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Radiation of southern African daisies: Biogeographic inferences for subtribe Arctotidinae (Asteraceae, Arctotideae)

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ABSTRACT

The majority of the approximately 80–90 species in subtribe Arctotidinae occur in southern Africa with the centre of diversity in the winter-rainfall region. Three species are restricted to afromontane eastern Africa and three species are endemic to Australia. To investigate biogeographic and phylogenetic relationships within Arctotidinae, sequence data from four cpDNA regions (*psbA-trnH*, *trnT-trnL* and *trnL-trnF* spacers and *trnL* intron) and the ITS nrDNA region for 59 Arctotidinae species were analyzed with parsimony and Bayesian-inference approaches. Eight well-supported major lineages were resolved. The earliest-diverging extant lineages are afromontane or inhabit mesic habitats, whereas almost all sampled taxa from the winter-rainfall and semi-arid areas have diverged more recently. Molecular dating estimated that the major clades diverged during the Miocene and Pliocene, which is coincident with the trend of increasing rainfall seasonality, aridification and vegetation changes in southwestern Africa. Trans-oceanic dispersal to Australia was estimated to have occurred during the Pliocene.

1. Introduction

Southern Africa is an important centre of floristic biodiversity. Approximately 24,000 species of vascular plants are recorded from the region, of which approximately 80% are endemic to southern Africa (Cowling and Hilton-Taylor, 1997). Almost 10% of this plant diversity is contributed by the Asteraceae (the daisy family), with approximately 250 genera and 2250 species recorded from the region (Koekemoer, 1996). Over 80% of these species are endemic to southern Africa (Cowling and Hilton-Taylor, 1997). The family has radiated in all of the recognized southern African biomes and is one of the more species-rich families in most, if not all, of these biomes.

The diversity and endemism of Asteraceae in southern Africa is focused in eight putative centres (Koekemoer, 1996). These centres are generally consistent with centres of endemism indicated by the southern African flora as a whole (e.g. see van Wyk and Smith, 2001). Despite being a major component of the flora, the evolution and biogeography of southern African Asteraceae is poorly investigated and well-resolved phylogenetic reconstructions are required to elucidate the origins and radiation of the Asteraceae lineages in the region (Galley and Linder, 2006). The only major southern African lineage of Asteraceae that has been investigated to any extent is the tribe Arctotideae, which has been the subject of both molecular (McKenzie et al., 2006a) and morphological (Karis 2006) phylogenetic investigations. However, the sampling in previous studies was not comprehensive and here we present the first well-sampled phylogenetic and biogeographic investigation of the predominantly southern African subtribe Arctotidinae to help to fill this gap in our knowledge.

Arctotideae is a small tribe containing about 215 species in 17 genera with an almost exclusively African distribution (Karis, 2007). Two subtribes are recognized with some authors placing other lineages in the tribe (for a brief review of the taxonomy and relationships of the tribe, see Funk et al., 2004). The subtribe Arctotidinae contains approximately 80–90 species currently classified into five genera (Karis, 2007). One species, *Haplocarpha scaposa*, has a wide distribution in montane and temperate southeastern Africa (e.g. Hilliard, 1977; Pope, 1992). Three species are indigenous to the high-altitude East African mountains (Mesfin Tadesse, 2004) and three species are endemic to Australia (Holland and Funk, 2006). The remaining species occur in southern Africa, with the centre of diversity in the Cape and Succulent Karoo Centres of Floristic Endemism (see van Wyk and Smith, 2001). Both areas occur in the winter-rainfall zone (Cowling, 1992; Dean and Milton, 1999; see Fig. 1) and are combined into a 'Greater Capensis' floristic region by some workers (Born et al., 2007). Because more than 50% of the species occur in the Cape Floristic Region (CFR), the Arctotidinae, and especially *Arctotis s.str.*, might represent a true 'Cape clade' *sensu* Linder (2003, 2005).

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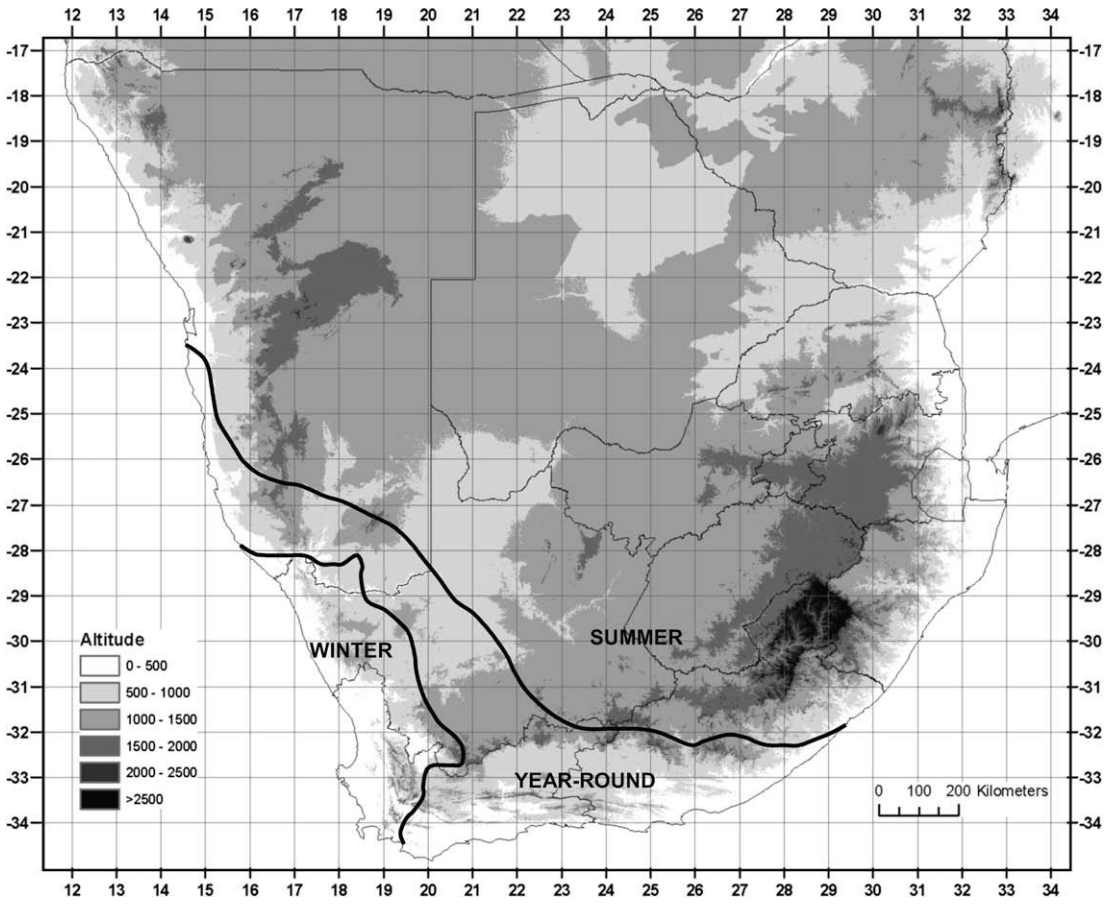

Fig. 1. A topographical map of southern Africa with the approximate boundaries of different rainfall regimes indicated (based on Chase and Meadows, 2007).

Understanding the factors responsible for the unusually high levels of floristic richness and endemism in the winter-rainfall region of southern Africa (and the CFR in particular) has attracted considerable interest (for reviews for the CFR, see Linder, 2003, 2005). Molecular dating has indicated that plant lineages have been recruited into the Cape flora since at least the Cretaceous and the onset of radiations in these lineages is indicated to range from the Oligocene to the Pliocene (Linder, 2005), so the extant diversity cannot be explained solely by vicariance or explosive speciation in response to one or more concurrent triggers. Furthermore, the southern African flora as a whole is indicated to have evolved from disparate sources, including descendants of Gondwanan ancestors (Goldblatt, 1978; Anderson et al., 1999), temperate southern African progenitors (Goldblatt, 1978), groups of Eurasian or tropical African origin (e.g. Meerow et al., 2003; Hurka et al., 2005; Mummenhoff et al., 2005), and Australasian ancestors (e.g. Linder et al., 2003). Therefore a thorough understanding of diversification processes in the southern African flora is likely to be best achieved by a taxon-focused, rather than a broad floristic, approach because of the individuality of taxon histories within a flora (Verboom et al., 2003).

