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RELATIONSHIPS OF THE MACARONESIAN AND MEDITERRANEAN FLORAS: MOLECULAR EVIDENCE FOR MULTIPLE COLONIZATIONS INTO MACARONESIA AND BACK-COLONIZATION OF THE CONTINENT IN *CONVOLVULUS* (CONVOLVULACEAE)[1]

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A molecular phylogenetic analysis of the Macaronesian endemic species of *Convolvulus* was undertaken using data from the nuclear ribosomal internal transcribed spacer (ITS) regions. The results of the analysis support two introductions into Macaronesia from distantly related clades within *Convolvulus* and a subsequent back-colonization to the continent from within one of the clades. Hypothesized relationships between Macaronesian species and New World taxa and between the Canarian endemic *C. caput-medusae* and the Moroccan *C. trabutianus* are refuted. Both Macaronesian clades are shown to have Mediterranean sister groups although one is predominantly western Mediterranean and the other predominantly eastern Mediterranean in distribution. The patterns of colonization into Macaronesia demonstrated by *Convolvulus* and also by other multiple colonizing genera conform to either a pattern of phylogenetic distinctiveness or a checkerboard distribution of island lineages. Both are consistent with the hypothesis that niche preemption is responsible for the limited number of colonizations into the region. A review of sister group relationships demonstrates that, in common with *Convolvulus*, most Macaronesian groups have sister groups distributed in the near-continent (i.e., western Mediterranean). Disjunct sister group relationships (including Eastern Mediterranean disjunctions) occur in only 18% of groups.

Key words: biogeography; Cabo Espichel; Canary Islands; Convolvulaceae; *Convolvulus*; *Jacquemontia*; Madeira; Oceanic Islands.

The flora of the Macaronesian region (Fig. 1) demonstrates a number of typical oceanic island characteristics including a high degree of endemism (20% overall, [Humphries, 1979]; 40% in the Canary Islands [Santos Guerra, 1999]) and a predominant woody habit among endemics (ca. 70% of Canary Island endemics are woody; Aldridge, 1979). However, whilst the Macaronesian region is typical of oceanic island systems in several respects, the region in general, and the Canary Islands in particular, differ markedly from more isolated Pacific island groups such as Hawaii, in that they are close to potential continental source areas. The easternmost Canary Islands are presently 95 km from the coast of North Africa and at periods during the last 20 million years (my) they have probably been as close as 65 km (García-Talavera, 1997, 1999). There are also more than 20 large volcanic sea mounts located between the Canary Islands, Madeira, Selvages, and the continent, several of which are presently less than 100 m below sea level. Recent dating studies have shown that some of the sea mounts in this area are at least 68 my old (Geldmacher et al., 2001),

and it is conceivable that they may have facilitated dispersal between the continent and islands by serving as "stepping stones" during glacial periods when sea levels were lower (García-Talavera, 1997).

The close proximity of the Macaronesian islands to the continent, the existence of potential stepping stones, and the age of the islands (ranging from 0.8 to 21 my old; Carracedo, 1994) raises the possibility that genera could have undergone multiple independent colonizations into the region. Back-colonization to the continent from Macaronesia could also have occurred, with the islands serving as a source area for continental endemics (Santos-Guerra, 1999; Park et al., 2001; Fuertes-Aguilar et al., 2002; Goertzen et al., 2002). Such relationships between island and continental floras are more complex than those generally hypothesized for other island oceanic island systems such as the Hawaiian and Galapagos archipelagos.

Recent molecular phylogenetic analyses of Macaronesian plant groups have provided valuable insights into the relationships of the region's endemics. Whilst each group seems to have a unique pattern of diversification in the region (Francisco-Ortega et al., 2001b), a number of more general patterns are starting to emerge. Multiple independent colonizations into the region may seem plausible but in the majority of genera so far investigated, the Macaronesian endemic taxa form a single monophyletic group. In most cases, the sister group of the Macaronesian clade is North African or western European and typically Mediterranean (e.g., *Argyranthemum* Webb ex Sch.Bip. (Asteraceae), Francisco-Ortega et al., 1997; *Isoplexis*
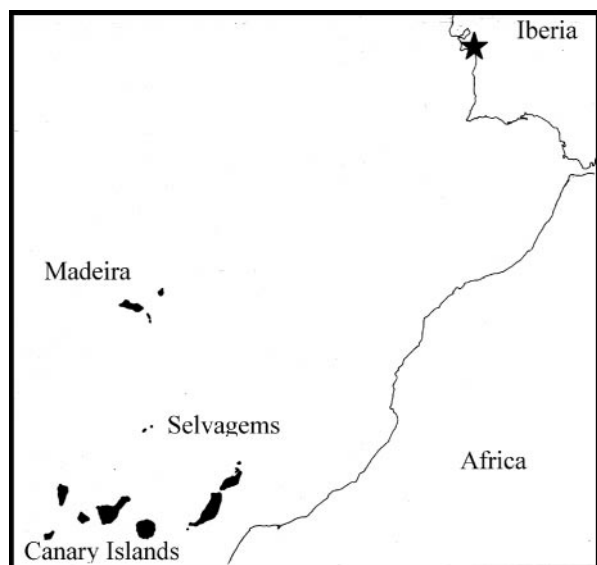
Fig. 1. The central Macaronesian region and the near-continent. The star indicates the position of Cabo Espichel in Portugal where *Convolvulus fernandesii* occurs. The Cape Verde Islands (the Macaronesian archipelago not shown in this figure) are situated off the coast of equatorial Africa, approximately 1200 km south from the Canary Islands.

(Lindl.) Loud. (Scrophulariaceae), Carvalho and Culham, 1998; *Bencomia* Webb & Berth. alliance (Rosaceae), Helfgott et al., 2000; *Sideritis* Tourn.ex Linn. (Lamiaceae), Barber et al., 2002; see also Santos-Guerra, 1999), although sister group relationships with taxa distributed elsewhere, notably the New World (e.g., Madeiran species of *Sedum* L. (Crassulaceae), van Ham and t'Hart, 1998; *Pericallis* D.Don (Asteraceae), Panero et al., 1999; *Bystropogon* L'Hérit. (Lamiaceae), Trusty et al., in press), East Africa (e.g., *Solanum* L. (Solanaceae), Olmstead and Palmer, 1997; *Camptoloma* Benth. (Scrophulariaceae), Kornhall et al., 2001) and southern Africa (e.g., *Phyllis* L. (Rubiaceae), Andersson and Rova, 1999) have also been revealed (Andrus et al., 2004).

Multiple congeneric colonizations into the region have been demonstrated for a number of genera investigated so far (Table 1) but documented examples of back-colonization of the continent from the Macaronesian region remain limited. Molecular phylogenetic analyses of *Euphorbia* L. subsect. *Pachyclade* (Boiss.) A.DC. (Euphorbiaceae; Molero et al., 2002) and *Teline* Medikus (Fabaceae; Percy and Cronk, 2002) are consistent with back-colonization of the continent but are equivocal. Back-colonization of the continent from Macaronesia is the most plausible explanation for the pattern of relationships of continental and island taxa in *Tolpis* Adans. (Asteraceae, Park et al., 2001; Moore et al., 2002). In *Aeonium* Webb & Berth. (Crassulaceae), the African representatives of the genus are nested within an otherwise exclusively Macaronesian clade, a pattern consistent with dispersal from Macaronesia to Africa (Mort et al., 2002).

In addition to those already investigated, multiple colonizations into Macaronesia have been hypothesized for a number of genera represented in the region by endemic taxa. These include *Viola* L. (Violaceae), *Limonium* Miller (Plumbaginaceae), *Salvia* L. (Lamiaceae), and *Senecio* L. (Asteraceae) (Fuertes-Aguilar et al., 2002). One genus for which both multiple colonizations into Macaronesia and back-colonization of

the continent have been hypothesized is *Convolvulus* L. (Convolvulaceae). Ten species of *Convolvulus* are endemic to the Canary Islands and one is endemic to Madeira and Desertas (Table 2). Of the remaining species found in Macaronesia, *C. arvensis* L., *C. althaeoides* L., and *C. farinosus* L. are considered introduced (Kunkel, 1991). The status of the weedy annual species *C. siculus* L., which is also widespread around the Mediterranean basin, is uncertain (Kunkel, 1991).

Whilst species with both herbaceous and woody habits occur in *Convolvulus*, all of the Macaronesian endemics are woody, thus demonstrating the typical insular woodiness associated with island endemism (e.g., Carlquist, 1974). Among the island endemic taxa, however, there is considerable morphological variation and taxonomic treatments of *Convolvulus* have placed the Macaronesian endemic species in two (Webb and Berthelot, 1840), three (Peter, 1891), or four (Sa'ad, 1967) different infrageneric groupings. Most recently, Mendoza-Heuer (1971, 1983) recognized two distinct groups of Macaronesian endemic *Convolvulus*, one comprising the endemic climbers and the other comprising the endemic non-climbers (Table 2). Furthermore, Mendoza-Heuer (1971, 1983) proposed that these two groups had resulted from two independent colonizations of the region.

Mendoza-Heuer (1971, 1983) was largely silent on the relationships of the endemic species of *Convolvulus* to other members of the genus but previous classifications have suggested a number of relationships that remain to be tested. These include a possible New World link for the Macaronesian endemic climbers (Choisy, 1845; Peter, 1891) and an eastern Mediterranean/Middle Eastern link for both *C. scoparius* L. (Sa'ad, 1967) and *C. floridus* L. (Peter, 1891). Additionally, Sa'ad (1967) proposed a North African link between the spiny endemic species *C. caput-medusae* Lowe and the North African spiny subshrub *C. trabutianus* Schweinf. & Muschler. This proposal was, however, vigorously challenged by Mendoza-Heuer (1971, 1983) who maintained, on the basis of floral morphology, that all three non-climbing Macaronesian endemic species formed a single group (Table 2, Group I).

A close relationship has also been proposed between the *C. canariensis* L. group of endemics (Table 2, Group IIa) and the Portuguese endemic *C. fernandesii* Pinto da Silva and Teles, which is confined to coastal cliffs at Cabo Espichel, in Portugal (Fig. 1). The scrambling woody habit of *C. fernandesii* is unlike any other species of *Convolvulus* distributed in the western Mediterranean, and Pinto da Silva and Teles (1981) hypothesized that *C. fernandesii* is a continental neo-endemic resulting from recent back-colonization of Cabo Espichel from the Macaronesian region. An alternative explanation, however, was provided by Bramwell and Bramwell (2001), who considered *C. fernandesii* and its putative relatives in the Macaronesian region to be relicts of a formerly widespread woody flora that covered the Mediterranean region during the Tertiary. As extinction occurred elsewhere in the Mediterranean following climate change in the Pleistocene, these woody taxa were able to survive in a few suitable refugial areas including Macaronesia and Cabo Espichel (e.g., Bramwell, 1972; Bramwell and Richardson, 1973; Aldridge, 1979; Sunding, 1979; Médail and Quezel, 1999; Bramwell and Bramwell, 2001).

There are therefore a number of competing hypotheses to explain the diversity and evolutionary history of *Convolvulus* in Macaronesia that remain to be tested. Potentially, *Convolvulus* demonstrates one of the most complex patterns of relationship in the region. There are very few genera for which

TABLE 1. Characteristics of genera for which multiple introductions of the Macaronesian endemic taxa is suggested by molecular data.

