

# Molecular Phylogeny and Biogeography of Tribe Anthemideae (Asteraceae), Based on Chloroplast Gene *ndhF*

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**Anthemideae (Asteraceae) is primarily a north temperate, Old World tribe of 109 genera and approximately 1740 species. We sequenced a 1200-bp portion of chloroplast gene *ndhF* for representative genera and subtribes and constructed a phylogeny for the tribe. There is support for monophyly of subtribes Chrysantheminae and Gonosperminae and for portions of some subtribes. However, our molecular phylogeny differs significantly from traditional classifications and from previously published morphological phylogenies of the tribe. Many South African genera from several different subtribes form a basal grade, indicating multiple, relictual lineages. Eurasian genera form a recently derived clade that includes the Mediterranean genera of the Iberian Peninsula and North Africa. There is little resolution or support for the placement of eastern Asian genera. Apparently, the tribe originated in the Southern Hemisphere, presumably in Africa, with the Eurasian and Mediterranean members being derived from a common ancestor.** © 2000 Academic Press

## INTRODUCTION

Anthemideae (Asteraceae, subfamily Asteroideae) is a tribe of 109 genera and 1740 species, which includes daisies, chrysanthemums, tarragon, and chamomile, as well as the sagebrushes, which dominate the cold deserts of the Great Basin in western North America and steppe-like communities worldwide. More than 50% of all Anthemideae species occur in five large core genera: *Achillea* (115 species), *Anthemis* (210 species), *Tanacetum* (150 species), *Artemisia* (390 species), and *Seriphidium* (130 species). The remaining 105 Anthemideae genera are small to medium in size, with 47 genera of 2–10 species each and 19 genera with 11–50 species each, such as *Cotula* and *Pentzia*; 39 genera are monotypic. The tribe is primarily Old World and north

temperate in distribution (Heywood and Humphries, 1977; Bremer and Humphries, 1993), with three major centers of diversity: the Mediterranean Region, south central Asia, and southern Africa. New World species, such as *Artemisia* and *Achillea*, are widespread; however, these genera are primarily palearctic in distribution. Other widespread genera, such as *Cotula* s.l., occur throughout the Southern Hemisphere, including South Africa, South America, and Australia. It is thought that the tribe originated in Eurasia, with an old Eurasian–African vicariance involved (Bremer, 1994).

The tribe Anthemideae is considered monophyletic, with most of the historically problematic genera previously transferred to other tribes (Heywood and Humphries, 1977; Bremer and Humphries, 1993). Both *Cotula* and *Ursinia* have been retained in the Anthemideae, despite considerable debate (Bentham, 1873; Robinson and Brettell, 1973; Jeffrey, 1978; Gadek *et al.*, 1989; Bruhl and Quinn, 1990, 1991; Bremer and Humphries, 1993; Kim and Jansen, 1995). Otherwise, placement of genera within Anthemideae remains relatively unchanged from historical taxonomic treatments (Bremer and Humphries, 1993). The tribe is characterized by the possession of dissected leaves (sometimes entire), a paleaceous pappus (sometimes absent), ecaudate or short-tailed anther appendages, truncate penicillate style branches with two parallel stigmatic lines, anthemoid pollen (Skvarla *et al.*, 1977; Skvarla and Turner, 1977; Vezey *et al.*, 1994), and an aromatic nature resulting from high concentrations of monoterpenes and sesquiterpenes (Greger, 1977; Heywood and Humphries, 1977; Bremer and Humphries, 1993; Bremer, 1994).

Many members of tribe Anthemideae possess reduced floral structures that are associated with modified breeding systems, making homology difficult to interpret (Heywood and Humphries, 1977). Consequently, some of the traditional classifications that were proposed for the tribe consist of subtribes and generic assemblages that are artificial and/or polyphyletic (Heywood and Humphries, 1977). The tribe has recently been monographed using a morphological phy-

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logeny based on a cladistic analysis of 184 characters (Bremer and Humphries, 1993), resulting in the recognition of 12 subtribes. This morphological phylogeny provides an excellent opportunity for reevaluation of relationships in tribe Anthemideae using molecular data as an independent assessment of phylogeny. The primary objective of this study was to construct a molecular phylogeny for tribe Anthemideae using sequences of the chloroplast gene *ndhF*. Specifically, this molecular-based phylogeny was used to (1) test the previously proposed classifications and make comparisons to the published morphological phylogeny and (2) examine evolutionary and biogeographic trends within the tribe.

## MATERIALS AND METHODS

The chloroplast gene *ndhF* has been used successfully to construct phylogenies at the intergeneric and interfamilial levels within the Gesneriaceae (Smith and Carroll, 1997), Bromeliaceae (Terry and Brown, 1996; Terry *et al.*, 1997a,b), and Acanthaceae (Scotland *et al.*, 1995), among others (Catalan *et al.*, 1997; Gaut *et al.*, 1997). Within the Asteraceae (Kim and Jansen, 1995), as well as within the angiosperms as a whole, the 3' end of *ndhF* is the least conserved region of the gene. Therefore, we used 1200-bp sequences of the 3' end of *ndhF* to construct a phylogeny for tribe Anthemideae at the intergeneric level.

Fifty genera were included, representing 10 of the 12 subtribes in Anthemideae (Table 1). Three additional genera, representing each of two sister tribes (*Aster*: Astereae and *Calendula*/*Osteospermum*: Calenduleae) were included as outgroups (Maddison *et al.*, 1984). Sister group relationships of tribes Astereae and Calenduleae to Anthemideae are well supported by morphological and molecular data (Jansen *et al.*, 1990, 1991; Kim *et al.*, 1992; Kim and Jansen, 1995; Mishler *et al.*, 1996; Karis, 1993; Bremer, 1995).

