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PALYNOLOGY OF TWENTY ONE SPECIES OF ECHEVERIA GENUS (CRASSULACEAE) FROM MEXICO – HIGH RESOLUTION STUDY

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ABSTRACT
Mexico has the largest diversity of genus *Echeveria*. To preserve the biological diversity of this highly endemic plant group, as well as to make its industrial production sustainable, the complete knowledge of their attributes is needed. Nevertheless, few taxonomic characteristics for the genus have been registered so far (little of its taxonomy is known, especially about its palynology); some species have not been described yet, and consequently they are not associated to any taxonomic name. The main aim of this paper is to provide data about its palynology. This work characterizes by Scanning Electron Microscope (SEM) the acetolysed pollen of species of the genus *Echeveria* (*Crassulaceae*). Twenty species of *Echeveria* were studied. The samples were subjected to acetolysis, and processed according to conventional method to be analyzed by SEM. The results revealed the following characteristics for *Echeveria* pollen: it presented isopolar symmetry; solitary free grain; regularly spheroidal shape, mostly with three compound apertures but sometimes with four; size range from small to medium; morphological features that indicate process of harmomegathy. Exine presented ornamentation typically regulate, and sub-layer structure not continuous (semitectate).

Keywords: Echeveria pollen, Crassulaceae, Mexico, Palinology, Harmomegathy.

INTRODUCTION

Echeveria is a large genus of Crassulaceae, with wide geographic distribution extending from Texas to Argentina. Mexico has the largest diversity of this group of plants in the World [1], with about 140 species, eighty-three percent of which are endemic to Mexico. Most of them are in danger of extinction and unfortunately, they have not been taxonomically identified yet. *Echeveria* plants have ornamental use and like cactus and orchids it has been illegally collected increasing the risk of extinction associated to endemic taxa. They have been appreciated since pre-Hispanic times not only as ornamental plants but also as traditional medicine. For example, Echeverias are considered cold-quality plants that are used to remove headaches, lower the body temperature and, as an antiseptic in wounds and sores, to relieve burns. In Mexico, many areas have been located where diversity of this species is concentrated but it is necessary more explorations because there are other regions without records. Pollen morphology of different genera of Crassulaceae family has been reported by several authors [2, 3, 4, 5, 6, 7, 8, 9, 10, 11]. Nonetheless, little and separate information has been obtained from Echeveria's palynology. For example, one of the earliest reports registers reticulate rugulate surfaces and lolongate endocolpium of *Echeveria sp.* and its hybrid *Cotyledon grandiflora* [12]. Subspherodial shape and several furrows delimit polygonal plates with pores at their corners are determined in apparently acetolysed pollen of *Echeveria gibbiflora* [13]. Tricolporate to tetracolporate with tiny margin in colpus, and scabrous sexine are

pointed in five not acetolysed species of *Echeveri*a [14]. Tricolpate, oblate and scabrate sculpture are reported for *E. marianae* in not acetolysed pollen [15].Finally, subspheroidal shape and possibly striate or reticulate ornamentation are determined for ten not acetolysed grains of *Echeveria* [16]. **64**

Little is known about the phylogenetic relationships in *Echeveria* and related genera due to lack of DNA sequences or other sources of data (palynological, anatomical, and ecological, among others) useful to establish homologies to infer relationships. In fact, genera like *Cremnophila*, *Graptopetalum*, *Thompsonella* and several species from *Sedum*, were recovered nested within *Echeveria* in phylogenetic studies [17]. Therefore, probably in future revisions there will be deep changes in the taxonomic status for taxa in these groups, like incorporation or removal of some species. In this paper acetolyzed pollen from a group of Echeveria´s species is analyzed by SEM, some of it is not yet formally described, to offer a set of information in order to begin a data base about its system and phylogeny. Besides, data obtained in this work could be useful for studies about the production of Echeveria's ornamental hybrids for sustainability purposes.

