

Molecular Phylogenetics of Crassulaceae

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ABSTRACT

Crassulaceae is the most species-rich family (ca. 1400 spp.) of the Saxifragales and the majority of its members are succulents. Great diversity of morphology, cytology and habit complicates systematics of the family and the relationships between species and genera remain poorly understood. Studies using various molecular markers placed Crassulaceae as one of Saxifragales crown groups and showed close relationships between the family and Haloragaceae, lacking any phenotypic background. Earlier molecular data analyses established a number of clades in the family and revealed a disagreement between the traditional taxonomic structure of Crassulaceae and the pattern of phylogenetic relationships between its members. In this paper, we review the major contributions to the phylogeny of Crassulaceae based on molecular data, with emphasis on the major clades established in the family, the clades' structure and polyphyly of some genera. We describe the areas of conflict and agreement between molecular phylogenies and stress that morphological characters provide little evidence for inferring relationships between taxa even at low taxonomic levels.

Keywords: clades, family taxonomic structure, molecular data, phylogenetic relationships

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INTRODUCTION

The morphologically diverse and systematically complex family Crassulaceae comprises approximately 1400 species currently classified in 33 genera (Eggli 2003). Most of its members are well adapted to xerophilic conditions although a number of aquatic species are known in the genus *Crassula*. The family includes no crop species but many are popular in horticulture as rocky garden, green roof and indoor plants.

Most of the species are perennial herbs, subshrubs or shrubs with succulent leaves, 5(3-32)-merous and radially symmetrical flowers having one or two whorls of sepals that are usually as many or twice as many as petals. The family has nearly cosmopolitan distribution with its members occurring predominantly in semiarid and mountainous habitats of the temperate and subtropical areas. Crassulaceae are particularly diverse in southern Africa, the suspected place of the family origin (Ham and 't Hart 1998), Mexico, Macaronesia, Mediterranean region and the Himalayas.

Crassulaceae are interesting from the physiological point of view because it is the only plant family where crassulacean acid metabolism (CAM), an ancient photosynthetic pathway that likely present in early vascular plants lived in seasonal pools in the Mesozoic era (200 Ma), occurs in both aquatic and terrestrial representatives (Keeley 1998; Keeley and Philip 2003).

The entity of the family has never been disputed except

for the genus *Penthorum*, once a member of the Crassulaceae (Candolle 1828a; Hutchinson 1973) that was then shifted to Saxifragaceae (Baillon 1871; Engler 1930; Takhtajan 1980; Cronquist 1991) or recognised as an independent monotypic family (Tieghem 1898; Thorne 1992, 2000). Traditionally Crassulaceae was considered a primitive rosoid because of its floral structure and embryological features and seen related to the Saxifragaceae (Baillon 1871; Takhtajan 1980; Dahlgren 1983; Cronquist 1991; Thorne 2000). Molecular phylogenetic studies confirmed this alliance, firmly placing Crassulaceae in the Saxifragales where it forms a clade together with Penthoraceae and Haloragaceae (Hoot *et al.* 1999; Qiu *et al.* 1999; Savolainen *et al.* 2000; Soltis *et al.* 2000; Fishbein *et al.* 2001; Fishbein and Soltis 2004). It was hypothesised that the clade diverged ca. 70 Ma and its major lineages are of the same age (Wikström *et al.* 2001; Crepet *et al.* 2004).

Apart from the distinct and easily recognizable appearance of the family members, definition of their monophyletic groups remains problematic because of diverse morphology, cytology, and habit (Ham and 't Hart 1998; Mort *et al.* 2001; Eggli 2003). By the end of 19th and the beginning of 20th centuries, several family concepts had been proposed (de Condolle 1828a, 1828b; Schönland 1891; Fröderström 1930, 1931) but only that of Berger (1930) was widely accepted (see brief review of 't Hart and Eggli 1995). He recognised six subfamilies and 33 genera in the Crassulaceae, these based on the number and arrangement of floral parts, the degree of sympetaly, and phyllotaxis. According

to Berger, the subfamilies compose two lineages, Crassula (Crassuloideae, Cotyledonoideae, and Kalanchoideae) and Sedum (Echeverioideae, Sedoideae, and Sempervivoideae) that are distinct in their distribution. Members of the Crassula lineage occur primarily in southern Africa, while those of the Sedum lineage are found predominantly in the Northern Hemisphere. Flowers with unfused corollas and a single whorl of stamens (haplostemonous) differentiated Crassuloideae from Cotyledonoideae and Kalanchoideae having fused corollas with pentamerous and tetramerous flowers, respectively. Echeverioideae were characterised by pentamerous more or less fused corollas, and Sempervivoideae were distinct in unfused, polymerous flowers. These five of the six families appeared to be morphologically as well as geographically more or less defined. However, characteristic of the largest subfamily Sedoideae has always been illusive. The largest crassulacean genus *Sedum* (ca. 500 spp.) was a core of the subfamily that also included other genera that could not be attributed to any of the subfamilies mentioned above. The artificial nature of Berger's system and particularly of his Sedoideae has been generally acknowledged (Fröderström 1930; Uhl 1961b, 1963; Ohba 1978; 't Hart 1982, 1991; Eggli *et al.* 1995), nevertheless, it has been followed in most regional floras.

In the later major classifications of the flowering plants, still based on phenotypical traits, number of the subfamilies was reduced to four (Tahtajan 1987) and then to three (Thorn 1992; Tahtajan 1997).

In the last two decades molecular phylogenetic analyses has advanced our understanding of the relationships between plants at different levels, challenged many long-standing hypotheses on plants evolution and significantly affected their systematics. Crassulaceae are not an exception in this respect. However, apart from the fact that the first Crassulaceae nucleotide sequences were deposited in the Genbank as early as in 1992 (Albert *et al.* 1992), phylogenetic relationships in the group are far from being well established. Here our attempt is to summarize recent studies that have addressed the evolution and taxonomy of Crassulaceae from a molecular phylogenetic perspective. We briefly discuss the family's closest relatives, the types of molecular data used to analyse relationships within Crassulaceae, the major clades established in the family and we point out discordance between family phylogeny and taxonomy, arguing that considerable work remains to be done before a convincing picture emerges.

CLOSEST RELATIVES OF CRASSULACEAE

The alliance of the family Crassulaceae to the order Saxifragales and its close relationship to the Saxifragaceae has been suggested by all modern authors (Takhtajan 1987; Cronquist 1991; Thorne 1992), supported by phenotypic characters (Grund, Jensen 1981; Hufford 1992) and further confirmed by earlier molecular phylogenetic studies (Chase *et al.* 1993; Morgan and Soltis 1993; Soltis *et al.* 1993, 1997; Soltis and Soltis 1997). Various hypotheses have been put forward on the closest relatives of the family in Saxifragales. Different data sets and markers show it to be either as a sister to Podostemaceae (Ueda *et al.* 1997), Cynomoriaceae (Nickrent *et al.* 2005) or to Haloragaceae (Morgan and Soltis 1993; Soltis and Soltis 1997) and it appears that the latter relationship is the best supported. Sisterhood of Crassulaceae and Podostemaceae established by *rbcL* sequence comparisons with relatively high significance was later refuted by combined SSU rDNA and *rbcL* analyses (Soltis *et al.* 1999) and attributed to possible long branch attraction and insufficient taxon sampling.

