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[1]

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The Gonosperminae (Asteraceae) are composed of three genera endemic to the Canary Islands (*Gonospermum* Less., and *Lugoa* DC.) and southern Africa (*Inulanthera* Källersjö), and they are considered an example of a floristic link between these two regions. Phylogenetic analyses of ITS sequences reveal that the Canarian genera are not sister to *Inulanthera* and do not support the monophyly of the Gonosperminae. These results, coupled with previous phylogenetic studies of other groups, suggest that many of the putative biogeographic links between Macaronesia and southeast Africa need to be evaluated by rigorous phylogenetic analyses. *Inulanthera* forms part of the basal southern African radiation of the Anthemideae, and therefore it is closely related to other taxa from this region. Maximum likelihood and weighted parsimony analyses support a monophyletic group in the Canary Islands, that includes *Lugoa, Gonospermum,* and three *Tanacetum* species endemic to the island of Gran Canaria. Bootstrap support for the monophyly of this Canarian group is weak, and it collapses in the strict consensus tree based on unweighted parsimony. *Lugoa* is nested within *Gonospermum,* and both interisland colonization among the western islands of La Gomera, El Hierro, La Palma and Tenerife, and radiation on the central island of Gran Canaria have been the major patterns of species diversification for these Canarian endemics.

Key words: African flora; Asteraceae; biogeography; floristic disjunctions; long-distance dispersal; Macaronesia; Natal; oceanic islands; plant evolution.

The use of morphological data to trace the continental relatives of species restricted to oceanic islands is often difficult because insular endemics are usually distinct from their continental ancestors (Givnish, 1998). Good examples of extremely divergent insular-continental sister taxa include the three genera (i.e., *Argyroxiphium* DC., *Dubautia* Gaud., and *Wilkesia* A. Gray) of the silversword alliance in Hawaii (Baldwin et al., 1991) and *Argyranthemum* Sch. Bip. (Asteraceae) in the Canary Islands and Madeira (Francisco-Ortega et al., 1997a, b). These insular genera are woody and show extraordinary levels of morphological and ecophysiological variation as a result of isolation and adaptation to most of the insular ecosystems of Hawaii and Macaronesia (Baldwin, 1997; Francisco-Ortega et al., 1997a). However, molecular data have facilitated the identification of continental relatives of morphologically divergent genera endemic to many oceanic islands. Several excellent examples of the utility of molecular phylogenies include *Scalesia* Arn. (Asteraceae) in the Galapagos Islands (Schilling, Panero, and Eliasson, 1994), *Ixanthus* Griseb. (Gentianaceae) in the Canary Islands (Struwe et al., 1998; Thiv, Struwe, and Kadereit, 2000), and *Hesperomannia* A. Gray (Asteraceae) in the Hawaiian Islands (Kim et al., 1998).

Seven major volcanic islands, which are situated in close proximity to the western Sahara coast comprise the Canary Islands. It has been suggested that many of the endemic species on these islands have a recent origin from the Mediterranean (Francisco-Ortega et al., 1997a, 1999; Struwe et al., 1998; Carvalho and Culham, 1998; Mort et al., 2000; Thiv, Struwe, and Kadereit, in press). In contrast, other endemic species have been linked to the boreotropical flora that existed in most of the northern Hemisphere during the Tertiary (Quézel, 1978; Mai, 1989). However, some Canarian endemics have been associated with a xerophytic flora that existed in most of the Saharan-Sahel belt during the late Miocene and Pliocene (Quezel, 1978, 1983). It has been suggested that this flora, known as the Rand Flora, originated in southeastern parts of South Africa in the Paleocene (Quézel, 1978; reviewed by Marrero, Almedia, and González-Martín, 1998). The Rand Flora replaced the tropical and temperate rain forests

that existed in the Sahara-Sahel belt during the Paleocene and Oligo-Miocene, respectively (Quézel, 1978). The existence of this flora is supported by the large number of disjunct taxa distributed along a band stretching from the Namibia desert to the Western Sahara via east Africa (Hamilton, 1974; Jürgens, 1997).

The Gonosperminae are one of the 12 currently recognized subtribes of the Anthemideae (Asteraceae) (Bremer and Humphries, 1993), and provide one of the putative examples of disjunct distributions between the Canary Islands and South Africa (Heywood and Humphries, 1977; Källersjö, 1985; Bremer and Humphries, 1993). The geographical distribution of members of this subtribe appears to support the hypothesis of a Tertiary link between the Canarian and the Rand Floras. The Gonosperminae (Table 1) are composed of two genera endemic to the Canary Islands [i.e., *Gonospermum* (four species) and *Lugoa* (one species)] and *Inulanthera*. The latter genus has ten species restricted to southern Africa (Table 1), although most of the species occur mainly in the Natal-Transvaal zone in northeastern South Africa. *Inulanthera* was formerly included in the South African genus *Athanasia*. However, it was elevated to generic level on the basis of chemical and morphological evidence (Källersjö, 1985).

