

# Phylogenetic position of *Echeveria heterosepala* (Crassulaceae): a rare species with diagnostic characters of *Pachyphytum*



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## Abstract

**Background:** *Echeveria* and *Pachyphytum* are two closely related Neotropical genera in the Crassulaceae. Several species in *Echeveria* possess characters cited as diagnostic for *Pachyphytum* such as a clearly defined stem, a nectary scale on the inner face of petals and as inflorescence a scorpioid cyme or cincinnus. *Pachyphytum* has been identified as monophyletic while *Echeveria* as polyphyletic in previous molecular phylogenetic analyses.

**Hypothesis:** The objective of this paper is to identify the phylogenetic position of a rare species with restricted distribution in *Echeveria*, *E. heterosepala* that possesses the diagnostic characters of *Pachyphytum* to better understand the generic limits between these two genera. We expect this species to be closely related to *Pachyphytum*.

**Methods:** Bayesian inference and Maximum Likelihood analyses were carried out using 47 taxa, including as ingroup, species of *Echeveria*, *Graptopetalum*, *Lenophyllum*, *Pachyphytum*, *Sedum*, *Thompsonella* and *Villadia* and as outgroup, species in *Dudleya*. Analyses were conducted based on plastid (*rpl16*, *trnL-F*) and nuclear (ETS, ITS) markers. Ancestral character reconstruction was carried out under a parsimony criterion based on the molecular trees retrieved by the phylogenetic analyses. Four morphological characters were considered: defined stem, type of inflorescence, nectary scale in petals and position of sepals.

**Results:** Accessions of *E. heterosepala* were retrieved in a well-supported clade group comprising exclusively *Echeveria* species. Therefore this species belongs to *Echeveria* although possessing characters similar to *Pachyphytum* and moreover it was not identified closely related to this genus. None of the traits considered have taxonomic importance. The node at the *Pachyphytum* clade identified unambiguous character states such as stem present, straight sepals, nectary scale on petals, however these character states were identified in the rest of the clades as well. Remarkably, the monophyly of *Pachyphytum* was corroborated, while *Echeveria* remains poorly understood.

**Keywords:** Acre clade, *Graptopetalum*, *Thompsonella*

## Resumen

**Antecedentes:** *Echeveria* y *Pachyphytum* son dos géneros Neotropicales cercanamente relacionados en Crassulaceae. Varias especies de *Echeveria* poseen caracteres citados como diagnósticos para *Pachyphytum*, tales como un tallo claramente definido, una escama nectarífera en la cara interna de los pétalos y una inflorescencia cimosa escorpioide, es decir un cincino. Filogenias moleculares previas han identificado a *Pachyphytum* como un grupo monofilético mientras que a *Echeveria* como polifilético.

**Hipótesis:** El objetivo de este trabajo es el de identificar la posición filogenética de una especie rara de distribución restringida en *Echeveria*, *E. heterosepala*, la cual posee los caracteres diagnósticos de *Pachyphytum* para entender mejor los límites de estos dos géneros. Nuestra hipótesis es que debería resultar cercanamente relacionada a *Pachyphytum*.

**Métodos:** Se llevaron a cabo análisis de inferencia Bayesiana y de Máxima Verosimilitud tomando en cuenta 47 taxa, incluyendo como grupo interno a especies de *Echeveria*, *Graptopetalum*, *Lenophyllum*, *Pachyphytum*, *Sedum*, *Thompsonella* y *Villadia* y como grupo externo a especies de *Dudleya*. Los análisis consideraron secuencias de ADN de cloroplasto (*rpl16*, *trnL-F*) y nuclear (ETS, ITS). También se llevó a cabo una reconstrucción de caracteres morfológicos ancestrales bajo el criterio de parsimonia tomando en cuenta los cladogramas de los análisis filogenéticos. Se codificaron cuatro caracteres morfológicos: tallo definido, tipo de inflorescencia, escama nectarífera en la cara interna de los pétalos y posición de los sépalos.

**Resultados:** Las muestras de *E. heterosepala* se identificaron en un clado bien soportado que comprendía únicamente especies de *Echeveria*, por lo que esta especie debe formar parte de este género, aunque posea caracteres diagnósticos de *Pachyphytum* y más aún no resultó cercanamente relacionada a este género. Ninguno de los caracteres codificados tuvo importancia taxonómica. Aunque el nodo del clado de *Pachyphytum* se caracterizó por caracteres no ambiguos, tales como tallo presente, pétalos rectos y presencia de escama nectarífera en los pétalos, estos caracteres sin embargo variaron en el resto de los clados. Es notable que la monofilia de *Pachyphytum* fue corroborada por este estudio mientras que las relaciones de *Echeveria* continúan sin entenderse.

**Palabras clave:** clado Acre, *Graptopetalum*, *Thompsonella*

## Author Contributions

Carlos Vázquez-Cotero, Pablo Carrillo-Reyes and Victoria Sosa collected samples, obtained the DNA sequences, performed analyses and wrote the paper

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*Echeveria* DC. is a genus in the Crassulaceae comprising approximately 140 species distributed in the New World, from Texas to Argentina with the highest diversity in the mountainous areas of southern Mexico (Moran 1967, Walther 1935, 1972, Kimmach 2003). It was split from *Cotyledon* by De Candolle in 1828 by including all New World species that have a lateral inflorescence. Since then, *Echeveria* has undergone few changes. *Oliveranthus* Rose and *Urbinia* Rose were two taxa segregated at the beginning of the last century (Britton & Rose 1903), however these genera were not approved by taxonomists. Berger (1930) considered *Thompsonella* and *Dudleya* Britton & Rose to be part of *Echeveria*, yet both genera were reestablished later (Clausen 1940, Moran 1951). In the last 50 years, the circumscription of *Echeveria* has remained unchanged and is divided into 17 series based on morphological and chromosomal evidence (Walther 1972, Kimmach 2003, Pilbeam 2008).

