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Cape diversification and repeated out-of-southern-Africa dispersal in paper daisies (Asteraceae–Gnaphalieae)

Nicola G. Bergh [a,b,*], H. Peter Linder [c]

[a] Compton Herbarium, Kirstenbosch Research Centre, South African National Biodiversity Institute, Private Bag X7, Newlands, Cape Town 7735, South Africa
[b] Bolus Herbarium, Botany Department, University of Cape Town, Private Bag, Rondebosch, Cape Town 7700, South Africa
[c] Institute of Systematic Botany, Zollikerstrasse 107, CH 8008 Zürich, Switzerland

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ABSTRACT

The large daisy tribe Gnaphalieae occurs in extra-tropical habitats worldwide, but is most diverse in southern Africa and in Australia. We explore the age and evolutionary history of the tribe by means of a phylogenetic hypothesis based on Bayesian analysis of plastid and nuclear DNA sequences, maximum likelihood reconstruction of ancestral areas, and relaxed Bayesian dating. Early diversification occurred in southern Africa in the Eocene–Oligocene, resulting in a grade of mostly Cape-centred lineages which subsequently began speciating in the Miocene, consistent with diversification times for many Cape groups. Gnaphalieae from other geographic regions are embedded within a southern African paraphylum, indicating multiple dispersals out of southern Africa since the Oligocene to Miocene which established the tribe in the rest of the world. Colonisation of Australia via direct long-distance *trans*-oceanic dispersal in the Miocene resulted in the radiation which produced the Australasian gnaphalioid flora. The similarly diverse regional gnaphalioid floras of Australasia and southern Africa thus exhibit very different temporal species accumulation histories. An examination of the timing and direction of *trans*-Indian Ocean dispersal events in other angiosperms suggests a role for the West Wind Drift in long-distance dispersal eastwards from southern Africa.

1. Introduction

The daisy tribe Gnaphalieae is represented on most continents but forms a significant component of dry- and cool-temperate floras in the Southern Hemisphere. This tribe of ca. 1240 species (Bayer et al., 2007) has two main centres of diversity: southern Africa, where one quarter of the ca. 2200 Asteraceae species are gnaphalioids (Koekemoer, 1996) and Australasia with nearly 500 species (Bayer et al., 2007). The remainder are found in South and Central America (ca. 100 species; Anderberg, 1991), the rest of Africa, the Mediterranean, Asia, and North America (Bayer et al., 2007).

In the morphological cladistic analysis of Anderberg (1991), the early-diverging lineages consist of taxa from both southern Africa and Australia. Bayer et al.'s (2000) molecular phylogenetic study did not include any Australasian taxa but indicated that the earliest diverging lineages contain southern African species from the near-endemic Cape subtribe Relhaniinae. This paraphyletic (Bayer et al., 2000) subtribe was identified by Linder (2003) as the tenth-largest Cape Floral Clade. Elucidation of the phylogenetic relationships in Relhaniinae is clearly key to understanding the evolutionary history of the tribe as well as to providing insight into the origins of the unique Cape Flora of southern Africa. Here we build on the analyses of Bayer et al. (2000, 2002) by constructing a phylogenetic hypothesis for the Gnaphalieae with special focus on members of the Relhaniinae, but including non-relhaniinoid taxa from southern Africa, Australia and the Northern Hemisphere. We ask, firstly, what such a phylogeny can tell us about the biogeographic history of the tribe and specifically, given that the two major centres of diversity are on either side of the Indian Ocean, about the timing and direction of *trans*-oceanic dispersal(s) in Gnaphalieae. Secondly, we ask whether there is a general pattern in *trans*-Indian Ocean plant dispersals, and what mechanisms could underly such a pattern.

2. Materials and methods

2.1. Sampling

Outgroup taxa from other tribes in the Asteraceae were included for molecular clock calibration (see below) and non-relhaniinoid taxa from the Gnaphalieae were selected to represent the

* Corresponding author. Address: Compton Herbarium, Kirstenbosch Research Centre, South African National Biodiversity Institute, Private Bag X7, Newlands, Cape Town 7735, South Africa. Fax: +27 (0)21761 4151.
E-mail address: bergh@sanbi.org (N.G. Bergh).

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widest possible taxonomic and geographic coverage (Table 1). Field collection of leaf material was carried out in South Africa or, for some taxa, DNA was extracted from herbarium material at BOL. We generated 67 new DNA sequences for southern African taxa and obtained the remainder from published studies.

2.2. DNA isolation, amplification and sequencing

Leaf material was collected directly into silica gel. Approximately 30 mg of dried leaf material was ground with sterilized sand and/or liquid nitrogen and total genomic DNA isolated using the CTAB method (Doyle and Doyle, 1987). Extracts from problematic samples were cleaned through QIAGEN® QIAquick cleaning columns. DNA was suspended and diluted in Tris–EDTA buffer. PCRs were performed on a Hybaid PCR Sprint thermal cycler (Fisher Scientific International).

The 3′ portion of the external transcribed spacer (ETS) of nuclear ribosomal DNA was amplified using the primers AST1 (Markos and Baldwin, 2001) and 18S-ETS (Baldwin and Markos, 1998). The chloroplast *psbA-trnH*^{GUG} spacer was amplified using the primers *psbA*-F and *trnH*-R of Sang et al. (1997). The chloroplast *trnL*^{UAA} intron and the *trnL*^{UAA}-*trnF*^{GAA} intergenic spacer were amplified together using the "c" and "f" primers of Taberlet et al. (1991). The reaction mixtures consisted of 5.0 mM $MgCl_2$, dNTPs at 0.1 mM each, primers at 0.5 μM, 0.3 μM and 0.33 μM (ETS, *psbA-trnH* and *trnL-F*, respectively) and 0.75 (*psbA-trnH* and *trnL-F*) or 1.0 (ETS) unit(s) of Bioline BioTaq®. The ETS mixture also contained 2% DMSO. Reaction volumes were made up to 25 μl (ETS) or 30 μl (*psbA-trnH* and *trnL-F*) with sterilized Millipore™ water, and included 3–4 μl of template DNA. Thermal profiles consisted of 2 min at 95 °C (ETS and *psbA-trnH*) or 97 °C (*trnL-F*) followed by 30 cycles of: (a) 94 °C (ETS and *psbA-trnH*) or 97 °C (*trnL-F*) for 1 min; (b) 1 min at 55 °C (ETS) or 52 °C (*trnL-F*); or 45 s at 54 °C (*psbA-trnH*) and (c) 2 min (ETS and *trnL-F*) or 1 min (*psbA-trnH*) at 72 °C. A final extension step at 72 °C lasted for 7 (ETS and *trnL-F*) or 8 (*psbA-trnH*) minutes.

Successfully amplified target DNA was cleaned in Microspin™ columns using GFX Band Purification Kits from Amersham Biosciences UK or in QIAGEN® QIAquick PCR purification columns. Cycle sequencing used the following thermal profile: 25 cycles of: 30 s at 96 °C, 90 s at 50 °C and 4 min at 60 °C in tubes containing 1–3 μl of cleaned target DNA, 0.16 μl of primer, 2.0 μl of ABI PRISM® BigDye® Terminator v3.1 cycle sequencing reaction mix and 0.1 μl of 20% DMSO made up to 10 μl with sterilized Millipore™ water. Sequenced fragments were visualised either on an ABI PRISM® 377 capillary DNA Sequencer or an ABI PRISM® 3100 Genetic Analyzer. Each region was sequenced in both directions using the original PCR primers; chromatograms were checked and assembled with Chromas software (version 1.45; Technelysium Pty. Ltd., Helensvale, Australia; Conor McCarthy, 1996) and Sequencher 4.5 (Gene Codes Corporation, 2005). Consensus sequences were aligned manually in MacClade 4.05 (Maddison and Maddison, 1992). Stretches of DNA that could not be unambiguously aligned across all taxa were recoded as missing data. Insertion/deletion (indel) events were coded independently as binary characters using the simple gap coding method of Simmons and Ochoterena (2000) implemented in Gapcoder (Young and Healy, 2003).

2.3. Phylogenetic analysis

Each of the four partitions (the *psbA-trnH* spacer; *trnL* intron; *trnL-trnF* spacer and the ETS) consisted of a stretch of nucleotides and the associated indel data and was initially analysed separately. Unweighted parsimony tree searching was conducted in PAUP ver 4.0 b10 (Swofford, 2002). Parsimony uninformative characters were excluded in order to standardize parsimony statistics. An initial shallow search was conducted with 10,000 random-addition replicates using NNI branch-swapping and saving only one tree per round of branch swapping. The resulting set of shortest trees was then subjected to TBR branch swapping while saving multiple trees. Node support was assessed via 1000 non-parametric bootstrap replicates, each replicate saving a maximum of 500 trees based on a simple addition sequence with the TBR branch-swapping algorithm.

Simultaneous Bayesian inference of nucleotide substitution parameters and topology was performed in MrBayes v3.1 (Huelsenbeck and Ronquist, 2001). Indel characters were analysed according to the restriction site (binary) model with ascertainment (coding) bias accounted for using the 'variable' option (Ronquist et al., 2005). The default prior and likelihood settings were used for all remaining parameters except the nucleotide substitution model which was set to the general time-reversible model with an inverse gamma distribution of rate variation across sites (GTR+I+Γ; see Results). For partitioned analysis, substitution model parameters and rates of substitution were allowed to vary across partitions using ratepr = variable and the 'unlink' command. The MCMC chain was run for up to 8,000,000 iterations, sampling parameters every 1000 iterations. Each analysis used one cold chain and up to 11 heated chains, with chain heating parameter values between 0.06 and 0.20. Longer runs and greater numbers of heated chains were required to achieve convergence for the analyses combining more than two gene partitions. Convergence of the Markov chain and assessment of 'burn-in' duration was determined by examining the average standard deviation of split frequencies and by comparing likelihood values, parameter estimates and traces between samples from two simultaneous runs starting from different random starting trees in Tracer v1.3 (Rambaut and Drummond, 2003). Additional assessment of convergence was based on the comparison of posterior probability (PP) consensus trees from different runs within and across analyses. At least three separate runs were conducted for each partition. Trees were visualized using the 'compute consensus' option in PAUP v4.0b10 (Swofford, 2002).

