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## A New Species of Echeveria (Crassulaceae) from Michoacán, Mexico

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Abstract—A new species, *Echeveria pistioides* (Crassulaceae) is described from south of Morelia in the State of Michoacán, Mexico. The species belongs to *Echeveria* ser. *Gibbiflorae* due to its short or acaulescent stems inconspicuous among the large basal leaves of rosettes, paniculiform inflorescence, conical-urceolate corolla, and tricolpate pollen grains. Within *Echeveria* ser. *Gibbiflorae*, the closest morphological affinities of *E. pistioides* are with *E. marianae* from which it differs in the glaucous leaves with a different shape and size, fewer cincinni, smaller flowers, and the absence of corolla appendages at the base of staminal filaments. The species grows on steep, humid slopes with *E*, *W*, or NW exposure and the general type of vegetation consists of oak and pine forest with mesophyllous elements.

Keywords-Morphology, scanning electron microscopy, Echeveria ser. Gibbiflorae, succulent plants, taxonomy.

Echeveria DC. (Crassulaceae) comprises ca. 140 species of which the majority have evolved in Mexico where the genus is characterized by a high degree of endemism (Uhl 1992; Thiede 1995; Meyrán and López-Chávez 2003; Pérez-Calix 2004). An exploration of the mountains south of Morelia during 2014-2015 revealed a new species which we name E. pistioides. The species belongs to Echeveria ser. Gibbiflorae (Baker) A. Berger (Berger 1930), which is characterized by large leaves, paniculiform thyrses, pedicellate flowers with pentagonal corollas, dark-colored stigmas (Walther 1972), and tricolpate pollen grains (Pérez-Calix 2004). Phylogenetic relationships within this apparently monophyletic group are largely unknown because only a few species were included in a recent molecular study (Carrillo-Reves et al. 2009). Historically, the series was separated into two informal subgroups useful for identification purposes (Walther 1972; García-Ruiz and Pérez-Calix 2007): one group of species characterized by acaulescent rosettes or having a short caudex, and the other group by a long caudex. The new species belongs to the former group, which includes, among others, E. dactylifera E. Walther (Walther 1972), E. novogaliciana J. Reyes, Brachet & O. González (Reyes et al. 2011a), and particularly E. marianae I. García & Costea, with whom this new species shares the closest morphological affinities.

#### MATERIALS AND METHODS

We conducted field work south of Morelia in the Municipality of Tzitzio during 2014-2015. In addition to herbarium specimens, flowers and leaves were fixed in FAA (Ruzin 1999) for morphological studies. Several living plants were collected with soil and cultivated in Jiquilpan, Michoacán for further study. We examined the basic morphology of both fresh and fixed flowers under a Nikon SMZ1500 stereomicroscope equipped with a PaxCam Arc digital camera and Pax-it 7.8 software (MIS Inc., Villa Park, Illinois). For scanning electron microscopy (SEM), we used hexamethydisilazane (HMDS) as an alternative for critical dry point (Wright et al. 2011). Fixed flowers were dehydrated using a series of ethanol steps (70%, 80%, 95%, and 100%; each step 10 minutes), immersed for 10 minutes in 1:1 ethanol: HMDS, and passed through three changes, each of 30 minutes in 100% HMDS. Samples were air dried and coated with 20 nm gold using an Emitech  $\hat{K}$  550 sputter coater. Micromorphological examination, measurements and pictures were taken at 10 kV using a Hitachi SU1510 variable pressure scanning electron microscope.

### TAXONOMIC TREATMENT

*Echeveria pistioides* I. García, I. Torres & Costea, sp. nov.— TYPE: MÉXICO. Michoacán: Municipio Tzitzio, aproximadamente 1 km al SE de Piedras de Lumbre, 1,950 m, ecotono entre el bosque de encino-pino y el bosque tropical caducifolio, con elementos de bosque mesófilo de montaña; 26 Nov 2015, *I. García & I. Torres 9136* (holotype: CIMI!; isotypes: DAO!, ENCB!, IEB!, MEXU!, MICH!).

