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Pollen characterization of ornamental species of *Mammillaria* Haw. (Cactaceae/Cactoideae)

Caracterização polínica de espécies ornamentais de Mammillaria *Haw. (Cactaceae/Cactoideae)*

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ABSTRACT
The pollen morphology of thirteen species of Cactaceae was studied: *M. backebergiana* F.G. Buchenau, *M. decipiens* Scheidw, *M. elongata* DC, *M. gracilis* Pfeiff., *M. hahniana* Werderm., *M. marksiana* Krainz, *M. matudae* Bravo, *M. nejapensis* R.T. Craig & E.Y. Dawson, *M. nivosa* Link ex Pfeiff., *M. plumosa* F.A.C. Weber, *M. prolifera* (Mill.) Haw, *M. spinosissima* var. "A Peak" Lem. and *M. voburnensis* Scheer. All analysed pollen grains are monads, with radial symmetry, medium size (*M. gracilis, M. marksiana, M. prolifera,* large), tricolpates (dimorphs in *M. plumosa* [3-6 colpus] and *M. prolifera* [3-6 colpus]), with circular-subcircular amb (quadrangular in *M. prolifera* and *M. plumosa* with six colpus). The pollen grains presented differences in relation to the shape and exine thickness. The exine was microechinate and microperforated. The pollen morphological data are unpublished and will aid in studies that use pollen samples. These pollen grains indicate ornamental cacti.
Keywords: cactus; Coryphunthanae; Palynology.

RESUMO
A morfologia polínica de 13 espécies de Cactaceae foi estudada: *M. backebergiana* F.G. Buchenau, *M. decipiens* Scheidw., *M. elongata* DC., *M. gracilis* Pfeiff., *M. hahniana* Werderm., *M. marksiana* Krainz, *M. matudae* Bravo, *M. nejapensis* R.T. Craig & E.Y. Dawson, *M. nivosa* Link ex Pfeiff., *M. plumosa* F.A.C. Weber, *M. prolifera* (Mill.) Haw., *M. spinosissima* var. "Un Pico" Lem. e *M. voburnensis* Scheer. Todos os grãos analisados eram mônades, com simetria radial, tamanho médio (*M. gracilis*, *M. marksiana*, *M. prolifera*, grande), tricolpados (dimorfos em *M. plumosa* [3-6 colpos] e *M. prolifera* [3-6 colpos]), com âmbito circular-subcircular (quadrangular em *M. prolifera* e *M. plumosa* quando com seis colpos). Os grãos de pólen apresentaram diferenças em relação à forma e espessura da exina. A exina é microequinada e microperfurada. Os dados morfológicos de pólen são inéditos e poderão auxiliar em estudos que utilizam amostras polínicas. Esses grãos de pólen indicam cactos ornamentais.
Palavras-chave: cactos; Coryphunthanae; Palinologia.

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INTRODUCTION

The Cactaceae include xerophytic and epiphytic plants, comprise over 2000 species and at least 125 genera, being considered a monophyletic family, sustained by several morphological characters and DNA sequence data (GARRALLA *et al.*, 2013).

Based on floral morphology, vegetative morphology, genetic sequences and palynologycal morphology, four subfamilies, Pereskiodeae, Opuntiodeae, Mahiuenoideae and Cactoideae, are

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currently recognized as natural groups (ANDERSON, 2001). The subfamily Cactoideae is the most differentiated and contains about 75% of the diversity of species within the family (HUNT, 1967; 1981; 1987). The monophyly of Cactoideae is sustained by a tiny leaf subtending each *areola,* succulent stems with *costa* (there are some exceptions), areolas without *gloquidia* and the extreme reduction or complete absence of leaves (WALLACE & GIBSON, 2002).

Into Cactoideae, the subtribe Coryphunthanae comprises the *genus Mammillaria* (figure 1) whose members are fairly small cacti, usually with distinctly globular-tuberculate-elongated stem morphology, colourfully spined, low growing, profusely flowering with small flowers arranged usually in a ring around the crown, succeeded by smooth juicy club shaped berries mostly of brilliant red color and plants either solitary or in massive mounds (HEYWOOD, 1985).

These traits are shared with other members of the "Mammilloid clade" which also share the presence of dimorphic areoles – the vegetative (spine-bearing) areole is borne on the tubercle apex while the flowering areoles are located in the axils of the tubercles (BUTTERWORTH *et al*., 2002).

