

# The Phylogenetic Position of Eastern Asian Sedoideae (Crassulaceae) Inferred from Chloroplast and Nuclear DNA Sequences

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**ABSTRACT.** The phylogenetic position of 74 taxa of Asian Sedoideae (Crassulaceae) was estimated based on analyses of the *trnL-trnF* intergenic spacer sequence of cpDNA and the ITS region, including the 5.8S rDNA sequence, of nDNA. Our analyses suggest that the subfamily Sedoideae is not monophyletic and the genus *Sedum* especially is highly paraphyletic. Recent studies based on cpDNA restriction-site variation and the *matK* region recognized seven clades of “major importance.” In our analyses, four major clades were recovered: *Kalanchoe*, *Sempervivum*, *Aeonium*, and *Acre*. All Asian *Sedum* (s.s.) tested are included in the *Acre* clade, and all other eastern Asian genera are in groups corresponding to the *Telephium* clade. The *Telephium* clade is split into four strongly supported subclades, *Rhodiola*, *Hylotelephium*, *Phedimus*, and *Umbilicus*, and is determined to be polyphyletic. All eastern Asian genera that were segregated from *Sedum* are separated in a lineage distinct from *Sedum* (s.s.). *Hylotelephium*, *Orostachys*, *Meterostachys*, and *Sinocrassula* are closely related, and *Hylotelephium* and *Orostachys* are polyphyletic. The infrageneric taxa within *Hylotelephium* and *Rhodiola* are indicated to be polyphyletic.

The Crassulaceae comprise about 1,500 species (Berger 1930) and has a nearly cosmopolitan distribution with centers of diversity in Mexico, South Africa, Macaronesia, and eastern Asian. Most species exhibit xerophytic adaptations (e.g., succulent leaves, a thick waxy cuticle, and crassulacean acid metabolism) and they inhabit primarily semiarid and mountainous habitats. The family has been considered a natural group and closely affiliated with the Saxifragaceae and Penthoraceae (Takhtajan 1980; Cronquist 1981; Thorne 1983) and recent molecular phylogenetic analyses support that assessment (Chase et al. 1993; Soltis et al. 2000). While the Crassulaceae are easily recognized, defining monophyletic groups within the family has been extremely difficult because the morphological features traditionally used for classification are highly homoplastic (Ham 1995). The most comprehensive treatment of the Crassulaceae is that of Berger (1930). He recognized six subfamilies and 33 genera in the family based on the number and arrangement of floral parts, the degree of sympetaly, and phyllotaxis. Because of its comprehensiveness and great practical value, Berger's classification has been the authoritative work on the family and has been the most widely followed despite the general conviction that it is highly artificial.

The core of the systematic problems in the Crassulaceae lies in the generic and infrageneric classification in subfamily Sedoideae. The Sedoideae comprise *Sedum*, the largest genus in the family with ca 470 species and nearly cosmopolitan in distribution, and several smaller genera (Table 1). Ideally, *Sedum* comprises the herbaceous, predominantly perennial, Crassulaceae with alternate and entire leaves with a single abaxial subapical hydathode, and 5-merous, obdiploste-

mous flowers with free petals ('t Hart and V. Bleij 2003). The genus encompasses a broad range of species, many of which appear transitional, thus the infrageneric taxonomy has been difficult. Cytological studies (e.g., Uhl 1948, 1963, 1978, 1992; 't Hart and Eggli 1988) and hybridization experiments (Uhl 1976, 1992; 't Hart 1991) with species of European and Mexican *Sedum* have been carried out across subfamilial boundaries within Echeverioideae and Cotyledonoideae. These studies clearly showed that species of *Sedum* possess a wide range of chromosome numbers due to rampant polyploidy and aneuploidy. Cytological criteria have not played a major role in systematic treatments of Sedoideae due to this high degree of diversity, except in some European groups. For example, the segregate genera *Prometheum* and *Rosularia* are distinguished from *Sedum* (s.s.) by their basic chromosome number ('t Hart 1995). Furthermore, cytological and crossing data are still insufficiently known in Asian Sedoideae. Consequently, opinions about the delimitation and infrageneric classification of *Sedum* are highly divergent. Various classifications of the subfamily have been proposed and there are two opposing viewpoints (Ohba 1978). One view retains *Sedum* as a catchall taxon and recognizes only a few additional genera under the Sedoideae (e.g., Praeger 1921; Fröderström 1930, 1931, 1932, 1936; Clausen 1959; 't Hart 1982). The other view segregates some groups as genera (e.g., *Rhodiola*, *Hylotelephium*, *Phedimus*) from *Sedum* on the basis of unique sets of morphological characters and recognizes a number of genera within the Sedoideae (e.g., Berger 1930; Borissova 1969; Ohba 1978; Table 1). It is necessary to resolve these conflicting viewpoints by testing the monophyly of genera separated

TABLE 1. The genera of Sedoideae recognized by Ohba (1978, 2000) with approximate number of species and world distribution.

<i>Sedum</i> —c.470 spp., N hemisphere, S America, N Africa
<i>Orostachys</i> —c.15 spp., C-E Asia
<i>Hylotelephium</i> —c.30 spp., N hemisphere
<i>Rhodiola</i> —c.60 spp., Himalaya, Siberia, Europe, N America
<i>Phedimus</i> —c.20 spp., Asia, Europe
<i>Sinocrassula</i> —c.3 spp., Himalaya
<i>Meterostachys</i> —1 sp., E Asia
<i>Pseudosedum</i> —c.10 spp., C Asia
<i>Rosularia</i> —c.20 spp., E Europe–Himalaya, Altai
<i>Prometheum</i> —c.8 spp., E Europe, Central Asia
<i>Perrierosedum</i> —1 sp., Madagascar

from *Sedum* and clarifying their phylogenetic relationships to *Sedum* (s.s.).

Molecular phylogenetic studies based on cpDNA restriction site data from 44 species of 19 genera and *trnL-F* intergenic spacer sequences from 49 species of 26 genera concluded that many of the subfamilies proposed by Berger (1930) are not monophyletic and detected seven major clades within Crassulaceae; viz., the *Crassula*, *Kalanchoe*, *Telephium*, *Sempervivum*, *Aeonium*, *Leucosedum*, and *Acre* clades (Ham 1995; 't Hart 1995; Ham and 't Hart 1998, 1999). In their analyses, *Sedum* is placed in five of seven major clades and is interpreted to be highly polyphyletic. Within these four clades, *Sedum* includes the least specialized species defined only by plesiomorphic features, forming a

basal paraphyletic grade within all four clades. The representatives of the eastern Asian genera (*Hylotelephium*, *Rhodiola*, and *Phedimus*) placed in *Sedum* by some authors are separated in a lineage distinct from *Sedum* (s.s.) and comprise the *Telephium* clade, which includes the genus *Umbilicus*, formerly placed in subfamily Cotyledonoideae. The other two genera from central Asia, *Rosularia* and *Prometheum*, also segregated from *Sedum*, are included in the *Leucosedum* clade. These results are largely confirmed in a recent study based on cpDNA *matK* sequence data from 112 species of 33 genera (Mort et al. 2001). Based on these results, 't Hart (1995) suggested a revised classification of the family (Fig. 1). In his new classification, only two subfamilies are upheld: Crassuloideae and Sedoideae. His Crassuloideae corresponds closely to Berger's Crassuloideae and his Sedoideae comprise the other five subfamilies of Berger (1930). 't Hart restricted *Sedum* to the species of the *Sempervivum*, *Aeonium*, *Leucosedum*, and *Acre* clade and advocated the retention of a united, albeit paraphyletic, *Sedum* for the sake of nomenclatural stability. In his classification, the *Telephium* clade is treated as one taxon, subtribe Telephinae. However, the *Telephium* clade receives only 25% bootstrap support in the analyses of Ham (1995) and Ham and 't Hart (1998). Mort et al. (2001) also did not recover the *Telephium* clade although there was a single indel that appeared to be diagnostic for the clade. Although these molecular phylogenetic studies provided initial

