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# PHYLOGENY OF SOUTH AFRICAN GNAPHALIEAE (ASTERACEAE) BASED ON TWO NONCODING CHLOROPLAST SEQUENCES[1]

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The Gnaphalieae are a group of sunflowers that have their greatest diversity in South America, Southern Africa, and Australia. The objective of this study was to reconstruct a phylogeny of the South African Gnaphalieae using sequence data from two noncoding chloroplast DNA sequences, the *trnL* intron and *trnL/trnF* intergenic spacer. Included in this investigation are the genera of the Gnaphalieae from the African basal groups, members of the subtribes Cassiniinae, Gnaphaliinae, and Relhaniinae, and African representatives from the large Old World genus *Helichrysum.* Results indicate that two Gnaphaloid genera, *Printzia* and *Callilepis,* should be excluded from the Gnaphalieae. In most trees the Relhaniinae s.s. (sensu stricto) and some of the basal taxa comprise a clade that is sister to the remainder of the tribe Gnaphalieae. The Relhaniinae, which are restricted to Africa, are not a monophyletic group as presently circumscribed, nor are the South African members of *Helichrysum,* the Cassiniinae and Gnaphaliinae. There is general agreement between our molecular analysis and that of morphology, particularly in the terminal branches of the trees.

**Key words:** Asteraceae; Cape Flora; chloroplast DNA; Gnaphalieae; noncoding sequence; phylogeny; Relhaniinae; South Africa.

The Gnaphalieae (paper daisies or everlastings) are a group of sunflowers that have their greatest diversity in South America, Southern Africa, and Australia. They are poorly represented in the Northern Hemisphere (e.g., *Antennaria* Gaertn., *Leontopodium* R. Brown ex Cass., and *Anaphalis* A. P. de Candolle). Phylogenetic relationships among the ~187 genera of the Gnaphalieae have been hypothesized through a recent morphology-based cladistic analysis (Anderberg, 1991a). However, the ubiquitous parallelisms in morphology that exist within the tribe Gnaphalieae, and indeed Asteraceae (Carlquist, 1976) as a whole, have made it difficult to find conservative (nonhomoplasious) characters that can be used reliably in phylogeny reconstruction. Based on his analysis, Anderberg (1991a) proposed that the tribe be composed of 15 monophyletic groups or subtribes, which is contrary to traditional taxonomic concepts in the Gnaphalieae as revised by Merxmüller, Leins, and Roessler (1977). Merxmüller, Leins, and Roessler (1977) recognized three subtribes in their Inuleae s.l. (sensu lato) and Anderberg has assembled his 15 taxa (subtribes and/or groups) from two of the subtribes of the Merxmüller, Leins, and Roessler (1977) classification, namely the Gnaphaliinae and the Athrixiinae.

Of considerable interest within the Gnaphalieae is the phylogenetic position and circumscription of the African subtribe Relhaniinae and its members. Several genera of this subtribe, *Oedera* L. for example, have historically been placed in other groups (e.g., Kosteletzky, 1833; Harvey, 1865; Dyer, 1975; Anderberg and Källersjö, 1988). This study, in part, was devised to resolve these questions.

Given the problems of nonhomologous morphological similarities (homoplasies) in the group, we have chosen to explore these relationships with a molecular approach. The objectives of our work were (1) to attempt to reconstruct the phylogeny of the South African Gnaphalieae using sequence data from two relatively short noncoding chloroplast DNA sequences, the *trnL* intron and *trnL/trnF* intergenic spacer and (2) to test the monophyly of the Relhaniinae and *Helichrysum* Mill. and assess their phylogenetic relationships to the basal groups of the Gnaphalieae. This spacer region has proven useful in resolving generic and tribal relationships in the Asteraceae (Bayer and Starr, 1998). Investigated in this paper are the genera of the Gnaphalieae from the basal African genera, *Anisothrix, Athrixia, Arrowsmithia, Callilepis, Pentatrichia,* and *Printzia,* members of the subtribe Relhaniinae, and representatives from the large Old World genus *Helichrysum* (~500 spp. worldwide). This study is the first part of an ongoing attempt to reconstruct the phylogeny of the Gnaphalieae on a worldwide basis.

***Recent analyses of the relationships of the Inuleae, Plucheeae, and Gnaphalieae***—The work of Anderberg (1989, 1991a, b, c) and Karis (1993) suggests that the

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tribe Inuleae (Asteraceae: Asteroideae) sensu Merxmüller, Leins, and Roessler (1977) should be considered as three separate tribal lineages: Inuleae s.s., Gnaphalieae, and Plucheeae. This ''three tribe'' system, based in part on a cladistic analysis of morphological characters, was later supported by two molecular analyses (Kim and Jansen, 1995; Bayer and Starr, 1998), but both studies indicated that the Inuleae s.l. was not a monophyletic lineage. In the *trnL* intron and *trnL/trnF* intergenic spacer analysis by Bayer and Starr (1998), the Inuleae s.s. and Plucheeae together form a clade sister to the remainder of the Asteroideae. Kim and Jansen (1995), using *ndhF*, also suggest a strong sister relationship of the Plucheeae and Inuleae within the Asteroideae, but the base of their topology was not sufficiently resolved to discern the sister relationships of that clade. The topological relationships identified by Bayer and Starr (1998) were almost identical to those described by Karis (1993) based on morphology. Therefore, the segregation of the Gnaphalieae from the Inuleae s.l. was warranted, as they do not form a monophyletic assemblage in any of the abovementioned analyses.

However, the sister relationships of the Gnaphalieae proposed by these studies remains controversial. Bayer and Starr (1998), based on the *trnL* intron and *trnL/trnF* spacer data, proposed that the Gnaphalieae are sister to a fourth tribe, the Senecioneae. Karis (1993), using morphology, revealed them as sister to a clade containing the tribes Astereae and Anthemideae. Jansen, Michaels, and Palmers' (1991) RFLP (restriction fragment length polymorphism) analysis described the Gnaphalieae as sister to the Inuleae (represented by *Inula* L.). Keeley and Jansen (1991) show them as sister to a clade consisting of the Inuleae and Plucheeae. Finally, Kim and Jansen's (1995) *ndhF* analysis have the Gnaphalieae in an unresolved clade containing the Calenduleae, Astereae, and Anthemideae.

## MATERIALS AND METHODS

***Fieldwork***—It was established that the greatest diversity could be sampled in the Western Cape Province of South Africa, so fieldwork focused on that region. We collected from as many Gnaphalieae, Senecioneae, and Calenduleae species as possible with a primary goal to collect material from the type species of each genus (Table 1). Leaves for DNA extraction were preserved in liquid CTAB/NaCl solution at ambient temperature and later at −20°C in the laboratory (Rogstad, 1992).

***Outgroup selection***—Outgroup taxa were selected on the basis of the analyses of Jansen and Palmer (1987, 1988), Bremer (1987, 1994), Kim et al. (1992), Kim and Jansen (1995), and Bayer and Starr (1998). Two genera of the Barnadesioideae, *Chuquiraga* Juss. and *Doniophyton* Wedd., were chosen as outgroups due to basal position in the Asteraceae of this subfamily in all the abovementioned studies. Their use as an outgroup for the remainder of the Asteraceae is now routine practice (Jansen et al., 1990; Jansen, Michaels, and Palmer, 1991; Keeley and Jansen, 1991; Bayer and Starr, 1998). Tribal circumscriptions and nomenclature are based on the treatment of the Asteraceae by Bremer (1994).

***Ingroup sampling of Cichorioideae and Asteroideae***—Sequences from representatives of the tribes of the Cichoriodieae and extra-Gnaphaloid members of the Asteroideae were taken from taxa sampled in a previous study of tribal relationships (Bayer and Starr, 1998). In addition, eight new taxa were sampled to broaden tribal representation, with emphasis on the Asteroideae: *Coreopsis tinctoria* Nutt. (Heliantheae: Coreopsidinae), *Dimorphotheca sinuata* DC. (Calenduleae), *Euryops virgineus* (L. f) Less. (Senecioneae), *Pentzia flabelliformis* Willd. (Anthemideae), *Pluchea dentex* R. Br. ex Benth. (Plucheeae), *Senecio lineatus* DC. (Senecioneae), and *S. pterophorus* DC. (Senecioneae) (Table 1). *Tarchonanthus trilobus* DC. (Mutisieae) was also sequenced as it has been recognized as the type genus of a new tribe, Tarchonantheae (Keeley and Jansen, 1991). Bentham (1873) included Tarchnanthus in the Inuleae s.l. A broad representation from the extra-Gnaphaloid Asteroideae was used so that the phylogenetic placement of several dubious members of the South African Gnaphalieae might be elucidated.

Except where noted, Anderberg (1991a) is followed throughout this work as a basis for nomenclature and classification of the Gnaphalieae. One member from each of the available recognized genera of the Gnaphalieae was sequenced, except for the large polymorphic and presumably polyphyletic genus *Helichrysum*, for which we sequenced 14 species. We followed the classification of Hilliard (1983) for *Helichrysum* and selected taxa belonging to 13 of her 30 informal morphological groups. Approximately 70% of the 49 genera of South African Gnaphalieae were sequenced in this study (Table 1). Fifty-three new sequences (72% of total) were generated for this study and have been submitted to GenBank (accession numbers are in Table 1). Voucher specimens for all samples are deposited in the herbaria cited in Table 1.