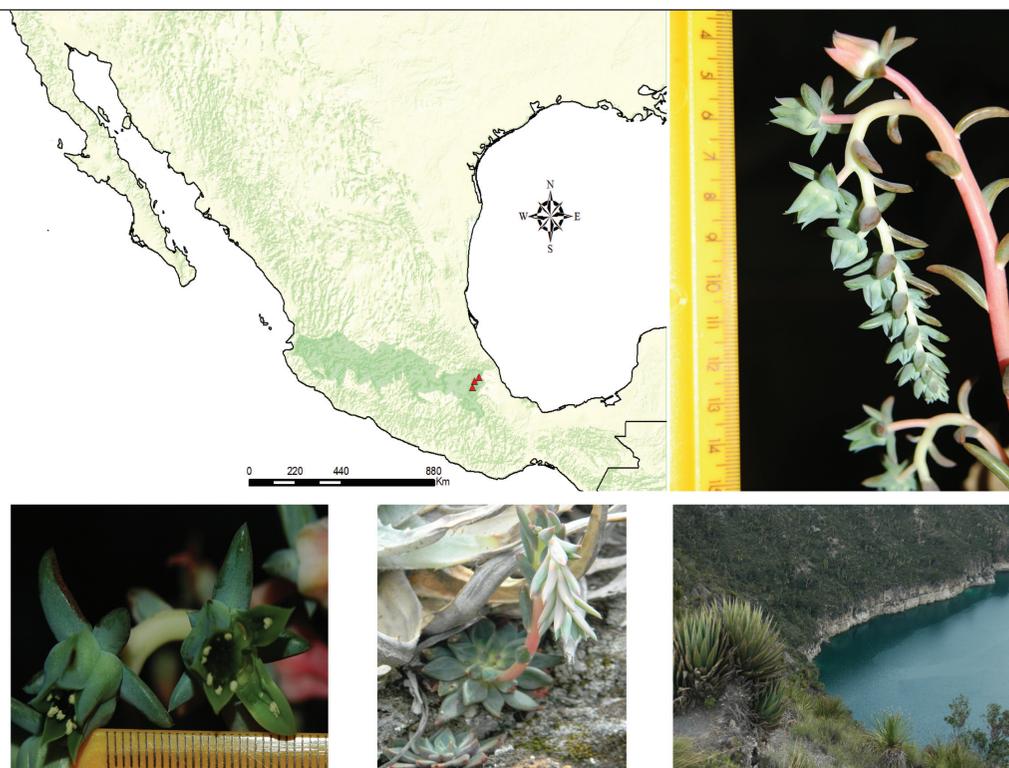
Plants of *Echeveria* have leaves arranged in rosettes, with variable type of inflorescence (lateral spike, raceme, cyme, scorpioid cyme or cincinnus, thyrsoïd). Flowers have mostly expanded succulent erect sepals, and bright colored succulent petals connate at the base (Kimmach 2003). The main traits used to recognize the 17 series are type of pubescence on the aerial stems, type of inflorescences and shape and length of the corolla (Walther 1972). However, most of the series are poly- or paraphyletic according to recent phylogenies, retrieved in clades embedded with species from *Cremanophila* Rose, *Graptopetalum* Rose, *Sedum* L. sect. *Pachysedum* H. Jacobsen, and *Thompsonella* Britton & Rose (Carrillo-Reyes et al. 2008, 2009).

*Pachyphytum* Link, Klotzsch & Otto is another genus in Crassulaceae mostly endemic to central Mexico comprising 19 described species, with a distribution centered on the Mexican Plateau, extending from southern Tamaulipas to northern Michoacán (von Poellnitz 1937, Moran 1963, 1989, 1991, García-Ruiz et al. 1999, Brachet et al. 2006, Martínez-Peralta et al. 2010). Most of the species in this genus have been described from a single locality (von Poellnitz 1937, Moran 1989, 1991, Brachet et al. 2006). Species grow in xerophytic scrub or less commonly in oak forest, and on vertical rock cliffs. Plants are characterized by a rock-dwelling habit, the leaves are terete, greenish or purple, sometimes conspicuously glaucous-farinose, the inflorescence is axillar, a scorpioid cyme or cincinnus, with somewhat imbricate succulent bracts. The flowers are pendant or rarely erect, pentamerous, succulent erect and appressed sepals sometimes surpassing the corolla, petals usually connate at the base, variously colored (white, greenish or reddish, or with maculae at the apex), with ten free stamens alternate or epipetalous and five nectaries. The fruits are erect to spreading and the seeds are ovoid and reticulate (von Poellnitz 1937, Uhl & Moran 1973, Thiede 2003, Thiede & Eggl 2007). Nevertheless, most authors coincide in recognizing scorpioid cymes or cincinnus, very succulent leaves and bracts and a nectary scale on the inner face of petals as the most important characters for distinguishing this genus (von Poellnitz 1937, Moran 1963, García-Ruiz et al. 1999, Brachet et al. 2006, Thiede & Eggl 2007). These similarities have led some authors to suggest that *Pachyphytum* might be included in *Echeveria* and be recognized as a section of the latter (Thiede 2003), however species of *Pachyphytum* have been retrieved in phylogenies in a well supported monophyletic group separate from *Echeveria* (Carrillo-Reyes et al. 2009).

One of the rarest species with restricted distribution in *Echeveria* possesses a nectary scale on the inner face of the corolla elements: *E. heterosepala* Rose (Figures 1, 2). It has been either considered in *Pachyphytum* or in *Echeveria* with a complex taxonomic history exemplifying the inadequate delimitation of these genera in Crassulaceae and the lack of diagnostic characters to distinguish genera. *E. heterosepala* was described by Rose (Bull. New York Bot. Gard. 3: 8. 1930) and later this species was considered to be the monotypic section *Echeveriopsis* of *Pachyphytum* (Walther 1931). This change was reverted by Moran (1960) who returned the species to *Echeveria*, creating the monotypic section *Chloranthae* in *Echeveria*, because *E. heterosepala* has more in common with *Echeveria* than with *Pachyphytum*. *E. heterosepala* is a rare species collected only in two areas of xerophytic scrub vegetation in the south of the Mexican Plateau, one of the populations in the surroundings of the Atexcac lake (Figure 1).

Here we include *Echeveria heterosepala* in a molecular phylogeny to identify its position as well as to determine whether the nectary scale on the inner face of petals can be diagnostic for *Pachyphytum*. We include in analyses species of *Echeveria*, *Graptopetalum* Rose, *Lenophyllum* Rose, *Pachyphytum*, *Thompsonella* Britton & Rose and *Villadia* Rose that have been retrieved

**Figure 1.** Distribution of *Echeveria heterosepala*, triangles indicate the localities where this species was collected. Inflorescence, flowers, rosette and habitat of Lake Atexcac, Puebla (19° 20' 1" N, 97° 27' 0" W) where it was collected are shown. The mountain chain Trans-Mexican Volcanic Belt is shaded in green. Images taken by Pablo Carrillo at Atexcac, Puebla on March 26th, 2015.



as closely related forming part of a clade known as the Acre Clade in the Crassulaceae (Carrillo-Reyes *et al.* 2009).

The objective of this paper is to identify the phylogenetic position of *Echeveria heterosepala* by means of molecular phylogenetic analyses and based on these results understand the evolution of the characters previously considered diagnostic for *Echeveria* and *Pachyphytum*.