Total DNA was isolated from fresh, frozen, and silica-gel-dried leaf material using the CTAB procedure of Doyle and Doyle (1987) as modified by Smith *et al.* (1991). *ndhF* was amplified using oligonucleotide primers that anneal to position 1024 in the coding region of the gene and in a spacer region approximately 607 nucleotides downstream (Kim and Jansen, 1995). Amplifications were done on a Perkin-Elmer Cetus DNA Thermocycler (Models 480 and 2400) using dGTP nucleotides from United States Biochemical (USB) and *Taq* polymerase (Perkin-Elmer). PCR parameters included the following: an initial denaturation for 3 min at 95°C; followed by 30 cycles of 95°C for 1 min, primer annealing at 45°C for 1 min, and 72°C extension for 1.5 min; with a final 7-min extension at 72°C.

Sequences were obtained from double-stranded PCR-amplified products as described by Gyllenstein (1989) using the Sanger dideoxy method (Sanger *et al.*, 1977).

The sequencing reactions were carried out as described in the USB-Sequenase kit protocol, with the cold shock modification described in Conti *et al.* (1993) and <sup>35</sup>S labeling. Amplification and sequencing primers (provided by R. Jansen), spaced approximately 300 bases apart, were used to obtain sequence from the 3' region of the gene. DNA fragments were separated on a 6% polyacrylamide gel and exposed to Kodak X-OMAT AR X-ray film (Sigma) for a minimum of 12 h. Approximately 1120 nucleotides from this region were sequenced for the 52 genera and deposited with GenBank (Table 1). Sequences were aligned to a template of an *Ursinia ndhF* sequence and entered manually into a computer file using MacClade version 3.04 (Maddison and Maddison, 1992). The aligned data matrix is available upon request to the senior author.

Using the software PAUP\* 4.0, sequence divergence values were calculated using the DISTANCE MATRIX option (Swofford, 1993, 1999). Maximum-parsimony analyses were performed on a 132-MHz Apple Macintosh PowerPC 9500 with 64 MB of RAM. For all analyses, the following search strategy was employed: 100 replicates with Maxtrees set to 5000 trees/replicate using random addition of sequences, tree bisection-reconnection (TBR) branch swapping, Steepest Descent, and MULPARS, with gaps treated as missing data. Initially, the data were analyzed using Fitch parsimony. The next analysis employed a step-matrix approach for character-state weighting of nucleotide sequence data (Albert and Mishler, 1992; Albert *et al.*, 1993). This model takes into account predicted mutational biases and assigns *a priori* weights (calculated from logarithmic probabilities) to transitions and transversions at each codon position. This model was used rather than empirically derived biases, since it is not prone to errors that result from circular reasoning. The actual weights applied are detailed in Albert *et al.* (1993). Strict and semistrict consensus trees were constructed for each analysis for the resulting equally most-parsimonious trees. One hundred bootstrap replications were performed to evaluate internal support for each node for both analyses, using TBR and Steepest Descent, with Maxtrees set to 5000 trees/replication (Sanderson, 1989; Wendel and Albert, 1992; Hillis and Bull, 1993; Felsenstein and Kishino, 1993).

## RESULTS

Sequence divergence among Anthemideae genera for the 1129-bp partial sequences of *ndhF* ranges from 0.2 to 7.6% between *Ajanía* and *Arctanthemum* and between *Chamaemelum* and both *Hippia* and *Soliva*, respectively. Several additional pairs of anthemid genera have values slightly higher than 0.2% or slightly lower than 7.6%. However, most pairwise comparisons are in the 2–6% range.

Of the 1129 characters in the data matrix (excluding

TABLE 1

Species Sampled, Voucher Information<sup>a</sup> and GenBank Accession No.

