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Palynological characterization of Crassulaceae J.St.-Hil.

Caracterização palinológica de Crassulaceae J.St.-Hil.

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ABSTRACT
Succulent plants are important from an ornamental and economic point of view and there is a scarcity of palynological studies, which is reflected in the uncertain taxonomy of this group. Thus, the present work aimed to study nine species of Crassulaceae: *Echeveria chroma* (hybrid bred by Renee O'Connell), *Echeveria pallida* E. Walther, *Echeveria pulidonis* E. Walther, *Graptopetalum macdougallii* Alexander, *Kalanchoe humilis* Britten, *Sedum clavatum* R.T. Clausen, *Sedum mexicanum* Britton, *Sedum mocinianum* Perez-Calix. e *Sedum treleasei*. Rose. The closed buds of succulents were preserved in acetic acid. The acetolysis was performed and microscopy slides were mounted, where 50 pollen grains of each species were photographed, being measured each view (polar and equatorial) in light microscopy, in micrometers (μm). Details were observed using SEM. The description of the grains showed a pattern of morphological characteristics in common: isopolar symmetry; grains in monads; small to medium size, regularly spheroidal shape, tricolporated, operculated opening and morphological characteristics that indicate a process of harmomegathy.
Keywords: morphology; ornamental plants; pollen; succulent plants.

RESUMO
As plantas suculentas são importantes do ponto de vista ornamental e econômico, e há escassez de estudos palinológicos, que se reflete na incerta taxonomia desse grupo. Assim, o presente trabalho visou estudar nove espécies de Crassulaceae: *Echeveria chroma* (híbrida criada por Renee O'Connell), *Echeveria pallida* E. Walther, *Echeveria pulidonis* E. Walther, *Graptopetalum macdougallii* Alexander, *Kalanchoe humilis* Britten, *Sedum clavatum* R.T. Clausen, *Sedum mexicanum* Britton, *Sedum mocinianum* Pérez-Calix. e *Sedum treleasei*. Rose. Os botões fechados das suculentas foram conservados em ácido acético. Foi realizada a acetólise e montaram-se lâminas de microscopia, onde foram fotografados 50 grãos de pólen de cada espécie, sendo medida cada vista (polar e equatorial) em microscopia óptica de luz, em micrômetros (μm). Observaram-se detalhes em MEV. A descrição dos grãos apresentou um padrão de características morfológicas em comum: simetria isopolar; grãos em mônades; tamanho pequeno a médio, forma regularmente esferoidal, tricolporados, abertura operculada e características morfológicas que indicam processo de harmomegatia.
Palavras-chave: morfologia; plantas ornamentais; pólen; suculentas.

INTRODUCTION

The Crassulaceae family, cosmopolitan, presents greater diversity in Central Asia, South Africa, the Mediterranean region and Mexico, and it is estimated that this family, composed of three subfamilies: Crassuloideae, Kalanchoideae and Sempervivoideae, includes around 1,500 species and 35 genera, of which the most important are: *Sedum* (350-500 species), *Crassula* (250 species), *Echeveria* (150 species) and *Kalanchoe* (100 species) (REYES SANTIAGO *et al*., 2011). Crassulaceae is one of the most important families of succulents, widely cultivated as ornamental plants and is probably the most cytologically complex family of angiosperms (CARRILLO-REYES *et al*., 2009).

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The so-called succulent plants are defined as plants that accumulate water in their tissues as they adapt to dry conditions, thus maintaining reserves during periods of absence of rain (EGGLI & NYFFELER, 2009). This water can be stored in the roots, stems, trunks and leaves, giving these plants a succulent appearance (EGGLI & NYFFELER, 2009). Succulent plants do not belong to a single plant family, but rather to more than 40 families, among which Crassulaceae stands out, but also Agavaceae, Cactaceae, among others (GRIFFITHS & MALES, 2017). According to Griffths & Males (2017), the diversity of anatomical and biochemical adaptations that allow succulents to maintain high water potential during drought include: mucilage, presence of large vacuoles, trichomes, diffuse and shallow roots, three-dimensional venations, thick and waxy cuticles, few stomata, high fiber content and high thermal capacity, among other ecophysiological strategies used by these plants. Around 3% to 5% of flowering plants are described as succulents - a small but significant and prominent proportion (EGGLI, 2012).

Pollen grains, microspores that are often present in large quantities in plants, differ taxonomically in shape and structure, and have nutritional, ecological and reproductive value (MOUGA & DEC, 2012). Gattuso & Gattuso (2021) show that the outer wall of the pollen grain (exine) is reinforced to prevent damage during grain transport, being composed of sporopollenin, and the inner layer (intine) is similar to that of a common plant cell.

Studies on the pollen morphology of succulent species are relevant considering their economic importance and the discrimination between species and varieties. Palynological data are needed for many taxa in this group, defined as functional and relevant in floricultural terms and with several genera in an imprecise taxonomic situation. In this context, the present study aims to collaborate with palynological knowledge, through the morphological characterization of Crassulacean pollen grains.

MATERIAL AND METHODS

Nine botanical species of Crassulaceae were studied, which originate from Mexico (genera *Echeveria, Graptopetalum* and *Sedum*) and are of African origin (*Kalanchoe*). The samples in the present study come from cultivation sites located in the region of Joinville, Santa Catarina, Brazil. The selected species lack palynological work and are: *Echeveria chroma* (hybrid created by Renee O' Connell), *Echeveria pallida* E. Walther, *Echeveria pulidonis* E. Walther, *Graptopetalum macdougallii* Alexander, *Kalanchoe humilis* Britten, *Sedum clavatum* R.T. Clausen, *Sedum mexicanum* Britton, *Sedum mocinianum* Pérez-Calix and *Sedum treleasei* Rose (figure 1).