MATERIALS AND METHODS

Pollen of twenty one species of *Echeveria* was obtained from the flowers of plants grown in a pot from the Botanical Garden of National Autonomous University of Mexico (UNAM). These species are: *E. calderoniae, E. fimbriata*, *E. gigantea, E. globulosa*, *E. gracilis, E. guerrerensis*, *E. halbingueri*, *E. juarezensis*, *E. longissima* var. *aztlatensis, E. olivacea*, *E. patriotica, E. perez-calixii*, *E. purhepecha*, *E. rosea*, *E. roseiflora, E. sp.* "chiltepec", *E. sp.* "fulgens blanca", *E. sp.* "magnifica", *E.* sp. "nanchititlense", *E. sp.* "yoloxensis", *E. waltheri*) "x" = provisional name. The grains were processed by standard method described by [18] and prepared for conventional SEM [19, 20, 21]. Pollen was placed on metallic stubs with carbon tape. Finally, the sample was coated with carbon, sputtered with gold and watched by SEM (JEOL-35CF) equipped with a tungsten filament, at the SEM laboratory from the Chemistry Faculty in UNAM. Measurements like Polar axis (P), Equatorial axis (E), grain size with their means (□) and standard deviation (SD) were calculated from 15 pollen grains per sample. In some species it was possible to notice the exine structure. The terminology used for pollen descriptions is the proposed by different authors [3, 18, 22].

RESULTS AND DISCUSSION

Morphology characteristic of pollen from Echeveria plant, Crassulaceae family (Tab. 1). Pollen unit is at maturity like solitary grains or monads although some immature grains are together as members of a meiotic pollen tetrad [Fig. 1A].

"The kind of aperture determined is formed by a membrane resistant to acetolysis".

It has a compound aperture since it consists of colpus and pore, and they are formed by a membrane resistance to acetolysis [Fig.1B]. Its size oscillates between small and medium [19 µm to 33 µm] [Table1] based on the length of polar axis. Grain shape from highest to lowest predominance is subprolate, prolate spheroidal, spheroidal, prolate and euoblate [Fig.1C-F]. Its exine surface has two types of sculpture: micro-rugulate and microestriate-rugulate. The former consists of elongate elements distributed irregularly over the pollen surface, longer than 1 µm; it is an combination intermediate of striate and reticulate architecture [19, 25] [Fig. 2A-F], and the thickness measure of the exine layer (from some species) varies from 0.6 µm. to 0.9 µm (Tab. 1).