The above evidence indicates that transcontinental and transoceanic dispersal has been important in the compilation of the present-day southern African flora. Indeed, there is increasing evidence for the importance of long-distance dispersal in determining extant plant biogeographic patterns worldwide (e.g. Price and Clague, 2002; de Queiroz, 2005). Phylogenetic reconstructions based on molecular data, in combination with molecular calibrations to estimate the date of divergence events, have provided strong evidence that in the Southern Hemisphere, following the breakup of Gondwana, transoceanic dispersal has been important in a diversity of animals (e.g. Vences et al., 2003; Schwarz et al., 2006) and plant groups (e.g. Baum et al., 1998; Mummenhoff et al., 2004; Cook and Crisp, 2005; Linder and Barker, 2005; Barker et al., 2007). Vicariance appears to have been much stronger in animals than plant groups (Sanmartín and Ronquist, 2004). Of particular significance to the present study, plant taxa with a disjunct southern African–Australian distribution, such as the Arctotidinae, are rare (Good, 1964; Thorne, 1972; Goldblatt, 1978), and as yet few of these taxa have been investigated in a phylogenetic context.

A previous molecular phylogenetic study of Arctotidinae examined sequence variation in five cpDNA regions (*ndhF*, *psbA-trnH*, *rps16*, *trnS-trnfM*, and *trnT-trnF*) and the ITS nrDNA region for 18 species representing the major morphological variation in the subtribe (McKenzie et al., 2006a). Strong incongruence between the prevailing taxonomy and evolutionary relationships were indicated. A clade comprising two *Haplocarpha* species (*H. nervosa* and *H. rueppellii*, both formerly segregated in *Landtia*) from the southern and East African mountain chain was sister to the rest of the subtribe, and the widely distributed *H. scaposa* was indicated to be an early divergence. The placement of the Australian *Cymbonotus lawsonianus* and East African *H. schimperi* in a well-supported clade along with south-eastern African *Arctotis* species provided evidence for dispersal from southern Africa to Australia and migration to East Africa, respectively. However, comprehensive sampling of extant lineages is required for confident phylogenetic reconstructions and to allow more accurate biogeographic inferences to be made. The primary objective of the present study was to reconstruct a species-level phylogenetic hypothesis for a comprehensive sample of Arctotidinae based on sequence data from four

cpDNA regions (the *psbA-trnH*, *trnT-trnL* and *trnL-trnF* intergenic spacers and *trnL* intron) and the ITS nrDNA region. The specific aims were to use the phylogeny to: (1) investigate biogeographic patterns at the continental and regional levels; (2) make a first attempt at dating the diversification of Arctotidinae; and (3) further explore congruence between species relationships and the taxonomic classification, focusing on the polyphyly of *Arctotis* and *Haplocarpha*.

2. Materials and methods

2.1. Taxon sampling

DNA sequences were obtained for 71 accessions from 52 species of Arctotidinae. These data were supplemented with published sequences for single accessions of 18 Arctotidinae and four species from the sister subtribe Gorteriinae (McKenzie et al., 2006a). Thus the ingroup comprised 89 accessions from 59 species of Arctotidinae, representing the five currently accepted genera, and the outgroup consisted of single accessions of four species of the sister subtribe Gorteriinae (Table 1).

It is essential to avoid nomenclatural and taxonomic confusion, and we thus adopt the following nomenclatural usage. Generic concepts as elucidated by Karis (2007) are adopted, and Lewin's (1922) infrageneric classification of *Arctotis* and Beauverd's (1915) infrageneric classification of *Haplocarpha* are followed. In addition, the following corrections of recent treatments by Beyers (2000) and names that appear in Germishuizen and Meyer (2003) are relevant. *Arctotis decurrens* is the correct name for *A. merxmuelleri* and *A. scullyi* (McKenzie et al., 2006b). Two specimens (*McKenzie 797/3* and *McKenzie 1124/1*) belong to a newly recognized species, *A. debensis* (McKenzie et al., 2006c). *Arctotis flaccida* is the correct name for *Arctotis* sp. '1' designated by Beyers (2000). *Arctotis semipapposa* (listed as a synonym of *A. flaccida* by Beyers, 2000) is a very different, morphologically distinct species (McKenzie et al., in press). *Arctotis* sp. '2' and sp. '4' were designated by Beyers (2000) and this usage is retained here. Five other undetermined *Arctotis* species herein are designated spp. 'A', 'B', 'C', 'D', and 'E' (see Table 1). A full revision of the taxonomy of southern African Arctotidinae by the first author is currently in progress.

2.2. DNA extraction, amplification, and sequencing

For most samples total genomic DNA was extracted from about 1 cm^2 of either fresh leaf material or leaves desiccated in silica gel using a CTAB extraction protocol (Doyle and Doyle, 1987). For *Cymbonotus maidenii* DNA was extracted from a herbarium specimen. Two non-coding cpDNA regions, the *psbA-trnH* intergenic spacer and *trnT-trnF* region (the *trnT-trnL* and *trnL-trnF* intergenic spacers and *trnL* intron), and one non-coding nrDNA region (ITS) were amplified and sequenced. The following primers were used for amplifying and sequencing: the primers A, B, C, D, E, and F of Taberlet et al. (1991) and Arct-trnL-R (ATT WTA TCR TTT CTG TAT CSG; previously unpublished) for the *trnT-trnF* region; psbA-F and trnH-R for the *psbA-trnH* intergenic spacer (Sang et al., 1997); ITS18 (Käss and Wink, 1997; modified by Beyra-Matos and Lavin, 1999), ITS26 (Käss and Wink, 1997), Chrysanth-5.8F and Chromo-5.8R (Barker et al., 2005), ITS1, ITS4, and ITS5 (White et al., 1990) for the ITS region. Attempts to amplify both cpDNA regions for the *C. maidenii* accession and the *psbA-trnH* spacer for one *Arctotis decurrens* sample (*Mucina 170903/20*) were unsuccessful. In some instances, each species is represented by multiple samples, in order to assess species monophyly.

Each 25 μl Polymerase Chain Reaction (PCR) solution contained 2.5 μl 10× PCR buffer (Bioline, London, UK), 1 μl 20 mM dNTPs (Bioline, London), 1 μl 0.1 μM solution of each forward and reverse primer, 0.2 μl BioTaq® DNA polymerase (5 U/μl, Bioline, London, UK) and 0.75–1.5 μl unquantified DNA extract. The volume of 50 mM $MgCl_2$ varied from 0.75–1.5 μl (1.5–3 mM) for *trnT-trnF* and 2–3 μl (4–6 mM) for *psbA-trnH*. Some reaction solutions contained 1.5–2 μl 0.1% bovine serum albumen. For some ITS amplification reactions, the solution differed in containing 5 μl 5× Colorless GoTaq® reaction buffer, 0.25 μl GoTaq® DNA polymerase (5 U/μl, Promega, Madison, WI) and no additional $MgCl_2$.

The DNA regions were amplified using a Hybaid PCR Sprint thermal cycler. The following parameters in the amplification reactions were standard for all regions: in the first reaction cycle, denaturing 95 °C, 45 s; primer extension 72 °C, 3 min; and the final extension cycle 72 °C, 10 min. The number of amplification cycles and primer annealing temperature differed for each region as follows; *psbA-trnH*: 25 cycles, 52 °C, 45 s; *trnT-trnL* spacer (A and B): 27–30 cycles, 52 °C, 45 s; *trnT-trnL* (A and D): 35 cycles, 52 °C, 45 s; *trnL-trnF* intron and spacer (C and F): 30 cycles, 52 °C, 45 s; *trnL-trnF* spacer (E and F): 25–30 cycles, 52 °C, 45 s; ITS: 30–33 cycles, 48–52 °C, 45 s. The PCR products were purified using the Wizard® SV Gel and PCR purification kit (Promega, Madison, Wisconsin) and resuspended in 25–30 μl nuclease-free water.

PCR products were sequenced in both forward and reverse directions. Sequencing reactions of the PCR products were performed using the BigDye® Terminator v. 3.1 cycle sequencing kit according to the manufacturer's instructions (Applied Biosystems, Foster City, California). The cycle-sequencing products were precipitated using the sodium acetate/EDTA protocol, and electrophoresed and resolved on an ABI Prism 3100 Genetic Analyzer at the Rhodes University DNA sequencing facility.

2.3. Sequence alignment

For each accession contiguous sequences were compiled with Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor) and edited visually. All sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/; accession numbers listed in Table 1). Sequences were aligned manually in MacClade 4.06 (Maddison and Maddison, 2000). Gaps were inserted manually based on visual inspection of the sequences. Alignments are deposited in TreeBASE (http://www.treebase.org/).

