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Phylogeny of Australian Gnaphalieae (Asteraceae) Based on Chloroplast and Nuclear Sequences, the *trnL* Intron, *trnL/trnF* Intergenic Spacer, *matK*, and ETS

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ABSTRACT. 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 Australian Gnaphalieae using sequence data from the *trnL* intron, *trnL/trnF* intergenic spacer, *matK,* and ETS. Included in this investigation are the Australian genera of the Gnaphalieae from the subtribes Cassiniinae, Gnaphaliinae, Angianthinae, and Loricariinae, and one to four genera from all tribes of the subfamily Asteroideae to serve as outgroups. Results indicate that the subtribes Angianthinae and Cassiniinae are non-monophyletic as currently circumscribed. There is also some evidence to suggest that the genera *Asteridea*, *Craspedia*, *Hyalosperma*, *Millotia*, and *Podolepis* are monophyletic, whereas *Calocephalus*, *Gnephosis*, *Myriocephalus* pro parte, *Ozothamnus*, *Siloxerus*, *Trichanthodium*, and *Xerochrysum* are non-monophyletic. A group of perennial shrubs and alpine cushion plants from southeastern Australia dominates the clade at the base of the Gnaphalieae. The more derived clades contain primarily herbaceous annual taxa, mainly from western Australia. Based on our results, it seems likely that initial colonization and diversification of the Australian Gnaphalieae occurred in the Bassian Floristic region in eastern New South Wales, Victoria, and Tasmania. Following diversification in eastern Australia, concurrent with the increasing aridity over the entire continent during the Miocene, a massive radiation in the Gnaphalieae occurred into the arid zone of South Australia and Western Australia.

The Gnaphalieae (paper daisies or everlastings) are a group of sunflowers that have their greatest diversity in Australia, southern Africa, and South America. Phylogenetic relationships among the currently recognized 187 genera of the Gnaphalieae have been hypothesized through a contemporary, morphology-based cladistic analysis (Anderberg 1991). Based on this analysis, Anderberg (1991) proposed that the tribe is composed of 11 monophyletic lineages, including six informal groups and five subtribes. The Australian flora contains approximately 84 endemic genera of Gnaphalieae, the majority of which (60 genera) reside within the endemic subtribe Angianthinae. The majority of the remaining genera (12 genera) are assigned to the subtribe Cassiniinae.

The ubiquitous parallelisms in morphology that exist within the tribe Gnaphalieae, and indeed Asteraceae as a whole (Carlquist 1976), have made it difficult to find conservative, homogenetic characters that can be used reliably in phylogenetic reconstruction. Given the problems of non-homologous morphological similarities (homoplasies) in the group, we have chosen to explore these relationships with a molecular approach. The objectives of our work are: (i) to attempt to reconstruct the phylogeny of the Australian Gnaphalieae using sequence data from two relatively short, non-coding chloroplast DNA sequences, the *trnL* intron and *trnL/trnF* intergenic spacer, as well as the maturase encoding plastid enzyme *matK,* and the external transcribed spacer (ETS) of nrDNA; and (ii) to test the monophyly of the Angianthinae and assess their phylogenetic relationships to the other Australian genera of the Gnaphalieae.

The *trnL* intron and *trnL/F* intergenic spacer regions have proven useful in resolving generic and tribal relationships in the Asteraceae (Bayer and Starr 1998; Bayer et al. 2000). To date, the ETS has been used mainly to study phylogeny within the Asteraceae (Baldwin and Markos 1998; Linder et al. 2000; Markos and Baldwin 2001), and the Fabaceae (Bena et al. 1998; Chandler et al. 2001). The *matK* coding region has been used widely in a number of plant groups [see Hilu and Laing 1997, for review], but to date, *matK* has been used only to a limited extent in the Asteraceae. The first study in Asteraceae to use *matK* was Mizukami et al. (1998), which examined relationships among five species of *Atractylodes* DC (Cardueae). More recently, Konishi et al. (2000) used *matK* to resolve relationships among species of *Podolepis* Labill. (Gnaphalieae). Due to the low levels of resolution and support in the resulting trees in these genus level studies, it was hoped that *matK* would, instead, be especially useful for reconstructing higher level phylogenies in the Asteraceae. In this paper, a phylogeny of 69 genera of Australian Gnaphalieae is presented using the above mentioned sequences. As this study is part of a larger study to investigate phylogeny and phytogeographic patterns in the Gnaphalieae, a possible phytogeographic scenario for Australian Gnaphalieae will be presented as part of the discussion of the phylogenetic analysis.

MATERIALS AND METHODS

Fieldwork. The greatest diversity of Australian Gnaphalieae lies in the southwest of Western Australia, an area well known for

its great botanical diversity (Beard et al. 2000). Fieldwork concentrated on this area, but was also conducted in the states of South Australia, New South Wales, and Victoria, as well as the Australian Capital Territory (Table 1). Whenever it was possible, material from the type species of the genus was chosen. New sequences for 102 taxa were generated. DNA of these taxa was extracted from fresh, air dried, or liquid preserved (CTAB/NaCl solution; Rogstad 1992) materials.

Outgroup Selection. Outgroup taxa were selected on the basis of the analyses of Kim and Jansen (1995), Jansen and Palmer (1987, 1988), Bremer (1987, 1994), Kim et al. (1992), and Bayer and Starr (1998), which all point to the monophyly of the subfamily Asteroideae. One to four genera of all the non-Gnaphalioid tribes of the Asteroideae [Astereae (2 genera), Anthemideae (1), Calenduleae (3), Heliantheae s.l. (4), Inuleae s.str. (1), Plucheeae (2), Senecioneae (2)] were chosen for the outgroups. Sequences from representatives of the tribes of the Asteroideae were taken primarily from taxa sampled in previous studies of tribal relationships (Bayer and Starr 1998; Bayer et al. 2000).

Tribal circumscriptions and nomenclature are based on the treatment of the Asteraceae by Bremer (1994), while (except where noted) Anderberg (1991) is followed as a basis for nomenclature and classification of the Gnaphalieae.

Ingroup Sampling of Gnaphalieae. Ideally, we would have liked to have sampled exemplars for all 87 genera of the Australian Gnaphalieae, but because some are either rare, grow in remote parts of Australia, or are annuals occurring only in random years, examination of all genera was not possible. However, at least one member from each of all the available recognized genera of the Gnaphalieae was sequenced. Anderberg (1991), Short (1989, 1990), and Wilson (1992a, 1992b, 1992c, 2001) have all provided comment on the circumscription and monophyly of most genera of the Gnaphalieae and some of their views were taken into consideration when selecting genera for detailed study of several morphologically polymorphic, and therefore potentially polyphyletic genera, including *Angianthus* J.C.Wendl., *Asteridea* Lindl., *Calocephalus* R.Br., *Craspedia* G.Forst., *Hyalosperma* Steetz, *Millotia* Cass., *Myriocephalus* Benth., *Ozothamnus* R. Br., *Podolepis*, *Siloxerus* Labill., *Trichanthodium* Sond. & F.Muell., and *Xerochrysum* Tsvelev, (= *Bracteantha* Anderb. and Haegi), for which several species were sequenced. The number of species in each of the genera that were sampled is provided in Table 1, as is the number of those species that were investigated. Approximately 82% of the 87 genera of Australian Gnaphalieae were sequenced in this study (Table 1). Three hundred and eighty-four new sequences (94% of total) were generated for this survey and have been submitted to GenBank (Table 1). The final matrix consists of 15 outgroup taxa and 87 ingroup members of the Australian Gnaphalieae. Voucher specimens for all taxa are deposited in the herbaria cited in Table 1.

DNA Isolation, Amplification, and Sequencing. Total DNA was isolated as outlined in Bayer et al. (1996). Recalcitrant DNAs were purified with Qiaquick® PCR Purification Columns (Qiagen Pty. Ltd., Clifton Hill, Victoria, Australia). 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 10X reaction buffer, 3 μl of 25 mM magnesium chloride solution, 4 μl of a 1.25 mM 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 50 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 *trnL/F* and *matK* 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 stands. The same PCR scheme was used for ETS, except that an annealing temperature of 55° C was used. Primer details (sequence and reference) are given in Table 2.

The *trnL/F* region was usually amplified as a single piece using primers ''c'' and ''f'' (Table 2) 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''. Likewise the *matK* region was sometimes amplified as a single large ~2.8 kb piece using primers *trnK*-3914F and *trnK*-2R, but in many cases it was amplified as two smaller separate regions using primer 1408F with *trnK*-2R and 1541R with *trnK*-3914F. The ~ 500 bp 3′ ETS region was amplified with any of three forward primers, AST-1, ETS1, or ETS2 and the reverse primer 18S-ETS (Table 2). In order to overcome problems of primer sequence divergence between members of the Astereae/Anthemideae and Gnaphalieae, as AST-1 is a primer that was developed for use in the Astereae and Anthemideae (Bruce Baldwin, pers. comm.), two new ''Gnaphalieae'' specific primers (ETS1 and ETS2) were developed, using the methodology described in Chander et al. (2001). Double-stranded PCR products were cleaned by column purification using Qiaquick® PCR Purification Columns (Qiagen Pty. Ltd., Clifton Hill, Victoria, Australia) prior to sequencing. Some intractable sequences were cloned before sequencing using pGEM®-T Easy Vector Systems (Promega Corporation, Madison, WI, U.S.A.).