Taxon	Voucher/Accession No.	GenBank
<i>Aaronsohnia pubescens</i> (Desf.) Bremer & Humphries	LW 95A–19A (MU, RAB)	AF153643
<i>Achillea millefolium</i> L.	LW, s.n. (UPS)	AF153633
<i>Ajania fruticulosa</i> (Ledeb.) Poljak.	YL, 95025 (IBSC)	AF153657
<i>Anacyclus clavatus</i> (Desf.) Pers.	LW, 95–6E (MU, SEVF)	AF153634
<i>Anthemis tinctoria</i> L.	LW, s.n. (UPS)	AF153636
<i>Arctanthemum arcticum</i> (L.) Tzvelev	NCP PI50226	AF153671
<i>Argyranthemum frutescens</i> (L.) Sch. Bip.	JO, 11 (TEX)	AF153637
<i>Artemisia vulgaris</i> L.	AK, S1181 (OKL)	AF153632
<i>Athanasia juncea</i> (L.) L.	LW & JP, 94–74 (MU, NBG)	AF153620
<i>Chamaemelum nobile</i> (L.) All.	LW, 95–30B (MU, SEVF)	AF153655
<i>Chrysanthemum coronarium</i> L.	LW, 95–13E (MU, SEVF)	AF153661
<i>Cladanthus arabicus</i> (L.) Cass.	LW, 95–20A (MU, RAB)	AF153654
<i>Coleostephus myconis</i> (L.) Rchb. F.	JO, s.n. (TEX)	AF153652
<i>Cotula lineariloba</i> (DC) Hilliard	SANBI 747/91	AF153644
<i>Crossostephium chinense</i> (L.) Makins	JL, GZ9501 (IBSC)	AF153664
<i>Cymbopappus adenosolen</i> (Hardy) Nord.	LW & JP, 94–79 (MU, NBG)	AF153658
<i>Eriocephalus africanus</i> L.	LW & JP, 94–25 (MU, NBG)	AF153645
<i>Eumorphia sericea</i> Wood & Evans	KEW 181–82.01783	AF153621
<i>Glossopappus macrotus</i> (Durieu) Burnat	LW, 95–26C (MU, SEVF)	AF153639
<i>Gonospermum canariense</i> (DC) Less.	MG, P4 (UPS)	AF153665
<i>Gymnopentzia bifurcata</i> Benth.	SANBI 977/90	AF153622
<i>Heteranthemis viscidehirta</i> Schott.	MAI-667584	AF153638
<i>Hippia pilosa</i> (Berg.) Druce	SANBI 107/91	AF153646
<i>Hymenolepis parviflora</i> (L.) DC	LW & JP, 94–33 (MU, NBG)	AF153623
<i>Ismelia carinata</i> (Schousboe) Sch. Bip.	GAT-38825	AF153653
<i>Kaschgaria komarovii</i> (Kraschen & Rubtzov) Polj.	KB, 3123 (UPS)	AF153631
<i>Lasiospermum bipinnatum</i> (Thunb.) Druce	SANBI 218/87	AF153624
<i>Lepidolopha</i> sp.	KB, 3109 (UPS)	AF153627
<i>Leucanthemella serotina</i> (L.) Tzvelev	NCP-PI502261	AF153659
<i>Leucanthemum vulgare</i> Lam.	KEW 086.70.00685	AF153640
<i>Lidbeckia vlokii</i> Kallersjö	LW & JP, 94–119 (MU, NBG)	AF153663
<i>Lonas annua</i> (L.) Vines & Druce	THM-6478	AF153651
<i>Lugoa revoluta</i> (Smith ex Link) DC	JO, 2 (TEX)	AF153660
<i>Matricaria matricarioides</i> Port. & Brit.	LW, s.n. (UPS)	AF153647
<i>Mauranthemum paludosum</i> (Poir.) Vogt & Oberpreiler	LW, s.n. (UPS)	AF153670
<i>Microcephala discoidea</i> (Ledeb.) Bremer & al.	KB, 3121 (UPS)	AF153668
<i>Nipponanthemum nipponicum</i> (Franchet ex Maxim) Kitam.	JY, 95100701 (TEX)	AF153662
<i>Oncosiphon grandiflorum</i> (Thunb.) Kallersjö	SANBI 343/87	AF153648
<i>Osmitopsis osmitoides</i> (Less.) Bremer	LW & JP, 94–80 (MU, NBG)	AF153642
<i>Pentzia dentata</i> (L.) OK.	LW & JP, 94–27 (MU, NBG)	AF153649
<i>Phymaspermum appressum</i> Bolus	LW & JP, 94–110 (MU, NBG)	AF153625
<i>Pseudohandelia umbellifera</i> (Boiss.) Tzvelev	KB 3113 (UPS)	AF153629
<i>Rhodanthemum arundanum</i> (Boiss.) Wilcox, Bremer & Humphries	LW, 95–16B (MU, RAB)	AF153641
<i>Santolina rosmarinifolia</i> L.	KEW 308–72.02859	AF153635
<i>Schistostephium umbellatum</i> (L.) Bremer & Humphries	LW & JP, 94–92 (MU, NBG)	AF153650
<i>Seriphidium tridentatum</i> (Nutt.) Weber	AK, 11872 (OKL)	AF153630
<i>Soliva anthemifolia</i> (Juss.) R. Br.	JL, GZ9503 (IBSC)	AF153667
<i>Tanacetum macrophyllum</i> Hemsley & Lace	KEW 000.69.18297	AF153628
<i>Tripleurospermum inodorum</i> (L.) Sch.Bip.	YL, 95031 (IBSC)	AF153656
<i>Ursinia pilifera</i> (P. Berg.) Poiret	LW & JP, 94–41 (MU, NBG)	AF153626
<i>Outgroup species</i>		
<i>Aster cordifolius</i> L.	RJ, 906 (MICH)	L39449
<i>Calendula arvensis</i> L.	LW, 95–13C (MU, RAB)	AF153619
<i>Osteospermum pinnatum</i> (Thunb.) Norlindh.	LW & JP, 94–40 (MU, NBG)	AF153669

<sup>a</sup> Vouchers are deposited at the following herbaria: Miami University (MU), University of Texas (TEX), University of Oklahoma (OKL), University of Michigan (MICH), Uppsala University (UPS), Academia Sinica (IBSC), National Botanic Garden—Kirstenbtsch, (NBG), Rabat University (RAB), and University of Seville (SEVF). Collectors are Linda Watson (LW), Jose Panero (JP), Javier Francisco-Ortega (JO), Mats Gustafsson (MG), Amy Kornkven (AK), Yeou-Ruenn Ling (YL), Jiang Lin (JL), Kare Bremer (KB), Jun Yokoyama (JY), and Robert Jansen (RJ). Accession numbers refer to: National Botanic Garden, South Africa (SANBI); Royal Botanic Gardens, Kew, England (KEW); Germplasm seedbank at Escuela Tecnica Superior de Ingenieros Agronomos, Madrid, Spain (MAI); Germplasm seedbank at North Central Regional Plant Introduction Station, USDA, Ames, IA, USA (NCP); Thompson and Morgan Inc., Jackson, NJ, USA (THM).



gaps), 318 were variable, resulting in 173 phylogenetically informative characters. In both searches, computer memory was exceeded when the maximum limit of 40,000 trees was reached. In both analyses, the equally most-parsimonious trees were 615 steps in length, and both had a consistency index (CI) of 0.657, a retention index (RI) of 0.738, and a rescaled consistency index (RC) of 0.485. The strict and semistrict consensus trees of both analyses are identical in topology, and, therefore, only one consensus tree is presented (Fig. 1).