The affinity of aquatic Haloragaceae to Saxifragales and its close relationship with Crassulaceae emerged only in molecular phylogenies and apparently it is not reflected by any phenotypic character (Soltis *et al.* 2005). Nevertheless, recent multigene analyses resolved Haloragaceae – Crassulaceae sistership with high significance (Fishbein *et al.* 2001; Fishbein and Soltis 2004) confirming earlier res-

ults based on *rbcL* along (Soltis and Soltis 1997). The clade occupies the crown position in the Saxifragales, often as a sister group of the Saxifragaceae alliance (Hoot *et al.* 1999; Soltis *et al.* 2000; Fishbein *et al.* 2001; Fishbein and Soltis 2004). It was shown that the genus *Penthorum* (Penthoraceae or Haloragaceae *s. l.*), once seen as a member of the Crassulaceae and morphologically the most similar to them, is in fact rather distant. Instead, *Aphanopetalum* and *Tetracarpaea*, both quite distinct from each other and Crassulaceae in morphology, occupy basal position in the Haloragaceae clade and thus share more recent ancestry.

Types of molecular data

Apart from the widely recognized complexity of relationships within Crassulaceae, surprisingly few attempts have been made to resolve this issue. The first assessment of crassulacean phylogeny based on chloroplast DNA restriction-site data and *trnL-F* intergenic spacer, both rather limited in taxon sampling, highlighted the problem and identified direction for further studies (Ham 1994; 't Hart 1995; Ham and 't Hart 1998). This work was followed by a broad (112 species of all major genera) analysis of chloroplast *matK* sequences (Mort *et al.* 2001). The phylogenetic tree presented by these authors was more detailed and significantly improved our understanding of the evolutionary trends in the family. Their gene tree generally agreed with the phylogenetic hypotheses put forward by Ham and 't Hart, many deep branches attained significance but the branching pattern within major lineages has remained partially unresolved and some of the branches were still under-sampled.

The recent study by Mayuzumi and Ohba (2004) aimed to resolve the phylogeny of East Asian Sedoideae but it also included representatives of other groups in the analyses. Incongruent taxon sampling with *trnL-F* chloroplast intergenic spacer (103 species) and nuclear ITS rDNA region (74 species) resulted in a combined analyses of 78 taxa, mostly from putative Telephium clade (see below). In all these phylogenies, the most complex genus *Sedum* was represented by a limited number of taxa and the relationships between its members remained unknown. To close this gap, Gontcharova and co-authors created a dataset that included 158 crassulacean ITS rDNA sequences where *Sedum* was represented by 65 species (15% of the total species number) and used it to reconstruct phylogeny within the family with nuclear-encoded marker (Gontcharova *et al.* in press). Their alignment was based on secondary structure models of ITS1 and ITS2 proposed for Crassulaceae (Gontcharova and Gontcharov 2004), easing search for homologous positions between rather divergent sequences (Coleman 2003).

Thus, there are only four studies to date that analyze crassulacean phylogeny at the family level. There is general agreement among them regarding the pattern of relationships within the group but the composition of some clades, particularly those forming crown assemblage of the family tree and their exact branching order, remains uncertain. Comparison of the three sequence-based phylogenies shows that neither coding (*matK*; Mort *et al.* 2001) nor non-coding sequences (*trnL-F* spacer and ITS rDNA; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press) provide sufficient phylogenetic signal to resolve deep branches of the tree significantly. Interestingly enough, the same clades, namely Telephium, Leucosedum and Sempervivum (see below), are the most problematic in this respect in three independent phylogenies (Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press). The question of whether this reflects their recent rapid radiation or limited resolving power of the markers used has yet to be answered.

Limited taxon sampling could also contribute to low resolution of the crassulacean tree (Pollock *et al.* 2002; Hillis *et al.* 2003). Although 28 of 33 currently recognized genera of putative non-hybrid origin (Eggli 2003) were rep-

resented in the analyses mentioned above, some clades, particularly the most complex and least resolved ones, are severely undersampled. The remaining five genera contain only a few species and basically are poorly studied. Some assumptions on their relationship to the rest of the family were made based on morphology (Eggl *et al.* 1995; 't Hart 1995; Eggl 2003) that, however, could be misleading in this group, an issue we discuss below. It might be expected that extended taxon sampling and a greater number of characters will lead to better resolution of the relationships within Crassulaceae.

Combined analyses of several markers have been used to address phylogenetic relationships within some crassulacean genera and clades (Mes *et al.* 1997, 2002; Fairfield *et al.* 2004; Mayuzumi and Ohba 2004; Mort *et al.* 2005, 2007). Generally, they improved the resolution of single loci analyses but also revealed incongruence between chloroplast and nuclear sequences attributed to cpDNA capture and/or hybridization (Mort *et al.* 2002, 2007). Although there is an indication that these processes may have played an important role in shaping the family (Prager 1929; Uhl 1993, 1994a, 1994b, 1994c; Nyffeler 1995; Mort *et al.* 2001; Eggl 2003), their extent has yet to be rigorously studied using molecular tools. Phylogenetic analyses of *Graptopetalum* and closely related taxa that combined several sequences from nuclear and chloroplast genomes have not turned up evidence of hybridization in the genus as was suspected (Uhl 1970; Acevedo-Rosas *et al.* 2004).

THE CURRENT STATUS OF THE PHYLOGENY OF CRASSULACEAE

The very first attempt to re-evaluate the traditional groupings in the family with molecular tools revealed significant contradiction between the emerging picture and Berger's (1930) system (Ham 1995). Chloroplast DNA restriction site data identified 7 clades in the family, *Crassula*, *Kalanchoe*, *Telephium*, *Sempervivum*, *Aeonium*, *Leucosedum*, and *Acre* (Ham 1995; Ham and 't Hart 1998; Fig. 1). Of these, only one, *Crassula*, corresponded to Berger's subfamily Crassuloideae, while others included members of at

least two subfamilies. Although the following studies, based on sequence comparisons, could not confirm the entity of some of the clades established, they have been referred to in the literature (Mort *et al.* 2001; Mayuzumi and Ohba 2004).

The *Crassula* clade was identified as a basal divergence in the family tree (Ham and 't Hart 1998; Fishbein *et al.* 2001; Mort *et al.* 2001; Fishbein and Soltis 2004; Hermsen *et al.* 2006) and this placement as well as the clade monophyly is supported not only by significance thresholds but also by a number of indels in the *matK* sequences although the latter feature appears to be homoplasious (Mort *et al.* 2001). Morphologically Crassuloideae are defined by haplostemonous androecia but this character is also homoplasious and typical for the distantly related genus *Sinocrassula* (Mayuzumi and Ohba 2004). Most of the *Crassula* members are confined to the southern Africa, with only the aquatic species having nearly cosmopolitan distribution.