Tests of data combinability such as the incongruence length difference (ILD) test have been shown to have a high type I error rate (Planet, 2006; Yoder et al., 2001; Barker and Lutzoni, 2002) and to be especially difficult to interpret when comparing more than two data partitions (Planet, 2006), and when partitions are represented by different numbers of taxa, as in our dataset. We therefore used a more straightforward approach to detecting incongruence amongst data partitions and compared individual trees for well-supported conflict (e.g. Wiens, 1998; Eldenäs and Linder, 2000) defined as a bootstrap value of $\geqslant$75% or a PP value $\geqslant$0.95.

2.4. Molecular age estimation

BEAST v1.4.6 (Drummond and Rambaut, 2007) was used to estimate divergence times from all gene regions simultaneously and using only nucleotide data. This software uses a 'relaxed phylogenetic' model, where topology and branch lengths are estimated simultaneously from the data. The topology, including placement of the root node, is not specified *a priori* (Drummond et al., 2006). The partitioned BEAST .xml input file (available on request from the corresponding author) was created with BEAUti v1.4.6 (Drummond and Rambaut, 2007) and edited manually to allow parameters to be estimated independently amongst data partitions. The substitution model parameters were the same as in MrBayes (GTR+I+Γ) and the gamma distribution was modelled with four categories. A log-likelihood ratio test rejected the strict clock model for our dataset ($p < 0.001$) so we implemented the relaxed Bayesian clock with rates for each branch drawn independently from a lognormal distribution (Drummond and Rambaut,

Table 1
Specimen collection and voucher information

Taxon	Voucher details (if collected for this study)		GenBank/EMBL Accession Nos.			
	Collector (and herbarium)	Collection locality	*psbA-trnH*	*trnL* intron	*trnL-trnF* spacer	ETS
Actinobole uliginosum (A. Gray) H. Eichler	—	—	—	AF141736	AF141824	AF319667
Amphiglossa callunoides DC.	Bergh 1452a (NBG)	Uitenhague	FM173155	—	—	FM173123
Amphiglossa corrudifolia DC.	Koekemoer 1291 (BOL)	Prince Albert	FM173156	—	—	FM173124
Amphiglossa tomentosa (Thunb.) Harv.	Bergh 1332 (NBG)	Cederberg	FM173157	FM173184	FM173184	FM173125
Angianthus micropodioides (Benth.) Benth.	—	—	—	AF141695	AF141783	AF319671
Anisothrix kuntzei O. Hoffm.in Kuntze	—	—	—	AF098859	AF100522	—
Argentipallium obtusifolium (Sond.) Paul G.Wilson	—	—	—	AF141730	AF141818	AF319674
Argyroglottis turbinata Turcz.	—	—	—	AF141692	AF141780	AF319675
Arrowsmithia styphelioides DC	—	—	—	AF098809	AF100472	—
Aster novae-angliae L	—	—	—	U82018	U82019	AF319676
Bryomorphe lycopodioides (Sch. Bip) Levyns	Bergh 1155 (NBG)	Matroosberg	FM173158	AF098820	AF100483	FM173126
Chionolaena lavandulifolia (Kunth.) Benth. & Hook.	—	—	—	AY143593	AY143593	—
Craspedia variabilis J. Everett & Doust	—	—	EF187694	AF141713	AF141801	EF187619
Decazesia hecatocephala F. Muell.	—	—	—	AF141752	AF141840	AF319695
Disparago ericoides (Berg.) Gaertn.	Bergh 1143 (NBG)	Houw Hoek	FM173159	AF098821	AF100484	FM173127
Dolichothrix ericoides (Lam.) Hilliard & Burtt.	Bergh 180 (NBG)	Cederberg	FM173160	AF098822	AF100485	FM173128
Edmondia sesamoides (L.) Hilliard	Bergh 1130 (NBG)	Jonaskop	FM173161	AF098844	AF100507	FM173129
Elytropappus rhinocerotis (L.f.) Less.	Bergh 3.3; Bergh M1 (NBG)	Kamiesberg	FM173162	FM173185	FM173185	FM173130
Ewartia catipes (DC) Beauverd	—	—	—	AF141698	AF141786	AF319701
Ewartia sinclairii Cheeseman	—	—	AY611218	—	—	—
Felicia filifolia Burtt Davy	—	—	—	AF318120	AF318929	AF319703
Fitzwillia axilliflora (W. Fitz. ex Ewart & Jean White) P. S. Short	—	—	—	AF141708	AF141796	AF319704
Haeckeria ozothamnoides F. Muell.	—	—	—	AF141725	AF141813	AF319710
Helianthus annuus L.	—	—	X60428	U82038	U82039	AF319711
Helichrysum asperum (Thunb.) Hilliard & B. L. Burtt	Bergh 1165 (NBG)	Cederberg	FM173163	FM173186	FM173186	FM173131
Helichrysum cylindriflorum (L.) Hilliard & B. L. Burtt	Bergh 1063 (NBG)	Cederberg	—	AF098839	AF100502	FM173132
Helichrysum dockeri (F. Muell.) Benth.	—	—	—	AF318111	AF318922	AF319665
Helichrysum felinum Less.	Bergh 1194 (NBG)	Namaqualand	FM173164	FM173187	FM173187	FM173133
Helichrysum lanceolatum Kirk.	—	—	EF187698	AY606889	AY606900	EF187647
Ixiolaena tomentosa Sond. & Muell.	—	—	—	AF141704	AF141792	AF319717
Jacobaea argunensis (Turcz.) B. Nord.	—	—	—	AF468169	AF468169	—
Jacobaea maritima (L.) Pelser & Meijden	—	—	AY155647	AF460158	AF460105	—
Lachnospermum fasciculatum Baill.	Bergh 1105 (NBG)	Jonaskop	FM173165	—	—	FM173134
Lachnospermum neglectum Schltr.	—	—	—	AF098824	AF100487	—
Langebergia canescens (DC) Anderb.	—	—	—	AF098828	AF100491	—
Leontopodium alpinum Cass.	Bergh 1349 (NBG)	Switzerland	FM173166	AF141733	AF141821	FM173135
Leucochrysum stipitatum (F. Muell.) Paul G. Wilson	—	—	—	AF141722	AF141810	AF319722
Leysera gnaphalodes (L.) L.	Bergh 1441 (NBG)	Malmsbury	FM173167	AF098810	AF100473	FM173136
Metalasia acuta P. O. Karis	Bergh 1301 (NBG)	Cederberg	FM173168	—	—	FM173137
Metalasia densa (Lam) Karis	Bergh 1266 (NBG)	Pearly Beach	FM173169	AF098848	AF100511	FM173138
Metalasia galpinii L. Bolus	Bergh 1276 (NBG)	Langeberg	FM173170	—	—	FM173139
Millotia perpusilla (Turcz.) P. S. Short	—	—	—	AF141757	AF141845	AF319762
Millotia tenuifolia Cass.	—	—	—	AF318124	AF318933	AF319727
Monoculus monstrosus (Burm. f.) B. Nord	—	—	—	U82048	U82049	AF319733
Oedera squarrosa (L.) Anderb. & Bremer	Bergh 1065 (NBG)	Cederberg	—	AF098812	AF100475	FM173140
Ozothamnus whitei (N. T. Burb.) Anderb.	—	—	—	AF141748	AF141836	AF319737
Pentatrichia petrosa Klatt.	—	—	—	AF098817	AF100480	—
Petalacte coronata D. Don.	—	—	—	AF098843	AF100506	—
Phaenocoma prolifera (L.) D. don	Bergh 1206 (NBG)	Struisbaai	FM173171	AF098825	AF100488	FM173141
Pithocarpa pulchella Lindl.	—	—	—	AF141756	AF141844	AF319739
Plecostachys serpyllifolia (Berg.) Hilliard & B. L. Burtt.	Bergh 1271 (NBG)	Riviersonderend	FM173172	AF098849	AF100512	FM173142
Pogonolepis stricta Steetz	—	—	—	AF141678	AF141766	AF319745
Polycalymma stuartii F. Muell. & Sond.	—	—	—	AF141726	AF141814	AF319746
Pycnosorus globosus Bauer ex Benth.	—	—	EF187719	AF141680	AF141768	EF187625
Quinetia urvillei Cass.	—	—	—	AF141716	AF141804	AF319750
Relhania calycina (L.f.) L'Herit.	Karis 650 (NBG)		FM173173	—	—	FM173143
Relhania fruticosa (L.) Bremer	—	—	—	AF098813	AF100476	—
Relhania pungens L'Herit.	Karis 876 (NBG)		FM173174	—	—	FM173144
Rhynchopsidium pumilum (L.f.) DC.	Karis 770 (NBG)		FM173175	AF098811	AF100474	FM173145
Rosenia glandulosa Thunb.	—	—	—	AF098815	AF100478	—
Senecio burchellii DC	Bergh 1110 (NBG)	Jonaskop	FM173176	—	—	FM173146
Senecio cordifolius L. f.	—	—	—	AY952916	AY952916	—
Senecio vulgaris L.	—	—	—	DQ208177	—	AF319755
Siloxerus multiflorus Nees	—	—	—	AF318127	AF318936	AF319756
Sondottia glabrata P. S. Short	—	—	—	AF141738	AF141826	AF319758
Stoebe aethiopica L.	Bergh 1040 (NBG)	Cederberg	FM173177	AF098845	AF100508	FM173147
Stoebe cinerea Thunb.	Bergh 1210 (NBG)	Du Toit's Kloof	FM173178	FM173188	FM173188	FM173148
Stoebe cryptophylla Baker	Gehrke AF281 (NBG)	Madagascar	FM173179	—	—	FM173149
Stoebe kilimandscharica O. Hoffm.	Gehrke AF076 (NBG)	Kenya	FM173180	—	—	FM173150
Stoebe muirii Levyns	Bergh 1263 (NBG)	De Hoop	FM173181	—	—	FM173151
Stoebe passerinoides Willd.	Hedderson 15894 (NBG)	La Réunion	FM173182	—	—	FM173152
Stuartina muelleri Sond.	—	—	EF187715	U82058	U82059	AF319760

