

A new species of *Echeveria* (Crassulaceae) from Durango, Mexico, supported by morphology and DNA diagnostic characters

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Abstract: *Echeveria kristenii* is described as a new species from Sierra Azul, Mezquital, Durango. The new species belongs to a sub-group of northwestern Mexican taxa within ser. *Gibbiflorae*. Within the series it is most similar to *E. dactylifera* and *E. novogaliciana* in its acaulescent habit, sessile, farinaceous and somewhat narrowed leaves, paniculate inflorescences with short cincinni, and the presence of finger-like appendages at the base of the epipetalous filaments. Characters that distinguish it from those species include the size of the rosette, the shape, color, and size of the leaves, and the color of the styles and nectary scales. Additionally, three diagnostic nucleotide characters were found in *rbcL*, *matK* and ITS2, three standard DNA barcoding regions, that differentiate the new species not only from its closest relatives, but from all other taxa of ser. *Gibbiflorae*.

Keywords: BOLD systems, diagnostic nucleotides, DNA barcoding, *Gibbiflorae*, taxonomy.

In 2009 the Botanical Garden of the National Autonomous University of Mexico (UNAM) started a research project aimed to develop a DNA barcoding reference library for the Mexican Crassulaceae in collaboration with the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Ontario, Canada. Since then, about 775 novel DNA sequences (*rbcL*, *matK* and ITS2) were generated and added to The Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007) for 474 Mexican Crassulaceae samples. The specimens were selected from living plant material cultivated in the greenhouses of the National Crassulaceae Collection belonging to the Botanical Garden of the Biological Institute of UNAM. The samples selected primarily belong to the genus *Echeveria* DC. (de Candolle, 1828), with 418 samples, and to a lesser extent to *Cremnophila* Rose (Britton & Rose, 1905), *Dudleya* Britton & Rose (Britton & Rose, 1903), *Graptopetalum* Rose

(Rose, 1911), *Lenophyllum* Rose (in Britton & Rose, 1904 & 1905), and *Pachyphytum* Link, Klotzsch & Otto (Klotzsch, 1841).

One of the main goals of the project is to use a character-based approach, combing data from both DNA barcodes and morphology, to discover and diagnose new species (Davis & Nixon, 1992; DeSalle et al., 2005; Sarkar et al., 2008; Nicolalde-Morejón et al., 2010; Goldstein & DeSalle, 2011; Jörger & Schrödl, 2014). DNA sequence data are often helpful for flagging cryptic species of Mexican Crassulaceae, which may become evident through phylogenetic analysis and/or through character-based barcoding approaches (Rach et al., 2008; Zou et al., 2011; Zou et al., 2016), and also provide molecular tools for the identification of previously described species (Reyes et al., 2020).

Echeveria series *Gibbiflorae* (Baker) Berger (Berger, 1930) has been a focus of our systematic

research because of the apparent underestimation of its constituent species and their taxonomically challenging tendency to display morphological overlap. Series *Gibbiflorae* has been recovered as a monophyletic group within an assemblage informally known as the “Echeveria Group,” which includes almost all Mexican Crassulaceae except for *Dudleya* and *Lenophyllum* (de la Cruz-López et al., 2019a). Within the Echeveria Group, series *Gibbiflorae* is closely related to *E. ser. Angulatae* E. Walther, *E. ser. Pruinosae* E. Walther, and *E. ser. Secundae* (Baker) Moran. With the inclusion of *E. guerrerensis* J. Reyes, O. González & Brachet (Reyes et al., 2011a) and *E. uxorium* Jimeno-Sevilla & Cházaro (Jimeno-Sevilla et al., 2012) and the exclusion of *E. semivestita* var. *floresiana* E. Walther (Walther, 1958) and *E. semivestita* Moran var. *semivestita* (Moran, 1954) based on the phylogenetic results of de la Cruz-López et al. (2019a), ser. *Gibbiflorae* now consists of 39 species (including the new species) and two heterotypic varieties. It is one of the most diverse series within *Echeveria*, equaled or exceeded only by *E. ser. Nudae* E. Walther and *E. ser. Racemosae* (Baker) Berger.

