UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

SISTEMÁTICA

CÓDIGOS DE BARRAS GENÉTICOS EN LA TAXONOMÍA INTEGRATIVA DE *ECHEVERIA* (CRASSULACEAE)

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

M. en C. LUIS EMILIO DE LA CRUZ LÓPEZ

TUTOR PRINCIPAL: DR. FRANCISCO ROBERTO VERGARA SILVA, INSTITUTO DE BIOLOGÍA, UNAM.

COMITÉ TUTORAL: DR. ABISAÍ JOSUÉ GARCÍA MENDOZA, INSTITUTO DE BIOLOGÍA, UNAM.

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UNAM POSGRADO

POSGRADO CIENCIAS BIOLÓGICAS

COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE BIOLOGÍA

OFICIO CPCB/1106/2022

ASUNTO: **Oficio de Jurado**

M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **22 de noviembre de 2021** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **DE LA CRUZ LOPEZ LUIS EMILIO** con número de cuenta **96251193** con la tesis titulada **"CÓDIGOS DE BARRAS GENÉTICOS EN LA TAXONOMÍA INTEGRATIVA DE *ECHEVERIA* (CRASSULACEAE)",** realizada bajo la dirección del **DR. FRANCISCO ROBERTO VERGARA SILVA,** quedando integrado de la siguiente manera:

Presidente:	DR. ROBERT ARTHUR BYE BOETTLER
Vocal:	DR. RAFAEL LIRA SAADE
Vocal:	DR. DAVID NAHUM ESPINOSA ORGANISTA
Vocal:	DRA. ANA LAURA WEGIER BRIUOLO
Secretario:	DR. JESÚS ALEJANDRO ESPINOSA DE LOS MONTEROS SOLÍS

Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 30 de noviembre de 2022

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

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Agradecimientos institucionales

Al Posgrado en Ciencias Biológicas, UNAM. Por haberme otorgado una formación de posgrado, por medio de sus cursos, y apoyos académicos de otra índole para la realización de esta tesis.

Al Consejo Nacional de Ciencia y Tecnología (CONACYT) por el otorgamiento de una beca de manutención por un periodo de cuatro años CVU: 365333 y el financiamiento del proyecto con clave: 247078 "Iniciativa interdisciplinaria para el aprovechamiento sustentable del género Echeveria (Crassulaceae), plantas con potencial hortícola para la producción en áreas rurales de México" otorgado al Dr. Francisco Vergara Silva.

A la UNAM a través de DGAPA-PAPIIT por el financiamiento del proyecto: IN212015, "Taxonomía integrativa y códigos de barras genéticos en Echeveria (Crassulaceae)", otorgado al Dr. Francisco Vergara Silva.

Al Dr. Francisco Vergara Silva por la dirección y asesoría para la elaboración de la tesis. Por su función como tutor principal y el financiamiento recibido de sus proyectos.

Al miembro del comité tutoral: Dr. Abisaí Josué García Mendoza por su asesoría en materia de taxonomía.

Al miembro del comité tutoral: Dr. Alejandro Espinosa de los Monteros Solís por sus recomendaciones en el área de filogenética molecular.

Al miembro invitado del comité tutoral: Dr. Pablo Carrillo Reyes por ser uno de los pioneros en estudios sistemáticos de Crassulaceae en México y por ofrecerme sus conocimientos en taxonomía y sistemática de *Echeveria*.

Agradecimientos a título personal

Al Biól. Jerónimo Reyes Santiago, curador de la Colección Nacional de Crasuláceas del jardín botánico de la UNAM, por permitirme la consulta de ejemplares vivos de *Echeveria*.

A los doctores Robert Bye, David Espinosa Organista, Alejandro Espinosa de los Monteros Solís, Rafael Lira Saade y la doctora Ana Wegier por su función como jurado de examen de grado.

A los investigadores(as) Alfonso Delgado, Gerardo Salazar, Carolina Granados, Lidia Cabrera, Alejandro Zaldívar, David Gernandt, Susana Magallón, Ricardo Sandoval, Helga Ochoterena, Ivalú Cacho e Hilda Flores por los cursos recibidos.

A la M. en C. Gabriela Espino Ortega por su apoyo en la toma de muestras y su apoyo en el trabajo de laboratorio.

A la Dra. Lidia Cabrera por su apoyo y supervisión en el Laboratorio de Sistemática Molecular del departamento de Botánica del Instituto de Biología.

A la M. en C. Ángeles Islas Luna por su apoyo durante el trabajo de campo, así como a Omar González Zorzano, Noemi y Andrea del staff del jardín Botánico de la UNAM.

A Julia Etter y Martin Kristen por proporcionar ejemplares de *Echeveria* y literatura especializada para la descripción de nuevas especies de *Echeveria*.

Dedicatoria

Esta tesis se dedica a mi hija Mariana Carolina de la Cruz Espino, quien se ha convertido en el motor que rige mi vida, incitándome a mejorar cotidianamente en diversos aspectos de la misma.

A mi esposa Gabriela Espino Ortega, quien me ha acompañado en las vicisitudes que ofrece la vida para un biólogo en el siglo XXI.

A mis padres Manuel de la Cruz Jasso y María Guadalupe López Negrete por todos los apoyos otorgados para la realización y conclusión de la tesis.

A mis hermanos Manuel y Diego por su acompañamiento cotidiano.

A todos los miembros de las familias de la Cruz y Espino por todas las muestras de apoyo recibidas.

Índice

Resumen

En esta tesis se presentan los resultados de un proyecto de investigación orientado a contribuir en el esclarecimiento de la sistemática y taxonomía del género *Echeveria* (Crassulaceae), un grupo de plantas suculentas ornamentales y cuya mayor diversidad se concentra en México. Este género ha sido pobremente estudiado desde diversas disciplinas (ecología, sistemática, biogeografía, entre otras) originando un desconocimiento general que imposibilita su taxonomía integrativa. Además de lo anterior, *Echeveria* y en general, toda la familia Crassulaceae, presentan una historia taxonómica compleja derivada de factores intrínsecos como diversos números cromosómicos, distintas formas de hábito, gran variación en la forma y color de las hojas, además de distintos tipos de inflorescencias, entre otros.

La pregunta principal planteada para abordar esta tesis: Dada su utilidad en sistemática y taxonomía integrativa, ¿Pueden los códigos de barras genéticos usarse para el esclarecimiento de problemas sistemáticos en *Echeveria*, en un contexto de taxonomía integrativa?. Para contestar la pregunta se propusieron los siguientes objetivos: 1) Determinar y asignar códigos de barras genéticos a las especies de *Echeveria*, 2) Utilizar los códigos de barras, así como otras fuentes de evidencia (morfología, biogeografía, entre otras) en la taxonomía integrativa del género, 3) Explorar sus relaciones filogenéticas infragenéricas, 4) Adicionar los códigos de barras genéticos de *Echeveria* a una librería de referencia (BOLD systems).

En una primera instancia, se evaluó el desempeño para discriminar entre especies de tres marcadores de ADN en una muestra de 52 spp. de 16 de los 17 grupos en los que se ha

dividido el género, empleando una metodología basada en caracteres diagnósticos conocida como CAOS. Las regiones fueron elegidas con base en un proyecto piloto anterior a esta tesis y correspondieron a las regiones genómicas del cloroplasto *matK* y *rbcL*, adicionalmente, se empleó un tercer marcador del genoma nuclear ITS2 que ha ganado reciente popularidad debido a su mayor capacidad de discernir entre especies. Los resultados de esta primera aproximación fueron muy promisorios, puesto que la combinación de los tres marcadores discriminó a nivel de especie al 100% de la muestra seleccionada con al menos un nucleótido diagnóstico (ver Capítulo 1).

Al inferir las relaciones de parentesco entre especies con base en la matriz combinada de *rbcL*, *matK* e ITS2 y con un incremento sustancial en el muestreo (153 taxones), se confirmó que *Echeveria* es parafilético puesto que incluye a los géneros *Cremnophila*, *Graptopetalum*, algunas especies de *Sedum* y *Thompsonella*. También se hizo evidente que la clasificación intuitiva predominante en la actualidad es altamente artificial y que los caracteres empleados para definir categorías infragenéricas son homoplásicos, en cambio, proponemos cambios en la composición de los grupos —e.g. el restablecimiento del género *Urbinia* separándolo de *Echeveria*, la creación de un nuevo género para la serie *Gibbiflorae* separado de *Echeveria*, así como la fusión de las series *Angulatae*, *Pruinosae* y *Secundae* en un nuevo género también separado de *Echeveria*— con base en los clados resultantes y los caracteres útiles para definirlos. En el Capítulo 2 se discuten también, las posiciones filogenéticas de especies *insertae sedis*, posiciones filogenéticas novedosas para un número sustancial de especies y su congruencia/incongruencia con la morfología.

La serie *Gibbiflorae*, uno de los grupos monofiléticos más numerosos resultantes en el análisis anterior, fue seleccionada para determinar la utilidad de los códigos de barras basados en caracteres a nivel de especie en una revisión taxonómico-integrativa. De manera similar a otros estudios en plantas, el porcentaje de identificaciones positivas se redujo considerablemente puesto que, únicamente el 55% de las especies seleccionadas presentaron caracteres diagnósticos moleculares. El árbol filogenético inferido para la serie *Gibbiflorae* indica tres subgrupos con alto soporte de probabilidad posterior, dos de los cuales pertenecen al grupo “sur de México”, mientras que el otro corresponde con el “noroeste de México” con base en su distribución geográfica. Así mismo, se señalan las afinidades geográficas, morfológicas y moleculares de dichos grupos (ver Capítulo 3).

Aún con las limitaciones del set de datos molecular, durante la revisión de la serie *Gibbiflorae* se identificaron 11 potenciales nuevas especies para el grupo, tres de las cuales han sido formalmente ilustradas y descritas en el Capítulo 4.

Abstract

This thesis presents the results of a research project oriented to contribute on the clarification of the systematics and taxonomy of genus *Echeveria* (Crassulaceae), an ornamental succulent plant group, whose greater diversity concentrates in Mexico. This genus has been poorly studied from different disciplines (ecology, systematics, biogeography, among others) originating from a lack of sufficient data to generate its integrative taxonomy. In addition, *Echeveria,* and Crassulaceae as a whole, present a complex taxonomic history derived by intrinsic factors such as diverse chromosomes numbers, different forms of habit, great variation in leaves shapes and color, besides of different inflorescence types.

The main question addressed in this thesis is: Given their utility in systematics and integrative taxonomy, can the DNA barcodes be used to clarify systematics problems of *Echeveria* from integrative taxonomy perspective?. To address this question, the following objectives were proposed: 1) To determine and to assign DNA barcodes to *Echeveria* species, 2) To employ the DNA barcodes, as well as others sources of evidence (morphology, biogeography, among others) on the integrative taxonomy of the genus, 3) To explore its infra-generic phylogenetic relationships, 4) To add the DNA barcodes of *Echeveria* to reference libraries (BOLD systems).

In first case, we evaluated the performance of three DNA markers to discriminate between 52 spp. representing 16 of the 17 groups of the genus that were derived using a methodology based on diagnostic characters known as CAOS. The regions were chosen based on a pilot project prior to this thesis and corresponded to *matK* and *rbcL* chloroplast genomic regions; in addition, the third marker used was ITS2, a nuclear genome which exhibits a

greater capability to discern between species. The results of this first approach were very promising since the combination of the three markers discriminated at the species level the 100% of the selected sample with at least one diagnostic nucleotide (see Chapter 1).

By inferring the phylogenetic relationships between species based on the combined matrix of *rbcL*, *matK* and ITS2 and with a substantial increase in sampling (153 taxa), it was confirmed that *Echeveria* is paraphyletic since it includes the genera *Cremnophila*, *Graptopetalum*, some *Sedum* species and *Thompsonella*. Also, it became clear that the currently predominant intuitive classification is highly artificial and that the characters used to define infrageneric groups are homoplastic. Instead, we propose changes in the composition of the genus —e.g., the re-establishment of the genus *Urbiniae* separating it from *Echeveria*, the foundation of a new genus for series *Gibbiflorae* separated of *Echeveria*, as well as the merge of *Angulatae*, *Pruinosae* and *Secundae* into a new genus also apart of *Echeveria*— based on the resulting clades and the useful characters to define them. In Chapter 2, we also discuss the phylogenetic positions of *insertae sedis* species, novel positions for a substantial number of species and their congruence/inconsistency with morphology.

The series *Gibbiflorae*, one of the most species-rich monophyletic groups resulting in the previous analysis, was selected to determine the usefulness at species-level character-based DNA barcodes in a taxonomic-integrative approach. As in other studies in plants, the percentage of positive identifications was considerably reduced since only 55% of the selected species presented molecular diagnostic characteristics. The phylogenetic tree inferred for series *Gibbiflorae* indicates three subgroups with high posterior probabilities support, two of which belong to the “southern Mexico” group, while the other one corresponds to the “northern Mexico” group, based on their geographical distribution. The geographic, morphological, and molecular affinities of these clades are discussed (see Chapter 3).

Even with the limitations of our molecular data set, 11 potential new species for the group were identified during the series *Gibbiflorae* review, three of which have been formally illustrated and described in Chapter 4.

Introducción

Antecedentes sistemáticos de Echeveria *DC*

La familia Crassulaceae incluye aproximadamente 1400-1500 especies agrupadas en 33–35 géneros (Berger, 1930; van Ham y ´t Hart, 1998; Eggli, 2003). Sus miembros son herbáceos, subarbustos o arbustos perennes o raramente anuales o bienales; presentan tallos y/u hojas muy suculentas, generalmente glabras o en menor frecuencia pubescentes a hirsutas; tienen flores pentámeras o (3–32)-meras, actinomorfas —simetría radial— (Eggli, 2003). Se distribuyen en casi todo el mundo con excepción de los polos, Australia y Polinesia, y son más raras en Sudamérica. Están adaptadas debido a sus órganos suculentos y su metabolismo ácido de las crasuláceas (CAM, por sus siglas en inglés) para ocupar diversos hábitats desde ambientes alpinos muy fríos, zonas templadas montañosas, bosques subtropicales, ambientes acuáticos (algunas especies de *Crassula* L.) hasta zonas áridas debido a la menor evapotranspiración y retención de agua en sus tejidos asociados a CAM (Winter y Smith, 1996) en un gradiente altitudinal muy amplio (Meyrán y López, 2003).

Análisis filogenéticos previos de Crassulaceae basados en secuencias de ADN han identificado a la familia como un grupo natural (monofilético), como uno de los grupos corona dentro del orden Saxifragales y cercana a las familias Haloragaceae y Penthoraceae (Fishbein y Soltis, 2004). Si bien, Crassulaceae ha sido recobrada como monofilética, la falta de ejemplares en colecciones tanto en fresco como herborizadas, aunado a la enorme diversidad fenotípica, citológica, su elevado número de especies, facilidad de hibridación en colecciones y la producción de numerosos cultivares, han propiciado que la taxonomía al interior de Crassulaceae sea históricamente complicada (Gontcharova y Gontcharov, 2009).

En particular, el género *Sedum* L. ha sido considerado como el más complejo dentro de la familia debido a su enorme diversidad de hábito, filotaxia de las hojas, diversos tipos de inflorescencia, grado de simpetalia de sus flores, entre otros caracteres, además de su distribución geográfica cosmopolita (Gontcharova y Gontcharov, 2009; Messerschmid et al., 2020) y por ser el género dentro de Crassulaceae en donde se transfieren aquellas especies que no tienen cabida en otros géneros (Uhl, 1963).

Los primeros análisis filogenéticos orientados a revelar las relaciones de parentesco al interior de la familia Crassulaceae basados en secuencias de ADN del cloroplasto *rbcL* y *MatK* (van Ham y ´t Hart, 1998; Mort et al., 2001), evidenciaron incongruencias con los sistemas clasificatorios tradicionales de la familia —i.e. Berger (1930), Eggli (2003)— y los patrones filogenéticos inferidos. Así mismo, la homoplasia exhibida en los caracteres morfológicos empleados para definir categorías taxonómicas intrafamiliares (Gontcharova y Gontcharov, 2009) han sido señalados como de poca utilidad para su empleo taxonómico y filogenético. Con base en los anteriores análisis, se redujeron las seis subfamilias establecidas por Berger (1930) a dos (Crassuloideae y Sedoideae), siendo Sedoideae la más numerosa y compleja de la familia.

Los árboles filogenéticos inferidos evidenciaron también, que la mayoría de las especies mexicanas (con excepción del género *Dudleya* Britton & Rose) y sudamericanas pertenecen al clado *Acre* dentro de la subfamilia Sedoideae (Tribu Sedeae, Subtribu Sedinae). Este último clado mayor fue objeto de un análisis filogenético mejor representado empleando secuencias de las regiones del cloroplasto *rps16* y *matK* y las regiones del genoma nuclear ETS e ITS (Carrillo-Reyes et al., 2009) en el que se señala que *Echeveria* no es monofilético —aunque con un muestreo limitado para este género—, puesto que se asocia con los géneros

Cremnophila Rose, *Graptopetalum* Rose, algunas especies de *Sedum* y *Thompsonella* Britton & Rose (Carrillo-Reyes et al., 2009) en oposición a la revisión realizada por A. Berger (1930) en donde *Echeveria* fue relacionado con *Altamiranoa* Rose, *Lenophyllum* Rose, *Pachyphytum* Link, Klotzsch & Otto y *Villadia* Rose ubicados en la subfamilia Echeverioideae con un criterio biogeográfico (basado en la distribución geográfica), así como morfológico (estambres obdiplostemonos de sus especies y sus corolas pentámeras). El estudio filogenético de Carrillo-Reyes et al. (2009) también reveló que varias de las categorías infragenéricas en las que se ha divido *Echeveria* no son monofiléticas, resultado que ha sido obtenido también en otros estudios con diferentes sets de datos (*ver* Nikulin et al., 2016; Vázquez-Cotero et al., 2017).

México es reconocido como un centro de diversificación para la familia Crassulaceae con más de 350 especies registradas, así como un centro de endemismo puesto que se registran 10 (11, si se considera *Altamiranoa*) de los 33–35 géneros totales para la familia, de los cuales *Cremnophila* (3 spp.), *Pachyphytum* (25 ssp.), *Reidmorania* Kimnach (1 sp.) y *Thompsonella* (8 ssp.) son géneros endémicos al país, mientras que *Villadia* (28 taxones) presenta un porcentaje de endemismo aproximado del 50%, *Dudleya* (38 ssp. y 30 subespecies) y *Lenophyllum* (7 ssp.), presentan niveles de endemismo superiores a 60%, en tanto que para *Echeveria* es de más del 83%. Otros géneros que se presentan en México corresponden con *Altamiranoa* —aunque sus características se traslapan con las de algunas especies de *Sedum* y en mayor medida con especies de *Villadia*, por lo que algunos autores lo incluyen en este último—, *Sedum* e incluso unas pocas especies de *Crassula* L. (Meyrán y López, 2003), otras tantas especies de *Crassula* y *Kalachoe* Adans han sido introducidas y son invasoras, principalmente en zonas del centro de México (Guerra, 2011).

De las crasuláceas registradas en México, casi la mitad corresponden al género *Echeveria*, siendo el más diverso (206 taxones) y el de mayor importancia económica del país. Su utilización como plantas ornamentales ha propiciado una industria creciente de cultivo y comercialización de especies —una gran proporción de manera ilegal—, así como la producción de numerosos cultivares e híbridos intra e intergenéricos identificándose como un género propicio para desarrollar nuevos cultivares ornamentales de interés comercial (Zúñiga y Carrodeguas, 2021).

La historia taxonómica y sistemática de *Echeveria* comenzó hace 229 años, las primeras especies de *Echeveria* en México en ser descritas se asociaron al género sudafricano *Cotyledon* L. por Cavanilles (1793) —*Cotyledon coccinea* Cav.— debido a la semejanza de sus rosetas, a sus flores pentámeras con dos verticilos de 5 estambres y por sus corolas tubulares. Posteriormente *C. coccinea* fungiría como tipo nomenclatural para el establecimiento formal del género *Echeveria* por parte de De Candolle (1828) dedicando el nombre del género al dibujante mexicano Atanasio Echeverría y Godoy, quien elaboró dibujos para el propio De Candolle (Moran, 1999), aunque éstos nunca se publicaron (Meyrán y López, 2003), al mismo tiempo que transfería a *C. gibbiflora* Moc. & Sessé ex DC. —*E. gibbiflora*—, *C. teretifolia* Lam. —probablemente correspondiente a *E. bifurcata* Rose, debido a lo incompleto de la ilustración (lectotipo)— y *C. caespitosa* Haw. —*Dudleya caespitosa* (Haworth) Britton & Rose— al nuevo género.

La separación de *Echeveria* de *Cotyledon* tiene bases biogeográficas —distribución en el continente americano vs. africano— y morfológicas tales como la posición de la inflorescencia —inflorescencias laterales vs. inflorescencias terminales—, el tipo de inflorescencia —cincinos, espigas, panículas, racimos vs tirsos o varios dicasios que terminan en un monocasio (van Jaarsveld, 2003)—.

Al incrementarse el número de especies registradas, en 1839 Schlechtendal realizó la primera división del género en grupos (Walther, 1972; Pilbeam, 2008), en la que separó a 22 especies en cinco grupos principales de acuerdo con el tipo de inflorescencia:

1) inflorescencia equilateral:

a. Caulescentes: *E. coccinea* (Cav.) DC. (actualmente en ser. *Echeveria*), *E. pubescens* Schltdl. (sinónimo de *E. coccinea*), *E. rosea* Lindl. (actualmente en ser. *Spicatae*).

b. Acaules: *E. racemosa* Cham. & Schltdl. (actualmente en ser. *Racemosae*), *E. lurida* Haw. (actualmente en ser. *Racemosae,* algunos autores la consideran sinónimo de *E. racemosa*, sin embargo, sus hojas son más angostas, canaliculadas y de coloraciones lilas), *E. mucronata* Schltdl. (actualmente en ser. *Mucronatae*), *E. peruviana* Meyen (actualmente en ser. *Racemosae*).

2) Inflorescencia secunda simple: *E. secunda* Booth ex Lindl. (actualmente en ser. *Secundae*).

3) Inflorescencia con dos o tres ramas: *E. bifida* Schltdl. (actualmente en ser. *Angulatae*), *E. scheeri* Lindl. (actualmente en ser. *Gibbiflorae,* aunque no se ha vuelto a colectar). —*E. scheeri* fue introducida al público por Frederick Scheer después de germinar unas semillas que le fueron proporcionadas por una persona anónima supuestamente de México, las plantas cultivadas por Scheer fueron descritas por John Lindley como nueva especie en 1845. Posteriormente E. Walther en su monografía realiza una descripción de *E. scheerii* basada en plantas cultivadas en el Strybing Arboretum del parque Golden Gate en San Francisco California, colectadas por Thomas MacDougall en el distrito Chontal en Oaxaca.

Aparentemente, *E. scheerii* no aparece en las notas de campo de T. MacDougall, por lo que es posible que Walther se refiriera a otra planta también de origen desconocido. Charles Uhl (2002) sugiere que *E. scheerii* puede tratarse de un híbrido de *E. juarezensis* E.Walther y otro parental desconocido, debido a lo anterior, *E. scheerii* se mantiene como un misterio taxonómico dentro de *Echeveria*—, *E. retusa* Lindl. (sinónimo de *E. fulgens* var. *fulgens* Lem.), *E. bracteolata* Link, Klotzsch, & Otto (sinónimo de *E. bicolor* E.Walther en ser. *Nudae*).

4) Inflorescencia paniculada: *E. gibbiflora* DC. (actualmente en ser. *Gibbiflorae*), E*. grandifolia* Haw. (sinónimo de *E. gibbiflora*), *E. acutifolia* Lindl. (actualmente en ser. *Gibbiflorae*), *E. campanulata* Kunze (sinónimo de *E. grandifolia* y ésta a su vez de *E. gibbiflora*).

5) Flores erectas: *Dudleya caespitosa*.

Posteriormente, al incrementarse aún más el número de nuevas especies, Eric (Edward) Walther estableció 14 grupos infrgenéricos (series) en su monografía (Walther, 1972); cabe indicar que la obra de Walther fue publicada después de su muerte (Meyrán y López, 2003):

Serie 1. *Paniculatae*, **Serie 2**. *Urceolatae*, **Serie 3**. *Secundae*, **Serie 4**. *Retusae* (actualmente incluida en ser. *Gibbiflorae*), **Serie 5**. *Gibbiflorae*, **Serie 6**. *Angulatae*, **Serie 7**. *Pruinosae*, **Serie 8**. *Nudae*, **Serie 9**. *Spicatae*, **Serie 10**. *Elatae* (actualmente incluida en ser. *Gibbiflorae*), **Serie 11**. *Racemosae*, **Serie 12**. *Mucronatae*, **Serie 13**. *Echeveria* y **Serie 14**. *Longistylae*.

Durante las décadas de la segunda mitad del siglo XX y las primeras décadas del siglo XXI se incrementó en gran medida el número de especies nuevas descritas por parte de diferentes botánicos especializados en este grupo, destacando Reid Moran, Myron Kimnach, Jorge Meyrán García, Jerónimo Reyes Santiago, Guillermo Pino (principalmente de especies sudamericanas), y en menor medida pero con igual importancia Emanuel Pérez Calix, Ignacio

García Ruiz, Ignacio Torres García, Gregorio Nieves Hernández, Antonio Vásquez García, Miguel Ángel Muñiz Castro, Miguel Cházaro Basáñez, Pablo Carrillo Reyes, David Jimeno Sevilla, Julia Etter y Martin Kristen, entre otros.

Gracias al trabajo de los autores mencionados y a aquellos anteriores a éstos, hasta la fecha se contabilizan 161 especies, 3 subespecies, 37 variedades y 6 formas de *Echeveria* (Etter y Kristen, 2022), mientras que, a septiembre del 2022, Kew Royal Botanical Gardens contabiliza 199 especies aceptadas (POWO, 2022) y su número sigue incrementándose a medida que se exploran con mayor profundidad tanto el Territorio Nacional, como regiones de centro y Sudamérica.

Los 199–207 taxones de *Echeveria* han sido agrupados en la más reciente clasificación (o subdivisión) del género en 17 series de acuerdo principalmente con los criterios de Walther (1972), Moran (1974) y con algunas modificaciones por parte de Kimnach (2003). Las series, las especies incluidas en cada serie y sus características más relevantes se enlistan a continuación de acuerdo con Kimnach (2003) y Pilbeam (2008):

1.- *Angulatae* E.Walther: Raíces frecuentemente tuberosas; tallos muy cortos (acaules); hojas pequeñas, delgadas; inflorescencias con uno o muchos cincinos; pétalos gruesos, agudamente aquillados. (11 taxones). Incluye a: *E. bifida* Schltdl., *E. bifurcata* Rose —algunos autores la colocan como sinónimo de *E. bifida*, sin embargo, sus flores son bicolores, mientras que en *E. bifida* las flores son de color rosa—, *E. lutea* Rose, *E. lyonsii* Kimnach, *E. rodolfoi* Mart.-Aval. & Mora-Olivo, *E. schaffneri* Rose, *E. shaviana* E.Walther, *E. strictiflora* A.Gray, *E. tamaulipana* Mart.-Aval., Mora-Olivo & M.Terry —esta especie es idéntica a *E. walpoleana*, además que su distribución geográfica es muy similar, debido a lo anterior podría considerarse como sinónimo— *, E. tenuis* Rose*, E. walpoleana* Rose.

2.- *Chloranthae* Moran: Tallo muy corto; inflorescencia con un cincino; pedicelos cortos, erectos; pétalos verdes con dos apéndices cilíndricos o planos a los costados de los filamentos epipétalos, pétalos obtusamente aquillados. Serie monotípica (1 especie): *E. heterosepala* Rose.

3.- *Ciliatae* Moran: Tallos cortos a más o menos cortos; hojas pubescentes o glabras; inflorescencia en racimo o panícula, generalmente pubescentes. (7 taxones). Incluye a: *E. derenbergii* J.A.Purpus, *E. globulosa* Moran, *E. setosa* var. *ciliata* (Moran) Moran, *E. setosa* var. *deminuta* J.Meyrán, *E. setosa* var. *minor* Moran, *E. setosa* var. *oteroi* Moran, *E. setosa* var. *setosa* Rose & J.A.Purpus.

4.- *Echeveria* E. Walther (incl. *Oliverella* Rose, *Oliveranthus* Rose, sect. *Oliveranthus* (Rose) A. Berger, Ser. *Vestitae* E. Walther): Tallos de mediana longitud a largos; hojas pubescentes a hirsutas; inflorescencia en espiga o racimo equilateral, frecuentemente pubescentes; flores generalmente rojizas con ápice amarillo. (12 taxones). Incluye a: *E. amphoralis* E.Walther, *E. carminea* Alexander, *E. coccinea* Cav., *E. harmsii* J.F.Macbr., *E. leucotricha* J.A.Purpus, *E. macrantha* Standl. & Steyerm., *E. pilosa* J.A.Purpus, *E. pringlei* var. *longisepala* Kimnach, *E. pringlei* var. *parva* Kimnach, *E. pringlei* var. *pringlei* (S.Watson) Rose, *E. pulvinata* Rose ex Hook.f., *E. zorzaniana* J.Reyes & Brachet.

5.- *Gibbiflorae* (Baker) A. Berger (Incl. ser. *Grandes* E.Walther and ser. *Retusae* E.Walther): Tallos cortos o de mediana longitud; hojas medianas a largas, glabras, anchas y planas o adelgazadas; inflorescencias cimoso-paniculadas (tirso), las ramificaciones de la inflorescencia en cincino, raramente pubescentes. (39 taxones). Incluye a: *E. acutifolia* Lindl*.*, *E. aurantiaca* Reyes, González-Zorzano & Brachet*, E. cante* Glass & Mend.-Garc., *E. cerrograndensis* A.Vázquez & Nieves*, E. coruana* I.García, D.Valentín & Costea, *E. crenulata* Rose*, E. dactylifera* E.Walther*, E. fimbriata* C.H.Thomps., *E. fulgens var. fulgens* Lem.*, E. fulgens var. obtusifolia* (Rose) Kimnach*, E. gibbiflora* DC.*, E. gigantea* Rose & J.A.Purpus*, E. grisea* E.Walther*, E. juarezensis* E.Walther*, E. kristenii* L.E.Cruz-López & J.Reyes*, E. longiflora* E.Walther*, E. lozanoi* Rose*, E. marianae* I.García & Costea, *E. michihuacana* L.E.Cruz-López, J.Reyes & Verg.-Silva*, E. munizii* Padilla-Lepe & A.Vázquez, *E. nayaritensis* Kimnach*, E. novogaliciana* J.Reyes, Brachet & O.González, *E. pallida* E.Walther*, E. patriotica* I.García & Pérez-Calix, *E. perezcalixii* Jimeno-Sevilla & P.Carrillo, *E. pistioides* I.García, I.Torres & Costea, *E. prunina* Kimnach y Moran*, E. purhepecha* I.García*, E.* roseiflora J.Reyes & O.González, *E. rubromarginata* Rose*, E. rulfiana* Jimeno-Sevilla, Santana Mich. & P.Carrillo, *E. scheerii* Lindl*.*, *E. semivestita* var*. floresiana* E.Walther*, E. semivestita* var*. semivestita* Moran*, E. sonianevadensis* A.Vázquez, Jimeno-Sevilla & I.García, *E. steyermarkii* Standl.*, E. subrigida* Rose*, E. triquiana* J.Reyes & Brachet, *E. xochipalensis* J.Reyes, L.E.Cruz-López & Verg.-Silva.

6.- *Longistylae* E. Walther: Tallos muy cortos; hojas glabras; inflorescencia con uno o dos cincinos; corola de hasta 3 cm de longitud, urceoladas; estilos de 2 cm de longitud. (4 taxones). Incluye a: *E. islasiae* J.Reyes & L.E.Cruz-López, *E. logissima* var. *aztatlensis* J.Meyrán, *E. longissima* subsp. *brachyatha* J.Reyes, Brachet & O.González, *E. longissima* var. *longissima* E.Walther.

7.- *Mucronatae* E.Walther: Raíces tuberosas: tallos muy cortos; hojas glabras, pequeñas; inflorescencias en espiga o sub-racemosas; pedicelos hasta 2 cm de longitud. (3 taxones). Incluye a: *E. mucronata* Schltdl., *E. pinetorum* Rose, *E. sessiliflora* Rose.

8.- *Nudae* E.Walther: Tallos largos; hojas glabras o papilosas; inflorescencia en racimo o con menor frecuencia espigada; flores frecuentemente rojizas; pétalos aquillados. (31) taxones). Incluye a: *E. alata* Alexander, *E. australis* Rose, *E. bicolor* var. *bicolor* E.Walther, *E. bicolor* var. *turumiquirensis* Steyerm., *E. brachetii* J.Reyes & O.González, *E. cerrateana* Pino & Kamm, *E. chapalensis* Moran & C.H.Uhl, *E. compressicaulis* Eggli & N.P.Taylor, *E. globuliflora* E.Walther, *E. goldmanii* Rose, *E. gracilis* Rose ex E.Walther, *E. guatemalensis* Rose, *E. johnsonii* E.Walther, *E. macdougallii* E.Walther, *E. maxonii* Rose, *E. montana* Rose, *E. multicaulis* Rose, *E. nebularum* Moran & Kimnach, *E. nodulosa* (Baker) Ed.Otto, *E. nuda* Lindl., *E. nuyooensis* J.Reyes & Islas, *E. pendulosa* Kimnach & C.H.Uhl, *E. procera* Moran, *E. quitensis* var. *cuencaensis* Pino & Kabir Montesinos, *E. quitensis* var. *quitensis* Lindl., *E. quitensis var. sprucei* Poelln., *E. recurvata* L.Carruth., *E. skinneri* E.Walther, *E. spectabilis* Alexander, *E. viridissima* E.Walther, *E. waltheri* Moran & J.Meyrán.

9.- *Occidentales* Moran: Raíces no tuberosas; tallos muy cortos; hojas pequeñas a medianas; inflorescencias cimosas, muy ramificadas apicalmente, con cincinos; corola rojiza. (2 especies). Incluye a: *E. affinis* E.Walther y *E. craigiana* E.Walther.

10.- *Paniculatae* A.Berger: Raíces no tuberosas; Tallos cortos; hojas pequeñas, glabras; inflorescencias cimoso-paniculadas, con cincinos; corola muy pequeña <8 mm. (2 especies). Incluye a: *E. amoena* De Smet ex E.Morren, *E. prolifica* Moran & J.Meyrán.

11.- *Pruinosae* E.Walther: Tallos cortos; hojas pequeñas a medianas, pruinosas a glaucas; inflorescencias 1 a 3 cincinos; pétalos gruesos, aquillados. (4 especies). Incluye a: *E. gudeliana* Véliz & García-Mend., *E. laui* Moran & J.Meyrán, *E. peacockii* Croucher ex T.Moore & Mast. —el nombre correcto de esta especie es *E. desmetiana* De Smet, *E. peacockii* es sinónimo de *Dudleya pulverulenta.* Debido a que en las principales monografías del género se emplea el nombre *E. peacockii* es que se mantienen aquí—, *E. runyonii* Rose ex E.Walther.

12.- *Racemosae* (Baker) A. Berger: Raíces tuberosas o fibrosas; tallos muy cortos o rara vez de mediana longitud; hojas generalmente glabras, con menor frecuencia pubescentes; inflorescencia racemosa. (49 taxones). Incluye a: *E. andicola* Pino, *E. argentinensis* var. *argentinensis* Hutchison ex Pino, R.Kiesling, W.Ale & Marquiegui, *E. argentinensis* var. *kieslingii* Pino, W.Ale & Marquiegui, *E. atropurpurea* (Baker) É.Morren, *E. bakeri* Kimnach, *E. ballsii* E.Walther, *E. bella* fa. *bella* Alexander, *E. bella* fa. *major* (E.Walther) Kimnach, *E. canaliculata* Hook., *E. carnicolor* E.Morren, *E. chazaroi* Kimnach, *E. chiclensis* var. *backerbergii* (Poelln.) Pino, *E. chiclensis* var. *cantaensis* Pino & Vilcapoma, *E. chiclensis* var. *chiclensis* (Ball) A.Berger, *E. chilonensis* (Kunze) E.Walther, *E. compressicaulis* Eggli & N.P.Taylor, *E. cuencaensis* Poelln., *E. cuscoensis* Pino, W.Galiano & P.Nuñez, *E. decumbens* var. *decumbens* Kimnach, *E. decumbens* var. *urubambensis* Pino, P.Nuñez & Kamm, *E. diffractens* Kimnach & A.B.Lau, *E. deltoidea* Pino & Vilcapoma, *E. eurychlamys* (Diels) A.Berger, *E. excelsa* (Diels)

A.Berger, *E. fruticosa* Pino, *E. helmutiana* Kimnach, *E. krahnii* Kimnach, *E. laresensis* Pino & Kamm, *E. megacalyx* E.Walther, *E. mondragoniana* J.Reyes & Brachet, *E. moranii* E.Walther, *E. ochoae* Pino & W.Galiano, *E. olivacea* Moran, *E. oreophila* Kimnach, *E. papillosa* Kimnach & C.H.Uhl, *E. penduliflora* E.Walther, *E. peruviana* Meyen, *E. racemosa* var. *citrina* Kimnach, *E. racemosa* var. *racemosa* Cham. & Schltdl., *E. saltensis* Pino, W.Ale & Marquiegui, *E. subcorymbosa* Kimnach & Moran, *E. uhli* subsp. *coelestis* J.Reyes & L.G.López, *E. uhli* subsp. *uhli* J.Meyrán, *E. utcubambensis* Hutchison ex Kimnach, *E. uxorium* Jimeno-Sevilla & Cházaro, *E. vulcanicola* Pino, Montesinos & Matusz, *E. westii* E.Walther, *E. whitei* Rose, *E. wurdakii* Hutchison ex Kimanch.

13.- *Secundae* (Baker) A. Berger: Raíces no tuberosas; tallos muy cortos a cortos; hojas glabras, pequeñas, generalmente glaucas; inflorescencias con 1 o 2 cincinos; flores rojizas con ápice amarillo; pétalos obtusamente aquillados. (6 taxones). Incluye a: *E. calderoniae* Pérez-Calix, *E. minima* J.Meyrán, *E. secunda* fa. *byrnesii* (Rose) Kimnach, *E. secunda* fa. *secunda* Booth ex Lindl., *E. subalpina* Rose & J.A.Purpus, *E. tolucensis* Rose.

14.- *Spicatae* (Baker) A.Berger: Tallos largos; hojas delgadas, glabras; inflorescencia densamente espigada o racemosa; sépalos frecuentemente más largos que la corola; pétalos obtusos, aquillados. (3 taxones). Incluye a: *E. pittieri* Rose , *E. rosea* Lindl., *E. tencho* Moran & C.H.Uhl.

15.- *Thyrsiflorae* Moran: Raíces tuberosas; tallos muy cortos; hojas glabras; inflorescencia tirsoide o sub-racemosa y determinada; flores amarillas o rojizas con tintes amarillos. (3 taxones). Incluye a: *E. paniculata* var. *maculata* (Rose) Kimnach, *E. paniculata* var. *paniculata* A.Gray, *E. platyphylla* Rose.

16.- *Urbiniae* E.Walther: Raíces no tuberosas; tallos muy cortos; hojas pequeñas, glabras, generalmente glaucas; inflorescencias secundo-racemosas (cincino); corola urceolada; pétalos no aquillados. (23 taxones). Incluye a: *E. agavoides* Lem., *E. angustifolia* E.Walther, *E. chihuahuensis* Poelln, *E. colorata* fa. *brandtii* Kimnach , *E. colorata* fa. *colorata* E.Walther, *E. cuspidata* var. *cuspidata* Rose, *E. cuspidata* var. *gemula* Kimnach, *E. cuspidata* var. *zaragozae* Kimnach, *E. elegans* Rose, *E. halbingeri* var. *goldiana* (E.Walther) Kimnach, *E. halbingeri* var. *halbingeri* E.Walther, *E. halbingeri* var. *sanchez-mejoradae* (E.Walther) Kimnach, *E. humilis* Rose, *E. lilacina* Kimanch & Moran, *E. pulidonis* E.Walther, *E. purpusorum* A.Berger, *E. simulans* Rose, *E. tobarensis* A.Berger, *E. tolimanensis* Matuda, *E. trianthina* Rose, *E. turgida* Rose, *E. unguiculata* Kimnach, *E. xichuensis* L.G.López & J.Reyes.

17.- *Valvatae* Moran: Raíces no tuberosas; tallos muy cortos; hojas delgadas, agudo-marginadas; inflorescencia con 1 o 2 cincinos; brácteas imbricadas; sépalos tan largos como la corola; pétalos valvados. (3 especies). Incluye a: *E. calycosa* Moran, *E. valvata* Moran, *E. yalmanantlanensis* A.Vázquez & Cházaro.

Si bien, la subdivisión del género y la ubicación de muchas de las especies en las respectivas series parece congruente en el esquema clasificatorio de kimnach (2003), en otra gran proporción se percibe como artificial, debido a que se han empleado características morfológicas que frecuentemente se presentan en otras especies o grupos de especies. Así como ejemplo, la serie *Pruinosae* que se estableció para dar cabida a especies de órganos aéreos muy pruinosos, excluye a muchas especies que presentan esta característica (*E. cante*, *E. colorata*, *E. tolimanesis*, entre otras) e incluso especies de otros géneros de Crassulaceae como *Cotyledon orbiculata* L., *Dudleya brittonii* Johans., *Pachyphytum oviferum* J.A.Purpus, entre muchas otras más.

La dificultad para establecer dentro de *Echeveria*, rasgos únicos que diferencien a las categorías infragenéricas durante la historia taxonómica y sistemática de *Echeveria* se debe a la homoplasia registrada en caracteres morfológicos y el probable origen híbrido y poliploide de varias de sus especies, entre otros. Adicionalmente, a la ausencia de un marco filogenético en donde se identifiquen aquellos caracteres compartidos entre un grupo de especies (sinapomorfías) y los caracteres que diferencien a especies o grupos de especies (autapomorfías).

Los pocos antecedentes filogenéticos señalan que *Echeveria* es polifilético y tiene una estrecha relación con los géneros mexicanos *Cremnophila*, *Graptopetalum*, algunas especies de *Sedum* y *Thompsonella*, con quienes comparte muchas de las características morfológicas y de manera similar que en *Echeveria*, sus relaciones filogenéticas no están del todo resueltas (Carrillo-Reyes et al., 2009).

A partir de los antecedentes filogenéticos, consideramos que el siguiente paso para comprender mejor la sistemática de *Echeveria* es ampliar el muestreo de sus especies de manera significativa, así como a todas sus subdivisiones (series) en un análisis filogenético molecular, así como especies de géneros afines. En segundo lugar, identificar aquellos caracteres moleculares y morfológicos que definan a los clados para obtener una clasificación acorde con relaciones de parentesco, en el contexto de la escuela de clasificación conocido como cladística (Williams et al., 2016; Morrone, 2016).

La iniciativa de los códigos de barras de la vida

La idea de utilizar un pequeño fragmento del genoma mitocondrial de los organismos como mecanismo de identificación rápida y precisa a nivel de especie fue propuesta por Hebert et al. (2003). Lo anterior generó entusiasmo por parte de diferentes grupos de investigación en muchos países convirtiéndose velozmente en una iniciativa global para generar bases de datos (librerías de referencia) con los códigos de barras genéticos de todas las especies que habitan la Tierra. En casi dos décadas posteriores a la postulación de los códigos de barras de la vida, más de seis millones de secuencias han sido agregadas en bases de datos como BOLD Systems (Ratnasingham y Hebert, 2007) y se han publicado desde 2004 al último día del año 2018 cerca de 3,756 artículos relacionados con diferentes aspectos de los códigos de barras genéticos (DeSalle y Goldstein, 2019).

Esta idea "novedosa" generó casi de inmediato una fuerte discusión que, al principio se radicalizó entre grupos de investigación que abrazaron y apoyaron esta idea y otros grupos que rechazaron contundentemente su utilización (Packer et al., 2009). Entre los puntos iniciales en conflicto se mencionaban, la autoría del concepto por parte de Hebert y colaboradores ya que, previamente se habían desarrollado y aplicado diferentes marcadores moleculares (AFLPs, RFLPs, mini y microsatélites) en investigaciones orientadas a establecer relaciones filogenéticas, delimitación de especies, identificación de especies (DeSalle y Goldstein, 2019), hibridación, dinámicas poblacionales, entre otras (Will et al., 2005). Por lo tanto, al grupo de investigación de la Universidad de Guelph podría atribuírsele únicamente la postulación y el uso del fragmento de aproximadamente 650 pb de la subunidad 1 del gen mitocondrial Citocromo Oxidasa (COI) que codifica para la citocromo oxidasa como locus identificador.

Algunos círculos de taxónomos señalaron también su preocupación referente a la desaparición o sustitución del sistema multidisciplinario e integrativo para el descubrimiento de especies nuevas que se realiza en la actualidad y del sistema taxonómico tradicional. La confusión se originó debido a algunos artículos (Tautz et al., 2003) que se asociaron a la literatura de códigos de barras genéticos que establecían el uso de secuencias de COI u otros fragmentos de Ácido Desoxirribonucleico (ADN) como única y suficiente evidencia para describir y nombrar nuevas especies (DeSalle et al., 2006). Sin embargo, una fortaleza que se adhiere a los códigos de barras genéticos es el protocolo de toma de muestras en donde se incluyen obligadamente datos adicionales como coordenadas georreferenciadas (Hubert y Hanner, 2015), fotografías del espécimen y el depósito del ejemplar en repositorios científicos (p. ej., herbarios). Este hecho permite la consulta y verificación del material en caso de dudas en la identificación.

Una de las aportaciones más interesantes de los códigos de barras es la posibilidad de identificar taxones en diferentes estadios de desarrollo (Lanteri, 2007). Es bien conocido que algunos estadios tempranos en insectos como huevos o larvas son casi imposibles de determinar a nivel de especie por medios morfológicos. En botánica, donde es común la necesidad de contar con ejemplares maduros y/o en floración para ser identificados con mayor precisión, ocurre un escenario similar, al abrirse la posibilidad de identificar estadios de desarrollo temprano (semillas y plántulas) o individuos o fragmentos estériles (hojas, madera y raíces).

La discusión anterior pasó a segundo término, y se enfocó en las virtudes y defectos metodológicos del sistema de identificación. Es frecuente en la literatura de códigos de barras genéticos la utilización de medidas de distancia tanto para distinguir entre especies (identificar) como para descubrir nuevas especies; entre los métodos más ampliamente utilizados, se encuentran BLAST o matrices de distancias empleadas para construir árboles con el criterio del vecino más cercano (Neighbor-Joining); lo que conlleva una serie de deficiencias que se confrontan con la sistemática filogenética y la taxonomía (DeSalle et al., 2009). En primer lugar, los valores de similitud no necesariamente indican a las especies más relacionadas en términos de ancestro/descendiente, en su lugar ofrecen una medida de similitud global. En segunda instancia, los valores de similitud siempre retribuyen a los más cercanos, nublando el replanteamiento de la hipótesis original .

Uno de los puntos más controversiales, y que ha sido reiteradamente criticado en la utilización de distancias por los productores códigos de barras genéticos, es la posibilidad de traslape de la variación genética interespecífica con la variación intraespecífica (DNA barcoding gap). Si bien, aunque basados en evidencias empíricas, los porcentajes de divergencia interespecífica han sido establecidos arbitrariamente (ej. aves 2.7%, insectos 3%, invertebrados 2%, vertebrados 4%). Algunos estudios han reportados niveles altos de traslape genético entre especies morfológicamente distintas, por ende, el DNA barcoding gap no puede aplicarse a estos grupos, convirtiéndolo en un criterio taxa-dependiente y subjetivo.

Otro de los puntos controversiales en el uso de códigos de barras implica su universalidad, es decir, que puedan emplearse para todos los seres vivos; adicionalmente, que los marcadores moleculares presenten flancos conservados que permitan emplear marcadores universales, que sean cortos (300-800 pb) para facilitar su amplificación y secuenciación en ambas direcciones, además de presentar altos niveles de variación nucleotídica para discriminar a nivel de especie. COI es uno de los marcadores que cumplen con estos criterios en grupos animales. Sin embargo, en las plantas tanto COI como el resto de las regiones del genoma mitocondrial han mostrado bajos niveles de divergencia, transferencia de genes al núcleo, así como rápidos re-arreglos del genoma.

Es debido a la poca utilidad de COI que el Consorcio para el Código de Barras de la Vida (CBOL por sus siglas en inglés) propuso utilizar fragmentos de los genes codificantes *rbcL* y *matK* como el núcleo del código de barras de las plantas terrestres (CBOL Plant Working Group, 2009). El planteamiento se generó después de evaluar en términos de universalidad a los primers, calidad de las secuencias obtenidas y poder de discriminación, a siete loci del cloroplasto candidatos, entre los que se encontraban genes (*matK*, *rbcL*, rpoB, rpoC1) y espaciadores intergénicos (psbK–psbI, atpF–atpH y *trnH*–*psbA*) en una muestra representativa de criptógamas, gimnospermas y angiospermas. Los primeros análisis de los marcadores propuestos por CBOL, sin embargo, mostraron que *rbcL* y *matK* presentaban en general, bajos niveles de diagnosis molecular (76% o inferiores) para distintos grupos vegetales (ej. *Zamia*, Zamiaceae: Cycadales; Nicolalde-Morejón et al., 2010) y que su utilidad como código de barras, particularmente en grupos complejos de reciente divergencia, sea cuestionada (Roy et al., 2010).

La búsqueda de marcadores moleculares más adecuados para las plantas en el contexto de los códigos de barras genéticos ha continuado en diferentes taxa vegetales y ha llegado a la inclusión de loci nucleares de los cuales, entre los más frecuentes, se encuentran a los espaciadores intergénicos (ITS, ITS2). Los marcadores nucleares han contribuido con poder discriminativo, en particular ITS2 ha sido propuesto reiteradamente como candidato a código de barras tanto en plantas o animales (Chen et al., 2010; Yao et al., 2010) y propuesto anteriormente en hongos, pero estas regiones, han agregado complejidades en cuanto a la factibilidad de amplificación.

Debido a la dificultad de encontrar una región con las cualidades esperadas en un código de barras genético, en botánica, se ha optado por el "Multi-locus approach" en donde se combinan dos o más loci para aproximar los requerimientos mencionados anteriormente, este enfoque es el que actualmente conlleva los mejores beneficios.

El único antecedente referente a la determinación de códigos de barras genéticos en Crassulaceae se enfocó en el género *Rhodiola*, obteniéndose hasta un 80.9% de identificación exitosa de las 55 especies del género analizadas, los criterios empleados fueron el porcentaje de divergencia intraespecífica e interespecífica y monofilia recíproca, utilizando cinco marcadores *rbcL*, *matK*, *trnH-psbA*, trnL-F e ITS (Zhang et al., 2015). A nivel individual, ITS fue el marcador con mayor resolución con el 66% de las especies identificadas.

Códigos de barras genéticos basados en caracteres

Dayrat (2005) introduce a la taxonomía integrativa como un sistema conciliador que contempla la colaboración de diversas disciplinas (filogeografía, ontogenia, etología, genética de poblaciones, morfología comparada, por mencionar algunas) para robustecer la delimitación de especies conocidas y el descubrimiento de nuevas especies; la taxonomía integrativa *sensu* Dayrat, aportó muy poco conceptual y metodológicamente puesto que propuso una serie de reglas orientadas a reducir la redundancia de nombres científicos. Muchos taxónomos previos a Dayrat, ya contrastaban datos provenientes de diferentes fuentes como parte de la rutina taxonómica, pero son Will y colaboradores (2005) quienes adoptan la incorporación de datos moleculares en la forma de códigos de barras genéticos a la taxonomía integrativa, aunque con reservas.

De acuerdo con DeSalle et al. (2005) existen problemas en la forma en que se incorporan los datos moleculares a la práctica taxonómica por parte de la iniciativa planteada por Hebert et al. (2003) y por lo tanto en la manera en la que se interpretan los códigos de barras genéticos. Tanto DeSalle y sus colaboradores (2005) como otros autores, refieren dos aspectos importantes en el empleo de los códigos de barras genéticos a aclarar para su correcto uso en taxonomía: 1) la identificación de especies y 2) el descubrimiento de especies. Ambas actividades requieren diferentes tipos y cantidades de datos.

Ya se han mencionado los obstáculos que ofrece el uso de distancias genéticas en taxonomía como el enmascaramiento de los caracteres homólogos al ser comprimidos en medidas de distancia, estas limitaciones desaparecen si el código de barras se usa como una fuente adicional de caracteres diagnosticables (Bergmann et al., 2009). Adicionalmente, DeSalle et al. (2005) refieren que el empleo de distancias genéticas en la iniciativa de los códigos de barras contradice a la práctica taxonómica tradicional puesto que ambas buscan el mismo fin, pero esta última está basada en caracteres y no en medidas de distancia.

La taxonomía integrativa *sensu* DeSalle y Goldstein (2005; 2011) "Character-based integrative taxonomy", es el marco de referencia que mejor concilia los métodos y conceptos de la taxonomía tradicional y las propiedades de los códigos de barras genéticos. El método basado en caracteres (Character-based DNA Barcoding) surge en medio de la discusión generada por los promotores de la taxonomía basada exclusivamente en ADN y de los códigos de barras genéticos, y la taxonomía clásica.

El algoritmo conocido como Sistema de Organización de Atributos Característicos (CAOS, por sus siglas en inglés; Sarkar et al., 2008), aunque no es el único, es el más ampliamente utilizado en el marco de los códigos de barras genéticos basados en caracteres. Ganó popularidad debido a su simple, pero poderosa estrategia de identificación de especies o incluso, poblaciones de especies (Bergmann et al., 2009), a través de la presencia o ausencia de caracteres diagnósticos discretos o combinaciones de estos caracteres en secuencias de ADN (Figura 1). Un código de barras de ADN basado en caracteres no es equivalente a un SNP (Single Nucleotide Polymorphisms), sin embargo, en otras disciplinas son empleados para la identificación de éstos como es el caso de genética de poblaciones o biomedicina, mientras que en el contexto del cladismo corresponden con los caracteres denominados autapomorfías (Williams, 2016).

Estos caracteres en la conceptualización de Sarkar y colaboradores (2008) se denominan atributos característicos (CA´s, por sus siglas en inglés). Los CA´s son identificados por medio un algoritmo, combinados y resumidos en una matriz para generar un código de barras específico para un taxón dado, bajo la premisa de que los miembros de un grupo taxonómico comparten atributos que están ausentes en otros grupos (Rach et al., 2008; Bergmann et al., 2009).

El algoritmo CAOS requiere de una estructura jerárquica previa que le sirva de guía para extraer los CA´s, esta guía jerárquica puede provenir de un fenograma como un árbol de distancias genéticas corregidas por el modelo Kimura de dos parámetros (KP2), aunque como DeSalle y sus colaboradores (2005) señalan, el empleo de distancias genéticas en los códigos de barras genéticos puede no ser lo más apropiado tanto para la identificación de especies como para el descubrimiento de las mismas puesto que se confronta con el empleo de caracteres por parte de otras disciplinas tales como la sistemática filogenética y la taxonomía tradicional (DeSalle et al., 2005;). El árbol filogenético es indispensable para CAOS para extraer atributos característicos de todos y cada uno de los nodos para las secuencias de ADN de los especímenes ingresados al análisis (DeSalle et al., 2005; Sarkar et al., 2008).

Los atributos característicos pueden ser de cuatro tipos (Sarkar et al., 2008), simples: puros o compuestos, o privados: puros o compuestos (Figura 1). Nótese que los atributos característicos son equivalentes a caracteres diagnósticos en el contexto de la taxonomía o equivalentes a sinapomorfías, autapmorfías, simplesiomorfías en el contexto cladístico (Kitching et al., 1998; Schuh, 2000; Morrone, 2016; Williams et al., 2016) dependiendo del estado de carácter en los sitios homólogos y si es compartido o no por otros taxa en las secuencias de ADN.

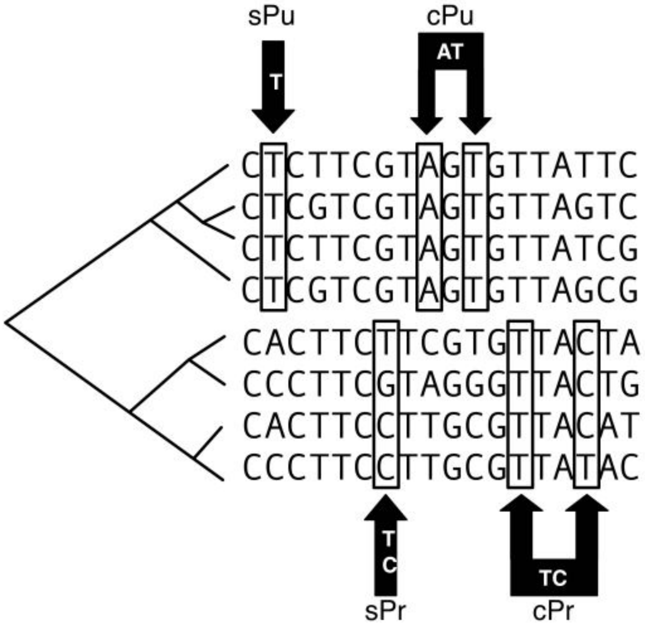

Figura 1. Characteristic Attribute Organization System (CAOS). Tipos de atributos característicos (CA´s) para un set hipotético de secuencias de ADN. Puros simples [sPu], Puros compuestos [cPu], Privativos simples [sPr] y Privativos compuestos. [cPr]. Ilustración tomada de Sarkar et al. (2008).

El empleo de los CA´s para delimitar o diagnosticar especies en el contexto de los códigos de barras genéticos es entonces muy compatible con la sistemática puesto que pueden designarse con la compañía de análisis filogenéticos basados también en caracteres (parsimonia, máxima verosimilitud o inferencia Bayesiana). CAOS de manera análoga a la taxonomía, busca identificar aquellos caracteres diagnósticos que diferencian a una especie de otra, útiles tanto para identificarlas como para proponer (acompañados de otros datos como morfológicos, ecológicos u otros) el descubrimiento de nuevas especies (DeSalle et al., 2005; DeSalle, 2006; DeSalle y Goldstein, 2019).

Así, los códigos de barras pueden utilizarse ya sea como una primera aproximación (que es el planteamiento original de la iniciativa) o última para delimitar especies, según sea el contexto. El reconocimiento de unidades genéticas discretas puede forzar al taxónomo a investigar y analizar otras fuentes de datos u otros aspectos importantes para delimitar especies, tales como el comportamiento de los organismos, análisis de coalescencia como el modelo GMYC de coalescencia, modelado de nicho ecológico, o reevaluar los ya recopilados para encontrar diagnosis que corrobore la hipótesis establecida inicialmente. En este sentido, DeSalle et al. (2005) proponen el círculo taxonómico (Figura 2) como una referencia en el proceder taxonómico, el cual está dirigido principalmente a los promotores de los códigos de barras genéticos, pero puede ser de carácter universal.

El método CAOS ha probado ser eficaz en la identificación y descubrimiento de especies en diferentes grupos de animales: odonatos (Rach et al., 2008), neogastrópodos (Zou et al., 2011), *Drosophila* (Yassin et al., 2010), peces (Cypriniformes, Chakraborty et al., 2017) y en géneros de plantas como *Decalepis* (Apocynaceae: Mishra et al., 2017) o Cícadas (Zamiaceae, Nicolalde-Morejón et al., 2010; Martínez-Domínguez et al., 2016).

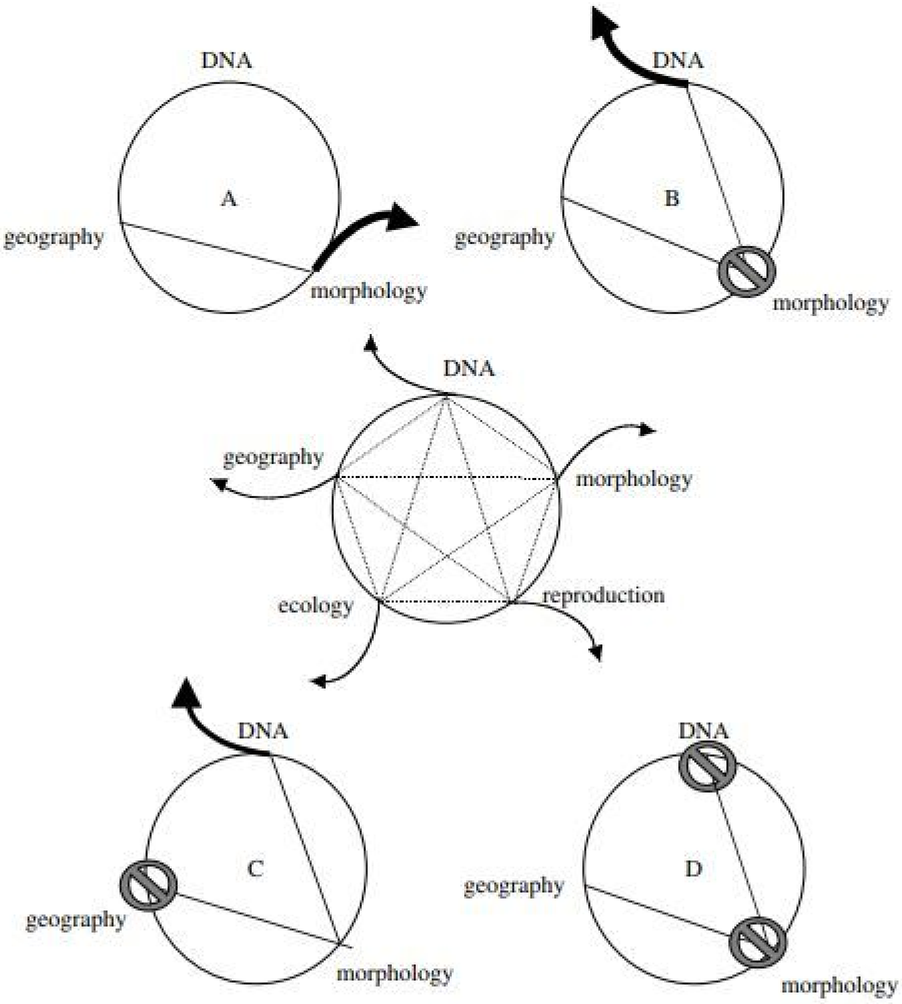

Figura 2. Círculo taxonómico (Tomado de DeSalle et al., 2005). Esquema muy simplificado de los distintos escenarios que pudieran presentarse en la práctica taxonómica actual. El escenario A ejemplifica la taxonomía tradicional en donde se plantea la hipótesis inicial que dos taxones son diferentes con base en una distribución geográfica distinta, al ser corroborada por diferencias en su morfología puede romperse el círculo. El escenario B refiere una hipótesis inicial basada en su distribución geográfica y corroborada por diferencias a nivel de ADN para romper el círculo, en este caso, la evidencia morfológica no apoya la diferenciación de entidades taxonómicas. El escenario C ejemplifica el descubrimiento de una especie simpátrica, basada en la corroboración de evidencia tanto a nivel morfológico como de ADN. El escenario D indica el fallo en la corroboración de las diferentes fuentes de evidencia para un taxón.

A pesar de su simpleza, CAOS ha demostrado obtener resultados similares o superiores a otros métodos más complejos empleados para asignar códigos de barras o delimitar especies entre los que se encuentran aquellos basados en coalescencia o en el cómputo de distancias genéticas (Rach et al., 2008; Zou et al., 2016a y 2016b).

Para el desarrollo de una librería de referencia de códigos de barras genéticos en grupos complejos como es el caso de *Echeveria*, consideramos que el "Character-Based DNA barcoding approach" es el método más adecuado, puesto que permite al mismo tiempo que se designan para los taxones de un grupo de especies, la realización de estudios sistemáticos y taxonómicos de las mismas.

Capítulo 1. Construyendo una librería de referencia de códigos de barras de *Echeveria*

Título: A pilot character-based DNA barcoding study in *Echeveria* (Crassulaceae): towards a DNA barcoding reference library for Mexican Crassulaceae

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Sometido a: Para ser sometido a *Proceeding Of The Royal Society B*

A pilot character-based DNA barcoding study in *Echeveria* (Crassulaceae): towards a DNA barcoding reference library for Mexican Crassulaceae

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Abstract

The genus *Echeveria* includes approximately 155 species, mostly endemic to México (83%) and frequently used as ornamentals worldwide. Difficulties in morphological character coding and scarcity of informative molecular data have restricted progress in the systematics of the 17 *Echeveria* series previously described through traditional taxonomic criteria and the species level taxonomy become a complex task. Supported by a Bayesian phylogenetic reconstruction on a molecular matrix of two standard chloroplast DNA regions (*matK* and *rbcL*) and one supplementary nuclear marker (*ITS2*), here we present a first effort towards DNA barcodes library of *Echeveria*, by representing 52 specimens and 16 taxonomic series. DNA diagnostics obtained from combined matrices of *matK*+ITS2 and *rbcL*+*matK*+ITS2 guaranteed 100% identification success for the specimen sampled but only if we consider phylogenetic relationships. Our results indicate that the current systematic classification of *Echeveria* requires revision and, suggest that future integrative taxonomy studies involving character-based DNA barcoding, morphology and biogeography might be feasible in *Echeveria*, as well as in other genera of Mexican Crassulaceae.

Key words: *Echeveria*, Mexican Crassulaceae*,* DNA barcoding, CAOS, Bayesian phylogenetics, integrative taxonomy

Introduction

Echeveria is the second most specious genus of the Acre clade in the family Crassulaceae (Saxifragales; Carrillo-Reyes et al., 2009) with approximately 155 species grouped into 17 series (Kimnach in Eggli, 2003; Pilbeam, 2008). Based on standard monographic treatments, individual specimens of *Echeveria* can be described as perennial, usually with fibrous or fusiform roots, caulescent, or with short stems. Their leaves are arranged in a compact or loose rosette; inflorescences are cincinni, spikes, panicles, or less frequently thyrsoid clusters, with few to many bracts similar to rosette leaves. Floral characters in the genus are generally more stable: the calyx has 5 parts, very small or as long as the corolla, united at the base, usually unequal, green or in different colors; the corolla is tubular-urceolate to tubular-campanulate, very angled to rounded; petals are erect, imbricated or valvate, and it presents one to three colors (usually yellow, white, red, orange, pink, or combinations thereof). The androecium in *Echeveria* flowers has 10 stamens, 5 of which are epipetalous, generally included in the corolla; the gynoecium has 5 free carpels, with nectary scales at the base of the carpels. Finally, the fruit is an aggregate of follicles, with many oblongs to elliptic seeds. The genus is distributed from southern United States (Texas) to northern Argentina; however, Mexico concentrates about 83% of the genus diversity (Etter & Kristen, 2007).

Besides the monograph of Kimnach (in Eggli, 2003) and the species update by Pilbeam (2008), *Echeveria* had been revised by Walther (1972). Along with the regional studies of Meyrán and López (2003), Jimeno-Sevilla (2008) and Espino and de la Cruz (2009), these works jointly indicate that supraspecific and infrageneric delimitation and inclusion of species in certain series are artificial.

Despite advances in the molecular phylogenetics of *Echeveria* (e.g. Carrillo-Reyes et al., 2009; Vázquez-Cotero et al., 2017), absence of a reliable reconstruction which includes the majority of species in the genus, as well as a comprehensive analysis of a number of morphological characters currently limits our understanding of the systematics and taxonomy in the genus and related taxa. Here we present results from the first stage of a molecular systematics project oriented to establish a DNA barcoding reference library for a group of Mexican species of *Echeveria*, exploring in parallel the phylogenetic relationships between these taxa under Bayesian criteria (Holder and Lewis 2003). Specifically, a phylogeny obtained with MrBayes was used as a guide tree for subsequent computation of diagnostic DNA characters with the Characteristic Attributes Organization System (CAOS) algorithm, an "automated systematic method for discovering conserved character states from cladograms (i.e. trees) or groups of categorical information" (Sarkar et al., 2008: 1256).

Like cladistic phylogenetic methods, CAOS only takes into account diagnostically informative characters, in analogy to the role that morphological diagnostic characters play in traditional taxonomy. This method, which has been successfully used for molecular identification of species in the three Mexican cycad genera (*Ceratozamia*, *Dioon* and *Zamia*; Nicolalde-Morejón et al., 2010 and 2011), constitutes an indispensable starting point for subsequent "integrative taxonomy" studies where character-based DNA barcodes are used in conjunction with biogeography, morphology and other sources of comparative biological information for inferential corroboration of species identity and further species discovery (DeSalle et al., 2005; Goldstein and DeSalle 2011; see also Padial et al., 2010).