(*continued on next page*)

Table 1 (*continued*)

Taxon	Voucher details (if collected for this study)		GenBank/EMBL Accession Nos.			
	Collector (and herbarium)	Collection locality	*psbA-trnH*	*trnL* intron	*trnL-trnF* spacer	ETS
Syncarpha canescens (L.) B. Nord.	Bergh 1222 (NBG)	Cape Town	FM173183	FM173189	FM173189	FM173153
Tagetes patula L.	—	—	—	U82060	U82061	AF319761
Vellereophyton dealbatum (Thunb.) Hilliard & B. L. Burtt.	Bergh 1256 (NBG)	Elim	—	AF098808	AF100471	FM173154

Localities listed without mention of a country are all in South Africa. Genbank Accession numbers starting with 'FM' were generated for this study and have not been previously published.

2007). A Yule prior (constant rate of speciation per lineage) was set for the branch lengths and the 'mean.Rate' parameter had a uniform prior between 0 and 0.1. The 'coefficient of variation' had a uniform prior between 0 and 1.0 and the 'covariance' prior was uniform between −1.0 and 1.0. Runs were initiated on random starting trees. Initially, several short BEAST runs were performed to examine the MCMC performance. After optimal operator adjustment as suggested by the output diagnostics, two final BEAST runs each of 10,000,000 iterations were performed. Convergence was assessed as for MrBayes using Tracer v1.3 (Drummond and Rambaut, 2007). After discarding the first 1,000,000 samples as burn-in, the trees and parameter estimates from the two runs were combined. The samples from the posterior were summarized on the maximum clade credibility tree which is the tree that has the maximum sum of posterior probabilities on its internal nodes (Drummond et al., 2007) using TreeAnnotator v1.4.6 (Drummond and Rambaut, 2007) with posterior probability limit set to 0.5 and summarizing mean node heights. These were visualized using Figtree v1.1.2 (Drummond et al., 2007). Means and 95% higher posterior densities (HPD) of age estimates were obtained from the combined outputs using Tracer v1.3. The 95% HPD represents the shortest interval that contains 95% of the sampled values from the posterior (Drummond et al., 2007).

Calibration nodes were defined via appropriate taxon sets which were not constrained to be monophyletic. We implemented prior age distributions with 'soft' bounds rather than 'hard' upper and lower cut-off bounds, as soft bounds allow the incorporation of more prior information about the timing of a divergence event (Yang and Rannala, 2006). Such prior distributions can be structured to better account for uncertainty both in the taxonomic placement of fossils and in the dates assigned to fossils or geological events, giving low but non-zero probabilities to times outside the most likely age boundaries. For example, since a fossil represents only the minimum age of the taxon (Hedges and Kumar, 2004), a distribution with a long 'tail' further back in time represents the diminishing probability that the divergence time may actually be much older than the fossil with greater accuracy than an arbitrary oldest cut-off age. In addition, some leeway around the youngest bound of the fossil age, rather than a hard cut-off, better incorporates the uncertainty associated with both the dating of the fossil and its assignment to a specific node in the tree. Hard, cut-off bounds assume that there is no error in either dating, taxonomic assignment of the fossil or correspondence between the fossil and the divergence it is assumed to represent. Hard cut-offs also have the drawback that when there is conflict amongst calibration points, age estimates for any 'bad' calibration points will be bound by the 'hard' limits of their priors, preventing the corrective influence of the (hopefully more accurate) remaining calibration points (Yang and Rannala, 2006). Allowing the analysis occasionally to sample states with lower probabilities will also result in larger confidence intervals, more accurately reflecting the error associated with divergence time estimates.

The calibration of the root node ('treemodel.Rootheight' in BEAST) corresponds to the crown age of the large subfamily Asteroideae and was informed by a previous molecular dating study (Kim et al., 2005) which estimated this node to range from 35 to 39 Ma based on a slow and a fast rate calibration of *ndhF* from other angiosperm families, and from 26 to 29 Ma based on NPRS dating calibrated with an outgroup fossil. Secondary calibration dates compound several sources of error and should be implemented and interpreted with caution (Graur and Martin, 2004). For this reason, we used as our prior for this node a normal distribution with a mean at 32.5 Ma, the midpoint of Kim et al.'s (2005) dates, and a range spanning two standard deviations around this mean (95% confidence interval of 14.1–50.9 Ma; Fig. 1a).

Ambrosia L. is a genus in tribe Heliantheae (represented in our tree by *Helianthus annuus*) and we used the earliest *Ambrosia*-type pollen, from the Beaverhead Basins flora of Montana (Leopold and MacGinitie, 1972; Becker, 1969), to calibrate the age of the node connecting *Helianthus* with *Tagetes* (tribe Tageteae). Becker (1969) and Graham (1996) state that this fossil is 22–30 (−35) Ma. Uncertainty in the age and imprecision in nodal assignment was accommodated by specifying a lognormal prior distribution that covered a wider time range and allowed the date to shift backwards in time (Fig. 1b). The 95% confidence interval for this prior lies between 16.9 and 44.1 Ma with the mean at 22.3 Ma.

The split between the range-restricted endemic limestone specialist *Stoebe muirii* and its putative sister *S. aethiopica* (N. G. Bergh et al., unpublished data) is likely to have occurred only after exposure of the Agulhas limestones (the Bredasdorp formation; Hendey, 1983). These were laid down during a series of marine transgressions from around 20 Ma up to 10 Ma (and according to some authors, as late as 3 Ma; Siesser and Dingle, 1981; Hendey, 1983) and only exposed after regression (Siesser, 1972) which may be related to continental uplift at around 5 Ma (Maud and Partridge, 1987). A normally-distributed prior is most appropriate for this kind of geological calibration (Ho, 2007) and we used such a distribution (Fig. 1c) with mean at 6.0 Ma, and 95% confidence interval between 3.1 and 8.9 Ma. The mean is shifted slightly backwards in time to allow for the possible occurrence of the habitat elsewhere before 5 Ma (e.g. the Elandsfontyn formation; Hendey, 1983). This prior also allows for the possibility that *S. muirii* may have speciated some time after the exposure of its habitat.

Stoebe passerinoides is endemic to high altitude heathlands on the volcanic island La Réunion where volcanism was initiated at ca. 5 Ma, building the massifs by ca. 2 Ma (McDougall, 1971; Gillot et al., 1994). The habitat may be older than La Réunion (Bell and Donoghue, 2005) so we used a normal prior distribution with a conservative 95% confidence interval between 1.8 and 7.7 Ma (mean of 4.75 Ma) for the split between this taxon and its sister *S. cryptophylla* from Madagascar. The older end of this range accommodates earlier volcanism in the Mascarene hotspot corresponding to the earliest estimated age of the shield volcano structure on the neighbouring island of Mauritius (McDougall and Chamalaun, 1969; Fig. 1d).

Given the potential errors associated with molecular age calibrations (Shaul and Graur, 2002; Heads, 2005), sensitivity to different calibration priors was tested using several alternative calibration schemes (Table 3). The original calibration settings described above are referred to as Scheme (a). We tested the influence of the root calibration by using an uninformative root prior in Scheme (b). Scheme (c) has a lognormal root prior allowing for

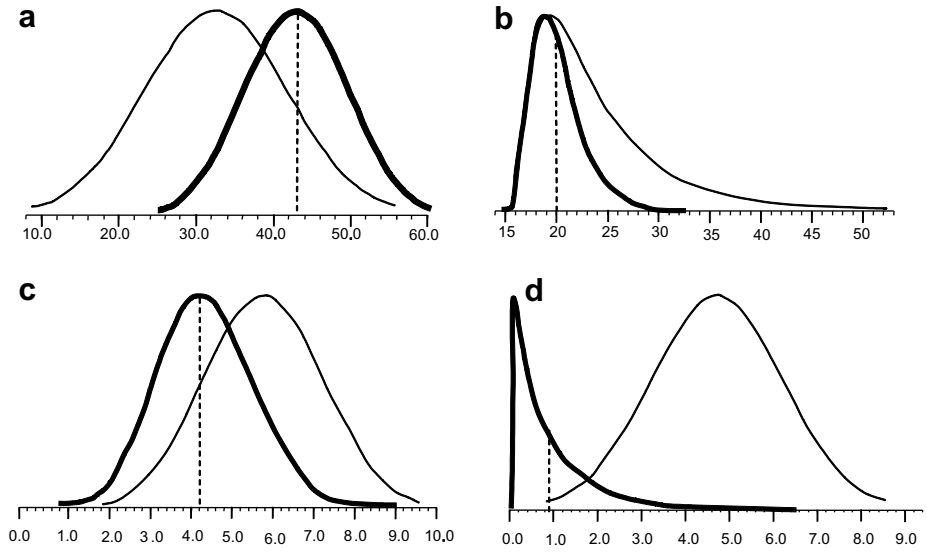

Fig. 1. Prior (thin line) and posterior (thick line) age distributions of calibration nodes in the BEAST uncorrelated relaxed clock analysis implementing calibration Scheme (a). The dashed line indicates the mean of the posterior distribution. The X-axis scale is in millions of years before present. The Y-axes are scaled so that the two curves are of equal height. (a) root node; (b) age of the split between *Helianthus* and *Tagetes*; (c) age of the limestone specialist *Stoebe muirii*; (d) age of La Réunion endemic *Stoebe passerinoides*.

the age to shift backwards in time with diminishing probability. Scheme (d) implements the narrower range of dates for the root node reported in Kim et al. (2005). The calibration for the *Helianthus–Tagetes* split is based on a Chadronian *Ambrosia* pollen grain (Wing, 1987). The end of the Chadronian corresponds to the Eo-Oligocene boundary (Prothero and Berggren, 1992). If free of stratigraphic error, this fossil might provide a more precise minimum age bound. Additionally, this divergence may pre-date the origin of the *Ambrosia*-type lineage (Funk et al., 2005) in which case the fossil is likely to be younger than the actual divergence. We explored the effect of this with a narrower lognormal prior distribution shifted to the older end of the original prior in Scheme (e). Scheme (f) omits the two geological calibrations.