A new species of *Echeveria* ser. *Gibbiflorae* was collected during a field trip by Julia Etter and Martin Kristen in the state of Durango, Mexico, in June of 2012. Some specimens were sent to the National Crassulaceae Collection belonging to the Biological Institute of UNAM for identification, cultivation, and observation. Initially, the plants were thought to be related to *E. dactylifera* E. Walther (Walther, 1972) and *E. novogaliciana* J. Reyes, Brachet & O. González (Reyes et al., 2011b) because of their morphological similarities, but a thorough analysis and comparison of living specimens of all taxa of ser. *Gibbiflorae* allowed us to diagnose a new species. The new species was included in the DNA barcoding library project “Crassulaceae in Mexico 3” in 2019, thus allowing us to assess the utility of selected barcode regions for the molecular differentiation of the species.

Description

Echeveria kristenii L.E. Cruz-López & J. Reyes., **sp. nov.**—Type: Mexico, Durango, municipality of Mezquitlan, La Flor, on the road between Yonora and La Guacamayita, to the east of El Charco Verde, 23°15'23.2"N, 104°38'8.9"W, 2730 m, 7 Jun 2012, *J. Etter & M. Kristen 3569*

(holotype: MEXU [!]; isotype: MEXU [!]). (Fig. 1.)

Diagnosis: *Echeveria kristenii* can be distinguished from *E. dactylifera* and *E. novogaliciana* by its smaller rosettes, shorter lilac-gray leaves, wider and shorter flowers, pinkish petals without any trace of yellow outside, pinkish-red nectary scales, dark purple styles, and by its larger appendages at the base of the epipetalous filaments (Fig. 2, Table 1).

Perennial herb, glabrous, solitary, acaulescent. Roots fibrous. Stems erect, 2–3 cm long and 1–2.5 cm in diameter, brown, with light brown leaf scars. Leaves in a spiral arrangement forming a compact rosette up to 35 cm in diameter, deltoid-lanceolate, lilac-gray, 4–15 cm long, 1.6–4.3 cm wide at the base, sessile, winged at the base, adaxial surface flat to slightly canaliculate abaxial surface keeled, very farinaceous, apex acute to acuminate, mucronate, margins entire. Inflorescences paniculate, 1–3 per rosette, 40–60 cm long or more and 0.7–1.4 cm wide at the base, with 7–18 cincinni, peduncles ascending, up to 30 cm in length, light pinkish-gray. Bracts 10–24, ascending, arranged in a spiral, deciduous, deltoid-lanceolate, 0.7–1.6 cm long, 0.3–0.9 cm wide, with acute apex, spurred, lilac-gray, very farinaceous. Bracteoles oblanceolate-lanceolate, 0.4–1.1 cm long, 0.15–0.22 cm wide, gray, farinaceous, spurred, apex acuminate; pedicels 0.8–1.5 cm long, 0.15–0.25 cm thick, light pink. Calyx 5-merous, the sepals fused at the base, lanceolate-deltoid, spreading to somewhat appressed to corolla, unequal, 0.7–1.4 cm long, 0.3–0.5 cm wide at the base and 0.2 cm thick, bluish-gray, apex acuminate, pruinose. Corolla tubular-urceolate, pentagonal in transection, 1.6–1.9 cm long, 1.2–1.6 cm wide at the base, 0.9–1.5 cm wide at the apex; segments elliptic-oblong, dorsally keeled, fused at the base, imbricate, apex acuminate and slightly reflexed, 0.5–0.7 cm wide at the base, pink, internally yellow with reddish margins and apex, thickened at the sides of the epipetalous filaments, with two finger-like appendages of 0.35 cm in length, pronounced nectar cavity. Androecium of 10 stamens, 5 antesepalous, 1.2–1.5 cm in length, 5 epipetalous slightly shorter; filaments whitish to pale yellow; anthers 0.15–0.3 cm in length, pale yellow. Gynoecium of 5 apocarpic carpels, 1.1–1.4 cm long, 0.3–0.4 cm wide at the base, nectary scales truncate-reniform, 0.28–0.35 cm wide and 0.15–0.25 cm tall, pinkish-red, ovaries white to pale yellow, styles dark purple, stigmas translucent to green. Fruit with 5 divergent follicles, with numerous reddish-brown seeds.

