Plant Syst. Evol. 248: 71–83 (2004)
DOI 10.1007/s00606-004-0190-7

Plant Systematics and Evolution
Printed in Austria

Phylogenetics and evolution of the Macaronesian members of the genus *Aichryson* (Crassulaceae) inferred from nuclear and chloroplast sequence data

K.N. Fairfield[1], **M.E. Mort**[1], and **A. Santos-Guerra**[2]

[1]Department of Ecology and Evolutionary Biology and the Museum of Natural History and Biodiversity Research Center, University of Kansas, Lawrence, Kansas, USA
[2]Jardin de Aclimatacion de la Orotava, Puerto de la Cruz, Tenerife, Canary Islands, Spain

Received May 1, 2003; accepted April 20, 2004

Abstract. *Aichryson* is a genus of annual or perennial herbs, comprising approximately 13 species. The genus is nearly endemic to Macaronesia, with the center of diversity on the Canary Islands. Previous studies indicate that *Aichryson* is monophyletic and sister to a clade comprising two other genera, *Monanthes* and *Aeonium*. However, phylogenetic relationships within the genus have yet to be investigated. Phylogenetic relationships were estimated for *Aichryson* using DNA sequence data from both the nuclear and chloroplast genome. Parsimony analyses were conducted using separate nuclear and chloroplast data sets, as well as a combined data set. These combined analyses provide a well-resolved topology that is used to investigate patterns of evolution within the genus. For example, although typically herbaceous in habit, two members display a woody growth form. Our analyses indicate that woodiness is a derived feature in *Aichryson* that has arisen once. Furthermore, the data presented suggest that the *Aichryson pachycaulon* group, a group of five subspecies, are in fact not monophyletic and that a reexamination of classification is necessary. The present study indicates that the biogeographical patterns exhibited by *Aichryson* appear to be rare, occurring in only one other known genus.

Key words: *Aichryson*, Crassulaceae, Canary Islands, Macaronesia, ITS sequences, *trn*L-*trn*F sequences, phylogeny.

Introduction

Crassulaceae are a diverse and systematically complex angiosperm family comprising 35 genera and approximately 1,500 species (Berger 1930). Members of this family typically are leaf-succulent herbs that occur in semi-arid habitats. The family is nearly cosmopolitan in distribution, with centers of taxonomic diversity in southern Africa, Madagascar, the Himalayas, Mexico, and Macaronesia.

The family has recently been the focus of several broad phylogenetic studies employing chloroplast DNA RFLPs (Ham and t'Hart 1998), *mat*K nucleotide sequence data (Mort et al. 2001), and nuclear ribosomal internal transcribed spacer (ITS1–5.8s–ITS2) sequence data (Mort. Botanical Society of America Meeting 2003, Abstract # 555). These independent estimates of phylogeny are quite similar to one another and resolve seven major lineages within the family, one of which (i.e. the Macaronesian Clade) comprises four genera, *Aeonium* Webb & Berth., *Greenovia* Webb & Berth., *Monanthes* Haw., and *Aichryson* Webb & Berth. that are largely endemic to Macaronesia (Azores, Canary Islands, Cape Verde Islands, Madeiran Islands, and the Salvage Islands.

Phylogenetic relationships within the Macaronesian Clade of Crassulaceae have been investigated using sequences of the *trn*L-*trn*F cpDNA intergenic spacer region (Mes and t'Hart 1994); nrDNA ITS (Mes and t'Hart 1996, Mort et al. 2002); an expanded cpDNA sequence data set (*mat*K, *trn*L-*trn*F, *psb*A-*trn*H), (Mort et al. 2002), as well as a combined cpDNA (*mat*K, *trn*L-*trn*F, *psb*A-*trn*H) and ITS data set (Mort et al. 2002). While each of these studies employed different data and taxon sampling, each resolved three major lineages in the Macaronesian Clade. The largely herbaceous genus *Aichryson* was basal and sister to a clade comprising a well-supported lineage of perennial *Monanthes* and a clade comprising *Aeonium* (including *Greenovia*). However, because the goals of these studies were primarily focused on estimating phylogeny for *Aeonium*, only a limited number of *Aichryson* specimens were included, and a robust estimate of phylogeny for this genus has yet to be investigated.

Aichryson comprises approximately 13 species of annual or perennial herbs, which are nearly endemic to Macaronesia. Among other members of the Macaronesian Clade of Crassulaceae, *Aichryson* species are characterized by having 6–12 parted flowers with digitate nectariferous glands, which are borne at the base of each carpel (Liu 1989). The monophyly of *Aichryson* is further supported by having a base chromosome number of $x = 15$ in the majority of species ($x = 17$ in *A. punctatum*; $x = 16$ in some forms of *A. pachycaulon*) (Bramwell 1977), whereas other members of the Macaronesian Clade (i.e. *Monanthes*, *Aeonium* o.k. *Greenovia*) have a base chromosome number of $x = 18$ (Uhl 1963). Most species of *Aichryson* are annual, herbs of moist habitats; however, two species are woody perennials: *A. tortuosum* (Lanzarote and Fuerteventura) and *A. bethencourtianum* (Fuerteventura). Mort et al. (2002) suggests that the woody, perennial habit is a derived feature; however because *A. bethencourtianum* has yet to be sampled it is unclear how many times this woody/perennial feature has arisen within *Aichryson*

Although present on many islands comprising Macaronesia, the highest level of species diversity within the genus occurs on the Canary Islands, where 10 species are endemic (Bramwell and Bramwell 1990); three additional species of *Aichryson* are found on Madeira and the Azores (Hansen and Sundig 1993). Of the ten species present on the Canary Islands, at least one species is found on each of the seven major islands (Fig. 1), with the lowest diversity of species (1) occurring on the island of Lanzarote and highest diversity on the island of La Palma (4). Excluding the subspecies of *A. pachycaulon*, only three species found on the Canarian Archipelago are single-island endemics and they include, *A. palmense* (La Palma), *A. bethencourtianum* (Fuerteventura), and *A. porphyrogennetos* (Gran Canaria).