## DISCUSSION

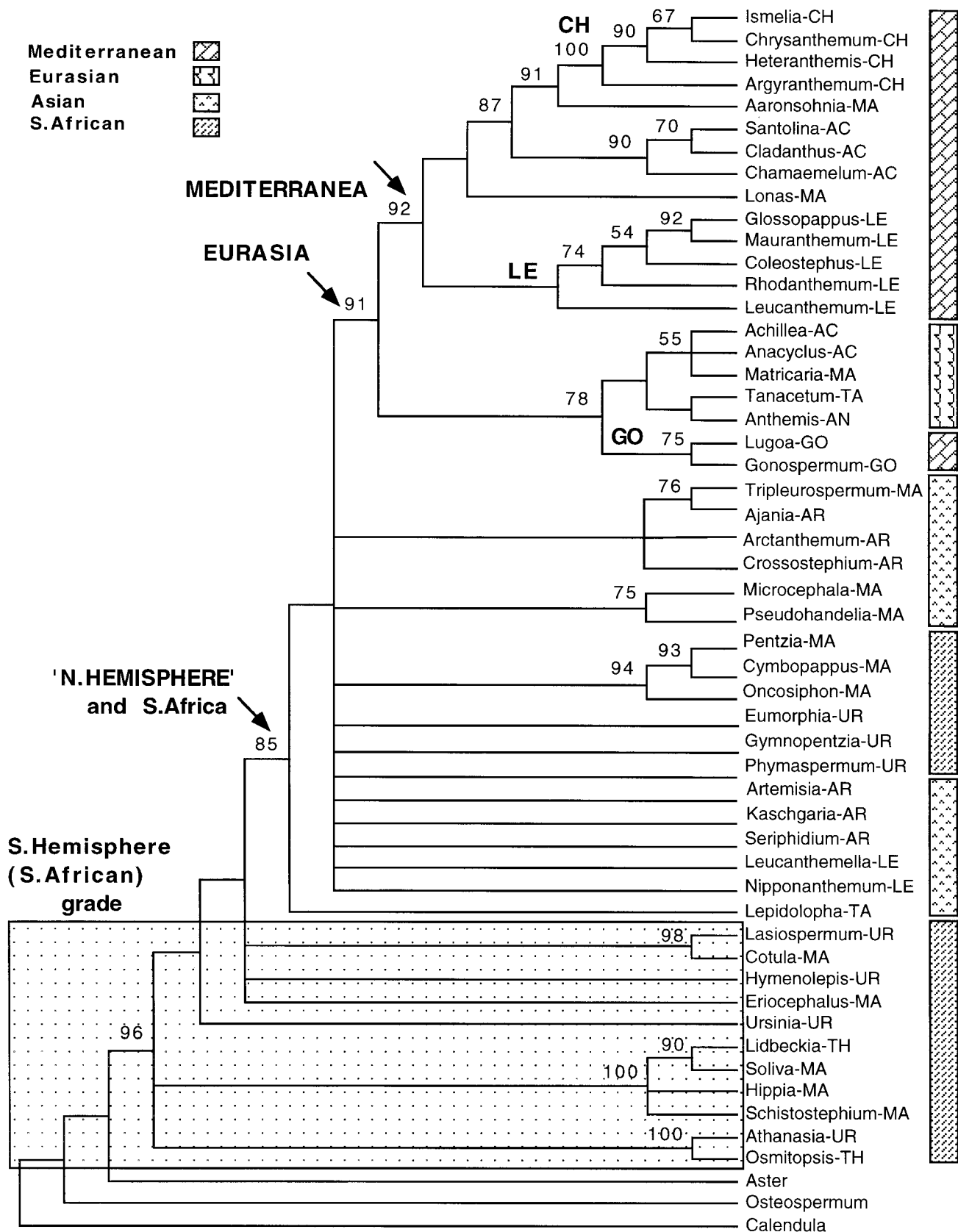
*Major lineages within tribe Anthemideae.* The *ndhF* phylogeny is composed of one major clade of Mediterranean and more widespread, northwestern Eurasian genera embedded within a large grade of eastern Asian and South African genera (Figs. 1 and 2) and a large basal grade of Southern Hemisphere (primarily South African) genera. In addition, there are several smaller subclades within the tree that are resolved and fairly well supported.

In the strict consensus tree, the basal grade (Fig. 1; *Lasiospermum* to *Osmitopsis*) is composed of 11 Southern Hemisphere genera, which occur primarily in South Africa. Members include several genera of subtribes Ursiniinae, Matricariinae, and Thaminophyllinae. The overwhelming majority of species in the genera of this large grade are South African. Within this basal grade, there are three resolved clades. Three genera (*Schistostephium*, *Hippia*, and *Lidbeckia*) that have strictly South African native distributions form one small clade along with *Soliva*. While *Soliva* is mostly South American, it also has widespread species that occur in both Australia and North America. A second, well-supported clade within this large basal grade consists of the largely South African genus *Lasiospermum* (with one species in Egypt) and *Cotula*. *Cotula* is a more widespread Southern Hemisphere genus, which has species that occur in South America, Australia, and South Africa (the *Cotula* species sampled for this study was collected in South Africa; Table 1). An additional clade is embedded within this large grade and contains the strictly South African genera *Athanasia* and *Osmitopsis*. This large basal grade of 11 South African/Southern Hemisphere genera most closely resembles the African complex of Heywood and Humphries (1977), which is largely composed of woody shrubs. Dispersal into the New World from these South African lineages occurred at least twice, with *Cotula* and *Soliva* placed in two different clades in the molecular phylogeny.