The double-stranded PCR products were then used as templates in cycle sequencing reactions. The *trnL/F* region employed four primers (Table 2) 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. *matK* sequencing was initially conducted using primers developed for Saxifragaceae by Johnson and Soltis (1994), but these primers did not work well for Asteraceae. New ''Asteraceae'' specific primers were developed by beginning with the sequencing primer 1470R of Johnson and Soltis (1994) (and its reverse complement), sequencing 300–400 bp regions and thereby developing new primers as each new segment of the sequence became known. The new sequencing primers, in order from the 5′ end of *matK* to near the 3′ end, are 1110R, 1240R, 1408F, 1541R, and 1694F (Table 2). For the ETS the terminal primers were used, i.e. AST-1, ETS1f, or ETS2 and the reverse primer 18S-ETS (Table 2). The double-stranded PCR products were sequenced using the dideoxy chain termination method (Sanger et al. 1977) with the use of the Big Dye Terminator RR Kit® (Perkin-Elmer Applied Biosystems, Wellesley, Massachusetts, U. S. A.) and an ABI automated sequencer in the Division of Plant Industry, CSIRO. Sequencing reactions for the *trnL/F* region and *matK* used 57° C annealing temperatures, whereas the ETS used 55° C annealing temperatures. The cycle sequencing protocol followed manufacturer's instructions. Sequences were assembled using Sequencher™ 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.).

Alignment of sequences proceeded by hand following the principles of noncoding sequence alignment discussed in Bayer et al. (2000). To improve homology assessment of the numerous insertions and deletions, gaps were positioned with consideration of mutational mechanisms that were likely to create 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 homogenetic. Several small regions of the *trnL* intron were eliminated from the analysis because homology assessments of the alignment could not be determined with confidence.

Sequence Data Analysis. Sequence data were analyzed using PAUP 4.0.0d55. (Swofford 1997). The data matrix initially consisted of 15 outgroup and 87 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). The three separate data sets (*matK, trnL/F,* and ETS) were analyzed as six separate analyses. The first analysis on each data set excluded all the coded indels, the second included all indels for that region and nucleotide characters. Strict and 50% majority rule trees were constructed for the set of equally most parsimonious cladograms.

Initial trees (not presented) were examined and ten ingroup taxa that were sister to taxa in the same genus were removed, to relieve computer memory constraints. As the separate analyses gave very similar topologies, the three matrices were combined for the final

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) and Bayer, Puttock, and Kelchner (2000). Voucher specimens are deposited in AD, ALTA, BRI, CANB, CANU, DNA, MEL, NSW, OS, NT, and PERTH. Data are presented in the following sequence: species, group affinity codes and (# of species in genus), collectors and numbers (voucher locations), geographic origin, GenBank accession numbers for *trnL/F* spacer, *trnL* intron, *matK* ETS. * = Type species of genus. A = Angianthinae, C = Cassiniinae, G = Gnaphaliinae, L = Loricariinae, M = Millotia group, and ? = unassigned. ‡ = Taxa removed from final analysis after monophyly of genus was evident. [1] This appears to be a new species of *Eriochlamys,* within the *E. behrii* Sond & F. Muell complex. It chiefly differs from typical *E. behrii* in having solitary, instead of groups of capitula, at the tips of the branches.

Acanthocladium dockeri F. Muell.*, C (1), Bayer & Chandler SA-99012 (CANB, AD, CHR), Australia: South Australia, AF318922, AF318111, AF318901, AF319665; *Acomis acoma* (F. Muell.) Druce*, A (3), Blake 21475 (CANB, BRI), Australia: Queensland, AF141850, AF141762, AF318906, AF319666; *Actinobole uliginosum* (A. Gray) H. Eichler*, A (4), Bayer WA-94011 (CANB, MEL, PERTH), Australia: Western Australia, AF141824, AF141736, AF151433, AF319667; *Ageratum houstonianum* Mill., N/A, Bayer GH-95011 (CANB), Mexico: Commercial source, U82013, U82012, AF151434, AF319668; *Ammobium alatum* R. Br.*, C (3), Bayer & Greber NSW-98005 (CANB), Australia: New South Wales, AF141795, AF141707, AF151435, AF319669; *Anemocarpa podolepidium* (F. Muell.) Paul G. Wilson, A (3), Lyne 1859 (CANB), Australia: New South Wales, AF141835, AF141747, AF151436, AF319670; *Angianthus micropodioides* (Benth.) Benth., A (15), *matK*–Bayer WA-94061 (CANB, MEL, PERTH), Australia: Western Australia, AF141783, AF141695, AF151437, AF319671; ‡*Angianthus preissianus* (Steetz) Benth., A (15), Bayer WA-94084 (CANB, MEL, PERTH), Australia: Western Australia, AF318923, AF318112, AF318918, AF319672; *Apalochlamys spectabilis* (Labill.) J. H. Willis*, C (1), Barnsley 1488 (CANB), Australia: South Australia, AF141829, AF141741, AF151438, AF319673; *Argentipallium obtusifolium* (F. Muell. & Sond.) Paul G. Wilson*, A (6), Bayer, Puttock, Breitwieser, & Ward VIC-97007 (CANB), Australia: Victoria, AF141818, AF141730, AF151439, AF319674; *Argyroglottis turbinata* Turcz.*, C (1), Bayer WA-94064 (CANB, MEL, PERTH), Australia: Western Australia, AF141780, AF141692, AF151440, AF319675; *Aster novae-angliae* L., N/A, Bayer AB-95003 (CANB), Canada: Commercial source, U82019, U82018, AF151441, AF319676; *Asteridea asteroides* (Turcz.) Kroner, A (7), Chandler 767 (CANB, CHR), Australia: Western Australia, AF318924, AF318113, AF318903, AF319677; ‡*Asteridea athrixioides* (Sond. & F. Muell.) Kroner, A (7), Chandler 794 (CANB, CHR), Australia: Western Australia, AF318925, AF318114, AF318904, AF319678; ‡*Asteridea nivea* (Steetz) Kroner, A (7), Bayer WA-94095 (CANB, MEL, PERTH), Australia: Western Australia, AF141791, AF141703, AF151442, AF319679

Bellida graminea Ewart*, A (1), Bayer WA-94131 (CANB, MEL, PERTH), Australia: Western Australia, AF141770, AF141682, AF151443, AF319680; *Blennospora drummondii* A. Gray*, A (2), Bayer WA-94013 (CANB, MEL, PERTH), Australia: Western Australia, AF141782, AF141694, AF151444, AF319681

Calendula officinalis L.*, N/A, Bayer GH-95009 (CANB), South Africa: Commercial source, U82021, U82020, AF151446, AF319684; *Calocephalus knappii* (F. Muell.) Ewart & Jean White, A (15), Chandler 675 (CANB), Australia: Western Australia, AF318927, AF318116, AF318907, AF319685; *Calocephalus multiflorus* (Turcz.) Benth., A (15), Bayer WA-94024 (CANB, MEL, PERTH), Australia: Western Australia, AF141784, AF141696, AF151447, AF319686; *Calomeria amaranthoides* Vent.*, A (1), Bayer & Greber NSW-98015 (CANB), Australia: New South Wales, AF141825, AF141737, AF151448, AF319687; *Cassinia longifolia* R.Br., C (22), Bayer & Burroughs NSW-94023 (CANB, NSW), Australia: New South Wales, AF141774, AF141686, AF151449, AF319688; *Cephalipterum drummondii* A. Gray*, A (1), Bayer WA-94036 (CANB, MEL, PERTH), Australia: Western Australia, AF141787, AF141699, AF151450, AF319689; *Cephalosorus carpesioides* (Turcz.) P. Short*, A (1), Bayer WA-94031 (CANB, MEL, PERTH), Australia: Western Australia, AF141807, AF141719, AF151451, AF319690; *Chrysocephalum semipapposum* (Labill.) Steetz, A (7), Bayer NSW-94007 (CANB, MEL, NSW), Australia: New South Wales, AF141789, AF141701, AF151452, AF319691; *Chthonocephalus pseudoevax* Steetz*, A (5), Crisp 6554 (CANB, MEL), Australia: Western Australia, AF141830, AF141742, AF151453, AF319692; ‡*Craspedia pleiocephala* F. Muell., A (11), Chandler 752 (CANB, CHR), Australia: Western Australia, AF318928, AF318117, AF318908, AF319693; *Craspedia variabilis* J. Everett & Doust, A (11), Bayer WA-94096 (CANB, MEL, PERTH), Australia: Western Australia, AF141801, AF141713, AF151455, AF319694

Decazesia hecatocephala F. Muell.*, A (11), George 10326 (CANB, PERTH), Australia: Western Australia, AF141840, AF141752, AF151456, AF319695; *Dimorphotheca sinuata* DC., N/A, Bayer & Puttock SAF-96148 (CANB, F, MO), South Africa: Northern Cape Province, AF100518, AF09855, AF318909, AF319696; *Dithyrostegia amplexicaulis* A. Gray*, A (2), Bayer WA-94030B (CANB, MEL), Australia: Western Australia, AF141838, AF141750, AF151457, AF319697

