

PHYLOGENETICS AND DIVERSIFICATION OF *COTYLEDON* (CRASSULACEAE) INFERRED FROM NUCLEAR AND CHLOROPLAST DNA SEQUENCE DATA¹

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Crassulaceae includes approximately 35 genera and 1500 species of leaf and stem succulent flowering plants. The family is nearly cosmopolitan in distribution, but is particularly diverse in southern Africa, where five genera comprising approximately 325 species are found. One of these genera, *Cotyledon*, includes 10 species that are largely confined to South Africa, where they are commonly found on rocky hillsides, coastal flats, and cliff faces. Species of *Cotyledon* are characterized by five-parted, pendulous, sympetalous flowers, but the genus is highly diverse in growth form, flower color and size, and leaf morphology. One particularly variable species, *C. orbiculata*, has been divided into five varieties based on leaf morphology and biogeography; however, the monophyly of this species as well as the relationships among the varieties have not previously been investigated. Parsimony analyses of a combined data set of DNA sequences from chloroplast and nuclear genome provided the first estimate of phylogeny for *Cotyledon*, and resulted in two minimum-length trees and a fully resolved phylogeny for the genus. Results indicate that *C. orbiculata* is not monophyletic and suggest the need for additional studies and a revised classification within the genus.

Key words: *Cotyledon*; *Cotyledon orbiculata*; Crassulaceae; nrDNA ITS; phylogeny; *psbA-trnH*; *trnL-trnF*; South Africa.

The flowering plant family Crassulaceae includes approximately 1500 species of leaf and stem succulent herbs and small shrubs within 35 genera. Most members of the family possess five-parted, apopetalous, actinomorphic flowers with 10 stamens in two whorls; however, this general floral pattern varies, with some genera having partially to completely fused petals and one genus, *Crassula*, having only five stamens (i.e., haplostemonous). The family is widespread and nearly cosmopolitan in distribution, but centers of taxonomic diversity are found in Macaronesia, Mexico, and southern Africa. Several recent phylogenetic studies on Crassulaceae using chloroplast DNA RFLPs (Ham and 't Hart, 1998), *matK* sequences (Mort et al., 2001), and sequences of the nrDNA internal transcribed spacers (Mort, 2002) and slightly different taxonomic sampling have resulted in topologies that are largely congruent. For example, each study strongly supports the monophyly and basal position of the *Crassula* clade and places this clade as sister to two major clades. The first clade comprises four genera from southern Africa and Madagascar: *Adromischus*, *Cotyledon*, *Kalanchoe* (including *Bryophyllum*), and *Tylecodon* (i.e., the *Kalanchoe* clade of Ham and 't Hart, 1998). The second clade comprises the remainder of the species in the family, including many familiar horticultural plants such as *Echeveria*, *Sempervivum*, and *Sedum*.

Phylogenetic relationships within the *Kalanchoe* clade of Crassulaceae have yet to be investigated in detail, but the *matK* study of Mort et al. (2001) does provide some insights into the monophyly and relationships among members of this clade. For example, each of the genera in this clade is recovered as monophyletic with very strong support (92–100% bootstrap). The two species of *Adromischus* sampled are sister to a clade that includes two subclades, one of which includes the eight species of *Kalanchoe* that were sampled, and the other comprises *Cotyledon* (three species sampled) and *Tylecodon* (three species sampled). In addition to the molecular data and similar distributions, the four genera present in this clade have long been considered closely related based on cytology (Baldwin, 1938; Uhl, 1948) and floral vascular patterns (Quimby, 1971). Furthermore, unlike most members of the family, these genera possess flowers that have fused (or partially fused) corollas. *Kalanchoe* is distinguished from the other genera by possessing four-parted rather than five-parted corollas, whereas species of *Adromischus* differ from the remaining genera by possessing spikelike inflorescences. A close relationship between *Tylecodon* and *Cotyledon* has been suggested based upon cytology (Uhl, 1948) and secondary chemistry (VanWyk and Winter, 1995); however, the two genera differ from one another in several features of growth form and morphology. For example, species of *Tylecodon* are typically deciduous herbs or shrublets with caudiciform bases and spirally arranged leaves, whereas *Cotyledon* species are evergreen, low-growing to erect shrubs that lack a caudiciform base and have decussate leaves. In addition, the flowers of *Tylecodon* species are erect and spreading, while those of *Cotyledon* species are pendent. The distribution of *Tylecodon* also coincides with the winter rainfall region, whilst *Cotyledon* can be found throughout the South African subregion in both the winter and summer rainfall regions (Van Jaarsveld, 2003).

As currently defined, *Cotyledon* includes 10 species that are

¹ Manuscript received 22 July 2004; revision accepted 7 April 2005.

Research support was provided by a Kansas EPSCoR First Award to MEM; by the Department of Ecology and Evolutionary Biology and the Natural History Museum and Biodiversity Research Center, University of Kansas; and by NSF DEB (0344883) to MEM. We thank John Trager and the Huntington Botanical Garden staff for providing plant materials and Jenny Archibald for field assistance, and Jenny Archibald, Priscilla Burgoyne, Daniel Crawford, Gideon Smith, and the comments of three anonymous reviewers for thoughtful input on this manuscript. This paper is dedicated to Charles Uhl in recognition of his numerous contributions to the understanding of the evolution of Crassulaceae.