Figure 1 – Species of *Mammillaria* of this study: 1) *M. backebergiana*, 2) *M. decipiens*, 3) *M. elongata*, 4) *M. gracilis*, 5) *M. hahniana*, 6) *M. marksiana*, 7) *M. matudae*, 8) *M. nejapensis*, 9) *M. nivosa*, 10) *M. plumosa*, 11) *M. prolifera*, 12) *M. spinosissima*, 13) *M. voburnensis.*

Mammillaria is distinct from the other genera of Coryphunthanae (*Coryphantha*, *Escobaria*, *Pelecyphora*, *Neolloydia* and *Ortegocactus*) in lacking an adaxial groove running from the vegetative areole, in some cases, along the entire length of the tubercle (BUTTERWORTH & WALLACE, 2004).

Distribution of the genus *Mammillaria* ranges from Venezuela and Colombia to the Southwestern United States, with maximal diversity and species richness in Mexico and it includes more than 200 species (BARTHLOTT & HUNT, 1993).

Pollen morphology of Cactaceae was first studied by Kurtz Jr. (1948; 1963) who continued over 110 genera and about 700 species as well as Leuenberger (1976) and some other regional reviews (HEUSSER, 1971; CUADRADO & GARRALLA, 2009; RAMÍREZ, 2012; BAUERMANN *et al.*, 2013; GARRALLA *et al.*, 2013; CANCELLI *et al.*, 2017) among others. The Cactaceae family is taxonomically difficult, presenting problems in the delimitation of genera and species, and the circumscription of many *taxa* shows inconsistences due to the variation of the morphological characters (JUDD *et al.*, 2009). As Palynology is considered a useful tool to discriminate between related species, this study aims to analyze species of *Mammilaria* in order to contribute to the pollen definition of the *genus,* whose data are scarce.

MATERIAL AND METHODS

Pollen grains of thirteen *taxa* were studied: *Mammillaria backebergiana* F.G. Buchenau, *M. decipiens* Scheidw, *M. elongata* DC, *M. gracilis* Pfeiff., *M. hahniana* Werderm., *M. marksiana* Krainz, *M. matudae* Bravo, *M. nejapensis* R.T. Craig & E.Y. Dawson, *M. nivosa* Link ex Pfeiff., *M. plumosa* F.A.C. Weber, *M. prolifera* (Mill.) Haw, *M. spinosissima* var. "A Peak" Lem. and *M. voburnensis* Scheer.

Samplings were done at the *Cactarium* of the Botanic Garden of Univille (University of the Region of Joinville), during the blooming months of the studied species. Closed floral buds were conserved in glacial acetic acid, with the botanic identification and the sampling date, in sealed bottles. The anthers of the floral buds were taken off and torn for the liberation of the pollen grains that were submitted to the acetolysis method (ERDTMANN, 1960). Recommendations of Salgado-Labouriau (1973) were followed with the utilization of Kisser's gelatin, closing the slides with paraffin. Measures and photographs of the pollen grains were taken in a delay of seven days, from the pollen slides, observed on the light microscope. Measures are presented in micrometers. The pollen grains of each species were photographed 25 times in each of the views, polar (P) and equatorial (E), utilizing all slides. The characterization of the form of the pollen grains was performed by calculating the ratio P/E. For each analyzed species, in relation to the size of the pollen grain, we verified, in both views, the maximum size, the minimum size, the average size and the standard deviation. The number and type of apertures was observed as well as details of the ornamentation of the exine. All details were registered and described according to the terminologies of Barth & Melhem (1988) and Punt *et al.* (2007). The observations took place under a light microscope Bioval with equipment Dino-Eye Microscope Eye-Piece Camera, associated with the software DinoCapture 2.0 at the University of the Region of Joinville and on a scanning electron microscope (SEM) of the University of the State of Santa Catarina (UDESC). Pollen slides were numbered and deposited at the pollen reference slides collection of Label-Bee Laboratory, at the University of the Region of Joinville. Measures were submitted to statistical analysis on Microsoft Excel.