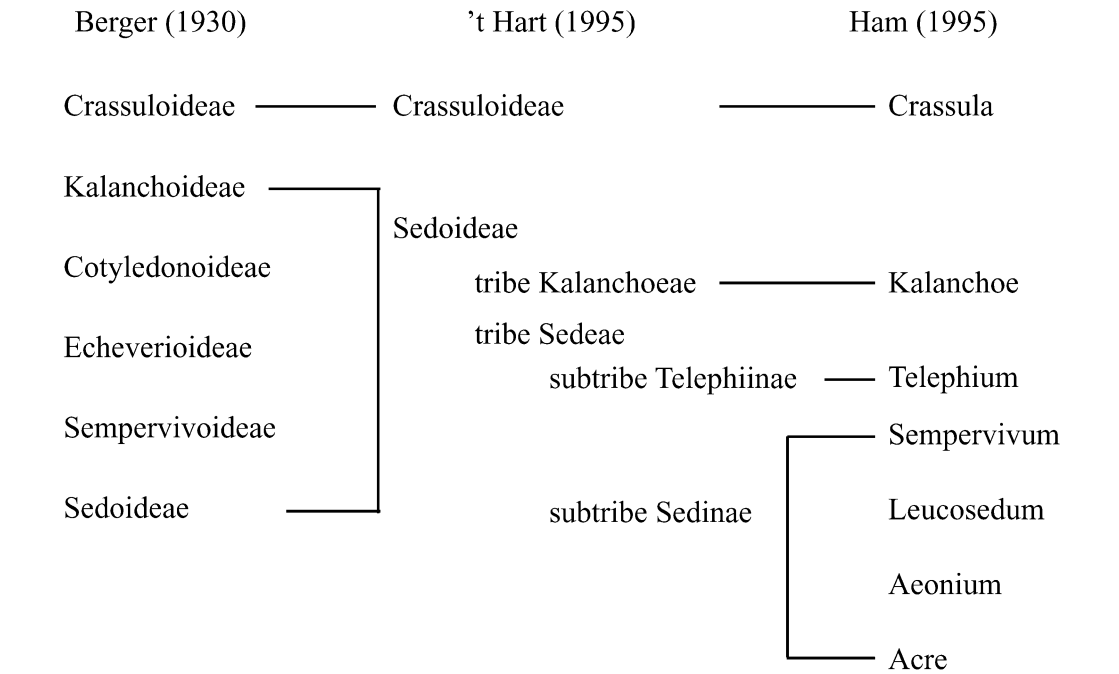


FIG. 1. Subfamilial classification of Crassulaceae by Berger (1930) and the revised classification by 't Hart (1995). The clades recognized by cpDNA restriction analysis (Ham 1995) are also indicated. 't Hart's (1995) revised classification is based on Ham (1995).

phylogenetic insights and a tentative classification of the Crassulaceae, generic boundaries and relationships in Sedoideae are still uncertain due to limited sampling within each genus.

About 300 species of Sedoideae are distributed in the eastern Asian region (Ohba 1978) and most genera segregated from *Sedum* are applied to eastern Asian species. This region can therefore be regarded as both a center of generic and infrageneric diversification and a center of distribution for the Sedoideae (s.s.). It is necessary to clarify the phylogenetic position of species described from this particularly rich area to fully understand the systematics of the family. In this study, we estimated the phylogeny of the Sedoideae on a broad scale across eastern Asia using sequence variation of the cpDNA *trnL-trnF* spacer and the internal transcribed spacer region (ITS) of the nuclear ribosomal repeat to clarify the phylogenetic position of the eastern Asian Sedoideae and determine their generic boundaries.

## MATERIALS AND METHODS

**Taxonomic Sampling, Selecting Sequences, and Defining Outgroups.** The most widely accepted classification within Sedoideae, first outlined by Ohba (1978), recognized 10 genera of Asian Sedoideae. Later, Ohba (2000) added another genus, *Pseudosedum*, to the subfamily. We follow that classification (Table 1). A list of taxa used in this study is given in Appendix 1. The 74 taxa sampled are from all seven of the eastern Asian genera plus two species of *Pseudosedum* from central Asia. We also sampled three species of *Umbilicus* of subfamily Cotyledonidae, because the genus is related to Asian Sedoideae according to analysis based on cpDNA restriction site (Ham 1995; Ham and 't Hart 1998) and *matK* sequence data (Mort et al. 2001).

We sequenced the *trnL* (UAA)-*trnF*(GAA) intergenic spacer of cpDNA (*trnL-F*) and the ITS regions including 5.8S gene of nuclear DNA. The *trnL-F* sequence was chosen for analysis because many studies have documented the utility of this spacer region for resolving phylogenetic relationships at infrafamilial and generic levels within the Crassulaceae ('t Hart 1995; Mes and 't Hart 1996; Mes et al. 1996; Kim et al. 1996; 't Hart and Alpinar 1999) and those results were largely consistent with the phylogeny based on cpDNA restriction site variation (Ham 1995; Ham and 't Hart 1998). To determine the phylogenetic position of the Asian Sedoideae within the family, we added 31 *trnL-F* sequences obtained in previous studies from across all six subfamilies, as well as all seven major clades recognized by Ham (1995), including two from other genera (*Rosularia* and *Prometheum*) of Sedoideae distributed mainly in central Asia. The ITS region has been successfully used for phylogenetic reconstruction at the generic and species level within Crassulaceae in several studies (e.g., Mes et al. 1996; Gehrig et al. 2001; Mort et al. 2002). We used those sequences, including the 5.8S gene sequence, to determine relationships among closely related genera of Sedoideae. Nine ITS sequences obtained by Gehrig et al. (2001) and Mort et al. (2002) also were added to the analyses.

Within Crassulaceae, a basal split between subfamily Crassuloideae and the other subfamilies was strongly supported by previous studies (Ham and 't Hart 1998; Mort et al. 2001). Therefore, in this study, two species (*Crassula multicava* and *Tillaea alata*) of Crassuloideae were used as outgroups for the analyses based on *trnL-F* sequences. The results obtained from the *trnL-F* sequence analyses indicated that all species of Sedoideae tested are included in a strongly supported clade with the sister group comprising

the Kalanchoideae. Therefore, four species of *Kalanchoe* were used as outgroups for the analyses based on ITS sequences.

**DNA Isolation, Amplification, and Sequencing.** Fresh leaves of individual samples of each taxon were dried in silica-gel, ground to a powder in liquid nitrogen, and used for total DNA isolation following a modified CTAB procedure for micropreparations (Doyle and Doyle 1987; herbarium specimens of some taxa were sampled when fresh material was unavailable. PCR amplification employed the universal primers B49873 and A50272 (Taberlet et al. 1991) for the *trnL-F* sequence, 5F and 4R (White et al. 1990) for the ITS sequence. The PCR program consisted of an initial 94°C heating step for three min, followed by 40 cycles at 94°C for one min, 50°C for one min, and 72°C for two min with an additional six min at 72°C following the final cycle. For purification, PCR products were run on agarose gels and then recovered with the help of QIAquick PCR Purification Kit (QIAGEN Corp.). Direct sequencing was performed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Inc.), using the PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.) for cycle sequencing reactions following the manufacturer's instructions. The same primer combinations used for amplification were also used for cycle sequencing. Double-stranded PCR products were sequenced in both directions and both strands were sequenced with a minimum overlap of ~80% of total sequence length.

**Phylogenetic Analyses.** Sequences were aligned with clustalX version 1.18 (Thompson et al. 1994) and were manually corrected. All phylogenetic analyses were conducted using PAUP\* version 4.0b10 (Swofford 1998). Parsimony analyses were conducted independently for the two datasets, *trnL-F* and ITS data, followed by an analysis of the combined data that included only those sampled for both datasets (78 in all). Before combining sequence data, separate phylogenetic analyses were conducted for resampled *trnL-F* and ITS data sets to test the congruence between the two datasets. There were no inconsistent tree topologies between the two data sets, so we combined them. We did not, however, assess the congruence between the two data sets because of a considerable polytomy in the *trnL-F* tree. The data sets are available on TreeBASE ([www.treeBASE.org](http://www.treeBASE.org); study accession number S1033; matrix accession numbers M1754, M1755, M1756). In all parsimony analyses all characters were weighted equally and indels were treated as missing data. Heuristic searches with MULTREES and TBR branch swapping were conducted. Starting trees were constructed using 1,000 replicates of random addition sequence. The number of trees retained in a replication was restricted to 1,000 because an excessive number of trees was generated for each replicate.