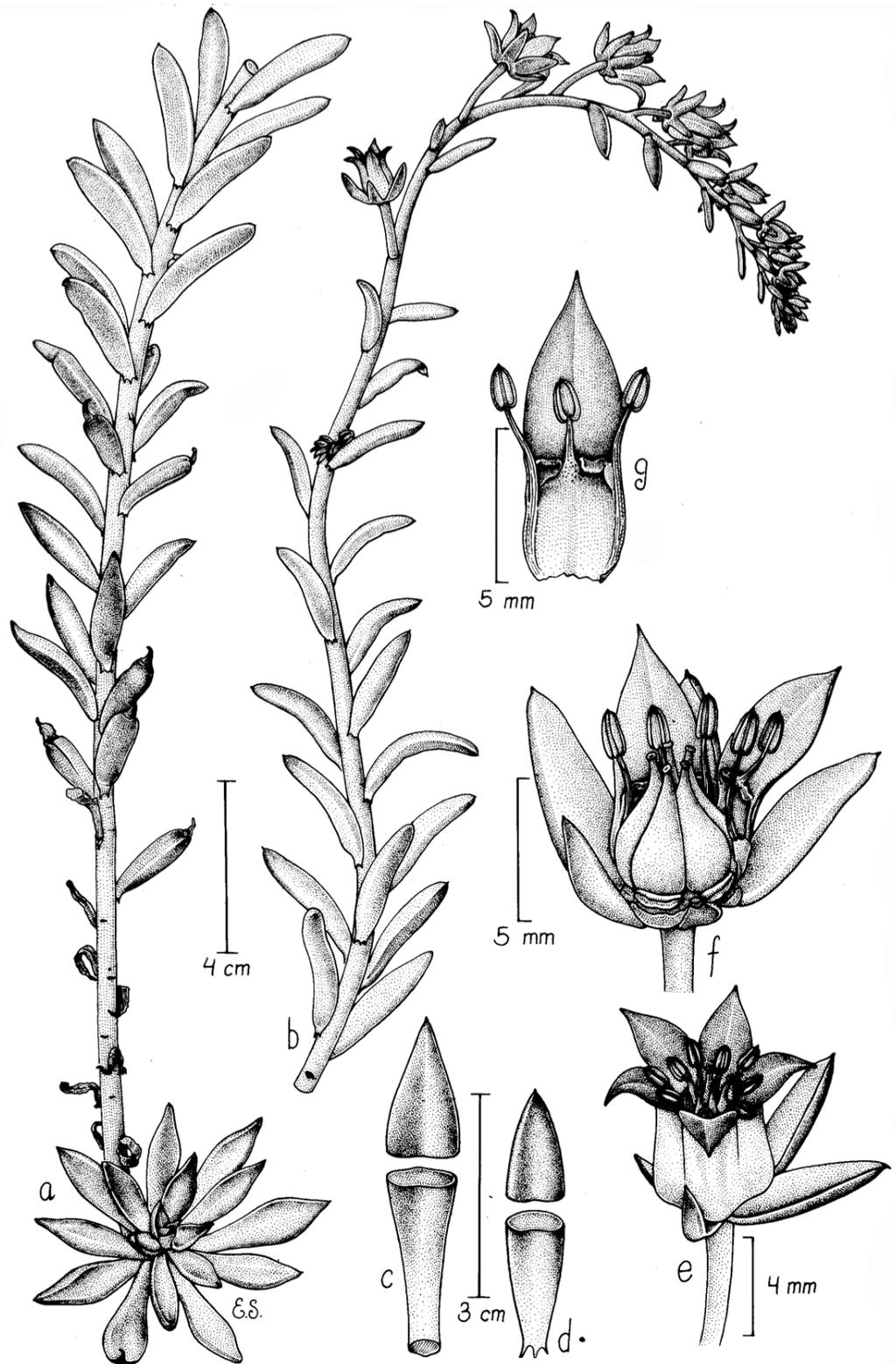
### Material and methods

**Taxon sampling.** We selected 47 taxa, representative species in the genera *Echeveria* (26 spp.), *Graptopetalum* (3 spp.), *Lenophyllum* (1 spp.), *Pachyphytum* (7 spp.), *Thompsonella* (2 spp.), *Sedum* (3 spp.) and *Villadia* (2 spp.) and based on previous phylogenetic analyses species in *Dudleya* (3 spp.) in the *Leucosedum* clade, were considered the outgroup (Carrillo-Reyes *et al.* 2008, 2009). Six *Echeveria* species with diagnostic characters attributed to *Pachyphytum* were included in the ingroup (*E. novogaliciana*, *E. rulfiana*, *E. dactylifera*, *E. mucronata* and *E. crassicaulis*) and *E. heterosepala*. Taxa, vouchers and GenBank accession numbers are listed in the Appendix 1.

**DNA, extraction, amplification and sequencing.** DNA was isolated using either a modified 2xCTAB method (Cota-Sánchez *et al.* 2006) or the DNeasy Plant MiniKit (Qiagen, Valencia, California) following the manufacturer's instructions. Two plastid (*rps16*, *trnL-F*) and two nuclear regions (ETS, ITS) were sequenced. Amplification and sequencing primers for ETS were 18S-ETS (Baldwin & Markos 1998) and ETS-IGSF (Acevedo-Rosas *et al.* 2004); for ITS the primers were ITS4 and ITS5 (White *et al.* 1990); for *rpS16*, *rpS16F* and *rpS16R* (Shaw *et al.* 2005); and for *trnL-F*, *trnL-c* and *trnL-f* (Taberlet *et al.* 1991). PCR products were purified with QIAquick columns (Qiagen, Valencia, USA) or ExoSAP-IT (Affymetrix, Santa Clara, USA), sequenced with the TaqBigDye Terminator Cycle Sequencing kit (Perkin Elmer Applied Biosystems, Foster City, USA) and processed on a 310ABI DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, USA). The sequences were edited in Sequencher 5.4.6 (Gene Codes) and aligned by MUSCLE (Edgar 2004) followed by manual refinement using BioEdit (Hall 1999).

**Phylogenetic analyses.** Phylogenetic analyses were conducted independently for three datasets,

**Figure 2.** *Echeveria heterosepala*. **A)** Rosette and lateral branch. **B)** Branch with scorpioid cyme or cincinnus. **C)** **D)** Petal nectary scale taken from the inner face of petals. **E)** Flower with all elements. **F)** Flower with petals removed showing ovaries and stamens. **G)** Detail of base of three stamens. Illustration hand drawn by Edmundo Saavedra.



ETS+ITS (nuclear data matrix), *trnL-F* and *rps16* (plastid data set), and for the combined data matrix. First, jModelTest 2.1.6 (Darriba *et al.* 2012) was used to identify the model of molecular evolution that best fit the three different matrices; the best models were GTR+G+I, GTR+G and GTR respectively under the Akaike Information Criterion (AIC). Maximum Likelihood (ML)

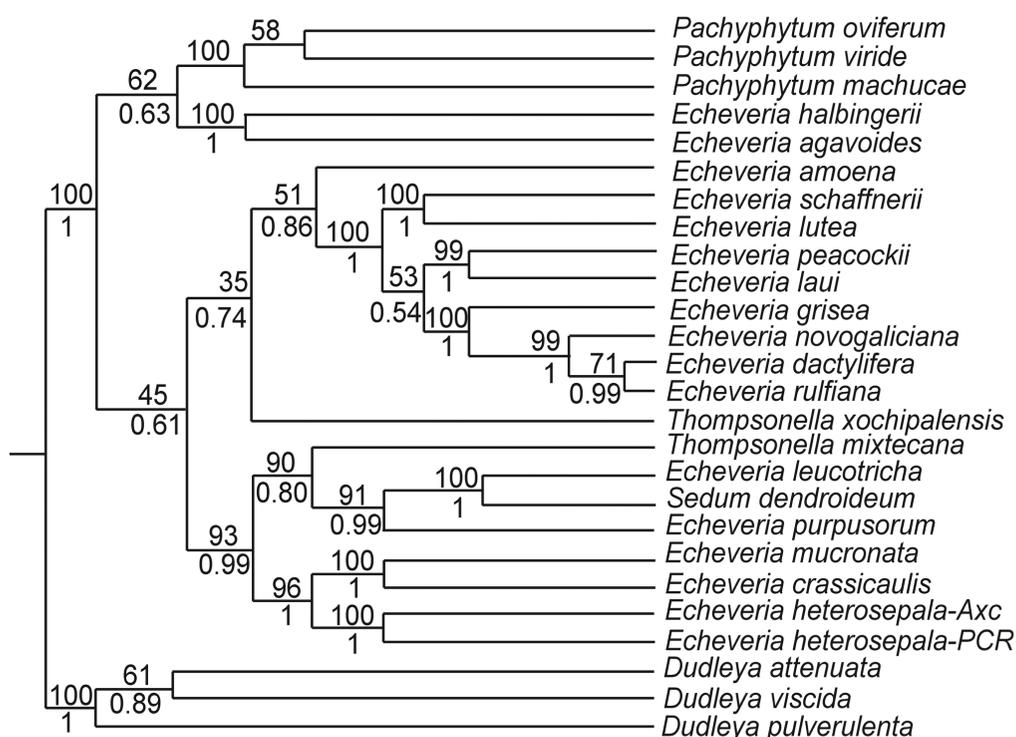
phylogenetic analyses were conducted with RAxML v7.0.4 (Stamatakis 2014). Clade support was assessed with 1,000 replicates of a nonparametric bootstrap analysis, also conducted with RAxML. Bayesian analyses were run in MrBayes v.3.2.2 (Huelsenbeck & Ronquist 2001), for every run one cold and three heated chains were set to run for 40 million generations, sampling one tree every 2,000 generations. Stationarity was determined by the likelihood scores for time to convergence, and sample points collected prior to stationarity were eliminated (25 %). Posterior probabilities for clade support were determined by a 50 % majority-rule consensus of the trees retained after burn-in.