2.4. Phylogenetic analyses

Because in flowering plants the chloroplast genome is usually inherited as a single unit without recombination (Soltis and Soltis, 1998), the cpDNA regions were combined into one data set. Three data sets were thus analyzed: the cpDNA data set; the ITS data set; and a 'total evidence' data set (Kluge, 1989; Nixon and Carpenter, 1996) with the cpDNA and ITS data sets combined. Congruence in the phylogenetic signal of the cpDNA and ITS data sets was assessed with the partition homogeneity (or ILD) test (Farris et al., 1994) using PAUP* 4.0b10 (Swofford, 2002) with 750 replicates, a maximum tree limit of 500, tree bisection–reconnection (TBR) branch swapping, with uninformative characters excluded following Lee (2001). Although conflict between the cpDNA and ITS data sets was indicated to be significant, the results must be interpreted with caution as the ILD test is an unreliable measure of data-set congruence in some circumstances (Ramírez, 2006). Under certain conditions this test can indicate significant incongruence between congruent data (Yoder et al., 2001; Hipp et al., 2004) and non-significance in cases of high incongruence ("hypercongruence" *sensu* Ramírez, 2006). As an alternative strategy, conflicting nodes were regarded to be incongruent if they each received parsimony bootstrap support (BS) > 70% (Mason-Gamer and Kellogg, 1996) or Bayesian posterior probabilities (PP) > 95% (Alfaro et al., 2003).

Table 1
Collection, voucher and GenBank accession information for sequences included in this study

Species[a]	Provenance of sample	Collector(s) and collection number	Herbarium[b]	GenBank accession numbers		
				ITS	*psbA–trnH*	*trnT–trnF*
Arctotheca calendula (L.) Levyns	South Africa: Western Cape	*McKenzie 808/3*	GRA	DQ444720	DQ444764	DQ444808
Arctotheca forbesiana (DC.) K. Lewin	South Africa: Western Cape	*McKenzie 1215/1*	GRA	**EU846328**	**EU846399**	**EU846468**
Arctotheca marginata Beyers	South Africa: Northern Cape	*Bosenburg 2*	NBG	**EU846329**	**EU846400**	**EU846469**
Arctotheca populifolia (P.J. Bergius) Norl.	South Africa: Eastern Cape	*Barker 1766*	GRA	**EU846330**	**EU846401**	**EU846470**
Arctotheca prostrata (Salisb.) Britten	South Africa: Eastern Cape	*Barker 1765*	GRA	**EU846331**	**EU846402**	**EU846471**
Arctotis acaulis L. 1	South Africa: Western Cape	*McKenzie 823/4*	GRA	DQ444721	DQ444765	DQ444809
Arctotis acaulis L. 2	South Africa: Western Cape	*McKenzie 827/1*	GRA	**EU846344**	**EU846414**	**EU846483**
Arctotis adpressa DC.	South Africa: Western Cape	*McKenzie 1365/1*	GRA	**EU846345**	**EU846415**	**EU846484**
Arctotis angustifolia L. var. *latifolia* Harv.	South Africa: Western Cape	*McKenzie 839*	GRA	**EU846346**	**EU846416**	**EU846485**
Arctotis arctotoides (L.f.) O. Hoffm. 1	South Africa: Western Cape	*Barker 1950*	GRA	**EU846334**	**EU846405**	**EU846474**
Arctotis arctotoides (L.f.) O. Hoffm. 2	South Africa: Eastern Cape	*McKenzie 855/1*	GRA	DQ444722	DQ444766	DQ444810
Arctotis argentea Thunb.	South Africa: Western Cape	*McKenzie 1250*	GRA	**EU846347**	**EU846417**	**EU846486**
Arctotis aspera L. var. *aspera* 1	South Africa: Western Cape	*McKenzie 844*	GRA	DQ444723	DQ444767	DQ444811
Arctotis aspera L. var. *aspera* 2	South Africa: Western Cape	*Samuel 41*	GRA	**EU846348**	**EU846418**	**EU846487**
Arctotis aspera L. var. *scabra* P.J. Bergius	South Africa: Western Cape	*McKenzie 1372*	GRA	**EU846349**	**EU846419**	**EU846488**
Arctotis auriculata Jacq.	South Africa: Northern Cape	*McKenzie 1285/1*	GRA	**EU846350**	**EU846420**	**EU846489**
Arctotis bellidifolia P.J. Bergius 1	South Africa: Western Cape	*Barker 1856*	GRA	**EU846351**	**EU846421**	**EU846490**
Arctotis bellidifolia P.J. Bergius 2	South Africa: Western Cape	*McKenzie 1060/1*	GRA	**EU846352**	**EU846422**	**EU846491**
Arctotis breviscapa Thunb.	South Africa: Western Cape	*McKenzie 1383/1*	GRA	DQ444724	DQ444768	DQ444812
Arctotis campanulata DC. var. *puberula* DC.	South Africa: Northern Cape	*McKenzie 1284*	GRA	**EU846353**	**EU846423**	**EU846492**
Arctotis canescens DC.	South Africa: Northern Cape	*McKenzie 1311/1*	GRA	**EU846354**	**EU846424**	**EU846493**
Arctotis debensis R.J. McKenzie 1	South Africa: Eastern Cape	*McKenzie 797/3*	GRA	**EU846335**	**EU846406**	**EU846475**
Arctotis debensis R.J. McKenzie 2	South Africa: Eastern Cape	*McKenzie 1124/1*	GRA	**EU846336**	**EU846407**	**EU846476**
Arctotis decurrens Jacq. 1	South Africa: Northern Cape	*McKenzie 1302/1*	GRA	**EU846356**	**EU846425**	**EU846495**
Arctotis decurrens Jacq. 2	South Africa: Northern Cape	*Mucina 170903/20*	GRA	**EU846355**	**—**	**EU846494**
Arctotis dregei Turcz. 1	South Africa: Western Cape	*McKenzie 815/2*	GRA	**EU846332**	**EU846403**	**EU846472**
Arctotis dregei Turcz. 2	South Africa: Western Cape	*McKenzie 834/4*	GRA	DQ444725	DQ444769	DQ444813
Arctotis elongata Thunb.	South Africa: Eastern Cape	*McKenzie 1085/1*	GRA	**EU846357**	**EU846426**	**EU846496**
Arctotis erosa Harv. 1	South Africa: Northern Cape	*McKenzie 1282*	GRA	**EU846358**	**EU846427**	**EU846497**
Arctotis erosa Harv. 2	South Africa: Northern Cape	*McKenzie 1290*	GRA	**EU846359**	**EU846428**	**EU846498**
Arctotis erosa Harv. 3	South Africa: Northern Cape	*Samuel 15*	GRA	**EU846360**	**EU846429**	**EU846499**
Arctotis erosa Harv. 4	South Africa: Western Cape	*Barker 1872*	GRA	**EU846361**	**EU846430**	**EU846500**
Arctotis fastuosa Jacq. 1	South Africa: Northern Cape	*McKenzie 1351*	GRA	**EU846362**	**EU846431**	**EU846501**
Arctotis fastuosa Jacq. 2	Namibia: Karas	*Mannheimer 2492*	GRA, WIND	**EU846373**	**EU846442**	**EU846512**
Arctotis flaccida Jacq. 1	South Africa: Western Cape	*McKenzie 1065/3*	GRA	**EU846363**	**EU846432**	**EU846502**
Arctotis flaccida Jacq. 2	South Africa: Western Cape	*McKenzie 1363/2*	GRA	**EU846364**	**EU846433**	**EU846503**
Arctotis graminea K. Lewin	South Africa: Western Cape	*McKenzie 824/1*	GRA	**EU846365**	**EU846434**	**EU846504**
Arctotis hirsuta (Harv.) Beauverd	South Africa: Western Cape	*McKenzie 1070/2*	GRA	**EU846366**	**EU846435**	**EU846505**
Arctotis hispidula (Less.) Beauverd	South Africa: Eastern Cape	*McKenzie 760/1*	GRA	**EU846337**	**EU846408**	**EU846477**
Arctotis incisa Thunb. 1	South Africa: Western Cape	*McKenzie 826/2*	GRA	**EU846367**	**EU846436**	**EU846506**
Arctotis incisa Thunb. 2	South Africa: Western Cape	*McKenzie 1380/1*	GRA	**EU846368**	**EU846437**	**EU846507**
Arctotis laevis Jacq.	South Africa: Western Cape	*McKenzie 1355/1*	GRA	**EU846369**	**EU846438**	**EU846508**
Arctotis lanceolata Harv. 1	South Africa: Western Cape	*McKenzie 835*	GRA	**EU846370**	**EU846439**	**EU846509**
Arctotis lanceolata Harv. 2	South Africa: Eastern Cape	*Ramdhani 616*	GRA	**EU846371**	**EU846440**	**EU846510**
Arctotis leiocarpa Harv.	Namibia: Karas	*Mucina 150704/7*	GRA	**EU846372**	**EU846441**	**EU846511**
Arctotis microcephala (DC.) Beauverd 1	South Africa: Eastern Cape	*McKenzie 784/3*	GRA	**EU846339**	**EU846409**	**EU846478**
Arctotis microcephala (DC.) Beauverd 2	South Africa: Eastern Cape	*McKenzie 1409*	GRA	**EU846340**	**EU846410**	**EU846479**