***DNA isolation, amplification, and sequencing***—Total DNA was isolated as outlined in Bayer, Hufford, and Soltis (1996). Recalcitrant DNAs were purified according to methods outlined in Gilmore, Weston, and Thomson (1993), except the amounts of components were scaled down for our purposes. The *trnL/F* region was amplified via the polymerase chain reaction (PCR) using Taq DNA polymerase. The PCR reaction mixture consisted of 5 μL of 20X reaction buffer, 6 μL of 25 mmol/L magnesium chloride solution, 16 μL of a 1.25 mmol/L dNTP solution in equimolar ratio, 25 pmol of each primer, 10–50 ng of template DNA, and 1.0 unit of polymerase in a total volume of 100 μL. The PCR samples were heated to 94°C for 3 min prior to the addition of DNA polymerase to denature unwanted proteases and nucleases. The double-stranded PCR products were produced via 30 cycles of denaturation (94°C for 1 min), primer annealing (48°C for 1 min), and extension (72°C for 2 min). A 7-min final extension cycle at 72°C followed the 30th cycle to ensure the completion of all novel strands.

The region was usually amplified as a single piece using primers ''c'' and ''f'' of Taberlet et al. (1991) to amplify across the *trnL* intron and *trnL/trnF* spacer. In some instances, recalcitrant DNA was amplified as two separate regions using primers ''c'' with ''d'' and ''e'' with ''f''. Double-stranded PCR products were cleaned by column purification using Wizard® PCR Preps DNA Purification System (Promega Corp., Madison, Wisconsin, USA) prior to sequencing.

The double-stranded PCR products were then used as templates in cycle sequencing reactions, which employed four primers (Taberlet et al., 1991) to sequence the two regions, including the terminal primers ''c'' and ''f'' and, in addition, the internal primers ''d'' and ''e'' when the region was amplified as two pieces. The double-stranded PCR products were sequenced using the dideoxy chain termination method (Sanger, Nicklen, and Coulson, 1977) with the use of the Big Dye Terminator RR Kit® (Perkin-Elmer Applied Biosystems, Norwalk, Connecticut, USA) and an ABI automated sequencer (Perkin-Elmer Applied Biosystems, Norwalk, Connecticut, USA) at CSIRO, Plant Industry. An annealing temperature of 57°C was used for primer ''f,'' while temperatures ranging from 60° to 62°C were employed for primers ''c,'' ''d,'' and ''e.'' The cycle sequencing protocol followed manufacturer's instructions. Sequences were assembled using Sequencher™ 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

The sequences were initially aligned using Clustal V vers. 1.4 (Higgins, Bleasby, and Fuchs, 1992), then adjusted manually (Swofford and

Olsen, 1990) following the principles of noncoding sequence alignment presented in Kelchner (unpublished data) and Kelchner and Clark (1997). To improve homology assessment of the numerous insertions and deletions, gaps were positioned with consideration of mutational mechanisms that likely created the observed length mutations. Indels were placed so as to minimize the number of inferred length mutations unless there was clear evidence that particular length mutation events were nonhomologous. The matrix was surveyed manually for the presence of possible hidden minute inversions (Kelchner and Wendel, 1996). The matrix is available from R.J.B. on request.

***Sequence data analysis***—Sequence data were analyzed using PAUP 4.0d64. (Swofford, 1997) on a MacIntosh G3. The data matrix consisted of two outgroup and 72 ingroup taxa. Phylogenetic reconstruction was performed on unweighted characters by heuristic searches with simple, closest, and furthest addition of taxa. Heuristic searches employing a random addition sequence of 100 replicates were also conducted to search for other islands of most parsimonious trees (Maddison, 1991). Indels were coded (present/absent) and three separate data sets were analyzed. The first excluded all the coded indels, the second included indel characters only, and the third was the complete matrix including all indels and nucleotide characters. Strict and 50% majority rule consensus trees (Margush and McMorris, 1981) were constructed for the set of equally most parsimonious cladograms (Fig. 1). Examining the distribution of phylogenetically informative characters (point mutations and indels) on the tree topologies was facilitated by MacClade version 3.0 (Maddison and Maddison, 1992).

Bremer support (''decay'' analysis; Bremer, 1988) was used to estimate the robustness of clades and were performed using a converse constraint (ENFORCE CONVERSE command) method (Baum, Sytsma, and Hoch, 1994). In this procedure, multiple heuristic TBR searches using a random addition sequence of 100 replicates were constrained to search for only those trees lacking an hypothesized clade present in the strict consensus tree of parsimony analyses. A simple subtraction of the shortest trees found in these searches from the most parsimonious tree found in parsimony analysis is equal to the Bremer support index for that clade. The amount of phylogenetic information in the parsimony analysis was assessed by use of the consistency index (C.I.; Kluge and Farris, 1969) and the retention index (R.I.; Farris, 1989).

Bootstrapping (Felsenstein, 1985) was considered an inappropriate measure of clade support for this data set due to the violation of its core assumptions by the following aspects of noncoding region molecular evolution (Kelchner, unpublished data): (1) nucleotides do not uniformly evolve independently in intergenic spacers and introns in the chloroplast; (2) mutations are not distributed randomly throughout chloroplast noncoding regions; and (3) the presence of many gaps in the data matrix make removal of indel characters essential, thus severely limiting the number of characters available for bootstrap resampling.

***Morphology***—MacClade was used to explore the distribution of a broad range of vegetative and floral characters on a tree with similar topology to that found in the molecular analysis. The morphological data matrix presented by Anderberg (1991a) and Anderberg (1992), but consolidated by Puttock (1994), was used for this purpose, using only the taxa we included in our analysis. These morphological character scorings, presented in Fig. 3, are predominantly from the matrices of Anderberg (1991a), Anderberg (1992), Anderberg and Bremer (1991); *Anderbergia* N. Norb. and *Langebergia* Anderb. were scored by R.J.B. and most other character states for other taxa were verified by R.J.B. Base chromosome numbers (Fig. 3) for each genus are from Anderberg (1991a). Distributions of genera were compiled from Lundgren (1972, 1974), Dyer (1975), Bremer (1976, 1978a, b), Kroner (1980), Hilliard and Burtt (1981), Hilliard (1983), Anderberg (1988, 1991a), and Nordenstam (1996), and their distributions are plotted (Fig. 3) against the phytogeographic biomes of Rutherford and Westfall (1994).

## RESULTS

***Sequence characteristics***—Length variation for the entire *trnL* intron ranged from a low of 419 nucleotides to a high of 470 nucleotides (Table 2). The proportion of nucleotide differences ranged from 0.0 to 6.7% between all species of Asteraceae (Table 2). The G/C content of the intron averages 35.0%. The complete *trnL/F* intergenic spacer ranges from 228 nucleotides in *Tagetes* L. to 402 nucleotides in length in the Asteraceae, but the mean length is 350 nucleotides. The great range in length may be somewhat misleading because *Tagetes* has a unique 82 nucleotide deletion; the next shortest sequence is 281 nucleotides. The proportion of nucleotide differences in the spacer is greater than that found in the intron ranging from 0.0 to 12.5% between all species of Asteraceae. Similar to the intron, the spacer has an average G/C content of 34.0% (Table 2). Within the Asteraceae, the proportion of nucleotide differences in the combined spacer and intron sequences ranged from 0.0 to 6.9% (Table 2). Total average G/C content is 34.5%. A total of 147 sites (14.0% of the sequence length) potentially provide phylogenetic information, but the other sites (86.0%) are either invariant or are strictly autapomorphic. Thirty-four indels (Table 2), ranging in length from one to 27 nucleotides, could be coded unambiguously for inclusion in the phylogenetic analysis. The spacer contains more phylogenetically informative indels than the intron does, although the ratios of potentially informative indels to potentially informative nucleotides (1:4.1 and 1:4.7, respectively) are very similar for the intron and spacer.

***Phylogenetic reconstruction***—Analyses of nucleotide characters only or coded indel characters only produced trees of similar topology to those retrieved when both nucleotide and indel characters were combined in one analysis. A 50% majority rule tree is presented in Fig. 1 and represents the analysis of combined nucleotide and coded indel characters. Branches not appearing in the strict consensus trees are indicated by dotted lines. The phylogenetic analysis of the sequence data excluding all indels yielded 366 equally parsimonious trees of 357 steps [consistency index (C.I.) = 0.58; retention index (R.I.) = 0.83; Fig. 1]. Island searches (Maddison, 1991) on the data sets did not reveal any islands of trees of shorter length. Also presented is one of the 366 most parsimonious trees illustrating that the Gnaphalieae s.l., as presently circumscribed, may not be monophyletic (Fig. 2). A reduced molecular tree, with all taxa for which morphological data are available, is presented in Fig. 3.