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TABLE 1. Morphological and phenological characteristics of the five recognized varieties of *Cotyledon orbiculata*. Information obtained from recent treatments of the genus (Tölken, 1985; Van Jaarsveld, 2003).

<i>C. orbiculata</i>	Flower color	Height	Leaf shape, orientation	Branching	Bark color	Flowering
var. <i>dactyloopsis</i>	Yellow	0.25 m	Linear-lanceolate, opposite and densely clustered	± branched, short spreading branches	Dark brown	Oct–Dec
var. <i>flanaganii</i>	Red/pink	0.75 m	Linear-terete, whorls of three	Sparsely branched, spreading	Pale	Nov–Jan
var. <i>oblonga</i>	Orange/red	0.8 m	Obovate-oblongate, opposite and densely clustered	Branching from base, decumbent	Pale	July–Sept
var. <i>orbiculata</i>	Deep red	0.2–0.3 m	Oblanceolate, obovate-orbicular, opposite	Highly branched, spreading	Pale	Nov–Jan
var. <i>spuria</i>	Orange/yellow	1 m	Oblanceolate, opposite	Highly branched, spreading	Pale	Nov–Jan

distributed primarily in South Africa. Members of this genus commonly inhabit rocky hillsides and cliff faces throughout the country, but are particularly speciose in the little Karoo and Drakensberg mountain regions. All members of the genus have five-parted flowers with fused corollas, but have considerable diversity in flower size, orientation of the corolla lobes (i.e., reflexed, recurved, or spreading), and flower color (i.e., red, orange, or yellow). In addition, members of *Cotyledon* are highly diverse in growth form and leaf morphology. One species in particular, *C. orbiculata*, is quite diverse in all of these features and has been divided into five varieties based primarily on leaf morphology and biogeography (Tölken, 1985, but also see Table 1). Two varieties of this species, *C. orbiculata* var. *orbiculata* and *C. orbiculata* var. *oblonga*, are widely distributed in southern Africa, whereas the other three varieties (i.e., *C. orbiculata* var. *dactyloopsis*, *C. orbiculata* var. *flanaganii*, and *C. orbiculata* var. *spuria*) are more narrowly distributed and present only in the country of South Africa (Fig. 1). In addition to differences in distribution, these varieties are also distinguished by flower size, shape, and color, growth form, and phenology (Table 1). In fact, in many features, the five varieties of *C. orbiculata* that have been described represent well the morphological diversity noted across the genus as a whole. The phylogeny of *Cotyledon*, as well as the monophyly of *C. orbiculata* and the relationship among the varieties, have not previously been investigated. A broad phylogenetic analysis of the genus will provide the necessary framework to assess the classification of the genus as well as to investigate patterns of morphological diversity and biogeographic distributions in *Cotyledon*. Here we present the first estimate of phylogeny for members of *Cotyledon* using DNA sequences from the nuclear and chloroplast genomes.

MATERIALS AND METHODS

Taxon sampling—Broad phylogenetic analyses of Crassulaceae place members of *Cotyledon* in a well-supported clade (i.e., *Kalanchoe* clade) with *Adromischus*, *Tylecodon*, and *Kalanchoe*, and place members of *Crassula* as sister to this clade (Mort et al., 2001). Two species of *Crassula* are included here as outgroups and to test the monophyly of *Cotyledon*; we sampled five members of *Tylecodon* and three species of *Adromischus*. All 10 species of *Cotyledon* currently recognized (Van Jaarsveld, 2003) were sampled, including four of the five varieties of *C. orbiculata* (Appendix). Individuals were either collected from field locations in South Africa, from documented collections at the Kirstenbosch Botanical Garden (Cape Town, South Africa), or from the Huntington Botanical Garden (San Marino, California, USA).

DNA extraction, amplification, and sequencing—Total DNA was extracted from a small amount (~10 mg) of silica-gel-dried leaf material or from fresh leaves using a modified CTAB method (Mort et al., 2001). Target DNA regions were amplified via PCR using the primer combinations N-nc18S10/C26A (ITS; Wen and Zimmer, 1996), *psbA/trnH* (*psbA-trnH* cpDNA spacer; Sang et al., 1997), and the universal primers C and F (*trnL-trnF* cpDNA spacer; Taberlet et al., 1991). Amplicons were purified using QIAquick PCR purification columns (Qiagen, Valencia, California, USA). Automated sequencing was accomplished using a Beckman-Coulter CEQ 8000 (Beckman-Coulter, Fullerton, California, USA) and the standard dye terminator cycle sequencing kits following the manufacturer's protocol, with half-volume reactions. For the *psbA-trnH* and *trnL-trnF* spacer regions, the same primers used for amplification were used for sequencing; sequences of ITS were generated using the primers ITS-1 and ITS-4 (White et al., 1990). Cycle sequencing reactions were purified using CleanSEQ (Agencourt Bioscience Corp., Beverly, Massachusetts, USA). All contigs were edited and assembled using Sequencher vers. 4.1 (GeneCodes, Ann Arbor, Michigan, USA).