The phylogeny of *Aichryson* has yet to be examined and the classification of the genus is unclear and in need of critical examination. One especially problematical species worthy of additional study is *A. pachycaulon*. This taxon (heretofore referred to as the *pachycaulon* group) has variously been recognized as a single species with five subspecies (Bramwell 1977, Hansen and Sundig 1993), or as several distinct species (A. Santos pers. com.). Generally speaking, this species complex has been defined by the absence of morphological features that define other members of *Aichryson*. For example, members of the *A. pachycaulon* group lack the distinctive pubescence possessed by several species of *Aichryson* and the lack of black punctate glands along the margins of the leaves separates this group from the other glaborous species *A. punctatum*. Each member of the *pachycaulon* group is endemic to a single island, with no two members present on the

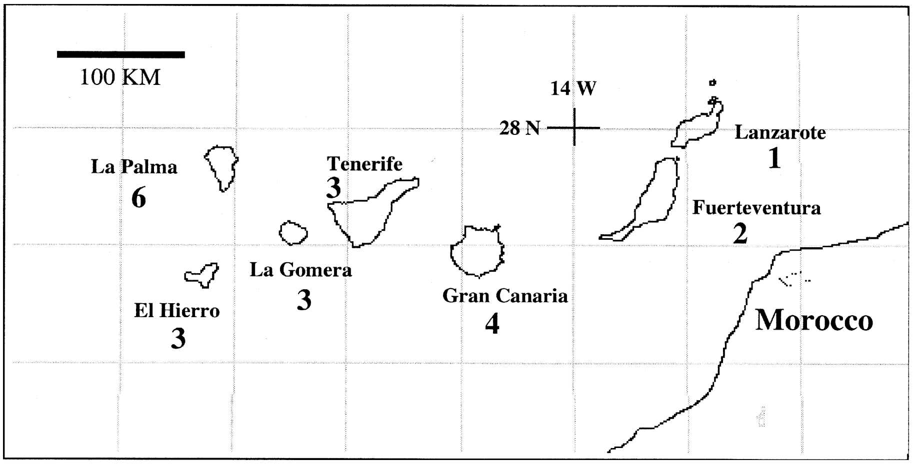

Fig. 1. Map of the Canary Islands with the number of *Aichryson* species present on each island indicated

same island. Within the *A. pachycaulon* group, taxa display a range of morphological and cytological variation (Table 2). For example, *A. pachycaulon* subsp. *pachycaulon* from Fuerteventura is erect in habit with erect leaves, stems, and branches, in contrast to the other taxa, which are sprawling in habit with divaricate branches and patent leaves (Praeger 1932). Flower size also differs between the taxa in this group ranging from 15 mm in diameter (*A. pachycaulon* subsp. *gonzalez-hernandezii*) to 6 mm in diameter (*A. pachycaulon* subsp. *parviflorum*) (Bramwell 1977). This relatively large range of flower diameter could be associated with pollinator interactions, but to date little is known regarding the pollination biology. Further investigation is needed to explain fully the large difference of flower diameter exhibited by the *pachycaulon* group.

As noted above, three species of *Aichryson* occur on other Macaronesian Archipelagos. *Aichryson villosum* occurs on Santa Maria (Azores) as well as the islands comprising the Madeiran archipelago. *Aichryson divaricatum* and *A. dumosum* occur only on the island of Madeira. This distributional pattern is not rare in Macaronesian taxa (Panero et al. 1999, Mort et al. 2002), but to date the biogeography of *Aichryson* has not been investigated in a phylogenetic context. In light of an estimate of phylogeny, it will be possible to ascertain the archipelago of origin, as well as patterns of inter-archipelago dispersal among members of *Aichryson*.

Estimates of phylogeny using molecular data are often difficult to obtain for insular taxa due to the lack of variation in molecular characters (reviewed by Baldwin et al. 1998). One approach to this problem is to combine data sets from multiple gene regions to achieve more robust estimates of phylogeny for island taxa. The utility of this approach has been demonstrated elsewhere (e.g. Mes and 't Hart 1996, Soltis et al. 1996, Baldwin 1997, Mort et al. 2002). We therefore generated multiple DNA sequence data sets, from both the nuclear and chloroplast genomes, to resolve relationships in *Aichryson*. The goals of our study were to (1) infer a phylogeny for *Aichryson* using broad taxon sampling. Using our estimate of phylogeny, we also (2) examine the evolution of the perennial, woody habit, (3) analyze the origin and evolution of the Madeiran members of *Aichryson*, and (4) assess the classification regarding the monophyly of the *pachycaulon* group.

Materials and methods

The majority of the species included in this study were collected from field locations; however

Table 1. Species of *Aichryson* sequenced for this study. Voucher specimens have been collected for all taxa, and have been deposited in the Marion Ownbey Herbarium (WS), McGregor Herbarium (KANU), or Jardin de Aclimatacion de la Orotava Herbarium (CI). One species was obtained from documented collections at the Huntington Library and Botanical Gardens (HBG), San Marino, CA. Abbreviations for species distributions are as follows: L(Lanzarote), F (Fuerteventura), GC (Gran Canaria), T (Tenerife), G (Gomera), P (La Palma), H (El Hierro), M (Madiera), AZ (Azores), MO (Morroco). Island abbreviations that are in bold are islands from which taxa were collected. Genbank accession numbers for each region sequenced are provided

Taxon	Island	Voucher	ITS	*trn*L-*trn*F
A. bethencourtianum Bolle	**F**	HBG	AY568347	AY568337
A. divaricatum (Ait.) Praeger	**M**	Fontinha s. n. (KANU)	AY568348	AY568338
A. dumosum (Lowe) Praeger	**M**	Fontinha s. n. (KANU)	AY568349	AY568339
A. laxum (Haw.) Bramw.	T,GC,P,**H**,G	Mort 1494 (WS)	AY082107	AY082225
A. pachycaulon Bolle	T, GC, P, F, G			
ssp. *gonzalez-hernandezii* (Kunk.) Bramw.	**G**	Santos s.n. (CI)	AY568351	AY568341
ssp. *immaculatum* (Webb ex Christ) Bramw.	**T**	Santos s.n. (CI)	AY568353	AY568343
ssp. *pachycaulon*	**F**	Santos s.n. (CI)	AY568356	AY568345
ssp. *parviflorum* (Bolle) Bramw.	**P**	Santos s.n. (CI)	AY568352	AY568342
ssp. *praetermisssum* Bramw.	**GC**	Mort 1404 (WS)	AY082107	AY082223
ssp. *praetermisssum* Bramw.	**GC**	Santos s.n. (CI)	AY568350	AY568340
A. palmense Webb ex Bolle	**P**	Mort 1482 (WS)	AY082104	AY082222
A. parlatorei Bolle	T, GC, **P**, F, G	Santos s.n. (CI)	AY568354	AY568344
A. porphyrogennetos Bolle	**GC**	Mort 1402 (WS)	AY082106	AY082224
A. punctatum (Chr. Sm. ex Buch) Webb et Berth.	T, GC, P, **H**, G	Mort 1495 (WS)	AY082103	AY082287
A. tortuosum (Ait.) Webb et Berth.	**L**, F	Santos s.n. (CI)	AY082102	AY082286
A. villosum (Ait.) Webb et Berth.	**M**, AZ	Fontinha s. n. (KANU)	AY568355	AY568346
Sedum jaccardianum Maire & Wilczek	MO	32211 (UT)	AY082100	AY082220
Sedum modestum Ball	MO	33112 (UT)	AY082101	AY082221

