discrepancy among the findings of this study and another description: while *D. hebdingii* is described as having reticulate ornamentation here (Fig. 2c), Halbritter (2016m) reports it as having microreticulate ornamentation.

The microreticulate pattern is recognized when the pollen grain has a reticulate pattern, but the lumina of the reticulum are smaller than 1 μm

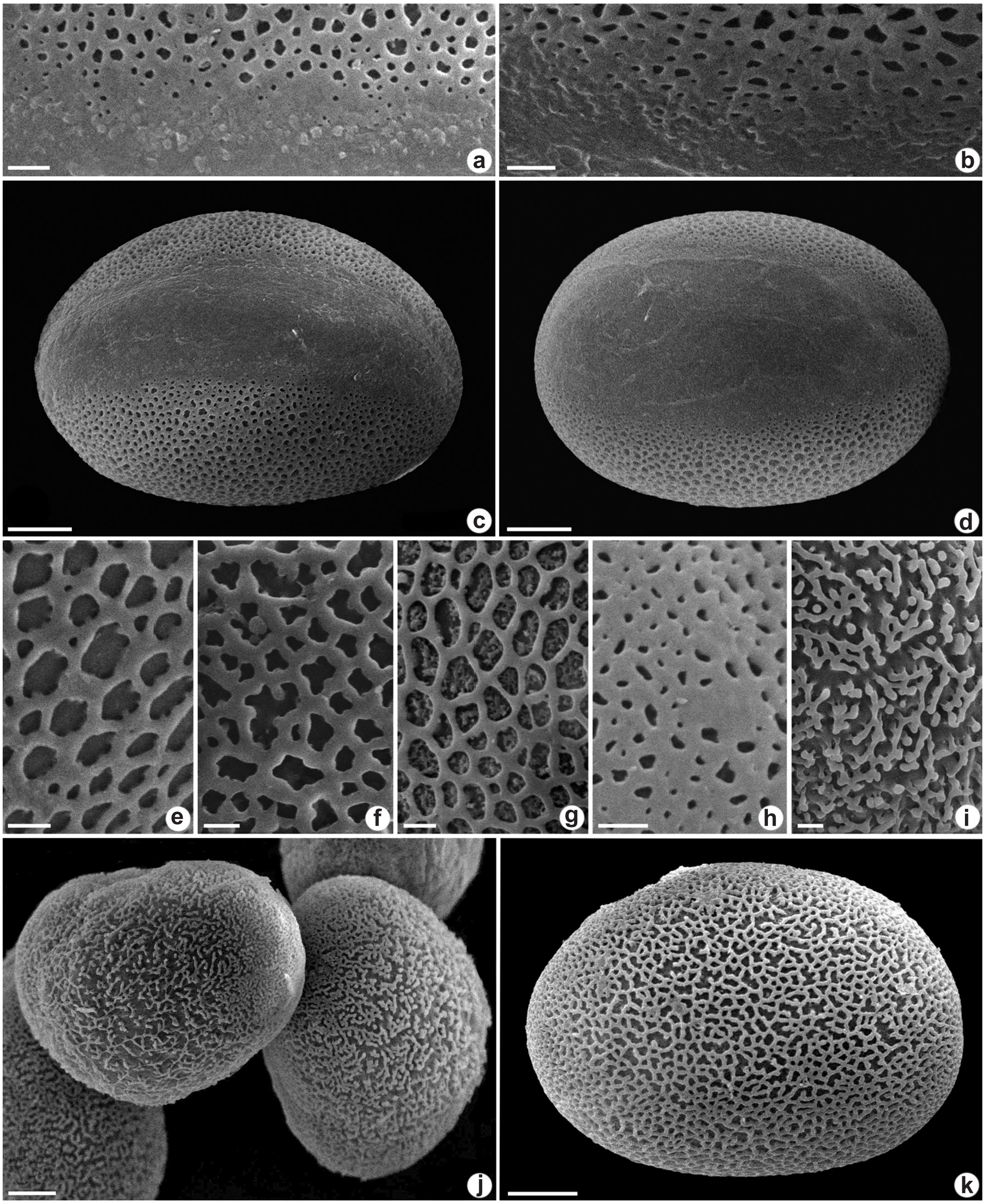

Figure 4 – a-k. Distinctive qualitative characters, obtained under scanning electron microscope (SEM) – a-b. in detail, aperture margin (note the difference between aperture margins) – a. margin characterized as "Other" type. *D. delicata*; b. well characterized *Puya* type margin. *D. maritima*; c-d. distal polar view, showing a simple sulcus type (note the small, but existing, difference between aperture margins, which makes it difficult to apply the typology proposed by Halbritter (1992) – c. margin characterized as "Other" type. *D. delicata*; d. *Puya* type margin. *D. domfelicianensis*; e-k. in detail, ornamentation – e-f. reticulate – e. *D. domfelicianensis*; f. *D. polyclada*; g. reticulate with free granules or bireticulate. *D. alba*; h. microreticulate. *D. agudensis*; i-j. reticulate with a discontinuous reticulum. *D. tomentosa* – i. in detail; j. general aspect (note how discontinuity of the reticulum is observed across the exine); k. reticulate with a partially discontinuous reticulum (note the difference in the extent of the reticulum discontinuity of the reticulum between "j" and "k"). *D. myriostachya.* Scale bar: a, b, e-i = 1 µm; c, d, j, k = 5 µm.