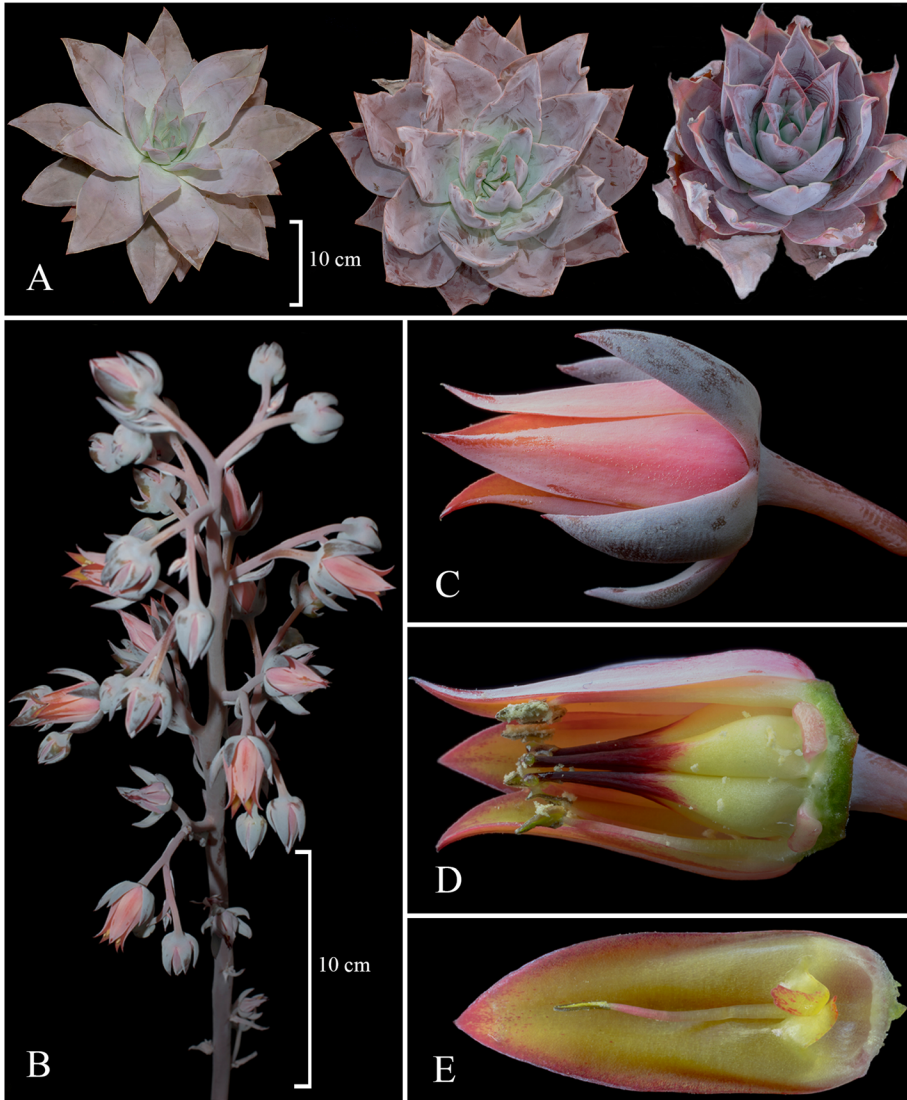


FIG. 1. *Echeveria kristenii*. A. Rosette and leaves, color and shape variations. B. Paniculate inflorescence. C. Calyx and corolla segments. D. Dissected flower, showing carpels and nectary scales. E. Inner view of petal, highlighting the appendages at the base of the epipetalous filaments. [Photographs by Luis E. de la Cruz-López taken from the holotype prior to conservation].