Phylogenetic analyses—Alignment of the DNA data was easily accomplished by eye using Se-AL version 1.0 (Rambaut, 1996); all gap characters (“-”) were scored as missing data (“?”); nonautapomorphic gaps were subsequently coded as either present (1) or absent (0) and included in our analyses. Parsimony analyses were conducted using PAUP* (Swofford, 1998) with all characters equally weighted. Analyses were first conducted on the individual nrDNA ITS and cpDNA data sets. Comparison of the resulting topologies revealed no instances of well-supported topological differences. Therefore, all data were combined into a single data matrix for subsequent parsimony analyses. Initial searches were conducted using 500 replicates of RANDOM taxon addition and NNI branch swapping. Each set of shortest trees from these initial searches was used for subsequent analyses employing TBR branch swapping. Relative support for the recovered clades was assessed using bootstrap analyses (Felsenstein, 1985) with 500 replicates, TBR branch swapping, and saving a maximum of 500 trees per replicate; decay values were determined using the computer program AutoDecay version 4.0 (Eriksson, 1999) with TBR branch swapping.

RESULTS

The combined ITS–cpDNA data set comprised 1931 nucleotide characters. Indels in the ITS data set were frequent, and most were 1–2 bp in length. These indels distinguished the ingroup from the outgroup, and none were inferred within the ingroup taxa sampled. Therefore, they were not included in our parsimony analyses. A total of 17 indels was inferred to align the *psbA-trnH* data, and 20 were inferred to align the *trnL-trnF* data; these were scored as present (“1”) or absent (“0”) and were included in our subsequent analyses. There-