The trees retrieved by Bayesian inference based on the nuclear data, the most complete data matrix, were utilized to understand character evolution. Ancestral characters were inferred by the parsimony method, using the command *trace character history* and the *unordered states* assumption was selected for categorical characters using Mesquite v.2.75 (Maddison & Maddison 2017). This parsimony method finds the ancestral states that minimize the number of steps of character change given the tree and observed character distribution. The characters and character states analyzed were: 1) Sepals: straight/curved. 2) Stem: present/absent. 3) Petal nectary scale: present/absent. 4) Type of inflorescence: raceme/cyme/panicle/spike.

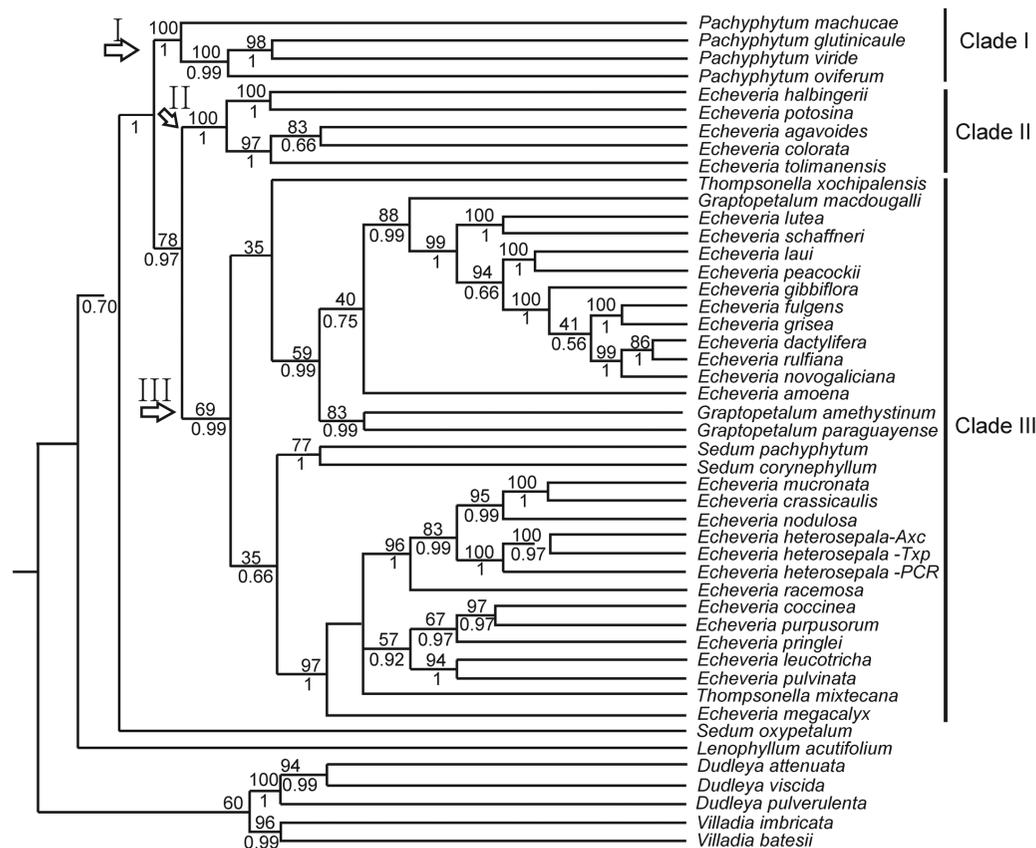
### Results

Analyses with plastid and combined (plastid + nuclear) DNA data matrices were performed with a reduced number of terminals, restricting them to taxa with complete sequences. The plastid data matrix included 25 taxa and 1,461 bp with 67 parsimony informative characters while the combined data matrix included 21 taxa with 2,536 bp and 362 parsimony informative characters. ML and Bayes inference with plastid data retrieved unresolved trees in which only the clade formed by the three species of *Dudleya*, the outgroup, received support (not shown). The combined data matrix retrieved a well supported clade formed by species of *Pachyphytum* (bootstrap bst = 100 % and posterior probabilities PP = 1), the two accessions of *Echeveria heterosepala* with complete sequences formed part of a well supported clade (bst = 93 %, PP = 0.99) comprising species of *Echeveria* and *Sedum dendroideum* (Figure 3).

**Figure 3.** Fifty percent majority rule consensus Bayesian tree based on combined data matrices (plastid *rps16+ trnL-F* and nuclear ETS+ITS). Percentage of bootstrap of Maximum Likelihood is indicated above branches and posterior probabilities of Bayesian inference is indicated below branches. Maximum likelihood analyses were performed in RAxML v7.0.4 (Stamatakis 2014) and Bayesian inference in MrBayes v.3.2.2 (Huelsenbeck & Ronquist 2001).



**Figure 4.** Fifty percent majority rule consensus Bayesian tree based on nuclear data matrix (ETS+ITS). Percentage of bootstrap of Maximum Likelihood is indicated above branches and posterior probabilities of Bayesian inference is indicated below branches. Maximum likelihood analyses were performed in RAxML v7.0.4 (Stamatakis 2014) and Bayesian inference in MrBayes v.3.2.2 (Huelsenbeck & Ronquist 2001).



The most complete was the nuclear data matrix including 47 taxa with 1,090 bp and 372 parsimony informative characters (Figure 4). ML and Bayesian analyses based on this data matrix retrieved species of *Pachyphytum* in a well supported Clade I (bst = 100, PP = 1). This clade was sister to a large clade with the rest of the ingroup species (bst = 78 %, PP = 0.97), which in turn were divided into two groups: one of them, Clade II comprised exclusively of *Echeveria* species (bst = 100 % PP = 1) and the remaining taxa were retrieved in Clade III (bst = 69 %, PP = 0.99) with species of *Echeveria* forming part of groups with *Thompsonella*, *Graptopetalum* and *Sedum*. *Lenophyllum acutifolium* was the sister group to the rest of ingroup species. Three three accessions of *E. heterosepala* formed part of a clade with *Echeveria* species such as *E. mucronata*, *E. crassicaulis*, *E. nodulosa* and *E. racemosa* (bst = 96 %, PP = 1) (Figure 4).