Eriochlamys Sond. & F. Muell. sp. nov.[1], A (2), Bayer NSW-94016 (CANB, NSW), Australia: New South Wales, AF141800, AF141712, AF151458, AF319698; *Erymophyllum glossanthus* P. G. Wilson, A (5), Bayer WA-94058 (CANB, MEL, PERTH), Australia: Western Australia, AF141802, AF141714, AF151459, AF319699; *Euryops virgineus* Less., N/A, Bayer and Puttock SAF-96237 (CANB), South Africa: Eastern Cape Province, AF100517, AF098854, AF318910, AF319700; *Ewartia catipes* (DC.) Beauverd, C (4), Breitwieser & Vogt 724 (CANU), Australia: Tasmania, AF141786, AF141698, AF151460, AF319701

Feldstonia nitens P. S. Short*, A (1), Bayer WA-94047 (CANB, MEL, PERTH), Australia: Western Australia, AF1417979, AF141709, AF1514615, AF319702; *Felicia filifolia* (DC.) Burtt-Davy subsp. *schaeferi* (Dinter) Grau, N/A, Bayer and Puttock SAF-96166 (CANB), South Africa: Northern Cape Province, AF318929, AF318120, AF318911, AF319703; *Fitzwillia axilliflora* (W. Fitz. ex Ewart & Jean White) P. S. Short*, A (1), Bayer WA-94028 (CANB, MEL, PERTH), Australia: Western Australia, AF141796, AF141708, AF151642, AF319704

Gaillardia aristata Pursh, N/A, Bayer GH-95006 (CANB), North America: Commercial Source, U82033, U82032, AF318912, AF319705; *Gilberta tenuifolia* Turcz.*, A (1), Bayer WA-94048 (CANB, MEL, PERTH), Australia: Western Australia, AF141809, AF141721, AF151464, AF319706; *Gilruthia osbornii* Ewart & Jean White*, A (1), Bayer WA-94054 (CANB, MEL, PERTH), Australia: Western Australia, AF141765, AF141677, AF151465, AF319707; *Gnephosis arachnoidea* Turcz., A (8), Bayer WA-94059 (CANB, MEL, PERTH), Australia: Western Australia, AF141803, AF141715, AF151466, AF319708; *Gnephosis intonsa* S. Moore, A (8), Chandler 864 (CANB, PERTH), Australia: Western Australia, AF318930, AF318121, AF318913, AF319709

TABLE 1. Continued.

Haeckeria ozothamnoides F. Muell., C (4), Donaldson, Edwards, & Sammon 989 (CANB), Australia: Victoria, AF141813, AF141725, AF151467, AF319710; *Helianthus annuus* L.*, N/A, Bayer GH-95007 (CANB), U.S.A.: Commercial source, U82039, U82038, AF151469, AF319711; *Helichrysum leucopsideum* DC., A (19), Chandler 750 (CANB), Australia: Western Australia, AF318931, AF318122, AF318914, AF319712; *Hyalochlamys globifera* A. Gray*, A (1), Ross 2771 (CANB, PERTH), Australia: Western Australia, AF141834, AF141746, AF151470, AF319713; *Hyalosperma cotula* (Benth.) P. G. Wilson, A (9), Bayer WA-94003 (CANB, MEL, PERTH), Australia: Western Australia, AF141777, AF141689, AF151471, AF319714; ‡*Hyalosperma demissum* (A. Gray) Paul G. Wilson, A (9), Bayer WA-94012 (CANB, MEL, PERTH), Australia: Western Australia, AF141776, AF141688, AF151472, AF319715

Inula helenium L.*, N/A, Bayer GH-95013 (CANB), Eurasia: Commercial source, U82041, U82040, AF151473, AF319716; *Ixiolaena tomentosa* Sond. & F. Muell., M (8), Bayer NSW-94017 (CANB, MEL, NSW), Australia: New South Wales, AF141792, AF141704, AF151474, AF319717; *Ixodia achillaeoides* R. Br.*, C (2), Canning & Corbett s.n. (CANB), Australia: South Australia, AF141812, AF141724, AF151475, AF319718

Lawrencella rosea Lindl.*, A (2), Bayer WA-94128 (CANB, MEL, PERTH), Australia: Western Australia, AF141788, AF141700, AF151476, AF319719; *Lemooria burkittii* (Benth.) P. S. Short*, A (1), Briggs 1006 (AD, CANB, MEL), Australia: South Australia, AF141819, AF141731, AF151477, AF319720; *Leptorhynchos scabrus* (Benth.) Haegi, A (10), Bayer WA-94102 (CANB, MEL, PERTH), Australia: Western Australia, AF141794, AF141706, AF151478, AF319721; *Leucochrysum stipitatum* (F. Muell.) Paul G. Wilson, A (5), Bayer WA-94133 (CANB, MEL, PERTH), Australia: Western Australia, AF141810, AF141722, AF151479, AF319722; *Leucophyta brownii* Cass.*, A (1), Bayer, Puttock, Breitwieser, & Ward SA-97006 (CANB), Australia: South Australia, AF141815, AF141727, AF151480, AF319723; *Matricaria matricarioides* (Less.) Port., N/A, Bayer AB-95005 (CANB), Canada: Alberta, U82047, U82046, AF151481, AF319724;

‡*Millotia eichleri* P. S. Short, M (6), Chandler 848 (CANB, CHR, PERTH), Australia: Western Australia, AF318932, AF318123, AF318915, AF319725; ‡*Millotia myosotidifolia* (Benth.) Steetz, M (6), Bayer WA-94075 (CANB, MEL, PERTH, Australia: Western Australia, AF141799, AF141711, AF151482, AF319726; *Millotia tenuifolia* Cass. var. *tenuifolia**, M (6), Chandler 942 (CANB, CHR, PERTH), Australia: Western Australia, AF318933, AF318124, AF318916, AF319727; ‡*Myriocephalus guerinae* F. Muell. [= *Polycalymma guerinae* (F. Muell.) Paul G. Wilson in ed.], A (8), Bayer WA-94045 (CANB, MEL, PERTH), Australia: Western Australia, AF141839, AF141751, AF151483, AF319728; *Myriocephalus morrisonianus* Diels [= *Polycalymma craspedioides* (W. Fitz.) Paul W. Wilson in ed.], A (8), Bayer WA-94050 (CANB, MEL, PERTH), Australia: Western Australia, AF141778, AF141690, AF151486, AF319729; ‡*Myriocephalus occidentalis* (F. Muell.) P. S. Short, A (8), Bayer & Wilson WA-94122 (CANB, MEL, PERTH), Australia: Western Australia, AF141771, AF141683, AF151484, AF319730; *Myriocephalus oldfieldii* (F. Muell.) Paul G. Wilson in ed., A (8), Bayer WA-94042 (CANB, MEL, PERTH), Australia: Western Australia, AF141772, AF141684, AF151485, AF319731

Odixia angusta (Wakefield) Orchard*, C (2), Briggs 966 (CANB, MEL, NSW), Australia: Tasmania, AF141775, AF141687, AF151487, AF319732; *Osteospermum clandestinum* (Less.) Norl., N/A, Bayer WA-94070 (CANB), Australia: Western Australia, U82049, U82048, AF151488, AF319733; *Ozothamnus bidwillii* (Benth.) Anderb., C (53), Telford 11025 (CANB, AD, PERTH), Australia: Queensland, AF141817, AF141729, AF151489, AF319734; *Ozothamnus diosmifolius* (Vent.) A. Cunn. ex DC, C (53), Bayer NSW-94008 (CANB), Australia: New South Wales, AF141773, AF141685, AF151490, AF319735; *Ozothamnus vagans* Anderb., Telford 12348 (CANB), Australia: New South Wales, AF141816, AF141728, AF151491, AF319736; *Ozothamnus whitei* (N.T. Burb.) Anderb. (= *Telfordia whitei* (N.T. Burb.) Puttock in ed.), C (53); Bayer, Cayzer, Chandler & Telford NSW-98072 (CANB), Australia: New South Wales, AF141836, AF141748, AF151517, AF319737

Parantennaria uniceps (F. Muell.) Beauverd*, C (1), Bayer & Greber NSW-98021 (CANB), Australia: New South Wales, AF141769, AF141681, AF151492, AF319738; *Pithocarpa pulchella* Lindl.*, A (2), Lepschi & Lally 2889 (PERTH), Australia: Western Australia, AF141844, AF141756, AF151493, AF319739; *Pleuropappus phyllocalymmeus* F. Muell.*, A (1), Briggs 1431 (CANB, AD, MEL), Australia: South Australia, AF141827, AF141739, AF151494, AF319740; *Pluchea dentex* R. Br. ex Benth., N/A, Short, Watanabe, Kosuge & Denda 4405 (AD, CANB, MEL, PERTH, TI), Australia: Western Australia, AF100521, AF098858, AF151495, AF319741; ‡*Podolepis canescens* Domin., A (20), Bayer WA-94010 (CANB, MEL, PERTH), Australia: Western Australia, AF141767, AF141679, AF151496, AF319742; *Podolepis lessonii* (Cass.) Benth., A (20), Chandler 853 (CANB, CHR), Australia: Western Australia, AF318934, AF318125, AF318917, AF319743; *Podotheca gnaphalioides* Grah., M (6), Bayer WA-94005 (CANB, MEL, PERTH), Australia: Western Australia, AF141798, AF141710, AF151497, AF319744; *Pogonolepis stricta* Steetz*, A (2), Bayer WA-94021B (CANB, MEL, PERTH), Australia: Western Australia, AF141766, AF141678, AF151498, AF319745; *Polycalymma stuartii* F. Muell. & Sond.*, A (1), Flowers & Donaldson 28 (CANB), Australia: South Australia, AF141814, AF141726, AF151499, AF319746; *Pterochaeta paniculata* Steetz*, A (1), Bayer WA-94074 (CANB, MEL, PERTH), Australia: Western Australia, AF141793, AF141705, AF151500, AF319747; *Pterygopappus lawrencii* Hook. f.*, L (1), Purdie 3439 (CANB), Australia: Tasmania, AF141832, AF141744, AF151501, AF319748; *Pycnosorus globosus* Bauer ex Benth.*, A (6), Telford & Nightingale 11814 (CANB), Australia: New South Wales, AF141768, AF141680, AF151502, AF319749