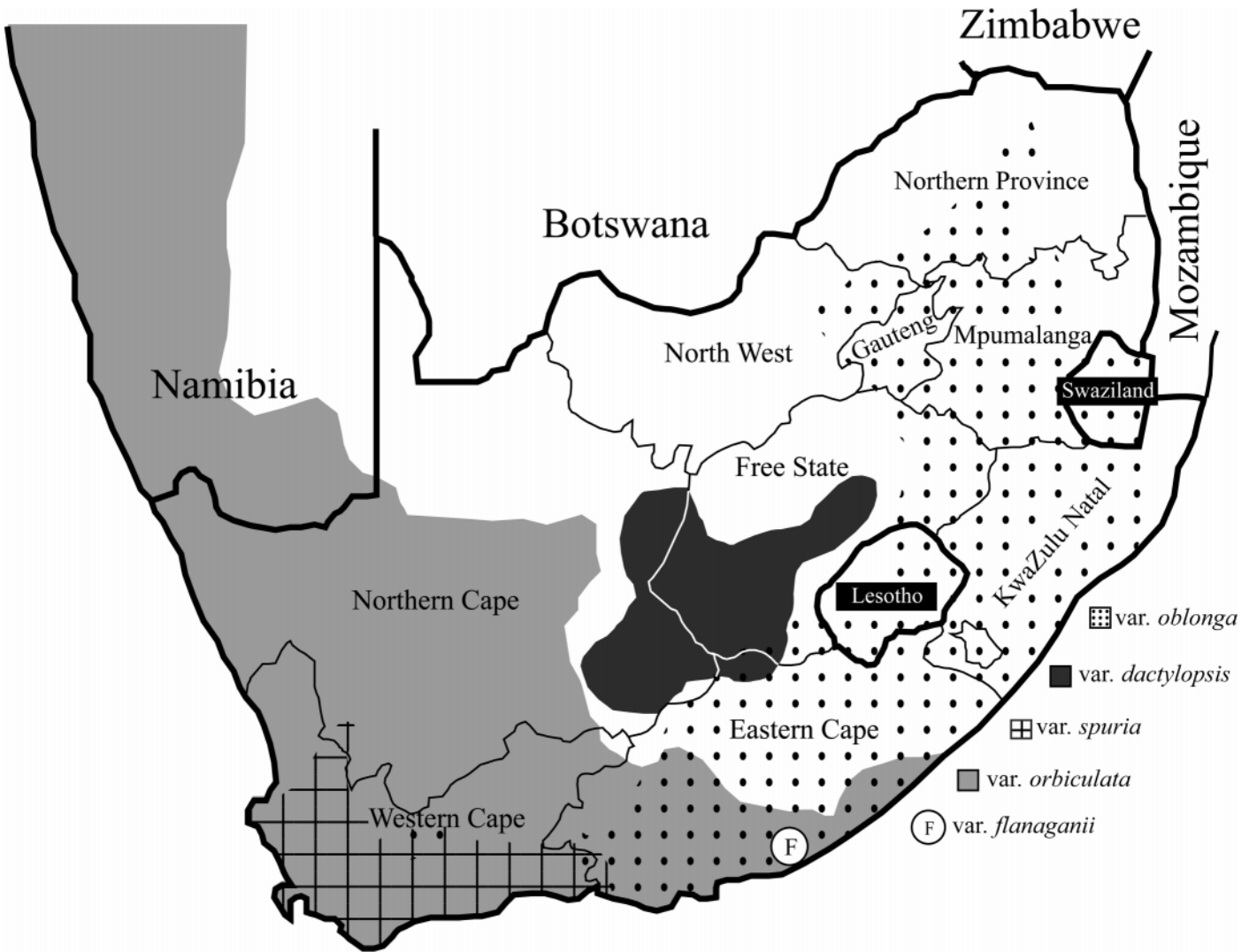


Fig. 1. Map of southern Africa with provinces of South Africa. Highlighted are the general distributions of the five varieties of *Cotyledon orbiculata*.

fore, the combined data set comprised 1968 characters, of which 1587 were constant and 298 characters were potentially parsimony informative. Parsimony analyses recover two minimum-length trees (CI = 0.8674; RI = 0.8960) of 475 steps in length. The strict consensus topology is highly resolved, and only one node joining two taxa collapses. One of these minimum-length trees is shown (Fig. 2).

Our analyses support the monophyly of *Adromischus* (100% bootstrap; decay = 21) and place this clade as sister to a well-supported clade (98% bootstrap; decay = 11) containing a monophyletic *Tylecodon* (92% bootstrap; decay = 5) and *Cotyledon* (100% bootstrap; decay = 7). Relationships within *Cotyledon* are fully resolved; however, most of the relationships recovered are weakly supported. Two major lineages are recovered within the genus. The first comprises two accessions of *C. adscendens* (72% bootstrap; decay = 1) that are sister to a clade containing two accessions each of *C. orbiculata* var. *oblonga* and *C. tomentosa*. The second major clade of *Cotyledon* includes three additional varieties of *C. orbiculata* as well as seven additional species of the genus. Within this clade, *C. orbiculata* var. *spuria* is basal and sister to two subclades. The first includes *C. cuneata*, which is sister to a clade

comprising *C. papillaris* and *C. elisseae*. The second comprises *C. campanulata* and *C. barbeyi* as sequential sisters, followed by *C. orbiculata* var. *orbiculata* and *C. orbiculata* var. *flanaganii*, which are sister to a clade comprised of *C. woodii* and *C. velutina*.

Noteworthy is that the different varieties of *C. orbiculata* are not monophyletic (Fig. 2). Analyses constraining *C. orbiculata* to form a clade resulted in a topology with a tree length of 487, which is 12 steps longer than the minimum-length trees recovered by our previous analyses.

DISCUSSION

Monophyly and position of Cotyledon in Crassulaceae—

The genus *Cotyledon* has been placed within a largely South African clade of Crassulaceae (the *Kalanchoe* clade of Ham and 't Hart, 1998). Historically, *Cotyledon* has served as a catch-all taxon for Old World Crassulaceae with sympetalous, five-parted corollas (Schönland, 1915). However, based upon cytotaxonomy (Baldwin, 1938; Uhl, 1948) and floral morphology (e.g., Quimby, 1971), as well as the aforementioned molecular data, it is now clear that many of the species for-