KEY		SPECIES	[3]P	[4]E	P/E	SIZE	[5]SHAPE GRAIN	APERTURE			EXINE		THICKNESS
[1]SEM	[2]JB		(µm)	(µm)	($?_{10}$)	[3]P	P/E	NUMBER	STRUCTURE	[6]L±STD (µm)	ORNAMENTATION	ESTRUCTURE	X±STD (µm)
41	JE-5096	*E. calderoniae* Pérez-Calix	26.5 ± 2.1	26.2 ±1.8	1.1	26.5	Prolate spheroidal	4	Colporate, operculate,	17.1±1.8	Microrugulate	Semitectate	0.63 ±0.06
25	JE-8794	*E. fimbriata* C.H. Thomps	30.2 ± 2.7	26.7 ±2	1.1	30.2	Prolate spheroidal	3	Colporate, operculate, syncolpate	25.2±2.5	Microestriate-rugulate		-
3	JE-6787	*E. gigantea* Rose & Purpus	24± 1	25.3 ±1.7	1.0	25.0	Spheroidal	3	Colporate, operculate, syncolpate	20.1±1.2	Microrugulate	-	-
42	JE-6110	*E. globulosa Moran*	19 ± 2.1	20.4 ±3.1	0.8	19.2	Enoblate	3 and 4	Colporate, operculate,	13.5±2.5	Microestriate-rugulate	-	-
45	JE-8667	*E. gracilis* Rose ex E. Walther	23.1 ± 1.7	20.8 ±1.5	1.1	23.1	Prolate spheroidal	3	Colporate, operculate	17.0±1.67	Microrugulate	-	-
24	JE-7521	*E. guerrerensis* J. Reyes, González- Zorzano et Brachet	23.4 ± 1.9	21 ±2.6	1.2	23.4	Subprolate	3	Colporate, operculate, syncolpate	17.7±2.1	Microrugulate	-	-
32	S/N	*E. halbingeri* var. E. Walther	22.7 ± 1.8	17.6 ±1.5	1.3	22.7	Subprolate	3	Colporate, operculate, syncolpate	18.6±2.0	Microestriate-rugulate	Semitectate	0.35 ±0.06
16	S/N	*E. juarezensis* E. Walther	27.2 ± 1.8	21.2 ±1.6	1.3	27	Subprolate	3	Colporate, operculate, quasi-syncolpate	22.4±1.8	Microrugulate	Semitectate	0.75 ± 0.11
59	S/N	*E. longissima* var. *Aztatlensis* J. Meyrán	32.6 ± 1.4	23.2 ±1.6	1.4	33	Prolate	3	Colporate, operculate, syncolpate	26.7±1.9	Microrugulate	-	-
18	JE-6402	*E.olivacea* Moran	23.5 ± 2.6	23.4 ±1.8	1.0	22	Spheroidal	3 and 4	Colpate or zonicolpate, operculate	11.6±2.7	Microestriate-rugulate	-	-
8	JE-6813	*E. patriotica* I. García & Perez-Calix	33.4 ± 2.6	30.8 ±2.6	1.1	33.4	Prolate spheroidal	3	Colporate, operculate, syncolpate	25.8±1.2	Microrugulate	-	-
22	PCR-6322	*E. perez-calixii* Jimeno-Sevilla & P. Carrillo	25.4 ± 1.8	22.4 ±1.4	1.2	25.4	Subprolate	3	Colporate, operculate quasi-syncolpate	21.2 ±1.9	Microrugulate	-	-
12	JE-8637	*E. purhepecha* I. García	25.5 ± 1.3	24.7 ±1.6	1.0	25.5	Spheroidal	3	Colporate, operculate, syncolpate	19.8±2.1	Microrugulate	-	-
67	JE-6369	*E. rosea* Lindl.	21.8 ± 1.6	18.2 ±1.5	1.2	22	Subprolate	3	Colporate, operculate, syncolpate	19.1±1.2	Microestriate-rugulate	-	-
9	GAS-6740	*E. roseiflora* J. Reyes & O González	22.9 ± 1.3	21.2 ±1.6	1.1	22.9	Prolate spheroidal	3	Colporate, operculate, slightly syncolpate	19±1.1	Microestriate-rugulate	-	-
29	JE-8578	*E. sp. "chiltepec"*	32 ± 2.3	26.5 ±2.1	1.2	32.0	Subprolate	3 and 4	Pantocolporate, or ring, operculate, syncolpate	25.6±4.2	Microestriate-rugulate	Semitectate	0.93 ±0.09
26	JE-8570	*E. sp. "fulgens blanca"*	30 ± 2.1	26.1 ±2.4	1.2	30.0	Subprolate	3 and 4	Colporate, operculate, syncolpate	24.4±6.7	Microestriate-rugulate	-	-
21	S/N	*E. sp. "magnifica"*	24.4 ± 3	23.6 ±2.6	1.0	24.4	Spheroidal	3	Colporate, operculate, syncolpate	18.8 ±1.4	Microestriate-rugulate	Semitectate	0.61 ± 0.06
11	JE-8061	*E. sp."nanchititlanensis"*	30.9 ± 2.6	25.8 ±2.3	1.2	31.0	Subprolate	3	Colporate, operculate, quasi-syncolpate	23.2±1.8	Microestriate-rugulate	-	-
40	JE-8769	*E. sp. "yoloxensis"*	25.8 ± 1.6	24.8 ±2.1	1.0	25.8	Spheroidal	3	Colporate, operculate, quasi-syncolpate	19.5±1.7	Microestriate-rugulate	Semitectate	0.43 ± 0.08
66	JE-6564	*E. waltheri* Moran & J. Meyrán	27.9 ± 1.7	22.8 ± 1.6	1.2	27.9	Subprolate	3	Colporate, operculate, quasi-syncolpate	21.9±2.6	Microestriate-rugulate	-	-

[1]SEM = Labotary of Scanning Electron Microscopy

[2]JB = Botanical Garden

[3]P = θ polar axis

[4]E= θ equatorial axis

[5]Shape grain= Subprolate, prolate spheroidal, oblate spheroidal, and suboblate pollen grain may be grouped togheter under the category of subspheroidal pollen.