Gonospermum fruticosum is widespread and occurs on three Canary Islands (i.e., La Gomera, El Hierro, and Tenerife). In contrast, *G. canariense, G. gomerae, G. elegans,* and *L. revoluta* are restricted to one island each on La Palma, La Gomera, El Hierro, and Tenerife, respectively. Two of the species (i.e., *G. elegans* and *G. canariense*) occur primarily in the pine forest (*Chamaecytiso angustifolii-Pinetea canariensis* Rivas Goday & Esteve ex Sunding). *Gonospermum canariensis* is relatively common on La Palma on northern and southern slopes, whereas *G. elegans* is rare and restricted to the low-altitude dry pine forests of southeastern El Hierro. The remaining species of the Gonosperminae are mainly found on northern slopes of the lowland scrub zone (*Oleo-Rhamnetea crenulatae* A. Santos). *Lugoa revoluta* occurs only on the northeastern tip of Tenerife, where it also thrives in the lowland scrub but at low elevation in areas close to the coastal and xerophytic belt [*Kleinio-Euphorbietea canariensis* (Rivas Goday & Esteve) A. Santos]. In contrast, *G. gomerae* tends to occur at higher elevation in subhumid zones that are closer to the laurel forest and heath belt (*Pruno-Lauretea azoricae* Oberdorfer ex Rivas-Martínez, Arnáiz, Barreno & A. Crespo), although the species is never found within the limits of the laurel forest. This species is not abundant and is only found in scattered populations on northeastern zones of La Gomera.

A cladistic analysis of the Anthemideae using morphological, cytological and chemical traits provided phylogenetic support for the monophyly of the Gonosperminae (Bremer and Humphries, 1993). A link between the Canarian and southern Africa genera was previously suggested by Heywood and Humphries (1977) and Källersjö (1985) on the basis of inflorescence, leaf, and cypsela characters. However, chemical studies of acetylenes supported a close relationship between the endemic Canarian genera and *Tanacetum* rather than with any southern African genera (Bohlmann, Burkhardt, and Zdero, 1973). Such a relationship was also suggested by Bremer and Humphries (1993), who indicated that it was likely that the Gonosperminae were related to part of the Tanacetinae.

Tanacetum, with ∼150 species, is one of the most poorly understood genera of Anthemideae (Heywood and Humphies, 1977; Bremer and Humphries, 1993). Three species of *Tana-*

cetum are endemic to the island of Gran Canaria. *Tanacetum oshanahanii* is extremely rare, with only 15 plants known from a single population on the northern slopes of the lowland scrub of Gran Canaria (Marrero, Febles, and Suárez, 1989). *Tanacetum ptarmiciflorum* is also extremely rare and is known only from two populations of the high-altitude pine forest of central Gran Canaria (Febles and Naranjo-Suárez, 1996a). *Tanacetum ferulaceum* occurs on southern slopes of the lowland scrub of Gran Canaria where scattered populations with few plants have been reported (Febles and Naranjo-Suárez, 1996b); however *T. ferulaceum* var. *latipinnum* (Svent.) G. Kunkel is restricted to northern slopes of this major ecological zone. Karyological, seed protein, and morphological studies also suggested that the Canarian *Tanacetum* species are so closely related to *Lugoa* and *Gonospermum* that they could be merged into a single endemic genus (Febles, Fernández-Peralta, and González-Aguilera, 1989a, b; Febles, 1990, 1996).

In a previous study (Francisco-Ortega et al., 1997b), we presented a molecular phylogeny of 32 genera of the Anthemideae using the internal transcribed spacers (ITS) of the nuclear ribosomal repeat (nrDNA). The ITS tree indicated that most taxa restricted to Europe, the Mediterranean, Macaronesia, and East Asia formed a strongly supported monophyletic group. Basal to this "Eurasian Clade" were several taxa from South Africa and the Far East. We also showed that none of the taxa of the "Eurasian Clade" has a 17–19 bp insertion in the ITS2 region. However, low bootstrap values did not enable the establishment of well-supported groups among most of the genera of the "Eurasian Clade."

In this paper, we present an ITS phylogeny of *Athanasia, Gonospermum, Inulanthera, Lugoa,* and a selection of Canarian, Eurasian, North American, and Mediterranean species of *Tanacetum* as well as several additional genera of the Anthemideae. The first objective of this study was to construct a phylogeny of the Gonosperminae to determine whether the Canarian taxa are more closely related to Eurasian species of *Tanacetum* and other Eurasian species, or whether they represent a biogeographic link to southern African through *Inulanthera*. The second objective was to reconstruct the evolutionary history of the insular endemics.

MATERIALS AND METHODS

Plant material—Thirty-seven species were included in the analysis (Table 1). The ingroup included all currently recognized species of *Gonospermum* and *Lugoa,* with multiple samples of the more common *G. fruticosum* from La Gomera (one population), El Hierro (two populations), and Tenerife (two populations). The type species of *Inulanthera* (i.e., *I. calva*) and *Athanasia* (i.e., *A. crithmifolia*), the three Canarian species of *Tanacetum,* and a selection of eight continental species of *Tanacetum* were also part of the ingroup. This selection of 11 *Tanacetum* species included a diverse geographic distribution of *Tanacetum,* including the type species (i.e., *T. vulgare*) plus species from Eurasia, the Mediterranean, and one species from the New World. The ingroup also included the South African *Cymbopappus,* because previous studies showed that the genus is sister to the Eurasian clade of the tribe (Francisco-Ortega et al., 1997b; Watson, Evans, and Boluarte, 2000). Thirteen species of ten Eurasian genera (i.e., *Achillea, Anthemis, Argyranthemum, Matricaria, Otospermum, Phalacrocarpum, Prolongoa, Rhodanthemum, Santolina, Tripleurospermum*) were also part of the ingroup (Francisco-Ortega et al., 1997b; Table 1). *Ursinia* was selected as the outgroup because both morphological (Bremer and Humphries, 1993) and molecular data (Kim and Jansen, 1995; Watson, Evans, and Boluarte, 2000) place it in a basal position in the Anthemideae.

