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Molecular phylogeny of the *Acre* clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*

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A B S T R A C T

The phylogenetic relationships within many clades of the Crassulaceae are still uncertain, therefore in this study attention was focused on the "*Acre* clade", a group comprised of approximately 526 species in eight genera that include many Asian and Mediterranean species of *Sedum* and the majority of the American genera (*Echeveria*, *Graptopetalum*, *Lenophyllum*, *Pachyphytum*, *Villadia*, and *Thompsonella*). Parsimony and Bayesian analyses were conducted with 133 species based on nuclear (ETS, ITS) and chloroplast DNA regions (*rpS16*, *matK*). Our analyses retrieved four major clades within the *Acre* clade. Two of these were in a grade and corresponded to Asian species of *Sedum*, the rest corresponded to a European–Macaronesian group and to an American group. The American group included all taxa that were formerly placed in the Echeverioideae and the majority of the American Sedoideae. Our analyses support the monophyly of three genera – *Lenophyllum*, *Thompsonella*, and *Pachyphytum*; however, the relationships among *Echeveria*, *Sedum* and the various segregates of *Sedum* are largely unresolved. Our analyses represents the first broad phylogenetic framework for *Acre* clade, but further studies are necessary on the groups poorly represented here, such as the European and Asian species of *Sedum* and the Central and South American species of *Echeveria*.

1. Introduction

Crassulaceae with approximately 1400 species is one of the most important groups of succulents that are widely cultivated as ornamentals because their leaves are aggregated into colorful rosettes. Members of the family are typically leaf-succulent herbaceous plants with flowers that are usually pentamerous, actinomorphic, and with 4–5 unfused, dehiscent carpels. The family exhibits highly complex cytological and chromosomal variation (Uhl, 1956, 1961b, 1963, 1992b), in fact, Crassulaceae is probably the most cytologically complex angiosperm family. The highest base chromosome number known for any dicot ($n = 270$) belongs to *Graptopetalum suaveolens* (Kimnach, 1978). Furthermore, it has been demonstrated that many species easily hybridize in culture (Uhl, 1961b, 1963) and there is evidence of hybrids in nature (Uhl, 1961a; Bañares, 1990; 't Hart et al., 1993).

Although the distribution of Crassulaceae is nearly worldwide, most species are found in five centers of diversity: Mexico (ca. 330 spp.), the Mediterranean basin (ca. 100 spp.), Macaronesia (ca. 63 spp.), southern Africa (ca. 250 spp.) and eastern Asia (ca. 300 spp.) (Webb, 1964; Ohba, 1978; Thiede and Eggli, 2007; Mort et al., 2002). Species usually grow in arid to semi-arid rocky and mountainous environments (Mort and Mori, 2004).

While the position of Crassulaceae has been well established in Saxifragales (APG II, 2003; Fishbein and Soltis, 2004; Soltis et al., 2007), the phylogenetic relationships within many clades of the family still remain uncertain ('t Hart, 1995; 't Hart and Eggli, 1995; Mort et al., 2001). The largely followed classification of Berger (1930) recognized six subfamilies, three of which are in the New World: Echeverioideae, Sedoideae and Crassuloideae. However, based on recent evidence, Thorne and Reveal (2007) recognized only two subfamilies: Crassuloideae and Sempervivoideae, and Thiede and Eggli (2007) proposed the recognition of a third subfamily, Kalanchoideae, with a reduced concept of Sempervivoideae. Following this classification, Crassuloideae is mostly restricted to southern Africa (except for a small group of aquatic *Crassula* species that are distributed worldwide), Kal-

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anchoideae is distributed in Africa and southern Asia and Sempervivoideae is widely distributed in the northern hemisphere. The latter subfamily has the greatest diversity, with approximately 975 species (Thiede and Eggli, 2007). Sempervivoideae has been subdivided into five tribes and Sedeae is the largest of these tribes with 640 species. Within the Sedeae, two groups have been repeatedly recovered by phylogenetic studies: the *Acre* clade and the *Leucosedum* clade (van Ham and 't Hart, 1998; Mort et al., 2001). The *Acre* clade is comprised of almost 526 species in eight genera including many Asian and Mediterranean species of *Sedum*, and the majority of the American genera (i.e., *Echeveria*, *Graptopetalum*, *Lenophyllum*, *Pachyphytum*, *Villadia*, and *Thompsonella*; Table 1). The mountains of central and southern Mexico are the main center of diversity for the *Acre* clade. However, there are other American genera not placed within this clade such as *Dudleya*, *Sedella* and *Sedum* subgen. *Gormania*, which have been placed in the *Leucosedum* clade. Additionally, few species from North America, (i.e., *Hylotelephium telephioides* (Michaux) H. Ohba, *Rhodiola integrifolia* Raf., *R. rhodantha* (A. Gray) H. Jacobsen, and *R. rosea* L.), are placed in the *Hylotelephium* clade.

Previous phylogenetic analyses have found a number of unresolved relationships within the *Acre* clade and the generic delimitation of most of the genera has been controversial (van Ham et al., 1994; van Ham, 1995; van Ham and 't Hart, 1998; Mort et al., 2001; Acevedo-Rosas et al., 2004a,b; Mayuzumi and Ohba, 2004; Carrillo-Reyes et al., 2008). Some of the genera are difficult to define morphologically, resulting in a lack of taxonomic resolution (e.g., Moran, 1942).

The most problematic and undoubtedly controversial taxon in the *Acre* clade is *Sedum*, the largest genus of the family, described by Linnaeus in 1753. At least 32 segregate genera have been published since then (Mort et al., 2001) and the most recent checklist of Crassulaceae includes 27 generic names as synonyms of *Sedum* ('t Hart and Bleij, 2003). Praeger (1921) recognized 10 sections within the genus and Berger (1930) recognized 22 of which ten are now known to be different genera or part of other groups (i.e., *Graptopetalum*, *Hasseanthus*, *Perrierosedum*, *Populisedum*, *Prometheum*, *Pseudorhodiola*, *Rhodiola*, *Sedella*, and *Telephium*; Sect. *Monanthella* is part of the *Leucosedum* clade and the status of Sect. *Telmissa* remains doubtful) (Eggli, 2003; Thiede and Eggli, 2007). The remaining 12 sections are part of the *Acre* clade, with the majority of species placed within *Sedum* sect. *Sedum* (e.g., Berger, 1930; Fu, 1965; 't Hart, 1991). Fröderström (1929–1935) proposed an alternative classification for *Sedum* based on geographic distribution and the type of fruit, proposing seven informally named groups. Fu (1965, 1974) described section *Filipes* and *Oreades*. Two another sections have been published, *Centripetalia* (Alexander, 1942) and *Craigia* (Clausen, 1943). Sections *Leptosedum* and *Dendrosedum* were merged under sect. *Fruticisedum* (Jacobsen, 1974; Uhl, 1980), and furthermore these sections together with *Pachysedum* were recognized as subgenus *Pachysedum* by Clausen (1943), who later also proposed the new subgenus *Sulcus* (Clausen, 1979).