Materials and methods

Sampled taxa

The present study is based on specimens corresponding to 46 currently valid *Echeveria* species, four synonym taxa (*E. chiapensis*, *E. crassicaulis*, *E. longipes*, *E. microcalyx*, of *E. rosea*, *E. mucronata*, *E. paniculata* var. *maculata*, *E. amoena*, respectively), and two specimens morphological allied to *E. gibbiflora* and *E. secunda* (*E. gibbiflora* complex and *E. secunda* complex) belonging to the "Colección Nacional de Crasuláceas", a taxonomic collection infrastructure custodied by the Jardín Botánico of the Instituto de Biología, Universidad Nacional Autónoma de México (JB-IBUNAM, located in Ciudad Universitaria, UNAM, Mexico City). Voucher information is summarized in **Table 1**. This sample of individual plants corresponds to 16 of the 17 series included in currently *Echeveria* classifications (Kimnach in Eggli, 2003) and represents approximately 30% of the genus diversity (Genbank accession numbers for these specimens can be consulted in **supplementary data** section). Some species not actually placed into series have been labeled as undefined (*E. cuicatecana* and *E. kimnachii*). Four species of *Pachyphytum* were selected as outgroups, based on previous phylogenetic information which placed this genus as a monophyletic sister group to *Echeveria* and the rest of the so-called "*Echeveria* group" (Carrillo-Reyes et al., 2009).

DNA extraction, amplification and sequencing

Total DNA was extracted from silica-gel dried tissue using 2X CTAB protocol (standard in Canadian Centre for DNA Barcoding (CCDB). DNA fragment amplifications was carried out using Platinum® Taq DNA Polymerase (Ivanova et al., 2006), the three pairs of primers for the selected regions and the PCR thermocycler program used are resume in **Table 2** according to CCDB standards. For *rbcL* and ITS2 sequences, amplification was performed in 12.5 µL final volumes with 6.25 µL of 10% Trehalose (Spiess et al., 2004). For *matK* 1.875 µL of 20% Trehalose was used. PCR products were visualized in pre-cast agarose E-gel® 96 from Invitrogen, the fragments were sequencing in the CCDB facilities. Accession numbers for all sequences are provided in **Table 3**.

Bayesian inference guide tree

Matrices of aligned sequences for each locus were downloaded from the BOLD Systems Database (http://www.boldsystems.org) as part of the first stage of the "Crassulaceae in Mexico" ("MKCRA" project), an ongoing collaboration between the Center for Biodiversity Genomics and Canadian Center for DNA Barcoding (University of Guelph) and UNAM (see authors list). Sequences of each molecular marker were concatenated in Mesquite v2.5 (Maddison & Maddison, 2008) to obtain an expanded matrix of 1821 bp in length.

After edition, the expanded matrix was analyzed with Bayesian criteria implemented in MrBayes v3.2 (Ronquist et al., 2012). Choice of molecular evolution model and better partition scheme for this analysis was obtained by PartitionFinder v1.1.1 (Lanfear et al., 2012) under the Bayesian Information Criterion (BIC), resulting in the following schemes: GTR+I+G for *matK* and r*bcL* 3rd codon positions, Jukes-Cantor for *rbcL* 1st and *rbcL* 2nd codon positions, and SYM+I+G for ITS2. Markov Monte Carlo analyses were then performed for 2,000,000 generations, using one cold and three hot chains sampled every 2,000 generations. Parameter convergence was visualized with Tracer v1.6. The first 25% of the saved trees was discarded, and a majority-rule consensus tree without branch lengths was obtained for further use and interpretation.

Character-based DNA barcoding

The CAOS protocol (Sarkar et al., 2008) requires a hierarchical database structure, which was provided by the Bayesian majority-rule consensus tree obtained previously. Polytomies in the tree were resolved in Mesquite v2.5 (Maddison & Maddison, 2008) according to morphological affinities, and the resulting tree was saved and incorporated into the composite matrix in NEXUS format. This file was then executed in the CAOS-Workbench (http://boli.uvm.edu/caos-workbench) through the P-Gnome program, which produces a "CAOS_attributesFile" and a "CAOS_groupFile". The latter files and an additional file containing a FASTA alignment were then loaded into the CAOS-Barcoder application (http://boli.uvm.edu/caos-workbench/caos_barcoder.php), which actually produces the file with the DNA diagnostics ("Characteristic Attributes" (CAs); Sarkar et al., 2008: 1257). From the seven xls files that CAOS outputs, only Total_barcode.xls which contains CAs without hierarchical arrangement and overview5.xls with CAs considering phylogenetic relationships (or hierarchical arrangement) were taking into account in the results (The two last files can be consulted in **Supplementary data**). Jörger and Schrödl (2014) provide further explanatory information for the use of the CAOS-

Workbench implementation of CAOS.

As a final step in the compilation of DNA diagnostic data for the CAOS analyses, only attributes with confidence values of 1.00 were used. Additionally, it should be noted that insertion-deletion events were not included if they corresponded to the beginning or end of alignments, as well as long missing data in some taxa due to sequencing uncertainty.

Results

Bayesian inference guide tree

The resulting majority-rule consensus tree shows a general topology in agreement with previous phylogenetic studies in *Echeveria* (Carrillo-Reyes et al., 2009; Vázquez-Cotero et al., 2017). *Pachyphytum* is recovered as monophyletic and sister to the rest of *Echeveria* species (**Figure 2**). Three main Echeverian clades are noticeable in the tree, one of them conformed by series *Urbinia* species (*E. agavoides* and *E. elegans*) *E. cuspidata* var. *cuspidata* also from *Urbiniae* is not nested within probably due long fragments of missing data in the alignment.

A second clade contains representatives for series *Chloranthae*, *Echeveria*, *Longistylae*, *Mucronatae*, *Nudae*, *Racemosae*, *Thyrsiflorae*, *Spicatae*, *Urbiniae* and *E. cuicatecana*. The third clade includes series *Angulatae*, *Gibbiflorae*, *Occidentales*, *Paniculatae*, *Pruinosae*, *Secundae*, *Urbiniae*, *Valvatae*, *E. kimnachii* and *E. semivestita* var. *semivestita* (**Figure 2**). Most of the shallow relationships were recovered with moderate to high support but in the deepest clades there is uncertainty (polytomies and low node support; **Figure 2**).

Character-based DNA barcoding in Echeveria *using CAOS*

Taken individually, the informative content of each DNA barcoding loci selected in the present study was variable. From the alignment alone (Total_barcode.xls file, **supplementary data**), without considering phylogenetic relationships, *rbcL* had the lowest diagnostic performance, with only six identified specimens (11.54 %) with at least one Characteristic Attribute (CA; Sarkar et al., 2008). In turn, the diagnostic capacity of *matK* was substantially higher than *rbcL*, providing correct identifications (with at least one CA) for 24 specimens (46.15 %). In contrast, *ITS2* contributed with slightly superior proportion of identifications (25 taxa = 48.08 %). The best results were obtained either by combining *matK*+*ITS2* or the three loci also guaranteed 73.08 % correct taxonomic identification of 52 *Echeveria* specimens, each corresponding to a valid binomial (**Figure 3**).

On the other hand, after consideration of the phylogenetic relationships (overview5.xls files, **supplementary data**) suggested by the guide tree, it was further possible to obtain the following identification numbers and percentages of specimens/species: first, 14 identifications (26.92 %) with *rbcL*; *matK* in turn allows 71.15 % identification (37 specimens/species), and ITS2 contributes with 40 species/specimens identified (76.92 %). Combinations of *matK*+ITS2 and the three markers guarantee 100 % discrimination of the sample (**Table 4**). No locus alone had the discriminative power of 100% in *Echeveria* species considered in the analysis (**Figure 4**).

Discussion

The lack of a phylogeny that includes all representatives of *Echeveria* is directly connected to the unreliable systematic classification currently available for the genus. Although is not the main goal for this first project step, here we explored its internal phylogenetic relationships under

Bayesian criteria. Using three molecular markers –*rbcL*, *matK* and *ITS2*– and a sample of 52 *Echeveria* species/specimens, the Bayesian phylogenetic tree obtained here only partially reflects the currently accepted classification for *Echeveria* (Kimnach in Eggli, 2003). Preserved as monophyletic groups, the series *Angulatae*, *Gibbiflorae*, *Paniculatae*, *Thyrsiflorae* and *Valvatae* showed high support values, whereas *Pruinosae* showed less statistical consistency. With respect to the aforementioned classification, the remaining series imply confusing phylogenetic relationships: among these, the series *Echeveria*, *Nudae*, *Mucronatae* and *Urbiniae* stand out due to their polyphyletic condition.

Although the Bayesian phylogenetic topology generated in this work includes only a sample of the diversity of the genus, it clearly highlights the need to reevaluate mainly morphological characters that have historically been used to assign series and delimit species. During subsequent stages of the University of Guelph / UNAM collaboration in the "Crassulaceae of Mexico" DNA barcoding project, phylogenetic relationships within *Echeveria* obtained during the guide tree-generation steps –mainly for the basal nodes– could be improved with the inclusion of additional loci.

Character-based DNA barcoding in Echeveria: *toward an integrative taxonomy of highly diverse genera of Mexican Crassulaceae*

Automated estimation of DNA barcodes in plant taxa has proven to be more complex task than in animal groups. Many loci studied in plant genomes usually do not comply with the ideal qualities sought in a DNA barcode: (i) ease of amplification, (ii) short sequences, and (iii) relatively high (taxonomic-systematic; phylogenetic) informative content (i.e. relatively high identification capacity). With the main aim of producing a first characterization of character-based DNA barcodes for this highly diverse Mexican Crassulaceae taxon, illustrating the 'multi-locus approach in botany' (Li et al., 2015), for *Echeveria* we have adopted the proposal from the Consortium for the Barcode of Life (CBOL Plant Working Group, 2009), by using the chloroplast coding regions *rbcL* and *matK* as the core of the barcode. However, we have confirmed that these two loci do not constitute a reliable molecular diagnostic tool, and therefore we adhere to published criticisms of their usefulness as DNA barcoding data sources (Roy et al., 2010; see also Nicolalde-Morejón et al., 2011). To attend the shortcomings of the core CBOL PWG DNA barcoding loci, here we have included the intergenic transcribed spacer (*ITS2*) as an additional, third DNA barcoding locus, given its relatively greater reliability in both animal and plant groups (Yao et al., 2010; Gu et al., 2013).

Goldstein & DeSalle (2011) have distinguished the different analytical underpinnings of the main methodological approaches to DNA barcoding. According to these authors, analytical strategies in DNA barcoding can be broadly divided in two groups: genetic distance-based and character-based methods. Both groups of methods require a hierarchical structure, which is normally provided by a branching diagram (e.g. a cladogram, dendrogram, phylogram, or other class of tree); but delimitation of species in genetic distance-based methods (i. e. neighbor-joining) rests exclusively on the criterion of reciprocal monophyly. In matrices of computed genetic distances, the percentage of divergence between lineages can be quantified; however, this has proven to be controversial (Zou et al., 2011), since the limits of interspecific genetic divergence, although based on empirical evidence, have been arbitrarily established (e.g. 2.7% for birds, 3% for insects, 2% for invertebrates, 4% for vertebrates, etc.). In addition, the requirement of no overlap between intraspecific variation and interspecific variation (the so-called "DNA barcoding gap") is a requirement that cannot always be met, because rates of nucleotide substitution vary between and within species, especially in recently divergent groups.

Given the aforementioned limitations in genetic distance-based methods, DNA barcoding based on the idea of suites of DNA characters, each of which can be individually diagnostic, is recently gaining popularity. Identification of biological entities on the basis of DNA diagnostics – that is, "character-based DNA barcoding" *sensu stricto* (DeSalle et al. 2005; Goldstein and DeSalle, 2011)– is determined by the presence of unique characteristic attributes in the DNA sequences that resembles the extraction of morphological characters traditionally used by morphology-based taxonomy to identify and differentiate species. The bioinformatic software CAOS implements a well-established character-based DNA barcoding method which computes DNA diagnostics (Sarkar et al., 2008). CAOS indispensably requires a 'guide tree' to function as a topological reference for the extraction of such diagnostic DNA character states for each group (clades), proceeding node by node until it reaches the terminals. As stated by Sarkar et al. (2008) (see also Jörger and Schrödl, 2014), it is advisable that the phylogenetic reconstruction is as complete and robust as possible to ensure the correct allocation of DNA barcodes.

CAOS-based DNA barcoding analyses in *Echeveria* indicate that *rbcL* contributes with a low number of CAs and ensures only 26.92% of correct identifications, based on at least one diagnostic site (CA). This result is consistent with a recent DNA barcoding for the Crassulaceae genus *Rhodiola* (Zhang et al., 2015). In contrast to *Rhodiola*, where 34-36% of species were diagnosed, the performance of *matK* in *Echeveria* is relatively better, with the percentage of correct identifications reaching 71.15%. Interestingly, the *Rhodiola* data were analyzed with genetic distance-based methods; it is likely that the use of character-based methods would prove to be more sensitive. Finally, the more variable internal transcribed spacer 2 (*ITS2*) provided the largest amount of DNA diagnostics in this study, allowing correct identification of 76.92% of the sampled *Echeveria* taxa. None of the loci employed in isolation had the ability to discriminate all entities; only the combination of the three loci, or the *matK*+*ITS2* pair, guaranteed 100% identification. Since the latter combination offers the best cost/benefit ratio, we consider these loci as the current best pair of candidates for DNA barcoding in *Echeveria*, and potentially in other Crassulaceae genera.

Systematics and taxonomy of *Echeveria* has been a complex task, which has been limited, primarily, by the lack of a complete phylogeny that reflects the evolutionary processes of the taxa that comprise the genus. The description of new species in *Echeveria* has been based historically on the biogeographical distribution of populations, the identification of ecological properties or phenological aspects, and the identification of morphologically diagnostic characters which, although useful as sources of evidence, in many cases they have led to taxonomical controversies and potential underestimations of species diversity.

The preliminary phylogenetic and DNA barcoding results described and interpreted in the present work suggest the need for modifications in the taxonomic status of a number of *Echeveria* species. In subsequent stages of our project on Mexican Crassulaceae, we plan to incorporate the conceptual framework of "integrative taxonomy" *sensu* DeSalle and collaborators (DeSalle et al., 2005; Goldstein and DeSalle, 2011) to inferentially support these changes, via the critical addition of biogeographical, morphological, and other sources of taxonomic/systematic information. In the following paragraphs, we provide a few examples of the potential application of this framework to the *Echeveria* case:

1. The series *Chloranthae* was designated for the single species *E. heterosepala* which has a unique character among all species of the genus consisting of the greenish petals coloration, whereas the

molecular phylogeny indicates that it is close to some of the *Mucronatae* species (*E. crassicaulis*) and *Thyrsiflorae* series (**Figure 1)** with which it shares the tuberous root type, the acaulescent rosette and the large number of bracts in the inflorescence, so the monotypic series *Chloranthae* may merge into series *Thyrsiflorae*. Vázquez-Cotero et al. (2017) also found a similar result with reference to phylogenetic position of *E. heterodsepala*.

2. *E. purpusorum* has been historically located in the series *Urbiniae*, while the molecular phylogeny approaches the species to the series *Longistylae* although with low support. Some characteristics unite *E. purpusorum* with the other *Longistylae* species: they are biogeographically distributed in the Mixteca Alta region (in Puebla-Oaxaca north-west limits), and present vegetative similarities –acaulescent rosette, greenish doted red to brown leaves with red margins– and floral characters –slightly globose corolla, with green sepals, red petals at base and yellowish at apex, and few flowers per floral branch– similar to those of the recently published *E. longissima* var. *brachyantha* (Reyes et al., 2015). The latter is characterized by its shorter corolla, relative to those of other taxa in the series; however, with respect to its closely relative, *E. longissima* var. *aztatlensis*, it shows nine DNA diagnostic differences in the three loci analyzed.

3. The series *Urbiniae* has been recovered as polyphyletic. The cases of *E. purpusorum* (mentioned above) and the pair *E. humilis* / *E. xichuensis* reflect that many of the morphological characters actually used to assign species into series are artificial and need to be reviewed. The latter two species were located into series *Urbiniae* by Kimnach (in Eggli, 2003), but they are not phylogenetically close –actually, they share more morphological attributes with *Angulatae* species than with *Urbiniae*–. Most of these differences lie in floral characters, such as longer and linear shaped sepals, angulated, noticeable keeled petals, tubular instead of urceolate corolla, and usually bigger nectary scales than *Urbiniae*.

4. *E. zorzaniana* and *E. carminea* are polytomic in the Bayesian tree, indicating uncertainty at the molecular phylogenetic terms. Few morphological characters actually distinguish these species (e.g. ovary color and stigma lobes; Reyes and Brachet, 2009), as well as shorter habit and thinner leaves. There is, however, a single diagnostic character state in the site 121 of the *ITS2* (**Table 4**), out of a total of 1821 sites. This suggests ‘raising a red flag’ (DeSalle, 2006) for potential synonymy; further character-based DNA barcoding and integrative taxonomy analyses might help to distinguish between these scenarios.

5. For the only two species of the series *Occidentales* (*E. affinis* and *E. craigiana*), their biogeographic distribution in Durango and Sinaloa overlaps. This pair of species mutually differs morphologically only in some vegetative characters (i. e. color, shape and size of leaves, and the size of the inflorescence), while at the molecular level they present a single difference in the site 241 of the ITS2 region (**Table 4**). Again, this scenario indicates the need to focus future efforts on additional data collection.

6. The description of *E. cuicatecana* generated controversy regarding the genus in which it belongs. Whereas authors of the original publication placed it in *Echeveria*, due to the absence of appendices in epipetalous filaments inside the petals (Reyes et al., 2004), Kimnach (2010) indicated that it belongs to *Pachyphytum*, based on several others morphological characteristics. However, in our Bayesian phylogenetic reconstruction, this species is nested within *Echeveria* and not with *Pachyphytum* (**Figure 1**), indicating its clear membership to *Echeveria*. The inclusion of *E. cuicatecana* in a specific series remains to be determined.

7. Three *Echeveria* taxa currently in synonymy –namely, *E. chiapensis*, *E. longipes* and *E. microcalyx* with *E. rosea*, *E. paniculata* var. *maculata* and *E. amoena,* respectively– do not exhibit any morphological differences and have no clear biogeographical diagnostic information.

However, differences at the molecular level (**Table 4**) may indicate that genetic variation within populations are present. In this regard, future inclusion of more individuals and populations might be required in order to strengthen taxonomic hypotheses in the genus, according to the 'taxonomic circle' rationale, one of the core ideas of the "integrative taxonomy" framework (DeSalle et al., 2005; Goldstein and DeSalle, 2011).

Acknowledgments

The present paper partially fulfills the requirements for the Ph. D. degree (Doctorado en Ciencias) of Luis Emilio de la Cruz López, awarded by the Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México (PCB-UNAM). The first and corresponding authors gratefully acknowledge support from the following financial sources: UNAM-Dirección de Asuntos del Personal Académico-Programa de Apoyo a Proyectos de Innovación e Investigación Científica (DGAPA-PAPIIT) Project IN212015; Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico) Postgraduate Scholarship program and CONACYT Project PDCPN 2014 247078; and PCB-UNAM. Special thanks to Dr. Patricia Escalante-Pliego (Zoology Department, IBUNAM) for establishing the initial contact between DNA barcoding researchers at the University of Guelph and our Crassulaceae research team at IBUNAM, and to M. Sc. Jorge Escutia-Sánchez for his technical support.

References

Carrillo-Reyes, P., Sosa, V., & Mort, M. E. (2009). Molecular phylogeny of the *Acre* clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*. *Molecular Phylogenetics and Evolution* 53: 267-276.

CBOL Plant Working Group (Hollingsworth, P. M., Forrest, L. L., et al.) (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America 106*:12794-12797.

DeSalle, R., Egan, M. G., & Siddall, M. (2005). The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B 360*: 1905-1916.

DeSalle, R. (2006). Species discovery versus species identification in DNA barcoding efforts: response to Rubinoff. *Conservation Biology 20*: 1545-1547-

Eggli, U. (2003). Illustrated handbook of succulent plants: Crassulaceae. *Springer-Verlag.*

Espino, O. G., & de la Cruz, L. L. E. (2009). Las Crasuláceas del Valle del Mezquital. Bachelor´s Degree Thesis. FES Zaragoza. UNAM.

Etter, J. & Kristen, M. (2017). The Crassulaceae database. www.crassulaceae.com

Goldstein, P. Z., & DeSalle, R. (2011). Integrating DNA barcode data and taxonomic practice: Determination, discovery, and description. *Bioessays 33*: 135–147.

Gu, W., Song, J., Cao, Y., Sun, Q., Yao, H., Wu, Q., Chao, J., Zhou, J., Xue, W., & Duan, J. (2013). Application of the ITS2 region for barcoding medicinal plants of Selaginellaceae in Pteridophyta. *PLoS ONE 8*: 1-8.

Holder, M., & Lewis, P. O. (2003). Phylogeny estimation: traditional and Bayesian approaches. *Nature Reviews Genetics 4*: 275-284.

http://www.boldsystems.org

http://www.barcodeoflife.org/content/about/what-dna-barcoding.

Ivanova, N., Grainger, C., Hajibabaei, M. (2006). Increased DNA barcode recovery using Platinum® Taq. *CCDB Advances, Methods Release 2*.

Jimeno-Sevilla, H. D. (2008). El género *Echeveria* (Crassulaceae) en Veracruz, México. Bachelor´s Degree Thesis. Facultad de Biología. Universidad Veracruzana.

Jörger, K. M., & Schrödl, M. (2014). How to use CAOS software for taxonomy? A quick guide to extract diagnostic nucleotides or amino acids for species descriptions. *Spixiana 37*.

Kimnach, M. (2010). The generic placement of *Echeveria cuicatecana*. *Cactus and Succulent Journal of America 82*: 125.

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution 29*:1695–1701.

Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2015). Plant DNA barcoding: from gene to genome. *Biological Reviews 90*: 157-166.

Maddison, W. P., & Maddison, D. R. (2008). Mesquite: a modular system for evolutionary analysis. Version 2.5 *http://mesquiteproject.org*.

Meyrán, J., & López, C. L. (2003). Las Crasuláceas de México. *Sociedad Mexicana de Cactología AC*.

Nicolalde-Morejón, F., Vergara-Silva, F., González-Astorga, J., & Setevenson, D. (2010). Character-based, population-level DNA barcoding in Mexican species of *Zamia* L. (Zamiaceae: Cycadales). *Mitochondrial DNA 21*: 51–59.

Nicolalde-Morejón, F., González-Astorga, J., Vergara-Silva, F., & Vovides, A. P. (2011). Perspectivas sistemáticas de Zamia (Zamiaceae) en Megaméxico: de la taxonomía alfa a los códigos de barras genéticos. *Revista Mexicana de Biodiversidad 82*:341-355.

Padial, J. M., Miralles, A., De la Riva, I. &Vences, M. (2010). The integrative future of taxonomy Frontiers in Zoology 7:16.

Pilbeam, J. (2008). The genus *Echeveria*. *The British Cactus & Succulent Society*.

Reyes, S. J. & Brachet, I. C. (2009). *Echeveria zorzaniana*, una nueva especie de la familia Crassulaceae para el estado de Oaxaca, México. C*actáceas y Suculentas Mexicanas 54*: 90-95.

Reyes, S. J., Brachet, I. C., González-Zorzano, O. & Islas, L. A. (2015). Four new taxa of the genus *Echeveria* from the State of Oaxaca, Mexico. *Haseltonia 21*: 80–91.

Reyes, S. J., Pérez, C. J. & Brachet, I. C. (2004). *Echeveria cuicatecana*, una nueva especie para el estado de Oaxaca, México. *Cactáceas y Suculentas Mexicanas 49*: 80-84.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology 61*: 1-4.

Roy, S., Tyagi, A., Shukla, V., Kumar, A., Singh, U. M., Chaudhary, L. B., Datt, B., Bag, S. K., Singh, P. K., Nair, N. K., Husain, T., & Tuli, R. (2010). Universal plant DNA barcode loci may not work in complex groups: A case study with Indian *Berberis* Species. *PLOS ONE 5*: 1-14.

Sarkar, I. N., Planet, P. J., & DeSalle, R. (2008). CAOS software for use in character-based DNA barcoding. *Molecular Ecology Resources 8*: 1256-1259.

Spiess, A. N., Mueller, N., Ivell, R. (2004). Trehalose is a potent PCR enhancer: Lowering of DNA melting temperature and thermal stabilization of Taq polymerase by the disaccharide trehalose. *Clinical Chemistry 50*:1256-1259.

Thiede, J. (2003). *Pachyphytum*. In: Eggli, U. Illustrated Handbook of Succulent Plants: Crassulaceae. *Springer*. 190-195.

Vázquez-Cotero, C., Sosa, V. & Carrillo-Reyes, P. 2017. Phylogenetic position of *Echeveria heterosepala* (Crassulaceae): a rare species with diagnostic characters of *Pachyphytum*. *Botanical Sciences 95*: 1-12.

Walther, E. (1972). *Echeveria*. *California Academy of Sciences*.

Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P. & Chen, S. (2010). Use of ITS2 region as the universal DNA barcode for plants and animals. *PLOS ONE 5*: 1-9.

Zhang, J. Q., Meng, S. Y., Wen, J., Rao, G. Y. (2015). DNA Barcoding of *Rhodiola* (Crassulaceae): A Case Study on a Group of Recently Diversified Medicinal Plants from the Qinghai-Tibetan Plateau. *PLoS ONE*. *10*: 1-15.

Zou, S., Li, Q., Kong, L., Yu, H., & Zheng, X. (2011). Comparing the Usefulness of Distance, Monophyly and Character-Based DNA Barcoding Methods in Species Identification: A Case Study of Neogastropoda. *PLoS ONE 6*. 1-8.

Figure legends

Figure 1a. Sampled *Echeveria* taxa. (1) *E. affinis*, (2) *E. gibbiflora* complex, (3) *E. secunda* complex, (4) *E. agavoides*, (5) *E. amoena*, (6) *E. bifida*, (7) *E. calderoniae*, (8) *E. calycosa*, (9) *E. cante*, (10) *E. carminea*, (11) *E. chapalensis*, (12) *E. chiapensis*, (13) *E. coccinea*, (14) *E. craigiana*, (15) *E. crassicaulis*, (16) *E. crenulata*, (17) *E. cuicatecana*, (18) *E. cuspidata* var. *cuspidata*, (19) *E. dactylifera*, (20) *E. elegans*, (21) *E. fulgens* var. *fulgens*, (22) *E. gibbiflora*, (23) *E. gigantea*, (24) *E. heterosepala*.

Figure 1b. Sampled *Echeveria* taxa. (25) *E. humilis*, (26) *E. kimnachii*, (27) *E. laui*, (28) *E. longiflora*, (29) *E. longipes*, (30) *E. longissima* var. *aztatlensis*, (31) *E. longissima* var. *brachyantha*, (32) *E. lutea*, (33) *E. microcalyx*, (34) *E. minima*, (35) *E. mucronata*, (36) *E. multicaulis*, (37) *E. nebularum*, (38) *E. nuda*, (39) *E. peacockii*, (40) *E. penduliflora*, (41) *E. pinetorum*, (42) *E. platyphylla*, (43) *E. purpusorum*, (44) *E. rosea*, (45) *E. roseiflora*, (46) *E. secunda*, (47) *E. semivestita* var. *semivestita*, (48) *E. subrigida*, (49) *E. triquiana*, (50) *E. valvata*, (51) *E. xichuensis*, (52) *E. zorzaniana*.

Figure 2. Bayesian majority-rule consensus tree. Actual series membership is indicated between brackets: A=*Angulatae*, C=*Chloranthae,* E=*Echeveria*, G=*Gibbiflorae,* ?=Indefinite, L=*Longistylae,* M=*Mucronatae,* N=*Nudae,* O=*Occidentales,* P=*Pruinosae,* Pan=*Paniculatae,* R=*Racemosae,* S=*Secundae,* Spi=*Spicatae,* T=*Thyrsiflorae,* U=*Urbiniae,* V=*Valvatae.* Circles in nodes designate posterior probabilities: green=0.9-1, blue=0.8-0.89, yellow=0.7-0.79, red<0.69. Dotted lines show polytomies output from MrBayes. Solid lines indicate the topology input in CAOS.

Figure 3. Percentage of taxa identified by locus and combinations thereof with at least one CA, without hierarchical arrangement.

Figure 4. Percentage of taxa identified by locus and combinations thereof with at least one CA,

considering topology of guide tree.

Table legends

Table 1. Geographic distribution of sampled taxa. Current series membership is indicated with a single-letter or three-letter code between brackets after each name: A=*Angulatae*, C=*Chloranthae,* E=*Echeveria*, G=*Gibbiflorae,* ?=Indefinite, L=*Longistylae,* M=*Mucronatae,* N=*Nudae,* O=*Occidentales,* P=*Pruinosae,* Pan=*Paniculatae,* R=*Racemosae,* S=*Secundae,* Spi=*Spicatae,* T=*Thyrsiflorae,* U=*Urbiniae,* V=*Valvatae.*

Table 2. Taxa, BOLD ID and Genbank accession numbers.

Table 3. Primer, sequence and Thermocycler program of oligonucleotides used to amplify DNA fragments.

Table 4. CAOS-based DNA diagnostics, according to majority-rule consensus guide tree.

Figure 1a.

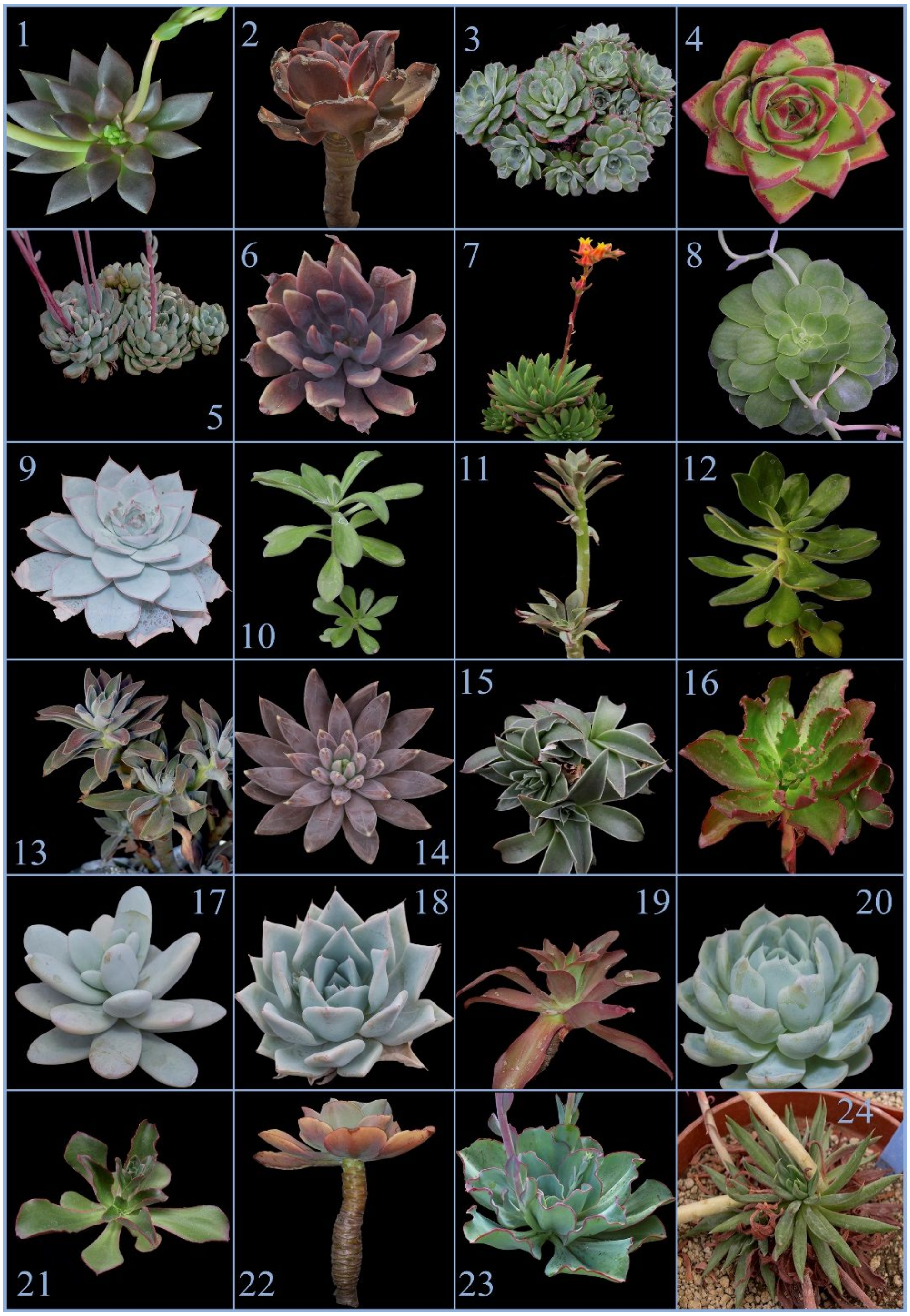

Figure 1b.

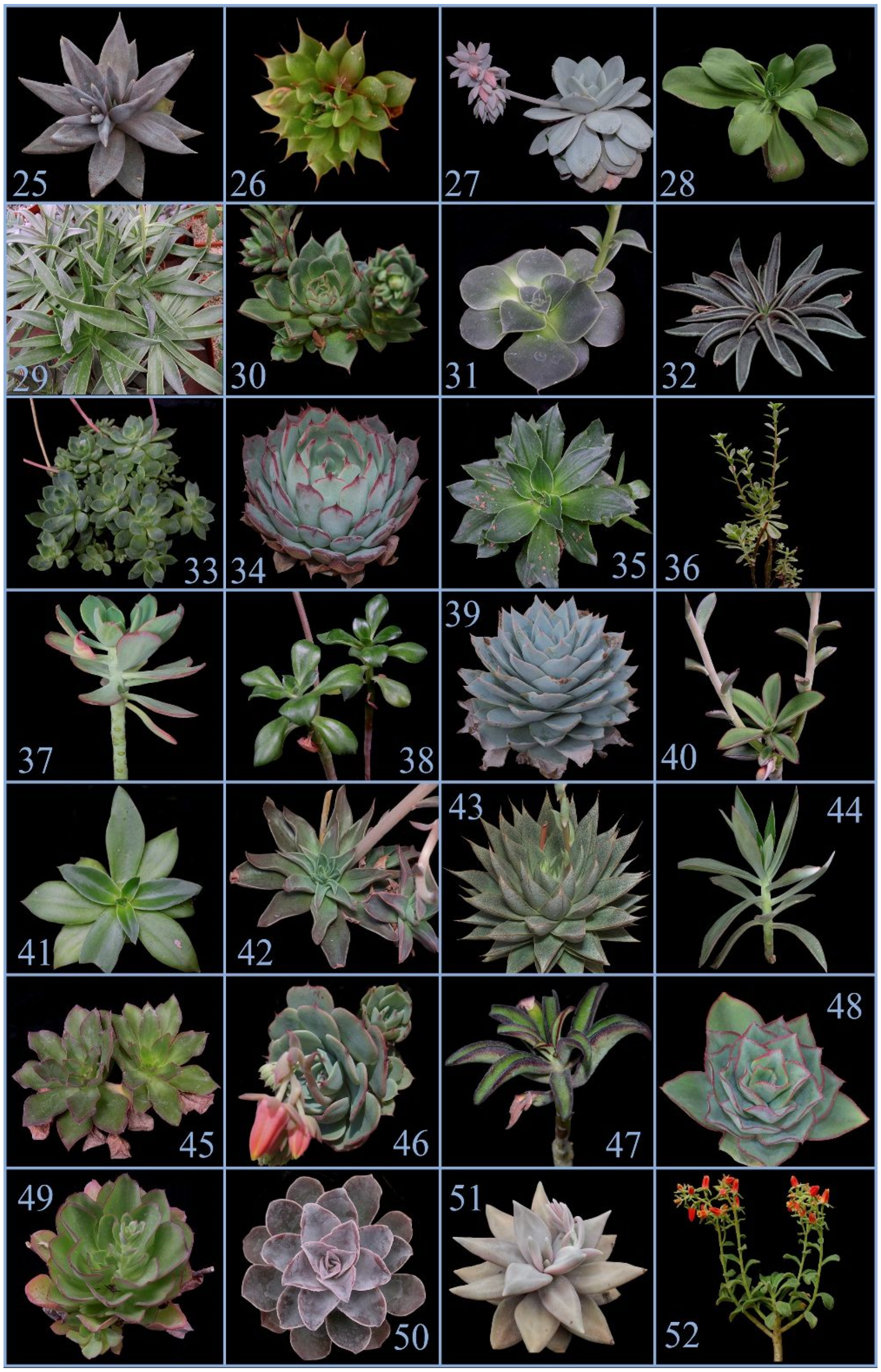
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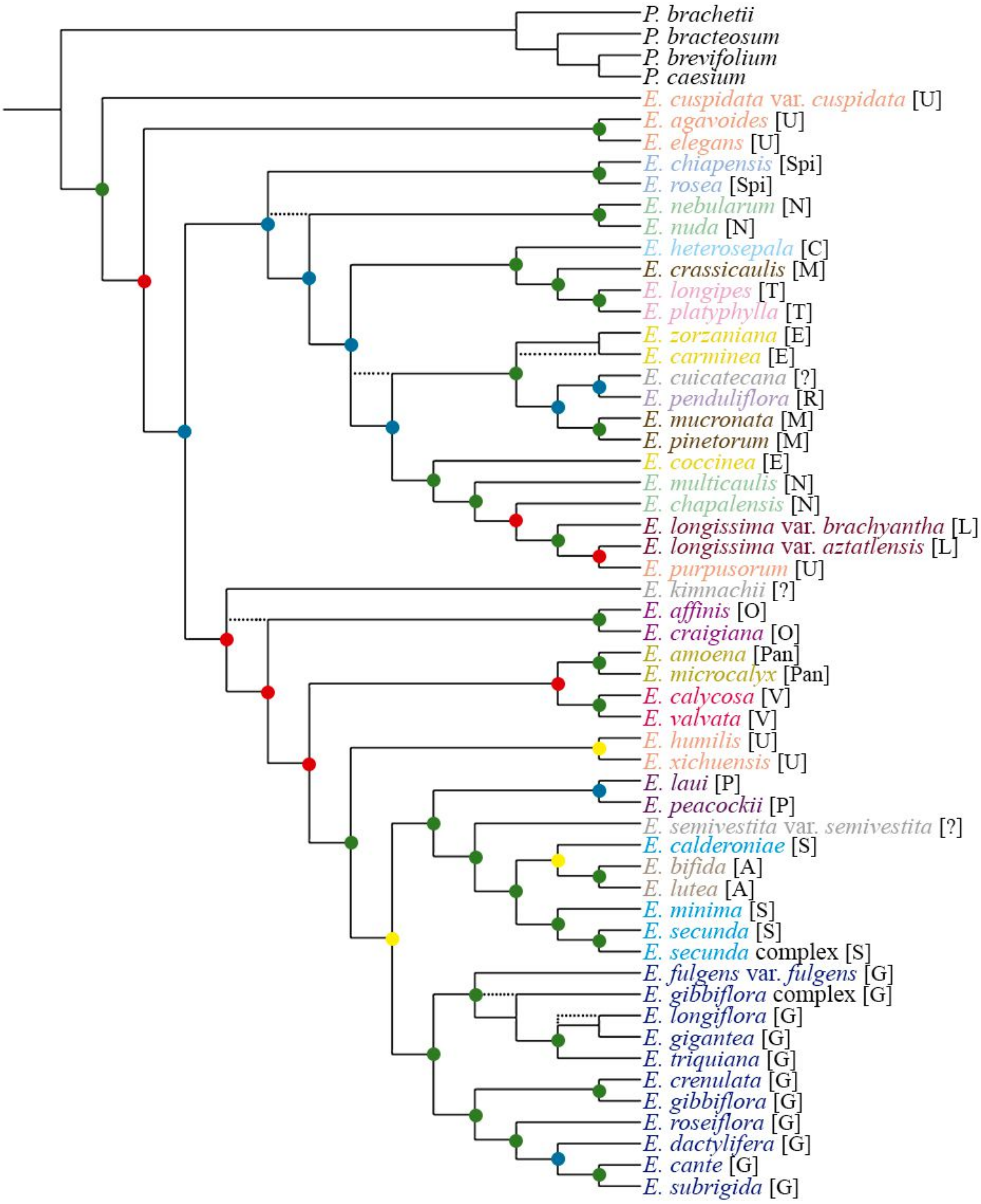
Figure 2.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P. bracteosum
P. brevifolium
P. caesium
E. cuspidata var. cuspidata [U]
E. agavoides [U]
E. elegans [U]
E. chiapensis [Spi]
E. rosea [Spi]
E. nebularum [N]
E. nuda [N]
E. heterosepala [C]
E. crassicaulis [M]
E. longipes [T]
E. platyphylla [T]
E. zorzaniana [E]
E. carminea [E]
E. cuicatecana [?]
E. penduliflora [R]
E. mucronata [M]
E. pinetorum [M]
E. coccinea [E]
E. multicaulis [N]
E. chapalensis [N]
E. longissima var. brachyantha [L]
E. longissima var. aztatlensis [L]
E. purpusorum [U]
E. kimnachii [?]
E. affinis [O]
E. craigiana [O]
E. amoena [Pan]
E. microcalyx [Pan]
E. calycosa [V]
E. valvata [V]
E. humilis [U]
E. xichuensis [U]
E. laui [P]
E. peacockii [P]
E. semivestita var. semivestita [?]
E. calderoniae [S]
E. bifida [A]
E. lutea [A]
E. minima [S]
E. secunda [S]
E. secunda complex [S]
E. fulgens var. fulgens [G]
E. gibbiflora complex [G]
E. longiflora [G]
E. gigantea [G]
E. triquiana [G]
E. crenulata [G]
E. gibbiflora [G]
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E. cante [G]
E. subrigida [G]

Figure 3.

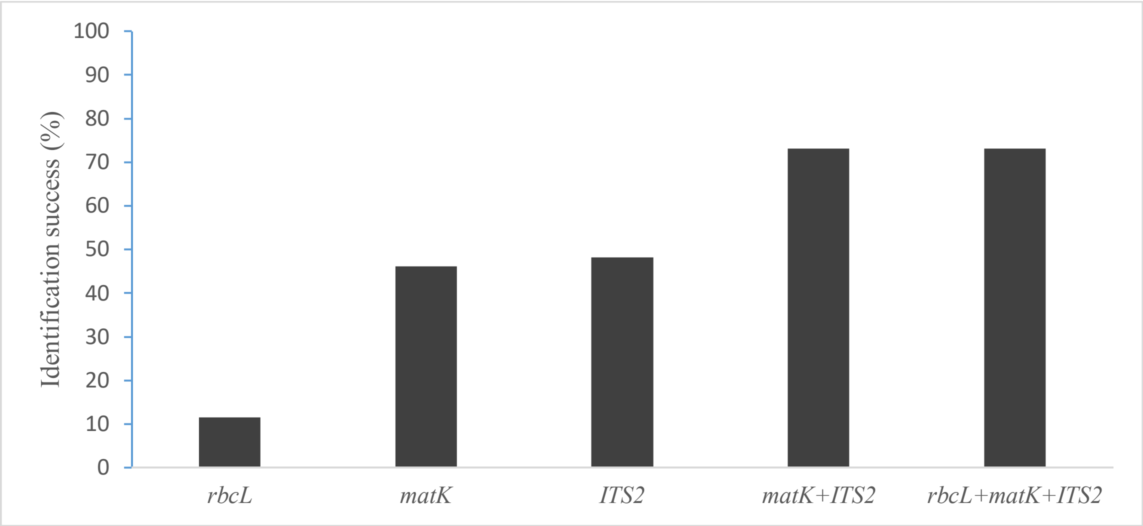

Figure 4.

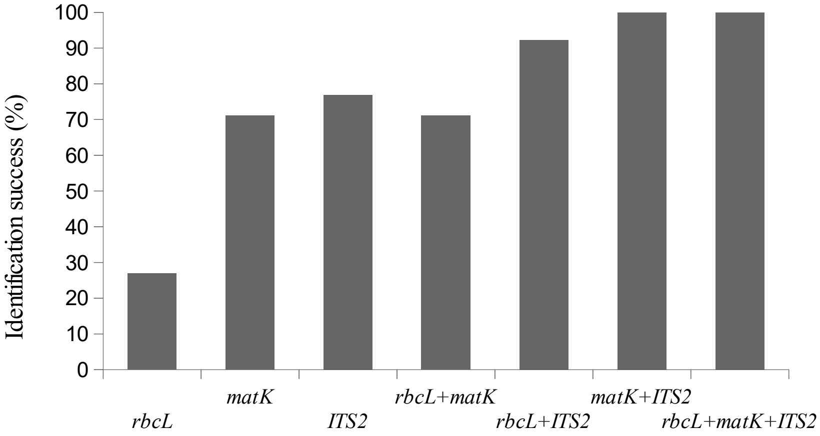

Table 1.

	Taxa	Distribution	Locality
1)	*E. affinis* [O]	Durango	Near Durango City
2)	*E. gibbiflora* complex [G]	Oaxaca	San Vicente del Vado
3)	*E. secunda* complex [S]	Hidalgo	Actopan
4)	*E. agavoides* [U]	Guanajuato	Highway 63 Ahualulco-Charcas
5)	*E. amoena* [Pan]	Puebla	Morelos Ravine
6)	*E. bífida* [A]	Hidalgo	Meztitlán
7)	*E. calderoniae* [S]	Aguascalientes	El Reparo
8)	*E. calycosa* [V]	Michoacán	Salto de Tzararacua
9)	*E. cante* [G]	Zacatecas	km 9 Fresnillo-Sombrerete Highway
10)	*E. carmínea* [E]	Oaxaca	km 35 between Concepción & Tenango
11)	*E. chapalensis* [N]	Michoacán	Saguayo
12)	*E. chiapensis* [Spi]	Oaxaca	El Botado Ravine
13)	*E. coccinea* [E]	Hidalgo	Xicuco Hill
14)	*E. craigiana* [O]	Chihuahua	Between Creel & San Juanito
15)	*E. crassicaulis* [M]	Distrito Federal	Tlalpan
16)	*E. crenulata* [G]	Estado de México	Mexicapa Ravine
17)	*E. cuicatecana* [?]	Oaxaca	San Juan Bautista Cuicatlán
18)	*E. cuspidata* var. *cuspidata* [U]	Nuevo León	El Enebro Ranch
19)	*E. dactylifera* [G]	Sinaloa	Espinazo del Diablo
20)	*E. elegans* [U]	Hidalgo	El Chico National Park
21)	*E. fulgens* var. *fulgens* [G]	Guerrero	Between Taxco & Ixcateopan
22)	*E. gibbiflora* [G]	Estado de México	km 51 Highway to Sultepec
23)	*E. gigantea* [G]	Oaxaca	Between Nduayaco & Texcatitlán
24)	*E. heterosepala* [C]	Oaxaca	Yutanino
25)	*E. humilis* [U]	Hidalgo	El Carrizal Ravine
26)	*E. kimnachii* [?]	Sinaloa	Tacuichamona Sierra
27)	*E. laui* [P]	Oaxaca	Cuicatlán
28)	*E. longiflora* [G]	Guerrero	Between Taxco & Ixcateopan
29)	*E. longipes* [T]	Hidalgo	Between Tula & Mixquiahuala
30)	*E. longissima* var. *aztatlensis* [L]	Oaxaca	San Miguel Aztatla
31)	*E. longissima* var. *brachyantha* [L]	Oaxaca	From Tlaxiaco to San Miguel El Grande
32)	*E. lutea* [A]	San Luis Potosí	Alvarez Sierra
33)	*E. microcalyx* [Pan]	Veracruz	Perote
34)	*E. minima* [S]	Hidalgo	Taxquillo
35)	*E. mucronata* [M]	Oaxaca	Ixtepeji
36)	*E. multicaulis* [N]	Guerrero	Cruz de Ocote
37)	*E. nebularum* [N]	Oaxaca	Comaltepec
38)	*E. nuda* [N]	Veracruz	km 231-232, Highway Orizaba-Puebla
39)	*E. peacockii* [P]	Puebla	Zapotitlán Salinas
40)	*E. penduliflora* [R]	Oaxaca	De la Y River
41)	*E. pinetorum* [M]	Oaxaca	Cuicatlán
42)	*E. platyphylla* [T]	Distrito Federal	Pedregal de San Ángel

43) *E. purpusorum* [U]	Oaxaca	Concepción Buenavista
44) *E. rosea* [Spi]	Veracruz	Between Carpinteros & Huayacocotla
45) *E. roseiflora* [G]	Jalisco	Juanacatlán Lagoon
46) *E. secunda* [S]	Estado de México	San Rafael
47) *E. semivestita* var. *semivestita* [?]	Querétaro	Ahuacatlán
48) *E. subrigida* [G]	San Luis Potosí	San Luis Potosí-Rio Verde Highway
49) *E. triquiana* [G]	Oaxaca	Juxtlahuaca
50) *E. valvata* [V]	Estado de México	Luvianos
51) *E. xichuensis* [U]	Guanajuato	6 km NO from Xichu
52) *E. zorzaniana* [E]	Oaxaca	San Miguel del Valle

Table 2.

Primer	**Sequence ´5 → 3´**	**Program**
rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC	94°C for 4 min; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min; final extension 72°C for 10 min.
rbcLa-R	GTAAAATCAAGTCCACCRCG	
MatK-1RKIM-f	ACCCAGTCCATCTGGAAATCTTGGTTC	94°C for 1 min; 35 cycles of 94°C for 30 sec, 52°C for 20 sec, 72°C for 50 sec; final extension 72°C for 5 min.
MatK-3FKIM-r	CGTACAGTACTTTTGTGTTTACGAG	
ITS2-S2F	ATGCGATACTTGGTGTGAAT	94°C for 5 min; 35 cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 45 sec; final extension 72°C for 10 min.
ITS4	TCCTCCGCTTATTGATATGC	

Table 3.

Species binomial / specimen	Process ID	rbcLa accession no.	ITS2 accession no.	matK accession no.
Echeveria affinis	MKCRA041-08	MG220490	MG217206	MG220546
Echeveria agavoides	MKCRA015-08	MG220440	MG217156	MG220496
Echeveria amoena	MKCRA044-08	MG220454	MG217170	MG220510
Echeveria bifida	MKCRA036-08	MG220474	MG217190	MG220530
Echeveria calderoniae	MKCRA030-08	MG220463	MG217179	MG220519
Echeveria calycosa	MKCRA026-08	MG220489	MG217205	MG220545
Echeveria cante	MKCRA046-08	MG220446	MG217162	MG220502
Echeveria carminea	MKCRA002-08	MG220436	MG217152	MG220492
Echeveria chapalensis	MKCRA005-08	MG220477	MG217193	MG220533
Echeveria chiapensis	MKCRA008-08	MG220439	MG217155	MG220495
Echeveria coccinea	MKCRA001-08	MG220471	MG217187	MG220527
Echeveria craigiana	MKCRA042-08	MG220470	MG217186	MG220526
Echeveria crassicaulis	MKCRA020-08	MG220441	MG217157	MG220497
Echeveria crenulata	MKCRA049-08	MG220469	MG217185	MG220525
Echeveria cuicatecana	MKCRA034-08	MG220468	MG217184	MG220524
Echeveria cuspidata	MKCRA018-08	MG220459	MG217175	MG220515
Echeveria dactylifera	MKCRA047-08	MG220445	MG217161	MG220501
Echeveria elegans	MKCRA014-08	MG220465	MG217181	MG220521
Echeveria fulgens	MKCRA050-08	MG220462	MG217178	MG220518
Echeveria gibbiflora	MKCRA053-08	MG220455	MG217171	MG220511
Echeveria gigantea	MKCRA054-08	MG220478	MG217194	MG220534
Echeveria heterosepala	MKCRA032-08	MG220473	MG217189	MG220529
Echeveria humilis	MKCRA038-08	MG220479	MG217195	MG220535
Echeveria kimnachii	MKCRA043-08	MG220443	MG217159	MG220499
Echeveria laui	MKCRA033-08	MG220456	MG217172	MG220512
Echeveria longiflora	MKCRA052-08	MG220467	MG217183	MG220523
Echeveria longipes	MKCRA040-08	MG220449	MG217165	MG220505
Echeveria longissima var aztatlensis	MKCRA024-08	MG220486	MG217202	MG220542
Echeveria longissima var. nov.	MKCRA025-08	MG220453	MG217169	MG220509
Echeveria lutea	MKCRA037-08	MG220461	MG217177	MG220517
Echeveria microcalyx	MKCRA045-08	MG220460	MG217176	MG220516
Echeveria minima	MKCRA029-08	MG220448	MG217164	MG220504
Echeveria mucronata	MKCRA021-08	MG220482	MG217198	MG220538
Echeveria multicaulis	MKCRA006-08	MG220476	MG217192	MG220532
Echeveria nebularum	MKCRA009-08	MG220485	MG217201	MG220541
Echeveria nuda	MKCRA004-08	MG220437	MG217153	MG220493
Echeveria penduliflora	MKCRA012-08	MG220458	MG217174	MG220514
Echeveria pinetorum	MKCRA019-08	MG220457	MG217173	MG220513
Echeveria platyphylla	MKCRA022-08	MG220491	MG217207	MG220547
Echeveria purpusorum	MKCRA016-08	MG220483	MG217199	MG220539
Echeveria rosea	MKCRA007-08	MG220438	MG217154	MG220494
Echeveria secunda	MKCRA028-08	MG220450	MG217166	MG220506
Echeveria semivestita	MKCRA055-08	MG220472	MG217188	MG220528
Echeveria sp.	MKCRA051-08	MG220488	MG217204	MG220544

Echeveria sp.	MKCRA003-08	MG220444	MG217160	MG220500
Echeveria sp.	MKCRA031-08	MG220452	MG217168	MG220508
Echeveria sp.	MKCRA056-08	MG220442	MG217158	MG220498
Echeveria sp.	MKCRA057-08	MG220487	MG217203	MG220543
Echeveria subrigida	MKCRA048-08	MG220481	MG217197	MG220537
Echeveria subsessilis	MKCRA035-08	MG220480	MG217196	MG220536
Echeveria valvata	MKCRA027-08	MG220466	MG217182	MG220522
Echeveria xichuensis	MKCRA039-08	MG220484	MG217200	MG220540
Pachyphytum brachetii	MKCRA069-08	MG220464	MG217180	MG220520
Pachyphytum bracteosum	MKCRA058-08	MG220447	MG217163	MG220503
Pachyphytum brevifolium	MKCRA073-08	MG220451	MG217167	MG220507
Pachyphytum caesium	MKCRA059-08	MG220475	MG217191	MG220531

Table 4. CAOS-based DNA diagnostics, according to majority-rule consensus guide tree.

	rbcL									*matK*										
Taxa/Position	**54**	**120**	**150**	**192**	**234**	**291**	**294**	**426**	**468**	**43**	**50**	**51**	**60**	**65**	**76**	**84**	**92**	**129**	**162**	**172**
E. affinis	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. aff. *gibbiflora*	C	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. aff. *secunda*	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. agavoides	T	A	T	A	T	C	G	A	T	G	T	A	C	T	G	C	A	A	C	C
E. amoena	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. bifida	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. calderoniae	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. calycosa	T	A	T	A	C	C	G	A	T	G	T	C	C	T	G	T	A	A	C	T
E. cante	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. carminea	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. chapalensis	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. chiapensis	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	T	C
E. coccinea	T	G	T	A	C	C	A	A	T	G	C	C	C	T	G	C	A	A	C	C
E. craigiana	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. crassicaulis	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	C	A	C	C
E. crenulata	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. cuicatecana	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. cuspidata var. *cuspidata*	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. dactylifera	T	A	T	A	C	C	G	A	T	G	C	C	C	G	A	C	A	A	C	C
E. elegans	T	G	T	A	C	C	G	A	C	G	T	C	C	T	G	C	A	A	C	C
E. fulgens var. *fulgens*	T	G	T	A	C	C	G	A	T	G	C	C	T	T	G	C	A	A	C	C
E. gibbiflora	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. gigantea	T	A	C	C	C	C	G	A	T	G	C	C	C	T	G	C	A	G	C	C
E. heterosepala	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. humilis	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. kinmachii	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. laui	T	G	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. longiflora	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. longipes	T	A	T	A	C	T	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. longissima var. *aztatlensis*	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. longissima var. *brachyantha*	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. lutea	T	A	T	A	C	T	G	A	T	G	T	C	C	T	G	C	A	A	C	C
E. microcalyx	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. minima	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. mucronata	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E, multicaulis	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. nebularum	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. nuda	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. peacockii	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. penduliflora	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. pinetorum	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. platyphylla	T	A	T	A	C	T	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. purpusorum	T	A	T	A	C	C	G	G	T	G	C	C	C	T	G	C	A	A	C	C
E. rosea	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. roseiflora	T	A	T	A	C	C	G	A	T	A	C	C	C	T	G	C	A	A	C	C
E. secunda	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. semivestita var. *semivestita*	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. subrigida	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. triquiana	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. valvata	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	T	A	A	C	C
E. xichuensis	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C
E. zorzaniana	T	A	T	A	C	C	G	A	T	G	C	C	C	T	G	C	A	A	C	C

Table 4 (continued).

	matK																			
Taxa/Position	**177**	**185**	**188**	**189**	**198**	**204**	**214**	**246**	**247**	**249**	**264**	**270**	**273**	**291**	**303**	**357**	**359**	**370**	**371**	**372**
E. affinis	G	T	C	C	A	G	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. aff. *gibbiflora*	G	G	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. aff. *secunda*	G	T	C	C	A	T	C	T	A	C	A	C	G	A	A	C	C	-	-	-
E. agavoides	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. amoena	G	T	C	C	A	T	C	T	G	C	C	C	G	A	A	C	C	-	-	-
E. bifida	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	C	C	-	-	-
E. calderoniae	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	C	C	-	-	-
E. calycosa	G	T	C	C	T	T	C	T	G	C	C	C	G	A	A	C	C	-	-	-
E. cante	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. carminea	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. chapalensis	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. chiapensis	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. coccinea	G	T	C	C	A	T	C	G	A	A	A	C	G	G	A	C	C	-	-	-
E. craigiana	G	T	C	C	A	G	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. crassicaulis	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. crenulata	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. cuicatecana	G	T	C	C	A	T	A	T	G	C	C	C	G	G	A	A	C	-	-	-
E. cuspidata var. *cuspidata*	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. dactylifera	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. elegans	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. fulgens var. *fulgens*	G	T	C	C	A	T	C	G	A	A	C	C	G	G	A	C	C	-	-	-
E. gibbiflora	G	T	C	C	A	T	C	T	G	C	C	C	G	G	C	A	C	-	-	-
E. gigantea	G	T	C	A	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. heterosepala	G	T	C	C	A	T	C	T	G	C	C	C	T	G	A	C	C	-	-	-
E. humilis	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. kinmachii	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. laui	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	C	C	-	-	-
E. longiflora	G	T	C	A	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. longipes	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	C	C	-	-	-
E. longissima var. *aztatlensis*	G	T	C	C	A	T	C	T	G	C	C	A	G	G	A	A	C	-	-	-
E. longissima var. *brachyantha*	G	T	C	C	A	T	C	T	G	C	C	A	G	G	A	C	C	-	-	-
E. lutea	G	T	C	C	A	T	C	T	A	C	C	C	G	A	A	C	C	-	-	-
E. microcalyx	G	T	C	C	A	T	C	T	G	C	C	C	G	A	A	C	C	-	-	-
E. minima	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	C	C	-	-	-
E. mucronata	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E, multicaulis	G	T	C	C	A	G	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. nebularum	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. nuda	T	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. peacockii	G	T	C	C	T	T	C	T	A	C	C	C	G	G	A	C	C	-	-	-
E. penduliflora	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. pinetorum	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. platyphylla	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	C	C	-	-	-
E. purpusorum	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. rosea	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. roseiflora	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. secunda	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	A	C	-	-	-
E. semivestita var. *semivestita*	G	T	C	C	A	T	C	T	A	C	C	C	G	G	A	A	A	-	-	-
E. subrigida	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	A	C	-	-	-
E. triquiana	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. valvata	G	T	C	C	A	T	C	T	G	C	C	C	G	A	A	C	C	G	A	A
E. xichuensis	G	T	A	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-
E. zorzaniana	G	T	C	C	A	T	C	T	G	C	C	C	G	G	A	C	C	-	-	-

Table 4 (continued).

	matK																			
Taxa/Position	**373**	**374**	**375**	**376**	**377**	**378**	**379**	**380**	**381**	**382**	**383**	**384**	**385**	**386**	**387**	**388**	**389**	**390**	**415**	**417**
E. affinis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. aff. *gibbiflora*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. aff. *secunda*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. agavoides	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	G
E. amoena	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. bifida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. calderoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. calycosa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. cante	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. carminea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. chapalensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. chiapensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. coccinea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. craigiana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. crassicaulis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. crenulata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. cuicatecana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. cuspidata var. *cuspidata*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. dactylifera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. elegans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. fulgens var. *fulgens*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. gibbiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. gigantea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. heterosepala	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. humilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	A
E. kinmachii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. laui	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. longiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. longipes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. longissima var. *aztatlensis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. longissima var. *brachyantha*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. lutea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. microcalyx	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. minima	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. mucronata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E, multicaulis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. nebularum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. nuda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. peacockii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. penduliflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. pinetorum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. platyphylla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. purpusorum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. rosea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. roseiflora	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. secunda	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. semivestita var. *semivestita*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. subrigida	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. triquiana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. valvata	G	T	G	T	T	T	T	C	T	A	T	T	C	A	A	A	A	A	C	G
E. xichuensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G
E. zorzaniana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	G

Table 4 (continued).

	matK																			
Taxa/Position	**429**	**434**	**435**	**466**	**468**	**486**	**503**	**541**	**564**	**566**	**586**	**595**	**600**	**604**	**606**	**630**	**632**	**664**	**680**	**687**
E. affinis	G	C	G	G	T	C	G	C	C	G	C	C	T	G	C	G	G	T	T	G
E. aff. *gibbiflora*	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. aff. *secunda*	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. agavoides	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. amoena	G	C	G	G	T	C	G	C	C	G	C	C	C	G	A	G	G	T	T	G
E. bifida	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. calderoniae	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. calycosa	G	C	A	G	T	C	G	C	C	G	G	C	C	G	C	G	G	T	T	G
E. cante	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. carminea	G	C	G	G	T	C	G	A	C	G	C	C	C	G	C	G	G	T	T	G
E. chapalensis	G	T	G	G	T	C	G	C	C	G	C	C	T	G	C	G	T	T	T	G
E. chiapensis	G	C	G	A	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. coccinea	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	T	T	T	G
E. craigiana	G	C	G	G	T	C	G	C	C	G	C	C	T	G	C	G	G	T	T	G
E. crassicaulis	G	C	G	G	T	C	G	A	C	G	C	C	C	G	C	G	G	T	T	G
E. crenulata	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. cuicatecana	G	C	G	G	T	T	G	A	C	G	C	C	C	G	C	G	G	T	T	G
E. cuspidata var. *cuspidata*	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. dactylifera	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. elegans	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. fulgens var. *fulgens*	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	T	T	T	G
E. gibbiflora	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. gigantea	G	C	A	G	T	C	G	C	C	G	C	C	C	G	C	G	G	G	T	G
E. heterosepala	G	C	G	G	T	C	G	C	T	G	C	C	C	G	C	G	G	T	T	G
E. humilis	G	C	G	G	T	C	G	C	C	G	C	C	C	A	C	G	G	T	T	G
E. kinmachii	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. laui	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	T	T	T	G
E. longiflora	G	C	A	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	T
E. longipes	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. longissima var. *aztatlensis*	G	T	G	G	T	C	G	C	C	G	C	C	C	G	C	G	T	T	T	G
E. longissima var. *brachyantha*	G	T	G	G	T	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. lutea	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. microcalyx	G	C	G	A	T	C	G	C	C	G	C	C	C	G	A	G	G	T	T	G
E. minima	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. mucronata	G	C	G	G	T	C	G	A	C	G	C	C	C	G	C	G	G	T	T	G
E, multicaulis	C	T	G	G	T	C	G	C	C	G	C	C	C	G	C	G	T	T	T	G
E. nebularum	G	C	G	G	T	C	G	C	C	G	C	T	C	G	C	G	G	T	T	G
E. nuda	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. peacockii	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	T	T	G	G
E. penduliflora	G	C	G	G	T	C	G	A	C	G	C	C	C	G	C	G	G	T	T	G
E. pinetorum	G	C	G	G	T	C	G	A	C	G	C	C	C	G	C	G	G	T	T	G
E. platyphylla	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. purpusorum	G	T	G	G	T	C	G	C	C	G	A	C	C	G	C	G	G	T	T	G
E. rosea	G	C	G	A	T	C	G	C	C	G	C	C	C	G	C	A	G	T	T	G
E. roseiflora	G	C	G	G	T	C	G	C	C	A	C	C	C	G	C	G	G	T	G	G
E. secunda	G	C	G	G	A	C	A	C	C	G	C	C	C	G	C	G	T	T	T	G
E. semivestita var. *semivestita*	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	T	T	T	G
E. subrigida	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. triquiana	G	C	A	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	T	G
E. valvata	G	C	G	G	T	C	G	C	C	G	G	C	C	G	C	G	G	T	T	G
E. xichuensis	G	C	G	G	T	C	G	C	C	G	C	C	C	G	C	G	G	T	G	G
E. zorzaniana	G	C	G	G	T	C	G	A	C	G	C	C	C	G	C	G	G	T	T	G

Table 4 (continued

	matK			ITS2																
Taxa/Position	**705**	**766**	**810**	**46**	**47**	**81**	**100**	**101**	**102**	**112**	**116**	**120**	**121**	**122**	**124**	**125**	**148**	**150**	**160**	**166**
E. affinis	A	C	C	C	G	G	-	-	C	C	A	G	C	G	A	T	C	C	T	A
E. aff. *gibbiflora*	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. aff. *secunda*	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. agavoides	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. amoena	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. bifida	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	G	C	C	T	A
E. calderoniae	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	A	C	T	A
E. calycosa	A	C	C	T	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. cante	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. carminea	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. chapalensis	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. chiapensis	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	A	C	C	T	A
E. coccinea	A	C	C	C	G	G	-	-	T	C	C	G	T	G	A	T	C	C	T	A
E. craigiana	A	C	C	C	G	G	-	-	C	C	A	G	C	G	A	T	C	C	T	A
E. crassicaulis	A	C	C	T	G	G	-	-	C	C	C	G	C	G	A	T	C	C	C	A
E. crenulata	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. cuicatecana	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. cuspidata var. *cuspidata*	A	C	C	C	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. dactylifera	A	C	C	C	A	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. elegans	A	C	A	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. fulgens var. *fulgens*	A	T	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. gibbiflora	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. gigantea	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. heterosepala	A	C	C	C	A	G	-	-	C	A	C	G	C	G	A	T	C	C	T	A
E. humilis	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. kinmachii	A	C	C	T	G	G	-	-	C	C	C	G	C	G	C	T	C	C	T	A
E. laui	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. longiflora	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. longipes	A	C	C	T	G	G	-	-	C	C	C	G	C	G	A	T	C	C	C	A
E. longissima var. *aztatlensis*	A	C	C	C	G	G	-	-	C	C	C	T	T	G	A	T	C	C	T	A
E. longissima var. *brachyantha*	A	C	C	C	G	G	-	-	C	C	C	T	T	G	A	T	C	C	T	A
E. lutea	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	G	C	C	T	A
E. microcalyx	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. minima	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. mucronata	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E, multicaulis	A	C	C	C	G	G	-	-	C	C	C	G	T	G	A	T	C	C	T	A
E. nebularum	A	C	C	T	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. nuda	A	C	C	C	G	A	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. peacockii	A	C	C	C	G	G	-	-	C	C	C	G	A	G	A	T	C	C	T	A
E. penduliflora	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	T	T	A
E. pinetorum	A	C	C	C	G	G	-	-	C	C	C	G	C	A	A	T	C	C	T	A
E. platyphylla	A	C	C	T	G	G	-	-	C	C	C	G	C	G	A	T	C	C	C	A
E. purpusorum	C	C	C	C	G	G	-	-	C	C	C	T	T	G	A	T	C	C	T	A
E. rosea	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	A	C	C	T	A
E. roseiflora	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	T
E. secunda	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. semivestita var. *semivestita*	A	C	C	C	G	G	C	C	C	C	C	G	C	G	A	T	C	C	T	A
E. subrigida	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. triquiana	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. valvata	A	C	C	T	G	G	-	-	C	C	C	G	C	A	A	A	C	C	T	A
E. xichuensis	A	C	C	C	G	G	-	-	C	C	C	G	C	G	A	T	C	C	T	A
E. zorzaniana	A	C	C	C	G	G	-	-	C	C	C	G	G	G	A	T	C	C	T	A

Table 4 (continued).