Genus and number of Macaronesian endemic species	No. of endemic Macaronesian colonization groups	Number of species per Macaronesian colonization group in the analysis	Relationships of Macaronesian colonization groups	Data source and analysis type	Reference
The *Asteriscus* Miller alliance (Asteraceae)[a,b]; 4 spp. + 1 subsp.	2	4[c] + 1 subsp.[c]	The four endemic species form a monophyletic group that is sister to North Africa taxon. The Canarian endemic *A. graveolens* subsp. *stenophyllus* is sister to the Moroccan *A. graveolens* subsp. *odorus*.	ITS, ETS, and *ndhF* combined; parsimony and ML	Goertzen et al., 2002
Euphorbia subsect. Pachyclade (Boiss.) A.DC. (Euphorbiaceae); 11 spp. + 1 subsp.	3	6 + 2 + 1 subsp. (not endemic)[d]	One Macaronesian group is sister to a Mediterranean clade. One unresolved clade has one Mediterranean species and six Macaronesian endemics. Another Macaronesian taxon (*E. balsamifera* subsp. *balsamifera*) is unresolved in a basal polytomy. Sampling was limited, however, and included only four continental taxa.	ITS; parsimony	Molero et al., 2002
Hedera L. (Araliaceae); 2 spp. + 1 subsp.	3	1 + 1 + 1 subsp.	ITS data support two independent colonizations from within the diploid clade, but resolution is inadequate to determine sister group relationships. A further colonization from the polyploid clade is supported. Chloroplast data are also consistent with three independent colonizations.	ITS; parsimony	Vargas et al., 1999a; Valcárel et al., 2003 (ITS); Ackerfield and Wen, 2003 (cpDNA)
Ilex L. (Aquifoliaceae)[b]; 2 spp.	2	1 + 1	*Ilex canariensis* is sister to the rest of the genus; *I. perado* nested within an East Asian clade.	*atpB-rbcL* spacer and *rbcL*; ITS and 5S spacer; parsimony	Cuénoud et al., 2000; Manen et al., 2002
Lavatera L. (Malvaceae); 2 spp.	2	1 + 1	*Lavatera phoenica* is sister to the *Lavatera* L.–*Malva* L. complex whilst *I. acerifolia* is sister to a Mediterranean species (ITS; 77% bootstrap) or a New World species (cpDNA data; 44% bootstrap).	ITS and RFLP; parsimony	Fuertes-Aguilar et al., 2002
Olea L. (Oleaceae); 1 nonmonophyletic subsp.[b,f]	2	1 subsp. + 1 subsp.[e]	*O. europaea* subsp. *cerasiformis* (Madeira) is sister to a clade comprising subsp. *europaea* (Med.) and subsp. *guanchica* (Canary Islands). Subsp. *guanchica* is resolved within subsp. *europaea*.	RAPD and ISSR; neighbor joining	Hess et al., 2000

TABLE 1. Continued.

Genus and number of Macaronesian endemic species	No. of endemic Macaronesian colonization groups	Number of species per Macaronesian colonization group in the analysis	Relationships of Macaronesian colonization groups	Data source and analysis type	Reference
Plantago L. (Plantaginaceae); 7 spp.	3	1 + 1 + 3	*Plantago subspathulata* is sister to a Mediterranean/European clade. *Plantago letopetala* is sister species to the cosmopolitan *P. lanceolata*. The remaining Macaronesian species form a paraphyletic group with two Mediterranean species although this relationship has low boostrap support (<50%).	ITS and *trnL-F*; parsimony	Rønsted et al., 2002
Pulicaria Gaertner (Asteraceae)[a,b], 2 spp.	2	1[c] + 1	*Pulicaria canariensis* is sister to a Moroccan species. The Cape Verdean *P. diffusa* is sister to a predominantly Mediterranean species.	ITS, parsimony	Francisco-Ortega et al., 2001b
Solanum L. (including the endemic genus *Normania* Lowe) (Solanaceae)[b]; 4 spp.	2	1[c] + 1	*Solanum vespertillo* is placed in a clade with East African species whilst *S. nava* (= *Normania*) is sister to the Mediterranean *S. herculeum*.	*ndhF* and ITS; parsimony	Bohs and Olmstead, 2001
Teline Medikus (Fabaceae)[a,b]; 12 spp.	2	5[c] + 7	Both the Macaronesian *T. linifolia* and *T. monspessulana* groups have western Mediterranean sister groups.	ITS; parsimony	Percy and Cronk, 2002

[a] Genus/generic alliance is not monophyletic.
[b] Nodes that support multiple introductions into Macaronesia are weakly supported (bootstrap value <75%).
[c] Sister taxon/clade have a predominantly or exclusively western Mediterranean distribution.
[d] *Euphorbia balsamifera* ssp. *balsamifera* is not endemic to the Canary Islands as it also occurs in Morocco and Mauritania.
[e] The two colonizations were to Madeira and the Canary Islands independently. Previously, the plants in these two areas had been treated as the same subspecies i.e., *O. europaea* ssp. *cerasiformis*.
[f] No indication of support for individual nodes is provided.

TABLE 2. Macaronesian endemic species of *Convolvulus* arranged according to the classification of Mendoza-Heuer (1971). *Convolvulus fernandesii*, which is endemic to Cabo Espichel, Portugal, was considered part of the *C. canariensis* group by Pinto da Silva and Teles (1982) and is therefore included in that group. Distribution data from Bramwell and Bramwell (2001) and Press and Short (1994) is also included.

Group	Species	Distribution									
		L	F	C	T	G	H	P	M	D	CE
I	*C. floridus* L.fil.	L	F	C	T	G	H	P			
I	*C. scoparius* L.fil.			C	T	G*					
I	*C. caput-medusae* Lowe		F	C							
IIa	*C. canariensis* L.			C	T	G	H	P			
IIa	*C. massonii* Dietr.								M	D	
IIa	*C. lopezsocasi* Svent.	L									
IIa	*C. volubilis* Link in Buch (= *C. diversifolius* Mendoza-Heuer)				T	G					
IIa	*C. fernandesii* Pinto da Silva and Teles										CE
IIb	*C. perraudierii* Coss.			C	T						
IIb	*C. subauriculatus* (Burchd.) Lindinger					G					
IIb	*C. fruticulosus* Desr.				T		H	P			
IIb	*C. glandulosus* (Webb) Hallier			C							

Notes: Group I: Plants woody, not scrambling, sepal apices from 3–5 mm long. Group II: Plants woody, more or less scrambling, sepals 5–12 mm long. Subgroup IIa (*Convolvulus canariensis* group): leaves large, generally more than 5 cm long; petiole distinct; sepals sometimes widening in their distal part. Subgroup IIb (*Convolvulus fruticulosus* group): leaves small, generally less than 5 cm long; petiole small and indistinct; sepals not widening in their distal part. Key to distributions: L, Lanzarote; F, Fuerteventura; C, Gran Canaria; T, Tenerife; G, La Gomera; H, El Hierro; P, La Palma; M, Madeira; D, Dererta Grande; CE, Cabo Espichel, Portugal. An asterisk indicates that an island record does not appear in Bramwell and Bramwell (2001) but plants of this species have been collected from the island by one of the authors (A. Santos-Guerra).

multiple introductions into Macaronesia and back-colonization of the continent have been hypothesized, and *Convolvulus* therefore presents a rare example with which to explore these phenomena and gain new insights into the evolution and relationships of the Macaronesian flora. To date, hypotheses concerning the relationships and evolution of Macaronesian *Convolvulus* have been based exclusively on intuitive assessments of morphology. In many groups, similar hypotheses have been refuted by the application of more rigorous analytical techniques (e.g., Bremer and Humphries, 1993) or new sources of data, notably DNA sequence data (e.g., Böhle et al., 1996; Kim et al., 1996; Francisco-Ortega et al., 1997; Helfgott et al., 2000; Mort et al., 2002). In this paper, therefore, a molecular phylogenetic analysis of relationships of the Macaronesian endemic *Convolvulus* is presented using data from the internal transcribed spacer regions (ITS) of nrDNA. Three specific questions are addressed: (1) How many endemic groups of *Convolvulus* occur in the Macaronesian region and how many colonizations into the region are necessary to explain the diversity among the endemic species? Is there any support for the Mendoza-Heuer (1983) hypothesis of two independent colonization events? (2) What are the closest continental relatives of the endemic species? We aim to test (a) the putative New World link between the island climbers (Table 2, Group II) and *C. nodiflorus* Desr. (Choisy, 1845; Peter, 1891); (b) the possible close relationship between the endemic spiny subshrub *C. caput-medusae*, *C. trabutianus* from North Africa and other spiny subshrubs (Sa'ad, 1967; cf. Mendoza-Heuer, 1971, 1983); and (c) the hypothesized relationships between *C. scoparius*, *C. floridus*, and Eastern Mediterranean/Arabian species (Choisy, 1845; Peter, 1891; Sa'ad, 1967) (3) What (and where) are the closest relatives of *C. fernandesii*? Is this species a neo-endemic and the result of speciation following back-colonization of Cabo Espichel from the Macaronesian region (Pinto da Silva and Teles, 1981) or is it a Tertiary relict (Bramwell and Bramwell, 2001)?

The results of the *Convolvulus* study are discussed in relation to other groups that demonstrate a complex colonization history in the region, to gain new insights into the processes underpinning the evolution of the Macaronesian endemic flora. The patterns of relationships of the Macaronesian and Mediterranean floras revealed by molecular data are also reviewed, and the implications of these data for our understanding of the evolution of the flora of these two regions are discussed.

MATERIALS AND METHODS

Taxon sampling—Fifty-nine accessions were included in the analysis (see Supplementary Data accompanying the online version of this article). Forty-three species of *Convolvulus* were sampled, with multiple accessions included for nine species. Nine of the 11 Macaronesian endemic species of *Convolvulus* were represented in the analysis together with *C. fernandesii* from Cabo Espichel in Portugal. *Convolvulus nodiflorus*, *C. trabutianus*, *C. cneorum* L., and *C. dorycnium* L. were included because a close relationship between these taxa and various Macaronesian species has been implied in previous classifications of *Convolvulus* (Choisy, 1845; Peter, 1891; Sa'ad, 1967). The remaining *Convolvulus* species included in the analysis represent a morphologically and geographically diverse sample of the genus from the Mediterranean and Western Asia.

Three species of *Calystegia* R.Br. were included in the study as this genus has been shown to be nested within a paraphyletic *Convolvulus* (Stefanović et al., 2002). Seven other species, representing related genera of Convolvulaceae, were included as outgroups on the basis of higher-level analyses (Manos et al., 2001; Stefanović et al., 2002).

Molecular methods—Total genomic DNA was extracted from ~0.1 g of dried leaf material (silica gel-dried or herbarium specimens) using a modified hexacyltrimethylammonium bromide (CTAB) micro-extraction protocol (Doyle and Doyle, 1987). Total extracted DNA was further purified, without precipitation, using GFX purification kits (Amersham Biosciences, Little Chalfont, UK).

Standard polymerase chain reaction (PCR) procedures were applied to amplify the ITS region together with part of the 17S and 26S gene region. For most taxa, the region was amplified as a single fragment using primers 17SE and 26SE (Sun et al., 1994). However, for problematic accessions, the region was amplified in two fragments using primers 17SE and ITS2 (White et al., 1990) to amplify the ITS 1 region and ITS3 (White et al., 1990) and 26SE to amplify the ITS 2 region. Betaine (1.2 mol/L) was added to prevent the formation of secondary structures in difficult templates and PCR reactions were carried out using a Techgene Thermal Cycler (Techne, Cambridge, UK)

using 30 cycles of: 1 min of denaturation at 94°C, 3 min of annealing at 49°C, 1 min of extension at 72°C, and 8 min final extension at 72°C. Amplification products were purified using GFX purification kits following the manufacturer's protocols.

Dideoxy cycle sequencing (28 cycles: 30 of denaturation at 95°C, 15 of annealing at 50°C, 4 min of extension at 60°C) with big dye terminators was performed in 10 μL volumes using a Hybaid Omnigene Thermal Cycler. Excess dye-labelled nucleotides from the sequence reactions were removed by standard ethanol/sodium acetate precipitation. Sequence products were subsequently resuspended and run on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, California, USA).

Sequence alignment and phylogenetic analyses—Sequence data were edited and assembled using Lasergene Navigator (DNAStar, Madison, Wisconsin, USA). Verified sequences were then aligned by eye in Se-Al (version 1.0al; Rambaut, 1996) prior to phylogenetic analysis. Gaps were not coded as informative characters for analysis because many were overlapping and consequently problematic for coding.

