

## PHYLOGENETIC RELATIONSHIPS AND EVOLUTION OF CRASSULACEAE INFERRED FROM *matK* SEQUENCE DATA<sup>1</sup>

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Chloroplast gene *matK* sequence data were used to estimate the phylogeny of 112 species of Crassulaceae sampled from 33 genera and all six recognized subfamilies. Our analyses suggest that five of six subfamilies recognized in the most recent comprehensive classification of the family are not monophyletic. Instead, we recovered a basal split in Crassulaceae between the southern African *Crassula* clade (Crassuloideae) and the rest of the family (Sedoideae). These results are compatible with recent studies of cpDNA restriction site analyses. Within Sedoideae, four subclades were also recovered: *Kalanchoe*, *Leucosedum*, *Acre*, and *Aeonium*; evidence also exists for a *Telephium* clade and *Sempervivum* clade. The genus *Sedum* is highly polyphyletic with representatives spread throughout the large Sedoideae clade. Sympetaly and polymorous flowers have arisen multiple times in Crassulaceae and thus are not appropriate characters upon which to base subfamilial limits, as has been done in the past. One floral character, haplostemy, appears to be confined to the well-supported *Crassula* clade. Our analyses suggest a southern African origin of the family, with subsequent dispersal northward into the Mediterranean region. From there, the family spread to Asia/eastern Europe and northern Europe; two separate lineages of European Crassulaceae subsequently dispersed to North America and underwent substantial diversification. Our analyses also suggest that the original base chromosome number in Crassulaceae is  $x = 8$  and that polyploidy has played an important role in seven clades. Three of these clades are exclusively polyploid (*Sempervivum* clade and two subclades within the *Kalanchoe* and *Aeonium* clades), whereas four (*Crassula*, *Telephium*, *Leucosedum*, and *Acre* clades) comprise both diploid and polyploid taxa. Polyploidy is particularly rampant and cytological evolution especially complex in the *Acre* clade.

**Key words:** character evolution; Crassulaceae; *matK*; phylogenetics.

Crassulaceae are a morphologically diverse and systematically complex angiosperm family comprising 35 genera and 1500 species (Berger, 1930). Members of the family are leaf-succulent, usually herbaceous, and often have five-parted, radially symmetrical flowers with two whorls of five stamens each. The family inhabits primarily semiarid habitats and is nearly cosmopolitan in distribution, with centers of diversity in Mexico, southern Africa, Macaronesia, and the Himalayas. The family has long been considered a natural group (e.g., Schönland, 1891; Berger, 1930), and recent molecular phylogenetic analyses of the angiosperms indicate that the family is monophyletic (e.g., Chase et al., 1993; Soltis et al., 1997). While Crassulaceae are easily recognized, defining monophyletic groups within the family has been extremely difficult because of extensive diversity in morphology, cytology, and habit. Not only are generic boundaries unclear, but relationships among genera are also poorly understood due to the frequent intergradation of morphological characters among taxa. Moran

(1942) stated that “if too much emphasis were placed on technical characters, the numerous exceptions and intergradations would necessitate the combination of genera until but six or only one genus remained.” In part, this morphological complexity may represent recurrent evolution of adaptations to xeric habitats.

The most comprehensive treatment of Crassulaceae is that of Berger (1930) who recognized 35 genera in six subfamilies (Fig. 1). These subfamilies have been placed into two lineages, a *Crassula* lineage including the three subfamilies (Crassuloideae, Cotyledonoideae, and Kalanchoideae) found predominantly in southern Africa, and a *Sedum* lineage including the three subfamilies (Echeverioideae, Sedoideae, and Sempervivoideae) found predominantly in the Northern Hemisphere (‘t Hart and Eggli, 1995). Within these two lineages, Berger circumscribed subfamilies based primarily on floral morphology. For example, within the *Crassula* lineage, Crassuloideae include species possessing a single whorl of stamens (haplostemonous) and unfused corollas, whereas Cotyledonoideae and Kalanchoideae include diplostemonous species with fused corollas. Berger distinguished Kalanchoideae from Cotyledonoideae based on the number of floral parts: Cotyledonoideae have five-merous flowers, whereas Kalanchoideae have four-merous flowers. In the *Sedum* lineage, Sempervivoideae all possess unfused, polymorous flowers, and the Echeverioideae have typically five-merous corollas that are partially to completely fused. Berger’s Sedoideae have been described as a “catch-all” taxon (Uhl, 1963) and include the large genus *Sedum*, as well as the remaining genera of Crassulaceae not eas-

<sup>1</sup> Manuscript received 5 February 1999; revision accepted 11 April 2000.

The authors thank Henk ‘t Hart for supplying plant material and helpful discussions and comments on early drafts of this manuscript; Leigh Johnson and Matthew Gitzendanner for technical assistance; Lowell Ahart, Dirk Al-bach, Mark Fishbein, Larry Hufford, Leigh Johnson, Jason Koontz, Michelle Mabry, and Dan Nickrent for plant collections; and the following botanical gardens for providing plant material: Missouri Botanical Garden, Kirstenbosch Botanical Garden, Huntington Botanical Garden, University of California, Berkeley Botanical Garden, and Santa Barbara Botanical Garden. This project was supported by DEB 9800887 to DES and MEM; Sigma Xi GIAR to MEM; CSSA to MEM; and a grant from the College of Sciences, WSU to MEM.

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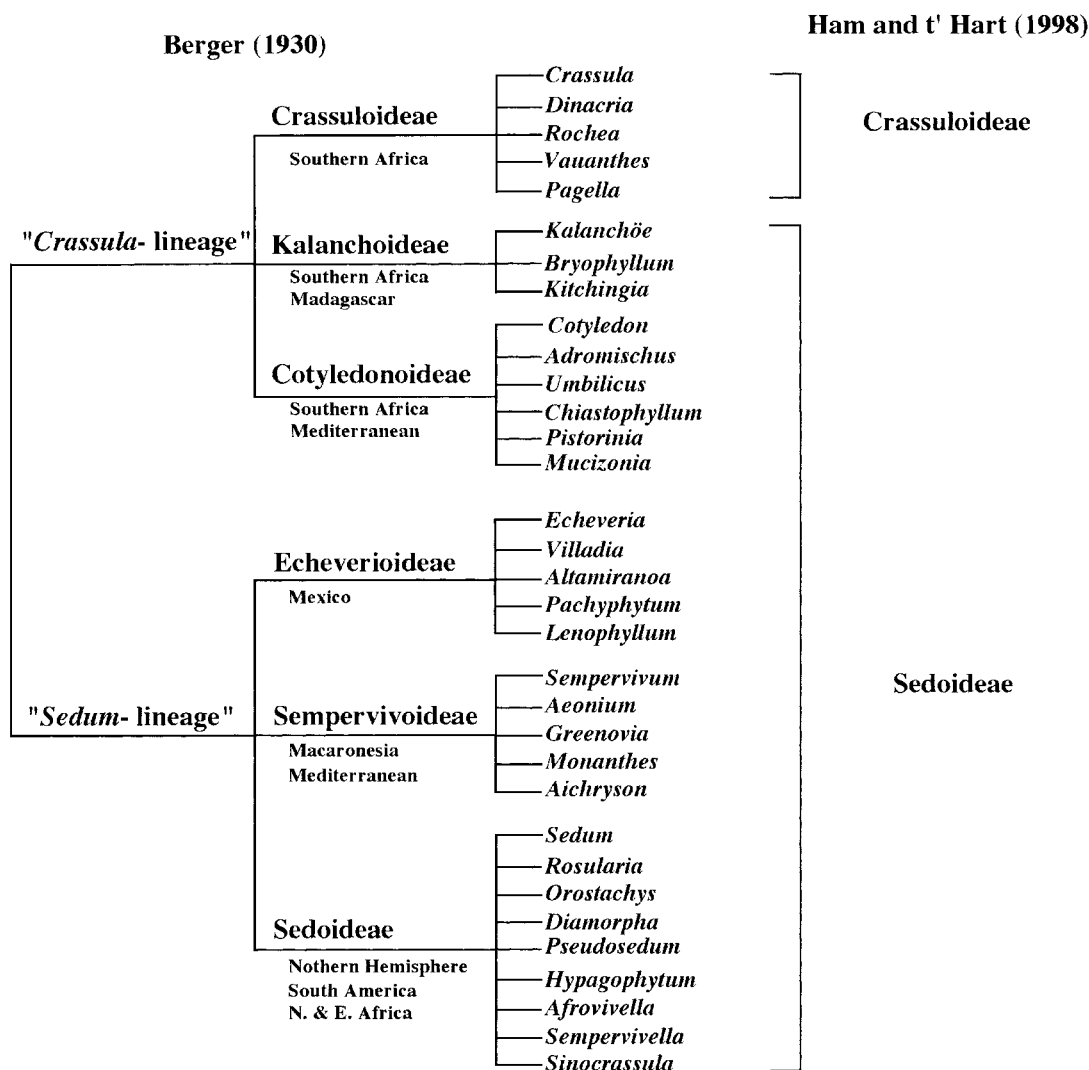


Fig. 1. Classification of Crassulaceae (redrawn from Ham and t' Hart, 1998) as proposed by Berger (1930). Major evolutionary lineages are in quotation marks. Biogeographic distributions of Berger's subfamilies are indicated on each line. Also indicated by brackets is the revised subfamilial classification of Crassulaceae proposed by Ham and t' Hart (1998).

ily placed in other subfamilies. Importantly, Sedoideae comprise taxa that display many morphological features used to circumscribe the other five subfamilies, including haplostemonous androecia, sympetalous corollas, and polymorous flowers.

A series of cytotaxonomic studies by Uhl has provided insights into subfamily circumscription, generic boundaries, and relationships among genera. In certain instances chromosomal data, in conjunction with other sources of data (e.g., biogeography) have indicated that Berger's subfamilies are largely artificial (e.g., Cotyledonoideae—Uhl, 1948; Sempervivoideae—Uhl, 1961a; and Sedoideae—Uhl, 1963). Chromosome studies have supported the monophyly of Crassuloideae (Uhl, 1963) and demonstrated that Kalanchoideae are a natural group closely related to Cotyledonoideae (Uhl, 1948). Although informative in some instances, chromosomal data have not been particularly useful for estimating boundaries of other taxa (e.g., Uhl, 1961a, 1963). For example, *Echeverioideae* and Mexican *Sedum* species possess a wide range of chro-

mosome numbers due to rampant polyploidy and/or aneuploidy (Uhl, 1961b, 1963, 1970).

*Sedum*, the largest genus of Crassulaceae, is cosmopolitan in distribution and encompasses much of the morphological diversity present in the family as a whole. A diverse array of chromosome morphologies and base chromosome numbers is also present in the genus. Due to this high degree of diversity, *Sedum* has a sordid taxonomic history. Berger included ~500 species in the genus; subsequent authors have named as many as 32 segregate genera (see t' Hart and Eggli, 1995). While several recent studies using morphology and DNA data have supported these segregate genera as discrete groups, the systematics of *Sedum* is still problematic and in need of additional investigation.