Quinetia urvillei Cass.*, A (1), Bayer WA-94105 (CANB, MEL, PERTH), Australia: Western Australia, AF141804, AF141716, AF151503, AF319750; *Quinqueremulus linearis* P. G. Wilson*, A (1), Cranfield 5832 (CANB, PERTH), Australia: Western Australia, AF141831, AF141743, AF151504, AF319751

Rhodanthe manglesii Lindl.*, A (43), Bayer WA-94033 (CANB), Australia: Western Australia, AF318935, AF318126, AF318919, AF319752; *Rutidosis leptorrhynchoides* F. Muell., A (9), Young QB-12-4 (CANB), Australia: New South Wales, AF141811, AF141723, AF151506, AF319753

TABLE 1. Continued.

Scyphocoronis major (Turcz.) Druce, M (2), Wilson 12093 (CANB), Australia: Western Australia, AF141846, AF141758, AF151508, AF319754; *Senecio vulgaris* L.*, N/A, Bayer AB-95006 (CANB), Canada: Alberta, U82053, U82052, AF151509, AF319755; *Siloxerus multiflorus* Nees in Lehm., A (3), Bayer WA-94076 (CANB, PERTH), Australia: Western Australia, AF318936, AF318127, AF318920, AF319756; *Siloxerus pygmaeus* (A. Gray) P. S. Short, A (3), Bayer WA-94089 (CANB, MEL, PERTH), Australia: Western Australia, AF141808, AF141720, AF151510, AF319757; *Sondottia glabrata* P. S. Short, A (2), Short 4347 (CANB, PERTH), Australia: Western Australia, AF141826, AF141738, AF151511, AF319758; *Streptoglossa cylindripes* (J. M. Black) Dunlop, N/A, Bayer WA-94049 (ALTA), Australia: Western Australia, U82057, U82056, AF151513, AF319759; *Stuartina muelleri* Sond.*, G (2), Bayer, Breitwieser, Puttock & Ward SA-97010 (CANB), Australia: South Australia, U82059, U82058, AF151514, AF319760

Tagetes patula L.*, N/A, Bayer s.n. (CANB), Mexico: Commercial source, U82061, U82060, AF151515, AF319761; *Toxanthes perpusilla* Turcz.*, M (3), Burbidge & Kanis 8082 (CANB), Australia: Western Australia, AF141845, AF141757, AF318921, AF319762; *Trichanthodium exilis* (W. Fitzg.) P. S. Short, A (4), Bayer WA-94060 (CANB, MEL, PERTH), Australia: Western Australia, AF141779, AF141691, AF151518, AF319763; *Trichanthodium skirrophorum* Sond. & F. Muell.*, A (4), Chandler, Monro & Donaldson 862 (CANB, CHR), Australia: Western Australia, AF318937, AF318128, AF318902, AF319764; *Triptilodiscus pygmaeus* Turcz.*, A (1), Bayer NSW-94012 (CANB, MEL, NSW), Australia: New South Wales, AF141823, AF141735, AF151519, AF319765

Waitzia acuminata Steetz, A (8), Bayer WA-94019 (CANB, MEL, PERTH), Australia: Western Australia, AF141790, AF141702, AF151520, AF319766

Xerochrysum bracteatum (Vent.) Tzvelev*, A (6), Donaldson 1991 (CANB, CHR, PERTH), Australia: Western Australia, AF318926, AF318115, AF318905, AF319682; *Xerochrysum viscosum* (DC.) R. J. Bayer, A (6), Bayer & Greber ACT-97001 (CANB), Australia: Australian Capital Territory, AF141805, AF141717, AF151445, AF319683

analysis. Tests of ''combinability'' of data sets, such as the incongruence length difference (ILD), commonly referred to as a partition homogeneity test, have been shown to yield spurious results (Yoder et al. 2001) in other groups. In fact Yoder et al. (2001) have recently recommended that the ''ILD never be used as a test of data partition combinability.'' As combinability tests have come under considerable criticism recently, they were not used to test the combinability of the data matrices. Thirty-six coded indels were included in the final analysis, which improved resolution and strengthened support in the resulting trees. Indels were scored as binary characters for use in analyses (Table 2), following the recommendations of Wojciechowski et al. (1993), with gaps treated as missing. Indels included only those potentially phylogenetically informative indels greater than 2 bp in length. This follows the advice of van Ham et al. (1994) and Lloyd and Calder (1991), who suggest that most of the homoplasy in indels is accounted for by very small (single basepair) indels.

For the final analysis, heuristic searches were carried out using 1000 random sequence addition replicates for which five trees were swapped on during each round. The resulting shortest trees were then used in a further round of random sequence addition using 100 replicates of heuristic searching and allowing 5000 trees to be saved for each replicate. The limit on the number of trees held at each step reduced the time spent searching on suboptimal trees. Successive approximations weighting (SAW; Farris 1969; Richardson et al. 2000) was then carried out on these shortest trees with a limit of 100 trees per replicate and 10 replicates per run. Characters were reweighted according to their rescaled consistency indices. The resulting trees from these ten replicates were then swapped on until completion (or until the 5000 tree limit was reached, as above) before the next round of weighting was begun. Additional rounds of SAW followed until tree lengths were the same in three consecutive rounds. This procedure was implemented to downweigh or eliminate characters that were highly homoplastic.

Five hundred bootstrap (Felsenstein 1985) replicates were then executed with the successive weights applied to estimate the robustness of clades. 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). MacClade was also used to calculate the number

TABLE 2. List of primer sequences and references used in this study.

Primer name	5′–3′ primer sequence	Reference (if applicable)
trnK-2R	ACC TAG TCG GAT GGA GTA G	Johnson and Soltis (1994)
trnK-3914F	GGG GTT GCT AAC TCA ACG G	Johnson and Soltis (1994)
1110R	TAT TCT GTT GAT ACA TTC G	Previously unpublished
1240R	CAG ATG AGC TGG GTA AGG T	Previously unpublished
1408F	CCT ATA TAC TTT TTA TGT ACG	Previously unpublished
1541R	GCT CCA GAA GAT GTT GAT CG	Previously unpublished
1694F	CTT TTG ATG AAT AAN TGG	Previously unpublished
c	CGA AAT CGG TAG ACG CTA CG	Taberlet et al. 1991
d	GGG GAT AGA GGG ACT TGA AC	Taberlet et al. 1991
e	GGT TCA AGT CCC TCT ATC CC	Taberlet et al. 1991
f	ATT TGA ACT GGT GAC ACG AG	Taberlet et al. 1991
18S-ETS	ACT TAC ACA TGC ATG GCT TAA TCT	Baldwin and Markos 1998
ETS1f	CTT TTT GTG CAT AAT GTA TAT ATA TAG GGG G	Linder et al. 2000
AST1	CGT AAA GGT GCA TGA GTG GTG T	Markos and Baldwin 2001
ETS1	CGC ATC GTT CGG TGC ATT CTG GG	Edward W. Cross, unpublished
ETS2	CAA CTT CCA CCT GGC ATA CCT CTT CA	Edward W. Cross, unpublished
18S-IGS	GAG ACA AGC ATA TGA CTA CTG GCA GGA TCA ACC AG	Baldwin and Markos 1998

TABLE 3. Sequence characteristics of the *trnL* intron, and *trnL/F* spacer non-coding regions, and the *matK* coding region sequenced in this study.

Sequence	*trnL/F* spacer	*trnL* intron	*matK* coding region	ETS	Combined
Length range (nucleotides)	341–434	412–448	1177–1225	372–474	2326–2541
Length mean (nucleotides)	363	434	1206	441	2463
Aligned length (nucleotides)	769	672	1289	553	3238
G + C content mean	32.5%	34.5%	32.0%	35.2%	N/A
Sequence divergence	0.0–12.9%	0.0–7.6%	0.0–13.7%	0.0–39.7%	0.08–14.3%
Number of variable sites	466/769 (61%)	177/627 (28%)	626/1289 (49%)	408/553 (74%)	1677/3543 (47%)
Number of potentially informative sites/total aligned length	149/769 (20%)	88/627 (14%)	345/1289 (27%)	305/553 (55%)	887/3543 (25%)
Number of constant sites/total aligned length	303/769 (39%)	450/627 (72%)	663/1289 (51%)	145/553 (26%)	1866/3453 (53%)
Number of autapomorphic sites/total aligned length	317/769 (41%)	89/627 (14%)	281/1289 (22%)	103/553 (19%)	790/3543 (22%)
Number of unambiguously aligned potentially informative indels	7	17	8	4	36
Indel size range (nucleotides)	5–9	3–14	3–9	3–17	3–17
Ratio of indels to potentially informative sites	1 : 21	1 : 5	1 : 43	1 : 76	1 : 25

of unambiguous steps per character optimized on one of the most parsimonious SAW trees (Fig. 2).