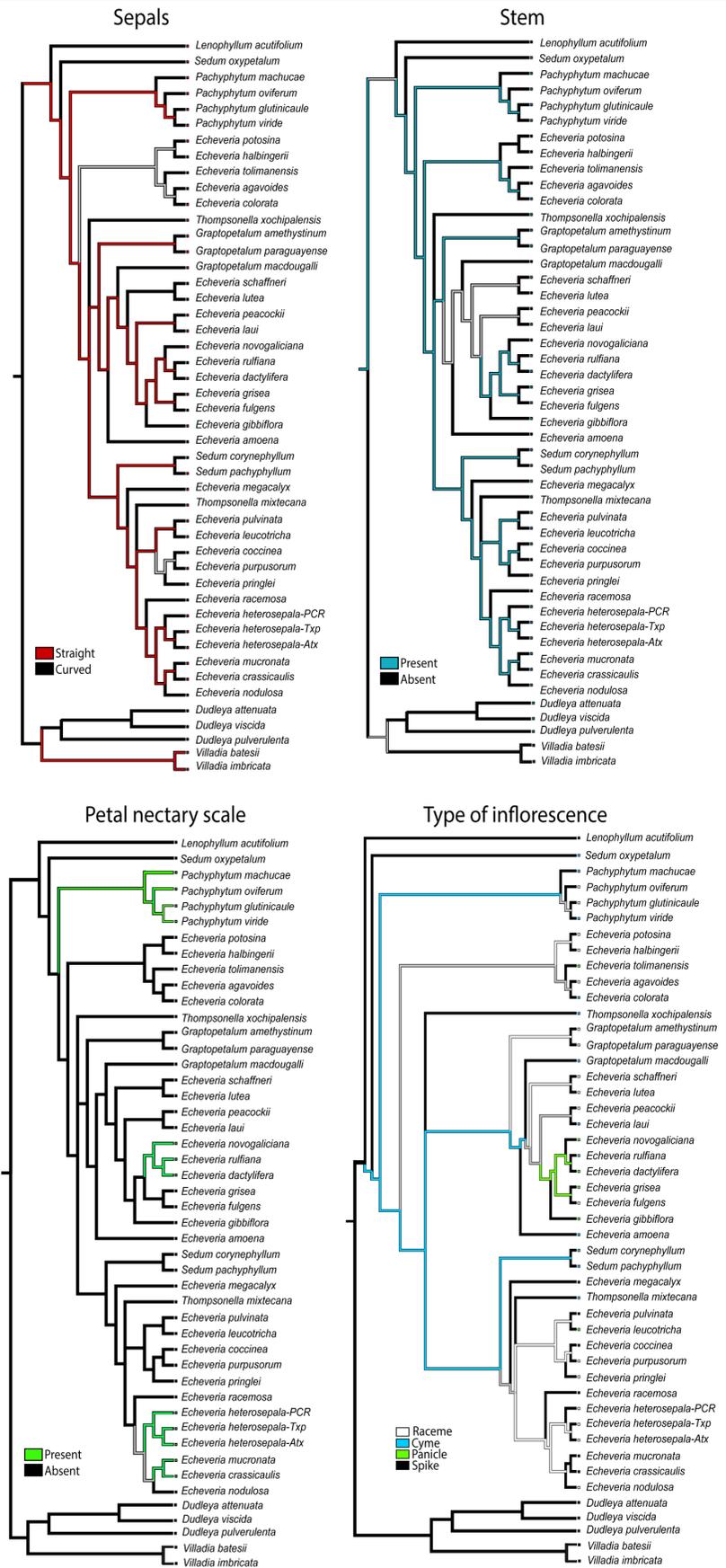
From the reconstruction of ancestral character states under parsimony, the clade formed by the species of *Pachyphytum* identified that unambiguous ancestral character states were: stem present, straight petals and petal scaly bract present (Figure 5). For the rest of clades the character states were reconstructed as ambiguous for the four selected traits (Figure 5).

## Discussion

Our study corroborated previous relationships identified by molecular phylogenies (Carrillo-Reyes *et al.* 2008, 2009): *Pachyphytum* was retrieved as a monophyletic group while species of *Echeveria* formed part of different clades with different genera. In this study we sequenced for the first time six species of *Echeveria* (*E. heterosepala*, *E. novogaliciana*, *E. rulfiana*, *E. dactylifera*, *E. mucronata* and *E. crassicaulis*) that have a nectary scale in petals, and based on previous sequences we selected seven species (*E. halbingeri*, *E. colorata*, *E. tolimanensis*, *E. lutea*, *E. peacockii* Baker, *E. laui*, *E. purpusorum*) with scorpioid cymes.

The only well supported clade formed exclusively by *Echeveria* species includes taxa of series *Urbinae*: *E. halbingeri*, *E. potosina*, *E. agavoides*, *E. colorata* and *E. tolimanensis*, they

**Figure 5.** Parsimony ancestral reconstruction for the four selected characters, the trees utilized for reconstruction were retrieved by the nuclear data matrix. It was conducted in Mesquite v.2.75 (Maddison & Maddison 2015).



share characters such as the lack of aerial stems and presence of urceolate flowers. Remarkably they do not share characters such like type of inflorescence. For example, *E. tolimanensis*, *E. agavoides* and *E. colorata* possess a cyme as inflorescence type while the rest of the species are characterized by a secund-racemose inflorescence.

The accessions of *Echeveria heterosepala* collected in Puebla, in Atexcac (Axc), Tenextepc (Txp) and Aljojuca (PCR) were retrieved in a well supported clade, closely related to species of *Echeveria* like *E. mucronata*, *E. crassicaulis*, *E. nodulosa* and *E. racemosa*. *E. mucronata* is an ornamental species with ample distribution from Arizona to Chiapas, *E. crassicaulis* grows along the Trans-Mexican Volcanic Belt, *E. nodulosa* from Puebla and Oaxaca while *E. racemosa* has been recorded in Puebla and Veracruz. *E. racemosa* is the type species of ser. *Racemosae* (Walther 1972).

Since *Pachyphytum* was described as a separate genus from *Echeveria*, the most relevant diagnostic morphological character has been the presence of a petal nectary scale. Even so, this scale has been observed on a number of species of *Echeveria*, such as *E. heterosepala* (Walther 1972) thus raising uncertainty about the utility of this character as diagnostic for differentiating these genera. Although a number of species in *Echeveria* classified in different series possess the petal nectary scale (*i.e.* the diagnostic character used for *Pachyphytum*), Moran (1960), argued that species in *Pachyphytum* have morphological similarities and should be recognized as a different genus. Our results suggest that neither the petal nectary scale nor the rest of the characters are exclusive to *Pachyphytum* and, with exception of a well supported clade of *Echeveria* that corresponded to series *Urbinae*, the rest of the species in this genus are embedded in clades with species in *Thompsonella*, *Sedum* and *Graptopetalum*. Our phylogenetic analyses found that *E. heterosepala* forms part of a clade comprised entirely by species of *Echeveria*, and in consequence this species belongs to *Echeveria*, not to *Pachyphytum*.