Table 2. Characteristics of the five subspecies comprising the *Aichryson pachycaulon* group. Abbreviations for distributions are as follows: *F* (Fuerteventura), *GC* (Gran Canaria), *T* (Tenerife), *G* (Gomera), *P* (La Palma), *H* (El Hierro); distributions and morphological characteristics taken from Bramwell (1977)

A. pachycaulon ssp.	Dist.	Flower Dia. (mm)	Leaf Margins	Chromosome No.
gonzalez- hernandezii (Kunk.) Bramw.	G	12–15	crenate	$n = 34$
immaculatum (Webb ex Christ) Bramw.	T	9–11.5	subentire	$n = 32$
pachycaulon (Bolle) Bramw.	F	9–12	subentire	$n = 34$
parviflorum (Bolle) Bramw.	P	<9	crenate	$n = 30$–32
praetermissum Bramw.	GC	10	crenate	$n = 34$ or $n = 17$

several taxa were obtained from botanical gardens (Table 1). Leaf material for several species of *Aichryson* (*A. bollei*, *A. brevipetalum*, and *A. bitumosum*) was unavailable and those species are not included in the present study. Previous broad phylogenetic studies of Crassulaceae (Ham and 't Hart 1998, Mort et al. 2001) have indicated that *Aichryson* is clearly monophyletic and sister to a clade comprising the *Monanthes*, *Greenovia* and *Aeonium*. Furthermore, these studies place several North African species of *Sedum* L. (Crassulaceae) as sister to Macaronesian clade; two of these species, *S. modestum* and *S. jaccardianum*, were used as outgroups for our analyses.

DNAs for all taxa were extracted using a modified CTAB buffer method (Mort et al. 2001). Previous phylogenetic studies of island plants have indicated that sequences from the nuclear ITS region are useful for phylogeny reconstruction at this level (e.g. Kim et al. 1996, Baldwin 1997, Mes et al. 1997, Mort et al. 2002). In addition, several regions in the chloroplast genome have shown utility for resolving relationships among taxa of relatively recent origin. One such region is *trn*L-*trn*F (Taberlet et al. 1991). Both the ITS region as well as the *trn*L-*trn*F region were sequenced for this study.

Target DNA regions were amplified using the primer combinations N-nc18S10/C26A (ITS; Wen and Zimmer 1996), and universal primers C/F (*trn*L-*trn*F; Taberlet et al. 1991). Automated sequencing was performed using the Dye Terminator Cycle Sequencing Kit (Beckman Coulter, Fullerton, CA), using half reactions; individual contigs were visualized on a Beckman Coulter CEQ 8000 genetic analysis system. With the exception of a small number of taxa in the ITS data set (where ITS1 and ITS4 primers were used; White et al. 1990), the same primer combinations used for PCR amplification were also used for cycle sequencing.

All sequences were edited using Sequencher (Gene Codes, Ann Arbor, MI). Sequences in the separate ITS and *trn*L-*trn*F data sets were easily aligned visually, using Se-Al (Rambaut 1996). All parsimony analyses were conducted using PAUP* (Swofford 1998), with all characters equally-weighted. Initial searches were conducted using 5000 replicates with RANDOM taxon addition, NNI branch swapping, and MULPARS, with five trees saved per replicate. This search strategy resulted in a pool of trees that was used as starting trees for subsequent searches using TBR branch swapping, saving a maximum of 5000 trees. Relative support was assessed using bootstrap analyses (Felsenstein 1985), with 500 replicates, TBR branch swapping, and a maximum of 500 trees saved per replicate. Separate phylogenetic analyses were conducted for both cpDNA and ITS. Upon inspection, no well-supported incongruences between the individual topologies were noted (i.e. hard incongruences; Mason-Gamer and Kellog 1996, Wendel and Doyle 1998). Therefore the data sets were combined and analyzed as described above. The phylogenetic distribution of annual/herbaceous versus perennial/woody habit was investigated. For each species, these features were determined by personal observation of the species occurring on the Canary Islands or by using recent monographs and floras (Kunkel 1977, Bramwell and Bramwell 1990) for species found elsewhere. Using MacClade (Maddison and Maddison 1992), data were traced onto the strict consensus tree resulting from parsimony analyses of a combined cpDNA/ITS data set. Biogeography was similarly investigated by plotting the insular distribution of each species onto the strict consensus tree.

Results

ITS sequence data. The aligned data matrix for ITS1-5.8S-ITS2 is 690 characters in length, of which 123 characters are variable and 64 are potentially parsimony-informative. A total of seven indels were observed in the ITS data set ranging in size from one to six base pairs. Of the seven indels inferred, three were autapomorphic. The presence/absence of these indels were not included in the analysis. Homoplasy in the data set was low as measured by CI = 0.9258 (including all characters), CI = 0.8111 (excluding uninformative characters), and RI = 0.8885. Parsimony analyses of these data recovered 126 minimum-length trees of 229 steps, all located on a single island. A strict consensus tree is provided (Fig. 2). Multiple searches using different sets of "starting trees" from our initial searches all resulted in topologies that were identical. Parsimony analyses recovered four clades receiving moderate to strong bootstrap support (Fig. 2). The first is a well-supported clade (99% bootstrap) comprising of *A. divaricatum* and *A. dumosum*, which is sister to a clade (75% bootstrap) of *A. bethencourtianum* and *A. tortuosum*. A third clade (65% bootstrap) is comprised of *A. villosum* and *A. pachycaulon* subsp.