	ITS2																			
Taxa/Position	**174**	**175**	**179**	**184**	**186**	**188**	**189**	**192**	**197**	**209**	**210**	**213**	**217**	**218**	**219**	**220**	**221**	**226**	**227**	**228**
E. affinis	T	G	C	A	A	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. aff. *gibbiflora*	A	G	C	A	A	A	C	C	T	-	A	A	T	T	T	C	G	C	G	A
E. aff. *secunda*	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	T	G	C	G	G
E. agavoides	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. amoena	T	G	C	A	A	A	C	C	C	-	A	A	C	T	T	T	G	C	A	G
E. bifida	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	T	G	C	G	A
E. calderoniae	C	G	C	A	A	A	C	C	C	-	A	A	C	A	T	T	T	C	T	A
E. calycosa	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	C	G	C	G	A
E. cante	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	A	G	C	G	G
E. carminea	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. chapalensis	C	G	T	A	C	A	C	C	A	-	A	A	C	T	T	C	G	C	G	A
E. chiapensis	C	A	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. coccinea	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	T	G	C	A	G
E. craigiana	T	G	C	A	A	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. crassicaulis	C	G	C	A	C	A	C	C	C	-	A	C	C	T	T	C	G	C	G	G
E. crenulata	C	G	C	A	A	A	C	C	C	-	A	A	T	T	T	C	G	C	G	G
E. cuicatecana	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	C	A
E. cuspidata var. *cuspidata*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	C	G	G
E. dactylifera	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	A	G	C	G	G
E. elegans	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. fulgens var. *fulgens*	A	G	C	A	A	A	C	C	T	-	A	A	T	T	T	C	G	C	G	A
E. gibbiflora	C	G	C	A	A	A	C	C	C	-	A	A	T	T	T	C	G	C	G	G
E. gigantea	A	G	C	A	A	A	C	C	T	-	A	A	T	T	T	C	G	C	G	A
E. heterosepala	C	G	C	A	C	A	C	C	C	-	A	T	C	T	T	C	G	C	G	G
E. humilis	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. kinmachii	C	G	C	A	G	A	C	C	T	-	G	A	C	T	T	C	G	C	G	G
E. laui	C	G	C	A	A	A	C	C	C	A	A	A	C	T	T	C	G	C	G	G
E. longiflora	A	G	C	A	A	A	C	C	T	-	A	A	T	T	T	C	G	C	G	A
E. longipes	C	G	C	A	C	A	C	C	C	-	A	C	C	T	T	C	G	C	G	G
E. longissima var. *aztatlensis*	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	T	G	C	G	A
E. longissima var. *brachyantha*	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	T	G	C	G	A
E. lutea	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	T	G	C	G	A
E. microcalyx	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	C	G	C	A	G
E. minima	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	T	G	C	G	G
E. mucronata	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	G	A
E, multicaulis	C	G	C	A	C	A	C	T	C	-	A	A	C	T	T	C	G	C	G	G
E. nebularum	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	T	G	C	G	G
E. nuda	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	T	G	A
E. peacockii	C	G	C	A	A	G	C	C	C	-	A	A	C	T	G	C	G	C	G	G
E. penduliflora	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	G	A
E. pinetorum	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	G	A
E. platyphylla	C	G	C	A	C	A	T	C	C	-	A	C	C	T	T	C	G	C	G	G
E. purpusorum	C	G	T	A	C	A	C	C	C	-	A	A	C	T	T	T	G	C	A	A
E. rosea	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. roseiflora	T	G	C	A	C	A	C	C	C	-	A	A	T	T	T	A	G	C	G	G
E. secunda	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	T	G	C	G	G
E. semivestita var. *semivestita*	C	G	C	A	A	A	C	C	C	-	A	A	C	T	T	T	G	C	G	G
E. subrigida	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	A	G	C	G	G
E. triquiana	A	G	C	A	A	A	C	C	T	-	A	A	T	T	T	C	G	C	G	A
E. valvata	C	G	C	G	A	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G
E. xichuensis	C	G	C	A	A	A	C	T	C	-	A	A	C	T	T	C	G	C	G	G
E. zorzaniana	C	G	C	A	C	A	C	C	C	-	A	A	C	T	T	C	G	C	G	G

Table 4 (continued).

	ITS2																			
Taxa/Position	**234**	**238**	**239**	**241**	**242**	**243**	**245**	**249**	**250**	**251**	**252**	**253**	**254**	**255**	**256**	**257**	**258**	**261**	**264**	**265**
E. affinis	A	C	A	T	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. aff. *gibbiflora*	A	C	A	C	G	C	A	C	A	C	C	C	G	G	C	C	C	G	C	G
E. aff. *secunda*	A	C	A	C	G	C	A	C	A	C	C	T	G	G	C	C	C	G	C	G
E. agavoides	A	C	G	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. amoena	A	C	A	C	G	C	G	C	G	C	C	C	G	T	T	C	C	G	C	G
E. bifida	A	C	A	C	G	C	G	C	A	C	C	T	G	G	C	T	C	G	C	G
E. calderoniae	A	C	A	C	G	C	G	C	A	C	C	T	G	G	C	C	C	G	C	A
E. calycosa	A	C	A	C	G	C	G	C	A	C	T	C	G	T	C	C	C	G	C	G
E. cante	A	C	A	C	G	C	G	C	A	C	C	C	G	G	T	C	C	G	C	G
E. carminea	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. chapalensis	A	T	A	C	G	T	G	A	G	C	C	C	A	T	C	C	C	A	C	A
E. chiapensis	A	C	A	C	G	C	C	C	A	C	C	C	A	T	C	C	C	G	C	G
E. coccinea	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. craigiana	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. crassicaulis	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	T	C	A	C	G
E. crenulata	A	C	A	C	G	C	G	C	A	C	C	C	G	G	C	C	C	G	C	G
E. cuicatecana	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. cuspidata var. *cuspidata*	A	C	A	C	G	C	G	C	G	C	C	C	A	T	C	C	C	G	C	G
E. dactylifera	A	C	A	C	G	C	G	C	A	C	C	C	G	G	C	C	C	G	C	G
E. elegans	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	-	C	G	C	G
E. fulgens var. *fulgens*	A	C	A	C	G	C	A	C	A	C	C	C	G	G	C	C	C	G	C	G
E. gibbiflora	A	C	A	C	G	C	G	C	A	C	C	C	G	G	C	C	C	G	C	G
E. gigantea	A	C	A	C	G	C	A	C	A	C	C	C	G	G	C	C	C	G	C	G
E. heterosepala	A	C	A	C	G	C	G	C	A	C	C	C	T	T	C	T	C	A	C	G
E. humilis	A	C	A	C	G	C	G	C	A	C	C	C	G	G	C	C	C	G	C	G
E. kinmachii	A	C	A	C	T	C	G	C	G	C	C	C	A	T	C	C	C	G	C	G
E. laui	A	C	A	C	G	C	G	C	A	T	C	T	T	G	C	C	C	G	C	G
E. longiflora	A	C	A	C	G	C	A	N	A	C	C	C	G	G	C	C	C	G	C	G
E. longipes	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	T	C	A	C	G
E. longissima var. *aztatlensis*	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. longissima var. *brachyantha*	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. lutea	A	C	A	C	G	C	G	C	A	C	C	T	G	G	C	T	C	G	C	G
E. microcalyx	A	C	A	C	G	C	G	C	G	C	C	C	G	T	T	C	C	G	C	G
E. minima	A	C	A	C	G	C	G	C	A	C	C	T	G	G	C	C	C	G	C	G
E. mucronata	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E, multicaulis	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. nebularum	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	A	C	G
E. nuda	T	C	A	C	A	C	G	C	A	C	C	C	A	T	C	C	C	A	C	G
E. peacockii	A	C	A	C	G	C	G	C	A	C	C	C	G	A	C	C	C	G	C	G
E. penduliflora	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	T	G
E. pinetorum	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. platyphylla	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	T	C	A	C	G
E. purpusorum	A	C	A	C	G	T	G	C	A	C	C	C	A	T	C	C	C	G	C	G
E. rosea	A	C	A	C	G	C	C	C	A	C	C	C	A	T	C	C	C	G	C	G
E. roseiflora	A	C	A	C	G	C	G	C	A	C	C	C	G	G	C	C	C	G	C	G
E. secunda	A	C	A	C	G	C	A	C	A	C	C	T	G	G	C	C	C	G	C	G
E. semivestita var. *semivestita*	A	C	A	C	G	C	G	C	A	C	C	T	G	G	C	C	C	G	C	G
E. subrigida	A	C	A	C	G	C	G	C	A	C	C	C	G	G	C	C	C	G	C	G
E. triquiana	A	C	A	C	G	C	A	C	A	C	C	C	G	G	C	C	T	G	C	G
E. valvata	A	C	A	T	G	T	G	C	A	C	C	G	G	T	C	C	C	G	C	G
E. xichuensis	A	C	A	C	G	C	G	C	A	C	C	C	G	G	C	C	C	G	C	A
E. zorzaniana	A	C	A	C	G	C	G	C	A	C	C	C	A	T	C	N	C	G	C	G

Table 4 (continued).

	ITS2																			
Taxa/Position	**267**	**268**	**274**	**276**	**282**	**284**	**285**	**286**	**287**	**288**	**290**	**292**	**293**	**295**	**296**	**298**	**300**	**301**	**329**	**378**
E. affinis	G	A	C	T	G	-	-	T	C	G	T	C	T	C	G	C	C	C	C	G
E. aff. *gibbiflora*	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. aff. *secunda*	A	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. agavoides	G	A	C	T	G	-	-	T	C	G	T	C	T	C	G	C	C	C	C	G
E. amoena	G	A	C	T	G	G	C	G	C	G	T	C	T	C	G	C	C	C	C	A
E. bifida	A	A	C	A	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. calderoniae	A	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. calycosa	G	A	C	T	G	-	-	G	T	G	T	C	T	C	G	T	C	C	C	G
E. cante	A	A	T	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. carminea	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. chapalensis	G	A	C	T	G	-	-	G	T	G	T	C	C	C	G	T	T	T	C	G
E. chiapensis	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. coccinea	G	A	C	T	G	-	-	G	C	A	T	C	T	C	G	C	C	C	C	G
E. craigiana	G	A	C	T	G	-	-	T	C	G	T	C	T	C	G	C	C	C	C	G
E. crassicaulis	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. crenulata	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. cuicatecana	A	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	T	C	C	G
E. cuspidata var. *cuspidata*	A	A	C	T	G	-	-	T	C	G	T	C	T	T	G	C	C	C	C	G
E. dactylifera	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. elegans	G	A	C	T	G	-	-	G	C	G	C	C	T	C	G	C	C	C	C	G
E. fulgens var. *fulgens*	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. gibbiflora	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. gigantea	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. heterosepala	G	A	C	T	G	-	-	G	T	G	T	C	T	C	G	C	C	C	C	G
E. humilis	G	A	C	T	G	-	-	G	C	G	T	T	T	C	G	C	C	C	C	G
E. kinmachii	A	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. laui	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	T	C	G
E. longiflora	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. longipes	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. longissima var. *aztatlensis*	G	A	C	T	G	-	-	G	C	G	T	T	T	C	G	T	C	T	C	G
E. longissima var. *brachyantha*	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	T	C	T	C	G
E. lutea	A	A	C	A	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. microcalyx	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	A
E. minima	A	A	C	T	G	-	-	G	C	G	T	A	T	C	A	C	C	C	C	G
E. mucronata	G	A	C	T	G	-	-	G	C	G	T	T	T	C	G	C	C	C	T	G
E, multicaulis	G	A	C	T	T	-	-	G	C	G	T	T	T	C	G	T	C	C	C	G
E. nebularum	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. nuda	G	A	C	T	A	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. peacockii	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	T	C	G
E. penduliflora	A	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. pinetorum	G	A	C	T	G	-	-	G	C	G	T	T	T	C	G	C	C	C	C	G
E. platyphylla	G	A	C	T	G	-	-	G	T	G	T	C	T	C	G	C	C	C	C	G
E. purpusorum	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. rosea	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. roseiflora	G	A	C	T	G	-	-	G	C	G	T	A	T	C	G	T	C	C	C	G
E. secunda	A	A	C	T	G	-	-	G	C	G	T	A	T	C	G	C	C	C	C	G
E. semivestita var. *semivestita*	A	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	T	C	A
E. subrigida	A	A	T	T	G	-	-	G	C	G	T	A	T	C	G	T	C	C	C	G
E. triquiana	G	A	C	T	G	-	-	G	T	G	T	A	T	C	G	C	C	C	C	G
E. valvata	-	G	C	T	G	-	-	G	C	G	T	C	T	T	G	T	C	C	C	G
E. xichuensis	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G
E. zorzaniana	G	A	C	T	G	-	-	G	C	G	T	C	T	C	G	C	C	C	C	G

Table 4 (continued).

	ITS2										
Taxa/Position	**398**	**407**	**434**	**435**	**438**	**439**	**440**	**443**	**444**	**446**	**447**
E. affinis	C	C	C	G	T	A	C	C	G	C	C
E. aff. *gibbiflora*	C	C	T	G	T	T	C	C	G	C	C
E. aff. *secunda*	C	C	T	G	T	C	C	C	G	C	C
E. agavoides	C	C	C	G	T	C	C	C	G	C	C
E. amoena	C	C	C	G	T	T	C	C	G	C	C
E. bifida	C	C	T	G	T	C	C	C	G	C	C
E. calderoniae	C	C	T	G	T	C	C	C	G	C	C
E. calycosa	C	T	C	G	T	C	T	C	G	C	C
E. cante	C	C	T	G	T	T	C	C	G	C	C
E. carminea	C	C	C	G	T	C	C	C	G	C	C
E. chapalensis	C	C	T	G	T	A	C	C	G	C	C
E. chiapensis	C	C	C	G	T	C	C	C	G	C	C
E. coccinea	C	C	C	G	T	C	T	C	G	T	C
E. craigiana	C	C	C	G	T	A	C	C	G	C	C
E. crassicaulis	C	C	C	G	T	C	C	C	G	C	C
E. crenulata	C	C	T	G	T	T	C	C	G	C	C
E. cuicatecana	C	C	C	A	T	C	C	C	G	C	C
E. cuspidata var. *cuspidata*	G	C	C	G	T	C	C	C	T	C	C
E. dactylifera	C	C	T	G	T	T	C	C	G	C	C
E. elegans	C	C	C	G	T	C	C	C	G	C	C
E. fulgens var. *fulgens*	C	C	T	G	T	T	C	C	G	C	C
E. gibbiflora	C	C	-	-	-	-	-	-	-	-	-
E. gigantea	C	C	T	G	T	T	C	C	G	C	C
E. heterosepala	C	C	C	G	T	T	C	C	G	C	T
E. humilis	C	C	T	G	T	C	C	C	T	C	C
E. kinmachii	C	C	C	G	T	C	C	C	G	C	C
E. laui	C	C	T	G	T	C	C	C	T	C	C
E. longiflora	C	C	T	G	T	T	C	C	G	C	C
E. longipes	C	C	C	G	T	C	C	C	G	C	C
E. longissima var. *aztatlensis*	C	C	C	G	T	C	C	C	G	C	C
E. longissima var. *brachyantha*	C	C	C	G	T	T	C	C	G	C	C
E. lutea	C	C	T	G	T	C	C	C	G	C	C
E. microcalyx	C	C	C	G	T	T	C	C	G	C	C
E. minima	C	C	T	G	T	T	T	C	G	C	C
E. mucronata	C	C	C	G	T	C	C	C	G	C	C
E, multicaulis	C	T	C	A	T	C	C	T	G	C	C
E. nebularum	C	C	C	G	T	C	C	C	G	C	C
E. nuda	C	C	T	G	T	C	C	C	G	C	C
E. peacockii	C	C	T	G	A	T	C	C	T	C	C
E. penduliflora	C	C	C	G	T	C	C	C	G	C	C
E. pinetorum	C	C	C	G	T	C	C	C	G	C	C
E. platyphylla	C	C	C	G	T	C	C	C	G	C	C
E. purpusorum	C	C	C	G	T	C	C	C	G	C	C
E. rosea	C	C	C	G	T	C	C	C	G	C	C
E. roseiflora	C	C	T	G	T	T	C	C	G	C	C
E. secunda	C	C	T	G	T	C	C	C	G	C	C
E. semivestita var. *semivestita*	C	C	T	G	T	T	C	C	G	C	C
E. subrigida	C	C	T	G	T	T	C	C	A	C	C
E. triquiana	C	C	T	G	T	T	C	C	G	C	C
E. valvata	C	T	T	G	T	C	T	C	A	C	C
E. xichuensis	C	C	T	G	T	C	C	C	T	C	C
E. zorzaniana	C	C	-	-	-	-	-	-	-	-	-

Capítulo 2. Relaciones filogenéticas de las *Echeveria* mexicanas

Título: Phylogenetic relationships of *Echeveria* (Crassulaceae) and related genera from Mexico, based on three DNA barcoding loci

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Publicado en: *Phytotaxa* 422 (1): 33-57.

URL: https://doi.org/10.11646/phytotaxa.422.1.3

Phytotaxa 422 (1): 033–057
https://www.mapress.com/j/pt/
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Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.422.1.3

Phylogenetic relationships of *Echeveria* (Crassulaceae) and related genera from Mexico, based on three DNA barcoding loci

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Abstract

Mexico is considered as diversification and endemism center of the genus *Echeveria*. Previous tree inference studies have shown the genus to be non-monophyletic in relation to other genera, but sampling for *Echeveria* has been poor, and has not allowed to understand the relationships within the genus. In this work, Bayesian and maximum likelihood phylogenetic analysis were performed, using a combined standard DNA barcoding loci matrix (*rbcL*, *matK* and ITS2) and GenBank ITS2 accessions for *Graptopetalum*, some *Sedum* and few *Echeveria* taxa. The selection of taxa encompasses all current infrageneric categories of the genus *Echeveria*, as well as species from genera previously associated with it. The inferred consensus trees suggested that *Echeveria* is paraphyletic. Instead, four main clades were retrieved within the "Echeveria group": Clade I includes exclusively *Pachyphytum* species. Clade II is formed by the majority of series *Urbiniae*; Clade III encompasses series *Chloranthae*, *Ciliatae*, *Echeveria*, *Mucronatae*, *Nudae*, *Racemosae*, *Thyrsiflorae*, *Pachyphytum cuicatecananum* and *Thompsonella*; Clade IV contains series *Angulatae*, *Gibbiflorae*, *Occidentales*, *Pruinosae*, *Secundae*, some *Urbiniae* species, *Valvatae*. genera *Cremnophila*, *Graptopetalum* and *Reidmorania*. *Pachyphytum* and *Thompsonella* were retrieved as monophyletic groups, but the first outside *Echeveria* while the latter inside. Results of our study suggest that *Echeveria* and the Echeveria Group require a redefinition.

Key words: morphological character, ornamental plant, synapomorphy, succulents

Introduction

Taxa belonging to the genus *Echeveria* DC. consist of perennial herbs with succulent leaves. The beauty of their rosettes and flowers places them as highly ornamental plants; in fact, they are cultivated for this purpose in Mexico and in other countries. The genus is distributed from the southern United States (Texas) to northern Argentina; however, Mexico concentrates about 83% of the genus diversity. These species can be found in semi-arid, temperate or subtropical regions along a wide elevation gradient and living very frequently on bare rocky formations.

Currently, 17 infrageneric groups are recognized: ser. *Angulatae* E.Walther is characterized by its pentagonal flowers, with keeled petals and by its scorpioid inflorescences; ser. *Chloranthae* Moran is recognized by its greenish flowers; ser. *Ciliatae* Moran groups plants with variable degree of ciliate leaves, inflorescences and flowers; ser. *Echeveria* E.Walther contains plants with medium to tall stems, with hairy organs (stems, leaves, inflorescences and flowers); ser. *Gibbiflorae* (Baker) A.Berger is characterized by its flat and wide leaves, paniculate-cymose inflorescences and pentagonal flowers; ser. *Longistylae* E.Walther groups plants with 1 to 2 cincinni, long corollas and styles; ser. *Mucronatae* E.Walther with tuberous roots, spicate to subracemose inflorescences; ser. *Nudae* E.Walther with caulescent or acaulescent plants, spicate to racemose inflorescences and large flowers (20–25 mm);

Accepted by Marie-Stéphanie Samain: 2 Oct. 2019; published: 21 Oct. 2019

ser. *Occidentales* Moran is recognized by its cymose-paniculate inflorescences and thin red petals; ser. *Paniculatae* A.Berger with cymose-paniculate inflorescences and thin yellow petals; ser. *Pruinosae* E.Walther consist of plants with very farinaceous organs (leaves, inflorescences and flowers); ser. *Racemosae* (Baker) Moran is characterized by its racemose inflorescences; ser. *Secundae* (Baker) A.Berger with acaulescent plants, cincinni inflorescences and keeled petals; ser. *Spicatae* (Baker) A.Berger is recognized by its dense spikes or raceme inflorescences and sepals often longer than the corolla; ser. *Thyrsiflorae* Moran groups plants with tuberous roots and thyrsoid or subracemose and determinate inflorescences; ser. *Urbiniae* E.Walther with cincinni inflorescences, urceolate corollas and round side petals; and ser. *Valvatae* Moran with cincinni inflorescences and valvate petals (Walther 1972; Pilbeam 2008).

The systematics and taxonomy of *Echeveria* and Crassulaceae J.St.-Hil. have been historically difficult because of intrinsic factors such as high homoplasy exhibited in morphological characters (Gontcharova & Gontcharov 2007), causing the current classification—as well as infrageneric sections of *Graptopetalum* Rose, *Pachyphytum* Link, Klotzsch & Otto and *Sedum* L.—to be perceived as artificial. Additionally, polyploidy (more than 50 different chromosomes numbers; Uhl 1992) is registered in *Echeveria,* increasing its taxonomic complexity. For some series, certain karyological patterns are observed, so for example, ser. *Angulatae* has haploid chromosomes numbers = 12, but numbers = 13 and 14 can also be found. For ser. *Gibbiflorae* the basic haploid number is = 27, but few species display numbers = 17, 28, 54, 81, 135, 108, 162 and 189 (Pilbeam 2008). The remaining 15 series own diverse chromosome numbers with no evident pattern.

Despite its species richness, high degree of endemism, and highly ornamental potential, there is an absence of phylogenetic framework—mainly due to scarcity of molecular information for many species—to contribute to the understanding of the relationships for the approximately 193 taxa, including species, subspecies, varieties and forms. Even *Echeveria* has been recovered in few preceding molecular phylogenetic analyses as paraphyletic nested with *Cremnophila* Rose, *Graptopetalum*, *Thompsonella* Britton & Rose and some representatives of sect. *Pachysedum* A.Berger from *Sedum* (van Ham & ’t Hart 1998; Mort *et al*. 2001; Acevedo-Rosas *et al*. 2004; Carrillo-Reyes *et al*. 2008; Carrillo-Reyes *et al*. 2009; Nikulin *et al*. 2016).

Previous phylogenetic studies on the former aggregate so-called “Echeveria group” (Thiede & Eggli 2007; Carrillo-Reyes *et al*. 2009) have sampled *Echeveria* very poorly, despite it being the most species-rich genus in the aggregate, therefore limiting the comprehension of the relationships within the genus, and its affinities with other Crassulaceae genera distributed in Mexico. These few prior analyses have shown that the majority of Crassulaceae from Mexico belong to the *Acre* clade—genus *Dudleya* Britton & Rose being part of the *Leucosedum* Fourr. clade—(Carrillo-Reyes *et al*. 2009) and that the “Villadia Rose group” is the sister clade of the “Echeveria group”.

In the present work, we explore the phylogenetic relationships for a more extensive sampling of the Mexican Crassulaceae taxa, focusing mainly on *Echeveria*, employing three standard DNA barcoding loci data generated in collaboration between the Botanical Garden of the Biology Institute of the National Autonomous University of Mexico (UNAM) and the Canadian Centre for DNA Barcoding (CCDB) also targeting the development of a DNA barcode reference library for Mexican *Echeveria*. Our main goals were to identify monophyletic clades within the “Echeveria group” and contrast them with current genera and infrageneric definitions. Moreover, we discuss briefly novel phylogenetic positions and morphological characters useful to circumscribe the resulting clades.

Materials and methods

Plant material

Plant material for this study was taken from the “Colección Nacional de Crasuláceas” (National Crassulaceae Collection), a facility of field collected living plants kept in greenhouses for their study, belonging to the Biology Institute of the National Autonomous University of Mexico (UNAM). Most of them have been collected by J. Reyes Santiago (Collection Curator) as well as Julia Etter and Martin Kristen. The nomenclature of the taxa is according to Kimnach (in Eggli 2003). Localities, field collection numbers, BOLD systems ID´s and GenBank accession numbers for this material are listed in Appendix 1.

Taxa sampled

Phylogenetic analysis included accessions for 226 taxa. The distribution of accessions was organized as follows: *Cremnophila* (6), *Echeveria* (153), *Graptopetalum* (20), *Pachyphytum* (22), *Sedum* (7), *Reidmorania* Kimnach (1), *Tacitus* Moran (1) and *Thompsonella* (10); as out-groups: *Dudleya* (2), *Lenophyllum* Rose (2) and *Villadia* (2); being

Dudleya the outer one. For the case of *Echeveria*, the sample corresponds to all 17 series in the current generic classification (Kimnach in Eggli 2003) and represents approximately 62.63% of the genus and 77.78% of the Mexican *Echeveria* species. We selected few specimens which are considered as synonymous of *E. amoena* De Smet ex É.Morren (*E. microcalyx* Britton &Rose), *E. bifida* Schltdl. (*E. bifurcata* Rose), *E. mucronata* Schltdl. (*E. crassicaulis* E.Walther), *E. paniculata* var. *maculata* Rose (Kimnach) (*E. longipes* E.Walther), *E. racemosa* Cham. & Schltdl. (*E. lurida* Haw.) and *E. rosea* Lindl. (*E. chiapensis* Rose ex Poelln.). Some species have not been currently placed into series, so they were labeled as uncertae sedis.

DNA extraction, amplification and sequencing

DNA fragments were sequenced in the Canadian Centre for DNA Barcoding (CCDB) facilities in Guelph, Canada, employing the following protocol: genomic DNA was extracted from silica-gel dried tissue using 2X CTAB protocol (standard in CCDB). DNA fragment amplifications were carried out using Platinum® Taq DNA Polymerase (Ivanova *et al*. 2006). The three pairs of primers for the selected DNA regions and the PCR thermocycler program used according to CCDB standards are resumed in Table 1. For *rbcL* and ITS2 sequences, amplification was performed in 12.5 µl final volumes with 6.25 µl of 10% Trehalose. For *matK*, 1.875 µl of 20% Trehalose was used. PCR products were visualized in pre-cast agarose E-gel® 96 from Invitrogen. Contig assembling and sequence edition was carried out in Geneious v.9 (http://www.geneious.com, Kearse *et al*. 2012). Sequences were uploaded to BOLD Systems database for further applications such as, reference library for DNA barcoding purposes.

TABLE 1. Primer sequence and thermocycler program for DNA fragments amplification.

Primer	**Sequence ´5 → 3´**	**Program**
rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC	94°C for 4 min; 35 cycles of 94°C for 30 secs, 55°C for 30 secs, 72°C for 1 min; final extension 72°C for 10 min.
rbcLa-R	GTAAAATCAAGTCCACCRCG	
MatK-1RKIM-f	ACCCAGTCCATCTGGAAATCTTGGTTC	94°C for 1 min; 35 cycles of 94°C for 30 secs, 52°C for 20 secs, 72°C for 50 secs; final extension 72°C for 5 min.
MatK-3FKIM-r	CGTACAGTACTTTTGTGTTTACGAG	
ITS2-S2F	ATGCGATACTTGGTGTGAAT	94°C for 5 min; 35 cycles of 94°C for 30 secs, 56°C for 30 secs, 72°C for 45 secs; final extension 72°C for 10 min.
ITS4	TCCTCCGCTTATTGATATGC	

Phylogenetic relationships reconstruction

Partial sequences of plasmid coding regions (*matK* and *rbcL*) as well as a noncoding nrDNA loci (ITS2) were included. The aligned sequences of each molecular marker were downloaded from the BOLD systems (Ratnasingham & Hebert 2007) database (Barcode of Life Data Systems. http://www.boldsystems.org) generated during the "Crassulaceae in Mexico" and "Crassulaceae in Mexico 2" (Appendix 1), where the authors of the manuscript are involved. Following minimal manual realignment edition, sequences of each molecular marker were concatenated in Mesquite v2.5 (Maddison & Maddison 2008) to obtain an expanded matrix of 1800 bp in length.

From the expanded matrix, a maximum likelihood (ML) analysis was performed on W-IQ-TREE through online implementation (Trifinopoulos *et al*. 2016). The alignment FASTA file was submitted to http://iqtree.cibiv.univie.ac.at/ with a partition file. The alignment matrix in NEXUS format was deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S23873). The evolution model was determinated automatically (Chernomor *et al*. 2016) selecting the auto detect box, resulting the following molecular evolution models: F81 (*rbcL* 1st), JC+I (*rbcL* 2nd), HKY+I (*rbcL* 3rd), TPM3u+R2 (*matK* 1st), K3Pu+R2 (*matK* 2nd and *matK*3rd) and TVMe+R3 (ITS2). For bootstrap (BS) analysis, we choose the ultrafast approximation (Minh *et al*. 2013) with 4,000 replicates. Finally, the 50% majority-rule consensus tree was used in the analysis.

Moreover, a Bayesian inference-based (BI) reconstruction for the same dataset was analyzed with MrBayes v3.2 (Ronquist *et al*. 2012). The choice of molecular evolution model and better partition scheme were obtained by PartitionFinder v1.1.1 (Lanfear *et al*. 2012) under the Bayesian Information Criterion (BIC), resulting the schemes: GTR+I+G = (*matK* and r*bcL* 3rd); Jukes-Cantor = (*rbcL* 1st and *rbcL* 2nd); SYM+I+G = (ITS2). Considering the adjustments required for the nucleotide substitution models, two independent runs of Markov Monte Carlo analysis was performed for 4,000,000 generations, using one cold and three hot chains sampled every 4,000 generations. Chain sampling and effective sampling sizes (ESS>300) were visualized and analyzed using Tracer v1.7 (Rambaut *et al*. 2018). The first 25% of the trees saved was discarded and the 50% majority-rule consensus tree without branch lengths was used to discuss relationships.

The maximum likelihood W-IQ-TREE consensus tree showed greater congruence with the observed species morphology, as well as with previous phylogenetic trees (Carrillo-Reyes *et al*. 2009; Vázquez-Cotero *et al*. 2017), comparted to the Bayesian inference consensus tree so, the ML tree was selected to show phylogenetic relationships of the Echeveria group. Node supports from the resulting ML and support for equivalent clades on BI consensus trees were combined and edited in Tree Graph2 v2.14.0-771 (Stöver & Müller 2010). The tree was edited in PhotoShop CS6 to fit the journal printsize.

Results

General relationships

The general consensus of two phylogenetic reconstruction criteria (ML and BI) shows four main clades within the "Echeveria group" with consistently high node supports from Bootstrap (BS) and/or Posterior Probabilities (PP). **Clade I** is exclusively composed by *Pachyphytum* species. **Clade II** clusters the majority of *Urbiniae* species from genus *Echeveria*. **Clade III** includes the following series from genus *Echeveria*: *Chloranthae*, *Ciliatae*, *Echeveria*, *Echeveria purpusorum* A.Berger from *Urbiniae*, *Longistylae, Mucronatae*, *Nudae*, *Racemosae*, *Spicatae* and *Thyrsiflorae*. Clade III contains also *Pachyphytum cuicatecanum* (J.Reyes, Joel Pérez & Brachet) Kimnach, and the genus *Thompsonella*. **Clade IV** groups the rest of *Echeveria* series: *Angulatae*, *Gibbiflorae*, *Occidentales*, *Paniculatae*, *Pruinosae*, *Secundae*, some species of *Urbiniae*, the genera *Cremnophila*, *Graptopetalum*, *Reidmorania* and *Tacitus*, *E. guerrerensis* J.Reyes, Brachet & O.González from series *Urbiniae*, *E. uxorium* Jimeno-Sevilla & Cházaro from series *Racemosae*, and *Sedum clavatum* R.T.Clausen; the latter species and *Graptopetalum* only from ITS2 GenBank data (Fig. 1).

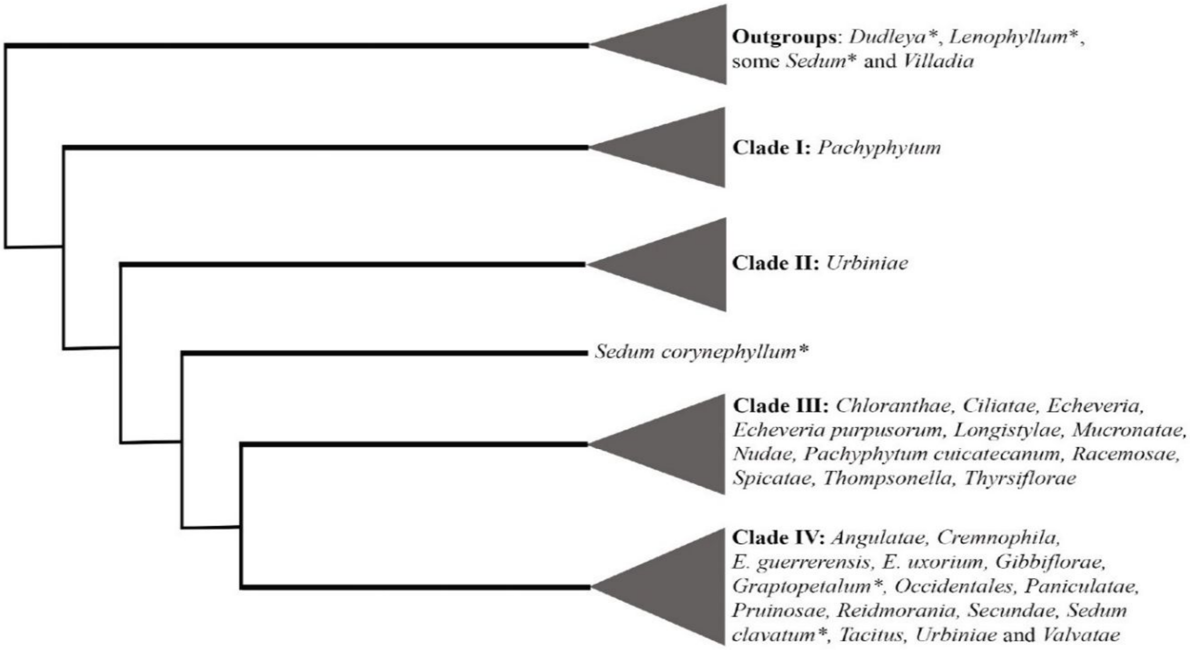

FIGURE 1. Simplified version of the maximum likelihood and Bayesian inference 50% majority-rule consensus trees for 226 terminals of combined dataset. Asterisk indicates groups/species with only ITS2 locus data from GenBank.

Detailed relationships

Clade I: This clade exclusively consists of *Pachyphytum* species (Fig. 2A**)** and is recovered with high support in both ML and BI reconstruction criteria (BS = 100, PP = 0.99, respectively). Within this clade uncertain relationships were found and shallow relationships within the genus were not solved, except for *P. fittkaui* Moran as sister species to *P. kimnachii* Moran. The node support for the former relationship is high (BS = 100 for ML consensus and PP = 1 for BI).

Clade II: Three main clades link *Echeveria* accessions, one of them exclusively consisting of ser. *Urbiniae* species. The type species of this series (*E. agavoides* Lem.) is nested in this clade, which is highly supported in both ML and BI analyses (BS =96 PP = 0.88, respectively Fig. 2A). The remaining resulting relationships range from not to high supported (BS = 54–100%) and show three subclades. The first one is composed by *E. cuspidata* var. *cuspidata* (Rose) Kimnach, *E. cuspidata* var. *zaragozae* (Rose) Kimnach, *E. chihuahuensis* von Poellnitz, *E. lilacina* Kimnach & Moran and *E. unguiculata* Kimnach is not supported in ML (BS = 54) and not retrieved by BI analysis (Fig. 2A). The second one includes *E. pulidonis* E.Walther, *E. elegans* Rose, *E. potosina* E.Walther, *E. halbingeri* var. *halbingeri* (E.Walther) Kimnach and *E. simulans* Rose and is well-supported (BS = 100, PP = 0.99). The last subclade contains *E. juliana* J.Reyes, O.González & Kristen, *E. tobarensis* A.Berger, *E. turgida* Rose, *E. tolimanensis* Matuda, *E. agavoides* and two accessions of *E. colorata* E.Walther with high support (BS = 96, PP = 0.93) and is sister to the prior subclade. The ITS2 GenBank accession for *E. colorata* is nested with our three loci *E. colorata* accessions (Fig. 2A).

Sedum corynephyllum: *Sedum corynephyllum* Fröd. from *Sedum* sect. *Pachysedum*, is recovered as sister species of two large main clades (III and IV) with high support (BS = 95) in the ML analysis; however, for the BI analysis consensus tree (not shown) this species is grouped in the clade IV, close to the genus *Graptopetalum*.

Clade III: This main clade is constituted by numerous and morphologically diverse taxa from different series of *Echeveria*, and even some species from *Sedum* and *Thompsonella*. From *Echeveria*, ser. *Chloranthae*, ser. *Ciliatae*, ser. *Echeveria*, ser. *Longistylae*, ser. *Mucronatae*, ser. *Nudae*, ser. *Racemosae*, ser. *Spicatae*, ser. *Thyrsiflorae*, *Echeveria cuicatecana*—not placed previously in any series—, *Echeveria purpusorum* from ser. *Urbiniae* and genus *Thompsonella* are nested here.

The clade is moderately supported in the ML analysis (BS = 78) and not supported by BI criteria (PP = 0.54, Fig. 2B). On the one hand, one of the subclades incorporates *E. rosea* and *E. chiapensis* from ser. *Spicatae*, *E. nuda* Lindl., *E. nebularum* Moran & Kimnach from ser. *Nudae*, *E. globulosa* Moran, *E. subcorymbosa* Kimnach & Moran, *E. megacalix* E.Walther, *E. mondragoniana* J.Reyes & Brachet, *E. chazaroi* Kimnach and *E. helmutiana* Kimnach from ser. *Racemosae*. This subclade was retrieved by W-IQ-TREE analysis with moderate support (BS = 70%); nonetheless, Bayesian analysis did not recover this subclade. The rest of the taxa are distributed in the trees in various subclades ranging from not supported to moderate support. However, some subclades with moderate to high support (at least in one of the two inference criteria) are mentioned, while *E. alata* Alexander, *E. viridissima* E.Walther, *E. globuliflora* E.Walther and *E. macdougallii* E.Walther are retrieved as closely allied. On the other hand, *E. lurida* Haw., *E. diffractens* Kimnach & A.B.Lau, *E. carnicolor* É.Morren, *E. canaliculata* Hook. from ser. *Racemosae* and *E. tencho* Moran & C.H.Uhl from ser. *Spicatae* form a subclade with moderate support (BS = 81, PP= 0.83) within Clade III. Another subclade comprehends *E. goldmanii* Rose, *E.* aff. *bella*, *E. bella* f. *major* (E.Walther) Kimnach and *E. sessiliflora* Rose (Fig. 2B). One of the subclades recovered with high support in the ML analysis (BS = 90) is composed by *E. heterosepala* Rose, *E. crassicaulis* E.Walther, *E. platyphylla* Rose, *E. longipes* E.Walther and *E. paniculata* var. *maculata* Rose (Kimnach).

The genus *Thompsonella* is retrieved as monophyletic within the clade III (BS = 100), although in the BI consensus tree (not shown) *T. mixtecana* J.Reyes & L.G.López does not group with the other *Thompsonella* species. The two accessions of *T. mixtecana* and the two of *T. sphatulata* Kimnach are each grouped together, but, in the case of *T. minutiflora* (Rose) Britton & Rose, *T. xochipalensis* M.Gual Diaz, S.Peralta & Pérez-Calix appears between the two accessions of the first species. The *Thompsonella* subclade is shown as sister to *E. moranii* E.Walther and *E. pringlei* (S.Watson) Rose and *E. pringlei* var. *parva* with moderate support (BS = 87). It should be noted that GenBank ITS2 accessions and sequences generated here group together in the trees, such is the case for *E. coccinea* (Cav.) DC. and *E. pulvinata* Rose with high support (100/1 and 98/1, respectively, Fig. 2B).

Clade IV: This clade is formed by the genera *Cremnophila*, *Graptopetalum*, *Reidmorania*, *Tacitus*, and *Sedum clavatum*, as well as elements of *Echeveria*. Clade IV has high support in ML and BI consensus trees (BS = 93, PP = 1). A part of genus *Graptopetalum* is recovered as non-monophyletic in the base of the clade with unclear relationships to *Reidmorania occidentalis* (Rose ex E.Walther) Kimnach, *Tacitus bellus* and ser. *Occidentales* (*E. affinis* E.Walther and *E. craigiana* E.Walther) and ser. *Valvatae* (*E. calycosa* Moran and *E. valvata* Moran) from genus *Echeveria* (Fig. 2C). The other part of *Graptopetalum* clusters together with *Sedum clavatum* in a poorly supported subclade. In turn, *Cremnophila* is shown as monophyletic and sister to ser. *Angulatae*, ser. *Gibbiflorae*, ser. *Secundae* and ser. *Pruinosae*, but barely supported (Fig. 2C). *Echeveria humilis* Rose ex Britton & Rose and *E. xichuensis* L.G.López & J.Reyes are sister species, but *E. trianthina* Rose is more closely related to the rest of the taxa than the previous two species.

Another subclade is constituted by ser. *Angulatae*, ser. *Pruinosae*, ser. *Secundae*, *E. semivestita* var. *floresiana* E.Walther and *E. semivestita* var. *semivestita* Moran—located by E.Walther in *Gibbiflorae*—. This subclade is well-supported in maximum likelihood and Bayesian inference (Fig. 2C). *Echeveria peacockii* Croucher (from ser.

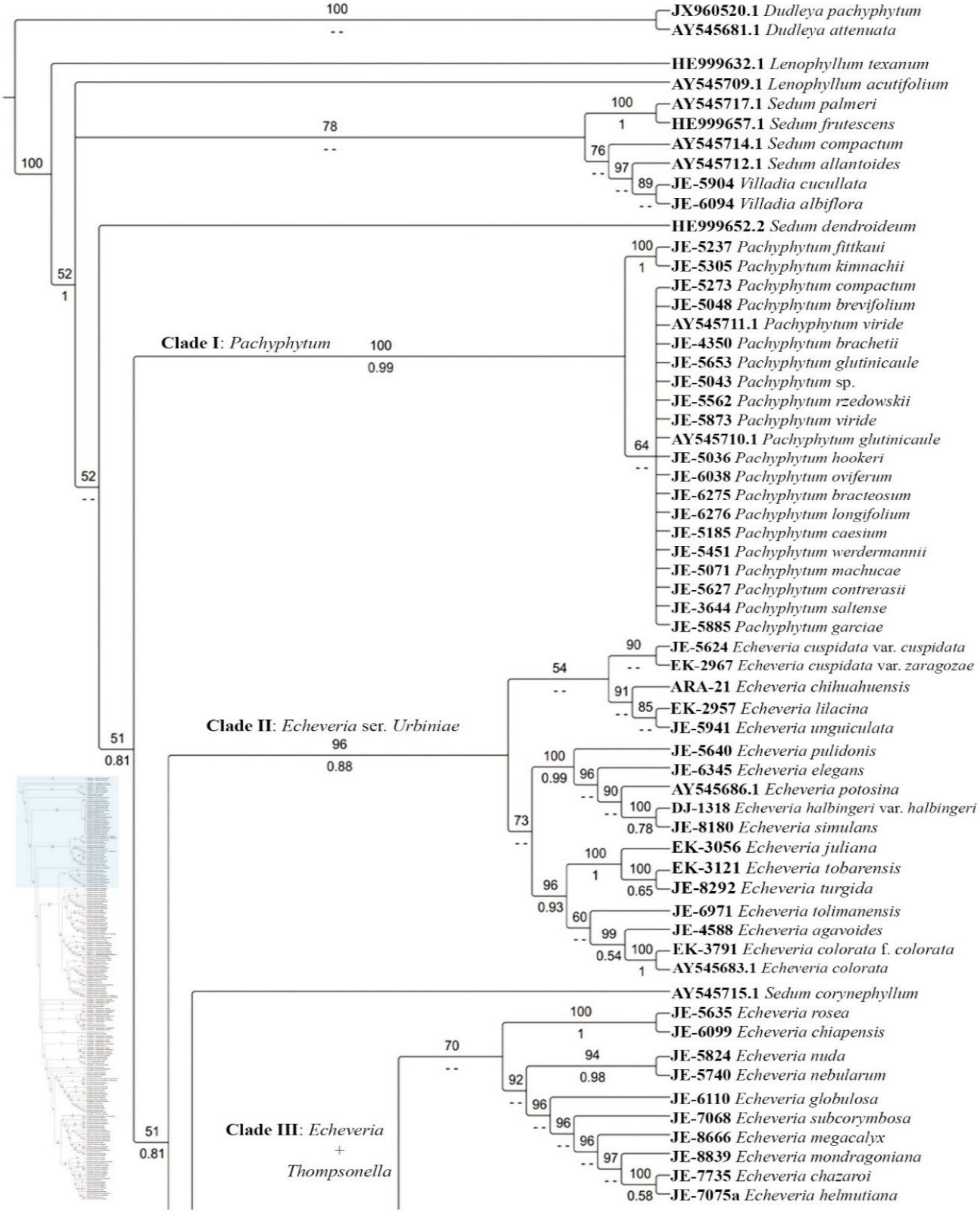

FIGURE 2A. Echeveria group (Clade I: *Pachyphytum*, Clade II: *Echeveria* ser. *Urbiniae* and Clade III: *Echeveria* plus *Thompsonella*), maximum likelihood 50% majority-rule consensus tree for combined dataset. Numbers above branches indicate bootstrap support (only values above 50% are shown). Numbers below branches designate Posterior Probabilities support (>0.50 are shown) from Bayesian-based reconstruction.

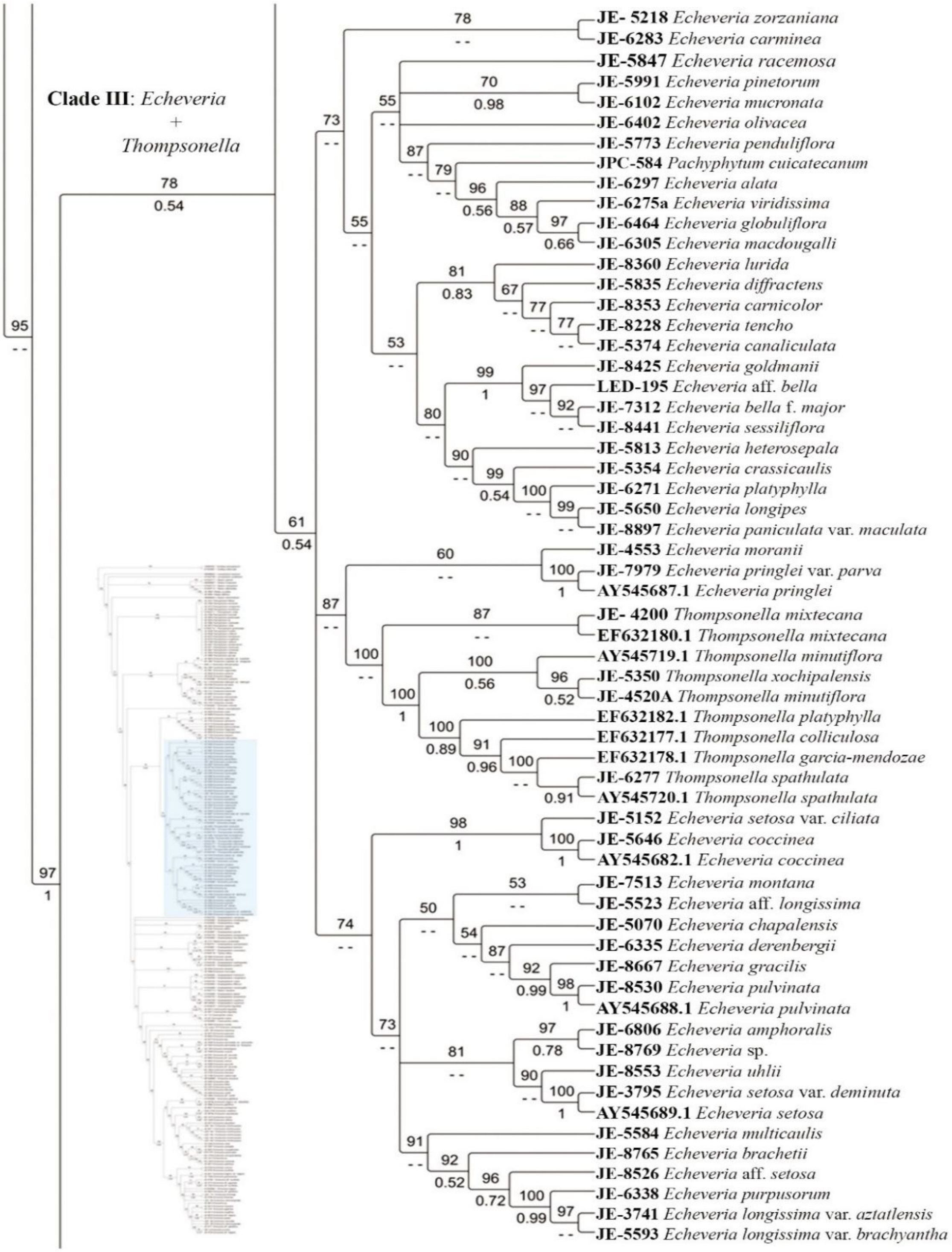

FIGURE 2B. Echeveria group (Clade III: *Echeveria* plus *Thompsonella*), maximum likelihood 50% majority-rule consensus tree for combined dataset (continuation). Numbers above branches indicate bootstrap support (only values above 50% are shown). Numbers below branches designate Posterior Probabilities support (>0.50 are shown) from Bayesian-based reconstruction.

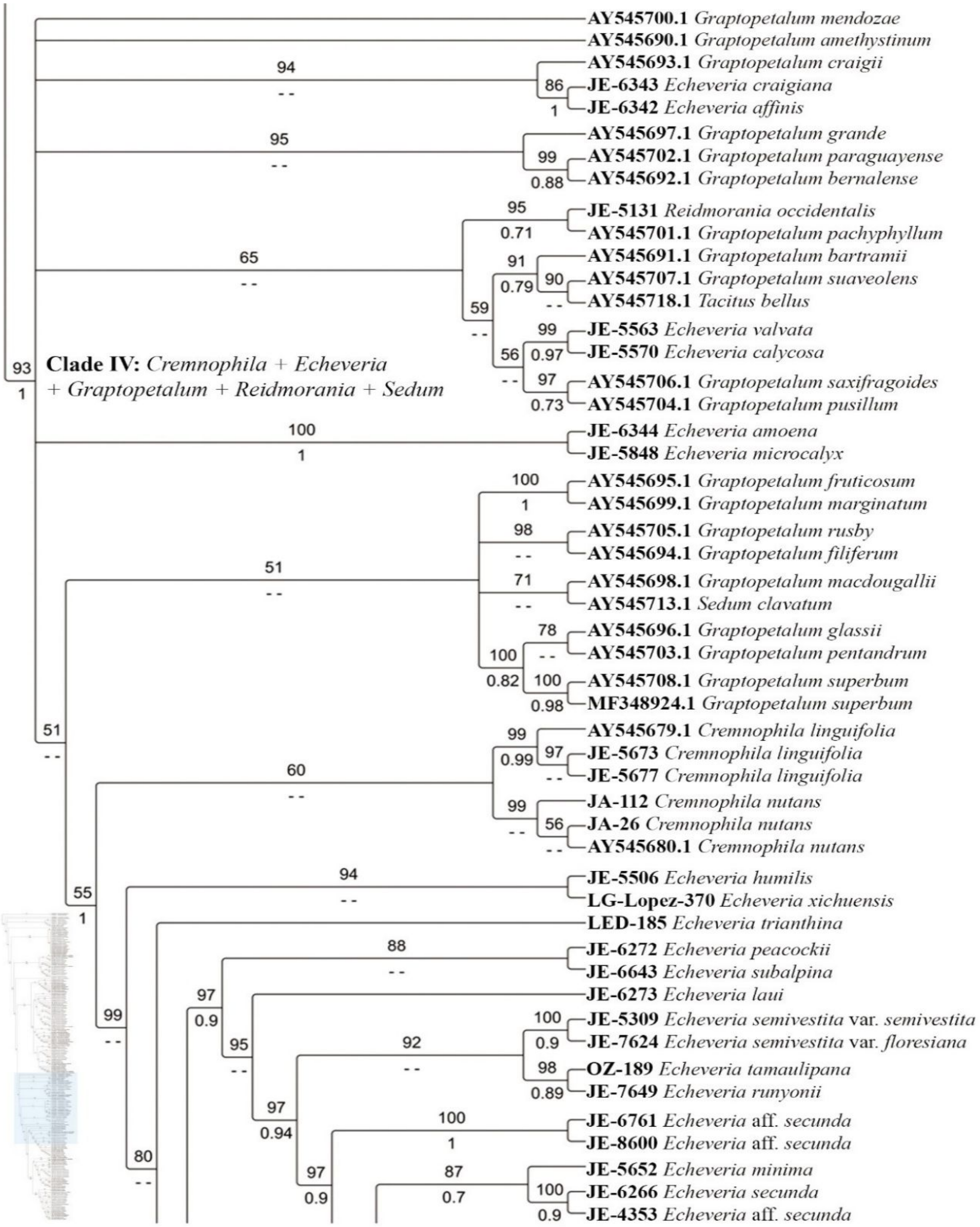

FIGURE 2C. Echeveria group (Clade IV: *Cremnophila*, *Echeveria*, *Graptopetalum*, *Reidmorania* and *Sedum*), maximum likelihood 50% majority-rule consensus tree for combined dataset (continuation). Numbers above branches indicate bootstrap support (only values above 50% are shown). Numbers below branches designate Posterior Probabilities support (>0.50 are shown) from Bayesian-based reconstruction.

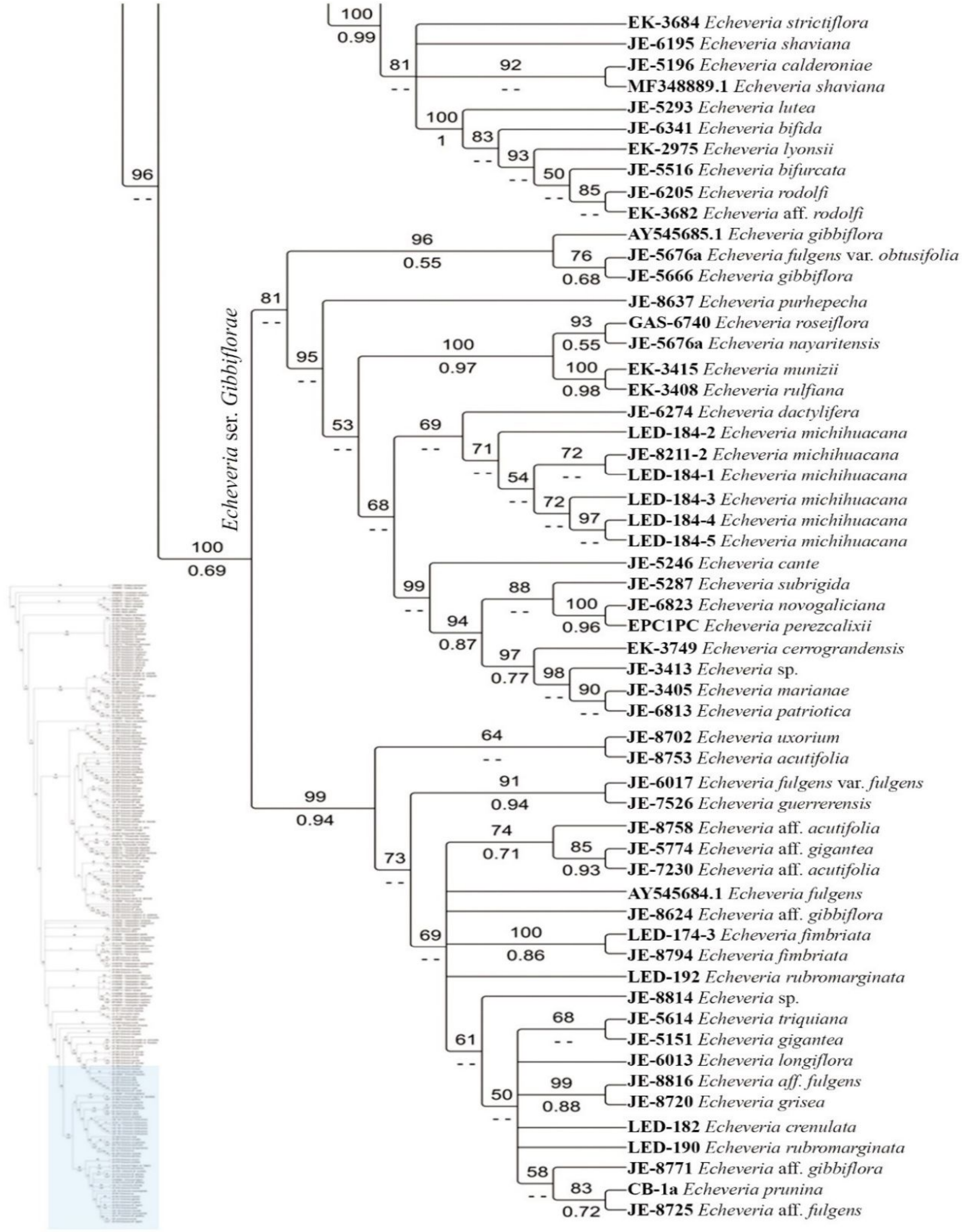

FIGURE 2D. Echeveria group (Clade IV, showing subclade *Echeveria* ser. *Gibbiflorae*), maximum likelihood 50% majority-rule consensus tree for combined dataset (continuation). Numbers above branches indicate bootstrap support (only values above 50% are shown). Numbers below branches designate Posterior Probabilities support (>0.50 are shown) from Bayesian-based reconstruction.

Pruinosae) is with the sister species of *E. subalpina* Rose & Purpus—placed in series *Secundae*—while *E. laui* Moran & J.Meyrán, is recovered as sister species for the rest of ser. *Angulatae*, ser. *Secunda* taxa and *E. semivestita* varieties. The two last varieties form a clade as sister to *E. tamaulipana* Mart.-Aval., Mora-Olivo & M.Terry and *E. runyonii* Rose ex E.Walther from *Angulatae*. On the other hand, ser. *Angulatae* taxa are grouped along with ser. *Pruinosae* and ser. *Secundae* taxa in the ML analysis (Fig. 2C–2D).

Species of ser. *Gibbiflorae* were recovered as monophyletic group. This clade is one of the most supported in ML inference criteria, but less so in BI, with BS/PP = 100/0.69, respectively.

Discussion

The consensus trees of our two inference criteria show *Echeveria* as non-monophyletic in agreement with previous studies (Carrillo Reyes *et al*. 2009; Nikulin *et al*. 2016; Vázquez-Cotero *et al*. 2017), since the genera *Cremnophila*, *Graptopetalum*, as well as some species from *Sedum* and *Thompsonella* are recovered within *Echeveria*. Moreover, infrageneric categories (series) from the latter genus are not monophyletic, although ser. *Gibbiflorae* and ser. *Urbiniae* are monophyletic. In the next section, we contrast and discuss the main clades and various subclades recovered here, as well as novel phylogenetic positions for some taxa obtained in our results against current classification proposal (Kimnach in Eggli 2003).

Clade I. The monophyly of this clade has been predicted in previous molecular phylogenetic analyses of the so-called "Acre Clade and Echeveria Group" even though the sampling was limited (Carrillo-Reyes *et al*. 2009; Vázquez-Cotero *et al*. 2017). The monophyly of this genus is confirmed in our results with high support and broader sampling (Fig. 2A). To this genus are associated cliff-dwelling plants with an evident caudex—sometimes larger than 1 m long and pendant—; very succulent obovate to clavate leaves; inflorescences in scorpioid cymes (cincinni); calyx segments erect, usually unequal, adpressed to corolla, shorter or longer than corolla. All species of this clade have structures called "appendages" on the inner face of the petals on the sides of the epipetalous filaments. The latter trait has been one of the diagnostic characters used to differentiate the genus *Pachyphytum*, despite the fact that several *Echeveria* species (*E. dactylifera* E.Walther, *E. heterosepala* Rose, *E. longissima* var. *longissima* E.Walther, *E. purhepecha* I.García, *E. rulfiana* Jimeno-Sevilla, Santana Mich. & P.Carrillo, among others) also present it. Different taxonomists use the inner petal appendages indistinctly for *Echeveria* and *Pachyphytum*, but in the latter, those are flat or membrane-like (Fig. 3A), while for the first, the appendages are generally cylindrical or finger-like shaped (Fig. 3B)—although, *E. heterosepala* may present both types of appendages (Fig. 3C). Anatomical studies are needed to establish the homology of this trait as J. Thiede (in Eggli 2003: 191) has stated.

Even though *Pachyphytum* is clearly monophyletic, its infrageneric relationships have not been resolved here because of the polytomy shown in the cladogram. This very low resolution might be due to very recent diversification of the species. The only relationship consistently recovered in the consensus trees within *Pachyphytum* is *P. fittkaui* as sister to *P. kimnachii*. This result favors the hypothesis that these two taxa could in fact belong to the same species, based on the shallow morphology differentiation (Fig. 3D) and biogeographic patterns.

Another significant result indicates that *Pachyphytum cuicatecanum* (J.Reyes, Joel Pérez & Brachet) Kimnach clearly does not belong to *Pachyphytum*, despite the similarity in many morphological traits (Fig. 3E). Its biogeography and the absence of appendages in *P. cuicatecanum*—not observed neither by Reyes *et al*. (2004) or Kimnach (2010); but the first author of the present study has observed in some *P. cuicatecanum* flowers a bulge in the sides of the epipetalous filaments that do not look like typical *Pachyphytum* appendages (Fig. 3F). This also suggests that this species is not related to *Pachyphytum*, instead it is located in a subclade of clade III along with *E. macdougalli*, *E. globuliflora*, *E. viridissima*, *E. alata* and *E. penduliflora* E.Walther (Fig. 2B).

Clade II. This clade contains the "true" *Urbiniae* species from the genus *Echeveria* and is recovered with high support (Fig. 2A). This series is characterized by acaulescent plants, compact rosettes, usually with thick leaves (Fig. 3G)—with exception maybe for *E. cuspidata* var. *cuspidata* and *E. lilacina* with thinner leaves—, scorpioid cymes (cincinni) with short pedicels or cymose inflorescences, usually with larger pedicels, the bracts are linear-lanceolate and fewer than in other series, the calyx segments are tiny, deltoid and frequently adpressed to petals, they have urceolate corollas and rounded petals (not keeled), nectary scales, in general, small and thin—compared to other series. Finally, the geographic distribution for this series is from central to northern Mexico (Chihuahua, Durango, Hidalgo, Jalisco, Michoacán, Nuevo León, San Luis Potosí, Sinaloa, Veracruz). This clade was also recovered in previous analyses (Nikulin *et al*. 2016; Vázquez-Cotero *et al*. 2017), but with more reduced sampling.

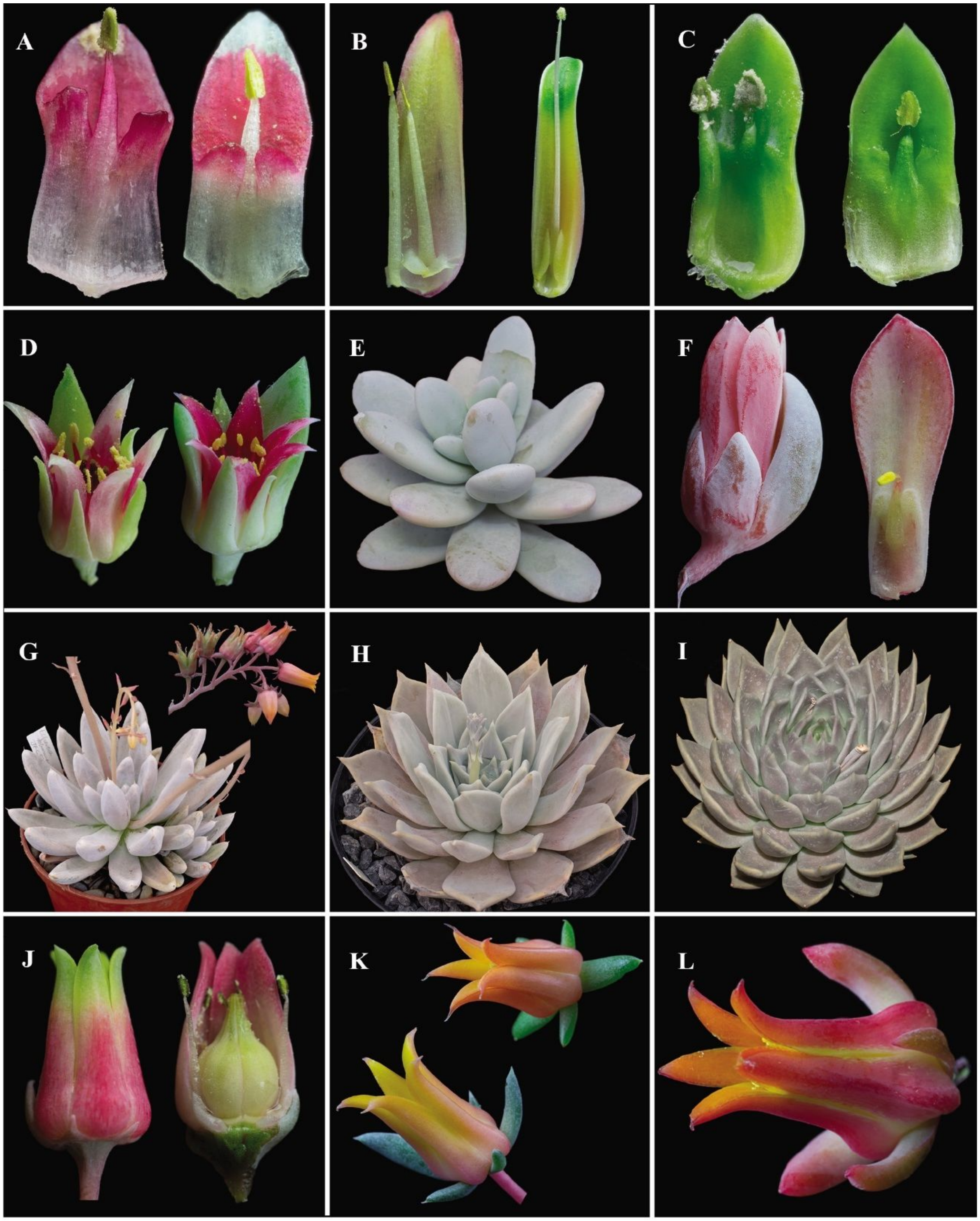

FIGURE 3. Morphological diversity in the Echeveria group. **A**. Inner face of petals of *P. bracteosum* (left), *P. oviferum* (right). **B**. Finger like appendages on *E. dactylifera* (left) and *E. longissima* var. *longissima* (right). **C**. Inner face of petals of *E. heterosepala*. **D**. Flowers of *P. kimnachi* (left) and *P. fittkaui* (right). **E**. Rosette of *P. cuicatecanum*. **F**. Flower of *P. cuicatecanum* (left), inner face of petal (right). **G**. Rosette and inflorescence of *E. tolimanensis*. **H.** *E. juliana*. **I**. *E. tobarensis*. **J**. Flower of *E. simulans* (left) and gynoecium of *E. juliana* (right). **K**. Flowers of *E. trianthina* (above) and *E. humilis* (below). **L**. Flower of *E. strictiflora*. (Photos: Luis Emilio de la Cruz).