Closed buds of the identified species were obtained, which were preserved in acetic acid and, for the processing, the buds were opened, the anthers removed, macerated, placed in acidic solutions and specific substances and subjected to centrifugation, in the acetolysis process (ERDTMAN, 1952). The acetolyzed pollen grains were mounted in glycerinated gelatin on thin slides for light microscopy (five slides per species) (ERDTMAN, 1960). The slides were placed in pollen slide collection of the Bee Laboratory (Label) at Univille-University of the Region of Joinville and subsequently observed and photographed under a light microscope (LM) (400x), using the Dino-Eye capture 2.0 software. The pollen grains of each species on the slides were photographed, performing 25 repetitions for each type of view (equatorial and polar), and the following parameters were measured, in micrometers (μm): equatorial diameter, polar axis and exine thickness (BARTH & MELHEM, 1988). The measurements were subsequently tabulated (Microsoft Excel), with the aim of obtaining the maximum, minimum, mean and standard deviation values. The following characteristics were verified: pollen unit, size, amb, symmetry, polarity, shape, openings and ornamentation.

The samples were also analyzed using Scanning Electron Microscopy (SEM) at the State University of Santa Catarina (Udesc). For this visualization, the buds that were stored in acetic acid were removed and the anthers were macerated on coverslips for mechanical release of the grains using tweezers and a drop of acetic acid. For image processing, the grains were metallized with gold-palladium, subjected to analysis and photographed.

For species for which it was not possible to perform SEM, fresh pollen slides were prepared with glycerin (BARTH & BARBOSA, 1971), then the anthers were macerated and, subsequently, a drop of fructose solution was added (50 ml of water for 10 g of fructose), followed by a drop of fuchsine solution (13 drops of dye for 20 ml of water). The fructose solution gives the appearance of fresh pollen, with a relatively turgid appearance, such that the grains appear naturally (BARTH, 1970a; 1970b). It is worth noting that, in this process, there is still the presence of the internal content of the grain, unlike the acetolysis process where this content is removed, which leads to an eventual slightly shriveled appearance of the exine (BARTH 1970c; 1970d). The fuchsine dye was added with the aim of obtaining a better visualization of aspects of the pollen grains (ALEXANDER, 1980).

The study took place from June 2022 to July 2023, at the University of the Joinville Region.