2.5. Ancestral area reconstruction

The fully-resolved BEAST maximum clade credibility tree was used for Maximum Likelihood (ML) ancestral area reconstruction. The advantages of this method over parsimony reconstruction are that it makes use of (in this case, time-proportional) branch lengths, and assigns relative probability values to nodal reconstructions. The Markov k-state one-parameter model (equal probability of change in both directions; Lewis, 2001) was implemented in Mesquite's Ancestral State Reconstruction Packages (Maddison and Maddison, 2006). Areas were coded as a multistate character as follows: 0—southern Africa, including Lesotho, Namibia, South Africa, Moçambique and Zimbabwe; 1—the highlands of Kenya, Tanzania, Uganda, Malawi and the DRC, Madagascar and La Réunion; 2—Eurasia including the Mediterranean region; 3—Central America; and 4—Australasia.

3. Results

3.1. Combining data partitions

We found several instances of incongruent taxon placement at well-supported nodes in trees from different data sets. These all involved tip nodes and taxa which were either composites of sequences from different specimens, or were represented by only one of the gene regions. We attribute the incongruence to missing data, conflict between data partitions, or errors in Genbank sequences. These taxa (*Anaphalis margaritacea* (L.) Benth. & Hook. f., *Anaxeton arborescens* (L.) Less., *Anaxeton asperum* (Thunb.) DC., *Athrixia capensis* Ker Gawl., *Gnaphalium declinatum* L. f., *Gnaphalium viscosum* Kunth., *Lasiopogon glomerulatus* (Harv.) Hilliard, *Pterygopappus lawrencii* Hook. f., *Toxanthes perpusilla* Turcz. and *Trichogyne ambigua* (L.) Druce) were removed from the analysis due to their potential to confound phylogenetic and branch length estimates, albeit at the expense of biogeographic and taxonomic coverage. Comparisons of support values showed no incongruence in the final 73-taxon data set.

3.2. Sequence characteristics and phylogenetic analyses

In the final alignment, individual regions are similar in length but differ greatly in their phylogenetic informativeness (Table 2). The ETS region provides nearly as many parsimony-informative (PI) characters as that of all the chloroplast data combined (262 versus 265) and nearly 45% of ETS characters are PI compared with an average of only ca. 15% for the plastid characters. Also, the ETS PI characters come mostly from nucleotide substitutions, while nearly a third of the plastid PI characters are derived from indel data. Given these facts, it is not surprising that the ETS region produces a better resolved and supported topology (Table 2) than any individual plastid partition. Combining the plastid data partitions reduces the proportion of resolved and supported nodes relative to the individual plastid regions, and combining ETS with the plastid data does the same relative to the ETS data alone (Table 2). This is likely to be due to the inclusion of an increased number of taxa with missing data. Partitioned Bayesian analysis with all regions gives the highest percentage of supported nodes (52.1%; Fig. 2). This may be due to the difference between Bayesian PP values and the bootstrap (Simmons et al., 2004; Erixon et al., 2003; Alfaro and Holder, 2006) or to the use of a nucleotide substitution model in the Bayesian analysis, which incorporates homoplasy as well as rate variation across sites (Huelsenbeck and Crandall, 1997).

Although the actual models chosen for each gene region by MrModeltest 2.2 (Nylander, 2004) are F81+G (*psbA-trnH*) and GTR+G (*trnL-trnF* intergenic spacer, *trnL* intron and ETS), a MrBayes run implementing these models returned the same topology and

Table 2
Taxa and character information for each gene region: number of included taxa, numbers of characters, parsimony informative (PI) characters, resolution and support for trees from each partition analysed separately, and from the combined analyses

	PLASTID					NUCLEAR	ALL
	psbA-trnH	*trnL* intron	*trnL-F* spacer	Combined *trnL-trnF* region	Combined plastid	ETS	
Total number of taxa	35	62	60	62	73	62	**73**
Number of ingroup taxa	32	53	53	53	63	55	**63**
Base-pairs in alignment	539	527	445	972	1511	486	**1997**
PI (%)	*65 (12.1)*	*44 (8.3)*	*75 (16.9)*	*119 (12.2)*	*184 (12.2)*	*223 (45.9)*	***407 (20.4)***
Indel characters	95	57	90	147	242	102	**344**
PI (%)	*37 (38.9)*	*19 (33.3)*	*25 (27.8)*	*44 (29.9)*	*81 (33.5)*	*39 (38.2)*	***120 (34.9)***
Total number of characters	634	584	535	1119	1753	588	**2463**
PI (%)	*102 (16.1)*	*63 (10.8)*	*100 (18.7)*	*163 (14.6)*	*265 (15.1)*	*262 (44.6)*	***527 (21.4)***
Parsimony statistics							
Tree length	166	125	222	364	530	886	**1467**
CI	0.69	0.62	0.61	0.58	0.61	0.5	**0.52**
RI	0.84	0.80	0.77	0.76	0.78	0.78	**0.77**
RC	0.57	0.49	0.47	0.44	0.48	0.39	**0.40**
No (%) of nodes resolved in strict consensus[a]	12 (36.4)	20 (33.3)	24 (41.4)	27 (45.0)	22 (31.0)	31 (51.7)	**28 (39.4)**
No (%) of nodes with BS $\geqslant$75[a]	11 (33.3)	5 (8.3)	11 (19.0)	16 (26.7)	17 (23.9)	21 (35.0)	**20 (28.2)**
No (%) of nodes with PP $\geqslant$0.95[a]	13 (39.4)	9 (15.0)	13 (22.4)	24 (40.0)	23 (32.4)	28 (46.7)	**37 (52.1)**

BS, bootstrap percentage; PP, posterior probability of a node in MrBayes analysis.
[a] Percentages of nodes are based on the maximum possible number of internal branches (n-2 where n is the number of taxa included in the analysis). Strict consensus, strict consensus of all shortest trees in parsimony analysis.

near-identical PP values to independant GTR+I+Γ models for each region. We used the latter in the final analysis as Bayesian inference is more robust to model overspecification than underspecification (Huelsenbeck and Rannala, 2004).

The BEAST maximum clade credibility tree with the highest posterior probabliity (Fig. 3) has the same topology as the MrBayes tree, but several nodes which received good support in the latter were not supported by the BEAST analysis. These were node B (the Gnaphalieae); nodes E and F, and node H (the Australasian taxa). Conversely, node L was well-supported in the BEAST analysis but only received a PP of 0.92 with MrBayes. Results may differ under the two models (i.e. unrooted vs. relaxed phylogenetic model; Drummond et al., 2006) but in this case the discrepancy appears to be due mainly to the exclusion of indel data from the BEAST analysis, as a MrBayes analysis without the indel data produced nodal PP values which were more similar to those of the BEAST analysis than of the original MrBayes analysis (results not shown).

3.3. Topology

Although tribe Gnaphalieae (node B, Fig. 2) is strongly supported in the MrBayes analysis (PP = 1.00), none of Anderberg's (1991) subtribes, indicated by uppercase letters after the taxon names in Fig. 2, are supported by the molecular data. The earliest-diverging gnaphalioid lineage (node K) was recovered and well-supported by all individual partitions and analyses and contains only southern African members of the paraphylum of early-diverging lineages from Anderberg (1991) termed by him the "basal group" and unassigned to any subtribe. We call this lineage the '*Relhania* clade' (Fig. 2). Non-southern African taxa placed by Anderberg in the unassigned "basal group" fall within later-diverging lineages descended from node G. The remaining taxa from Relhaniinae *sensu* Anderberg fall into one of two well-supported (each PP = 1.0) clades (the '*Metalasia* clade' and the '*Stoebe* clade') that, together with the *Relhania* clade, form a largely southern African grade at the base of the Gnaphalieae. *Stoebe* species from the ericaceous belt associated with afroalpine-type habitats (*S. kilimandscharica*, *S. passerinoides* and *S. cryptophylla*) form a clade with PP of 1.0.

The clade defined by node E (termed the 'rest of the Gnaphalieae' in Fig. 2, with PP = 1.0) is sister to the *Stoebe* clade and contains taxa from Eurasia, Central America and Australasia, embedded in a mostly southern African paraphylum (Fig. 3). As found by previous authors (eg. Bayer et al., 2000; Galbany-Casals et al., 2004), sampled members of the large, cosmopolitan genus *Helichrysum* are not monophyletic. The southern African *Plecostachys* forms a well-supported (PP = 0.96) clade with the Eurasian *Leontopodium alpinum* and *Chionolaena lavandulifolia* from Mexico. Node F (PP = 0.95) indicates that the southern African taxa *Edmondia* and *Syncarpha* from Anderberg's (1991) Gnaphaliinae are most closely related to the Australasian taxa. Node G represents a large clade with unsupported internal relationships and genera occurring only in Australasia. Despite lack of support at this node, a more exclusive Australasian clade (node H) is well-supported, indicating probable monophyly of this regional sample representing 15 of the 20 clades recovered in the consensus tree of Bayer et al. (2002). Although none of their clades was supported by bootstrap percentages $\geqslant$50%, this is our best attempt at broad phylogenetic sampling of the Australasian Gnaphalieae.