Two subgenera are currently recognized: *Gormania* and *Sedum* ('t Hart and Bleij, 2003; Thiede and Eggli, 2007), the former was originally proposed as a separate genus by Britton (Britton and Rose, 1903). Subgenus *Gormania* has 110 species, which according to recent phylogenetic studies belong to the *Aeonium*, the *Sempervivum* and the *Leucosedum* clades (van Ham and 't Hart, 1998; Mort et al., 2001; Thiede and Eggli, 2007). Approximately 320 of the species ascribed to subgen. *Sedum* belong to the *Acre* clade (van Ham and 't Hart, 1998; Mort et al., 2001).

Among the American segregate genera of *Sedum* are *Sedastrum* and *Corynephyllum* (Rose in Britton and Rose, 1905), the first includes plants with numerous stems arising from dense basal rosettes and carpels with a concavity behind the scales, while the latter includes shrubby species with lateral inflorescences and flowers with a calyx larger than the corolla (Rose in Britton and Rose, 1905). While these genera are differentiated based on these morphological features, there is controversy over their recognition (Clausen, 1943; 't Hart and Bleij, 2003; Thiede and Eggli, 2007). By contrast, *Lenophyllum* (Britton and Rose, 1904), a small group from northeastern Mexico and southern USA with decussate leaves, thyrsoid inflorescences, and a putative base chromosome number of 11, has been clearly recognized as a separate genus from *Sedum* (Berger, 1930; Moran, 1994; Uhl, 1996; Thiede and Eggli, 2007). *Villadia* and *Altamiranoa*, two additional genera segregated from *Sedum*, have a broad distribution from the southern USA to South America. These taxa have alternate, small leaves, corollas that are fused basally, and thyrsoid and cymous inflorescences, respectively (Britton and Rose, 1903). Some authors include the species of *Altamiranoa* in *Sedum*, section *Fruticisedum* Berger (Moran, 1996; Thiede and 't Hart, 1999). *Lenophyllum*, *Villadia* and *Altamiranoa*, were initially placed within Berger's (1930) Echeverioideae, but were later transferred to

Table 1
Comparison of several classifications of the taxa comprising the *Acre* clade and *Sedum* subgen. *Gormania*.

Genus	Infrafamiliar classification				Number of species	Distribution	References
	Berger (1930)	Walther (1936)	't Hart (1995)	Thiede and Eggli (2007)			
Cremnophila Rose	Sedoideae/ Echeverioideae	Sedoideae/ Echeverioideae			2	S Mexico	Moran (1978)
Echeveria DC.	Echeverioideae	Echeverioideae			±145	From S USA to Argentina	Walther (1972), Kimnach (2003)
Graptopetalum	Sedoideae	Echeverioideae			19	From S USA to S Mexico	Acevedo-Rosas et al. (2004a,b)
Lenophyllum	Sedoideae	Sedoideae	Sedoideae	Sempervivoideae	7	SE USA and NE Mexico	Moran (1994)
Pachyphytum	Echeverioideae	Echeverioideae	Tribe Sedeae	Tribe Sedeae	17	C Mexico	Thiede and Eggli (2007)
Sedum subgen. *Sedum*	Sedoideae	Sedoideae	Subtribe Sedinae	*Acre* clade	±330	S USA, Mexico, S America, Eurasia and Asia	't Hart and Bleij (2003)
Villadia	Echeverioideae	Sedoideae			±25	S USA, Mexico, Guatemala, Peru	Thiede (2003)
Thompsonella	Echeverioideae (Sect. of *Echeveria*)	Echeverioideae			9	S Mexico	Moran (1992), Carrillo-Reyes et al. (2008)
Sedum subgen. *Gormania*	Sedoideae	Sedoideae	Sedoideae	Sempervivoideae	±110	N. America, Europa, East	't Hart and Bleij (2003)
			Tribe Sedeae Subtribe Sedinae	Tribe Sedeae *Leucosedum* clade		Africa	

Sedoideae (Walther, 1936). However, phylogenetic analyses place them together with several American species of *Sedum* (van Ham and 't Hart, 1998; Mort et al., 2001).

Cremnophila was previously described as an independent monotypic genus from *Sedum*, but its position has been debated. Moran (1978), based on cytological evidence gathered by Uhl (1976b),