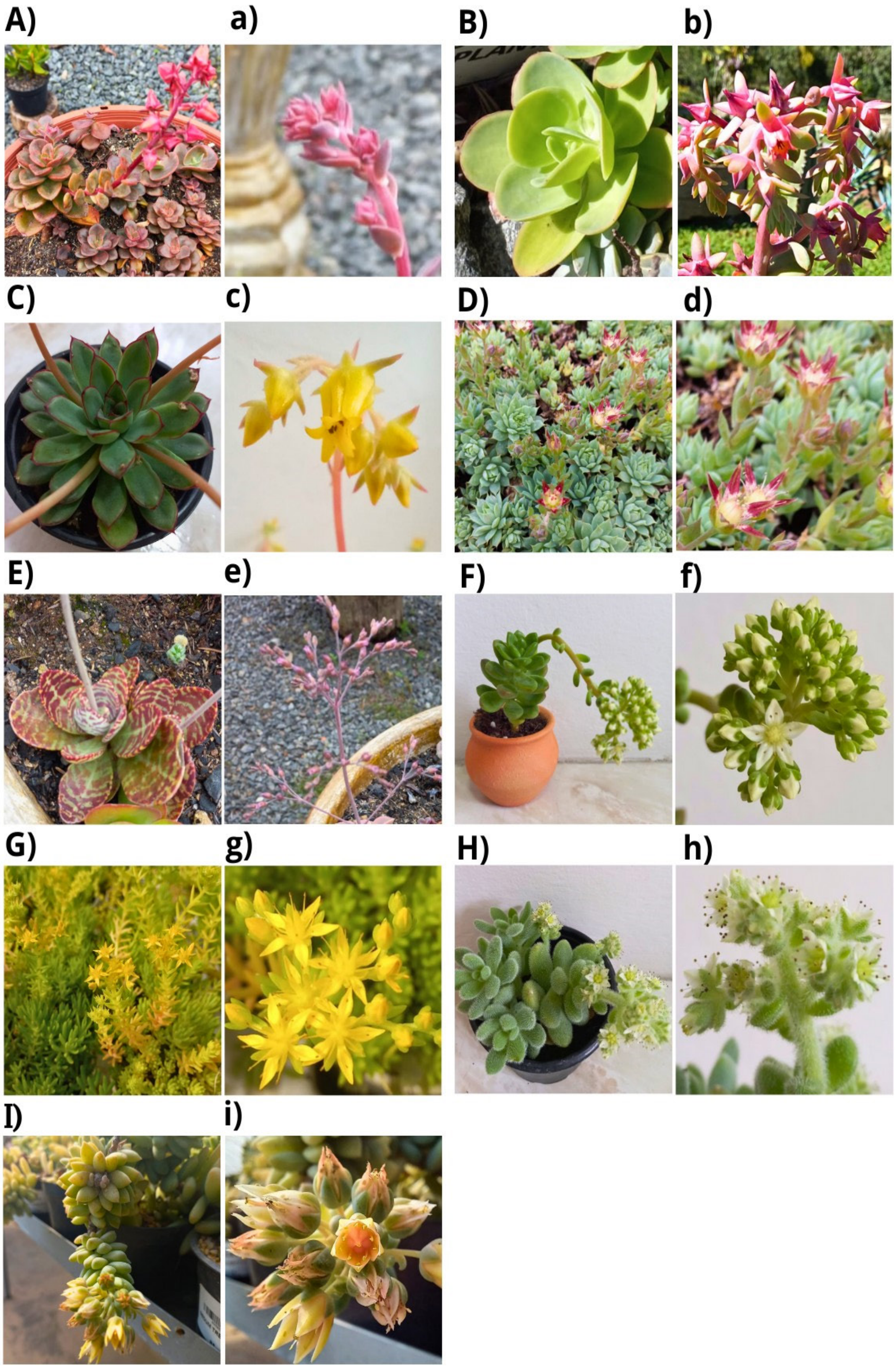


Figure 1 – Photographs of the plant species on the left (A-I) (uppercase letters), followed by photographs of their flowering on the right (a-i) (lowercase letters). (Crassulaceae) A/a) *Echeveria chroma*; B/b) *Echeveria pallida*; C/c) *Echeveria pulidonis*; D/d) *Graptopetalum macdougallii*; E/e) *Kalanchoe humilis*; F/f) *Sedum clavatum*; G/g) *Sedum mexicanum*; H/h) *Sedum mocinianum*; I/i) *Sedum treleasei*. Source: primary.

RESULTS

The descriptions of the studied species are below (table 1 and figure 2):

1 – *Echeveria chroma*

Description: monad, medium size, circular amb, radial symmetry, isopolar, prolate-spheroidal shape.
Apertures: tricolporate.
Exine: average thickness 1,58 (minimum 1,24; maximum 2,01; standard deviation 0,21), ornamentation psilate.
Measurements: average polar axis 31,68 (minimum 27,18; maximum 36,33; standard deviation 2,30); average equatorial diameter 28,12 (minimum 21,41; maximum 28,12; standard deviation 3,71).

2 – *Echeveria pallida*

Description: monad, medium size, circular amb, radial symmetry, isopolar, oblate-spheroidal shape.
Apertures: tricolporate.
Exine: average thickness 2,29 (minimum 1,72; maximum 3,18; standard deviation 0,33), ornamentation psilate.
Measurement: average polar axis 31,19 (minimum 29,57; maximum 33,45; standard deviation 1,08); average equatorial diameter 33,46 (minimum 25,76; maximum 40,88; standard deviation 5,00).

3 – *Echeveria pulidonis*

Description: monad, medium size, circular amb, radial symmetry, isopolar, prolate-spheroidal shape.
Apertures: tricolporate.
Exine: average thickness 2,16 (minimum 1,81; maximum 2,62; standard deviation 0,24), ornamentation psilate.
Measurement: average polar axis 27,92 (minimum 25,53; maximum 33,66; standard deviation 1,83); average equatorial diameter 25,98 (minimum 22,45; maximum 29,35; standard deviation 1,80).

4 – *Graptopetalum macdougallii*

Description: monad, medium size, circular amb, radial symmetry, isopolar, subprolate shape.
Apertures: tricolporate.
Exine: average thickness 2,13 (minimum 1,72; maximum 2,57; standard deviation 0,23), ornamentation rugulate.
Measurements: average polar axis 28,59 (minimum ,97; maximum 30,43; standard deviation 1,19); average equatorial diameter 22,98 (minimum 20,81; maximum 25,45; standard deviation 1,22).

5 – *Kalanchoe humilis*

Description: monad, medium size, subtriangular amb, radial symmetry, isopolar, prolate-spheroidal shape.
Apertures: tricolporate.
Exine: average thickness 1,63 (minimum 1,28; maximum 2,07; standard deviation 0,23), ornamentation psilate.
Measurements: average polar axis 25,49 (minimum 21,98; maximum 32,65; standard deviation 2,86); average equatorial diameter 24,21 (minimum 18,85; maximum 28,87; standard deviation 2,20).

6 – *Sedum clavatum*

Description: monad, small size, circular amb, radial symmetry, isopolar, subprolate shape.
Apertures: tricolporate.
Exine: average thickness 1,56 (minimum 1,31; maximum 1,85; standard deviation 0,14), ornamentation rugulate.
Measurements: average polar axis 22,41 (minimum 20,27; maximum 28,39; standard deviation 1,72); average equatorial diameter 16,97 (minimum 12,08; maximum 20,68; standard deviation 1,51).

7 – *Sedum mexicanum*

Description: monad, small size, circular amb, radial symmetry, isopolar, subprolate shape.
Apertures: tricolporate.
Exine: average thickness 1,90 (minimum 1,56; maximum 2,53; standard deviation 0,22), ornamentation rugulate.
Measurements: average polar axis 20,50 (minimum 18,83; maximum 22,00; standard deviation 0,85); average equatorial diameter 15,66 (minimum 14,25; maximum 17,47; standard deviation 0,94).

8 – *Sedum mocinianum*

Description: monad, small size, subcircular amb, radial symmetry, isopolar, subprolate shape.
Apertures: tricolporate.
Exine: average thickness 1,54 (minimum 1,15; maximum 2,21; standard deviation 0,28), ornamentation rugulate.
Measurements: average polar axis 21,78 (minimum 12,54; maximum 26,18; standard deviation 2,71); average equatorial diameter 18,57 (minimum 15,75; maximum 22,07; standard deviation 1,79).