The central position of *Sedum* with regard to the evolution of Crassulaceae has been stressed by t' Hart (1982). In his classification, *Sedum* is subdivided into three large, geographically defined sections, which are hypothesized to have given rise to the genera endemic to each respective region. However,

this hypothesis is yet to be tested rigorously ('t Hart and Eggli, 1995). Clearly, to understand fully the systematics of Crassulaceae it will be necessary to define the limits of *Sedum* and the segregate genera already named, as well as to test more rigorously the monophyly of the lineages proposed by 't Hart (1982).

Using phylogenetic analyses of cpDNA restriction site data, Ham and 't Hart (1998) suggested recognizing two subfamilies, Crassuloideae and the rest of Crassulaceae (Sedoideae), as well as seven clades of "major importance" and concluded that many of the subfamilies proposed by Berger (1930) are not monophyletic. In addition, their analyses placed the 23 species of *Sedum* analyzed in five of the seven major clades recovered, clearly illustrating the polyphyly of *Sedum*. Although their study provided initial phylogenetic insights for Crassulaceae, some caution is warranted. For example, only 19 of the 35 genera of Crassulaceae were sampled, and in many instances, only a single species of each genus was included. Furthermore, many of the nodes recovered, especially the deeper nodes, received bootstrap support below 50%.

Not only are generic boundaries uncertain, but also the number of major groups (e.g., subfamilies) is not yet evident. Berger's (1930) subfamilies have been shown to be polyphyletic (e.g., Uhl, 1963; Ham and 't Hart, 1998); Thorne (1983) and Takhtajan (1997) each suggested recognizing three subfamilies in Crassulaceae (Crassuloideae, Kalanchoideae, and Sedoideae), whereas Ham and 't Hart (1998) recognized two (Crassuloideae and Sedoideae sensu lato). However, the topology presented by Ham and 't Hart (1998) is consistent with the subfamilies of Thorne (1992) and Takhtajan (1997). Thus, despite the use of numerous sources of data, systematic relationships within Crassulaceae remain enigmatic. We have employed comparative sequencing of the chloroplast gene *matK* to provide a comprehensive, family-level estimate of phylogeny for Crassulaceae. Our goals were to: (1) infer phylogenetic relationships on a broad scale across Crassulaceae and (2) investigate the distribution of several morphological and cytological characters often used to define major groups within Crassulaceae, using our phylogenetic hypothesis as an evolutionary framework.

## MATERIALS AND METHODS

**Taxon sampling**—Using Berger's (1930) classification of Crassulaceae as a guide, taxa were sampled from 30 of 35 recognized genera (Table 1). For the large and systemically complex genera (e.g., *Sedum*, *Crassula*, *Semprevivum*, and *Kalanchoe*), multiple species were included, and with the exception of *Bryophyllum*, *Thompsonella*, and the monotypic *Tacitus*, at least two species from each genus were sampled. A total of 112 ingroup taxa, representing 33 genera (several of which were not recognized by Berger; see Table 1) of Crassulaceae was sequenced for this study. Recent phylogenetic analyses of the angiosperms (Chase et al., 1993; Soltis et al., 1997) indicate that Crassulaceae are a member of Saxifragales and are closely related to *Penthorum*, *Tetracarpaea*, and *Pterostemon* (Soltis and Soltis, 1997). The latter three taxa were previously sequenced for *matK* and were used as outgroups.

**DNA extraction, amplification, and sequencing**—DNAs for all taxa (Table 1) were isolated using a modified CTAB buffer method (Doyle and Doyle, 1987). Leaves of Crassulaceae were desiccated on silica gel for a minimum of 1 wk prior to DNA extraction. Leaf material (~0.7 g) was ground in liquid nitrogen and mixed with 5 mL of 4× CTAB (110 mmol/L) buffer; extractions were incubated at 60°C for 2 h.

The chloroplast gene *matK* is ~1550 base pairs long and encodes a maturase used in RNA splicing (Neuhaus and Link, 1987; Wolfe, Morden, and

Palmer, 1992). *matK* was chosen for analysis because many studies have documented the utility of this gene for resolving phylogenetic relationships at a variety of taxonomic levels, from closely related species to the family level (e.g., Johnson and Soltis, 1994, 1995; Soltis et al., 1996; Hilu and Liang, 1997; Kron, 1997).

PCR (polymerase chain reaction) amplification employed the primer combinations *trnK*-3914F and *trnK-psbA*-R (Johnson and Soltis, 1994). Manual sequencing followed Johnson and Soltis (1994, 1995) and used the sequencing primers *trnK*-710F, *matK*-1470R, *matK*-1470F, and *matK*-2000R (Johnson and Soltis, 1994; Soltis et al., 1996). The same primer combinations, as well as a primer designed specifically for Crassulaceae, *matK*-1800R (5'-AGT TGA CTC CGT ACA ACB GAA-3'), were used for automated sequencing. Automated sequencing was performed on an ABI 377 automated sequencer following the general methods outlined in Soltis and Soltis (1997), and employed the PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, California, USA).

**Phylogenetic analyses**—Sequences in this matrix range from 1121 to 1145 bp in length and provide a data set of 1202 bp after alignment. Alignment was easily accomplished visually; there are occasional indels in *matK*, all in multiples of three. Indel length ranged from 3 to 12 bp (Table 2). All gap characters ("—") were scored as missing data ("?"), rather than a fifth character; following parsimony analyses of only base substitutions, the phylogenetic distribution of indels was explored by plotting the indels onto the shortest trees.

Parsimony analyses were conducted using PAUP\* (Swofford, 1998). Large data sets can pose special problems during phylogenetic analyses. To analyze as much tree space as possible, initial searches were conducted using 1000 replicates with RANDOM taxon addition, NNI branch swapping, and MULPARS, with five trees saved per replicate; all characters were equally weighted. This strategy allowed us to obtain a pool of "starting trees." The shortest trees from these initial searches were used as starting trees for subsequent searches with TBR branch swapping and saving a maximum of 5000 minimum-length trees. Relative support for clades was assessed by using bootstrap analyses (Felsenstein, 1985), with 5000 replicates of fast bootstrapping as implemented in PAUP\* (Swofford, 1998). Fast bootstrapping has recently been shown to be a slightly more conservative estimate of internal support than full heuristic bootstrap analyses (Mort et al., 2000); this approach is well suited for analysis of large data sets.

**Character evolution**—Berger (1930) defined subfamilies of Crassulaceae primarily based on three floral features, haplostemonous androecia, sympetalous corollas, and polymorous flowers, in concert with biogeography. The evolution of these floral traits was investigated by tracing the character states for terminal taxa onto the strict consensus of the shortest trees using MacClade (Maddison and Maddison, 1992). Biogeography was similarly investigated by plotting the distribution of each species onto the strict consensus tree. Morphological data and biogeographic distributions for taxa included in our analyses were obtained from previous studies (Praeger, 1921; Quimby, 1971; Clausen, 1975; Spongberg, 1978; Stephenson, 1994; 't Hart and Eggli, 1995). Chromosomal evolution was similarly investigated. Base chromosome numbers were obtained from the extensive data collected by Uhl (1948, 1961a, b, 1963), as well as from numbers reported elsewhere (Baldwin, 1935, 1937; Moore, 1973). A simplified topology for Crassulaceae presenting only the major relationship among clades was constructed for analysis of chromosome evolution. This approach was used because not all of the species included in the phylogenetic analyses have chromosome numbers reported. In other cases, close relatives of taxa included in our analyses have base chromosome numbers that have been reported. Included on this topology as sister to *Crassula* is *Tillaea*, which was not analyzed in the present study, but was strongly supported as sister to the *Crassula* clade by Ham and 't Hart (1998). Base chromosome numbers were then traced onto this simplified topology to provide initial insights into chromosomal evolution in Crassulaceae. When several alternative base chromosome numbers are apparent, they are provided. The distribution of polyploidy was also explored using this summary topology for Crassulaceae.

## RESULTS

**Phylogenetic analyses**—Of the 1202 characters analyzed, 953 are variable, and 599 are potentially parsimony informative. Pairwise sequence divergence among taxa sampled ranged from 0.0080 to 0.2311, with an average divergence of 0.1073 (0.1024 among ingroup taxa only). Five separate searches employing TBR branch swapping were performed using different sets of five starting trees each from our initial NNI searches. Each search resulted in a pool of 5000 minimum-length trees each of 2621 steps (each with C.I. = 0.515 and R.I. = 0.816), with an identical strict consensus topology. An additional search, with unlimited MAXTREES, was also conducted, which likewise resulted in a large number (12 000 trees before the memory was exceeded) of trees of 2621 steps. The high number of most parsimonious trees recovered during our analyses is likely due to large polytomies in the *Aeonium* and *Acre* clades. Results of one of these searches are summarized as a strict consensus tree (Fig. 2).

Phylogenetic analyses of *matK* sequence data strongly support the monophyly of Crassulaceae, relative to the outgroups used. Parsimony analyses recover a topology that is in general agreement with that of Ham and 't Hart (1998); indicated on our cladogram are the seven clades of "major importance" discussed by these authors. Strongly supported by *matK* sequences are the *Crassula* (99%), *Kalanchoe* (94%), and *Aeonium* (100%) clades. The *Acre* clade receives low support (66%). The *Leucosedum* clade is recovered in our analyses, but receives low support (<50%). The *Telephium* and *Sempervivum* clades of Ham and 't Hart (1998) are not resolved by *matK* sequences.

**Distribution of indels**—To align the *matK* sequences of 112 ingroup taxa and three outgroups, it is necessary to infer 16 indels (Table 2). Previous studies (e.g., Johnson and Soltis, 1994, 1995; Plunkett et al., 1996, 1997; reviewed by Soltis and Soltis, 1998) indicate that indels in *matK* are often phylogenetically informative. Comparison of the taxonomic distribution of indels to the results of our phylogenetic analyses suggests that ten of these indels are phylogenetically informative (indels A, B, D, E, J, K, L, M, O, P); five are autapomorphic (indels F, G, H, I, and N); and one (indel C) is informative, but appears to be homoplasious. The distribution of indels, other than those that are autapomorphic, is shown by plotting them onto the strict consensus tree (Fig. 2).