Arctotis muricata Thunb.	South Africa: Western Cape	*McKenzie 1059/1*	GRA	**EU846374**	**EU846443**	**EU846513**
Arctotis perfoliata (L.f.) O. Hoffm. 1	South Africa: Western Cape	*McKenzie 822*	GRA	**EU846341**	**EU846411**	**EU846480**
Arctotis perfoliata (L.f.) O. Hoffm. 2	South Africa: Western Cape	*McKenzie 825/1*	GRA	DQ444726	DQ444770	DQ444814
Arctotis pinnatifida Thunb.	South Africa: Western Cape	*McKenzie 777/1*	GRA	**EU846375**	**EU846444**	**EU846514**
Arctotis reptans Jacq.	South Africa: Western Cape	*McKenzie 1205/2*	GRA	**EU846376**	**EU846445**	**EU846515**
Arctotis revoluta Jacq. 1	South Africa: Western Cape	*McKenzie 1071/2*	GRA	**EU846377**	**EU846446**	**EU846516**
Arctotis revoluta Jacq. 2	South Africa: Western Cape	*Samuel 42*	GRA	**EU846378**	**EU846447**	**EU846517**
Arctotis rotundifolia K. Lewin	South Africa: Western Cape	*McKenzie 1389/1*	GRA	**EU846379**	**EU846448**	**EU846518**
Arctotis scapiformis Thell. 1	South Africa: KwaZulu-Natal	*Ramdhani 508*	GRA	**EU846342**	**EU846412**	**EU846481**
Arctotis scapiformis Thell. 2	South Africa: Eastern Cape	*White 41*	GRA	**EU846343**	**EU846413**	**EU846482**
Arctotis semipapposa (DC.) Beauverd 1	South Africa: Western Cape	*McKenzie 811/1*	GRA	**EU846380**	**EU846449**	**EU846519**
Arctotis semipapposa (DC.) Beauverd 2	South Africa: Western Cape	*McKenzie 843/1*	GRA	**EU846381**	**EU846450**	**EU846520**
Arctotis stoechadifolia P.J. Bergius 1	South Africa: Western Cape	*McKenzie 892/1*	GRA	**EU846382**	**EU846451**	**EU846521**
Arctotis stoechadifolia P.J. Bergius 2	South Africa: Western Cape	*McKenzie 1074/1*	GRA	**EU846383**	**EU846452**	**EU846522**
Arctotis sulcocarpa K. Lewin	South Africa: Northern Cape	*McKenzie 1281/1*	GRA	DQ444728	DQ444772	DQ444816
Arctotis venusta Norl.	South Africa: Free State	*McKenzie 875/1*	GRA	DQ444729	DQ444773	DQ444817
Arctotis verbascifolia Harv. 1	South Africa: Western Cape	*Barker s.n.*	GRA	**EU846384**	**EU846453**	**EU846523**
Arctotis verbascifolia Harv. 2	South Africa: Western Cape	*McKenzie 1234/1*	GRA	**EU846385**	**EU846454**	**EU846524**
Arctotis sp. '2'	South Africa: Western Cape	*Barker 1865*	GRA	DQ444727	DQ444771	DQ444815
Arctotis sp. '4'	South Africa: Western Cape	*McKenzie 1075*	GRA	**EU846386**	**EU846455**	**EU846525**
Arctotis sp. 'A'	South Africa: Northern Cape	*McKenzie 1296/1*	GRA	**EU846387**	**EU846456**	**EU846526**
Arctotis sp. 'B'	South Africa: Northern Cape	*McKenzie 1300/1*	GRA	**EU846388**	**EU846457**	**EU846527**
Arctotis sp. 'C' 1	South Africa: Northern Cape	*McKenzie 1336/1*	GRA	**EU846389**	**EU846458**	**EU846528**
Arctotis sp. 'C' 2	South Africa: Northern Cape	*Samuel 31*	GRA	**EU846390**	**EU846459**	**EU846529**
Arctotis sp. 'D' 1	South Africa: Northern Cape	*McKenzie 1339/1*	GRA	**EU846391**	**EU846460**	**EU846530**
Arctotis sp. 'D' 2	South Africa: Northern Cape	*Samuel 35*	GRA	**EU846392**	**EU846461**	**EU846531**
Arctotis sp. 'E'	South Africa: Northern Cape	*Samuel 20*	GRA	**EU846393**	**EU846462**	**EU846532**
Cymbonotus lawsonianus Gaudich. 1	Australia: Australian Capital Territory	*Bayer ACT-05001*	GRA	DQ444730	DQ444774	DQ444818
Cymbonotus lawsonianus Gaudich. 2	Australia: New South Wales	*Weston 2486*	NSW	**EU846333**	**EU846404**	**EU846473**
Cymbonotus maidenii (Beauverd) A.E. Holland & V.A. Funk	Australia: New South Wales	*Radunz s.n.*, 5.ii.1982	NSW	**EU846338**	**—**	**—**
Dymondia margaretae Compton	South Africa: Western Cape ex hort.	*Barker 1780*	GRA	DQ444731	DQ444775	DQ444819
Haplocarpha lanata Less.	South Africa: Western Cape	*McKenzie 845/1*	GRA	DQ444732	DQ444776	DQ444820
Haplocarpha lyrata Harv.	South Africa: Eastern Cape	*Barker 1767*	GRA	DQ444733	DQ444777	DQ444821
Haplocarpha nervosa (Thunb.) Beauverd 1	South Africa: Eastern Cape	*McKenzie 970*	GRA	DQ444734	DQ444778	DQ444822
Haplocarpha nervosa (Thunb.) Beauverd 2	South Africa: Eastern Cape	*McKenzie 1192*	GRA	**EU846323**	**EU846394**	**EU846463**
Haplocarpha parvifolia (Schltr.) Beauverd	South Africa: Western Cape	*Mucina 290805/13*	GRA	**EU846327**	**EU846398**	**EU846467**
Haplocarpha rueppellii (Sch. Bip.) Beauverd 1	Ethiopia: Bale	*Barker 1906*	ETH	DQ444735	DQ444779	DQ444823
Haplocarpha rueppellii (Sch. Bip.) Beauverd 2	Kenya: Central Province	*Namaganda, Abdillahi & Nakamatle 1746*	EA	**EU846324**	**EU846395**	**EU846464**
Haplocarpha scaposa Harv. 1	South Africa: Eastern Cape	*Barker 1772*	GRA	DQ444736	DQ444780	DQ444824
Haplocarpha scaposa Harv. 2	South Africa: KwaZulu-Natal	*Ramdhani 519*	GRA	EU846325	EU846396	EU846465
Haplocarpha schimperi (Sch. Bip.) Beauverd 1	Ethiopia: Arsi	*Barker 1899*	ETH	DQ444737	DQ444781	DQ444825
Haplocarpha schimperi (Sch. Bip.) Beauverd 2	Kenya: Central Province	*Namaganda, Abdillahi & Nakamatle 1748*	EA, GRA	**EU846326**	**EU846397**	**EU846466**
Outgroup taxa:						
Berkheya carduoides (Less.) Hutch.	South Africa: Eastern Cape	*Barker 1924*	GRA	DQ444716	DQ444760	DQ444804
Cuspidia cernua (L.f.) B.L. Burtt	South Africa: Eastern Cape	*Barker 1896*	GRA	DQ444717	DQ444761	DQ444805
Gazania krebsiana Less.	South Africa: Eastern Cape	*Barker s.n.*	GRA	DQ444718	DQ444762	DQ444806
Hirpicium echinus Less.	South Africa: Northern Cape	*McKenzie 861*	GRA	DQ444719	DQ444763	DQ444807

GenBank numbers in bold are new submissions.

[a] Duplicate samples of a species are numbered sequentially.

[b] EA, East African Herbarium, Nairobi; ETH, National Herbarium of Ethiopia, Addis Ababa; GRA, Selmar Schonland Herbarium, Grahamstown; NBG, Compton Herbarium, Cape Town; NSW, National Herbarium of New South Wales, Sydney; WIND, National Herbarium of Namibia, Windhoek.