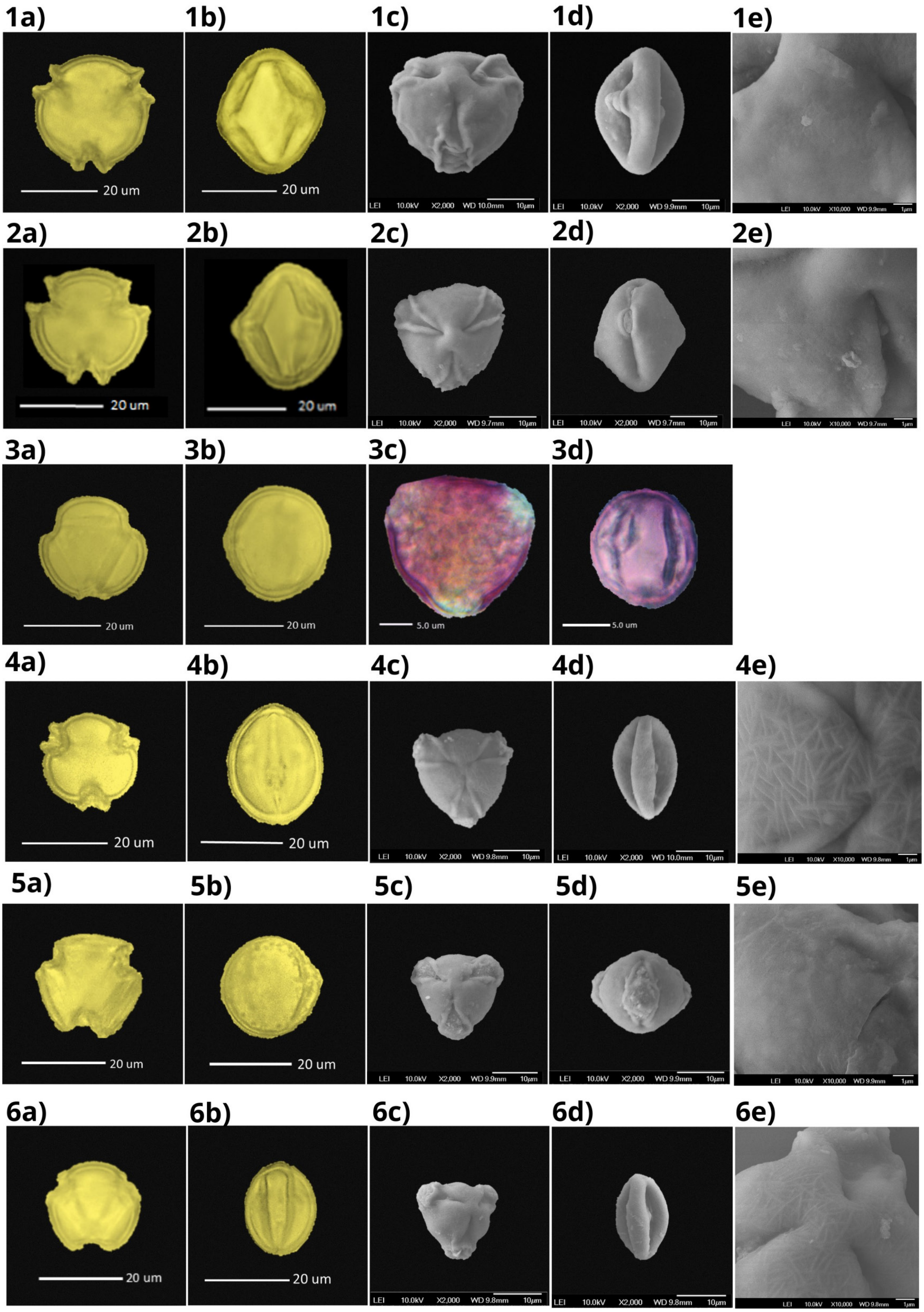
9 – *Sedum treleasei*

Description; monad, small size, circular amb, radial symmetry, isopolar, prolate-spheroidal shape.
Apertures: tricolporate.
Exine: average thickness 1,58 (minimum 1,02; maximum 2,50; standard deviation 0,35), ornamentation rugulate.
Measurements: average polar axis 22,30 (minimum 20,62; maximum 25,56; standard deviation 1,50); average equatorial diameter 20,62 (minimum 18,03; maximum 22,92; standard deviation 1,23).

It is worth mentioning that for species 3 – *Echeveria pulidonis*, 7 – *Sedum mexicanum* and 9 – *Sedum treleasei*, the exine ornamentation was visualized only in LM.

Table 1 – Morphological data of the pollen grains of the crassulaceans species analyzed. Presentation of data: minimum, (average), maximum and standard deviation respectively. All measurements were done in micrometers (µm).