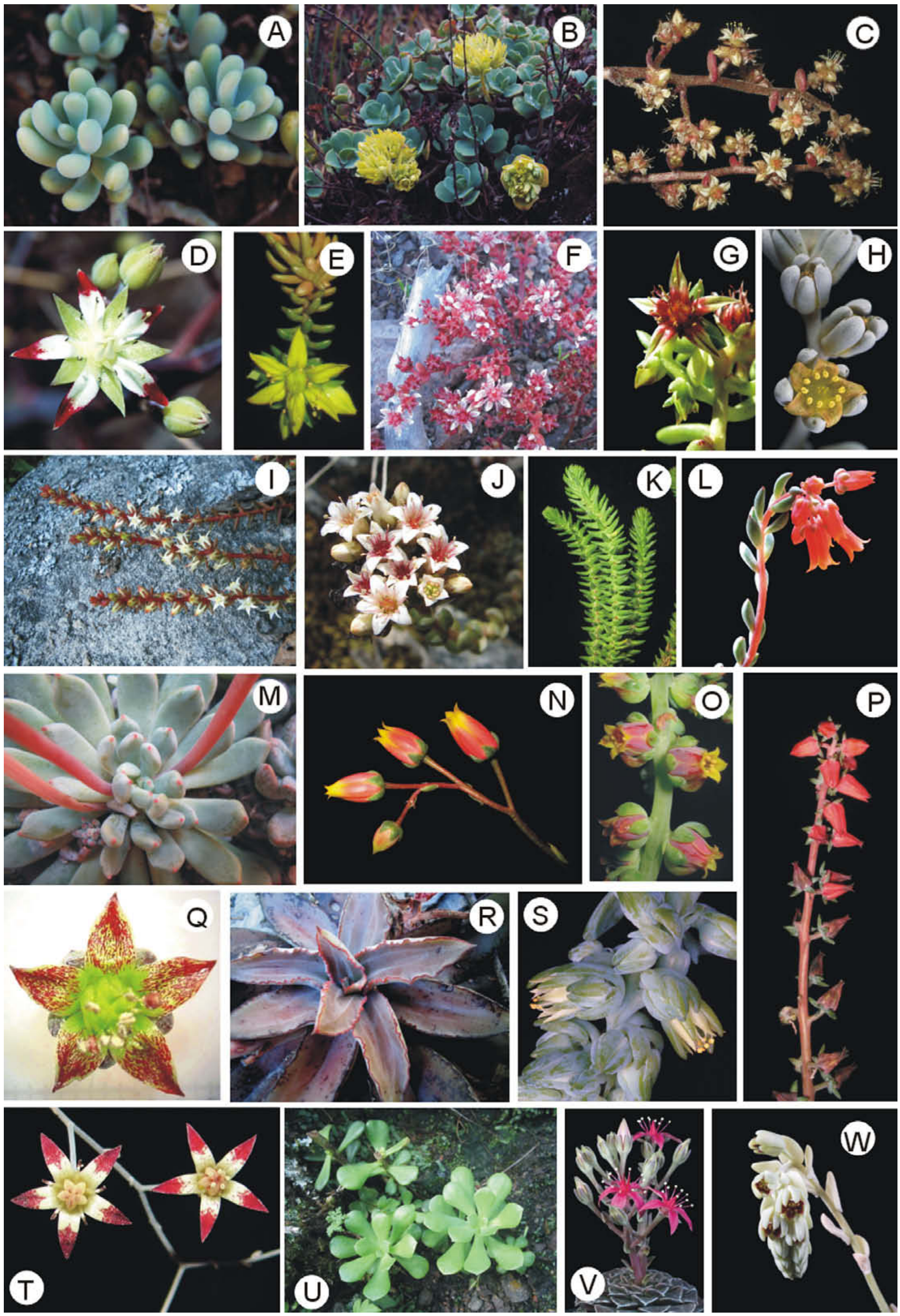

Fig. 1. Morphological variation among American representatives of the *Acre* clade. (A) *Sedum allantoides*; (B) *S. obcordatum*; (C) inflorescences of *S. hemsleyanum*; (D) flower of *S. allantoides*; (E) inflorescence of *S. greggii* ssp. *angustifolium*; (F) *S. vinicolor*; (G) flowers of *S. chloropetalum*; (H) flowers of *Lenophyllum guttatum*; (I) inflorescences of *Villadia recurva*; (J) inflorescence of *V. reniformis*; (K) branches of *V. jurgensenii*; (L) inflorescence of *Echeveria amoena*; (M) rosette of *E. amoena*; (N) inflorescence of *E. purpusorum*; (O) inflorescence of *E. megacalyx*; (P) inflorescence of *E. racemosa*; (Q) flower of *Thompsonella platyphylla*; (R) rosette of *T. nellydiegoae*; (S) flowers of *Cremnophila nutans*; (T) flowers of *Graptopetalum pentandrum*; (U) *G. fruticosum*; (V) *Graptopetalum bellum*; (W) inflorescence of *Pachyphyum werdermanii*.

resurrected the genus and expanded it by including one species of *Echeveria*. *Cremnophila* has been recognized by a number of authors (Meyrán, 1988; Stephenson, 1994; Eggli, 2003; Meyrán and López, 2003), but Thiede and Eggli (2007) consider this taxon part of *Sedum*.

Echeveria is the second largest genus in the *Acre* clade; the genus is comprised of about 145 species that are distributed from the southern USA to northern Argentina. *Echeveria* is recognized by its lateral inflorescences and fleshy flowers with partially fused lobes (Kimnach, 2003). Species of this genus were originally assigned to *Cotyledon*, but later, the New World species with lateral inflorescences were transferred to the *Echeveria* (De Candolle, 1828). Although some genera like *Oliveranthus* and *Urbinia* were segregated from *Echeveria* (Britton and Rose, 1903, 1905), they are now considered part of *Echeveria* and the genus is now divided into 17 series (Walther, 1972; Kimnach, 2003). Recent phylogenetic studies have retrieved species of *Echeveria* clustered with representative taxa of *Cremnophila*, *Graptopetalum*, *Pachypythum*, and *Thompsonella* as well as species of *Sedum* sect. *Pachysedum* (van Ham and 't Hart, 1998; Mort et al., 2001; Acevedo-Rosas et al., 2004a,b; Mayuzumi and Ohba, 2004; Carrillo-Reyes et al., 2008) and the assemblage has been informally named the "*Echeveria* group" (Thiede and Eggli, 2007). Furthermore, these studies indicated that *Echeveria* is paraphyletic, because species of *Graptopetalum*, and *Sedum* sect. *Pachysedum* are nested within the same clade (Acevedo-Rosas et al., 2004a; Carrillo-Reyes et al., 2008). *Graptopetalum* is a small genus of the "*Echeveria* group" from Mexico and the southern USA. Its limits still need to be defined since it was retrieved embedded with representative taxa in *Echeveria* series *Gibbiflorae*, *Cremnophila*, *Sedum* sect. *Pachysedum* and with the monotypic *Tacitus* (Acevedo-Rosas et al., 2004a,b). *Thompsonella* is endemic to Mexico; a genus of *Echeveria*-like plants possessing thyrsoid inflorescences and small flowers with petals with minute red lines (Britton and Rose, 1909; Moran, 1992; Carrillo-Reyes et al., 2008). *Pachyphytum*, a group of approximately 17 species from central Mexico, is recognized by its thick leaves, lateral inflorescences and scale-like appendages on the internal face of the corolla lobes and has been accepted as separate genus from *Sedum* (Britton and Rose, 1905; Berger, 1930; Thiede, 2003).

Thus the *Acre* clade includes two main controversial groups, one allied to *Sedum* and another to *Echeveria*, with segregate genera of uncertain status. The main objective of this paper is to investigate the relationships among the *Acre* clade focusing on New World taxa, in order to better understand the limits of these genera based on analyses of chloroplast and nuclear DNA sequence data.

2. Materials and methods

2.1. Taxon sampling

From the approximately 550 species of the *Acre* clade, 133 representative taxa were selected to represent all the genera of the Acre clade as well as the morphological variation and geographical distribution of the clade. Based on previous phylogenetic analyses of the family (van Ham and 't Hart, 1998; Mort et al., 2001), *Sedum jaccardianum* and *S. modestum* from the *Aeonium* clade as well as two *Dudleya* species from the *Leucosedum* clade were selected as outgroups. *Sedum jaccardianum*, the most distantly related taxon, was used for rooting. Taxa, vouchers and GenBank accession numbers are listed in Table 1; 133 sequences of ETS, ITS and *rpS16* are newly reported for this study. Nomenclature follows Eggli (2003).

2.2. DNA methods and sequence alignment

DNA was extracted from fresh-frozen or silica-gel dried tissues with the DNeasy Plant Mini kit (Qiagen, California, USA). Amplification and sequencing primers were for ETS, 18S-ETS (Baldwin and Markos, 1998) and ETS-IGSf (Acevedo-Rosas et al., 2004a), for ITS, N-nc18S10 and C26A (Wen and Zimmer, 1996), and for rpS16: rps16F and rps16R (Shaw et al., 2005). PCR fragments were purified using QIAquick columns (Qiagen, Valencia, California, USA) according to the manufacturer's protocols. PCR products were sequenced in both directions using the BigDye Terminator Mix (Perkin Elmer Applied Biosystems, Foster City, California, USA) on an ABI 310 automated DNA sequencer (Perkin Elmer Applied Biosystems, California, USA). Sequences of the cpDNA gene *matK* were obtained from GenBank. Contigs were edited and assembled using Sequencher 4.1 (Gene Codes, Ann. Arbor, MI). Alignment of the DNA sequences was mostly unambiguous and was done by eye using Se-Al version 1.0 (Rambaut, 1996).