Parsimony analyses were performed using PAUP*. For each data set, a heuristic search was conducted with 1000 simple taxon-addition replicates, TBR branch swapping, and the MULTREES option in effect in order to ensure that multiple islands of equally parsimonious trees were found (Maddison, 1991). Further heuristic searches employed 1000 random taxon-addition replicates, holding one tree at each step, TBR branch swapping, with the MULTREES and STEEPEST DESCENT options in effect, for each replicate saving at most one tree $\geqslant$ the tree length from the simple heuristic search, followed by a complete heuristic search on the saved trees with a maximum tree limit of 100,000. In all analyses no shorter trees were recovered than from the simple-addition search. Uninformative characters were excluded before all analyses and all characters were equally weighted and unordered. Parsimony analyses were repeated with unambiguous insertion/deletion events (indels) greater than 2 bp in length recoded as a binary (presence/absence) character, following Bayer et al. (2002), using the 'simple indel coding' method of Simmons and Ochoterena (2000). Parsimony bootstrap assessments for each data set were carried out using 1000 replicates in PAUP*. The ITS data set was analyzed twice. In an initial analysis, all sites were considered, while a second analysis excluded four ambiguously aligned regions (aligned bp 70–79, 208–232, 256–262, and 479–497). Relative branch support was assessed using nonparametric bootstrap analysis (Felsenstein, 1985) with 1000 replicates, TBR branch swapping and a maximum tree limit of 500.

Bayesian inference (BI) analyses using Markov Chain Monte Carlo methods (Yang and Rannala, 1997) were performed using MrBayes 3.1.1 (Huelsenbeck and Ronquist, 2001). Prior to analysis, sequences were partitioned into coding, exon, intron, and intergenic spacer regions based on sequences from the chloroplast genomes of *Lactuca sativa*, *Nicotiana sylvestris*, and *N. tabacum* from GenBank, and the ITS secondary structure model for the Asteraceae of Goertzen et al. (2003). Eleven partitions were defined. The *psbA-trnH* sequence comprised the *psbA-trnH* spacer, 53 bp at the 3′ end of the *psbA* gene, and 32 bp at the 5′ end of the *trnH* gene. The *trnT-trnF* region comprised the *trnL* intron, the flanking *trnL* 5′ and 3′ exons, and the *trnT-trnL* and *trnL-trnF* intergenic spacers. The ITS region comprised the 5.8S gene and the flanking internal transcribed spacers ITS1 and ITS2 (Baldwin, 1992). An optimal nucleotide-substitution model for each partition (Table 2) was selected for use in the BI analyses using the Akaike information criterion with MrModeltest 2.2 (J.A.A. Nylander, Uppsala University, Uppsala).

Two independent BI analyses each with four Markov chains, three heated and one cold, starting from a random tree were run simultaneously for 1×10^6 generations with trees sampled every 100 generations. The trees sampled prior to stabilization of the log-likelihood value were discarded as 'burn-in' samples; the burn-in varied from 23,000 generations for the ITS data analysis, 30,000 for the cpDNA analysis and 40,000 for the total-evidence analysis. The remaining trees from the simultaneous runs were combined and used to generate a 50% majority rule consensus tree and to determine the PP for each node. The consensus trees from the Bayesian inference analyses are deposited in TreeBASE (http://www.treebase.org/; submission No. SN3970).

Table 2
Nucleotide-substitution model for each partition specified in the Bayesian inference analyses

Partition	Model[a]	Rate variation across sites
psbA	JC	Equal
psbA–trnH spacer	GTR	Gamma
trnH	JC	Equal
trnT–trnL spacer	GTR	Gamma
trnL 3′ exon	JC	Equal
trnL	GTR	Equal
trnL 5′ exon	JC	Equal
trnL–trnF spacer	GTR	Gamma
ITS1	SYM	Gamma
5.8S	GTR	Proportion invariable + gamma
ITS2	SYM	Gamma

[a] GTR, General Time Reversible; JC, Jukes-Cantor; SYM, Symmetrical.

2.5. Molecular dating

Ideally, dating of molecular phylogenies requires either reliable calibration points or a known rate of molecular evolution, but unfortunately obtaining reliable calibration points to date phylogenetic reconstructions within Asteraceae is problematic due to the family's poor fossil record. Consequently, divergence dates were estimated from the ITS data set utilizing ITS mutation rates published for other Asteraceae taxa following the methodology of S Howis (unpublished data). Three mutation rates ('slow', 'average', and 'fast') were used. Mutation rates published for Asteraceae range from 2.51×10^{-9} substitutions per site per year ($s\,s^{-1}\,y^{-1}$; 'slow') in *Eupatorium* (Schmidt and Schilling, 2000) to $7.83 \times 10^{-9}\,s\,s^{-1}\,y^{-1}$ ('fast') in *Robinsonia* (Sang et al., 1995). These rates span much of the range of rates published for angiosperms as a whole (see Kay et al., 2006). An 'average' rate ($4.58 \times 10^{-9}\,s\,s^{-1}\,y^{-1}$) was calculated based on the rates for four Asteraceae taxa listed by Kay et al. (2006); this rate was virtually identical to the average rate ($4.59 \times 10^{-9}\,s\,s^{-1}\,y^{-1}$) for the 10 herbaceous angiosperm taxa listed by the same authors.

The ITS data set and cladogram were reduced to 50 terminals to exclude duplicates of species with almost identical sequences. Log-likelihood ratio tests were performed to compare the likelihoods of obtaining the same topology with or without a clock assumption. An appropriate evolutionary model was selected with MrModeltest 2.2. Maximum likelihood analyses were performed with PAUP* with and without a clock enforced and the difference in log-likelihoods was tested by means of a χ^2 test. As the assumption of a strict molecular clock was rejected, divergence dates were estimated using a relaxed clock with Bayesian inference and MCMC procedures implemented in BEAST 1.4.7 (Drummond and Rambaut, 2007). A relaxed clock model allows the among-branch evolution rate to vary. In addition, BEAST has the advantage of permitting specification of a number of evolutionary models in a single framework (Drummond and Rambaut, 2007). Consistent with the MrModeltest results, a General Time Reversible nucleotide-substitution model with gamma + invariant sites was used. A relaxed molecular clock with branch substitution rates drawn from a lognormal distribution, auto-optimization of the operators, no topological constraints and a constant speciation rate per lineage (i.e. Yule tree prior) with a uniform prior distribution was used. Rates were sampled every 1000 cycles from 50,00,000 MCMC steps with a burn-in of 500,000 cycles. Running the analysis for 100,00,000 steps had no impact on the estimated divergence dates (data not presented). For all analyses two independent runs were performed, the log files were combined to check for convergence on the same distribution and to ensure adequate sample sizes, and viewed using Tracer v.1.4 (distributed with BEAST). Divergence dates estimated when branch substitution rates were drawn from an exponential distribution were considerably older and with much wider 95% highest posterior density (HPD) limits than those derived from a lognormal distribution, and in some instances the lower HPD bound extended well beyond the probable origin of the Asteraceae of c. 60 Mya. Therefore analyses utilising a lognormal distribution were the more plausible and are only considered herein.

2.6. Dispersal–vicariance analysis

To infer the ancestral areas of the basal and internal nodes, a dispersal–vicariance analysis was performed with DIVA 1.1 (Ronquist, 1997). A fully dichotomized tree (a requirement of DIVA) was created based on a neighbor-joining (NJ) tree produced using PAUP*. As can be seen from Figs. 2 and 3, not all clades were fully resolved, so use of a NJ approach obviated a subjective resolution of species-level relationships. We acknowledge that there are many possible permutations of species relationships in poorly resolved clades. However, in this study lack of resolution affected primarily the 'core Arctotis' clade, all members of which are from the Fynbos or Succulent Karoo regions, and exploration of alternative relationships is unlikely to affect the overall result of the DIVA analysis. It is emphasised that the basal and internal nodes of the NJ tree, which were the nodes of interest, were identical to those in the maximum parsimony and Bayesian total-evidence phylogenies.

The selection of unit areas of endemism used in DIVA analyses can be problematic, as different workers may not agree on boundaries of regions of endemism, and some areas might be viewed as subsets of larger areas. For this analysis, we mostly used areas that correspond to vegetation units, namely afromontane, Fynbos, Nama-Karoo, Succulent Karoo, and Savanna biomes, as well as the widely recognized Albany Centre of Floristic Endemism (Cowling and Hilton-Taylor, 1997). We thus chose to differentiate between the Fynbos and Succulent Karoo, and not consider them as part of a larger floristic region (as suggested by Born et al., 2007, for example). Australia was treated as a single area. Analyses were run without limiting the number of ancestral area optimizations.