N	Species	Polar axis (P)	Equatorial diameter (E1)	Exine thickness	Shape
1	*Echeveria chroma*	27,18 (31,68) 36,33 2,30	21,41 (28,12) 28,12 3,71	1,24 (1,58) 2,01 0,21	Prolate-spheroidal
2	*Echeveria pallida*	29,57 (31,19) 33,45 1,08	25,76 (33,46) 40,88 5,00	1,72 (2,29) 3,18 0,33	Oblate-spheroidal
3	*Echeveria pulidonis*	25,53 (27,92) 33,66 1,83	22,45 (25,98) 29,35 1,80	1,81 (2,16) 2,62 0,24	Prolate-spheroidal
4	*Graptopetalum macdougallii*	25,97 (28,59) 30,43 1,19	20,81 (22,98) 25,45 1,22	1,72 (2,13) 2,57 0,23	Subprolate
5	*Kalanchoe humilis*	21,98 (25,49) 32,65 2,86	18,85 (24,21) 28,87 2,20	1,28 (1,63) 2,07 0,23	Prolate-spheroidal
6	*Sedum clavatum*	20,27 (22,41) 28,39 1,72	12,08 (16,97) 20,68 1,51	1,31 (1,56) 1,85 0,14	Subprolate
7	*Sedum mexicanum*	18,83 (20,50) 22,00 0,85	14,25 (15,66) 17,47 0,94	1,56 (1,90) 2,53 0,22	Subprolate
8	*Sedum mocinianum*	12,54 (21,78) 26,18 2,71	15,75 (18,57) 22,07 1,79	1,15 (1,54) 2,21 0,28	Subprolate
9	*Sedum treleasei*	20,62 (22,30) 25,56 1,50	18,03 (20,62) 22,92 1,23	1,02 (1,58) 2,50 0,35	Prolate-spheroidal



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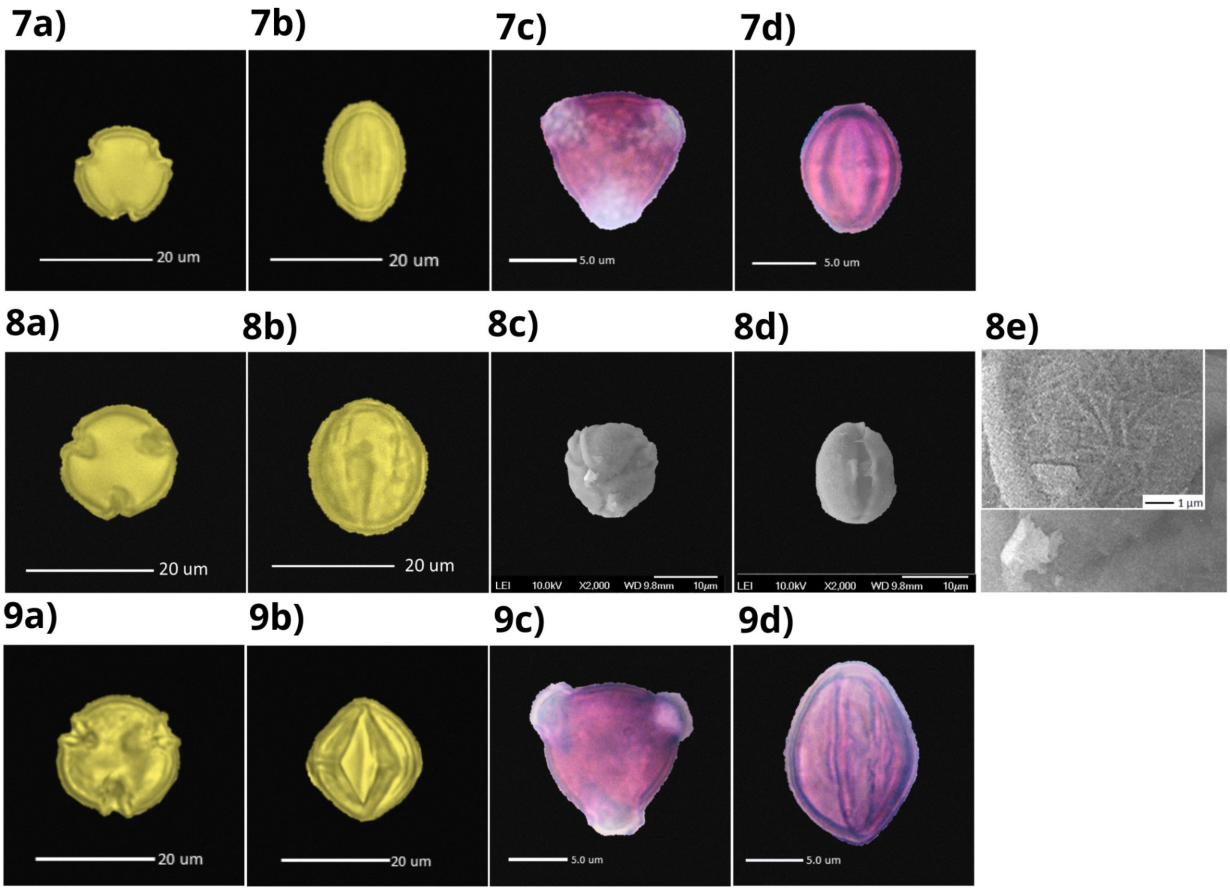

Figure 2 – Images of the pollen grains of the succulent species in LM (a – polar view; b – equatorial view) and SEM (c – polar view; d – equatorial view; e – exine). 1a-1e) *Echeveria chroma*; 2a-2e) *Echeveria pallida*; 3a-3d) *Echeveria pulidonis*; 4a-4e) *Graptopetalum macdougallii*; 5a-5e) *Kalanchoe humilis*; 6a-6e) *Sedum clavatum*; 7a-7d) *Sedum mexicanum*; 8a-8e) *Sedum mocinianum*; 9a-9d) *Sedum treleasei*. The images in purple are of pollen grains stained fresh with fucsine. The image 8e focused on the ornamentation of the exine in *S. mocinianum* with high contrast. Source: primary.