TABLE 1.   List of taxa and source of plant material. Vouchers are deposited at ORT, except where indicated. Botanic gardens and herbaria are coded according to Heywood, Heywood, and Jackson (1990) and Holmgren, Holmgren, and Barnett (1990). Number of species, according to Bremer and Humphries (1993), in each genus is indicated in parentheses. Type species are indicated by "*". Isolations from material preserved in silica gel are indicated by ¶.

Taxon	Voucher and germplasm origin	Geographical distribution of taxa	GenBank accession no. (ITS1/ITS2)[a]
Achillea L. (115)		Europe and temperate Asia	
A. millefolium L.	From local nursery, Kim 91-207, TEX	Europe and Asia,	GBAN-AF155265/AF155302
Anthemis L. (211)		Europe, Asia, and N. Africa	
A. arvensis L.	Ex. hort at TEX from germplasm collection of NCY (#466), TEX	Europe, N. Africa, and W. Asia	GBAN-AF155266/AF155303
A. cretica L.	Ex. hort at TEX from germplasm collection of NCY (#1148), TEX	Mediterranean, Middle East, and Iran	GBAN-AF155267/AF155304
A. maritima L.	Ex. hort at TEX from germplasm collection of BORD (#10/92), TEX	SW Europe and N. Africa	GBAN-AF155268/AF155305
A. tinctoria L.	Ex. hort at TEX from germplasm collection of TO (s.n.), TEX	Europe, W. and C. Asia	GBAN-AF155269/AF155306
Argyranthemum Sch. Bip. (24)		Canary Islands and Madeira	
A. foeniculaceum (Willd.) Sch. Bip.	Ex. hort at TEX from germplasm collection of ORT (ORT-9), TEX	Tenerife	GBAN-AF155270/AF155307
Athanasia L. (39)		South Africa	
A. crithmifolia (L.) L.	Ex. hort at ORT from germplasm collection of NBG (s.n.), Franc.-Ort., 97-73, TEX	South Western Cape Region	GBAN-AF155241/AF155278
Cymbopappus B. Nord. (4)		South Africa	GBAN-AF155242/AF155279
C. adenosolen (Harvey) B. Nord.	Cape province, South Africa, L. Watson 94-79, MU	Cape Region	
Gonospermum Less. (4)		Canary Islands	
¶*G. canariense* (DC.) Less.	Mazo, La Palma, A. Santos & Franc. Ort. 97-51	La Palma	GBAN-AF155243/AF155280
¶*G. elegans* (Cass.) Less.	Hesperillas, El Hierro, A. Santos & Franc. Ort. 97-53	El Hierro	GBAN-AF155244/AF155281
*¶*G. fruticosum* (Link) Less.	La Vega, La Gomera, A. Santos & Franc. Ort. 97-28	El Hierro, La Gomera, and Tenerife	GBAN-AF155245/AF155282
	Frontera, El Hierro, A. Santos & Franc. Ort. 97-45		GBAN-AF155249/AF155286
	Mocanal, El Hierro, A. Santos & Franc. Ort. 97-47		GBAN-AF155248/AF155285
	Güimar, Tenerife, A. Santos & Franc. Ort. 97-49		GBAN-AF155246/AF155283
	El Palmar, Tenerife, A. Santos & Franc. Ort. 97-107		GBAN-AF155247/AF155284
¶*G. gomerae* Bolle	Agulo, La Gomera, A. Santos & Franc. Ort. 97-44	La Gomera	GBAN-AF155250/AF155287
Inulanthera Källersjö (10)		South Africa, Lesotho, Angola, Zimbabwe, and Madagascar	
*¶*I. calva* (Hutch.) Källersjö	Drakensberg, Natal, C. Carbutt 419, TEX	Natal, Transvaal, and Swaziland	GBAN-AF155251/AF155288
Logoa DC. (1)		Canary Islands	
*¶*L. revoluta* (Link) DC.	Taganana, Tenerife, Franc.-Ort. 98-24, FTG	Tenerife	GBAN-AF155252/AF155289
Matricaria L. (7)		N. hemisphere	
M. recutita L.	Ex. hort at ORT from seeds provided by J.L. Hudson Seedsman (#MATI2), Redwood City, California, TEX	Europe and temperate Asia	GBAN-AF155271/AF155308
Otospermum Willk. (1)		Spain, Portugal, Morocco, Algeria, and Tunisia	
O. glabrum (Lag.) Willk.	Casaseca et al., 10-Abril-1980, SALA	Spain, Portugal, Morocco, Algeria, and Tunisia	GBAN-AF155272/AF155309
Rodanthemum R.H. Wilcox, K. Bremer & Humphries (12)		Spain, Morocco, and Algeria	
R. gavanum (Cosson & Durieu) R.H. Wilcox, K. Bremer & Humphries	Ex. hort at TEX from seeds provided by Town Farm Nursery (s.n.), Cleveland, England, TEX	Morocco	GBAN-AF155275/AF155312
Phalacrocarpum (DC.) Willk.		Spain and Portugal	
P. hoffmannseggii (Brot.) Lainz (2)	Casaseca, 11-Abril-1977, SALA	Spain and Portugal	GBAN-AF155273/AF155310
Prolongoa Boiss. (1)		Spain	
P. hispanica G. López & C.E. Jarvis	Rico et al., 8-Julio-1985, SALA	Spain	GBAN-AF155274/AF155311