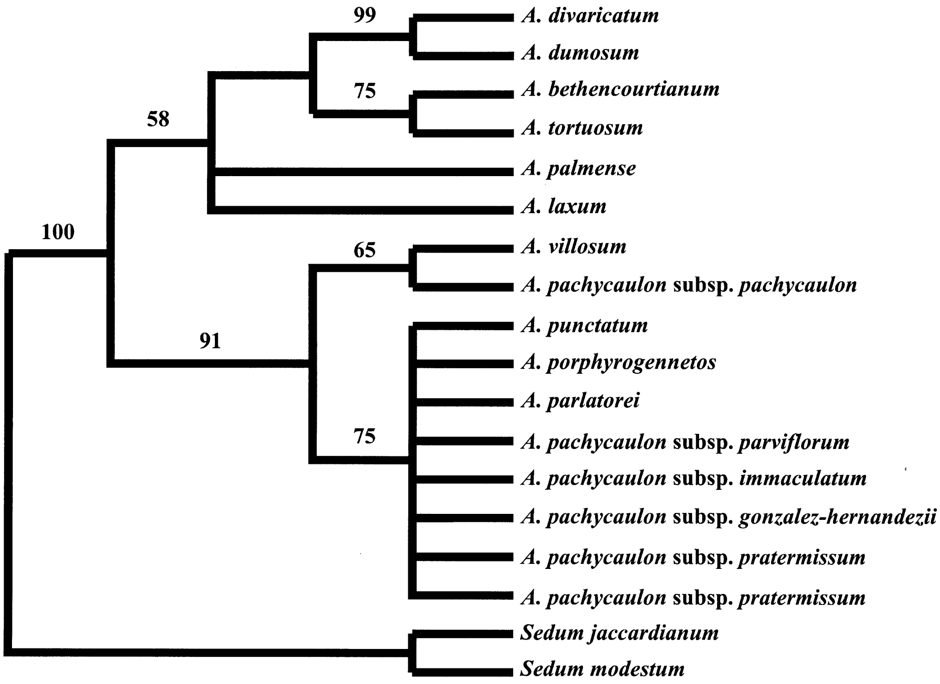

Fig. 2. Strict consensus of 5000 minimum-length trees resulting from parsimony analyses of ITS sequence variation (229 steps; C.I. = 0.9258). Relative support for the clades recovered is indicated by bootstrap values (greater than 50%) using 500 replicates of bootstrapping with TBR branch swapping

pachycaulon. Sister to this clade is a large clade (75% bootstrap) composed of *A. pachycaulon* subsp. *parviflorum*, *A. pachycaulon* subsp. *immaculatum*, two individuals of *A. pachycaulon* subsp. *praetermissum*, *A. pachycaulon* subsp. *gonzalez-hernandezii*, *A. punctatum*, *A. porphyrogennetos*, and *A. parlatorei*. Although recovered with moderate to high support, phylogenetic relationships within the clade are not resolved robustly.

***trn*L-*trn*F sequence data.** The aligned data matrix for the *trn*L-*trn*F spacers/intron is 898 characters in length, of which 48 characters are variable and 21 are potentially parsimony-informative. A total of six indels were observed in the cpDNA data set ranging in size from one to nine base pairs. Of the six indels inferred, four were autapomorphic. The presence/absence of these indels were not included in the analysis. Homoplasy in the data set is low as measured by CI = 0.9726 (including all characters), CI = 0.9200 (excluding uninformative characters), and RI = 0.9474. Parsimony analyses of these data recovered 73 minimum-length trees of 73 steps, all located on a single island. Parsimony analyses recovered four moderate to well-supported clades (Fig. 3). The first is comprised of *A. divaricatum* and *A. dumosum* (87%). Sister to this strongly supported clade is *A. villosum*. There is moderate support for the grouping of *A. pachycaulon* subsp. *pachycaulon*, *A. pachycaulon* subsp. *immaculatum*, and *A. benthencourtianum*. A third well-supported clade (88%) is comprised of *A. pachycaulon* subsp. *parviflorum*, two individuals of *A. pachycaulon* subsp. *praetermissum*, *A. porphyrogennetos*, and *A. parlatorei*. Finally, there is moderate support (79% bootstrap) for a clade is comprised of *A. pachycaulon* subsp. *gonzalez-hernandezii* and *A. punctatum*. Three species, *A. tortuosum*, *A. palmense*, and *A. laxum*, are unresolved in our cpDNA analyses.

Combined analyses. The combined ITS/cpDNA data set includes 1588 characters, of which 256 characters were variable and 85 were potentially parsimony-informative. Parsimony searches retrieved 294 minimum-length

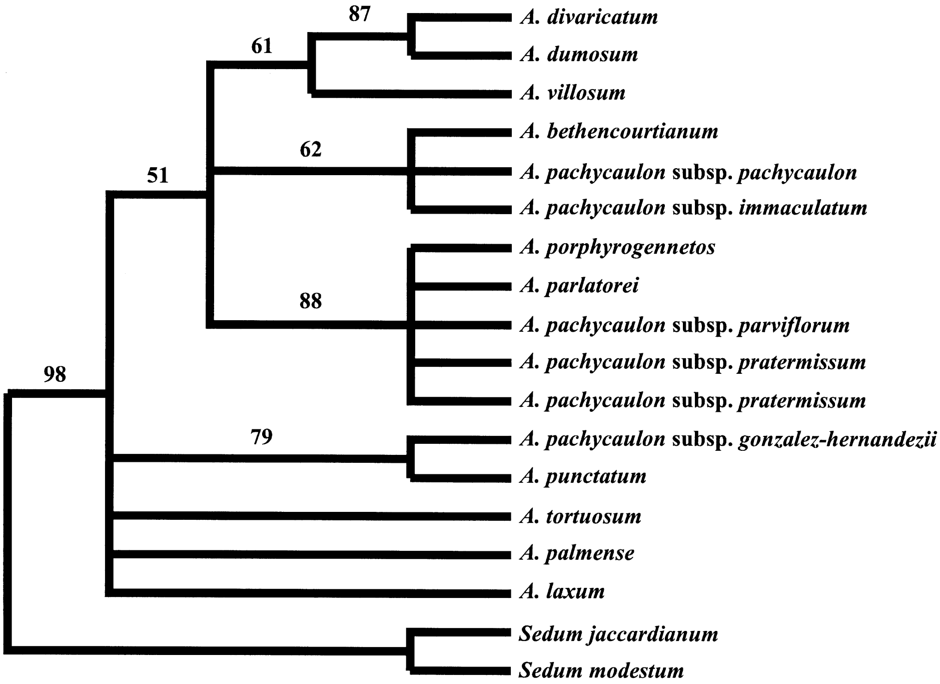

Fig. 3. Strict consensus of 5000 minimum-length trees resulting from parsimony analyses of cpDNA sequence variation (73 steps; C.I. = 0.9200). Relative support for the clades recovered is indicated by bootstrap values (greater than 50%) using 500 replicates of bootstrapping with TBR branch swapping