It is important to highlight that, in the figures, some pollen grains are found dehydrated, which provides a relatively withered appearance, as it is possible to observe in the SEM images (figure 2) of the following species: *Echeveria chroma* (1c and 1d), *Graptopetalum macdougallii* (4d) and *Sedum clavatum* (6d).

Supplementary images of SEM pollen grains from some succulent species are shown in figure 3.

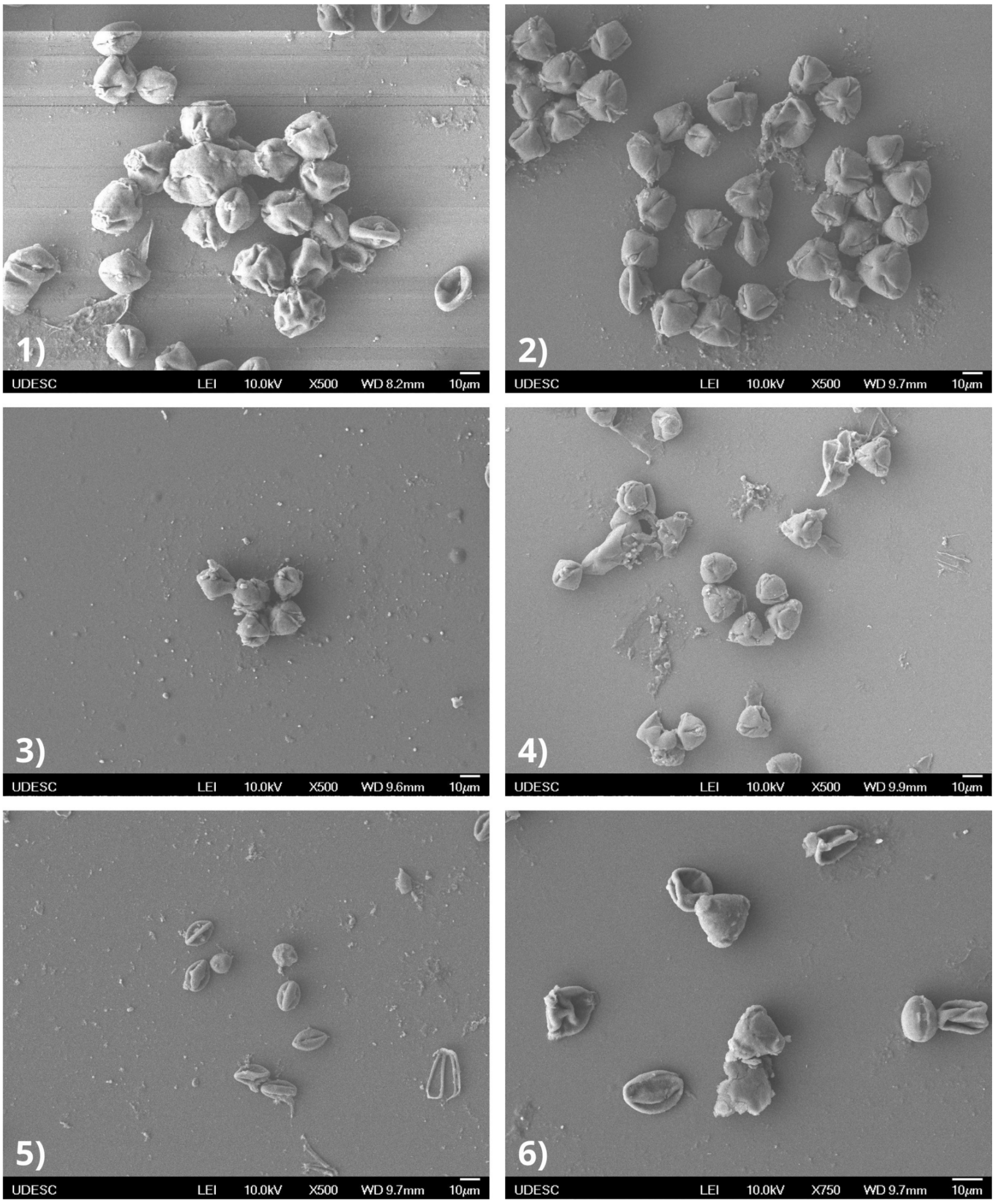

Figure 3 – Supplementary images of the pollen grains in SEM of some species of succulents. 1) *Echeveria chroma*; 2) *Echeveria pallida*; 3) *Graptopetalum macdougallii*; 4) *Kalanchoe humilis*; 5) *Sedum clavatum*; 6) *Sedum moicianum. Source*: primary.

DISCUSSION

In general, the pollen grains of the taxa studied here, belonging to the Crassulaceae family, presented grains in monads, small to medium in size, with circular, subcircular or subtriangular amb, isopolar, with small polar area, prolate-spheroidal, subprolate or oblate-spheroidal in shape, tricolporate, with openings at the angles of the grain contour in polar view, sometimes with circular, operculate openings, with psilate or rugulate ornamentation. Some observed morphological characteristics indicate a harmomegathy process.

Echeveria

Echeveria is a large genus of Crassulaceae (150 species), with a wide geographic distribution, extending from Texas to Argentina (REYES SANTIAGO *et al.*, 2011), distributed mainly in Mexico (127 species), being one of the most important of the aforementioned country, since 97.2% of its species are endemic there (VILLASEÑOR, 2016).