TABLE 1.   Continued.

Taxon	Voucher and germplasm origin	Geographical distribution of taxa	GenBank accession no. (ITS1/ITS2)[a]
Santolina L. (8)		Mediterranean	
*S. chamaecyparissus L.	Ex. hort at ORT from seeds provided by J.L. Hudson Seedsman (s.n.); Redwood City, California, TEX	Mediterranean	GBAN-AF155276/AF155313
Tanacetum L. (152)		Mediterranean, Eurasia, and N. America	
T. cinerariifolium (Trevir.) Sch. Bip.	Ex. hort at ORT from germplasm collection of BESN (s.n.), Franc.-Ort. 98-231	SE and E. Europe, Caucasus, Asia from Turkey to C. Asia, and China	GBAN-AF155253/AF155290
T. corymbosum (L.) Sch. Bip.	Ex. hort at ORT from germplasm collection of GOET (s.n.), Franc.-Ort. 98-165	Morocco, Algeria, most of Europe except N. parts, Turkey, Caucasus, and Central Asia	GBAN-AF155254/AF155291
¶*T. ferulaceum* (Webb) Sch. Bip.	Ex. hort at ORT from germplasm collection of LPA (s.n.), Franc.-Ort. 97-74	Gran Canaria	GBAN-AF155255/AF155292
T. huronense Nutt.	Ex. hort at ORT from germplasm collection of ALTA (s.n.), Franc.-Ort. 98-368	Alaska, Canada, and Michigan	GBAN-AF155256/AF155293
T. macrophyllum (Waldst. & Kit.) Sch. Bip.	Ex. hort at ORT from germplasm collection of GOET (s.n.), Franc.-Ort. 98-166	Central and SE Europe, Turkey, and Caucasus	GBAN-AF155257/AF155294
T. microphyllum DC.	Ex. hort at TEX from germplasm collection of MA (s.n.), TEX	Spain and Portugal	GBAN-AF155258/AF155295
T. oshanahanii Marrero Rodr., Febles & C. Suárez	Ex. hort at ORT from germplasm collection of LPA (s.n.), Franc.-Ort. 97-94	Gran Canaria	GBAN-AF155259/AF155296
T. parthenium (L.) Sch. Bip.	Ex. hort at ORT from germplasm collection of GOET (s.n.), Franc.-Ort. 98-167	N. Africa, SE and E. Europe, SW Asia from Turkey to Caucasus, Iran, Afghanistan, and C. Asia	GBAN-AF155261/AF155297
T. pseudoachillea Winkler.	Ex. hort at ORT from germplasm collection of BESN (s.n.), Franc.-Ort. 98-234	C. Asia	GBAN-AF155260/AF155300
T. ptarmiciflorum (Webb) Sch. Bip.	Ex. hort at ORT from germplasm collection of LPA (#303), Franc.-Ort. 97-65	Gran Canaria	GBAN-AF-155262/AF155298
*T. vulgare L.	Kladno, Czech Republic, Bohuslávek 989, TEX	Most of Europe and temperate Asia	GBAN-AF155263/AF155299
Tripleurospermum Sch. Bip. (38)		Europe and temperate Asia	
T. perforatum (Mérat) Lainz	Ex. hort at TEX from seeds provided by Thompson & Morgan, Inc. (#6131), Franc.-Ort. TRIPLE, TEX	Europe and temperature Asia	GBAN-AF155277/AF155314
Ursinia Gaertn. (114)		South Africa, Namibia, Botswana, and Ethiopia	
U. anthemoides (L.) Poir.	Ex. hort at TEX from seeds provided by Thompson & Morgan, Inc. (#7319), Franc.-Ort. URSANTH, TEX	South Africa	GBAN-AF155264/AF155301

[a] The prefix GNAN- has been added to link the online version of *American Journal of Botany* to GenBank, but is not part of the actual accession number.

DNA isolation, PCR amplification, and sequencing—Most of the total genomic DNAs were isolated from plants grown in the greenhouses of TEX or ORT. Isolations of some taxa used plant material collected from wild populations and stored in silica gel (Table 1) or from herbarium specimens (i.e., *Tanacetum vulgare*). The CTAB (cetyltrimethylammonium bromide) technique of Doyle and Doyle (1987) was used, followed by purification by ultracentrifugation in CsCl-ethidium bromide gradients. Strategies for PCR (Polymerase Chain Reaction) amplification followed protocols previously used with members of the tribes Anthemideae (Francisco-Ortega et al., 1997b) and Inuleae (Francisco-Ortega et al., 1999). PCR products were purified using the QIA-quick PCR purification kit (Qiagen Inc., Valencia, California, USA) and automated sequencing followed the methods outlined in Francisco-Ortega et al. (1999).