Phylogenetic analyses of the data were performed using PAUP* Beta version 4.0b5 (Swofford, 1998). To determine the effect of outgroup alignment on the ingroup topology two analyses were performed: the first included all accessions whereas the second included only the ingroup taxa (*Convolvulus* + *Calystegia*). All parsimony analyses were simultaneous and unconstrained with character state changes unordered and weighted equally (Nixon and Carpenter, 1993). An heuristic search comprising 1000 random replicates was performed with accelerated transformation (ACCTRAN), saving all minimal trees (MULPARS), and tree bissection and reconnection (TBR) options. Trees were rooted on the most distal outgroup (*Jacquemontia mexicana*) based on knowledge from previous phylogenetic analyses (Stefanović et al., 2002).

The ensemble consistency index (CI; Kluge and Farris, 1969) and retention index (RI; Farris, 1989) were calculated in PAUP*. The robustness of clades in the strict consensus tree was evaluated by nonparametric bootstrap analysis (Felsenstein, 1985) and by computing decay values (Bremer, 1988). Bootstrap values were determined from 100 bootstrap replicates each comprising 100 random stepwise addition heuristic searches with TBR branch swapping, saving two trees per replicate. Decay analysis was conducted using AutoDecay (Eriksson, 1998) in conjunction with PAUP*. For each of the decay constraint trees, a heuristic analysis with 10 replicates and TBR swapping was performed.

RESULTS

Sequence alignments—The aligned data matrix was 781 base pairs (bp) in length. Alignment of the ingroup taxa required 15 gaps between 1 and 3 bp long. An additional 21 gaps of between one and 20 bp were necessary to align the outgroup taxa. Of the 781 characters in the final matrix, 373 were variable and 289 potentially informative for parsimony analysis. Removing outgroup taxa from the matrix resulted in 300 variable characters and 227 parsimony-informative characters.

Parsimony analyses—Parsimony analysis of the complete data set (including outgroups) resulted in 672 most parsimonious trees (length = 1232 steps; CI [excluding uninformative characters] = 0.491; RI = 0.800). Analysis of ingroup taxa alone also resulted in 672 trees but of length 719 steps (CI [excluding uninformative characters] = 0.545; RI = 0.869). Both analyses resulted in identical sets of ingroup topologies. Bootstrap and decay values obtained for the ingroup-only analysis were also similar to those obtained from analysis of the complete data set, and as inclusion of outgroups did not affect either the ingroup topology or the general levels of support, in the remainder of this paper, we confine our discussion to the results from the analysis of the entire data set.
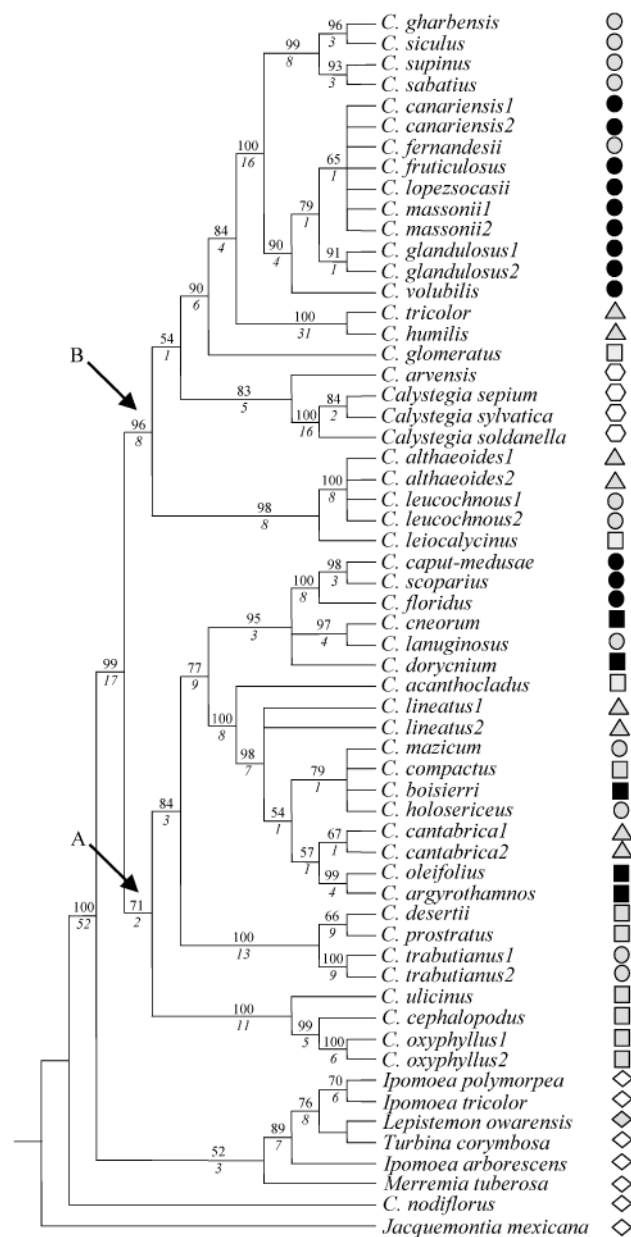

Fig. 2. Strict consensus tree of 672 most parsimonious trees from parsimony analysis of internal transcribed spacer (ITS) nrDNA. Bootstrap percentages and Bremer support values are shown above and below branches, respectively. Clades I and II correspond to Macaronesian Clades I and II discussed in the text. Arrows A and B indicate the two major lineages within *Convolvulus*. Symbols indicate species distributions: filled circle, Macaronesia; shaded circle, western Mediterranean; filled square, eastern Mediterranean; shaded square, western Asia; shaded triangle, widespread in Mediterranean and adjacent areas; open diamond, New world; shaded diamond, Tropical Africa; open hexagon, widespread.

Figure 2 shows the strict consensus tree from the complete analysis with bootstrap and decay values indicated and species distributions provided. One phylogram is shown in Fig. 3. The analysis results in a strongly supported group comprising *Convolvulus* (excluding the New World *C. nodiflorus*) and *Calystegia* (hereafter the *Convolvulus* clade; Fig. 2, bootstrap = 99%, decay value = 17). Within this clade, two clades are resolved corresponding to a clade of perennial nonclimbing

C. gharbensis
C. siculus
C. supinus
C. sabatius
C. canariensis1
C. canariensis2
C. fernandesii
C. fruticulosus
C. lopezsocasii
C. massonii1
C. massonii2
C. glandulosus1
C. glandulosus2
C. volubilis
II
C. tricolor
C. humilis
C. glomeratus
B
C. arvensis
Calystegia sepium
Calystegia sylvatica
Calystegia soldanella
C. althaeoides1
C. althaeoides2
C. leucochnous1
C. leucochnous2
C. leiocalycinus*
C. caput-medusae*
C. scoparius
C. floridus
C. cneorum
C. lanuginosus
C. dorycnium
C. acanthocladus*
C. lineatus1
C. lineatus2
C. mazicum
C. holosericeus
C. compactus
C. boisierri
C. cantabrica1
C. cantabrica2
C. oleifolius
C. argyrothamnos
A
C. desertii
C. prostratus
C. trabutianus1*
C. trabutianus2*
C. ulicinus*
C. cephalopodus
C. oxyphyllus1*
C. oxyphyllus2*
Ipomoea polymorpea
Ipomoea tricolor
Lepistemon owarensis
Turbina corymbosa
Ipomoea arborescens
Merremia tuberosa
C. nodiflorus
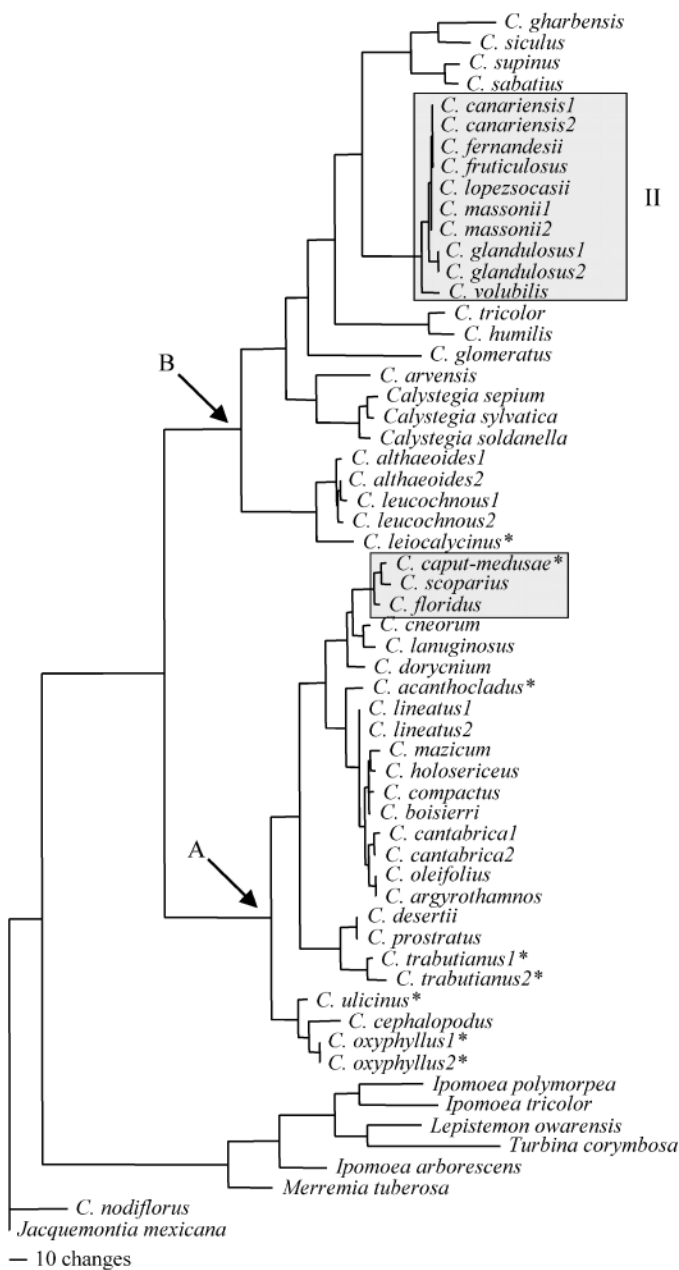Jacquemontia mexicana

— 10 changes

Fig. 3. One most parsimonious phylogram selected at random from the 672 most parsimonious solutions. Clades I and II correspond to Macaronesian Clades I and II discussed in the text. Arrows A and B indicate the two major lineages within *Convolvulus*. Species highlighted with an asterisk were placed in section *Acanthocladi* by Sa'ad (1967).

species, most of which are shrubs and subshrubs with sessile leaves (Fig. 2, clade A; bootstrap = 71%; decay value = 2) and a clade of annual or perennial herbs and suffrutescent plants typically with a climbing or trailing habit and petiolate leaves (Fig. 2, clade B; bootstrap = 96%; decay value = 8).

The Macaronesian endemic species of *Convolvulus* are resolved as two distinct clades (Fig. 2, clades I and II) with one Macaronesian clade occurring in each of clades A and B. Clade I (Fig. 2, bootstrap = 100%, decay value = 8) comprises the erect shrubs belonging to Mendoza-Heuer's (1971) Section I (Table 2) and is resolved within the larger clade of

nonclimbing species (clade A). Within clade I, *C. floridus* (the only species in this clade found on all of the Canary islands) is resolved as sister group to the other two species (bootstrap = 98%; decay value = 3). Clade II (Fig. 2, bootstrap = 90%, decay value = 4) comprises the endemic climbers (Table 2, Section II) together with the Portuguese endemic *C. fernandesii*. This clade is nested within clade B comprising *Convolvulus* species that typically possess a climbing or trailing habit. Phylogenetic structure within the Macaronesian clade II is weakly supported but *C. volubilis* Link. and *C. glandulosus* (Webb) Sa'ad are resolved as successive sister species to the rest of the clade (Fig. 2, bootstrap values = 79% and 65%, respectively; decay value of one in both cases). From Fig. 3, it is apparent that there are very short branch lengths in much of the Macaronesian clade II.

A close relationship with Mediterranean species for both Macaronesian clades is supported in the analysis. Thus, clade I is resolved in a clade with *C. dorycnium* (E. Mediterranean), *C. cneorum* (E. Mediterranean), and *C. lanuginosus* Desr. (W. Mediterranean) (Fig. 2, bootstrap = 95%; decay value = 3), and clade II is resolved as sister group to a clade comprising *C. gharbensis* Batt. & Pit., *C. supinus* Coss. & Kral., *C. sabatius* Viv. (all western Mediterranean), and *C. siculus* L. (circum-Mediterranean) (Fig. 2, bootstrap = 100%; decay value = 16).