Within *Urbiniae*, the resulting internal relationships are, in most cases, in agreement with morphological evidence in the following sense: 1) the two *E. cuspidata* varieties sampled are nested together, they differ only in few characters such as the shape of the leaves and the length of the corolla; 2) *E. chihuahuensis*, even though displaying a rosette shape similar to *E. agavoides* or *E. colorata*, its scorpioid cyme inflorescences and flowers are more related morphologically to those found in *E. cuspidata*; 3) *E. lilacina* is also related to the abovementioned species by its flower morphology and geographic distribution; 4) *E. pulidonis*, *E. elegans*, *E. potosina*, *E. halbingeri* var. *halbingeri* and *E. simulans* are grouped together in a well-supported subclade within *Urbiniae*, which is supported by morphology, with respect to rosette and leaf shape and similar type of inflorescences (scorpioid cymes); 5) another well-supported subclade indicates that *E. juliana* (Fig. 3H)—recently described—, *E. tobarensis* (Fig. 3I)—recently rediscovered by J. Etter and M. Kristen—, *E. tolimanensis, E. agavoides*, and *E. colorata* are closely related, they share the ovate-deltoid shape of leaves and rosettes, cymose inflorescences with longer pedicels and typical *Urbiniae* corollas, small urceolate flowers, tiny sepals, small nectary scales and bottle shaped apocarpous gynoecium (Fig. 3J), styles and stigmas adpressed very close to each other. In turn, *E. turgida* seems not to match at all in this subclade, since it is known to have scorpioid inflorescences, oblong leaves and slightly longer sepals than *E. agavoides* and *E. colorata*.

Other species previously assigned to *Urbiniae* clearly do not belong here. In the case of *E. purpusorum*, it resembles more the species of ser. *Longistylae* than those of *Urbiniae* regarding the green with reddish-brown dotted leaves—mainly on the abaxial side of the leaves—, short and few flowered inflorescences (cincinni), and it is distributed in the "Tehuacán-Cuicatlán" Biosphere Reserve on the border of Puebla and Oaxaca states in Mexico, where *Longistylae* is also located and no other *Urbiniae* representatives are found. Phylogenetic relationships inferred in this work confirm its relatedness to this series (Fig. 2B). Similarly, the assignment to *Urbiniae* of *E. humilis*, *E. trianthina* (Fig. 3K), and *E. xichuensis* does not correspond to morphology and phylogenetics, as these are more similar to *Angulatae* species than to *Urbiniae*, example.g., the inflorescences resemble to those found in *E. bifida*, *E. lyonsii* Kimnach or *E. rodolfoi* Mart.-Aval. & Mora-Olivo (published as *E. rodolfi*) in having cincinni—sometimes bifurcated—, larger spreading linear-deltoid sepals and keeled petals—as in *E. strictiflora* A.Gray (Fig. 3L). Our phylogenetic analyses indicate that these neither belong to *Angulatae* nor *Urbiniae*, but are sister to *Angulatae*, *Gibbiflorae*, *Pruinosae* and *Secundae* (Fig. 2C).

Until now, *Urbiniae* has been considered as part of *Echeveria*, but due to its monophyletic status, this clade could be considered as a separate genus. Other phylogenetic studies have also recovered *Urbiniae* as a monophyletic group (see Carrillo-Reyes *et al*. 2009; Nikulin *et al*. 2016 and Vázquez-Cotero *et al*. 2017) and apparently, neither *Cremnophila*, *Graptopetalum*, *Pachyphytum*, *Sedum*, *Thompsonella* and even other *Echeveria* series taxa could be nested here. *Echeveria agavoides*—the type species of ser. *Urbiniae*—was designated to genus *Urbinia* by Rose in 1903 and then transferred to *Echeveria* again by A. Berger (1930), although with another name (*E. oscura*). The genus *Urbiniae* relationships could be clarified in posterior analyses that involve more DNA loci information.

Sedum corynephyllum is recovered as sister to two main clades III and IV which corresponds to the rest of *Echeveria* species as well as genera *Cremnophila*, *Graptopetalum*, *Reidmorania* and some *Sedum* species (Fig. 2A). This is congruent with the analysis of Nikulin *et al*. (2016), although in the study by Vázquez-Cotero *et al*. (2017), *Sedum corynephyllum* is close to *S. pachyphyllum* Rose in their clade III. More specimens including lateral inflorescences from *Sedum* (sect. *Pachysedum* and sect. *Palmeri* C.H.Uhl) are needed in future sampling to confirm whether this can be a clade within the Echeveria group (Carrillo-Reyes *et al*. 2009).

Sedum corynephyllum resembles some *Pachyphytum* taxa rather than *Echeveria* in having an evident stem with clavate leaves (Fig. 4A), thick adpressed sepals and tubular corolla (Fig. 4B). However, the paniculate-cymose inflorescences, greenish-yellow sepals, yellow petals and the absence of inner face appendages approaches it to *Echeveria*, especially to *E. prolifica* Moran & J.Meyrán (Fig. 4C).

Clade III. This main clade contains the type species of the genus *Echeveria*—*E. coccinea* (Cav.) DC. (Fig. 4D), and it is evident that the location of species into series is highly artificial in this part of the "Echeveria group". The morphological homoplasy is more noticeable for ser. *Echeveria*, ser. *Mucronatae,* ser. *Nudae* and ser. *Racemosae*, as our phylogeny shows them to be non-monophyletic. Hence, the current classification does not match at all to consensus phylogenetic trees for this clade. Nevertheless, more resolution is needed in order to resolve and identify monophyletic groups, as well as circumscribe them in this clade. Additionally, all Central and South American species were not sampled here, and we hypothesize that they may correspond to this main clade, so future analyses should consider these. Some shifts in phylogenetic positions and morphological affinities in the recovered subclades are discussed next.

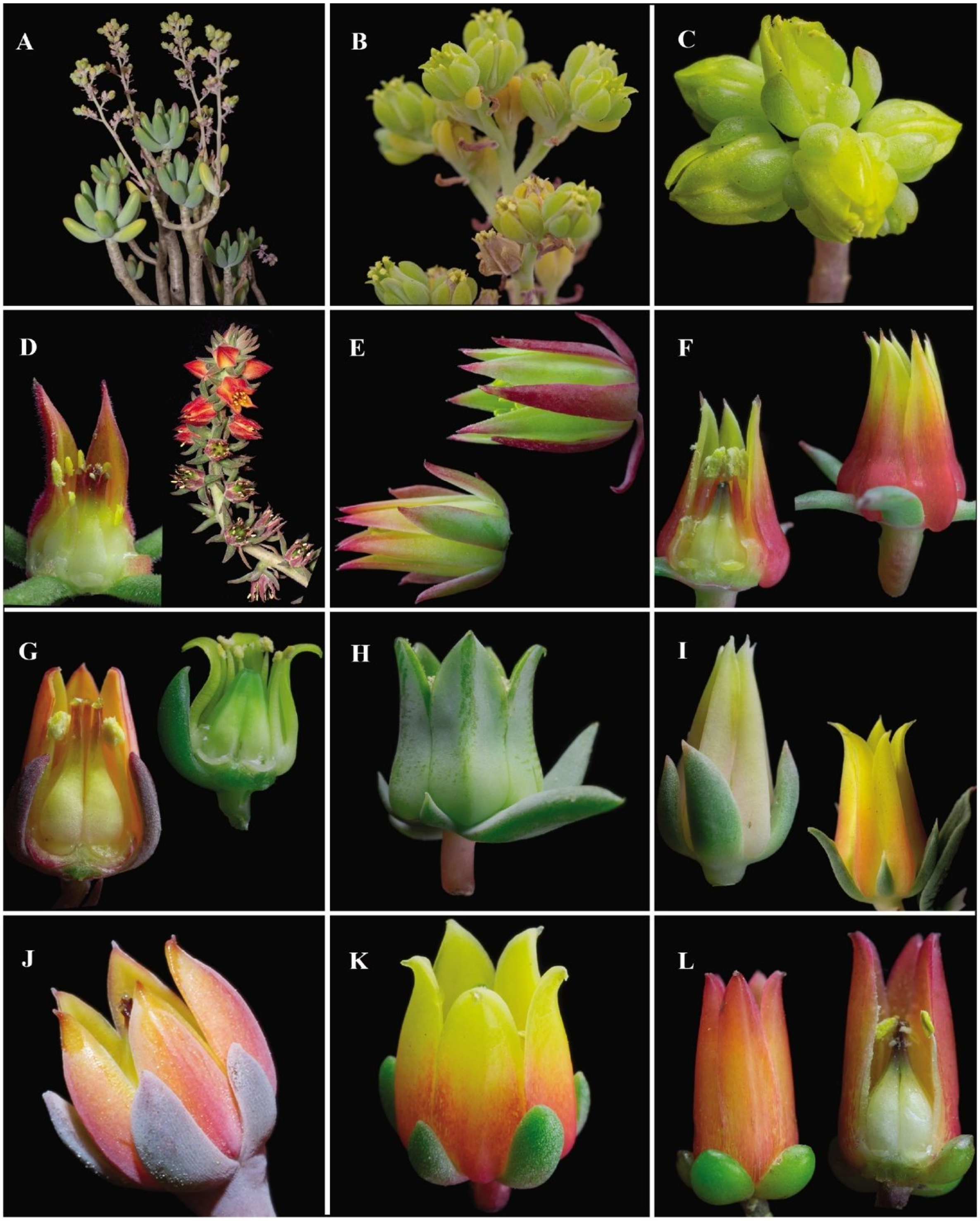

FIGURE 4. Morphological diversity in the Echeveria group (continuation). *S. corynephyllum*. **B**. Flowers of *S. corynephyllum*. **C**. Flowers of *E. prolifica*. **D**. *E. coccinea* flower (left), *E. coccinea* inflorescence (right). **E**. *E. rosea* (above), *E. tencho* (below). **F**. *E. nuda* inner view of flower (left), *E. nuda* flower (right). **G**. *E. helmutiana* (left), *E. megacalyx* (right). **H**. *E. heterosepala*. **I**. *E. platyphylla* (left), *E. mucronata* (right). **J**. *E. sessiliflora*. **K**. *E. bella*. **L**. *E. goldmanii* flower (left), *E. goldmanii* inner view of flower (right). (Photos: Luis Emilio de la Cruz).

For series *Spicatae*, consensus trees indicate that *E. tencho* is not closely related to *E. rosea*, despite their very similar vegetative and flower traits (Fig. 4E). Rather *E. tencho* is recovered in a subclade along with taxa from ser. *Racemosae*: *E. canaliculata*, *E. carnicolor*, *E. diffractens* and *E. lurida* (Fig. 2B). In turn, *E. rosea* and *E. chiapensis* are nested together as we expected, since they are considered synonymous and their morphological differentiation is almost impossible to establish, despite their very wide geographic distribution. Future analysis must sample *E. pitteri* Rose to elucidate its relatedness to this series. Moreover, ser. *Nudae* is retrieved as non-monophyletic since its taxa are distributed along the various subclades recovered, *E. nuda* (Fig. 4F)—the type species of ser. *Nudae*—and *E. nebularum* are grouped together as morphology indicates. Nevertheless, they are closely related to species from ser. *Racemosae* [*E. subcorymbosa*, *E. megacalyx* (Fig. 4G), *E. chazaroi* and *E. helmutiana* (Fig. 4G)] in a well-supported subclade (BS = 92); this result points out the artificial nature of the ser. *Nudae*.

In another sense, the series *Chloranthae* was designated for the single species *E. heterosepala* which has a unique character among all species of the genus consisting of greenish petals coloration (Fig. 4H), whereas the molecular phylogenetics indicates that it is close to some of the *Mucronatae* species (*E. crassicaulis*) and ser. *Thyrsiflorae* (*E. platyphylla* Rose (Fig. 4I) and *E. paniculata* var. *maculata*). In Vázquez-Cotero *et al.* (2017), *E. heterosepala* is close to *E. crassicaulis* and *E. mucronata* (Fig. 4I), since neither *E. paniculata* nor *E. platyphylla* were sampled—with which it shares the tuberous root type, the acaulescent rosette and the substantial number of bracts in the inflorescence, so the monotypic series *Chloranthae* could merge into series *Thyrsiflorae*, even when *E. heterosepala* have a scorpiod cyme inflorescences. Another point to highlight is that some taxonomists consider *E. crassicaulis* as synonymous of *E. mucronata* because of very close morphological relatedness; nonetheless, according to our results *E. crassicaulis* is closer to *E. platyphylla* and *E. paniculata* var. *maculata*, while *E. mucronata* is closer to *E. pinetorum* Rose—a revision is recommended since these later taxa could be synonymous.

Echeveria sessiliflora Rose (Fig. 4J), currently located in ser. *Mucronatae*, in our W-IQ-TREE and MrBayes trees, is grouped with *E. bella* f. *major* (E.Walther) Kimnach (Fig. 4K) and *E.* aff. *bella* (collected Near Tenejapa, Chiapas) from ser. *Racemosae*. Although *E. sessiliflora* has tuberous root as those found in ser. *Mucronatae*, the acaulescent habit, the shape and dimensions of the rosette and flowers, the proliferous habit, their chromosomes numbers (n = 15) and their geographic distribution associate these tree taxa along with *E. goldmanii* Rose, even when the latter species possesses a long caudex and its flower morphology resembles that of ser. *Racemosae* flowers (Fig. 4L).

Homoplasy in morphological characters is found also in ser. *Echeveria*. While some of its members are nested together, such as *E. carminea* Alexander (Fig 5. A) and *E. zorzaniana* J.Reyes & Brachet (Fig. 5B). Contrarily, *E. amphoralis* E.Walther, *E. coccinea*, *E. pringlei* var. *parva* Kimnach and *E. pulvinata* Rose were recovered in different sections of the Clade III, although with insufficient resolution of the trees. Presence of trichomes on stems, leaves, sepals and petals may represent symplesiomorphic characters and are not useful for delimiting series. Instead, free styles and stigmas at anthesis, white ovaries and nectary scales and finally, large reddish flowers may act as synapomorphies for ser. *Echeveria*.

The ser. *Racemosae* is also retrieved as polyphyletic in our trees, *E. racemosa*—the type species—is grouped along with species from ser. *Mucronatae*, *Nudae* and *Racemosae* in a barely supported subclade, while other *Racemosae* taxa are arranged in other poorly supported subclades joined with ser. *Ciliatae*, ser. *Echeveria*, ser. *Spicatae*, and ser. *Mucronatae* taxa. The confusing relationships resulting in ser. *Racemosae* can be due, in part, to the absence of *rbcL* and *matK* data for *E. racemosa* and *E. canaliculata*. Another possible reason for this incoherence may be the lack of Central and South American species, as many, if not all, species from this region belong to ser. *Nudae* and ser. *Racemosae*.

The ser. *Racemosae* can be recognized by its papillose leaves (Fig. 5D–F), racemose inflorescences (Fig. 5G), tubular, sometimes gibbous corollas and somewhat keeled petals (Fig. 5H), with apocarpous carpels and connate styles and stigmas at anthesis. These traits can be found in *E. canaliculata, E. carnicolor*, *E. diffractens*, *E. goldmanii*, *E. lurida*—considered synonymous of *E. racemosa* by Kimnach—, *E. moranii*, *E. olivacea* Moran, *E. penduliflora* and *E. racemosa*. Nonetheless, many taxa assigned to ser. *Racemosae* display somewhat different characteristics (*E. bella*, *E. chazaroi*, *E. helmutiana*, *E. megacalyx*, *E. mondragoniana*, *E. subcorymbosa* and *E. uhlii* J.Meyrán), e.g., the leaves are not as papillose as *E. racemosa*—at least for some taxa—(Fig. 5I), their pedicels are shorter (Fig. 5J)—except for *E. subcorymbosa*—, and the corollas are sometimes bicolored and tubular-campanulate to globose shaped (Fig. 5K).

Within Clade III, the genus *Thompsonella* is recovered as a monophyletic group (Fig 2A). In the analysis of Vázquez-Cotero *et al.* (2017), *T. mixtecana* does not group with the rest of the genus. Yet, the monophyletic status of *Thompsonella* was found by Carrillo-Reyes *et al.* (2008; 2009) and as in our topology, the genus is located in the Clade III even when they do not differentiate between clade III and clade IV in their results. The morphology of *Thompsonella* is somewhat different from *Echeveria*, particularly in reference to the degree of sympetalic corollas:

FIGURE 5. Morphological diversity in the Echeveria group (continuation). **A**. *E. carminea*, **B**. *E. zorzaniana*. **C**. *E. amphoralis* flower inner view. **D**. *E. olivacea*. **E**. *E. lurida*. **F**. *E. carnicolor*. **G**. Inflorescences of ser. *Racemosae*, *E. diffractens* (left), *E. olivacea* (right). **H**. Flowers of ser. *Racemosae*, *E. racemosa* (left), *E. morani* (right). **I**. *E. mondragoniana*. **J**. *E. chazaroi* inflorescence (left), *E. mondragoniana* inflorescence (right). **K**. *E. uhli* flower (left) and *E. mondragoniana* (right). **L**. *Thompsonella nellydiegoae* flower (below), *T. platyphylla* flower (above). (Photos: Luis Emilio de la Cruz).

in *Echeveria* the petals are fully erect and fused at the base, while in *Thompsonella*, petals are fused at their base but extended from the first third of the corolla (Fig. 5L). Additionally, the inner face of petals is colored in shades of red or purple stripes instead of yellowish, pink, white or orange as in the majority of *Echeveria* species. Similarities in rosette and leaves are the only relatedness between these genera; however, former analyses also recover *Thompsonella* along with *Echeveria* (Vázquez-Cotero *et al.* 2017), even when more DNA information is required to fully solve this clade, we hypothesize that *Thompsonella* can be considered as series of *Echeveria*.

Clade IV. This main clade is constituted by the genera *Cremnophila*, *Graptopetalum*, *Reidmorania*, *Sedum clavatum*, *Tacitus*, and from the genus *Echeveria*, ser. *Angulatae*, ser. *Gibbiflorae*, ser. *Occidentales,* ser. *Paniculatae*, ser. *Pruinosae*, ser. *Secundae*, ser. *Valvatae*, and *E. humilis*, *E. semivestita* var. *floresiana*, *E. semivestita* var. *semivestita*, *E. trianthina*, *E. xichuensis*. First branching in the clade, genus *Graptopetalum* is closely related to ser. *Occidentales* (*E. affinis* and *E. craigiana*), ser. *Paniculatae* (*E. amoena* and *E. microcalyx*), ser. *Valvatae* (*E. calycosa* and *E. valvata*), from the genus *Echeveria*, *Sedum clavatum*, *Tacitus* (see Acevedo-Rosas *et al.* 2004) and *Reidmorania*, but it is not possible to establish their relationships due to lack of resolution (Fig. 2C). The somewhat controversial identity of *Reidmorania occidentalis* (Fig. 6A) as belonging to *Echeveria* (*E. kimnachi*, Meyrán &Vega, 1998), *Graptopetalum* (*G. occidentale* Rose ex E.Walther perhaps) or the new genus *Reidmorania* has started to reveal; our analysis indicates that it is related to *G. pachyphyllum* Rose (Fig. 6B) with moderate to high support BS = 95, PP = 0.71). Hence, it is very likely that this taxon belongs to *Graptopetalum*, even when it displays yellowish petal coloration vs. whitish one in the majority of taxa in this genus.

As mentioned above, *Graptopetalum* taxa are gathered along with *E. affinis* and *E. craigiana* (Fig. 6C) from ser. *Occidentales* of genus *Echeveria*, *Graptopetalum bellum* (Moran & J.Meyrán) D.R.Hunt (Fig. 6D)—or *Tacitus bellus* according to some authors—and *S. suaveolens*—or *G. suaveolens* (Kimnach) R.T.Clausen—as Acevedo-Rosas *et al.* (2004) found. On the one hand, in the case of ser. *Occidentales*, the morphological characters that associate them with *Graptopetalum* are cymose inflorescences and red petal coloration (as in *G. bellum*). There is no association with other series of *Echeveria*, and their geographic distribution also suggests a close relationship. On the other hand, semi-erect petals without transversal reddish stains segregate them from this genus; therefore, the phylogenetic evidence of series *Occidentales* related to *Graptopetalum* must be considered in future taxonomic revisions.

Ser. *Valvatae* [*E. calycosa* and *E. valvata* (Fig. 6E)] is another series of *Echeveria* related to *Graptopetalum* (Fig. 2C). No phenotypical character associates ser. *Valvatae* with genus *Graptopetalum*, except maybe for the acaulescent habit, rosette shape and length and leaves shape, their geographical distribution and flower morphology also does not relate them. This is the first time that ser. *Valvatae* is related to *Graptopetalum*, since previous studies had not sampled any representative of this series.

The genus *Cremnophila* is recovered as a monophyletic group, although with low support, as sister clade of *Angulatae*, *Gibbiflorae*, *Pruinosae* and *Secundae*. Currently, for *Cremnophila* three species are known, but the recently described species *C. tlahuicana* J.Reyes & Ávila & Brachet (2015) is difficult to differentiate from *C. linguifolia* (Lem.) Moran (Fig. 6F). In fact, an accession of *C. tlahuicana* from Barranca de Mexicapa, labeled as JE-5677, groups with samples of *C. linguifolia* from Malinalco and Barranca de Mexicapa is one of the localities referred to *C. linguifolia* (Meyrán & López 2003). A detailed morphological revision is recommended to find whether they are synonymous.

One of the most supported and resolved clades is constituted by *Angulatae*, *Pruinosae* and *Secundae* series—at least in ML criteria—from the genus *Echeveria*. The cases of *E. humilis*, E. *trianthina* and *E. xichuensis* were discussed earlier. In this subclade, ser. *Pruinosae* is paraphyletic because *E. peacockii* (Fig. 6G) is nested with *E. subalpina* located in *Secundae*, and *E. laui* as sister species of the rest of *Angulatae* and *Secundae* (Fig. 2C). One of the most significant results is that *E. semivestita* varieties—historically difficult to locate in any series—were recovered close to *E. tamaulipana* from *Angulatae.* These two taxa (*E. semivestita* var. *semivestita* and *E. semivestita* var. *floresiana*) are rare morphologically speaking, because of their evident stem, hirsute narrow leaves, paniculate inflorescences and their *Angulatae* like flowers. This morphological complexity caused E. Walther to locate them in *Gibbiflorae*, perhaps giving priority to their paniculate inflorescences. Our analyses indicate that they are closer to *E. tamaulipana* and *E. runyonii*—*E. walpoleana* was not sampled, it is very similar to *E. tamaulipana*—with which they share the shape and color of the leaves, although with hirsute leaves and conspicuous caudex. Hence, based on our results, they could be placed in *Angulatae*.

The rest of the clade exclusively comprises *Angulatae* and *Secundae* species. Some specimens of the *E. secunda* Booth ex Lindl. complex from Tuxpan in the state of Michoacán, Mexico, are not directly related to the rest of *Secundae* (Fig. 6G). Moreover, *E. calderoniae* Pérez-Calix—formerly placed in *Secundae*—is closer to typical *Angulatae* species. In fact, the linear leaves of *E. calderoniae* are similar to those that characterize to *E. bifurcata*. Although it has been considered synonymous to *E. bifida*, we cannot be certain about this assumption, as shown in our

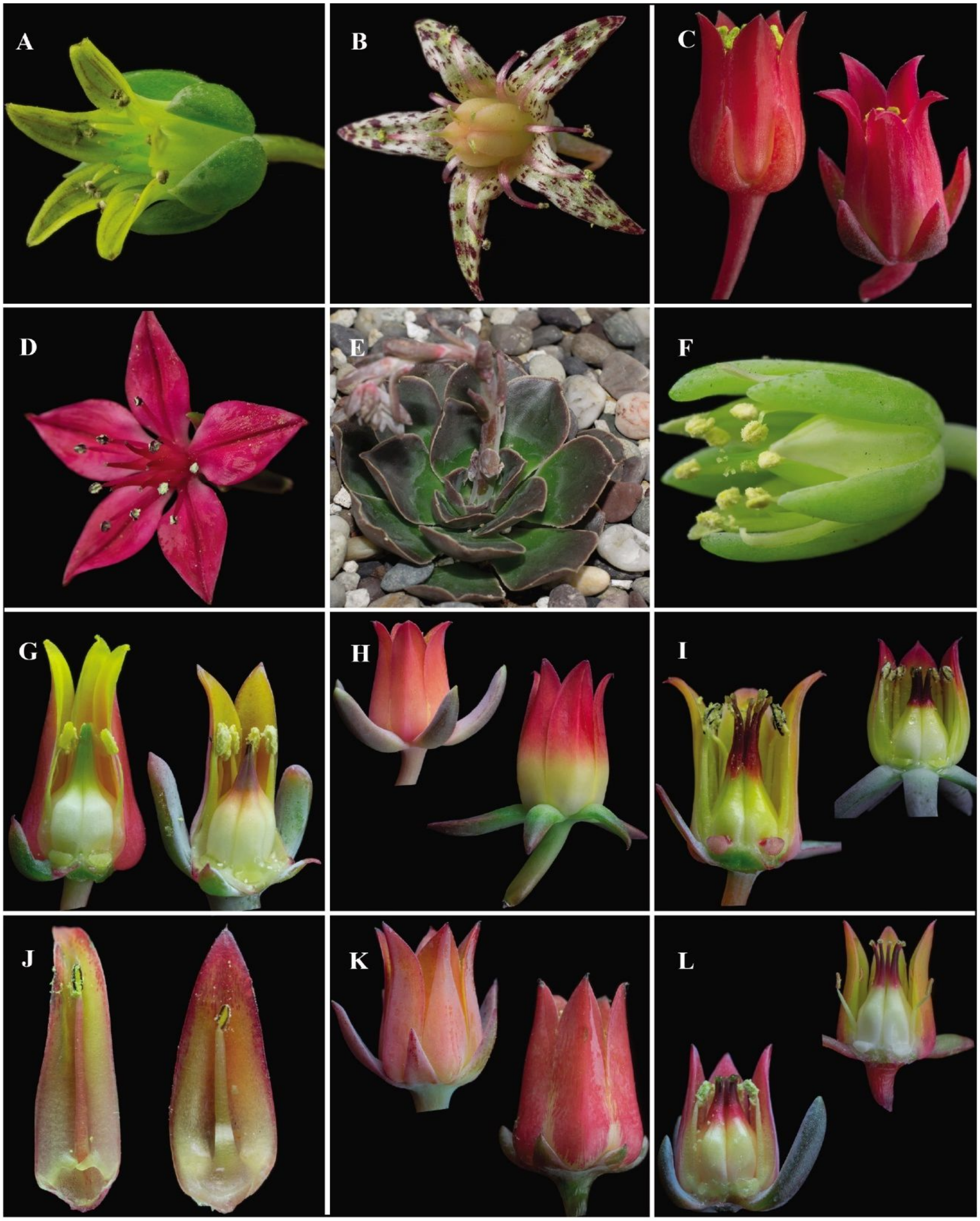

FIGURE 6. Morphological diversity in the Echeveria group (continuation). **A**. *Reidmorania occidentalis* flower. **B**. *Graptopetalum pachyphyllum* flower. **C**. *E. affinis* flower (left), *E. craigiana* flower (right). **D**. *Tacitus bellus* (*G. bellum*) flower. **E**. *E. valvata* rosette. **F**. *Cremnophila linguifolia* flower. **G**. *E. secunda* inner view of flower (left), *E. peacockii* inner view of flower (right). **H**. *E. perezcalixii* flower (left), *E. patriotica* flower (right). **I**. *E. subrigida* flower (left), *E. nayaritensis* flower (right). **J**. Inner face of petals, *E. novogaliciana* (left) and *E. purhepecha* (right). **K**. *E. juarezensis* flower (left), *E. grisea* flower (right). **L**. Dissected flowers of *E. prunina* (below) and *E. crenulata* (above). (**Photos**: Luis Emilio de la Cruz).

trees, where *E. bifurcata* is closer to *E. lyonsii* rather than to *E. bifida*. Additionally, morphological differences can be found in the flowers, *E. schaffneri* Rose ex Britton & Rose and *E. walpoleana* (not sampled), and the flowers even are pentagonal as in the ser. *Secundae*, as well as ser. *Angulatae* species are well known to have pentagonal flowers. Given the resulting phylogenetic relationships here recovered, it seems reasonable to group the three series and *E. semivestita* varieties in a single series, regardless of different chromosomes numbers found for these series—x=12 for *E. bifida*, *E. lutea* Rose, *E. schaffneri*, *E. strictiflora*; x=13 for *E. shaviana* E.Walther, *E. walpoleana*; x=14 for *E. runyonii* Rose ex E.Walther; x=15 for *E. peacockii*; x=17 for *E. semivestita* var. *semivestita* and *E. semivestita* var. *floresiana*; x=28 for *E. minima* J.Meyrán; x=30/32 for *E. secunda* and *E. secunda* var. *byrnesii* (Rose) Kimnach; x=34 for *E. subalpina* and ca. x=60 for *E. tolucensis* Rose (Pilbeam 2008).

The monophyly for *Gibbiflorae* has been suggested in previous phylogenetic studies (Carrillo-Reyes *et al*. 2009; Vázquez-Cotero *et al*. 2017), although with a less representative sample (17.4% and 20%, respectively). Instead, our sampling for this series includes approximately 80% of *Gibbiflorae* alpha diversity and confirms the monophyly of the group. It corresponds to *Gibbiflorae sensu* Moran (1974)—with the addition of *E. guerrerensis* and *E. uxorium*—. Likewise, *Gibbiflorae* is divided in two well-supported clades (81/- - and 99/0.94, for bootstrap and Posterior Probabilities, respectively), one of which integrates northwestern Mexico taxa and the other one is composed by southwestern species. About the dichotomy in this group, in the taxonomic history of *Gibbiflorae*, some efforts have been made to recognize series *Retusae* E.Walther apart from *Gibbiflorae*, as some taxonomists have proposed that acaulescent versus caulescent trait could separate both series (García-Ruíz & Pérez-Calix 2007). However, based on our phylogenetic hypothesis, both traits are present indistinctly in these groups. E.g., in taxa from northwestern Mexico, *E. gibbiflora* DC., *E. munizii* Padilla-Lepe & A.Vázquez, *E. patriotica* I.García & Pérez-Calix (Fig. 6H), *E. purhepecha*, *E. rulfiana* and *E.* sp. develop a stem, whereas *E. cante* Glass & Mend.-Garc., *E. cerrograndensis* A.Vázquez & Nieves, *E. dactylifera*, *E. fulgens* var. *obtusifolia* (Rose) Kimnach, *E. marianae* I.García & Costea, *E. novogaliciana* J.Reyes, Brachet & O.González, *E. perezcalixii* Jimeno-Sevilla & P.Carrillo (Fig. 6H), *E. roseiflora* J.Reyes & O.González and *E. subrigida* Rose are known to be acaulescent. Similarly, the southwestern species, such as *E. guerrerensis*, *E. rubromarginata* Rose, *E. prunina* Kimnach & Moran and *E. triquiana* J.Reyes & Brachet are acaulescent plants, while *E. acutifolia* Lindl., *E. crenulata* Rose, *E. fimbriata* C.H.Thomps., *E. fulgens* var. *fulgens* Lem., *E. gigantea* Rose & J.A.Purpus, *E. longiflora* E.Walther, among others, have an evident stem.

No morphological character has been found that is exclusive for both clades within *Gibbiflorae*, but some trends are discussed here. On the one hand, the clade from northwestern Mexico tends to have larger flowers—more than 1.5 cm, except for *E. fulgens* var. *obtusifolia* and *E. purhepecha*—, the styles are frequently larger, the nectary scales usually are pinkish or reddish and less frequently yellowish or cream colored (Fig. 6I). Finally, many of the species in this clade have finger-like appendages on the inner side of the petals on the sides of the epipetalous filaments, e.g., *E. coruana* I.García , D.Valentín & Costea, *E. dactylifera*, *E. marianae*, *E. nayaritensis* Kimnachi, *E. novogaliciana* (Fig. 6J), *E. patriotica*, *E. perezcalixii*, *E. purhepecha* (Fig. 6J), *E. roseiflora* and *E. rulfiana* (Luis E. de la Cruz, pers. obs.). On the other hand, the southwestern Mexican species of *Gibbiflorae* possess smaller flowers (Fig. 6K), shorter styles, nectary scales which are frequently whitish (Fig. 6L) and less commonly yellowish, and finally, none of the species have appendages on the inner face of petals.

Shallow phylogenetic relationships within *Gibbiflorae* were not fully resolved here, so discussion about them will be treated in posterior opportunities. Nevertheless, two novel phylogenetic positions are underlined, in the first place *E. uxorium* was located in series *Racemosae* by Jimeno-Sevilla *et al*. (2012) based on the inflorescence type, which does not form cincinni. However, according to our analyses it must be placed in *Gibbiflorae*. One morphological character that associates *E. uxorium* with *Gibbiflorae* is the free styles and stigmas in anthesis—although in *E. uxorium* this is not very obvious—, while *Racemosae* species have both styles and stigmas joined in anthesis. Another novel phylogenetic position refers to *E. guerrerensis*, it was previously placed in *Urbiniae* by J.Reyes *et al*. (2011), but similar to the previous species, it is recovered in *Gibbiflorae*, and also presents free styles and stigmas that characterize all *Gibbiflorae* species, even though some ser. *Echeveria* and ser. *Nudae* species show this character too.

Conclusions

The results of our study indicate that the current infrageneric classification of genus *Echeveria* is highly artificial, many of the morphological characters used to circumscribe series are homoplasic and are not useful from a taxonomical point of view. The paraphyletic status of *Echeveria* along with other Mexican genera suggests a complex diversification history and that the "Echeveria Group" requires a redefinition. This is the first most comprehensive phylogenetic analysis for *Echeveria* and can be viewed as an important first step for a phylogeny-based classification. We suggest the separation of ser. *Urbiniae* from *Echeveria* and the re-establishment of the genus *Urbinia*. The genus *Echeveria* only includes series *Chloranthae*, *Ciliatae*, *Echeveria*, *Longistylae*, *Mucronatae*, *Nudae*, *Racemosae*, *Spicatae* and *Thyrsiflorae*, and the genus *Thompsonella* must be included as series in genus *Echeveria*. Ser. *Occidentales*, *Paniculatae* and *Valvatae* are somewhat related to genus *Graptopetalum*. Ser. *Angulatae*, *Pruinosae* and *Secundae* must be joined in one series. *Gibbiflorae* is monophyletic and is the sister clade of ser. *Secundae*. More DNA data are needed in order to solve shallow relationships of the "Echeveria Group", phylogenomic studies are very welcome in future revisions of the Mexican Crassulaceae.

Acknowledgements

Financial support for this work was provided by UNAM-DGAPA-PAPIIT [project IN212015, "Taxonomía integrativa y códigos de barras genéticos en *Echeveria* (Crassulaceae)" awarded to F. V.-S.] and CONACYT [Project 247078, "Iniciativa interdisciplinaria para el aprovechamiento sustentable del género *Echeveria* (Crassulaceae), plantas con potencial hortícola para la producción en áreas rurales de México" awarded to F. V.-S.] and Project [LANABIO: 232746 "Laboratorio Nacional de Biodiversidad"]. The first author acknowledges reception of graduate studies scholarships from Posgrado en Ciencias Biológicas, UNAM and CONACYT. We also thank Julia Etter and Martin Kristen for the plant material provided to extend the sampling for the study. Special thanks to the anonymous reviewer and editor Dr. Marie-Stéphanie Samain for the valuable comments that improved substantially the paper.

References

Acevedo-Rosas, R., Camero, K., Sosa, V. & Pell, S. (2004) A molecular phylogenetic study of *Graptopetalum* (Crassulaceae) based on ETS, ITS, *rpl16* and *trn*L-F nucleotide sequences. *American Journal of Botany* 91: 1099–1104. https://doi.org/10.3732/ajb.91.7.1099

Carrillo-Reyes, P., Sosa, V. & Mort, M.E. (2008) *Thompsonella* and the "Echeveria group" (Crassulaceae): phylogenetic relationships based on molecular and morphological characters. *Taxon* 57: 863–874. https://doi.org/10.1002/tax.573015

Carrillo-Reyes, P., Sosa, V. & Mort, M.E. (2009) Molecular phylogeny of the *Acre* clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*. *Molecular Phylogenetics and Evolution* 53: 267–276. https://doi.org/10.1016/j.ympev.2009.05.022

Chernomor, O., von Haeseler, A. & Minh, B.Q. (2016) Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 65: 997–1008. https://doi.org/10.1093/sysbio/syw037

García, R.I. & Pérez-Calix, E. (2007) Una especie nueva de *Echeveria* (Crassulaceae) originaria del estado de Jalisco, México. *Acta Botánica Mexicana* 78: 125–132. https://doi.org/10.21829/abm78.2007.1035

Gontcharova, S.B. & Gontcharov, A.A. (2007) Molecular phylogenetics of Crassulaceae. *Genes, Genomes and Genomics* 1: 40–46.

Ivanova, N.V., Dewaard, J.R & Hebert, P.D.N. (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6: 998–1002. https://doi.org/10.1111/j.1471-8286.2006.01428.x

Jimeno-Sevilla, H.D., Carrillo-Reyes, P., Pérez-Calix, E. & Cházaro-Basáñez, M.J. (2012) Additions to the Crassulaceae of the State of Veracruz, Mexico. *Haseltonia* 18: 140–152. https://doi.org/10.2985/026.018.0115

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kimnach, M. (2003) Echeveria. *In*: Eggli, U. (Ed.) *Illustrated handbook of succulent plants*: *Crassulaceae*. Springer–Verlag, Berlin, pp. 103–128.

Kimnach, M. (2010) The generic placement of *Echeveria cuicatecana*. *Cactus and Succulent Journal of America* 82: 125.

https://doi.org/10.2985/015.082.0306

Lanfear, R., Calcott, B., Ho, S. & Guindon, S. (2012) PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution* 29: 1695–1701.
https://doi.org/10.1093/molbev/mss020

Maddison, W.P. & Maddison, D.R. (2008) Mesquite: a modular system for evolutionary analysis. Version 2.5. Available from: http://mesquiteproject.org (accessed 21 October 2019)

Meyrán, J. & López, C.L. (2003) *Las Crasuláceas de México*. Sociedad Mexicana de Cactología AC, Ciudad de México, 234 pp.

Meyrán, J. & Vega, R. (1998) *Echeveria kimnachii*, una nueva especie de Sinaloa. *Cactáceas y Suculentas Mexicanas* 43: 6–9.

Minh, B.Q., Nguyen, M.A.T. & von Haeseler, A. (2013) Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution* 30: 1188–1195.
https://doi.org/10.1093/molbev/mst024

Moran, R. (1974) Echeveria. *In*: Jacobsen, H. (Ed.) *Lexicon of succulent plants*. Bland-Ford Press, London, pp. 184–186.

Mort, M.E., Soltis, D.E., Soltis, P.S., Francisco-Ortega, J. & Santos-Guerra, A. (2001) Phylogenetic Relationships and Evolution of Crassulaceae Inferred from *MATK* Sequence Data. *American Journal of Botany* 88: 76–91.
https://doi.org/10.2307/2657129

Nikulin, V.Y., Gontcharova, S.B., Stephenson, R. & Gontcharov A.A. (2016) Phylogenetic relationships between *Sedum* L. and related genera (Crassulaceae) based on ITS rDNA sequence comparisons. *Flora* 224: 218–229.
https://doi.org/10.1016/j.flora.2016.08.003

Pilbeam, J. 2008. *The genus Echeveria*. The British Cactus & Succulent Society, Essex, 333 pp.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G. & Suchard, M.A. (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* 67: 901–904.
https://doi.org/10.1093/sysbio/syy032

Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7, 355–364.
https://doi.org/10.1111/j.1471-8286.2007.01678.x

Reyes, S.J., Pérez, C.J. & Brachet, I.C. (2004) *Echeveria cuicatecana*, una nueva especie para el estado de Oaxaca, México. *Cactáceas y Suculentas Mexicanas* 49: 80–84.

Reyes, S.J., Brachet, I.C. & González-Zorzano, O. (2011) *Echeveria guerrerensis* (Crassulaceae) una nueva especie para el estado de Guerrero, México. Cactáceas y Suculentas Mexicanas 56: 75–81.

Reyes, S.J., Ávila, S.M. & Brachet, I.C. (2015) Una nueva especie del género *Cremnophila* (Crassulaceae) en el Estado de México, México. *Cactáceas y Suculentas Mexicanas* 60: 19–28.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology* 61: 539–42.
https://doi.org/10.1093/sysbio/sys029

Stöver, B.C. & Müller, K.F. (2010) TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* 11: 1–9.
https://doi.org/10.1186/1471-2105-11-7

Thiede, J. (2003) *Pachyphytum*. *In*: Eggli, U. (Ed.) *Illustrated Handbook of Succulent Plants: Crassulaceae*. Springer, Berlin, pp. 190–195.

Thiede, J. & Eggli, U. (2007) Crassulaceae. *In*: Kubitzki, K. (Ed.) *The Families and Genera of Vascular Plants 9*. Springer, Hamburg, pp. 83–118.
https://doi.org/10.1007/978-3-540-32219-1_12

Trifinopoulos, J., Nguyen, L.T., von Haeseler, A. & Minh, B.Q. (2016) W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Reserch* 44: 232–235.
https://doi.org/10.1093/nar/gkw256

Ulh, C,H. (1992) Polyploidy, dysploidy, and chromosomes pairing in *Echeveria* (Crassulaceae) and its hybrids. *American Journal of Botany* 79: 556–566.
https://doi.org/10.2307/2444868

van Ham, R.C.H.J. & t´Hart, H. (1998) Phylogenetic Relationships in the Crassulaceae Inferred from Chloroplast DNA Restriction-Site Variation. *American journal of botany* 85. 123–134.
https://doi.org/10.2307/2446561

Vázquez-Cotero, C., Sosa, V. & Carrillo-Reyes, P. (2017) Phylogenetic position of *Echeveria heterosepala* (Crassulaceae): a rare species with diagnostic characters of *Pachyphytum*. *Botanical Sciences* 95: 515–526.
https://doi.org/10.17129/botsci.1190

Walther, E. (1972) *Echeveria*. California Academy of Sciences, San Francisco, 426 pp.

Appendix 1. Voucher information, field number, Mexico state distribution, locality, herbarium, ITS2 accession, *rbcL* accession, *matK* accession and BOLD systems ID are listed in this order.

Crassula connata: MF963799.1; ***Crassula pellucida***: LN878931.1; ***Crassula rupestris***: LN878932.1; ***Cremnophila linguifolia***: *J. Reyes 5673*, Estado de México, Malinalco, MEXU, MG521431.1, MG521705.1, MG521585.1, JE-5673; ***Cremnophila linguifolia***: *J. Reyes 5677*, Estado de México, Barranca de Mexicapa, MEXU, MG521476.1, MG521752.1, MG521629.1, JE-5677; ***Cremnophila linguifolia***: AY545679.1; ***Cremnophila nutans***: *J. Ávila 26*, Morelos, Near Tepoztlán, MEXU, MG521426.1, MG521700.1, MG521580.1, JA-26; ***Cremnophila nutans***: *J. Ávila 112*, Morelos, Near Tepoztlán, MEXU, MG521498.1, MG521774.1, MG521649.1, JA-112; ***Cremnophila nutans***: AY545680.1; ***Dudleya attenuata***: AY545681.1; ***Dudleya pachyphytum***: JX960520.1; ***Echeveria acutifolia***: *J. Reyes 8753*, Oaxaca, A 6 km de la desviación a la carretera Huatulco al poblado de San Isidro Chacalapa, A 500m del Rio Chacalapilla, MEXU, MG521466.1, MG521742.1, MG521619.1, JE-8753; ***Echeveria*** **aff.** ***acutifolia***: *J. Reyes 8758-1*, Oaxaca, La Pedrera, cerca de la Ventosa y NE de Nizanda, MEXU, MG521477.1, MG521753.1, MG521630.1, JE-8758-1; ***Echeveria*** **aff.** ***bella***: *L. E. de la Cruz 195*, Chiapas, 2 Kilómetros al noreste de Tenejapa, MEXU, MG521434.1, MG521708.1, MG521588.1, LED-195; ***Echeveria*** **aff.** ***fulgens***: *J. Reyes 8816*, Guerrero, Km 23,800, camino a Olinalá, MEXU, MG521514.1, MG521791.1, MG521663.1, JE-8816; ***Echeveria*** **aff.** ***gibbiflora***: MG521487.1, JE-8624-2; ***Echeveria*** **aff.** ***gigantea***: *J. Reyes 5774*, Oaxaca, San Vicente del Vado, MEXU, MG217158.1, MG220442.1, MG220498.1, JE-5774; ***Echeveria*** **aff.** ***secunda***: *J. Reyes 4353*, Hidalgo, Actopan, MEXU, MG217168.1, MG220452.1, MG220508.1, JE-4353; ***Echeveria affinis***: *J. Reyes 6342*, Durango, Cerca de la Cd. de Durango, MEXU, MG217206.1, MG220490.1, MG220546.1, JE-6342; ***Echeveria agavoides***: *J. Reyes 4588*, Guanajuato, km 25 carretera 63 Ahualulco-Charcas, MEXU, MG217156.1, MG220440.1, MG220496.1, JE-4588; ***Echeveria alata***: *J. Reyes 6297*, Oaxaca, Santa María Ecatepec, MEXU, MG521823.1, MG521691.1, JE-6297; ***Echeveria amoena***: *J. Reyes 6344*, Puebla, Cañada Morelos, MEXU, MG217170.1, MG220454.1, MG220510.1, JE-6344; ***Echeveria amphoralis***: *J. Reyes 6806*, Oaxaca, 4 Km al sur de Vicente Guerrero, terracería a Textitlán, MEXU, MG521471.1, MG521747.1, MG521624.1, JE-6806; ***Echeveria bella*** **f.** ***major***: *J. Reyes 7312*, Chiapas, Km 60 carretera San Cristóbal de las Casas-Tuxtla Gutiérrez, Zinacantán, MEXU, MG521468.1, MG521744.1, MG521621.1, JE-7312; ***Echeveria bifida***: *J. Reyes 6341*, Hidalgo, entrada el Pueblo de Meztitlán, MEXU, MG217190.1, MG220474.1, MG220530.1, JE-6341; ***Echeveria bifurcata***: *J. Reyes 5516*, Hidalgo, Cerca de Ixmiquilpan, MEXU, MG521517.1, MG521794.1, MG521665.1, JE-5516; ***Echeveria brachetii***: *J. Reyes 8765*, Oaxaca, Camino San Pedro Yolox-San Juan Quiotepec, MEXU, MG521435.1, MG521709.1, MG521589.1, JE-8765; ***Echeveria calderoniae***: *J. Reyes 5196*, Aguascalientes, El Reparo, MEXU, MG217179.1, MG220463.1, MG220519.1, JE-5196; ***Echeveria calycosa***: *J. Reyes 5570*, Michoacán, Salto de Tzararacua, MEXU, MG217205.1, MG220489.1, MG220545.1, JE-5570; ***Echeveria canaliculata***: *J. Reyes 5374*, Veracruz, Monte Blanco, MEXU, MG521483.1, MG521759.1, JE-5374; ***Echeveria cante***: *J. Reyes 5246*, Zacatecas, km 9 carretera Fresnillo-Sombrerete, MEXU, MG217162.1, MG220446.1, MG220502.1, JE-5246; ***Echeveria carminea***: *J. Reyes 6283*, Oaxaca, km 35 entre Concepción y Tenango, MEXU, MG217152.1, MG220436.1, MG220492.1, JE-6283; ***Echeveria carnicolor***: *J. Reyes 8353*, Veracruz, Barranca de Tenampa, MEXU, MG521501.1, MG521777.1, JE-8353; ***Echeveria cerrograndensis***: *J. Etter & M. Kristen 3749*, Jalisco, Mirador los 7 Palos, Tolimán, MEXU, MG521450.1, MG521724.1, MG521603.1, EK-3749; ***Echeveria chapalensis***: *J. Reyes 5070*, Michoacán, Cañada de Agua, MEXU, MG217193.1, MG220477.1, MG220533.1, JE-5070; ***Echeveria chazaroi***: *J. Reyes 7735*, Oaxaca, Cerca de Yosonicaje, Santa María Yucuhiti, MEXU, MG521499.1, MG521775.1, MG521650.1, JE-7735; ***Echeveria chiapensis***: *J. Reyes 6099*, Oaxaca, Cañada el Botado, MEXU, MG217155.1, MG220439.1, MG220495.1, JE-6099; ***Echeveria chihuahuensis***: *A. Gutiérrez 21*, Chihuahua, A 1.3 km antes de la desviación a Uriachi, Terraceria Cascada Basaseachic a San Juanito, Basaseachic, MEXU, MG521480.1, MG521756.1, MG521633.1, ARA-21; ***Echeveria coccinea***: *J. Reyes 5646*, Hidalgo, Cerro Xicuco, MEXU, MG217187.1, MG220471.1, MG220527.1, JE-5646; ***Echeveria coccinea***: AY545682.1; ***Echeveria colorata***: *J. Etter & M. Kristen 3791*, Jalisco, 4 km NO de Mixtlán, Carretera Mascota-Ameca, Mixtlán, MEXU, MG521515.1, MG521793.1, EK-3791; ***Echeveria colorata***: AY545683.1; ***Echeveria craigiana***: *J. Reyes 6343*, Chihuahua, Entre Creel y San Juanito, MEXU, MG217186.1, MG220470.1, MG220526.1, JE-6343; ***Echeveria crassicaulis***: *J. Reyes 5354*, Ciudad de México, La Cima, MEXU, MG217157.1, MG220441.1, MG220497.1, JE-5354; ***Echeveria crenulata***: *J. Reyes 5676a*, Estado de México, Barranca de Mexicapa, MEXU, MG217185.1, MG220469.1, MG220525.1, JE-5676a; ***Echeveria crenulata***: *L. E. de la Cruz 182*, Morelos, Km 11 carretera Xochitlán-Ocuituco, Yecapixtla, MEXU, MG521420.1, MG521694.1, MG521574.1, LED-182; ***Echeveria cuicatecana***: *J. Pérez 584*, Oaxaca, Cuicatlán, MEXU, MG217184.1, MG220468.1, MG220524.1, JPC-584; ***Echeveria cuspidata*** **var.** ***cuspidata***: *J. Reyes 5624*, Nuevo León, Rancho El Enebro, MEXU, MG217175.1, MG220459.1, MG220515.1, JE-5624; ***Echeveria cuspidata*** **var.** ***zaragozae***: *J. Etter & M. Kristen 2967*, Nuevo León, Cañón cerca de La Joya de

Alardín, General Zaragoza, MEXU, MG521448.1, MG521722.1, MG521601.1, EK-2967; ***Echeveria dactylifera***: *J. Reyes 6274*, Sinaloa, Espinazo del Diablo, MEXU, MG217161.1, MG220445.1, MG220501.1, JE-6274; ***Echeveria derenbergii***: *J. Reyes 6335*, Oaxaca, Cañada Grande entre San Antonio Abad-La Mexicana, Santiago Ihuitlán Plumas, MEXU, MG521497.1, MG521773.1, MG521648.1, JE-6335; ***Echeveria diffractens***: *J. Reyes 5835*, Veracruz, Km 10 Oeste de Palma Sola, Alto Lucero, MEXU, MG521465.1, MG521741.1, MG521618.1, JE-5835; ***Echeveria elegans***: *J. Reyes 6345*, Hidalgo, Parque Nacional `El Chico`, MEXU, MG217181.1, MG220465.1, MG220521.1, JE-6345; ***Echeveria fimbriata***: *L. E. de la Cruz 174-3*, Morelos, Cerro de la Cruz, Noeste a 1.5 Km de San Juan Tlacotenco, MEXU, MG521442.1, MG521716.1, MG521595.1, LED-174-3; ***Echeveria fimbriata***: *J. Reyes 8794*, Morelos, Cerro de la Cruz, Noreste a 1.5 Km de San Juan Tlacotenco, MEXU, MG521486.1, MG521762.1, MG521638.1, JE-8794; ***Echeveria fulgens* var. *fulgens***; *J. Reyes 6017*, Guerrero, La Mora, MEXU, MG217178.1, MG220462.1, MG220518.1, JE-6017; ***Echeveria fulgens* var. *fulgens***: AY545684.1; ***Echeveria gibbiflora***: *J. Reyes 5666*, Estado de México km 51 carretera a Sultepec, MEXU, MG217171.1, MG220455.1, MG220511.1, JE-5666; ***Echeveria gibbiflora***: AY545685.1; ***Echeveria gigantea***: *J. Reyes 5151*, Oaxaca, Entre Nduayaco y Texcatitlán, MEXU, MG217194.1, MG220478.1, MG220534.1, JE-5151; ***Echeveria globuliflora***: *J. Reyes 6464*, Estado de México, Cerro Jilotepec, Cerca de San Pedro Jilotepec, MEXU, MG521507.1, MG521783.1, MG521656.1, JE-6464; ***Echeveria globulosa***: *J. Reyes 6110*, Oaxaca, Rio de las Chaparritas, Rio abajo de Guadalupe Carrizal, Villa Diaz Ordaz, MEXU, MG521462.1, MG521737.1, MG521614.1, JE-6110; ***Echeveria goldmanii***: *J. Reyes 8425*, Chiapas, La Cueva a 500 metros al este del Km 191.2 de la carretera Comitán-Cd. Cuauhtémoc, sur de la Trinitaria, La trinitaria, MEXU, MG521508.1, MG521784.1, JE-8425; ***Echeveria gracilis***: *J. Reyes 8667*, Oaxaca, Carretera de Teotitlán-Huautla, Teotitlán de Flores Magón, MEXU, MG521523.1, MG521801.1, JE-8667; ***Echeveria grisea***: *J. Reyes 8720*, Guerrero, Km 34, carretera Paso Morelos-Iguala, Huitzuco de los Figueroa, MEXU, MG521491.1, MG521767.1, MG521642.1, JE-8720; ***Echeveria guerrerensis***: *J. Reyes 7526*, Guerrero, Carretera 134, Zihuatanejo-Cd Altamirano, MEXU, MG521536.1, MG521816.1, MG521684.1, JE-7526; ***Echeveria halbingeri* var. *halbingeri***: *D. Jimeno-Sevilla 1318*, Veracruz, Ojo de agua, Barranca de Santiago, Huayacocotla, MEXU, MG521460.1, MG521735.1, MG521612.1, DJ-1318; ***Echeveria helmutiana***: *J. Reyes 7075a*, Oaxaca, Terracería Juxtlahuaca-Yucunicoco, Santiago Juxtlahuaca, MEXU, MG521504.1, MG521780.1, MG521653.1, JE-7075a; ***Echeveria heterosepala***: *J. Reyes 5813*, Oaxaca, El Cebollal, MEXU, MG217189.1, MG220473.1, MG220529.1, JE-5813; ***Echeveria humilis***: *J. Reyes 5506*, Hidalgo, Barranca El Carrizal, MEXU, MG217195.1, MG220479.1, MG220535.1, JE-5506; ***Echeveria juliana***: *J. Etter & M. Kristen 3056*, Sinaloa, Carretera Tayoltita-San Ignacio, San Ignacio, MEXU, MG521430.1, MG521704.1, MG521584.1, EK-3056; ***Echeveria kimnachii***: *J. Reyes 5131*, Sinaloa, Estancia de los García, MEXU, MG217159.1, MG220443.1, MG220499.1, JE-5131; ***Echeveria laui***: J. Reyes 6273, Oaxaca, norte de Quiotepec, MEXU, MG217172.1, MG220456.1, MG220512.1, JE-6273; ***Echeveria lilacina***: *J. Etter & M. Kristen 2957*, Nuevo León, Montemorelos, Primer cañón a la derecha después de La Merced, Galeana, MEXU, MG521445.1, MG521719.1, MG521598.1, EK-2957; ***Echeveria longiflora***: *J. Reyes 6013*, Guerrero, Cruz Verde, MEXU, MG217183.1, MG220467.1, MG220523.1, JE-6013; ***Echeveria longipes***: J. Reyes 5650, Hidalgo, Cerro Xicuco, MEXU, MG217165.1, MG220449.1, MG220505.1, JE-5650; ***Echeveria longissima* var. *aztatlensis***: *J. Reyes 3741*, Oaxaca, San Miguel Aztatla, MEXU, MG217202.1, MG220486.1, MG220542.1, JE-3741; ***Echeveria longissima* var. *brachyantha***: *J. Reyes 5593*, Oaxaca, Cerro Yucunino, MEXU, MG217169.1, MG220453.1, MG220509.1, JE-5593; ***Echeveria lurida***: *J. Reyes 8360*, Veracruz, Pedregal de Almolonga, Xalapa, MEXU, MG521438.1, MG521712.1, MG521592.1, JE-8360; ***Echeveria lutea***: *J. Reyes 5293*, San Luis Potosí, Las Rusias, MEXU, MG217177.1, MG220461.1, MG220517.1, JE-5293; ***Echeveria lyonsii***: *J. Etter & M. Kristen 2975*, Tamaulipas, Miquihuana-Las Joyas, cerca de La Peña, Miquihuana, MEXU, MG521427.1, MG521701.1, MG521581.1, EK-2975; ***Echeveria macdougalli***: *J. Reyes 6305*, Oaxaca, Cerro de la flor, 3 Km al oeste de Santiago Lachiguiri-El Porvenir, Santiago Lachiguiri, MEXU, MG521470.1, MG521746.1, MG521623.1, JE-6305; ***Echeveria marianae***: *J. Etter & M. Kristen 3405*, Jalisco, Valle de Juárez, MEXU, MG521478.1, MG521754.1, MG521631.1, EK-3405; ***Echeveria megacalyx***: *J. Reyes 8666*, Oaxaca, Carretera de Teotitlán-Huautla, Teotitlán de Flores Magón, MEXU, MG521506.1, MG521782.1, MG521655.1, JE-8666; ***Echeveria michihuacana***: *J. Reyes 8211-2*, Michoacán, Km. 179 de la carretera Cd. Hidalgo-Morelia, Mil Cumbres, MEXU, MG521479.1, MG521755.1, MG521632.1, JE-8211-2; ***Echeveria michihuacana***: *L. E. de la Cruz 184-1*, Michoacán, Carretera Morelia-Cd. Hidalgo, MEXU, MG521500.1, MG521776.1, MG521651.1, LED-184-1; ***Echeveria michihuacana***: *L. E. de la Cruz 184-2*, Michoacán, Carretera Morelia-Cd. Hidalgo, MEXU, MG521472.1, MG521748.1, MG521625.1, LED-184-2; ***Echeveria michihuacana***: *L. E. de la Cruz 184-3*, Michoacán, Carretera Morelia-Cd. Hidalgo, MEXU, MG521440.1, MG521714.1, LED-184-3; ***Echeveria michihuacana***: *L. E. de la Cruz 184-4*, Michoacán, Carretera Cd. Hidalgo-Jeráhuaro de Juárez, MEXU, MG521425.1, MG521699.1, MG521579.1, LED-184-4; ***Echeveria michihuacana***: *L. E. de la Cruz 184-5*, Michoacán, Carretera Cd. Hidalgo-Jeráhuaro de Juárez, MEXU, MG521421.1, MG521695.1, MG521575.1, LED-184-5; ***Echeveria microcalyx***: *J. Reyes 5848*, Veracruz, entre Cruz Blanca y La Mina, MEXU, MG217176.1, MG220460.1, MG220516.1, JE-5848; ***Echeveria minima***: *J. Reyes 5652*, Hidalgo, Puerto Juárez, MEXU, MG217164.1, MG220448.1, MG220504.1, JE-5652; ***Echeveria mondragoniana***: *J. Reyes 8839*, Oaxaca, Nevería, Terracería a Santo Domingo Ozolotepec. A 100 m del Criadero de truchas, Santo Domingo Ozolotepec, MEXU, MG521458.1, MG521733.1, JE-8839; ***Echeveria montana***: *J. Reyes 7513*, Guerrero, Entre el Edén y Puerto del Gallo, Chilpancingo de los Bravo, MEXU, MG521449.1, MG521723.1, MG521602.1, JE-7513; ***Echeveria moranii***: *J. Reyes 4553*, Oaxaca, Km. 70-72 carretera Oaxaca-Tehuantepec, San Pedro Totolapan, MEXU, MG521495.1, MG521771.1, MG521646.1, JE-4553; ***Echeveria mucronata***: *J. Reyes 6102*, Oaxaca, Corral de Piedra a Nuevo Zoquiapan, MEXU, MG217198.1, MG220482.1, MG220538.1, JE-6102; ***Echeveria multicaulis***: *J. Reyes 5584*, Guerrero, Filo de Caballo a Yextla, MEXU, MG217192.1, MG220476.1, MG220532.1, JE-5584; ***Echeveria munizii***: *J. Etter & M. Kristen 3415*, Colima, 2 Km antes de la Hacienda San Antonio, Carretera Comala-San Antonio, MEXU, MG521520.1, MG521798.1, MG521669.1, EK-3415; ***Echeveria nayaritensis***: *J. Reyes 5676a*, Jalisco, Rio Otates, Km 29 Carretera Ameca-Talpa, Guachinango, MEXU, MG521525.1, MG521804.1, JE-5676a; ***Echeveria nebularum***: *J. Reyes 5740*, Oaxaca, Cueva de los Perros, MEXU, MG217201.1, MG220485.1, MG220541.1, JE-5740; ***Echeveria novogaliciana***: *J. Reyes 6823*, Jalisco, Cerro Colli, sureste del Estadio de Futbol de las Chivas, Zapopan, MEXU, MG521436.1, MG521710.1, MG521590.1, JE-6823; ***Echeveria nuda***: *J. Reyes 5824*, Veracruz, km 231-232, carretera Orizaba-Puebla, MEXU, MG217153.1, MG220437.1, MG220493.1, JE-5824; ***Echeveria olivacea***, *J. Reyes 6402*, Oaxaca, Cerro San Pedro, San Miguel Tenango, MEXU, MG521464.1, MG521739.1, MG521616.1, JE-6402; ***Echeveria paniculata* var. *maculata***: *J. Reyes 8897*, Guanajuato, La Cuesta, MEXU, MG521490.1, MG521766.1, MG521641.1, JE-8897; ***Echeveria patriotica***: *J. Reyes 6813*, Michoacán, Entre Palo Dulzal y Palo Herrado carretera Quitupán a Santa María del Cerro, MEXU, MG521456.1, MG521731.1, MG521609.1, JE-6813; ***Echeveria peacockii***: *J. Reyes 6272*, Puebla, Cerro Cuthá, MEXU, MG217196.1, MG220480.1, MG220536.1, JE-6272; ***Echeveria penduliflora***: *J. Reyes 5773*, Oaxaca, Entre el Vado y San Sebastián de las Grutas, MEXU, MG217174.1, MG220458.1, MG220514.1, JE-5773; ***Echeveria perezcalixii***: *P. Carrillo-Reyes s/n*, Zacatecas, cerca de Teúl González, MEXU, MG521538.1, MG521818.1, MG521686.1, EPC1PC; ***Echeveria pinetorum***: *J. Reyes 5991*, Oaxaca, 1 km al oeste de Reyes Pápalos, MEXU, MG217173.1, MG220457.1, MG220513.1, JE-5991; ***Echeveria platyphylla***: *J. Reyes 6271*, Ciudad de México, Reserva Ecológica del Pedregal de San Ángel, MEXU, MG217207.1, MG220491.1, MG220547.1, JE-6271; ***Echeveria potosina***: AY545686.1; ***Echeveria pringlei***: AY545687.1; ***Echeveria pringlei* var. *parva***: *J. Reyes 7979*, Jalisco, Barranca Colimilla. Entre Tonalá y Zapopan, Tonalá, MEXU, MG521496.1, MG521772.1, MG521647.1, JE-7979; ***Echeveria prunina***: *C. Betespadier s/n*, Chiapas, Chiapa de Corzo, MEXU, MG521541.1, MG521821.1, MG521689.1, CB-1a; ***Echeveria pulvinata***: *J. Reyes 8530*, Oaxaca, Arriba de la Montaña Roja a 3 Km de Apoala. Al Suroeste de Apoala. Santiago Apoala, MEXU, MG521510.1, MG521786.1, MG521658.1, JE-8530; ***Echeveria pulvinata***: AY545688.1; ***Echeveria purhepecha***: *J. Reyes 8637*, Michoacán, a un costado NE del panteón de San Juan Nuevo Parangaricutiro, MEXU, MG521481.1, MG521757.1, MG521634.1, JE-8637; ***Echeveria purpusorum***: *J. Reyes 6338*, Oaxaca, Cañada Grande, MEXU, MG217199.1, MG220483.1, MG220539.1, JE-6338; ***Echeveria rodolfi***: *J. Reyes 6205*, Tamaulipas, Terracería paso de la muerte rumbo a Gertrudis, Hidalgo, MEXU, MG521513.1, MG521790.1, MG521662.1, JE-6205; ***Echeveria rosea***: *J. Reyes 5635*, Veracruz, Entre Carpinteros y Huayacocotla, MEXU, MG217154.1, MG220438.1, MG220494.1, JE-5635; ***Echeveria roseiflora***: *G. Salazar 6740*, Jalisco, Entre Juanacatlán y Mascota, MEXU, MG217203.1, MG220487.1, MG220543.1, GAS-6740; ***Echeveria rubromarginata***: *L. E. de la Cruz 190*, Tlaxcala, Desviación de la autopista San Martin Texmelucan-Tlaxcala rumbo a Huiloapan, MEXU, MG521439.1, MG521713.1, MG521593.1, LED-190; ***Echeveria rubromarginata***: *L. E. de la Cruz 192*, Veracruz, Autopista 50D Esperanza-Orizaba, MEXU, MG521503.1, MG521779.1, LED-192;; ***Echeveria rulfiana***: *J. Etter & M. Kristen 3408*, Jalisco, Sayula-San Gabriel, Ciudad Venustiano Carranza, MEXU, MG521447.1, MG521721.1, MG521600.1, EK-3408; ***Echeveria runyonii***: *J. Reyes 7649*, Tamaulipas, Ejido el Gavilán a Silva Vázquez, San Carlos, MEXU, MG521452.1, MG521726.1, JE-7649; ***Echeveria secunda***: *J. Reyes 6266*, Estado de México, San Rafael, MEXU, MG217166.1, MG220450.1, MG220506.1, JE-6266; ***Echeveria semivestita* var. *floresiana***: *J. Reyes 7624*, Tamaulipas, Cerca de los Acahuales, carretera Tula-Cd. Mante, cerca del Puerto, Tula, MEXU, MG521543.1, MG521824.1, MG521692.1, JE-7624; ***Echeveria semivestita* var. *semivestita***: *J. Reyes 5309*, Querétaro, Ahuacatlán, MEXU, MG217188.1, MG220472.1, MG220528.1, JE-5309; ***Echeveria sessiliflora***: *J. Reyes 8441*, Chiapas, a 1.5 Km de la desviación al Puerto de San Lorenzo, de la carretera Las Rosas-Teopisca, Venustiano Carranza, MEXU, MG521469.1, MG521745.1, MG521622.1, JE-8441; ***Echeveria setosa***: AY545689.1; ***Echeveria setosa* var. *ciliata***: *J. Reyes 5152*, Oaxaca, a 24.5 Km Carretera Huajuapan de León-Tamazulapan, San Andrés Dinicuiti, MEXU, MG521537.1, MG521817.1, MG521685.1, JE-5152; ***Echeveria setosa* var. *deminuta***: *J. Reyes 3795*, Oaxaca, Rio Elitte, al norte de San Pedro Nopala, San Pedro Nopala, MEXU, MG521533.1, MG521813.1, MG521681.1, JE-3795;