Morphology. LucID Professional for Windows® (CSIRO Publishing; Collingwood, Australia) was used to explore the distribution of a broad range of vegetative and floral characters on the trees. The morphological data matrix presented by Anderberg (1991) and Anderberg (1992), but consolidated by Puttock (1994), and emended and enlarged by Bayer et al. (2000) was used for this purpose, using only the taxa included in this analysis.

RESULTS

Sequence Characteristics. Length variation for the entire *trnL/F* intergenic spacer over all taxa ranged from a low of 341 nucleotides to a high of 434 nucleotides (Table 3). The proportion of nucleotide differences ranged from 0.0–12.9% between all species of Asteroideae (Table 3). The G/C content of the spacer averages 32.5%. The complete *trnL* intron ranges from 412 nucleotides to 448 nucleotides in length in the Asteroideae. The proportion of nucleotide differences in the intron is less than that found in the spacer ranging from 0.0–7.6% between all species of Asteroideae. Similar to the spacer, the intron has an average G/C content of 34.5% (Table 3). The average total length of *matK* in the Angiosperms is ~1500 bp (Hilu and Liang 1997). In this study a range of between 1177 and 1225 bp of the more variable 5′ end of the coding region was sequenced (Table 3). The average G/C content is 32.0% and sequence divergence ranges from 0.0 to 13.7%. ETS is the most variable sequence in the study, as sequence divergence ranges from 0.0 to 39.7%. Only a portion of the 3′ end of the ETS was sequenced and it ranged in length from 372 to 474 bp long (G/C content averaged 35.2%). Within the Asteroideae, the proportion of nucleotide differences in the combined spacer and intron sequences ranged from 0.08 to 14.3% (Table 3).

A total of 887 sites (25% of the sequence length) are potentially phylogenetically informative. Thirty-six indels (Tables 3) ranging in length from three to 17 nucleotides, could be coded unambiguously for inclusion in the phylogenetic analysis. The percent of phylogenetically informative sites (Table 3) ranges from 14% in the *trnL* intron to 55% in ETS, whereas the number of autapomorphic sites varies from 14% in the *trnL* intron to 41% in the *trnL/F* spacer. Interestingly, the *matK* coding region has a higher percent of potentially informative sites (27%) than either of the chloroplast spacer regions (Table 3). The ETS has the fewest (4) unambiguously aligned potentially informative indels, whereas the *trnL* spacer has the greatest (17) number (Table 3).

Phylogenetic Reconstruction. Analyses of nucleotide characters only produced trees of similar topology to those retrieved when both nucleotide and indel characters were combined in one analysis. Analyses of indel characters alone were not attempted. A 50% majority rule tree (from which a strict tree topology can also be observed) 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 applying SAW, and including all indels, yielded 2089 equally parsimonious SW trees of 508 steps [consistency index (C.I.) = 0.64; retention index (R.I.) = 0.80; Fig. 1]. Also presented is one of the most parsimonious SAW trees illustrating the number of Fitch changes along branches (total length 3122, C.I. = 0.43, R.I. = 0.50) (Fig. 2).

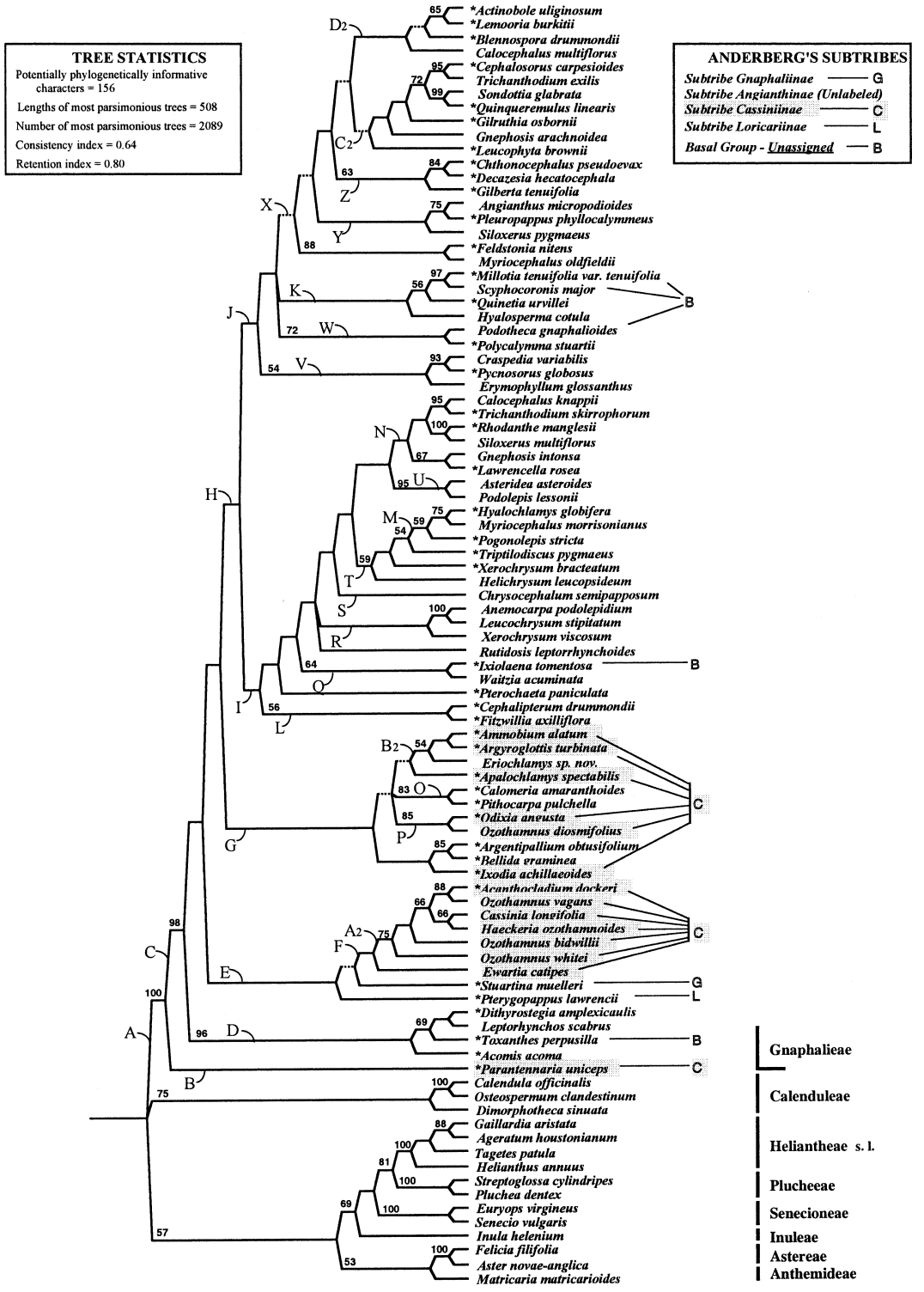

FIG. 1. The 50% majority rule consensus tree of 2089 equally parsimonious trees resulting from phylogenetic analysis of sequence data of the *trnL* intron, the *trnL/F* intergenic spacer, *matK* coding region, and ETS using all informative nucleotide characters and indels. Branches that did not appear in the strict consensus tree are indicated by dashed lines. Bootstrap values > 50% are indicated above the branches. Species names preceded by an asterix indicate that this taxon is type species of the genus. Terminal taxon names represent tribes of the Asteroideae. Subtribal group names following the circumscription of Anderberg (1991): unlabeled = Angianthinae, C = Cassiniinae, G = Gnaphaliinae, L = Loricariinae, B = Anderberg's basal group of the Gnaphalieae. Clades that are discussed in the text are labeled with capital letters.