trees of 307 steps that were all located on a single island. Homoplasy in the combined data set appears to be low with CI = 0.9218 (including all characters), CI = 0.8000 (excluding uninformative characters), and RI = 0.7082. Analyses of the ITS/cpDNA data set yielded a topology (Fig. 4) that was similar to that obtained from the analyses of the individual ITS and cpDNA data sets. A total of six clades receiving bootstrap support of 50% or greater were recovered in the combined ITS/cpDNA analyses. Similar to the separate analyses, a clade comprised of *A. divaricatum* and *A. dumosum* is recovered with strong bootstrap support (100%). *Aichryson villosum* and *A. pachycaulon* subsp. *pachycaulon* form a weakly-supported clade (50%). A large well-supported (96%) clade comprised of *A. pachycaulon* subsp. *parviflorum*, two individuals of *A. pachycaulon* subsp. *praetermissum*, *A. porphyrogennetos*, and *A. parlatorei* is recovered, although relationships within the clade are poorly resolved. A grouping of the same five taxa is also recovered in the cpDNA analyses and these taxa are part of a larger clade in the ITS analyses. A clade receiving strong support (92%) comprised of *A. punctatum* and *A. pachycaulon* subsp. *gonzalez-hernandezii* is sister to *A. pachycaulon* subsp. *immaculatum*; this clade is also present in the cpDNA analyses. A clade of *A. laxum* and *A. palmense* is only recovered in the combined analyses and is moderately supported (71%). A clade, which is comprised of *A. bethencourtianum* and *A. tortuosum*, receives moderate support (69%). This relationship is also recovered in the analyses of the ITS data set.

Discussion

Our analyses recover six major clades within *Aichryson* (Fig. 4). The monophyly of this genus is strongly supported (100%) as has been previously indicated in other studies of the Macaronesian Clade (Ham and 't Hart 1998; Mort et al. 2001, 2002). Morphological

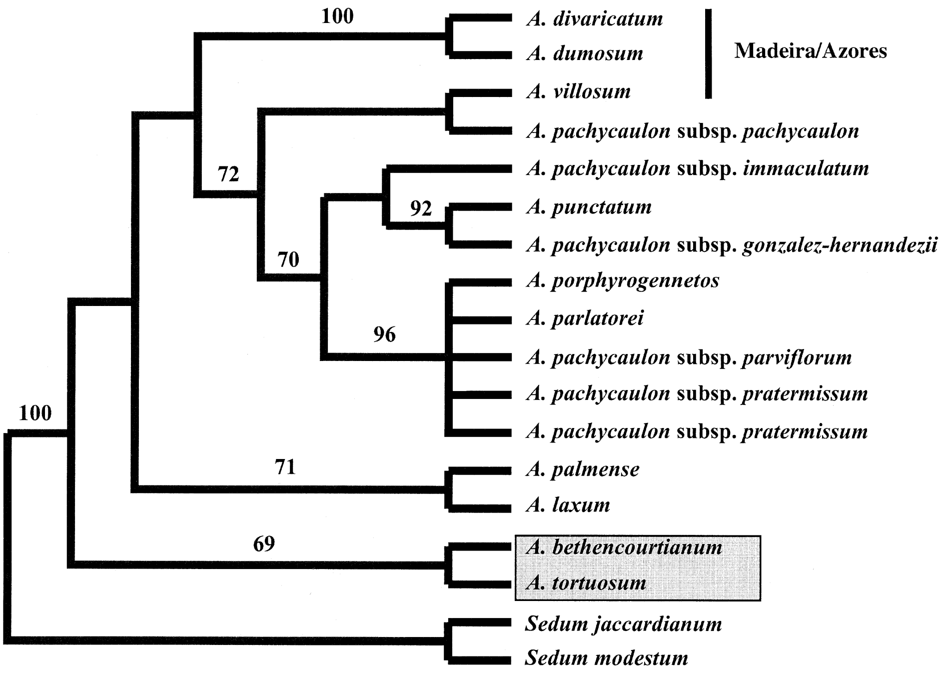

Fig. 4. Strict consensus of 5000 minimum-length trees resulting from parsimony analyses of combined DNA sequence variation (307 steps; C.I. = 0.9218). Relative support for the clades recovered is indicated by bootstrap values (greater than 50%) using 500 replicates of bootstrapping with TBR branch swapping. The shaded box highlights those species of *Aichryson* that display a woody/perennial habit

characters (i.e. large digitate nectary glands) which are not found among other genera in the Macaronesian Clade, a base chromosome number of $x = 15$ in most taxa, as well as an annual, herbaceous habitat (except for *A. bethencourtianum* and *A. tortuosum*), also are apparent synapomorphies supporting the monophyly of *Aichryson* (Mort et al. 2002).

Within *Aichryson* two lineages are recovered that correspond to habit and growth form. The first lineage comprises species that display a woody/perennial habit (*A. bethencourtianum* and *A. tortuosum*). These two species are perennial sub-shrubs and can be distinguished from one another by leaf form. *Aichryson tortuosum* has sessile leaves, whereas *A. bethencourtianum* has leaves that are petiolate (Bramwell 1977). The second lineage recovered consists of the herbaceous, annual members of the genus. Within this lineage, five clades receiving moderate to strong support are resolved. Of the three *Aichryson* species that are not endemic to the Canarian Archipelago, two of these species (*A. divaricatum* and *A. dumosum*) form a well-supported clade (100%). These two species display similar growth forms and are both glabrous. In addition to these morphological similarities, both *A. divaricatum* and *A. dumosum* are endemic to the island of Madeira. Unlike *A. divaricatum* and *A. dumosum*, *A. villosum*, the other non Canary Island endemic, occurs on all three islands comprising the Madeiran Archipelago as well as on the island of Santa Maria (Azores). *Aichryson villosum* differs from *A. divaricatum* and *A. dumosum* in leaf vestiture by being densely pubescent. *A. villosum* forms a weakly supported clade (50% bootstrap) with *A. pachycaulon* subsp. *pachycaulon*. Unlike *A. villosum*, *A. pachycaulon* subsp. *pachycaulon* is glabrous and shares no obvious morphological similarities with *A. villosum*. Chromosomal differences also occur between these two taxa. *A. villosum* has a base chromosome number of $x = 30$ (Uhl 1963), whereas *A. pachycaulon* subsp.

pachycaulon has a base chromosome number of $x = 17$ (Bramwell 1977).