2.3. Phylogenetic analysis

To evaluate the effect of missing data on the topology, two analyses were performed: an expanded analysis including 137 taxa and a second analysis with only 35 taxa which have three DNA regions (i.e., ETS, ITS, and *rps16*). Parsimony searches were conducted with TNT (Goloboff et al., 2003) using only potentially informative characters. Gaps were coded as missing. "Traditional" searches were carried out with a total of 1000 random sequences using TBR branch swapping and holding 50 trees, followed by more extensive analyses employing TBR and holding 10,000 trees. The most parsimonious trees (MPT) found were saved and a strict consensus topology was calculated using the "Nelsen" option in TNT (Goloboff et al., 2003). Bootstrap support (Felsenstein, 1985) was estimated with 1000 replicates with TBR branch swapping. A Bayesian analysis was also conducted to determine additional clade support given by posterior probabilities (PP). The best-fit molecular model evaluation was selected using Modeltest version 3.7 (Posada and Crandall, 1998), which finds the best fit according to the Akaike information criterion (Akaike, 1974), the TVM + G model was found to be the most appropriate for the combined data matrix. Four Markov chains starting with a random tree were run simultaneously for 1,000,000 generations with MrBayes v. 3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and trees were sampled every 100th generation. The run was set to stop if topological convergence was reached between two runs, as determined by a standard deviation in split frequencies lower than 0.01. Trees were imported into the program PAUP* (Swofford, 2001), and a majority rule consensus tree was computed after discarding the first 25% of the total number of trees, which were saved prior to MCMC convergence.

3. Results

The expanded matrix including all 133 taxa of the *Acre* clade and the four outgroup species comprised 3555 base pairs, of which 963 (27.08%) were potentially parsimony informative. Parsimony analyses recovered 440 MPTs ($L = 5009$, C.I. = 0.35, R.I. = 0.62). The strict consensus tree (Fig. 2) retrieved the *Acre* clade as monophyletic (bootstrap [bts] 61%), and found the *Leucosedum* clade, represented by *Dudleya* as its sister group.

Our analyses recovered four major clades within the *Acre* clade. Two of these are in a grade and correspond to the Asian species of *Sedum*, the rest correspond to a European–Macaronesian group and to an American group.

Asian representatives of *Sedum* are recovered as polyphyletic and formed two clades that are sequentially sister to the remaining members of the *Acre* clade. The first branching clade is comprised of all accessions from *Sedum* sect. *Oreades* and many members of *Sedum* sect. *Sedum*. The second Asian clade includes only representatives of *Sedum* sect. *Sedum* in a clade with moderate support (bts

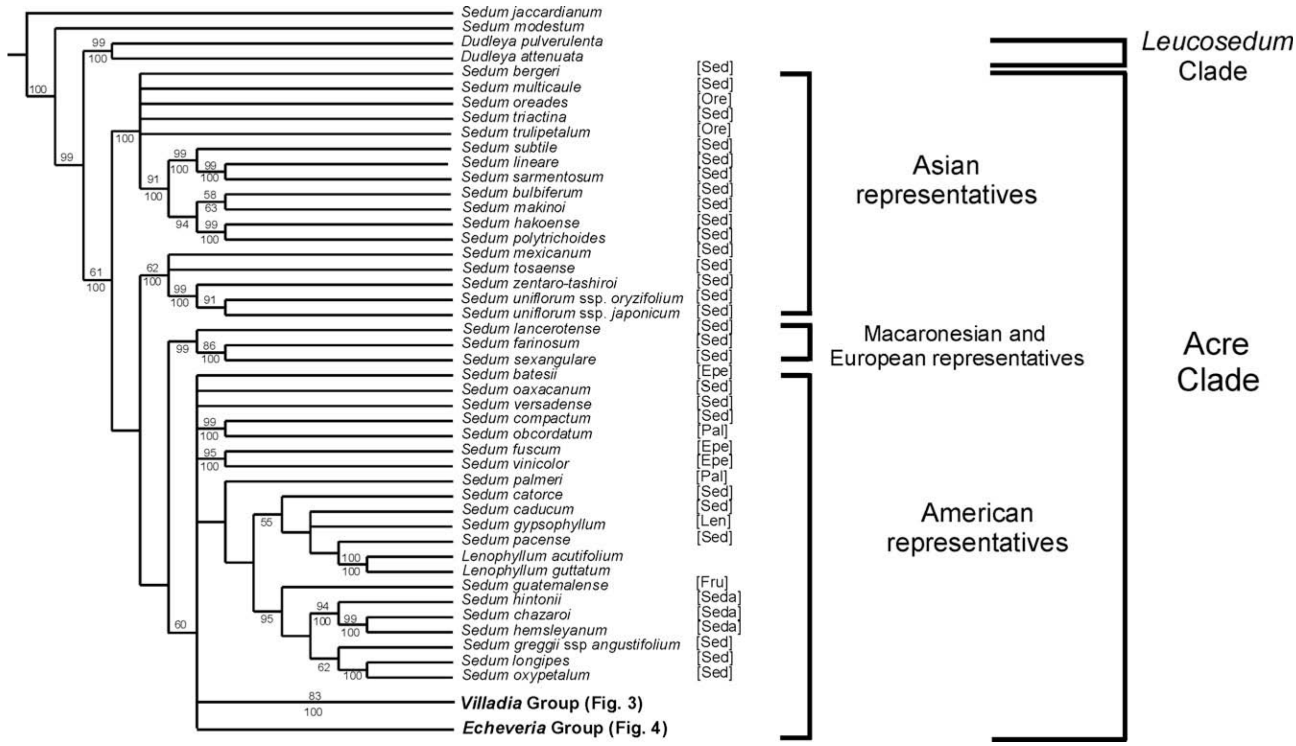

Fig. 2. Strict consensus of 440 MPT retrieved by expanded data matrix (L = 5671 steps, C.I. = 0.34, R.I. = 0.59). Bootstrap values over 50% are indicated above branches. Bayesian posterior probabilities over 89% are indicated below branches. Abbreviations for sections of *Sedum* are: Epe., *Epeteium*; Fru., *Fruticisedum*; Len., *Lenophyllopsis*; Ore., *Oreades*; Pal., "Palmeri"; Sed., *Sedum*; Seda., *Sedastrum*; Topological relationships within the *Villadia* and *Echeveria* groups are found in Figs. 3 and 4.