The exclusion of some unsampled species may affect the results of a DIVA analysis, just as they can affect phylogenetic interpretations. In the present study, the majority of the unsampled taxa are *Arctotis* species and have CFR distributions and, based on morphological data, it is predicted they will be members of the 'core Arctotis' clade. This would not affect the outcome of the DIVA analysis.

3. Results

3.1. Phylogenetic analyses

The sequence characteristics and variability of each DNA region and the combined datasets are summarized in Table 3. For each data set parsimony and BI generated almost identical phylogenetic reconstructions. Consequently, in the following discussion, the method of analysis is only specified when a phylogenetic arrangement is in conflict. In all analyses, the 'Landtia' clade (comprising *Haplocarpha nervosa*, *H. rueppellii*, and *H. parvifolia*) was sister to the rest of the Arctotidinae with maximum BS and PP support. *Haplocarpha scaposa* and *Dymondia margaretae* were placed on subsequently diverging, well-supported monotypic branches. The remaining taxa were placed in four strongly supported clades: a large 'core Arctotis' clade; a 'Cymbonotus' clade (composed of *Cymbonotus lawsonianus*, *H. schimperi* and species from *Arctotis* sect. *Austro-orientales*); a clade consisting of the two sampled species from *Arctotis* sect. *Anomalae*; and an *Arctotheca*–'core Haplo-

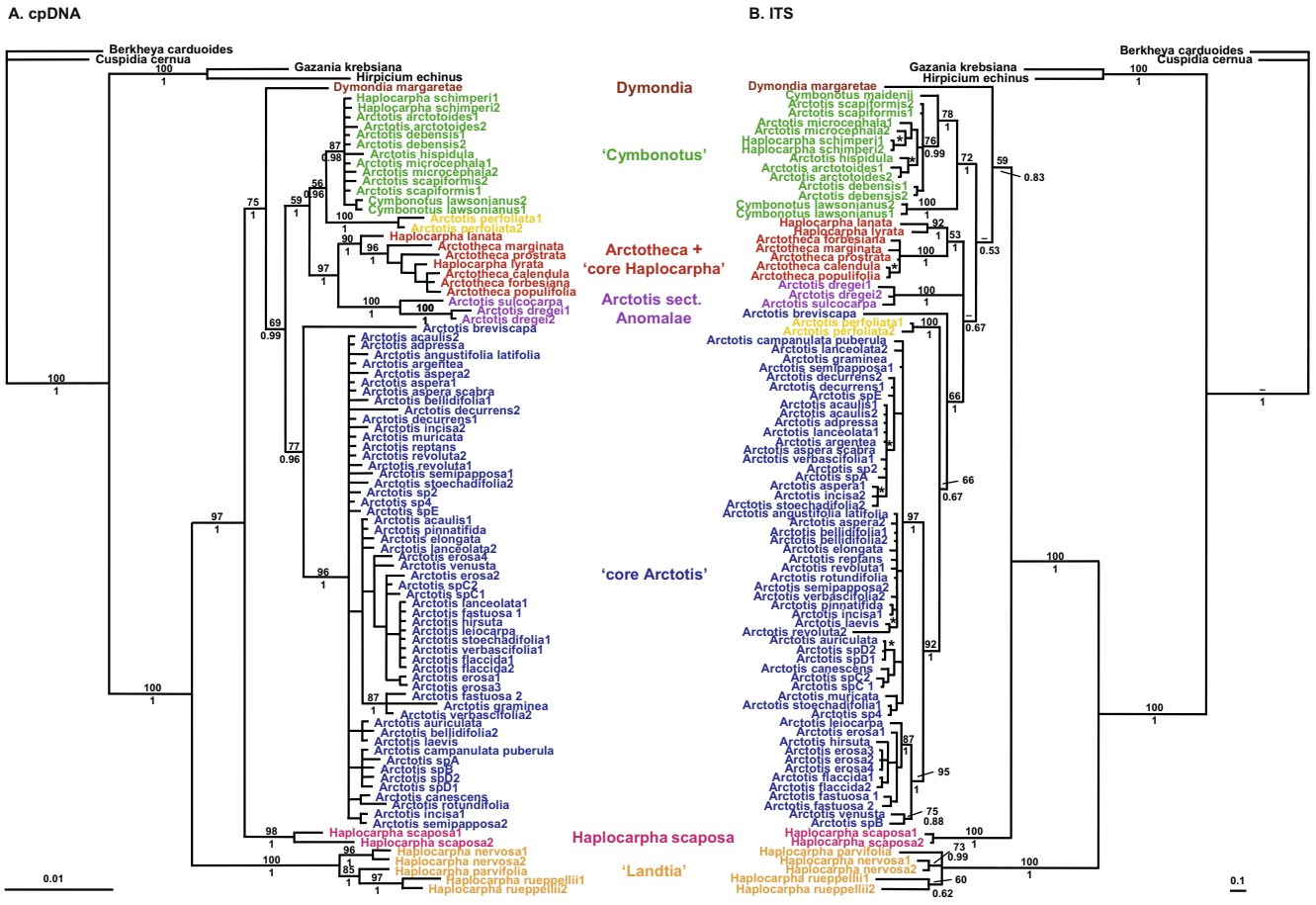

Fig. 2. Phylograms obtained from Bayesian-inference analyses. (A) cpDNA data set; (B) ITS data set. Support values above the branches are bootstrap percentages, and below the branches are Bayesian posterior probabilities. For terminal clades, support values are only given for nodes receiving bootstrap percentages > 75% and Bayesian posterior probabilities > 0.95.

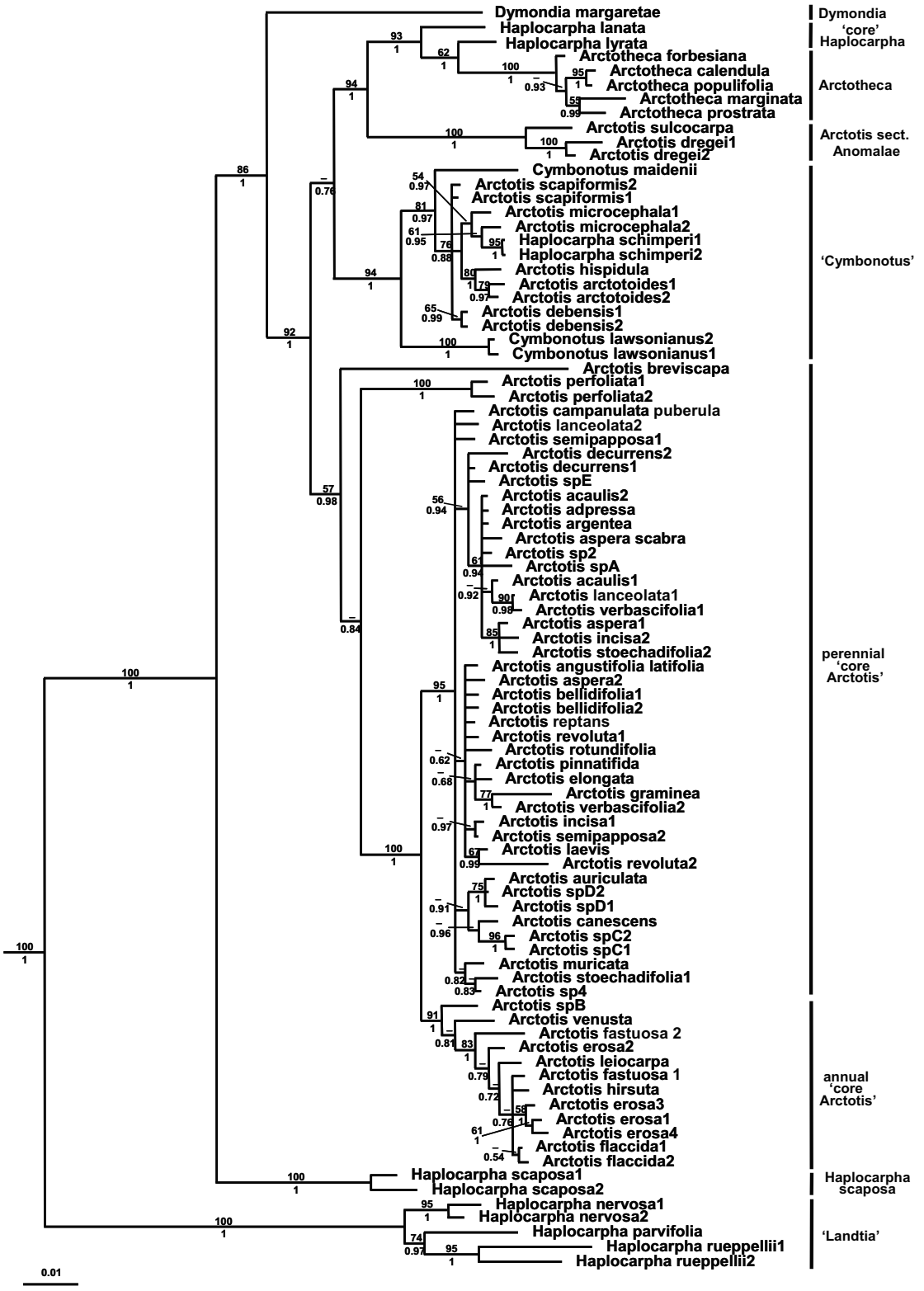

Fig. 3. Phylogram obtained from Bayesian-inference analysis of the total evidence. Support values are as in Fig. 2.

carpha' clade (comprising all *Arctotheca* species, *H. lanata* and *H. lyrata*). Relationships amongst these four clades varied depending on the data set and method of analysis.