3.4. Estimates of divergence times

3.4.1. Parameters of the BEAST analysis

We report the mean (and 95% HPD) of parameter estimates combined from at least two separate BEAST analyses (minus the burn-in fraction). All parameter estimates are based on at least 450 (and often many more) independent samples from the posterior as estimated by Tracer v1.3. The mean number of new lineages arising from a single parent lineage per million years (Yule.birthrate) was 0.074 (0.068–0.110). The mean number of substitutions per site per million years (mean.Rate) across the whole tree was estimated o be 0.0018 (0.0011–0.0026). The mean branch rate under the relaxed clock model (ucld.mean) across all data partitions was 0.0020 (0.0017–0.0030) substitutions per site per million years and the standard deviation of this parameter (ucld.stdev) was 0.80 (0.67–0.95). The relative closeness of this estimate to 1.0 suggests rate heterogeneity across lineages (Drummond et al., 2007) which is confirmed by the non-zero value for the parameter 'coefficient of variation' (Drummond et al., 2007), estimated to be 0.82 (0.66–0.99). The parameter which measures the average autocorrelation of rates of evolution from parent to daughter lineages (covariance) was estimated to be 0.19 (0.03–0.37). If this value spans zero, daughter branches typically have rates which are very different to parent branches (Drummond et al., 2007) so our data-

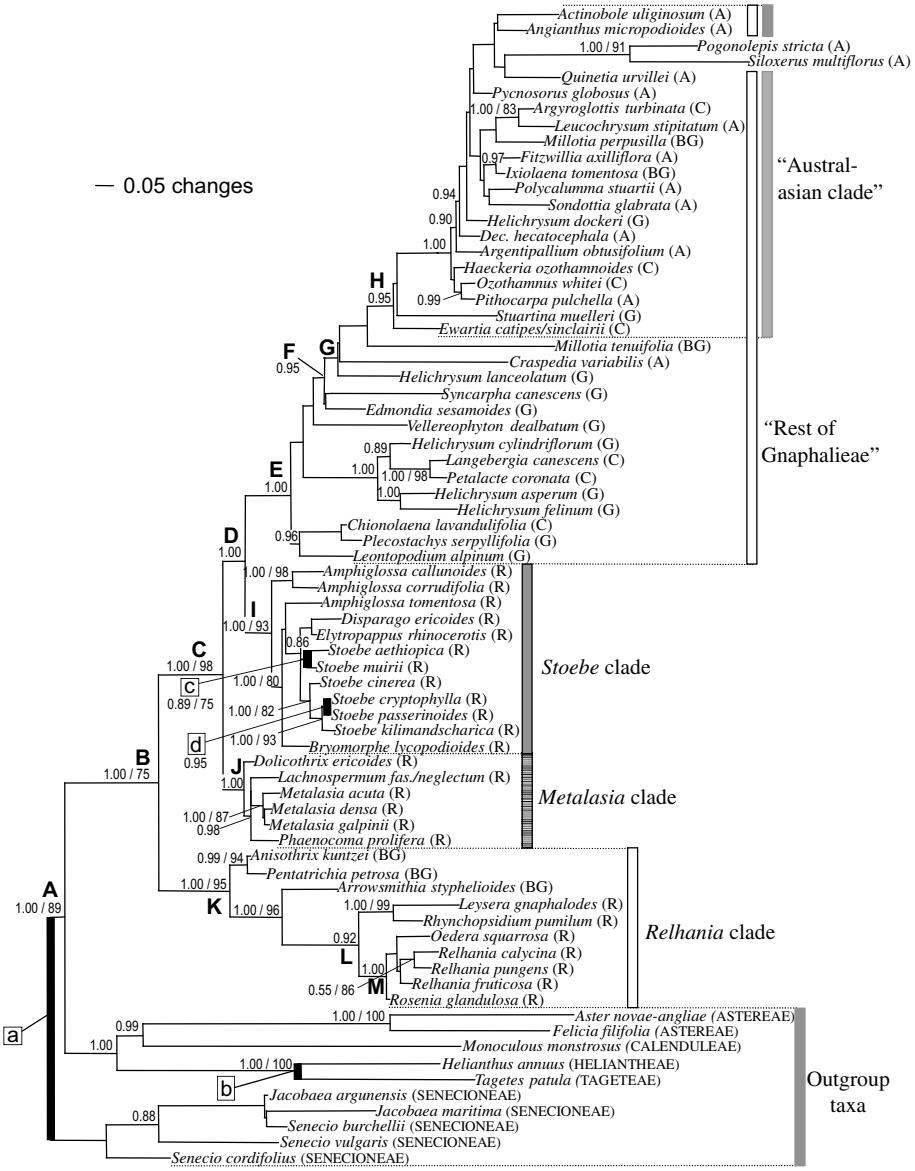

Fig. 2. Phylogram of one of the trees from MrBayes analysis of all data partitions. Numbers above branches indicate posterior probability (PP) values followed by bootstrap values. These are only shown for PP values ⩾0.85 and/or bootstrap values ⩾75%. Upper-case letters identifying nodes are referred to in the text. Lower-case letters in boxes indicate the calibration nodes (thick black lines) as they are referred to in Fig. 1. Bracketed upper-case letters after the species names indicate subtribal affiliation according to Anderberg (1991): (A) Angianthinae, (BG) "basal group"; (C) Cassiniinae, (G) Gnaphaliinae, (R) Relhaniinae.

set may exhibit some degree of rate autocorrelation (Drummond, personal communication).

3.4.2. Prior versus posterior distributions of calibration nodes

The posterior distributions of calibration node ages are shown in Fig. 1. The posterior distribution most closely echoes the prior distribution for the age of the *Helianthus–Tagetes* split (Fig. 1b) but the others show a strong influence of their co-calibration points. The posterior distribution shifted backwards in time relative to the prior for the root node (Fig. 1a) and forward in time for the ages of the limestone endemic *Stoebe muirii* (Fig. 1c) and La Réunion endemic *Stoebe passerinoides* (Fig. 1d). The directions of the two latter shifts are consistent with geological calibrations representing oldest bounds on a node age. The calibration node for *S. passerinoides* is the only case where the shape of the posterior distribution is very different from that of the prior, perhaps due to the data and remaining calibrations overwhelming the prior in support of a very recent age.

3.4.3. Sensitivity analysis

When there is no constraint on the root node, the tree age is pushed backwards in time by the other calibrations, as shown in Scheme (b), with the root estimated to be over 80 Ma. Scheme (c) produced an intermediate set of divergence ages. Scheme (d), assuming no error in the dates from Kim et al. (2005), resulted in younger divergences across the tree. With Scheme (e), the narrower and older *Ambrosia* prior pushed this node and the root backwards in time but the remaining divergences became slightly younger. The youngest overall ages were obtained with Scheme (f) which omitted the two geological calibrations.

3.4.4. Mean age estimates for nodes of interest

The root node (the Asteroideae crown group) is estimated to be of Eocene age, with the majority of samples from the posterior falling in the Mid- to Late-Eocene (Table 4). The 95% HPD for the age of Gnaphalieae spans the entire Eocene and Oligocene, with the mean in the Late Eocene. The estimated crown group ages of the three