Our reconstruction of ancestral characters indicates that the scale on petals has arisen independently four times, in the clades of *Pachyphytum*, *Echeveria novogaliciana*, *E. heterosepala* and *E. crassicaulis*. The rest of the characters were reconstructed arising multiple times as shown in Figure 5. Petal elaborations, like the nectary scale, have been associated to diverse floral biological functions in angiosperms, mostly related to attracting pollinators (Endress & Matthews 2006). Ontogeny of nectary scale has been recorded only in two genera in Crassulaceae. In *Pachyphytum* the nectary scale resulted of petals having a ventral lobe and the petal is fused with the stamen of the same radius (Leinfellner 1954), while in *Sedum* the nectary scale potentially corresponds to a staminode (De Craene & Smets 2001). Probably different origin of nectary scale in petals of the different taxa studied here is the explanation for finding this character arising multiple times, and in consequence it cannot have taxonomic significance. However this hypothesis has to be tested.

Polyploidy has been reported in both *Echeveria* and *Pachyphytum* as well as in *Graptopetalum*, *Lenophyllum* and *Sedum* (see Table 1 and references herein). The basic chromosome number in the Acre clade is 10 and in the *Leucosedum* clade is 7 (Mort *et al.* 2001). All studied taxa in the Acre clade are polyploids, as well as the outgroup *Dudleya* that belongs to the *Leucosedum* clade. Excepting species of *Graptopetalum* with the highest known number of chromosomes in Crassulaceae, *Pachyphytum hookeri* is remarkable having reports of 160 chromosomes and moreover the rest of studied species in this genus have high numbers of chromosomes as well (see Table 1 and references herein). Polyploidy has been associated with complex evolutionary processes. For instance, it has been described that diploid and tetraploid plant species could be genetically differentiated but morphologically similar (Stark *et al.* 2011). Furthermore, polyploidy has been recognized as a mechanism for sympatric speciation (Otto & Whitton 2000) and it has been identified as well that speciation events in angiosperms can be accompanied by ploidy increase (Wood *et al.* 2009). Future studies may show whether polyploidy is the result of rapid speciation without morphological changes in the case of *Pachyphytum* and *Echeveria* or whether other causes correlated or not to polyploidy such like population isolation by the mountains of the Mexican Trans-Volcanic Belt promoted rapid speciation.

The lack of definition of generic limits in *Echeveria* has already been identified based on molecular phylogenies (Mort *et al.* 2001, Carrillo-Reyes *et al.* 2009). To understand its limits, additional sampling of *Sedum* from Europe and Asia should be considered, as species in this

Table 1. Species considered in analyses for which there are chromosome counts.

Species	Chromosome number	Reference
<i>Dudleya attenuata</i> (S.Watson) Moran	17	Uhl & Moran 1953
<i>D. pulverulenta</i> Britton & Rose	17	Uhl & Moran 1953
<i>D. viscida</i> (S.Watson) Moran	34	Uhl & Moran 1953
<i>Echeveria amoena</i> De Smet ex É.Morren	33,66	Uhl 1982, 1992
<i>E. coccinea</i> (Cav.) DC.	23,25	Uhl 1963
<i>E. colorata</i> E.Walther	27	Uhl 1982
<i>E. fulgens</i> Lem.	27,162	Walther 1972
<i>E. gibbiflora</i> DC.	27,49, 51, 54	Funamoto & Yuasa 1989
<i>E. grisea</i> E.Walther	27	Uhl 1982
<i>E. megacalyx</i> E.Walther	20	Uhl 1992b
<i>E. nodulosa</i> (Baker) Ed.Otto	16	Uhl 1961
<i>E. pringlei</i> (S.Watson) Rose	23	Uhl 1992b
<i>E. pulvinata</i> Rose	23+2	Uhl 1992b
<i>E. purpusorum</i> A.Berger	27	Uhl 1982
<i>E. racemosa</i> Cham. & Schtdl.	18	Uhl 1982
<i>Graptopetalum amethystinum</i> E.Walther	34,35	Uhl 1970
<i>G. macdougallii</i> Alexander	64-66, 192, 244, 245	Uhl 1970
<i>G. paraguayense</i> (N.E.Br.) E.Walther	68	Uhl 1970
<i>Lenophyllum acutifolium</i> Rose	22,44	Uhl 1996
<i>Pachyphytum glutinicaule</i> Moran	33, 66, 99	Uhl & Moran, 1999
<i>P. hookeri</i> A.Berger	32, 64, ±128, ±160	Uhl 2001
<i>P. kimnachii</i> Moran	±33	Uhl & Moran, 1999
<i>P. oviferum</i> J.A. Purpus	33	Uhl & Moran, 1999
<i>P. viride</i> E.Walther	33	Uhl & Moran, 1999
<i>Sedum corynephyllum</i> Fröd.	34,68	Uhl 1978
<i>S. oxypetalum</i> Kunth.	29	Uhl 1980
<i>Sedum</i> L.	34	Uhl 1978

genus appear embedded in *Echeveria* clades and mainly New World species have only been sequenced. Until now the *Echeveria* species included in analyses are mostly from Mexico; further collections from Central and South American need to be added. For *Pachyphytum* approximately ten species require to be considered as well. Novel primers for plastid genomes have been designed as an effective and feasible strategy for phylogenomics in many groups of angiosperms (Yang *et al.* 2014). Broader phylogenetic analyses should consider these primers for sequencing plastid data (current plastid markers utilized show little variation) and a more ample sampling. These analyses will help to finally understand limits not only of *Echeveria* and *Sedum* but also of *Pachyphytum*, to determine whether they should be split in several genera or not.

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### Literature cited

Acevedo-Rosas R, Cameron K, Sosa V, Pell S. 2004. A molecular phylogenetic study of *Graptopetalum* (Crassulaceae) based on ITS, ETS, *rpl 16* and *trn L-F* nucleotide sequences. *American Journal of Botany* **91**: 109-1104.