Echeveria shaviana: *J. Reyes 6195*, Tamaulipas, Cerca de Dulces Nombres, Hidalgo, MEXU, MG521429.1, MG521703.1, MG521583.1, JE-6195; ***Echeveria shaviana***: MF348889.1; ***Echeveria simulans***: *J. Reyes 8180*, Nuevo León, 1.5 km E de Iturbide, carretera Matehuala-Linares, Iturbide, MEXU, MG521489.1, MG521765.1, MG521640.1, JE-8180; ***Echeveria* sp.**: *J. Etter & M. Kristen 3413*: Jalisco, Nevado de Colima, Tuxpan, MEXU, MG521422.1, MG521696.1, MG521576.1, EK-3413; ***Echeveria* sp.**: *J. Etter & M. Kristen 3682*, Nuevo León, Cuesta de Mamulique, km 63 de la carretera libre Monterrey-Ciénega de Flores-Laredo, Salinas Victoria, MEXU, MG521451.1, MG521725.1, MG521604.1, EK-3682; ***Echeveria* sp.**: *J. Reyes 6761*, Michoacán, Peña la Orolicua a 1 Km al Oeste de Rincón de Sánchez, Tuxpan, MEXU, MG521441.1, MG521715.1, MG521594.1, JE-6761; ***Echeveria* sp.**: *J. Reyes 7230*, Oaxaca, a 2 Km al sur de Guadalupe Carrizal, Terracería a San Miguel del Valle, MEXU, MG521542.1, MG521822.1, MG521690.1, JE-7230; ***Echeveria* sp.**: *J. Reyes 8526*, Oaxaca, Peña de los nueve Chuparosas, Apoala, MEXU, MG521423.1, MG521697.1, MG521577.1, JE-8526; ***Echeveria* sp.**: *J. Reyes 8600*, Michoacán, Ejido Patámbaro, Tuxpan, MEXU, MG521457.1, MG521732.1, MG521610.1, JE-8600; ***Echeveria* sp.**: *J. Reyes 8624*, Michoacán, Km 263 de la carretera Arteaga-Uruapan, MEXU, MG521487.1, MG521763.1, JE-8624; ***Echeveria* sp.**: *J. Reyes 8725*, Guerrero, Km 7 al Oeste de Amojiteca, rumbo a Omiltemi, Chilpancingo de los Bravo, MEXU, MG521433.1, MG521707.1, MG521587.1, JE-8725; ***Echeveria* sp.**: *J. Reyes 8769*, Oaxaca, cerca de San Pedro Yolox, MEXU, MG521534.1, MG521814.1, MG521682.1, JE-8769; ***Echeveria* sp.**: *J. Reyes 8771-1*, Oaxaca, Terracería entre San Juan Quiotepec-Malinaltepec, MEXU, MG521532.1, MG521811.1, MG521679.1, JE-8771-1; ***Echeveria* sp.**: *J. Reyes 8814*, Guerrero, Cañada La Loma, al Noroeste de Tlapa de Comonfor, MEXU, MG521485.1, MG521761.1, MG521637.1, JE-8814; ***Echeveria* sp.**: *J. Reyes 8823*, Guerrero, Cerro Cistepec, Ca. de 6 km N de Cualac, Cualac, MEXU, MG521463.1, MG521738.1, MG521615.1, JE-8823; ***Echeveria strictiflora***: *J. Etter & M. Kristen 3684*, Nuevo León, Ojo de Agua, Mina, MEXU, MG521459.1, MG521734.1, MG521611.1, EK-3684; ***Echeveria subalpina***: *J. Reyes 6643*, Puebla, a 1 Km de El Sabinal-Cd. Serdán, Chalchicomula de Sesma, MEXU, MG521502.1, MG521778.1, MG521652.1, JE-6643; ***Echeveria subcorymbosa***: *J. Reyes 7068*, Oaxaca, 500 m al norte de la Laguna Encantada, San Sebastián Tecomaxtlahuaca, MEXU, MG521511.1, MG521787.1, MG521659.1, JE-7068; ***Echeveria subrigida***: *J. Reyes 5287*, San Luis Potosí, El Puerto, MEXU, MG217197.1, MG220481.1, MG220537.1, JE-5287; ***Echeveria tamaulipana***: *O. González Z. 189*, Tamaulipas, del Pueblo de la Reforma rumbo a la Reja, Victoria, MEXU, MG521526.1, MG521805.1, MG521674.1, OZ-189; ***Echeveria tencho***: *J. Reyes 8228*, Veracruz, Ejido Belén Chico, a 4 millas NE de San Andrés Tuxtla, MEXU, MG521540.1, MG521820.1, MG521688.1, JE-8228; ***Echeveria tobarensis***: *J. Etter & M. Kristen 3121*, Durango, Rancho Los Sauces cerca de Mina Tovar, Tepehuanes, MEXU, MG521424.1, MG521698.1, MG521578.1, EK-3121; ***Echeveria tolimanensis***: *J. Reyes 6971*, Hidalgo, Mina Lomo de Toro, Zimapán, MEXU, MG521519.1, MG521796.1, MG521667.1, JE-6971; ***Echeveria trianthina***: *L. E. de la Cruz 183*, Hidalgo, Barranca de Tolantongo, MEXU, MG521524.1, MG521802.1, MG521672.1, LED-185; ***Echeveria triquiana***: *J. Reyes 5614*, Oaxaca, Santiago Naranja, MEXU, MG217204.1, MG220488.1, MG220544.1, JE-5614; ***Echeveria turgida***: *J. Reyes 8292*, Coahuila, Km 198.5 carretera Torreón-Saltillo, Parras, MEXU, MG521527.1, MG521806.1, JE-8292; ***Echeveria uhlii***: *J. Reyes 8553*, Oaxaca, Cañada del cerro Pericón, Nopala, San Pedro Nopala, MEXU, MG521437.1, MG521711.1, MG521591.1, JE-8553; ***Echeveria unguiculata***: *J. Reyes 5941*, San Luis Potosí, San Antonio de la Ordena, Villa de Guadalupe, MEXU, MG521446.1, MG521720.1, MG521599.1, JE-5941; ***Echeveria uxorium***: *J. Reyes 8702*, Puebla, a 500 m pasando el poblado de Zacatepec a Tlacotepec, San Sebastián Tlacotepec, MEXU, MG521467.1, MG521743.1, MG521620.1, JE-8702; ***Echeveria valvata***: *J. Reyes 5563*, Estado de México, Luvianos, MEXU, MG217182.1, MG220466.1, MG220522.1, JE-5563; ***Echeveria viridissima***: *J. Reyes 6275a*, Oaxaca, cerro el Mojón, oeste de San Juan Ozolotepec, colindante a San Pedro Mixtepec, cerca del cerro Siempreviva, Santo Domingo Ozolotepec, MEXU, MG521512.1, MG521788.1, MG521660.1, JE-6275a; ***Echeveria waltheri***: *J. Reyes 8574*, Estado de México, Km. 70 de la carretera libre Ixtapan de la Sal a Toluca, a 200 metros de Llano de la Unión, MEXU, MG521516.1, JE-8574; ***Echeveria xichuensis***: *L. López 370*, Guanajuato, 6 km NO de Xichu, MEXU, MG217200.1, MG220484.1, MG220540.1, LG Lopez-370; ***Echeveria zorzaniana***: *J. Reyes 5218*, Oaxaca, San Miguel del Valle, MEXU, MG217160.1, MG220444.1, MG220500.1, JE-5218; ***Graptopetalum amethystinum***: AY545690.1; ***Graptopetalum bartramii***: AY545691.1; ***Graptopetalum bernalense***: AY545692.1; ***Graptopetalum craigii***: AY545693.1; ***Graptopetalum filiferum***: AY545694.1: ***Graptopetalum fruticosum***: AY545695.1: ***Graptopetalum glassii***: AY545696.1; ***Graptopetalum grande***: AY545697.1; ***Graptopetalum macdougallii***: AY545698.1; ***Graptopetalum marginatum***: AY545699.1; ***Graptopetalum mendozae***: AY545700.1; ***Graptopetalum pachyphyllum***: AY545701.1; ***Graptopetalum paraguayense***: AY545702.1; ***Graptopetalum pentandrum***: AY545703.1; ***Graptopetalum pusillum***: AY545704.1; ***Graptopetalum rusbyi***: AY545705.1; ***Graptopetalum saxifragoides***: AY545706.1; ***Graptopetalum suaveolens***: AY545707.1; ***Graptopetalum superbum***: MF348924.1; ***Graptopetalum superbum***: AY545708.1; ***Lenophyllum acutifolium***: AY545709.1; ***Lenophyllum texanum***: HE999632.1; ***Pachyphytum brachetii***:

J. Reyes 4350, Hidalgo, Actopan; MEXU; MG217180.1, MG220464.1, MG220520.1, JE-4350; ***Pachyphytum bracteosum***: *J. Reyes 6275*, Hidalgo, Laguna de Meztitlán, MEXU, MG217163.1, MG220447.1, MG220503.1, JE-6275; ***Pachyphytum brevifolium***: *J. Reyes 5048*, Guanajuato, Los Picones, MEXU, MG217167.1, MG220451.1, MG220507.1, JE-5048; ***Pachyphytum caesium***: *J. Reyes 5185*, Aguascalientes, Barranca Montoro, MEXU, MG217191.1, MG220475.1, MG220531.1, JE-5185; ***Pachyphytum compactum***: *J. Reyes 5273*, Querétaro, La Cañada, MEXU, MG521432.1, MG521706.1, MG521586.1, JE-5273; ***Pachyphytum contrerasii***: *J. Reyes 5627*, Jalisco, Mirador Dr. Atl, MEXU, MG521484.1, MG521760.1, MG521636.1, JE-5627; ***Pachyphytum fittkaui***: *J. Reyes 5237*, Guanajuato, Los Jofres, MEXU, MG521528.1, MG521807.1, MG521675.1, JE-5237; ***Pachyphytum garciae***: *J. Reyes 5885*, Querétaro, El Zapote, MEXU, MG521488.1, MG521764.1, MG521639.1, JE-5885; ***Pachyphytum glutinicaule***: *J. Reyes 5653*, Hidalgo, Puerto Juárez, MEXU, MG521461.1, MG521736.1, MG521613.1, JE-5653; ***Pachyphytum glutinicaule***: AY545710.1; ***Pachyphytum hookeri***: *J. Reyes 5036*, Guanajuato, Cerro Gordo, MEXU, MG521539.1, MG521819.1, MG521687.1, JE-5036; ***Pachyphytum kimnachii***: *J. Reyes 5305*, San Luis Potosí, Cerro Agujón, MEXU, MG521530.1, MG521809.1, MG521677.1, JE-5305; ***Pachyphytum longifolium***: *J. Reyes 6276*, Hidalgo, entre Gilo y Almolón, MEXU, MG521419.1, MG521693.1, MG521573.1, JE-6276; ***Pachyphytum machucae***: *J. Reyes 5071*, Michoacán, Cañada de Agua, MEXU, MG521492.1, MG521768.1, MG521643.1, JE-5071; ***Pachyphytum oviferum***: *J. Reyes 6038*, San Luis Potosí, El Cajón, MEXU, MG521444.1, MG521718.1, MG521597.1; JE-6038; ***Pachyphytum rzedowskii***: *J. Reyes 5562*, Michoacán, 1.5 km NO de Puente Grande, MEXU, MG521494.1, MG521770.1, MG521645.1, JE-5562; ***Pachyphytum saltense***: *J. Reyes 3444*, Zacatecas, El Salto, MEXU, MG521521.1, MG521799.1, MG521670.1, JE-3644; ***Pachyphytum* sp.**: *J. Reyes 5043*, Guanajuato, Las Presitas, MEXU, MG521518.1, MG521795.1, MG521666.1, JE-5043; ***Pachyphytum viride***: *J. Reyes 5873*, Guanajuato, entrada al Pueblo de Coconoxtle, MEXU, MG521535.1, MG521815.1, MG521683.1, JE-5873; ***Pachyphytum viride***: AY545711.1; ***Pachyphytum werdermannii***: *J. Reyes 5451*, Tamaulipas, Cañón de Nogales, MEXU, MG521509.1, MG521785.1, MG521657.1, JE-5451; ***Sedum allantoides***: AY545712.1; ***Sedum clavatum***: AY545713.1; ***Sedum compactum***: AY545714.1; ***Sedum corynephyllum***: AY545715.1; ***Sedum dendroideum***: HE999652.2; ***Sedum frutescens***: HE999657.1; ***Sedum palmeri***: AY545717.1; ***Tacitus bellus***: AY545718.1; ***Thompsonella minutiflora***: *J. Reyes 4520a*, Puebla, Cerro Cuthá, MEXU, MG521522.1, MG521800.1, MG521671.1, JE-4520a; ***Thompsonella minutiflora***: AY545719.1; ***Thompsonella mixtecana***: *J. Reyes 4200*: Oaxaca, La Esperanza, MEXU, MG521531.1, MG521810.1, MG521678.1, JE-4200; ***Thompsonella spathulata***: *J. Reyes 6277*, Oaxaca, MEXU, MG521529.1, MG521808.1, MG521676.1, JE-6277; ***Thompsonella spathulata***: AY545720.1; ***Thompsonella xochipalensis***: *J. Reyes 5350*, Guerrero, El Tanque, MEXU, MG521475.1, MG521751.1, MG521628.1, JE-5350; ***Villadia albiflora***: *J. Reyes 6094*, Oaxaca, entre San Vicente y Anama, MEXU, MG521453.1, MG521727.1, MG521605.1, JE-6094; ***Villadia cucullata***: *J. Reyes 5904*, San Luis Potosí, desviación a Jaujal, MEXU, MG521454.1, MG521728.1, MG521606.1, JE-5904.

Capítulo 3. Taxonomía integrativa basada en caracteres en la serie *Gibbiflorae*

Título: Phylogenetic relationships and character-based DNA barcoding on the integrative taxonomy of ser. *Gibbiflorae* (*Echeveria*, Crassulaceae)

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Para ser sometido a: *Phytokeys*

Phylogenetic relationships and character-based DNA barcoding on the integrative taxonomy in ser. *Gibbiflorae* (*Echeveria*, Crassulaceae)

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Abstract

Ser. *Gibbiflorae* (*Echeveria*, Crassulaceae) consists of 39 taxa distributed mainly in Mexico. The taxonomy of the group is a complex task because of its very variable morphological characteristics, as well as the limited DNA data available. We generate and use DNA sequences of *rbcL*, *matK* and ITS2 to infer the phylogenetic relationships of ser. *Gibbiflorae* with Bayesian inference criterion and to extract DNA diagnostic nucleotides to complement morphology in the review the ser. *Gibbiflorae* in character-based DNA barcoding approach. *Gibbiflorae* is retrieved as monophyletic group, however shallow relationships were not solved. The majority-rule consensus tree shows one sub-group of norther Mexico´s species composed by 20 taxa. Character-based DNA barcoding approach and morphology comparisons identified eleven potential new species for the series.

Key words: Bayesian inference, CAOS, Paniculate inflorescences

Resumen

La serie *Gibbiflorae* (*Echeveria*, Crassulaceae) consiste en 39 taxones distribuidos principalmente en México. La taxonomía del grupo es un tatarea difícil debido a sus muy variables caracteres morfológicos, así como a las limitadas secuencias de ADN disponibles. Nosotros generamos y empleamos secuencias de *rbcL*, *matK* e ITS2 para inferir las relaciones filogenéticas de la serie *Gibbiflorae* mediante el criterio de inferencia Bayesiana y para extraer nucleótidos diagnósticos que complementen a la morfología en la revisión de la serie en un contexto de códigos de barras basado en caracteres. *Gibbiflorae* fue recobrado como grupo monofilético, sin embargo, las relaciones someras no fueron resueltas. El árbol de consenso de mayoría muestra un subgrupo de especies del noroccidente de México que está compuesto por 20 especies. Los códigos de barras basados en caracteres y la comparación morfológica identificaron once potenciales nuevas especies para esta serie.

Palabras clave: CAOS, Inferencia bayesiana, Inflorescencias paniculadas

Introduction

The series *Gibbiflorae* (*Echeveria*, Crassulaceae, Saxifragales) consists of succulent perennial plants whose beauty provides them with a high ornamental value. Several of its species are among the largest of the genus —up to 80 cm in rosette diameter and 2.5 m in height including stem, rosette, and inflorescence— and have physiological adaptations such as Crassulacean Acid Metabolism, as well as morphological features such as succulent stems and leaves, which makes them very resistant to sunlight and drought. The ser. *Gibbiflorae* has a wide distribution range mainly in Mexico, but one species extends up to Guatemala (Walther, 1972; Pilbeam, 2008). In this large geographic area, they are found in temperate forests, tropical deciduous forests and in semi-arid environments (Walther, 1972; Pilbeam, 2008).

Despite the high degree of endemism exhibited (97.3%) and its economic potential as ornamental plants, both the series *Gibbiflorae* and the genus *Echeveria* have been poorly studied from different approaches (phylogenetics, ecology, biogeography, among others). The lack of interest in the systematics of the group has caused a limited understanding of the entities that compose the genus and the relationships within, therefore, the taxonomy of both, the series and the genus has been historically complex, that is for example, the definition of *Gibbiflorae* has been modified several times in the history of the group and the inclusion of the species in this infrageneric group has been, in occasions, controversial.

Since *Echeveria* was included in genus *Cotyledon*, *Gibbiflorae* already has been established as series, later it was transferred to *Echeveria* by A. Berger (1930). Since then, the definition of *Gibbiflorae* has been confusing. Eric Walther (1972) for instance, define the series as: glabrous, big to very big plants; with erect, mainly unbranched stems; few medium to large leaves, usually with thick petiole, channeled upwards, keeled below, with thin wings to the sides of the petiole; inflorescences always paniculate, with long rachis and three or more racemose-secund branches; lower bracts relatively big and very similar to the leaves but smaller; short pedicels or as long as 10 mm; unequal sepals ascending to extended, deltoid, linear or oblanceolate, most acute; corolla to 15–22 mm long or more, cylindric-urceolate to campanulate, pentagonal, pink-reddish to scarlet and yellow, more or less glaucous-pruinose, petals strongly keeled, and pronounced nectary cavities; big nectary scales, thick, truncated; dark styles, red to almost black.

In his monograph, E. Walther recognize 14 taxa in ser. *Gibbiflorae*: *E. acutifolia* Lindl., *E. crenulata* Rose, *E. dactylifera* E.Walther, *E. fimbriata* C.H.Thomps., *E. gibbiflora* DC., *E. gigantea* Rose & J.A.Purpus, *E. grandifolia* Haw., *E. grisea* E.Walther, *E. longiflora* E.Walther, *E. pallida* E.Walther, *E. palmeri* Rose, *E. rubromarginata* Rose, *E. subrigida* Rose and *E. violescens* E.Walther, being *E. gibbiflora* the series type. E. Walther also, had established ser. *Retusae* (1959) whose definition is very similar to *Gibbiflorae* and therefore, a very close relationship between these two series is presumed, leading the author to comment that "the first could be considered a smaller version of the second". Ser. *Retusae* is defined as: plants usually glabrous, leaves rarely puberulous or with ciliate margins; small to medium-sized rosettes; stem evident but short, simple or sometimes stoloniferous from base; leaves either numerous and crowded, or very few, relatively small, not over 15 cm long nor 7 cm broad, obtuse, retuse to acute, somewhat narrowed to base but scarcely petiolate, entire or lacerate, sometimes ciliate when young or puberulous when old, green or glaucous, or tinged or edged with red; inflorescence cymose-racemose, mostly with 2-4 branches, less often simple or subpaniculate; peduncle mostly stout, erect, with numerous broad and flat bracts; pedicels evident, 3mm long or more; sepals ascending or spreading; corolla medium sized, rarely large, scarlet or crimson, to coral or rose-red; petals blunty keeled, hollowed within at base; styles mostly dark; nectaries thick. The type species of ser. *Retusa* is *E. fulgens* Lem., and includes: *E. acutifolia*, *E. fulgens* Lem., *E. juarezensis* E.Walther, *E. lozani* Rose, *E. obtusifolia* Rose, *E. rubromarginata*, *E. sayulensis* E.Walther, *E. scheerii* Lindl., *E. semivestita* var. *floresiana* E.Walther, *E. semivestita* var. *semivestita* Moran, *E. steyemarkii* Standl. y *E. stolonifera* Otto.

The combination of the previous series results in 24 taxa —it should be noted that *E. acutifolia* and *E. rubromarginata* are listed in both series—, of which *E. palmeri* have become synonymous to *E. subrigida*. *E. grandifolia* although it is an accepted name, Kimnach (2003) has reduced it to a synonym of *E. gibbiflora*. *E. sayulensis* E.Walther and *E. stolonifera* (Baker) Otto are recognized as hybrids (Walther, 1972; Uhl, 1989), *E. scheerii* Lindl., remains as a mystery due the origin of the plant is not known and it has never been collected again, supposedly from the Chontal region of Oaxaca, Mexico (Walther, 1972). *E. semivestita* var. *floresiana* and *E. semivestita* var. *semivestita* although both Walther (1972) and Kimnach (2003) include them here, most likely belong to ser. *Angulatae* (de la Cruz-López et al. 2019); while the validity of *E. violescens* has been questioned as it is reported from Saltillo, Coahuila, far exceeding the limits of the known distribution of all *Gibbiflorae* . The elimination of these names decreases the number of species to 16. Since the publication of E. Walther´s monograph, 22 new species of *Gibbiflorae* have been published. *E. guerrerensis* Reyes, González-Zorzano & Brachet (Reyes et al., 2011) and *E. uxorium* (Jimeno-Sevilla et al., 2012) were located in ser. *Urbiniae* and ser. *Racemosae* respectively, but we now known that they belong to *Gibbiflorae* (de la Cruz-López et al. 2019). *E. gudeliana* (Véliz & García-Mendoza, 2011) from ser. *Pruinosae* is another species that could better be placed in *Gibbiflorae*, due to its morphological affinities to this series — caulescent, paniculate inflorescences vs acaulescent, cincinnus inflorescences of *Pruinosae*—. *Gibbiflorae* then, includes a total of 38 taxa making it one of the most alpha rich series of the genus competing with ser. *Racemosae* and ser. *Nudae*.

The taxonomic complexity of *Gibbiflorae* is exacerbated by intrinsic factors to consider, including the karyological complexity found in the group, whose basic haploid chromosomal number is n = 27 and varies up to n = 189 in the cases of *E. cant*e and *E. fulgens* var. *fulgens*, respectively (Pilbeam, 2008), and even show different chromosomal numbers within the same species such as *E. fimbriata*, *E. fulgens* var. *fulgens* and *E. fulgens* var. *obtusifolia* (Pilbeam, 2008), which may indicate a rapid and complex diversification that includes hybridization. Another factor that has contributed to the detriment of the *Gibbiflorae* taxonomy is the great phenotypic variation present in their vegetative structures, which make difficult to differentiate entities by means of morphological diagnostic characters. If we add to the problems mentioned above, the lack of a representative phylogeny, as well as the existence of the "*E. fulgens*" complex originated, among other causes, by the lack of type locality and an insufficient description in its protologue and finally, that the morphological characters used to assign the species in a given series and to differentiate them from other species are shown as symplesiomorphic, at least in previous studies carried out in the "Echeveria Group" (Carrillo-Reyes et al., 2009) then the taxonomy of *Gibbiflorae* becomes difficult to handle with traditional means.

Morphology has been the main criteria for differentiating species and assigning them to series in *Echeveria*, although the use of morphological characters is a frequent and valid practice to establish species hypotheses, in both *Gibbiflorae* and the genus as a whole, has proved to be insufficient in many cases. For this reason, the integrative taxonomy *sensu* DeSalle and collaborators (2005) and Goldstein and DeSalle (2011) is perceived as the adequate framework to review the systematics of complex groups such as *Gibbiflorae*, it offers a greater potential since, it conjugates the DNA key diagnostic characters (Character-based DNA barcoding) along with the morphology and/or another source of evidence that either corroborate or refute species hypothesis by using the graphical conceptual scheme known as the 'taxonomic circle' (DeSalle et al., 2005).

The so-called Characteristic Attributes Organization System (CAOS), in an analogous way as the traditional taxonomy, identifies those unique nucleotides in DNA sequences present in the analyzed taxa, allowing their differentiation at the molecular level (Sarkar et al., 2008; Nicolalde-Morejón et al., 2010). Despite their simplicity, character-based methods such as CAOS or others, have shown to match others more complex parametric methods as those based on coalescence to delimit species (see for example Zou et al. 2011), and overcome genetic distances-based or those based on phylogenetic trees (Zou et al., 2011).

Here we discuss the first molecular phylogenetic analysis of ser. *Gibbiflorae*, using *rbcL* and *matK* chloroplast regions proposed as the standard barcode core of life for land plants (CBOL Plant Working Group, 2009) and previously employed in systematic studies in Crassulaceae, as well as the nuclear intergenic spacer ITS2, which has shown a greater degree of discrimination in both plants and animals (Chen et al., 2010; Gu et al., 2013). In parallel, we used these markers to identify molecular diagnostic characters that conjugated to morphological ones, can contribute to strengthen the hypotheses of known species, as well as for the identification of potential new entities on integrative taxonomy approach *sensu* DeSalle and collaborators, additionally, DNA barcoding reference library was built for this series of genus *Echeveria*.

Materials and methods

Taxon sampled

Thirty-six taxa were selected for the analysis, of a total of thirty-eight formally described, which corresponds to 92.3% of the total of the series. Live specimens were collected in the distribution range of the series and stored in the “Colección Nacional de Crasuláceas” belonging to the Botanical Garden of the Instituto de Biología of the “Universidad Nacional Autónoma de México” (UNAM). *E. scheeri* was excluded, because have not been collected again since their description in 1845 and could be of artificial origin. And in the cases of *E. gudeliana* and *E. steyermarkii*, their collection in Guatemala was not possible. In contrast, some specimens were included whose morphological attributes differ from the above entities and represent potential new species.

For each species, three to eight individuals were considered for molecular analyzes, except for *E. juarezensis*, *E. patriotica* I.García & Pérez-Calix, *E. purhepecha* I.García, *E. rulfiana* Jimeno-Sevilla, Santana Mich. & P.Carrillo and *E. uxorium* Jimeno-Sevilla & Cházaro with two; and *E. aurantiaca* Reyes, González-Zorzano & Brachet, *E. prunina* Kimnach & Moran and *E. sonianevadensis* A.Vázquez, Jimeno-Sevilla & I.García with one, due difficulties on amplification and sequencing of the DNA fragments. As external groups for phylogenetic analysis, representative species of the remaining 16 series that compose *Echeveria* were selected, as well as representatives of *Pachyphytum*, previously established as sister group of *Echeveria* (Carrillo-Reyes et al. 2009) as outer outgroup.

Genomic DNA extraction, PCR amplification and DNA sequencing

Tissue taken from the leaf epidermis was previously dried on silica gel and then 30 mg was pulverized on porcelain mortars using liquid nitrogen. A Thermo Fisher PureLink Genomic DNA Purification Kit was used, following the manufacturer's protocol to extract the genomic DNA.

The sequences selected correspond to the chloroplast coding regions *rbcL* and *matK* suggested by the CBOL Plant Working Group (2009) as the nucleus of the DNA barcode in angiosperms. Due to previous reports in other groups of plants that refer to the low discriminative level of these regions, we include the ITS2 region of the nuclear ribosomal complex as a complement to increase discrimination since this nuclear intergenic spacer has been proposed as an excellent candidate as bar code in groups of plants and animals (Chen et al., 2010; Yao et al., 2010). The oligonucleotides used are in accordance with the standards of the Canadian Center for Barcodes (CCDB). The polymerase chain reaction to amplify the selected genomic regions was carried out by the Qiagen Taq PCR Core Kit following the manufacturer's instructions in volumes of 12.5 µL. PCR products were verified by electrophoresis on 1% agarose gels stained with RedGel and visualized in an ultraviolet camera. Forward and reverse PCR primers used were: rbcLa-F (5´ATGTCACCACAAACAGAGACTAAAGC3´), rbcLa-R (5´GTAAAATCAAGTCCACCRCG3´), MatK-1RKIM-f (5´ACCCAGTCCATCTGGAAATCTTGGTTC3´), MatK-3FKIM-r (5´CGTACAGTACTTTTGTGTTTACGAG3´), ITS2-S2F (5´ATGCGATACTTGGTGTGAAT3´), ITS4 (5´TCCTCCGCTTATTGATATGC3´).

The amplified fragments were sequenced using the Sanger technique in the Centre for Biodiversity Genomics & Biodiversity Institute of Ontario, University of Guelph, Canada. The resulting chromatograms were edited in Geneious v.10.0.9 (http://www.geneious.com, Kearse et al., 2012) to obtain the DNA sequences. Voucher information, BOLD systems ID´s and GenBank accession numbers of the sampled species are summarized in Appendix 1.

Phylogenetic analysis

The aligned sequences of each molecular marker were downloaded from BOLD systems database (Barcode of Life Data Systems. http://www.boldsystems.org) generated during the "Crassulaceae in Mexico", "Crassulaceae in Mexico2" and "Crassulaceae in México3" projects (see Table S1.xls), where the authors of the manuscript are involved. After slightly manual re-alignment and edition, sequences of each molecular marker were concatenated in Mesquite v3.61 (Maddison & Maddison, 2019) to obtain an expanded matrix of 1679 bp in length, we also incorporated 10 morphological characters described in Table 1.

Table 1. Characters and character states for the morphological partition.

Character: state
1. Habit: **0**=acaulescent, **1**=caulescent
2. Filament appendixes: **0**=reduced, **1**=evident
3. Appendixes shape: **0**=reduced, **1**=cylindrical, **2**=membranous
4. Inflorescence type: **0**=cincinnous, **1**=spike, **2**=raceme, **3**=panicle, **4**=cyme, **5**=thyrse
5. Corolla length: **0**=<1 cm, **1**=1.01–1.5 cm, **2**=1.51-2.0 cm, **3**=2.01–2.5, **4**=>2.51
6. Corolla transversal shape: **0**=pentagonal, **1**=cylindrical
7. Corolla base color: **0**=yellow, **1**=orange, **2**=pink, **3**=red, **4**=cream, **5**=green
8. Corolla apex color: **0**=yellow, **1**=orange, **2**=pink, **3**=red, **4**=cream, **5**=green
9. Nectary scales color: **0**=white, **1**=yellow, **2**=red, **3**=orange, **4**=pink
10. Styles color: **0**=yellow, **1**=green, **2**=orange, **3**=red, **4**=purple

The coding regions were partitioned into first, second and third codon positions, not so for the ITS2 (non-coding) region. From the expanded matrix, a Bayesian inference phylogenetic analysis was performed on MrBayes (Huelsenbeck & Ronquist, 2001). The nucleotide substitution models for DNA partitions were GTR+I+G and for morphology partition was the Mk model. Two independent Markov Monte Carlo analyses was run for 2,000,000 generations each, using one cold and three hot chains sampled every 2,000 generations. Parameter convergence was visualized with Tracer v1.6 (Rambaut et al., 2014). The first 25% of the saved trees was discarded, and a majority-rule consensus tree without branch lengths was obtained, the tree was edited in TreeGraph2 v. 2.15 (Stöver & Müller, 2010) and PhotoShop was used to merge a distribution map onto phylogenetic tree of ser. *Gibbiflorae*. The alignment matrix in NEXUS format was deposited in TreeBase.

Extraction of molecular diagnostic sites

The identification of molecular diagnostic characters in the DNA sequences were obtained through CAOS using the previously inferred cladogram. This guide tree was incorporated into the NEXUS file of aligned sequences in Mesquite v3.61 (Maddison & Maddison, 2019) as established by the protocol for the use of CAOS (Jörger and Schrödl, 2014). The resulting CAOS_TotalBarcoder file, which contains the diagnostic sites for each taxon, was used to the construction of the diagnostics sites matrixes, of those characters, the "simple pure" diagnostic characters according to the designation of DeSalle et al. (2005) and Sarkar et al. (2008) with confidence values >0.6 were considered.

Morphological characters comparisons

Living specimens of ser. *Gibbiflorae* were compared to find morphological diagnostic characters useful to delimit its species. All organs were considered; however, special attention was made on floral characters. Photographs were taken to construct comparative figures and tables of the most relevant characteristics.

Results

Phylogenetic analysis

Bayesian inference majority-rule consensus tree based on combined DNA and morphology datasets, indicates that ser. *Angulatae*, ser. *Pruinosae* and ser. *Secundae* are the sister clade of ser. *Gibbiflorae* with high support (pp = 1), and *Gibbiflorae* is recovered as monophyletic group with high support (pp = 1, Fig. 1). Two sub-groups within *Gibbiflorae* are evident based on their geographical distribution in Mexico, the "southern Mexico group" contains a total of 21 taxa divided in two sub-clades retrieved with moderate to high support (pp = 0.93 and pp = 1, respectively), the first sub-clade includes *E. aurantiaca*, *E. guerrerensis* and *E. longiflora* as closely related, while the second sub-clade groups *E. acutifolia*, *E. crenulata*, *E. fimbriata*, *E. fulgens* var. *fulgens*, *E. fulgens* var. *obtusifolia*, *E. gibbiflora*, *E. gigantea*, *E. grisea*, *E.* sp. "cupreata", *E.* sp. JE-7130, *E. pallida*, *E. prunina*, *E. rubromarginata*, *E. triquiana* and *E. xochipalensis*. The dataset does not have the enough resolution to completely solve the relationships within "southern Mexico group", this is more evident for *E. juarezensis*, *E.* sp. "magnifica", *E. uxorium* and some *E. fulgens* var. *fulgens* and *E. gibbiflora* specimens.

The "north-western Mexico group" consists of 20 taxa and is retrieved with moderate support (pp = 0.9), within this sub-group, three sub-clades are recovered with moderate to high support, one of them corresponds to specimens morphologically related to *E. fulgens* var. *fulgens* (JE-8061), another one includes to *E. lozanoi*, *E. munizii*, *E. nayaritensis*, *E. purhepecha*, *E. roseiflora* and *E. rulfiana*. The last sub-clade within "north-western group": *E. cante*, *E. cerrograndensis*, *E. coruana*, *E. dactylifera*, *E. marianae*, *E. michihuacana*, *E. novogaliciana*, *E. patriotica*, *E. perezcalixii*, *E. pistioides*, *E. sonianevadensis*, *E.* sp. "EK-3569" and *E. subrigida*

(Fig. 1). Within "north-western group" few sister species relationships were retrieved, *E. michihuacana* to *E. pistioides*, *E. coruana* to *E. patriotica* and both to *E. sonianevadensis*, while *E. novogaliciana* are sister to *E. perezcalixii* and finally, *E. dactylifera* to *E*. sp. EK-3569.

Character-based DNA barcoding

Simple pure molecular diagnostic characters allow the identification of 50 percent of the sampled taxa. At least one diagnostic site was found for *E. acutifolia*, *E. aurantiaca*, *E. cante*, *E. cerrograndensis*, *E. coruana*, *E. dactylifera*, *E. fimbriata*, *E. fulgens* var. *obtusifolia*, *E. guerrerensis*, *E. longiflora*, *E. marianae*, *E. nayaritensis*, *E. pallida*, *E. patriotica*, *E. prunina*, *E. roseiflora, E. subrigida* and *E. triquiana*. In addition, nine more taxonomic units presented diagnostic nucleotides: *E. gigantea* (JE-5151/*E*. aff. *gibbiflora* JE-87331-1/ JE-7548-1), *E*. sp. (EK-3569), *E*. aff. *fulgens* (JE-8592-1/JE-8392-1), *E*. aff. *acutifolia* (JE-8758/JE-6745), *E*. sp. (JE-8061), *E*. sp. (JE-8786/JE-8762), *E*. sp. (JE-8771), *E*. aff. *fulgens* (JE-8816), and *E*. sp. (JE-6371), Table 2.

Discussion

Phylogenetic relationships of Gibbiflorae

Series *Angulatae*, *Pruinosae* and *Secundae* are the sister clade of ser. *Gibbiflorae* as previous results suggests (de la Cruz-López et al. 2019). *Gibbiflorae* is recovered with high posterior probabilities support as monophyletic group however, our dataset (*rbcL*, *matK* and ITS2) do not have the enough resolution to fully resolve its inner relationships, nevertheless, three clades are evident. One of the sub-clades is composed by *E. aurantiaca*, *E. guerrerensis* and *E. longiflora* from the Mexican states of Guerrero and Morelos. The second clade includes center-southwestern Mexico (Chiapas, Ciudad de México, Estado de México, Guerrero, Morelos, Oaxaca, Puebla, Tlaxcala and Veracruz) taxa: *E. crenulata*, *E. gigantea*, *E. grisea*, *E. prunina*, *E. rubromarginata*, *E. triquiana*, *E. xochipalensis* and taxons morphologically close to *E. acutifolia*, *E. fulgens* var. *fulgens*, *E. gibbiflora* and *E. gigantea*.

The third clade groups north-western Mexico (Aguascalientes, Colima, Durango, Estado de México, Guanajuato, Hidalgo, Jalisco, Michoacán, Nayarit, San Luis Potosí, Sinaloa and Zacatecas) taxa: *E. cante*, *E. cerrograndensis*, *E. coruana*, *E. dactylifera*, *E. lozanoi*, *E. marianae*, *E. michihuacana*, *E. munizii*, *E. nayaritensis*, *E. novogaliciana*, *E. patriotica*, *E. perezcalixii*, *E. pistioides*, *E. purhepecha*, *E. sonianevadensis*, *E. roseiflora*, *E. rulfiana*, *E. subrigida* and taxa morphologically associated to *E. fulgens* (JE-8061, JE-6744, JE-8592, JE-8392, EK-4192) and *E*. aff. *novogaliciana* (EK-3569).

E. fulgens var. *fulgens*, *E. gibbiflora*, *E. juarezensis* and *E. uxorium* shown uncertain relationships within *Gibbiflorae*.

Integrative taxonomy of Gibbiflorae

We start from 36 previously established species hypothesis, remembering that *E. gudeliana*, *E. scheeri* and *E. steyermarkii* were not included in the sampling. Bayesian inference phylogenetic analysis recovers 36 clusters from which, 27 agree with starting hypothesis, these corresponds to *E. acutifolia*, *E. aurantiaca*, *E. cante*, *E. cerrograndensis*, *E. coruana*, *E. crenulata*, *E. dactylifera*, *E. fimbriata*, *E. fulgens* var. *obtusifolia*, *E. guerrerensis*, *E. longiflora*, *E. marianae*, *E. michihuacana*, *E. munizii*, *E. nayaritensis*, *E. novogaliciana*, *E. pallida*, *E. patriotica*, *E. perezcalixii*, *E. pistioides*, *E. prunina*, *E. purhepecha*, *E. roseiflora*, *E. rulfiana*, *E.*

sonianevadensis, *E. subrigida* and *E. triquiana*; although *E. aurantiaca* and *E. sonianevadensis* with a single individual.

Character-based DNA barcoding recovers 26 molecular units, from which 17 match with the starting established species hypotheses: *E. acutifolia*, *E. aurantiaca*, *E. cante*, *E. cerrograndensis*, *E. coruana*, *E. dactylifera*, *E. fimbriata*, *E. fulgens* var. *obtusifolia*, *E. guerrerensis*, *E. longiflora*, *E. marianae*, *E. nayaritensis*, *E. pallida*, *E. prunina*, *E. roseiflora*, *E. subrigida* and *E. triquiana*. Conversely, *E. crenulata*, *E. fulgens* var. *fulgens*, *E. gibbiflora*, *E. gigantea*, *E. grisea*, *E. juarezensis*, *E. lozanoi*, *E. michihuacana*, *E. munizii*, *E. novogaliciana*, *E. perezcalixii*, *E. pistioides*, *E. purhepecha*, *E. rubromarginata*, *E. rulfiana*, *E. sonianevadensis*, *E. uxorium* and *E. xochipalensis* did not presented diagnostic characters. The low-resolution problem may be due, regardless the limited number of loci, a rapid diversification of the genus.

Taxonomic treatment

Ser. *Gibbiflorae* (Baker) Berger *In*. Engler, Nat. Pflanzenf. ed. 2. Vol. 18a, p. 474, 1930; E. Walther Leafl. West. Bot., vol. 9. Pp. 3,4. 1959.
(Incl. ser. *Grandes* E. Walther and ser. *Retusae* E. Walther)
Cotyledon gibbiflorae Baker,
Type: *Echeveria gibbiflora*

Glabrous. Acaulescents or caulescents. Small to large sized rosettes. Stems erect or somewhat postrate, frequently unbranched. Leaves few, mainly wide and flat or less frequently ensiform, usually with thick petiole, channeled upwards, keeled below, frequently with thin wings to the sides of the petiole, margins fimbriate, entire, undulate, crenate or crenulate, often with reddish margin. Inflorescences paniculiform, with long rachis and three or more scorpioid cymes branches (cincinni), sometimes with a single cincinni. Bracts relatively big and very similar to the leaves but smaller (the basal ones), the upper bracts narrower. Pedicels short or as long as 1.5 cm. Sepals unequal ascending to extended, deltoid, linear or oblanceolate, mostly acute. Corolla to 1.0–2.4 cm long, tubular-urceolate to tubular-campanulate, pentagonal in transection, pink, orange, reddish and yellow or combinations thereof, more or less farinaceous. Petals strongly keeled, with pronounced nectary cavities. Carpels with free styles. Ovaries whitish, cream colored or yellowish. Nectary scales big, thick, truncated, colored in white, cream, yellowish, orange or reddish. Styles in red or purple (never yellow, orange or green as in other series).

1) ***Echeveria acutifolia*** Lindl. Type: From a Garden in Oaxaca, Mexico, *Hartweg 749* (CGE). Caulescent with sub-rhomboid acute leaves, concave at the apex; dense cylindrical panicles with 3–4 flowered branches; acute sepals. **Distribution**: Oaxaca. **Notes**: There is a lot of confusion regarding this species. There is no type locality in the wild, additionally, the iconotype in Lindley´s protologue refers more to *E. crenulata* Rose from Morelos, Mexico. Our sampled *E. acutifolia* labeled as JE-8753 was collected near Huatulco in the coast of Oaxaca Mexico and is described briefly as: caulescent; medium sized rosettes (up to 30 cm); pale green leaves somewhat farinose; few branched panicles; tubular pink flowers with orange apex (FIG. 2); without epipetalous appendices; white ovaries, red-wine styles sometimes greenish below; white to cream reniform nectary scales (FIG. 4). It is morphologically and biogeographically related to: *E. pallida*, from which can be distinguished by its less flowered panicles; phylogenetically related

to unknown. Molecularly can be identified by the nucleotides C (Cytosine) and A (Adenine) on the sites 266 and 255 of *matK* and ITS2 regions respectively, against G (Guanine) in both cases of the rest of *Gibbiflorae* taxa (Table 1).

2) ***Echeveria aurantiaca*** J.Reyes, O.González & Brachet. Type: Mexico. Guerrero. Tepacoya, 17 km to the North of Taxco, on the road to San Gregorio, 18° 37' 33" N y 99° 34' 57" O, 1585 m. Dec. 8, 2007. *J. Reyes 6012* (Holotype MEXU).
Acaulescent; small sized rosettes (up to 17 cm); green to brown oblanceolate-obovate leaves, margins entire or crenate, not pruinose; few branched paniculate inflorescences or single branched cincinnous; calyx green; corolla tubular-urceolate, short (1.1 cm long); petals orange (FIG. 2); without epipetalous appendixes; yellow ovaries, wine-red styles, white-hyaline reniform nectary scales (FIG. 4). **Distribution**: Guerrero, only known from the type locality. **Notes**: Morphologically related to *E. fulgens* var. *fulgens* and *E. fulgens* var. *obtusifolia*, from whom differs by its orange flowers (*E. fulgens* and *E. obtusifolia* have pink and red flowers, respectively), biogeographically and phylogenetically related to *E. guerrerensis* and *E. longiflora*. Molecularly can be identified by the insertion event of the nucleotide Adenine (A) on the site 108 of ITS2 region against a gap of the rest of *Gibbiflorae* taxa (Table 1).

3) ***Echeveria cante*** Glass & Mend.-Garc., Type: Mexico. Zacatecas, between Sombrerete and Fresnillo, ca. 40 km NW of Fresnillo, 1 km near west of the Highway 45, Sierra of Chapultepec, near and around rocks on the top of the mountains.
Acaulescent; medium to large sized rosettes (up to 40 cm); oblanceolate, very farinose leaves, pink entire margins or sometimes crenated; farinaceous panicles with few cincinni; tubular-urceolate corollas with keeled farinaceous petals (FIG. 2); without epipetalous appendices; white ovaries; wine-red long styles; white-hyaline, reniform-truncate nectary scales sometimes with pink stains (FIG. 4). **Distribution**: Zacatecas, only known from the type locality. **Notes**: Morphologically related to *E. subrigida* from which differs by its more farinaceous leaves, pink flowers (vs yellowish-orange) and by its white nectary scales (vs red). Biogeographically related to *E. dactylifera*. Phylogenetically related to unknown but belongs to the northwestern Mexico taxa. Molecularly can be distinguished by the nucleotide T (Thiamine) at the site 252 of the ITS2 region vs the nucleotide C (Cytosine) of all *Gibbiflorae* species (Table 1).

4) ***Echeveria cerrograndensis*** A.Vázquez & Nieves. Type: Mexico. Colima: Minatitlán, Lagunitas-El Terrero road, 19°25'8.79"N, 103°55'7.07"W, 2200 m, *G. Nieves Hernández, Miguel Cházaro, Julia Etter, Raúl López, Ignacio Contreras s.n.* (holotype IBUG).
Acaulescent; small to medium sized rosettes (up to 20 cm); pale green to purple obovate-oblanceolate leaves, somewhat farinaceous, margins entire or undulate; short (up to 35 cm) and few flowered inflorescences; pink-orange tubular-urceolate corollas with keeled petals (FIG. 2); without epipetalous appendices; white ovaries; short wine-red styles; red-pink reniform nectary scales (FIG. 4). **Distribution**: only known from the type locality. **Notes**: Morphologically related to *E. fulgens* var. *fulgens* and *E. perezcalixii*, from which differs by its red nectary scales (vs yellow) also, *E. roseiflora* can be related to *E. cerrograndensis*, however, differs by its pale pink vs pink-orange corollas, respectively. Biogeographically is related to *E. munizii*. Phylogenetically related to unknown, belongs to northwestern Mexico group. Molecularly, *E. cerrograndensis* have the nucletides T/C (three individuals have T and one C) vs the nucleotide C in the sites 398 and 564 of the *matK* region from the rest of *Gibbiflorae* taxa.

5) ***Echeveria coruana*** I.García, D.Valentín & Costea. Type: Mexico. Municipality of Ziracuaretiro, northwest side of San Andrés Corú, 1730 m, *I.García & M.García 9138* (CIMI). Acaulescent or with short stem (3–8 cm long); small sized rosettes up to 18 cm long; leaves linear-oblanceolate to spathulate, pseudopetiolate, pale green to dark green, sometimes tinged with brown at the apex; inflorescences 1 to 3 panicles per rosette, up to 74 cm long, 3–7 flowers per cincinni; sepals green, deltoid-lanceolate; corolla tubular-urceolate, up to 2 cm long; petals bicolored, white to cream at the base third, red in the middle and apex, with two very small appendices on the sides of the epipetalous filaments; ovaries white at the base, yellowish above, purple styles, reniform and yellow nectary scales. **Distribution**: Only known from the type locality in Michoacán. **Notes**: Morphologically, biogeographically and phylogenetically related to *E. patriotica*, from which differs by the acaulescent vs caulescent habit, its narrowed leaves and by its yellow vs red nectary scales. Molecularly can be identified not only from *E. patriotica*, but for all *Gibbiflorae* taxa by the presence of the nucleotides A vs C and T vs G in the sites 230 and 128 of *rbcL* and ITS2 regions, respectively (Table 1).

6) ***Echeveria crenulata*** Rose. Type: Mexico. Morelos, near Cuernavaca, *Rose & Painter 790* (Holotype: US).
Caulescent; broadly obovate, pale green or light bluish, pseudo-petiolate leaves, margins often crenulate reddish or purple colored; bracts as basal leaves but smaller; inflorescences in short and compact panicles, lateral branches short and few flowered; sepals unequal, spreading, acute; corolla to 1.5 cm long, reddish in the base, yellowish at the apex (Fig. 2); with ovaries and nectary scales, reddish styles (Fig. 4). **Distribution**: Estado de México (Chalma), Morelos (Cuernavaca, near Yecapixtla). **Notes**: this species is frequently confused with *E. fulgens* var. *fulgens*, *E. fulgens* var. *obtusifolia* and *E. rubromarginata* which are distributed in nearby areas, or with other crenulated leaves taxa. The description of Rose matches our sampled specimens from Yecapixtla, Morelos, but E. Walther´s description is also correct, the discrepancies in the measures of the plants may be to the fact that in many *Gibbiflorae* species, the summer leaves are bigger than the winter leaves to resist the drought season and even can be of different color and shape. These variability in length, color and shape of the leaves make it difficult to use them in taxonomy. Morphologically *E. crenulata* is related to *E. fulgens* varieties as mentioned above, however differs by having a conspicuous caudex, looser rosettes (the distance of the leaves insertion in the stems is more spaced), more branched paniculate inflorescences (*E. fulgens* varieties have one to four longer branches), and the yellowish corolla apex. Biogeographically, is related to *E. fulgens* varieties and *E. rubromarginata*. Phylogenetically is related to unknown, belong to the southern Mexico group. Molecularly it can´t be identify by our dataset.

7) ***Echeveria dactylifera*** E. Walther. Type: Mexico. Sinaloa: on the road Mazatlán to Durango, near the limits of Sinaloa and Durango (holotype CAS).
Acaulescent; big sized rosettes (up to 60 cm); green with red margins or reddish oblanceolate leaves, margins often entire, adaxial surface frequently channeled, abaxial surface keeled; inflorescences in short branched panicles; deltoid acute sepals; tubular-urceolate big corollas (up to 2.5 cm long), pink; petals keeled with yellowish apex and margins, somewhat recurved at the apex, filaments with two finger-like appendixes at the base; white ovaries, yellowish nectary scales and red long styles. **Distribution**: Durango, Jalisco, Sinaloa. **Notes**: Morphologically and biogeographically is related to *E. novogaliciana* and the potentially new species *E.* sp. EK-3569 from which differs by the color and shape of the leaves and by its yellow vs orange and pink

nectary scales respectively. Phylogenetically is related to *E.* sp. EK-3569 from Durango, Mexico. Molecularly can be identify by the presence of the nucleotide A on the site 47 of the ITS2 region (three out of four individuals have the A and one any of the four nucleotides represented by an N) against the G of the rest of the *Gibbiflorae* taxa.

8) ***Echeveria fimbriata*** C.H.Thomps. Type: Mexico. Morelos: Sierra de Tepoztlán, near San Juanico Tlacotenco *Trelease 1905*. (Clonotype: US).
Caulescent; medium sized rosettes (up to 30 cm); leaves with fimbriate margins; paniculate, few branching inflorescences; tubular flowers, pinkish-orange petals; white ovaries, wine-red styles and white nectary scales. **Distribution**: Only known from the type locality in Morelos. **Notes**: Morphologically and biogeographically, close to *E. fulgens* var. *fulgens*, from which differs by its fimbriate leaves. Phylogenetically related to unknown, belong to the southern Mexico group. Molecularly it can be identified by the presence of the nucleotide G in the site 273 of the *matK* region versus A and the nucleotide G in the site 245 of the ITS2 region against C.

9) ***Echeveria fulgens* var. *fulgens*** Lem. Type: Mexico. No designate. Neotype: Illustration in Le Jardin Fleur. volume 3, plate 244, 1855.
Acaulescent or with short stem; green or glaucous leaves, with entire to crenate margins, the margins can vary from reddish, hyaline or yellowish; paniculate inflorescences with one to four long cincinni; pinkish-orange medium sized tubular flowers (1–1.5 cm); white ovaries, purple styles and white, hyaline or yellow nectary scales. **Distribution**: Estado de México, Guerrero, Michoacán, Morelos, Oaxaca. **Notes**: this is the most confusing species in the series and maybe for the whole genus, the Lemaire´s protologue (1845) mention its green leaves, not glaucous, however, the description of E. Walther (1972) refers to a plant with glaucous leaves. The short protologue description, it's very wide occurrence, and the lack of type locality also contributes to the confusing associated to this name. We have observed a lot of variation in the leaves, specially, in shape, color, margins, and in the color of the nectary scales (white, hyaline, yellow and red). Additionally, some populations of this species are located phylogenetically in the north-western Mexico taxa (with red nectary scales), while others belong to south Mexico (with white, hyaline or yellowish nectary scales), in the transition zone between these two Mexican zones (Estado de México, south of Michoacán, Morelos, north of Guerrero) is where is most difficult to differentiate the specimens. According to Uhl (2002) *E. fulgens* can be a cluster of several species. Morphologically related to *E. aurantiaca*, *E. cerrograndensis*, *E. fimbriata*, *E. fulgens* var. *obtusifolia*, *E. guerrerensis*, *E. juarezensis*, *E. michihuacana*, *E. pistioides*, *E. purhepecha*, *E. sonianevadensis*, *E. triquiana* and *E. xochipalensis*. Molecularly it can´t be identify by our dataset, however, specimens labeled as *E. fulgens* var. *fulgens* JE-8061 from Estado de México can be differentiated by the presence of the nucleotide A versus C in the site 739 of the *matK* region and *E. fulgens* var. *fulgens* JE-8816 from Guerrero by the presence of the nucleotide G against C in the site 245 of the ITS2 region.

10) ***Echeveria fulgens* var. *obtusifolia*** (Rose) Kimnach. Type: Mexico. Morelos: Canyon near Cuernavaca, Morelos, 3,150 m.
Acaulescent, small sized rosettes (up to 15 cm in diameter); leaves frequently crenate or crenulate, green with red or brown margins, short paniculate inflorescences with few flowers; tubular short corolla (1.5 cm long); reddish petals. **Distribution**: Estado de México, Morelos. **Notes**: this species is often confused with *E. crenulata*, however, *E. fulgens* var. *obtusifolia* have

a very short stems, while *E. crenulata* have a conspicuous caudex and more branched panicles, additionally, the later have a bicolored (pink at the base and yellow at the apex) corolla pattern, in turn, *E. fulgens* var. *obtusifolia* display a red or dark pink flowers. M. Kimnach (1998) reduced *E. obtusifolia* to variety of *E. fulgens* considering the few differences between both, we think that *E. fulgens* var. *obtusifolia* must be reestablished as species (*E. obtusifolia*). It is related morphologically to *E. fulgens* var. *fulgens* and *E. triquiana* from which is difficult to differentiate. Molecularly, can be identificateted by the presence of the nucleotide T in the site 588 of *matK* region against G, and the nucletiode C in the site 286 of the ITS2 region against T in all *Gibbiflorae* taxa.

11) ***Echeveria gibbiflora*** DC. Type: Not designate. Neotype: DeCandolle, Memoire Sur la Famille des Crassulacees, plate 5, 1828.
Caulescent, stems up to 70 cm long; medium to large sized rosettes (up to 50 cm in diameter); flat oblanceolate leaves, sometimes up curved, green or violet with red margins; large paniculate inflorescences with many branches; pinkish-orange flowers; gibbous keeled petals; white ovaries and nectary scales, with purple styles. **Distribution**. Ciudad de México, Estado de México, Guerrero, Michoacán, Morelos, Oaxaca. **Notes**: populations of this species from Guerrero and Oaxaca are distinct morphologically from the typical *E. gibbiflora*, and may represent different species, however the limitations of our dataset do not allow to differentiate them. It is related to *E. gigantea* from which differs by its green vs bluish leaves and its pink-orange vs pink petals Phylogenetically related to unknown, belong to the southern Mexico group. Molecularly it can´t be identify by our dataset.

12) ***Echeveria gigantea*** Rose & J.A.Purpus. Type: Mexico. Puebla: Cerro de la Yerba, near San Luis Atolotitlán, Puebla. Purpus, 07/414, (holotype: US).
Caulescent, stems up to 60 cm long; large sized rosettes (up to 80 cm in diameter); bluish obovate-oblanceolate or obcordate leaves, with pink margins, the margins sometimes undulate to crenate; paniculate inflorescences, up to 1 m long, with long branching cincinni; phosphorescent pink flowers; keeled petals; white ovaries and nectary scales, purple styles. **Distribution**: Puebla, Oaxaca. **Notes**: variability is found across its wide geographical range, morphologically related to *E. gibbiflora* and *E. rubromarginata*. Phylogeneticlly related to unknown, belong to the southern Mexico group. Molecularly it can´t be identify by our dataset.

13) ***Echeveria grisea*** E. Walther. Type: Mexico. Guerrero. Cañón de la Mano, near Iguala. E. Walther, 35/1 (CAS, no. 251051).
Acaulescent or with short stems (up to 10 cm log); small to medium sized rosettes (up to 20 cm in diameter); leaves obovate-spathulate, grayish-green or tinged in purple, farinaceous, margins entire to undulate; paniculate inflorescences with few circinate branches; tubular corollas; pink keeled petals; white ovaries, purple styles and white nectary scales sometimes with pink edges. **Distribution**: Guerrero, Michoacán. **Notes**: morphologically related to *E. fulgens* var. *fulgens* and *E. guerrerensis* from which differs by its gray farinaceous leaves. Phylogenetically related to unknown, belong to the southern Mexico group. Molecularly it can´t be identify by our dataset.

14) ***Echeveria gudeliana*** Véliz & García-Mend. Type: Guatemala. Huehuetenango: Municipality Nentón, road to Gracias a Dios, 1074 m, *M.Véliz & L.Velásquez MV 22564* (holotype: BIGU).
Caulescent, stems up to 20 cm long; medium sized rosettes (up to 30 cm in diameter);

oblanceolate farinaceous leaves; paniculate inflorescences with 3–4 cincinni; tubular corollas; pink keeled petals, farinaceous; white to cream ovaries, pale yellow nectary scales and purple styles. **Distribution**: Guatemala, Mexico: Chiapas. **Notes**: this species was located in the ser. *Pruinosae* by Véliz & García-Mendoza, however, its characteristics agree with ser. *Gibbiflorae*, specially, its paniculate inflorescences and the purple free styles, in ser. *Pruinosae*, the inflorescences are cincinni, the styles are pale red and adpressed each other. Unfortunately, its collection for this study was not possible. It is related biogeographically and morphologically to *E. steyermarkii* regarding its short stems, its leaves with three longitudinal veins, paniculate farinaceous inflorescences and flowers and by its pink flowers. Phylogenetically related to unknown (not sampled), belong probably to the southern Mexico group.

15) ***Echeveria guerrerensis*** J.Reyes, Brachet & O.González. Type: Mexico, Guerrero: Municipality Zihuatanejo, ca. 2 km through the road east of Ejido "El Bálsamo", Km 110, highway Cd. Altamirano-Zihuatanejo. 1850 m. *G. Salazar, Carolina Granados & Daniel Burguete No. 7478* (Holotype: MEXU).
Acaulescent; small rosettes (up to 18 cm in diameter); leaves spathulate sometimes obcordate, glaucous tinged lilac with pink margins, the margins entire to crenate; inflorescences panicles with two or more cincinni or sometimes single; tubular-urceolate corolla, short (1.1–1.5 cm long); keeled orange-pink petals; white ovaries, purple styles, white-hyaline nectary scales. **Distribution**: Guerrero. **Notes**: this species was located in ser. *Urbiniae* by Reyes et al. (2011), however, a phylogenetic analysis locate it in ser. *Gibbiflorae* (Cruz-López et al., 2019). Morphologically is related to *E. fulgens* var. *fulgens* and *E. grisea*. Molecularly can be identified by the presence of the nucleotides C and T in the sites 472 and 323 of the *rbcL* and *matK* regions, respectively, against G and C of the rest of the *Gibbiflorae* taxa sampled.

16) ***Echeveria juarezensis*** E. Walther. Type: Cultivated plant at University of California Botanical Garden (no. 56. 7911), Thomas MacDougall, no. B-72, from Ixtepeji, Sierra de Juárez, Oaxaca, Mexico (CAS, no. 409864; isotypes: UC, US).
Caulescent or with stems up to 8 cm tall; leaves obovate-cuneate, corge green to lettuce green, mucronate thick and rigid, deeply concave above, beneath rounded and somewhat keeled; inflorescences in short (20 cm) panicles of three secund racemes; corolla scarlet, pentagonal, conoid-urceolate, 1.2 cm long; white ovaries, styles oxblood-red. **Distribution**: Oaxaca?. **Notes**: apparently, T. MacDougall bought the plant at Oaxaca City market, where told him that the plant came from Ixtepeji, we search for specimens at Ixtepeji with no success, instead we collected specimens at La Cumbre from Sierra de San Felipe in the nearby area, these specimens are referred in the literature as *E*. sp. "Cumbre" (Pilbeam, 2008). This *E*. sp. "Cumbre" may correspond to *E. juarezensis* even when the E. Walther´s protologue do not match at all the characteristics of the La Cumbre specimens, particularly in reference to the length of the stems. It can be describe briefly as: acaulescent (in the wild) or caulescent (up to 10 cm long in cultivation); small to medium sized rosettes (20–35 cm); pale green obovate to oblanceolate leaves, sometimes farinaceous and concave, entire to crenulate pink margins; paniculate inflorescences with few cincinni branches; numerous bracts; sepals sometimes larger than flower, green to pinkish-orange; corolla tubular-urceolate, pentagonal; petals pinkish-orange, keeled; ovaries and nectary scales white, styles purple. Morphologically is close to *E. fulgens* varieties, from which cannot be differentiated easily, except for the longer sepals and by its geographical distribution. Molecularly can´t be identified by our dataset.

17) ***Echeveria longiflora*** E. Walther. Type: From cultivated plant at Strybing Arboretum, Golden Gate Park, San Francisco, C. Halbinger in Ciudad de México, type collected by E. Walther in 1950 (CAS, no. 354990).
Caulescent; erect steam, simple; leaves obovate-orbicular up to 15 cm long, 8 cm broad, green somewhat glaucous; inflorescences one or two panicles, with three elongated cincinii; corolla long and somewhat narrowed, 2.2 cm long, 1.3 cm broad at the base; petals keeled, pale vinaceous-lilac; ovaries seafoam-yellow; nectary scales pale yellowish, styles reddish. **Distribution**: Guerrero (without type locality). **Notes**: Morphologically, this species is very similar to *E. gibbiflora* from which differs by its longer and narrower flowers and the lesser branching inflorescences. Our sampled specimens came from near Taxco in the state of Guerrero. Phylogenetically related to *E. aurantiaca* and *E. guerrerensis* from which differs by its evident stems (*E. aurantiaca* and *E. guerrerensis* have a very short stems). Molecularly can be identified by the presence of the nucleotide T in the sites 24 and 730 of the *matK* region against the nucleotide C of the rest of the *Gibbiflorae* taxa.

18) ***Echeveria lozanoi*** Rose. Type: Mexico. Jalisco, mountains near Etzatlán, *C. G. Pringle y F. L. Lozano 11890*, 1905 (US, num. 460734).
From Rose´s protologue: Acaulescent; leaves forming a dense rosette, lying flat upon the ground, lanceolate or strap-shaped, 10 cm long, 2-4 cm broad, the central one's copper colored; inflorescences short panicles, 3-4 dm long; corolla light copper colored. **Distribution**: Jalisco. **Notes**: A poorly known species, several attempts to re-collect this species in the type locality have failed, our sampled specimens came from near Ayutla, Jalisco and match the description in Rose protologue. We expand the description as follows: leaves sometimes undulate or crenulate, channeled, dark brown or brown with copper shades; sepals linear-deltoid, brown; corolla tubular-urceolate, pale pink at the base, orange at the apex; petals slightly recurved at the apex, keeled; petals with two white finger-like appendixes at the base of the epipetalous filaments; carpels with white ovaries at the base, green above, red styles, green stigmas, light yellow nectary scales. Phylogenetically and biogeographically related to *E. munizii*, *E. nayaritensis*, *E. roseiflora* and *E. rulfiana*. Morphologically is closer to *E. roseiflora*, since *E. munizii*, *E. nayaritensis* and *E. rulfiana* have evident stems but shares with the former taxa the yellowish nectary scales. Differs from *E. roseiflora* by its bicolored (light pink at base, orange at the apex vs pale pink) flowers and the color of the nectary scales (yellow vs red). Molecularly cannot be identified by our dataset.

19) ***Echeveria marianae*** I.García & Costea. Type: Mexico. Jalisco: Municipality of Valle de Juárez, Ojo de Agua canyon to the east of Mazamitla, 2460 m, *I. García & M. Costea 8732* (holotype: CIMI!).
Acaulescent; glabrous; rosettes up to 40 cm; leaves obovate to lanceolate, light green to yellow-green, not glaucous, acute to apiculate, margins with a thin red line, crenulate in young leaves; inflorescences paniculate, solitary or double, up to 100 cm long, with 7-10 cincinni; corolla urceolate-campanulate, 15-20 cm long, pale yellow-whitish at the base, orange in the rest with dark-orange to reddish tips; petals with two finger-like appendixes at the base of the epipetalous filaments; white ovaries, purple styles sometimes reddish below, green stigmas, pinkish-red nectary scales. **Distribution**: Jalisco. **Notes**: Morphologically it is related to *E. subrigida* from which shares the corolla coloration pattern and the color of the nectary scales (pinkish-red), however, it can be differentiated from *E. subrigida* by its widest farinaceous leaves and by the

absence of appendixes on the epipetalous filaments in this latter. Can be associated also, morphologically and geographically with *E. dactylifera* and *E. novogaliciana* by their acaulescent habit, narrowed leaves, and the presence of epipetalous appendixes, and can be differentiated because *E. marianae* have red nectary scales vs yellow (*E. dactylifera*) and salmon (*E. novogaliciana*). Molecularly it can be identified by the presence of the nucleotide A (adenine) on the site 228 of the *matK* chloroplast region vs the presence of C (cytosine) of the rest of the *Gibbiflorae* taxa.

20) ***Echeveria michihuacana*** L.E.Cruz-López, Reyes & Verg.-Silva. Type: Mexico. Michoacán. Municipality of Ciudad Hidalgo, along the Mexico 15 highway from Morelia to Ciudad Hidalgo, near Mil Cumbres, 2564 m, *Luis E. de la Cruz-López 184* (MEXU).
Caulescent; stems erect or somewhat decumbent, 2–30 cm long; leaves forming a lax rosette up to 50 cm in diameter, obovate to lanceolate-oblanceolate, green with red margins or completely reddish in the dry period, pseudo-petiolate and winged at the base, only the apical leaves slightly pruinose, apex obtuse to rounded, mucronate, margins entire to crenate, reddish; inflorescence 1–3 panicles per rosette, 50–120 cm long or more; sepals fused at the base, linear–deltoid, reflexed, unequal, green; corolla conoid-tubular in bud, tubular-urceolate at anthesis, rounded to slightly pentagonal in transection, 1.8–2.15 cm long, 1.6–1.95 cm wide at the base, 0.9–1.25 cm wide at the apex, segments ovate-lanceolate, dorsally slightly keeled, fused at the base, imbricate, apex acute and slightly reflexed, 6.5–8 mm wide at the base, orange or pink, internally yellow with reddish margins and apex, somewhat thickened at the sides of the epipetalous filaments, nectar cavity reduced; androecium included, stamens 10, 5 episepalous, 1.4–1.7 cm in length, 5 epipetalous slightly shorter, filaments yellow, anthers 1.5–3 mm in length, pale yellow; gynoecium included, carpels 5, free, 1.4– 1.7 cm long, 3–3.8 mm wide at the base, nectary scales lunate-reniform, 4 mm wide and 1.5–2 mm tall, deep red, ovaries white to pale yellow, styles wine-red to dark purple, stigmas green; fruit with 5 divergent follicles, with numerous reddish seeds. **Distribution**: Michoacán. **Notes**: Morphologically, biogeographically and phylogenetically related to *E. pistioides*, our molecular dataset cannot differentiate both species, however, pronounced morphological differences such as: its evident stem vs acaulescent, narrowed and longer leaves vs obovate-obcordate, its red or green with red margins vs glaucous farinose leaves, ovate-deltoid vs elliptic-petals and by its white vs yellow ovaries, among others, indicates that are two distinct species.

21) ***Echeveria munizii*** Vázquez-García, Nieves-Hernández, Padilla-Lepe, Nuño-Rubio & Cházaro-Basáñez. Type: Mexico. Colima: Carretera de Comala a la Hacienda San Antonio, 2 km antes de la Hacienda de San Antonio, 1325 m, 19°26’21.1”N, 103°42’32.4”W, 12 de octubre de 2012, *Miguel A. Muñiz-Castro, J. Antonio Vázquez-García, Jesús Padilla-Lepe 1137* (holotype IBUG; isotypes MEXU, IEB).
Epiphytic perennial with lax rosette; stem erect to decumbent, 25–40 cm; medium sized rosettes up to 30 cm; leaves oblanceolate to obovate, green to olive or brownish green; inflorescence cymose-paniculate with 10–11 flowers in lateral circinate branches; sepals equal, lanceolate; corolla urceolate, 1.5 cm long, 1.0–1.1 cm wide; petals pink at the base to orange at the apex, ovate, concave, acute at the apex; white ovaries, yellowish and reniform nectary scales, purple styles. **Distribution**: Only known from the type locality in Colima. **Notes**: Phylogenetically belongs to a sub-clade of the “northwestern” group, which includes: *E. lozanoi*, *E. nayaritensis*, *E. roseiflora* and *E. rulfiana*. Of this group, *E. munizii* is geographically and morphologically

closer to *E. rulfiana*, from which is difficult to differentiate (maybe by the absence vs presence of epipetalous appendices, respectively). Molecularly, cannot be identified by our dataset.