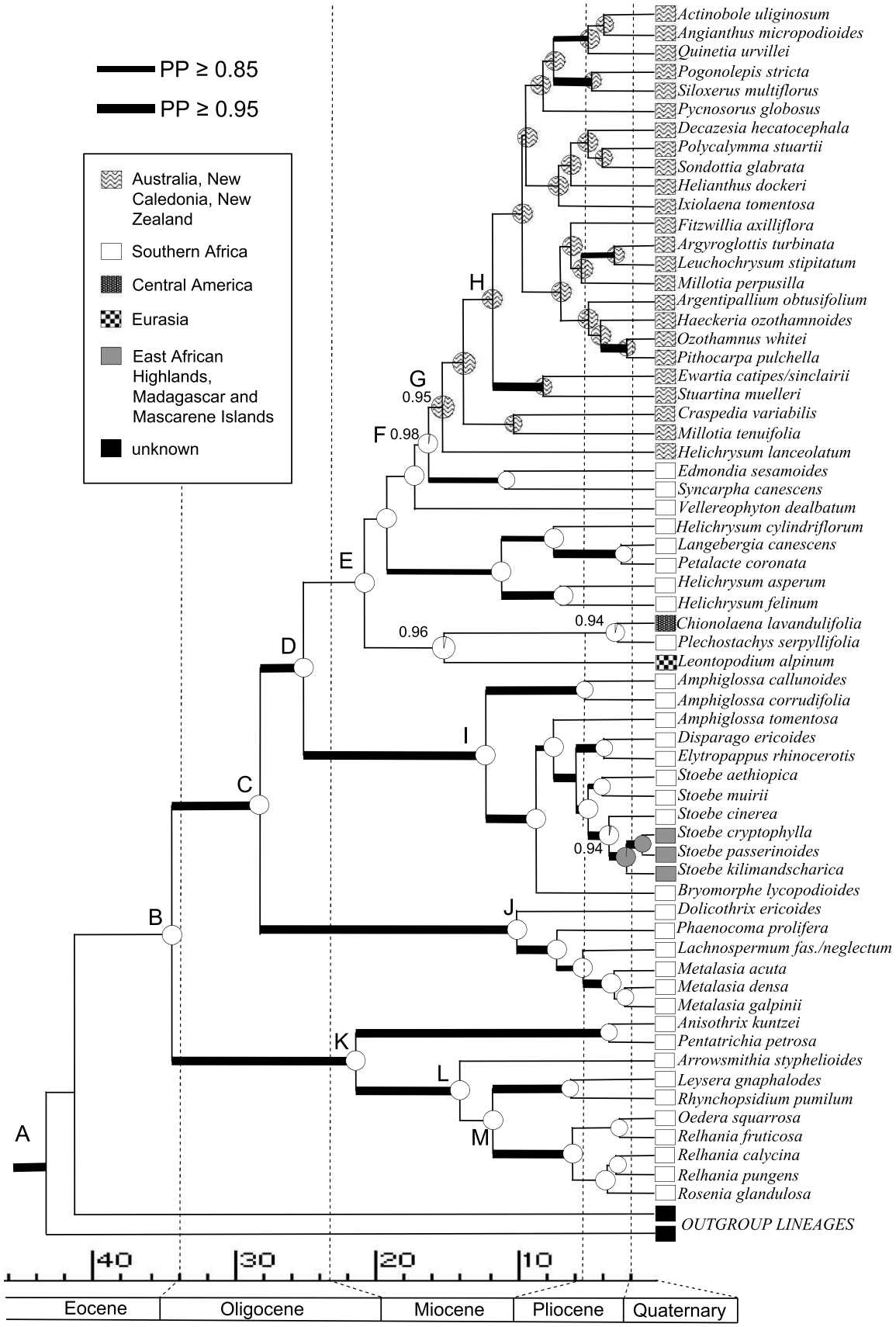

Fig. 3. Maximum clade credibility tree from relaxed phylogenetic analysis in BEAST. The timescale is in millions of years before present with ages from Gradstein et al. (2004). Squares represent geographic areas of extant taxa; circles represent geographical areas of ancestral taxa as inferred by maximum likelihood (ML) reconstruction. Numbers next to nodes indicate the ML probability of that state if it is less than 0.999. Line thickness indicates branch support.

Table 3
Results of the dating sensitivity analyses indicating prior distributions (PRIORS) on calibration nodes, and posterior distributions (POSTERIORS) on calibration nodes and two other nodes of interest (crown age of Gnaphalieae, and oldest age of the Australasian clade [node F])

	PRIORS	POSTERIORS	PRIORS	POSTERIORS
	(a) As described in Methods		*(b) As for Scheme (a) but with an uninformative prior on the root node*	
Root	N: 32.5 (14.1–50.9)	43.0 (29.3–56.6)	U: 0–1000	85.5 (36.7–145.2)
Ambrosia fossil	logN: 22.3 (16.9–44.1)	20.1 (15.8–25.8)	logN: 22.3 (16.9–44.1)	46.4 (33.5–67.8)
Gnaphalieae	—	34.5 (20.6–52.3)	—	72.2 (29.1–127.9)
Node F	—	15.6 (9.1–22.2)	—	31.3 (13.2–53.8)
Stoebe muirii	N: 6.0 (3.1–8.9)	4.2 (1.9–6.7)	N: 6.0 (3.1–8.9)	5.6 (3.0–8.3)
Stoebe passerinoides	N: 4.75 (1.8–7.7)	0.9 (5.4E-5–2.7)	N: 4.75 (1.8–7.7)	1.7 (5.2E-4–4.2)
	(c) As for Scheme (a) but with a logN prior allowing for a much older root node		*(d) As for Scheme (a) but with a narrower root prior corresponding closely to the range of estimates in Kim et al. (2005)*	
Root	logN: 33.1 (30.1–87.5)	57.2 (34.9-82.8)	N: 32.5 (26.2–38.8)	34.7 (28.8–40.3)
Ambrosia fossil	logN: 22.3 (16.9–44.1)	21.3 (15.9–28.6)	logN: 22.3 (16.9–44.1)	19.1 (15.9–23.3)
Gnaphalieae	—	48.3 (25.6–73.9)	—	28.5 (19.5–38.0)
Node F	—	21.3 (11.2–32.7)	—	13.4 (9.1–18.2)
Stoebe muirii	N: 6.0 (3.1–8.9)	4.9 (2.4–7.5)	N: 6.0 (3.1–8.9)	3.8 (1.7–6.0)
Stoebe passerinoides	N: 4.75 (1.8–7.7)	1.3 (1.4E-2–3.3)	N: 4.75 (1.8–7.7)	0.7 (1.9E-6–2.2)
	(e) As for Scheme (d) but Ambrosia with a narrower and older prior age distribution		*(f) As for Scheme (d) but leaving out the geological calibrations*	
Root	N: 32.5 (26.2–38.8)	37.2 (33.6–41.7)	N: 32.5 (26.2–38.8)	33.6 (27.7–39.4)
Ambrosia fossil	logN: 36.3 (33.9–41.7)	35.5 (33.3–37.9)	N: 22.3 (16.9–44.1)	19.1 (15.9–23.3)
Gnaphalieae	—	22.7 (15.6–30.1)	—	26.1 (17.2–35.9)
Node F	—	11.5 (7.1–15.6)	—	12.0 (7.9–16.5)
Stoebe muirii	N: 6.0 (3.1–8.9)	3.4 (1.5–5.7)	—	2.3 (0.6–4.1)
Stoebe passerinoides	N: 4.75 (1.8–7.7)	0.6 (9.5E-6–1.9)	—	0.2 (1.9E-7–0.8)

Values are in millions of years before present and represent the mean and (in brackets) 95% HPD for each distribution. The initial calibration scheme (a) is shown for comparative purposes. For the priors, 'N' indicates a normal, 'logN' a lognormal, and 'U' a uniform distribution.

Table 4
Divergence age estimates in millions of years before present

Node	Description	Mean	95% HPD
A	Root node	43.0	29.3–56.6
B	Gnaphalieae crown age	34.5	20.6–52.3
C	Gnaphalieae excluding *Relhania* clade	27.2	16.3–40.7
D	*Stoebe* clade + rest	24.1	13.9–34.9
E	Rest of Gnaphalieae (excluding *Stoebe* clade)	20.0	12.0–29.3
F	Australasian clade stem age	15.6	9.1–22.1
G	Australasian clade crown age	14.6	8.3–20.6
I	*Stoebe* clade crown age	12.2	5.3–20.0
J	*Metalasia* clade crown age	9.4	3.3–17.2
K	*Relhania* clade crown age	21.0	9.9–33.9
L	Sublade containing *Relhania*	13.6	6.4–22.3

Nodes are labelled as in Figs. 2 and 3. Values are the mean and 95% HPD.

clades containing the former Relhaniinae are all in the Miocene. Although the *Relhania* clade is older than the *Stoebe* or *Metalasia* clades, the subclade containing *Relhania* (node L) also has a Miocene crown age. Node F falls in the Miocene and the radiation of the Australasian clade is estimated to have begun by the Mid-Miocene.

3.5. Ancestral area reconstruction

Conditional probabilities for ancestral states inferred with maximum likelihood represent the mapping confidence given the biogeographical data input into the analysis, and were all greater than or equal to 0.94 (those below 0.99 are indicated on Fig. 3). Biogeographic conclusions are least certain for more sparsely-sampled parts of the tree (i.e. the non-Relhaniinae). The earliest ancestral nodes of the Gnaphalieae (B–E) all optimize to southern Africa. The ancestors of the *Relhania*, *Metalasia* and *Stoebe* clades also optimize to southern Africa. There are four independent dispersals out of southern Africa: one to the afroalpine region in *Stoebe*, one to Australasia between nodes F and G, to the Americas by *Chionolaena* and to Eurasia by *Leontopodium*.

4. Discussion

4.1. Topology and systematics

4.1.1. Tribe Gnaphalieae

We present a robustly-supported phylogenetic hypothesis for relationships within Gnaphalieae, including representatives of 47 (25%) of the 185 currently-recognized genera in the tribe (Bayer et al., 2007) and members of all of Anderberg's (1991) subtribes except Loricariinae (see below). Our analysis provides insight into relationships amongst regional Gnaphalieae from all continents, albeit with very sparse sampling from Eurasia and the Americas. Within the two largest areas of diversity, sampling is fairly representative for the Australian species but better for southern Africa.

The tribe experienced its early diversification in southern Africa, resulting in at least three near-endemic basal lineages and a southern African paraphylum. Taxa from the rest of Africa, Eurasia, the Americas and Australiasia are embedded within this paraphylum, indicating multiple dispersals out of southern Africa after the initial diversification, apparently followed in many cases by radiations in other regions. For the southern African taxa, our topology is consistent with that of Bayer et al. (2000) but our addition of more DNA sequence data improved resolution and support. Relationships amongst the Australian and New Zealand taxa are poorly-resolved in our tree, and due to different taxon sampling cannot readily be compared with those found in previous analyses (Bayer et al., 2002; Breitweiser et al., 1999; Breitweiser and Ward, 2003). In our tree, the species from Australasia are monophyletic, although support is lacking at node G. None of the above-mentioned studies included sufficient outgroup taxa to test for monophyly of the Australasian gnaphalioids.

Most of the South American taxa were placed by Anderberg (1991) in the subtribes Gnaphaliinae and Loricariinae. Although we included many taxa from the Gnaphaliinae, we could not obtain sequences of any member of Loricariinae except *Pterygopappus lawrencii* from Tasmania. Both Bayer et al. (2002) and Breitweiser

et al. (1999) found good support for the inclusion of this species with the Australasian taxa. We were able to confirm this, but removed *P. lawrencii* from our final analysis as its position was unstable within the Australasian clade.