Our analyses recover a large well-supported clade (96%) comprised of *A. pachycaulon* subsp. *parviflorum*, two individuals of *A. pachycaulon* subsp. *praetermissum*, *A. porphyrogennetos*, and *A. parlatorei*. Two taxa within this clade, *A. porphyrogennetos* and *A. parlatorei*, share several morphological characters and can be found on the same island (Gran Canaria). Both *A. porphyrogennetos* and *A. parlatorei* are densely pubescent and have an obovate leaf lamina (i.e. broadest at or above the middle of the blade) as opposed to the more typical ovate shaped leaf (Bramwell 1977). Another feature that has been used in distinguishing these two taxa is that *A. porphyrogennetos* has a purple hue throughout the entire plant whereas *A. parlatorei* is only purple throughout the stem; other species of *Aichryson* lack this feature completely. Sister to this large clade is a clade (92%) comprised of *A. punctatum* and *A. pachycaulon* subsp. *gonzalez-hernandezii*, with *A. pachycaulon* subsp. *immaculatum* as sister to this subclade. Several suites of morphological and chromosomal characters are shared between these two taxa. Both *A. punctatum* and *A. pachycaulon* subsp. *gonzalez-hernandezii* are glabrous and have rhomboidal to broadly ovate leaves (Bramwell 1977). *Aichryson punctatum* has a base chromosome number of $x = 17$ and has been hypothesized to be one of the parents of the putative allopolyploid *A. pachycaulon* subsp. *gonzalez-hernandezii*, which has a chromosome number of $n = 34$ (Bramwell 1977). This hypothesis is plausible and will be one of the foci of our future work within *Aichryson*.

Evolution of habit. The evolution of a woody habit within insular plant groups is a subject of considerable interest among island plant biologists. Over the years, two views have prevailed regarding the origin and evolution of insular woodiness. One view is that woody insular taxa are actually relicts of extinct continental species (Bramwell 1972, 1976), and the other hypothesis is that woody island plants are derived from herbaceous continental ancestors (i.e. insular woodiness; Carlquist 1965, 1974; Baldwin 1997; reviewed by Baldwin et al. 1998). Many molecular phylogenetic studies of insular plants provide support for Carlquist's view of insular woodiness in endemic plant lineages. For example, insular woodiness is documented in the Hawaiian silversword alliance (Baldwin et al. 1990, Baldwin 1997), the Hawaiian lobelioids (Givnish et al. 1996), and *Dendroseris* D. Don (Asteraceae) and *Robinsonia* DC. (Asteraceae) from the Juan Fernandez Islands (Crawford et al. 1993, Sang et al. 1994). Although Carlquist's view of insular woodiness appears to prevail in most island plant lineages, there are also clear examples of relictual woodiness island plant lineages (Carlquist 1990, Tucker and Douglas 1996). Recent molecular phylogenetic analyses of several nearly entire endemic Macaronesian genera (i.e. *Sonchus* L. [Asteraceae], *Echium* L. [Boraginaceae], *Argyranthemum* Webb ex Schultz–Bip [Asteraceae], and *Aeonium* [Crassulaceae]) have indicated that a woody habit has evolved from herbaceous continental ancestors (Kim et al. 1996, Böhle et al. 1996, Francisco-Ortega et al. 2002, Mort et al. 2002). Although not as common within Macaronesian genera, a woody relictual habit has been documented in Canarian *Lavatera* L. [Malvaceae] (Ray 1995).

Within *Aichryson*, the evolution of a woody/perennial habit has arisen once (*A. bethencourtianum*, *A. tortuosum*; see Fig. 4). The taxa that share this derived feature are sister to the herbaceous/annual members of the genus. This finding is congruent with previous analyses of the Macaronesian Crassulaceae (Mort et al. 2002). Previous authors, based primarily on the woody habit, have suggested placing *A. tortuosum* and *A. bethencourtianum* in a separate genus, *Macrobia* (Kunkel 1977). However, due to the morphological (i.e. presence of digitate nectary glands) and cytological ($x = 15$) similarity of these species with the herbaceous members of *Aichryson*, this treatment has not been widely adopted. Our data support Kunkel's view that *A. bethencourtianum* and *A. tortuosum* are closely related, but also strongly support the monophyly of

Aichryson as a whole (Fig. 4). Considering the similarities in morphology and cytology, it is our opinion that *A. tortuosum* and *A. bethencourtianum* should at this time be retained within *Aichryson* and not placed in the segregate genus *Macrobia*.

Classification of the *Aichryson pachycaulon* group. Recent taxonomic treatments of *Aichryson pachycaulon* (Bramwell 1977) recognize five subspecies. Each subspecies is endemic to a single island and grows in similar habitats (usually mesic) on each island. Though many of the taxa in the *pachycaulon* group share morphological similarities such as the lack of pubescence differences can be observed. For example, floral diameter ranges from <9 mm to 15 mm in this group, and in one subspecies black glands can be found on the leaf margins. Chromosome number differences also occur within this group with haploid numbers ranging from $n = 17$ to 32, and 34 (Bramwell 1977).

Our analyses place the taxa comprising the *pachycaulon* group in three separate clades (Fig. 4). The first is a weakly supported clade (50%) composed of *A. villosum* and *A. pachycaulon* subsp. *pachycaulon*. The remaining taxa comprising the *pachycaulon* group are placed in either a large well-supported clade (96%), which includes *A. pachycaulon* subsp. *parviflorum*, two individuals of *A. pachycaulon* subsp. *praetermissum*, *A. porphyrogennetos*, and *A. parlatorei*, or in a well-supported sister clade (92%) including *A. pachycaulon* subsp. *gonzalez-hernandezii* and *A. punctatum*. Sister to *A. pachycaulon* subsp. *gonzalez-hernandezii* and *A. punctatum* is *A. pachycaulon* subsp. *immaculatum*.

Bramwell (1977), based primarily upon chromosome numbers and morphology, suggested that *A. punctatum* might be one of the parentals of the putative tetraploid species *A. pachycaulon* subsp. *gonzalez-hernandezii* and the putative tetraploid species *A. pachycaulon* subsp. *praetermissum*. He also suggested that *A. punctatum* could also be the parent of *A. pachycaulon* subsp. *immaculatum* and *A. pachycaulon* subsp. *parviflorum*. *Aichryson laxum*, *A. parlatorei*, and *A. palmense* were also considered to be possible parents of *A. pachycaulon* subsp. *immaculatum* and *A. pachycaulon* subsp. *parviflorum* (Bramwell 1977). Our combined analyses recover a strongly supported clade (92%) comprising *A. punctatum* and *A. pachycaulon* subsp. *gonzalez-hernandezii* with *A. pachycaulon* subsp. *immaculatum* sister to this clade. Thus, these data suggest that *A. punctatum* is closely related to *A. pachycaulon* subsp. *gonzalez-hernandezii* and *A. pachycaulon* subsp. *immaculatum*. However, *A. punctatum* does not appear to be closely related to *A. pachycaulon* subsp. *praetermissum*, *A. pachycaulon* subsp. *parviflorum*, or *A. pachycaulon* subsp. *pachycaulon* as has been previously suggested (Bramwell 1977).