[6] Lenght average of ten measurements

Position, number, shape, and structure of the aperture of Echeveria pollen. The aperture position is interradial, with reference to the pollen tetrad stage, because it goes parallel to the polar axis [Fig. 1A]; it meets at points where only two grains of the tetrad are in contact, and with the distal pole of pollen facing away from the meiotic tetrad position and equidistant on equator. With respect to number of aperture, most of the pollen grains have three apertures, except *E. calderoniae* that has four (Fig. 3A). Moreover some species have both three and four colpi [*E. olivaceae*, *E. sp.* "fulgens blanca", *E. sp.* "chiltepec", *E. globulosa*] [Fig. 3B-F; Tab. 1]. Its shape consists of a furrow called colpus; this presents a thin or thickened margin termed "operculum"[Fig. 4B-C]. The function of this operculum has been related to the protection of the delicate aperture from pathogens and/or dehydration, particularly in taxa from dry habitats [24]. The presence of this operculum in pollen of Echeveria coincides with that reported for: E. coccinea, *E. gibbiflora*, *E. mucronata*, and *E. secunda* [14]. The structure of colpus contains combined sulcus and pore (colporate) which has already been documented for *E. gibbiflora* [13], and *E. secunda* [14]. Furthermore, most of the species present in greater or lesser degree the syncolpated character [Tab. 1], which consists of two or more colpi that are fused at the end [Fig.3F]. These colpi can sometimes form a spiral [Fig. 3D].

Position with respect to pollen grain. Most of the Echeveria pollen has elongated apertures with one of their axis crossing the equator at right angle, which are named colpi. Except for two species that have a different kind of aperture position: *E. olivaceae* has zonicolpate colpi [Fig. 3D] which is situated at the equator [Fig. 3C] and *E. sp*. "Chiltepec" that has a pantocolporate and ring aperture [Fig. 3E, 4A; respectively] which is distributed regularly over the whole surface.

Exine structure. Grains in some *Echeveria* species were broken and it was possible to reveal their thin exine composed by sexine and nexine. The exine microstructure of pollen in this study has a thickness that varies from 0.35 µm to nearly 1µm [Table 1]. Frequently the internal sexine is two double-layered and consists of internal columellae covered by a roof-like layer or tectum. Those rod-like elements in Echeveria pollen form a discontinuous layer with some free columnellae [Fig. 4D-F], characteristic of semitectate grains. However more studies of the pollen wall by SEM or Transmission Electron Microscope (TEM) are required to realize phylogenetic potential of wall morphology of *Echeveria* pollen.

The ornamentation determined here [microrugulate and microestriate rugulate] generally is associated with biotic pollination [26]. But in this work, it is proposed that it may also be a morphological or structural adaptation. It has been found that the rugulate architecture provides **194** additional flexibility to the pollen wall that helps it adjusts to volume changes [27]. Such modifications occur in the pollination because they dehydrate when the grain is released from the anther and rehydrate when it falls to the stigma and absorbs water from the stigmatic cell [28]. Specifically, most of the pollen studied here has three colpi and only few have four apertures. It is known that pollen with three colpi lives more than