The spiny North African species *C. trabutianus*, which was considered closely related to the Macaronesian endemic *C. caput-medusae* by Sa'ad (1967), is resolved as sister group to a clade comprising the Western Asian species *C. desertii* Hochst. and *C. prostratus* Forsk. (Fig. 2, bootstrap = 100%; decay value = 13). The analysis also suggests that *Convolvulus nodiflorus* is erroneously placed in the genus as this species is placed in a basal trichotomy with the outgroup *Jacquemontia mexicana* (Fig. 2, bootstrap = 100%; decay value = 52).

DISCUSSION

Multiple colonizations of the Macaronesian region—The results presented in this paper support the hypothesis of Mendoza-Heuer (1983) that the Macaronesian endemic species of *Convolvulus* are the result of two independent colonizations into the region.

Macaronesian Clade I is nested in a derived position within the *Convolvulus* clade that comprises perennial nonclimbing species, most of which are shrubs and subshrubs with sessile leaves (Fig. 2, clade A). Macaronesian clade II is nested within the clade of annual or perennial herbs and suffrutescent plants typically with a climbing or trailing habit and petiolate leaves (Fig. 2, clade B). It is therefore apparent that the two colonizations of Macaronesia by *Convolvulus* were from distantly related lineages with distinct morphologies.

Several other plant genera have also undergone multiple independent colonizations into Macaronesia and information on these genera is summarized in Table 1. It should be noted that two of the genera included in Table 1, namely *Pulicaria* Gaertner and *Teline* Medikus, have been shown to be nonmonophyletic and that low bootstrap values (<75%) for relevant nodes provide only weak phylogenetic support for multiple introductions in several other genera (see Table 1). Of the remaining examples, however, several demonstrate a similar pattern to *Convolvulus* (Fig. 2), in which the Macaronesian endemic congeners are distantly related. In *Plantago* L., for example, the three colonizations occurred from within three dif-

ferent subgenera (Rønsted et al., 2002), whilst the three island colonization groups of *Euphorbia* subsect *Pachyclade* (Molero et al., 2002) and the two island colonization groups in *Solanum* L. (Bohs and Olmstead, 2001) are, in each case, morphologically and phylogenetically distinct. Indeed, some authors have treated one of the Macaronesian colonization groups in *Solanum* as a separate genus (*Normania* Lowe) because of its morphological distinctiveness (Bohs and Olmstead, 2001). In *Lavatera* L., the two introductions into Macaronesia are not only morphologically distinct but the phylogenetic position of the Macaronesian species suggests that there was one recent and one ancient colonization into the region (Fuertes-Aguilar et al., 2002). The temporally distinct colonizations hypothesized for these taxa supports the more general conclusion that the Macaronesian region includes both endemics linked to an ancient Tertiary flora and others that have a more recent origin (e.g., Helfgott et al., 2000; Francisco-Ortega et al., 2001b). A similar scenario is also consistent with the molecular study of *Ilex* L. (Manen et al., 2002) in which the two Macaronesian species may have been the result of colonization events at very different times although the results are weakly supported.

Olea L., *Hedera* L., and the *Asteriscus* Miller alliance are the only genera for which a pattern of multiple colonization into Macaronesia by distantly related congeners does not appear to apply. In *Hedera*, one Macaronesian colonization was from the polyploid clade whilst two other colonizations occurred from within the diploid clade. Within the latter, relationships remain poorly resolved, and it is conceivable that the two colonizations were from closely related lineages. It is notable, however, that ITS data suggest that the three colonizations of Macaronesia by *Hedera* occurred independently to the Canaries, Madeira, and the Azores. Similarly, in *Olea*, whilst Macaronesia was colonized independently on two occasions by the same species (*O. europaea* L.), the two colonizations were to the Canaries and Madeira independently. Thus, whilst the Macaronesian lineages in *Olea* and *Hedera* might not be distantly related, in each case they conform to a ''checkerboard distribution'' (Diamond, 1975), i.e., they are geographically isolated within the region.

The *Asteriscus* alliance is the only example to date for which multiple colonization of the same archipelago by closely related congeners is a plausible interpretation of the results of molecular phylogenetic analyses. One introduction resulted in *A. graveolens* Less. subsp. *stenophyllus* (Link) Greuter, and the second introduction resulted in a clade comprising *A. sericeus* (L.fil.) DC. and *A. intermedius* (dc.) Pit. & Proust. However, some of the relationships of continental and island taxa in this complex remain weakly supported and further data are necessary to robustly establish the number of island colonizations in the group.

Further support for multiple introductions into Macaronesia for some genera comes from the patterns of interspecific hybridization between Macaronesian endemics. Interspecific hybrids are common within genera or groups of genera that have radiated in the islands following a single colonization event (Francisco-Ortega and Santos-Guerra, 2001). For example, hybrids are known to occur easily among several species of the *Aeonium* alliance, *Argyranthemum*, the *Bencomia* alliance, the *Gonospermum* Less. alliance, *Echium* L., *Pericallis*, *Sideritis* L., and the woody *Sonchus* L. alliance (reviewed by Francisco-Ortega and Santos-Guerra, 2001). Species of these genera form monophyletic groups in Macaronesia and hybrids within each of these assemblages have been detected both in the wild

and in cultivation (Hansen and Sunding, 1993; Francisco-Ortega and Santos-Guerra, 2001). In contrast, there are no reports for hybrids between members of the two *Convolvulus* clades, between *Ilex canariensis* Poir. and *I. perado* Ait. or between *Euphorbia balsamifera* Ait. and any of the species of the *E. dendroides* L. clade even though congeneric species overlap in their distribution range and, in some instances, are cultivated in public gardens of the islands. It is unknown to what extent hybridization occurs between species from different colonization events of *Asteriscus*, *Hedera*, *Lavatera*, *Olea*, *Plantago*, *Pulicaria*, *Teline*, and *Solanum*.

A pattern of phylogenetic distinctiveness and/or a checkerboard distribution pattern appears to apply to most, if not all, examples of multiple congeneric endemic Macaronesian plant lineages examined to date. Other genera for which multiple introductions have been hypothesized to account for the Macaronesian endemics include *Limonium* Mill. (Plumbaginaceae), *Salvia* L. (Labiatae), and *Viola* L. (Violaceae) (Fuertes-Aguilar et al., 2002), *Dracaena* L. (Agavaceae; Marrero et al., 1998), and *Hypericum* L. (Guttiferae; Robson, 1977). *Artemisia* L. (Asteraceae), *Campanula* L. (Campanulaceae), *Helianthemum* Miller (Cistaceae), and *Senecio* L. (Asteraceae) also probably conform to this pattern. Nearly all of these genera have a broad continental distribution, are species-rich and ecologically and morphologically diverse and it is conceivable that explicit phylogenetic analyses of many of these groups will demonstrate a similar pattern of phylogenetically distinct congeneric Macaronesian lineages.

Those genera listed in Table 1 that are monophyletic, together with *Convolvulus* and those genera for which multiple introductions are hypothesized but have yet to be rigorously examined, total approximately 20. This is significantly more than the number of multiple introductions demonstrated for other volcanic oceanic islands, notably Hawaii (one: *Rubus* L., Howarth et al., 1997) and the Galapagos Islands (one: *Gossypium* L., Wendel and Percival, 1990). However, bearing in mind the close proximity of the Macaronesian islands to the mainland, it represents a small proportion of the total number of genera with endemic Macaronesian representatives (approximately 232; Humphries, 1979).

The limited number of introductions into island groups such as Hawaii has often been attributed to the extreme difficulty of dispersing to geographically isolated archipelagos during the short period of time such young islands have been in existence (e.g., Carlquist, 1995). This explanation does not appear to be satisfactory for the Macaronesian region since several islands are of considerable age (e.g., Carracedo, 1994) and many Macaronesian endemic groups appear capable of dispersing over the distances necessary to colonize Macaronesia from the continent. In *Convolvulus*, for example, the Macaronesian climber clade (Clade II) is distributed in both the Canary Islands and Madeira (Fig. 2), suggesting dispersal over approximately 400 km following initial colonization of the region (Fig. 1). In other genera (e.g., *Aichryson* Webb & Berth., *Euphorbia*, *Pericallis*, and *Tolpis* that occur in both the Azores and Canaries; *Echium* and *Sonchus* that occur in the Cape Verdes and Canaries), dispersal over greater distances is consistent with their distribution in the region. Difficulty of dispersal to the Macaronesian region, particularly for groups with sister groups distributed in the near-continent may therefore be an inadequate explanation for the limited number of introductions to the region.

A more significant factor limiting the number of successful

TABLE 3. Summary of phylogenetic studies for species endemic to the Macaronesian Islands which are sister to or thought to be closely related to species with a Mediterranean distribution.

Genus	No. Macaronesian endemic species (no. included in the study)	Reference	Notes
Adenocarpus DC. (Fabaceae)[a]	3 (3)	Percy and Cronk, 2002; ITS; Käss and Wink, 1995, 1997; *rbcL*.	Unresolved polytomy with a Mediterranean species (ITS) or sister to a Mediterranean clade (*rbcL*).
Aeonium Webb & Berthel. alliance (Crassulaceae) (four genera)[b]	63 (53)	Mort et al., 2001, 2002	Sister to a western Mediterranean species. At least two back-colonizations from Macaronesia to Africa have occurred.
Androcymbium Willd. (Colchicaceae)	2 (2)	Caujapé-Castells et al., 1999	Sister to a western Mediterranean clade.
Arbutus L. (Ericaceae)[c]	1 (1)	Hileman et al., 2001	Sister species to a clade of two Mediterranean species.
Argyranthemum Sch. Bip (Asteraceae)[d]	24 (24)	Francisco-Ortega et al., 1995, 1997	Sister to a Mediterranean clade.
Artemisia L. (Asteraceae)	3 (1)	Torrell et al., 1999	Unresolved polytomy with western Mediterranean species.
Avena L. (Poaceae)[e]	1 (1)	Alicchio et al., 1995	Sister to a Mediterranean species distributed in Morocco and Syria.
Bellis L. (Asteraceae)	1 (1)	Fiz et al., 2002	Sister to a Mediterranean species.
Bencomia Webb & Berthel. (Rosaceae) alliance (three genera)[d]	8 (8)	Helfgott et al., 2000	Unresolved polytomy with two Mediterranean species.
Beta L. (Chenopodiaceae)[e,f]	2 (2)	Santoni and Bervillé, 1992	Polytomy with a western Mediterranean species.
Chamaecytisus Link (Fabaceae)	1 (1)	Käss and Wink, 1995, 1997; Badr et al., 1994	Sister to a Mediterranean clade.
Cheirolophus Cass. (Asteraceae)	15 (10)	Susanna et al., 1999	Sister to a western Mediterranean clade.
Echium L. (Boraginaceae)[c]	27 (22)	Böhle et al., 1996	Sister to a Mediterranean clade.
Erucastrum (DC.) C. Presl (Brassicaceae)[c]	1 (1)	Warwick and Black, 1993	Sister to a species with a Mediterranean–Canarian distribution.
Genista L. (Fabaceae)[c]	2 (2)	Percy and Cronk, 2002	Sister to a widespread Mediterranean–European species.
Gonospermum Less. alliance (includes the three endemic species of *Tanacetum*) (three genera)[d]	8 (8)	Watson et al., 2000; Francisco-Ortega et al., 2001a	Unresolved clade with three genera with a predominantly Mediterranean distribution (ITS data) or sister to a clade with five genera with a predominantly Mediterranean distribution (*ndhF* data).
Hypochaeris L. (Asteraceae)	1 (1)	Cerbah et al., 1998	Sister to an eastern Mediterranean species.
Isoplexis (Lindl.) Benth. (Scrophulariaceae)[d]	4 (3)	Carvalho and Culham, 1998	Sister to a western Mediterranean species.
Ixanthus Griseb. (Gentianaceae)[d]	1 (1)	Thiv et al., 1999	Sister to a Mediterranean species.
Pinus L. (Pinaceae)	1 (1)	Krupkin et al., 1996; Liston et al., 1999; Geada-López et al., 2002	Conflicting hypotheses: resolved as either sister to a Mediterranean clade (RFLP of cpDNA and ITS[g]) or placed in an unresolved polytomy with a Mediterranean and a Himalayan species (four cpDNA regions)[g].
Plocama Aiton (Rubiaceae)[d,g]	1 (1)	Andersson and Rova, 1999	Sister to a species with a predominantly Mediterranean distribution.
Ranunculus L. (Ranunculaceae)[c,d]	1 (1)	Johansson, 1998	Unresolved polytomy with 11 Eurasian species (inadequately resolved).
Reichardia Roth (Asteraceae)	3 (1)	Kim et al., 1996	Sister to a Mediterranean species.
Sambucus L. (Caprifoliaceae)	2 (2)	Eriksson and Donoghue, 1997; Trusty et al., 2001 (www.2001.botanyconference.org)	The two endemic taxa are paraphyletic to a widespread species.
Sideritis L. (Lamiaceae)	24 (24)	Barber et al., 2002	Sister to a western Mediterranean species.
Sinapidendron Lowe (Brassicaceae)[d]	5 (2)	Warwick and Black, 1993	Sister to clade with a Mediterranean–Canarian distribution.
Spartocytisus Webb & Berthel. (Fabaceae)[d]	2 (1)	Cubas et al., 2002	Sister to a Mediterranean clade.
Tinguarra Parl (Apiaceae)	2 (2)	Downie et al., 2000a	Nested within a clade of western Mediterranean species.
Todaroa Parl (Apiaceae)[d]	1 (1)	Downie et al., 2000b	Nested within a clade of western Mediterranean species.