Sequence aligment and phylogenetic reconstructions—Boundaries of both

ITS1 and ITS2 were determined by comparison to published sequences (Baldwin, 1992; Francisco-Ortega et al., 1997b). Multiple alignment of ITS sequences was performed using CLUSTAL X (Thompson et al., 1997) with minor manual adjustments made. All phylogenetic analyses were performed using version 4.0d64 of PAUP* (Swofford, 1999) with gaps treated as missing. In an additional analysis, gaps were also treated as separate binary characters (absence/presence), which did not effect the resulting phylogenies (data not shown).

An initial phylogenetic reconstruction was done with weighted parsimony analyses using transition:transversion ratios (ts/tv) estimated from the Maximum Likelihood (ML) analysis (see below, ts/tv = 1.9) and weights of 1.0 (unweighted parsimony), 1.1, 1.5, and 2.0 (Swofford et al., 1996). All parsimony analyses were undertaken using heuristic searches with ACCTRAN, MULPARS, and the TBR options of PAUP using 100 random entries (Maddison, 1991). Support for monophyletic groups was evaluated using 1000

bootstrap replicates (Felsenstein, 1985) with ACCTRAN, MULPARS, and TBR options employed in a heuristic search using one random addition sequence of taxa. Limitations of computer memory necessitated restricting the maximum numbers of trees saved to 2000 in each replicate. Consistency Index (CI) (Kluge and Farris, 1969) and Retention Index (RI) (Farris, 1989) were also computed.

An additional phylogenetic reconstruction was obtained by ML method using the HKY85 model (Hasegawa, Kishino, and Yano, 1985) with empirical base frequencies. Among-site rate variation was assessed by the Γ-distribution model with estimation of shape parameter. The model for nucleotide substitution was based on estimation of the transition:tranversion ratios. Due to limitations in computer memory, the ML search was undertaken with a reduced number of taxa, including all species of *Gonosperminae* and *Tanacetum* and all taxa from South Africa. A heuristic search with a single random entry and the ACCTRAN, MULPARS, and TBR was calculated. Weighted (weights of 1.1, 1.5, and 2.0) and unweighted parsimony analyses were also undertaken with this reduced data set. Heuristic searches and estimation of bootstrap supports for these maximum parsimony analyses were calculated, as indicated above, for the data matrix which included all the taxa. The goals of this second analysis were to test the monophyly of the Canary Island taxa and to reconstruct their evolutionary history.

RESULTS

Our previous ITS phylogeny of Anthemideae identified an indel of 17–19 bp in ITS2 (Francisco-Ortega et al., 1997b), which is also present in the new taxa sequenced for this study. All species from southern Africa have this insertion, which is absent from the remaining taxa of the tribe. An aligned data matrix has been deposited in FTG and is available upon request from the senior author.

Phylogenetic analyses—The strict consensus trees based on the four weighted parsimony analyses have identical topologies (Fig. 1). There is a strong correlation between the geographical distribution of the taxa and this topology. Southern African species are in a basal position, while all Eurasian species and the Canarian members of the Gonosperminae are in a derived position sister to the South African genus *Cymbopappus* (bootstrap support of 92%). The ITS phylogeny suggests that the Gonosperminae are not monophyletic, and that the subtribe does not represent a biogeographic link between southern Africa and the Canary Islands. The Canarian Gonosperminae appear more closely related to Canarian *Tanacetum* species than to *Inulanthera*. The Canary Island taxa of *Tanacetum* and the Gonosperminae form a monophyletic lineage although this group is not strongly supported by the bootstrap value (40%). Three major lineages are evident in the Canary Islands, including the basalmost lineage of the three *Tanacetum* species endemic to Gran Canaria. The second lineage includes two *Gonospermum* species endemic to La Palma and El Hierro while *G. fruticosum, G. gomerae,* and monotypic *Lugoa* form the third lineage.

The major difference between the tree topologies from the weighted and unweighted parsimony analyses concerns the monophyly of the Canary Island species. Trees based on unweighted parsimony do not support the monophyly of the island taxa, whereas all weighted analyses weakly support monophyly of this group (Fig. 1). The parsimony analyses do not resolve the identity of the continental relatives of the Canarian endemics, with most of the branches having low bootstrap support and collapsing in the strict consensus tree using unweighted parsimony.