22) ***Echeveria nayaritensis*** Kimnach. Type: Mexico. Nayarit: ca. 1 milla NW of Ahuacatlán, ladera de lava a lo largo de la carretera 15. 3,500 m, *M. Kimnach 1912* (Holotype: HNT). Caulescent, stems up to 20 cm long; medium sized rosettes (20 cm in diameter), lax; leaves oblanceolate, light brown to lilac-purple colored, canaliculate, acuminate to acute at apex; inflorescences in panicles up to 40 cm long, with circinated branches, each with 3–5 flowers, long pedicels (up to 1.5 cm long); sepals reflexed, deltoid-ovate, green to purplish-green; corolla tubular-urceolate, up to 1.5 cm long; petals elliptic-ovate, reflexed at the apex, yellow at the base and orange-red at the apex, with tiny appendices at the base of the epipetalous filaments; white ovaries, yellow nectary scales and short wine-red styles. **Distribution**: South of Nayarit and northwest of Jalisco. **Notes**: Phylogenetically belongs to a sub-clade of the “northwestern” Mexico group, along with *E. lozanoi*, *E. munizii*, *E. roseiflora* and *E. rulfiana*. A close relationship with *E. patriotica* and *E. coruana* was presumed based on the flower pattern coloration, however our molecular dataset places them distantly related. Molecularly, is distinct from all *Gibbiflorae* species by the presence of the nucleotide G vs A in the site 22 of the *matK* region.

23) ***Echeveria novogaliciana*** J.Reyes, Brachet & O.Gonzáles. Type: Mexico, Aguascalientes, Municipality of San José de Gracia, Agua Zarca, near Milpillas, 2434 m, *J. Reyes, C. Brachet, A. Gutiérrez de la Rosa JE-5184* (MEXU).
Acaulescent; large sized rosettes (up to 60 cm in diameter); leaves lanceolate to deltoid-ensiform, channeled, acuminate, orange, lilac or flesh colored, farinose, margins entire sometimes denticulate; inflorescences in panicles, erect, up to 80 cm long, 4–7 flowers per cincinni; corolla tubular-campanulate, up to 2.5 cm in length; petals ovate-oblong, reflexed at the apex, pink with orange margins, with two finger-like appendices in the sides of the epipetalous filaments; white ovaries, wine-red styles, salmon-pink elliptic nectary scales. **Distribution**: Aguascalientes, Jalisco. **Notes**: Phylogenetically, closer o *E. perezcalixii*, morphologically similar to *E. dactilifera* and to a potential new species (EK-3569) from Durango. It is different from the aforementioned taxa by its ensiform channeled leaves and by its salmon-pink vs yellow and pink nectary scales. Molecularly, can be differentiated from all *Gibbiflorae* taxa except for *E. perezcalixii* (Table 2).

24) ***Echeveria pallida*** E.Walther. Type: From cultivated plants at Ciudad de México and cultivated in the Golden Gate Park, San Francisco (CAS, no. 251053).
Caulescent, stem simple or branching up to 40 cm long; medium sized lax rosettes up to 40 cm in diameter; leaves obovate to oblanceolate, pale green with pink entire margins; inflorescences in erect panicles up to 50 cm in length, much branched, cincinni with up to 15 flowers each; sepals very unequal, the longest to 2 cm long, pale green; corolla tubular to campanulate, 1.7 cm in length; petals gibbous at the base, ovate-oblong, pink, with prominent nectar cavity; white ovaries, wine-red styles, white nectary scales. **Distribution**: unknown, probably from Oaxaca and Chiapas, where it is cultivated and sale. **Notes**: phylogenetically related to unknown, belongs to the “southern Mexico” group. Morphologically, related to *E. acutifolia* and *E. gigantea*. From *E. gigantea* differs by its broad obovate leaves, pale green vs bluish leaves and by its pink flowers. From *E. acutifolia*, is difficult to differentiate (broader bracts, much more flowered panicles). Molecularly, can be identified by the presence of the nucleotide C against T in the site 529 of the

rbcL region, our sampled specimens came from a nursery near Betania, Chiapas.

25) ***Echeveria patriotica*** I.García & Pérez-Calix. Type: Mexico. Jalisco. Municipality of Mazamitla, Plan de Cervantes, km 14.5 highway to junction Quitupan-Valle de Juárez, to Santa María del Oro; 5 km straight to the south of the ranch El Tigre. 2290 m; 19°51'15" N; 102°56'50" W.
Caulescent, stems up to cm long, simple sometimes branched; medium sized rosettes (up to 42 cm in diameter), lax; leaves oblong-obovate to spatulate, pseudopetiolate, channeled at the base, green, reddish-brown sometimes purple tinged; inflorescences paniculated, up to 100 cm long, cincinni with few flowers (1–8); corolla tubular-conoid, up to 2.4 cm in length; petals elliptic-oblong, keeled, slightly reflexed at the apex, yellowish at the first tird, red at the apex; white ovaries, purple styles, dark red nectary scales. **Distribution**: Jalisco. **Notes**: Phylogenetically, geographically and morphologically related to *E. coruana*, particularly, regarding the flower characteristics, and from which differs by its evident stem (caulescent vs acaulescent), its wider leaves, longer flowers (2.4 vs 2.0 cm) and by the color of the nectary scales (dark red vs yellow). Molecularly, it can be segregated from *E. coruana* by the presence of the nucleotide C vs A in the site 230 of the *rbcL* chloroplast region. From all *Gibbiflorae* taxa (except *E. coruana*) E. patriotica can be differentiated by the presence of the nucleotide G against T in the site 693 of the matK region (Table 2).

26) ***Echeveria perezcalixii*** Jimeno-Sevilla & P.Carrillo. Type: Mexico. Nayarit, Municipality of La Yesca, road from Plan del Muerto-La Virgen to hydroelectric dam of La Yesca, 1510 m, 21°13′54″N, 104°03′23″W, *D.Jimeno-Sevilla, A.Albalat & M.Cházaro 671* (holotype: IEB).
Acaulescent; medium sized rosettes (up to 25 cm in diameter), lax; leaves oblanceolate-spatulate, psedopetiolate, channeled at the base, light brown to lilac-gray, farinaceous, margins white-hyaline, often crenulate; inflorescences paniculate, up to 48 cm in length, long pedicels (2.0 cm); corolla tubular-urceolate, up to 1.5 cm in length; petals oblong, keeled, orange-pink, with two short conical appendices on the sides of the epipetalous filaments; white ovaries, wine-red styles, yellow nectary scales. **Distribution**: Jalisco, Nayarit, Jalisco. **Notes**: related to *E. fulgens* var. *fulgens* regarding the shape of the leaves, its short flower, orange-pink petals and by its yellow nectary scales, however, Bayesian inference consensus tree indicates that are distantly related, in turn *E. perezcalixii* is closer to *E. novogaliciana*. Molecularly can be distinguished from all *Gibbiflorae* taxa (exept from *E. novogaliciana* and *E*. aff. *acutifolia* JE-8758) by the presence of the nucleotide T against C in the site 221 of the ITS2 region.

27) ***Echeveria pistiodes*** I.García, I.Torres & Costea. Type: Mexico. Michoacán. Municipality of Tzitzio, c.a 1 km to the southeast of Piedras de Lumbre, 1950 m, *I.García & I.Torres 9136* (holotype: CIMI).
Acaulescent; large sized rosettes (up to 50 cm in diameter), lax; leaves obcordate to obovate-spatulate, glaucous or emerald-green, farinaceous, margins entire sometimes with reddish line; inflorescences in panicles up to 90 cm long, 3–4 secondary cincinni; sepals somewhat appressed; corolla tubular-urceolate, up to 1.6 cm long; petals ovate-elliptic, keeled, light orange at the base, pink in the middle and red at the apex; cream colored ovaries, purple styles, dark red nectary scales. **Distribution**: Michoacán. **Notes**: phylogenetically, biogeographically and morphologically related to *E. michihuacana*, from which differs by the acaulescent habit (vs caulescent), its wider and frequently glaucous farinaceous leaves (vs green without farina), its

shorter inflorescences and flowers, its flower coloration pattern (light orange at the base, orange-pink at the middle and red at the apex vs uniform reddish), and by its yellow vs white ovaries. Molecularly, it can´t be distinguished from *E. michihuacana*, their DNA sequences are very similar, in fact, they share a deletion event at the site 247 of the ITS2 region, which makes them very closely related. This deletion event can be used to identified *E. pistioides* from others *Gibbiflorae* taxa.

28) ***Echeveria prunina*** Kimnach & Moran. Type: Mexico. Chiapas: over rocks at el Mirador Cuesa, El Sumidero, near Tuxtla Gutiérrez, ca. 4000 m. *T. MacDougall B-138* (holotype: HNT). Acaulescent or with short stem (up to 10 cm long); lax small sized rosettes (15 cm in diameter); leaves oblanceolate-spatulate, pseudopetiolate, brownish-green to bluish-lilac, farinaceous; inflorescences short panicles or cincinni, up to 30 cm long, with few bracts similar to the rosette leaves but shorter; sepals unequal, somewhat appressed to corolla tube, gray, farinaceous; corolla tubular, up to 1.3 cm in length; petals oblong-lanceolate, acute, pink; ovaries cream colored, wine-red styles, cream translucid nectary scales. **Distribution**: Chiapas. **Notes**: poorly known species, phylogenetically closer to unknown, belong to the "southern Mexico" group. Morphologically related to E. grisea, from which differs by its shorter and less flowered inflorescences and by its geographical distribution. Molecularly can be distinguished from all *Gibbiflorae* taxa by the presence of the nucleitode C vs A in the site 162 of the matK region and the nucleotide A vs G in the site 218 of the ITS2 region. Our sampled specimen came from Chiapa de Corzo, Chiapas, due PCR amplification problems only one individual was sampled.

29) ***Echeveria purhepecha*** I.García. Type: Mexico. Michoacán. Municipality of Nuevo Parangaricutiro ca. 0.5 km to the north of Nuevo San Juan Parangaricutiro. 1,900 m; 19º 25´ 18´´ N; 102º 07´ 43´´ O. *I.García & J.A.Machuca 4491* (holotype: IEB).
Caulescent, stems up to 11 cm in length; small sized rosettes (up to 12 cm in diameter); leaves oblanceolate, pseudopetiolate, channeled at the base, dark green, margin entire with red line; inflorescences in short panicles, up to 30 cm in length; bracts few, elliptic-oblong, ascending, somewhat appressed to the floral stem; corolla tubular-conoid, 1.3 cm long; petals keeled, light orange at the base, orange in the middle and red at the base, with two short appendices at the base of the epipetalous filaments; white ovaries, purple styles, cream colored nectary scales. **Distribution**: Michoacán. **Notes**: phylogenetically it is located in a sub-clade of the "northwestern Mexico" group along *E. lozanoi*, *E. rulfiana*, *E. nayaritensis*, *E. roseiflora* and *E. munizii*. Morphologically is related to *E. fulgens* var. *fulgens*, particularly, the specimens labeled as JE-8031 from Estado de México, which may represent a potential new species. Molecularly it can´t be differentiated by or dataset.

30) ***Echeveria roseiflora*** J.Reyes & O.Gonzáles. Type: Mexico. Jalisco, Municipality of Mascota, 2 km from Juanacatlán to Mascota road to la Laguna de Juanacatlán. 2150 m, *G. Salazar & Miguel Angel Soto 7249* (holotype: MEXU).
Acaulescent; small sized rosettes (up to 20 cm in diameter), lax; leaves obovate-oblanceolate or strap-shaped, green, yellowish, purplish or reddish, margins frequently crenate, pink; inflorescences in panicles, up to 55 cm long; corolla tubular-urceolate, 1.6 cm in length; petals reflexed at the apex, ovate-oblong, pale pink, with two short appendices of the inner face of the petals; white ovaries, purple styles, red nectary scales. **Distribution**: Jalisco. **Notes**: phylogenetically located in a sub-clade of the "northwestern Mexico" group along with *E.*

lozanoi, *E. rulfiana*, *E. nayaritensis* and *E. munizii*. Of these group, *E. roseiflora* is morphologically closer to *E. lozanoi* regarding their acaulescent habit, strap shaped leaves and the shape of the flowers, in turn, it can be segregated by its pale pink (vs cooper) flowers and by its red (vs pale yellow) nectary scales. Molecularly can be distinguished by the presence of the nucleotide G (two out of three individuals) vs A of the rest of the *Gibbiflorae* taxa.

31) ***Echeveria rubromarginata*** Rose. Type: Mexico. Veracruz, over rocks near Orizaba, C. A. Purpus, 1903/R:930 (US no. 399848).
Acaulescent or with short stem, up to 10 cm long; medium sized rosettes (up to 40 cm in diameter); leaves obovate to oblanceolate, bluish with pink entire or ondulate margins, somewhat farinaceous; inflorescences in panicles up to 70 cm long, cincinni with 4–15 flowers; corolla tubular-campanulate, up to 1.7 cm in length; petals ovate-lanceolate, pink at the base, orange at the apex, keeled; white ovaries, wine-red styles, white nectary scales. **Distribution**: Puebla, Tlaxcala, Veracruz. **Notes**: phylogenetically related to unknown, morphologically and geographically related to *E. gigantea* regarding their bluish leaves with pink margins, the shape of the inflorescence and their white nectary scales, conversely, can be differentiated from *E. gigantea* by the acaulescent vs caulescent habit and by its pink-orange (vs phosphorescent pink) flowers. Molecularly can´t be distinguished by our dataset.

32) ***Echeveria rulfiana*** Jimeno-Sevilla, Santana Mich. & P.Carrillo. Type: Mexico. Jalisco, Municipality of San Gabriel, Hill to the wets of Apango, ravine over lava rocks, 2063 m, D. *Jimeno S., F. J. Santana M., D. Vergara R. y M. Castañeda 1336* (holotype: IEB).
Caulescent, stems erect to pendants, up to 135 cm in length; medium sized rosettes (up to 40 cm in diameter); leaves oblanceolate, canaliculate, pseudopetiolate, margin entire, hyaline-white; inflorescences in panicles, 1 per rosette, up to 70 cm long; corolla tubular-urceolate, up to 2 cm in length; petals lanceolate-oblong, orange-pink, keeled, reflexed at the apex, with two finger-like appendices on the inner face of the petals; white ovaries at the base, yellowish above, wine-red long styles, yellow nectary scales. **Distribution**: Jalisco. **Notes**: phylogenetically belongs to a sub-clade of the "northwestern Mexico" group along with *E. lozanoi*, *E. nayaritensis*, *E. roseiflora* and *E. munizii*, being this last one species, from which is morphologically closer (see *E. munizii* notes). Molecularly can´t be identified by our dataset.

33) ***Echeveria sonianevadensis*** A.Vázquez, Jimeno-Sevilla & I.García. Type: Mexico. Jalisco. Municipality of Tuxpan, to the north of the road of Las Trojitas ravine, 3253 m, *Miguel Muñiz-Castro, Rosa Murguía-Araiza & Jesús Padilla-Lepe 1205* (holotype: IBUG).
Caulescent, stems up to 25 cm long; medium sized rosettes (up to 30 cm in dimeter), lax; leaves oblanceolate, green with reddish margins; inflorescences in panicles, up to 45 cm long, with 1–3 cincinni; corolla tubular-conoid, up to 1.8 cm in length; petals oblong, keeled, orange-red with yellow keel; white ovaries, purple styles, dark red nectary scales. **Distribution**: Colima and Jalisco limits. **Notes**: poorly known species due very recent discovery, we had access only to single specimen. Phylogenetically related to *E. coruana* and *E. patriotica*. Geographically related to *E. cerrograndensis*, *E. munizii* and *E. rulfiana*. Morphologically similar to *E. fulgens* var. *obtusifolia*, from which can be easily distinguished by its longer stems, and its red nectary scales. Molecularly can´t be identified by our dataset.

34) ***Echeveria steyemarkii*** Standl. Type: Guatemala. Dept. Zacapa, between Santa Rosalía de

Mármol and San Lorenzo. 1,200–1,600 m, *Julian A. Steyermark 43145* (type in Herb. Field Mus.).
From E. Walther´s description. Acaulescent or with short stem, up to 5 cm long; medium sized rosettes (up to 30 cm in diameter); leaves oblanceolate, persistent when dried, margin entire, at apex rounded to acute, pseudopetiolate, with three parallel longitudinal veins; inflorescences one or several, 5 to 20 cm. tall, of a simple, or sometimes 2-branched secund raceme; racemes with three to five flowers each, pedicels 5 to 15 mm long, somewhat turbinate below calyx; sepals unequal, 4 to 8 mm. long, free to base or somewhat connate, spreading to reflexed at anthesis, ovate-oblong, to deltoid, acute; corolla to 1.1 cm long to 8 mm thick near base when pressed; petals narrowly lanceolate, erect, keeled on back, slightly spreading at the acuminate apex. **Distribution**: Guatemala. **Notes**: poorly known species, more field work needed, this species is geographically and morphologically related to *E. gudeliana* regarding their short stems, rosettes sizes, oblanceolate leaves, their three parallel leaves veins, reddish flowers. It is possible that *E. gudeliana* and *E. steyermarkii* belongs to the same species, but more field work and DNA sequences are needed in order to reveal this hypothesis.

35) ***Echeveria subrigida*** Rose. Type: Mexico, over rock at Tultenango canyon. Pringle, 1892/4326. (GH, type; BR,F,-MEXU,P,PH,UC,US,W).
Acaulescent; medium sized rosettes (up to 40 cm in diameter, or more); leaves obovate-oblanceolate, pale green or glaucous, very farinose, margins entire to crenate-undulate, frequently with red line margin; inflorescences in narrow panicles, up to 1 m long, with up to 12 cincinni branches, up to 8 flowers each; sepals linear-lanceolate; corolla tubular-campanulate, 1.7–2.2 cm long; petals elliptic-oblong, keeled, pink-orange at the base, yellow at the apex, without epipetalous appendices; yellow ovaries, wine-red styles, pinkish to dark red nectary scales. **Distribution**: Estado de México, Guanajuato, Hidalgo, Michoacán, San Luis Potosí, Tlaxcala. **Notes**: phylogenetically belongs to a sub-clade of the northwestern Mexico group along with *E. marianae*, *E. cerrograndensis*, *E. cante*, *E. soninevadensis*, *E. patriotica*, *E. coruana*, *E. novogaliciana*, *E. perexcalixii*, *E. dactylifera* and *E*. sp. EK-3569. In this group *E. subrigida* is morphologically closer to *E. marianae* and *E. cante*. From *E. marianae* is distinguished by its broader glaucous leaves (*E. marianae* have narrower and frequently green leaves), and the absence of epipetalous appendices (*E. marianae* have two appendices). From *E. cante* can be differentiated by its orange-yellow flowers (vs pink) and the color of the nectary scales (dark red vs white sometimes with pinkish stains). Molecularly can be identified by the presence of the nucleotide T/C (four out of six individuals have the T) vs C in the site 49 of the *matK* region and by the presence of the nucleotide T vs C in the site 294 of the ITS2 region.

36) ***Echeveria triquiana*** J. Reyes & Brachet. Type: Mexico. Oaxaca. Municipality of Santiago Juxtlahuaca, Agua Fría, 7.7 km south of Naranjos, along the road Juxtlahuaca to Tlaxiaco and Putla, 1889 m. *J. Reyes & C. Brachet 5614* (Holotype: MEXU).
Acaulescent or with short stem (up to 10 cm long); medium sized rosettes (up to 30 cm in diameter); leaves obovate-oblanceolate, green or tinged reddish-brown, margins entire to crenate, red; inflorescences in panicles, up to 60 cm long, 3–6 cincinni; corolla tubular-urceolate, up to 1.3 cm long; petals lanceolate, pinkish-orange; yellow ovaries, purple styles, white nectary scales. **Distribution**: Oaxaca. **Notes**: phylogenetically related to unknown, morphologically related to *E. fulgens* var. *fulgens* from which is difficult to differentiate. Molecularly can be identified from all Gibbiflorae taxa (except from *E. nayaritensis* ad *E*. aff. *gibbiflora* JE-6371) by the presence of

the nucleotide T vs C in the site 254 of the ITS2 nuclear genome region.

37) **Echeveria uxorium** Jimeno-Sevilla & Cházaro-Basáñez. Type: Mexico. Veracruz. Municipality of Amatlán de los Reyes, Summit of the hill Rostro Divino, 2 km NE of the bus Terminal of Córdova City. *D. Jimeno-Sevilla, M. Cházaro & A. Albat 245* (holotype: IEB). Caulescent, stem up to 45 cm long or more; medium sized rosettes (up to 30 cm in diameter), lax; leaves oblanceolate to oblanceolate-obovate, green or reddish-brown, cuneate, channeled, margins entire to lacerate-undulate; inflorescences in thyrsoid racemes, much branched, up to 65 cm long, 1–8 flowers per cincinnus, with many bracts; corolla tubular-conoid, up to 1.5 cm in length; petals lanceolate, keeled, reddish-orange; white ovaries, wine-red styles, white nectary scales. **Distribution**: Oaxaca, Veracruz. **Notes**: this species was originally located in ser. *Racemosae*, however, as is mentioned in the authors discussion, *E. uxorium* can be related to ser. *Gibbiflorae* too. Previous phylogenetic analysis confirms that this species belongs to Gibbiflorae (Cruz-López et al, 2019). Our data set does not have sufficient resolution to determine its closest relatives but, is grouped in the south Mexico group. Morphologically is related to *E. fulgens* regarding its short reddish-orange corollas, white ovaries and white nectary scales, conversely, can be distinguished by its longer stems and by its much branching thyrsoid racemes. Molecularly can´t be identified by our dataset.

38) **Echeveria xochipalensis** J.Reyes, L.E.Cruz-López & Verg.-Silva. Type: Mexico. Guerrero. Municipality of Eduardo Neri, about 2 kms southwest of La Laguna, along the road from Filo de Caballo to Xochipala, 1960 m, *J. Reyes 8132* (holotype: MEXU).
Caulescent, stem up to 7 cm, with white leaf scars; small sized rosettes (up to 20 cm in diameter), lax; leaves spatulate, rigid, pseudopetiolate, green, lilac-gray, or copper colored with pinkish hues, margin entire, yellow to hyaline in apical leaves; inflorescences in panicles, up to 70 cm with up to 4 cincinni; corolla tubular-urceolate, 1.2–1.25 cm long; petals lanceolate, keeled, slightly reflexed, pink at the base, orange at the apex; white ovaries, wine-red styles, white nectary scales. **Distribution**: Guerrero. **Notes**: phylogenetically related to unknown, belongs to south Mexico group, morphologically similar to *E. fulgens* varieties, from which differs by its rigid, spatulate and variable in color leaves and by its white nectary scales. Molecularly can´t be identified by our dataset.

Literature cited

Berger, A. (1930) Crassulaceae. *En*: Engler, A. y Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien*, ed. 2, 18a. W. Engelmann, Leipzig, pp. 352–483.

Bergmann, T., Hadrys, H., Breves, G. y Schierwater, B. (2009) Character-based DNA barcoding: a superior tool for species classification. *Berliner und Münchener Tierärztliche Wochenschrift* 122: 446–450.

Bergmann, T., Rach, J., Damm, S., DeSalle, R., Schierwater, B. y Hadrys, S. (2013) The potential of distance-based thresholds and character-based DNA barcoding for defining problematic taxonomic entities by CO1 and ND1. *Molecular Ecology Resources* 13: 2–13. DOI: 10.1111/1755-0998.12125

Candolle, A. P. (1828) Crassulaceae. *En*: A.P. de Candolle, A.P. y de Candolle A.L.P.P. (Eds.), *Prodromus Systematis Naturalis Regni Vegetabilis* 3. Treuttel & Würtz, París, pp. 382–414.

Carrillo-Reyes, P., Sosa, V. y Mort, M. E. (2009) Molecular phylogeny of the *Acre* clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*. *Molecular Phylogenetics and Evolution* 53: 267–276. DOI: 10.1016/j.ympev.2009.05.022.

CBOL Plant Working Group. (2009) A DNA Barcode for Land Plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 12794-12797. DOI: http://dx.doi.org/10.1073/pnas.0905845106

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. y Leon, C. 2010. Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE* 5: 1–8.

De la Cruz-López, L. E., F. Vergara-Silva, S. J. Reyes, O. G. Espino, P. Carrillo-Reyes & M. Kuzmina. 2019. Phylogenetic relationships of Echeveria (Crassulaceae) and related genera from Mexico, based on three DNA barcoding loci. Phytotaxa 422: 33–57.

DeSalle, R. Egan, M. G. y Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B* 360: 1905–1916.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology And Evolution* 29.

Etter, J. y Kristen, M. 2015. Crassulaceae. Data base. www.crassulaceae.com.

Fujita, M. K, Leaché, A. D, Burbrink, F. T., McGuire, J. A., & Moritz, C. 2012. Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology and Evolution* 27.

García R, I. y Pérez-Calix, E. 2007. Una especie nueva de *Echeveria* (Crassulaceae) originaria del estado de Jalisco, México. Acta Botánica Mexicana 78: 125-132.

García R, I. 2011. Nueva especie de *Echeveria* del centro-occidente de Michoacán, México. *Revista Mexicana de Biodiversidad 82*.

García-Ruiz, I., Torres, I., y Costea, M. 2016. A New Species of *Echeveria* (Crassulaceae) from Michoacán, México. *Systematic Botany 41*.

Goldstein, P. Z. y DeSalle, R. (2011) Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. *Bioessays* 33: 135–147.

Gu, W., Song, J., Cao, Y., Sun, Q., Yao, H., Wu, Q., Chao, J., Zhou, J., Xue, W., & Duan, J. 2013. Application of the ITS2 region for barcoding medicinal plants of Selaginellaceae in Pteridophyta. *PLOS ONE 8*.

Huelsenbeck, J. P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754-755.

Jimeno-Sevilla, H. D. & Carrillo-Reyes, P. 2010. *Echeveria perezcalixii*, una especie nueva del occidente de México. *Brittonia 62*.

Jimeno-Sevilla, H. D., P. Carrillo-Reyes., E. Pérez-Calix. & M. J. Cházaro-Basáñez. 2012. Additions to the Crassulaceae of the State of Veracruz, Mexico. Haseltonia 18: 140–152.

Jörger, K. M. y Schrödl, M. (2014) How to use CAOS software for taxonomy? A quick guide to extract diagnostic nucleotides or amino acids for species descriptions. *SPIXIANA* 37: 21–26.

Katoh, K. y Standley, D. M. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Molecular Biology and Evolution 30*.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies & A. Drummond. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649.

Kimnach, M. (2003) Echeveria. *En*: Eggli, U. (Ed.) *Illustrated handbook of succulent plants*: *Crassulaceae*. Springer–Verlag, Berlin, pp. 103–128.

Lanfear, R. Calcott, B. Ho, S. Y. & Guindon, S. 2012. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution 29*:1695–1701.

Lemaire, A.C. (1845) *Echeveria fulgens*: Crassulacées & Obilicees. Hortus Vanhoutteanus 1: 8.

Maddison, W. P. and D.R. Maddison. 2019. Mesquite: a modular system for evolutionary analysis. Version 3.61 http://www.mesquiteproject.org

Meyrán, J. y López C, L. 2003. Las crasuláceas de México. *Sociedad Mexicana de Cactología, A.C.*

Moran, R. 1974. Division of the genus Echeveria into series. In: Jacobsen, H. (ed.). Lexicon of succulent plants. *Blandford Press*.

Nicolalde-Morejón, F., Vergara-Silva, F., González-Astorga, J. y Stevenson, D. W. (2010) Character-based, population-level DNA Barcoding in Mexican species of *Zamia* L. (Zamiaceae: Cycadales). *Mitochondrial DNA* 21: 51-59.

Pilbeam, J. 2008. *The genus Echeveria*. The British Cactus & Succulent Society, Essex, 333 pp.

Rach, J., DeSalle, R., Sarkar, I. N., Schierwater, B. y Hadrys, H. 2008. Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. *Proceedings of the Royal Society B* 275: 237–247.

Rambaut, A., Suchard, M. A., Xie, D. & Drummond, A. J. 2014. Tracer v1.6, Available from http://beast.bio.ed.ac.uk/Tracer.

Ratnasingham, S. y Hebert, P.D.N. (2007) BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7, 355–364. DOI: 10.1111/j.1471-8286.2006.01678.x

Reyes-Santiago, J. & González-Zorzano, O. 2010. Echeveria roseiflora (Crassulaceae) una nueva especie para el estado de Jalisco, México. *Cactáceas y Suculentas Mexicanas 55*.

Reyes S, J., Brachet I, C., González-Zorzano, O. 2011. Echeveria guerrerensis (Crassulaceae) una nueva especie para el estado de Guerrero, México. Cactáceas y Suculentas Mexicanas 56(3): 75–81.

Sarkar, I. N., Planet, P. J. y DeSalle, R. (2008) caos software for use in character-based DNA barcoding. Molecular Ecology Resources 8: 1256–1259. DOI: https://doi.org/10.1111/j.1755-0998.2008.02235.x

Stöver, B.C., Müller, K.F. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* **11**. https://doi.org/10.1186/1471-2105-11-7

Véliz P, M. E. & García-Mendoza, A. J. 2011. Echeveria gudeliana, nueva especie de Crassulaceae de Mesoamérica. International Cactus-adventures 91: 1–7.

Walther, E. (1972) *Echeveria*. California Academy of Sciences, San Francisco, 426 pp.

Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P. y Chen, S. (2010) Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS ONE 5*: 1–9.

Yang, Z. 2015. The BPP program for species tree estimation and species delimitation. *Current Zoology 61*.

Zhang J-Q, Meng S-Y, Wen J, Rao G-Y (2015) DNA Barcoding of *Rhodiola* (Crassulaceae): A Case Study on a Group of Recently Diversified Medicinal Plants from the Qinghai-Tibetan Plateau. *PLoS ONE*. 10(3): 1-15.

Zou, S., Q. Li., L. Kong., H. Yu., X. Zheng. 2011. Comparing the Usefulness of Distance, Monophyly and Character-Based DNA Barcoding Methods in Species Identification: A Case Study of Neogastropoda. PLoS ONE 6: 1–8.

Zou, S., Fei, C., Wang, C., Gao, Z., Bao, Y., He, M. y Wang, C. 2016. How DNA barcoding can be more effective in microalgae identification: a case of cryptic diversity revelation in Scenedesmus (Chlorophyceae). *Scientific Reports 6*.

Zou, S, Fei, C., Song, J., Bao, Y., He, M., y Wang, C. 2016. Combining and Comparing Coalescent, Distance and Character-Based Approaches for Barcoding Microalgaes: A Test with Chlorella-Like Species (Chlorophyta). *PLoS ONE 11*.

Table captions

Table 2. Simple pure diagnostic characters for ser. *Gibbiflorae* on *rbcL*, *matK* and ITS2 DNA regions.

		rbcL						*matK*												
	Taxa	**151**	**193**	**230**	**472**	**479**	**529**	**22**	**24**	**49**	**93**	**154**	**162**	**168**	**179**	**228**	**266**	**273**	**300**	**323**
1	*E. acutifolia*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	**C**	A	C	C
2	*E. aurantiaca*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
3	*E. cante*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
4	*E. cerrograndensis*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
5	*E. coruana*	T	T	**A**	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
6	*E. crenulata*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
7	*E. dactylifera*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
8	*E. fimbriata*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	**G**	C	C
9	*E. fulgens* var. *fulgens*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
10	*E. fulgens* var. *obtusifolia*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
11	*E. gibbiflora*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
12	*E. gigantea*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
13	*E. grisea*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
14	*E. guerrerensis*	T	T	C	**C**	C	T	A	C	C	A	A	A	T	A	C	G	A	C	**T**
15	*E. juarezensis*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
16	*E. longiflora*	T	T	C	G	C	T	A	**T**	C	A	A	A	T	A	C	G	A	C	C
17	*E. lozanoi*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
18	*E. marianae*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	**A**	G	A	C	C
19	*E. michihuacana*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
20	*E. munizii*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
21	*E. nayaritensis*	T	T	C	G	C	T	**G**	C	C	A	A	A	T	A	C	G	A	C	C
22	*E. novogaliciana*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
23	*E. pallida*	T	T	C	G	C	**C**	A	C	C	A	A	A	T	A	C	G	A	C	C
24	*E. patriotica*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
25	*E. perezcalixii*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
26	*E. pistioides*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
27	*E. prunina*	T	T	C	G	C	T	A	C	C	A	A	**C**	T	A	C	G	A	C	C
28	*E. purhepecha*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
29	*E. roseiflora*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
30	*E. rubromarginata*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
31	*E. rulfiana*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
32	*E. sonianevadensis*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
33	*E. subrigida*	T	T	C	G	C	T	A	C	**T/C**	A	A	A	T	A	C	G	A	C	C
34	*E. triquiana*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
35	*E. uxorium*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
36	*E. xochipalensis*	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
37	*E. gigantea* JE-5151	**C**	**C**	C	G	C	T	A	C	C	**G**	A	A	T	A	C	G	A	C	C
38	*E.* sp EK-3569	T	T	C	G	**T/N**	T	A	C	C	A	**G**	A	T	A	C	G	A	C	C
39	*E. fulgens* JE-8592-1/JE-8392-1	T	T	C	G	C	T	A	C	C	A	A	A	**G**	**C**	C	G	A	**T**	C
40	*E.* sp. JE-8061	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
41	*E.* sp. JE-8786/JE-8762	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
42	*E.* sp. JE-8758	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
43	*E.* sp. JE-8771	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
44	*E.* sp. JE-8816	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C
45	*E.* sp. JE-6371	T	T	C	G	C	T	A	C	C	A	A	A	T	A	C	G	A	C	C

Table 2. Simple pure diagnostic characters for ser. *Gibbiflorae* on *rbcL*, *matK* and ITS2 DNA regions (**continuation**).

		matK												ITS2				
	Taxa	**398**	**431**	**446**	**463**	**564**	**588**	**628**	**676**	**693**	**730**	**739**	**774**	**47**	**108**	**128**	**156**	**218**
1	*E. acutifolia*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
2	*E. aurantiaca*	C	T	A	A	C	G	T	A	T	C	C	C	G	**A**	G	C	G
3	*E. cante*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
4	*E. cerrograndensis*	**T/C**	T	A	A	**T/C**	G	T	A	T	C	C	C	G	-	G	C	G
5	*E. coruana*	C	T	A	A	C	G	T	A	**G**	C	C	C	G	-	**T**	C	G
6	*E. crenulata*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
7	*E. dactylifera*	C	T	A	A	C	G	T	A	T	C	C	C	**A/N**	-	G	C	G
8	*E. fimbriata*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
9	*E. fulgens* var. *fulgens*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
10	*E. fulgens* var. *obtusifolia*	C	T	A	A	C	**T/G**	T	A	T	C	C	C	G	-	G	C	G
11	*E. gibbiflora*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
12	*E. gigantea*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
13	*E. grisea*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
14	*E. guerrerensis*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
15	*E. juarezensis*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
16	*E. longiflora*	C	T	A	A	C	G	T	A	T	**T**	C	C	G	-	G	C	G
17	*E. lozanoi*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
18	*E. marianae*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
19	*E. michihuacana*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
20	*E. munizii*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
21	*E. nayaritensis*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
22	*E. novogaliciana*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
23	*E. pallida*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
24	*E. patriotica*	C	T	A	A	C	G	T	A	**G**	C	C	C	G	-	G	C	G
25	*E. perezcalixii*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
26	*E. pistioides*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
27	*E. prunina*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	**A**
28	*E. purhepecha*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
29	*E. roseiflora*	C	T	**G/A**	**G/A**	C	G	T	A	T	C	C	C	G	-	G	C	G
30	*E. rubromarginata*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
31	*E. rulfiana*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
32	*E. sonianevadensis*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
33	*E. subrigida*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
34	*E. triquiana*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
35	*E. uxorium*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
36	*E. xochipalensis*	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
37	*E. gigantea* JE-5151	C	T	A	A	C	G	**G**	A	T	C	C	C	G	-	G	C	G
38	*E.* sp. EK-3569	C	T	A	A	C	G	T	A	T	C	C	**T**	G	-	G	C	G
39	*E. fulgens* JE-8592-1/JE-8392-1	C	**C**	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
40	*E.* sp. JE-8061	C	T	A	A	C	G	T	A	T	C	**A**	C	G	-	G	C	G
41	*E.* sp. JE-8786/JE-8762	C	T	A	A	C	G	T	**C**	T	C	C	C	G	-	G	C	G
42	*E.* sp. JE-8758	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	**T**	G
43	*E.* sp. JE-8771	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
44	*E.* sp. JE-8816	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G
45	*E.* sp. JE-6371	C	T	A	A	C	G	T	A	T	C	C	C	G	-	G	C	G

Table 2. Simple pure diagnostic characters for ser. *Gibbiflorae* on *rbcL*, *matK* and ITS2 DNA regions (**continuation**).

		ITS2									
	Taxa										
		221	**225**	**237**	**245**	**247**	**252**	**254**	**255**	**286**	**294**
1	*E. acutifolia*	C	A	C	C	C	C	C	**A**	T	C
2	*E. aurantiaca*	C	A	C	C	C	C	C	G	T	C
3	*E. cante*	C	A	C	C	C	**T**	C	G	T	C
4	*E. cerrograndensis*	C	A	C	C	C	C	C	G	T	C
5	*E. coruana*	C	A	C	C	C	C	C	G	T	C
6	*E. crenulata*	C	A	C	C	C	C	C	G	T	C
7	*E. dactylifera*	C	A	C	C	C	C	C	G	T	C
8	*E. fimbriata*	C	A	C	**G/C**	C	C	C	G	T	C
9	*E. fulgens* var. *fulgens*	C	A	C	C	C	C	C	G	T	C
10	*E. fulgens* var. *obtusifolia*	C	A	C	C	C	C	C	G	**C**	C
11	*E. gibbiflora*	C	A	C	C	C	C	C	G	T	C
12	*E. gigantea*	C	A	C	C	C	C	C	G	T	C
13	*E. grisea*	C	A	C	C	C	C	C	G	T	C
14	*E. guerrerensis*	C	A	C	C	C	C	C	G	T	C
15	*E. juarezensis*	C	A	C	C	C	C	C	G	T	C
16	*E. longiflora*	C	A	C	C	C	C	C	G	T	C
17	*E. lozanoi*	C	A	C	C	C	C	C	G	T	C
18	*E. marianae*	C	A	C	C	C	C	C	G	T	C
19	*E. michihuacana*	C	A	C	C	-	C	C	G	T	C
20	*E. munizii*	C	A	C	C	C	C	C	G	T	C
21	*E. nayaritensis*	C	A	C	C	C	C	**T**	G	T	C
22	*E. novogaliciana*	**T**	A	C	C	C	C	C	G	T	C
23	*E. pallida*	C	A	C	C	C	C	C	G	T	C
24	*E. patriotica*	C	A	C	C	C	C	C	G	T	C
25	*E. perezcalixii*	**T**	A	C	C	C	C	C	G	T	C
26	*E. pistioides*	C	A	C	C	-	C	C	G	T	C
27	*E. prunina*	C	**C**	C	C	C	C	C	G	T	C
28	*E. purhepecha*	C	A	C	C	C	C	C	G	T	C
29	*E. roseiflora*	C	A	C	C	C	C	C	G	T	C
30	*E. rubromarginata*	C	A	C	C	C	C	C	G	T	C
31	*E. rulfiana*	C	A	C	C	C	C	C	G	T	C
32	*E. sonianevadensis*	C	A	C	C	C	C	C	G	T	C
33	*E. subrigida*	C	A	C	C	C	C	C	G	T	**T**
34	*E. triquiana*	C	A	C	C	C	C	**T**	G	T	C
35	*E. uxorium*	C	A	C	C	C	C	C	G	T	C
36	*E. xochipalensis*	C	A	C	C	C	C	C	G	T	C
37	*E. gigantea* JE-5151	C	A	C	C	C	C	C	G	T	C
38	*E.* sp. EK-3569	C	A	C	C	C	C	C	G	T	C
39	*E. fulgens* JE-8592-1/JE-8392-1	C	A	C	C	C	C	C	G	T	C
40	*E.* sp. JE-8061	C	A	C	C	C	C	C	G	T	C
41	*E.* sp. JE-8786/JE-8762	C	A	C	C	C	C	C	G	T	C
42	*E.* sp. JE-8758	**T**	A	C	C	C	C	C	G	T	C
43	*E.* sp. JE-8771	C	A	**T**	C	C	C	C	G	T	C
44	*E.* sp. JE-8816	C	A	C	**G**	C	C	C	G	T	C
45	*E.* sp. JE-6371	C	A	C	C	C	C	**T**	G	T	C

Figure captions

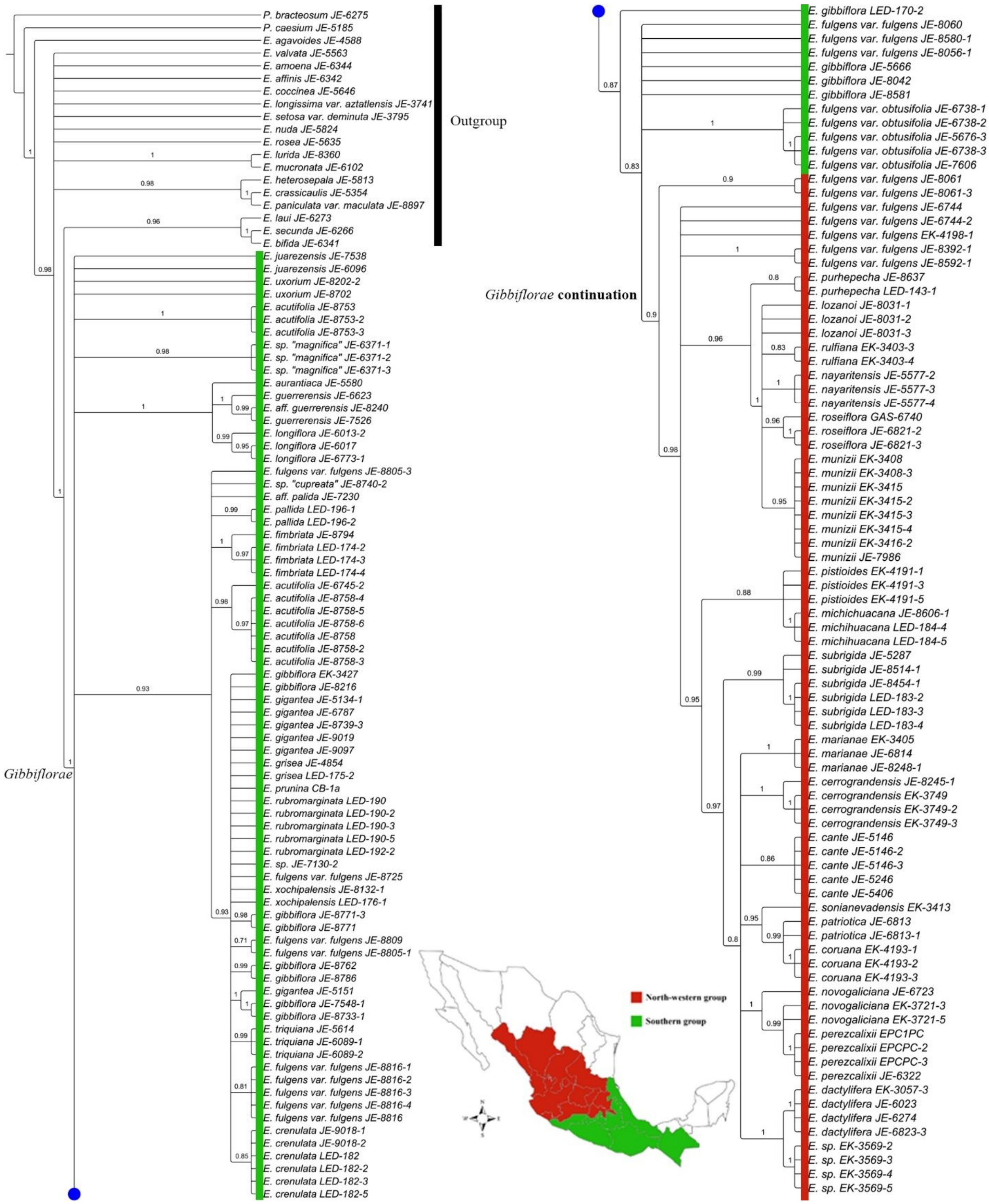

Figure 1. Bayesian inference majority-rule consensus cladogram based on combined data of 36 *Gibbiflorae* taxa. Node numbers above the branches indicate posterior probabilities (only pp > 0.8 are shown). Green lines correspond to "southern Mexico group", red line indicate "north-western Mexico group". Map box show the approximate distribution of the two ser. *Gibbiflorae* groups. Blue circles below and above figures indicates linking point between halves of the tree.

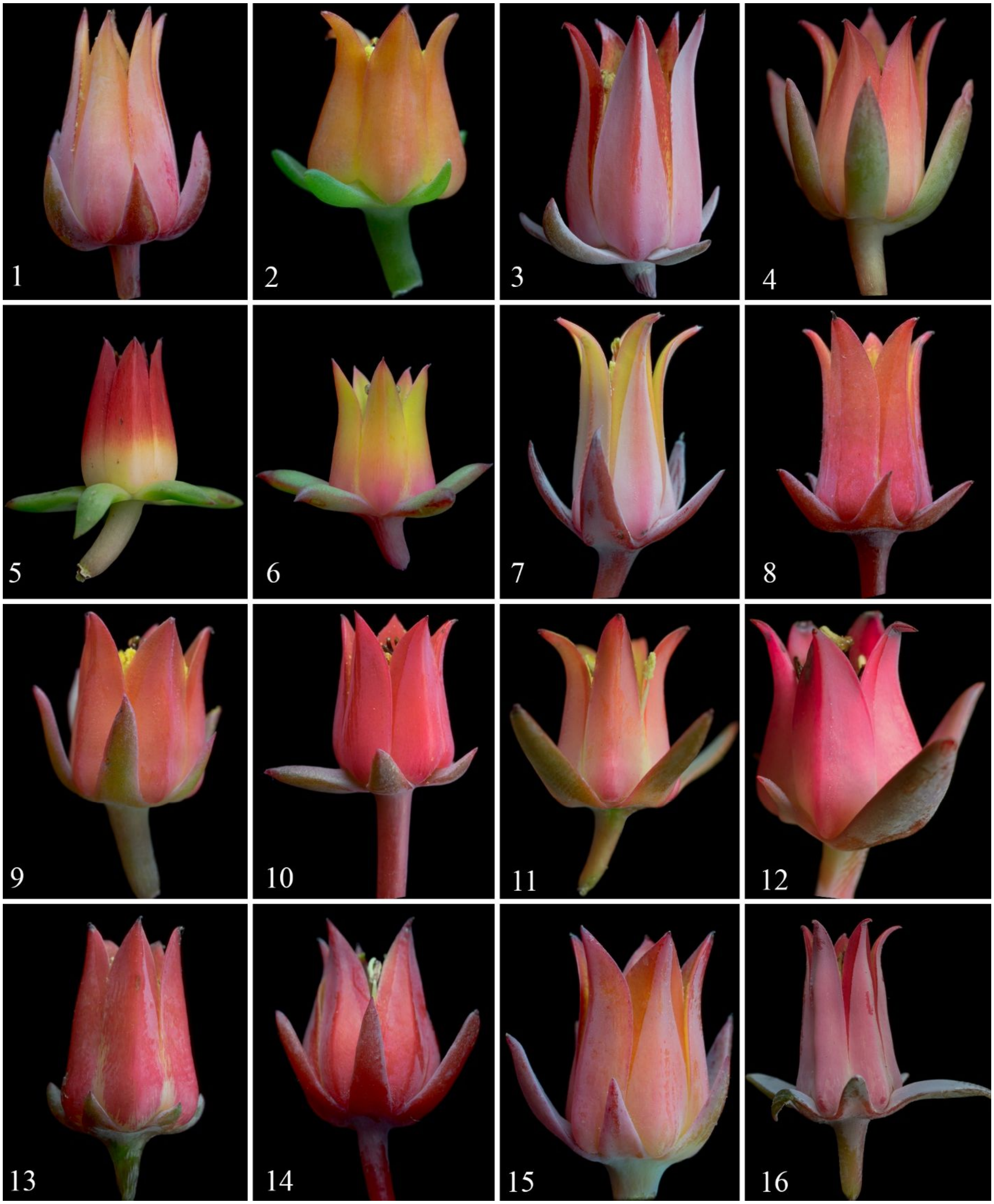

Figure 2. External flower morphology in ser. *Gibbiflorae*. **1**. *E. acutifolia*, **2**. *E. aurantiaca*, **3**. *E. cante*, **4**. *E. cerrograndensis*, **5**. *E. coruana*, **6**. *E. crenulata*, **7**. *E. dactylifera*, **8**. *E. fimbriata*, **9**. *E. fulgens* var. *fulgens*, **10**. *E. fulgens* var. *obtusifolia*, **11**. *E. gibbiflora*, **12**. *E. gigantea*, **13**. *E. grisea*, **14**. *E. guerrerensis*, **15**. *E. juarezensis*, **16**. *E. longiflora*.

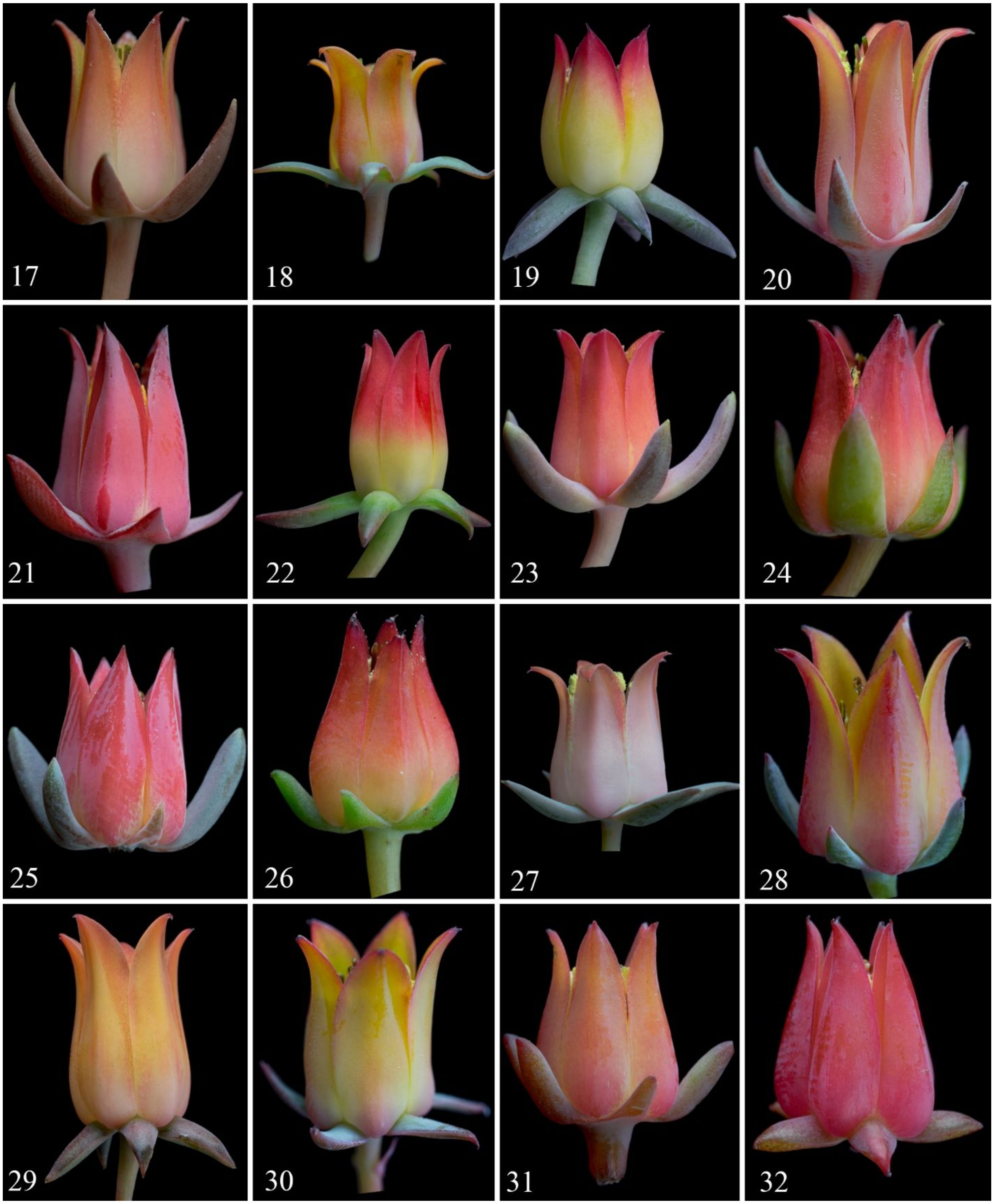

Figure 2. External flower morphology in ser. *Gibbiflorae* (**continuation**). **17**. *E. lozanoi*, **18**. *E. marianae*, **19**. *E. nayaritensis*, **20**. *E. novogaliciana*, **21**. *E. pallida*, **22**. *E. patriotica*, **23**. *E. perezcalixii*, **24**. *E. pistioides*, **25**. *E. prunina*, **26**. *E. purhepecha*, **27**. *E. roseiflora*, **28**. *E. rubromarginata*, **29**. *E. rulfiana*, **30**. *E. subrigida*, **31**. *E. triquiana*, **32**. *E. uxorium*.

Figure 3. Internal flower morphology in ser. *Gibbiflorae*. **1**. *E. acutifolia*, **2**. *E. aurantiaca*, **3**. *E. cante*, **4**. *E. cerrograndensis*, **5**. *E. coruana*, **6**. *E. crenulata*, **7**. *E. dactylifera*, **8**. *E. fimbriata*, **9**. *E. fulgens* var. *fulgens*, **10**. *E. fulgens* var. *obtusifolia*, **11**. *E. gibbiflora*, **12**. *E. gigantea*, **13**. *E. grisea*, **14**. *E. guerrerensis*, **15**. *E. juarezensis*, **16**. *E. longiflora*.

Figure 3. Internal flower morphology in ser. *Gibbiflorae* (**continuation**). **17**. *E. lozanoi*, **18**. *E. marianae*, **19**. *E. nayaritensis*, **20**. *E. novogaliciana*, **21**. *E. pallida*, **22**. *E. patriotica*, **23**. *E. perezcalixii*, **24**. *E. pistioides*, **25**. *E. prunina*, **26**. *E. purhepecha*, **27**. *E. roseiflora*, **28**. *E. rubromarginata*, **29**. *E. rulfiana*, **30**. *E. subrigida*, **31**. *E. triquiana*, **32**. *E. uxorium*.

Capítulo 4. Tres especies nuevas de *Echeveria* ser. *Gibbiflorae* para México

Título: *Echeveria michihuacana* (Crassulaceae), a new species from Michoacán, Mexico

Autores: Luis Emilio de la Cruz-López, Jerónimo Reyes Santiago y Francisco Vergara Silva

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Publicado en: *Brittonia* 71 (1): 25-31, 16 de enero de 2019.

URL: https://doi.org/10.1007/s1222

Título: *Echeveria xochipalensis* (Crassulaceae), A New Species from Guerrero, Mexico

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Publicado en: *Phytotaxa 399* (1): 14-24, 25 de marzo de 2019.

URL: http://dx.doi.org/10.11646/phytotaxa.399.1.2

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

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Publicado en: *Brittonia 73*(4): 1–9, 28 de septiembre de 2021.

URL: http://dx.doi.org/10.1007/s12228-021-09684-7

Echeveria michihuacana *(Crassulaceae), a new species from Michoacán, Mexico*

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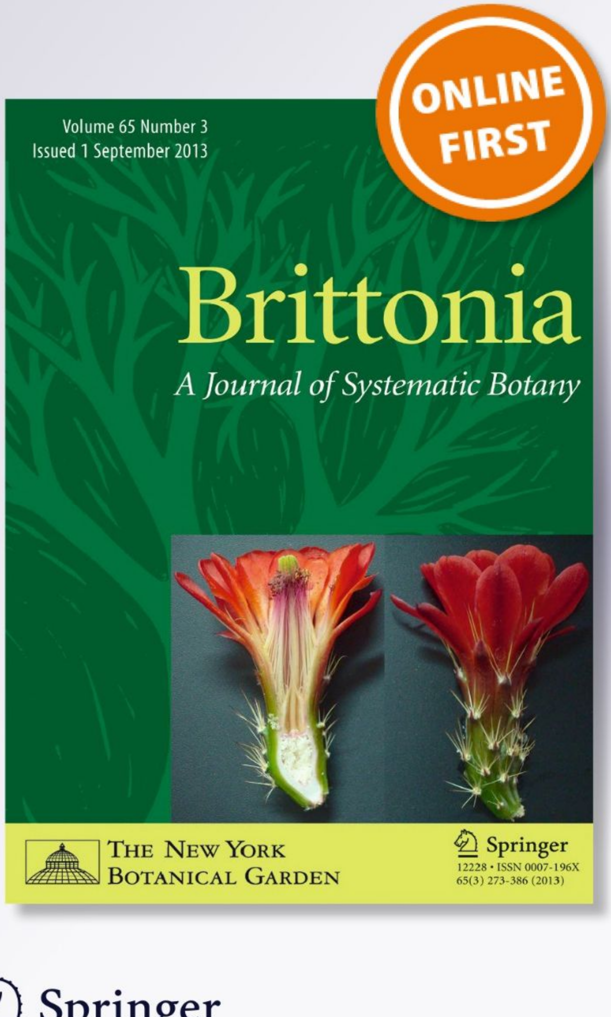
Brittonia

ISSN 0007-196X

Brittonia
DOI 10.1007/s12228-018-9558-9



Echeveria michihuacana (Crassulaceae), a new species from Michoacán, Mexico

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Abstract. **Echeveria michihuacana** (Crassulaceae), a new species for science, is described and illustrated. The new taxon is related to a group of species of *Echeveria* series *Gibbiflorae* from western Mexico, including *Echeveria dactylifera*, *Echeveria fulgens*, *Echeveria patriotica*, and particularly the recently published *Echeveria pistioides*. With the last species, *E. michihuacana* is similar in the shape and coloration of floral structures, as well as in its geographical distribution. In contrast, it differs from all of the aforementioned related species in having an evident stem, larger panicles and flowers, deep-red nectary scales, and absence of appendages on the inner side of the petals.

Keywords: Ciudad Hidalgo, *Gibbiflorae*, Mil Cumbres, taxonomy.

Resumen. Se describe e ilustra a **Echeveria michihuacana** (Crassulaceae) como nueva especie para la ciencia. El nuevo taxón está relacionado con un grupo de especies de *Echeveria* de la serie *Gibbiflorae* del occidente de México, incluyendo *Echeveria dactylifera*, *Echeveria patriotica*, y en particular, con la recientemente publicada *Echeveria pistioides*. Con esta última comparte la forma y coloración de las estructuras de la flor, así como su distribución geográfica. En contraste, difiere de los taxones relacionados por presentar un tallo evidente, panículas y flores más largas, escamas nectaríferas rojo intenso y por la ausencia de apéndices en la cara interna de los pétalos.

The genus *Echeveria* DC. (Crassulaceae) is divided into 17 series based on morphological affinities (Kimnach, 2003). The lack of a comprehesive phylogeny for *Echeveria* results in poor understanding of relationships within and among the series of the genus, especially within *E.* ser. *Gibbiflorae* (Baker) A.Berger. This situation hinders the correct assignment of species to infrageneric groups since relationships can only be inferred by similarity. A genus-wide phylogeny and other data (i.e. DNA barcodes, ecology, palynology) are also needed to better establish the limits of wide-ranging, variable species complexes, such as the one centered on *E. fulgens* Lem., which is broadly distributed in Mexico.

Series *Gibbiflorae* (incl. *E.* ser. *Grandes* E.Walther) is characterized by medium to large sized rosettes with broad and flat, sometimes linear-deltoid shaped leaves, inflorescences in panicles or thyrsoid clusters, and tubular (sometimes tending toward urceolate or campanulate) flowers with free styles and stigmas at anthesis, reddish to deep-purple styles, and relatively large, whitish, yellowish or reddish nectary scales (Kimnach, 2003; Meyrán & López, 2003; Pilbeam, 2008).

Brittonia, DOI 10.1007/s12228-018-9558-9
ISSN: 0007-196X (print) ISSN: 1938-436X (electronic)

In December of 2015, during a revisionary study of series *Gibbiflorae* by our research team at the Jardín Botánico of the Instituto de Biología, UNAM (JB-IBUNAM), specimens of an unidentified species of *Echeveria* were collected in three geographical areas in the municipality of Ciudad Hidalgo in the state of Michoacán. The plants were cultivated in greenhouses at the JB-IBUNAM to measure and count their structures while comparing them with living material and herbarium specimens of related species at the MEXU herbarium (e.g. *E. dactylifera* E.Walther, *E. fulgens* Lem. and *E. patriotica* I. García & Pérez-Calix) and the protologue of *E. pistioides* I. García, I. Torres & Costea, (García-Ruiz et al., 2016). This morphological comparison allowed the identification of morphological traits to diagnose a new species.

Species description

Echeveria michihuacana de la Cruz-López, Reyes & Vergara-Silva, **sp. nov**. Type: Mexico, Michoacán, mpio. Ciudad Hidalgo, along the Mexico 15 highway from Morelia to Ciudad Hidalgo, near Mil Cumbres, 19°38′39.17”N, 100°47′10.21”W, 2564 m, Sep 2015 [−fl], *L. E. de la Cruz L. 184* (holotype: MEXU; isotype: MEXU). (Fig. 1, 2).

Diagnosis: *Echeveria michihuacana* is morphologically similar to *E. dactylifera*, *E. patriotica*, *E. pistioides* and, in some aspects, the *E. fulgens* complex. Morphologically, the species can be differentiated from these related taxa by having an evident caudex (2–30 cm long), large (4–30 cm) and often crenulated leaves, larger inflorescences, conoid-tubular flowers, red petals, deep red nectary scales, and the lack of epipetalous appendages.

Perennial herb, glabrous, solitary or with lateral shoots, caulescent, main roots somewhat thickened, secondary roots fibrous; stems erect or somewhat decumbent, 2–30 cm long and 1–3.5 cm in diameter, reddish brown at base and green at apex, with light brown leaf scars; leaves in a spiral arrangement forming a lax rosette up to 50 cm in diameter, obovate to lanceolate-oblanceolate, green with red margins or completely reddish in the dry period, 4–30 cm long, 1.6–3.3 cm wide at the base and 2.3–10 cm wide at widest point below the apex, pseudo-petiolate and winged at the base, adaxial surface canaliculate, abaxial surface keeled, only the apical leaves slightly pruinose, apex obtuse to rounded, mucronate, margins entire to crenate, reddish; inflorescence 1–3 panicles per rosette, 50–120 cm long or more and 1.8 cm wide at the base, with 2–5 cincinni each, peduncles ascending, up to 20 cm in length, light pink to reddish; bracts 10–30, ascending, arranged in a spiral, deciduous, oblanceolate, 1.7–9.7 cm long, 0.6–4.6 cm wide, with obtuse apex, spurred, green with red margins; bracteoles lanceolate, 0.5–1.1 cm long, 1.5–4 mm wide, green with pink dyes, spurred, apex acuminate; pedicels 3–8 mm long, 2.5–3 mm thick, pink; calyx of 5 sepals, these fused at the base, linear–deltoid, reflexed, unequal, 0.7–1.6 cm long, 3.5–6 mm wide at the base and 2 mm thick, green, apex acuminate, pink, slightly pruinose; corolla conoid-tubular in bud, tubular-urceolate at anthesis, rounded to slightly pentagonal in transection, 1.8–2.15 cm long, 1.6–1.95 cm wide at the base, 0.9–1.25 cm wide at the apex, segments ovate-lanceolate, dorsally slightly keeled, fused at the base, imbricate, apex acute and slightly reflexed, 6.5–8 mm wide at the base, orange or pink, internally yellow with reddish margins and apex, somewhat thickened at the sides of the epipetalous filaments, nectar cavity reduced; androecium included, stamens 10, 5 episepalous, 1.4–1.7 cm in length, 5 epipetalous slightly shorter, filaments yellow, anthers 1.5–3 mm in length, pale yellow; gynoecium included, carpels 5, free, 1.4–1.7 cm long, 3–3.8 mm wide at the base, nectary scales lunate-reniform, 4 mm wide and 1.5–2 mm tall, deep red, ovaries white to pale yellow, styles wine-red to dark purple, stigmas green; fruit with 5 divergent follicles, with numerous reddish seeds.

Distribution and habitat.—The new taxon is known only from three localities in the eastern part of the state of Michoacán. It is common in roadside *Pinus* sp. forest along the road from Morelia to Ciudad Hidalgo (near Mil Cumbres and Huajúmbaro) and at two nearby localities on the road from Ciudad Hidalgo to Ucareo (near Los Azufres).

Phenology. —Flowering occurs from October to January.

Etymology.—The specific epithet refers to the word “michihuacán,” the Nahuatl word for Michoacán, to which the taxon seems to be

Fig. 1. *Echeveria michihuacana*. **A.** Habit. **B.** Young leaves. **C.** Stem and reddish winter leaves. (Photographs by L. E. de la Cruz-López.)

restricted. In Nahuatl, "michihuacán" means "lugar de pescadores" (fishermen's place).

Additional specimens examined. MEXICO. Michoacán: Mpio. Hidalgo, along the highway from Ciudad Hidalgo to Jeráhuaro de Juárez, 5 km SW of Los Azufres, 19°45′50.12"N, 100°41′45.99"W, 2646 m, *L. E. de la Cruz 184–5* (MEXU); Mpio. Hidalgo, along the highway from Ciudad Hidalgo to Jeráhuaro de Juárez, 8 km SW of Los Azufres, 19°45′21.84"N, 100°41′15.145"W, 2521 m, *L. E. de la Cruz 184–6* (MEXU).

The new species is placed in *Echeveria* series *Gibbiflorae* due to its possession of diagnositic characters for the series such as inflorescences organized in simple secund racemes or more frequently paniculate-thyrsoid clusters, styles reddish, wine-red or deep purple (never yellow or green), and the styles and stigmas free at anthesis (Moran, 1974; Kimnach, 2003; Meyrán & López, 2003; Pilbeam, 2008). Some authors (García-Ruíz & Pérez-Calix, 2007) segregate series *Retusae* E. Walther from series *Gibbiflorae*, and the new taxon might be better placed in the former series. However, the main distinction between the two series (i.e., acaulescent habit in series *Retusae* vs. caulescent habit in series *Gibbiflorae*) appears to be an artificial distinction.