***Topology of major clades***—A consensus of all trees indicates that the Asteroideae is monophyletic (Fig. 1; clade L) and sister to members of the subfamily Cichorioideae, as represented by the tribes Cardueae, Mutisieae, Vernonieae, Arctoteae, and Lactuceae. Bremer support index (BSI) of 2, and synapomorphies (SYN) of 4 lend support to the monophyly of the group (Fig. 1). As in the previous study of Bayer and Starr (1998), in most cases tribes that were represented by more than one genus [i.e., the Anthemideae (clade M), Calenduleae (clade J), Helianthieae s. l. (clade G), Plucheeae (Clade O), Lactuceae (clade N), Senecioneae (clade I)] are monophy-

TABLE 1. Collections of Asteraceae newly sequenced for this study. Documentation for previously sequenced taxa, used as part of the outgroup in this study, can be found in Bayer and Starr (1998). Presented are species, group affinities (one or three letter codes), collectors (location of voucher), geographic origin, and GenBank accession numbers. Voucher specimens are deposited in AD, CANB, F, MEL, MO, NBG, PERTH, and TI. GenBank accession numbers for the sequences (intron, spacer) are given. * = Type species of genus. ANT = Anthemideae, A = *Athrixia* group, CAL = Calenduleae, C = Cassiniinae, G = Gnaphaliinae, HEL = Heliantheae, M = *Macowania* group, MUT = Mutisieae, P = *Pentatrichia* group, PLU = Plucheeae, R = Relhaniinae, SEN = Senecioneae.

| Species | Affinity | Collectors and numbers (voucher location) | Geographic origin | GenBank numbers (intron, spacer)[a] |
|---|---|---|---|---|
| 1) *Anaxeton arborescens* (L.) Less.* | C | Bayer & Puttock SAF-96035 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098826, GBAN-AF100489 |
| 2) *Anderbergia elsiae* B. Nord.* | C | Bayer & Puttock SAF-96254 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098827, GBAN-AF100490 |
| 3) *Anisothrix kuntzei* O. Hoffm.* | P | Bayer & Puttock SAF-96271 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098859, GBAN-AF100522 |
| 4) *Arrowsmithia styphelioides* DC.* | M | Bayer & Puttock SAF-99223 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098809, GBAN-AF100472 |
| 5) *Athrixia capensis* Ker-Gawl.* | A | Bayer & Puttock SAF-96287 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098818, GBAN-AF100481 |
| 6) *Bryomorphe lycopodioides* (Sch. Bip. ex Walp.) Levyns.* | R | Bayer & Puttock SAF-96057 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098820, GBAN-AF100483 |
| 7) *Callilepis laureola* DC.* | P | Bayer & Puttock SAF-96212 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098857, GBAN-AF100520 |
| 8) *Coreopsis tinctoria* Nutt. | HEL | Bayer FL-97003 (CANB) | U.S.A.: Florida | GBAN-AF098851, GBAN-AF100514 |
| 9) *Dimorphotheca sinuata* DC. | CAL | Bayer & Puttock SAF-96148 (CANB, F, MO) | South Africa: Northern Cape | GBAN-AF098855, GBAN-AF100518 |
| 10) *Disparago ericoides* (Berg.) Gaertn.* | R | Oliver 10980 (NBG, CANB) | South Africa: Western Cape | GBAN-AF098821, GBAN-AF100484 |
| 11) *Dolichothrix ericoides* (Lam.) Hilliard & B. L. Burtt* | R | Bayer & Puttock SAF-96252 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098822, GBAN-AF100485 |
| 12) *Edmondia sesamoides* (L.) Hilliard* | G | Bayer & Puttock SAF-96066 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098844, GBAN-AF100507 |
| 13) *Elytropappus rhinocerotis* (L. f.) Less. | R | Bayer & Puttock SAF-96018 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098823, GBAN-AF100486 |
| 14) *Euryops virgineus* (L. f.) Less. | SEN | Bayer & Puttock SAF-96237 (CANB, F, MO) | South Africa: Eastern Cape | GBAN-AF098854, GBAN-AF100517 |
| 15) *Helichrysum acrophilum* Bolus | G | Bayer & Puttock SAF-96118 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098836, GBAN-AF100499 |
| 16) *Helichrysum allioides* Less. | G | Bayer & Puttock SAF-96214 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098832, GBAN-AF100495 |
| 17) *Helichrysum argyrophyllum* DC. | G | Bayer & Puttock SAF-96230 (CANB, F, MO) | South Africa: Eastern Cape | GBAN-AF098837, GBAN-AF100500 |
| 18) *Helichrysum aureum* (Houtt.) Merrill | G | Bayer & Puttock SAF-96191 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098838, GBAN-AF100501 |
| 19) *Helichrysum cephaloideum* Less. | G | Bayer & Puttock SAF-96199 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098833, GBAN-AF100496 |
| 20) *Helichrysum cylindriflorum* (L.) Hilliard & B. L. Burtt | G | Bayer & Puttock SAF-96138 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098839, GBAN-AF100502 |
| 21) *Helichrysum dasyanthus* (Willd.) Sweet | G | Bayer & Puttock SAF-96014 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098840, GBAN-AF100503 |
| 22) *Helichrysum dregeanum* Sond. & Harv. | G | Bayer & Puttock SAF-96196 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098831, GBAN-AF100494 |
| 23) *Helichrysum leontonyx* DC. | G | Bayer & Puttock SAF-96179 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098829, GBAN-AF100492 |
| 24) *Helichrysum odoratissimum* (L.) Sweet | G | Bayer & Puttock SAF-96189 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098834, GBAN-AF100497 |
| 25) *Helichrysum patulum* (L.) D. Don | G | Bayer & Puttock SAF-96006 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098830, GBAN-AF100493 |
| 26) *Helichrysum populifolium* DC. | G | Bayer & Puttock SAF-96222 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098835, GBAN-AF100498 |
| 27) *Helichrysum tricostatum* (Thunb.) Less. | G | Bayer & Puttock SAF-96165 (CANB, F, MO) | South Africa: Northern Cape | GBAN-AF098841, GBAN-AF100504 |
| 28) *Helichrysum zwartsbergense* Bolus | G | Bayer & Puttock SAF-96101 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098842, GBAN-AF100505 |
| 29) *Lachnospermum neglectum* Schltr. | R | Bayer & Puttock SAF-96106 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098824, GBAN-AF100487 |
| 30) *Langebergia canescens* (DC.) Anderb.* | C | Bayer & Puttock SAF-96250 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098828, GBAN-AF100491 |
| 31) *Lasiopogon glomeratus* (Harv.) Hilliard | G | Bayer & Puttock SAF-96180 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098819, GBAN-AF100482 |
| 32) *Leysera gnaphalodes* (L.) L.* | R | Bayer & Puttock SAF-96021 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098810, GBAN-AF100473 |
| 33) *Metalasia densa* (Lam.) P. O. Karis | R | Bayer & Puttock SAF-96001 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098848, GBAN-AF100511 |
| 34) *Oedera squarrosa* (L.) Anderb. & K. Bremer | R | Bayer & Puttock SAF-96112 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098812, GBAN-AF100475 |
| 35) *Oreoleysera montana* (Bolus) Bremer* | R | Esterhuysen 8480 (NBG) | South Africa: Western Cape | GBAN-AF098814, GBAN-AF100477 |
| 36) *Pentatrichia petrosa* Klatt.* | P | Williamson 4163 (NBG) | South Africa: Northern Cape | GBAN-AF098817, GBAN-AF100480 |

TABLE 1. Continued.

| Species | Affinity | Collectors and numbers (voucher location) | Geographic origin | GenBank numbers (intron, spacer)[a] |
|---|---|---|---|---|
| 37) *Pentzia flabelliformis* Willd. | ANT | Bayer & Puttock SAF-96275 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098856, GBAN-AF100519 |
| 38) *Petalacte coronata* (L.) D. Don* | C | Bayer & Puttock SAF-96002 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098843, GBAN-AF100506 |
| 39) *Phaenocoma prolifera* (L.) D. Don* | R | Bayer & Puttock SAF-96045 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098825, GBAN-AF100488 |
| 40) *Plecostachys serpyllifolia* (Berg.) Hilliard & B. L. Burtt* | G | Bayer & Puttock SAF-96049 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098849, GBAN-AF100512 |
| 41) *Pluchea dentex* R. Br. ex Benth. | PLU | Short et al. (AD, CANB, MEL, PERTH, TI) | Australia: Western Australia | GBAN-AF098858, GBAN-AF100521 |
| 42) *Printzia polifolia* (L.) Hutch.* | A | Bayer & Puttock SAF-96284 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098816, GBAN-AF100479 |
| 43) *Relhania fruticosa* (L.) K. Bremer* | R | Bayer & Puttock SAF-96294 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098813, GBAN-AF100476 |
| 44) *Rhynchopsidium pumilum* (L. f.) DC. | R | Bayer & Puttock SAF-96122 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098811, GBAN-AF100474 |
| 45) *Rosenia glandulosa* Thunb.* | R | Goldblatt & Manning 10528 (MO, NBG) | South Africa: Western Cape | GBAN-AF098815, GBAN-AF100478 |
| 46) *Senecio lineatus* DC. | SEN | Bayer & Puttock SAF-96246 (CANB, MO, F) | South Africa: Western Cape | GBAN-AF098852, GBAN-AF100515 |
| 47) *Senecio pterophorus* DC. | SEN | Bayer & Puttock SAF-96003 (CANB, MO, F) | South Africa: Western Cape | GBAN-AF098853, GBAN-AF100516 |
| 48) *Stoebe aethiopica* L.* | R | Bayer & Puttock SAF-96068 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098845, GBAN-AF100508 |
| 49) *Syncarpha gnaphaloides* (L.) DC.* | G | Bayer & Puttock SAF-96269 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098846, GBAN-AF100509 |
| 50) *Tarchonanthus trilobus* DC. | MUT | Bayer & Puttock SAF-96211 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098860, GBAN-AF100523 |
| 51) *Tenrhynea phylicifolia* (DC.) Hilliard & B. L. Burtt* | G | Bayer & Puttock SAF-96213 (CANB, F, MO) | South Africa: Kwazulu Natal | GBAN-AF098850, GBAN-AF100513 |
| 52) *Trichogyne ambigua* (L.) Druce*[ab] | G | Bayer & Puttock SAF-96024 (CANB, F, MO) | South Africa: Western Cape | GBAN-AF098847, GBAN-AF100510 |
| 53) *Vellereophyton dealbatum* (Thunb.) Hilliard & B. L. Burtt* | G | Bayer WA-94100 (CANB, MEL, PERTH) | Australia: Western Australia[c] | GBAN-AF098808, GBAN-AF100471 |