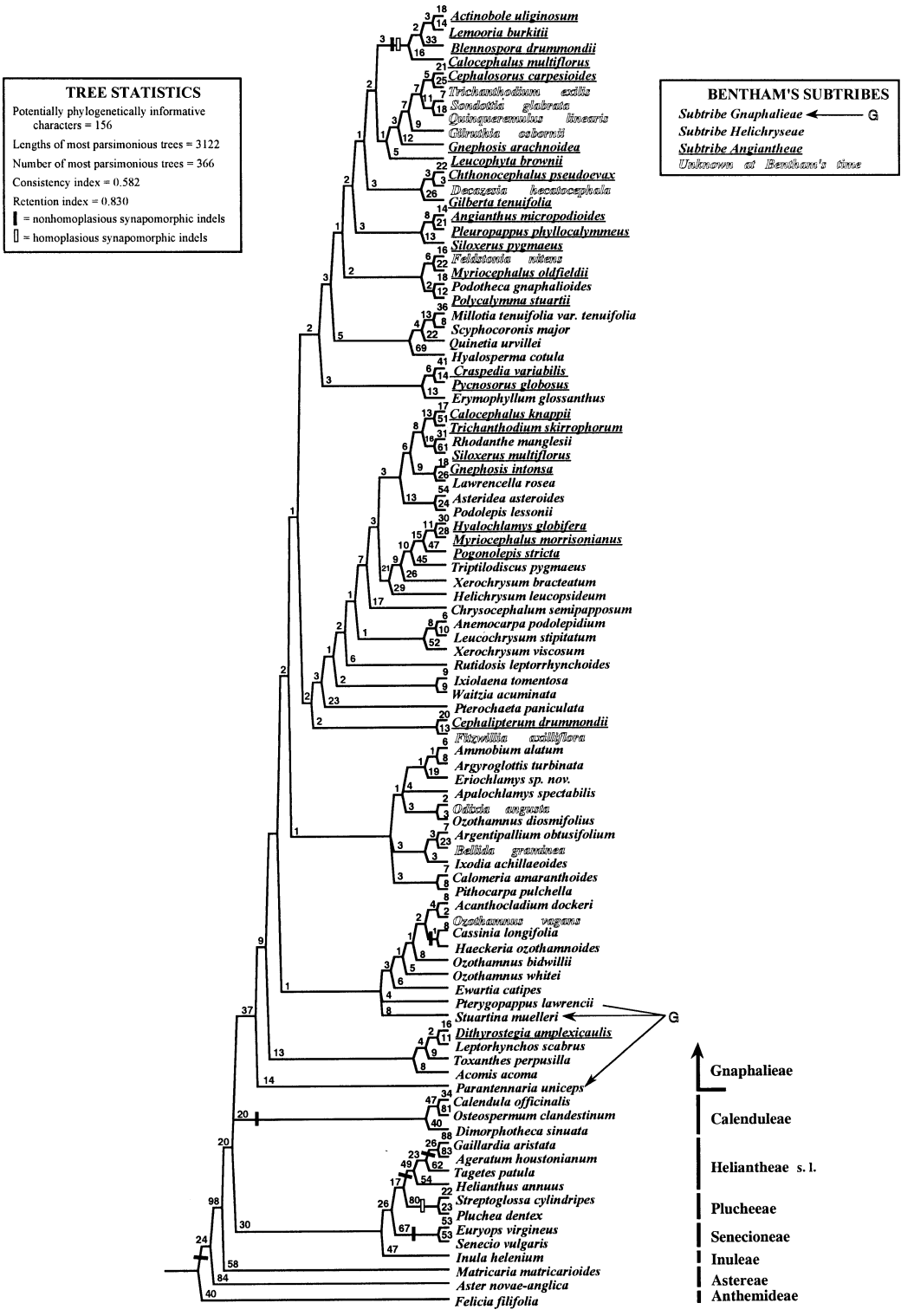

FIG. 2. One of 2089 equally parsimonious trees of length 502 resulting from phylogenetic analysis of sequence data of the *trnL* intron, the *trnL/F* intergenic spacer, the *matK* coding region, and ETS using all informative nucleotide characters and indels. The tree gives the number of apomorphies (including indels) above the branches and the distribution of homoplastic and homogenetic indels on the branches. Subtribal group names following the circumscription of Bentham (1867): underlined = Angiantheae, italics but no underlining = Helichryseae, G = Eugnaphalieae, taxon names in outline font = Taxa that were unknown at the time of Bentham.

Bootstrap values are given in Fig. 1 and the branch positions of synapomorphic indels are shown in Fig. 2.

Topology of Major Clades. A consensus of all trees indicates that the Gnaphalieae are a strong monophyletic lineage (Figs. 1, 2; Clade A; synapomorphies (SYN) = 37; bootstrap value (BSV) = 100%). They are sister to other tribes of the subfamily Asteroideae, but it is still uncertain as to which specific tribe they are sister (Figs. 1, 2). 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 Astereae (BSV = 100%), Calenduleae (SYN = 20; BSV = 75%), Helianthieae s.l. (SYN = 17; BSV = 100%), Plucheeae (SYN = 80; BSV = 100%), and Senecioneae (SYN = 67; BSV = 100%) are strong monophyletic groups (Figs. 1, 2). Generally, tribal support throughout the topologies (Figs. 1, 2) is high, with most clades being supported by multiple synapomorphies. Within the Gnaphalieae are several major clades including Clades E, G, I, and J and although most of these branches do not enjoy a great deal of support, the branches do appear in the strict consensus trees (Fig. 1). However, there is much more support for some of the smaller lineages nested within the major clades in the trees (Figs. 1, 2).

Discussion

The Australian Gnaphalieae have a long history of taxonomic change since the first description of an Australian species, *Richea glauca* [= *Craspedia glauca* (Labill.) Spreng.] by Labillardière (1800). Major works on the Gnaphalieae of Australia include the work of Brown (1817), de Candolle (1838), Lessing (1832), Gray (1852), Turczaninow (1851), and Steetz (1845). The first major taxonomic revision of the group was put forth by George Bentham in ''Flora Australiensis'' (1867). He circumscribed the tribe into three subtribes, Eugnaphalieae, Helichryseae, and Angiantheae, containing 35 genera in all. The majority of the species belonged to a few large genera such as *Angianthus*, *Calocephalus*, *Cassinia* R.Br., *Gnephosis* Cass., *Helichrysum* Mill., *Helipterum* DC. ex Lindl., *Myriocephalus*, and *Podolepis* (Bentham 1867). Between the time of Bentham's monograph (1867), and the early 1980's, most botanists accepted Bentham's treatment, and during the 115 year period, 1867–1982, only ten new genera were described. Most recently it has been recognized that many of the large genera are polyphyletic and these large genera have been recircumscribed. As a result, thirteen monotypic and ditypic genera, along with four polytypic genera have been described in the past 20 years (Short 1989, 1990; Short and Wilson 1990; Wilson 1992a, 1992b, 1992c, 2001). Currently there are 84, primarily endemic, Australian genera in the Gnaphalieae encompassing ~475 species. The genera, as a whole, are mostly non-speciose, with only ten of the genera having more than ten species and a third (28 genera) being monotypic. The purpose of this study was to begin to look at the phylogenetic relationships of the genera and examine the monophyly of the currently recognized subtribal groups.

Major Clades Within the Gnaphalieae. If we examine the Gnaphalieae lineage of the tree (Fig. 1), beginning at the base of the group (Clade A) and proceeding toward the top of the tree (Clade D_2), a number of variably supported groups are evident. The stem group of the Gnaphalieae, as represented in this analysis, is Clade B, which is a single taxon lineage consisting of the monotypic *Parantennaria* Beauverd, a genus of the dioecious, alpine cushion plants. It is sister to the remainder of the well-supported main group of the Gnaphalieae (Clade C; SYN = 9; BSV = 98). Clade D is a well supported clade (SYN = 13; BSV = 96%). These herbaceous taxa have discoid, mostly homogamous, capitula that are many-flowered, and synflorescences that are solitary or have only a few capitula together. A group of perennial shrubs or alpine cushion plants dominates the weakly supported Clade E (SYN = 1), including a number of taxa belonging to Anderberg's (1991) '*Cassinia*' group of the Cassiniinae. The next clade (Clade G; SYN = 1) consists largely of woody or herbaceous taxa primarily from eastern Australia. Synapomorphies for this group include simple, discoid heads with numerous florets, with more than one row of phyllaries, which do not enclose the florets.

Clade H, although weakly supported (SYN = 1), consists primarily of herbaceous, annual (sometimes perennial) taxa mainly from Western Australia. It is composed of two weakly supported large sister clades (Clades I and J; Figs. 1, 2). This is the main Angianthinae clade and all genera of the clade belong to this subtribe, except *Ixiolaena* Benth., *Millotia*, *Podotheca* Cass., and *Scyphocoronis* A.Gray. The taxa in Clade I are generally simple headed, with the exception of members of subclades L, M, and most of subclade N, which have secondary capitula. As well, there is a transition from homogamy to heterogamy within over half of the genera of Clade I, including members of *Asteridea*, *Chrysocephalum* Walp., *Ixiolaena*, *Lawrencella* Lindl., *Podolepis*, *Pterochaeta* Steetz, *Rutidosis* DC., and *Triptilodiscus* Turcz. (Fig. 1). As presently circumscribed, *Calocephalus*, *Gnephosis*, *Myriocephalus*, *Siloxerus*, and *Xerochrysum*, are non-monophyletic, as species from these genera appear in at least two different places in Clade H. Clade J consists primarily of taxa with homogamous, secondary capitula, except for members of Clade K, *Gilruthia* Ewart in Ewart, Jean White, & B.Rees, and *Erymophyllum* Paul G. Wilson, which have simple, homogamous capitula.