TABLE 3.   Continued.

Genus	No. Macaronesian endemic species (no. included in the study)	Reference	Notes
Tolpis Adanson (Asteraceae)[c]	14 (14)	Park et al., 2001; Moore et al., 2002	Sister group of the Macaronesian clade is widespread. Mediterranean species of *Tolpis* are nested within a Macaronesian clade.
Vaccinium L. (Ericaceae)	2 (2)	Powell and Kron, 2002	Sister to eastern Mediterranean species.
Vierea Webb & Berthel. (Asteraceae)[d,g]	1 (1)	Francisco-Ortega et al., 2001b	Sister to a western Mediterranean species.

[a] Study based on neighbor joining analysis.
[b] Two genera of this alliance are endemic.
[c] Genus is polyphyletic or paraphyletic.
[d] Endemic genus or endemic alliance of genera.
[e] Bootstrap value unreported.
[f] Study based on UPGMA analysis.
[g] Relationship supported by a bootstrap value less than 75%.

colonizations into the region is likely to be establishment following initial dispersal of propagules to the islands (Carlquist, 1974) as competitors already in residence may effectively "close" an island system to future potential colonizers (MacArthur and Wilson, 1967; Whittaker, 1998). Silvertown (2004) recently discussed dispersal and establishment barriers in relation to the colonization of Macaronesia. Using published molecular phylogenies of a number of plant groups, Silvertown (2004) demonstrated that in genera that had undergone a single colonization of the region, the mean number of species per endemic group was seven times greater than that for genera that had undergone multiple colonizations into the region. He therefore hypothesized that groups with many species are more effective at excluding repeat colonizations by relatives than groups with fewer species since larger groups should preempt more of the niche or habitat space available for colonization.

The pattern of relationships of congeneric Macaronesian lineages discussed in this paper is also consistent with this hypothesis: the patterns of phylogenetic distinctiveness or checkerboard distributions of those genera that have undergone multiple introductions into the region support the hypothesis that niche preemption has played an important role in restricting the number of new colonizations into Macaronesia. Multiple congeneric colonization of Macaronesia appears only to have occurred when either (1) different archipelagos within the region have been colonized independently or (2) when the colonizing groups are sufficiently phylogenetically, morphologically, and, presumably, ecologically distinct that they are not in direct competition.

Whilst the sample of genera in Table 1 is limited, it is interesting to note that Macaronesian endemic animal groups are also consistent with this pattern. Phylogenetic analyses have shown that several animal genera have undergone multiple independent colonization into the Macaronesian region namely *Calathus* Bonelli (Coleoptera; Emerson et al., 1999), *Dysdera* Latreille (Aracnida; Arnedo et al., 2001), *Tarentola* Gray (Reptilia; Carranza et al., 2002), and possibly *Chalcides* Laurenti (Reptilia; Brown and Pestano, 1998) (although the precise relationships of the Macaronesian representatives of the latter remain to be robustly resolved). In all of these examples, the independent Macaronesian lineages demonstrate a checkerboard distribution and are confined either to different archipelagos or to different islands within an archipelago.

Back-colonization of the continent from Macaronesia— Results presented in this paper place *C. fernandesii* from Cabo Espichel in Portugal in an unresolved terminal polytomy within the Macaronesian climber group (Fig. 2, Clade II). This is consistent with the view expressed by Pinto de Silva and Teles (1981) that *C. fernandesii* forms part of the Macaronesian climber clade. The placement of *C. fernandesii* in a late branching position in the Macaronesian climber clade and the extremely short branches within this clade (Fig. 3) furthermore support the hypothesis of Pinto de Silva and Teles (1981) that *C. fernandesii* is a continental neo-endemic of recent origin, resulting from back-colonization of the continent from Macaronesia (a minimum distance of ca. 1200 km). The traditionally held view of this distribution as relictual (e.g., Bramwell and Bramwell, 2001) is not supported.

The coastal area near Lisbon where *C. fernandesii* occurs is among the most floristically diverse areas of the Iberian peninsula (Lobo et al., 2001). The Portuguese endemic *Euphorbia pedroi* Molero and Rovira, which is part of the Mediterranean-Macaronesian pachycaul group within *Euphorbia* (Molero et al., 2002), also occurs in this area and whilst molecular phylogenetic data have been unable to robustly resolve the relationships within this clade (Molero et al., 2002), an origin for this species by back-colonization from Macaronesia to Portugal remains a plausible hypothesis.

Recent back-colonization of Portugal from Macaronesia may have been facilitated by the volcanic seamounts located between the Macaronesian archipelagos and the continent. These could have served as stepping stones during glacial periods when sea levels were lower (García-Talavera, 1997). In the case of the Hawaiian archipelago, palaeogeographic reconstructions have been integrated with phylogenetic hypotheses to gain new insights into the evolution of the Hawaiian biota (Price and Clague, 2002) and a similar approach for the complex Macaronesian region may shed further light onto the possible role of sea mounts in the colonization history of the Cabo Espichel region.

Sister group relationships of Macaronesian Convolvulus and their systematic implications—A Mediterranean sister group relationship is suggested for both Macaronesian clades in the analysis (Fig. 2). Macaronesian clade I is placed in an unresolved polytomy with *C. dorycnium*, *C. cneorum*, and *C.*

lanuginosus, which lends some support to the suggestions of Peter (1891) and Sa'ad (1967) that *C. floridus* and *C. scoparius*, respectively, are closely related to *C. dorycnium. Convolvulus dorycnium* is a shrub up to 1 m high, distributed in the eastern Mediterranean whilst *C. cneorum* is a suffrutescent plant up to 30 cm high, also distributed in the eastern Mediterranean, and *C. lanuginosus* is a subligneous plant to 30 cm high with a western Mediterranean distribution. The sister group of the Macaronesian erect endemic species is therefore predominantly, though not exclusively, central/eastern Mediterranean. Woodiness may be interpreted as a plesiomorphy for Macaronesian clade I, although *C. floridus* in particular shows a general increase in size and woodiness when compared with its close continental relatives.

Sa'ad (1967), in her revision of *Convolvulus* from the Canary Islands, Mediterranean, and Near and Middle East, treated *C. trabutianus* from Morocco as conspecific with the Canarian spiny subshrub *C. caput-medusae*. The expanded *C. caput-medusae* sensu lato (s.l.) was placed in section *Acanthocladi* that comprised all spinescent subshrubs. This suggestion was challenged by Mendoza-Heuer (1983), and the results presented in this paper demonstrate unequivocally that *C. caput-medusae* is more closely related to other Canarian endemics (*C. floridus* and *C. scoparius*) than it is to *C. trabutianus* from Morocco (Fig. 3). The sample of section *Acanthocladi* included in the analysis suggests that the group is highly polyphyletic (Fig. 3) and that the spines of species from the Canary Islands, the western Mediterranean, and the eastern Mediterranean are non-homologous. Whilst taxon sampling in the present analysis does not allow us to propose a new infrageneric classification of *Convolvulus*, it does highlight the need for both a careful reexamination of putative morphological homologies and for further work on the infrageneric classification of the group.

Macaronesian clade II, comprising the climbing species, is resolved as sister group to a clade of erect or trailing herbs that are distributed in the western Mediterranean (*C. supinus*, *C. sabatius*, and *C. gharbensis*) or more generally in the Mediterranean and adjacent regions (*C. siculus*) (Fig. 2). Whilst *C. siculus* and *C. gharbensis* are entirely herbaceous, *C. supinus* and *C. sabatius* are herbs that are slightly woody at the base. The prominently woody habit of the Macaronesian climbers however, is a synapomorphy for this clade and provides a further clear example of derived insular woodiness (Carlquist, 1974). The short branch lengths within this clade (Fig. 3) suggest that the Macaronesian climbers may have undergone a recent and rapid radiation.

Choisy (1845) and Peter (1891) placed the Macaronesian climbers in section *Strophoaulos* Don. within an informal group comprising all climbers that were at least slightly woody at the base. The other representatives of this group were New World. Choisy's (1845) and Peter's (1891) circumscription of genera within Convolvulaceae differed significantly from currently accepted generic concepts within the family and several species included in section *Strophoaulos* were later transferred to *Jacquemontia* Choisy. The only non-Macaronesian species in section *Strophoaulos* currently retained within *Convolvulus* is *C. nodiflorus* (Stearn, 1969), a robust climber reaching 6 m in height, distributed in tropical America. The results presented in this paper demonstrate that *C. nodiflorus* is not closely related to the Macaronesian climbers and does not appear to belong to *Convolvulus*. Indeed, its placement in an unresolved basal trichotomy with *Jacquemontia mexicana*, the outgroup

taxon in the present analysis, suggests that the earlier placement of this species in *Jacquemontia* may be correct.

Stefanović et al. (2002) demonstrated that *Convolvulus* and *Jacquemontia* are distantly related in Convolvulaceae. This result contrasted markedly with earlier taxonomic treatments that had considered the two genera closely related in the tribe Convolvuleae. The two genera have been distinguished on the basis of pollen morphology (tricolpate in *Convolvulus*, pantocolpate in *Jacquemontia*) and stigma morphology (elongate in *Convolvulus*, flattened and tongue-like in *Jacquemontia*) although several species, including *C. nodiflorus* and *C. rozynskii* (Standley) Lewis and Oliver, have been placed at different times in either *Convolvulus* or *Jacquemontia*. Stearn (1969) demonstrated that *C. nodiflorus* possesses tricolpate pollen and as a result, suggested that this species belonged in *Convolvulus*. However, the molecular data strongly disagree with this placement. Further work is necessary to clarify the circumscription and diagnosis of these two genera.

General patterns of sister-group relationships of the Macaronesian flora—The Macaronesian climber group of *Convolvulus* is one of a number of groups for which a close relationship with highly disjunct taxa has been hypothesized. Whilst molecular phlylogenetic analyses support several such sister group relationships (see below), others have been strongly refuted (Andrus et al., 2004), notably the endemic genus *Argyranthemum* (Francisco-Ortega et al., 1996), the *Bencomia* alliance (Helfgott et al., 2000), the *Gonospermum* alliance (Francisco-Ortega et al., 2001a), and *Echium* (Böhle et al., 1996) in addition to the Macaronesian climber group of *Convolvulus*. All of these groups have been shown to have their closest relatives in the Mediterranean. Indeed, molecular phylogenetic analyses suggest that the sister groups of most Macaronesian endemic taxa examined to date have a predominantly Mediterranean distribution. Those genera for which the results of molecular cladistic analyses are consistent both with (1) a single colonization of Macaronesia and (2) a Mediterranean or predominantly Mediterranean sister group for the Macaronesian clade are summarized in Table 3. From this table it can be seen that there is at least some molecular support for a Mediterranean sister group for the endemic Macaronesian clades of 32 genera/alliances.