Supporting the monophyly of Crassulaceae is a 9-bp (base pair) deletion (indel B). The monophyly of the genus *Crassula* is supported by a 12-bp deletion (indel J). Another insertion found in *Crassula* (indel C) is considered homoplasious. This 6-bp insertion is shared by all species of *Crassula* and *Umbilicus* included in our analyses; these taxa are well separated from one another in the topology of Crassulaceae (Fig. 2). When the taxa possessing indel C are constrained to form a clade and phylogenetic analyses are repeated (see above), the minimum-length trees obtained are 2649 steps in length. This large increase in tree length (28 steps) supports the conclusion that indel C is homoplasious. As noted above, analyses of *matK* sequences do not recover the *Telephium* clade of Ham and 't Hart (1998). Rather, the three component subclades of taxa representing the *Telephium* clade form a polytomy (Fig. 2); hence our data do not contradict the potential monophyly of this clade. Significantly, a 6-bp insertion (indel D) is shared by all members of the *Telephium* clade, and if indels are in-

cluded in phylogenetic analyses, the *Telephium* clade is recovered (Fig. 2), but receives low bootstrap support (<50%). Within the *Telephium* clade, a 3-bp insertion (indel E) is shared by *Orostachys* and *Sinocrassula*. Indel O, a 6-bp deletion, is shared by all members of Crassulaceae sampled except *Crassula* and two clades of *Sedum* (*S. fusiforme*, *S. lancerotense*, and *S. nudum*; and *S. oryzafolium*—*S. urvillei*). The sequence of six nucleotides in indel O in these two *Sedum* clades is identical (although the fourth position of the indel is polymorphic), but differs from the corresponding 6-bp sequence observed for *Crassula* (Table 2). Based on the shortest trees and base composition of the indel, we infer that deletion "O" occurred following the divergence of the *Crassula* clade; this was followed by a reinsertion at the same position in these two clades of *Sedum*. An additional 6-bp insertion (indel P) is unique to the *Sedum fusiforme*, *S. lancerotense*, *S. nudum* clade. Other indels that support major clades revealed in our analyses include indel L, which is shared by all members of the *Acre* clade, and indels A and M, which support the monophyly of the *Aeonium* clade.

## DISCUSSION

**Monophyly of Crassulaceae**—Broad phylogenetic analyses of *rbcL* sequences (Chase et al., 1993; Morgan and Soltis, 1993) and 18S rDNA sequences (Soltis and Soltis, 1997; Soltis et al., 1997) indicate that the Crassulaceae are part of a Saxifragales clade. Our analyses of *matK* sequences for 112 species likewise indicate that Crassulaceae are a strongly supported monophyletic group (Fig. 2). Also supporting the branch leading to Crassulaceae is a 9-bp deletion (Table 2, indel B). Given these molecular data, as well as the numerous morphological features that unite the family, there is little dispute that Crassulaceae are monophyletic.

Within Crassulaceae is a well-supported basal split between the *Crassula* clade and the rest of the family. A similar basal dichotomy was noted by Ham and 't Hart (1998), and these two clades correspond to the two subfamilies that they recognize: Crassuloideae and Sedoideae. We will follow this demarcation of the family into Crassuloideae and Sedoideae herein (Fig. 2). Within the Sedoideae clade, a well-supported dichotomy exists between the *Kalanchoe* clade and the remainder of the clade. A number of additional subclades (i.e., *Leucosedum*, *Acre*, and *Aeonium* clades) that largely correspond to those recovered by Ham and 't Hart (1998) are present in the remainder of Sedoideae. Strong support for a relationship among the *Aeonium*, *Acre*, *Leucosedum*, and *Sempervivum* subclades is also apparent (Fig. 2).

The taxonomic composition and support for these clades will be discussed in detail below. For clarity, we applied to clades the same names that have been used by Ham and 't Hart (1998); however, the monophyly of several of these clades (i.e., *Telephium* and *Sempervivum*) is not strongly supported by either *matK* or cpDNA restriction site analyses (see Ham and 't Hart, 1998).

**Crassula clade**—The *matK* sequence data indicate that the first-branching lineage in Crassulaceae is *Crassula*, in agreement with the results from cpDNA restriction site analyses (Ham and 't Hart, 1998). *Crassula*, along with four other genera (*Dinacria*, *Pagella*, *Rochea*, and *Vauanthes*), were placed by Berger (1930) in Crassuloideae. More recently Tölken (1977, 1985) placed all five of these genera in a broadly de-

TABLE 1. Species of Crassulaceae sequenced for *matK*. Included are the subfamilies to which Berger (1930) assigned each species. Asterisk (\*) indicates genera that were not recognized by Berger. Voucher specimens for all species collected by the primary author have been deposited in the Marion Ownbey Herbarium (WS). Botanical garden abbreviations are as follows: Huntington Botanical Garden (HBG), Kirstenbosch Botanical Garden (KBG), Missouri Botanical Garden (MO), Rancho Santa Anna Botanical Garden (RSA), Royal Botanic Gardens at Kew (KEW), University of Washington (UW), University of Utrecht (UT), and University of Bonn (BBG).

Taxon	Subfamily	Voucher	GenBank no. <sup>a</sup>
<i>Adromischus caryophyllus</i>	Cotyledonoideae	KBG 305/86	GBAN-AF115576
<i>Adromischus maculatus</i>	Cotyledonoideae	MO-U6638	GBAN-AF115575
<i>Aeonium goochiae</i>	Sempervivoideae	<i>Mort</i> 1480	GBAN-AF115579
<i>Aeonium nobile</i>	Sempervivoideae	<i>Mort</i> 1459	GBAN-AF115582
<i>Aeonium percarneum</i>	Sempervivoideae	<i>Mort</i> 1400	GBAN-AF115581
<i>Aeonium sedifolium</i>	Sempervivoideae	<i>Mort</i> 1384	GBAN-AF115583
<i>Aeonium simsii</i>	Sempervivoideae	<i>Mort</i> 1406	GBAN-AF115585
<i>Aeonium spathulatum</i>	Sempervivoideae	<i>Mort</i> 1378	GBAN-AF115584
<i>Aeonium tabuliforme</i>	Sempervivoideae	<i>Mort</i> 1377	GBAN-AF115577
<i>Aeonium undulatum</i>	Sempervivoideae	<i>Mort</i> 1401	GBAN-AF115580
<i>Aeonium vestitum</i>	Sempervivoideae	<i>Mort</i> 1397	GBAN-AF115586
<i>Aeonium virginianum</i>	Sempervivoideae	<i>Mort</i> 1395	GBAN-AF115578
<i>Aichryson laxum</i>	Sempervivoideae	KEW 081-79-06674	GBAN-AF115588
<i>Aichryson punctatum</i>	Sempervivoideae	<i>Mort</i> 1495	GBAN-AF115587
<i>Bryophyllum</i> sp.	Kalanchoideae	<i>Mort</i> 1595	GBAN-AF115589
<i>Cotyledon orbiculata</i>	Cotyledonoideae	<i>Mort</i> 1341	GBAN-AF115591
<i>Cotyledon tomentosus</i>	Cotyledonoideae	KBG 1482/38	GBAN-AF115592
<i>Cotyledon velutinosus</i>	Cotyledonoideae	KBG 0035/70	GBAN-AF115593
<i>Crassula crenulata</i>	Crassuloideae	BBG 05128	GBAN-AF115598
<i>Crassula deceptor</i>	Crassuloideae	MO 840772	GBAN-AF115597
<i>Crassula falcata</i>	Crassuloideae	MO 882392	GBAN-AF115594
<i>Crassula fascicularis</i>	Crassuloideae	UW n.v.	GBAN-AF115596
<i>Crassula marnierana</i>	Crassuloideae	<i>Morgan</i> 2152	GBAN-AF115600
<i>Crassula orbicularis</i>	Crassuloideae	UW 124	GBAN-AF115601
<i>Crassula rupestris</i>	Crassuloideae	MO 800755	GBAN-AF115602
<i>Cremnophylla nutans</i>	Sedoideae*	UW 120	GBAN-AF115603
<i>Dudleya candelabrum</i>	Echeverioideae*	RSA 15534	GBAN-AF115605
<i>Dudleya greenei</i>	Echeverioideae*	RSA 10158	GBAN-AF115606
<i>Dudleya viscida</i>	Echeverioideae*	RSA 17710	GBAN-AF115604
<i>Echeveria fulgens</i>	Echeverioideae	<i>Mort</i> 1596	GBAN-AF115607
<i>Echeveria pumilla</i>	Echeverioideae	<i>Mort</i> 1597	GBAN-AF115608
<i>Echeveria rosea</i>	Echeverioideae	<i>Brunner</i> 2038	GBAN-AF115609
<i>Graptopetalum fruticosum</i>	Echeverioideae*	UW 217	GBAN-AF115610
<i>Graptopetalum macdougallii</i>	Echeverioideae*	UW n.v.	GBAN-AF115611
<i>Greenovia aurea</i>	Sempervivoideae	<i>Mort</i> 1428	GBAN-AF115614
<i>Greenovia diplocycla</i>	Sempervivoideae	<i>Mort</i> 1496	GBAN-AF115613
<i>Greenovia dondentralsis</i>	Sempervivoideae	MO 841246	GBAN-AF115612
<i>Hylotelephium ewersii</i>	Sedoideae*	KEW 197-80-01945	GBAN-AF115615
<i>Hylotelephium telephium</i>	Sedoideae*	<i>Soltis &amp; Soltis</i> 2560	GBAN-AF115669
<i>Jovibarba arenaria</i>	Sempervivoideae*	KEW 184-42-08087	GBAN-AF115617
<i>Jovibarba heuffellii</i>	Sempervivoideae*	KEW 436-53-43001	GBAN-AF115616
<i>Kalanchoë daigremontiana</i>	Kalanchoideae	<i>Mort</i> 1610	GBAN-AF115618
<i>Kalanchoë integrifolia</i>	Kalanchoideae	BBG 05164	GBAN-AF115619
<i>Kalanchoë scapigera</i>	Kalanchoideae	BBG 02434	GBAN-AF115620
<i>Kalanchoë synsepala</i>	Kalanchoideae	MO 771895	GBAN-AF115621
<i>Kalanchoë uniflora</i>	Kalanchoideae	BBG 05175	GBAN-AF115623
<i>Kalanchoë zimbabwensis</i>	Kalanchoideae	MO 850883	GBAN-AF115622
<i>Kitchingia gracilipes</i>	Kalanchoideae	BBG 02121	GBAN-AF115624
<i>Lenophyllum acutifolium</i>	Echeverioideae	<i>Mort</i> 1598	GBAN-AF115625
<i>Monanthes amygdros</i>	Sempervivoideae	UW 892	GBAN-AF115627
<i>Monanthes anagensis</i>	Sempervivoideae	<i>Mort</i> 1391	GBAN-AF115626
<i>Monanthes ictérica</i>	Sempervivoideae	<i>Santos s.n.</i>	GBAN-AF115629
<i>Monanthes polyphylla</i>	Sempervivoideae	UW 208	GBAN-AF115628
<i>Orostachys fimbriata</i>	Sedoideae	<i>Mort</i> 1593	GBAN-AF115631
<i>Orostachys</i> sp.	Sedoideae	<i>Brunner</i>	GBAN-AF115630
<i>Pachyphytum compactum</i>	Echeverioideae	UW 2209	GBAN-AF115632
<i>Pachyphytum kinnachii</i>	Echeverioideae	UCB 67-0716	GBAN-AF115633
<i>Parvisedum pumillum</i>	Sedoideae	<i>Ahart s.n.</i>	GBAN-AF115634
<i>Phedimus hybridum</i>	Sedoideae*	<i>Mort</i> 1599	GBAN-AF115663
<i>Phedimus kamchaticum</i>	Sedoideae*	UT 33168	GBAN-AF115653
<i>Phedimus stolonifera</i>	Sedoideae*	UT 31379	GBAN-AF1156654
<i>Rosularia pallida</i>	Sedoideae	UW n.v.	GBAN-AF115635
<i>Rosularia serrata</i>	Sedoideae	BBG 05642	GBAN-AF115636
<i>Sedum bulbiferum</i>	Sedoideae	UT 32795	GBAN-AF115652
<i>Sedum burrito</i>	Sedoideae	UW 593	GBAN-AF115655

TABLE 1. Continued.