The major clades in the cpDNA phylogeny were strongly supported by both phylogenetic methods, but within-clade relationships were poorly resolved (Fig. 2A). The interior nodes linking *Dymondia margaretae*, 'core Arctotis', an *Arctotis* sect. *Anomalae*–*Arctotheca*–'core Haplocarpha' clade, and a 'Cymbonotus'–*Arctotis perfoliata* clade were weakly to moderately supported by parsimony bootstrap (BS = 59–77%), but well supported in the BI analysis (PP = 0.96–1). *Arctotis perfoliata* was placed sister to the 'Cymbonotus' clade with weak support (BS = 56%, PP = 0.96). *Arcto-*

Table 3
Summary of statistics from parsimony analyses of each DNA fragment and the combined datasets

Characteristic	*psbA-trnH*	*trnT-trnF*	Combined cpDNA	ITS	Total evidence
Length range (nucleotides)	330–495	1348–1463	1716–1950	632–653	–
Aligned length (nucleotides)	563	1593	2155	688	2843
Number of indels recoded (number that are parsimony informative)	20 (12)	31 (20)	51 (32)	6 (4)	57 (36)
Number of variable sites	83 (14.7%)	195 (12.2%)	278 (12.9%)	275 (40.0%)	553 (19.5%)
Number of parsimony informative sites	48 (8.5%)	94 (5.9%)	142 (6.6%)	206 (29.9%)	348 (12.2%)
Number of most parsimonious trees	—	—	100 000	92 524	94 496
Tree length (steps)	—	—	268	611	872
CI	—	—	0.743	0.622	0.606
RI	—	—	0.928	0.865	0.872

The statistics are from parsimony analyses in which indels were recoded. Recoded indels are excluded from the data for sequence length, variability and parsimony informativeness.

tis breviscapa was placed sister to the 'core Arctotis' clade with moderate support (BS = 77%, PP = 0.96). Although the rest of the 'core Arctotis' clade was strongly supported (BS = 96%, PP = 1), relationships within the clade were poorly resolved. *Arctotis* sect. *Anomalae*, *Arctotheca* and 'core Haplocarpha' formed a strongly supported clade (BS = 97%, PP = 1). A 'core Haplocarpha' clade was not retrieved. *Haplocarpha lanata* was sister to the rest of the *Arctotheca*–'core Haplocarpha' clade, and *H. lyrata* was nested within the *Arctotheca* clade but with little support (BS = 58%, PP = 0.78).

The ITS phylogeny (Fig. 2B) differed from the cpDNA phylogeny in the following respects: the internal branches linking the *Arctotheca*–'core Haplocarpha', *Arctotis* sect. *Anomalae*, 'Cymbonotus' and 'core Arctotis' clades were poorly supported or unresolved, but relationships within the major clades were somewhat better resolved. 'Core Haplocarpha' and *Arctotheca* formed strongly supported sister clades. *Cymbonotus lawsonianus* was sister to the rest of the 'Cymbonotus' clade (BS = 72%, PP = 1). The two *Cymbonotus* species formed a basal grade to the southern and eastern African species in this clade. *Arctotis perfoliata* was placed close to the base of the 'core Arctotis' clade but with poor support (BS = 66%, PP = 0.67). The 'core Arctotis' clade was differentiated into highly supported annual and perennial lineages (BS = 97%, PP = 1, and BS = 95%, PP = 1, respectively).

Exclusion of four ambiguously aligned regions reduced the ITS data set to 632 bp, of which 238 bp (37.7%) were variable and 174 bp (27.5%) were parsimony informative. Parsimony analysis yielded 1526 MPTs of 487 steps (CI 0.616, RI 0.867). The strict consensus tree topology differed to that obtained from the complete ITS data analysis in that the *Arctotis* sect. *Anomalae* clade was placed sister to the 'core Haplocarpha' clade but with no BS support (BS < 50% from 100 replicates).

The ILD test indicated significant conflict existed between the cpDNA and ITS data sets ($p = 0.001342$). Visual comparison of tree topologies revealed incongruence in the placement of *A. perfoliata* and *H. lyrata* between the cpDNA and ITS trees (Fig. 2A and B), but the placement of both species was weakly supported by one data set. The placement of *A. perfoliata* in the cpDNA phylogeny was strongly supported (BS = 100%, PP = 1), but only weakly supported in the ITS tree (BS = 66%, PP = 0.67). In contrast, the placement of *H. lyrata* was strongly supported (BS = 92%, PP = 1) by the ITS data, but its placement within the *Arctotheca* clade from the cpDNA data received conflicting support (BS = 58%, PP = 1). Placement of the 'Cymbonotus' clade also differed in the cpDNA and ITS trees, but overall support was low (BS = 59%, PP = 1 in the cpDNA phlyogeny; BS < 50%, PP = 0.53 in the ITS phylogeny) and the data sets were thus combined.

Both analytical methods yielded phylogenies with improved resolution from the total evidence data set (Fig. 3) than was obtained in the analyses of the separate data sets (Fig. 2A and B). The basal nodes were well supported (BS $\geqslant$ 86%, PP = 1). The placement of the 'Cymbonotus' clade sister to the *Arctotheca*–'core Haplocarpha'–*Arctotis* sect. *Anomalae* clade, and the 'core Arctotis' node were poorly supported, but the other internal nodes were well supported. The placement of *A. perfoliata* within the 'core Arctotis' clade received little support (BS < 50%, PP = 0.84). Highly supported annual and perennial clades were resolved within 'core Arctotis' (BS = 99%, PP = 1).

Exclusion of *A. perfoliata* and *H. lyrata* from total-evidence analyses had no impact on the remaining tree topology, but support for the 'core Arctotis' clade improved considerably (BS = 83% from 200 bootstrap replicates), whereas the 'Cymbonotus'–*Arctotis* sect. *Anomalae*–*Arctotheca*–'core Haplocarpha' clade remained weakly supported (BS = 56%).

3.2. Molecular dating

Divergence dates estimated with the 'slow' and 'average' mutation rates resulted in considerably older mean divergence dates with wider 95% HPD ranges than dates estimated with the 'fast' mutation rate (Table 4). Based on the mean estimated dates, the Landtia clade (node 1) was estimated to have diverged some time during the early Oligocene to late Miocene epochs, but the crown of extant species (node 15) is of much more recent origin (Fig. 4 and Table 4). The three major extant clades—'core Arctotis', 'Cymbonotus', and *Arctotis* sect. *Anomalae*–'core Haplocarpha'–*Arctotheca* (nodes 4, 5, and 6, respectively)—were indicated to have diverged in a rapid radiation during the mid or late Miocene. *Cymbonotus lawsonianus* (node 9) was indicated to have diverged within the 'Cymbonotus' clade during the late Miocene to late Pliocene. Radiation of the *Arctotis arctotoides* species complex (node 10) was dated to the late Pliocene at the earliest. Within this complex, *Arctotis microcephala* and *H. schimperi* (node 11) were estimated to have diverged during the late Pliocene or Pleistocene.