Topology of Minor Clades Within the Gnaphalieae. Although many of the major clades in the tree suffer from a lack of support, many of the smaller clades

have moderate to strong support. Indeed stronger conclusions can be reached with regard to phylogenetic relationships in the minor clades than the major ones. *Angianthus preissianus* (Steetz) Benth., *Asteridea athrixioides* (Sond. & F.Muell.) Kroner, *Asteridea nivea* (Steetz) Kroner, *Craspedia pleiocephala* F.Muell., *Hyalosperma demissum* (A. Gray) Paul G. Wilson, *Millotia eichleri* P.S.Short, *Millotia myosotidifolia* (Benth.) Steetz, *Myriocephalus guerinae* F. Muell., *Myriocephalus occidentalis* (F. Muell.) P. S. Short, and *Podolepis canescens* Domin. (Table 1) were removed from the analysis because they became superfluous, as they came out as sister to another species of their genus. Therefore, there is some evidence suggesting that the genera *Asteridea*, *Craspedia*, *Hyalosperma*, *Millotia*, *Myriocephalus*, and *Podolepis* are monophyletic, at least in part.

Acomis F. Muell., *Dithyrostegia* A. Gray, *Leptorhynchos* Less., and *Toxanthes* Turcz. (Clade D; SYN = 13; BSV = 96%) are herbaceous taxa that have discoid, mostly homogamous, many-flowered capitula and synflorescences that are solitary or with only a few capitula together. They also have inner and outer phyllaries that are monomorphic, transparent, and brownish in color. In Clade E, *Pterygopappus* Hook.f. and *Ewartia* Beauverd are two genera of alpine cushion plants from the eastern Australian mainland and Tasmania. Anderberg (1991) placed dioecious *Pterygopappus* in his subtribe Loricariinae along with four other genera, whereas *Ewartia* was placed in Cassiniinae. They are primarily dioecious cushion plants or shrublets with strongly imbricate leaves. Currently, *Pterygopappus* is in a clade with a number of genera from the Cassiniinae, and only with the inclusion of other Loricariinae in future analyses can the monophyly of the Loricariinae be evaluated. At this point, its affinities rest with the shrubby Cassiniinae from eastern Australia. Species of *Ozothamnus* form a non-monophyletic group in this clade (Clade E), and other species of *Ozothamnus* appear in other clades, thus the genus, as presently circumscribed, is polyphyletic. The one anomalous taxon in Clade E is *Stuartina* Sond., which is annual and herbaceous, and the only member of subtribe Gnaphaliinae in our analysis, and as with the Loricariinae, more members of the Gnaphaliinae need to be added to the analysis before the monophyly of this subtribe can be assessed. It is typical of many Gnaphaliinae, but differing from close relatives like *Euchiton* Cass. by lacking a pappus. *Stuartina* is sister to Clade F, which consists of *Ewartia*, and the Clade A_2 genera *Ozothamnus* (pro parte), *Cassinia*, *Haeckeria* F. Muell., and *Acanthocladium* F. Muell.(Fig. 1). Like *Parantennaria* (Clade B), *Ewartia* is a dioecious, alpine cushion plant native of the high mountains of south eastern Australia. Clade A2, consists of a cohesive group (SYN = 1; BSV=75%) of eastern Australian shrubs or subshrubs with simple heads and multiple series of papery phyllaries of more or less equal length, the heads being usually heterogamous with either paleate or epaleate receptacles. In fact, the presence or absence of paleae on the receptacle has often been used as a generic delimiter in this group. The taxonomic history of this group, including *Acanthocladium*, *Cassinia*, *Haeckeria*, and *Ozothamnus*, reveals that these taxa, at Bentham's time (1867) or later, all belonged to *Helichrysum* (sect. *Ozothamnus*), *Humea* Sm. (= *Calomeria* Vent.) or *Cassinia*, and they are all currently classified in subtribe Cassiniinae (sensu Anderberg 1991). The main differences among the genera are the absence of pappus in *Haeckeria*, a lack of receptacular paleae in *Ozothamnus*, and the presence of both in *Cassinia*. Anderberg has placed all these genera, except for *Acanthocladium*, in his informal ''*Cassinia*'' group. Although *Acanthocladium* [= *Helichrysum dockeri* (F.Muell) Benth. of *Helichrysum* section *Ozothamnus* of Bentham (1867)] was not included in the ''*Cassinia*'' group, it was close to it in Anderberg's cladistic analysis of morphology (Anderberg 1991), and aside from its apomorphy of heavily-armed thorny branches, its flowering heads seem to fit well into this group. It is not unreasonable to consider *Acanthocladium* as a member of that group because it has all the characterisitics of the group as defined by Anderberg (1991).

Clade G is a mixture of taxa from the Cassiniinae and Angianthinae from both eastern and western Australia. In general they are perennial (except *Bellida* Ewart) herbs or shrubs, with simple, many-flowered, homogamous capitula. Additionally, the pappus bristles of the group, when present, are unusual in that the bristles have shorter, instead of longer, teeth apically. In Clade B2, *Ammobium* R. Br.and *Argyroglottis* Turcz. are united by their slightly clawed phyllaries and their white radiating phyllary tips. The similarity of *Pithocarpa* Lindl. and *Calomeria* (Clade O; SYN = 3; BSV = 83%) was first put forth by Bentham (1867), who noted they both lacked pappus, a feature shared with *Ammobium*, *Eriochlamys* Sond. & F.Muell. in Sond., *Odixia* Orchard, and *Ixodia* R.Br. of Clade G. They both have loose groups of numerous simple, homogamous, discoid heads. The style branches of the florets are truncate, they lack a pappus and the cypselae are obovoid (*Gnephosis* type, sensu Anderberg 1991). These two genera are possibly related to *Apalochlamys* Cass., which is also part of Clade G, as it shares the above set of characters with *Calomeria* and *Pithocarpa*. The sister relationship between *Ozothamnus diosmifolius* (Vent.) DC. and *Odixia* in Clade P is not unexpected, as both *Odixia* and *Ixodia* are apomorphic segregates of the ''*Cassinia*'' group. The primary difference between the sister taxa in Clade P is the shape of the receptacle, the receptacle being conical in *Odixia* and flat in *Ozothamnus*.

Clade I contains a large number of well-supported, small subclades. The morphologically dissimilar *Ce-*

phalipterum A. Gray and *Fitzwillia* P. S. Short are sister taxa in Clade L, and are united by the presence of secondary heads and solitary synflorescences (Fig. 1). Clade Q, containing *Ixiolaena* and *Waitzia* J. C. Wendl., is sister to the single lineage containing *Pterochaeta* (Fig. 1). It is not surprising to find *Pterochaeta* to be close to *Waitzia*, as this monotypic genus was formerly part of *Waitzia* (Bentham 1867). Anderberg (1991) placed *Ixiolaena* in his basal group of genera, unassigned to a subtribe; however, our analysis shows it associated with *Waitzia* (SYN = 2; BSV = 64%), well within a group the Angianthinoid taxa of Clade I. Bentham (1867) noted the similarity of both *Ixiolaena* and *Waitzia* to *Helichrysum*; *Waitzia* differing from *Helichrysum* primarily in its beaked achenes and *Ixiolaena* differing in it herbaceous, not papery, phyllaries.

Much of the remainder of Clade I consists of simple headed, papery-bracted genera that were erected from the previous *Helichrysum* and *Helipterum*. *Helichrysum*, in the traditional sense, consisted of species occurring, throughout Africa, Eurasia, and Australia. However, it has long been recognized that the genus is a polyphyletic assemblage of unrelated taxa. Over the years, a number of genera have been proposed to accommodate some of the variation in *Helichrysum* s.l. (see review in Bayer 2001). The African-Australian genus *Helipterum* DC. has likewise been recognized as polyphyletic, as well as the name being illegitimate (Wilson 1989). Australian species of the former *Helipterum* have been transferred to *Rhodanthe* Lindl., *Hyalosperma*, *Leucochrysum* (DC.) Paul G. Wilson, *Anemocarpa* Paul G.Wilson, *Gilberta* Turcz, among others. Clade M (SYN = 15; BSV = 59%), which is a subclade of Clade T (SYN = 2; BSV = 59), has three annual taxa with well-developed secondary heads, including *Pogonolepis* Steetz, *Hyalochlamys* A. Gray, and *Myriocephalus morrisonianus* Diels. Looking now at the final subclades of Clade I, Subclade U (SYN = 13; BSV = 95%) contains the genera *Asteridea* and *Podolepis* (Fig. 1). These two taxa are both perennial with simple, homogamous capitula, but their most obvious synapomorphy is their joint possession of radiate heads. Radiate heads are rare in the Gnaphalieae and the only Australian Gnaphalioid genera to contain them are *Asteridea* and *Podolepis*. Clade N (SYN = 6) contains a mixture of annual genera from Anderberg's (1991) ''*Angianthus* sensu amplo'' group with more or less densely-amassed secondary heads, such as *Calocephalus*, *Trichanthodium* (formerly *Gnephosis*), *Siloxerus*, and *Gnephosis*, along with two genera that have simple, papery-bracted, capitula—*Rhodanthe* (formerly *Helipterum*) and *Lawrencella* [formerly *Helichrysum* sect. *Lawrencella* (Lindl.) Benth.]. The placement of *Rhodanthe* and *Lawrencella* is somewhat enigmatic because they have simple heads with papery radiating laminae and are situated in a clade with taxa with secondary heads without radiating phyllary tips, although they are near clades containing members of the former *Helipterum* and *Helichrysum*, in which they were previously placed.