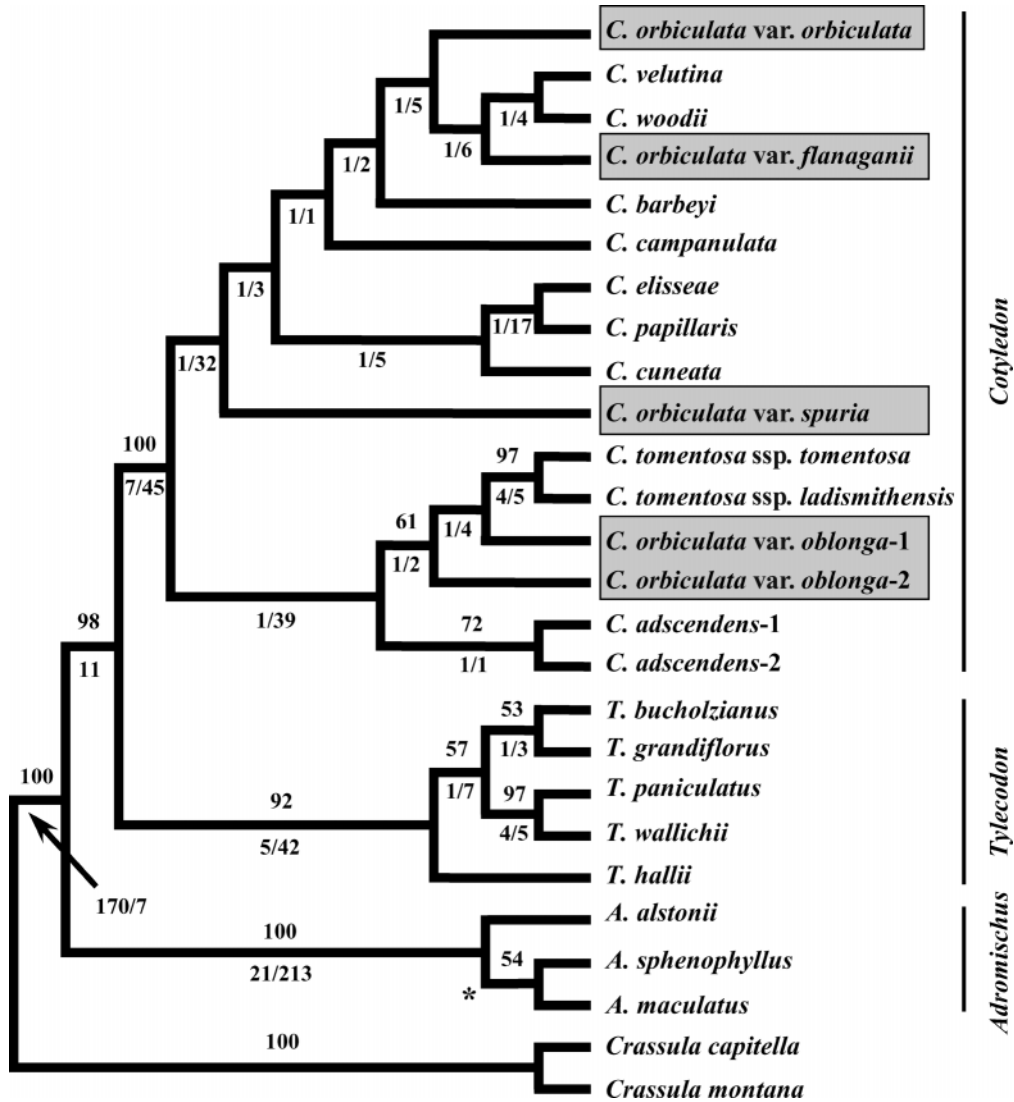


Fig. 2. One of two minimum-length trees (length = 475 steps, CI = 0.8674, RI = 0.8960) inferred from parsimony analyses of a combined ITS/cpDNA data set; the single node that collapses in the strict consensus topology is indicated by an asterisk (*). Bootstrap support greater than 50% is indicated above the branches; decay values and branch lengths, respectively, are indicated below each branch. Shaded boxes highlight the varieties of *Cotyledon orbiculata* sampled for this study.

merly placed within *Cotyledon* are best assigned to other genera. Thus, the current delimitation of the genus includes 10 species that are distinguished from *Tylecodon* by their persistent, opposite (rarely whorled) vs. deciduous, spirally arranged leaves. Furthermore, members of *Cotyledon* have inflorescences of one to many pendent flowers, in contrast to the typically erect flowers seen in species of *Tylecodon*. Previous investigations of Crassulaceae as a whole based upon parsimony analyses of cpDNA restriction sites (Ham and 't Hart, 1998), *matK* sequences (Mort et al., 2001), and nrDNA ITS sequences (Mort, 2002) have supported the monophyly of the genera comprising the *Kalanchoe* clade (i.e., *Adromischus*, *Cotyledon*, *Kalanchoe*, and *Tylecodon*). Furthermore, these studies provided topologies that are congruent and placed *Tylecodon* as sister to *Cotyledon*, with *Kalanchoe* and *Adromischus* as sequential sisters to the *Cotyledon* plus *Tylecodon* clade. However, it is important to note that these family-level studies of

Crassulaceae have had only limited taxonomic sampling of the genera comprising this clade.

Our analyses strongly support the monophyly of *Adromischus*, *Tylecodon*, and *Cotyledon* (Fig. 2). As with previous studies, *Tylecodon* and *Cotyledon* form a well-supported lineage, with *Adromischus* sister to this clade. Because the goals of the present study were to produce the first estimate of phylogeny for *Cotyledon*, we did not include sequences from *Kalanchoe*; however, previous studies of Crassulaceae have strongly supported the monophyly of the genus (Mort et al., 2001). The monophyly of *Kalanchoe* is also supported by a unique base chromosome number of $x = 17$ and a number of morphological features (e.g., four-parted sympetalous flowers).

Phylogenetic relationships within *Cotyledon*—Parsimony analyses of the combined chloroplast and nuclear DNA sequence data provide a highly resolved phylogeny for the 16

taxa of *Cotyledon* sampled (Fig. 2). Prior to the present study, phylogenetic relationships had not been investigated in *Cotyledon*, and the most recent treatment of the genus does not include any hypotheses of evolutionary relationships (Van Jaarsveld, 2003). Therefore, we will confine our discussion to the results reported here and use the estimate of phylogeny to examine morphological diversity and biogeographic distributions in *Cotyledon*. Our analyses recover two major lineages within *Cotyledon*. The first includes two accessions each of three species, *C. adscendens*, *C. tomentosa*, and *C. orbiculata* var. *oblonga*; the second major clade comprises three varieties of *C. orbiculata* as well as seven additional *Cotyledon* species.