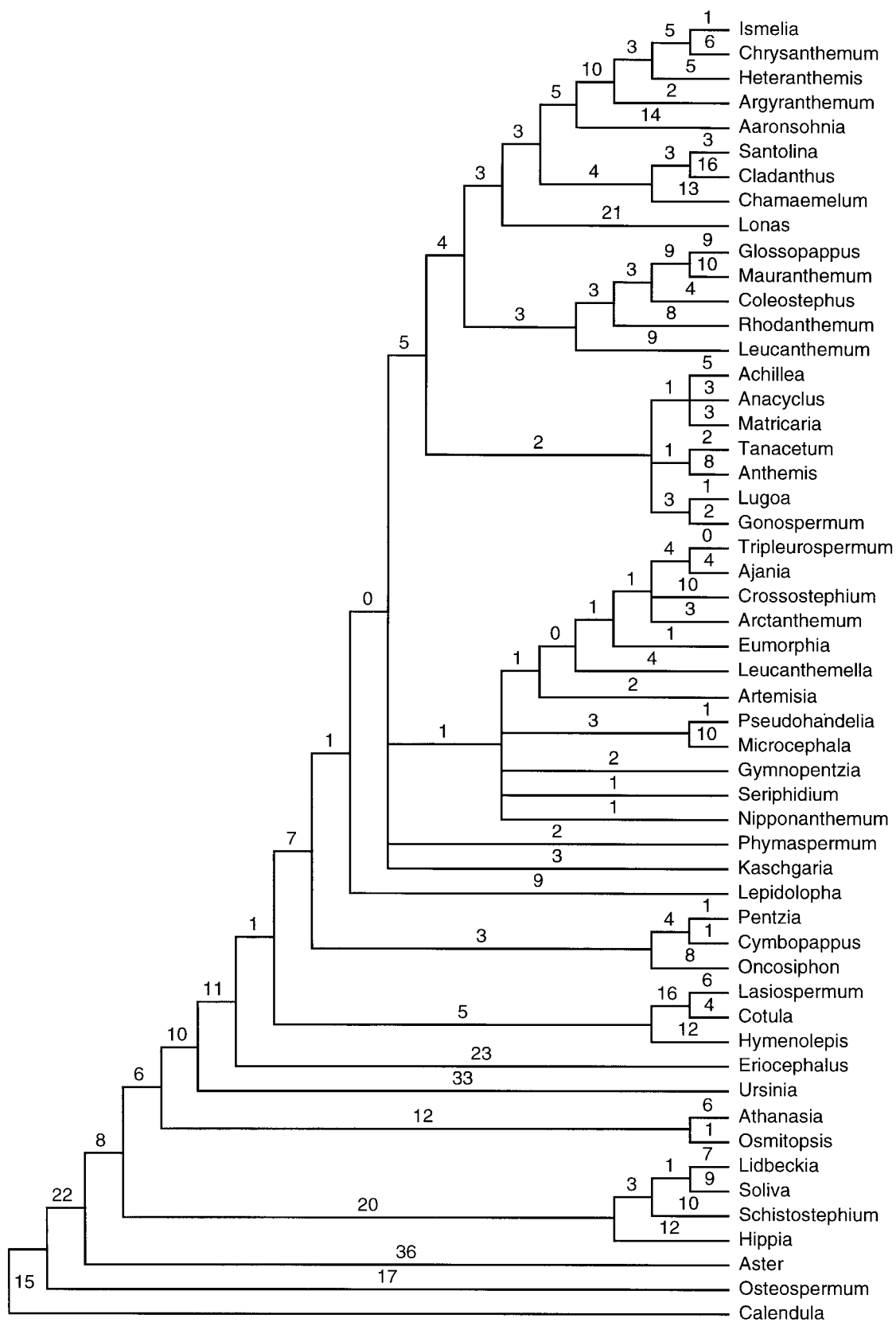
A large clade of mostly Northern Hemisphere genera (Fig. 1) contains several large, well-supported clades and several smaller subclades, as well as numerous polytomies. This mostly Eurasian clade also contains six South African and several far eastern Asian genera, with unresolved placements. The largest subclade is

composed of genera which are more southern Mediterranean in distribution and occur on the southern Iberian Peninsula and in North Africa. This clade includes subtribe Chrysantheminae (CH), large portions of both subtribes Leucantheminae (LE) and Achilleinae, and two genera of Matricariinae (*Lonas* and *Aaronsohnia*). The most strongly supported clade is composed of the four genera of Mediterranean–Eurasian subtribe Chrysantheminae, with *Aaronsohnia* (subtribe Matricariinae) sister to the Chrysantheminae. *Aaronsohnia* occurs primarily in North Africa and the middle East but was considered in the past to be more closely related to *Matricaria* and *Adenoglossa* than to the Chrysantheminae. Some members of subtribe Achilleinae are sister to the Chrysantheminae–*Aaronsohnia* clade and include *Santolina*, *Cladanthus*, and *Chamaemelum*. All three of these Achilleinae genera have a Mediterranean distribution centered in Spain, Portugal, Morocco, and Algeria. The North African genus *Lonas* (Matricariinae) is sister to this large clade. Interestingly, the large widespread genus *Achillea* falls outside of this Mediterranean clade and likely represents an earlier radiation. Some members of subtribe Leucantheminae are sister to the Achilleinae–Chrysantheminae clade, including *Glossopappus*, *Mauranthemum*, *Coleostephus*, *Rhodanthemum*, and *Leucanthemum*. All of these genera also have a Mediterranean distribution, except for *Leucanthemum*, which is primarily Mediterranean but widespread throughout Eurasia. Interestingly, the Asian members of Leucantheminae (*Leucanthemella* and *Nipponanthemum*) occur outside of this clade, collapsed along with numerous other Asian genera for which there is no resolution in the *ndhF* tree. The Asian genera and three South African genera (*Eumorphia*, *Phymaspermum*, and *Gymnopentzia*) are collapsed at the base of the “Northern Hemisphere” clade. Within this unresolved region of the tree, there are three smaller clades that include some members of subtribes Artemisiinae and Matricariinae. The best-supported clade in this region of the tree consists of genera of South African Matricariinae.