- Baldwin BG, Markos S. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* **10**: 44-463. DOI: 10.1006/mpev.1998.0545
- Berger A. 1930. Crassulaceae. In: A. Engler & Prantl K. Eds. Die Natürlichen Pflanzenfamilien. 352-483. Leipzig: K. Verlag. DOI: 10.5962/bhl.title.4635
- Brachet IC, Reyes-Santiago J, Mondragón-Larios R. 2006. *Pachyphytum saltensis* Brachet, Reyes & Mondragón, una nueva especie para el estado de Zacatecas. *Cactáceas y Suculentas Mexicanas* **51**: 46-51.
- Britton NL, Rose JN. 1903. New or noteworthy North American Crassulaceae. *Bulletin of the New York Botanical Garden* **3**: 1-45.
- Carrillo-Reyes P, Sosa V, Mort ME. 2008. *Thompsonella* and the “*Echeveria* group” (Crassulaceae), phylogenetic relationships based on molecular and morphological characters. *Taxon* **57**: 863-874.
- Carrillo-Reyes P, Sosa V, Mort ME. 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.mpev.2009.05.022
- Clausen RT. 1940. Studies in Crassulaceae: *Villadia*, *Altamiranoa* and *Thompsonella*. *Bulletin of the Torrey Botanical Club* **67**: 195-198.
- Cota-Sánchez JH, Remarchuk K, Ubayasena K. 2006. Ready-to-use DNA extracted with a CTAB method adapted for herbarium specimens and mucilaginous plant tissue. *Plant Molecular Biology Reporter* **24**: 161-167. DOI: 10.1007/BF029114055
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772. DOI:10.1038/nmeth.2109
- De Candolle AP. 1828. *Prodromus Systematis Naturalis Regni Vegetabilis* **3**: 381-414.
- De Craene LP, Smets EF. 2001. Staminodes: their morphological and evolutionary significance. *Botanical Review* **67**: 351-402. DOI:10.1007/BF02858099
- Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792-1797. DOI: 10.1093/nar/gkh340
- Endress PK, Matthews ML. 2006. Elaborate petals and staminodes in eudicots: diversity, function and evolution. *Organisms Diversity and Evolution* **6**: 257-293. DOI:10.1016/j.ode.2005.09.005
- Funamoto T, Yuasa H. 1989. Chromosome studies of the genus *Echeveria* (Crassulaceae). Part II. Somatic chromosome numbers of the remaining taxa. *Research Institute of Evolutionary Biology Science Repository* **6**: 16-29.
- García-Ruiz I, Glass C, Cházaro-Basáñez M. 1999. *Pachyphytum machucaae* (Crassulaceae) una nueva especie de Michoacán, Mexico. *Acta Botanica Mexicana* **47**: 9-14.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. <<http://www.mbio.ncsu.edu/bioedit/bioedit.html>> (accessed November 11, 2016)
- Huelsenbeck JP, Ronquist F. 2001. MrBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754-755. DOI: 10.1093/bioinformatics/17.8.754.
- Kimmach M. 2003. *Echeveria*. In: U. Eggli ed. *Illustrated Handbook of Succulent Plants, Crassulaceae*, Berlin: Springer Verlag, 103-128.
- Leinfellner W. 1954. Beiträge zur Kronblattmorphologie. III. Die Kronblätter der gattung Pachphytum. *Österreichische Botanische Zeitschrift* **101**: 586-591. DOI: 10.1007/BF01284373
- Maddison WP, Maddison DR. 2017. Mesquite: a modular system for evolutionary analysis. Version 3.2. <<http://mesquiteproject.org>>
- Martínez-Peralta C, Mancilla R, Altamirano-Vázquez HG, Aguilar-Morales G. 2010. Características poblacionales de *Pachycereus weberi* y su relación con polinizadores en la comunidad Dominguillo en el Valle de Tehuacán-Cuicatlán, Puebla-Oaxaca. *Cactáceas y Suculentas Mexicanas* **55**: 85-94.
- Moran R. 1951. A revision of *Dudleya*. PhD Thesis. University of California, Berkeley.
- Moran R. 1960. *Echeveria heterosepala* Rose. *Cactáceas y Suculentas Mexicanas* **5**: 75-80.
- Moran R. 1963. *Pachyphytum brevifolium* Rose and *P. glutinicaule*, a new species from Hidalgo, Mexico. *Cactus and Succulent Journal* **15**: 35-41.
- Moran R. 1967. *Echeveria procera*, a new species from Oaxaca, Mexico. *Cactus and Succulent Journal* **39**: 182-185.
- Moran R. 1989. *Pachyphytum bracteosum* Klotsch. *Cactus and Succulent Journal* **61**: 119-124.
- Moran R. 1991. *Pachyphytum longifolium* Rose. *Cactus and Succulents Journal* **63**: 261-265.
- Mort ME, Solits DE, Soltis PS, Francisco-Ortega J, Santos-Guerra A. 2001. Phylogenetic relationships and evolution of the Crassulaceae inferred from *matK* sequence data. *American Journal of Botany* **88**: 76-91.
- Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* **34**: 401-437. DOI: 10.1146/annurev.genet.34.1.401
- Pilbeam J. 2008. *The genus Echeveria*. British Cactus and Succulent Society, London.
- Seqencher v.5.4.6 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI, USA.

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- Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling E, Small RL. 2005. The tortoise and the hare II: relative utility of 21 non-coding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* **92**: 142-166. DOI: 10.3732/ajb.92.1.142
- Stamakakis A. 2014. RaxML Version 8: A tool for phylogenetic analysis and post analysis of large phylogenies. *Bioinformatics* **9**. DOI: 10.1093/bioinformatics/btu033
- Stark C, Michalski SG, Babik W, Winterfeld G, Durka W. 2011. Strong genetic differentiation between *Gymnadenia conopsea* and *G. densiflora* despite morphological similarity. *Plant Systematics and Evolution* **293**: 213-226. DOI:10.1007/s00606-011-0439-x
- Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* **17**: 1105-1109. DOI: 10.1007/BF00037152
- Thiede J. 2003. *Pachyphytum*. In Egli U. ed. *Illustrated Handbook of Succulent Plants: Crassulaceae*. Berlin: Springer. 190-195. DOI: 10.1007/978-3-642-55874-0
- Thiede J, Egli U. 2007. Crassulaceae. In Kubitzki K. ed. *The Families and Genera of Vascular Plants*. Hamburg: Springer. 83-118.
- Thiers B. 2017 [continuously updated]. *Index Herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih>
- Uhl CH. 1961. The Crassulaceae and cytotaxonomy. *Cactus and Succulent Journal (US)* **48**: 225-229.
- Uhl CH. 1963. Chromosomes and phylogeny of the Crassulaceae. *Cactus and Succulent Journal* **35**: 3-7.
- Uhl CH. 1970. The chromosomes of *Graptopetalum* and *Thompsonella* (Crassulaceae). *American Journal of Botany* **57**: 1115-1121.
- Uhl CH. 1978. Chromosomes of Mexican *Sedum* II. Section *Pachysedum*. *Rhodora* **80**: 491-
- Uhl CH. 1980. Chromosomes of Mexican *Sedum* III. Section *Centripetalia*, *Fructisedum* and other woody species. *Rhodora* **82**: 377-402.
- Uhl CH. 1982. The problem of ploidy in *Echeveria* (Crassulaceae) I. Diploidy in *E. ciliata*. *American Journal of Botany* **69**: 843-854.
- Uhl CH. 1992. Polyploidy, and chromosome pairing in *Echeveria* and its hybrids. *American Journal of Botany* **79**: 556-566.
- Uhl CH. 1992b. Polyploidy, dysploidy, and chromosomes pairing in *Echeveria* (Crassulaceae) and its hybrids. *American Journal of Botany* **79**: 556-566.
- Uhl CH. 1996. Chromosomes and polyploidy in *Lenophyllum* (Crassulaceae). *American Journal of Botany* **83**: 216-220.
- Uhl CH. 2001. Hybrids of *Pachyphytum hookeri* (Crassulaceae) and their chromosomes. *Haseltonia* **8**: 63-84.
- Uhl CH, Moran R. 1953. The cytotaxonomy of *Dudleya* and *Hasseanthus*. *American Journal of Botany* **40**: 492-502.
- Uhl CH, Moran R. 1973. The chromosomes of *Pachyphytum* (Crassulaceae). *American Journal of Botany* **60**: 648-656.
- Uhl CH, Moran R. 1999. Chromosomes of *Villadia* and *Altamiranoa* (Crassulaceae). *American Journal of Botany* **86**: 387-397.
- von Poellnitz K. 1937. The genus *Pachyphytum*. *The Cactus and Journal* **5**: 72-75.
- 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, California.
- White TJ, Bruns TD, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal genes for phylogenetics In: Innis MA, Gelfand, DH, Sninsky, JJ, White TJ, eds. *PCR Protocols*. San Diego, California: Academic Press. 315-322.
- Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH. 2009. The frequency of polyploid speciation in vascular plants. *Proceedings of the National Academy of Sciences* **106**: 13875-13879. DOI: 10.1073/pnas.0811575106
- Yang JB, Li DZ, Li HT. 2014. Highly effective sequencing whole chloroplast genomes of angiosperms by nine novel universal primer pairs. *Molecular Ecology Resources* **14**: 1024-1031. DOI: 10.1111/1755-0998.1251

## Appendix 1

Taxa included in the analyses, voucher information, locality and GenBank accession numbers for ITS, ETS, *rps16* and *trnL-F*. Sequences not obtained are designated by —; \* indicates sequence previously deposited in GenBank; HBG= accession from the Huntington Botanical Garden; herbarium acronyms follow Thiers (2016).