RESULTS

Results are presented on tables 1 and 2 and figures 2A, 2B and 2C (where MEV means SEM). All pollen grains are monads, with radial symmetry, isopolar. Size is medium (*M. gracilis*, *M. marksiana*, *M. prolifera*, large). All species are 3-colpate, with dimorphic grains in *M. plumosa* (3-6 colpus) and *M. prolifera* (3-6 colpus). Amb varies between circular and subcircular (quadrangular in *M. prolifera* and *M. plumosa* when with six colpus). Form and exine thickness are oblate-spheroidal (*M. backebergiana* [P=44,14 µm; E=49,46 µm; ex.=1,72], *M. decipiens* [P=39,61 µm; E=44,93 µm; ex.=2,63], *M. elongata* [P=44,52 µm; E=48,28 µm; ex.=2,84], *M. gracilis* [P=46,25 µm; E=51,72 µm; ex.=3,13], *M. hahniana* [P=42,19 µm; E=47,51 µm; ex.=2,21], *M. matudae* [P=40,21 µm; E=45,56

µm; ex.=2,53], *M. spinosissima* [P=43,21 µm; E=48,82 µm; ex.=2,54], *M. voburnensis* [P=41,32 µm; E=45,48 µm; ex.=2,59]) and suboblate (*M. marksiana* [P=43,77 µm; E=51,58 µm; ex.=3,5], *M. nejapensis* [P=39,70 µm; E=45,49 µm; ex.=2,09], *M. nivosa* [P=40,83 µm; E=49,28 µm; ex.=2,83], *M. plumosa* [P=42,19 µm; E=50,36 µm; ex.=3,1], *M. prolifera* [P=53,73 µm; E=63,76 µm; ex.=3,37]). Exine is rugulate or microechinate and microperforate in the analyzed species.

Table 1 – Morphometric data of the pollen grains from analyzed species. Caption: P = polar diameter; E = equatorial diameter; P/E: polar equatorial rate. Average, minimum and maximum measures for exine thickness.

	P/E	Form	Exine thickness medium (minimum-maximum)	Apertures
M. backebergiana	0,89	Oblate-spheroidal	1,72 (1,24 – 2,29)	3 – Colpus
M. decipiens	0,88	Oblate-spheroidal	2,63 (2,05 – 3,24)	3 – Colpus
M. elongata	0,92	Oblate-spheroidal	2,84 (1,76 – 3,85)	3 – Colpus
M. gracilis	0,89	Oblate-spheroidal	3,13 (2,34 – 4,04)	3 – Colpus
M. hahniana	0,88	Oblate-spheroidal	2,21 (1,24 – 2,69)	3 – Colpus
M. marksiana	0,84	Suboblate	3,38 (2,59 – 4,69)	3 – Colpus
M. matudae	0,88	Oblate-spheroidal	2,53 (1,67 3,39)	3 – Colpus
M. nejapensis	0,87	Suboblate	2,09 (1,41 – 2,69)	3 – Colpus
M. nivosa	0,82	Suboblate	2,83 (1,46 – 3,92)	3 – Colpus
M. plumosa	0,83	Suboblate	3,1 (2,34 3,69)	3-6 Colpus
M. prolifera	0,84	Suboblate	3,37 (2,64 – 4,34)	3-6 Colpus
M. spinosissima	0,88	Oblate-spheroidal	2,54 (2,14 – 2,91)	3 – Colpus
M. voburnensis	0,9	Oblate-spheroidal	2,59 (1,89 – 3,74)	3 – Colpus

Table 2 – Morphometric data of the pollen grains from analyzed species. Caption: P = polar diameter; E = equatorial diameter. Average, minimum and maximum measures for P and E.

	Exine ornamentation	*Ambitus*	P medium (minimum-maximum)	E medium (minimum-maximum)	Size
M. backebergiana	Microperfurate microechinate	Circular	44,14 (35,28 – 51,02)	49,46 (37,31 – 60,63)	Medium
M. decipiens	Microperfurate microechinate	Circular	39,61 (36,31 – 43,12)	44,93 (41,82 – 47,61)	Medium
M. elongata	Microperfurate microechinate	Subcircular	44,52 (36,95 – 53,99)	48,28 (41,89 – 57,31)	Medium
M. gracilis	Rugulate	Circular	46,25 (43,90 – 50,22)	51,72 (48,85 – 58,91)	Large
M. hahniana	Microperfurate microechinate	Circular	42,19 (35,32 – 48,03)	47,51 (42,33 – 52,64)	Medium
M. marksiana	Microperfurate microechinate	Subcircular	43,77 (37,43 – 53,60)	51,58 (42,99 – 58,62)	Medium
M. matudae	Microperfurate microechinate	Circular	40,21 (35,47 – 44,68)	45,56 (40,87 – 50,13)	Medium
M. nejapensis	Rugulate	Circular	39,70 (37,08 – 44,43)	45,49 (42,99 – 49,41)	Medium