A group of Eurasian genera is sister to the Mediterranean clade and contains subtribe Gonosperminae (GO), as well as four large, relatively widespread genera that occur in Eurasia and/or are holarctic in distribution (*Tanacetum*, *Anthemis*, *Achillea*, and *Matricaria*) and the smaller, predominantly North African genus *Anacyclus*. The sister group relationship of these widespread, holarctic genera to the Mediterranean clade is well supported. However, support for the monophyly and the internal nodes of the widespread clade is somewhat weak (78% bootstrap), and conclusions about relationships among them are tenuous. Further, *Tanacetum* is a large polyphyletic complex, and interpretations based on the placement of one representative species are unwarranted.



**FIG. 1.** Strict consensus tree of 40,000 equally most-parsimonious trees, based on partial sequences of chloroplast gene *ndhF*. Numbers above the lines are bootstrap values based on 100 TBR replicates of 5000 maximum trees/replicate. The two-letter codes above the nodes and following taxa indicate subtribal affinity (sensu Bremer and Humphries, 1993); CH, Chrysantheminae; MA, Matricariinae; AC, Achilleinae; LE, Leucantheminae; TA, Tanacetinae; AN, Anthemidinae; GO, Gonosperminae; AR, Artemisiinae; UR, Ursiniinae; TH, Thaminophyllinae.



**FIG. 2.** One of 40,000 equally most-parsimonious trees (615 steps in length), based on partial sequences of chloroplast gene *ndhF*. Numbers above the lines are branch lengths; CI = 0.657, RI = 0.738, RC = 0.485, excluding autapomorphies.

Thus, it appears that the basal grade of genera that are largely South African in distribution are relictual members of tribe Anthemideae, which may indicate that the anthemid lineage originated in southern Africa rather than Eurasia. The eastern Asian (and some South African) members of the Anthemideae are the next basal-most group, and a single dispersal into Europe likely occurred from these Asian–South African ancestors, followed by a large radiation, particularly in the Mediterranean Region of southern Europe and North Africa. It appears that if a vicariant event occurred that separated South African from Eurasian members, it more likely involved the far eastern Asian rather than the western Eurasian or European ancestors. However, additional resolution in the basal portion of the “Northern Hemisphere” clade is necessary for evaluating the alternative hypotheses of vicariance vs long-distance dispersal for explaining this disjunction.

The closest sister tribe to Anthemideae is the Astereae, which is the second largest tribe in the Asterales, composed of over 3000 species. Like the Anthemideae, Astereae appears to have originated in the Southern Hemisphere, likely in South Africa, with subsequent dispersals and radiations into the Northern Hemisphere (Noyes and Rieseberg, 1999). While diversity of the Anthemideae has remained primarily in the Old World and proliferated throughout Eurasia, Astereae became cosmopolitan in distribution with major lineages established worldwide.

**Phylogeny and classification.** Early classifications of tribe Anthemideae were clearly artificial and based on only one or two morphological characteristics. For example, Lessing (1832) and Hoffmann (1894) recognized two subtribes based on the presence or absence of receptacular scales, and Bentham (1873) subdivided the tribe into six groups based on discoid vs radiate capitula (Heywood and Humphries, 1977). Later, Poljakov (1967) recognized six subtribes based on capitular characters. This treatment, however, was basically that of Bentham's with somewhat greater detail devoted to the Artemisiinae (Heywood and Humphries, 1977).

Classifications that were constructed to reflect natural relationships in tribe Anthemideae were based on a broader set of characters, with Reitsebrecht (1967) using cypsel anatomy to recognize seven provisional groups. In an attempt to understand evolutionary relationships, Heywood and Humphries (1977) examined a more extensive array of data that included carpology, embryology, and phytochemistry, and they recognized six generic assemblages. Four of these assemblages were centered around the large core genera of the tribe (i.e., *Artemisia*, *Achillea*, *Anthemis*, and *Tanacetum*), and two were centered on moderate-sized genera (*Ursinia* and *Cotula*, and respective allies). The *Chrysanthemum* complex was composed of five large genera (*Tanacetum*, *Argyranthemum*, *Chrysanthemum*, *Leu-*

*canthemum*, and *Dendranthema*) and several smaller genera. The *Anthemis* assemblage contained *Chamaemelum*, *Tripleurospermum*, and *Matricaria*. The “Cotuleae” group was composed of *Cotula*, *Soliva*, and *Nanathea*, in addition to several genera that have since been excluded from the Anthemideae. The *Artemisia* group contained the large genus *Artemisia*, as well as many smaller segregate genera. An African complex was also recognized, which included *Ursinia* and its allies, as well as *Osmitopsis*, *Hippia*, and *Pentzia*, among others. *Achillea* was considered to be distantly related to most other genera in Anthemideae, with the possible exceptions of *Handelia* and/or *Santolina*. The creation of a new classification was not the major intent of the Heywood and Humphries review (1977), with emphasis placed on evolutionary patterns of transitions from one generic assemblage to the next.