Support for individual nodes was assessed with bootstrap values (Felsenstein 1985) and decay indices (Bremer 1988; Donoghue et al. 1992). For the bootstrap analyses PAUP\* was set to run 1,000 replicates with ten replicates of random addition sequence and NNI branch swapping, saving a maximum of 1,000 trees per random addition replicate. Decay values were obtained using AutoDecay 4.0 (Eriksson 1999). In the reverse constraint runs for the decay analyses PAUP\* was set to run 10 replicates of random addition sequence with TBR branch swapping, saving 1000 trees in each replicate.

Although we only present results from the parsimony analyses, alternative methods (neighbor joining and maximum likelihood based on various substitution models) produced similar results that did not affect the conclusions reached (data not shown).

## RESULTS

***trnL-F* Data.** The 103 sequences obtained from across all six subfamilies of Crassulaceae vary from 249 to 294 bp in length, and the aligned sequences exhibit a total length of 380 sites. Insertions/deletions (indels) range from 1–22 bp in length and are scattered throughout the region. Most of the indels exhibit homoplasy or are autapomorphic and not phylogeneti-

cally useful in the context of the taxa sampled for this study. In the MP analysis, 157 of 380 sites are constant, 81 variable sites are parsimony-uninformative, and 142 sites are parsimony-informative. A total of 86,000 equally parsimonious trees was found by heuristic searches. Those trees have a length of 505 steps, CI = 0.69, and RI = 0.89. The strict consensus tree of those trees is presented in Fig. 2, along with bootstrap percentages and decay values.

The general topology and major lineages within Crassulaceae nearly agree with the results based on cpDNA restriction-site variation (Ham 1995; Ham and 't Hart 1998) and the *matK* (Mort et al. 2001). In our analyses, the *Kalanchoe* (74% bootstrap, decay 26), *Aeonium* (92% bootstrap, decay 10), *Sempervivum* (100% bootstrap, decay 15), and *Acre* (bootstrap 94%, decay 23) clades were recovered, but the *Telephium* and *Leucosedum* clades of Ham (1995) were not resolved (<50% bootstrap). The *Kalanchoe* clade was the first-branching lineage of the ingroup and the remaining ingroup forms a clade with 95% bootstrap and decay value of 39. The *Acre*, *Leucosedum*, *Sempervivum*, and *Aeonium* clades comprise one clade (64% bootstrap, decay 2). Ham's *Telephium* clade is split into four strongly supported subclades, which we refer to by the major genus in each clade, the *Hylotelephium*, *Rhodiola*, *Phedimus*, and *Umbilicus* clades. The *Hylotelephium* clade (99% bootstrap, decay 11) includes four genera, *Sino-crassula*, *Meterostachys*, *Orostachys*, and *Hylotelephium*. The *Rhodiola* clade (76% bootstrap, decay 5) includes two genera *Rhodiola* and *Pseudosedum*. The *Phedimus* (84% bootstrap, decay 6) and *Umbilicus* clade (100% bootstrap, decay 9) clades comprise only one genus each, *Phedimus* and *Umbilicus* respectively. The relationships between the four subclades of the *Telephium* 'clade' were not resolved. All species of Asian Sedoideae are included in either the *Telephium* group or the *Acre* clade.

**ITS Data.** The data set includes 74 taxa of eastern Asian Sedoideae, three of *Umbilicus*, five taxa from Macaronesia, and four of *Kalanchoe* as the outgroup. The sequences varied from 649 to 688 bp in length and the aligned sequences contained a total length of 776 sites. Positions 286–295, 477–515, and 650–691 were excluded from the analysis because those regions could not be aligned easily because of excessive variation in indels. A total of 642 sites were used for analyses. Except for the excluded regions, indels range from 1–3 bp in length and are scattered throughout the region. The indels are autapomorphic and are not phylogenetically useful. In the MP analysis, 274 sites are constant, 60 variable sites are parsimony-uninformative, and 308 sites are parsimony-informative. A total of 874 equally parsimonious trees were found, having a length of 1,542 steps, CI of 0.44, and RI of 0.83. The strict con-

sensus tree of the 776 trees is presented with bootstrap percentages and decay values in Fig. 3.

Six clades are recognized with strong support by bootstrap and decay values, corresponding to the *Hylotelephium*, *Rhodiola*, *Phedimus*, *Umbilicus*, *Aeonium*, and *Acre* clades in the *trnL-F* trees. Within the ingroup, the first split separated the *Hylotelephium* clade with 97% bootstrap and a decay value of 63. The remaining five subclades form one clade that is supported strongly by decay analyses (decay 30), but weakly by bootstrap (52%). This clade is separated into two clades, one comprising the *Umbilicus*, *Rhodiola*, and *Phedimus* clades with 68% bootstrap and a decay value of 31. The *Rhodiola* clade (93% bootstrap, decay 12) and the *Phedimus* clade (100%, decay 28) form a strongly supported clade (99% bootstrap, decay 31), which is sister to the *Umbilicus* clade. The other comprises the *Aeonium* (100% bootstrap, decay 23) and *Acre* clades (100% bootstrap, decay 51) and is supported by 89% bootstrap and a decay value of 30.

In the *Hylotelephium* clade, the genera *Hylotelephium* and *Orostachys* are not monophyletic. The genus *Rhodiola* comprises a weakly supported clade (41% bootstrap, 3 decay) that is sister to a clade comprising the genus *Pseudosedum* of the *Rhodiola* clade.

**Combined Data.** Before combining, each data set was reduced to 78 taxa to achieve homogeneity between data sets. Because of the large difference in the number of taxa between the original data set and the reduced one, we conducted phylogenetic analyses again for each resampled data set to test congruence against the original one. Both in *trnL-F* and ITS data sets, the topology of the strict consensus tree based on the reduced data set is almost equivalent with that based on the original one. There was no inconsistency in tree topology between resampled *trnL-F* and ITS.

The combined *trnL-F*/ITS data set includes 989 sites (347 from *trnL-F*, 642 from ITS), of which 396 sites (93 from *trnL-F*, 303 from ITS) are parsimony-informative. Parsimony analyses recovered 6,738 minimum-length trees of 1,723 steps (CI = 0.49, RI = 0.84). The strict consensus of these trees is presented with bootstrap percentages and decay values in Fig. 4. The topology of the strict consensus tree based on the combined data set is identical to that based on the ITS data set, but many nodes are more strongly supported.

## DISCUSSION

### *Phylogenetic Position of Eastern Asian Sedoideae.*

Previous studies based on cpDNA restriction site data and *trnL-F* sequences detected seven major clades within the Crassulaceae; viz., the *Crassula*, *Kalanchoe*, *Telephium*, *Sempervivum*, *Aeonium*, *Leucosedum*, and *Acre* clades (Ham 1995; 't Hart 1995; Ham and 't Hart 1998). Within the Crassulaceae, a well-supported basal split exists between the *Crassula* clade and the rest of the