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	Exine ornamentation	*Ambitus*	P medium (minimum-maximum)	E medium (minimum-maximum)	Size
M. nivosa	Microperfurate microechinate	Circular	40,83 (33,38 – 43,92)	49,28 (45,87 – 53,35)	Medium
M. plumosa	Rugulate	Subcircular-quadrangular	42,19 (38,94 – 47,41)	50,36 (47,86 – 52,58)	Medium
M. prolifera	Microperfurate microechinate	Circular-quadrangular	53,73 (46,07 – 61,19)	63,76 (55,57 – 73,74)	Large
M. spinosissima	Rugulate	Circular	43,21 (39,43 – 51,04)	48,82 (43,03 – 53,06)	Medium
M. voburnensis	Microperfurate microechinate	Circular	41,32 (38,38 – 47,94)	45,48 (41,49 – 55,63)	Medium

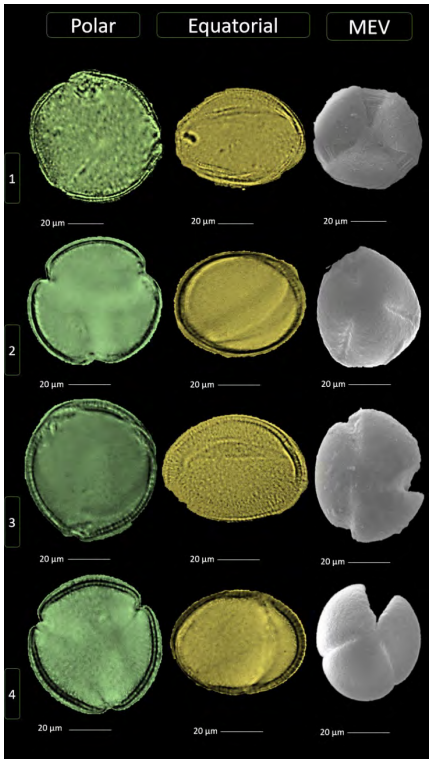

Figure 2A – Pollen grains of the studied species: 1) *M. backebergiana*, 2) *M. decipiens*, 3) *M. elongate*, 4) *M. gracilis*.

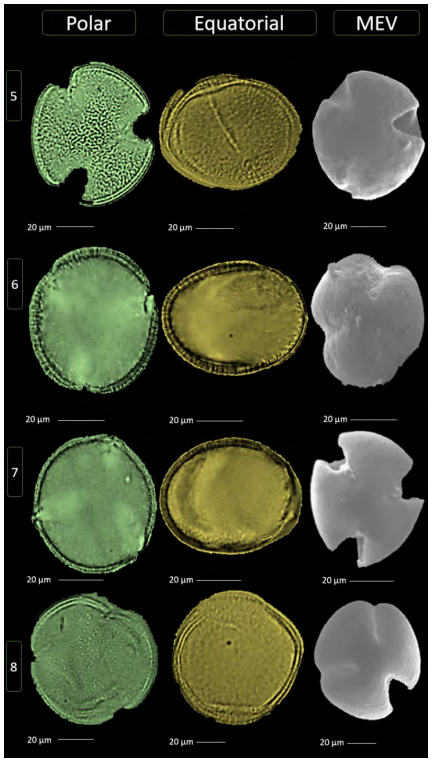

Figure 2B – Pollen grains of the studied species: 5) *M. hahniana*, 6) *M. marksiana*, 7) *M. matudae*, 8) *M. nejapensis.*

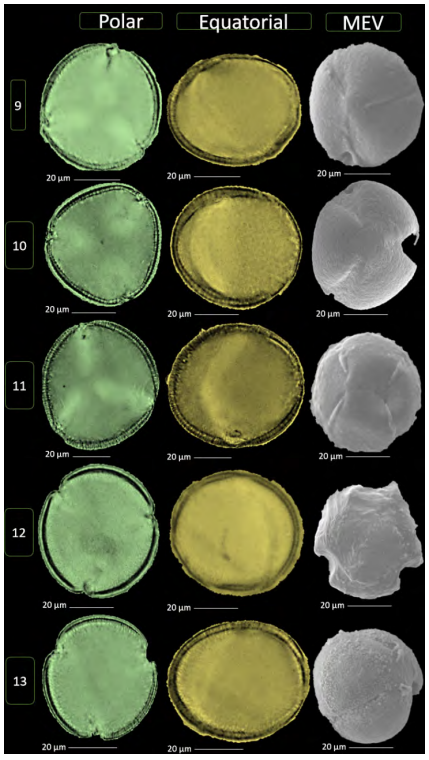

Figure 2C – Pollen grains of the studied species: 9) *M. nivosa*, 10) *M. plumosa*, 11) *M. prolifera*, 12) *M. spinosissima*, 13) *M. voburnensis*.