Clade J contains a number of moderate to well supported small subclades. *Erymophyllum*, *Craspedia*, and *Pyncosorus* form Clade V (SYN = 3; BSV = 54%). The morphological connection between *Erymophyllum* (formerly *Helipterum*) and the *Craspedia—Pycnosorus* Benth. clade (SYN = 6; BSV = 93%) is obscure, although Anderberg (1991) points to possible relationships of certain *Rhodanthe* species (formerly *Helipterum*) to *Craspedia*. In contrast, the relationship between *Craspedia* and *Pyncosorus* is obvious, as *Pycnosorus* is a segregate genus from *Craspedia*. Clade K presents a well-supported group (SYN = 5) of four taxa. *Hyalosperma* (formerly *Helipterum*) seems an unlikely member of this group, but its sister clade consisting of *Quinetia* Cass. sister to *Millotia* and *Scyphocoronis*, in our opinion, is morphologically sound, as they all have cartilaginous phyllaries, which are rare among Australian Gnaphalieae. *Millotia*, *Scyphocoronis*, and *Toxanthes* all have phyllaries in a single series and corollas that are curved at anthesis, and the curved corolla is shared by *Podotheca*, in the neighboring Clade W. Anderberg (1991) also suggests the close relationship of *Millotia*, *Scyphocoronis*, and *Toxanthes* (Clade D), stating that the primary differences among the genera are characteristics of the pappus. These taxa, along with *Podotheca* (Clade W), were placed by Anderberg (1991) in his group of genera unassigned to a subtribe, but it seems at least *Millotia*, *Scyphocoronis*, and *Podotheca* might best be placed in a redefined Angianthinae. *Millotia*, *Scyphocoronis*, and *Toxanthes* were all united into a single genus, *Millotia* s.l. by Short (1995). Clade X (Fig. 1) is a group of Western Australian annuals (except *Leucophyta* R. Br.), with yellow-floreted, homogamous, secondary heads (except *Gilberta* and *Gilruthia*). Most of these taxa also have phyllaries of equal length throughout the heads, and the stereomes are mostly undivided. All of these genera (excepting *Gilberta*, *Myriocephalus*, and *Quinqueremulus* Paul G. Wilson), are part of Anderberg's (1991) ''*Angianthus*'' group. The structure of this clade is very similar to that based on morphology presented by Anderberg (1991, fig. 13). Clade Y (SYN = 3) contains *Angianthus*, *Pleuropappus* F. Muell., and *Siloxerus*, which all have opposite leaves (at least basally), transparent involucral bracts, and obovoid (*Gnephosis* type) cypselae. In fact, *Angianthus* and *Pleuropappus* are very similar morphologically, the primary difference between the genera being the pappus of bristles in the former and scales in the latter. Bentham (1867) included the monotypic *Pleuropappus* within his *Angianthus*. However, both Short (1983) and Anderberg (1991) recognize it as generically distinct. *Chthonocephalus* Steetz, *Decazesia* F. Muell., and *Gilberta* (Clade Z; SYN = 3; BSV = 63%) are united by the morphological syna-

pomorphies of having untailed anthers with concave apical appendages, truncate style branches, brownish or hyaline phyllaries, and cypselae that are much shorter than their corollas. Taxa in Clade C2 (SYN = 1) all share the common features of unclawed, transparent bracts, in which the inner bracts are not much shorter than the outer ones. Additionally, all these taxa have cypselae that lack a pappus, a feature that is not common in the Gnaphalieae. Finally, Clade D2 (SYN = 3; indels = 2; Fig. 1) contains *Actinobole* Fenzl ex Endl., *Lemooria* P. S. Short (formerly *Angianthus*), *Blennospora* A.Gray (formerly *Calocephalus*), and *Calocephalus* and these taxa are united by the common possession of plumose pappus bristles that are distinctly connate at the base.

In summary, results of this analysis indicate that the subtribes Angianthinae and Cassiniinae are non-monophyletic as currently circumscribed (Anderberg 1991). The Angianthinae might be made monophyletic with some recircumscription, by transfering *Ixiolaena*, *Millotia*, *Podotheca*, and *Scyphocoronis* into it, and the exclusion of taxa such as *Calomeria* and *Pithocarpa* would make the large Clade H (Fig. 1) a monophyletic redefined Angianthinae. There is some indication that the genera *Asteridea*, *Craspedia*, *Hyalosperma*, *Millotia*, *Myriocephalus*, and *Podolepis* are monophyletic, whereas *Calocephalus*, *Gnephosis*, *Myriocephalus*, *Ozothamnus*, *Siloxerus*, *Trichanthodium*, and *Xerochrysum*, as presently circumscribed, are non-monophyletic. Groups of perennial shrubs or alpine cushion plants from eastern Australia dominate the clades at the base of the Gnaphalieae clade. The other clades consist primarily of herbaceous annual (sometimes perennial) taxa mainly from western Australia, but one subclade (I) has taxa with primarily simple, both heterogamous and homogamous capitula and the other subclade (J) consists primarily of taxa with homogamous, secondary capitula. There is general agreement between our molecular analysis with that of morphology (Anderberg 1991), particularly in the terminal branches of the trees.

Phytogeography and Character Evolution of the Gnaphalieae in Australia. Anderberg's phylogeny (1991) 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. If this is the case the ancestors of Australian Gnaphalieae may have dispersed to Australia from South Africa, along the Trans Indian Ocean biogeographic track proposed by Crisp et al. (1999). Most Gnaphalieae occur in dry, open habitats and, based on our results, it seems feasible that initial colonization and diversification of the Australian Gnaphalieae occurred in the temperate Bassian biotic region (Schodde 1989) in eastern New South Wales, Victoria, and Tasmania (Fig. 3). The first Gnaphalieae to colonize Australia might therefore have been the shrubby Cassiniinae occurring as understory in temperate *Eucalyptus* L'Herit forests, like those taxa in Clade F, and to some degree in Clade G. Following diversification in eastern Australia and concurrent with the increasing aridity over the entire continent during the Miocene (17–18 m.y.b.p.), a massive radiation in the Gnaphalieae may have occurred into the arid, Eryean Zone of South and Western Australia (Fig. 3). This is a common biogeographic pattern in many plant and animal groups in Australia (Schodde 1989) and our cladistic relationships are consistent with this pattern. We noted similar patterns with regard to the Gnaphalieae in Southern Africa (Bayer et al. 2000). The basal elements are shrubby perennial species growing in the mountains of the Western Cape Province and there have been massive speciation events in the Cape Flora, likely beginning in the Pliocene. This has led to the possible rapid and recent radiation of herbaceous and annual species, such as those in the genus *Helichrysum*, both in the Cape region and in the mountains of Kwazulu Natal, the Orange Free State, Eastern Cape, Lesotho, and Madagascar.

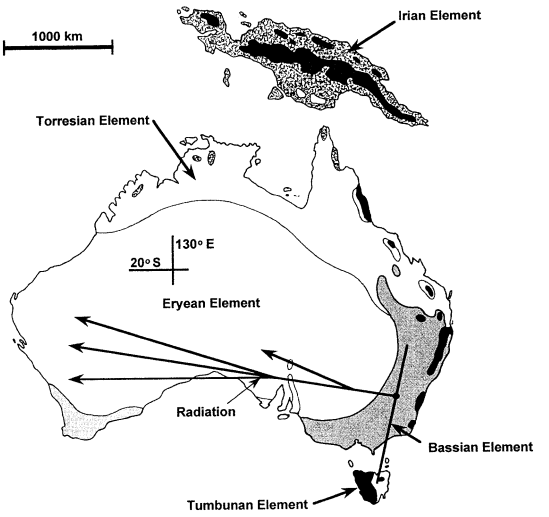

FIG. 3. Map of Australia and New Guinea showing Australasian biotic regions (redrawn after Schodde 1989). The five biotic regions, Bassian, Eryean, Irian, Torresian, and Tumbunan, are indicated. The onset of the aridity of the Miocene (17–18 m.y.b.p.), was the likely impetus for the eastern Bassian Gnaphalieae to undergo an adaptive radiation into the Eryean biotic zone of South Australia and Western Australia.

Besides the switch from perennial duration to annual in several lineages, there are a number of apparent evolutionary trends within the Gnaphalieae, such as a switch from homogamy to heterogamy occurring in at least two lineages (Clades I and E). Radiate heads appear to have arisen only once in the Australian Gna-

phalieae, in *Asteridea* and *Podolepis* (Clade U). Secondary heads appear to have arisen several times in the Australian Gnaphalieae, particularly in Clades M, N, V, and X. However, secondary heads may not be homologous across Australian Gnaphalieae as there are several types of secondary-heads, and their evolution and development is very complex (Claßen-Bockhoff 1992, 1996). When the transparency of the involucral bracts is considered, it is the transparent ones that are often associated with secondary heads, whereas opaque ones are more often allied with simple capitula. Other evolutionary trends in morphology and breeding systems may become more evident as more taxa and characters are added to this developing data set.

Although this paper brings us somewhat closer to understanding relationships among groups of Gnaphalieae, there is much left to be accomplished. The next logical step in our work will be to combine the sequence matrix from this study with the African one (Bayer et al. 2000), and include additional taxa from South America, North America, and Eurasia in order to study phylogeny and phytogeography on a worldwide tribal level.

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