In Berger's system the subfamily included five genera but current classification has merged four of them (*Dinacria*, *Pagella*, *Rochea*, and *Vauanthes*) with *Crassula* (Tölken 1977, 1985; Eggl *et al.* 1995; Jaarsveld 2003). The validity of this treatment has yet to be explicitly tested with molecular tools. *Crassula* s.l. is a species rich (ca. 200) and morphologically diverse genus with up to 20 sections recognised (Tölken 1977, 1985; Jaarsveld 2003). Although at least some sections are distinct in vegetative, flower and inflorescence morphology, habit and distribution, the phylogenetic significance of these characters is unknown. The status of one section (formerly genus), *Tillaea*, comprised of dwarf, mostly annual aquatic plants, has been already challenged. It was resolved as a sister to the rest of *Crassula* (Ham 1995; Ham and 't Hart 1998) with a high divergence between the two branches. This result was acknowledged as a possible argument for restoring *Tillaea* to generic rank and to recognise it as the first divergence in the family following earlier phylogenetic hypotheses (Schönland 1891; Fröderström 1930; Uhl 1963; 't Hart 1995; Ham and 't Hart 1998). However, in our opinion, it only confirms the monophyly of the two entities but it does not resolve their taxonomic rank.

The *Kalanchoe* clade comprises genera from two traditi-

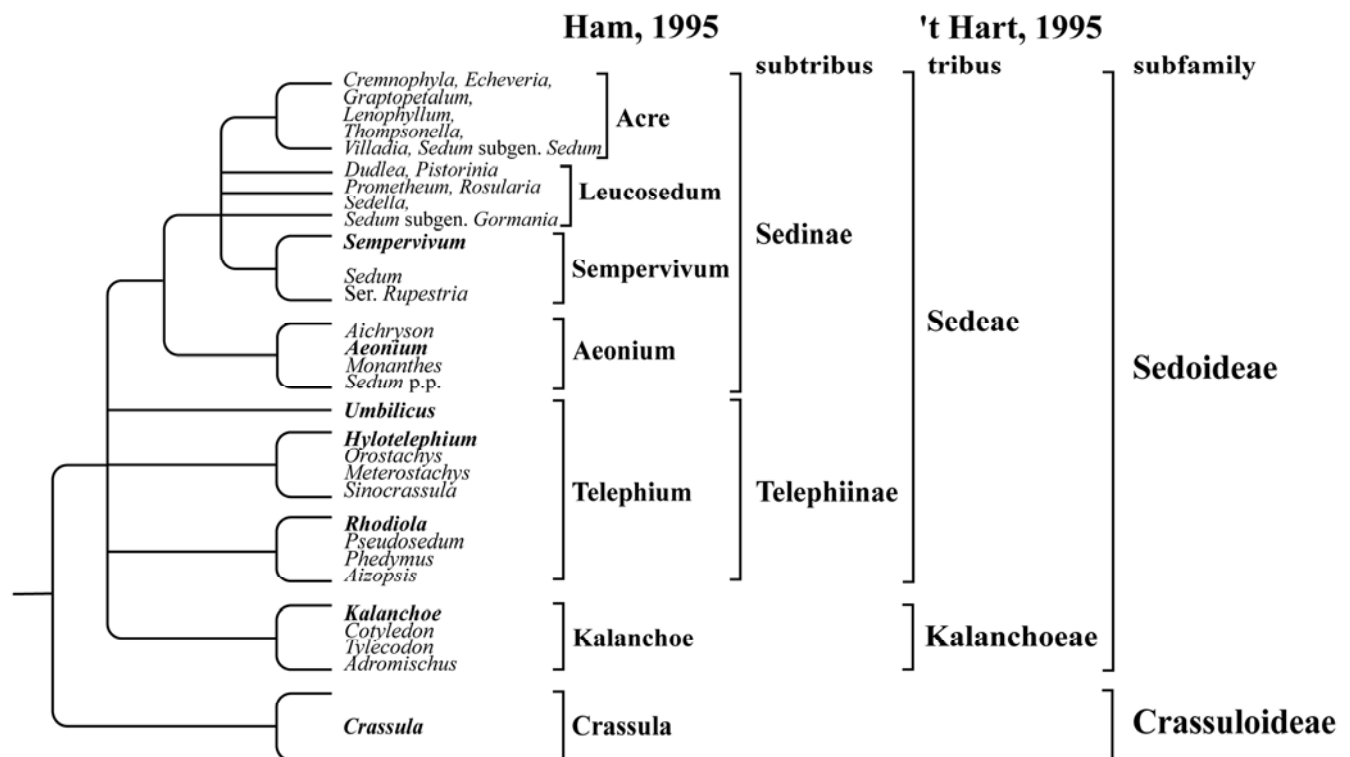


Fig. 1 Simplified phylogenetic relationships in the family Crassulaceae based on nucleotide sequence comparisons (Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press). Clades designation and taxonomic structure of the family is in accordance with Ham (1995) and 't Hart (1995). Taxa nominant for the clades are shown in bold.

onal subfamilies: all of the Kalanchoideae (the genus *Kalanchoe* s.l.) and a portion of the Cotyledonoideae. Fused corollas, base chromosome number ($x = 9$), chromosome morphology and mostly southern African distribution are common for the clade members (Baldwin 1938; Uhl 1948; Quimby 1971; Mort *et al.* 2001). There is general agreement between phylogenetic markers regarding clade monophyly and its internal structure but not to its position in the tree. Restriction site data and chloroplast markers significantly position it as the second divergence in the family clade (Ham 1995; 't Hart 1995; Ham and 't Hart 1998; Mort *et al.* 2001) but in ITS rDNA phylogeny some members of the putative Telephium clade (see below) presided it although with very low support (Gontcharova *et al.* in press). The clade was recognised as the tribe Kalanchoideae 't Hart (1995; Fig. 1).

The monophyletic nature of the genera composing the clade appears to be well established (Gehrig *et al.* 2001; Mort *et al.* 2001, 2005; Gontcharova *et al.* in press). *Adromischus* was identified as its basal divergence followed by a *Cotyledon/Tylecodon* pair having *Kalanchoe* as a sister (Mort *et al.* 2001). Thus molecular data confirmed the Baldwin (1938) hypothesis, based on the cytotaxonomy, on a close relationship between otherwise morphologically distinct *Kalanchoe*, *Adromischus* and *Cotyledon*. However, it is unlikely that *Kalanchoe* has an allopolyploid origin with *Cotyledon* and *Crassula* being its parental taxa as has been suggested (Baldwin 1938). Although this assertion has not been rigorously tested, comparison of the topologies, based on nuclear (ITS rDNA; Gontcharova *et al.* in press) and chloroplast (*matK*; Mort *et al.* 2001) sequences, reveals no discrepancy between them regarding branching pattern in the clade, suggesting evolution from a single ancestor.