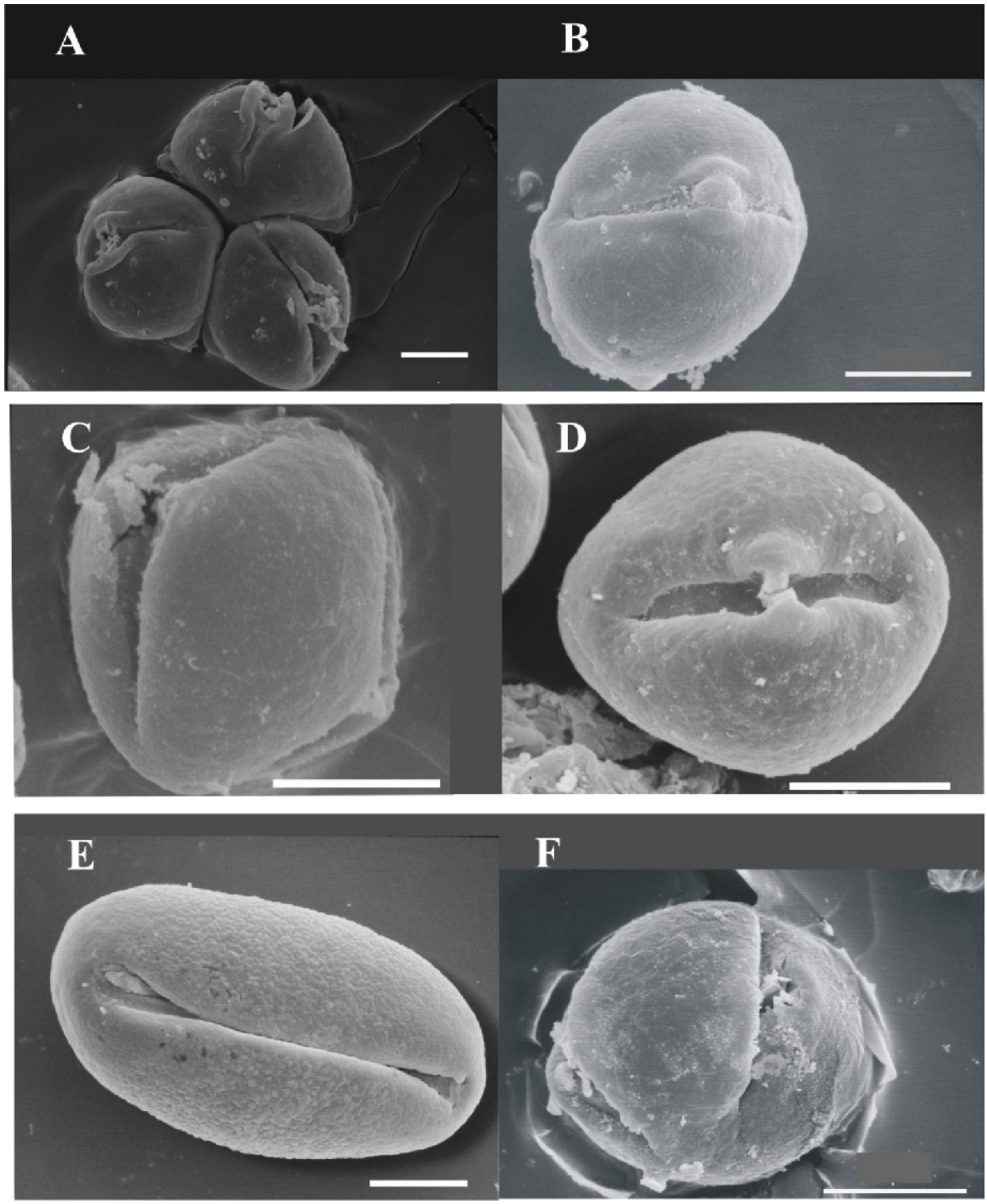

Fig. 1. Micrographs by SEM of *Echeveria* pollen in equatorial view. **A**, Meiotic tetrad of *E. juarezensis*; **B**, Colporate aperture of *E. gigantea*; **C**, Subprolate shape of *E. guerrerensis*; **D**, Prolate spheroidal shape of *E. gracilis*; **E**, Characteristic folded aperture as a consequence of harmomegathy of *E, longissima*; **F**, Euoblate shape of *E. globulosa*. Scale bar = 10 µm.