Taxon	Subfamily	Voucher	GenBank no. <sup>a</sup>
<i>Sedum clavatum</i>	Sedoideae	HBG 23982	GBAN-AF115660
<i>Sedum commixtum</i>	Sedoideae	UW 124	GBAN-AF115656
<i>Sedum dasyphyllum</i>	Sedoideae	<i>Soltis &amp; Soltis 2557</i>	GBAN-AF115657
<i>Sedum farinosum</i>	Sedoideae	UT 29006	GBAN-AF115658
<i>Sedum furfuraceum</i>	Sedoideae	HBG 47681	GBAN-AF115659
<i>Sedum fusiforme</i>	Sedoideae	UT 29010	GBAN-AF115638
<i>Sedum gracile</i>	Sedoideae	UT 31384	GBAN-AF115650
<i>Sedum hemsleyanum</i>	Sedoideae	<i>Brunner 2079a</i>	GBAN-AF115661
<i>Sedum hispanicum</i>	Sedoideae	MO 1987-1140	GBAN-AF115662
<i>Sedum jaccardianum</i>	Sedoideae	UT 32219	GBAN-AF115637
<i>Sedum laconicum</i>	Sedoideae	UT 31101	GBAN-AF115642
<i>Sedum lancerotense</i>	Sedoideae	<i>Mort 1533</i>	GBAN-AF115641
<i>Sedum lydium</i>	Sedoideae	UT 32532	GBAN-AF115643
<i>Sedum magellense</i>	Sedoideae	UT 30908	GBAN-AF115644
<i>Sedum modestum</i>	Sedoideae	UT 31627	GBAN-AF115639
<i>Sedum morrisonense</i>	Sedoideae	UT 36400	GBAN-AF115651
<i>Sedum multiceps</i>	Sedoideae	UT 25256	GBAN-AF115645
<i>Sedum nudum</i>	Sedoideae	UT 28996	GBAN-AF115646
<i>Sedum oaxaenicum</i>	Sedoideae	HBG 72161	GBAN-AF115664
<i>Sedum obcordatum</i>	Sedoideae	HBG 79360	GBAN-AF115665
<i>Sedum oryzifolium</i>	Sedoideae	UT 33173	GBAN-AF115647
<i>Sedum rubrotinctum</i>	Sedoideae	<i>Mort 1600</i>	GBAN-AF115666
<i>Sedum rupestre</i> ssp. <i>rupestre</i>	Sedoideae	BBG 13951	GBAN-AF115667
<i>Sedum sarmentosum</i>	Sedoideae	UT 33152	GBAN-AF115649
<i>Sedum sediforme</i>	Sedoideae	UT 15429	GBAN-AF115640
<i>Sedum sexangular</i>	Sedoideae	UT 9630	GBAN-AF115668
<i>Sedum ternatum</i>	Sedoideae	MO 951462	GBAN-AF115670
<i>Sedum urvillei</i>	Sedoideae	UT 31685	GBAN-AF115648
<i>Sempervivella alba</i>	Sedoideae	BBG 15409	GBAN-AF115677
<i>Sempervivum ciliosum</i>	Sempervivoideae	UT 31473	GBAN-AF115676
<i>Sempervivum italicum</i>	Sempervivoideae	HBG	GBAN-AF115672
<i>Sempervivum giuseppi</i>	Sempervivoideae	<i>Mort 1602</i>	GBAN-AF115673
<i>Sempervivum mettianum</i>	Sempervivoideae	BBG 06101	GBAN-AF115674
<i>Sempervivum minimum</i>	Sempervivoideae	UT 31371	GBAN-AF115675
<i>Sempervivum tectorum</i>	Sempervivoideae	<i>Mort 1601</i>	GBAN-AF115671
<i>Sinocrassula indica</i>	Sedoideae	<i>Cody s.n.</i>	GBAN-AF115679
<i>Sinocrassula yunnanensis</i>	Sedoideae	UW 888	GBAN-AF115678
<i>Tacitus bellus</i>	Echeverioideae*	UW s.n.	GBAN-AF115680
<i>Thompsonella minutiflora</i>	Echeverioideae*	HBG 72129	GBAN-AF115681
<i>Tylecodon ventricosus</i>	Cotyledonoideae	KBG 321/69	GBAN-AF115682
<i>Tylecodon wallichii</i>	Cotyledonoideae	KBG 499/69	GBAN-AF115590
<i>Tylecodon</i> sp.	Cotyledonoideae	KBG	GBAN-AF115683
<i>Umbilicus heylandianus</i>	Cotyledonoideae	<i>Mort 1407</i>	GBAN-AF115685
<i>Umbilicus rupestris</i>	Cotyledonoideae	UW n.v.	GBAN-AF115684
<i>Villadia imbricata</i>	Echeverioideae	UW 949	GBAN-AF115686

<sup>a</sup> The prefix GBAN- has been added to all GenBank numbers to link the online version of *American Journal of Botany* to GenBank, but is not part of the actual accession number.

finer *Crassula*, or *Crassula* s.l. (sensu lato) that comprises ~200 species distributed primarily in southern Africa. Morphologically, *Crassula* s.l. differs from the remainder of Crassulaceae by possessing haplostemonous flowers.

We sampled nine species of *Crassula*, including a species formerly placed within *Rocrea*. Samples of species placed within the other closely related genera (*Pagella*, *Dinacria*, and *Vauanthes*) could not be obtained. Based on the samples employed, our sequence data strongly support the monophyly of *Crassula*. Also supporting the monophyly of this clade is a unique 12-bp deletion (indel J). The position of *Crassula* as sister to the remainder of Crassulaceae is also supported by a 6-bp deletion (indel O) shared by all Crassulaceae except *Crassula* and two small clades of *Sedum* (see above for discussion); *Crassula* shares the same base composition as the outgroup taxa in this 6-bp region.

*Tillaea*, which Berger (1930) recognized as a section of

*Crassula*, was not included in our analyses. The analyses of Ham and 't Hart (1998) place *Tillaea* as a lineage distinct from *Crassula*; cpDNA divergence between *Tillaea* and *Crassula* is greater than the divergence among many other taxa of undisputed generic status. However, a long branch does not necessarily imply that *Tillaea* is distinct from *Crassula*. As currently circumscribed, *Tillaea* includes 20 species, with a nearly worldwide distribution. This genus of diminutive, semi-aquatic plants differs from *Crassula* in a number of morphological characters, including fruit dehiscence and ovule number ('t Hart and Eggli, 1995). However, Schönland (1916, cited in Spongberg, 1978) concluded that "no sharp line" can be drawn between section *Tillaea* and other sections of *Crassula*. Although we obtained plant material of *T. erecta*, attempts to obtain DNA from this material were unsuccessful. Thus, it remains to be demonstrated whether *Tillaea* is distinct from *Crassula*.

TABLE 2. Insertion/deletion events (indels) observed in *matK* sequences following alignment. Indels are labeled alphabetically; these letters correspond to those mapped onto the strict consensus topology (Fig. 2). Dashes represent missing bases associated with indels. Dots in the sequence below the reference taxa (*Tetracarpaea*, *Pterostemon*, and *Penthorum*) indicate that the same nucleotide present in the reference taxon is also present in the species containing the indel. Letters other than those representing nucleotides are standard IUC ambiguity codes and indicate bases that are polymorphic in that taxon.

Indel	Taxa	Pos.	Sequence region
A	<i>Aeonium</i> spp.	51	CAACATAAC --- TTCCTATAC
	<i>Aichryson</i> spp.		..CTTTGAC <b>GAC</b> ..T.....
	<i>Greenovia</i> spp.		..CTTTGAC <b>GAC</b> ..T.....
	<i>Monanthes</i> spp.		..CTTTGAC <b>GAT</b> ..T.....
	<i>Sedum modestum</i>		..CTTTGAC <b>GAC</b> ..A.....
	<i>Sedum jaccardianum</i>		..CTTTGAC <b>GAC</b> ..T.....
B	Crassulaceae	102	TATGATCAT <b>GTTTTAAAT</b> AGATCC
C	<i>Crassula</i> spp.	138	C.....AT ----- .....
	<i>Umbilicus</i> spp.		AATGTGG ----- GTTATGA
			..... <b>ATGTGG</b> .....
D/E	<i>Hylotelephium</i> spp.	219	TCYRC ----- TAA --- TGATTCT
	<i>Orostachys fimbriata</i>		..TTA. <b>TAATAC</b> ... <b>TGG</b> ..GT...
	<i>Sinocrassula</i> spp.		..TTA. <b>TACTAC</b> ... <b>TGG</b> ..GT...
	<i>Phedimus</i> spp.		..TTA. <b>TACTAY</b> ... --- ..GT...
	<i>Umbilicus</i> spp.		..TTA. <b>TACTRC</b> ... --- ..GT...
	<i>Orostachys</i> sp.		..TTA. ----- ... <b>TGG</b> ..GT...
F	<i>Monanthes anagensis</i>	339	CCCTA ----- SAAAG
G		357	..T.T <b>GAAAGCTTT</b> GAAAG
	<i>Pterostemon</i>		AAAAAA <b>GAA</b> ATAGTC
	<i>Penthorum</i>		...G.. --- ...A.T
	<i>Tetracarpaea</i>		...G.. --- ...AGA
	Crassulaceae		...V.H --- M.MD.M
H	<i>Crassula deltoidea</i>	585	KAATT --- RKARTC
	Other Crassulaceae		..TC.A <b>GTT</b> GG.A.A
I		603	..B.. --- GGRARA
	<i>Pterostemon</i>		CTTATT <b>ACTTCA</b> ACTCCAAAGAAA
	<i>Penthorum</i>		..... ----- ...A.....
	<i>Tetracarpea</i>		..... ----- .....
	Crassulaceae		H..M.. ----- M.A.K.....
J	<i>Crassula</i> spp.	627	AAATCCAT <b>TTCTTTTTC</b> AAAAAG
K		636	.....A.G ----- .....
	<i>Cotyledon</i> spp.		TTTTTT <b>TTCAAAAAG</b> GAATCAAAGAT
L		642	..... ----- .....C..A..
	<i>Cremnophylla nutans</i>		TTTTTCAA --- AASGAATC
	<i>Echeveria</i> spp.		.....T... <b>TTTTAA</b> ..G.....
	<i>Graptopetalum</i> spp.		
	<i>Pachyphytum</i> spp.		
	<i>Sedum burito</i>		
	<i>Sedum clavatum</i>		
	<i>Sedum farinosum</i>		
	<i>Sedum furfuraceum</i>		
	<i>Sedum fusiforme</i>		
	<i>Sedum hemsleyanum</i>		
	<i>Sedum laconicum</i>		
	<i>Sedum lancerotense</i>		
	<i>Sedum multiceps</i>		
	<i>Sedum nudum</i>		
	<i>Sedum oryzifolium</i>		
	<i>Sedum rubrotinctum</i>		
	<i>Sedum sexangulare</i>		
	<i>Sedum ternatum</i>		
	<i>Sedum urvillei</i>		
	<i>Thompsonella minutiflora</i>		
	<i>Villadia imbricata</i>		
	<i>Sedum bulbiferum</i>		.....T... <b>CTTTAA</b> ..G.....
	<i>Sedum morrisonensis</i>		
	<i>Sedum sarmentosum</i>		
	<i>Sedum oxoconicum</i>		.....T... <b>GATTAA</b> ..G.....