Distribution and habitat.—*Echeveria kristenii* is known only from the type locality in the state of Durango, Mexico. It inhabits rocky areas among *Pinus-Quercus* forest (Fig. 3 & Fig. 4).

Etymology.—The specific epithet is dedicated to Martin Kristen, a Crassulaceae enthusiast, and, together with Julia Etter, editor and administrator of the www.crassulaceae.com website. He has also made significant contributions to science, collecting plant material for the National Collection of Crassulaceae at the Botanical Garden of UNAM

(IB-UNAM) and the Mexican National Herbarium (MEXU), including the collection of this new species.

Discussion

Echeveria kristenii is placed in ser. *Gibbiflorae* because of its medium-sized rosettes, flat, sessile leaves, paniculate inflorescences, pentagonal flowers with keeled petals, and dark purple styles (Walther, 1972; Pilbeam, 2008). Independent



FIG. 2. Morphological differences between selected species of *Echeveria*. **A.** *Echeveria kristenii*. **B.** *E. dactylifera*. **C.** *E. novogaliciana*. From top to bottom, rosettes; flowers, lateral view; flowers, oblique view; flowers with two petals removed, lateral view; petal and stamen(s). Note differences in shape and/or color of rosettes, leaves, corolla and calyx segments, ovaries, styles, nectary scales, filaments, and anthers. [A, holotype collection; B, *J. Reyes* 6274 (MEXU); C, *J. Reyes* 6823 (MEXU); photographs by Luis E. de la Cruz-López].

molecular phylogenetic analysis based on *rbcL*, *matK* and ITS2 DNA sequence data confirms its placement within ser. *Gibbiflorae* (de la Cruz-López et al., in press) in a sub-group informally called “Northwestern Mexico” that also includes the

following 18 species: *E. cante* Glass & Mend.-Garc. (Glass & Mendoza, 1997), *E. cerrograndensis* A.Vázquez & Nieves (Nieves-Hernández et al., 2014), *E. coruana* I.García, D.Valentín & Costea (García-Ruiz et al., 2016a), *E. dactylifera* E.Walther,

TABLE 1. MORPHOLOGICAL AND GEOGRAPHICAL COMPARISON BETWEEN *ECHEVERIA KRISTENII*, *E. DACTYLIFERA*, AND *E. NOVOGALICIANA*.

Character	<i>E. kristenii</i>	<i>E. dactylifera</i>	<i>E. novogaliciana</i>
Rosette diameter	Up to 35 cm	Up to 60 cm	Up to 60 cm
Leaf length	4–15 cm	6–26 cm	12–30 cm
Leaf width at the base	1.6–4.3 cm	2.6–6 cm	1.0–3 cm
Leaf color	Lilac–gray	Green, reddish	Orange-brown, reddish
Leaf shape	Deltoid-lanceolate	Lanceolate-oblancoolate	Linear-deltoid
Leaf adaxial surface texture	Flat to slightly channeled	Channeled	Channeled
Leaf apex shape	Acute	Acute	Acuminate
Inflorescence length	40–60 cm	40–100 cm	53–76 cm
Corolla length	1.6–1.9 cm	1.8–2.3 cm	1.8–2.1 cm
Corolla width at the base	1.2–1.6 cm	1.0–1.3 cm	0.9–1.4 cm
Corolla shape	Tubular-urceolate	Urceolate-campanulate	Urceolate-campanulate
Petal length	1.6–1.9 cm	1.8–2.3 cm	1.8–2.1 cm
Petal width at the base	0.5–0.7 cm	0.3–0.58 cm	0.37–0.5 cm
Petal shape	Elliptic-oblong	Lanceolate-oblancoolate	Lanceolate-oblancoolate
Petal color	Pink	Light Pink	Pink
Petal margins color	Pink	Yellow	Orange
Epipetalous appendix length	0.35 cm	0.2 cm	0.2 cm
Styles color	Dark purple	Blood-red	Blood-red
Nectary scales color	Pinkish-red	Yellow	Light orange
Geographical distribution	Durango	Durango, Jalisco	Aguascalientes, Jalisco