(Punt *et al.* 2007). The observed cases also exhibit heterobrochate characteristics. The ornamentation is shown in Figure 4h, and the measurements of the lumina are provided in Table S1 (available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>). The presence of this ornamentation in the genus supports other findings in the literature (Halbritter 2016j; PalDat 2000; Souza *et al.* 2004).

The reticulate with free granules or bireticulate pattern exhibits reticulate ornamentation with the deposition of exine elements in the lumina of the reticulum (*D. alba*). The methods used in this study did not allow for a definitive determination of whether it is a case of bireticulate ornamentation (Punt *et al.* 2007). Bireticulate ornamentation is characterized by a suprareticulum supported by a microreticulate tectum. In the case demonstrated in Figure 4g, such morphology is possible but not clearly demonstrated. There are no descriptions of bireticulate pollen grains within Bromeliaceae, which suggests that the present interpretation may be incorrect. Another possibility is the deposition of free exine elements in the lumen region of the reticulum, which has been observed in several other genera in Bromeliaceae, such as *Aechmea* Ruiz & Pav., *Acanthostachys* Klotzsch, *Billbergia* Thunb., *Canistropsis* (Mez) Leme, *Neoregelia* L.B.Sm., *Ronnbergia* É.Morren & Andre, *Quesnelia* Gaudich., and *Tillandsia* L. (PalDat 2000). However, in most cases, the exine deposition in the lumen does not resemble that observed in *D. alba*. For example, the deposition in *Billbergia euphemiae* É.Morren (Halbritter 2019b) consists of isolated granules that are independent of each other. In *D. alba* (Fig.4g), the elements appear to be interconnected, although the extent of connectedness is not visible. *Aechmea incompta* Leme & H.Luther (Halbritter 2019a) is the closest example to the observed case, where the deposition in the lumen of the reticulum is consistent and described as free columellae. Further studies employing more in-depth observation methods should be conducted to better understand the characteristics of these elements.

The reticulate with a partially discontinuous reticulum pattern is characterized as reticulate, but with scattered discontinuities throughout the reticulum, as shown in Figure 4k. This trait was observed in only one species (*D. myriostachya*) and is consistent with other genera in Bromeliaceae, such as *Pitcairnia*, *Quesne*lia, and *Vriesea* Lindl. (PalDat 2000).

Finally, the reticulate with a discontinuous reticulum pattern was found in only one species (*D. tomentosa*) and exhibits generalized discontinuities throughout the entire pollen grain (Figures 4i, j). The precise definition as a reticulum may be questionable, as there is no continuity in exine deposition that forms a clearly distinguishable reticulum, nor a delimited formation of lumina. However, the term was chosen here considering that the dominant pattern found among pollen grains of the genus is reticulate, and a reticulum can be inferred. There is only one species of Bromeliaceae reported to exhibit this characteristic, which also belongs to the subfamily Pitcairnioideae: *Pitcairnia albiflos* Herb. (Halbritter 2016l). The description of this species also refers to it as reticulate, despite the generalized discontinuity in exine deposition and the constant absence of delimited lumina. It is important to note that the observation of discontinuity characteristics in the reticulum, as described here - either restricted to some regions or in a generalized manner - only occurs in the genera *Dyckia* and *Pitcairnia*, which may indicate a specific derivation within Pitcairnioideae. However, more specific studies may identify other groups with this characteristic and incorporate it into the discussion of the evolutionary derivation of ornamentation and/or its application in taxonomy.

Intine

The intine of all analyzed species has the same characteristics, as shown in Figures 3a, 3c, and 3f-i. Intine measurements are presented in Table S2 (available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>). Figure 3f reveals three distinct layers of the intine, identified by staining with different intensities using Basic Fuchsin. In Figure 3g, the intine is identified by a more intense staining with Ruthenium Red, while in Figures 3a, 3c, and 3h, Toluidine Blue is used for identification. Finally, Figure 3i demonstrates the intine through fluorescence microscopy using Calcofluor White, a specific reagent for highlighting cellulose, a compound exclusively found in the intine (O'Brien & McCully 1981; Nepi & Franchi 2000).