TABLE 2. Continued.

Indel	Taxa	Pos.	Sequence region
M	<i>Aeonium</i> spp.	798	GAAAAAAAA AAA --- CATCTTG
	<i>Aichryson</i> spp.		..... ----- .T.....
	<i>Greenovia</i> spp.		
	<i>Monanthes</i> spp.		
	<i>Sedum modestum</i>		
	<i>Sedum jaccardianum</i>		
N	Other Crassulaceae	810	.RD...DW AAAAAA .T.....
			ATCTTGT AGAAGTCTT TGCTAAT
O	<i>Crassula orbicularis</i>	825	.AA.... ----- .TA....
	<i>Crassula</i> spp.		TAAT GATTTT CAGACCATCC
	<i>Sedum fusiforme</i>		.... AATTTT ..A....AG.
	<i>Sedum lancerotense</i>		RTT. TCTRTT ..A....AG.
	<i>Sedum nudum</i>		
	<i>Sedum oryzifolium</i>		
P	<i>Sedum urvillei</i>	1038	.... ----- ..A....AG.
	Other Crassulaceae		CTTTAT ----- GGGCTATTT
	<i>Sedum fusiforme</i>		.G.... GTTTAT ...T.....
	<i>Sedum lancerotense</i>		
	<i>Sedum nudum</i>		

**Kalanchoe clade**—Our analyses reveal a strongly supported *Kalanchoe* clade that includes six genera, *Adromichus*, *Tylecodon*, *Cotyledon*, *Bryophyllum*, *Kitchingia*, and *Kalanchoe* (Fig. 1). This *Kalanchoe* clade encompasses Berger's Kalanchoideae, which consists of three genera (*Bryophyllum*, *Kitchingia*, and *Kalanchoe*), as well as three genera of Berger's Cotyledonoideae (*Adromichus*, *Cotyledon*, and *Tylecodon*). Hence, this single clade illustrates well the lack of correspondence between monophyletic groups and the six traditionally recognized subfamilies. The limits of *Bryophyllum*, *Kitchingia*, and *Kalanchoe* have been much debated among systematists, with some recognizing three genera (e.g., Berger, 1930) and others two genera (e.g., 't Hart and Eggli, 1995) or even a single genus (Baldwin, 1938). Analyses of *matK* sequence data place *Kitchingia* and *Bryophyllum* within *Kalanchoe* with strong support, suggesting that it may be more appropriate to recognize the single genus *Kalanchoe*. However, greater taxon density is needed to resolve fully the boundaries of these genera.

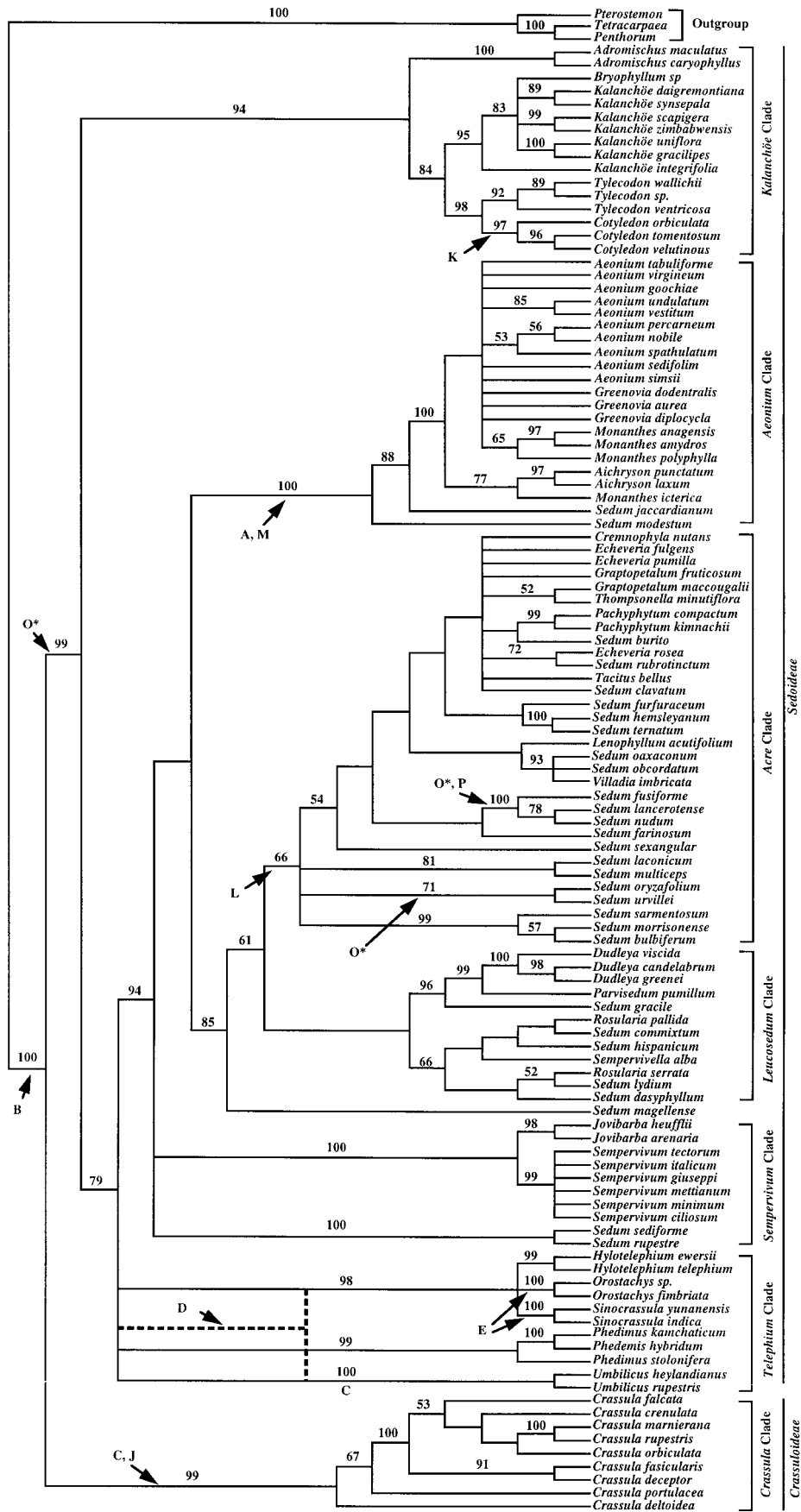
Representatives of the *Kalanchoe* clade all possess flowers with fused corollas and are mostly distributed in southern Africa. The close relationship between *Kalanchoe* and certain genera of Cotyledonoideae, such as *Adromischus* and *Cotyledon*, was suggested by Baldwin (1938), who, based on cytology, hypothesized that the *Kalanchoe* subfamily was an allopolyploid derivative involving "Cotyledon-like" and "Crassula-like" ancestors. Uhl (1948) similarly suggested that Kalanchoideae are polyploids derived from "Cotyledon-like" ancestor(s), but he excluded the role of a "Crassula-like" taxon. Uhl further suggested that *Adromischus* and *Cotyledon* were closely related to *Kalanchoe*, but that several other genera (e.g., *Umbilicus*, *Mucizonia*, and *Pistorinia*) placed within the Cotyledonoideae by Berger (1930) were not closely related to *Cotyledon* or *Adromischus*. While the proposed polyploid origin of Kalanchoideae remains to be tested more rigorously, our data do suggest that a *Cotyledon*-like taxon could have been the maternal parent, contributing the chloroplast genome to Kalanchoideae.

**Telephium clade**—When only base substitutions are considered, *matK* sequence data are inconclusive with regard to monophyly of the *Telephium* clade. However, taxa in the *Telephium* clade share a 6-bp insertion (indel D, Table 2), and if this indel is coded as an additional character and included in parsimony analyses, the *Telephium* clade is monophyletic, but receives bootstrap support <50%. This clade was also weakly supported (bootstrap value of 25%) using cpDNA restriction site data (Ham and 't Hart, 1998).

Our analyses of *matK* sequence data resolve and strongly support three subclades within the *Telephium* clade: (1) *Umbilicus*, a genus placed by Berger (1930) in Cotyledonoideae; (2) *Orostachys* and *Sinocrassula*, both of Berger's Sedoideae, and *Hylotelephium*, a segregate genus of *Sedum*; and (3) *Phedimus*, still another segregate of *Sedum*. Hence, this clade again illustrates well the problems of the traditional delineation of subfamilies in Crassulaceae.

*Umbilicus* is primarily Mediterranean in distribution and, like other members of Berger's Cotyledonoideae, has five-parted flowers with fused corollas. However, this genus has a base chromosome number of  $x = 24$ , whereas other Cotyledonoideae have  $x = 9$ . In addition, the chromosome morphology and biogeography of *Umbilicus* differ from Cotyledonoideae (Uhl, 1948). Although the closest relative of this genus within the *Telephium* clade is unresolved, our analyses support Uhl's conclusion that *Umbilicus* is not allied with other Cotyledonoideae.

A subclade within the *Telephium* clade comprising *Hylotelephium*, *Sinocrassula*, and *Orostachys* is also strongly supported. Within this subclade a sister-group relationship between *Orostachys* and *Sinocrassula* is supported by a 3-bp insertion (indel E) that is unique to these genera. Taxa in this subclade share a primarily Asian distribution, ranging from southwestern China (*Sinocrassula*) to central Asia (*Orostachys* and *Hylotelephium*). *Orostachys* is particularly noteworthy in that most species were originally described as members of *Cotyledon*, but various authors have placed these taxa within



*Sempervivum*, *Sedum*, *Umbilicus*, and *Crassula* (reviewed by Uhl, 1948).