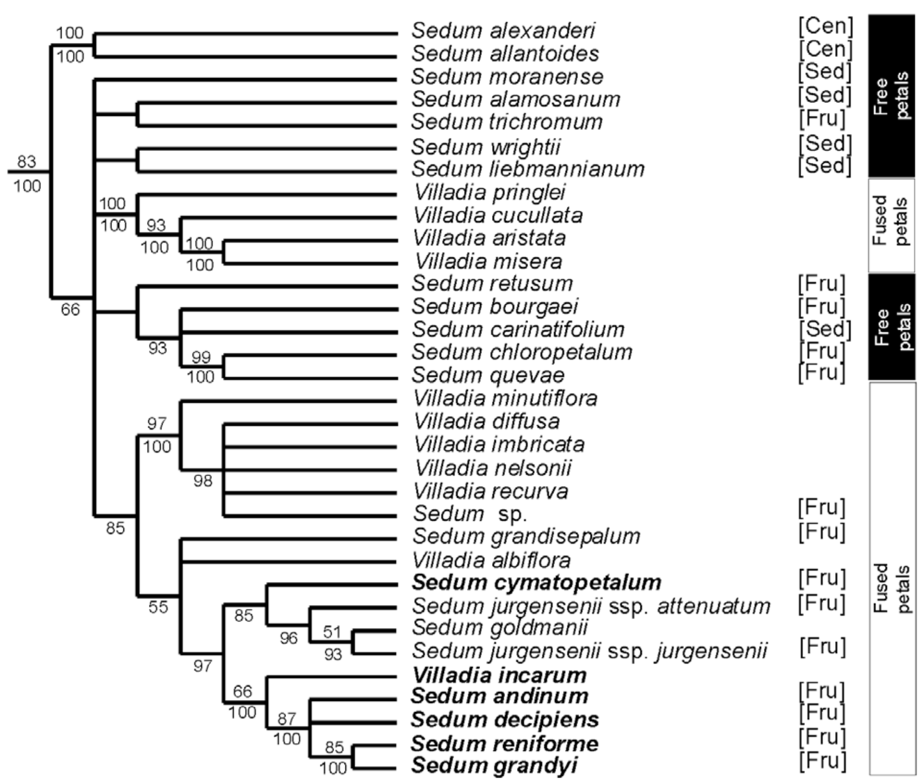

Fig. 3. The "*Villadia* Group". Strict consensus of 440 MPT retrieved by expanded data matrix (L = 5671 steps, C.I. = 0.34, R.I. = 0.59). Bootstrap values over 50% are indicated above branches. Bayesian posterior probabilities over 89% are indicated below branches. Taxa in bold font are representatives from South America. Abbreviations for sections of *Sedum* are: Cen., *Centripetalia*; Fru., *Fruticisedum*; Sed., *Sedum*.

64%) that includes *S. mexicanum*, *S. tosaense*, *S. zentaro-tashiroi* and the two sampled subspecies of *S. uniflorum* (bts < 50%). The European and Macaronesian species of *Sedum* included in this analysis formed a clade (bts < 50%), which was sister to a weakly supported clade (bts 60%) of the American representatives of the *Acre* clade. Within the latter clade, a basal polytomy was recovered, which includes, among others, two subclades: the *Echeveria* group (bts 96%) and a lineage (i.e., the *Villadia* group) that includes *Villadia* species and 21 *Sedum* species (bts 83%). The remaining American representatives of the *Acre* clade are placed in a polytomy that includes species of *Lenophyllum*, representatives of the "*Sedum palmeri* group" (Uhl, 1980), *Sedum* sects. *Epeteium*, *Sedastrum*, and *Lenophyllopsis*, some representatives of sect. *Sedum* (including all accessions of biennial species), and a few representatives of *Sedum* sect. *Fruticisedum*.

The "*Villadia* group" includes some species of *Sedum* sect. *Sedum*, the majority of species of *Sedum* sect. *Fruticisedum*, and all accessions of *Villadia* and *Sedum* sect. *Centripetalia* that were sampled for the present study. Within this clade, *Sedum* sect. *Centripetalia* is strongly supported as monophyletic (bts 100%; Fig. 3). However, *Villadia* as well as *Sedum* sect. *Fruticisedum* were retrieved as paraphyletic. The "*Echeveria* group" was subdivided into two clades, one of them with all the species of *Pachyphytum* (bts 99%). The second clade (bts 84%) was comprised of *Echeveria*, *Thompsonella*, *Graptopetalum* and *Sedum* sect. *Fruticisedum*, but most of these taxa were retrieved as polyphyletic. Also placed within this clade were the two species of *Cremnophila*, which