A second strongly supported clade (96%) recovered consists of *A. parlatorei*, *A. porphyrogennetos*, two individuals of *A. pachycaulon* subsp. *praetermissum*, and *A. pachycaulon* subsp. *parviflorum*. Although the relationships within this clade are unresolved, inferences can be made as to previous hypotheses regarding taxa comprising this clade. Firstly as mentioned above, *A. punctatum* is not closely related to *A. pachycaulon* subsp. *parviflorum* as has been previously suggested (Bramwell 1977). Secondly, *A. parlatorei* can be considered to be more closely related to *A. pachycaulon* subsp. *parviflorum* than to *A. pachycaulon* subsp. *immaculatum*. *Aichryson porphyrogennetos*, which has never been considered as closely related to members of the *Aichryson pachycaulon* group, appears to be a close relative to at least a portion of this group. *Aichryson palmense* and *A. laxum* form a clade (71%) that is distinct from the members of the *pachycaulon* group. Bramwell (1977) hypothesized that *A. pachycaulon* subsp. *immaculatum* and *A. pachycaulon* subsp. *parviflorum* were possible progeny of *A. palmense* or *A. laxum*, but the data presented here do not support this hypothesis. Finally, *A. pachycaulon* subsp. *pachycaulon*, which does not form a sister relationship to any other member of the *pachycaulon* group is found to be more closely related to *Aichryson villosum* with weak support (50%). This relationship is somewhat surprising, in that no obvious morphological or cytological character seem to unite this group.

Biogeography. The combined cpDNA and ITS analyses provide the first estimate of phylogeny for *Aichryson* and permit an investigation of the biogeographical patterns displayed by the extant members of *Aichryson*. Our data suggest that the islands of Fuerteventura and Lanzarote were the first islands to be colonized by *Aichryson*. This scenario would seem to be most likely due to the fact that the two closest relatives of *Aichryson*, *Sedum jaccardianum* and *Sedum modestum*, occur in Morocco, which is approximately 100 km from Fuerteventura.

Recent phylogenetic studies of other genera of the Canary Islands reveal a close biogeographic affinity between the five western-most islands of the Canary Archipelago and Madeira (e.g. Panero et al. 1999, Mort et al. 2002). The sister relationship of *A. villosum* (Madeira, Azores) and *A. pachycaulon* subsp. *pachycaulon* (Fuerteventura) suggest a biogeographic link between the eastern-most Canary Islands. In fact, our data suggest two independent dispersal events from the Canary Archipelago to the islands comprising Madeira. However, these conclusions are preliminary and additional data and increased taxon sampling is required to assess the biogeographic patterns more robustly. This pattern of biogeography seems to be relatively rare, and appears to have been reported in only one other known genus, (*Crambe*; Brassicaceae) (Francisco-Ortega et al. 2002).

Patterns of biogeography for the Canary Island endemic *Aichryson* are much more complex than that of the Madeiran taxa. As mentioned above, the two eastern-most islands were the first to be colonized by *Aichryson*, with subsequent dispersal to the five western-islands, but the dispersal patterns to these islands remains unclear. In part, the limited ability to assess biogeographic patterns among the Canary Island members of Aichryson is due to the low resolution/support for relationships among closely related species as well as limited taxonomic sampling at this time. The focus of future efforts will be to collect individuals from multiple populations that occur on separate islands. Patterns of gene flow between these populations will then be investigated using hyper-variable DNA markers (i.e. ISSR's, and AFLP's) to ascertain the patterns of biogeography exhibited by *Aichryson*. In any case, what can be established is that the island of La Palma is the center of diversity for *Aichryson*. This pattern differs from other genera comprising the Macaronesian clade of Crassulaceae in which the larger, more centrally located island of Tenerife is the center of diversity.

The authors would like to thank Susana Fontinha of the Madeiran Botanical Garden for plant material of Madeiran *Aichryson*; Drs. D. Crawford and J. Archibald, as well as two anonymous reviewers for helpful comments; Eastern Illinois Undergraduate Honors Research Fund, and University of Kansas Plant Biology Research Award. Additional financial support for this work was provided by the Department of Ecology and Evolutionary Biology and the Museum of Natural History and Biodiversity Research Center, University of Kansas and a Kansas NSF EPSCoR First Award (NSF 32171) to MEM.

References

Baldwin B. G., Kyhos D. W., Dvorak J. (1990) Chloroplast DNA evolution and adaptive radiation in the Hawaiian silversword alliance (Compositae-Madiinae). Ann. Missouri Bot. Gard. 77: 96–109.

Baldwin B. G. (1997) Adaptive radiation of the Hawaiian silver-sword alliance; congruence and conflict of phylogenetic evidence from molecular and non-molecular investigations. In: Givnish T. J., Systma K. J. (eds.) Molecular evolution and adaptive radiation. Cambridge University Press, New York, pp. 103–128.

Baldwin B. G., Crawford D. J., Francisco-Ortega J., Kim S., Sang T., Stuessy T. (1998) Molecular phylogenetic insights into the origin and evolution of island plants. In: Soltis D. E., Soltis P. S., Doyle J. J. (eds.) Molecular systematics of plants

II. Kluwer Academic Publishers, Boston, pp. 410–441.

Berger A. (1930) Crassulaceae. In: Engler A., Prantl K. (eds.) Die Natürlichen Pflanzenfamilien. Duncker and Humblot, Berlin, pp. 352–483.

Böhle U.-R., Hilger H. H., Martin W. F. (1996) Island colonization and evolution of the insular woody habit in *Echium* L. (Boraginaceae). Proc. Natl. Acad. Sci. USA 93: 11740–11745.

Bramwell D. (1972) Endemism in the flora of the Canary Islands. In: Valentine D. H. (ed.) Taxonomy, phytogeography, and evolution. Academic Press, London, pp. 141–159.

Bramwell D. (1977) The subspecies of *Aichryson pachycaulon* Bolle (Crassulaceae) and their probable origin. Bot. Macar. 4: 105–111

Bramwell D., Bramwell Z. I. (1990) In: Bramwell D., Bramwell Z. I. (eds.) Flores silvestres de las Islas Canarias. Editorial Rueda, Madrid.

Carlquist S. (1965) In: Carlquist S. (ed.) Island life: a natural history of the islands of the world. Natural History Press, New York.