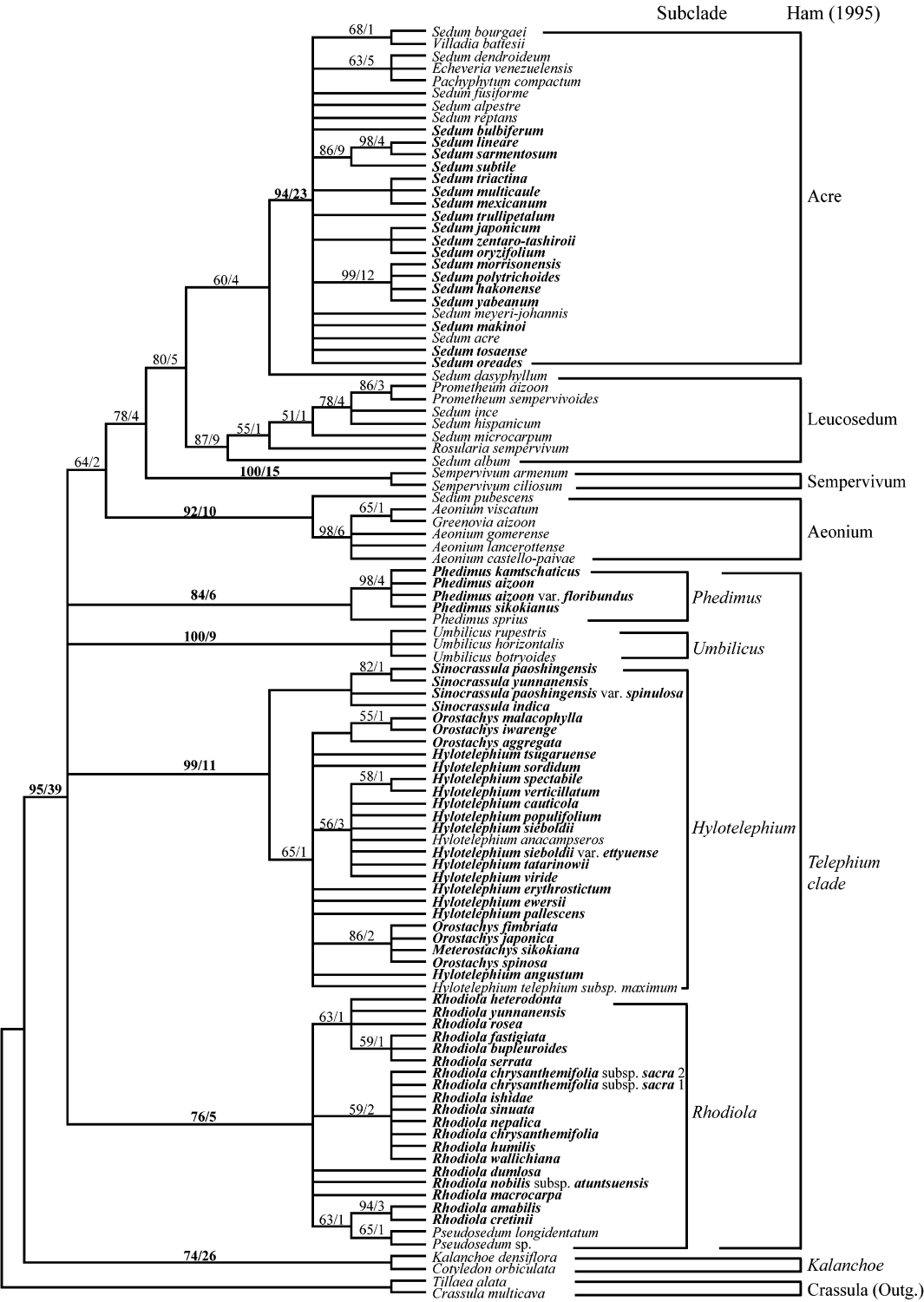
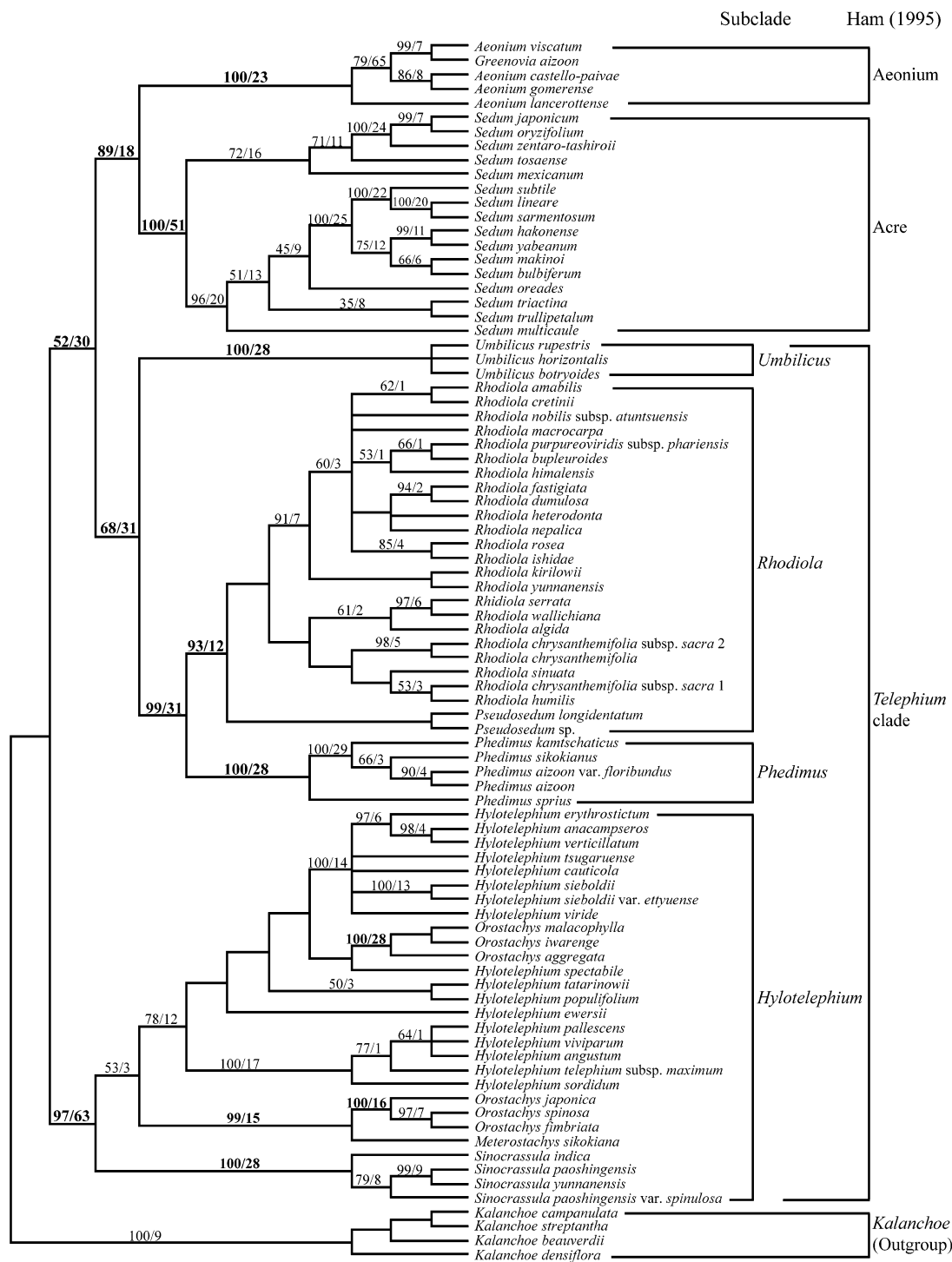


FIG. 2. The strict consensus tree of 86,000 MP trees based on *trnL-F* sequences of 103 Crassulaceae species, using *Crassula multicaeva* and *Tillaea alata* as outgroups. Bootstrap/Decay support values are indicated at corresponding nodes. Major clade names are those applied previously by Ham (1995). The east Asian species are marked in bold.