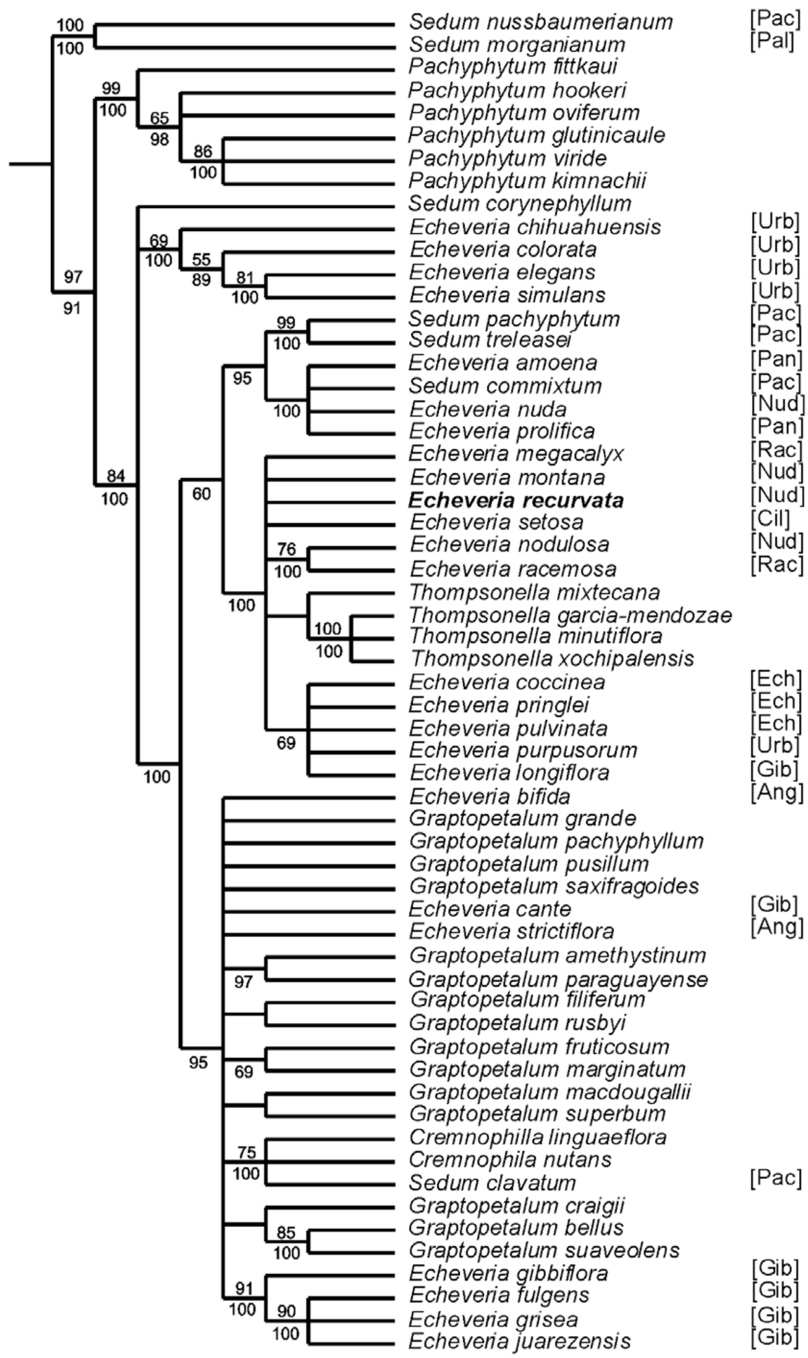

Fig. 4. The "*Echeveria* Group". Strict consensus of 440 MPT retrieved by expanded data matrix (L = 5671 steps, C.I. = 0.34, R.I. = 0.59). Bootstrap values over 50% are indicated above branches. Posterior Bayesian probabilities over 89% are indicated below branches. Taxa in bold font are representatives from South America. Abbreviations for series of *Echeveria* are: Ang., *Angulatae*; Cil., *Ciliatae*; Gib., *Gibbiflorae*, Nud., *Nudae*; Pan., *Paniculatae*; Rac., *Racemosae*; Urb., *Urbiniae*. Abbreviations for sections of *Sedum* are: Pac., *Pachysedum*; Pal., "Palmeri".

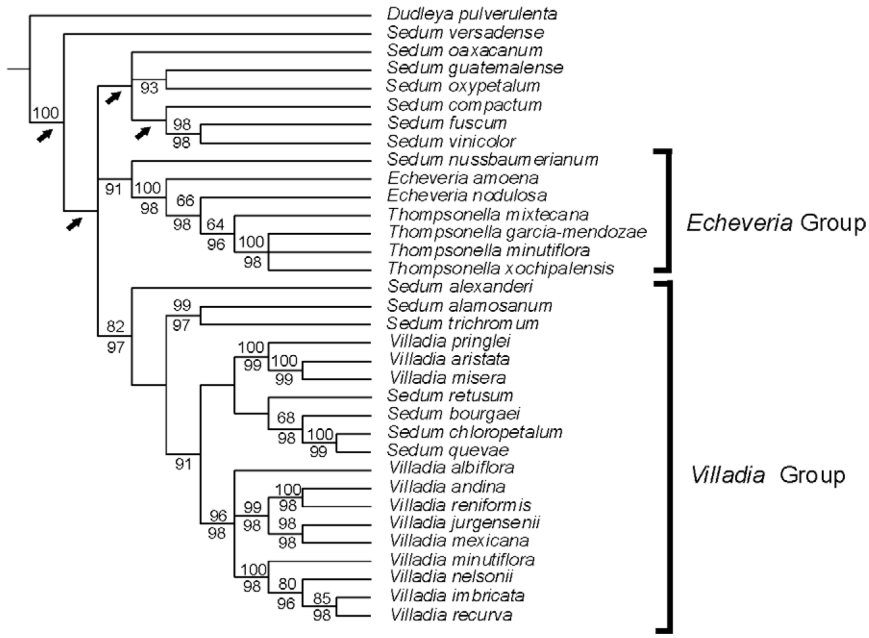

Fig. 5. Strict consensus of 26 MPT retrieved by combined cpDNA + nrDNA data set (L = 1612 steps, C.I. = 0.49, R.I. = 0.58). Bootstrap values over 50% are indicated above branches. Bayesian posterior probabilities over 89% are indicated below branches. Arrows indicates clades that were not recovered by Bayesian inference.

formed a clade (bts 75%) with *Sedum clavatum* (Fig. 4). Many of the relationships within the large clade received moderate to strong support; however, it is noteworthy that there was a high number of polytomies, especially within the *Graptopetalum* clade.

The second set of phylogenetic analyses of 35 taxa, employing chloroplast (*rps16*) and nuclear markers (ETS + ITS), retrieved 26 MPT (*L* = 1602 steps, C.I. = 0.49, R.I. = 0.59). Although only American representatives were included in this analysis, the strict consensus tree (Fig. 5) is largely congruent with the expanded analysis. More clades received support and the *Echeveria* group and the *Villadia* group received a high degree of support (bts 100%). Bayesian analysis recovered a very similar topology differing only in the position of *Sedum versadense*, *S. compactum* and *S. fuscum*, and in the presence of a basal polytomy (Fig. 2).

4. Discussion

The *Acre* clade was still retrieved even with a larger sampling than that of previous phylogenetic studies (Kim et al., 1996; van Ham and 't Hart, 1998; Mort et al., 2001). However, support for the *Acre* clade (61%) is low compared with analyses by van Ham and 't Hart (1998), and similar to the results of Mort et al. (2001). As discussed by the latter, this might be attributable to the greater number of taxa sampled. In addition the clade is also defined by morphological characters such as glabrous plants or if pubescence is present this is of non-glandular trichomes, with reticulate-papillate seeds, and by the occurrence of piperidine alkaloids very often replacing tannins (Stevens et al., 1995; Stevens, 1995; Thiede and Eggli, 2007).

4.1. Asian group

Most of the segregated genera from *Sedum* are distributed in eastern Asia, one of the major centers of diversification in the tribe Sedeae (Stephenson, 1994; Ohba, 1995). Our analyses suggest that this area is the center of origin of the *Acre* clade with a subsequent migration to Europe and North America. Mayuzumi and Ohba (2004) found that the eastern Asian representatives of the *Acre* clade formed a monophyletic group; however, they included only Asian representatives and failed to include taxa from Macaronesia, Europe or America in their sampling. Mort et al. (2001) similarly retrieved the Asian species of *Sedum* in a basal polytomy in the *Acre* clade. Our analysis suggests that the Asian representatives of *Sedum* are paraphyletic and neither *Sedum* sect. *Sedum* nor *Sedum* sect. *Oreades* (Fu, 1974) were supported as monophyletic. Representatives of the former are in a grade in two main groups, while the rest are placed in a basal polytomy within the first branching clade. To determine the taxonomic status of *Sedum* it is necessary to increase the sampling of Asian representatives of this genus, concentrating on the unsampled *Sedum* sect. *Filipes*.