[a] The prefix GBAN- has been added to link the online version of *American Journal of Botany* to GenBank, but is not part of the actual accession number.
[b] The designated type of *Trichogyne* Less. is *T. laricifolia* (Lam.) Less., which is a taxonomic synonym of *T. ambigua* (L.) Druce.
[c] *Vellereophyton dealbatum* is native to South Africa, but adventive in Australia.

letic. The only exception to this is the Cardueae in the Cardueae/Mutisieae clade (K). *Tarchonanthus* L. is part of the Cardueae/Mutisieae clade (K) lending support to Bremer's (1994) placement of the genus in the Mutisieae.

Generally, tribal support throughout the topology (Fig. 1) is high, with most clades being supported by multiple synapomorphies, from three in the Helianthieae s. l. (G) to 20 in the Anthemideae (clade M). The Gnaphalieae, in the sense of Anderberg (1991a), are not monophyletic in this analysis because the genera *Callilepis* DC. and *Printzia* Cass. are associated with the Astereae (Fig. 1; clade H). Although *Callilepis* is not in clade H with *Aster* L. and *Printzia* (SYN = 6), it does share an insertion with *Aster* (Fig. 1, arrow). Additionally, in several shortest trees the basal group of the Gnaphalieae (Figs. 1, 2; clade F) becomes sister to other tribes, such as the Astereae, Anthemideae, and Heliantheae (Fig. 2; clade G, H). Clade F contains members of the Relhaniinae s.s. and some of Anderberg's (1991a) ''basal'' group of the Gnaphalieae. The Gnaphalieae in a broad sense (clade Q) are not well supported (SYN = 2), as the group does not appear in all most parsimonious trees.

***Topology of clades within the Gnaphalieae s. l. (clade Q)***—The following considers the Gnaphalieae in a broad sense (clade Q) to include the members of the Relhaniinae and some of Anderberg's ''basal'' group (clade F), and the Gnaphalieae s.s. as clade P (Fig. 1). Beginning at the base of the group (clade F) and proceeding systematically toward the top of the tree (clade T) a number of well-supported groups are evident. The basal group of the Gnaphalieae as represented in this anlayis is clade F, which contains three taxa from Anderberg's ''basal'' group (BG), *Pentatrichia* Klatt, *Anisothrix* O. Hoffm., and *Arrowsmithia* DC. and the Relhaniinae s.s. (R). *Pentatrichia* and *Anisothrix* are sister taxa and together form a clade that is sister to the strongly supported (SYN = 4; BSI = 6) clade F' in which *Arrowsmithia* is sister to the Relhaniinae s.s. A strong relationship of *Leysera* L. to *Rhynchopsidium* DC and A. DC. (SYN = 5; BSI = 6) is evident, and they are sister to a clade containing *Rosenia* Thunberg, *Relhania* L'Her, and *Oedera*.

The Gnaphalieae s.s. (clade P) are strongly supported (SYN = 5; BSI = 4) with *Trichogyne* Less. sister to the rest of the group (Fig. 1). Next is clade E containing shrubby members of the Relhaniinae s. l., *Metalasia* R. Br., *Phaenocoma* D. Don, *Lachnospermum* Willd., and *Dolichothrix* Hilliard and B. L. Burtt as well as with *Athrixia* Ker-Gawl. *Athrixia* is the only disparate member of this clade, having been placed as part of the basal group in Anderberg's (1991a) analysis (Fig. 1). *Lasiopogon* Cass., a member of Anderberg's subtribe Gnaphaliinae, is sister to clade U, showing more sequence affinites to this clade than to other members of the Gnaphaliinae, such as *Vellereophyton* Hilliard and B. L. Burtt (Fig. 1). Clade D (SYN = 1; BSI = 2) contains four genera of ericoid-leaved shrubs, *Bryomorphe* Harv., *Disparago* Gaertn., *Elytropappus* Cass., and *Stoebe* L., which are currently members of the Relhaniinae of Anderberg (1991a). *Vellereophyton* is part of a polytomy that comprises other members of the Gnaphaliinae of clade C, including *Plecostachys* Hilliard and B. L. Burtt and *Tenrhynea* Hilliard and B. L. Burtt. Also part of this poly-

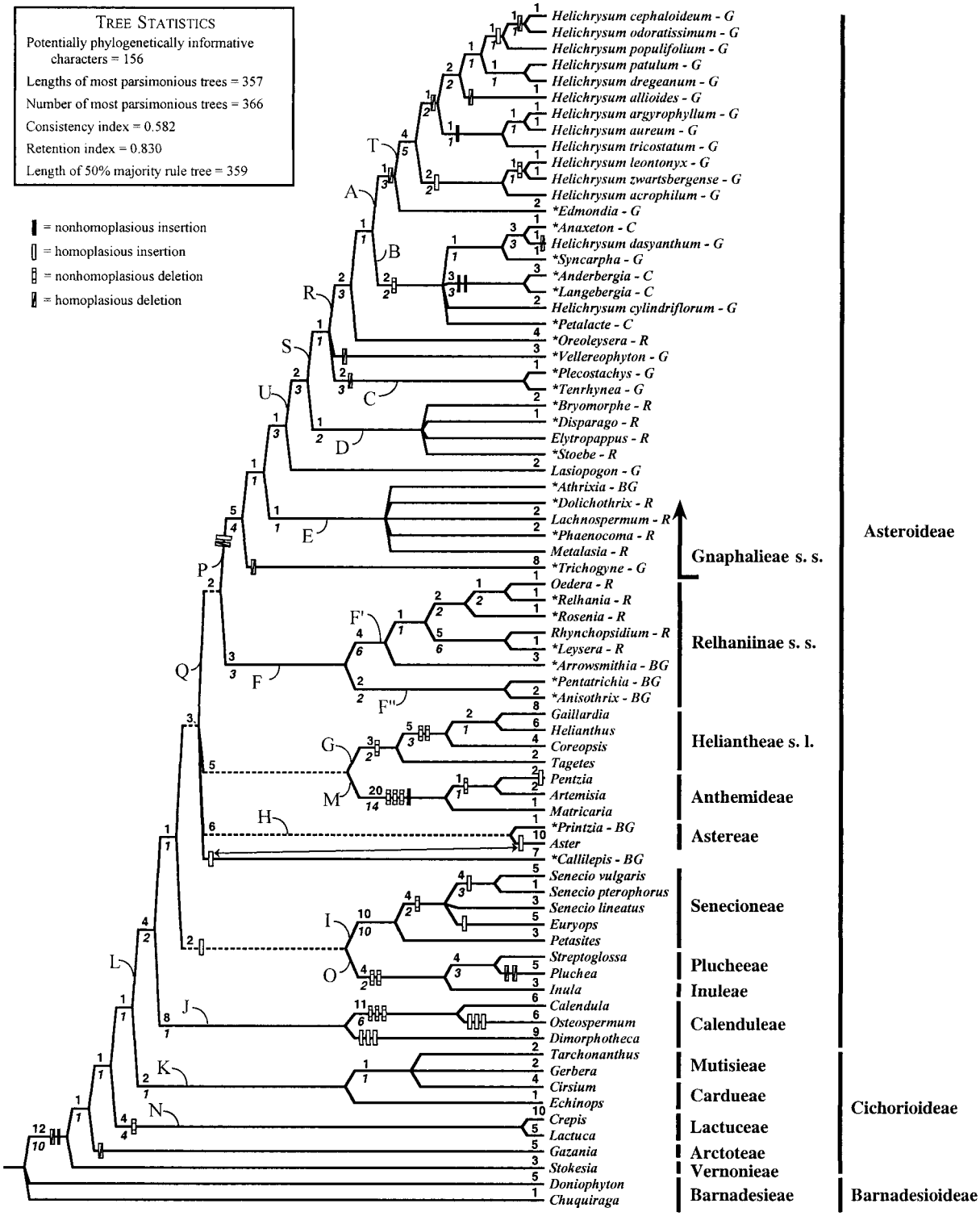


Fig. 1. The 50% majority rule consensus tree of 366 equally parsimonious trees resulting from phylogenetic analysis of sequence data of the *trnL* intron and the *trnL/F* intergenic spacer using all informative nucleotide characters and indels. Branches that did not appear in the strict consensus tree are indicated by dashed lines. The tree gives the number of apomorphies (including indels) above the branches, decay index values in italics below each branch. Phylogenetically informative insertions and deletions are shown on the branches. Taxon labels are from left to right: genera, tribes, and subfamilies. Generic names preceded by an asterisk indicate instances where the type species of the genus was used to represent that genus in the analysis. Abbreviations following terminal taxon names represent subtribe or group names following the circumscription of Anderberg (1991): G = Gnaphaliinae, C = Cassiniinae, R = Relhaniinae, BG = Anderberg's basal group of the Gnaphalieae. Arrow shows indel shared by *Callilepis* and *Aster*. Clades that are discussed in the text are labeled with capital letters.