Within the former clade, there is moderate support (72% bootstrap; decay = 1) for a grouping of the two accessions of *C. adscendens* sampled for this study. This species is narrowly distributed and inhabits coastal dunes (dune thicket vegetation) east of Port Elizabeth in the Eastern Cape Province of South Africa. Tölken (1985, p. 7) noted that this species "might be considered a hybrid between *C. velutina* and *C. woodii*," but discounted this hypothesis because of the narrow distribution of *C. adscendens* and its absence in other areas where the proposed parental species are sympatric. Although *C. adscendens* does share some morphological features with its putative parental species, it is easily distinguished from other species in the genus by its scandent or scrambling growth form, a feature that is unique for the genus. Furthermore, our DNA sequence data suggest that *C. adscendens* is not closely related to either of these putative parentals.

Next branching in this clade are two accessions of *C. orbiculata* var. *oblonga*, which are sequential sisters to two accessions of *C. tomentosa*. *Cotyledon orbiculata* var. *oblonga* is widespread in eastern South Africa, Swaziland, and Mozambique (Fig. 1). This variety of *C. orbiculata* is very diverse in leaf morphology and vestiture across its range, but its basally branched, decumbent growth form and corolla with a distinct bulge in the center differentiates it from the other varieties of *C. orbiculata* (Table 1). Despite this distinctive floral morphology, our analyses suggest that this variety is not monophyletic; however, this result is only weakly supported (<50% bootstrap; decay = 1). Two subspecies (i.e., subsp. *ladismithensis* and subsp. *tomentosa*) have been recognized within *C. tomentosa*. This species is a small, dwarf shrub and possesses leaves that are often dorsiventrally compressed with several apical teeth and are densely pubescent to tomentose. This species is distributed primarily in the Succulent Karoo of the Western Cape Province and Eastern Cape Province. The two subspecies differ in a number of morphological features. For example, *C. tomentosa* subsp. *tomentosa* is a highly branched, small shrub (to 11 cm), with slender branches and orange-red flowers having recurved petals. *Cotyledon tomentosa* subsp. *ladismithensis* is a larger shrub (to 25 cm) with only a few, rigid branches. The flowers of this latter subspecies also have recurved petal lobes, but are yellow to light orange. Both subspecies have been included in the present study, and our analyses strongly support (97% bootstrap; decay = 4) the monophyly of this species. No obvious morphological similarities unite this species with *C. orbiculata* var. *oblonga*; however, there is moderate support (61% bootstrap; decay = 1) for the relationship among these species.

Three of the four varieties of *C. orbiculata* sampled in this study are placed within the second major clade recovered by our analyses, although not as sister taxa. One of these taxa, *C. orbiculata* var. *spuria* is placed (decay = 1) basal in this clade.

This variety is confined to the Western Cape Province and is completely within the distribution of the widespread *C. orbiculata* var. *orbiculata* (Fig. 1). Similar to the latter variety, *C. orbiculata* var. *spuria* possesses oblanceolate leaves and is a highly branched decumbent to erect shrub. *Cotyledon orbiculata* var. *spuria* is, however, typically larger (up to 1 m), has orange to yellow flowers rather than the deep red flowers of var. *orbiculata* (Table 1), and inhabits lower slopes, often in depressions along river banks with low vegetation. Despite this difference in habitat, putative hybrids between vars. *orbiculata* and *spuria* have been reported in areas where they are in close proximity (see Tölken, 1985).

Although not strongly supported (<50% bootstrap; decay = 1), a clade containing three dwarf shrubs (*C. papillaris*, *C. elisseae*, and *C. cuneata*) is present in both minimum-length trees. *Cotyledon papillaris* is distributed from Namibia through Namaqualand and extends into the Eastern Cape of South Africa, where it grows as a decumbent shrub, reaching only about 10 cm in height. The flowers of *C. papillaris* are distinctive in the genus by being yellow-green in color, often with purple spots. Similar to *C. papillaris*, *C. elisseae* is a dwarf shrub (reaching 20 cm), but differs from the former in its upright, rounded growth form and deep red flowers. This species also differs in its distribution, being confined to the Western Cape Province, where it is present in the Succulent Karoo and subtropical thicket. Our analyses suggest a close relationship between these species (decay = 1) and place *C. cuneata* as sister to this clade. *Cotyledon cuneata* is a decumbent, dwarf shrub (up to 30 cm) that is widespread in western South Africa with a range similar to that of *C. orbiculata* var. *orbiculata* (Fig. 1). The flowers of *C. cuneata* are yellow to yellow-green in color and are cylindrical to urceolate, as opposed to the tubular shape displayed by most species of *Cotyledon*. In addition, the flowers of *C. cuneata* are highly viscid on the outside of the corolla.