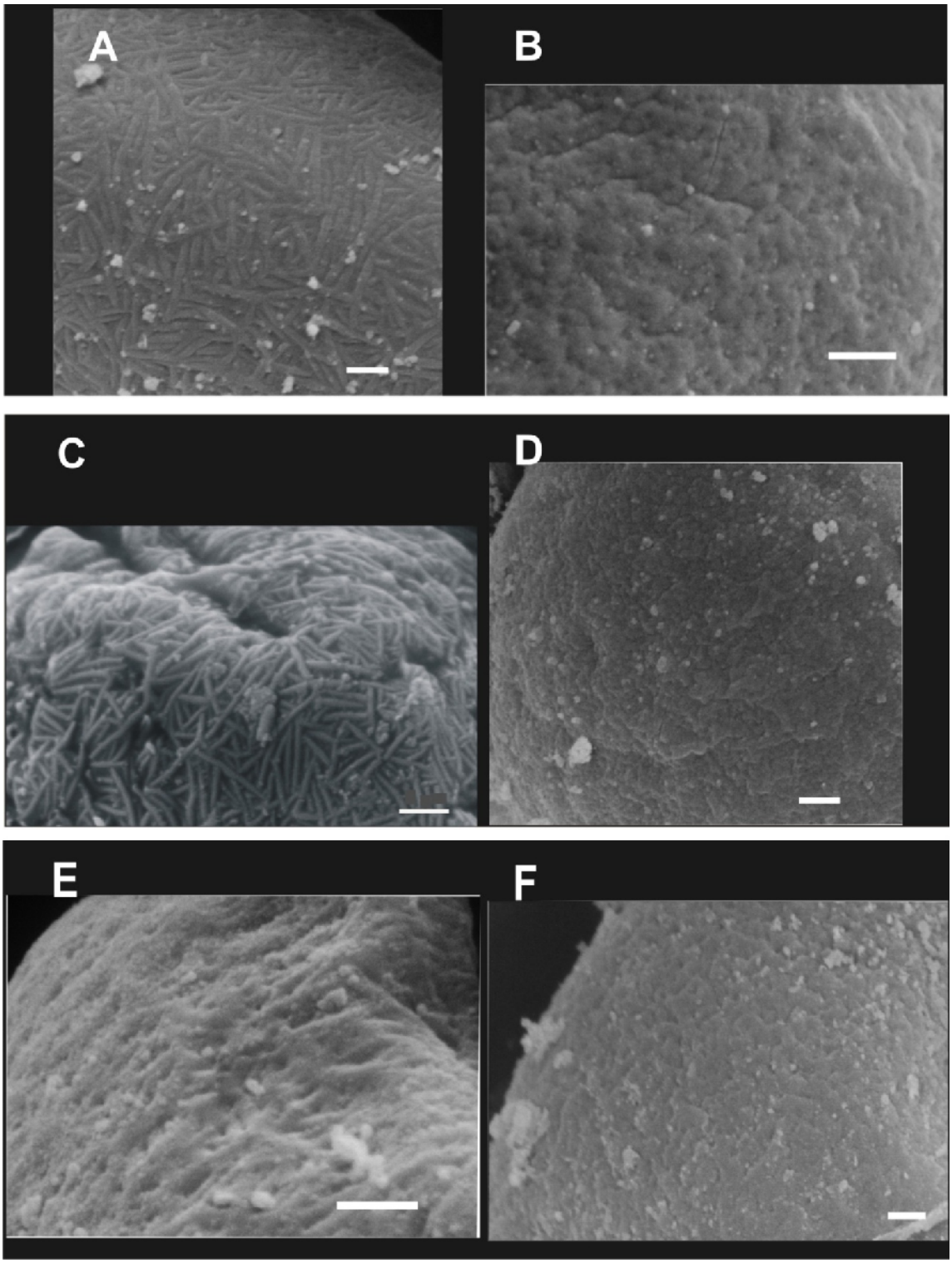

Fig. 2. Micrographs by SEM of the ornamentation from *Echeveria* pollen. **A**, Microestriate-rugulate exine sculpture of *E. fimbrata*; **B**, Microrugulate exine sculpture of *E. gracilis*; **C**, Microestriate-rugulate exine sculpture *E. rosea*; **D**, Microrugulate exine sculpture of *E. perez-calixii*; **E**, Microestriate-rugulate exine sculpture *E. sp.* "*yoloxensis*"; **F**, Microrugulate exine sculpture of *E. purepecha*. Scale bar = 1 μm.