Similar morphologically to *E. marianae* in the absent or short caudex, calyx with unequal sepals and carinate petals, but differing in having pruinose leaves, 8–9 (12) cm wide, obcordate, obovate-spatulate to widely obovate with cuneate base and rounded to truncate, emarginated or bilobed apex; 3–4 cincinni, and flowers 12–16 mm long with petals 10–15 mm long, light-pink at the base and pink-orange to reddish in the rest, lacking corolla appendages.

Perennial herb, glabrous, caudex somewhat prostrate, 2-15 cm long and 1.5-2 cm in diameter; rosette lax, 14-40 (50) cm in diameter with 18-26 leaves; young leaves white-pruinose to glaucous, mature leaves light-green to emerald green remaining white-glaucous towards the base, blade obcordate, obovatespatulate to widely obovate, 16-25 × 8-9 (12) cm, thickness of blade at the base 8-9 mm, base cuneate, apex rounded to truncate, emarginated or bilobed, mucronate, margins entire with or without a thin, red-colored line; inflorescence 1(2-3) paniculiform thyrse(s), 30-60 (90) cm long and 5.8-9.5 (11) cm wide, with 3-4 secondary axes (cincinni), each with 4-8 flowers; bracts of the main inflorescence axis spiralled, 8-17, oblong-obovate to oblanceolate, glaucous, caducous, 2-6 (9)  $\times$  1-2 (8) cm, base auriculate, apex obcordate to rounded, mucronate; bracts of cincinni linear-lanceolate to narrow-elliptic, 9-18 × 3-8 mm, base auriculate; pedicels 5-16 mm long and 1.4-1.7 (2) mm thick. Flowers 12-16 mm long and 9.5-11 mm in diameter; calyx gamosepalous, the tube 1-2 mm long, lobes unequal, spreading, triangularlanceolate, whitish-green, 3.5-9 (11) × 4-6 mm; corolla pentagonal-conical in bud, cylindrical-urceolate at anthesis, petals imbricate, oblong-lanceolate with a concavity at the base corresponding to the nectaries,  $10-15 \times 4-6$  mm, tips mucronate and sometimes recurved, external color cream to



FIG. 1 *Echeveria pistioides*. A–B. Habitat. C. Leaf rosettes and developing inflorescences. D. Leaves. E. Habit. F–G. Inflorescence. H–I. Flower viewed from different positions. J–K. Flower opened to show stamens and nectaries. L. Cross-section through FAA fixed ovary, revealing nectaries; white color is due to pigment extraction by the ethanol. Scale bar = 1 mm.

light pink at the base, pink-orange to reddish in the rest, internal color cream at the base, pale pink to orange in the rest; epipetalous filaments (including the anthers) 9-10.5 mm long, without corolla appendages at the base; episepalous filaments (including anthers) 10–11.5 mm long; anthers  $1.5 \times 1$  mm, brown in the floral button, brown with reddish lines before anthesis and yellow at anthesis; pollen grains most commonly tricolpate, oblate to oblate-spheroidal in equatorial view and triangular in polar view but sometimes 4- or even 5-colpate, spherical or rectangular;  $28-30 \times 17-20 \ \mu m$ , tectum imperforate, scabrate; pollen grains eventually agglutinate into large masses; nectaries rectangular, reniform or bilobed,  $1.6-2.2 \times$ 0.8–1 mm, cherry-red colored; ovary with 5 carpels,  $10-13 \times$ 4 mm, white-yellowish; carpels 5,  $9.5-10.5 \times 4.5$  mm, ovary whitish-yellow; styles (including the stigmas) 4.5–5 mm long, reddish at the base and dark red in the rest with the tips dark brown; stigma globose; fruit suberect follicles,  $5-12 \times 2.5$  mm; seeds numerous, oblong to obovate, brown, reticulate,  $0.6-1 \times$ 0.3-0.5 mm; reticulum size 30-50 µm. Figures 1-2.

*Etymology*—The specific epithet alludes to the leaf and rosette resemblance with species of aquatic genus *Pistia* L. (water cabbage, Araceae).