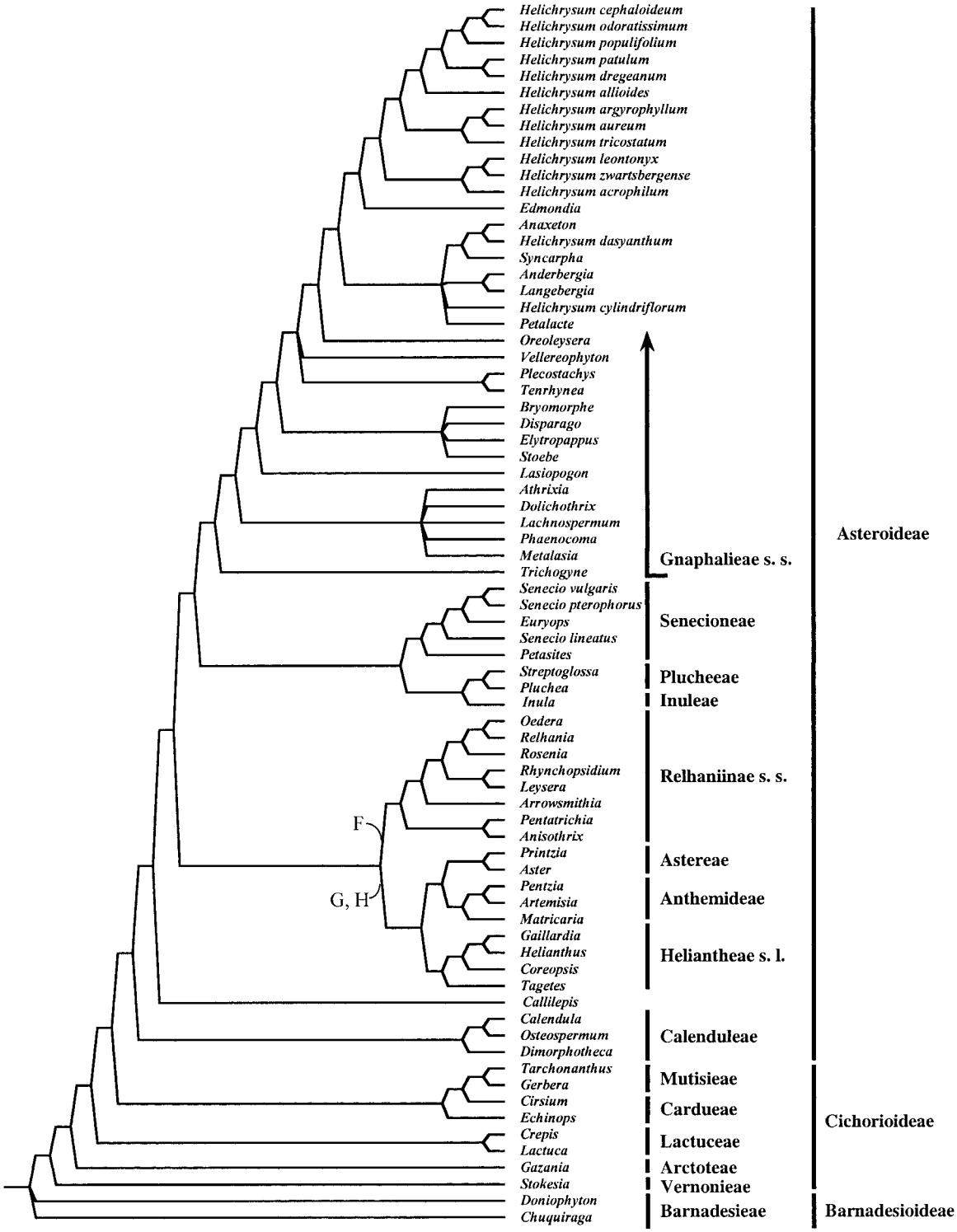


Fig. 2. One shortest tree of 366 equally parsimonious trees of length 357 resulting from phylogenetic analysis of sequence data of the *trnL* intron and the *trnL/F* intergenic spacer using all informative nucleotide characters and indels. It shows a different topology from the strict and majority rule consensus trees in that the Relhaniinae s.s. (clade F) are not sister to the rest of the Gnaphalieae. Clades that are discussed in the text are labeled with capital letters.

TABLE 2. Sequence characteristics of the *trnL/F* spacer, *trnL* intron, and combined *trnL − trnL/F* noncoding region sequenced in this study.

| Sequence characteristic | *trnL/F* spacer | *trnL* intron | Combined (*trnL* intron + *trnL/F* spacer) |
|---|---|---|---|
| Length range (nucleotides) | (228) 281–402 | 419–470 | (661) 700–861 |
| Length mean (nucleotides) | 350 | 442 | 792 |
| Aligned length (nucleotides) | 497 | 557 | 1054 |
| G + C content mean | 34.0 | 35.0 | 34.5 |
| Sequence divergence | 0–12.5% | 0–6.7% | 0–6.9% |
| Number of variable sites | 216/497 (43%) | 131/557 (24%) | 347/1054 (33%) |
| Number of potentially informative sites/total aligned length | 86/497 (17%) | 61/557 (11%) | 147/1054 (14%) |
| Number of constant sites/total aligned length | 281/497 (57%) | 426/557 (76%) | 707/1054 (67%) |
| Number of autapomorphic sites/total aligned length | 130/497 (26%) | 70/557 (14%) | 200/1054 (19%) |
| Number of unambiguously aligned indels | 21 | 13 | 34 |
| Indel size range (nucleotides) | 1–27 | 1–24 | 1–27 |
| Ratio of indels to potentially informative sites | 1:4.1 | 1:4.7 | 1:4.3 |

tomy is a large clade (R) in which *Oreoleysera* K. Bremer, currently in subtribe Relhaniinae, is sister to the last two Clades A and B, consisting primarily of members of the Cassiniinae and Gnaphaliinae, respectively. Clade B contains four members of Anderberg's Cassiniinae, *Petalacte* D. Don, *Langebergia, Anderbergia,* and *Anaxeton* Gaertn., as well as *Syncarpha* DC. and two species of *Helichrysum, H. cylindriflorum* (L.) Hilliard and Burtt and *H. dasyanthum* (Willd.) Sweet, all three of the subtribe Gnaphaliinae (Fig. 1). In clade A, *Edmondia* Cass., which was previously part of both *Helichrysum* and *Helipterum* DC. (Hilliard, 1983; Nordenstam, 1989), is sister to a well-supported lineage (SYN = 4; BSI = 5) of 12 South African *Helichrysum* species representing 11 of Hilliard's informal groups (Hilliard, 1983).

## DISCUSSION

The most diverse family of flowering plants in Southern Africa are the Asteraceae, with 174 genera, 80 of which are endemic, and 2072 endemic species (Goldblatt, 1978). Most of the genera of the South African Gnaphalieae are confined to or have their greatest diversity in the Cape floristic region (Fig. 3). The fact that Anderberg's (1991a) basal group of the Gnaphalieae is restricted in distribution to southern Africa suggests the original radiation of the group took place in South Africa.

Anderberg's (1991a) ''basal'' group of the Gnaphalieae and the Relhaniinae consists primarily of genera that Merxmüller, Leins, and Roessler (1977) had placed in the subtribe Athrixiinae of their Inuleae. Merxmüller, Leins, and Roessler (1977) were ''disturbed'' by this subtribe, realizing it was not well defined and had a number of disparate members that did not seem to fit in the group, or any other group for that matter. One of the most anomalous of these genera is *Callilepis,* and indeed, the phylogenetic relationships proposed in Fig. 1 indicate *Callilepis* is not included in the Gnaphalieae (clade Q).

The affinities of *Callilepis* are not clear from our analysis, as it is part of a polytomy in the majority rule topology (Fig. 1). However, it does share a single base pair deletion with *Aster* (Fig. 1, arrow), which could point to an affinity there. Its heads are radiate, the pappus consists of a few awns, and the style branches have stigmatic hairs localized near the apices, characteristics that are unlike most of the Gnaphalieae. It shares an unusual chemical character with *Atractylis* L. (Cardueae) (Candy et al., 1977), but that may be convergence as most evidence points to Asteroideae affinities for *Callilepis,* not Cichorioideae. In another chemical investigation Bohlmann and Zdero (1982) discovered additional compounds in *Callilepis* indicating no clear relationships of *Callilepis* to members of the Athrixiinae sensu Merxmüller, Leins, and Roessler (1977). As we continue to add sequence data to our data set the phylogenetic position of *Callilepis* will hopefully be clarified; however, at present our evidence indicates *Callilepis* is probably not a member of the Gnaphalieae.