The results summarized in Table 3, together with *Convolvulus* and those in Table 1, provide a useful basis with which to reexamine general patterns of sister-group distributions for Macaronesian endemic clades. In addition to those groups detailed in these tables, molecular data have resolved the sister-group relationships of a further seven clades. In each case, a non-Mediterranean sister group is supported for the Macaronesian endemic representatives of the genus: *Camptoloma* Benth. (East Africa; Scrophulariaceae; one species; Kornhall et al., 2001); *Pericallis* (Asteraceae; New World; 15 species; Panero et al., 1999); Madeiran *Sedum* clade (New World; Crassulaceae; two species; van Ham and t'Hart, 1998); *Ocotea* Aubl. (South Africa; Lauraceae; one species; Chanderbali et al., 2001); *Phyllis* L. (South Africa; Rubiaceae; one species; Andersson and Rova, 1999); Madeiran species of *Saxifraga* L. (Eurosiberia; two species; Vargas et al., 1999b); Canarian species of *Silene* L. (Eurosiberia; seven species; Clement et al., 1997).

The Macaronesian endemic groups accounted for in Table 1 (25 groups) and Table 3 (32 groups), together with *Convolvulus* and the seven genera described above, represent 64 Ma-

caronesian groups that have been subject to molecular phylogenetic analyses to date. Of these, however, eight have sister taxa that are either (1) imprecisely known because of conflicting phylogenies (*Pinus canariensis*), (2) inadequately resolved (*Hedera* [three clades]; *Ranunculus* L.) or (3) established on the basis of extremely limited sampling of continental species (*Euphorbia* [three clades]). Excluding these clades from consideration, the sister-group relationships of 56 endemic Macaronesian clades have been resolved using molecular data. The distributions of the sister taxa of these groups are summarized in Table 4.

An estimate of the number and proportion of Macaronesian species whose continental closest relatives are distributed in each of the areas considered is also provided in Table 4. On the basis of a number of recent works on the Macaronesian flora (Santos-Guerra and Francisco-Ortega, 1990; Brochmann et al., 1997; Jardim and Francisco, 2000; Santos-Guerra, 2001; Schäffer, 2003), we estimate that the total number of species endemic to Macaronesia is approximately 874, and the analyses summaried in Table 4 account for 276 of these, i.e., 32% of all Macaronesian endemic species.

As can be seen from Table 4, 71% of Macaronesian clades examined have a Mediterranean, or predominantly Mediterranean, sister group, and these clades comprise 80% of the Macaronesian endemic species subject to molecular phylogenetic analysis. Thus, both in terms of number of clades and species diversity, the Mediterranean element of the Macaronesian flora is the dominant element and floristic links with other remote regions account for only a relatively minor component of the endemic plant biodiversity.

In this respect, *Convolvulus* conforms to the general pattern of relationships of the Macaronesian flora as both Macaronesian clades have Mediterranean sister groups. It is notable however, that the sister taxa of the two Macaronesian *Convolvulus* clades differ in their areas of distribution within the Mediterranean basin with the sister group of the climber clade (Fig. 2, Clade 2) predominantly western Mediterranean and the sister group of the erect clade (Fig. 2, Clade 1) predominantly eastern Mediterranean in distribution.

Of the clades considered in Tables 3 and 4, 18 (32%) have an exclusively or predominantly western Mediterranean distribution (i.e., Spain, Portugal, Morocco, Algeria, Tunisia, and South France) and 19 (34%) have a sister group distributed in both eastern and western Mediterranean basins, whereas only three (5% of all clades) have an exclusively or predominantly eastern Mediterranean distribution (*Hypochaeris* L., *Vaccinium* L., and the erect *Convolvulus* clade [Fig. 2, clade 1]). Relationships between Macaronesian taxa and those distributed in the western Mediterranean, i.e., in the near-continent, therefore account for 66% of the groups examined and are far more abundant than disjunctions between Macaronesia and the eastern Mediterranean.

The disjunct relationships hypothesized for some Macaronesian groups has been used to support the hypothesis that much of the Macaronesian flora is relictual in nature. However, this view has been challenged by many molecular phylogenetic studies during the last 10 years, and Table 4 illustrates that disjunctions to the Eastern Mediterranean, the New World, southern Africa, eastern Africa, or eastern Asia account for only 19% of Macaronesian groups studied in molecular analyses to date (see also Andrus et al., in press).

It seems plausible that the Macaronesian flora does indeed comprise lineages that diverged from their closest continental

TABLE 4.   Summary of sister group relationships of Macaronesian groups.

Area	No. Macaronesian clades with sister groups distributed in the area	Percentage of Macaronesian clades with sister groups distributed in the area[a]	No. Macaronesian species with sister groups distributed in the area	Percentage of Macaronesian species with sister groups distributed in the area[a]
1. Mediterranean	40	71	210 + 3 subspecies	80
[a. Western Mediterranean]	[18]	[32]		
[b. Eastern Mediterranean]	[3]	[5]		
[c. Widespread in Mediterranean]	[19]	[34]		
2. New World	2	4	17	6
3. South Africa	2	4	2	<1
4. East Africa	2	4	2	<1
5. East Asia	1	2	1	<1
6. Eurosiberia	2	4	9	3
7. Widespread	7	13	21	8
8. Inadequately resolved	4	—	4	—
9. Conflicting relationships	1	—	1	—
10. Poor sampling	3	—	9	—
Total	64	—	276[b]	—
Total (excluding 8—10 above)	56	—	262	—

[a] Groups for which the results of cladistic analyses are either inadequately resolved, conflicting, or poorly sampled were excluded from the calculations.
[b] Endemic subspecies excluded from the calculation.

relatives at different times and that some lineages may be ancient (e.g., Fuertes-Aguilar et al., 2002). Macaronesian lineages with sister taxa distributed in different areas (such as the two *Convolvulus* lineages) may be the result of temporally distinct waves of colonization into Macaronesia that reflect the complex geological and climatic history of the region. The apparently congruent patterns of sister group relationships with taxa distributed in the near-continent also need close scrutiny as these may conceal ''pseudo-congruent'' patterns sensu Cunningham and Collins (1994; see Donoghue and Moore, 2003). Macaronesian clades with sister groups distributed in the near-continent include both groups that have radiated spectacularly in the islands and evolved distinctive island traits (e.g., *Aeonium* alliance, *Argyranthemum*, the *Convolvulus* climbers, *Sideritis*) and groups that show little or no evidence of island radiation (e.g., *Avena* L., *Androcymbium* Willd., *Erucastrum* C.Presl.). It is conceivable that the Macaronesia–Mediterranean area relationship specified by these groups may be pseudo-congruent in that they may have resulted from diversification from continental sister groups at different times and, presumably, with different underlying causes.

As Donoghue and Moore (2003) note, knowledge of the timing of events distinguishes those groups that diversified during the same time period, and therefore might have experienced the same causal events, from those that diversified during different time periods and require different causal explanations. The application of biogeographic approaches that attempt, either explicitly or implicitly, to incorporate temporal information on the diversification of Macaronesian lineages from their continental relatives may provide new insights into the extremely complex patterns of relationships between the Macaronesian islands and continental floras that are well illustrated by groups such as *Convolvulus*.

LITERATURE CITED

ACKERFIELD, J., AND J. WEN. 2003. Evolution of *Hedera* (the ivy genus, Araliaceae): insights from chloroplast DNA data. *International Journal of Plant Sciences* 164: 593–602.

ALDRIDGE, A. E. 1979. Evolution within a single genus: *Sonchus* in Macaronesia. *In* D. Bramwell [ed.], Plants and islands, 279–293. Academic Press, London, UK.

ALICCHIO, R., L. ARANCI, AND L. CONTE. 1995. Restriction fragment length polymorphism based phylogenetic analysis of *Avena* L. *Genome* 38: 1279–1284.

ANDERSSON, L., AND J. H. E. ROVA. 1999. The *rps16* intron and the phylogeny or the Rubiodeae (Rubiaceae). *Plant Systematics and Evolution* 214: 161–186.

ANDRUS, N., J. TRUSTY, A. SANTOS-GUERRA, R. K. JANSEN, AND J. FRANCISCO-ORTEGA. 2004. Using molecular phylogenies to test phytogeographical links between East/South Africa, Sourthern Arabia and the Macaronesian islands: a review, and the case of *Vierea* and *Pulicaria* section *Vieraeopsis* (Asteraceae). *Taxon* 53: 333–346.

ARNEDO, M. A., P. OROMI, AND C. RIBERA. 2001. Radiation of the spider genus *Dysdera* (Araneae, Dysderidae) in the Canary Islands: cladistic assessment based on multiple data sets. *Cladistics* 17: 313–353.

BADR, A., W. MARTIN, AND U. JENSEN. 1994. Chloroplast DNA restriction site polymorphism in Genisteae (Leguminosae) suggests a common origin for European and American lupines. *Plant Systematics and Evolution* 193: 95–106.

BARBER, J. C., J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, K. G. TURNER, AND R. K. JANSEN. 2002. Origin of Macaronesian *Sideritis* L. (Lamioideae: Lamiaceae) inferred from nuclear and chloroplast sequence datasets. *Molecular Phylogenetics and Evolution* 23: 293–306.

BÖHLE, U. R., H. H. HILGER, AND W. F. MARTIN. 1996. Island colonization and evolution of the insular woody habit in *Echium* L (Boraginaceae). *Proceedings of the National Academy of Sciences, USA* 93: 11 740–11 745.

BOHS, L., AND R. G. OLMSTEAD. 2001. A reassessment of *Normania* and *Triguera* (Solanaceae). *Plant Systematics and Evolution* 228: 33–48.

BRAMWELL, D. 1972. Endemism in the flora of the Canary Islands. *In* D. H. Valentine [ed.], Taxonomy phytogeography and evolution, 141–159. Academic Press, London, UK.

BRAMWELL, D., AND Z. I. BRAMWELL. 2001. Wild flowers of the Canary Islands, 2nd ed. Editorial Rueda, Madrid, Spain.

BRAMWELL, D., AND I. B. K. RICHARDSON. 1973. Floristic connections between Macaronesia and the East Mediterranean region. *Monographiae Biologicae Canariensis* 4: 118–125.

BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795–803.

BREMER, K., AND C. J. HUMPHRIES. 1993. Generic monograph of the Asteraceae: Anthemideae. *Bulletin of the Natural History Museum (London), Botany Series* 23: 71–177.

BROCHMANN, C., O. H. RUSTAN, W. LOBIN, AND N. KILIAN. 1997. The endemic vascular plants of the Cape Verde Islands, W Africa. *Sommerfeltia* 24: 1–356.

BROWN, R. P., AND J. PESTANO. 1998. Phylogeography of skinks (*Chalcides*) in the Canary Islands inferred from mitochondrial DNA sequences. *Molecular Ecology* 7(9): 1183–1191.

CARLQUIST, S. J. 1974. Island biology. Columbia University Press, New York, New York, USA.

CARLQUIST, S. J. 1995. Introduction. *In* W. L. Wagner and V. A. Funk [eds.], Hawaiian biogeography: evolution of a hotspot archipelago, 1–13. Smithsonian Institution Press, Washington, D.C., USA.

CARRACEDO, J. C. 1994. The Canary Islands: an example of structural control on the growth of large oceanic-island volcanoes. *Journal of Volcanology and Geothermal Research* 60: 225–241.

CARRANZA, S., E. N. ARNOLD, J. A. MATEO, AND P. GENIEZ. 2002. Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 23: 244–256.

CARVALHO, J. A., AND A. CULHAM. 1998. Conservation status and preliminary results on the phylogenetics of *Isoplexis* (Lindl.) Benth. (Scrophulariaceae), an endemic Macaronesian genus. *Boletim do Museo Municipal do Funchal Suplemento* 5: 109–127.