Within series *Gibbiflorae*, preliminary phylogenetic analyses (de la Cruz-López et al., unpubl. data) and morphology suggest the existence of

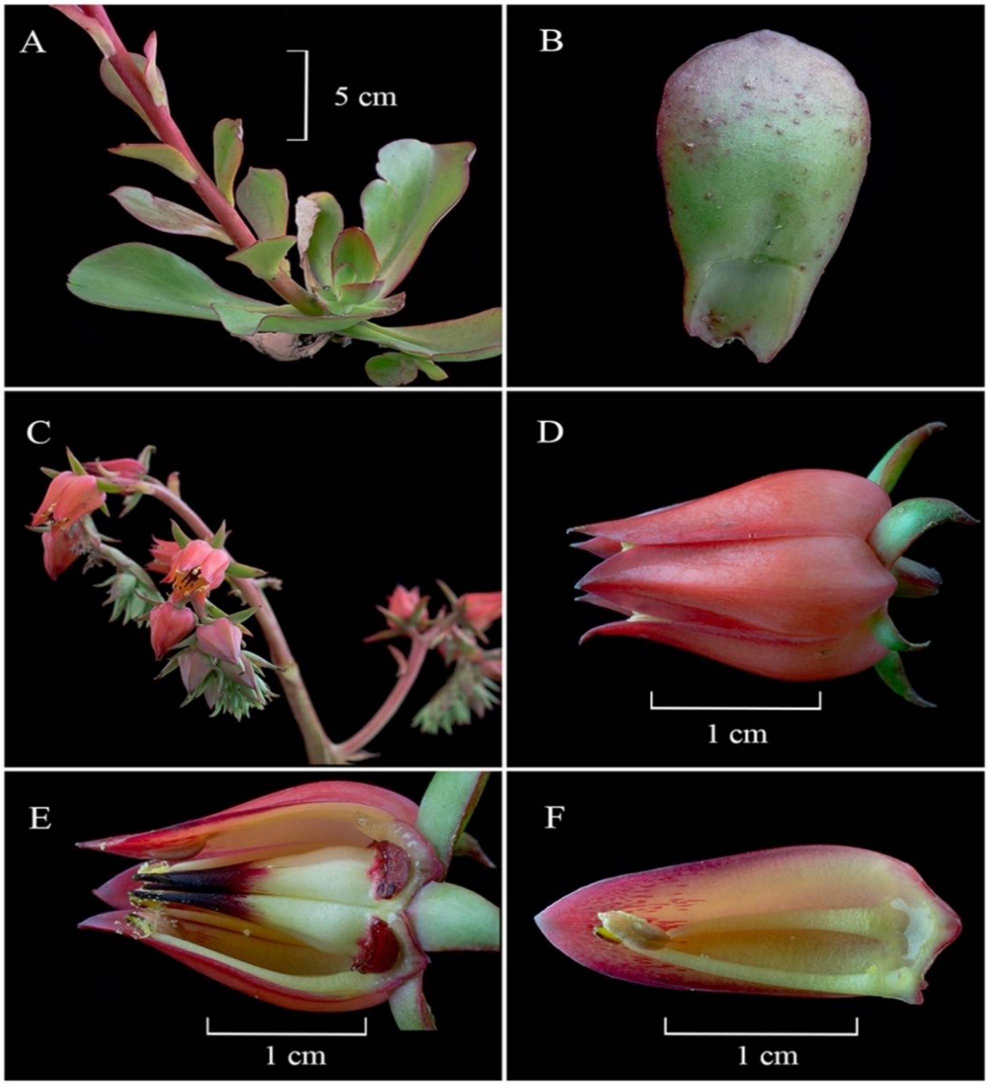

FIG. 2. *Echeveria michihuacana*. **A.** Rosette. **B.** Bracts. **C.** Detail of inflorescence. **D.** Lateral view of flower. **E.** Internal view of flower, highlighting carpels and deep red nectary scales. **F.** Inner face of petals without appendages in epipetalous filaments. (Photographs by L. E. de la Cruz-López.)

two main groups. One group is formed by species from southern Mexico with relatively small flowers and usually white or yellow ovaries and nectary scales. The second group, to which *E. michihuacana* seems to be more closely allied, is composed of species from western Mexico with relatively large flowers and carpels and usually yellow or reddish nectary scales, and includes *E. cante* Glass & Mend.-Garc., *E. cerrograndensis* A.Vázquez & Nieves, *E. dactylifera* E.Walther, *E. novogaliciana* J.Reyes, Brachet & O.González, *E. marianae* I.García & Costea, *E. munizii* Padilla-Lepe & A.Vázquez, *E. patriotica* I.García & Pérez-Calix, *E. perezcalixii* Jimeno-Sevilla & P.Carrillo, *E. pistioides* I.García, I.Torres & Costea, *E. purhepecha* I.García, *E. roseiflora* J.Reyes & O.González, *E. rulfiana* Jimeno-Sevilla, Santana Mich. & P.Carrillo and *E. subrigida* Rose.

Among the species of the "western Mexico group" of series *Gibbiflorae*, *E. michihuacana* resembles *E. dactylifera*, with which it shares large rosettes and green, red-margined leaves that tend to become completely red in winter

(November to February). *Echeveria michihuacana* differs from *E. dactylifera* in its tubular-urceolate (vs. tubular-campanulate) corolla, deep red (vs. yellow) nectary scales, purple (vs. reddish) styles, and in lacking appendages in the epipetalous filaments (vs. appendages present) (Table I; Fig. 3). *Echeveria michihuacana* is also morphologically related to *E. patriotica* I. García & Pérez-Calix in terms of floral dimensions, especially in the length of the corolla (Table I), stamens (1.7 cm vs 1.4–1.6 cm) and carpels (2 cm vs 1.2–2 cm), but the former is easily distinguished by having a pinkish to orange-red

TABLE I. MORPHOLOGICAL COMPARISON OF SPECIES RELATED TO *E. MICHIHUACANA*.*

Character/Taxa	*E. michihuacana*	*E. dactylifera*	*E. fulgens*	*E. patriotica*	*E. pistioides*
Stem					
Length	2–30 cm	Up to 9 cm	5–25 cm	(18)–20 cm	2–15 cm
Rosette					
Diameter	Up to 50 cm	35.5 cm	Up to 30 cm	10–(26)–35 cm	14–50 cm
Leaves					
Color of mature leaves	Green, red in dry season	Green to reddish	Glaucous	(Yellowish), reddish	Light green to emerald green, glaucous at base
Shape	Obovate to lanceolate–oblanceolate	Lanceolate–oblanceolate	Obovate to oblanceolate	Oblong–obovate to spatulate	Obovate–spatulate, widely obovate, obcordate
Margin	Entire, crenate or crenulate	Entire	Crenate or crenulate	Entire	Entire, crenulate in young leaves
Margin color	Red	Red or hyaline	Redish or hyaline	(Hyaline), reddish	Same as leaves or red, less frequently hyaline
Corolla					
Length	1.8–2.15 cm	2.3 cm	1–1.6 cm	1.8–(1.9)–2.0–(2.3) cm	1.2–1.6 cm
Width at base	1.6–1.95 cm	1.0 cm	0.8–1 cm	(1.2)–1.4 cm	0.95–1.1 cm
Lateral shape	Conoid–tubular	Tubular–urceolate	Tubular	Cylindrical–urceolate	Pentagonal–conical in bud, cylindrical–urceolate at anthesis
Frontal shape	Cylindrical–pentagonal	Cylindrical–pentagonal	Pentagonal	(Cylindrical–pentagonal)	Cylindrical
Outer petal color	Orange–pink at base, reddish at apex	Pink at base, yellow at margin and apex	Orange–red	White to cream at base, orange-red at apex	Cream to light pink at base, pink–orange to reddish in the rest
Petal inner face appendices	Absent	Present (cylindrical)	Absent	Present (cylindrical)	Absent
Nectaries					
Nectary length	1.5–2 mm	3 mm	1–1.5 mm	1 mm	1 mm
Nectary shape	Lunate–reniform	Lunate–reniform	Reniform	Reniform	Rectangular, reniform or bilobed
Nectaries color	Deep red	Yellow	Yellow or whitish	Red–purple	Cherry red
Flowering time	October–January	December	October–January	October–January	November–January
Geographic distribution	Center of Michoacán	Aguascalientes, Durango, Sinaloa	Estado de México, Michoacán, Morelos	South of Jalisco	Center of Michoacán

*Measurements in brackets for *E. patriotica* were taken from García-Ruiz and Pérez-Calix (2007). Measurement data for *E. pistioides* was taken from García-Ruiz et al., (2016).

Fig. 3. Comparison of rosette and flower morphology in *Echeveria michihuacana* and related species. **A.** *E. michihuacana*. **B.** *E. dactylifera*. **C.** *E. fulgens*. **D.** *E. patriotica*. **E.** *E. pistioides*. (Photographs by L. E. de la Cruz-López.)

corolla (vs. bicolored, white or cream at the base and reddish in the upper half), as well as by the absence of appendages on the inner side of the petals (Fig. 3A, D).

The flowers of *E. michihuacana* are most similar to those of *E. pistioides*. The two species share a tubular-conoid corolla in bud and tubular-urceolate corolla at anthesis, pinkish-orange petals, deltoid-elongate ovaries, deep red to purple styles, green stigmas, deep red nectary scales, and the lack of appendages on the inner side of the petals (Fig. 3A,.E; Table I). On the other hand, the new species differs from *E. pistioides* particularly in the caudex 2–30 cm long (vs. 2–15 cm), the leaves oblanceolate (vs. widely obovate), the inflorescences less strikingly pruinose, and often somewhat longer (50–120 cm vs. 30–90 cm), and the flowers 1.8–2.15 cm long (vs. 1.2–1.6 cm).

The new species has been mistakenly identified as *E. fulgens* Lem. in herbaria (e.g., *C. Delgadillo 12*, MEXU), and indeed some characters of *E. michihuacana* are shared with the *E. fulgens* complex, such as the presence of a stem, oblanceolate leaves with red or brown-reddish margins that are frequently ondulate-crenate, and the reddish flowers. However, *E. michihuacana* differs from the *E. fulgens* complex by having deep-red (vs. yellow or whitish) nectary scales (Walther, 1972; Pérez-Calix, 2008), as well as by the additional characters listed in Table I.

Acknowledgments

Financial support was provided by UNAM-DGAPA-PAPIIT [project IN212015] and CONACYT [project 247078]. The first author received graduate studies scholarships from Posgrado en Ciencias Biológicas, UNAM and CONACYT. We thank an anonymous reviewer and Dr. Joachim Thiede for helpful comments on the manuscript.

Literature cited

García-Ruiz, I. & E. Pérez-Calix. 2007. Una especie nueva de *Echeveria* (Crassulaceae) originaria del Estado de Jalisco, México. Acta Botánica Mexicana 78: 125–132.

García-Ruiz, I., I. Torres & M. Costea. 2016. A new species of *Echeveria* (Crassulaceae) from Michoacán, México. Systematic Botany 41: 966–970.

Kimnach, M. 2003. *Echeveria*. *In*: Eggli, U. (ed.). Illustrated handbook of succulent plants: Crassulaceae. Springer-Verlag, Berlin.

Meyrán, J. & L. López. 2003. Las crasuláceas de México. Sociedad Mexicana de Cactología, A.C. México, D.F., México.

Moran, R. 1974. *Echeveria*. Pp. 184–186. *In:* H.Jacobsen (ed.). Lexicon of succulent plants. Blandford Press, London.

Pérez-Calix, E. 2008. Crassulaceae. Pp. 1–141. *In*: J. Rzedowski & G. C. de Rzedowski (eds.). Flora del Bajío y de regiones adyacentes. Fas. 156. Instituto de Ecología-Centro Regional del Bajío. CONACYT & CONABIO. Pátzcuaro, Michoacán, México.

Pilbeam, J. 2008. The genus *Echeveria.* The British Cactus & Succulent Society, Essex, UK.

Walther, E. 1972. *Echeveria*. California Academy of Sciences, San Francisco.

APPENDIX 1. Specimens examined of four species of *Echeveria* that are closley related to *E. michihuacana*; all of the cited collections are from Mexico.

***Echeveria dactylifera*: SINALOA**: Durango-Mazatlán highway, Espinazo del Diablo, *J. Reyes S. 6274* (MEXU).

***Echeveria fulgens*: ESTADO DE MÉXICO**: Mpio. Amanalco, volcanic rocks 1 Km south of San Juan Amanalco, 2340 m, Quercus scrub, *A. García-Mendoza* et al. *6123*, 8 Dec 1995 (MEXU); **Michoacán**: Mpio. Zitácuaro, 3.78 km north of San Felipe de Los Alsati. 19°31′20.1”N, 100°21′ 59.7”W, 2147 m, pine-oak forest, *D. Álvarez* et al. *14,683*, 17 Nov 2014; between Cd. Hidalgo and Mil Cumbres, km 232, old México-Nogales highway, *C. Delgadillo 12*, 15 Dec 1965 (MEXU); mpio. Zacapu, west of Angostura, matorral pedregoso, *A. Grimaldo 422*, 18 Nov 1988 (MEXU); mpio. Uruapan, malpaís (lava fields) of Capacúaro, forest of Quercus, 2250 m, *E. Pérez-Calix 3544*, 9 Dec 1996 (MEXU); mpio. Pátzcuaro, 2 km southeast of Cerro del Estribo, oak forest, 2230 m, *J. Espinosa 2436*, 8 Dec 1986 (MEXU); mpio. Zacapu, El Pinal, near Santa Gertrudis, oak foresto n basaltic lava stream, 2100 m, *H. Díaz y A. Grimaldo 4498*, 6 Nov 1987 (MEXU); **Morelos:** Mpio. Huitzilac, ± 1 km southwest of Huitzilac, pine-oak forest, on volcanic rocks, 2550 m, *E. Pérez-Calix & I. García 3966*, 25 Oct. 1999 (MEXU).

***Echeveria patriotica*: Michoacán**: between Palo Dulzal and Palo Herrado along road from Quitupan to Santa María del Cerro, 2322 m, *J. Reyes S. 6813* (MEXU).

***Echeveria pistioides*: Michoacán**: Mpio. Tzitzio, aproximadamente 1 km al SE de Piedras de Lumbre, 1950 m, *J. Etter y M. Kristen 4191* (MEXU).

Phytotaxa 399 (1): 014–024
https://www.mapress.com/j/pt/
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Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.399.1.2

Echeveria xochipalensis (Crassulaceae), a new species from Guerrero, Mexico

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Abstract

Echeveria xochipalensis, from the state of Guerrero, Mexico, is described as a new species to science. It is related to species of ser. *Gibbiflorae*, particularly to both *E. fulgens* varieties, from which it is distinguished by its green to lilac-gray spatulate leaves, although they commonly are coppery tinged; the leaves are rigid and with entire margin (not crenate), the margin is yellow-hyaline; bracts oblanceolate with rigid consistency; corolla pink at base and orange at the apex and whitish filaments, ovaries and nectary scales.

Key words: *Echeveria*, *Gibbiflorae*, succulents, white leaf scars, Xochipala

Resumen

Se describe e ilustra a *Echeveria xochipalensis* del estado de Guerrero, México, como nueva especie para la ciencia. Se relaciona con las especies de la ser. *Gibbiflorae*, en particular con las dos variedades de *E. fulgens*, de las cuales se diferencia por sus hojas espatuladas de color verde a lila-grisáceas, aunque con frecuencia cobrizas, rígidas y con margen entero (no crenado), éste amarillo-hialino; brácteas oblanceoladas de consistencia rígida; corola de color rosa con ápice naranja y filamentos, ovarios y nectarios blanquecinos.

Introduction

In the genus *Echeveria* A. P. de Candolle (1828: 401), ser. *Gibbiflorae* (Baker) A.Berger (1930: 472) consists of approximately 36 described species, and while the limits for the majority of these taxa are more or less evident, *E. fulgens* Lemaire (1845: 8) shows a great uncertainty in its taxonomic delimitation. This is due to the insufficiency of diagnostic characters in the original description by C. Lemaire and therefore, E. Walther (1972: 159) was forced to re-describe *E. fulgens* to complete its description, also it lacks a specified type locality. In addition, there is an enormous morphological and morphometric variation found in specimens from different localities in Mexico which have all been associated with this name. Recently, new species in this series have been published which would probably have been associated with *E. fulgens*, but whose comparison has allowed their differentiation based on morphological diagnostic characters, biogeographical and phenological comparisons among others: *E. aurantiaca* J.Reyes, O.González & Brachet (Reyes *et al*. 2011: 70), *E. cerrograndensis* A.Vázquez & Nieves (Nieves-Hernández *et al*. 2014: 248), *E. munizii* Padilla-Lepe & A.Vázquez (Vázquez-García *et al*. 2014: 166), *E. perezcalixii* Jimeno-Sevilla & P.Carrillo (Jimeno-Sevilla & Carrillo-Reyes 2010: 303), *E. pistioides* I.García, I.Torres & Costea (García-Ruiz *et al*. 2016: 966), *E. purhepecha* I.García (García-Ruiz 2011: 63) and *E. roseiflora* J.Reyes & O.González (Reyes & González-Zorzano 2010: 22). From this perspective, the *E. fulgens* complex has started to break down into smaller and better diagnosable units. Following this trend, *E. xochipalensis* from specimens collected in the municipality of Eduardo Neri, in the Mexican state of Guerrero, is proposed as a new species to science. The description of the new taxon is based on morphological and geographical evidence.

Accepted by Marie-Stéphanie Samain: 8 Feb. 2019; published: 25 Mar. 2019

Material and Methods

Through explorations made to the state of Guerrero, Mexico, during the revision of the genus *Echeveria* and, in particular, of ser. *Gibbiflorae*, specimens of this last series were located near the town of La Laguna on the road from Xochipala to Filo de Caballos. Several specimens were collected and deposited in the herbarium MEXU, while others were kept as living plants cultivated in greenhouses of the "Colección Nacional de Crasuláceas", belonging to the "Instituto de Biología, Universidad Nacional autónoma de México". Following comparisons with related species (herbarium and cultivated plants), morphological differences between them were identified.

Taxonomic treatment

Echeveria xochipalensis J.Reyes, de la Cruz-López & Vergara-Silva. *sp. nov*. (FIGS. 1–3)

Echeveria xochipalensis can be distinguished from the *E. fulgens* complex, by the white leaf scars on the stems, the entire leaf margins, adaxially channeled at the base and pseudo-petiolate, leaf color frequently in hues of copper, the yellowish to hyaline leaf margin, pinkish-orange corolla instead of orange-reddish, the whitish filaments, instead of yellowish in the two *E. fulgens* varieties, as well as whitish ovaries and nectary scales, instead of yellowish ones (Table 1). It also differs from *E. crenulata*, by its less branched inflorescences, shorter pedicels and pink-orange (vs pink-yellow) corolla.

Type:—MEXICO, Guerrero, municipality Eduardo Neri, 1960 m, about 2 kms southwest of La Laguna, along the road from Filo de Caballo to Xochipala, 11 November 2012, *J. Reyes 8132* (holotype, MEXU!).

FIGURE 1. *E. xochipalensis*. **A**. Habit. **B**. Steam detail with white leaf scars. **C**. Leaf color variation. Photos: Luis Emilio de la Cruz.

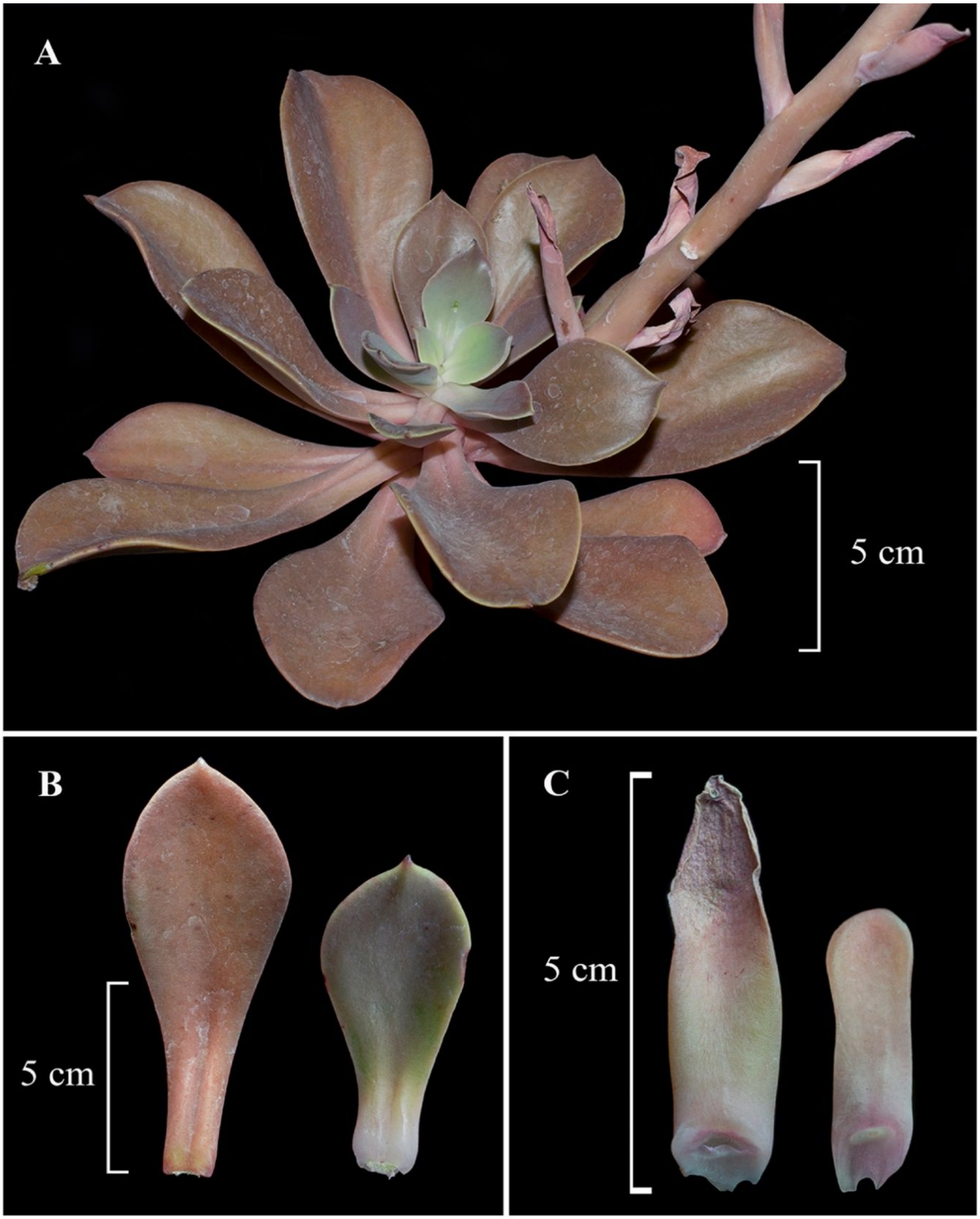

FIGURE 2. *Echeveria xochipalenesis*. **A**. Rosette detail. **B**. Pseudo-petiolate and spatulate leaves. **C**. Variation in length and form of floral stem leaves. Photos: Luis Emilio de la Cruz.

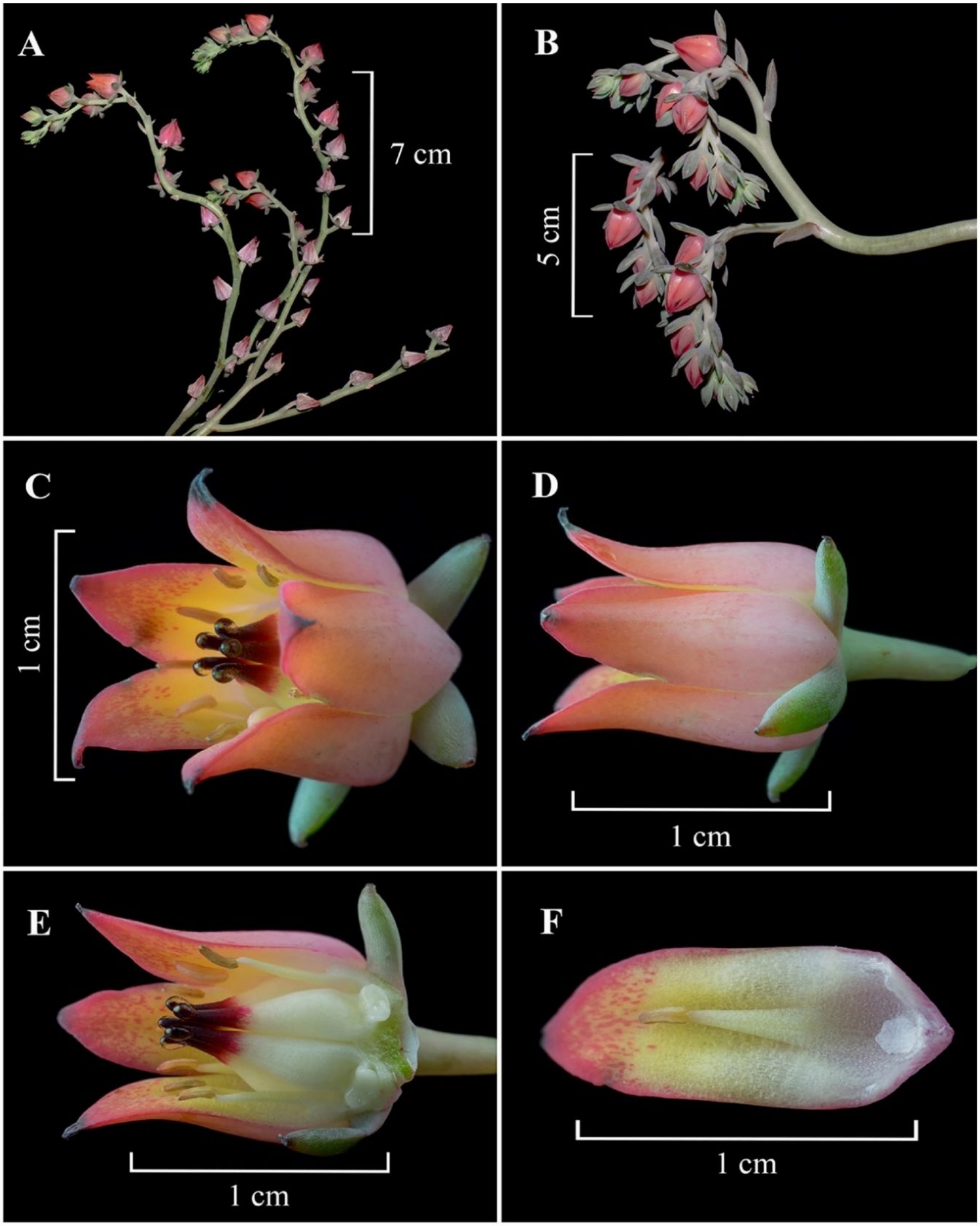

FIGURE 3. *Echeveria xochipalensis*. **A**. Inflorescence. **B**. Detail of inflorescence. **C**. Front view of the corolla and calyx segments. **D**. Side view of the corolla. **E**. Dissected flower, view of the androecium and gynoecium segments. **F**. Internal view of the petal. Photos: Luis Emilio de la Cruz.

TABLE 1. Morphological comparison between *E. xochipalensis*, *E. crenulata* and *E. fulgens* varieties.

Character/Taxon	*E. xochipalensis sp. nov.*	*E. crenulata*	*E. fulgens* var. *fulgens*	*E. fulgens* var. *obtusifolia*
Stem				
Length	Up to 7 cm	Up to 30 cm	5–25 cm	Up to 5 cm
Rosette				
Diameter	20 cm	Up to 40 cm	Up to 30 cm	Up to 20 cm
Leaves				
Shape	Spatulate	Oblanceolate–obovate	Obovate to oblanceolate	Spatulate, widely obovate
Base of the leaves	Pseudo-petiolate	Pseudo-petiolate	Subsessile, winged	Pseudo-petiolate, winged
Length	2.5–11.5 cm	4–20 cm	4–15 cm	15.5–6.5 cm
Color	Green, copper, lilac–gray, reddish	Light green	Light green to Glaucous	Green
Margin	Entire	Entire to crenulate	Crenate or crenulate	Ondulate, crenate, crenulate
Margin color	Yellowish or hyaline	Reddish	Reddish or hyaline	Hyaline
Inflorescence				
Number of inflorescences	1–4	1–2	1–3	1–3
Length	50–70 cm	Up to 90 cm	Up to 70 cm	Up to 30 cm
Bracts				
Consistency	Rigid	Soft	Soft	Soft
Corolla				
Length	1.2–1.25 cm	1.4–1.7 cm	1–1.6 cm	1–1.3 cm
Shape	Tubular, slightly urceolate	Tubular	Tubular	Tubular
Color	Orange–pink	Pink–Yellow	Orange–red	Reddish
Filaments				
Color	White	White	Yellow	Yellow
Carpels				
Ovaries color	Whitish	White	Light yellow	White
Styles color	Wine–red	Wine–red	Wine–red to deep purple	Wine–red
Stigmas color	Green	Green	Green	Green
Nectaries color	White	White	Yellow or whitish	White
Nectaries shape	Elliptic–reniform	Reniform	Reniform	Elliptic–reniform
Geographical distribution	Guerrero	Morelos, Estado de México	Estado de México, Morelos, Michoacán	Estado de México, Morelos

Plant perennial, glabrous, solitary. Main roots somewhat thickened, secondary ones fibrous. Stem erect, simple, up to 7 cm tall, 1–1.3 cm in diameter, brown-reddish at the base and green to pinkish at the apex, with white leaf scars. Rosette lax, up to 20 cm in diameter. Leaves spatulate, pseudo-petiolate, basal leaves green, lilac-gray, or copper colored with pinkish hues, apical leaves greenish, 2.5–11.5 cm long, 1.7–4 cm wide, adaxial surface channeled at base, abaxial surface slightly keeled, only the apical leaves slightly pruinose, apex obtuse to rounded, shortly mucronate, color reddish, margin entire, yellow to hyaline in apical leaves. Floral stem erect, panicle axillary, 1–4 per rosette or more, 50–70 cm long, 1–1.35 cm wide at the base with oblanceolate leaves, quickly deciduous, rigid, ascending, 2–5 cm long, 0.5–0.7 cm wide, spurred, margin entire, apex obtuse, reddish with orange hues. Inflorescence paniculate with up to 4 cincinni of up to 25 cm long, light green, slightly pruinose, bracteoles lanceolate, 0.5–0.7 cm long, 0.17–0.22 cm wide, green to yellowish, spurred, apex acuminate, slightly pruinose. Pedicels 0.5–0.6 cm long, 0.2 cm thick, pale green, slightly pruinose. Calyx 5 sepals, basally fused, deltoid-lanceolate, unequal, 0.5–0.8 cm long, 0.2–0.4 cm wide at the base, 0.2 cm thick, olive green, apex acute, slightly pruinose. Corolla pentagonal in frontal view, slightly urceolate in lateral view, 1.2–1.25 cm long, 0.9–1 cm wide at the base, petals lanceolate, dorsally keeled,

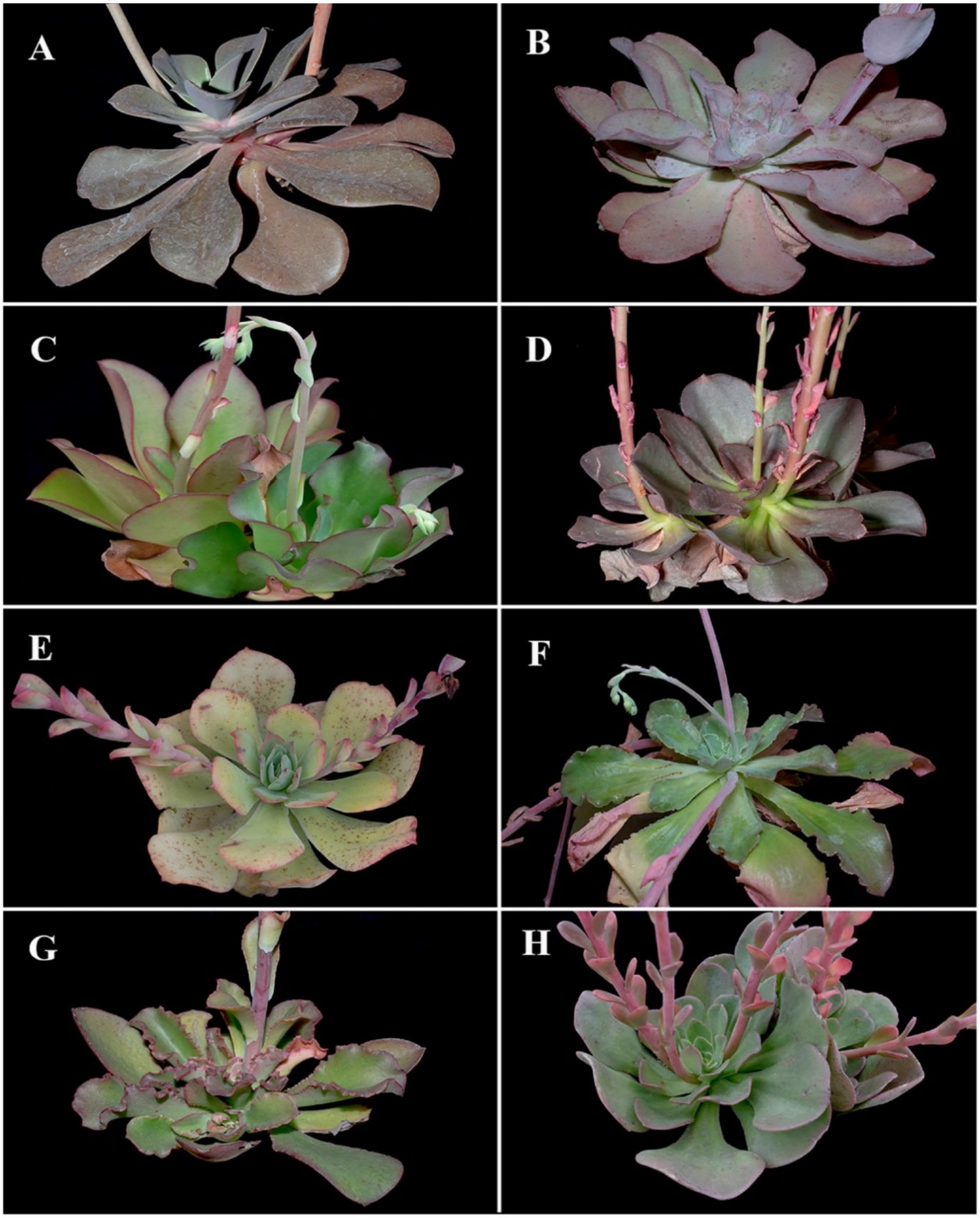

FIGURE 4. Diversity in habit and morphology of leaves inside the *E. fulgens* complex. **A**. *E. xochipalensis*, **B–H**. Specimens from different localities associated to *E. fulgens*. Photos: Luis Emilio de la Cruz.

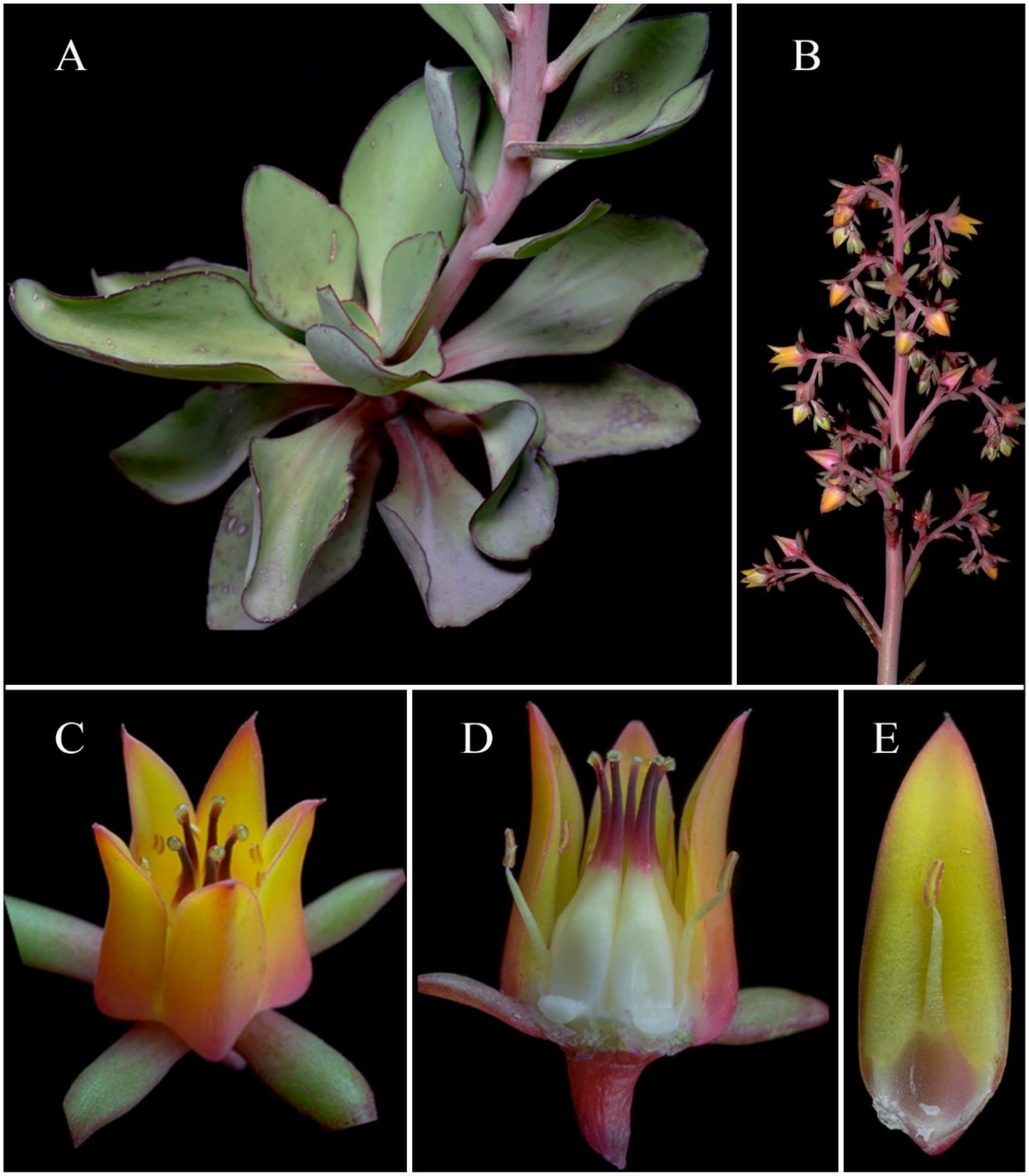

FIGURE 5. *Echeveria crenulata*. **A**. Rosette. B. Inflorescence. **C**. Flower. **D**. Dissected flower, view of ovaries and nectaries. **E**. Internal view of petal. Photos: Luis Emilio de la Cruz.

united at the base, imbricate, apex acute, slightly deflexed, 0.4 cm wide at the base, pink at the base, orange at the apex, internally tricolored, white at the base, yellow in the middle and reddish at the apex, nectary cavity prominent; androecium shorter than corolla tube, stamens 10, 5 antisepalous, 0.8 cm long, 5 epipetalous, slightly shorter, filaments white with slight yellowish hue, anthers 0.2 cm long, yellow; gynoecium with 5 carpels, free, 0.75–1 cm long, 0.25–0.3 cm wide at the base, nectaries reniform, 0.18–0.2 cm wide, 0.1–0.15 cm tall, white, ovaries white, styles wine red, stigmas wine-red with green apex; fruits follicles with numerous reddish seeds (Figs. 1–3).

Phenology:—Flowers from October to December in habitat.

Distribution and habitat:—*Echeveria xochipalensis* is only known from the type locality. It grows in *Quercus magnoliifolia* Née (1801: 268) forest, somewhat disturbed by adjoining areas of maize fields; other associated species are *Lysiloma acapulcensis* Kunth (Bentham) (1844: 83), *Juniperus flaccida* Schlechtendal (1838: 495), *Ipomoea murucoides* Roemer & Schultes (1819: 248), *Gnaphalium* sp., *Salvia* spp., among others. Further field work is needed in order to find other localities for the present new taxon.

Etymology:—The specific epithet makes reference to the village of Xochipala in the state of Guerrero, Mexico.

Discussion

The new taxon belongs to series *Gibbiflorae* which is characterized by glabrous plants with short or more or less long stems; medium-sized to large rosettes, more or less lax; wide and flat leaves sometimes with a pseudo-petiolate or sessile base; inflorescences one or more, scorpioid cymes or more frequently paniculate-cymose; corollas of 1–3 cm in length, gibbous or not, segments pruinose or not farinose; styles wine colored or purple (never yellow, orange or green; Luis E. de la Cruz-López, pers. obs.) and free styles and stigmas at anthesis (Moran 1974, Kimnach in Eggli 2003, Meyrán & López 2003, Pilbeam 2008). Some authors recognize series *Retusae* E. Walther (1972: 150) where the new taxon would be better positioned since it shares many of its own characteristics with *E. fulgens* var. *fulgens* and *E. fulgens* var. *obtusifolia* (Rose) Kimnach (1997: 51), particularly with reference to the reproductive structures (FIG. 5. B–H). However, the delimitation of both ser. *Retusae* and ser. *Gibbiflorae* is still not clearly established, partly because of the lack of a molecular phylogeny reflecting the relationships in *Echeveria*, supporting the location of lineages that would allow a phylogeny-based classification of the genus and its infrageneric categories. For this reason, we chose to use the criteria of Moran (1974), Kimnach (in Eggli 2003) and Meyrán & López (2003), including the members of ser. *Retusae* in ser. *Gibbiflorae*, at least until these groups are properly defined.

Echeveria xochipalensis, as well as *E. fulgens* var. *fulgens* and *E. fulgens* var. *obtusifolia*, have a short but evident stem, where the leaves are arranged in a more or less loosely arranged rosette (FIG. 1. A), but unlike other specimens of the *E. fulgens* complex, the stem is erect and exhibits large white leaf scars (FIG. 1. B). Moreover, the leaves are spatulate instead of obovate or oblanceolate as in *E. fulgens* (FIG. 2. B, Table 1). The pseudo-petiolate leaf trait from *E. xochipalensis* is rare in *E. fulgens* even when they can be narrowed at the base (sessile), but leaves are frequently flat and winged (FIG. 4. B–H). Leaves and bracts in the new species present rigid consistency, instead of soft and fragile as in both *E. fulgens* varieties. Another trait that differentiates it from other species is the entire, hyaline or yellowish margins, since both *E. fulgens* varieties usually displays wavy, crenate or crenulated leaves. Additionally, variation in leaf color was observed in *E. xochipalensis*, coppery or purple-grayish hues or rosettes with completely light green leaves are common, although in the dry season the leaves can become completely reddish, unlike the *E. fulgens* complex where leaf color variation is restricted to glaucous or greenish-yellowish hues (Table 1).

In summary, the vegetative characteristics of the new species resemble even more *E. crenulata* Rose (1911: 295) than *E. fulgens*, since the leaves are arranged more or less loose on the stem, by its pseudo-petiolate adaxially channeled leaves (FIG. 5, Table 1), which differs from *E. xochipalensis* by panicle inflorescences and pink–yellow (at base and apex, respectively) corolla (FIG. 5). However, the inflorescences of the new taxon and those of *E. fulgens* are very similar in length and form as well as in the dimensions and shape of the bracts, but the consistency of the latter ones in *E. xochipalensis* is rigid, similar to the one of the leaves (Table 1). The flowers also relate it to the *E. fulgens* complex; they are very similar, particularly with respect to the length, width, tubular shape and the orange coloration of the petals, but it has to be noted that the color of both ovaries and nectaries are whitish instead of yellowish (FIG. 6. B–H), as mentioned in the description of *E. fulgens* in E. Walther's monography (1972). Additionally, filaments in *E. xochipalensis* are white versus yellow in *E. fulgens* var. *fulgens* (FIG. 6) and *E. fulgens* var. *obtusifolia* (FIG. 7).

Additional specimens examined

E. crenulata: **MEXICO**. **Morelos**: municipality Yecapixtla, along the sides of the road, km 11 of Xochitlán-Ocuituco highway near Xochitlán, 1736 m, 18°52´50.74"N, 98°48´48.2"W, 1 January 2016, *L. E. de la Cruz-López 182* (MEXU).

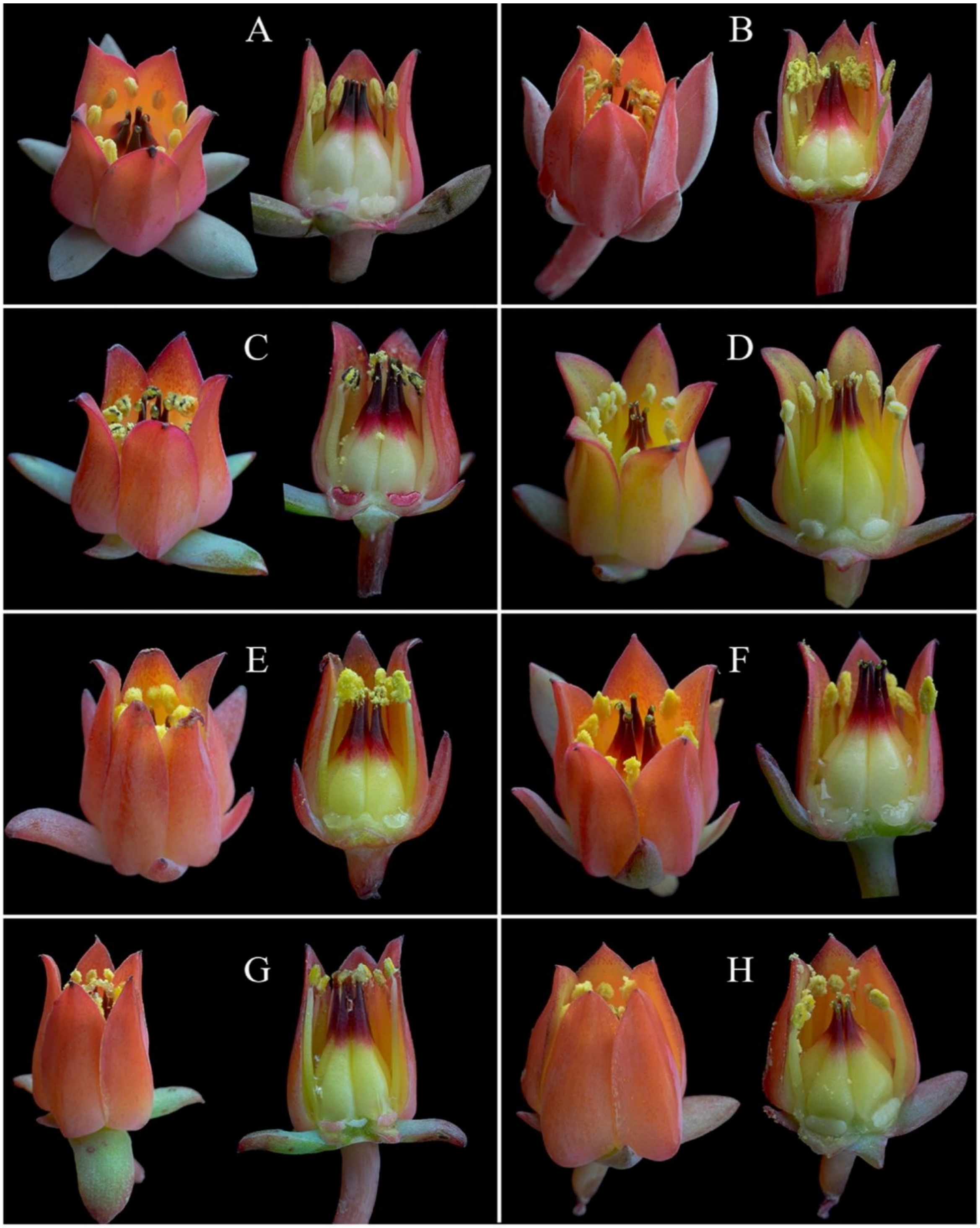

FIGURE 6. Morphological diversity in floral structures of representatives of the *E. fulgens* complex. **A**. *E. xochipalensis*, **B–H**. Specimens from different localities associated to *E. fulgens*. Photos: Luis Emilio de la Cruz.

FIGURE 7. Morphological comparison between: **A**. *E. xochipalensis* and **B**. *E. fulgens* var. *obtusifolia*. Photos: Luis Emilio de la Cruz.

E. fulgens: **MEXICO**. **Estado de México**: municipality Amanalco, volcanic rocks 1 km south of San Juan Amanalco, 2340 m, *Quercus* scrub, 8 December 1995, *A. García-Mendoza et al. 6123* (MEXU); **Michoacán**: municipality Ciudad Hidalgo, km 232 between Cd. Hidalgo and Mil Cumbres, old México-Nogales highway, 15 December 1965, *C. Delgadillo 12* (MEXU); municipality Pátzcuaro, 2 km southeast of Cerro del Estribo, oak forest, 2230 m, 8 December 1986, *J. Espinosa 2436* (MEXU); municipality Uruapan, malpaís (lava fields) of Capacúaro, forest of *Quercus*, 2250 m, 9 December 1996, *E. Pérez-Calix 3544* (MEXU); municipality Zacapu, El Pinal, near Santa Gertrudis, 2100 m, oak forest on basaltic lava stream, 6 November 1987, *H. Díaz y A. Grimaldo 4498* (MEXU); west of Angostura, matorral pedregoso, 18 November 1988, *A. Grimaldo 422* (MEXU); municipality Zitácuaro, 3.78 km north of San Felipe de Los Alzati, pine-oak forest, 2147 m, 19°31´20.1”N, 100°21´59.7”W, 17 November 2014, *D. Álvarez et al. 14683* (MEXU).

E. fulgens var. *obtusifolia*: **MEXICO**. **Morelos**. municipality Huitzilac, ± 1 km southwest of Huitzilac, on volcanic rocks, 2550 m, pine-oak forest, 25 October 1999, *E. Pérez-Calix* & *I. García 3966* (MEXU).

Acknowledgments

The authors thank to CONACyT for financial support during projects 247078, “Iniciativa interdisciplinaria para el aprovechamiento sustentable del género *Echeveria* (Crassulaceae), plantas con potencial hortícola para la producción en áreas rurales de México” awarded to F. V.-S., and 232746, awarded to Laboratorio de Biodiversidad. We also thank DGAPA-PAPIIT, UNAM, for financial support of project IN212015, “Taxonomía integrativa y códigos de barras genéticos en *Echeveria* (Crassulaceae)”, awarded to F. V.-S. L. E. de la Cruz acknowledges the Posgrado en Ciencias Biológicas, UNAM, and CONACyT for graduate scholarships. Finally, we thank Julia Etter and Martin Kristen for advice on the English translation.

References

Bentham, G. (1844) Notes on Mimoseae. *London Journal of Botany* 3: 83–84.

Berger, A. (1930) Crassulaceae. *In*: Engler, A. & Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien*, ed. 2, 18a. W. Engelmann, Leipzig, pp. 352–483.

Candolle, A.P. (1828) Crassulaceae. *In*: A.P. de Candolle, A.P. & de Candolle, A.L.P.P. (Eds.) *Prodromus Systematis Naturalis Regni Vegetabilis* 3. Treuttel & Würtz, Paris, pp. 382–414.

García-Ruiz, I. (2011) Nueva especie de *Echeveria* (Crassulaceae) del centro-occidente de Michoacán, México. *Revista Mexicana de Biodiversidad* 82: 63–67.

García-Ruiz, I., Torres, I. & Costea, M. (2016) A New Species of *Echeveria* (Crassulaceae) from Michoacán, Mexico. *Systematic Botany* 41: 966–970.
https://doi.org/10.1600/036364416X694071

Jimeno-Sevilla, H.D. & Carrillo-Reyes, P. (2010) *Echeveria perezcalixii* (Crassulaceae), una especie nueva del occidente de México. *Brittonia* 62: 303–308.
https://doi.org/10.1007/s12228-010-9137-1

Kimnach, M. (2003) Echeveria. *In*: Eggli, U. (Ed.) *Illustrated Handbook of Succulent Plants*: *Crassulaceae*. Springer-Verlag. Berlin, pp. 103–128.

Lemaire, A.C. (1845) Echeveria fulgens: Crassulacées & Obilicees. *Hortus Vanhoutteanus* 1: 8.

Meyrán, J. & López, L. (2003) *Las crasuláceas de México*. Sociedad Mexicana de Cactología, A.C., Ciudad de México, 234 pp.

Moran, R. (1974) *Echeveria*. *In*: Jacobsen, H. (Ed.) *Lexicon of Succulent Plants*. Blandford Press, pp. 184–186.

Née, L. (1801) Quercus magnoliaefolia. *Anales de Ciencias Naturales* 3: 268.

Nieves-Hernández, G., Vázquez-García, J.A., Muñiz-Castro, M.A. & Cházaro-Basáñez, M. (2014) *Echeveria cerrograndensis* (Crassulaceae) a new species from eastern calcareous Sierra de Manantlán, Colima, México. *Phytotaxa* 172: 247–255.
https://doi.org/10.11646/phytotaxa.172.3.5

Pilbeam, J. (2008) *The genus Echeveria*. The British Cactus & Succulent Society. Essex, UK, 333 pp.

Reyes, S.J. & González-Zorzano, O. (2010) *Echeveria roseiflora* (Crassulaceae) una nueva especie para el estado de Jalisco, México. *Cactáceas y Suculentas Mexicanas* 55: 19–26.

Reyes, S.J., Gónzalez-Zorzano, O. & Brachet, I.C. (2011) *Echeveria aurantiaca* (Crassulaceae), una nueva especie del estado de Guerrero, México. *Cactáceas y Suculentas Mexicanas* 56: 68–74.

Roemer, J.J & Schultes, J.A. (1819) Ipomea murucoides. *Systema Vegetabilium* 4: 248.

Schlechtendal, D.F.L. (1838) Juniperus flaccida. *Linnaea* 12: 495.

Vázquez-García, J.A., Nieves-Hernández, G., Padilla-Lepe, J., Nuño-Rubio, A.T. & Cházaro-Basáñez, M. (2014) *Echeveria munizii* (Crassulaceae) a new species of epiphyte from tropical Volcán de Colima, México. *Phytotaxa* 191: 165–171.
https://doi.org/10.11646/phytotaxa.191.1.11

Walther, E. (1972) *Echeveria*. California Academy of Sciences. San Francisco, 426 pp.

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

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10.1007/s12228-021-09684-7

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 Mezquital, 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.

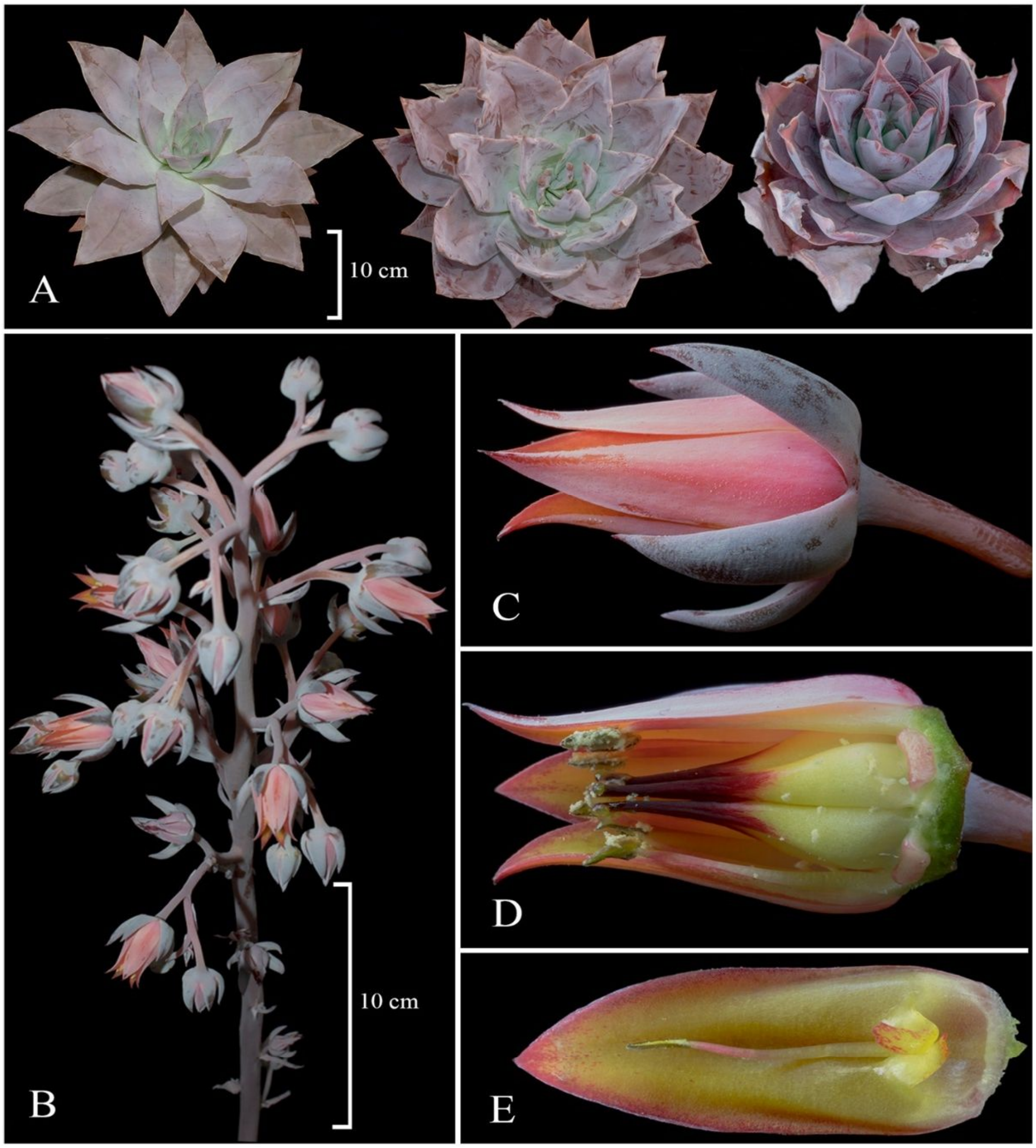

Fig. 1. *Echeveria kristenii.* **A.** Rosette and leaves, color and shape variations. **B.** Paniculate inflorescence. **C.** Calyx and corolla segments. **D.** Dissected flower, showing carpels and nectary scales. **E.** Inner view of petal, highlighting the appendages at the base of the epipetalous 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	*E. kristenii*	*E. dactylifera*	*E. novogaliciana*
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-oblanceolate	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-oblanceolate	Lanceolate-oblanceolate
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* Kimnach (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, paniculate 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	*E. kristenii*	*E. dactylifera*	*E. novogaliciana*
***rbcL*: 479**	**T/N**	C	C
matK: 29	G	G	**T**
matK: 40	A	A	**G**
matK: 100	C	C	**G**
***matK*: 154**	**G**	A	A
***matK*: 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	*rbcL*	*matK*	ITS2
E. kristenii	EK-3569-2	MT733085	MT733095	MT733105
E. kristenii	EK-3569-3	MT733086	MT733096	MT733106
E. kristenii	EK-3569-4	MT733087	MT733097	MT733107
E. kristenii	EK-3569-5	MT733088	MT733098	MT733108
E. novogaliciana	EK-3721-3	MT733089	MT733099	MT733109
E. novogaliciana	EK-3721-5	MT733090	MT733100	MT733110
E. novogaliciana	JE-6823-3	MT733091	MT733101	MT733111
E. novogaliciana	JE-6823	MG521710.1	MG521590.1	MG521436.1
E. dactylifera	JE-5287-3	MT733092	MT733102	MT733112
E. dactylifera	JE-6023	MT733093	MT733103	MT733113
E. dactilifera	EK-3057-3	MT733094	MT733104	MT733114
E. dactylifera	JE-6274	MG220445.1	MG220501.1	MG217161.1

without any trace of yellow outside (vs. lanceolate-oblanceolate 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.

Acknowledgements

We thank the Consejo Nacional de Ciencia y Tecnología (CONACyT) for financial support of the project 247078, "Iniciativa interdisciplinaria para el aprovechamiento sustentable del género *Echeveria* (Crassulaceae), plantas con potencial hortícola para la producción en áreas rurales de México" (awarded to F. V.-S.), and project 232746 (awarded to the Laboratorio de Biodiversidad). We also thank DGAPA-PAPIIT, UNAM, for financial support of the project IN212015, "Taxonomía integrativa y códigos de barras genéticos en *Echeveria* (Crassulaceae)" (awarded to F. V.-S.), and L. E. de la Cruz acknowledges the Posgrado en Ciencias Biológicas, UNAM, and CONACyT for graduate scholarships. We are also grateful to Julia Etter and Martin Kristen for the plant material provided. Finally, we thank two anonymous reviewers and the editorial team of Brittonia for their helpful comments and suggestions on the manuscript.

Literature cited

Berger, A. 1930. Crassulaceae. *In*: A. Engler & K. Prantl (eds.), Die natürlichen Pflanzenfamilien, ed. 2, 18a: 352–483. Wilhelm Engelmann, Leipzig.

Britton, N. L. & J. N. Rose. 1903. New or noteworthy North American Crassulaceae. Bulletin of the New York Botanical Garden 3: 1–45.

Britton, N. L. & J. N. Rose. 1905. Crassulaceae. *In*: N. L. Britton & J. N. Rose (eds), North American Flora 22: 7–74. Bulletin of the New York Botanical Garden, New York.

Candolle, A. P. 1828. Crassulaceae. *In*: A. P. de Candolle & A. L. P. P. de Candolle (eds.), Prodromus Systematis Naturalis Regni Vegetabilis 3: 382–414. Treuttel & Würtz, Paris.

Davis, J. I. & K. C. Nixon. 1992. Populations, genetic variation, and the delimitation of phylogenetic species. Systematic Biology 41: 421–435.

de la Cruz-López, L. E., F. Vergara-Silva, S. J. Reyes, O. G. Espino, P. Carrillo-Reyes & M. Kuzmina. 2019a. Phylogenetic relationships of *Echeveria* (Crassulaceae) and related genera from Mexico, based on three DNA barcoding loci. Phytotaxa 422: 33–57.

de la Cruz-López, L. E., S. J. Reyes & F. Vergara-Silva. 2019b. *Echeveria michihuacana* (Crassulaceae), a new species from Michoacán, Mexico. Brittonia 71: 25–31.

DeSalle, R., M. G. Egan & M. Siddall. 2005. The unholy trinity: taxonomy, species delimitation and DNA barcoding. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences 360: 1905–1916.

García-Ruiz, I. 2011**.** Nueva especie de *Echeveria* (Crassulaceae) del centro-occidente de Michoacán, México. Revista Mexicana de Biodiversidad 82: 63–67.

García-Ruiz, I., D. Valentín-Martínez, P. Carrillo-Reyes & M. Costea. 2016a. Taxonomic and floristic novelties for *Echeveria* (Crassulaceae) in Central Michoacan, Mexico. PhytoKeys 75: 1–13.

García-Ruiz, I., I. Torres & M. Costea. 2016b. A new species of *Echeveria* (Crassulaceae) from Michoacán, Mexico. Systematic Botany 41: 966–970.

García-Ruiz, I. & M. Costea. 2014. *Echeveria marianae* (Crassulaceae), a new species from Jalisco, México. Phytotaxa 170: 35–40.

García-Ruiz, I. & E. Pérez-Calix. 2007. Una especie nueva de *Echeveria* (Crassulaceae) originaria del estado de Jalisco, México. Acta Botánica Mexicana 78: 125–132.

Glass, C. & M. Mendoza-García. 1997. *Echeveria cante*, a new species from the mountains of Zacatecas. Cactus and Succulent Journal of America 69: 239–243.

Goldstein, P. Z. & R. DeSalle. 2011. Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. BioEssays 33: 135–147.

Jimeno-Sevilla, H. D. & P. Carrillo-Reyes. 2010. *Echeveria perezcalixii* (Crassulaceae), una especie nueva del occidente de México. Brittonia 62: 303–308.

Jimeno-Sevilla, H. D., P. Carrillo-Reyes, E. Pérez-Calix & M. J. Cházaro-Basáñez. 2012. Additions to the Crassulaceae of the state of Veracruz, Mexico. Haseltonia 18: 140–152.

Jimeno-Sevilla, H. D., M. F. J. Santana & P. Carrillo-Reyes. 2015. Dos especies nuevas de Crassulaceae del sur de Jalisco, México. Acta Botánica Mexicana 110: 71–88.

Jimeno-Sevilla, H. D., J. A. Vázquez-García, M. A. Muñiz-Castro, I. García-Ruiz, G. Hernández-Vera & C. J. Villa. 2019. *Echeveria sonianevadensis* (Crassulaceae), una nueva especie del Volcán Nevado de Colima del occidente de México y una clave para la serie *Gibbiflorae*. Brittonia 71: 156–165.

Jörger, K. M. & M. Schrödl. 2014. How to use CAOS software for taxonomy? A quick guide to extract diagnostic nucleotides or amino acids for species descriptions. Spixiana 37: 21–36.

Kimnach, M. 1979. A new *Echeveria* from Nayarit, Mexico. Cactus andSucculent Journal of America 51: 207–209.

Klotzsch, **J. F.** 1841. Beschreibung einer neuen mexikanischen Pflanze *Pachyphytum bracteosum*. Allgemeine Gartenzeitung 9: 9–11.

Moran, R. 1954 A new species of *Echeveria* from Hidalgo, Mexico. Cactus and Succulent Journal of America 16: 174–176.

Nicolalde-Morejón, F., F. Vergara-Silva, J. González-Astorga & D. Stevenson. 2010. Character-based, population-level DNA barcoding in Mexican species of *Zamia* L. (Zamiaceae: Cycadales). Mitochondrial DNA 21: 51–59.

Nieves-Hernández, G., J. A. Vázquez-García., M. A. Muñiz-Castro & M. Cházaro-Basáñez. 2014. *Echeveria cerrograndensis* (Crassulaceae) a new species from eastern calcareous Sierra de Manantlán, Colima, México. Phytotaxa 172: 247–255.

Pilbeam, J. 2008. The genus *Echeveria*. The British Cactus and Succulent Society, Essex.

Rach, J., R. DeSalle, I. N. Sarkar, B. Schierwater & H. Hadrys. 2008. Character-based DNA barcoding allows discrimination of genera, species and populations in O-donata. Proceedings of the Royal Society B: Biological Sciences 275: 237–247.

Ratnasingham, S. & P. D. N. Hebert. 2007. BOLD: The Barcode of Life Data System (www.barcodinglife.org). Molecular Ecology Notes 7: 355–364.

Reyes, S. J., I. C. Brachet. & O. Gónzalez-Zorzano. 2011a. *Echeveria guerrerensis* (Crassulaceae) una nueva especie para el estado de Guerrero, México. Cactáceas y Suculentas Mexicanas 56: 75–81.

Reyes, S. J., I. C. Brachet. & O. Gónzalez-Zorzano. 2011b. *Echeveria novogaliciana*, una nueva especie de la familia Crassulaceae para los estados de Aguascalientes y Jalisco, México. Cactáceas y Suculentas Mexicanas 56: 82–95.

Reyes, S. J. & O. Gónzalez-Zorzano. 2010. *Echeveria roseiflora* (Crassulaceae) una nueva especie para el estado de Jalisco, México. Cactáceas y Suculentas Mexicanas 55: 19–26.

Reyes, S. J., Etter K., M. Kristen. & L. E. de la Cruz-López. 2020. *Pachyphytum huastecanum* (Crassulaceae), a new species from the Huasteca Canyon, Nuevo León, México. Cactus and Succulent Journal 92: 141–147.

Rose, J. N. 1903. *Echeveria subrigida*. *In*: N. L. Britton & J. N. Rose (eds.), New or Noteworthy North American Crassulaceae. Bulletin of the New York Botanical Garden 3: 1–45.

Rose, J. N. 1904. *Lenophyllum* Rose, gen. Nov. *In*: N. L. Britton & J. N. Rose (eds), *Lenophyllum*, a new genus of Crassulaceae. Smithsonian Miscellaneous Collections 47: 159–162, pl. XX.

Rose, J. N. 1911. *Graptopetalum*, a new genus. Contributions from the United States National Herbarium 13: 296.

Sarkar, I. N., P. J. Planet & R. DeSalle. 2008. CAOS software for use in character-based DNA barcoding. Molecular Ecology Resources 8: 1256–1259.

Vázquez-García, J. A., G. Nieves-Hernández, J. Padilla-Lepe, A. T. Nuño-Rubio & M. Cházaro-Basáñez. 2014. *Echeveria munizii* (Crassulaceae) a new species of epiphyte from tropical Volcán de Colima, México. Phytotaxa 191: 165–171.

Walther, E. 1958. *Echeveria semivestita* var. *floresiana*. Cactus and Succulent Journal of America 30: 108–109.

Walther, E. 1972. *Echeveria*. California Academy of Sciences, San Francisco.

Zou, S., Q. Li, L. Kong, H. Yu, & X. Zheng. 2011. Comparing the usefulness of distance, monophyly and character-based DNA barcoding methods in species identification: a case study of Neogastropoda. PLoS ONE 6: 1–8.

Zou, S., C. Fei, J. Song, Y. Bao, M. He & C. Wang. 2016. Combining and comparing coalescent, distance and character-based approaches for barcoding microalgaes: a test with *Chlorella*-like species (Chlorophyta). PLoS ONE 11(4): 1–24.

Appendix

SPECIMENS EXAMINED OF *ECHEVERIA DACTLIFERA* 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).

Discusión general y perspectivas

Desarrollo de una librería de referencia de códigos de barras genéticos de Echeveria

En el capítulo 1 de esta tesis se establecieron los procedimientos que se llevaron a cabo para el desarrollo de una librería de referencia de códigos de barras genéticos para ser empleada como herramienta de identificación rápida y precisa de las especies mexicanas del género *Echeveria* (Crassulaceae). En paralelo al desarrollo de la librería se realizaban estudios sistemáticos y taxonómicos para este grupo de plantas suculentas ornamentales, cuya historia carece de marco filogenético y ausencia generalizada de información molecular de sus especies.

De manera inédita para *Echeveria*, en esta tesis se secuenciaron tres regiones genómicas para una proporción importante de las especies que componen al grupo. Siguiendo la sugerencia del Consorcio para el Código de Barras de la Vida empleamos a *rbcL* y *matK* como núcleo del código de barras (CBOL Plant Working Group, 2009) para las especies mexicanas de *Echeveria* e incluimos a la región del genoma nuclear ITS2 que ha sido probado y recomendado como una región complementaria útil para diferenciar especies (Chen et al., 2010; Yao et al., 2010; Han et al., 2013; Al-Juhani, 2019) en el contexto de los códigos de barras genéticos.