The only other species possessing cylindrical to urceolate shaped flowers, *C. campanulata*, is sister to a grouping of four species (*C. velutina*, *C. woodii*, *C. barbeyi*, and two varieties of *C. orbiculata*) that all share an erect growth form. *Cotyledon campanulata* is a decumbent, dwarf shrub (up to 20 cm) with yellow flowers that, like *C. cuneata*, are viscid on the outside of the corolla. However, *C. campanulata* has smaller flowers and is much more narrowly distributed, present only in a small region of the Eastern Cape Province of South Africa. *Cotyledon barbeyi* is sister (decay = 1) to the remainder of the erect shrubs. This species is a large (up to 2 m) shrub that grows primarily in the Northern Province of South Africa and Swaziland, but ranges as far north as Ethiopia. Throughout its range, this species displays a high degree of diversity in leaf size, shape, and pubescence. Only one accession of this species was included here, and therefore the monophyly of this highly diverse taxon cannot be tested. Future work on the phylogeny and patterns of evolution of *Cotyledon* should include multiple populations of *C. barbeyi* to test the monophyly of this species and to examine the patterns of diversification in this taxon.

Cotyledon orbiculata var. *orbiculata* is another morphologically diverse and widespread taxon that overlaps the distribution of three other varieties of *C. orbiculata* (Fig. 1). *Cotyledon orbiculata* var. *orbiculata* is a large, decumbent to erect shrub (up to 1 m, but often 20–30 cm) that has deep red flowers, although as noted by Tölken (1985), the flowers often appear orange due to a thick, waxy bloom on the outside of

the corolla tube. This variety is extremely variable in leaf morphology and many other morphological features (as described later), and many local forms have been recognized (see Van Jaarsveld, 2003). In fact, the high degree of variation noted in this taxon makes it difficult to assess morphological affinities with other *Cotyledon* species. Our analyses place the single accession of this variety as sister (decay = 1) to a clade including *C. orbiculata* var. *flanaganii* and two additional species of *Cotyledon*.

Cotyledon orbiculata var. *flanaganii* is a narrowly distributed taxon (Fig. 1) that differs from other *Cotyledon* species by having leaves arranged in whorls of three. This variety is present on hillsides around the Kei River in the Eastern Cape Province, where it typically inhabits gravelly slopes in scrub vegetation. Recently, this species was found in the Mzimvubu River Valley about 150 km to the northeast of Kei Mouth, although it is uncertain whether this population is natural or due to an escape from cultivation. Our analyses place *C. orbiculata* var. *flanaganii* as sister to a clade containing two additional species of *Cotyledon* that share a similar, although somewhat broader, distribution with *C. orbiculata* var. *flanaganii*. One of these species, *C. velutina*, is a large shrub (up to 3 m tall and the largest species in the genus *Cotyledon*) and, like *C. orbiculata* var. *flanaganii*, is only sparsely branched and is common in scrub vegetation. The other, *C. woodii*, is a highly branched erect shrub that reaches heights up to 1.5 m. *Cotyledon woodii*, however, differs from the other species in this clade in a number of features. For example, this species typically inhabits forest margins or sheltered ravines (i.e., kloofs) and has an inflorescence that is reduced to a single flower; this species also has a longer flowering period than other species of *Cotyledon* in this clade (December to April as opposed to November to December) and, as Tölken (1985) notes, it is possible to find flowering individuals year round.

Phylogenetic status of *Cotyledon orbiculata*—The *C. orbiculata* species complex includes five varieties that display an extensive degree of morphological variation in leaf form and pubescence, flower color, growth form, and habitat preference. The high degree of diversity and intergradation in morphology between varieties led Tölken (1979) to describe *C. orbiculata* as the “most complex species” in Crassulaceae and further state that the taxonomy of this species was greatly in need of revision. As circumscribed by Tölken (1985), *C. orbiculata* now includes as many as 11 species that have been recognized historically. Defining the limits of the varieties in this species complex is very difficult; Tölken (1979) states that if the range of variation in leaf and other morphological features is taken into account, there is extensive overlap between the five varieties of *C. orbiculata*. While providing an overview of the degree of variation within and among the varieties, Tölken (1979) does not indicate the morphological features that are distinctive (i.e., synapomorphic) for *C. orbiculata*, other than noting that in areas of sympatry, putative hybrids between varieties have been reported. The reproductive biology of *Cotyledon* has yet to be thoroughly investigated, and therefore, the retention of cross compatibility between varieties (or species) cannot be discussed in detail. Future research efforts should assess the levels of cross-compatibility in *Cotyledon*, especially between morphologically divergent populations of *C. orbiculata*.

Four of the five varieties of *Cotyledon orbiculata* were sampled for the present study, including two accessions of the

widespread *C. orbiculata* var. *oblonga*. The only variety not included here was the narrowly distributed *C. orbiculata* var. *dactylopsis*. Our parsimony analyses do not support the monophyly of this species. The two accessions of *C. orbiculata* var. *oblonga* sampled for the present study are placed with moderate support (61% bootstrap; decay = 1) in a clade with *C. tomentosa* and well removed from the three other varieties included in the present study (Fig. 2). Furthermore, the two accessions of *C. orbiculata* var. *oblonga* are not supported as being monophyletic; however, this result is only weakly supported (decay = 1). This result is somewhat surprising considering that this variety of *C. orbiculata* is somewhat distinctive within this group; *C. orbiculata* var. *oblonga* is a decumbent shrub that flowers in winter (July to September), whereas the other varieties flower in summer (November to January) and are more or less erect shrubs (Table 1).