CAUJAPÉ-CASTELLS, J., R. K. JANSEN, J. PEDROLA-MONFORT, AND N. MEMBRIVES. 1999. Chloroplast DNA restriction site phylogeny of the genus *Androcymbium* (Colchicaceae). *Systematic Botany* 24: 581–597.

CERBAH, M., T. SOUZA-CHIES, M. J. JUBIER, B. LEJEUNE, AND S. SILJAK-YAKOVLEV. 1998. Molecular phylogeny of the genus *Hypochaeris* using internal transcribed spacers of nuclear rDNA: inference for chromosomal evolution. *Molecular Biology and Evolution* 15: 345–354.

CHANDERBALI, A. S., H. VAN DER WERFF, AND S. S. RENNER. 2001. Phylogeny and historical biogeography of *Lauraceae*: evidence from the chloroplast and nuclear genomes. *Annals of the Missouri Botanical Garden* 88: 104–134.

CHOISY, J. D. 1845. Convolvulaceae. *In* A. P. P. De Candolle [ed.], Prodromus systematis naturalis regni vegetablis, vol. 9, 325–461. Paris, France.

CLEMENT, J. S., J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, AND R. K. JANSEN. 1997. Relationships of the Canary Islands endemic species of *Silene* (Caryophyllaceae) based on nuclear ribosomal internal transcribed spacer sequences. *American Journal of Botany* 84(Supplement): 182.

CUBAS, P., C. PARDO, AND H. TAHIRI. 2002. Molecular approach to the phylogeny and systematics of *Cytisus* (Leguminosae) and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (trnL-trnF intergenic spacer). *Plant Systematics and Evolution* 233: 223–242.

CUÉNOUD, P., M. A. D. MARTINEZ, P. A. LOIZEAU, R. SPICHIGER, S. ANDREWS, AND J. F. MANEN. 2000. Molecular phylogeny and biogeography of the genus *Ilex* L. (Aquifoliaceae). *Annals of Botany* 85: 111–122.

CUNNINGHAM, C. W., AND T. M. COLLINS. 1994. Developing model systems for molecular biogeography: vicariance and interchange in marine invertebrates. *In* B. Schierwater, G. Streit, P. Wagner, and R. DeSalle [eds.], Molecular ecology and evolution: approaches and applications, 405–433. Birkhäuser Verlag, Basel, Switzerland.

DIAMOND, J. M. 1975. Assembly of species communities. *In* M. L. Cody and J. M. Diamond [eds.], Ecology and evolution of communities, 342–344. Harvard University Press, Cambridge, Massachusetts, USA.

DONOGHUE, M. J., AND B. R. MOORE. 2003. Toward an integrative historical biogeography. *Integrative and Comparative Biology* 43: 261–270.

DOWNIE, S. R., D. S. KATZ-DOWNIE, AND K. SPALIK. 2000a. A phylogeny of Apiaceae tribe Scandiceae: evidence from nuclear ribosomal DNA

internal transcribed spacer sequences. *American Journal of Botany* 87: 76–95.

DOWNIE, S. R., M. F. WATSON, K. SPALIK, AND D. S. KATZ-DOWNIE. 2000b. Molecular systematics of Old Wold Apioideae (Apiaceae): relationships among some members of tribe Peucedaneae sensu lato, the placement of several island-endemic species, and resolution within the apioid super-clade. *Canadian Journal of Botany* 78: 506–528.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid isolation procedure for small quantities of leaf tissue. *Phytochemical Bulletin* 19: 11–15.

EMERSON, B. C., P. OROMI, AND G. M. HEWITT. 1999. MtDNA phylogeography and recent intra-island diversification among Canary Island Calathus beetles. *Molecular Phylogenetics and Evolution* 13: 149–158.

ERIKSSON, T. 1998. AutoDecay, version 4. Department of Botany, Stockholm University, Stockholm, Sweden.

ERIKSSON, T., AND M. J. DONOGHUE. 1997. Phylogenetic relationships of *Sambucus* and *Adoxa* (Adoxoideae, Adoxaceae) based on nuclear ribosomal ITS sequences and preliminary morphological data. *Systematic Botany* 22: 555–573.

FARRIS, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417–419.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FIZ, O., V. VALCARCEL, AND P. VARGAS. 2002. Phylogenetic position of Mediterranean Astereae and character evolution of daisies (*Bellis*, Asteraceae) inferred from nrDNA ITS sequences. *Molecular Phylogenetics and Evolution* 25: 157–171.

FRANCISCO-ORTEGA, J., J. C. BARBER, A. SANTOS-GUERRA, R. FEBLES-HERNANDEZ, AND R. K. JANSEN. 2001a. Origin and evolution of the endemic genera of *Gonosperminae (Asteraceae: Anthemideae)* from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. *American Journal of Botany* 88: 161–169.

FRANCISCO-ORTEGA, J., R. K. JANSEN, D. J. CRAWFORD, AND A. SANTOS-GUERRA. 1995. Chloroplast DNA evidence for intergeneric relationships of the Macaronesian endemic genus *Argyranthemum* (Asteraceae). *Systematic Botany* 20: 413–422.

FRANCISCO-ORTEGA, J., R. K. JANSEN, AND A. SANTOS-GUERRA. 1996. Chloroplast DNA evidence of colonization, adaptive radiation, and hybridization in the evolution of the Macaronesian flora. *Proceedings of the National Academy of Sciences, USA* 93: 4085–4090.

FRANCISCO-ORTEGA, J., S.-J. PARK, A. SANTOS-GUERRA, A. BENABID, AND R. K. JANSEN. 2001b. Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. *Biological Journal of the Linnean Society* 72: 77–97.

FRANCISCO-ORTEGA, J., AND A. SANTOS GUERRA. 2001. Genes y conservación de plantas vasculares. *In* J. M. Fernández-Palacios and J. L. Martín-Esquivel [eds.], Naturaleza de las Islas Canarias. Ecología y conservación, 357–365. Publicaciones Turquesa, Santa Cruz de Tenerife, Spain.

FRANCISCO-ORTEGA, J., A. SANTOS-GUERRA, A. HINES, AND R. K. JANSEN. 1997. Molecular evidence for a Mediterranean origin of the Macaronesian endemic genus *Argyranthemum (Asteraceae)*. *American Journal of Botany* 84: 1595–1613.

FUERTES-AGUILAR, J., M. F. RAY, J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, AND R. K. JANSEN. 2002. Molecular evidence from chloroplast and nuclear markers for multiple colonizations of *Lavatera* (Malvaceae) in the Canary Islands. *Systematic Botany* 27: 74–83.

GARCÍA-TALAVERA, F. 1997. Las Canarias orientales y la vecina costa africana en el Holoceno. *Eres* 7: 55–63.

GARCÍA-TALAVERA, F. 1999. La Macaronesia. Consideraciones geológicas, biogeográficas y palaeoecológicas. *In* J. M. Fernández-Palacios, J. J. Bacallado, and J. A. Belmonte [eds.], Ecología y cultura en Canarias, 39–63. Múseo de la Ciencia, Cabildo Insular de Tenerife, Santa Cruz de Tenerife, Canary Islands, Spain.

GEADA-LÓPEZ, G., K. KAMIYA, AND K. HARADA. 2002. Phylogenetic relationships of *Diploxylon* pines (subgenus *Pinus*) based on plastid sequence data. *International Journal of Plant Sciences* 163: 737–747.

GELDMACHER, J., K. HOERNLE, P. VAN DEN BOGAARD, G. ZANKL, AND D. GARBE-SCHÖNBERG. 2001. Earlier history of the ≥70-Ma-old Canary hotspot based on the temporal and geochemical evolution of the Selvagen Archipelago and neighbouring seamounts in the eastern North Atlantic. *Journal of Volcanology and Geothermal Research* 111: 55–87.

GOERTZEN, L. R., J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, J. P. MOWER,

C. R. LINDER, AND R. K. JANSEN. 2002. Molecular systematics of the *Asteriscus* alliance (Asteraceae: Inuleae) II: combined nuclear and chloroplast data. *Systematic Botany* 27: 815–823.

HANSEN, A., AND P. SUNDING. 1993. Flora of Macaronesia: checklist of vascular plants. 4th revised ed. *Sommerfeltia* 17: 1–295.

HELFGOTT, D. M., J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, R. K. JANSEN, AND B. B. SIMPSON. 2000. Biogeography and breeding system evolution of the woody *Bencomia* alliance (Rosaceae) in Macaronesia based on ITS sequence data. *Systematic Botany* 25: 82–97.

HESS, J., J. W. KADEREIT, AND P. VARGAS. 2000. The colonization history of *Olea europaea* L. in Macaronesia based on internal transcribed spacer 1 (ITS-1) sequences, randomly amplified polymorphic DNAs (RAPD), and intersimple sequence repeats (ISSR). *Molecular Ecology* 9: 857–868.

HILEMAN, L. C., M. C. VASEY, AND V. T. PARKER. 2001. Phylogeny and biogeography of the Arbutoideae (Ericaceae): implications for the Madrean-Tethyan hypothesis. *Systematic Botany* 26: 131–143.

HOWARTH, D. G., D. E. GARDNER, AND C. W. MORDEN. 1997. Phylogeny of *Rubus* subgenus *Idaeobatus* (Rosaceae) and its implications toward colonization of the Hawaiian Islands. *Systematic Botany* 22: 433–441.

HUMPHRIES, C. J. 1979. Endemism and evolution in Macaroensia. *In* D. Bramwell [ed.], Plants and islands, 171–200. Academic Press, London, UK.

JARDIM, R., AND D. FRANCISCO. 2000. Flora Endémica de Madeira. Múchia Publições, Funchal, Portugal.

JOHANSSON, J. T. 1998. Chloroplast DNA restriction site mapping and the phylogeny of *Ranunculus* (Ranunculaceae). *Plant Systematics and Evolution* 213: 1–19.

KÄSS, E., AND M. WINK. 1995. Molecular phylogeny of the Papilionoideae (family Leguminosae): *rbc*L gene sequences versus chemical taxonomy. *Botanica Acta* 108: 149–162.

KÄSS, E., AND M. WINK. 1997. Phylogenetic relationships in the Papilionoideae (family Leguminosae) based on nucleotide sequences of cpDNA (*rbc*L) and ncDNA (ITS1 and 2). *Molecular Phylogenetics and Evolution* 8: 65–88.

KIM, S. C., D. J. CRAWFORD, J. FRANCISCO-ORTEGA, AND A. SANTOS-GUERRA. 1996. A common origin for woody *Sonchus* and five related genera in the Macaronesian islands: molecular evidence for extensive radiation. *Proceedings of the National Academy of Sciences, USA* 93: 7743–7748.

KLUGE, A. G., AND J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* 18: 1–32.

KORNHALL, P., N. HEIDARI, AND B. BREMER. 2001. Selagineae and Manuleeae, two tribes or one? Phylogenetic studies in the Scrophulariaceae. *Plant Systematics and Evolution* 228: 199–218.

KRUPKIN, A. B., A. LISTON, AND S. H. STRAUSS. 1996. Phylogenetic analysis of the hard pines (*Pinus* subgenus *Pinus*, Pinaceae) from chloroplast DNA restriction site analysis. *American Journal of Botany* 83: 489–498.

KUNKEL, G. W. H. 1991. Flora y vegetacion del archipiélago Canario: tratado florístico. Segunda parte: dicotiledoneas. Edirca, Las Palmas de Gran Canaria, Spain.

LISTON, A., W. A. ROBINSON, D. PINERO, AND E. R. ALVAREZ-BUYLLA. 1999. Phylogenetics of *Pinus* (Pinaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *Molecular Phylogenetics and Evolution* 11: 95–109.

LOBO, J. M., I. CASTRO, AND J. C. MORENO. 2001. Spatial and environmental determinants of vascular plant species richness distribution in the Iberian Peninsula and Balearic Islands. *Biological Journal of the Linnean Society* 73: 233–253.

S MACARTHUR, R. H., AND E. O. WILSON. 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey, USA.