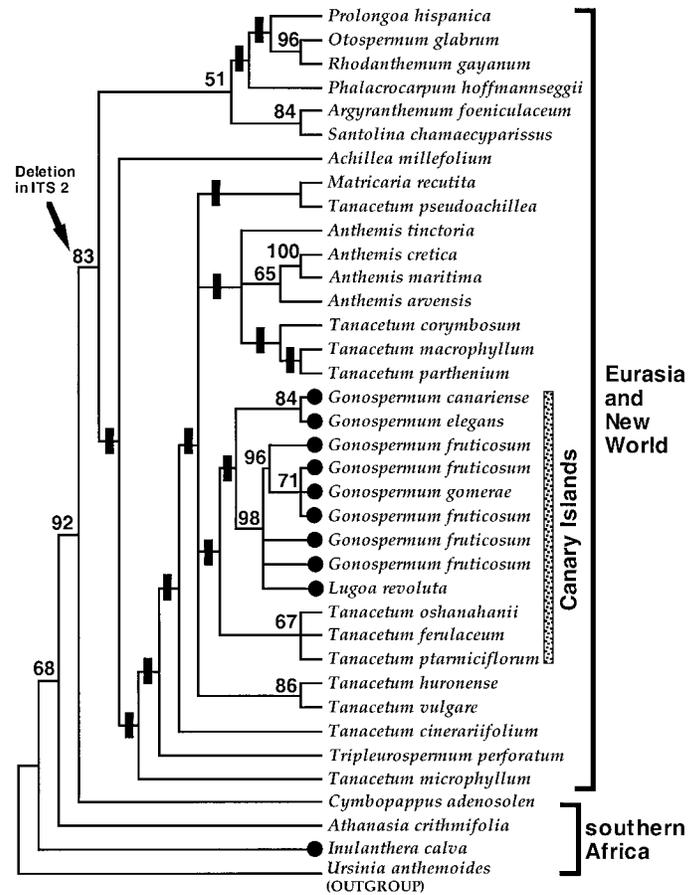The ML analysis of the data set that includes all species of

Fig. 1. Strict consensus tree of 1637 ITS trees (817 steps, CI = 0.5141, excluding autapomorphies; RI = 0.5361) obtained after weighted parsimony analysis of 37 taxa of the Anthemideae. Tranversions were weighted over transitions by a factor of 1.9:1.0. Bootstrap values higher than 50% are indicated above each branch. Branches that collapse in the strict consensus tree of 52 000 trees (601 steps, CI = 0.5077, excluding autapomorphies; RI = 0.5235) and that were obtained using unweighted parsimony are indicated with a solid bar. Closed circles indicate those species that belong to subtribe Gonosperminae. The two major geographical groups are also indicated.

Gonosperminae, Tanacetum, and representative South African Anthemideae yields one tree with a −Ln likelihood of 2182.447 (not shown). The transition:transversion ratio is 1.9, and the Γ distribution has a shape parameter of 0.71. This tree has a similar topology to the strict consensus trees based on weighted parsimony (Fig. 2). The Canarian taxa form a monophyletic group with three major clades which is weakly supported (54% bootstrap).

DISCUSSION

Taxonomic implications—The ITS data do not support the monophyly of the Gonosperminae. This study, combined with previous molecular phylogenies that included other subtribes of the Anthemideae (Francisco-Ortega et al., 1997b; Watson, Evans, and Boluarte, 2000), suggests that the current subtribal classification of Bremer and Humphries (1993) must be re-evaluated. These molecular phylogenies of the Anthemideae indicate that Chrysantheminae is the only monophyletic subtribe. The lack of support for the current subtribal classification of the Anthemideae is also apparent in molecular phylogenies
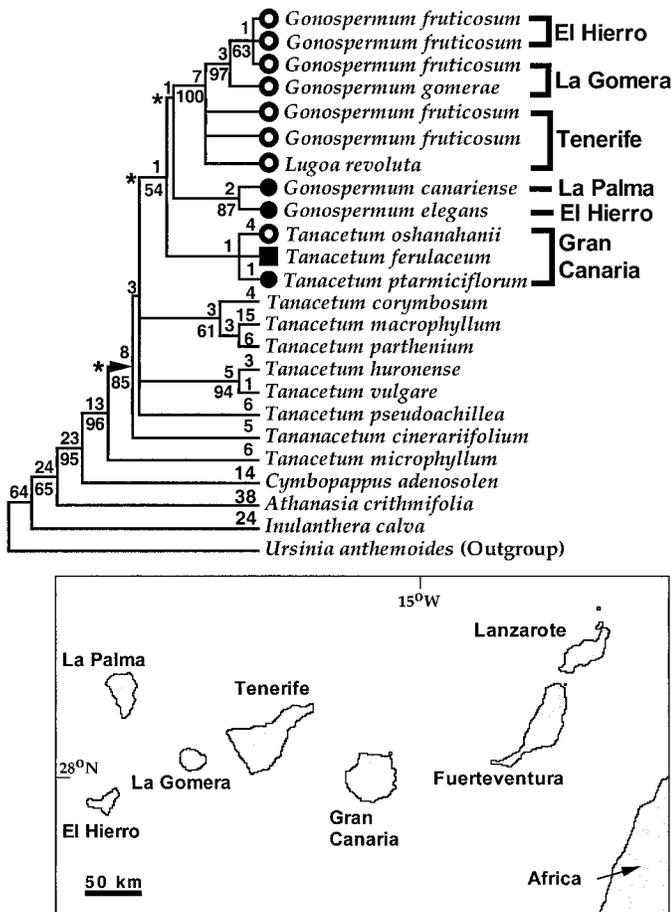
Fig. 2. One of the 1404 shortest ITS trees from the unweighted parsimony analysis (301 steps, CI = 0.636, excluding autapomorphies; RI = 0.700) of all species of Gonosperminae, *Tanacetum* and South Africa. The three branches that collapse in the strict consensus tree are indicated by asterisks. The branch that collapses in the four weighted parsimony analyses is indicated by an arrow. Numbers of changes are indicated above each branch. Bootstrap values higher than 50% are indicated below each branch. Insular distribution of Canarian endemics is indicated. Predominant ecological zones for each of the Canarian endemics are coded as follows: closed circle = pine forest; open circle = lowland scrub with a northern orientation and under the influence of the NE trade winds, closed square = lowland scrub with a southern orientation and not under the influence of the NE trade winds. A map of the Canary Islands is also shown.