***Sempervivum* clade**—Ham and 't Hart's analyses of cpDNA restriction sites recovered a clade comprising *Sempervivum* and several Eurasian *Sedum* species, including *S. sediforme*, *S. mooneyi*, and *S. assyriacum*. Although their *Sempervivum* clade was only weakly supported (bootstrap of 17%), it suggested a close relationship between *Sempervivum* and *Sedum* section *Rupestre*, a relationship previously suggested by Jacquuin (1770) and Uhl (1961a). While our analyses do not contradict the monophyly of the *Sempervivum* clade, it is not resolved on our strict consensus topology.

*Jovibarba* and *Sempervivum* form a clade (bootstrap of 100%); and each is monophyletic (bootstrap of 98 and 99%, respectively). Berger (1930) considered *Jovibarba* to be a section of *Sempervivum*. In contrast, Parnell (1991) recognized *Jovibarba* as a genus distinct from *Sempervivum*. *Jovibarba* and *Sempervivum* have similar chromosome morphology and overlapping base chromosome numbers (Uhl, 1961a) suggesting that they are closely related, but these data are inconclusive regarding whether they should be treated as distinct genera.

***Leucosedum* clade**—Taxa in the *Leucosedum* clade are distributed throughout the arid southwestern United States, Mexico, and Europe. This biogeographically widespread clade is weakly supported (bootstrap <50%) by our analyses. In contrast, it is one of the most strongly supported clades (bootstrap of 82%) in analyses of cpDNA restriction sites (Ham and 't Hart, 1998). In part, this difference in support may be due to differences in taxon sampling. For example, our data set includes 12 species with nearly equal sampling of Mexican and European taxa, whereas that of Ham and 't Hart sampled 11 species, primarily from Europe.

Although the *Leucosedum* clade does not receive strong support in our analyses, two subclades were recovered, each with moderate to high bootstrap support. One subclade includes *Dudleya*, the monophyly of which is strongly supported (bootstrap of 100%), and its sister taxa *Parvisedum* followed by *Sedum gracile* (bootstrap of 96%). In addition, a subclade of mostly European Sedoideae, including *Rosularia*, several species of *Sedum* (e.g., *S. dasyphyllum*, *S. lydium*, and *S. hispanicum*), and *Sempervivella* was recovered, with bootstrap support of 66%. *Rosularia* appears polyphyletic, which was also suggested by Ham and 't Hart (1998).

***Acre* clade**—This clade comprises Echeverioideae and species of *Sedum* from around the world (Fig. 2), including *S. nudum*, *S. fusiforme*, and *S. lancerotense* from Macaronesia; *S. furfuraceum*, *S. burito*, and *S. clavatum* from Mexico; *S. urvillei*, and *S. oryzifolium* from Asia; and *S. multiceps* from Europe. Placed within this clade are taxa that are among the most variable and confusing taxa based on chromosome data, including *Pachyphytum*, *Graptopetalum*, *Echeveria*, *Lenophyllum*, and the Mexican species of *Sedum* (see below).

The support for the *Acre* clade in our analyses is low (boot-

strap of 66%) when compared to the analyses of Ham and 't Hart (bootstrap of 100%). This is likely attributable to the small taxon sampling of Ham and 't Hart compared to the present study (13 vs. 32 taxa). Ham and 't Hart included a single species of each of only three genera of Echeverioideae, whereas our data set includes eight genera of Echeverioideae (four of five genera recognized by Berger), four additional genera described since Berger's treatment (*Dudleya*, *Graptopetalum*, *Thompsonella*, and *Tacitus*), and a greater density of Mexican *Sedum* species. The topology recovered in our analyses is not in conflict with that of Ham and 't Hart (1998), but contains a number of unresolved polytomies. That *matK* provides few characters to resolve many of the relationships within this clade might indicate that this clade is of relatively recent origin. Also relevant to this low resolution is the substantial intergeneric and inter-subfamilial crossability found among many of the taxa in the *Acre* clade (Spongberg, 1978; Uhl, 1989, 1994). It is, therefore, likely that frequent chloroplast exchanges have occurred among members of this clade, affecting a plastid-based phylogeny.

Several well-supported subclades within the *Acre* clade were recovered by our analyses. There is strong support (bootstrap of 100%) for a clade of *Sedum* species (*S. nudum*, *S. fusiforme*, and *S. lancerotense*) from Macaronesia and Africa and a clade comprising *S. ternatum* and *S. hemsleyanum* (bootstrap of 100%), both native to North America. Also well supported is the monophyly of *Pachyphytum* (bootstrap of 99%). The *Acre* clade comprises approximately one-third of the taxonomic diversity of Crassulaceae, and while our study expands upon the phylogenetic hypothesis of Ham and 't Hart (1998), much more phylogenetic work is needed to resolve fully the generic boundaries and relationships within this large clade.

***Aeonium* clade**—This clade comprises four genera of Sempervivoideae (*Aeonium*, *Aichryson*, *Greenovia*, and *Monanthus*) that are largely endemic to Macaronesia. These genera were placed by Berger in Sempervivoideae because, like *Sempervivum*, these taxa all possess, in varying degrees, polymorous flowers. Our analyses strongly support the monophyly of the *Aeonium* clade and place two African species of *Sedum*, *S. modestum* and *S. jaccardianum*, as sister to the four Macaronesian genera. In addition to analyses of base substitutions, two indels (A and M) support the monophyly of this clade. The sister relationship between the Macaronesian genera and some African *Sedum* species was suggested by Uhl (1961a) and has recently been supported by cpDNA restriction sites (Ham and 't Hart, 1998), as well as RAPD analyses and ITS sequence data (Mes, 1995). Analyses of *matK* sequences also indicate that the Macaronesian genera are not closely related to the *Sempervivum* clade, as proposed by Berger (1930).

Most authors (e.g., Lems, 1960; Uhl, 1961a; Liu, 1989; Mes, Wijers, and 't Hart, 1997; Ham 't Hart, 1998) agree that the four Macaronesian genera are closely related, but the relationships among and limits of these genera have been debated. *Aichryson* differs from the rest of these genera in having base chromosome numbers of  $x = 15$ , 16, or 17, and, with

←

Fig. 2. One of 5000 minimum length trees recovered from parsimony analyses of *matK* sequences for 112 members of Crassulaceae and three outgroup taxa. Numbers above each branch are support values resulting from 5000 replicates of "fast" bootstrap analyses; numbers below the lines are branch lengths. The phylogenetic distributions of indels in *matK* are indicated as letters below the clades in which they are found. Clade names are those applied previously by Ham and 't Hart (1998).

one exception, the genus comprises annuals. The other genera, with one exception, *Monanthes ictERICA*, are perennials. *Monanthes* shares a basic number of  $x = 18$  with *Aeonium* and *Greenovia*, but differs by having large, petaloid nectary scales. *Greenovia* differs from *Aeonium* in several respects, including 20–35 merous flowers (vs. 12–18 merous flowers), carpels partially sunken into the receptacle, and placentation type.

Mes (1995) suggested that *Greenovia* is derived from within *Aeonium*. However, there is little support for these conclusions as measured by their bootstrap and decay analyses. Also, these conclusions must be considered tentative as the authors included only a single species of *Greenovia*. The placement of *Monanthes ictERICA* (annual habit) has also been debated. Recent phylogenetic analyses (Mes, Wijers, and 't Hart, 1997) indicate that this species is imbedded within a clade of *Aichryson* species.

Bootstrap support for relationships within the *Aeonium* clade is generally low. However, there is moderate support for the association of *Monanthes ictERICA* with species of *Aichryson* (bootstrap of 77%). In addition, several small clades are resolved and receive moderate bootstrap support. The results of our analyses are in agreement with a recent radiation of the *Aeonium* clade in Macaronesia. Phylogenetic analyses of the *Aeonium* clade employing both additional taxa and gene sequences are currently in progress (Mort et al., unpublished data).

**Character evolution**—Berger (1930) relied largely on three floral characters to define subfamilies in Crassulaceae: haplostemonous androecia, sympetalous flowers, and polymeric flowers. It has been suggested elsewhere (e.g., Ham and 't Hart, 1998) that these characters have evolved independently a number of times in Crassulaceae. However, the distribution of these characters has not been investigated in a broad phylogenetic context. Therefore, we traced the distribution of these features onto our strict consensus cladogram (Fig. 3) using MacClade (Maddison and Maddison, 1992). We chose to use the strict-consensus topology because we recovered a large number of minimum-length trees (5000 trees). However, this approach is not without problems, for the strict-consensus tree is a summary of all trees obtained and does not reflect the exact relationships portrayed by any one of the minimum-length trees. Since our goal is to examine the distribution of floral features, and not necessarily the patterns of evolution, we feel that the strict-consensus tree best summarizes the overall results of our phylogenetic analyses. In addition, biogeography was investigated by plotting broadly defined distributions for terminal taxa onto this same cladogram.

**Haplostemy**—Haplostemonous flowers (with a single whorl of stamens) characterize Berger's (1930) Crassuloideae. A haplostemonous androecium has arisen once and is confined to the *Crassula* clade (Fig. 3). However, haplostemy has been reported in *Sedum* (see Uhl, 1963) and *Hypagophytum* (Uhl, 1961a). Species of these genera that display this floral feature were not included in our analyses. Thus, it remains uncertain whether this condition truly arose only once. Furthermore, the

ontogeny and homology of this character have yet to be investigated. Additional taxonomic sampling, as well as detailed morphological investigation of this character, are necessary to address fully the evolution of haplostemy.

**Sympetaly**—Sympetaly, in concert with biogeography and the number of floral parts, was used by Berger (1930) to define three distinct subfamilies: Kalanchoideae, Cotyledonoideae, and Echeverioideae. Sympetaly, however, is not entirely confined to these taxa, but is also present in several genera of Berger's (1930) Crassuloideae and Sedoideae, as well as several different lineages of *Sedum* (Uhl, 1963). Taxa displaying any fusion of petals were scored as sympetalous. This strategy likely underestimates the complexity of floral fusion as not all fused corollas are necessarily the end result of the same developmental pathway (e.g., Erbar, 1991). However, this approach illustrates the distribution of this character and serves as a starting point to further phylogenetically based investigations of the evolution of sympetaly in Crassulaceae.