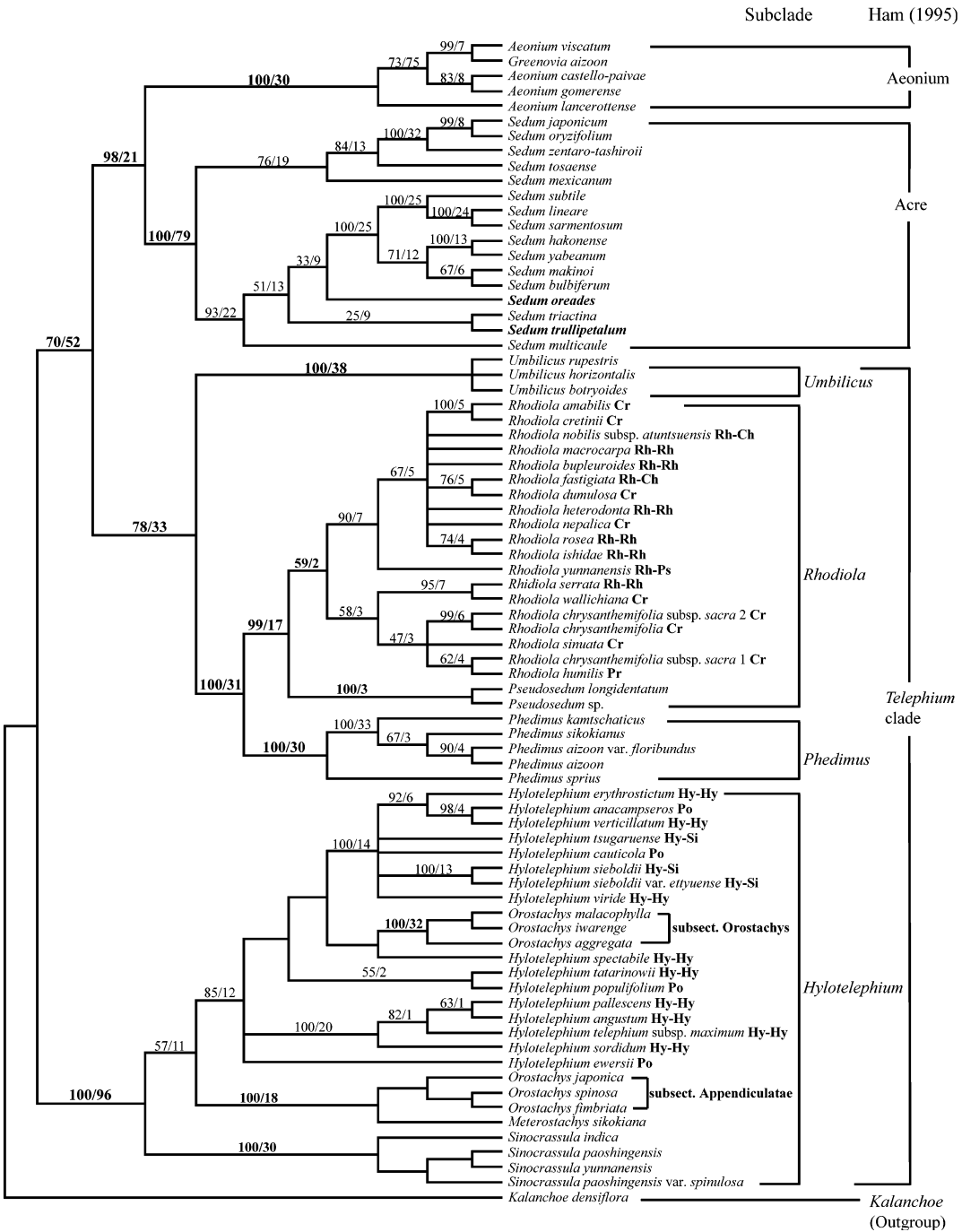


FIG. 4. The strict consensus tree of 6,738 MP trees based on combined data, *trnL-F* and ITS, from eastern Asian Sedoideae, genus *Umbilicus*, Macaronesian species, and *Kalanchoe densiflora* as the outgroup. Bootstrap/Decay support values are indicated at corresponding nodes. Major clade names are those applied previously by Ham (1995). Two species of *Sedum* sect. *Oreades* are marked in bold. Abbreviations for infrageneric taxa are as follows: genus *Rhodiola*; Cr (subgen. *Crassipedes*), Pr (subgen. *Primuloides*), Rh-Rh (subgen. *Rhodiola* sect. *Rhodiola*), Rh-Ch (subgen. *Rhodiola* sect. *Chamaerhodiola*), Rh-Ps (subgen. *Rhodiola* sect. *Pseudorhodiola*); genus *Hylotelephium*; Po (sect. *Populisedum*), Hy-Hy (sect. *Hylotelephium* ser. *Hylotelephium*), Hy-Si (sect. *Hylotelephium* ser. *Sieboldia*).

family, and the two clades correspond to the two subfamilies that 't Hart (1995) recognized. These results are largely confirmed with a recent study based on cpDNA gene *matK* sequence data (Mort et al. 2001), although they did not recover the *Telephium* and *Leucosedum* clades. They also recognized a well-supported dichotomy between the *Kalanchoe* clade and the remainder of the *Sedoideae* clade; those clades correspond to tribes Kalanchoeae and Sedeae recognized by 't Hart (1995).

In our study, the general topology and major lineages within the Crassulaceae nearly agree with the results of those previous studies, except for the monophyly of the *Telephium* clade. All of the species of Asian *Sedum* (s.s.) tested are included in the *Acre* clade, and all other Asian genera are included in subclades corresponding to Ham's *Telephium* clade. 't Hart (1995) treated the *Telephium* clade as one taxon, subtribe Telephinae, under tribe Sedeae and characterized it by having flat, dentate leaves and tuberous roots or thickened rhizomes. In our results Ham's *Telephium* clade is divided into four strongly supported subclades, the *Hylotelephium*, *Rhodiola*, *Phedimus*, and *Umbilicus* clades. The relationships between subclades are not resolved in the *trnL-F* tree. In the ITS and combined data trees, those four subclades do not comprise one clade and the *Sedeae* clade splits at the base into the *Hylotelephium* clade and a remaining clade. The remaining clade, however, is relatively weakly supported by bootstrap values (ITS data 52%, combined data 70%), but is supported strongly by decay values (30 decay, 52 decay). These results suggest that the *Telephium* clade is polyphyletic.

Because of the high frequency of parallelism in Crassulaceae it is impossible to characterize the major clades clearly by a single morphological character or cytological feature, except for the *Crassula* clade (Ham and 't Hart 1998; Mort et al. 2001). The basal split within the *Sedeae* clade and the *Hylotelephium* clade and the remaining clade also is not supported by morphological or cytological features, but flowering phenology is diagnostic. All species in the *Hylotelephium* clade bloom in autumn, whereas those in the remaining clade bloom from spring to early summer. Mort et al. (2001) suggested a southern African origin for the family, with subsequent dispersal northward into the Mediterranean region, from which the family spread to Asia, Eastern Europe, and northern Europe. Flowering season is significant because the point of major phylogenetic divergence in the *Sedeae* clade corresponds to that of the geographical dispersal of the family from the southern to the northern hemisphere across the equator. The feature is not as clear in species distributed mainly near the equator or in high mountains, but there is a possibility that the clades can be

characterized by long-day flowering or short-day flowering.

Although we tested a broad range of species of Asian *Sedoideae*, we could not examine the species included in the *Leucosedum* and *Sempervivum* clades in the ITS and combined data analyses. Much more phylogenetic work is needed to resolve fully the phylogenetic relationships of the *Telephium* group in the family. The taxonomic implications of each subclade are discussed below.

**THE RHODIOLA CLADE.** The genus *Rhodiola*, containing primarily alpine plants, is mainly distributed in the Himalaya and SW China. The genus was distinguished from *Sedum* by Linnaeus (1753) because the plants are dioecious. Later, *Rhodiola* was reduced to a synonym within *Sedum* (Scopoli 1777; De Candolle 1828; Endlicher 1839; Schönland 1890; Berger 1930; Fröderström 1930) until Nakai (1938) and Borissova (1939) clarified the differences between these two genera. They distinguished *Rhodiola* from *Sedum* by the well-developed rhizomes and by the annual flowering stems arising from the axils of the scaly radical leaves. Our analyses using a broad range of species indicate that species of *Rhodiola* comprise one clade, which is well-separated from *Sedum* (s.s.). This result supports the classification that treats *Rhodiola* as a genus distinct from *Sedum* (s.s.).

The genus *Rhodiola* is a highly derived group and previous studies recognized some taxa under *Rhodiola* as subgenera, sections, or series (e.g., Schönland 1890; Berger 1930; Fröderström 1930; Borissova 1939; Praeger 1921; Ohba 1975). Ohba (1975) recognized four subgenera under *Rhodiola* based on floral characters (unisexual or hermaphrodite), radical leaves, and inflorescences; i.e., subg. *Rhodiola*, *Primuloides*, *Crassipedes*, and *Clementsia*. Furthermore, he recognized four sections within subg. *Rhodiola* and three sections within subg. *Primuloides*. We tested representatives of three subgenera and the distributions of the infrageneric taxa are noted in the combined tree (Fig. 3). Although subgenus *Rhodiola* has been distinguished from other subgenera by being dioecious, it is polyphyletic with respect to subgenus *Crassipedes*. This result suggests that dioecy has arisen multiple times within *Rhodiola*. Section *Chamaerhodiola* and *Rhodiola* in subg. *Rhodiola* are also shown to be polyphyletic. These results suggest the necessity for a new infrageneric classification of the genus *Rhodiola*.

No studies have discussed the relationship of *Rhodiola* and *Pseudosedum* morphologically. This is because *Pseudosedum* is distinct in having petals fused basally and species distributed mainly in central Asia, while *Rhodiola* is mainly Himalayan. The analysis based on cpDNA restriction site data showed that *Rhodiola* and *Pseudosedum* are closely related ('t Hart 1995). Our results indicate that *Rhodiola* and *Pseudosedum* comprise

a strongly supported clade (the *Rhodiola* clade). One morphological character, presence of bract scales on the flowering stems, may characterize this clade. Although the phylogenetic relationship between *Rhodiola* and *Pseudosedum* is not fully resolved, *Pseudosedum* might better be reduced to a synonym of *Rhodiola* because of the very close relationships of the two genera. Additional taxonomic sampling is needed to resolve fully the boundaries between these genera.