E. lozanoi Rose (Britton & Rose, 1905), *E. marianae* I.García & Costea (García-Ruiz & Costea, 2014), *E. michihuacana* L.E.Cruz-López, Reyes & Verg.-Silva (de la Cruz-López et al., 2019b), *E. munizii*

Padilla-Lepe & A.Vázquez (Vázquez-García et al., 2014), *E. nayaritensis* Kimmach (1979), *E. novogaliciana*, *E. patriotica* I.García & Pérez-Calix (García-Ruiz & Pérez-Calix, 2007),



FIG. 3. Habitat of *Echeveria kristenii* in Sierra Azul, Durango. [Photograph by J. Etter and M. Kristen].



FIG. 4. *Echeveria kristenii* growing in bare rocks areas among *Pinus-Quercus* forest. [Photograph by J. Etter and M. Kristen].

E. perezcalixii Jimeno-Sevilla & P.Carrillo (Jimeno-Sevilla & Carrillo-Reyes, 2010), *E. pistioides* I.García, I.Torres & Costea (García-Ruiz et al., 2016b), *E. purhepecha* I.García (García-Ruiz, 2011), *E. roseiflora* J.Reyes & O.González (Reyes & González-Zorzano, 2010), *E. rulfiana* Jimeno-Sevilla, Santana-Mich. & P.Carrillo (Jimeno-Sevilla et al., 2015), *E. sonianevadensis* A.Vázquez, Jimeno-Sevilla & I.García (Jimeno-Sevilla et al., 2019), and *E. subrigida* (Robinson & Seaton) Rose (in Britton & Rose, 1903). A Bayesian phylogenetic analysis (de la Cruz-López et al., in press) indicates that *E. dactylifera* is its most closely related species, although it is morphologically much more similar to *E. novogaliciana*.

The new species shares traits with the abovementioned taxa, such as acaulescent habit, sessile, farinaceous and somewhat narrowed leaves, panicle inflorescences with short cincinni, flowers with keeled petals, and the presence of finger-like appendages on the base of the epipetalous filaments. Conversely, *Echeveria kristenii* can be differentiated from *E. dactylifera* and *E. novogaliciana* by its smaller rosettes (up to 35 cm vs. 60 cm); lilac-gray leaves (vs. green, red or orange-brown), with the adaxial surface flat or slightly canaliculate (vs. conspicuously canaliculate; wider (1.2–1.6 vs. 1.0–1.3 / 0.9–1.4 cm) and shorter flowers (1.6–1.9 vs. 1.8–2.3 / 1.8–2.1 cm); elliptic-oblong and pink petals

TABLE 2. TEN DIAGNOSTIC MOLECULAR CHARACTER DIFFERENCES IN DNA BARCODE REGIONS AMONG *Echeveria kristenii*, *E. dactylifera*, AND *E. novogaliciana*. CHARACTERISTIC ATTRIBUTES (CA'S SENSU DAVIS & NIXON, 1992) ARE INDICATED IN BOLD.

DNA Region: site	<i>E. kristenii</i>	<i>E. dactylifera</i>	<i>E. novogaliciana</i>
<i>rbcL</i>: 479	T/N	C	C
<i>matK</i> : 29	G	G	T
<i>matK</i> : 40	A	A	G
<i>matK</i> : 100	C	C	G
<i>matK</i>: 154	G	A	A
<i>matK</i>: 774	T	C	C
ITS2: 47	G	A	G
ITS2: 228	C	C	T
ITS2: 273	A	G	A
ITS2: 280	T	C	T

TABLE 3. GENBANK ACCESSION NUMBERS FOR THE DNA BARCODE SEQUENCES OF TAXA OF *ECHEVERIA* COMPARED IN THIS STUDY.