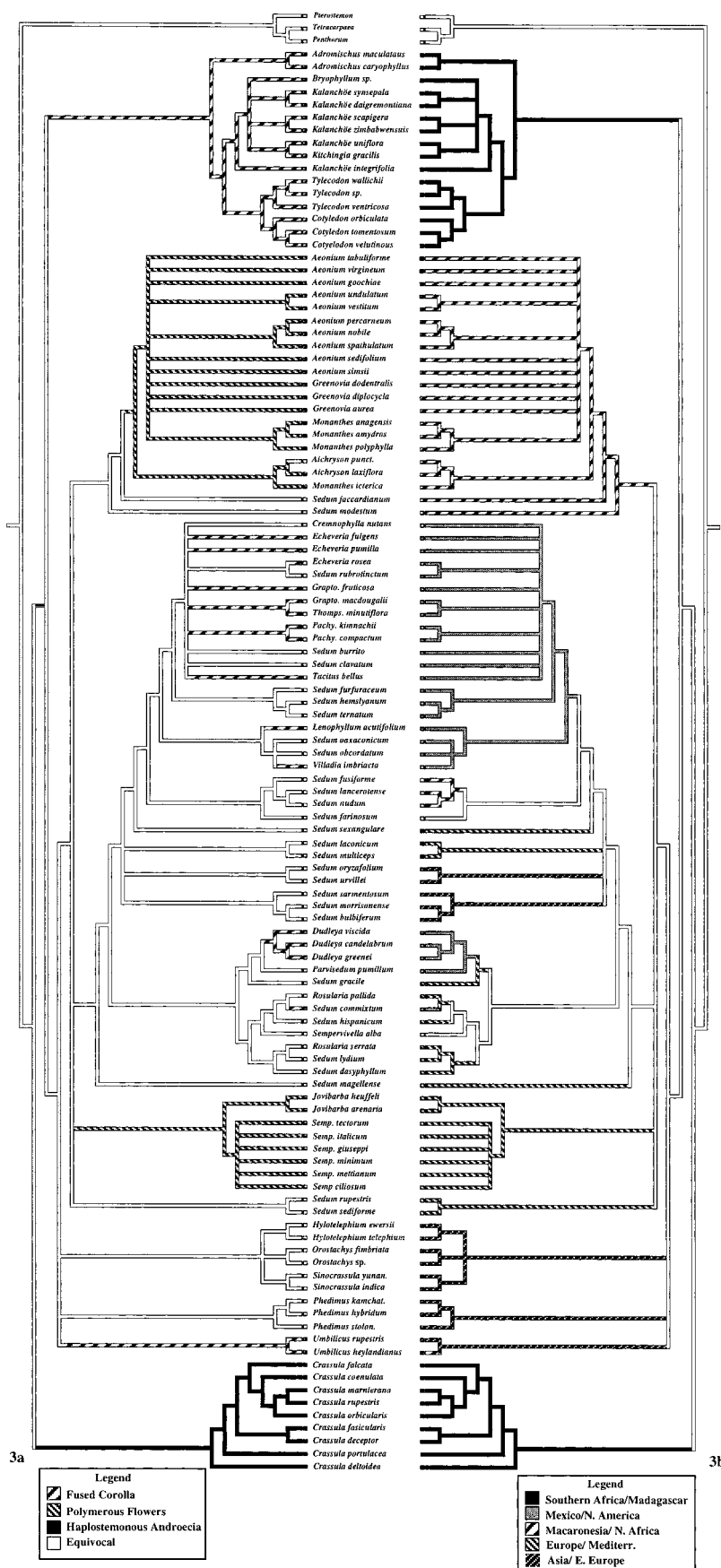
The results of our analyses indicate that sympetaly has arisen in a minimum of five separate lineages of Crassulaceae with no apparent reversals (Fig. 3a). A single origin of sympetaly can be hypothesized for the *Kalanchoe* clade. Additional origins are inferred for *Umbilicus* (*Telephium* clade), as well as two separate origins in the *Leucosedum* clade. Within the *Acre* clade sympetaly is widespread. Our analyses indicate a minimum of two origins of sympetaly in this clade; however, many of these relationships are unresolved or weakly supported.

Not included in our analyses are several taxa known to display sympetaly (e.g., *Rochea*). Therefore, it is possible that this character is even more widespread in Crassulaceae than suggested by our analyses. We conclude that sympetaly is a widespread condition in Crassulaceae that has evolved in parallel in at least five lineages and represents a grossly homoplasious character.

**Polymeric flowers**—The typical number of floral parts in Crassulaceae, as well as the outgroups, is four or five. Members of our Crassuloideae, as well as the early-branching taxa in Sedoideae, possess five-parted flowers. MacClade reconstructs five-parted flowers as the ancestral floral condition in Crassulaceae. We define polymeric flowers as those flowers possessing at least twice this number of floral parts (i.e., ten or greater). Species with polymeric flowers were assigned by Berger (1930) to Sempervivoideae. Our analyses indicate that this subfamily is polyphyletic; two genera (*Jovibarba* and *Sempervivum*) form a well-supported clade that is removed from the remainder of polymeric taxa (*Aeonium* clade). Thus, polymeric flowers appear to have evolved in two separate and well-supported lineages (Fig. 3a).

**Biogeography**—Crassulaceae are nearly cosmopolitan in distribution due to several widespread taxa (e.g., *Tillaea*), but the family has discrete centers of taxonomic diversity, including southern Africa, Mexico, Macaronesia, the Mediterranean region, and the Himalayas (Spongberg, 1978). Within each of these centers of diversity are found genera of Crassulaceae

Fig. 3. Strict consensus topology of 112 species of Crassulaceae and three outgroup taxa based on parsimony analyses of *matK* sequence data. (a) Phylogenetic distribution of three floral features traditionally used to define subfamilies in Crassulaceae. (b) Phylogenetic distribution of broadly defined geographic regions inhabited by each species.



endemic to that region. In addition, in many of these regions (especially those in the Northern Hemisphere), there are species that have been assigned to *Sedum*. As noted above, 't Hart (1982) has hypothesized that *Sedum* is a geographically widespread, paraphyletic genus. He further hypothesized that certain species of *Sedum* appear to have given rise to other genera in the family that are confined to specific biogeographic regions.

Ham and 't Hart (1998) noted two major biogeographic inferences from their cpDNA-based tree. First, Crassulaceae appear to have originated in either southern Africa or the Mediterranean region. Second, Crassulaceae have reached the Macaronesian islands a minimum of three times. Furthermore, review of their topology indicates that *Sedum* species are placed in five of the seven major clades removed.

Our analyses also place species of *Sedum* in five of the seven major clades recovered (Fig. 3); only the *Crassula* and *Kalanchoe* clades lack *Sedum* species. Thus, *Sedum* as currently defined is grossly polyphyletic and in need of taxonomic revision. As predicted by 't Hart (1982), many of the species of *Sedum* included in our analyses generally group together with genera that are confined to a single biogeographical region (Fig. 3b). For example, *S. modestum* and *S. jaccardianum* from north Africa are sister to the genera endemic to Macaronesia, and the Mexican species of *Sedum* are placed in a clade of genera endemic to this same region (viz. *Pachyphyllum* and *Lenophyllum*). However, it is important to note that *matK* is maternally inherited. Because of the potential for hybridization and chloroplast capture, additional sequences from the nuclear genome (or other sources of data) should be analyzed before too many generalizations concerning *Sedum* are made.

As noted, phylogenetic analyses of *matK* sequences strongly support the *Crassula* clade (Crassuloideae) as sister to a large Sedoideae clade; within the latter the *Kalanchoe* clade is sister to the remaining Sedoideae. Taxa comprising the *Crassula* and *Kalanchoe* clades are confined to southern Africa and Madagascar (Fig. 3b), a distribution that suggests a southern African origin of Crassulaceae. However, to test this hypothesized southern African origin of the family, additional analyses employing a broader sampling of taxa from Saxifragales is needed. Regardless of the origin of the family, it appears that the first major diversification event occurred in southern Africa. We hypothesize that from southern Africa the family spread through the Mediterranean region and into eastern Europe and Asia (e.g., *Sempervivum* and *Leucosedum* clades), and species from northern Africa dispersed to Macaronesia, where they subsequently diversified (*Aeonium* clade). North America was reached by Crassulaceae at least two times: once by the ancestor of the clade comprising *Parvisedum* and *Dudleya*, and at least once by the ancestor of a core subclade in the *Acre* clade. These biogeographic patterns are in agreement with those described by Ham and 't Hart (1998).

Included in the Macaronesian Crassulaceae flora are four genera of Sempervivoideae (*Aeonium*, *Aichryson*, *Greenovia*, and *Monanthes*), several species of *Sedum*, and one species of *Umbilicus* (Santos-Guerra, 1983; Bramwell and Bramwell, 1990). Ham and 't Hart (1998) suggest, based on cpDNA restriction site analyses, that Crassulaceae arrived in Macaronesia a minimum of three times. Likewise, our analyses indicate that Crassulaceae have reached the Macaronesian islands three times (Fig. 3b): once by the ancestor of a clade of three species of *Sedum* (*S. nudum*, *S. lancerotense*, and *S. fusiforme*), once by the ancestor of the core of the *Aeonium* clade, and by *Umbilicus*. It is noteworthy that the progenitor of the Macaronesian *Sedum* species appears to be from Mexico, whereas *Umbilicus* and the core of the *Aeonium* clade is of northern African/Mediterranean origin.

**Chromosome numbers**—Chromosome morphology and base chromosome numbers have been extensively studied in Crassulaceae (e.g., Baldwin, 1935, 1937; Uhl, 1948, 1961a, 1963, 1995). Although chromosome numbers are highly variable in most genera and a few species, the patterns of chromosomal evolution are not the same in all groups of Crassulaceae, and thus may have "major phylogenetic significance" (Uhl, 1961). Previously published chromosome counts were used to infer the base number for the major clades recovered in our phylogenetic analyses; these numbers were then plotted onto a simplified topology that we produced to examine in a preliminary fashion the distribution and possible evolution of both base chromosome number and polyploidy (Fig. 4).

The original base chromosome number for Crassulaceae appears to be  $x = 8$ . This is based on the fact that the closest outgroup, *Penthorum*, has  $2x = 16$  and *Tillaea*, the sister to *Crassula* (Ham 't Hart, 1998), also has  $2x = 16$ . A decrease in base chromosome number to  $x = 7$  occurs in *Crassula*.

MacClade also reconstructs  $x = 8$  as the base number for the large Sedoideae clade (Fig. 4). Within the Sedoideae clade, the base chromosome number increases to  $x = 9$  in the *Kalanchoe* clade. Several genera of the *Kalanchoe* clade (*Adromischus*, *Cotyledon*, *Tylecodon*) have  $2x = 18$ . However, all species of *Kalanchoe*, *Bryophyllum*, and *Kitchingia* have either  $x = 17$  or 18 (or a multiple thereof). Base chromosome number, as well as chromosome morphology suggest that the subclade of *Kalanchoe*, *Bryophyllum*, and *Kitchingia* is of polyploid origin (Baldwin, 1938) and is likely derived from a tetraploid "Cotyledon-like" taxon (Uhl, 1963). Our data are in agreement with this hypothesis, suggesting that either the ancestor or some extinct member of the *Cotyledon*/*Tylecodon* subclade was the maternal parent of these polyploids. Another base chromosome number increase from  $x = 8$  to  $x = 12$  and higher has occurred in the *Telephium* clade. Chromosome evolution in this clade is complex and still unclear. The clade comprises three subclades (Fig. 2). The first subclade comprises *Hylotelephium*, *Orostachys*, and *Sinocrassula*; these taxa share a base chromosome number of  $x = 12$ . A second subclade corresponds to *Phedimus*, which has a base number of  $x = 16$ . *Umbilicus* ( $x = 24$ ) forms the third subclade. Because analyses of *matK* sequences do not resolve the relationships among these subclades, it is not possible to infer the ancestral chromosome number for this clade. Regardless, the base chromosome numbers of  $x = 16$  and  $x = 24$  are likely the result of polyploidy. However, the origin of  $x = 12$  is unclear. It could represent aneuploid increase from  $x = 8$  or decrease from a polyploid ancestor with  $x = 16$ . The *Telephium* clade, therefore, represents still another episode of polyploidy in Crassulaceae.