Another taxon conflicting with Anderberg's (1991) Gnaphalieae is *Printzia.* On seeing *Printzia* for the first time in the field, we (R.J.B. and C.F.P.) were impressed by its resemblance to species of *Aster,* and interestingly the type species of *Printzia, P. polifolia* (L.) Hutch. (Table 1), was originally described by Linnaeus as *Aster polifolius* L. (Kroner, 1980). In our analysis, *Printzia* and *Aster* form a monophyletic group supported by six synapomorphies in 97% of the most parsimonious trees. Additionally, in a study of the phytochemistry of *Printzia,* Bohlmann and Zdero (1978) found compounds that were identical to those found in members of the Astereae, specifically to species of *Solidago* L. Given the evidence, we feel the affinities of *Printzia* lie with the Astereae, not the Gnaphalieae.

The Relhaniinae s.s. and some ''basal'' group taxa [the core Athrixiinae of Merxmüller, Leins, and Roessler (1977)] comprise clade F. In the majority of most parsimonious trees, this group is sister to the remainder of the tribe Gnaphalieae (Fig. 1), but in some it is sister to a clade containing the Anthemideae and Helianthieae s. l. (Fig. 2; clades G, H). It is interesting to note that historically *Oedera* has most often been considered as a member of the Anthemideae (Anderberg and Källersjö, 1988), although it was originally placed in the Inuleae by Linnaeus (1771). The primary reason for lodging *Oedera* with the Anthemideae is the lack of tailed anthers, truncate and apically penicillate style branches, and pappus of scales (Anderberg and Källersjö, 1988). These are features that are shared among other members of clade F (Fig. 3), but some are relatively uncommon in the Gnaphalieae s.s. A similarity among *Oedera* and *Leysera* and *Relhania* was noted by Anderberg and Källersjö (1988), who argued that removal of *Oedera* to the Inuleae and later Gnaphalieae (Anderberg, 1991a) was better than

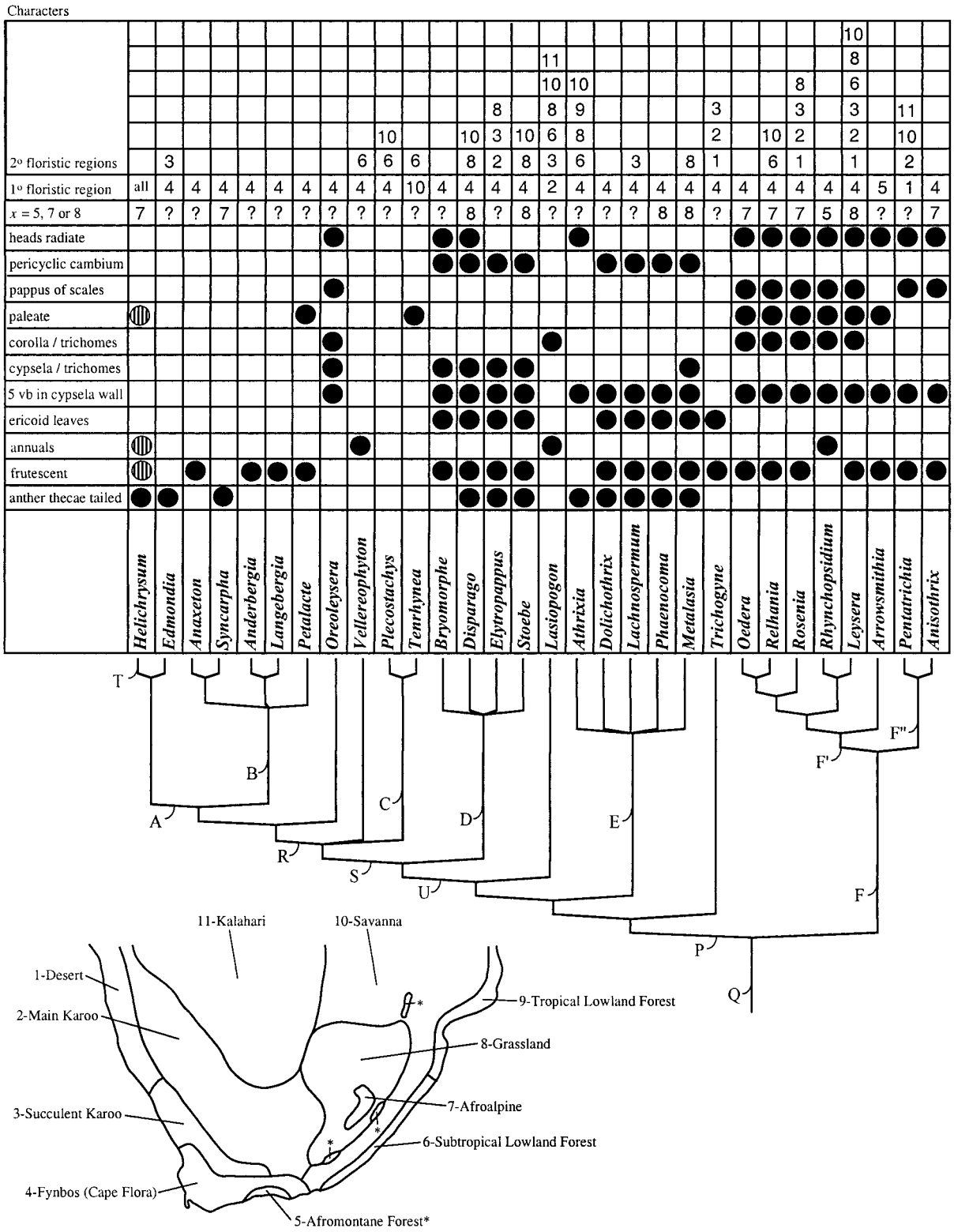


Fig. 3. Distribution of 11 morphological characters, base chromosome numbers, and geographic distribution plotted against a reduced 50% majority rule tree (Fig. 1). A solid dot indicates that all or the majority of taxa possess that character state; a partially filled dot indicates that the character state is polymorphic and present only in some of species of the genus. Alternative character states for characters include: heads discoid; cambium not pericyclic; pappus scales absent; receptacle epaleate; corolla and cypselae without trichomes; 2 (1 or 3) vascular bundles in the cypsela wall; leaves nonericoid; plants perennial; plants herbaceous; and anther thecae not distinctly tailed. Primary (1°) floristic region is that where all of most species of the genus occur, 2° floristic regions are those where a minority of the species occur. *Helichrysum* occurs in all regions. The coded numbers under the floristic regions correspond to the regions on the inset map, which follows one presented in Rutherford and Westfall (1994). Clades that are discussed in the text are labeled with capital letters.

moving *Leysera* and *Relhania* to the Anthemideae. At this time, there appear to be two alternative hypotheses as to the relationships of this group, either it is the basal group of the Gnaphalieae or it might be a separate tribe itself, i.e., the Relhanieae Kostel., as has been proposed in the distant past by Kosteletzky (1833).

The Relhaniinae s.s. do not appear to be part of the Anthemideae, but may be sister to it (Fig. 2). There are a number of morphological features (Fig. 3) that hold the *Relhaniinae* s.s. itself rudimentarily together, including frutescent growth habit, a pappus of scales, five vascular bundles in the cypsela wall, and paleate receptacles. Chromosome numbers (Fig. 3) in the group appear to be based on $x = 7$, with aneuploid increase in *Leysera* to $x = 8$ and a decrease to $x = 5$ in the annual genus *Rhynchopsidium.* In clade F′′ we have *Pentatrichia* and *Anisothrix* as sister taxa. Anderberg (1988, 1991a) alluded to the morphological similarity of these two taxa, and they are unusual among the Gnaphalieae for having leaves mostly with dentate margins [excepting *A. integra* (Compton) Anderb.]. The Gnaphalieae almost exclusively have leaves with entire margins. *Anisothrix* has some of the rare leysseral derivative compounds also found in *Relhania, Leysera,* and *Macowania* Oliv. (Zdero, Bohlmann, and Anderberg, 1991).

Within the Relhaniinae s.s., *Arrowsmithia* is sister to the traditionally (Anderberg and Bremer, 1991) recognized members of the *Relhania* group. It shares a number of morphological features with the Relhaniinae, namely radiate heads, paleate receptacles, and frutescent habit (Fig. 3), and is also strongly supported as a member of the group based on our molecular data (Fig. 1). The brownish color, texture, and size of the phyllaries of *Arrowsmithia* are very similar to members of the Relhaniinae (R.J.B., personal observations). *Arrowsmithia* is a monotypic genus that has been included within *Macowania* (Kroner, 1980), and the close relationship of these taxa has been recognized by others (Hilliard and Burtt, 1985; Anderberg, 1991a). A chemical investigation of *Macowania* (Bohlmann and Zdero, 1977) showed that two compounds isolated from this genus were also found in *Leysera gnaphalodes* L. Harvey (1865) was perhaps the first to note the similarity in ''aspect'' between *Arrowsmithia* and *Relhania.* Although Anderberg (1991a) had *Arrowsmithia* as part of his basal group, quite removed from the Relhaniinae, our current results, the morphological characteristics mentioned above, and the phytochemical evidence of Bohlmann and Zdero (1977) indicate a probable sister relationship to the Relhaniinae s.s.