DISCUSSION

The studied species show a conservative pattern as nearly all grains display the same arrangement, with few or minor variations, mainly in the size (medium and large), amb (circular, subcircular and quadrangular) and form (suboblate, oblate-spheroidal). The ornamentation of the exine is also very uniform (regulate or microechinate/microperforate) in the analyzed species.

According to Kurtz Jr. (1948), pollen grains of *Mammillaria vivipara* Engelm. vary from 43 to 50 µm, *M. deserti* Engelm. from 52 to 54 µm, *M. microcarpa* Engelm. 53-54 µm, *M. alversonii* (Coulter) Zeissold 58-59 µm, *M. arizonica* Engelm. 60-63 µm. Buchner & Halbritter (2011) published that *Mammillaria micromeris* grains are in monads, large (51-100 µm), colpate, isopolar, spheroidal, with circular amb, aperture membrane ornamented, exine perforate, microechinate, eutectate and with Ubisch bodies present. For Pollen (2018), *Mammillaria magnimamma* is medium sized (38.9-43.7 µm), tricolpate, psilates, scabrates, verrucate or with microverrucate sculptures, roundish amb to slightly triangular, spheroidal, isopolar, with apertured membranes grainy and *M. toluca* is medium (36.5-41.2 µm), tricolpate, psilate, scabrate, verrucate or with microverrucate sculptures, circular to slightly triangular amb, spheroidal, isopolar, the aperture membranes grainy, with medium polar field.

Miesen *et al*. (2015) stated that the pollen grains of Cactaceae are subspheroidal, prolate or oblate, apolar or isopolar, tricolpate or periporate, with a relatively large diameter (40-100 µm). According to Kurtz Jr. (1963), the palynologycal characteristics of the subtribe Coryphunthanae include diameter of 33-70 micrometers, number of furrows 3 and sculpturing of the exine pitted.

The great homogeneity observed in this work points to a pattern in pollen morphology of *Mammillaria* that remains, despite that fact that the *genus* presents ornamental interest and has been hybridized many times.

Early classifications included in *Mammillaria* members of some now separated genera, like *Coryphantha* and *Ariocarpus*. Added to this, there is the fact that a collection of names was created by earlier commercial plant collectors. As a result, the large and diverse genus *Mammillaria* has seen multiple attempts to subdivide the species in it into smaller groups within the genus or attempts to split it into multiple genera for better understanding of the plants' relationship. Consequently, some genera (*Dolichothele, Krainzia, Mammillopsis* and others) have been merged back into *Mammillaria* and others, like *Escobaria* and *Mammilloydia,* were confirmed as separate. Following the reorganization of the genus *Opuntia* by Wallace and Dickie (2002) into a number of segregate genera, the genus *Mammillaria* has taken precedence as the most species-rich genus in the cactus family. Modern estimates of species numbers vary greatly depending upon circumscription at both the generic and specific levels and thus, of 181 species recognized by Pilbeam (1999), Hunt (1999) accepts 145 species.

The core group of *Mammillaria* seems to be driven by a strong morphological convergence and, as currently circumscribed, the genus *Mammillaria* is polyphyletic on a number of levels, being likely to be split into two large genera, one of them possibly including certain species of other closely related genera like *Coryphantha*, *Ortegocactus* and *Neolloydia* (BUTTERWORTH & WALLACE, 2004). Mattagajasingh *et al.* (2006) found considerable polymorphism in *Mammilaria* and state that finding wide genetic distances reveals that there is relatively high genetic variation among the species of the genus.

Pollen morphology provides additional useful information to clarify differences and affinities between *taxa* may help to establish phylogenetic order. Once a well-supported phylogeny has been produced, assessments of morphology can be utilized along with phylogenetic information to yield a reliable infrageneric classification within *Mammillaria*. Till then, conclusions regarding *Mammillaria* must be viewed with caution. Anyway, these pollen grains, when found in pollinic samples, may indicate ornamental cacti of Mammilaria. *Mammillaria* is a source of interest for collectors. Its proper identification and an accurate system of classification is required for academic interest, conservation and for successful breeding programs.

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