Base chromosome numbers for the *Sempervivum* clade are  $x = 16, 17, 18, 19, 28$ . Within this clade, *Jovibarba* and *Sempervivum* form a clade and have base chromosome numbers of  $x = 19$  and  $x = 16$ –19, respectively. The *Sempervivum* clade likely represents another instance of polyploid increase from  $x = 8$ , coupled with aneuploidy. Another episode of polyploidy is suggested for *Sedum* section *Rupestre*, which has  $x = 28$ . Relationships within the *Sempervivum* clade are un-

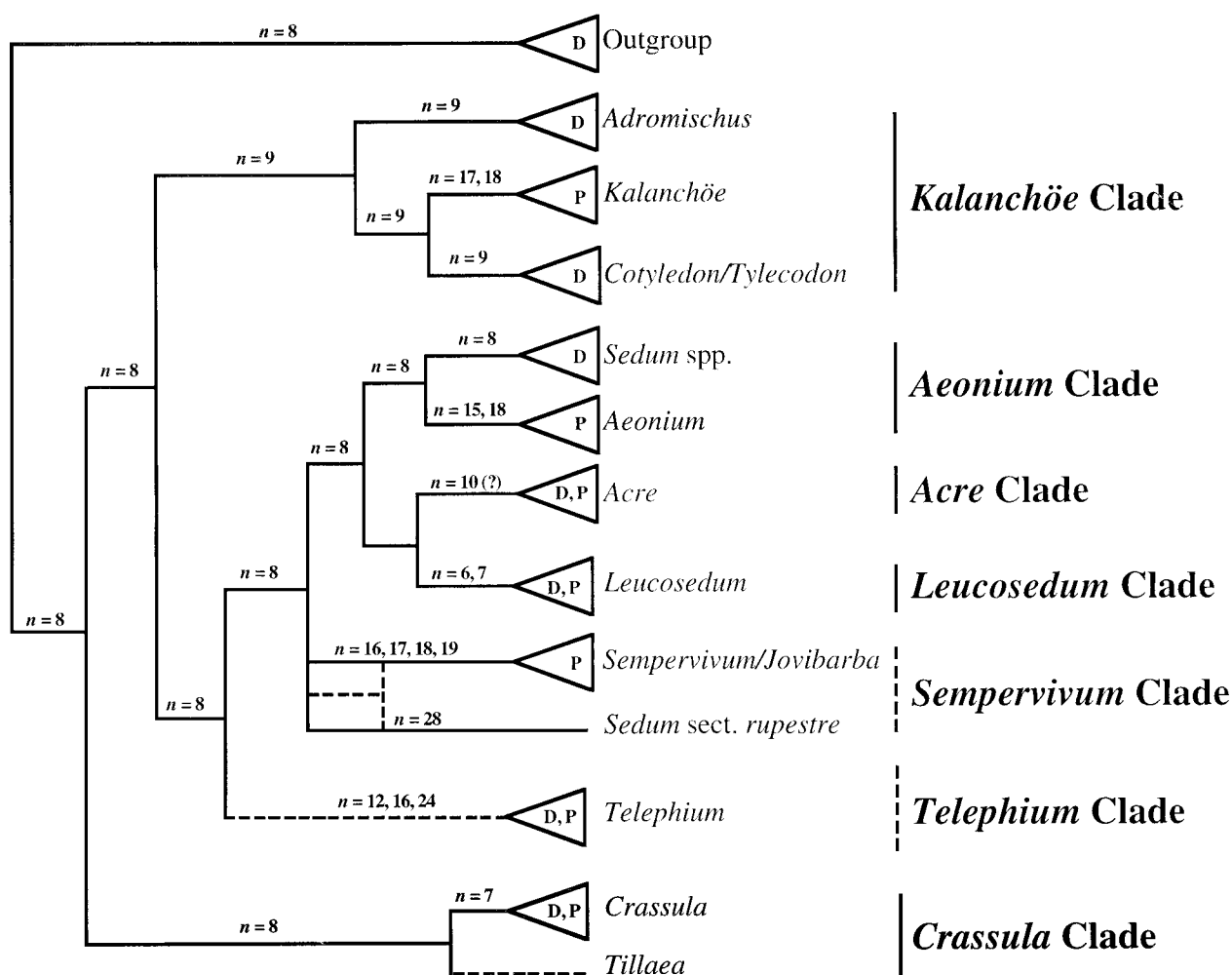


Fig. 4. Simplified summary topology representing the relationships among major clades in Crassulaceae. Previously named clades that were neither recovered nor contradicted by our analyses of matK sequences are indicated by dashed lines and brackets. Included as sister to *Crassula* is *Tillaea* (indicated by a dashed line) following Ham and 't Hart (1998). Base chromosome numbers reconstructed using MacClade are indicated above each branch. The occurrence of diploid (D) and polyploid (P) species in each clade is indicated in each triangle.

clear, and additional work is needed to resolve the cytological evolution of this clade.

The *Acre* and *Leucosedum* clades are sister groups; their ancestral base chromosome number is equivocal based on our MacClade reconstruction (Fig. 4). Within the *Leucosedum* clade most taxa are diploid with  $2x = 12, 14$  ( $x = 6, 7$ ). Two subclades are found within this clade (Fig. 2). The first comprises taxa that have base chromosome numbers of either  $x = 6$  or  $7$ , with the exception of *S. dasyphyllum*, which has  $x = 14$ . Likewise, the first branching members of the second subclade have  $x = 6$ . However, *Dudleya*, also in this subclade, has  $x = 17$  (or a multiple thereof), which suggests another polyploid event in Crassulaceae.

Taxa within the *Acre* clade display a wide array of chromosome numbers, ranging from  $x = 6$  to  $x = 270$  (or greater); polyploidy appears to be widespread in this clade (e.g., Uhl, 1963, 1970, 1993; Federov, 1969; Moore, 1973; Uhl and Moran, 1973). Because of this common polyploidy, the base number of many genera is often not apparent, which greatly complicates inferences of cytological evolution. However, when examined in a phylogenetic context, it is possible to make

several general inferences regarding chromosomal evolution in the *Acre* clade.

The early-branching members of the *Acre* clade comprise a number of small clades that are primarily Mexican species of *Sedum*. These groups are highly variable in base chromosome number. Uhl (1961b) reported 45 different base numbers for 60 *Sedum* species from this region; however,  $x = 10$  is most common. In the remainder of the *Acre* clade polyploidy appears to be very common, and chromosomal evolution is especially complex. For example, species of *Pachyphytum* have  $x = 31-33$ , with polyploids of  $x = 66$  and  $x = 128$  reported (Uhl and Moran, 1973). The base number for *Graptopetalum* is  $x = 30-35$ , with two species forming polyploids of  $x = 240$  to  $275$  (Uhl, 1970). Polyploidy and aneuploidy have likely played a major role in the evolution of this clade.

In the *Aeonium* clade, *Sedum modestum* and *S. jaccardianum* are sister to the Macaronesian Sempervivoideae. These species of *Sedum* share a base chromosome number of  $x = 8$ ; the base number for many of the remaining species in the *Aeonium* clade is  $x = 18$ , indicating that the core of this clade

is polyploid, apparently derived from diploid ancestors with  $x = 8$ .

**Summary**—Relationships in Crassulaceae have been a focus of study for a number of years, and students of the family have employed a variety of tools to unravel these relationships. Analyses of *matK* sequence data provide additional insights into the evolution of this family. The results of our analyses are in agreement with those of Ham and 't Hart (1998) in that two major clades are recovered: a *Crassula* clade (Crassuloideae) and a large clade comprising six subclades (Sedoideae). Thorne (1983) recognized three subfamilies: Crassuloideae, Kalanchoideae (including Cotyledonoideae), and Sedoideae. Our topology is also in basic agreement with this treatment in that within the large Sedoideae clade there are two clades, one corresponding to Thorne's Kalanchoideae, and one (with five subclades) that corresponds to Thorne's Sedoideae. Five of the seven clades of major interest named by Ham and 't Hart (1998) are recovered by our analyses: the *Crassula*, *Kalanchoe*, *Acre*, *Leucosedum*, and *Aeonium* clades. In many instances these clades are also supported by other sources of data (e.g., cpDNA restriction sites, base chromosome number, biogeography). Two clades recognized by Ham and 't Hart (1998), the *Telephium* and *Sempervivum* clade, are not contradicted by our analyses, but receive bootstrap support below 50%. If indels are included in our analyses, the *Telephium* clade is recovered, but no indels support the *Sempervivum* clade. Phylogenetic relationships are largely unresolved in the *Acre* and *Aeonium* clades, possibly suggesting a relatively recent radiation of these lineages.

This study also provides initial insights into character evolution and biogeography in Crassulaceae. Two floral features often used to define subfamilies of Crassulaceae, sympetaly and polymorous flowers, have arisen independently in several lineages. A third floral character, haplostemy, is confined to the *Crassula* clade. Crassulaceae appear to have arisen in southern Africa, from where the family spread northward into the Mediterranean region. From there, the family spread to Asia/eastern Europe and northern Europe, giving rise to a number of biogeographically confined genera. Two separate lineages of European Crassulaceae subsequently dispersed to North America and underwent substantial diversification. The northern African Crassulaceae subsequently dispersed to the Macaronesian islands where the genera *Aeonium*, *Aichryson*, *Greenovia*, and *Monanthes* arose. In addition, Crassulaceae reached the Macaronesian islands at least two additional times. Finally, these analyses support 't Hart's (1982) hypothesis that *Sedum* is a geographically widespread, polyphyletic taxon, with species having close affinities for genera that are confined to a single biogeographic region. However, while the current study includes 29 species of *Sedum* as well as several genera recently segregated from *Sedum* (e.g., *Hylotelephium*, *Phedimus*), additional taxon sampling, as well as sequence data from the nuclear genome, are needed to address relationships within this genus.

Our studies suggest that the base chromosome number for Crassulaceae was  $x = 8$  with a reduction to  $x = 7$  in *Crassula* and  $x = 6, 7$  in the *Leucosedum* clade. Polyploidy is widespread in the family and has played a role in the evolution of seven major clades of Crassulaceae. Three of these clades are exclusively polyploid (*Sempervivum* clade, and subclades of the *Kalanchoe* and *Aeonium* clades), whereas four clades (*Crassula*, *Telephium*, *Leucosedum*, and *Acre* clades) comprise

both diploid and polyploid taxa. Polyploidy has been particularly common and cytological evolution especially complex in the *Acre* clade.

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