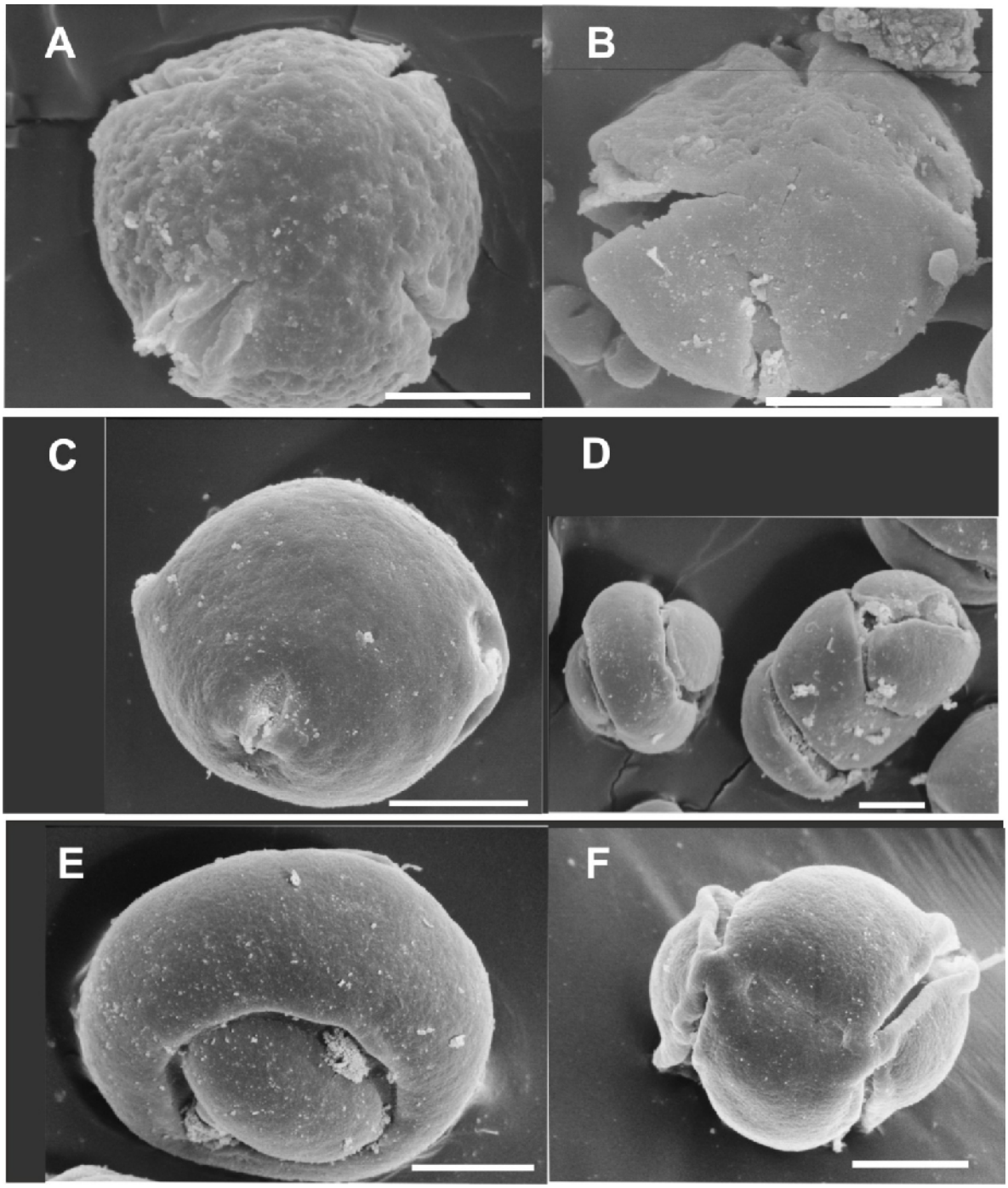

Fig. 3. Micrographs by SEM of *Echeveria* pollen. **A**, Tetracolpade grain of *E. calderoniae*; **B**, Tetracolporate grain of *E. globulosa*; **C**, Tetracolporate grain of *E. olivaceae*; **D**, Syncolpate and spiraled grains of *E. sp*. "*chiltepec*"; **E**, Ring aperture of *E. sp.* "chiltepec"; **F**, Tetracolporate grain of *E. sp*. "*fulgens blanca*". Scale bar = 10 µm.