Carlquist S. (1974) In: Carlquist S. (ed.) Island biology. Columbia University Press, New York.

Carlquist S. (1990) Wood anatomy and relationships of Lactoridaceae. Amer. J. Bot. 77: 1498–1505.

Crawford D. J., Stuessy T. F., Cosner M. B., Haines D. W., Silva M. (1993) Ribosomal and chloroplast DNA restriction site mutation and the radiation of *Robinsonia* (Asteraceae: Senecioneae) on the Juan Fernandez Islands. Plant Syst. Evol. 184: 233–239.

Felsenstein J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Francisco-Ortega J., Fuertes-Aguilar J., Kim S., Santos-Guerra A., Crawford D. J., Jansen R. K. (2002) Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on the internal transcribed spacer sequences of nuclear ribosomal DNA. Amer. J. Bot. 89: 1984–1990.

Givnish T. J., Knox E., Patterson T. B., Hapeman J. R., Palmer J. D., Systma K. J. (1996) The Hawaiian lobelioids are monophyletic and underwent a rapid initial radiation roughly 15 million years ago. Amer. J. Bot. (Suppl. 6) 83: 159.

Ham R. C. H. J. Van., 't Hart H. (1998) Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA restriction-site variation. Amer. J. Bot. 85: 123–134.

Hansen A., Sundig P. (1993) Flora of Macaronesia. Checklist of vascular plants. Sommerfeltia 17: 92–105.

Kim S., Crawford D. J., Jansen R. K. (1996) Phylogenetic relationships among genera of the subtribe Sonchinae (Asteraceae): evidence from ITS sequences. Syst. Biol. 21: 421–432.

Kunkel G. (1977) *Macrobia*, nuevo nombre genérico en Crasuláceas Canarias. Cuad. Bot. Canar. 28: 35–37.

Liu H. Y. (1989) Systematics of *Aeonium* (Crassulaceae). Special Publication No. 3, Nat. Mus. of Nat. Sci. Taichung, Taiwan.

Maddison W. P., Maddison D. R. (1992) MacClade: analysis of phylogeny and character evolution. Sinauer, Sunderland, MA.

Mason-Gamer R. J., Kellogg E. A. (1996) Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae. Syst. Biol. 45: 524–545.

Mes T. H. M., 't Hart H. (1994) *Sedum surculosum* and *Sedum jaccardianum* (Crassulaceae) share a unique 70 bp deletion in the chloroplast DNA *trn*L (UAA) -*trnF* (GAA) intergenic spacer. Plant Syst. Evol. 193: 213–221.

Mes T. H. M., 't Hart H. (1996) The evolution of growth-forms in the Macaronesian genus *Aeonium* (Crassulaceae) inferred from chloroplast DNA RFLPs and morphology. Molec. Ecol. 5: 351–363.

Mes T. H. M., Wijers G. J., t'Hart H. (1997) Phylogenetic relationships in *Monanthes* (Crassulaceae) based on morphological, chloroplast, and nuclear DNA variation. J. Evol. Biol. 10: 193–216.

Mort M. E., Soltis D. E., Soltis P. S., Francisco-Ortega J., Santos-Guerra A. (2001) Phylogenetic relationships and evolution of Crassulaceae inferred from *mat*K sequence data. Amer. J. Bot. 88: 76–91.

Mort M. E., Soltis D. E., Soltis P. S., Francisco-Ortega J., Santos-Guerra A. (2002) Phylogenetics and evolution of the Macaronesian Clade (Crassulaceae) inferred from nuclear and chloroplast sequence data. Syst. Bot. 27: 271–288.

Panero J. L., Francisco-Ortega J., Jansen R. K., Santos-Guerra A. (1999) Molecular evidence for multiple origins of woodiness and a new world biogeographic connection of the Macaronesian island endemic *Pericallis* (Asteraceae: Senecioneae). Proc. Natl. Acad. Sci. USA 96: 13886–13891.

Praeger R. L. (1932) An account of the *Sempervivum* group. Roy. Hort. Soc. London

Rambaut A. (1996) Se-Al, sequence alignment program v1. d1. University of Oxford.

Ray M. F. (1995) Systematics of *Lavatera* and *Malva* (Malvaceae, Malveae): a new perspective. Plant Syst. Evol. 198: 29–53.

Sang T., Crawford D. J., Kim S.-C., Stuessy T. F. (1994) Radiation of the endemic genus *Dendroseris* (Asteraceae) on the Juan Fernandez Islands: evidence from sequences of the ITS regions of the nuclear ribosomal DNA. Amer. J. Bot. 81: 1494–1501.

Soltis P. S., Soltis D. E., Weller S. G., Sakai A. K., Wagner W. L. (1996) Molecular phylogenetic analysis of the Hawaiian endemic *Schidea* and *Alsinodendron* (Caryophyllaceae). Syst. Bot. 21: 365–380.

Swofford D. L. (1998) PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4.0. Sinauer, Sunderland, MA.

Taberlet P., Gielly L., Pautou G., Bouvet J. (1991) Universal primers for amplification of three noncoding regions of chloroplast DNA. Pl. Molec. Bio. 17: 1105–1109.

Tucker S. C., Douglas A. W. (1996) Floral structure, development, and relationships of paleoherbs: *Saruma*, *Cabomba*, *Lactoris*, and selected Piperales. In: Taylor D. W., Hickey L. J. (eds.) Flowering plant origin, evolution, and phylogeny. Chapman Hall, New York, pp. 141–175.

Uhl C. H. (1963) Chromosomes and phylogeny of the Crassulaceae. Cactus and Succulent Jour. of Amer. 35: 80–84.

Wen J., Zimmer E. A. (1996) Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of the nuclear ribosomal DNA. Molec. Phylogenet. Evol. 6: 167–177.

Wendel J. F., Doyle J. J. (1998) Phylogenetic incongruences: window into genome history and molecular evolution. In: Soltis D. E., Soltis P. S., Doyle J. J. (eds.) Molecular systematics of plants II. Kluwer Academic Publishers, Boston, pp. 265–296.

White T. J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Gelfand D., Sninsky J., White T. (eds.) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp. 315–322.

Addresses of the authors: Kristopher N. Fairfield, Mark E. Mork (e-mail: memort@ku.edu), Department of Ecology and Evolutionary Biology and the Museum of Natural History and Biodiversity Research Center, University of Kansas, Lawrence, Kansas 66045, USA. Arnoldo Santos-Guerra, Jardin de Aclimatacion de la Orotava, Calle Retama Número 2, Puerto de la Cruz, Tenerife, Canary Islands, E-38040, Spain.