Anderberg (1991) placed the Loricariinae as (unsupported) sister to Relhaniinae, based on morphological cladistic analysis. However, our results and those of Bayer et al. (2000, 2002) show that the subtribes of Anderberg (1991) are not monophyletic. Relhaniinae is clearly not monophyletic, while the grade of southern African lineages descended from node E in Figs. 1 and 2 consists of both Gnaphaliinae and Cassiniinae. The Australasian clade (node G) contains members of Gnaphaliinae, Cassiniinae, the "basal group" and Angianthiinae. Elucidation of subtribal relationships will require greater sampling from across the tribe (Bayer et al., 2007), and especially from poorly-sampled regions such as South and Central America. Greater geographic sampling for several large, widely-distributed and possibly polyphyletic genera will also be key to full understanding of gnaphalioid relationships (i.e. *Anaphalis* [ca. 110 spp.], *Gnaphalium* [ca. 80 spp.], *Helichrysum* [ca. 600 spp.], and *Pseudognaphalium* [ca. 90 spp.]).

4.2. Divergence times and biogeography

4.2.1. Calibration schemes and age estimates

The sensitivity analysis indicated that age estimates rely heavily on the root node calibration, which was derived secondarily from the previous dating exercises of Kim et al. (2005). Our estimate of an Eocene age for the stem divergence between Gnaphalieae and other members of subfamily Asteroideae (root node, A) is older than would be predicted by authors who estimate that the family as a whole is of Eocene or even Oligocene age (Raven and Axelrod, 1974; Graham, 1996; Bremer and Gustafsson, 1997; Funk et al., 2005; Anderberg et al., 2007). However, this age for the family is based largely on the absence of pre-Oligocene fossils, and Turner (1977) and Muller (1970) argue that the Oligo-Miocene fossil presence of Asteraceae indicates not the origin but the increase in abundance of the family, since the modern tribes are all represented in their current geographic positions. The youngest age estimate for our root node was obtained with calibration scheme (f), but there appears to be no strong fossil evidence to either contradict or support an age of 43 Ma or even older (calibration schemes b and c) for node A.

4.2.2. Age of the Gnaphalieae

The crown age of tribe Gnaphalieae (node B) is estimated to be 34.5 (20.6–51.3) Ma, indicating that early diversification was in the Late Eocene. All calibration schemes except for Scheme (b) with no constraint on the root, place this diversification at or after the peak in global temperatures around 50 Ma (Zachos et al., 2001) which presaged global climatic cooling and, in Africa, the subsequent expansion of open habitats together with the development of arid-adapted vegetation (Bobe, 2006).

4.2.3. Early divergence in Gnaphalieae: the southern African lineages

Early diversification of the tribe occurred in southern Africa and produced the lineages which Anderberg (1991) assigned to the Relhaniinae. This subtribe is well-represented in our tree, as the eight unsampled genera share well-established morphological synaporphies with genera in the *Metalasia* clade or the clade subtended by node M (Anderberg, 1991; Karis, 1990). Thus further sampling is unlikely to change the inference of a southern African location for these early divergences. However, several unsampled Mediterranean and Eurasian genera from Anderberg's "basal group" (*Athrixia*, *Phagnalon* and Cass. and *Aliella* Qaiser & Lack) are affiliated with *Anisothrix* and *Pentatrichia* in the *Relhania* clade (Montes-Moreno et al., unpublished; Fig. 2). Since this lineage represents the earliest split in the tribe, inclusion of these taxa in ancestral area analysis might indicate a different geographic origin for the Gnaphalieae. Another factor influencing the inferred point of origin of the tribe is the geographic distribution of the sister group. The sister relationship of Gnaphalieae remains unclear (Funk et al., 2005; Bayer et al., 2007) and once established, might produce novel insights into where the tribe arose. However, whether Gnaphalieae arose in southern Africa or elsewhere, the region has clearly played a major role in its evolution. In this regard, there are parallels with the geographic history of several related lineages in Asteraceae. The splits at the base of subfamily Asteroideae leading to the tribes Anthemideae, Senecioneae [especially subtribes Senecioninae and Othonninae] and Calenduleae, as well as the more distantly-related *Corymbium* and Cichorioideae s.s. are all postulated to have had a southern African origin and/or diversification (Funk et al., 2005; Oberprieler, 2005; Pelser et al., 2007; Himmelreich et al., 2008). Most of these groups have substantial diversity within the region but have also dispersed out of southern Africa and independently radiated in the Mediterranean, Australia and the Americas. The southern African region has thus been an important cradle of diversification for a large part of the daisy family, as well as an 'evolutionary springboard' from which multiple lineages colonized the rest of the world.

The *Relhania* group of genera (node L), the *Stoebe* clade and the *Metalasia* clade are all Cape-centred, with crown ages in the Late Miocene. This is the period during which the radiations of many Cape clades were initiated (Linder, 2005), e.g. Cape *Indigofera* (13.1 Ma; Schrire et al., 2003), *Ehrharta* (9.8 Ma; Verboom et al., 2003); *Muraltia* clade II (19.3; Forest et al., 2007). All these groups have substantial endemism in summer-arid and/or fire-prone habitats and it is possible that their diversification around this time is linked to the opening of these habitats.

4.2.4. Dispersals out of the southern African region

After initial diversification, there were multiple dispersals out of southern Africa. Several of these have been captured in our tree but there are additional, independent dispersal events which we have not sampled, suggesting that the observed events (discussed below) under-represent the true number. For example, the genera *Leysera* and *Ifloga* each have a southern African-centred distribution with one or more species in the Mediterranean region (Bremer, 1978; Trisos et al., unpublished), indicating two additional dispersal events to the Mediterranean region. Directional dispersal from southern Africa to the Mediterranean may have been especially prevalent in Gnaphalieae: if we assume, as is indicated by our analysis, a southern African root for the tree of Mediterranean *Helichrysum* produced by Galbany-Casals et al. (2004), their phylogeny indicates at least five dispersal events from southern Africa to the Mediterranean Region, and one from there to Asia.

Four of the species of the otherwise Cape-centred *Stoebe* clade occur in the ericaceous vegetation which characterizes the lower margin of the afroalpine zone (Hedberg, 1970). These species, *S. kilimandscharica* from east Africa; *S. passerinoides* from Réunion, and the Madagascan *S. cryptophylla* and *S. pachyclada* Humbert, form a clade (if, as is likely on morphological grounds, the two Madagascan species are sisters; Koekemoer, 2002) and thus derive from a single initial dispersal event out of the Cape (Fig. 3). Our dating indicates a recent dispersal to the afroalpine region between 2.0 (0.4–4.0) Ma and 3.5 (1.1–6.0) Ma. This is consistent with the results of Galley et al. (2006), who showed overwhelmingly unidirectional dispersal from the Cape to the Afromontane and afroalpine regions during the last 17 Ma. An allopatric speciation model following dispersal seems most probable in *Stoebe*, as each sub-afroalpine region (mainland Africa, Madagascar and La Réunion) has its own endemic species or species pair. The estimated age of 0.9 Ma for *S. passerinoides* is consistent with the 2 Ma age

of La Réunion's alpine zone postulated by McDougall (1971) and Gillot et al. (1994).

Three other dispersals out of Africa are evident (Fig. 3). Dispersal to Eurasia is inferred for the ancestor of *Leontopodium* and this event is potentially as old as the node uniting *Leontopodium* with *Plecostachys* and *Chionolaena*, dated to the middle Miocene. Dispersal to Central and South America by *Chionolaena* appears to have been more recent, in the Pliocene or later (Fig. 3). The reconstruction indicates that the common ancestor of *Chionolaena* and *Plecostachys* was located in southern Africa, implying dispersal directly from Africa to Central America. However, sparse sampling of Eurasian and American taxa means that we cannot exclude dispersal to the Americas via Europe or elsewhere. With no information on the monophyly of the South American taxa, we also cannot say whether the dispersal by *Chionolaena* represents the founding of the South American everlasting flora (a 'single origin' hypothesis) or whether it is only one of many colonisations of that continent, perhaps from multiple different regions and at different times ('multiple origins' hypothesis). To our knowledge, the only other South American taxon which has been included in molecular phylogenetic analysis is *Lucilia araucana* from Argentina, placed by Anderberg (1991) in the Gnaphaliinae. In the analysis of Breitweiser et al. (1999), *L. araucana* was strongly supported as sister to a New Zealand species, which together with our results supports the 'multiple origins' hypothesis for Gnaphalieae from the Americas.

4.2.5. Trans-Indian Ocean dispersal

The gnaphalioid taxa of Australia and New Zealand appear to have been founded by a single dispersal event from Africa, since they all share a common ancestor at node G (Figs. 2 and 3), and this lends support to a 'single origin' hypothesis for the Australasian taxa (Bayer et al., 2002).

Our results support direct long-distance dispersal to Australia across the Southern Indian Ocean, because the Australasian taxa are embedded within a grade of African lineages (nodes E–F; Fig. 3) not within a Eurasian clade as might be expected if they reached Australia via stepping-stone dispersal through Asia. The same geographic pattern was recovered by Galbany-Casals et al. (2004) in a study of mainly Mediterranean gnaphalioids. Although the taxa they sampled are mostly different from those in the present study, these authors recovered clades which match those found in our tree, and showed, with good support (BS $\geqslant$ 80% at all relevant nodes), that Australian and New Zealand taxa are sister to species from the Cape, while Eurasian species are embedded in a separate southern African grade. If the Australian species are the result of such long-distance dispersal, we might expect them to have inherited the ability to disperse long distances, and indeed Australian gnaphalioids have clearly achieved the ca. 2000 km oceanic crossing from Australia to New Zealand multiple times (e.g. Breitweiser et al., 1999; Breitweiser and Ward, 2003).