Phylogenetic relationships have been assessed in some detail for two of the four clade members. Analyses of relationship within the third large crassulacean genus *Kalanchoe* based on ITS rDNA sequence comparisons confirmed its monophyly and identified three major monophyletic lineages that are generally consistent with their distribution pattern and partially with their morphology (Gehrig *et al.* 2001). Accelerated evolutionary rates in ITS rDNA sequences characterize the genus *Kalanchoe*, where an average sequence divergence (*p*-distance) accounts for 10% (Gontcharova *et al.* in press). The presence of numerous rather extensive indels in ITS and a somewhat elevated GC content (>60%) are also typical for *Kalanchoe*. In contrast to that sequence divergence is significantly lower ($\leq 1\%$) in *Adromischus*, *Cotyledon* and *Tylecodon* and it appears that similar trend holds for the chloroplast non-coding sequences as well. Mort *et al.* (2005) analysed a fairly long (1931 nt) combined data set of chloroplast (*psbA-trnH* and *trnL-trnF*) and nuclear (ITS rDNA) sequences to estimate *Cotyledon* phylogeny but were unable to resolve most branches within an otherwise robust genus clade.

The putative Telephium clade is composed of genera (former infrageneric taxa of *Sedum*) having predominantly Eastern Asian distribution and *Umbilicus* occurring in the Mediterranean region (Ham 1995; Ham and 't Hart 1998). Although the clade was resolved with very low significance (25%), it was readily accepted by the crassulacean specialists, recognized as the subtribe Telephiinae and is rather loosely defined by five-merous flowers with free petals, flat, dentate leaves and tuberous roots or thickened rhizomes ('t Hart 1995). Further studies based on chloroplast *matK* gene (Mort *et al.* 2001), *trnL-F* intergenic spacer ('t Hart 1995; Mayuzumi and Ohba 2004) and nuclear ITS rDNA sequences (Mayuzumi and Ohba 2004; Gontcharova *et al.* 2006) could not confirm its entity. Instead, in all these analyses the putative clade members were grouped into 3 or 4 significant lineages. Two of them, *Rhodiola* and *Phedimus*, showed strong affinity to each other (Mayuzumi and Ohba 2004) and are recognised as a clade *Rhodiola* by Gontcharova *et al.* (2006). Weakly supported sister relationships were established between this pair and *Umbilicus* but only with datasets that did not include the most basal crassula-

cean lineage, the genus *Crassula* (Mayuzumi and Ohba 2004; Gontcharova *et al.* 2006). In global analyses of the family phylogeny based on ITS rDNA sequence comparisons *Umbilicus* was positioned apart from *Rhodiola* and *Phedimus* with weak significance (Gontcharova *et al.* in press).

The affinity of one more lineage, Hylotelephium-clade composed of the genera *Hylotelephium*, *Orostachys*, *Metorostachys* and *Sinocrassula*, also remains uncertain. In all phylogenies Hylotelephium and *Rhodiola* clades formed unresolved polytomy (Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* 2006). It could not be ruled out that these clades have common ancestry but it is likely that their divergence was fast and could not be traced with the markers applied.

Molecular data fully confirmed the distinctness of *Hylotelephium*, *Orostachys*, *Rhodiola*, *Phedimus* and *Aizopsis* from *Sedum* where they were formerly classified. At the same time, the pattern of relationships between (e.g. robust polyphyletic clades *Hylotelephium* + *Orostachys* and *Rhodiola* + *Pseudosedum*) and within these genera (e.g. polyphyly of *Hylotelephium* and *Rhodiola* infrageneric taxa) revealed with molecular data are somewhat unexpected and is not reflected by their phenotypic traits (Mayuzumi and Ohba 2004; Gontcharova *et al.* 2006).

Significant in most of the analyses crown assemblage of the crassulacean tree, currently recognised in the system as subtribe Sedinae ('t Hart 1995; Egli 2003), is composed of four remaining clades, *Aeonium*, *Sempervivum*, *Leucosedum* and *Acre*. The genus *Sedum* s.str. is represented in each of them and constitutes the bulk of *Acre* and *Leucosedum* clades, demonstrating its highly polyphyletic nature. In addition, the subtribe includes ca. 16 genera and accounts for more than 50% of family diversity ('t Hart 1995; Egli 2003). Although composition of the subtribe is more or less defined, the branching order of its clades remains, to a large extent, tentative. Non-coding chloroplast (*trnL-F* spacer; Mayuzumi and Ohba 2004) and nuclear (ITS rDNA; Gontcharova *et al.* in press) sequences identified *Aeonium* clade as its basal divergence, followed by *Sempervivum* clade. Paraphyletic or non-significant in most phylogenies *Leucosedum* and the most species-rich *Acre* clades form a sister group of the latter.

Although Sedinae are well supported by molecular data, morphologically the group is very heterogeneous and its phenotypic circumscription is almost impossible. This holds for its major clades as well. Perhaps, *Aeonium* is defined best of all by molecular data (significance thresholds and indels in *matK* and ITS rDNA sequences; Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press), by its distribution (mostly Macaronesia) and by its polymerous flowers. However, the latter feature is shared by the distantly related in molecular phylogeny *Sempervivum* clade. Molecular phylogenetic studies elucidated the relationship within *Aeonium* clade, analyzed the evolution of its morphological traits and diversification in the genera composing the clade and confirmed its Macaronean origin from a North African predecessor (Mes *et al.* 1997; Jorgensen and Frydenberg 1999; Mort *et al.* 2001; Jorgensen 2002; Mort *et al.* 2002; Fairfield *et al.* 2004). Mes (1995a, 1995b) recognized a number of North African *Sedum* species that may give a rise to *Aeonium* clade and grouped them into three putatively related series. However, exact relationships between these taxa and their relationship to Macaronesian genera has yet to be studied.

The *Sempervivum* clade is another crassulacean group that could not be unambiguously characterised by any phenotypic character and its composition has yet to be defined. Its nominant genus *Sempervivum* is rather distinct in gross morphology, habit and distribution and undoubtedly represent a monophyletic lineage in the Sedinae ('t Hart *et al.* 2003). There is some controversy regarding the taxonomic status of *Jovibarba*, a section of *Sempervivum* or separate genus (Berger 1930; Parnell and Favarger 1992; Egli *et al.* 1995; 't Hart and Bleij 1999), but in any case the two taxa

are closely related and form a robust clade (Mort *et al.* 2001). More uncertain is a relationship between *Sempervivum* and some *Sedum* species. It was suggested that *Sempervivum* evolved from a polyploid *Sedum* lineage (Favarger *et al.* 1968) and the *Sedum* series *Rupestris* was seen as its relative (Uhl 1961a). Indeed, this alliance was established in restriction site data as well as ITS rDNA analyses although with no significance (Gontcharova *et al.* in press; Ham and 't Hart 1998). In *matK* sequence comparisons *Sedum* series *Rupestris* and *Sempervivum* did not show strong affinity to each other as well (Mort *et al.* 2001). Morphological similarity between the two lineages is not pronounced except for polymerous flowers, feature that likely arose several times in the family. Both, the *Sempervivum* and the *Sedum* series *Rupestris* are likely to be of polyploids but they differ in base chromosome numbers, $x = 16-19$ and $x = 28$, respectively (Mort *et al.* 2001).