**THE PHEDIMUS CLADE.** Species of *Phedimus* have also generally been treated as members of *Sedum*. They are morphologically very similar to *Sedum* (s.s.), but differ slightly by having well developed rhizomes and dentate, flat leaves. 't Hart (1995) and Ohba et al. (2000) treated plants with these features as *Phedimus*, based both on the morphological characters and on the results of Ham's cpDNA restriction site analysis using only a few species. In our study, the species of *Phedimus* are separated in a lineage distinct from *Sedum* (s.s.) and comprise one strongly supported clade (the *Phedimus* clade). Our results support the monophyly of *Phedimus* and its exclusion from *Sedum*.

**THE HYLOTELEPHIUM CLADE.** *Hylotelephium*, *Orostachys*, *Meterostachys*, and *Sinocrassula* comprise one clade (the *Hylotelephium* clade) in this study. *Hylotelephium* has generally been treated as a synonym of *Sedum*. Ohba (1977) pointed out that *Hylotelephium* differs clearly from the other species of *Sedum* by having stipitate or attenuate ovaries, flat broad leaves, compound corymbose inflorescences, and non-yellow petals. He treated the group as a distinct genus, *Hylotelephium*. In our study, the separation of the species of *Hylotelephium* as distinct from *Sedum* (s.s.) is supported.

*Orostachys* has a spadix-like inflorescence and a conspicuous rosette of crowded cauline leaves, whereas *Hylotelephium* has corymbose inflorescences and lacks rosette leaves. Because of these differences, the two genera have generally been thought to be distantly related, but Ohba (1978, 1995) suggested a close relationship based on the fact that both genera have apocarpous ovaries with a stipitate or attenuate base. The monotypic *Meterostachys* is characterized by its 'cymose-paniculate' inflorescence and petals fused basally (Nakai 1935). *Meterostachys* resembles *Orostachys* in having a conspicuous rosette and reddish white petals and was reduced to *Orostachys* by Ohwi (1953). Ohba (1978) treated *Meterostachys* as distinct from *Orostachys* because of the floral features mentioned above. The results from the ITS and combined data analyses indicate not only that these three genera are closely related, but also that *Orostachys* is polyphyletic relative to a paraphyletic *Hylotelephium*. *Orostachys* is divided into two groups corresponding to the series *Appendiculatae* and *Eappendiculatae* recognized by Borissova (1939). Series *Appendiculatae* comprises a clade with a species of *Meterostachys* and series *Eappendiculatae* comprises a

clade with some species of *Hylotelephium*. The former clade is characterized by spines at the apex of the leaves while the latter is characterized by entire leaves. This character, cuspidate or muticous leaves, is expected to have utility in a new generic classification. Ohba (1977) recognized two sections, *Hylotelephium* and *Populisedum*, in the genus *Hylotelephium* based mainly on the point of insertion of the flowering stems. He also divided section *Hylotelephium* into series *Hylotelephium* and *Sieboldii* based on the angle of flowering stems. The distribution of these infrageneric taxa in the combined analysis is shown in Fig. 3. Section *Populisedum* and series *Hylotelephium* are indicated to be polyphyletic. Because of low resolution, the monophyly of series *Sieboldii* is not resolved. To reconstruct a new generic system, much more phylogenetic work is needed to resolve fully generic and infrageneric boundaries.

*Sinocrassula*, with a narrow distribution from the Himalaya to southwestern China, comprises one clade that splits from the base of the *Hylotelephium* clade. The monophyly of the genus is supported. The genus has haplostemonous flowers, which characterize Berger's (1930) *Crassuloideae* and the *Crassula* clade recognized by previous phylogenetic studies (Ham 1995; 't Hart 1995; Ham and 't Hart 1998, 1999; Mort et al. 2001). However, Mort et al. (2001) incorrectly noted that the character has arisen only once in the family and is confined to the *Crassula* clade. As in Mort et al. (2001), our results also indicate a distant relationship between *Sinocrassula* and the *Crassula* clade. These results indicate that haplostemonous flowers have evolved in two separate and well-supported lineages. Ohba (1975) transferred a few species of *Sinocrassula* to *Orostachys* and treated them as section *Schoenlandia*. Although these species also have haplostemonous flowers, they are distinguished from *Sinocrassula* by having stipitate or attenuate ovaries, racemose inflorescences, and basally connate petals (Ohba 1975). Although we did not sample these species in our analyses, it is possible to consider an additional origin of haplostemonous flowers in the family depending on the phylogenetic position of the species of *Schoenlandia*. As Mort et al. (2001) noted, the ontogeny and homology of this character have yet to be investigated.

**THE ACRE CLADE.** In previous phylogenetic analyses, the *Acre* clade comprises the Echeverioideae and species of *Sedum* (s.s.) from around the world (Ham 1995; 't Hart 1995; Ham and 't Hart 1998; Mort et al. 2001). In our *trnL-F* tree, the members of the clade are not in conflict with previous studies, but most of the relationships within the clade could not be reconstructed because of unresolved polytomies. 't Hart (1995) and Kim et al. (1996) characterized the *Acre* clade by the occurrence of alkaloids, the ornamentation of the seeds, which have a reticulate testa, and the absence of glandular hairs.

Previous studies have shown that *Sedum* (s.s.) is highly polyphyletic and spread throughout the large *Sedeae* clade (Ham 1995; 't Hart 1995; Ham and 't Hart 1998; Mort et al. 2001). Our findings based on *trnL-F* data are in agreement. However, all Asian species of *Sedum* (s.s.) sampled in this study are confined to the *Acre* clade. Fu and Ohba (2001) divided Asian *Sedum* (s.s.) into three sections; sect. *Sedum*, *Oreades*, and *Filipes*. They distinguished sect. *Oreades* from sect. *Sedum* by lacking gibbous carpels. In this study we sampled only two species of sect. *Oreades* (*S. oreades* and *S. trulipetalum*). Our analyses based on ITS and combined data indicate that sect. *Sedum* and *Oreades* are both paraphyletic with only weak support.

It is estimated that the *Acre* clade comprises approximately one-third of the taxonomic diversity of the Crassulaceae (Mort et al. 2001). The distribution of critical characters such as testa ornamentation and the occurrence of alkaloids is still insufficiently known in the family (see Kim et al. 1996). Much more phylogenetic, morphological and phytochemical work are needed to resolve fully the generic boundaries and relationships within this large clade.

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## APPENDIX 1

Voucher, literature, and GenBank accession information for all taxa included in this study; n/a: region not sequenced for this taxon. The subfamilies are according to Berger (1930).

## Subfamily Sedoideae

*Orostachys* Fisch. - *O. aggregata* (Makino) H. Hara: Japan, Mayuzumi et al. C00012 (TI); *trnL-F* AB089734, ITS AB088574. *O. fimbriata* (Turcz.) A. Berger: China, S. Akiyama 105571 (TI); *trnL-F* AB089719, ITS AB088578. *O. ivarenge* (Makino) H. Hara: Japan, K. Midorikawa 1998 (TI); *trnL-F* AB089733, ITS AB088573. *O. japonica* (Maxim.) A. Berger: Japan, Mayuzumi et al. C00027 (TI); *trnL-F* AB089720, ITS AB088576. *O. malacophylla* (Pall.) Fisch.: Japan, Mayuzumi et al. C00011 (TI); *trnL-F* AB089732, ITS AB088572. *O. spinosa* (L.) A. Berger: Russia, No. 97 1570/0 (ZSS); *trnL-F* AB089718, ITS AB088577.