MANEN, J.-F., M. C. BOULTER, AND Y. NACIRI-GRAVEN. 2002. The complex history of the genus *Ilex* L. (Aquifoliaceae): evidence from the comparison of plastid and nuclear DNA sequences and from fossil data. *Plant Systematics and Evolution* 235: 79–98.

MANOS, P. S., R. E. MILLER, AND P. WILKIN. 2001. Phylogenetic analysis of *Ipomoea*, *Argyreia*, *Stictocardia*, and *Turbina* suggests a generalized model of morphological evolution in morning glories. *Systematic Botany* 26: 585–602.

MARRERO, A., A. S. ALMEIDA, AND M. GONZALEZ-MARTIN. 1998. A new species of the wild dragon tree, Dracaena (Dracaenaceae) from Gran Canaria and its taxonomic and biogeographic implications. *Botanical Journal of the Linnean Society* 128: 291–314.

MEDAIL, F., AND P. QUEZEL. 1999. The phytogeographical significance of SW Morocco compared to the Canary Islands. *Plant Ecology* 140: 221–244.

MENDOZA-HEUER, I. 1971. Aportacion al conocimiento del genero *Convolvulus* en la zona Macaronesica. *Cuadernos de Botanica Canaria* 12: 22–34.

MENDOZA-HEUER, I. 1983. El genero *Convolvulus* en Macaronesia. In Comunicacoes apresentadas ao 2 congresso internacional pro flora Macaronesica, Funchal, 19–25 de Junho de 1977, 23–30. Congresso Internacional Pró Flora Macaronésica, Funchal, Portugal.

MOLERO, J., T. GARNATJE, A. ROVIRA, N. GARCIA-JACAS, AND A. SUSANNA. 2002. Karyological evolution and molecular phylogeny in Macaronesian dendroid spurges (*Euphorbia* subsect. *Pachycladae*). *Plant Systematics and Evolution* 231: 109–132.

MOORE, M. J., J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, AND R. K. JANSEN. 2002. Chloroplast DNA evidence for the roles of island colonization and extinction in *Tolpis* (Asteraceae: Lactuceae). *American Journal of Botany* 89: 518–526.

MORT, M. E., D. E. SOLTIS, P. S. SOLTIS, J. FRANCISCO-ORTEGA, AND A. SANTOS-GUERRA. 2001. Phylogenetic relationships and evolution of Crassulaceae inferred from *mat*K sequence data. *American Journal of Botany* 88: 76–91.

MORT, M. E., D. E. SOLTIS, P. S. SOLTIS, J. FRANCISCO-ORTEGA, AND A. SANTOS-GUERRA. 2002. Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. *Systematic Botany* 27: 271–288.

NIXON, K. C., AND J. M. CARPENTER. 1993. On outgroups. *Cladistics* 9: 413–426.

OLMSTEAD, R. G., AND J. D. PALMER. 1997. Implications for the phylogeny, classification and biogeography of *Solanum* from cp-DNA restriction site variation. *Systematic Botany* 22: 19–29.

PANERO, J. L., J. FRANCISCO-ORTEGA, R. K. JANSEN, AND A. SANTOS-GUERRA. 1999. Molecular evidence for multiple origins of woodiness and a New World biogeographic connection of the Macaronesian Island endemic *Pericallis* (Asteraceae: Senecioneae). *Proceedings of the National Academy of Sciences, USA* 96: 13886–13891.

PARK, S. J., E. J. KOROMPAI, J. FRANCISCO-ORTEGA, A. SANTOS-GUERRA, AND R. K. JANSEN. 2001. Phylogenetic relationships of *Tolpis* (Asteraceae: Lactuceae) based on *ndh*F sequence data. *Plant Systematics and Evolution* 226: 23–33.

PERCY, D. M., AND Q. C. B. CRONK. 2002. Different fates of island brooms: contrasting evolution in *Adenocarpus*, *Genista*, and *Teline* (Genisteae, Fabaceae) in the Canary Islands and Madeira. *American Journal of Botany* 89: 854–864.

PETER, A. 1891. Convolvulaceae. In A. Engler and K. Prantl [eds.], Die natürlichen pflanzenfamilien, vol. 4, part 3a, 1–40. Wilhelm Engelmann, Leipzig, Germany.

PINTO DA SILVA, A. R., AND A. N. TELES. 1981. *Convolvulus fernandesii* Pinto da Silva et Teles, um notavel endemismo portugues de origem macaronesica. *Boletim do Sociedade Broteriana* (Series 2)54: 233–237.

POWELL, E. A., AND K. A. KRON. 2002. Hawaiian blueberries and their relatives—a phylogenetic analysis of *Vaccinium* sections *Macropelma*, *Myrtillus* and *Hemimyrtillus* (Ericaceae). *Systematic Botany* 27: 768–779.

PRESS, J. R., AND M. J. SHORT. 1994. Flora of Madeira. HMSO (Her Majesty's Stationary Office), London, UK.

PRICE, J. P., AND D. A. CLAGUE. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society of London, Series B* 269: 2429–2435.

RAMBAUT, A. 1996. Se-Al, Sequence alignment editor version 1.0, alpha 1. University of Oxford, Oxford, UK.

ROBSON, N. B. K. 1977. Studies in the genus *Hypericum* L. (Guttiferae). 1. Infrageneric classification. *Bulletin of the British Museum of Natural History (Botany)* 5: 291–355.

RØNSTED, N., M. W. CHASE, D. C. ALBACH, AND M. A. BELLO. 2002. Phylogenetic relationships within *Plantago* (Plantaginaceae): evidence from nuclear ribosomal ITS and plastid *trnL-F* sequence data. *Botanical Journal of the Linnean Society* 139: 323–338.

SA'AD, F. 1967. The *Convolvulus* species of the Canary Islands, the Mediterranean region and the near and Middle East. *Medelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht* 281: 1–288.

SANTONI, S., AND A. BERVILLÉ. 1992. Characterization of the nuclear ribosomal DNA units and phylogeny of *Beta* L. wild forms and cultivated beets. *Theoretical and Applied Genetics* 83: 533–542.

SANTOS-GUERRA, A. 1999. Origen y evolución de la flora de Canarias. In J. M. Fernández-Palacios, J. J. Bacallado, and J. A. Belmonte [eds.], Ecología y cultura en Canarias, 107–129. Organismo Autónomo de Museos y Centros—Cabildo de Tenerife, Santa Cruz de Tenerife, Spain.

SANTOS-GUERRA, A. 2001. Flora vascular nativa. In J. M. Fernández-Palacios and J. L. Martín-Esquivel [eds.], Naturalez de las Islas Canarias. Ecología y conservación, 185–198. Publicaciones Turquesa, Santa Cruz de Tenerife, Spain.

SANTOS-GUERRA, A., AND J. FRANCISCO-ORTEGA. 1990. Endemismos macaronésicos en las Islas Canarias: estado de conservación. In J. E. Hernández-Bermejo, M. Clemente, and V. H. Heywood [eds.], Conservation techniques in botanic gardens, 191–196. Koeltz, Konigstein, Germany.

SCHÄFFER, H. 2003. Chorology and diversity of the Azorean flora. Dissertationes Botanicae Band 374. Borntraeger, Berlin, Germany.

SILVERTOWN, J. 2004. The ghost of competition past in the phylogeny of island endemic plants. *Journal of Ecology* 92: 168–173.

STEARN, W. T. 1969. Research: the scanning electron microscope. *Report on the British Museum (Natural History)* 1966–1968: 41–46.

STEFANOVIC, S., L. KRUEGER, AND R. G. OLMSTEAD. 2002. Monophyly of the Convolvulaceae and circumscription of their major lineages based on DNA sequences of multiple chloroplast loci. *American Journal of Botany* 89: 1510–1522.

SUN, Y., D. Z. SKINNER, G. H. LIANG, AND S. H. HULBERT. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32.

SUNDING, A. 1979. Origins of the Macaronesian flora. In D. Bramwell [ed.], Plants and islands, 13–40. Academic Press, London, UK.

SUSANNA, A., T. GARNATJE, AND N. GARCIA-JACAS. 1999. Molecular phylogeny of *Cheirolophus* (Asteracea: Cardueae-Centaureinae) based on ITS sequences of nuclear ribosomal DNA. *Plant Systematics and Evolution* 214: 147–160.

SWOFFORD, D. L. 1998. PAUP*: phylogenetic analysis using parsimony and other methods, version 4.0β8 for Macintosh PPC. Sinauer, Sunderland, Massachusetts, USA.

THIV, M., L. STRUWE, AND J. W. KADEREIT. 1999. The phylogenetic relationships and evolution of the Canarian laurel forest endemic *Ixanthus viscosus* (Aiton) Griseb. (Gentianaceae): evidence from *mat*K and ITS sequences, and floral morphology and anatomy. *Plant Systematics and Evolution* 218: 299–317.

TORRELL, M., N. GARCIA-JACAS, A. SUSANNA, AND J. VALLES. 1999. Phylogeny in *Artemisia* (Asteraceae, Anthemideae) inferred from nuclear ribosomal DNA (ITS) sequences. *Taxon* 48: 721–736.

TRUSTY, J., R. G. OLMSTEAD, D. J. BOGLER, A. SANTOS-GUERRA, AND J. FRANCISCO-ORTEGA. In press. Using molecular data to test a biogeographic connection of the Macaronesian genus *Bystropogon* (Lamiaceae) to the New World: a case of conflicting phylogenies. *Systematic Botany*.

TRUSTY, J. L., A. SANTOS-GUERRA, T. ERIKSSON, AND J. FRANCISCO-ORTEGA. 2001. Biodiversity, conservation, and molecular phylogenies in the laurel forest of the Canary Islands: the case of a critically endangered taxon of *Sambucus*. Botany 2001, Annual Meeting of the Botanical Society of America, August 12–16, 2001, Albuquerque, New Mexico. www.2001.botanyconference.org

VALCÁREL, V., O. FIZ, AND P. VARGAS. 2003. Chloroplast and nuclear evidence for multiple origins of polyploids and diploids of *Hedera* (Araliaceae) in the Mediterranean basin. *Molecular Phylogenetics and Evolution* 27: 1–20.

VAN HAM, R. C. H. J., AND H. T'HART. 1998. Phylogenetic relationships in the Crassulaceae inferred from chloroplast DNA restriction-site variation. *American Journal of Botany* 85: 123–134.

VARGAS, P., H. A. MCALLISTER, C. MORTON, S. L. JURY, AND M. J. WILKINSON. 1999a. Polyploid speciation in *Hedera* (Araliaceae): phylogenetic and biogeographic insights based on chromosome counts and ITS sequences. *Plant Systematics and Evolution* 219: 165–179.

VARGAS, P., C. M. MORTON, AND S. L. JURY. 1999b. Biogeographic patterns in Mediterranean and Macaronesian species of *Saxifraga* (Saxifragaceae) inferred from phylogenetic analyses of ITS sequences. *American Journal of Botany* 86: 724–734.

WARWICK, S. I., AND L. D. BLACK. 1993. Molecular relationships in subtribe Brassicinae (Cruciferae, tribe Brassiceae). *Canadian Journal of Botany* 71: 906–918.

WATSON, L. E., T. M. EVANS, AND T. BOLUARTE. 2000. Molecular phylogeny and biogeography of tribe Anthemideae (Asteraceae), based on chloroplast gene *ndh*F. *Molecular Phylogenetics and Evolution* 15: 59–69.

WEBB, P. B., AND S. BERTHELOT. 1840. Histoire naturelle des Iles Canaries: phytographia Canariensis, Section 3. Bureaux, Paris, France.

WENDEL, J. E., AND A. E. PERCIVAL. 1990. Molecular divergence in the Galapagos Islands-Baja California species pair *Gossypium klotzchianum* and *G. davidsonii*. *Plant Systematics and Evolution* 171: 99–115.

WHITE, T. J., T. BRUNS, S. LEES, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. Innis, D. Gelfanc, J. Sninsky, and T. White [eds.], PCR protocols: a guide to methods and applications, 315–322. Academic Press, San Diego, California, USA.

WHITTAKER, R. J. 1998. Island biogeography. Ecology, evolution, and conservation. Oxford University Press, Oxford, UK.