4.2. American species

The American representatives of the *Acre* clade were retrieved as a monophyletic group, and their sister group consisted of European and Macaronesian representatives. As indicated by Mort et al. (2001), the New World clade includes all the taxa of Echeverioideae and the majority of representative taxa of Sedoideae sensu Berger (1930). Among the clades recovered by our analyses, the vast majority of morphological and cytological variation is found in this clade. Among morphologically variable attributes are habit, degree of succulence, leaf arrangement, inflorescence type and position, and floral morphology (e.g., fusion of petals and the size, shape and color of nectarial appendages; (Fig. 1). Our analyses suggest that the American clade is sister to the European–Macaronesian clade, and that migrations to South America occurred at least three times independently in *Echeveria* and *Villadia* from Mexico. Most of the genera of the *Acre* clade are exclusive to the New World, only *Sedum* also occurs in Europe and Asia. Our analysis retrieved an unresolved relationship at the base of the American clade that involves only *Sedum* species.

We sampled three species of *Sedum* sect. *Epeteium* (i.e., *S. batesii*, *S. fuscum*, and *S. vinicolor*), a section characterized by a biennial habit. These plants are tiny and delicate, with thin succulent leaves. Chromosome numbers for most of them are low compared to other the Mexican Crassulaceae (Uhl, 1976a). Whether they represent the ancestral state among the American representatives or not, as well as their monophyly, cannot yet be addressed because the current analyses place them in a polytomy (Fig. 2).

With the exception of *Lenophyllum*, *Pachyphytum* and *Thompsonella*, the majority of the genera in the *Acre* clade were not retrieved as monophyletic. These three genera are easily differentiated from *Sedum* and *Echeveria* by several morphological characters, such as decussate leaves and thyrsoid inflorescences in *Lenophyllum*, fused corollas with nectarial appendages arranged in cincinni in *Pachyphytum*, and thyrsoid inflorescences with red-lineolated petals in *Thompsonella* (Uhl, 1970, 1996; Uhl and Moran, 1973; Moran, 1992). The two species of *Lenophyllum* sampled were recovered as monophyletic with strong support (bts 100%; Fig. 2), which along with the *Villadia* and the *Echeveria* groups forms one of the few well supported clades in the *Acre* clade. *Lenophyllum* is endemic to northeastern Mexico and southern Texas; Moran (1994) suggested that *Lenophyllum* is closely related to *Echeveria*, but our results rather suggest a relationship with *Sedum pacense* and with some other species of *Sedum*, with which *Lenophyllum* shares its geographical range (Northeast Mexico) and a preference for limestone soils (i.e., *Sedum calcicola*, *S. caducum*, and *S. catorce*).

4.3. The Villadia group

Our analyses also recovered a clade (i.e., the "*Villadia* Group") that includes *Villadia*, *Sedum* sect. *Centripetalia* and most of the accessions of *Sedum* sect. *Fruticisedum*. Previous phylogenetic analyses did not identify this clade. This group has two centers of diversity: southern Mexico and the Central Andes (Peru to northern Argentina). *Sedum* sect. *Centripetalia* was proposed to include two species: *S. allantoides* and *S. alexanderi* (Alexander, 1942), these species share morphological and cytological features, such as a sub-shrubby habit, succulence and large leaves, compound pleiochasium inflorescences, distally maculate free petals, and basic chromosomal number $x = 29$ (Moran, 1966; Uhl, 1980). Our analyses recovered this section with strong support (bts 100%) as the sister to the rest of representatives of the "*Villadia* group" (Fig. 3). Given its distinctive morphology and the strong support for its monophyly, *Sedum* sect. *Centripetalia* could be recognized as a separate genus.

The rest of the species of the "*Villadia* group", including all accessions of *Villadia* were in a polytomy, which precludes any conclusions regarding the taxonomic status of these genera (Fig. 3). When *Villadia* was described, another closely related genus, *Altamiranoa* was also named. The former was proposed for plants with thyrsoid inflorescences, whereas the latter was for those with cymous inflorescences (Rose in Britton and Rose, 1903). The recognition and status of *Villadia* and *Altamiranoa* has been highly controversial and is still entirely based on the type of inflorescence (Baehni, 1937; Walther, 1938; Clausen, 1940; Moran, 1996; Thiede and 't Hart, 1999). Our results suggest that *Villadia* is not monophyletic because the taxa from this genus formed two distinct and well supported groups. One of these clades includes species from central and northern Mexico (*Villadia aristata*, *V. cucullata*, *V. misera*, and *V. pringlei*) with thyrsoid inflorescences and basal rosettes. The other *Villadia* clade includes species with caulinar rosettes and variable inflorescences from southern Mexico and South America. This latter clade included all accessions previously placed in *Altamiranoa* (i.e., *Sedum grandisepalum*, *S. jurgensenii*, *S. goldamnii*, *S. decipiens*, *S. andinum*, and *S. grandyi*), our results suggest these taxa would be better considered members of *Villadia*. These species have the same distribution as the rest of the genus and share morphological features such as leaf arrangement, terminal inflorescences, and small tubular flowers. All South American species of Sedoideae sensu Berger (1930) were placed in this group. The Peruvian species (i.e., *Sedum grandyi*, *S. decipiens*, *S. reniforme*, *Villadia andina*, and *V. incarum*) were grouped in a clade (bts 66%) despite having different types of inflorescences. This is contrary to the criterion of placing species with cymous inflorescences into *Sedum* (Moran, 1996; Thiede and 't Hart, 1999; Pino, 2006). The only other South American species, *Sedum cymatopetalum*, from Bolivia and northern Argentina was placed with the Mexican *Villadia jurgensenii* and *V. mexicana* (formerly *Altamiranoa*). A close relationship between *Sedum* sect. *Fruticisedum* and *Villadia* was previously suggested based on morphological and cytological similarities (Uhl, 1980; Uhl and Moran, 1999). Our analyses place most of the accessions of *Sedum* sect. *Fruticisedum* (i.e., *S. bourgaei*, *S. chloropetalum*, *S. quevae*, *S. retusum*, and *S. trichromum*) within the "*Villadia* group". Most of them share leaf type and arrangement with *Villadia*; however, *S. guatemalense* and *S. oxypetalum*, also of section *Fruticisedum* are not placed within the clade corresponding to the "*Villadia* group", which suggests that section *Fruticisedum* is also paraphyletic.

4.4. The Echeveria group

This group was recognized by Thiede and Eggli (2007) as a clade including *Echeveria*, *Graptoptalum*, *Pachyphytum*, *Thompsonella* and *Sedum* sect. *Pachysedum*. The main center of diversity for the "*Echeveria* group" is southern Mexico. *Echeveria* is the only genus from this group that is found in Central and South America, although the diversity of this genus decreases towards southern latitudes. Our analyses recovered a clade corresponding to the "*Echeveria* Group" but only with weak support (<50%) and within this clade only *Thompsonella* and *Pachyphytum* were recovered as monophyletic (Fig. 4).