using the chloroplast gene *ndhF* and a wider taxonomic sampling of taxa (Watson, Evans, and Boluarte, in press).

One major taxonomic implication of the ITS phylogeny is that the morphological synapomorphies (i.e., leaves large with many rounded lobes and pappus of teeth projected from the ribs) of the Gonosperminae are either plesiomorphic to the Canary Island taxa or they have originated twice in taxa from distant geographic regions. However, detailed morphological studies of the Canarian taxa of the Gonosperminae reveal that there is considerable variation for these traits (R. Febles, Jardín Botánico Viera y Clavijo, unpublished data). *Gonospermum gomerae* does not have leaves with rounded lobes. Cypselas without a pappus are found in individuals of *G. fruticosum* from El Hierro and in *G. canariense* and in *G. elegans*. Both *Inulanthera* and *Gonospermum* have their capitula in corymbs, a feature that has also been used to suggest a morphological connection between the Canarian and South African members

of the Gonosperminae (Källersjö, 1985). This trait, however, is extremely variable in the tribe and seems to be of little taxonomic value for assessing intergeneric or intertribal relationships.

The ITS phylogeny does not support the Canary Island endemic *Lugoa* as a distinct genus, because it is nested within *Gonospermum*. This placement agrees with morphological data; both genera have scales at the base of each floret. The major morphological difference between these genera is that *Lugoa* has radiate capitula, whereas those of *Gonospermum* are discoid. The endemic species of *Tanacetum* also have radiate capitula. However, this character has been demonstrated to be highly plastic in many Asteraceae genera, and is controlled by a single gene (Ford and Gottlieb, 1990).

Biogeographic implications—There are several examples of plant groups with a disjunct distribution between the Macaronesian islands and southern or eastern Africa. Morphological comparisons suggested that these taxa are natural groups and that they represent floristic links between the Macaronesian archipelagos and Africa (reviewed in Bramwell, 1972, 1978, 1985; Sunding, 1979). The best known examples include: *Echium* L. (Boraginaceae) from Macaronesia and *Lobostemum* Lehm. (Boraginaceae) from South Africa (Bramwell, 1973; Pérez de Paz and Pardo, 1994); the Canarian endemic *Phyllis* L. (Rubiaceae) and the predominantly South African genera *Anthospermum* L. and *Galopina* Thunb. (Rubiaceae) (Puff, 1982); *Parolinia* Webb (Brassicaceae) from the Canaries and the mostly east African *Diceratella* Boiss. (Brassicaceae) (Bramwell, 1985); and the three Canarian genera of the *Bencomia* Webb & Berthel. alliance (Rosaceae) (i.e., *Bencomia, Dendriopoterium* Svent., and *Marcetella* Svent.), which have been associated with arborescent east African genera of the tribe Sanguisorbeae (i.e., *Hagenia* J.F. Gmel. and *Cliffortia* L.) (Bramwell, 1985). However, few phylogenetic analyses have been produced that test these putative relationships between the Canary Islands and Africa. Two recent chloroplast DNA (cpDNA) phylogenies within the Rubiaceae (Bremer, 1996; Andersson and Rova, 1999) support a relationship between the Canarian endemic genus *Phyllis* and *Anthospermum, Galopina* and *Nenax* Gaertn.

In other cases, molecular phylogenies do not support putative biogeographic connections between the Canary Islands and south or east Africa. A combined ITS and cpDNA phylogeny of *Echium* strongly supports a close relationship between the Macaronesian and predominantly Mediterranean species of this genus (Böhle, Hilger, and Martin, 1996) rather than to the South African genus *Lobostemum* Lehm. (Bramwell, 1973; Pérez de Paz and Pardo, 1994). The origin of the *Bencomia* alliance provides another example in which a relationhip between the floras of Macaronesia and distant regions of Africa is not supported by phylogenetic studies. The three endemic genera in the Canary Islands are part of a clade formed mostly by Mediterranean species of *Sanguisorba* L. and *Sarcopoterium* Spach. (Helfgott et al., 2000).

We are not suggesting that biogeographical links between the floras of the Macaronesian islands and south and east Africa do not exist. However, in many cases these putative relationships are not based on a phylogenetic framework and, therefore, may not reflect true biogeographical connections. An example of the utility of phylogenetic studies for identifying floristic affinities between the Macaronesian islands and east Africa is provided by the two Canarian endemic species

of *Solanum* L. (Solanaceae) (i.e., *S. lidii* Sunding and *S. vespertilio* Aiton). Morphological characters place these two taxa with the Mexican species *S. tridynamum* Dunal [*Solanum* sect. *Nycterium* (Vent.) Dunal], suggesting a floristic connection between the New World and Macaronesia (Whalen, 1984). However, a cpDNA restriction site phylogeny (Olmstead and Palmer, 1997) showed that the Canarian taxa are sister to east African *Solanum*, providing one of the clearest cases of a biogeographical link between Macaronesia and East Africa.