The polygeneric *Leucosedum* clade was one of the best supported in restriction site data analyses (Ham 1994; Ham and 't Hart 1998) but further sequence-based phylogenies resolved it with no significance (Mort *et al.* 2001) or as a paraphyletic entity (Mayuzumi and Ohba 2004; Gontcharova *et al.* in press). The putative clade accommodates the *Sedum* subgen. *Gormania* and a number of genera that were seen as evolved from this lineage both, in N America (e. g. *Dudleya*, *Sedella*) and Eurasia (e. g. *Rosularia*, *Prometheum*, *Pistorinia*; 't Hart 1982, 1991; 't Hart *et al.* 1999). This problematic assemblage accounting for ca. 200 species classified in 5 to 7 genera has been little studied with molecular tools and thus the relationships between its members are poorly understood. In addition to *Gormania* species, a number of the subgenus *Sedum* members were significantly placed into several *Leucosedum* lineages (Mort *et al.* 2001; Gontcharova *et al.* in press) making them highly eclectic. It is currently almost impossible to make any conclusion regarding clade phylogenetic status, composition, biogeography and morphology.

In ITS rDNA sequence comparisons the taxa composing *Leucosedum* lineages were the most divergent (the average p -distance >15%) and this may reflect either accelerated evolutionary rates in the group or very distant relationships between its members (Gontcharova *et al.* in press).

The Acre clade is the most species and genera rich in the family. It is expected to accommodate ca. 500 species currently classified in 7 genera (Eggl 2003). These are members of *Sedum* subgen. *Sedum* and most American endemic genera, formerly classified in the subfamily Echeverioideae (Berger 1930). Entity of the clade is well-established in all analyses (Ham 1995; Ham and 't Hart 1998; Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press) but some uncertainty remains regarding affinity of non-American subgen. *Sedum* species that could belong to some of *Leucosedum* lineages (see above). At the same time there is yet no indication so far that some of the subgen. *Gormania* member may ally to the Acre clade.

The internal structure of the clade was unclear until recently and only a limited number of subclades were established. It was shown that members of the former Echeverioideae genera constitute several crown subclades but their relationships with a bulk of *Sedum* taxa could not be analysed because of a limited taxon sampling (Mort *et al.* 2001; Acevedo-Rosas *et al.* 2004). Comparisons of a large number of *Sedum* ITS rDNA sequences allowed Gontcharova *et al.* (in print) to elucidate the relationships within the genus and particularly in the Acre clade. It was shown that the clade is split into two major subclades. One significant lineage is comprised N American taxa and was presided by several Macaronesian *Sedum* species while the other united only Eurasian species. In American subclade *Sedum* taxa were arranged in a number of supported groups forming an unresolved basal polytomy while former Echeverioideae genera were placed into a large polyphyletic crown assemblage again having *Sedum* species at the base. Eurasian species of the genus were also arranged into several significant

lineages but their assemblage gained no support (Gontcharova *et al.* in press). It was suggested that elevated evolutionary rates (average p -distance 15%) and still limited taxon sampling (21 spp.) could be responsible for the lack of support for the Eurasian subclade. In contrast to that, the sequence divergence in American lineage was twice as lower apart from the fact that it included representatives of as many as 7 conventional genera. Relatively low sequence divergence (Acevedo-Rosas *et al.* 2004; Gontcharova *et al.* in press) and the presence of numerous intergeneric hybrids (Eggl 2003) raises questions about the importance of the morphological characters used to differentiate them.

Thus, the phylogenetic structure of the family Crassulaceae is far from being firmly established. Of the seven clades that emerged in early cp-DNA restriction site data only four were confirmed as monophyletic entity by further nucleotide sequence based studies, Crassula, Kalanchoe, Aeonium and Acre (Fig. 1). In three clades, namely Telephium, *Sempervivum* and *Leucosedum*, several monophyletic lineages were identified (e.g. *Hylotelephium*, *Rhodiola*, *Umbilicus*, *Sempervivum*, etc., see above) showing little if any affinity to each other. Question whether these results represent hard or soft polytomies remains unclear and should be further addressed with more comprehensive taxon sampling and markers.

Although it was convincingly shown that the traditional system of the Crassulaceae is artificial, its modern revision ('t Hart 1995) remains to be deficient as well since the monophyly of some new intrafamilial taxa has been contended. The recognition of two subfamilies, Crassuloideae, identical to that in Berger system and Sedoideae that unites his remaining five subfamilies, does not contradict the phylogenetic structure of the family (Fig. 1). It is likely that the name *Sempervivoideae* Arnott is more correct because it has taxonomic priority over *Sedoideae* (Thorn 2000). In any case, the internal structure of the *Sedoideae*, namely entity of the tribes *Sedeae* and *Kalanchoeae*, has been already questioned, at least in nuclear ITS rDNA phylogeny (Gontcharova *et al.* in press). The status of the sutribe *Telephiinae* is also problematic because all phylogenetic analyses failed to establish it as a clade (Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press). It could be perhaps accepted as a paraphyletic taxon but in that case we need some objective reason for drawing that conclusion.

Now we come to the issue of the morphological heterogeneity of the newly established taxa. Basically, none of them could be characterised unambiguously by any phenotypic synapomorphy or at least a unique combination of characters. Neither flower and inflorescence nor vegetative morphology could be used to diagnose crassulaceae suprageneric taxa or clades because it was shown that most of them evolved independently a number of times in the family (Ham and 't Hart 1998; Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press).

Unresolved phylogeny and high phenotypic homoplasy hampers any attempt to reconstruct morphology evolutionary trends in Crassulaceae. Autapomorphic characters could be established at least for some genera but even at this low taxonomic level homoplasy in morphology is involved. *Sedum* is particularly notorious in this respect. Characterised as a "catch-all" taxon (Uhl 1963) that accommodated species not fitting diagnoses of other genera now it nests a number of morphologically distinct in some features taxa (e.g. former members of Echeverioideae and *Sempervivoideae*; Mort *et al.* 2001; Mayuzumi and Ohba 2004; Gontcharova *et al.* in press) forming a conglomerate that accounts for almost a half of the species diversity of the family. Its future split into smaller monophyletic and hopefully uniform entities is likely but they are yet to be identified with molecular tools and characterised phenotypically.

Another point of disagreement between the morphology and phylogeny in the genera definition is *Telephiinae* lineages. A recent studies revealed an add relationship between *Hylotelephium* and *Orostachys* as well as *Rhodiola* and *Pseudosedum* that are distinct from each other morphologi-

cally but were shown to form polyphyletic clades (Mayuzumi and Ohba 2004; Gontcharova *et al.* 2006). Common ovaries morphology suggested possible relatedness of *Hylotelephium* and *Orostachys* (Ohba 1978, 1995) but the genera were treated as distinct because of pronounced differences in habit and inflorescence morphology.