The most recent classification of tribe Anthemideae is based on a phylogeny derived from a cladistic analysis of 184 characters (Bremer and Humphries, 1993), in which 12 subtribes are recognized. Most characters are morphological (96%), with over 80% related to reproductive biology, including capitula, floret, and cypsel characters. However, secondary compounds and chromosome numbers are also used. Of the five large core genera, *Achillea* and *Anthemis* are relatively homogeneous in morphology and are considered monophyletic with well-circumscribed generic boundaries (Bremer and Humphries, 1993). *Tanacetum* is the most problematic genus and has recently been used as the taxonomic “garbage can” for the Anthemideae. It currently serves as the recipient of most of the species formerly accommodated in *Chrysanthemum* (presently containing two species). *Seriphidium* is sometimes treated as a subgenus of *Artemisia*, along with several smaller genera that have recently been segregated, making *Artemisia* most likely paraphyletic (Kornkven *et al.*, 1998, 1999). There is little congruence of the cladistically based, morphological classification to any of the previous taxonomic classifications, with the historic generic assemblages and subtribes being variously divided among the 12 newly created subtribes. Bremer and Humphries (1993) acknowledge that some of the newly recognized subtribes are not monophyletic (including Tanacetinae) and consider several subtribes as “provisionally monophyletic” by the inclusion of some problematic genera that, when examined in greater detail, could eventually change the current subtribal delimitations. These subtribes include Cancriniinae, Artemisiinae, Achilleinae (with the uncertain inclusion of *Santolina*), Leucantheminae, Anthemidinae, and Matricariinae. There appears to be strong support for monophyly of many subtribes, however, including Ursiniinae, Gonosperminae, Chrysantheminae, Handeliinae, and Thaminophyllinae. Unfortunately, the morphological dataset does not resolve the basal nodes within the tribe; so, sister group relationships among subtribes remain basically unknown.



While the *ndhF* data strongly support the monophyly of Anthemideae (96% bootstrap; Fig. 1), in general, the molecular phylogeny is largely incongruent with the morphological phylogeny of Bremer and Humphries (1993), as well as with all other previously proposed classifications for the tribe (Reitbrecht, 1967; Poljakov, 1967; Heywood and Humphries, 1977). There is little support for monophyly of most subtribes or generic assemblages, and there is substantial disagreement with previously proposed sister group relationships of genera and subtribes (Fig. 1).

Only two small subtribes, Gonosperminae and Chrysantheminae, are supported as monophyletic (100 and 78% bootstrap support, respectively; GO and CH of Fig. 1). Subtribe Gonosperminae is composed of three small genera (*Gonospermum*, *Lugoa*, and *Inulanthera*), of which both *Gonospermum* and *Lugoa* (five species total) are endemic to the Canary Islands. *Inulanthera*, which is found in southwestern Africa, was formerly included in *Athanasia* (Kallersjö, 1986, 1991). A close relationship of Gonosperminae to subtribe Tanacetinae was previously proposed (Bremer and Humphries, 1993), which is also supported, in part, by the molecular phylogeny. However, the molecular data do not support a relictual pattern (e.g., basal placement) for the Gonosperminae, as previously hypothesized (Bremer and Humphries, 1993). The most strongly supported clade (100% bootstrap; CH of Fig. 1) in the *ndhF* phylogeny is composed of the four genera of subtribe Chrysantheminae, with *Ismelia* and *Chrysanthemum* sister to each other (67% bootstrap), *Heteranthemis* sister to both genera (90% bootstrap), and *Argyranthemum* in the most basal position (100% bootstrap). The close relationship among these four genera was previously recognized (Heywood and Humphries, 1977). All four genera are Mediterranean in distribution, with *Argyranthemum* restricted to Macaronesia, *Chrysanthemum* widespread throughout Eurasia, and *Heteranthemis* and *Ismelia* occurring primarily on the Iberian Peninsula and in North Africa. The *ndhF* data support the recognition of *Ismelia* as a segregate genus of *Chrysanthemum* (formerly *C. carinatum*), concordant with morphology (Bremer and Humphries, 1993) and ITS sequence data (Francisco-Ortega *et al.*, 1997). A Mediterranean origin has been proposed for *Argyranthemum* (Francisco-Ortega *et al.*, 1997). However, previous studies have not recognized *Aaronsohnia* (Matricariinae), a genus of the Mediterranean and Mideast, as sister to the Chrysantheminae (91% bootstrap).

Other than support for monophyly of subtribes Gonosperminae and Chrysantheminae, there is little support for monophyly of the remaining subtribes (Fig. 1). However, there is support for large portions of some subtribes, which helps to further delineate the "provisionally monophyletic" subtribes (sensu Bremer and Humphries). For example, members of subtribe Achilleinae (*Santolina*, *Cladanthus*, and *Chamaemelum*; ex-

cluding *Achillea*) form a strongly supported clade (90% bootstrap; Fig. 1) that is sister to the Chrysantheminae (87% bootstrap). The widespread genus *Achillea*, however, occurs outside of this clade. Some members of subtribe Leucantheminae form a strongly supported clade (LE of Fig. 1), sister to the Chrysantheminae–Achilleinae clade. Although placement of *Leucanthemella* and *Nipponanthemum* are collapsed at the base of a large clade, both appear to have sister group relationships outside of the Leucantheminae.

Artemisiinae is the largest subtribe in Anthemideae, containing over 600 species in 18 genera, with the overwhelming majority of species in 2 genera: *Artemisia* and *Seriphidium* (often treated as a subgenus of *Artemisia*). Additionally, there are numerous, small (often monotypic) segregate genera in this subtribe. Some members of Artemisiinae (Fig. 1) show a close sister group relationship in the molecular phylogeny; however, this clade also includes *Tripleurospermum* (subtribe Matricariinae). Placements of *Artemisia*, *Seriphidium*, and *Kaschgaria* are outside of this weakly supported clade.