Within the Relhaniinae s.s. (Fig. 1) we find a strong sister relationship between *Leysera* and *Rhynchopsidium,* a relationship also indicated by the morphological cladistic analysis of Anderberg and Bremer (1991). *Rhynchopsidium* had once been part of *Leysera* (Bremer, 1978a), but was removed from *Leysera* based on a number of apomorphies, including annual habit. In the remaining clade we find *Oedera* as sister to *Relhania* and they in turn sister to *Rosenia* (Fig. 1; clade F′). A similar relationship was demonstrated by Anderberg and Bremer (1991); in their analysis *Rosenia* is somewhat more removed from *Oedera* and *Relhania.*

The only member of the Relhaniinae s.s. that is not part of the group in clade F′ in our analysis is the monotypic genus *Oreoleysera.* Instead, *Oreoleysera* is in a monotypic clade that is sister to clade R, containing *Helichrysum* species and elements of the South African Cassininae (Fig. 1). Bremer (1978b) removed *Leysera montana* Bolus from the genus *Leysera* and placed in its own genus, Oreoleysera, because he felt the genus was nonmonophyletic when the species was included. *Oreoleysera montana* (Bolus) K. Bremer is an alpine cushion plant from the Cape Region and as such has highly reduced and modified morphology. *Leysera* has a pappus of dentoid scales (Fig. 3) and plumose pappus bristles, while *Oreoleysera* has small narrow scales and nonplumose bristles. There has been some question as to the homology of those scales, with Bremer (1978b) indicating they are absent, or at least are similar to those seen in *Leysera.* Later, Anderberg and Bremer (1991) reversed the earlier statement, claiming they are present. Additionally, *Oreoleysera* is epaleate, whereas all the Relhaniinae s.s. (including *Arrowsmithia;* Fig. 3, clade F′) are paleate. The analyses of Anderberg (1991a) and Puttock (1994) show *Oreoleysera* as sister to the rest of the Relhaniinae s.s. *Oreoleysera* has probably been misplaced in the Relhaniinae due to its reduced morphology and alpine habit convergence.

*Trichogyne* was incorporated into the genus *Ifloga* Cass. by Hilliard and Burtt (1981), but Anderberg (1991a) disagreed and reinstated *Trichogyne* to represent eight species in South Africa, leaving six other South African and Saharo-Sindian species in *Ifloga.* Anderberg's cladistic analysis of morphology (Anderberg, 1991a) shows *Ifloga* and *Trichogyne* in a strongly supported sister relationship. Traditionally this group has been placed in the subtribe Filaginae of the Inuleae s.l. (Bentham, 1873), but Merxmüller, Leins, and Roessler (1977) put it in the Gnaphaliinae (*Gnaphalium* L.—*Helichrysum* group). Anderberg (1991a) follows the lead of Merxmüller, Leins, and Roessler (1977), also positioning the group in his Gnaphaliinae. Hilliard and Burtt (1981) comment that *Ifloga* and *Trichogyne* remain isolated members of the Gnaphaliinae, implying the Filaginae affiliation is more convincing. Our analysis shows *Trichogyne* as sister to clade E, a group that shares the apomorphies of shrubby habit, small ericoid leaves, small few-flowered heads (as in *Metalasia*), one row of barbellate–plumose pappus bristles, and epaleate receptacles (Fig. 3). Therefore, we believe the affinities of *Trichogyne* may lie with the members of clade E.

In clades E and D (Fig. 1) are the members of Anderberg's Metalasia group that we surveyed. These are the remaining elements of Anderberg's (1991a) Relhaniinae s.l. The only enigmatic member of either clade is *Athrixia,* of Anderberg's basal group, in clade E (Fig. 1). It differs from other members of clade E by having radiate heads and nonericoid leaves, but radiate heads do appear in *Bryomorphe* and *Disparago* in the other Metalasia group, clade D (Fig. 3). *Athrixia* aside, the two clades (D and E) of Anderberg's (1991a) Metalasia group share a number of apomorphies (Fig. 3), including shrubby habit, ericoid leaves, five vascular bundles in the cypsela wall, pericyclic cambium, and possibly a base chromosome number of $x = 8$. All members of clade E except *Lachnospermum* share papery, opaque, dimorphically colored phyllaries that are a color other than hyaline or brown,

as well as pappus bristles that are barbellate or scabrous. The members of clade D, in contrast, all have plumose pappus bristles (except *Bryomorphe,* which is barbellate) and cartilaginous, transparent, monomorphically colored involucral bracts that are hyaline or brown in color. Even though Anderberg's Metalasia group as a whole seems fairly cohesive morphologically, we have found it paraphyletic or polyphyletic as it does not form a clade on the topology (Figs. 1 and 3). *Phaenocoma* and *Stoebe* share a similar phytochemistry (Bohlmann and Suwita, 1978), further uniting Anderberg's (1991) Metalasia group. The taxa included within clades D and E (Fig. 3) correspond to Anderberg's (1991a) groups based on morphology, with the exception that *Bryomorphe* was sister to the other two clades of the Metalasia group in his study. *Bryomorphe,* like *Oreoleysera,* is an alpine cushion plant; subsequently it has highly reduced and convergent morphology presumably driven by the alpine environment, hence its phylogenetic position may have been obscured by homoplasious morphological characters. *Lasiopogon* is in a rather unusual position, allied to the two Metalasia clades, but sharing few morphological apomorphies with them (Fig. 3). It is a small annual in subtribe Gnaphaliinae and is placed close to *Trichogyne* by Anderberg.

On the whole, in clade S we see a trend, except in *Oreoleysera,* toward the following charater states: nonericoid leaves, 2 (1–3) vascular bundles in the cypsela wall, cypselae with trichomes, absence of pericyclic cambium, and perhaps a tendency toward a base chromosome number of $x = 7$ (Fig. 3). *Plecostachys* and *Tenrhynea* are sister taxa in our analysis (Fig. 1) and in Anderberg's (1991a). They share a number morphological apomorphies with *Vellereophyton,* such as lack of distinctly tailed anthers and herbaceous habit (Fig. 3). All three belong to Anderberg's (1991a) subtribe Gnaphaliinae, and both *Plecostachys* and *Vellereophyton* are recent segregates out of the large, polyphyletic genus *Gnaphalium.* Anderberg's cladistic analysis of morphology shows the three genera as being fairly closely related and this conclusion is supported by our molecular analysis.

Clade B consists of all the South African members of Anderberg's (1991a) Cassiniinae (Anaphalis group), as well as a few members of the Gnaphaliinae, *Syncarpha,* and two species of *Helichrysum* s. l. *Anaxeton, Petalacte, Langebergia,* and *Anderbergia* represent a cohesive group on morphological grounds in Anderberg's (1991a) analysis. In fact, *Langebergia* and *Anderbergia* were recently created segregate genera from former *Petalacte* species (Anderberg, 1991a; Nordenstam, 1996). The strong sister relationship of *Langebergia* and *Anderbergia* cannot be denied, based on both morphological and molecular grounds (Fig. 1; SYN = 3, BSI = 3, and they share two large nonhomoplasious insertions).

*Syncarpha,* which was resurrected by Nordenstam (1989) to accommodate misaligned species of *Helipterum* and *Helichrysum,* has similarities to the Anaphalis group. They all have monochromous, opaque phyllaries, epaleate receptacles (except *Petalacte*), and barbellate (infrequently plumose) pappus. Anderberg's analysis (1991a) did not test the relationships between the Anaphalis group and *Syncarpha,* but Puttock (1994) showed them to be nested in a polytomy of members of the Gnaphaliinae and sister to a clade containing *Syncarpha.* Perhaps *Syncarpha* lies within the Anaphalis group in the Cassiniinae rather than with the Gnaphaliinae; however, its affinities remain unclear until more species can be evaluated.

The presence of the two species of *Helichrysum* in clade B is supportive of the idea that *Helichrysum* is a polyphyletic genus. *Helichrysum dasyanthum* is sister to *Anaxeton,* and they in turn are sister to *Syncarpha.* An usual synapomorphy it shares with *Syncarpha* is that of fimbrilliferous receptacles, which are rare in the Gnaphalieae, appearing only in *Syncarpha, Edmondia,* and some species of *Helichrysum* [absent from *H. orientale* (L.) Gaertn., the type of the genus]. *Helichrysum dasyanthum* bears a morphological resemblance to *Anaxeton* in that it is a small shrub with leaves up to 30 mm long, the heads are born in terminal corymbs and are heterogamous, and the phyllaries are opaque to semi-opaque (Hilliard, 1983). The pappus bristles are barbellate, and the florets, especially the pistillate ones, are covered with glandular hairs near the apex. Lundgren (1972) commented on the problems of generic delimitation in the Gnaphalieae, but it was his opinion that *Anaxeton* was a comparatively well-defined genus. However, it may be that the generic concept of *Anaxeton* could be expanded to include *H. dasyanthum* and other *Helichrysum* species.