Traditionally, the intine is known to be a thin layer and usually exhibits greater thickening in the aperture region (Nepi & Franchi 2000), as observed in Figures 3a, 3c, 3f, 3g, and Table S2 (available on supplementary material <https://doi.org/10.6084/m9.figshare.25474912.v1>). However, the enhanced thickening in this particular region is not apparent when using only the specific cellulose identification reagent (Fig. 3i). This is because there is an equally thickened layer of cellulose present beneath the entire exine, including the aperture zone. Beneath the opening of the exine (sulcus), the expanded structure of the intine allows for the identification of distinct sublayers, each characterized by unique chemical compositions. Toluidine Blue and Basic Fuchsin stain all the intine sublayers, including the inner cellulosic/hemicellulosic layer, in the aperture region but with varying colors and intensities (Figs. 3f, i). Ruthenium Red indicates the presence of pectic acids, displaying an intense pink color (Fig. 3g). These findings are consistent with the existing literature, demonstrating evident stratification of the intine in the aperture region, with pectins located more externally (Fig. 3g) and cellulose is exclusively positioned internally (Fig. 3i). Future analysis employing transmission electron microscopy methods will undoubtedly uncover stratifications of the intine within the inter-aperture regions. This is attributed to the significant compaction of the intine in this specific area, which cannot be discerned using light microscopy images to differentiate strata.

Dyckia selloa complex

Based on the provided data, we can make groups of species by examining similarities in their morphological characters. Here is a possible grouping based on them:

Group 1: this group consists of several species with similar characteristics. They have relatively smaller polar axis measurements, moderate to large major equatorial diameters, and similar size ratios (P/E and e/E). Their shapes are predominantly oblate, and they exhibit similar extension lumes reticulum measurements. The exine ornamentation is generally reticulate, and the aperture type is monosulcate. This group includes *D. agudensis*, *D. domfelicianensis*, *D. hebdingii*, *D. maritima*, *D. myriostachya*, *D. nigrospinulata*, *D. polyclada*, *D. retroflexa*, and *D. selloa*.

Group 2: this group consists of species with similar characteristics, but they differ slightly from those in Group 1. They have larger polar axis and major equatorial diameter measurements compared to Group 1, and their size ratios (P/E and e/E) are slightly different. The shapes of the pollen grains are primarily oblate, and the extension lumes reticulum measurements are within a similar range. The exine ornamentation shows variations such as free granules or bireticulate patterns. The aperture type is mostly monosulcate, but there is an additional category labeled "*Other*". This group includes *D. alba*, *D. delicata*, and *D. tomentosa*.

Group 3: this group consists of a single species, *D. rigida*. It has distinctive morphological characteristics compared to the other species. It has a relatively larger major equatorial diameter, a smaller size ratio (e/E), and an oblate-spheroidal shape. The extension lumes reticulum measurement is different, and the exine ornamentation is described as reticulate. The aperture type is monosulcate, similar to the other groups.

The categorizations mentioned are solely derived from the provided data on pollen grain morphology. However, considering the findings in Troleis *et al.* (2023) regarding leaf morphology and leaf anatomy of the same species, which propose two distinct groups within this complex, additional factors such as genetic analysis and ecological traits, when analyzed collectively, could enhance the precision of the groupings and yield a more comprehensive comprehension of the interrelationships among the species.

Based on the characteristics of pollen grains from various species of the genus *Dyckia*, belonging to the *selloa* complex, several conclusions can be drawn:

Size Variation: the studied *Dyckia* species exhibit a range of pollen sizes. Some species have small-sized pollen grains (*e.g.*, *D. agudensis* and *D. domfelicianensis*), while others have medium-sized grains (e.g., *D. alba* and *D. selloa*).

Shape Consistency: the pollen grains of the studied *Dyckia* species are predominantly oblate or oblate-spheroidal in shape. This consistent shape indicates a common characteristic within the genus.

Aperture Type: all the species analyzed have a monosulcate aperture type, which means they have a single furrow in their pollen grains. This is a shared feature among the *Dyckia* species.

Exine Ornamentation: the exine ornamentation of the pollen grains varies within grouping. The grains exhibit different patterns such as reticulate, microreticulate, reticulate with free granules or bireticulate, reticulate with a partially discontinuous reticulum and reticulate with a discontinuous reticulum. This suggests some level of variation in exine ornamentation among the species.

Aperture Margin Type: the aperture margin type also varies within the studied *Dyckia* species.

Some species have an aperture margin characterized as the "*Puya* type" (*e.g.*, *D. agudensis* and *D. domfelicianensis*), while others have an "*Other*" type margin (*e.g.*, *D. alba*, *D. delicata*, and *D. selloa*). This indicates a diversity in the characteristics of the aperture margin within the genus.

Based on these observations, it can be concluded that the studied species of *Dyckia* exhibit both similarities and variations in their pollen characteristics. The shared features are the shape consistency and monosulcate aperture type. However, the variations in size, exine ornamentation, and aperture margin type indicate some level of divergence among the species. Further analysis and study would be needed to determine the exact relationship and grouping of these *Dyckia* species within the genus.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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