Homoplasy of morphological characters was documented at the low taxonomic levels (subgenera and sections) in *Hylotelephium* and *Rhodiola* (Mayuzumi and Ohba 2004; Gontcharova *et al.* 2006) and comprehensive studies with other crassulacean genera are awaited. The same studies suggested that phylogenetic and taxonomic importance of some phenotypic features should be re-evaluated. Mayuzumi and Ohba (2004) revealed that the subsections of the genus *Orostachys*, *Appendiculatae* and *Orostachys*, distinct only in a shape of the leaf apex (cuspidate and blunt, respectively), in fact are distant from each other and subsect. *Orostachys* is embedded into the genus *Hylotelephium* while subsect. *Appendiculatae* is close to the genus *Meterostachys*. Indeed, *Orostachys* subsect. *Appendiculatae* and *Meterostachys* share cuspidate leaves but differ in cymose-paniculate versus spadix-like inflorescence and basally fused petals. Flower and inflorescence morphology always was an important feature in plant taxonomy but it appears that in Crassulaceae it is more labile, perhaps even at the genus level.

CONCLUSION

Our understanding of phylogenetic relationships in the family Crassulaceae is still incomplete after almost 15 years of studies with molecular tools. Detailed investigations are still needed to clarify status of some suprageneric taxa (e.g. Sedeae, Kalanchoeae and Telephiinae), putative clades (e.g. Acre, Leucosedum and Sempervivum) and most crassulacean genera. Establishment of their monophyletic entities will facilitate analysis of the complex morphology evolution and diversification in the family and eventually will lead to a classification system that adequately reflects relationships in the group.

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REFERENCES

- Acevedo-Rosas R, Camero K, Sosa V, Pell S (2004) A molecular phylogenetic study of *Graptopetalum* (Crassulaceae) based on ETS, ITS, *rp16*, and *trnL-F* nucleotide sequences. *American Journal of Botany* **91**, 1099-1104
- Albert VA, Williams SE, Chase MW (1992) Carnivorous plants: phylogeny and structural evolution. *Science* **257**, 1491-1495
- Baillon H (1871) Saxifragaceae. *Histoire des Plantes* **3**, 325-464
- Baldwin JT (1938) *Kalanchoe*: the genus and its chromosomes. *American Journal of Botany* **25**, 572-579
- Berger A (1930) Crassulaceae. In: Engler A, Prantl K (Eds) *Die Natürlichen Pflanzenfamilien*, (Vol 18a, 2nd Edn), Wilhelm Engelmann Verlag, Leipzig, Germany, pp 352-485
- Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price RA, Hills HG, Qiu Y-L, Kron KA, Rettig JH, Conti E, Palmer JD, Manhart JR, Sytsma KJ, Michaels HJ, Kress WJ, Karol KG, Clark WD, Hedren M, Gaut BS, Jansen RK, Kim K-J, Wimpee CF, Smith JF, Furnier GR, Strauss SH, Xiang Q-Y, Plunkett GM, Soltis PS, Swensen SM, Williams SE, Gadek PA, Quinn CJ, Eguiarte LE, Golenberg E, Learn GH, Graham SW, Barrett SCH, Dayanandan S, Albert VA (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcl*. *Annals of the Missouri Botanical Garden* **80**, 528-580
- Coleman AW (2003) ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in Genetics* **19**, 370-375
- Crepet WL, Nixon KC, Gandolfo MA (2004) Fossil evidence and phylogeny: the age of major angiosperm clades based on mesofossil and macrofossil evidence from Cretaceous deposits. *American Journal of Botany* **91**, 1666-1682
- Cronquist A (1991) *An Integrated System of Classification of Flowering Plants*, Columbia University Press, NY, 1262 pp
- Dahlgren R (1983) General aspects of angiosperm evolution and macrosystematics. *Nordic Journal of Botany* **3**, 119-149
- de Candolle AP (1828a) Crassulaceae. In: De Candolle AP (Ed) *Prodromus Systematis Naturalis Regni Vegetabilis* (Vol 3), Truettel and Wurtz, Paris, France, pp 381-414
- de Candolle AP (1828b) *Mémoire sur la Famille des Crassulacées*, Truettel and Wurtz, Paris, 60 pp
- Eggli U (2003) *Illustrated Handbook of Succulent Plants: Crassulaceae*, Springer, Berlin, 458 pp
- Eggli U, 't Hart H, Nyffeler R (1995) Towards a consensus classification of the Crassulaceae. In: 't Hart H, Eggli U (Eds) *Evolution and Systematics of the Crassulaceae*, Backhuys Publishers, Leiden, The Netherlands, pp 173-192
- Engler A (1930) Saxifragaceae. In: Engler A, Prantl K (Eds) *Die Natürlichen Pflanzenfamilien* (Vol 18a, 2nd Edn), Wilhelm Engelmann Verlag, Leipzig, Germany, pp 74-226
- Fairfield KN, Mort ME, Santos-Guerra A (2002) Phylogenetics and evolution of the Macaronesian members of the genus *Aichryson* (Crassulaceae) inferred from nuclear and chloroplast sequence. *Plant Systematics and Evolution* **248**, 71-83
- Favarger C, Maeder AM, Zésiger F (1968) Hybrides interspécifiques et intergénériques chez les Jovibarbes. *Archiv der Julius Klaus-Stiftung Vererbungs-forschung, Sozialanthropologie und Rassenhygiene* **43**, 18-30
- Fishbein M, Hibsich-Jetter C, Soltis DE, Hufford L (2001) Phylogeny of Saxifragales (angiosperms, eudicots): analysis of a rapid, ancient radiation. *Systematic Biology* **50**, 817-847
- Fishbein M, Soltis DE (2004) Further resolution of the rapid radiation of Saxifragales (Angiosperms, Eudicots) supported by mixed-model Bayesian analysis. *Systematic Botany* **29**, 883-891
- Fröderström MH (1930) The genus *Sedum* L., 1. *Acta Horticulturae Gothoburgensis* **5** (app.), 1-75
- Fröderström MH (1931) The genus *Sedum* L., 3. *Acta Horticulturae Gothoburgensis* **6** (app.), 1-125
- Gehrig H, Gaubmann O, Marx H, Schwarzzott D, Kluge M (2001) Molecular phylogeny of the genus *Kalanchoe* (Crassulaceae) inferred from nucleotide sequences of the ITS-1 and ITS-2 regions. *Plant Science* **160**, 827-835
- Gontcharova SB, Artyukova EV, Gontcharov AA (2006) Phylogenetic relationships among members of the subfamily Sedoideae (Crassulaceae) inferred from the ITS region sequences of nuclear rDNA. *Russian Journal of Genetics* **42**, 654-661
- Gontcharova SB, Gontcharov AA (2004) Sequence and secondary structure evolution of ITS rDNA in the family Crassulaceae. In: Kondo K (Ed) *Evolutionary Botany of Certain Plant-Groups of the East Eurasian Floristic Element Related to Japanese Flora on the Basis of Chromosomes*, Hiroshima University, Hiroshima, Japan, pp 32-34
- Gontcharova SB, Stephensen R, Gontcharov AA (2007) Analysis of phylogenetic relationships in the fam. Crassulaceae based on comparisons of ITS region of nuclear rDNA. *Botanical Zhurnal*, in press
- Grund C, Jensen U (1981) Systematic relationships of Saxifragales revealed by serological characteristics of seed proteins. *Plant Systematics and Evolution* **137**, 1-22
- Ham RCHJ (1994) *Phylogenetic implications of chloroplast DNA. variation in the Crassulaceae*. PhD thesis, University of Utrecht, 153 pp
- Ham RCHJ (1995) Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA variation. In: 't Hart H, Eggli U (Eds) *Evolution and Systematics of the Crassulaceae*, Backhuys Publishers, Leiden, The Netherlands, pp 16-29
- Ham RCHJ, 't Hart H (1998) Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA restriction-site variation. *American Journal of Botany* **85**, 123-134
- 't Hart H (1982) The white-flowering *Sedum* species. 1. Principles of a phylogenetic classification of the Sedoideae (Crassulaceae) and the position of the white-flowering *Sedum* species. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C* **87**, 401-411
- 't Hart H (1991) Evolution and classification of the European *Sedum* species (Crassulaceae). *Flora Mediterranea* **1**, 31-61
- 't Hart H (1995) Classification of the Crassulaceae. In: 't Hart H, Eggli U (Eds) *Evolution and Systematics of the Crassulaceae*, Backhuys Publishers, Leiden, The Netherlands, pp 159-172
- 't Hart H, Bleij B (1999) Nieuwe Namen in *Sempervivum* Sect. *Jovibarba* (Crassulaceae). *Succulenta* **78**, 35-42
- 't Hart H, Bleij B, Zonneveld B (2003) *Sempervivum*. In: Eggli U (Ed) *Illustrated handbook of Succulent Plants: Crassulaceae*, Springer, Berlin, Germany, pp 332-349
- 't Hart H, Ham RDHJ, Stevens JF, Elema ET, van der Klis H, Gadella TWJ (1999) Biosystematic, molecular and phytochemical evidence for the multiple origin of sympetaly in Eurasian Sedoideae (Crassulaceae). *Biochemical Systematics and Ecology* **27**, 407-426
- Hermesen EJ, Nixon KC, Crepet WL (2006) The impact of extinct taxa on understanding the early evolution of Angiosperm clades: an example incorporating fossil reproductive structures of Saxifragales. *Plant Systematics and Evolution* **260**, 141-169
- Hillis DM, Pollock DD, McGuire JA, Zwickl DJ (2003) Is sparse taxon sampling a problem for phylogenetic inference? *Systematic Biology* **52**, 124-126
- Hoot SB, Magallo SN, Crane PR (1999) Phylogeny of basal eudicots based on