The pollen grains of the species of the genus *Echeveria* studied here had morphologically similar results to each other and to other studied species of the same genus (https://pollen.tstebler.ch/MediaWiki/index.php?title=Artenliste; González-Mancera *et al*., 2018), with regard to the thickness of the exine, which here revealed approximate values between the taxa in focus, to the amb close to circular and to the tricolporate openings.

It is worth mentioning that González-Mancera *et al*. (2018) cited the presence of three compound openings, with pollen grains sometimes having four openings. However, this last characteristic was not observed with the *Echeveria* specimens in the present work. The same mentioned authors also point out the *operculum* (thickened margin at the opening (*sensu* Iversen & Troels-Smith, 1950)) of the pollen grains they studied and which are configured as thickenings in the exine, covering most of the openings, for protection against pathogens and dehydration These structures were observed in the present study in the species *Echeveria chroma, E. pallida* and *E. pulidonis.*

Echeveria is a polyploid genus, with a great diversity of species and morphologies, of special interest in cytogenetic research as it presents a variety of chromosome numbers and ploidy levels (endopolyploidy) (PALOMINO *et al.*, 2021), with the formation of cells with very large nuclei, a consequence of endopolyploidization. Endopolyploidization can be triggered by stress caused by environmental changes, such as too little or too much light, temperature changes, water stress, etc., as part of a response that allows plants to adapt to these conditions and overcome physical or genomic data resulting from stress (UHL, 2006). As morphological differences within species, however, are not all consistently correlated with differences in ploidy and as different chromosomal variants cannot always be reliably identified by their morphology, a study on a possible correlation between variations in pollen morphology and ploidy levels can contribute to knowledge about the taxonomy of the genus and the evolution of species, in addition to the fact that this information can provide useful knowledge for biotechnology, conservation and floriculture programs.

Graptopetalum

Graptopetalum is a small genus from the southern United States and Mexico, with 20 species, found mainly in semi-arid vegetation from Arizona, in the United States, to Oaxaca, in Mexico (UHL, 1970). It is a member of the Acre clade (of Crassulaceae), which includes species from genera such as *Cremnophila* Rose, *Echeveria* DC., *Pachyphytum* Link, Klotzsch & Otto, *Sedum* L., *Tacitus* Moran & Meyra´n, and *Thompsonella* Britton & Rose (MORT *et al.,* 2001). The genus *Graptopetalum* has limits that still need to be defined, since it was recovered and incorporated into taxa such as *Echeveria, Cremnophila, Sedum,* among others (CARRILLO-REYES *et al.*, 2009). A phylogenetic analysis of *Graptopetalum* using morphological characters does not support the monophyly of the genus unless certain species of *Sedum* are transferred to *Graptopetalum.* Thus, palynological knowledge about the genus can help clarify the taxonomic delimitation of the taxon.

One species of *Graptopetalum* has been studied palynologically, *G. bellum* (Halbritter & Buchner, 2016; https://pollen.tstebler.ch/MediaWiki/index.php?title=Artenliste#G), with which *G. macdougalii,* the species here studied, shares most of the characteristics.

The *operculum* (again *sensu* Iversen & Troels-Smith, 1950) were observed in the present study in *Graptopetalum macdougallii* (figure 2). The relative similarity of *Graptopetalum* with *Echeveria* is expected, since *Graptopetalum* was previously included within *Echeveria* and other taxa, being recognized as an independent genus only later (ACEVEDO-ROSAS *et al.*, 2004). Furthermore, the ornamentation of *G. macdougalii* (rugulate) was confirmed in electron microscopy, an ornamentation already pointed out in other palynological analyzes of the genera where *Graptopetalum* was previously designated (CARRILLO-REYES *et al.*, 2009).

Graptopetalum macdougallii is a species of cytological interest as it has very high chromosomal numbers (the species has a type collection with n = 192, with n = 244 having been recorded in another collection, apparently of the same species) (UHL, 1961). As two ancient species of *Sedum* were transferred to *Graptopetalum (G. craigii* and *G. suaveolens*) (ACEVEDO-ROSAS *et al.*, 2004), knowledge about *Graptopetalum* helps, in addition, to evaluate the group *''Sedum sensu lato''*, this last one having not yet been completely resolved in terms of circumscription. *Graptopetalum* has already had its name placed on the Mexican red list of threatened species (SEMARNAT, 2002). In short, understanding pollen morphology in *Graptopetalum* can lead to a better understanding of the relationships of this group and assist in the conservation of the species.

Kalanchoe

The genus *Kalanchoe* includes around 144 species (SHAW, 2008) whose center of diversity is Madagascar, with around 67 species, with many others occurring in southern and eastern Africa and southeast Asia and two of the three *Kalanchoe* species of Taiwan, *K. garambiensis* and *K. gracilis* are endemic (AKULOVA-BARLOW, 2009).