The remaining varieties of *C. orbiculata* are all placed within a second large clade within *Cotyledon*, but not as sister taxa. Two of these varieties (*C. orbiculata* vars. *flanaganii* and *spuria*) are more narrowly distributed than either *C. orbiculata* vars. *oblonga* or *orbiculata*. *Cotyledon orbiculata* var. *flanaganii* is placed as sister to *C. velutina* and *C. woodii*. While this species is morphologically distinctive by having linear-terete leaves that are arranged in whorls of three, no obvious morphological features unite this species with *C. velutina* or *C. woodii*. As noted, *C. orbiculata* var. *orbiculata* is a geographically widespread and morphologically variable taxon. For example, across its range, leaves vary in shape from oblanceolate to orbicular, may be terete or more or less flattened, and their vestiture varies from completely glabrous to densely pubescent. Furthermore, Tölken (1985) notes that across its range, this variety has flowers that vary in color from bright red to orange or pink. The combinations of these features that vary across the range of *C. orbiculata* var. *orbiculata* have led to the recognition of numerous local forms (Van Jaarsveld, 2003). Future investigations of *Cotyledon* should sample more populations of *C. orbiculata* var. *orbiculata* that represent the range of morphological diversity and geographic range for this variety. This approach will allow a much more thorough assessment of the status of this variety, as well as provide the necessary framework to investigate patterns of morphological diversification in this taxon as well as across the genus as a whole.

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APPENDIX. Taxa included in the present study, including voucher information and GenBank accession numbers. Accession numbers for species obtained from the collections at the Huntington Botanical Garden (San Marino, California, USA) and Kirstenbosch Botanical Garden (Cape Town, South Africa) are designated by HBG and KBG, respectively. Species that were not sequenced for a particular DNA region are indicated by “—.”

Taxon; Voucher; Accession numbers: ITS; *psbA-trnH*; *trnL-trnF*.

Adromischus Lemaire

- A. alstonii* (Schönland & Baker fil.) C.A. Smith; HBG 17383; AY692316;—; AY692286.
- A. maculatus* (Salm-Dyck) Lemaire; HBG 69133; AY692317;—; AY692287.
- A. sphenophyllus* C.A. Smith; HBG 70042; AY692318;—; AY692288.
- Cotyledon* L.
- C. adscendens* R.A. Dyer; Van Jaarsveld, s.n.; AY596329; AY596344; AY692291.
- C. adscendens* R.A. Dyer; Van Jaarsveld, s.n.; AY596330; AY596345;—.
- C. barbeyi* Schweinfurth; KBG 17212; AY596335; AY596350; AY692292.
- C. campanulata* Marloth; Van Jaarsveld, s.n.; AY596324; AY596339; AY692293.
- C. cuneata* Thunberg; Van Jaarsveld, s.n.; AY596331; AY596346; AY692294.
- C. eliseae* Van Jaarsveld; HBG 17212; AY596323; AY596338; AY692295.
- C. orbiculata* var. *flanaganii* (Schönland & Baker) Tölken; VPHS 21; AY596332; AY596347; AY692296.
- C. orbiculata* var. *oblonga* (Haworth) De Candolle; KBG 958/90; AY596333; AY596348; AY692297.
- C. orbiculata* var. *oblonga* (Haworth) De Candolle; Van Jaarsveld, s.n.; AY596334; AY596349; AY692298.

- C. orbiculata* var. *orbiculata* L.; HBG 77962; AY596325; AY596340; AY692299.
- C. orbiculata* var. *spuria* (L.) Tölken; KBG 17714; AY596337; AY596352; AY692300.
- C. papillaris* L.; Williamson, 10/94; AY596336; AY596351; AY692301.
- C. tomentosa* subsp. *ladismithensis*; HBG 69296; AY692311;—; AY692302.
- C. tomentosa* subsp. *tomentosa*; HBG 87298; AY692312; ; AY692303.
- C. velutina* Hooker; HBG 65572; AY596327; AY596342; AY692304.
- C. woodii* Schönland & Baker; HBG 67807; AY596328; AY596343; AY692305.
- Crassula* L.
- C. capitella* Thunberg; KBG 16848; AY596321;—; AY692289.
- C. montana* Thunberg; KBG 16686; AY596322;—; AY692290.
- Tylecodon* Tölken
- T. bucholzianus* (Schult & Stephan) Tölken; HBG m.v. 5283;—; AY692283; AY692306.
- T. grandiflorus* (Burman fil.) Tölken; HBG 54361;—;—; AY692307.
- T. hallii* Tölken; HBG 49205; AY692315; AY692285; AY692308.
- T. paniculatus* (Linné fil.) Tölken; HBG 17604; AY692313;—; AY692309.
- T. wallichii* (Harvey) Tölken; HBG 54089; AY692314; AY692284; AY692310.