Taxon, Voucher, Mexico, GenBank accessions: ITS, ETS, *rps16*, *trnL-F*

*Ingroup* – *Echeveria agavoides* Lem. Mexico, Zacatecas, Pinos. L.F. Colín-Nolasco 848 (IBUG), MF818300, MF818281, MF818237, MF818258; *Echeveria amoena* De Smet ex E. Morren, \*EF632172, \*EF632151, \*EF632189, —; *Echeveria coccinea* (Cav.) DC., \*AY545682, \*AY540512, —, —; *Echeveria colorata* E. Walther, \*AY545683, \*AY540513, —, —; *Echeveria crassicaulis* E. Walther, Mexico; *D. Jimeno-Sevilla 1359* (XAL), MF818302, MF818281, MF818249, MF818259; *Echeveria dactylifera* E. Walther, Mexico, Durango, El Salto; J.A. Pérez de la Rosa s.n. (IBUG), Mexico, MF818303, MF818283, MF818250, MF818260; *Echeveria fulgens* Lem., \*AY545684, \*AY540514, —, \*AY540553; *Echeveria gibbiflora* DC., \*AY545685, \*AY540515, —, \*AY540554; *Echeveria grisea* E. Walther, P. Carrillo-Reyes, s.n. (IBUG), MF818304, \*EF632153, MF818246, MF818261; *Echeveria halbingeri* E. Walther, Mexico, *D. Jimeno-Sevilla 268* (XAL), MF818305, MF818284, MF818238, MF818262; *Echeveria heterosepala* Rose ex Britton & Rose, Mexico, Puebla, Atexcac, Guadalupe Victoria; Vázquez-Cotero 1 (XAL), MF818306, MF818285, MF818244, MF818263; Puebla, Tenextepec; Vázquez-Cotero 2 (XAL), MF818307, MF818286, MF818245, MF818264; Mexico, Puebla, Aljojuca, P. Carrillo-Reyes 8136 (IBUG), MF818308, MF818287, MF818248, MF818265; *Echeveria lauii* Moran & J.Meyrán, cultivated, MF818309, MF818288, MF818239, MF818266; *Echeveria leucotracha* J.A.Purpus, Mexico, Puebla, Caltepec, D. Cabrera-Toledo & F. Nicolalde 6 (IBUG), MF818310, MF818289, MF818240, MF818267; *Echeveria lutea* Rose, Mexico, San Luis Potosí, Villa Hidalgo, E. Ruiz-Sánchez 90 (IBUG) MF818311, MF818290, MF818241, MF818268; *Echeveria megacalyx* E. Walther, \*FJ753916, \*EF632154, —, —; *Echeveria mucronata* Schldl., Mexico, Querétaro, Querétaro, A. Rodríguez et al. 6799 (IBUG) MF818312, MF818298, MF818251, MF818269; *Echeveria nodulosa* Otto, \*EF632173, EF632156, EF632190, —; *Echeveria novogaliciana* J.Reyes, Brachet & O.González, Mexico, Jalisco, Zapopan, D. Jimeno-Sevilla s.n. (XAL), MF818313, MF818291, MF818252, MF818270; *Echeveria peacockii* Baker, Mexico, Puebla, Caltepec, A. Rodríguez & C. Briseño-Avena 3905 (IBUG); MF818314, MF818292, MF818242, MF818271; *Echeveria pringlei* (S. Watson) Rose, \*AY545687, \*AY 540517, —, \*AY540555; *Echeveria potosina* E. Walther, Mexico, Jalisco, Guadalajara, P. Carrillo-Reyes & M. Harker 206 (IBUG), MF818315, MF818293, —, —; *Echeveria pulvinata* Rose, \*AY545688, \*AY540518, —, —; *Echeveria purpurorum* A. Berger, P. Carrillo-Reyes & D. Cabrera-Toledo 4472 (XAL), \*FJ753919, FJ753872, MF818243, MF818272; *Echeveria racemosa* Cham. & Schldl., \*FJ753920, \*EF632157, —, —; *Echeveria rulfiana* Jimeno-Sevilla, Santana Mich & P. Carrillo, Mexico, Jalisco, San Gabriel, P. Carrillo-Reyes & J.M. Carrillo 6336 (IBUG), Mexico, MF818316, MF818294, MF818253, MF818273; *Echeveria schaffneri* Rose, Mexico, Zacatecas, Concepción del Oro, J.A. Pérez de la Rosa 2091 (IBUG), MF818301, MF818295, MF818247, MF818274; *Echeveria tolimanensis* Matuda, Mexico, Hidalgo, Pacula, A. Castro-Castro 641-A (IBUG) MF818317, MF818296, —, —; *Graptopetalum amethystinum* E. Walther, \*AY545690, \*AY40519, —, \*AY540556; *Graptopetalum macdougalli* Alexander, \*AY545698, \*AY40527, —, \*AY540564; *Graptopetalum paraguayense* (N.E. Brown) E. Walther, \*AY545792, \*AY540531, —, \*AY540568; *Pachyphytum fittkaui* Moran, \*FJ753925, —, —, —; *Pachyphytum glutinicaule* Moran, \*AY5456710, \*AY40539, —, —; *Pachyphytum hookeri* (Salm-Dyck) A. Berger, \*FJ753926, —, —, —; *Pachyphytum kimmachii* Moran, \*FJ753927, —, —, —; *Pachyphytum machucae* I.García, Glass & Cházaro, Mexico, Michoacán, Pajacuarán, I. García-Ruiz 4497 (IEB) MF818318, MF818297, MF818254, MF818275; *Pachyphytum oviferum* J.A. Purpus, HBG-48603, Mexico, FJ753928, MF818299, MF818255, MF818276; *Pachyphytum viride* E. Walther, \*AY545711, \*AY540540, MF818256, MF818277; *Sedum pachyphyllum* Rose, \*FJ753960, \*FJ753893, —, —; *Sedum corynephyllum* Rose, \*AY5456715, \*AY40544, —, —; *Thompsonella mixtecana* J. Reyes & L. López, \*EF632180, \*EF632162, \*EF632196, MF818278; *Thompsonella xochipalensis* M. Gual Diaz, S.Peralta & Pérez-Calix, \*EF632184, \*EF632166, \*EF632199, MF818279.

*Outgroup* – *Dudleya candelabrum* Rose ex Britton & Rose, \*JX960502, \*JX960466, —, \*JX960544; *Lenophyllum acutifolium* Rose, \*AY5459709, \*AY40538, —, —; *Sedum oxypetalum* Kunth, \*FJ753958, \*FJ753891, \*FJ753856, —; *Villadia batesii* (Hemsl.) Baehni & J.F.Macbr., \*EF63218, \*EF632168, MF818257, MF818280; *Villadia imbricata* Rose, \*EF632187, \*EF632170, \*EF632203, —.