The affinities of *Helichrysum cylindriflorum* are less obvious. It is a small shrub with large numbers of tiny heads arranged in corymbs or panicles, the phyllaries are semi-opaque, the pappus is of barbellate bristles, the corolla is glandular at the apex, and the receptacle is epaleate. Since it is part of a polytomy in this analysis, its relationships within clade B are difficult to assess. Perhaps further sampling of taxa within this clade could provide resolution to this problem.

In clade A, *Edmondia* is sister to a well-supported clade of 12 *Helichrysum* species representing 11 of Hilliard's informal groups (Hilliard, 1983). *Helichrysum* and *Edmondia* seem quite distinct genera with a large number of morphological differences. The leaves of *Edmondia* are concave to involute and pubescent abaxially only, while in *Helichrysum* they are flat and pubescent on both surfaces. In *Edmondia* the capitula are solitary or few, whereas in *Helichrysum* they are many and generally in flat-topped corymbs or panicles. The pappus of *Edmondia* is dimorphic and the bristles cohere in a ring, whereas in *Helichrysum* the bristles are monomorphic and freely separate from each other when the cypselae are dispersed. *Edmondia* is a lineage that is strongly differentiated from the remainder of *Helichrysum* s.s.

The lineage containing the majority of the *Helichrysum* species (clade T) is *Helichrysum* s.s., at least until we can determine the affinities of the type of the genus, *H. orientale.* Out of the 30 groups of *Helichrysum* recognized by Hilliard (1983), we collected and sampled members of 13 of these groups. The apparent polyphyly of *Helichrysum* in this study illustrates the need for more sequencing and evaluation of the genus. In a large, polymorphic genus such as *Helichrysum* it is often difficult to find synapomorphies that will unite all the taxa. However, the combination of characters used to circumscribe the genus are smooth, honey-combed, or fimbrilliferous receptacles, glandular hairs on the abaxial surface of the corolla lobes, anthers generally possessing apical append-

ages, style branches truncate or penicillate, cypselae generally glabrous or with small hairs, pappus usually of scabrous, barbellate, or plumose bristles, and bases free or coherent only by the presence of patent cilia (Hilliard, 1983). It is difficult to assess relationships of taxa within this clade, as we need to sequence many more species before we can draw any meaningful conclusions. However, there are some interesting trends within this clade that are worthy of discussion. The basal group consists of *H. acrophilum* Bolus (Hilliard's group 17), *H. leontonyx* DC (group 4), and *H. zwartsbergense* Bolus. (group 4). It is also interesting to note that both *H. leontonyx* and *H. zwartsbergense* belong to the same group (group 4) of Hilliard (1983). This is the only case where we have included two species from the same morphological group, and they are sister taxa in our analysis. Both are small-leaved annuals or weak perennials, with small heads bearing phyllaries that do not radiate. The next resolved clade of *Helichrysum* contains *H. tricostatum* (Thunb.) Less. (group 11), *H. aureum* (Houtt.) Merrill (group 30), and *H. argyrophyllum* DC. (group 29). Similarities between *H. argyrophyllum* and the other two species are not obvious, but there are some resemblances between *H. tricostatum* and *H. aureum.* Both of these species have large showy, yellow, radiating phyllaries and heterogamous heads, receptacles that are shortly honey-combed, pistillate flowers with a conspicuous limb, and glabrous cypselae. The similarity among *H. allioides* Less., *H. dregeanum* Sond. and Harv., and *H. patulum* (L.) D. Don is also not immediately obvious, but both *H. allioides* and *H. patulum* have fimbrilliferous receptacles. On the other hand, both *H. dregeanum* and *H. patulum* are small-leaved perennials with numerous small heads and scabrid pappus bristles joined by patent cilia. *Helichrysum populifolium* DC is a taxonomically isolated species (Hilliard, 1983) with its large cordate leaves and large, open, paniculate synflorescences. *Helichrysum cephaloideum* Less. and *H. odoratissimum* (L.) Sweet share a number of apomorphies such as heads in compact corymbose, to almost globose, panicles, yellow phyllaries, and fimbrilliferous receptacles.

A summary of the general morphological trends in the Gnaphalieae correlating with the molecular tree in Fig. 3 follows. The basal group (clade F) consists mostly of shrubs with radiate heads, paleate receptacles, pappus of scales, and untailed anthers. The next group of major clades (D and E) are mostly shrubs with nonradiate heads, pericyclic cambia, ericoid leaves, epaleate receptacles and distinctly tailed anthers. The final clade (S) represents mostly herbaceous taxa that have nonericoid leaves, nonradiate heads, 2–3 vascular bundles in their cypsela walls, pappus of bristles, epaleate receptacles, nonpericyclic cambium, and untailed anthers. There is general agreement of taxon relationships between our molecular analysis and that based on morphology by Anderberg (1991a), with respect to Anderberg's small informal groups within his subtribes. However, our analysis differs from Anderberg's in that the Relhaniinae are not supported as a monophyletic group, nor is there strong evidence for the overall subtribal classification of Anderberg (1991a). The subtribes will no doubt need further realignment.

***Phytogeography of the Gnaphalieae in South Africa***—Anderberg's phylogeny (1991a) indicates that the basal members of the Gnaphalieae belong to genera that are confined to Africa, particularly South Africa. Historical biogeography would therefore suggest that South Africa is the place of origin of the tribe, followed by radiation to other continents, especially Australia and South America. Since we have not included any extra-African members of the Gnaphalieae in our analysis, it is difficult to corroborate this scenario. However, we can summarize its general phytogeographic patterns in South Africa.

Massive speciation events in the Cape Flora likely began during the Pliocene (Goldblatt, 1978) beginning ~7 million years before present. The Cape Flora probably has its principal origins from temperate and tropical Africa (Goldblatt, 1978) and secondarily from Eurasia. It is thought that very few of the taxa had Austral origins from South America or Australia (Goldblatt, 1978), as scant genera are shared between the Cape and these two continents.

Of the genera of Gnaphalieae s.l. we surveyed, almost all are endemic to southern Africa (except *Helichrysum, Lasiopogon, Leysera,* and *Stoebe*), and a large proportion of these are restricted to the Cape Flora (Fig. 3). This is not surprising as the Cape Flora is renowned for its species richness and high degree of endemism (95.8% of the species are endemic; Wiemarck, 1941; Oliver, Linder, and Rourke, 1983). This high species richness and endemism have been linked to its unusual infertile soils, extreme climatic fluctuations in the past, and its current Mediterranean climate with a strong winter rainfall pattern (Goldblatt, 1978). A few genera, including *Pentatrichia, Lasiopogon,* and *Tenrhynea,* occur only in areas outside the Cape Flora (Fig. 3).

The Relhaniinae s.s. (clade F) are most diverse in the Cape region, except for the genus *Pentatrichia* of Namibia and northwestern South Africa, and *Arrowsmithia* of the Afromontane forest of the Eastern Cape. It is only the most diverse genera that have extended their ranges beyond the Cape; those that are restricted entirely to the Cape region tend to be less speciose or monotypic genera, such as *Phaenocoma* and *Anisothrix.* In clade P, *Trichogyne* is sister to the rest of the Gnaphalieae s.s. and it has a distribution that is restricted to the Cape, karoo, and desert biomes of western South Africa (Fig. 3). In clades D and E, the Metalasia group consists of genera that are most diverse in the Cape region, but many also occur outside the Cape, where they are represented often by one or a few widespread species. *Stoebe* has extended its range to the Mascarene Islands, but several other genera such as *Dolichothrix, Bryomorphe,* and *Phaenocoma* are narrow endemics. Clade C, containing *Plecostachys* and *Tenrhynea,* represents a group that is distributed around the coast of South Africa from the Cape through to the subtropical lowland forest and savanna biomes of Kwazulu Natal. *Oreoleysera* and its sister clade, clade B, including *Helichrysum dasyanthum* and *H. cylindriflorum,*, are restricted to the Cape region and perhaps represent a recent radiation in the South African Gnaphalieae lineage, as they represent the most derived group on our topology. The clade containing *Edmondia* and *Helichrysum* s.s. (clade A) is unique for in its great diversity. *Edmon-*

*dia* is a genus of three species endemic to the Cape region, but *Helichrysum* is found across South Africa and has ~250 species there. This has likely been the result of the possible rapid and recent radiation of *Helichrysum*, both in the Cape region and in the mountains of Kwazulu Natal, the Orange Free State, Eastern Cape and Lesotho. The genus is particularly diverse in the alpine (afroalpine zone) of these eastern mountains and is the predominant genus of Gnaphalieae there (Fig. 3). Unfortunately, it is difficult to discuss anything about the phytogeographic origins of *Helichrysum* until the recircumscription of this genus is complete.

The distribution of the South African Gnaphalieae discussed above seems to indicate that this tribe radiated in the Cape Flora region. Perhaps the expansion and contraction of the fynbos and karoo created islands of diversity leading to the formation of endemic taxa, as proposed by Anderberg (1991a). Ongoing investigations should provide further information about the origin, distribution, and diversity of the Gnaphalieae.

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