Circumscription of Ursiniinae is also problematic with respect to the molecular phylogeny. Traditionally, subtribe Ursiniinae is defined by the possession of furanosesquiterpenes (Greger, 1977) and micromorphological characters of the florets, cypselas, anthers, and pappus (Kallersjö, 1986, 1991). However, it has been suggested that the plesiomorphic nature of Ursiniinae morphology makes support for its monophyly difficult (Bremer and Humphries, 1993). The large basal grade in the molecular phylogeny contains genera previously placed in subtribe Ursiniinae (*Ursinia*, *Lasiospermum*, *Hymenolepis*, and *Athanasia*), in addition to genera from other subtribes. The Ursiniinae genera occur in three distinct clades in the *ndhF* phylogeny (Fig. 1). A close relationship between *Osmitopsis* (Thaminophyllinae) and *Athanasia* (Ursiniinae) is strongly supported (100% bootstrap). On the basis of unique anthers in *Osmitopsis*, placement within the tribe itself was previously questioned (Bremer and Humphries, 1977). The remaining Ursiniinae genera examined in this study (*Gymnopentzia*, *Eumorphia*, and *Phymasperma*) have sister group relationships outside of this grade. However, their placement is unresolved and unsupported.

The most problematic subtribe, with respect to the molecular phylogeny, is Matricariinae. It has representative genera occurring in many different clades, scattered among different subtribes throughout the molecular phylogeny. Matricariinae is one of the largest subtribes in the Anthemideae (25 genera and 250 species) and lacks any morphological synapomorphies to support its monophyly (Bremer and Humphries, 1993). However, two subclades that are supported by synapomorphies in the morphological phylogeny correspond to genera centered primarily in either the Southern Hemisphere or the Northern Hemisphere (Bremer



and Humphries, 1993; Kallersjö, 1988). In the molecular phylogeny, members of the Southern Hemisphere subclade of Matricariinae (*Soliva*, *Hippia*, *Schistotephium*, *Cotula*, and *Eriocephalus*) are variously embedded with other non-Matricariinae genera within a basal grade of Southern Hemisphere (primarily South African) genera. A second South African subclade is embedded in the primarily Northern Hemisphere clade of the molecular phylogeny, including *Pentzia*, *Cymbopappus*, and *Oncosiphon*. The Northern Hemisphere Matricariinae genera (*Matricaria*, *Aaronsohnia*, *Lonas*, *Microcephala*, *Tripleurospermum*, and *Pseudohandelia*) also have sister group relationships outside of this basal grade and do not occur as a monophyletic group. Interestingly, the basal placement of members of subtribes Matricariinae and Thaminophyllinae was not previously recognized, with both subtribes being placed in a relatively derived position in the morphological phylogeny (Bremer and Humphries, 1993).

Subtribe Tanacetinae is also problematic in that both genera sampled (*Tanacetum* and *Lepidolopha*) are in two distantly related clades. However, Tanacetinae and *Tanacetum* are both considered polyphyletic and in need of revision. Therefore, any conclusions regarding the placement of these taxa is unwarranted.

*Summary of biogeographic and taxonomic implications.* The molecular data clearly illustrate the influence of biogeography on phylogenetic relationships within the Anthemideae. The distinction between the Northern and the Southern Hemisphere genera was not previously recognized in the past, with the exception of cypsela morphology (Heywood and Humphries, 1977; Reitsebrecht, 1967). It should be noted that this biogeographic pattern is also independently supported by nuclear sequences (internal transcribed spacers) and chloroplast DNA restriction site data (Francisco-Ortega *et al.*, 1997; Watson *et al.*, unpubl. data), indicating that the observed pattern reflects true relationships among taxa.

The basal position of the South African genera suggests that tribe Anthemideae probably had its origin in the Old World/Southern Hemisphere, because multiple (presumably relictual) lineages comprise this large grade. This is in contrast to previous ideas that the tribe originated in Eurasia. The relictual nature of these genera explains the historically anomalous placement of *Ursinia*, which is morphologically distinct from most other Anthemideae genera. *Cotula* and *Soliva* likely represent separate dispersals into the New World from Old World ancestors, as they are in two separate clades. However, it is less certain if the more widespread distribution of *Cotula* represents an historically widespread Southern Hemisphere distribution or more recent secondary dispersal. The Mediterranean clade is clearly monophyletic and probably represents a recent radiation from a single dispersal event from a north temperate, Eurasian ancestor. Colonization of Macaron-

esia occurred at least twice, with the Gonosperminae and *Argyranthemum* (Chrysantheminae) in two different clades. Many of the large and widespread genera are sister to the Mediterranean clade and do not occur within the relictual/basal lineages from the Southern Hemisphere, suggesting that these genera may be relatively recent.

With few exceptions (e.g., Chrysantheminae and Gonosperminae), subtribal boundaries within Anthemideae need to be reexamined in light of the molecular data. Matricariinae needs major revision. The remaining "provisionally monophyletic" subtribes (sensu Bremer and Humphries) need better circumscription. For example, exclusion of a few genera from some subtribes, such as the Achilleinae and Leucantheminae, would result in the remainder of each subtribe being relatively intact.

Sister group relationships among monophyletic and "provisionally monophyletic" subtribes are better resolved in the molecular phylogeny than in the morphological phylogeny. In the morphological consensus tree, all basal nodes among subtribes are collapsed, providing virtually no information on relative placement of genera and subtribes within the tribe or relationships among them. However, in the molecular tree, some trends are apparent, although some portions of the tree are only weakly supported and/or form grades rather than clades. For example, subtribal members of Ursiniinae, Southern Hemisphere Matricariinae, as well as Thaminophyllinae, all occur in a basal position within the tree. Furthermore, a derived position and close relationship of Chrysantheminae and Leucantheminae is supported, as previously proposed (Bremer and Humphries, 1993), although some members of Achilleinae and Matricariinae share a more recent common ancestor with Chrysantheminae. In an effort to understand the patterns of evolution of this relatively recent tribe in the Asteraceae, we are currently evaluating additional molecular markers to further clarify relationships in the unresolved portions of the phylogenetic tree, examining major clades more extensively, and reevaluating morphology as it relates to homology and convergent evolution.

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