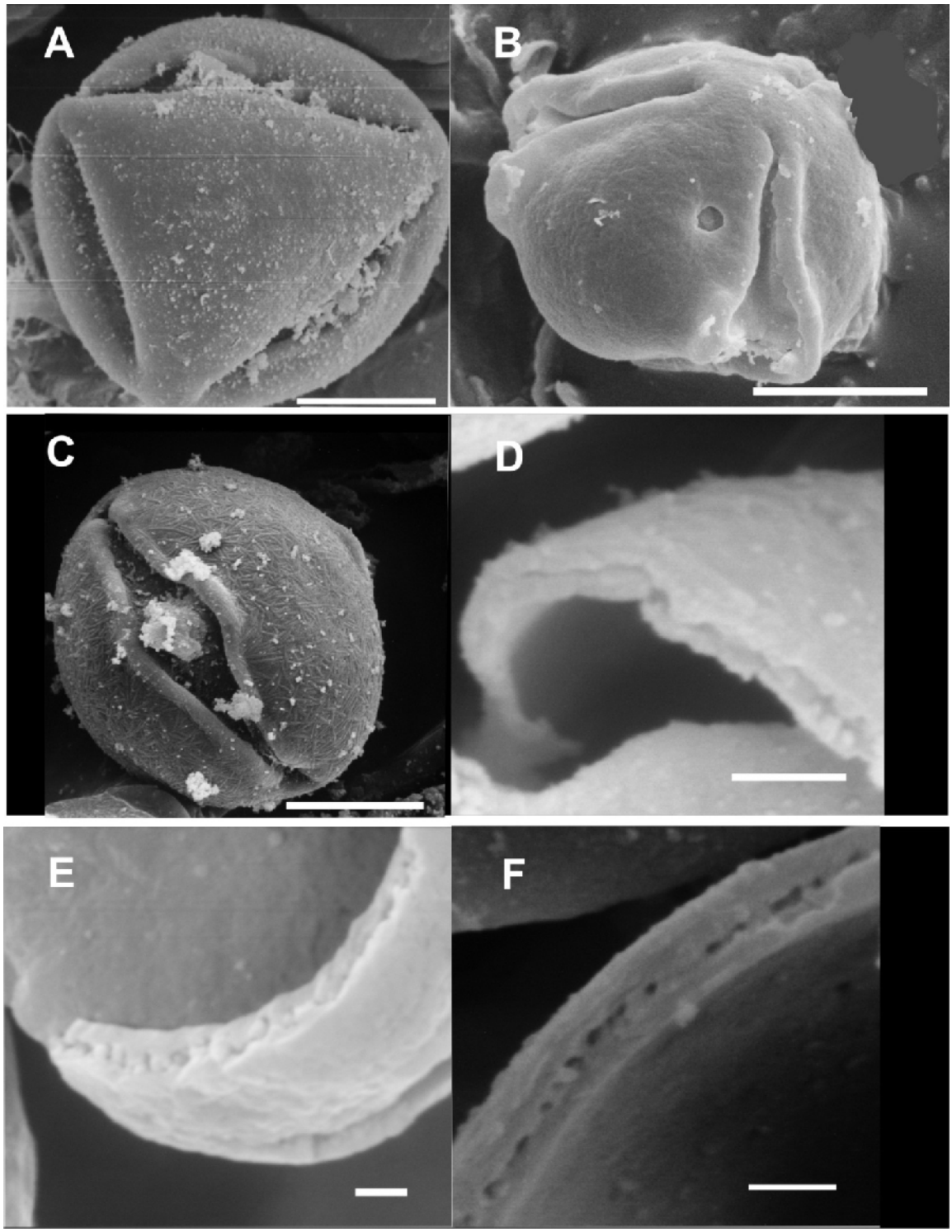

Fig. 4. Micrographs by SEM of Echeveria pollen. **A**, Pantocolpate aperture of *E. sp*. "*chiltepec*"; **B**, thickened margin in aperture (operculum) from tricolporate pollen in polar view of *E. gigantea*; **C**, Microestriate-rugulate ornamentation and operculate colpus of *E. fimbriate*. Scale bar = 10 µm; **D**, Semitectate exine microstructure of *E. calderoniae*; **E**, *E. juarezensis*; **F**, *E. magnifica*. Scale bar= 1 µm.

the other one, but pollen with four colpi germinates faster in the stigma [28]. Maybe the grains with four colpi are an adaptive transition that helps pollen to survive in dry environments. Another relevant aspect detected in Echeveria pollen is that it presents morphological features that indicate a process of harmomegathy, which 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 [28]. Finally, it is proposed in this paper that the colpus patterns (number, shape, and ornamentation) determined here might imply a trade-off between survival and germination of the Echeveria pollen. The coloporate aperture shape detected here is suggested as one of those morphologic adaptations. One of the most widespread harmomegatic systems involves composed aperture, where the endoapertural areas are more elastic than the rest of the colpus [29]. The thickened margin (operculum) found in Echeveria pollen is another character suggested here as adaptive specialization for the function of harmomegathy. The Operculum is a more compact and resistant area than the membrane of the colpus. Consequently, the rugulate exine ornamentation in Echeveria pollen is also proposed here also as an evidence of harmomegathy. The exine sculpture can influence the way in which the deformation of the pollen wall takes place during dehydration [12]. Finally, the last feature proposed as a harmomegathic adaptation in Echeveria pollen is the semitectate exine. It has been shown that the discontinuous exine, semitectate ultrastructure provides a great resistance to compression force [30].

CONCLUSIONS

Study of Echeverria pollen proposes the idea that the species studied are closely related. The Palynological features which they have in common are: (1) isopolar pollen, (2) medium sized to small pollen, (3) tricolporate aperture, (4) operculate aperture, (5) microrugulate exine, (6) semitectated exine, (7) microrugulate ornamentation, and (8) morphologic characters of harmomegathy. Nevertheless of the twenty species studied *E. olivaceae*, *E. sp*. "magnifica", *E. sp.* "fulgens blanca", *E. sp.* "chiltepec", *E. calderoniae* and *E. globulosa* could be separated, by presenting both tricolporate and tetracolporate pollen grain.

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