The pollen grain of the *Kalanchoe* species in the present study was compatible with other *Kalanchoe* species: *K. densiflora* and *K. pinnata* (HEIGL, 2020; AUER, 2021), with the exception of ornamentation. On the other hand, Stebler (2023) palynologically described 13 species of *Kalanchoe* (*K. ballyi*, *K. blossfeldiana*, *K. germanae*, *K. lobata*, *K. marnieriana*, *K. orgyalis*, *K. pinnata* (*Bryophyllum pinnatum*), *K. pubescens*, *K. pumila*, *K. rodandi-bonapartei*, *K. tetraphylla*, *K. uniflora*, *K. zimbabwensis*) whose differences between them and with the species of the present work were in the presence or absence of an *operculum*, in the ornamentation and in the shape of the pore. RCPol (2023) shows the pollen grains of the species *K. blossfeldiana*, *K. delagoensis*, *K. fedtschenkoi*, *K. longiflora*, *K. pinnata*, which follow the characteristics of *Kalanchoe*, in general. Thus, in short, the analysis of the *Kalanchoe* species already studied shows a certain uniformity, with reservations in the ornamentation of the exine and the shape of the pollen.

The pollen grain of the *Kalanchoe* species in the present study (*K. humilis*) showed compatibility with other Crassulaceans, although some pollen grains revealed a tendency to have a triangular amb.

Sedum

Sedum L., is the largest genus of the subfamily Sempervivoideae and also of Crassulaceae, contains around 470 species (around 50% of the total number of species in the family) and is the most taxonomically complex of Crassulaceae (GONZÁLEZ-MANCERA *et al.*, 2018). Apparently, it has a primitive nature, as it has some of the least specialized groups of species within the Sempervivoideae subfamily, the latter characterized by basic elements of the Crassulaceae family (SARWAR & QAISER, 2012). According to Messerschmid *et al.* (2020), there are five major lineages (clades) in Sempervivoideae: Telephium, Petrosedum, Sempervivum Jovibarba, Aeonium, Leucosedum + Acre. In the five clades, with the exception of the Sempervivum clade, *Sedum* species never form a monophyletic group but are more or less spread across the respective phylogenetic trees (e.g., Acre and Leucosedum clades) or form a basal gradation (e.g., Aeonium clade). Thus, *Sedum*, in its current circumscription, is actually a highly polyphyletic (or paraphyletic) taxon. It is considered that the high level of homoplasy (convergent phenotypic characteristics) of growth forms, vegetative and reproductive characteristics traditionally used to delineate crassulacean genera, makes the phenotypic characterization of *Sedum* difficult and complicates the reconstruction of trends in morphological evolution (NIKULIN *et al*., 2016).

The pollen grains of the species of the genus *Sedum* studied here (*S. clavatum, S. mexicanum, S. mocinianum, S. treleasei*) were small, this being the common size pattern in this genus, circular in amb, subprolate to prolate-spheroidal in shape, with rugulate ornamentation, which was only possible to be observed with electron microscopy, an ornamentation already pointed out in other palynological analyzes of this genus as Qaiser *et al.* (2015) state that the most common exine pattern in this genus is striated-rugulate and, in addition to this, psilate to sub-psilate. For the species *S. mexicanum* and

S. treleasei, the images were analyzed only in LM. The four species studied here are distributed in Mexico, with *S. mexicanum* reaching as far as Colombia. Observing the work of Messerschmid *et al.* (2020), the species *S. clavatum* and *S. treleasei* are included in the Echeveria clade and the species *S. mexicanum* and *S. mocinianum*, in the Acre clade.

In general, the pollen grains of the genera studied here presented a similar pattern of morphological characteristics, which includes operculated opening and morphological characteristics of harmomegathy. *Opercula* are configured as thickenings in the exine, covering part of the openings, to protect against pathogens and grain dehydration, with the most likely hypothesis for their existence being an adaptation against grain dehydration, as these plants were adapted to a xerophytic environment (GONZÁLEZ-MANCERA *et al.*, 2018). Harmomegathy is a process of morphological and physiological adaptations of pollen grains in arid environments, by means of which the pollen wall changes its shape to accommodate variations in cytoplasm volume caused by hydration changes (DAJOS *et al.*, 1991). Gontachatrova & Gontcharov (2004) state that the morphological line developed by the pollen grain of succulents matches the arid environments in which they had to adapt. The Crassulaceae family is the most representative in Mexico, due to the high endemism of most of its species and the role it plays in the structure and function of the country's inhospitable ecosystems (TOLEDO, 1988).

Crassulaceae pollen was first characterized by Erdtman (1952) and later by several authors, as being radially symmetric, isopolar, suboblate to oblate-spheroidal, prolate-spheroidal or subprolate, rarely prolate, tricolporate, trizonocolporate. generally triangular, three-lobed or circular in polar view and elliptical or broadly elliptical in equatorial view, with striate-rugulate to psilate exine rarely reticulate-rugulate.

According to Qaiser *et al.* (2015), Crassulaceae is a medium-sized family, probably the most cytologically complex, with highly intricate cytological and chromosomal variations (UHL, 1961) and, in the same way, the morphology of the family's pollen is also quite heterogeneous as it shows a considerable variation, particularly in the ornamentation of the exine and the class of pollen shape, being considered a eurypolinic family.

It is worth noting that similar pollen grains are found in families such as Rosaceae and Saxifragaceae (STEBLER, 2020).

CONCLUSION

Although the Crassulaceae are easily recognizable, identifying monophyletic groups within the family is very difficult due to the wide range of morphological, cytological and habit traits and, thus, the generic limits and the relationship between the genera are not clear due to the frequent intergradation of morphological aspects between taxa.

The environmental conditions in which these plants developed put pressure on their morphology, and this can be observed in their reproductive structures (such as the pollen grain), with reproduction being a primary factor of extreme importance for the continuity of the species.

The present study seeks to provide palynological data that support knowledge about morphology, aiming to contribute to systematics.

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