Pachyphytum is endemic to dry areas in central Mexico and distinguished by scorpioid inflorescences, very succulent leaves and bracts and a nectary scale in the inner face of the petals, although these characters have also been noted from some *Echeveria* species such as *E. heterospela* Rose, *E. longissima* E. Walther, and *E. procera* Moran (Moran, 1967; Walther, 1972). Thiede (2003), based on morphology and cytological evidence gathered by Uhl (1996), hypothesized that *Pachyphytum* species would be nested within *Echeveria*, and placed close to *Echeveria* series *Urceolatae*. Furthermore, he indicated that the genus could be recognized as a section of *Echeveria*. Nevertheless, our analyses clearly supported *Pachyphytum* as an independent genus (Fig. 4) and placed the genus as the sister group of the rest of the "*Echeveria* group". Thus our results suggest that urceolate corollas and nectary scales in the inner surface of petals are homoplastic characters.

Thompsonella is endemic to southern Mexico and characterized by thyrsoid inflorescences and red-lined petals. We recovered a clade with the three species of *Thompsonella* that were included, but this clade is only weakly supported (bts < 50%), although a previous study of the genus using both morphological and molecular data supported its monophyly (Carrillo-Reyes et al., 2008).

Thiede and Eggli (2007) placed the species of *Sedum* sect. *Pachysedum*, a group with free petals, in the "*Echeveria* group". Our results retrieved two species of this section as the sister group to this clade and the rest spread throughout the groups in *Echeveria* and *Cremnophila*. Therefore, further sampling of sect. *Pachysedum* is required to determine its status.

Our results retrieved a supported subclade (bts 75%) formed by the two species of *Cremnophila* and *Sedum clavatum*. The close relationship of *Cremnophila* with *S. clavatum* was suggested in previous studies (Uhl, 1976b; Acevedo-Rosas et al., 2004a; Carrillo-Reyes et al., 2008). Although there are differences in floral morphology, *S. clavatum* shares with *Cremnophila* such characters as a lateral inflorescence, cliff-dwelling habit, and very succulent leaves, as well as having the same geographical range (south-central Mexico) and chromosome number ($n = 33$) (Uhl, 1976b; Moran, 1978). The inclusion of *S. clavatum* along with some other representatives of section *Pachysedum* in *Cremnophila* might be reasonable (Uhl, 1978), but further studies including more species of section *Pachysedum* from central-southern Mexico, such as *Sedum oculiense* Meyrán and *S. orbatum* Moran and Meyrán would be useful in the circumscription of *Cremnophila.*

Unresolved relationships persisted in *Echeveria* and *Graptopetalum.* These two genera were retrieved as paraphyletic in our analyses. The relationships of the latter have been already discussed by Acevedo-Rosas et al. (2004a,b). In *Echeveria* most of the representative taxa in series *Gibbiflorae* were retrieved in a well supported clade (bts 91%) (except *Echeveria longiflora*). Further analyses should include representatives of several series of the genus that have not been previously sampled, such as *Chloranthae*, *Longistylae*, *Mucronatae*, *Occidentales*, *Pruinosae*, *Secundae*, *Spicatae*, *Thyrsiflorae*, and *Valvatae*.

4.5. Paraphyly of Sedum

Since its establishment by Linnaeus, *Sedum* has had diffuse limits and has been difficult to circumscribe based on morphological characters. Phylogenetic analyses have shown that the genus is paraphyletic (van Ham and ’t Hart, 1998; Mort et al., 2001; Acevedo-Rosas et al., 2004a,b; Carrillo-Reyes et al., 2008) and that it has been defined by plesiomorphic attributes. Elucidating the limits and relationships of *Sedum* is the main challenge in the systematics of Crassulaceae. Our analyses placed *Sedum* species throughout the *Acre* clade embedded in clades with species of *Cremnophila*, *Graptopetalum*, *Echeveria*, *Lenophyllum* and *Villadia*, but there is a general lack of resolution and support for many of these placements. Therefore, to define the monophyletic segregate genera of *Sedum*, additional data and increased taxonomic sampling is needed. With further analyses the status of *Corynephyllum*, *Altamiranoa* and *Cremnophila* will become clear. However, *Sedum* sect. *Centripetalia* (see above) is perhaps a monophyletic group with clear diagnostic characters that merits generic recognition. Our analysis placed the three accessions of *Sedum* sect. *Sedastrum* (i.e, *Sedum chazaroi*, *S. hemsleyanum*, and *S. hintonii*) grouped in clade with strong support (Fig. 2). This section is defined by characters such as dense basal rosettes formed by usually pubescent leaves, paniculate inflorescences and a nectarial cavity (Clausen, 1943; Uhl, 1992a). *Sedum* sect. *Sedastrum* is comprised of six species (Pérez-Calix, 1998; Carrillo-Reyes and Lomelí-Sención, 2008) distributed from northern Mexico to Central America (Clausen, 1943; Uhl, 1992a). Thus, this is another group that could be recognized as a genus separate from *Sedum.*

4.6. Evolution of sympetaly

The degree of fusion of the corolla lobes has been widely used as a diagnostic character at generic or higher levels in Crassulaceae. Together with distribution patterns, position of leaves and number of floral elements, sympetaly was among the most important features taken into account by Berger (1930) for defining subfamilies in Crassulaceae (Mort et al., 2001). However, cytological evidence indicated that corolla characters have only limited taxonomic value in Crassulaceae (Uhl, 1978). New phylogenetic approaches likewise demonstrated multiple origins for sympetaly. For example, ’t Hart et al. (1999) found that sympetaly evolved independently at least eight times among the European Crassulaceae. Mort et al. (2001) found that sympetaly, a widespread character within the *Acre* clade, arose in at least five lineages of Crassulaceae. Our analyses indicate that sympetaly has arisen independently at least two times, once in the *Echeveria* group and once in the *Villadia* group, the only two clades with representatives in South America. Mort et al. (2001) noted that no reversals occurred, but in contrast, our results found that at least three reversions occurred in the ‘‘*Echeveria* group” involving representatives of *Sedum* sect. *Pachysedum*. Although sympetaly is a highly homoplastic character, it could be a useful diagnostic character in the recognition of groups such as *Echeveria*, *Graptopetalum*, *Pachyphytum*, *Villadia*, and *Thompsonella*. Sympetaly gives stability to floral architecture and provides an enormous potential for the diversification of flowers (Endress, 2001). In the *Acre* clade, sympetaly may have allowed for novel interactions with pollinators that facilitated the radiation of Crassulaceae in Mexico and the colonization of the Andes cordillera.

Although our analysis recovered a broad overview of the *Acre* clade, the first to date, further studies are necessary, particularly those that focus on the groups poorly represented here such as the European and Asian species of *Sedum*, as well as Central and South American species of *Echeveria*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.05.022.

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