Our analysis dates the dispersal to Australia between 14.6 (8.3–20.6) and 15.6 (9.1–22.1) Ma, in the Miocene (Fig. 3; Table 4) with the oldest estimate (Table 3, Scheme [b]) at 31.3 (13.2–53.8). This is consistent with the fossil record which suggests that Asteraceae arrived in Australia well after the isolation of the continent more than 35 Ma (Crisp et al., 2004; Stover and Partridge, 1973; Martin, 1973). Novel evidence of long-distance *trans*-oceanic dispersal has opened new discussion on the mechanisms involved (Sanmartín and Ronquist, 2004; Knapp et al., 2005; de Queiroz, 2005; Sanmartín et al., 2007). Long-distance dispersal by wind has been implicated in the distribution of Southern Hemisphere extra-tropical lichens, bryophytes and pteridophytes (Muñoz et al., 2004) and may be facilitated by the westerly winds, which increase in force with greater latitude in the Southern Ocean ('roaring forties', 'furious fifties' and 'shrieking sixties'). Although seeds in Gnaphalieae can be small and light, and possess a pappus which might enhance wind dispersal, it is unknown whether they could be blown across the ca. 8000 km of ocean separating Africa from Australia. Another possibility is oceanic rafting, which would be facilitated by the West Wind Drift (WWD), a large surface current flowing from west to east in the Southern Ocean. West Wind Drift would take roughly a year to float objects between South Africa and Australia (Waters and Roy, 2004). Tolerance to sea-water in the seeds of Gnaphalieae is unknown, but flotation for a year seems unlikely. Other mechanisms are possible, such as rafting above the water on floating vegetation, or attachment to the feet or feathers of migrating birds, as shown for *Lepidium* (Brassicaceae, Table 5; Carlquist, 1983; Mummenhoff et al., 2004). *Lepidium* has mucilaginous seeds, as does *Zygophyllum*, another successful colonizer of Australia from Africa (Beier et al., 2003; Table 5). Inter-continental dispersal in *Sarcocornia* (Table 5) and well-dispersed relatives in the Chenopodiaceae is likely to be facilitated by their adaptations to dispersal by salt water and water birds (Kadereit et al., 2006). However, long-distance *trans*-oceanic dispersal has been inferred for many terrestrial organisms that have no obvious adaptations to facilitate such dispersal (e.g. de Queiroz, 2005; Sanmartín et al., 2007; Muñoz et al., 2004; Barker et al., 2007). We surveyed the literature for other Southern Hemisphere plant groups which exhibit *trans*-Indian Ocean disjunction, in which the direction of dispersal could reasonably be inferred and for which dated phylogenetic hypotheses are available. We measured the age of each dispersal event as the mean of the estimated ages of the nodes at either end of the branch along which the disjunction occurred (Table 5). In 10 angiosperm families (including our Gnaphalieae example), we found evidence for 17 disjunctions whose estimated ages ranged from 0.5 to 55.9 Ma (Table 5). This rules out Gondwanan vicariance scenarios because Africa had separated from other Gondwanan land masses by 100 Ma (Scotese et al., 2008). If the original study did not provide evidence for the direction of dispersal across the Indian Ocean, we inferred this from the phylogeny using a strict parsimony criterion (minimizing the number of dispersals). Nine of the dispersal events are from Australasia to Africa and occurred between 1.8 and 55.9 Ma (mean and standard deviation 29.6 ± 21.3 Ma). The remaining eight are in the opposite direction and occurred between 0.5 and 15.1 Ma (6.5 ± 5.2 Ma). These means are significantly different (*t*-test, $p = 0.007$). Thus dispersals from Australia to Africa have been ongoing since the Eocene, while movements in the opposite direction have only occurred since the start of the Miocene epoch. There are several other groups which exhibit dispersal eastwards across the Indian Ocean from southern Africa: the grass genus *Pentaschistis* (Galley and Linder, 2007); *Wurmbea* in the Colchicaceae (Case et al., 2008); *Gunniopsis* in the Aizoaceae (Klak et al. 2003); *Cymbonotus* (Asteraceae; Funk et al., 2007); *Leptinella* (Asteraceae; Himmelreich et al., 2008) and Senecioneae (Asteraceae; 4 dispersals from Africa to Australasia; Pelser et al., 2007). These were not included in our analysis because age estimates for these events are not yet available. However, these dispersals are all likely to be Miocene or younger (Vinnersten and Bremer, 2001; personal communications from A. Case, C. Klak, V. Funk and P. Pelser).

Assuming that this pattern is not due to biased extinction or different ages of continental habitats, the fact that eastwards dispersal occurs only after the Oligocene might implicate the West Wind Drift (WWD) in these events, because full establishment of this surface circumpolar current only occurred after the deepening of oceanic passageways separating Antarctica from South America ca. 41 Ma (Scher and Martin, 2001) and from Australia ca. 35 Ma (Stickley et al., 2004). The WWD is independently invoked in the gnaphalioid dispersal if, as proposed by Bayer et al. (2002), initial colonization was to the Bassian biotic region of southeastern Australia and Tasmania. This is because WWD is likely to raft

Table 5
Mean ages and likely directions of *trans*-Indian Ocean dispersals

Inferred direction of movement	Family	Phylogeny and age estimate based on:	Taxa involved	Age estimate (Ma)[a]	Reference
From Australasia to Africa	Poaceae	Chloroplast and nuclear DNA sequences	Ehrharteae	38.7	Verboom et al. (2003) Verboom et al. (2008)
	Proteaceae	Chloroplast DNA sequences and fossil-calibrated relaxed Bayesian clock	*Adenanthos*–Leucadendrinae	34.25	Sauquet et al. (unpublished)
			Aulax–*Petrophile*	55.95	
	Iridaceae	Chloroplast DNA sequences; NPRS calibrated with an estimate from a prior analysis	*Patersonia*–*Geosiris*	51	Goldblatt et al. (2002)
	Restionaceae	Chloroplast DNA sequences	Basal split	52.57	Linder et al. (2003) Verboom et al. (2008)
	Cyperaceae	Chloroplast DNA sequences	Reticulate-sheathed *Tetraria* clade	9.2	Verboom (2006) Verboom et al. (2008)
			Capeobolus–*Cyathocoma*	1.86	
			Non-reticulate-sheathed *Tetraria* clade	12.76	
			Neesenbeckia punctoria	10.22	
From Southern Africa to Australasia	Chenopodiaceae	Chloroplast and nuclear DNA sequences; strict clock analysis calibrated with Chenopodiaceae macrofossils and fossil pollen	*Sarcocornia*	2.9	Kadereit et al. (2005, 2006)
	Brassicaceae	ITS sequences and a local molecular clock calibrated using fossil data	*Lepidium*	0.55	Mummenhoff et al. (2004)
	Zygophyllaceae	Chloroplast DNA sequences and relaxed Bayesian clock	*Zygophyllum*	4.53	Bellstedt et al.(2008)
	Cyperaceae	Chloroplast DNA sequences	*Trianoptiles*–*Carpha*	13.03	Verboom (2006) Verboom et al. (2008)
	Asteraceae	This study	Australasian *Gnaphalieae*	15.1	This study
	Asteraceae	Nuclear ITS DNA sequences; NPRS dating	*Leptinella*–*Soliva*–*Cotula*	7.7	Oberprieler (2005) Himmelreich et al. (2008)
	Geraniaceae	Chloroplast, mitochondrial and nuclear DNA sequences	*Pelargonium havlase*–*P. australe* clade	6.11	Bakker et al. (2004, 2005) Verboom et al. (2008)
	Poaceae	Chloroplast and nuclear DNA sequences	*Rytidosperma*	1.77	Verboom et al. (2006) Verboom et al. (2008)

Molecular age estimates were derived from the original reference, in which case the methods are outlined in the third column, or from the relaxed Bayesian analyses of Verboom et al. (2008).

[a] Dispersal of a disjunct clade could have occurred anywhere along the branch between the stem and crown nodes. Where age estimates existed for both stem and crown nodes, we used the mean of the total range (ie the range from the youngest estimate for the crown node to the oldest estimate for the stem node). If stem node age was not available we took the oldest estimated age for the crown node.

propagules from southern Africa not to the closest landfall (Western Australia) but to these south-easternmost regions (New South Wales and Tasmania; Waters and Roy, 2004). This raises the possibility of oceanic rafting as a primary factor in long-distance Angiosperm colonization of Australia from southern Africa.

The two main centres of Gnaphalieae diversity, southern Africa and Australia, have similar numbers of species but apparently very different histories of species accumulation. In southern Africa, species diversity may have been accumulating since the Eocene, while in Australia the diversity appears to be the result of rapid radiation from a single colonizing ancestor in the Miocene. This suggests that speciation/extinction ratios amongst the two regions are very different and opposite to those for the Restionaceae (Linder et al., 2003).

4.3. Conclusion

Tribe Gnaphalieae may have originated in southern Africa in the Eocene and this region has served as a cradle of gnaphalioid diversification, accounting for the large number of species represented in the southern African region. Members of the former subtribe Relhaniinae fall into the three earliest-diverging lineages of the tribe. These all had southern African ancestors, and the Cape-centred clades all began diversifying in the Miocene. A well-sampled phylogenetic hypothesis for the tribe and its closest relatives, including taxa from the Americas, is required for further biogeographic insight and to provide the framework for future evolutionary studies. However, the present study indicates that dispersal out of southern Africa has been ongoing since the early Miocene. Only one successful dispersal event around 15 Ma is required to explain the presence of the tribe in Australasia, and diversity here may be the result of rapid radiation. West-to-east *trans*-Indian Ocean dispersal appears to have been occurring only since the Miocene, potentially implicating the WWD in long-distance plant dispersal.

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