*Hylothelephium* H. Ohba - *H. anacampseros* (L.) H. Ohba: South Europe, HRT-5356 (UT); *trnL-F* AB089730, ITS AB088563. *H. angustum* (Maxim.) H. Ohba: Russia, M. Suzuki et al. 1996 (TI); *trnL-F* AB089722, ITS AB088560. *H. caudicola* (Praeger) H. Ohba: Japan, Mayuzumi et al. C00014 (TI); *trnL-F* AB089729, ITS AB088566. *H. erythrostictum* (Miq.) H. Ohba: Japan, Mayuzumi C00064 (TI); *trnL-F* AB089716, ITS AB088556. *H. everusii* (Ledeb.) H. Ohba: Russia, M. Wakabayashi et al. 1993 (TI); *trnL-F* AB089737, ITS AB088570. *H. pallescens* (Freyen) H. Ohba: Japan, H. Ohba (TI); *trnL-F* AB089736, ITS AB088558. *H. populifolium* (Pall.) H. Ohba: Russia, M. Wakabayashi et al. 1993 (TI); *trnL-F* AB089727, ITS AB088571. *H. sieboldii* (Hook.) H. Ohba: Japan, Mayuzumi C00022 (TI); *trnL-F* AB089725, ITS AB088567. *H. sieboldii* var. *ettyunense* (Tomida) H. Ohba: Japan, Mayuzumi C00023 (TI); *trnL-F* AB089726, ITS AB088568. *H. sordidum* (Maxim.) H. Ohba: Japan, S. Kato C00068 (TI); *trnL-F* AB089717, ITS AB088562. *H. spectabile* (Boreau) H. Ohba: Japan, Mayuzumi C00003 (TI); *trnL-F* AB089723, ITS AB088575. *H. tatarinowii* (Maxim.) H. Ohba: China, H. Ohba 1976 (TI); *trnL-F* AB089731, ITS AB088557. *H. telephium* (L.) H. Ohba subsp. *maximum* (L.) H. Ohba: N. Armenia, H. Ohba 1999 (TI); *trnL-F* AB089738, ITS AB088561. *H. tsugaruense* (Hara) H. Ohba: Japan, K. Midorikawa 1992 (TI); *trnL-F* AB089735, ITS AB088565. *H. verticillatum* (L.) H. Ohba: Japan, H. Ikeda 2000 (TI); *trnL-F* AB089724, ITS AB088564. *H. viride* (Makino) H. Ohba: Japan, Mayuzumi C00015 (TI); *trnL-F* AB089728, ITS AB088569. *H. viviparum* (Maxim.) H. Ohba: Korea, J. Murata et al. 21006 (TI); *trnL-F* n/a, ITS AB088559.

*Sinocrassula* A. Berger - *S. indica* (Decne.) A. Berger: Bhutan, H. Hara et al. 1967 (TI); *trnL-F* AB089740, ITS AB088580. *S. paoshingensis* (S.H. Fu) H. Ohba et al.: China, Wu et al. 103555 (TI); *trnL-F* AB089741, ITS AB088581. *S. paoshingensis* var. *spinulosa* H. Ohba et al.: China, Akiyama et al. 105516 (TI); *trnL-F* AB089739, ITS AB088583. *S. yunnanensis* (Franch.) A. Berger: China, Mayuzumi C00115 (TI); *trnL-F* AB089742, ITS AB088582.

*Meterostachys sikokiana* (Makino) Nakai: Japan, Mayuzumi et al. C00028 (TI); *trnL-F* AB089721, ITS AB088579.

*Rhodiola* L. - *R. algida* (Ledeb.) Fisch. & C.A. Mey.: Russia, M. Wakabayashi et al. 1993 (TI); *trnL-F* n/a, ITS AB088608. *R. amabilis* (H. Ohba) H. Ohba: Nepal, F. Miyamoto et al. T19592550 (TI); *trnL-F* AB089760, ITS AB088587. *R. bupleuroides* (Hook. f. & Thomson) S.H. Fu: Nepal, F. Miyamoto et al. T19420236 (TI); *trnL-F* AB089750, ITS AB088592. *R. chrysanthemifolia* (H. Lév.) S.H. Fu

subsp. *chrysanthemifolia*: China, Wu et al. 103573 (TI); *trnL-F* AB089756, ITS AB088604. *R. chrysanthemifolia* subsp. *sacra* (Raym.-Hamet) H.Ohba 1: Nepal, E Miyamoto et al. TI9420171 (TI); *trnL-F* AB089752, ITS AB088606. 2: China, Wu et al. 103546 (TI); *trnL-F* AB089746, ITS AB088603. *R. cretinii* (R.-Hamet) H.Ohba: Nepal, E Miyamoto et al. TI9596251 (TI); *trnL-F* AB089761, ITS AB088588. *R. dumulosa* (Franch.) S.H.Fu: Bhutan, E Miyamoto 9361658 (TI); *trnL-F* AB089744, ITS AB088595. *R. fastigiata* (Hook.f. & Thoms.) S.H.Fu: Nepal, E Miyamoto et al. TI9592280 (TI); *trnL-F* AB089749, ITS AB088594. *R. heterodonta* (Hook.f. & Thoms.) Boriss.: Nepal, J.D.A.Stainton 4312 (TI); *trnL-F* AB089745, ITS AB088596. *R. himalensis* (D.Don) S.H.Fu: Nepal, E Miyamoto et al. TI9596339 (TI); *trnL-F* n/a, ITS AB088593. *R. humilis* (Hook.f. & Thomson) S.H.Fu: Bhutan, S.Akiyama 9209420 (TI); *trnL-F* AB089755, ITS AB088611. *R. ishidae* (Miyabe & Kudo) H.Hara: Japan, K.Midorikawa 1997 (TI); *trnL-F* AB089751, ITS AB088600. *R. kirilowii* (Regel) Maxim.: China, Wu et al. 103481 (TI); *trnL-F* n/a, ITS AB088601. *R. macrocarpa* (Praeger) S.H.Fu: China, L.Sung 12230 (TI); *trnL-F* AB089759, ITS AB088590. *R. nepalica* (H.Ohba) H.Ohba: Nepal, Stainton et al. 2045 (TI); *trnL-F* AB089754, ITS AB088598. *R. nobilis* (Franch.) S.H.Fu subsp. *atuntsuensis* (Praeger) H.Ohba: China, Wu et al. 103528 (TI); *trnL-F* AB089758, ITS AB088589. *R. purpureoviridis* (Praeger) S.H.Fu. *R. nobilis* subsp. *phariensis* (H.Ohba) H.Ohba: Nepal, E Miyamoto et al. TI9440132 (TI); *trnL-F* n/a, ITS AB088591. *R. rosea* L.: Japan, K.Midorikawa 1999 (TI); *trnL-F* AB089743, ITS AB088599. *R. serrata* H.Ohba: China, T.Yoshida 1997 (TI); *trnL-F* AB089747, ITS AB088597. *R. sinuata* (Edgew.) S.H.Fu: Bhutan, S.Akiyama 9209417 (TI); *trnL-F* AB089753, ITS AB088605. *R. wallichiana* (Hook.) S.H.Fu: Nepal, E Miyamoto et al. TI9440087 (TI); *trnL-F* AB089757, ITS AB088607. *R. yunnanensis* (Franch.) S.H.Fu: China, Wu et al. 1999 (TI); *trnL-F* AB089748, ITS AB088602.

*Pseudosedum* A.Berger - *Pseudosedum longidentatum* Boriss.: Kazakhstan, M.Nakao 73 (TI); *trnL-F* AB089762, ITS AB088609. *Pseudosedum* sp.: Tajikistan, M.Ogisu 2000 (TI); *trnL-F* AB089763, ITS AB088610

*Phe'dimus* Rafin. - *P. aizoon* (L.) 't Hart: Japan, Mayuzumi C00092 (TI); *trnL-F* AB089766, ITS AB088615. *P. aizoon* L.var. *floribundus* (Nakai) H. Ohba: Japan, Mayuzumi et al. C00010 (TI); *trnL-F* AB089767, ITS AB088614. *P. kamtschaticus* (Fisch.) 't Hart: Japan, K.Midorikawa 1999 (TI); *trnL-F* AB089765, ITS AB088612. *P. sikokianus* (Maxim.) 't Hart: Japan, Mayuzumi C00083 (TI); *trnL-F* AB089768, ITS AB088613. *P. spurius* (M.Bieb.) 't Hart: Russia, Mayuzumi C00114 (TI); *trnL-F* AB089764, ITS AB088616.