A partir de un proyecto piloto anterior a esta tesis (Gernandt et al., 2011), se evaluó el desempeño de estos tres marcadores moleculares para diferenciar a una muestra de 52 especies representantes de 16 de las 17 series —con excepción de *Ciliatae*— en las que se subdivide al género en las más recientes monografías de Kimnach (2003) o Pilbeam (2008), mediante la identificación de atributos característicos en una matriz de secuencias concatenada y alineada.

Para la identificación de los caracteres diagnósticos moleculares se empleó el algoritmo CAOS (Characteristic Attributes Organization System, por sus siglas en inglés) desarrollado por Sarkar y colaboradores (2008) el cual identifica aquellos caracteres —en este caso nucleótidos: adenina, citosina, guanina y timina o también la ausencia de estos— y los sitios en el alineamiento en los cuales se presentan, es decir, en sitios homólogos. Estos caracteres que en la conceptualización de Sarkar y colaboradores (2008) se denominan Atributos Característicos (CA´s, Characteristic Attributes, por sus siglas en inglés), en otras disciplinas se emplean para identificar SNPs (Single Nucleotide Polymorphisms) en el caso de genética de poblaciones (Aitken et al., 2004), o pueden ser denominados autapomorfías en el contexto del cladismo (Kitching et al., 1998; Schuh, 2000; Morrone, 2016; Williams et al., 2016; Brower y Schuh, 2021).

El algoritmo CAOS requiere de una estructura jerárquica que le sirva de guía para extraer los CA´s, esta guía jerárquica puede provenir de un fenograma como un árbol de distancias genéticas corregidas por el modelo Kimura de dos parámetros (KP2) aunque como DeSalle y sus colaboradores (2005) señalan, el empleo de distancias genéticas en los códigos de barras genéticos puede no ser lo más apropiado tanto para la identificación de especies como para el descubrimiento de las mismas, puesto que se confronta con el empleo de caracteres por parte de otras disciplinas tales como la sistemática filogenética y la taxonomía tradicional (DeSalle et al., 2005; Rach et al, 2008).

DeSalle y colaboradores (2005) también sugieren el empleo de árboles filogenéticos inferidos con cualquiera de los criterios que se basan en caracteres, tales como parsimonia, máxima verosimilitud o inferencia bayesiana, como jerarquías para extraer los caracteres derivados (apomorfías) compartidos (sinapomorfías) o únicos (autapomorfías) por los taxa en el análisis en cada uno de los nodos de la topología del árbol filogenético.

De esta manera análoga a la taxonomía, uno de los objetivos de la tesis consistió en identificar aquellos caracteres diagnósticos que diferencian a una especie de otra, útiles tanto para identificarlas como para proponer (acompañados de otros datos morfológicos, ecológicos o de otra índole) el descubrimiento (DeSalle et al., 2005; DeSalle, 2006; DeSalle y Goldstein, 2019) de nuevas especies en *Echeveria*.

En la primera etapa del proyecto (Capítulo 1) se infirió un árbol de consenso de mayoría con criterio bayesiano tanto para explorar sus relaciones filogenéticas, como para la extracción de los CA´s de las especies muestreadas. En esta etapa el 100% de los taxones representados presentaron al menos un atributo característico con los cuales pueden ser identificadas (Ver Tabla 4 del Capítulo 1). La alta capacidad de identificación de especies por parte del set de datos molecular en la primera etapa del proyecto puede atribuirse a la "lejanía" de las especies muestreadas que, de acuerdo con las monografías del género, pertenecen a categorías infragenéricas distintas de *Echeveria*. Desde la primera etapa del proyecto también se hizo evidente que estas categorías (series) correspondían solo parcialmente con los esquemas clasificatorios del género empleados en la actualidad (ver Kimnach, 2003; Pilbeam, 2008).

Al incrementar el número de especies de crasuláceas secuenciadas en la segunda etapa de desarrollo de la librería, se recuperaron cuatro clados principales. Para el clado 1 que corresponde con el género *Pachyphytum*, 11 de las 20 especies muestreadas (55%) presentan atributos característicos en las secuencias concatenadas de ADN mediante los cuales pueden ser identificadas (ver Reyes et al., 2019; 2020).

En el segundo clado principal (Clado II), el número de taxones que pueden ser identificados por medio de CA´s en la matriz combinada (*rbcL*+*matK*+ITS2) es de 12 de las 16 muestreadas (75%) y estos corresponden con *E. cuspidata* var. *cuspidata*, *E. cuspidata* var. *zaragozae*, *E. lilacina*, *E. unguiculata*, *E. elegans*, *E. potosina*, *E. juliana*, *E. tobarensis*, *E. turgida*, *E. tolimanensis*, *E. agavoides* y *E. colorata*. Mientras que *E. chihuahuensis*, *E. pulidonis*, *E. halbingeri* var. *halbingeri* y *E. simulans* no presentaron CA´s.

Dentro del clado principal III (Clado III), el género *Thompsonella* presenta un porcentaje de identificación de especies de 62.5% por parte del set molecular. En tanto que, para el resto de los taxones que pertenecen al género *Echeveria*, el porcentaje de identificación con al menos un atributo característico es del 51%. Cabe señalar que para este grupo de especies el porcentaje fue de los más bajos para los clados recuperados, lo que siguiere que se requiere mayor número de loci tanto para resolver las relaciones filogenéticas de este Clado III, como para incrementar el número de especies identificadas en la librería de referencia de códigos de barras genéticos.

Dentro del Clado IV, el número de identificaciones para *Graptopetalum* por parte del set molecular—incluidos *Reidmorania*, *Sedum suaveolens* y *Tacitus*— es del 88%. Este resultado es sobresaliente tomando en cuenta que únicamente se dispuso de secuencias de ITS2 para casi todas las especies de este grupo.

El 100% de las especies de *Cremnophila* —solo dos especies— pueden identificarse por medio de atributos característicos por parte de la matriz combinada de (*rbcL*+*matK*+ITS2) al interior del Clado IV.

Otro de los grupos (clados) que fueron recuperados dentro del Clado IV, corresponde con especies de las series: *Angulatae*, *Pruinosae*, *Secundae* y las dos variedades de *E.*

semivestita. Dentro de la perspectiva de la librería de referencia de códigos de barras genéticos, el número de taxones que pueden ser identificados con los marcadores empleados para este subclado particular del Clado IV es de 63.2%.

Nótese que los grupos (clados) con mayor incertidumbre filogenética indicada por bajos valores de soporte (Bootstrap/probabilidades posteriores) o por politomías en las topologías de los diferentes árboles filogenéticos recobrados en la tesis, corresponden con los grupos con menor número de especies identificadas por CAOS. Por el contrario, los grupos con mayor resolución en los árboles filogenéticos presentan mayor número de especies identificadas con al menos un atributo característico —Characteristic Attributes, de acuerdo con la terminología de Sarkar et al (2008)—. Lo anterior sugiere que existe una relación directa entre la resolución del árbol filogenético inferido y la presencia de atributos característicos extraídos por CAOS, BLOG 2.0 (Weitschek et al., 2013) u otros programas bioinformáticos orientados a estos fines.

Esto cobra mayor sentido puesto que la inferencia filogenética —en especial el cladismo— depende de estos mismos atributos característicos (sinapomorfías, autapomorfías) en sitios homólogos para inferir las relaciones de parentesco entre taxa seleccionados.

Así, para construir una librería de referencia de códigos de barras genéticos basados en caracteres de cualquier grupo de organismos, es recomendable inferir árboles filogenéticos de preferencia también basados en caracteres (parsimonia, máxima verosimilitud e inferencia Bayesiana) tal y como es sugerido por los autores del algoritmo CAOS (Sarkar et al., 2008) tanto para determinar el número de loci requeridos para abarcar el 100% de discriminación de las especies que componen al grupo (identificación), como para la correcta comparación entre grupos taxonómicos.

Aunque los desarrolladores del algoritmo CAOS no lo mencionan explícitamente, se puede asumir que el programa bioinformático extrae las autapomorfías de los clados recuperados de cada uno de los nodos para el árbol filogenético introducido al algoritmo. Existen diferentes programas bioinformáticos con los cuales se pueden extraer las sinapomorfías y autapomorfías de los árboles filogenéticos (TNT, Winclada, entro otros). Sin embargo, CAOS además de extraer CA´s, ofrece una propuesta clasificatoria de los taxa muestreados con base en estos caracteres (Sarkar et al, 2008) lo que resulta muy conveniente en el contexto taxonómico.

Esta compatibilidad entre la sistemática filogenética, la taxonomía basada en morfología, y el algoritmo CAOS para extraer caracteres moleculares únicos entre especies o grupos de especies es lo que vuelve atractivo el empleo de CAOS como herramienta de identificación de especies establecidas previamente ya sea por métodos morfológicos o de cualquier otro tipo, así como para identificar potenciales nuevas especies que pueden ser corroboradas por otras fuentes de evidencia en un contexto taxonómico-integrativo (Bergmann et al., 2008). En tanto que los métodos basados en distancias pueden conflictuarse con los métodos filogenéticos debido a su naturaleza fenética (DeSalle, 2006).

A pesar de su simpleza, CAOS ha demostrado obtener resultados similares o en ocasiones superiores a otros métodos empleados para asignar códigos de barras o delimitar especies entre los que se encuentran métodos basados en coalescencia, basados en el cómputo de distancias genéticas, u otros (Bergmann et al., 2008; Rach et al., 2008; Zou et al., 2016a y 2016b).

La capacidad discriminativa de los marcadores elegidos fue aceptable para los casos de *matK* e ITS2, sin embargo, el locus *rbcL* resultó ser demasiado conservado para ser empleado en la iniciativa de los códigos de barras. Este último puede ser descartado para su empleo en *Echeveria* y géneros afines, y ser sustituido por loci con mayor grado de informatividad filogénica observada en otros grupos, tales como regiones intergénicas y no codificantes del genoma del cloroplasto: *trnL-F* (García et al., 2013), *trnH-psbA*, *atpF-atpH*, *psbK-I* (Nicolalde-Morejón et al., 2010), entre otros; y regiones nucleares como ITS (Sonnante et al., 2003), y ETS (Carrillo-Reyes et al., 2009).

Relaciones filogenéticas de Echeveria

Debido a los resultados de la primera etapa, el Capítulo 2 de la tesis se orientó fundamentalmente a explorar con mayor profundidad las relaciones de parentesco de *Echeveria*, para el análisis filogenético se incluyeron 153 taxones de *Echeveria* correspondientes al 77.78% de las especies de distribución geográfica en México, así como a 73 taxones representantes de los siete géneros que antecedentes filogenéticos (Carrillo-Reyes et al., 2009) han señalado como cercanos a *Echeveria*.

En el Capítulo 2 de la tesis se presenta la filogenia molecular más representativa de *Echeveria* y géneros relacionados publicada hasta el año 2019. El muestreo consistió en 226 taxones los cuales se enumeran a continuación: *Cremnophila* (6), *Dudleya* (2), *Echeveria* (153), *Graptopetalum* (20), *Lenophyllum* (2), *Pachyphytum* (22), *Sedum* (7), *Reidmorania* (1), *Tacitus* (1), *Thompsonella* (10) y *Villadia* (2). Los árboles de consenso de mayoría de máxima verosimilitud e inferencia bayesiana resultantes están en concordancia general con estudios filogenéticos independientes con diferentes sets de datos moleculares (Carrillo-Reyes et al., 2009; Nikulin et al., 2016; Vázquez-Cotero et al., 2017), aunque estos últimos con muestreos de especies menos representativos para *Echeveria*.

De acuerdo con los resultados se confirma que *Echeveria* no es monofilético como los antecedentes filogenéticos han señalado (Carrillo-Reyes et al., 2009; Vázquez-Cotero, 2017). Derivado de estos análisis se recuperan cuatro clados principales y algunas especies en grados dentro del "Grupo *Echeveria*" que es un clado informalmente nombrado dentro del clado *Acre* (Mort et al., 2001; Carrillo-Reyes et al., 2009).

El clado más tempranamente divergente de acuerdo con la polaridad de los grupos externos elegidos (*Dudleya*, *Lenophyllum*, algunos *Sedum* y *Villadia*) corresponde con el género *Pachyphytum* (Clado I) que es recobrado como monofilético con altos valores de Bootstrap y probabilidad posterior. Aunque se presentan sinapomorfías moleculares que agrupan a las especies del género y autapomorfías que segregan a este grupo con respecto a otros grupos de crasuláceas mexicanas, los tres marcadores empleados no presentaron la resolución suficiente para revelar las relaciones de parentesco al interior del mismo.

Antecedentes filogenéticos con otros marcadores moleculares también han recobrado a *Pachyphytum* como monofilético (Carrillo-Reyes et al., 2009; Vázquez-Cotero et al., 2017) con un muestreo taxonómico limitado. Algunos esfuerzos se han realizado para revelar las relaciones al interior del género, I. García Ruíz (2003) empleando marcadores AFLP´S señala que se identifican dos grupos dentro del género basados en su similitud genética, sin embargo, estos no corresponden con las secciones de *Pachyphytum* establecidas por Moran (1968). Las relaciones de *Pachyphytum* han sido históricamente difíciles de resolver, debido posiblemente a su muy reciente tiempo de diversificación.

Las especies de *Pachyphytum* presentan características morfológicas que pueden representar sinaporfías para el grupo como son: sufrutescentes, tallos suculentos largos, hojas muy suculentas dispuestas laxamente a lo largo del tallo, con una roseta apical más densa, inflorescencias en cincino (cima escorpiode), sépalos adpresos a la corola, estilos y estigmas libres y apéndices a los lados de los filamentos epipétalos —todas las especies presentan apéndices planos a membranosos en la cara interna de los pétalos, otras especies de *Echeveria* también los presentan pero estos son cilíndricos (dactyloides)—.

Uno de los resultados importantes para la clasificación del género que se presenta en esta tesis, es que la especie primero descrita como *Echeveria cuicatecana* J.Reyes, Joel Pérez & Brachet y posteriormente transferida a *Pachyphytum* —*Pachyphytum cuicatecanum* (J.Reyes, Joel Pérez & Brachet) Kimnach— por compartir muchas de sus características no es recobrada en este Clado I, más bien en el Clado III en donde se anidan especies de *Echeveria* y *Thompsonella*. La ausencia de apéndices a los costados de los filamentos epipétalos, adicionalmente a su distribución geográfica en la zona de la Cañada en Oaxaca en donde no se registran otras especies de *Pachyphytum* justifican su exclusión del género.

El segundo de los clados principales recuperados (Clado II) corresponde con la enorme mayoría de especies de la serie *Urbiniae*. Este clado es recuperado con alto valor de Bootstrap (98%) y moderado soporte de probabilidad posterior (0.88). Las principales características morfológicas de este grupo de especies son: el hábito acaulescente, hojas generalmente muy suculentas, inflorescencias en cincino para un grupo y paniculado-cimosas en otro grupo de especies, sépalos deltoides generalmente muy cortos, corolas urceoladas, pétalos redondeados dorsalmente, estilos y estigmas adpresos entre sí, nectarios angostos generalmente pequeños. Este clado también ha sido recobrado en previos estudios con alto soporte (Vázquez-Cotero et al., 2017) aunque con un reducido número de especies muestreadas.

Cuatro de las especies que en monografías y tratamientos del género han sido ubicadas en *Urbiniae*, son recobradas en clados distintos al Clado II, tales son los casos de *E. purpusorum* que es recobrada como especie hermana de las variedades de *E. longissima* en el Clado III. Tanto la morfología como la biogeografía apoyan la transferencia de *E. purpusorum* a la serie *Longistylae*, pues comparte con estas últimas la morfología y su distribución geográfica como se detalla en el capítulo 2. Los otros casos revelados aquí se refieren a *E. humilis* y *E. xichuensis* que son recobradas como especies hermanas siendo el clado hermano de *E. trianthina* —otra especie previamente ubicada en *Urbiniae*—, los géneros *Cremnophila*, *Graptopetalum*, *Reidmorania*, *Sedum clavatum* y las series *Angulatae*, *Secundae*, *Pruinosae* y *Gibbiflorae*. Algunos caracteres de *E. humilis*, *E. trianthina* y *E. xichuensis* están en correspondencia con *Urbiniae* como son: su hábito acaulescente, sus hojas muy suculentas y sus inflorescencias en cincino —aunque también las inflorescencias en cincino se presentan en *Angulatae*— sin embargo, sus sépalos son más largos y sus pétalos son aquillados dorsalmente, estas características se presentan en *Angulatae*. La distribución geográfica de estas especies está en correspondencia tanto con *Angulatae* como con *Urbiniae*. La exclusión de *E. humilis*, *E. purpusorum*, *E. trianthina* y *E. xichuensis* de *Urbiniae* está justificada sólo parcialmente por la morfología. La posición filogenética de estas cuatro especies no tiene precedentes en otros análisis filogenéticos previos a esta tesis debido al pobre muestreo de *Urbiniae* como se puede observar en Vázquez-Cotero et al. (2017).

Sedum corynephyllum Fröd es recobrado solitario en un grado hermano de los clados III y IV. Es posible que el muestreo más amplio de especies de *Sedum*, principalmente de aquellos con inflorescencias laterales de las secciones *Pachysedum* y *Palmeri* recobren un clado aquí en futuros análisis tal y como ha sido sugerido por Carrillo-Reyes et al. (2009).

Debido a que *Sedum* es altamente polifilético es posible que los *Sedum* de México formen varios clados —posiblemente generándose nuevos géneros— como se aprecia en filogenias independientes a esta tesis (Messerschmid et al., 2020).

El Clado III está conformado por 71 taxones, entre las cuales la mayoría son pertenecientes a únicamente a 9 de las 17 series previamente establecidas y resumidas en las monografías de Kimnach (2003) y Pilbeam (2008) para el género *Echeveria*: *Chloranthae*, *Ciliatae*, *Echeveria*, *Longistylae*, *Mucronatae*, *Nudae*, *Racemosae*, *Spicatae* y *Thyrsiflorae*, así como a *Echeveria purpusorum* de *Urbiniae*, *Pachyphytum cuicatecanum* —discutidos anteriormente— y al género *Thompsonella*.

Si bien este clado fue recobrado con bajo a moderado soporte tanto de Bootstrap como de probabilidad posterior, en árboles de consenso de mayoría de inferencia bayesiana con dos matrices concatenadas de los marcadores *rps16*+*trnL-F*+ETS+ITS y ETS+ITS (Vázquez-Cotero et al., 2017) se recobra una topología similar, pero con un muy reducido muestreo de especies. A pesar de observarse dos clados más o menos diferenciados Vázquez-Cotero et al. (2017) no hacen una separación entre estos debido a los bajos valores de soporte dentro de su Clado III. Aunque en el capítulo 2 de la tesis se presenta el mismo problema de soporte de Bootstrap y probabilidad posterior, consideramos que al incrementarse la información molecular los clados puedan obtener mayor apoyo de los caracteres y definirse con mayor claridad.

En este Clado III se incluye al género *Thompsonella*, este último como grupo monofilético con alto soporte de Bootstrap a diferencia de un antecedente filogenético con respecto a la no monofilia del género debido a que *T. xochipalensis* Gual, S.Peralta & Pérez-Calix se anida en otro clado (Vázquez-Cotero et al., 2017), en los árboles generados aquí se anida con el resto de *Thompsonella*. Ya se había identificado previamente a este pequeño grupo de siete especies como monofilético al interior de *Echeveria* (Carrillo-Reyes et al., 2008; 2009). Los árboles de consenso de mayoría presentados en el capítulo 2 de la tesis indican una cercana relación de *Thompsonella* con especies de las series *Echeveria* —*E. pringlei* y *E. pringlei* var. *parva*— y *Racemosae* —*E. moranii*—. Las implicaciones sistemáticas y taxonómicas de estos resultados son relevantes puesto que *Thompsonella* está más cercanamente relacionado a *Echeveria* que incluso otras series como: *Angulatae*, *Gibbiflorae*, *Pruinosae*, *Secundae* y *Urbiniae* como se aprecia también en un análisis independiente orientado específicamente para conocer las relaciones de *Thompsonella* (Carrillo-Reyes et al., 2008).

Es posible que, al incorporar mayor número de caracteres moleculares, *Thompsonella* sea recobrado al exterior de *Echeveria* como géneros hermanos, por lo pronto nuestro set de datos lo recobran al interior de *Echeveria*.

La especie tipo del género *Echeveria* (*E. coccinea*) se encuentra ubicada en este Clado III por lo que nomenclaturalmente este clado corresponde con el género *Echeveria*. Aunque algunos pocos subclados concuerdan con el esquema clasificatorio de Kimnach (2003), todas las series que componen al mismo son recobradas como polifiléticas o parafiléticas.

La falta de resolución para este clado por parte del set molecular (*rbcL*+*matK*+ITS2) es más evidente para las series *Nudae* y *Racemosae*, dos de las series más especiosas dentro de *Echeveria*, la topología de los árboles las muestra como altamente polifiléticas. En el capítulo 2 de la tesis se discute con mayor profundidad que los caracteres que se han empleado para definir a estas dos series son altamente artificiales, así por ejemplo, el único carácter que diferencia a la ser. *Echeveria* de la ser. *Nudae* es la presencia de pubescencia en los órganos aéreos vs órganos glabros, en tanto que comparten muchos rasgos tales como: caulescentes, hojas generalmente de color verde con márgenes rojizos, filotaxia laxa, inflorescencias en espiga o racimo y pétalos rojizos o naranjas con ápice amarillo —las excepciones son *E. chapalensis* y *E. waltheri* que presentan pétalos rosas y blanquecinos, respectivamente—, estas series podrían fusionarse en futuras revisiones.

Con base en la observación y comparación de la morfología de especímenes de la serie *Racemosae* se aprecian al menos dos grupos de especies con características disruptivas, el primer grupo presenta plantas acaules o caulescentes, con hojas papilosas, brácteas que se desprenden muy fácilmente y corolas relativamente largas en relación con su ancho; en este grupo se encuentra la especie tipo de la serie *E. racemosa*, además de *E. atropurpurea*, *E. canaliculata*, *E. carnicolor*, *E. diffractens*, *E. lurida*, *E. olivacea* y *E. penduliflora*. En el segundo grupo, las plantas son acaulescentes, hojas no papilosas, brácteas que no se desprenden, corolas cortas y más anchas, en este grupo se encuentran *E. chazaroi*, *E. derenbergii*, *E. globulosa*, *E. helmutiana*, *E. megacalyx*, *E. mondragoniana*, *E. subcorymbosa* y *E. uhli*. Los árboles inferidos en el capítulo 2 de la tesis, reflejan parcialmente estas observaciones de la morfología. En la medida que se incorpore información molecular adicional, estos grupos podrían revelarse y segregar al segundo grupo de *Racemosae*.

Tanto la factible fusión de las ser. *Echeveria* y ser. *Nudae* y la división de la ser. *Racemosae* no han sido planteadas con anterioridad en ninguna de las monografías de *Echeveria*, por lo que se considera uno más de los aportes de esta tesis. Cabe indicar que, si bien la representatividad de estas dos series fue significativa para las especies mexicanas, en posteriores análisis es indispensable el muestreo de las especies de Centroamérica y Suramérica de *Nudae* y *Racemosae* —la gran mayoría de las especies de *Echeveria* al sur de

México pertenecen a estas dos series—.

En este Clado III también se confirmó que *E. heterosepala*, una especie que fue transferida al género *Pachyphytum* por E. Walther (1931), renombrada por él mismo (E. Walther, 1935) y transferida de nuevo a *Echeveria* por R. Moran (1960) creando la serie monotípica *Chloranthae*, no se anida con *Pachyphytum*. A partir del análisis filogenético de Vázquez-Cotero et al. (2017) orientado específicamente para determinar la ubicación de esta especie se establece que se relaciona con *E. crassicaulis*, *E. mucronata*, *E. nodulosa* y *E. racemosa*.

Debido a la inclusión de un mayor número de especies y de todos los grupos infragenéricos de *Echeveria*, en los árboles inferidos en esta tesis es recobrada en un subclado compuesto por *E. heterosepala* como la más tempranamente divergente, *E. crassicaulis* (sinónimo de *E. mucronata* perteneciente a la serie *Mucronatae*), *E. platyphylla* (perteneciente a la serie *Thyrsiflorae*), *E. longipes* E.Walther (probable sinónimo de *E. paniculata* var. *maculata*) y *E. paniculata* var. *maculata* (perteneciente a la serie *Thyriflorae*). Este pequeño subclado en donde se ubica *E. heterosepala* y donde se agrupan a especies de tres series de *Echeveria* podría fusionarse reduciéndose aún más sus grupos infragenéricos.

El cuarto clado principal (Clado IV) recobrado en los árboles consenso de mayoría contiene 106 taxones, siendo el más numeroso y corresponde con el clado más recientemente divergente de acuerdo con la polaridad de los grupos externos. En este clado se recupera al género *Graptopetalum* formando subclados y grados con *Reidmorania occidentalis* (Rose ex E.Walther), *Echeveria craigiana*, *Echeveria affinis*, *Echeveria valvata*, *Echeveria calycosa*, *Graptopetalum bellum* (Moran & J.Meyrán) D.R.Hunty —descrito como *Tacitus bellus*— y *Sedum clavatum*. Si bien no era la intensión de esta tesis, las relaciones de *Graptopetalum* no fueron resueltas en este análisis, es importante subrayar que, para este género únicamente se emplearon secuencias de ITS descargadas de GenBank generadas posiblemente del análisis de Acevedo-Rosas et al. (2004), aparente mente no se han generado secuencias de *rbcL* y *matK* para este grupo.

Incorporar secuencias de especies de *Graptopetalum* y otros géneros relacionados para inferir las relaciones de parentesco en *Echeveria*, se fundamenta en estudios previos en donde frecuentemente se recobran embebidas en *Echeveria* o viceversa (ver Mort et al., 2001; Acevedo-Rosas et al., 2004; Carrillo-Reyes et al., 2008; Carrillo-Reyes et al., 2009; Nikulin et al., 2016; Messerschmid et al., 2020).

Graptopetalum ha sido recobrado como no monofilético previamente a esta tesis (Acevedo-Rosas et al., 2004), independientemente de obtener el mismo resultado, los aportes del capítulo 2 con relación a la sistemática de *Graptopetalum* y *Echeveria* se refieren a que algunas especies de este último género parecen estar más relacionadas con el primero. Más específicamente, las dos especies de la serie *Occidentales*: *E. affinis* y *E. craigiana* y las dos especies de la serie *Valvatae*: *E. calycosa* y *E. valvata* de *Echeveria* con *Graptopetalum*.

Esta cercanía filogenética no había sido observada o asumida en trabajos taxonómicos o filogenéticos antecedentes a esta tesis, aún y cuando existen bases biogeográficas (en el caso de ser. *Occidentales*) y morfológicas para presumir su cercana relación. Entre las similitudes en rasgos morfológicos de *Occidentales* y *Graptopetalum* se pueden mencionar: similitudes en las rosetas —principalmente con el grupo acaulescente de *Graptopetalum*— *G. bartramii* Rose, *G. bellum* (Moran & J.Meyrán) D.R.Hunt, *G. filiferum* (S.Watson) J.Whitehead, *G. glasii* Acev.-Rosas & Cházaro, *G. macdougalli* Alexander, *G. marginatum* Kimnach & Moran, *G. pusillum* Rose, *G. rusbyi* Rose, *G. saxifragoides* Kimnach y *G. sinaloensis* Vega—, similitud en la forma de las hojas, el tipo de inflorescencia cimosa y sus pétalos delgados.

Aunque los pétalos de *E. affinis* y *E. craigiana* son erectos y forman un tubo, a diferencia de los pétalos de *Graptopetalum* en donde son extendidos, existen otras especies tales como *G. bellum*, *G. mendozae* Glass & Cházaro, *R. occidentalis* Kimnach, *S. suaveolens* Kimnach —recobrado filogenéticamente dentro de *Graptopetalum*— con grados de simpetalia y coloraciones de los pétalos distintos a la mayoría de las especies. Las características disruptivas de estas especies dentro del género son debidas posiblemente a cambios en el síndrome de polinización de estas especies como señalan Acevedo-Rosas y colaboradores (2004).

Reidmorania occidentalis, primero descrita como *Echeveria kimnachi* J.Meyrán & Vega y posteriormente renombrada al crearse un género monotípico nuevo para ella por M. Kimnach es recobrada como cercana a *G. pachyphyllum* Rose. La inclusión de *R. occidentalis* en una filogenia molecular es inédita hasta la realización de esta tesis. La posición filogenética, así como las similitudes de sus rosetas y sus afinidades biogeográficas con *Graptopetalum* justifican su inclusión en *Graptopetalum*, como en los casos similares de *Tacitus bellus* y *Sedum suaveolens* revelados por Acevedo-Rosas y colaboradores (2004).

Para el caso de las especies de la serie *Valvatae*, la posible relación de estas con *Graptopetalum* tampoco había sido inferida o contemplada a través de su historia taxonómica, lo anterior debido a que ninguna de las tres especies que componen a la serie *Valvatae* habían sido incluidas en filogenias moleculares de crasuláceas mexicanas con anterioridad, además de que la relación biogeográfica y la asociación de su morfología con *Graptopetalum* es más difícil de establecer —tal vez únicamente semejanzas en la roseta—. Aunque *Valvatae* es recobrada cercanamente con *G. saxifragoides* y *G. pusillum* con bajos valores de Bootstrap, en posteriores inferencias se debe considerar el incremento de caracteres moleculares para establecer con mayor claridad su posición filogenética.

El género *Cremnophila* es recobrado en el Clado IV como monofilético, aunque con bajos valores de Bootstrap. En este Clado IV también se incluye a *Sedum clavatum* R.T.Clausen, que ha sido recobrado anteriormente anidado en *Cremnophila* (Carrillo-Reyes et al., 2009), aquí se anida con *Graptopetalum macdougallii* en un subclado con bajo soporte, se necesita más información molecular para determinar si *S. clavatum* pertenece o no a *Cremnophila* como su morfología y su distribución geográfica lo sugieren.

Otra de las novedades que aporta el análisis filogenético de esta tesis se refiere a que las especies *E. humilis*, *E. trianthina* y *E. xichuensis* que han sido históricamente ubicadas en *Urbiniae* son recobradas en este Clado IV —como se discutió anteriormente en la sección referente al Clado I—. Son recobradas en un subclado y un grado hermanos de las series *Angulatae*, *Pruinosae* y *Secundae* y de la serie *Gibbiflorae* de *Echeveria*.

Siguiendo con los subclados más recientemente divergentes del Clado IV, uno de estos incluye a las series *Angulatae*, *Pruinosae* y *Secundae* recobrado con alto soporte de Bootstrap y moderado soporte de probabilidad posterior. Este clado es hermano de ser. *Gibbiflorae* y las tres series que lo componen muestran un estatus de parafiléticas o polifiléticas. Con base en los cladogramas resultantes y a la comparación y análisis de su morfología, estas series pueden fusionarse junto con las dos variedades de *E. semivestita* —ubicadas históricamente en *Gibbiflorae*— para crear un nuevo género independiente de *Echeveria*. Entre las características que pueden agrupar a las especies de *Angulatae*, *Pruinosae* y *Secundae*, se pueden mencionar el hábito acaulescente, inflorescencias en cincino —en ocasiones bifurcados—, sépalos linear-lanceolados, agudos a acuminados, pétalos aquillados dorsalmente, corolas pentagonales en vista frontal, y estilos y estigmas adpresos entre sí.

Echeveria semivestita var. *floresiana* y *E. semivestita* var. *semivestita*, no solo presentan una morfología contrastante con las series mencionadas anteriormente, sino con cualquiera de las 17 series en las que se divide al género. Entre sus rasgos morfológicos que contrastan con las otras especies de este subclado se pueden enlistar: a su hábito caulescente, tallos, hojas y brácteas hirsutas a pubescentes, inflorescencias paniculadas y sépalos azulosos. Debido a sus características disruptivas, E. Walther (1972) la ubicó en la ser. *Gibbiflorae* debido a sus inflorescencias paniculadas y desde entonces se mantiene en esta serie. Nuestro análisis filogenético las ubica más bien cercanas a *E. tamaulipana* y *E. runyonii* y deben transferirse a este grupo creándose un nuevo género.

Finalmente, el clado más recientemente divergente del análisis filogenético de *Echeveria* y géneros afines está constituido por especies de la ser. *Gibbiflorae* con algunas adiciones y exclusiones. Este subclado fue recuperado con alto valor de Bootstrap y bajos valores de probabilidad posterior. La enorme mayoría de sus constituyentes son especies que han sido atinadamente ubicadas aquí. Los taxones que se excluyen de *Gibbiflorae* a partir de los resultados de esta tesis son las dos variedades de *E. semivestita* —discutidas en párrafos anteriores—, mientras que las adiciones corresponden con *E. guerrerensis* y *E. uxorium* ubicadas en *Urbiniae* (Reyes et al., 2011) y *Racemosae* (Jimeno-Sevilla et al., 2012), respectivamente y recobradas ahora en *Gibbiflorae*. Como en la mayoría de las categorías infragenéricas de *Echeveria* que se discuten anteriormente, *Gibbiflorae* ha sido pobremente representada en análisis filogenéticos previos e independientes a esta tesis. En el capítulo 2 de esta tesis se incluyó una muestra muy representativa de *Gibbiflorae* y se recuperó con alto soporte de Bootstrap.

Por las razones antes expuestas, el set de datos moleculares empleado en esta tesis fue insuficiente para resolver las relaciones filogenéticas someras de *Echeveria* y géneros relacionados, por lo tanto, aún existe mucha incertidumbre sobre la composición de algunos grupos. Lo anterior señala la necesidad de generar nuevas secuencias de ADN de distintos loci para ser combinados con *matK* e ITS2 generados aquí para mejorar la resolución en los árboles filogenéticos para el "Grupo Echeveria" y resolver las relaciones filogenéticas que el set de datos no pudo resolver en este proyecto.

En síntesis, con base en los resultados presentados en esta tesis, se propone la modificación del esquema clasificatorio empleado en la actualidad para el género *Echeveria*, el cual contiene varias inconsistencias en relación con la interpretación que distintos taxónomos han realizado de su morfología y el marco filogenético aproximado aquí y en otros estudios independientes con distintos sets de datos moleculares.

Una de las opciones para resolver la circunscripción de *Echeveria* es incluir a 16 de las 17 series —con excepción de *Urbiniae* que puede ser reestablecido como género como Rose había propuesto con anterioridad (en Britton y Rose, 1903)— al esquema clasificatorio actual además de los géneros *Cremnophila*, *Graptopetalum*, *Reidmorania* y muchas de las especies de *Sedum*. La propuesta de Messerschmid y colaboradores (2020) va incluso más allá, integrando a todas las especies de los clados mayores *Leucosedum* y *Acre* de la tribu Sedeae es decir, 750 especies de 14 géneros: *Afrovivella*, *Cremnophila*, *Dudleya*, *Echeveria*, *Graptopetalum*, *Lenophyllum*, *Pachyphytum*, *Pistorinia*, *Prometheum*, *Rosularia*, *Sedella*, *Sedum* (una gran proporción), *Thompsonella* y *Villadia* en *Sedum*. La propuesta de estos autores si bien está basada en el reconocimiento del clado como monofilético, generaría la desaparición de los géneros antes mencionados y que la nueva definición de *Sedum* sea muy amplia.

En nuestra racionalización, tanto el clado *Acre* como *Leucosedum* pueden fraccionarse en varios géneros, al menos para el "Grupo Echeveria", una vez que se incremente el número de loci empleados para inferir las relaciones filogenéticas y el incremento en el muestreo de *Sedum* de México, los grupos podrían revelarse con mayor claridad y podrían definirse reinterpretando su morfología y su correlación con su biogeografía en un esquema taxonómico más fácil de implementar.

Revisión taxonómica de ser. Gibbiflorae *(*Echeveria*, Crassulaceae)*

Debido a la pobre representatividad de *Gibbiflorae* en análisis filogenéticos de Crassulaceae previos y por la probable subestimación de sus especies con base en la observación y comparación de especímenes en fresco custodiados en la "Colección Nacional de Crasuláceas" del Jardín Botánico de la UNAM realizadas por parte del autor de esta tesis, se decidió revisar a *Gibbiflorae* desde la perspectiva de la taxonomía integrativa *sensu* DeSalle y colaboradores (2005), DeSalle (2006), Bergman y colaboradores (2009), Goldstein y DeSalle (2011).

En este sentido, en el Capítulo 3 de la tesis se presentan los resultados de una primera y novedosa revisión de *Gibbiflorae* en el sentido que en la historia taxonómica tanto de *Gibbiflorae* como de *Echeveria* nunca se habían incorporado caracteres moleculares a la sistemática/taxonomía de estos grupos. El muestreo de taxones consistió en 1–7 individuos de las 36 especies reconocidas, no se incluyeron a *E. gudeliana*, *E. scheerii* y *E. steyermarkii* por las razones expuestas en el Capítulo 3.

Al set de datos molecular se incorporaron 10 caracteres morfológicos —principalmente florales— considerados como potencialmente informativos con base en observaciones previas realizadas por el autor de la tesis. El árbol de consenso de mayoría basado en la matriz combinada (ADN y morfología) recobra a *Gibbiflorae* como grupo monofilético con alto soporte de probabilidad posterior. Al interior, las relaciones filogenéticas de esta serie de *Echeveria* muestran muchas politomías causadas por la baja resolución del set de datos.

Entre los resultados más destacables se menciona la identificación de un clado más recientemente divergente con moderado a alto soporte de probabilidad posterior, en el cual son recobradas especies de distribución geográfica predominantemente noroccidental —tomando como referencia el Eje Neovolcánico Transversal hacia el norte de México— en tanto que, el resto de las especies se distribuye al sur de México.

El resultado anterior es relevante para la sistemática y taxonomía de *Gibbiflorae* ya que E. Walther (1972) estableció a la serie *Retusae* segregándola de la primera por considerar que presentan características morfológicas disruptivas, sin embargo, como se ha reiterado en diferentes secciones de esta tesis los rasgos definidos por Walther para separar a los grupos son homoplásicos y de poca utilidad taxonómica. García y Pérez-Calix (2007) consideran que la serie puede dividirse en dos grupos, el primero conformado por plantas con tallo corto, hojas sésiles con ápice agudo —*E. cante*, *E. dactylifera* y *E. subrigida*—. El segundo grupo estaría formado por plantas caulescentes, hojas pseudopecioladas y flores con nectarios pálidos —*E. acutifolia*, *E. crenulata*, *E. fimbriata*, *E. gibbiflora*, *E. violescens*, *E. gigantea*, *E. grisea*, *E. longiflora*, *E. pallida*, *E. rubromarginata* y *E. nayaritensis*—. La división de *Gibbiflorae* por parte de García y Pérez-Calix (2007) está en correspondencia con los resultados de esta tesis —con excepción de *E. nayaritensis*—. Sin embargo, al considerarse todas las especies reconocidas hasta la fecha, los caracteres morfológicos establecidos por García y Pérez-Calix (2007) fallan para definir a los grupos.

Más bien *Gibbiflorae* parece dividirse en dos grupos principales que pueden definirse primordialmente con base a su distribución geográfica y por algunas generalidades de su morfología floral como por ejemplo, la predominancia de colores amarillos o rojos en los nectarios ubicados en la base de los carpelos u otros, como se discute con mayor profundidad en el Capítulo 3 de esta tesis.

En adición a lo anterior, *Gibbiflorae* puede ser segregado de *Echeveria* con base en los resultados filogenéticos de esta tesis para conformar un nuevo género ya que posee apomorfías moleculares y rasgos morfológicos para definirlo tales como: plantas de talla mediana a grande, inflorescencias predominantemente paniculadas, flores de 0.9–2.5 cm de longitud, flores tubulares a urceoladas, estilos y estigmas libres, estilos rojizos a púrpuras y nectarios relativamente grandes.

Durante el análisis integrativo de *Gibbiflorae* se identificaron 11 potenciales nuevas especies dos de las cuales no fueron apoyadas por atributos característicos por lo que corresponden al escenario A del círculo taxonómico de DeSalle (2005), en donde tanto la distribución geográfica como la morfología apoyan el planteamiento de la hipótesis del descubrimiento de una nueva especie. El escenario A del círculo taxonómico mencionado anteriormente también puede ser referido como taxonomía tradicional y ha sido el escenario mayoritariamente implementado en la historia taxonómica de *Echeveria* y otros grupos de crasuláceas mexicanas. El descubrimiento de nuevas especies por medio de la identificación de diferencias morfológicas y geográficas (o biogeográficas) es una práctica válida y muy frecuente en taxonomía.

En este sentido, en el Capítulo 4 de esta tesis se describen como nuevas especies de *Gibbiflorae* a *Echeveria michihuacana* L.E.Cruz-López, J.Reyes & Verg.-Silva y *E. xochipalensis*

J.Reyes, L.E.Cruz-López & Verg.-Silva con base en la identificación de diferencias morfológicas respecto de las especies conocidas del grupo. En el caso de *E. michihuacana*, como se aprecia en el árbol de consenso de mayoría de inferencia bayesiana en el Capítulo 3 de la tesis, está relacionada con *E. pistioides* como su especie hermana sin embargo, puede ser diferenciada de ésta última por presentar un tallo conspicuo (vs acaulescente), por sus hojas no pruinosas (vs pruinosas), verdes (vs glaucas), hojas frecuentemente onduladas a crenuladas vs enteras, por sus ovarios blancos vs ovarios amarillentos, por sus pétalos agudos vs obtuso, entre otros (de la Cruz-López et al., 2019).

La descripción de *E. xochipalensis* como nueva especie de *Gibbiflorae*, también está basada en diferencias morfológicas con respecto a especímenes del complejo de *E. fulgens*. Difiere de los anteriores por presentar un tallo conspicuo, hojas marcadamente pseudopecioladas, más angostas, rígidas, con marcado polimorfismo en el color de las hojas de verde claro, rojizo a marrón-púrpura (Reyes et al., 2019). En el Capítulo 3 de la tesis no es posible identificar a la especie hermana de *E. xochipalensis*, aunque la morfología sugiere similitudes con el complejo de *E. fulgens*. De manera similar a *E. michihuacana*, el set de datos molecular fue insuficiente para revelar atributos característicos para este taxón en particular y en general para casi la mitad de las especies de *Gibbiflorae*.

Las restantes nueve especies potencialmente nuevas si presentaron diferencias en sitios homólogos en la matriz de ADN combinada. Una de estas especies que el Capítulo 3 de la tesis fue etiquetada como *E*. sp EK-3569, fue propuesta como nueva especie en el Capítulo 4 de la tesis. *Echeveria kristenii* L.E.Cruz-López & J.Reyes fue descrita como una especie distinta de *E. dactylifera* y *E. novogaliciana* a partir de la identificación de diferencias morfológicas, geográficas y la identificación de diferencias en sitios homólogos (CA´s) en las secuencias generadas de los tres marcadores moleculares elegidos (de la Cruz-López et al., 2021). En el árbol inferido para *Gibbiflorae* (Capítulo 3), *E. kristenii* es la especie hermana de *E. dactylifera* sin embargo, también presenta similitudes morfológicas con *E. novogaliciana* por lo que se compara también con esta última.

Aunque queda pendiente la descripción de las restantes ocho potenciales nuevas especies identificadas en la revisión de *Gibbiflorae*, así como la resolución del complejo de *E. fulgens*, la descripción de *E. kristenii* constituye un parteaguas en la historia taxonómica de *Echeveria* puesto que en conjunción con *E. islasiae* (Reyes y de la Cruz-López, 2021) constituyen las únicas especies de *Echeveria* en describirse acompañadas de información molecular en la historia taxonómica de más de 229 años del género.

El aprovechamiento de Echeveria en el contexto del protocolo de Nagoya

Un último aspecto adicional que debe discutirse en esta tesis se relaciona con el Protocolo de Nagoya adoptado por los países adscritos al Convenio Sobre la Diversidad Biológica de Las Naciones Unidas (Convenio Sobre la Diversidad Biológica, 2011). Este protocolo fue acordado durante la décima reunión de la Conferencia de las Partes, celebrada en la ciudad de Nagoya en Japón el día 29 de octubre de 2010.

Al tratarse de plantas reconocidas como recurso biológico/genético con uso ornamental (Zúñiga y Carrodeguas, 2021), la utilización de las especies de *Echeveria* y géneros relacionados debe adherirse a los lineamientos establecidos por el protocolo referido entre los que se encuentran, que cada una de las partes adopte las medidas necesarias —legislativas, administrativas o políticas— para garantizar la repartición equitativa de los beneficios (Convenio Sobre la Diversidad Biológica, 2011).

Plantas, animales, microorganismos, genes, enzimas u otras moléculas orgánicas derivadas, entre otros, que son empleados en muy diversos ámbitos socioeconómicos, frecuentemente son monopolizados sin compartir beneficio alguno hacia los países y localidades que han conservado el recurso (Greiber et al., 2013).

Aunque el tratado entró en vigor para México el 12 de octubre de 2014, se evidenció la ausencia de legislaciones secundarias mexicanas que acompañen los objetivos de este acuerdo internacional (Hernández, 2019). La problemática anterior cobra mayor relevancia debido a que México cuenta con un mosaico cultural muy amplio que abarca localidades de pueblos originarios con conocimientos tradicionales, aprovechamiento sustentable y conservación de sus recursos genéticos. Sin embargo, muy frecuentemente estas comunidades son las últimas en obtener beneficios al haber conservado el recurso.

En relación con los resultados derivados de esta tesis, en donde se generó información de fragmentos de regiones genómicas con la finalidad de emplearse como un medio de identificación de las especies y como descriptivos de los mismos —al menos una fracción muy pequeña de su genoma— el Protocolo de Nagoya y la legislación necesaria cobran gran relevancia ya que podrían contener la biopiratería que puede derivarse de la información generada en esta tesis. Las secuencias de ADN generadas aquí fueron almacenadas en bases de datos especializadas, públicas y de acceso global como BOLD systems (Ratnasingham y Hebert, 2007) y GenBank (Sayers et al., 2022). La publicación de la información genética es un requisito en trabajos científicos, sin embargo, esto incrementa el riesgo de patentes y apropiación de los recursos genéticos por parte de empresas, países, personas físicas o morales o de cualquier índole sin el consentimiento de los propietarios del recurso, que en nuestra racionalización corresponde a aquellos que lo han custodiado, conservado y utilizado de manera sustentable hasta nuestros días.

En este sentido, el establecimiento de Unidades de Manejo Ambiental (UMAs) consideradas en la Ley General de Vida Silvestre promovida por la Secretaría de Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP, 2000) en las comunidades y localidades dónde se ubican los recursos genéticos —en este caso, especies de *Echeveria* u otras crasuláceas— pueden coadyubar a distribuir de manera más equitativa los beneficios del aprovechamiento sostenible de los mismos.

Conclusiones

- — Los resultados presentados en esta tesis señalan la necesidad de modificar la actual clasificación de *Echeveria* establecida primordialmente por Moran (1974) y Kimnach (2003).
- — El género *Pachyphytum* es monofilético, debe excluirse a *P. cuicatecanum* y transferirlo de nuevo a *Echeveria*.
- — La serie *Urbiniae* de *Echeveria* debe reestablecerse como género independiente (*Urbinia*) como Rose (en Britton y Rose, 1903) había propuesto.
- — El género *Echeveria* debe ser reducido a únicamente 111 taxones pertenecientes a 9 de las 17 series reconocidas: *Ciliatae*, *Echeveria*, *Longistylae*, *Mucronatae*, *Nudae*, *Racemosae*, *Spicatae* y *Thyrsiflorae.*
- — *Chloranthae* debe ser incorporada a la serie *Mucronatae*.
- — *Echeveria purpusorum* debe transferirse de ser. *Urbiniae* a ser. *Longistylae*.
- — Con base en los resultados de esta tesis, *Thompsonella* debe incluirse en *Echeveria*.
- — Las series *Angulatae*, *Pruinosae* y *Secundae* y las dos variedades de *E. semivestita*

deben fusionarse y segregarse de *Echeveria* para formar un nuevo género independiente.

- Se sugiere la incorporación de las series *Occidentales* y *Valvatae* de *Echeveria* al género *Graptopetalum*.
- *Echeveria amoena* y *E. prolifica* deben transferirse a *Sedum*.
- Se propone la creación de un nuevo género para la ser. *Gibbiflorae* independiente de *Echeveria*.
- El set de datos empleado únicamente puede identificar al 51% de las especies de *Echeveria*.
- La combinación de los marcadores *rbcL*, *matK* e ITS2 únicamente pueden identificar a 17 de las 36 especies reconocidas (47.22%) para *Gibbiflorae* con al menos un atributo característico.
- Al interior de *Gibbiflorae*, se identificaron 11 potenciales nuevas especies con base en su morfología, 9 de las cuales son apoyadas por atributos característicos moleculares. Lo que incrementaría el porcentaje de identificaciones a (55%).
- Para lograr la meta de una librería de referencia de códigos de barras con el 100% de identificación de especies de Echeveria, se deben secuenciar al menos dos regiones genómicas adicionales y ser descartado el empleo de *rbcL* en esta iniciativa.
- México requiere de una legislación especializada para proteger sus recursos genéticos y ofrecer beneficios a las comunidades propietarias de los mismos.

Referencias bibliográficas

Acevedo-Rosas, R., Camero, K., Sosa, V. & Pell, S. (2004) A molecular phylogenetic study of *Graptopetalum* (Crassulaceae) based on ETS, ITS, *rpl16* and *trn*L-F nucleotide sequences. *American Journal of Botany* 91: 1099–1104. DOI: 10.3732/ajb.91.7.1099.

Aitken, N., Smith, S., Schwarz, C. y Morin, P. A. (2004). Single nucleotide polymorphism (SNP) discovery in mammals: a targeted-gene approach. Molecular Ecology 13: 1423–1431.

Al-Juhani, W. S. (2019). Evaluation of the capacity of the DNA barcode ITS2 for identifying and discriminating dryland plants. Genetics and Molecular Research 18: 1–18.

Berger, A. (1930) Crassulaceae. *En*: Engler, A. y Prantl, K. (Eds.) *Die natürlichen Pflanzenfamilien*, ed. 2, 18a. W. Engelmann, Leipzig, pp. 352–483.

Bergmann, T., Hadrys, H., Breves, G. y Schierwater, B. (2009) Character-based DNA barcoding: a superior tool for species classification. *Berliner und Münchener Tierärztliche Wochenschrift* 122: 446–450.

Britton, N.L. y Rose, J.N. (1903). New or noteworthy North American Crassulaceae. Bulletin of the New York Botanical Garden 3: 1–45.

Brower, A. V. Z. y Schuh, R. T. (2021). *Biological systematics: Principles and applications*, 3rd *. Edition*. Ithaca: Cornell University Press.

Candolle, A. P. (1828) Crassulaceae. *En*: A.P. de Candolle, A.P. y de Candolle A.L.P.P. (Eds.), *Prodromus Systematis Naturalis Regni Vegetabilis* 3*.* Treuttel & Würtz, París, pp. 382–414.

Carrillo-Reyes, P., Sosa, V. & Mort, M.E. (2008) *Thompsonella* and the "Echeveria group" (Crassulaceae): phylogenetic relationships based on molecular and morphological characters. *Taxon* 57: 863–874.

DOI: 10.1002/tax.573015.

Carrillo-Reyes, P., Sosa, V. & Mort, M.E. (2009) Molecular phylogeny of the *Acre* clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*. *Molecular Phylogenetics and Evolution* 53: 267–276.

DOI: 10.1016/j.ympev.2009.05.022.

Cavanilles, A. J. (1793) Cotyledon coccinea. *Icones et Descriptiones Plantarum* 2: 54–55.

CBOL Plant Working Group. (2009) A DNA Barcode for Land Plants. *Proceedings of the National Academy of Sciences of the United States of America* 106: 12794-12797. DOI: http://dx.doi.org/10.1073/pnas.0905845106

Chakraborty, M., Dhar, B. y Ghosh, S. K. (2017) Design of character-based DNA barcode motif for species identification: A computational approach and its validation in fishes. *Molecular Ecology Resources* 17: 1359–1370. DOI: https://doi.org/10.1111/1755-0998.12671

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y. y Leon, C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PLoS ONE* 5: 1–8.

Convenio Sobre la Diversidad Biológica. (2011). Protocolo de Nagoya sobre el acceso a los recursos genéticos y participación justa y equitativa en los beneficios que se deriven de su utilización al convenio sobre la diversidad biológica. Secretaría del Convenio sobre la Diversidad Biológica, Naciones Unidas, Montreal, Quebec, Canadá.

Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85: 407–415.

De la Cruz-López, L. E., Reyes S, J. y Vergara-Silva, F. (2019). *Echeveria michihuacana* (Crassulaceae), a new species from Michoacán, Mexico. *Brittonia* 71: 25–31.

De la Cruz-López, L. E., Reyes S, J. y Vergara-Silva, F. (2021). A new species of Echeveria (Crassulaceae) from Durango, Mexico, supported by morphology and DNA diagnostic characters. *Brittonia* 73: 1–9.

DeSalle, R. Egan, M. G. y Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B* 360: 1905–1916.

DeSalle, R. (2006) Phenetic and DNA taxonomy; a comment on Waugh. *BioEssays* 29: 1289–1290.

DeSalle, R. y Goldstein, P. (2019) Review and Interpretation of Trends in DNA Barcoding. *Frontiers in Ecology and Evolution 7*: 1–11.

Eggli, U. (2003). Illustrated handbook of succulent plants: Crassulaceae. *Springer-Verlag.*

Etter J y Kristen M. (2022). Crassulaceae database. www.crassulaceae.com.

Fishbein, M y Soltis, D. E. (2004) Further resolution of the rapid radiation of Saxifragales (Angiosperms, Edicots) supported by mixed-model Bayesian Analysis. *Systematic Botany* 29: 883–891.

García A, M. A., Terrazas, T., Segura L, O., Arias, S., Vibrans, H. y López-Mata, L. (2013). Caracterización molecular de tres especies de *Hylocereus* (Cactaceae) presentes en México. *Revista Fitotecnia Mexicana* 36: 13–22.

García R, I. (2003). Relaciones interespecíficas del género *Pachyphytum* (CRASSULACEAE), empleando marcadores genéticos AFLP. Tesis de Maestría. Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima.

García R, I. y Pérez-Calix, E. (2007). Una especie nueva de *Echeveria* (Crassulaceae) originaria del estado de Jalisco, México. Acta Botánica Mexicana 78: 125–132.

Gernandt, D., Salazar, G., Vergara, F., Arias, S., Sosa, V., García, A., Reyes, J., Cabrera, R. y Rosas, P. (2011). Código de barras genético de cinco grupos críticos de la flora de México. Instituto de Ecología. Instituto de Biología. Universidad Nacional Autónoma de México. Bases de datos SNIB-CONABIO, proyecto GE021. México, D. F.

Goldstein, P. Z. y DeSalle, R. (2011). Integrating DNA barcode data and taxonomic practice: determination, discovery, and description. *Bioessays* 33: 135–147.

Gontcharova, S. B y Gontcharov, A. A. (2009) Molecular phylogeny and systematics of flowering plants of the family Crassulaceae DC. *Molecular Biology* 43: 794–803.

Greiber, T., Peña M. S., Åhrén, M., Nieto C. J., Chege K. E., Cabrera M. J., Oliva, M. J., Perron-Welch, F., Ali, N. y Williams, C. (2013). Guía Explicativa del Protocolo de Nagoya sobre Acceso y Participación en los Beneficios. UICN, Gland, Suiza. 402 pp.

Guerra G, A. (2011). Evaluación del éxito clonal en una especie invasora: *Kalanchoe delagoensis*. Tesis de licenciatura en Biología. Facultad de Ciencias. Universidad Nacional Autónoma de México.

Han, J., Zhu, Y., Chen, X., Liao, B., Yao, H., Song, J., Chen, S., y Meng, F. (2013). The Short ITS2 Sequence Serves as an Efficient Taxonomic Sequence Tag in Comparison with the Full-Length ITS. *BioMed Research International* 2013: 1–7.

Hebert, P., Cywinska, A., Ball, S. L., y Dewaard, J. (2003) Biological identification through DNA barcodes. *Proceedings of the Royal Society of London B* 270: 313–321.

Hernández O, S. R. (2019). El protocolo de Nagoya en México: un análisis legal del cumplimiento y el papel de los protocolos comunitarios bioculturales. *Revista de la Facultad de Derecho de México* 69: 611–644.

Hubert, N. y Hanner, R. (2015). DNA Barcoding, species delineation and taxonomy: a historical perspective. *DNA Barcodes* 3: 44–58.

Jimeno-Sevilla, H. D., Carrillo-Reyes, P., Pérez-Calix, E. y Cházaro-Basáñez, M. J. (2012). Additions to the Crassulaceae of the State of Veracruz, Mexico. *Haseltonia* 18: 140–152.

Kimnach, M. (2003). Echeveria. *En*: Eggli, U. (Ed.) *Illustrated handbook of succulent plants*: *Crassulaceae*. Springer–Verlag, Berlin, pp. 103–128.

Kimnach, M. (2010) The generic placement of *Echeveria cuicatecana*. *Cactus and Succulent Journal of America* 82: 125.

Kitching, I. J., Forey, P. L., Humphries, C. J. y Williams, D. M. (1998). Cladistics: The Theory and Practice of Parsimony Analysis. 2nd edition. Oxford University Press. Londres.

Lanteri, A. A. (2007). Código de barras del ADN y sus posibles aplicaciones en el campo de la Entomología. *Revista de la Sociedad Entomológica Argentina* 66: 15–25.

Lindley, J. (1845). *Echeveria scheerii*. Edwards´s Botanical Register 31: 27.

Martínez-Domínguez, L., Nicolalde-Morejón, F., Vergara-Silva, F. y Stevenson, D. W. (2016). Integrative taxonomy of Mexican cycads: biogeography, morphology and DNA barcoding corroborate a new sympatric species in Ceratozamia (Zamiaceae). *Phytotaxa* 268: 25–45. DOI: http://dx.doi.org/10.11646/phytotaxa.268.1.2

Messerschmid T, F. E., Klein, J. T., Kadereit, G., Kadereit, J. W. (2020). Linnaeus's folly – phylogeny, evolution and classification of Sedum (Crassulaceae) and Crassulaceae subfamily Sempervivoideae. *Taxon* 69: 1–35.

Meyrán, J. y López C, L. (2003). *Las Crasuláceas de México*. Sociedad Mexicana de Cactología AC, Ciudad de México, 234 pp.

Mishra, P., Kumar, A., Sivaraman, G., Ashutosh, K. S., Kaliamoorthy, R., Slater, A. y Velusamy, S. (2017). Character-based DNA barcoding for authentication and conservation of IUCN Red listed threatened species of genus *Decalepis* (Apocynaceae). *Scientific Reports* 7**:** 1–12. DOI: https://doi.org/10.1038/s41598-017-14887-8

Moran R. (1960). *Echeveria heterosepala* Rose. *Cactáceas y Suculentas Mexicanas* 5: 75-80.

Moran, R. (1968). New subgeneric groups in *Echeveria* and *Pachyphytum*. *Cactus and Succulent Journal of America* 40: 36–42.

Moran, R. (1974). Echeveria. *In*: Jacobsen, H. (Ed.) *Lexicon of succulent plants*. Bland-Ford Press, London, pp. 184–186.

Moran R. (1999). The name of Echeveria. *Cactus & Succulent Journal of America* 71: 301-306.

Morrone, J. (2016). El lenguaje de la cladística. Facultad de Ciencias, Universidad Nacional Autónoma de México. pp. 100.

Mort, M. E., Soltis, D. E., Soltis, P. S., Francisco-Ortega, J. y Santos-Guerra, A. (2001). Phylogenetic Relationships and Evolution of Crassulaceae Inferred from *MATK* Sequence Data. *American Journal of Botany* 88: 76–91. DOI: 10.2307/2657129.

Nicolalde-Morejón, F., Vergara-Silva, F., González-Astorga, J. y Stevenson, D. W. (2010). Character-based, population-level DNA Barcoding in Mexican species of *Zamia* L. (Zamiaceae: Cycadales). *Mitochondrial DNA* 21: 51-59.

Nikulin, V. Y., Gontcharova, S. B., Stephenson, R. y Gontcharov A. A. (2016). Phylogenetic relationships between *Sedum* L. and related genera (Crassulaceae) based on ITS rDNA sequence comparisons. *Flora* 224: 218–229. DOI: 10.1016/j.flora.2016.08.003.

Packer, L., Gibbs, J., Sheffield, C. y Hanner, R. (2009). DNA barcoding and the mediocrity of morphology. *Molecular Ecology Resources* 9: 42–50.

Padial, J. M., Miralles, A., De la Riva, I. &Vences, M. (2010). The integrative future of taxonomy. *Frontiers in Zoology* 7:16.

Pilbeam, J. (2008). The genus Echeveria. The British Cactus & Succulent Society.

POWO (2022). "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 30 September 2022.

Rach, J., DeSalle, R., Sarkar, I. N., Schierwater, B. y Hadrys, H. (2008). Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. *Proceedings of the Royal Society B* 275: 237–247.

Ratnasingham, S. y Hebert, P.D.N. (2007). BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes* 7, 355–364. DOI: 10.1111/j.1471-8286.2006.01678.x

Reyes S, J., Brachet I, C. y González Z, O. (2011). *Echeveria guerrerensis* (Crassulaceae) una nueva especie para el estado de Guerrero, México. *Cactáceas y Suculentas Mexicanas* 56: 75–81.

Reyes S, J., de la Cruz-López, L. E. y Vergara-Silva, F. (2019). *Echeveria xochipalensis* (Crassulaceae), a new species from Guerrero, Mexico. *Phytotaxa* 399: 14–24.

Reyes S, J., de la Cruz-López, L. E., Kuzmina, M. y Vergara-Silva, F. (2020). Morphological and molecular diagnostic characters reveal a new species of *Pachyphytum* (Crassulaceae). *Haseltonia* 26: 14–22.

Reyes S, J., Etter, J., Kristen, M. y de la Cruz-López, L. E. (2020). *Pachyphytum huastecanum* (Crassulaceae), a new species from the Huasteca Canyon, Nuevo León, Mexico. *Cactus and Succulent Journal* 92: 141–149.

Reyes S, J. y de la Cruz-López, L. E. (2021). *Echeveria islasiae*, una nueva especie de la serie *Longistylae* (Crassulaceae). *Brittonia* 73: 363–373.

Roy, S., Tyagi, A., Shukla, V., Kumar, A., Singh, U. M., Chaudhary, L. B., Datt, B., Bag, S. K., Singh, P. K., Nair, N. K., Husain, T. y Tuli, R. (2010). Universal plant DNA barcode loci may not work in complex groups: a case study with Indian Berberis species. *PLoS ONE 5*: 1–14.

Sarkar, I. N., Planet, P. J. y DeSalle, R. (2008). CAOS software for use in character-based DNA barcoding. *Molecular Ecology Resources 8*: 1256–1259. DOI: https://doi.org/10.1111/j.1755-0998.2008.02235.x

Sayers E. W., Bolton E. E., Brister J. R., Canese K., Chan J., Comeau D. C., Connor R., Funk K., Kelly C., Kim S., Madej T., Marchler-Bauer A., Lanczycki C., Lathrop S., Lu Z., Thibaud-Nissen F., Murphy T., Phan L., Skripchenko Y., Tse T., Wang J., Williams R., Trawick B. W., Pruitt K. D., Sherry S. T. (2022). Database resources of the national center for biotechnology information. *Nucleic Acids Research* 50: D20-D26.

Schuh, R. T. (2000). Biological Systematics: Principles and Appplications. Cornell University Press. New York.

SEMARNAP. Secretaría de Medio Ambiente, Recursos Naturales y Pesca. (2000). Ley General de Vida Silvestre. Diario Oficial de la Federación.

Sonnante, G., Galasso, I. y Pignone, D. (2003). ITS Sequence Analysis and Phylogenetic Inference in the Genus *Lens* Mill. *Annals of Botany* 91: 49–54.

Talavera, G., Lukhtanov, V., Pierce, N. E. y Vila, R. (2022). DNA barcodes combined with multilocus data of representative taxa can generate reliable higher-level phylogenies. Systematic Biology 71: 382–395.

Tautz, D., Arctander, P., Minelli, A., Thomas, R. H. y Vogler, A. P. (2003). A plea for DNA taxonomy. *TRENDS in Ecology and Evolution* 18: 70–74.

Uhl C. H. (1963). Chromosomes and phylogeny of the Crassulaceae. *Cactus and Succulent Journal 35*: 80–84.

Uhl. C. H. (2002). Chromosomes and hybrids of *Echeveria* (Crassulaceae). VII. Series *Gibbiflorae* (Baker) Berger. *Hasseltonia* 9: 121–145.

Van Ham, R. C. H. J. y T´Hart, H. (1998). Phylogenetic Relationships in the Crassulaceae Inferred from Chloroplast DNA Restriction-Site Variation. *American Journal of Botany* 85. 123–134. DOI: 10.2307/2446561.

Van Jaarsveld, E. (2003). *Cotyledon*. *En*: Eggli, U. (Ed.) *Illustrated handbook of succulent plants*: *Crassulaceae*. Springer–Verlag, Berlin, pp. 103–128.

Vázquez-Cotero, C., Sosa, V. y Carrillo-Reyes, P. (2017). Phylogenetic position of *Echeveria heterosepala* (Crassulaceae): a rare species with diagnostic characters of *Pachyphytum*. *Botanical Sciences* 95: 515–526. DOI: http://dx.doi.org/10.17129/botsci.1190.

Walther, E. (1931). Genus *Pachyphytum*. *Cactus and Succulent Journal* 3: 9–13.

Walther, E. (1935). Notes on the genus *Echeveria*. *Cactus and Succulent Journal* 7: 69–72.

Walther, E. (1972). *Echeveria*. California Academy of Sciences, San Francisco, 426 pp.

Weitschek, E., Van Velzen, R., Felici, G. y Bertolazzi, P. (2013). BLOG 2.0: a software system for character-based species classification with DNA Barcode sequences. What it does, how to use it. *Molecular Ecology Resources* 13: 1–4.

Will, K. W., Mishler, B. D. y Wheeler, Q. D. (2005). The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology* 54: 844–851.

Williams, D., Schmitt, M., Wheeler, Q. (2016). The Future of Phylogenetic Systematics: The legacy of Willi Hennig. The Systematics Association, Cambridge University Press, United Kingdom.

Winter K. y Smith, J. A. C. (1996). An introduction to Crassulacean Acid Metabolism. Biochemical Principles and Ecological Diversity. *Ecological Studies* 114: 1–13.

Yao, H., Song, J., Liu, C., Luo, K., Han, J., Li, Y., Pang, X., Xu, H., Zhu, Y., Xiao, P. y Chen, S. (2010). Use of ITS2 region as the universal DNA barcode for plants and animals. *PLoS ONE 5*: 1–9.

Yassin, A., Markow, T. A., Narechania, A., O´Grady, P. M. y DeSalle, R. (2010). The genus Drosophila as a model for testing tree- and character-based methods of species identification using DNA barcoding. *Molecular Phylogenetics and Evolution* 57: 509-517. DOI: https://doi.org/10.1016/j.ympev.2010.08.020.

Zhang, J. Q., Meng, S. Y., Wen, J. y Rao, G. Y. (2015). DNA Barcoding of *Rhodiola* (Crassulaceae): A case study on a group of recently diversified medicinal plants from the Qinghai-Tibetan plateau. *PLoS ONE* 10: 1–15.

Zimmerman, S. J., Aldridge, C. L. y Oyler-McCance, S. J. (2020). An empirical comparison of population genetic analyses using microsatellite and SNP data for a species of conservation concern. *BMC Genomics* 21: 1–16.

Zou, S., Li, Q., Kong, L., Yu, H. y Zheng, X. (2011) Comparing the Usefulness of Distance, Monophyly and Character-Based DNA Barcoding Methods in Species Identification: A Case Study of Neogastropoda. *PLoS ONE* 6: e26619. DOI: https://doi.org/10.1371/journal.pone.0026619

Zou, S, Fei, C., Song, J., Bao, Y., He, M., y Wang, C. (2016a). Combining and Comparing Coalescent, Distance and Character-Based Approaches for Barcoding Microalgaes: A Test with Chlorella-Like Species (Chlorophyta). *PLoS ONE 11*: 1–12.

Zou, S., Fei, C., Wang, C., Gao, Z., Bao, Y., He, M. y Wang, C. (2016b). How DNA barcoding can be more effective in microalgae identification: a case of cryptic diversity revelation in *Scenedesmus* (Chlorophyceae). *Scientific Reports* 6: 1–12.

Zúñiga O, A. y Carrodeguas G, A. (2021). *Echeveria* (Crassulaceae): Potencial para la mejora genética como ornamental. *Avances en Investigación Agropecuaria* 25: 57–81.