**Notes**—The morphological similarities and differences between *E. pistioides* and *E. marianae* are summarized in the diagnosis of the former species, and presented in detail in Table 1. The absence of corolla appendages at the base of epipetalous stamens of *E. pistioides* provides an easy separation from *E. marianae*, but their nectaries are similar in regard to the numerous stomata present on their epidermis (Fig. 2C). Although the populations of the two species are located at ca. 200 km distance from one another, they grow in similar wet and shaded habitats (see Distribution and Ecology). It is possible that the water regime plays an important role in the diversification of Echeveria (Walther 1972). However, in the absence of a phylogenetic study, it is not possible to determine whether the similarities between *E. pistioides* and *E. marianae* are the result of convergent evolution or a consequence of close evolutionary relationships. A molecular study for ser. Gibbiflorae with more extensive sampling than that of Carrillo-Reyes et al. (2009) is necessary to understand evolutionary relationships among the numerous members of this group (Walther 1972), including several recently described species (e.g. Reves et al. 2011a; García-Ruiz and Costea 2014; Jimeno-Sevilla et al. 2015). The two species also have a different phenology: E. pistioides flowers from November to January, E. marianae from August to October. The rosette and leaf shape of E. pistioides resemble superficially those of E. guerrerensis J. Reyes, O. González & Brachet; however, the latter species has different inflorescence and floral traits and it was placed provisionally in ser. Urbinae Walther (Reves et al. 2011b).

**Distribution and Ecology**—*Echeveria pistioides* is currently known from one large population in the vicinity of Piedras de Lumbre, Mpio. Tzitzio, Michoacán, at elevations between 1,850 and 1,950 m. This geographical location is part of the southern foothills of the Trans-Mexican Volcanic Belt within Balsas River Basin. The species is saxicole and grows in shaded habitats that maintain humidity even during the dry season, for example, in wet ravines or margins of streams, with E, W, and NW exposure. The substrate is thin and



FIG. 2 Scanning electron microscopy of *Echeveria pistioides*, pollen, nectary, and seed. A–B. Pollen (arrows indicate 4-colpate pollen grains). C. Nectary surface (arrows indicate stomata). D–E. Seed morphology. Scale bars embedded in the images.