Taxa	Voucher ID	<i>rbcL</i>	<i>matK</i>	ITS2
<i>E. kristenii</i>	EK-3569-2	MT733085	MT733095	MT733105
<i>E. kristenii</i>	EK-3569-3	MT733086	MT733096	MT733106
<i>E. kristenii</i>	EK-3569-4	MT733087	MT733097	MT733107
<i>E. kristenii</i>	EK-3569-5	MT733088	MT733098	MT733108
<i>E. novogaliciana</i>	EK-3721-3	MT733089	MT733099	MT733109
<i>E. novogaliciana</i>	EK-3721-5	MT733090	MT733100	MT733110
<i>E. novogaliciana</i>	JE-6823-3	MT733091	MT733101	MT733111
<i>E. novogaliciana</i>	JE-6823	MG521710.1	MG521590.1	MG521436.1
<i>E. dactylifera</i>	JE-5287-3	MT733092	MT733102	MT733112
<i>E. dactylifera</i>	JE-6023	MT733093	MT733103	MT733113
<i>E. dactylifera</i>	EK-3057-3	MT733094	MT733104	MT733114
<i>E. dactylifera</i>	JE-6274	MG220445.1	MG220501.1	MG217161.1

without any trace of yellow outside (vs. lanceolate-oblongate and (light) pink with yellow or orange margins and apex), pinkish-red nectary scales (vs. yellow in *E. dactylifera* and light orange in *E. novogaliciana*), dark purple styles (vs. blood-red), and larger appendages on the base of the epipetalous filaments (3.5 Vs. 2 mm long) (Fig. 2). Specimens examined of *E. dactylifera* and *E. novogaliciana* are cited in [Appendix](#).

Searches of sequences of the sampled loci in the BOLD systems database for character-based DNA barcoding (Davis & Nixon, 1992; DeSalle et al., 2005; Sarkar et al., 2008; Goldstein & DeSalle, 2011) retrieved a total of ten nucleotide differences between the taxa compared (Table 2). Three sites stand out because they are pure “characteristic attributes” (CA’s) in the terminology of Davis & Nixon, (1992) for *Echeveria kristenii*, not only to differentiate it from *E. dactylifera* and *E. novogaliciana* but also from all other taxa of ser. *Gibbiflorae*. These sites correspond to site 479 in the aligned matrix for the *rbcL* and sites 154 and 774 in the aligned matrix for *matK* (Table 2; for GenBank accession numbers of the DNA sequences compared, see Table 3).

To our knowledge, this is the first use of character-based DNA barcoding to help diagnose a new species of *Echeveria*, not only contributing to our taxonomic conclusion, but also providing a useful molecular tool to aid the identification of the new species.

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Appendix

SPECIMENS EXAMINED OF *ECHEVERIA DACTYLIFERA* AND *E. NOVOGALICIANA*

Echeveria dactylifera. **MEXICO. Jalisco:** Municipality of Bolaños, southwest of San Martín de Bolaños, 21°53'10.6"N, 103°50'10.7"W, 1975 m, 3 Jun 2012, *J. Etter & M. Kristen 3543* (MEXU). **Durango:** Durango-Mazatlán Highway, Espinazo del Diablo, 23°39'38.24"N, 105°45'46.75"W, 2078 m, *J. Reyes 6274* (MEXU).

Echeveria novogaliciana. **MEXICO. Jalisco:** municipality of Zapopan, El Colli Hill, southwest of Las Chivas soccer stadium, 20°39'45.7"N, 103°27'39.3"W, 1872 m, 28 Nov 2010, *J. Reyes 6823* (MEXU). **Zacatecas:** Municipality of Jalpa, Km 17.4 of the Jalpa-Tlaltenango highway, 21°39'35.3"N, 103°7'17.6"W, 1971 m, 2 Nov 2012, *J. Etter & M. Kristen 3721* (MEXU).