Interisland colonization and adaptive radiation—Very few studies of Macaronesian plants have identified monophyletic groups of species restricted to single islands (Francisco-Ortega et al., 1996a, b; Kim et al., 1996; Panero et al., 1999; Mort et al., in press). In most cases, interisland colonization between similar ecological zones has been the primary pattern of species diversification including the *Aeonium* Webb & Berthel. alliance (Crassulaceae), *Argyranthemum*, *Crambe* L. (Brassicaceae), *Pericallis* D.Don (Asteraceae), and the woody *Sonchus* L. alliance (Asteraceae). The only exceptions are in *Argyranthemum* and the woody *Sonchus* alliance in Madeira, where monophyletic assemblages of species occur on the same island but in different ecological zones. The Madeiran archipelago is situated ~1000 km north of the Canaries, making dispersal between these two archipelagos much more difficult than among the Canary Islands. Therefore, it is not surprising that the Madeiran endemics of *Argyranthemum* and *Sonchus* form distinct monophyletic groups.

The ITS phylogeny of *Gonospermum, Lugoa,* and the Canarian species of *Tanacetum* enables an evaluation of the relative importance of interisland colonization and insular radiation in the evolution of Canary Island endemics. The three species of *Tanacetum* from the central island of Gran Canaria show insular radiation into three major ecological zones (Fig. 2). This is the first known case of a monophyletic plant group which has experienced several ecological shifts on a single island of the Canarian archipelago. In contrast, populations of *Gonospermum* and *Lugoa* from the western islands of La Gomera, El Hierro, La Palma, and Tenerife are correlated with major ecological zones (Fig. 2). The two endemics of the pine forest of La Palma and El Hierro form a monophyletic group. This pattern is also evident for the three lowland scrub species from different islands. Thus, the major mode of species diversification in the western Canary Islands is interisland colonization between the same ecological zones.

Within the broad limits of the lowland scrub, there is some degree of ecological specialization. *Lugoa* primarily occurs at low-elevation stands of this major ecological zone, but is restricted to the northeastern peninsula of Anaga. In contrast, *G. gomerae* tends to grow at higher elevations, and scattered populations are found in northeastern La Gomera. Likewise *G. elegans* is associated with the low-elevation dry pine forests of southern El Hierro, whereas *G. canariensis* has a broader ecological distribution and occurs in both the dry and subhumid pine forests of La Palma.

Conclusions—Molecular phylogenies are providing new perspectives to our understanding of the origin and evolution of the Macaronesian flora. The disjunct distribution between Macaronesia-northwest Africa and east Africa for many plant groups [e.g., *Aeonium, Campylanthus* Roth (Scrophulariaceae), *Canarina* L. (Campanulaceae), *Erucastrum* C. Presl. (Brassicaceae), *Euphorbia balsamifera* (Euphorbiaceae), *Hemicrambe fruticulosa* Webb (Brassicaceae), and ''draco tree'' *Dracaena* (Dracaenaceae)] clearly indicate that there are floristic links between the Canaries and distant regions of Africa (Sunding, 1979; Bramwell, 1985). Molecular phylogenies have confirmed these biogeographical patterns in some groups, but have rejected it for others. These phytogeographic connections cannot be extended to other elements of the flora until phylogenetic studies include all of the putative continental relatives. The same kind of generalization cannot be made regarding the evolutionary history of Macaronesian endemics. We have shown that the Canarian endemics of *Gonospermum, Lugoa* and *Tanacetum* have experienced both adaptive radiation (*Tanacetum*) and interisland colonization (*Gonospermum-Lugoa*). This pattern has also been reported for the Canarian and Madeiran species of *Argyranthemum* and *Sonchus* (Kim et al., 1996; Francisco-Ortega et al., 1997a). In *Argyranthemum,* hybridization has also been an important evolutionary process (Francisco-Ortega et al., 1997a).

In other groups such as the *Aeonium* alliance (Crassulaceae) (Uhl, 1961; Bramwell, 1977; Liu, 1989), *Dactylis glomerata* L. (Poaceae) (Lumaret, 1997), *Lotus* L. subgen. *Pedrosia* Lowe (Fabaceae) (Ortega, 1976), *Sideritis* L. (Lamiaceae) (Marrero, 1992), and *Tolpis* Adanson (Jarvis, 1980) changes in chromosome numbers also have been reported; however, this represents a small fraction of the endemic flora of the islands. Although chromosomal changes have played a role in the evolution of some groups, adaptive radiation, interisland colonization, and hybridization appear to be more important in species diversification of the endemic Macaronesian flora. The emerging picture from molecular phylogenies is that each group has unique evolutionary patterns in the islands. These patterns or modes can only be revealed after rigorous phylogenetic, morphological, ecological, and cytological studies.

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