Character	Echeveria pistioides	Echeveria marianae
Caudey	Inconspicuous	Acaulescent or inconspicuous
Length x diameter (cm)	$2-15 \times 15-2$	A=6 × 3=4
Rosette	2 10 × 1.5 2	- 0 × 0 +
diameter (cm)	14-40 (50)	30-40
Leaves		00 10
Color	Young leaves white-pruinose to glaucous: mature leaves	Not glaucous: light green to yellow-green
	light-green to emerald green, remaining white-glaucous towards the base	regardless of age
Shape	Obovate-spatulate to widely obovate, obcordate, base cuneate, apex rounded to truncate emarginated or bilobed, mucronate	Obovate-oblanceolate; base amplexicaulous;apex acute to mucronate
Length (cm)	16–25	5–22 (24)
Width (cm)	8–9 (12)	3–7.5
Margin/Border	Margin entire with or without a thin, red-colored line border, at the base, sometimes hyaline; young leaves sometimes crenulate	Margin entire or lobed in the distal 1/2; border with a thin, red line; young leaves ± crenulate
Inflorescence		
Number of main axes	1–2 (3)	1–2
Length (cm)	30-60 (90)	80-90 (100)
Width at the base (cm)	5.8–9.5 (11)	6–13
Number of cincinni	3–4	6–8
Number of flowers per cincinnus	4-8	1–6
Shape	Oblong-obovate to oblanceolate	Oblong to ovate-lanceolate
Length (cm)	2-6 (9)	2–6.2
Width (cm)	1–2 (8)	0.5–1.5
Pedicel		
Length × diameter (mm)	$5-16 \times 1.4-1.7$ (2)	5-9 × 2-3
Flower length (mm)	12-16	15-20
Flower width (mm)	9.5-11	10–11 Come dia ta distribute a come da come cond
Sepais (calyx)	Spreading, unequal	Spreading to slightly recurved; unequal
Jongth v width (mm)	$25.0(11) \times 4.6$	
Color	3.5-9 (11) × 4-0 Wibitish groop	9-15 × 5-0
Petals (corolla)	wildsi-green	Light green
Corolla shape	Pentagonal-conical in bud: cylindrical- urceolate at anthesis	Pentagonal-conical in bud: broadly urceolate
corona snape	Tenagonal contear in bad, cymanear arceolae ar anneois	at anthesis
Petal shape	Oblong-lanceolate, carinate, mucronate	Oblong-lanceolate, carinate
Petal length $\times$ width (mm)	$10-15 \times 4-6$	15–20 × 6–7
External color	Cream to light pink at the base, pink-orange to reddish in the rest (including the tips)	Whitish-yellow at the base, orange in the rest, with the tips reddish;
Internal color	Cream at the base, pale pink in the median part and orange-reddish at the tips	Pale white-yellow at the base, yellow-orange in the rest, tips reddish
Corolla appendages	Absent	(1) 2; lanceolate, oblanceolate or conical, ascendant or convergent
Nectaries		
Shape	Rectangular, reniform or bilobate	Reniform
Length (mm)	1.6–2.2	2.5–3.3
Width (mm)	1	1
Color	Cherry-red	Pink with reddish margins
Stamens	10 11 <b>-</b>	10.10
Episepalous filaments + anther length (mm)	10-11.5	10–12
Epipetalous filaments + anther length (mm)	9–10.5	7–9
Anther length (mm)	1.5	2-2.5
Anther color	Brown with red lines in flower button, yellow at anthesis	Pink-reddish to yellow
Gynoecium/carpels		10.10 4
Length × width (mm)	9.5–10.5 × 4.5	$10-13 \times 4$
rruit See de	Fources semierect to ascendant, $5-12 \times 2.5$ mm	Fources semierect, $8.5 \times 2.8$ mm
Flowering	November-January	August-October
Geographical distribution	Center of Michoacán, S of Morelia	SE of Jalisco

TABLE 1. Comparison of *Echeveria pistioides* and *E. marianae* (García-Ruiz and Costea 2014). For a comparison of the latter species with *E. novogaliciana* and *E. dactylifera*, see García and Costea (2014).

clayey, on steep slopes or ledges of eroded and unstable rock walls. The plants growing rarely in sunnier microhabitats have leaf margins bordered by a thin red line, a feature that was not observed in the individuals occurring in the more typical shaded conditions. The steep slopes/ledges forming the habitat of *E. pistioides* are colonized by bryophytes, and herbaceous angiosperms such as *Arisaema* Mart. sp. and *Valeriana* L. sp. The general type of vegetation consists of oak and pine forest with mesophyllous elements such as *Clethra* L., *Oreopanax* Decne. & Planch., *Carpinus caroliniana* Walter, Hedyosmum mexicanum Cordem ex Baill. Dominant and companion species are Pinus leiophylla Schltdl. & Cham., Quercus obtusata Bonpl., Sabal pumos (Kunth) Burret, Bursera hintonii Bullock, Lysiloma acapulcense Benth., Agave cupreata Trel. & A. Berger, A. inaequidens K. Koch, and Clusia salvinii Donn. Sm.

*Pollination Biology*—Based on field observations, flowers are visited by hummingbirds.

Phenology—Flowers from November to January.

**Preliminary Conservation Status**—Although the species is locally abundant, with thousands of individuals present, it is apparently found only in a limited area SE of Piedras de Lumbre (area of occupancy less than 100 km<sup>2</sup>. Therefore, based on the IUCN (2016) criteria B2 biii, we preliminarily assigned this species a Critically Endangered (CR) rank. More field research will be carried out in the future to search for other populations.

Additional Specimens Examined—MÉXICO. Michoacán: Municipio Tzitzio, aproximadamente 1 km al SE de Piedras de Lumbre, 1,950 m, ecotono entre el bosque de encino-pino y el bosque tropical caducifolio con elementos de bosque mesófilo de montaña, 26 Oct 2015, *I. García & I. Torres 9132* (CIMI!).

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