- three molecular datasets: *atpB*, *rbcL*, and 18S nuclear ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* **86**, 1-32
- Hufford L** (1992) Rosidae and their relationships to other nonmagnoliid dicotyledons: a phylogenetic analysis using morphological and chemical data. *Annals of the Missouri Botanical Garden* **79**, 218-248
- Hutchinson J** (1973) *The Families of Flowering Plants*, Oxford University Press, Oxford
- Jorgensen T** (2002) The importance of phylogeny and ecology in microgeographical variation in the morphology of four Canarian species of *Aeonium* (Crassulaceae). *Biological Journal of the Linnean Society* **76**, 521-533
- Jorgensen T, Frydenberg HJ** (1999) Diversification in insular plants: inferring the phylogenetic relationship in *Aeonium* (Crassulaceae) using ITS sequences of nuclear ribosomal DNA. *Nordic Journal of Botany* **19**, 613-621
- Keeley JE** (1998) Photosynthetic pathway diversity in a seasonal pool community. *Functional Ecology* **13**, 106-118
- Keeley JE, Philip WR** (2003) Evolution of CAM and C₄ carbon-concentrating mechanisms. *International Journal of Plant Sciences* **164**, 55-77
- Mayuzumi S, Ohba H** (2004) The phylogenetic position of East Asian Sedoideae (Crassulaceae) inferred from chloroplast and nuclear DNA sequences. *Systematic Botany* **29**, 587-598
- Mes THM** (1995a) *Origin and Evolution of the Macaronesian Sempervivoideae*. PhD Thesis, Utrecht University, Utrecht, 215 pp
- Mes THM** (1995b) Phylogenetic and systematic implications of chloroplast and nuclear spacer sequence variation in the Macaronesian Sempervivoideae and related Sedoideae. In: 't Hart H, Eggli U (Eds) *Evolution and Systematics of the Crassulaceae*, Backhuys Publishers, Leiden, The Netherlands, pp 30-44
- Mes THM, Wijers GJ, 't Hart H** (1997) Phylogenetic relationships in *Monanthes* (Crassulaceae) based on morphological, chloroplast and nuclear DNA variation. *Journal of Evolutionary Biology* **10**, 193-216
- Morgan DR, Soltis DE** (1993) Phylogenetic relationships among members of Saxifragaceae *sensu lato* based on *rbcL* sequence data. *Annals of the Missouri Botanical Garden* **80**, 631-660
- Mort ME, Archibald JK, Randle CP, Levens ND, O'Leary TR, Topalov K, Wiegand CM, Crawford DJ** (2007) Inferring phylogeny at low taxonomic levels: utility of rapidly evolving cpDNA and nuclear ITS loci. *American Journal of Botany* **94**, 173-183
- Mort ME, Levens N, Randle CP, van Jaarsveld E, Palmer A** (2005) Phylogenetics and diversification of *Cotyledon* (Crassulaceae) inferred from nuclear and chloroplast DNA sequence data. *American Journal of Botany* **92**, 1170-1176
- Mort ME, Soltis DE, Soltis PS, Francisco-Ortega J, Santos-Guerra A** (2001) Phylogenetic relationships and evolution of Crassulaceae inferred from *matK* sequence data. *American Journal of Botany* **88**, 76-91
- Mort ME, Soltis DE, Soltis PS, Francisco-Ortega J, Santos-Guerra A** (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. *Systematic Botany* **27**, 271-288
- Nickrent DL, Der JP, Anderson FE** (2005) Discovery of the photosynthetic relatives of the "Maltese mushroom" *Cynomorium*. *BMC Evolutionary Biology* **5**, 38
- Nyfelner R** (1995) Hybridization in *Monanthes*. In: 't Hart H, Eggli U (Eds) *Evolution and Systematics of the Crassulaceae*, Backhuys Publishers, Leiden, The Netherlands, pp 76-88
- Ohba H** (1978) Generic and infrageneric classification of the Old World Sedoideae (Crassulaceae). *Journal of the Faculty of Science, University of Tokyo: Section III* **12**, 139-198
- Ohba H** (1995) Systematic problems of Asian Sedoideae. In: 't Hart H, Eggli U (Eds) *Evolution and Systematics of the Crassulaceae*, Backhuys Publishers, Leiden, The Netherlands, pp 153-158
- Parnell J, Favarger C** (1992) *Sempervivum*. In: Tutin TG, Heywood UH, Burges NA, Valentine DH, Walters SM, Webb DA (Eds) *Flora Europaea*, (Vol 1, 2nd Edn) Cambridge, Cambridge University Press, UK, pp 425-429
- Pollock DD, Zwickl DJ, McGuire JA, Hillis DM** (2002) Increased taxon sampling is advantageous for phylogenetic inference. *Systematic Biology* **51**, 664-671
- Prager LR** (1929) *Semperviva* of the Canary Islands area. *Proceedings of the Royal Irish Academy: Section B* **38**, 454-499
- Qiu YL, Lee J, Bernasconi-Quadroni F, Soltis DE, Soltis PS, Zanis M, Zimmer EA, Chen ZD, Savolainen V, Chase MW** (1999) The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature* **402**, 404-407
- Quimby MW** (1971) *Floral morphology of Crassulaceae*. PhD Thesis, University of Mississippi, Starkville, 42 pp
- Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, Bayer C** (2000) Phylogenetics of flowering plants based upon a combined analysis of plastid *atpB* and *rbcL* gene sequences. *Systematic Biology* **49**, 306-362
- Schönlund S** (1891) Crassulaceae. In: Engler A, Prantl K (Eds) *Die Natürlichen Pflanzenfamilien* (Vol 3, 2nd Edn), Verlag Wilhelm Englemann, Leipzig, Germany, pp 23-38
- Soltis DE, Morgan DR, Grable A, Soltis PS, Kuzoff R** (1993) Molecular systematics of Saxifragaceae *sensu stricto*. *American Journal of Botany* **80**, 1056-1081
- Soltis DE, Mort ME, Soltis PS, Hibsich-Jetter C, Zimmer EA, Morgan D** (1999) Phylogenetic relationships of the enigmatic angiosperm family podostemaceae inferred from 18S rDNA and *rbcL* sequence data. *Molecular Phylogenetics and Evolution* **11**, 261-272
- Soltis DE, Soltis PS** (1997) Phylogenetic relationships in Saxifragaceae *sensu lato*: A comparison of topologies based on 18S rDNA and *rbcL* sequences. *American Journal of Botany* **84**, 504-522
- Soltis DE, Soltis PS, Chase MW, Mort ME, Albach DC, Zanis M, Savolainen V, Hahn WH, Hoot SB, Fay MF, Axtell M, Swensen SM, Prince LM, Kress WJ, Nixon KC, Farris JS** (2000) Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbcL*, and *atpB* sequences. *Botanical Journal of the Linnean Society* **133**, 381-461
- Soltis DE, Soltis PS, Endress PK, Chase MW** (2005) *Phylogeny and Evolution of Angiosperms*, Sinauer Associates Inc., Sunderland, 370 pp
- Soltis DE, Soltis PS, Nickrent DL, Johnson LA, Hahn WJ, Hoot SB, Sweere JA, Kuzoff RK, Kron KA, Chase MW, Swensen SM, Zimmer EA, Chaw S-M, Gillespie LJ, Kress WJ, Sytsma KJ** (1997) Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* **84**, 1-49
- Tahtajan A** (1987) *Systema Magnoliifitov*, Nauka, Moscow, 439 pp
- Takhtajan A** (1980) Outline of the classification of flowering plants (Magnoliophyta). *Botanical Review* **46**, 225-359
- Takhtajan A** (1997) *Diversity and Classification of Flowering Plants*, Columbia University Press, NY, 643 pp
- Thorne RF** (1992) Classification and geography of the flowering plants. *Botanical Review* **58**, 225-348
- Thorne RF** (2000) The classification and geography of the flowering plants: Dicotyledons of the class angiospermae (Subclasses magnoliidae, ranunculidae, caryophyllidae, dilleniidae, rosidae, asteridae, and lamiidae). *Botanical Review* **66**, 441-647
- van Tieghem P** (1898) Sur le genre *Penthoraceae* considéré comme type d'une famille nouvelle les Penthoracées. *Journal de Botanique* **12**, 150-154
- Tölken HR** (1977) A revision of the genus *Crassula* in Southern Africa. *Contributions from the Bolus Herbarium* **8**, 1-595
- Tölken HR** (1985) Crassulaceae. In: Leistner OA (Ed) *Flora of Southern Africa* (Vol 14), Botanical Research Institute, Pretoria, South Africa, pp 1-230
- Ueda K, Hanyuda T, Nakano A, Shiuchi T, Seo A, Okubo H, Hotta M** (1997) Molecular phylogenetic position of Podostemaceae, a marvelous aquatic flowering plant family. *Journal of Plant Research* **110**, 87-92
- Uhl CH** (1948) Cytotaxonomic studies in the subfamilies Crassuloideae, Kalanchoideae, and Cotyledonoideae of the Crassulaceae. *American Journal of Botany* **35**, 695-706
- Uhl CH** (1961a) The chromosomes of the Sempervivoideae (Crassulaceae). *American Journal of Botany* **48**, 114-123
- Uhl CH** (1961b) Some cytotaxonomic problems in the Crassulaceae. *Evolution* **15**, 375-383
- Uhl CH** (1963) Chromosomes and phylogeny of the Crassulaceae. *Cacthus and Succulent Journal (U.S.)* **35**, 80-84
- Uhl CH** (1970) Chromosomes of *Graptopetalum* and *Thompsonella* (Crassulaceae). *American Journal of Botany* **85**, 123-134
- Uhl CH** (1993) Intergeneric hybrids in the Mexican Crassulaceae. I. *Lenophyllum*. *Cacthus and Succulent Journal (U.S.)* **65**, 271-273
- Uhl CH** (1994a) Intergeneric hybrids in the Mexican Crassulaceae. II. *Dudleya*. *Cacthus and Succulent Journal (U.S.)* **66**, 74-80
- Uhl CH** (1994b) Intergeneric hybrids in the Mexican Crassulaceae. III. *Thompsonella*. *Cacthus and Succulent Journal (U.S.)* **66**, 175-179
- Uhl CH** (1994c) Intergeneric hybrids in the Mexican Crassulaceae. IV. *Villadia*. *Cacthus and Succulent Journal (U.S.)* **66**, 214-217
- van Jaarsveld EJ** (2003) *Crassula*. In: Eggli U (Ed) *Illustrated Handbook of Succulent Plants: Crassulaceae*, Springer, Berlin, Germany, pp 32-84
- Wikström N, Savolainen V, Chase MW** (2001) Evolution of the angiosperms. Calibrating the tree. *Proceedings of the Royal Society B: Biological Sciences* **268**, 2211-2220