*Sedum* L. - *S. bulbiferum* Makino: Japan, L.Niu 1999 (TI); *trnL-F* AB089776, ITS AB088628. *S. hakonense* Makino: Japan, Mayuzumi C00005 (TI); *trnL-F* AB089777, ITS AB088625. *S. japonicum* Miq.: Japan, Mayuzumi et al. C00030 (TI); *trnL-F* AB089784, ITS AB088617. *S. lineare* Thunb.: Japan, Mayuzumi C00120 (TI); *trnL-F* AB089773, ITS AB088623. *S. makinoi* Maxim.: Japan, Mayuzumi C00086 (TI); *trnL-F* AB089779, ITS AB088627. *S. mexicanum* Britt.: Japan, Mayuzumi C00001 (TI); *trnL-F* AB089783, ITS AB088621. *S. morrisonensis* Hayata: Taiwan, HRT-31048 (UT); *trnL-F* Kim et al. (1996), ITS n/a. *S. multicaule* Lindl.: Nepal, E Miyamoto et al. TI9596136 (TI); *trnL-F* AB089782, ITS AB088631. *S. oreades* (Decne.) Raym.-Hamet: Nepal, E Miyamoto et al. TI9420140 (TI); *trnL-F* AB089788, ITS AB088632. *S. oryzifolium* Makino: Japan, Mayuzumi C00016 (TI); *trnL-F* AB089786, ITS AB088618. *S. polytrichoides* Hemsl.: Korea, HRT-32797 (UT); *trnL-F* Kim et al. (1996), ITS n/a. *S. sarmentosum* Bunge: Japan, Mayuzumi C00008 (TI); *trnL-F* AB089774, ITS AB088624. *S. subtile* Miq.: Japan, A.Shimizu et al. 1999 (TI); *trnL-F* AB089775, ITS AB088622. *S. tosaense* Makino: Japan, A.Iwamoto 2000 (TI); *trnL-F* AB089787, ITS AB088620. *S. triactina* A.Berger: Nepal, E Miyamoto et al. TI9596091 (TI); *trnL-F* AB089780, ITS AB088629. *S. trullipetalum* Hook.f. & Thomson: Nepal, E Miyamoto et al. TI9420132 (TI); *trnL-F* AB089781, ITS AB088630. *S. yabeaenum* Makino: Japan, Mayuzumi et al. C00029 (TI); *trnL-F* AB089772, ITS AB088626. *S. zentaro-tashiroi* Makino:

Japan, Ohba 1998 (TI); *trnL-F* AB089785, ITS AB088619. *S. acre* L.: Turkey, HRT-30869 (UT); *trnL-F* Ham et al. (1994) / GNB-X71990, ITS n/a. *S. album* L.: Spain, HRT-29329 (UT); *trnL-F* Ham et al. (1994) / GNB-X71991, ITS n/a. *S. alpestre* Vill.: Turkey, HRT-30915 (UT); *trnL-F* Kim et al. (1996), ITS n/a. *S. bourgaei* Hemsl.: Mexico, HRT-21588 (UT); *trnL-F* Kim et al. (1996), ITS n/a. *S. dasyphyllum* L.: Italy, HRT-17579 (UT); *trnL-F* Kim et al. (1996), ITS n/a. *S. dendroideum* Moc. & Sessé: Mexico, HRT-28227 (UT); *trnL-F* Kim et al. (1996), ITS n/a. *S. fusiforme* Lowe: Portugal, HRT-29011 (UT); *trnL-F* Kim et al. (1996), ITS n/a. *S. hispanicum* L.: Turkey, HRT-30865 (UT); *trnL-F* 't Hart and Alpinar (1999), ITS n/a. *S. ince* 't Hart & Alpinar: Turkey, HRT-31987 (UT); *trnL-F* 't Hart and Alpinar (1999), ITS n/a. *S. meyeri-johannis* Engl.: Kenya, H.Ohba 990131 (TI); *trnL-F* AB089778, ITS n/a. *S. microcarpum* (Smith) Schönland: Israel, HRT-31112 (UT); *trnL-F* 't Hart and Alpinar (1999), ITS n/a. *S. pubescens* Vahl: Tunisia, HRT-31627 (UT); *trnL-F* Mes et al. (1996) / X80551, ITS n/a. *S. reptans* R.T.Clausen: Mexico, HRT-30661 (UT); *trnL-F* Kim et al. (1996), ITS n/a.

*Rosularia sempervivum* (M. Bieb.) A.Berger: Turkey, HRT-31674 (UT); *trnL-F* 't Hart and Alpinar (1999), ITS n/a.

*Prometheum* H.Ohba - *Prometheum aizoon* (Fenzl) 't Hart: Turkey, HRT-31740 (UT); *trnL-F* 't Hart and Alpinar (1999), ITS n/a. *Prometheum sempervivoides* (M. Bieb.) H.Ohba: Turkey, HRT-31153 (UT); *trnL-F* 't Hart and Alpinar (1999), ITS n/a.

#### Subfamily Crassuloideae

*Crassula multicaea* Lem.: South Africa, HRT-30474 (UT); *trnL-F* Ham et al. (1994) / X71984, ITS n/a.

*Tillaea alata* Viv.: Israel, HRT-31113 (UT); *trnL-F* Ham et al. (1994) / X71993, ITS n/a.

#### Subfamily Kalanchoideae

*Kalanchoe* Adan. - *K. beauverdii* Raym.-Hamet: Madagascar, voucher not cited; *trnL-F* n/a, ITS Gehrig, H. et al. (2001) / AJ231305. *K. campanulata* Baill.: Madagascar, voucher not cited; *trnL-F* n/a, ITS Gehrig, H. et al. (2001) / AJ231309. *K. densiflora* Rolfe 1: Kenya, HRT-30772 (UT); *trnL-F* Ham et al. (1994) / X71986, ITS n/a. 2: Africa, voucher not cited; *trnL-F* n/a, ITS Gehrig, H. et al. (2001) / AJ231334. *K. streptantha* Baker: Madagascar, voucher not cited; *trnL-F* n/a, ITS Gehrig, H. et al. (2001) / AJ231322.

#### Subfamily Cotyledonoideae

*Cotyledon orbiculata* L.: South Africa, HRT-32688 (UT); *trnL-F* Ham et al. (1994) / X71983, ITS n/a.

*Umbilicus* DC. - *Umbilicus botryoides* A.Rich.: Kenia, H.Ohba (TI); *trnL-F* AB089771, ITS AB088586. *Umbilicus rupestris* Dandy: Saudi Arabia, H.Ohba and T.Miyazaki 1451 (TI); *trnL-F* AB089769, ITS AB088584. *Umbilicus horizontalis* DC.: Saudi Arabia, H.Ohba and T.Miyazaki 1403 (TI); *trnL-F* AB089770, ITS AB088585.

#### Subfamily Sempervivoideae

*Sempervivum armerum* Boiss. & Huet: Turkey, HRT-30411 (UT); *trnL-F* Ham et al. (1994) / X71992, ITS n/a. *Sempervivum ciliosum* Craib: Greece, HRT-31473 (UT); *trnL-F* Ham et al. (1994) / X74287, ITS n/a.

*Aeonium castello-paivae* Bolle: Canary, Mort 1519 (WS); *trnL-F* Mort et al. (2002) / AY082236, ITS Mort et al. (2002) / AY082127. *Aeonium gomerense* (Praeger) Praeger: Canary, Mort 1454 (WS); *trnL-F* Mort et al. (2002) / AY082242, ITS Mort et al. (2002) / AY082133. *Aeonium lancerottense* (Praeger) Praeger: Canary, Mort 1518 (WS); *trnL-F* Mort et al. (2002) / AY082250, ITS Mort et al. (2002) / AY082143. *Aeonium viscatum* Webb ex C.Bolle: Canary, Mort 1432 (WS); *trnL-F* Mort et al. (2002) / AY082299, ITS Mort et al. (2002) / AY082154.

*Greenovia aizoon* Bolle: Canary, Mort 1425 (WS); *trnL-F* Mort et al. (2002) / AY082229, ITS Mort et al. (2002) / AY082112.

#### Subfamily Echeveroideae

*Echeveria venezuelensis* Rose: Venezuela, HRT-30445 (UT); *trnL-F* Ham et al. (1994) / X71985, ITS n/a.

*Pachyphytum compactum* Rose: Mexico, HRT-30620 (UT); *trnL-F* Kim et al. (1996), ITS n/a.

*Villadia batesii* (Hemsl.) Rose: Mexico, HRT-29606 (UT); *trnL-F* Kim et al. (1996), ITS n/a.