3.3. Biogeography and dispersal–vicariance analysis

The two basalmost lineages in Arctotidinae have a wide geographic distribution (Fig. 4). The 'Landtia' clade grows largely at higher altitudes, and is distributed from the CFR (*H. parvifolia*) to southeastern Africa (*H. nervosa*) and afromontane regions of the East African mountain chain (*H. nervosa* and *H. rueppellii*). *Haplocarpha scaposa* is widely distributed in submontane and montane southeastern Africa and tropical eastern Africa. DIVA analysis indicated that Arctotidinae might have an afromontane origin, but the possible ancestral area of the widespread *H. scaposa* was equivocal. Furthermore, the accurate assessment of the ancestral area requires a resolved phylogeny of the sister group and use of a more distant outgroup. The ancestral area of all other extant Arctotidinae was indicated to be in the fynbos (i.e., in the CFR). The *Arctotheca*–'core Haplocarpha' clade was indicated to have originated in fynbos, consistent with the centre of diversity in the CFR, with subse-

Table 4
Estimated divergence dates for nodes of interest from BEAST analysis using ITS mutation rates published for other Asteraceae taxa

Node	Slow	ESS	Average	ESS	Fast	ESS
1	32.6 ± 0.17 (21.7–44.0)	1128.463	27.2 ± 0.11 (17.6–38.6)	2894.552	10.2 ± 0.05 (6.8–13.9)	1128.886
2	18.6 ± 0.14 (13.1–24.7)	479.403	11.8 ± 0.06 (8.2–15.9)	1157.966	5.9 ± 0.03 (4.2–7.8)	1027.545
3	17.3 ± 0.11 (12.7–22.5)	565.099	10.7 ± 0.05 (7.5–14.0)	1087.701	5.5 ± 0.03 (4.0–7.1)	966.825
4	16.1 ± 0.1 (11.7–20.7)	545.097	9.3 ± 0.04 (6.9–12.2)	1035.193	5.1 ± 0.03 (3.8–6.7)	836.345
5	16.1 ± 0.1 (11.8–20.9)	556.745	9.3 ± 0.04 (6.7–12.2)	1077.755	5.1 ± 0.03 (3.6–6.6)	859.889
6	13.8 ± 0.1 (9.4–18.8)	658.419	8.0 ± 0.05 (5.6–10.6)	823.279	4.4 ± 0.03 (3.0–6.0)	749.412
7	9.4 ± 0.07 (5.7–13.6)	947.15	5.3 ± 0.03 (3.1–7.5)	1827.21	3.0 ± 0.02 (1.8–4.3)	1453.303
8	3.9 ± 0.03 (1.4–6.9)	3391.419	2.0 ± 0.01 (0.8–3.5)	4844.536	1.2 ± 0.01 (0.44–2.2)	2792.901
9	9.6 ± 0.07 (5.5–14.0)	1051.392	5.4 ± 0.03 (3.0–8.0)	1957.358	3.0 ± 0.02 (1.7–4.4)	1478.561
10	3.7 ± 0.03 (1.9–6.0)	1120.797	1.7 ± 0.01 (0.9–2.7)	1258.726	1.2 ± 0.01 (0.59–1.9)	1229.467
11	2.3 ± 0.02 (0.7–4.4)	2013.626	1.1 ± 0.01 (0.3–2.2)	2001.685	0.73 ± 0.01 (0.20–1.4)	1606.189
12	10.6 ± 0.06 (7.4–13.9)	801.202	6.0 ± 0.03 (4.2–8.1)	1102.589	3.4 ± 0.02 (2.4–4.4)	987.303
13	9.4 ± 0.05 (6.5–12.5)	918.283	5.0 ± 0.02 (3.5–6.9)	1533.579	3.0 ± 0.02 (2.0–4.0)	1040.264
14	6.8 ± 0.04 (4.6–9.1)	1055.801	3.4 ± 0.01 (2.3–4.6)	1800.349	2.2 ± 0.01 (1.5–2.9)	1043.336
15	9.7 ± 0.07 (5.4–14.1)	1125.109	5.6 ± 0.03 (3.5–8.1)	1423.365	3.0 ± 0.02 (1.7–4.4)	974.539

Results are from two independent analyses combined. For the node numbers see Fig. 3. Values (in My) are the mean divergence date ± SD and the 95% highest posterior density range. Slow = 2.51×10^{-9} s s^{-1} y^{-1} (*Eupatorium*; Schmidt and Schilling, 2000); average = mean of rates for four Asteraceae taxa listed by Kay et al. (2006); fast = 7.83×10^{-9} s s^{-1} y^{-1} (*Robinsonia*; Sang et al. 1995). Effective sample size (ESS) = the number of effectively independent samples from the posterior distribution.

quent extension into Namaqualand (*A. calendula* and *A. marginata*), the Nama-Karoo and Succulent Karoo (*A. calendula*) and the Albany hotspot in southeastern South Africa (*A. populifolia*, *A. prostrata* and *H. lyrata*). The *Arctotis* sect. *Anomalae* lineage is centred in the CFR and Succulent Karoo, and extends into the Nama-Karoo (an undescribed species) and the Albany region (*A. dregei*). The 'Cymbonotus' clade was indicated to have migrated from fynbos to the Albany hotspot, savanna and afromontane southeastern Africa (*Arctotis* sect. *Austro-orientales* p.p.) and to have dispersed to Australia (*C. lawsonianus* and *C. maidenii*). An origin in the fynbos is implicated for the 'core Arctotis' clade, with subsequent independent radiations in the Succulent Karoo in both the perennial and annual clades.

4. Discussion

The eight major lineages retrieved in the present investigation are congruent with those obtained in a previous study (McKenzie et al., 2006a), which was based on a smaller sample of taxa. Our results confirm the polyphyly of *Arctotis* and *Haplocarpha* as the genera are presently circumscribed. *Haplocarpha* species are segregated between four clades and *Arctotis* species are placed in three well-resolved lineages. A molecular phylogenetic study by Funk et al. (2007) included sequence data for two species not included in the present study, namely *Haplocarpha oocephala* (the sample was incorrectly named *H. lanata*) and *Cymbonotus preissianus*, as well as both cpDNA (*ndhF* and *trnL-F*) and ITS data for *C. maidenii*. *Haplocarpha oocephala* was placed on a monotypic lineage diverging between the 'Landtia' and *H. scaposa* lineages, and *C. preissianus* and *C. maidenii* were in the same clade as *C. lawsonianus*. The inclusion of a comprehensive sample of taxa in the present study has clarified the phylogenetic placement of several species whose placement was previously considered equivocal (McKenzie et al., 2006a). *Arctotheca calendula* was shown to belong unequivocally in the *Arctotheca* clade. In all analyses *Arctotis breviscapa* was placed sister to the rest of the 'core Arctotis' clade. The sample of *H. lyrata* was indicated to possess a chloroplast haplotype shared with *Arctotheca*, whereas the ITS data resolved a monophyletic 'core Haplocarpha'. The conflicting placement of two accessions of *Arctotis perfoliata* sister to the 'Cymbonotus' clade in the cpDNA phylogeny and near-basal within the 'core Arctotis' clade from ITS data confirms the findings of McKenzie et al. (2006a).

Using the criteria of Mason-Gamer and Kellogg (1996) and Alfaro et al. (2003), 'hard' incongruence (the conflicting placement of a taxon or clade is strongly supported in phylogenies derived from different data sets) was not apparent for any taxon or clade in the present study. Although the placement of *Arctotis perfoliata* and *Haplocarpha lyrata* conflicted between the cpDNA and ITS phylogenies, support was high for one data set only (cpDNA for *A. perfoliata*, ITS for *H. lyrata*). Exclusion of both species from analyses did not affect the resulting tree topology (data not presented), indicating the overall topologies reflect strong phylogenetic signal in the data. Both *A. perfoliata* samples possessed a unique 93 bp deletion in the *psbA-trnH* intergenic spacer and an additional seven unique 1 bp substitutions (three each in the *trnT-trnF* and ITS1 spacers and one in the *psbA-trnH* spacer). In morphology *A. perfoliata* possesses a combination of distinctive traits of the 'Cymbonotus' and 'core Arctotis' clades (McKenzie et al., 2005; R.J. McKenzie, unpublished data), which might reflect an ancestral hybridization event occurring during the evolution of *A. perfoliata*. However, incongruence between phylogenetic hypotheses might also reflect lineage sorting of ancestral polymorphisms, paralogy, lateral gene transfer, or erroneous phylogenetic reconstruction (Sang and Zhong, 2000). Sampling of additional accessions and DNA regions is needed to further resolve the phylogenetic relationships and evolutionary histories of *A. perfoliata* and *H. lyrata* and to establish what factors might account for the phylogenetic uncertainty.

The ITS data resolved highly supported annual and perennial clades within the 'core Arctotis' clade, in contrast to the cpDNA data, but this might reflect the fewer informative cpDNA characters or a need for additional cpDNA data, rather than hard incongruence in the phylogenetic signal. The annual and perennial *Arctotis* clades were well-supported in the total-evidence analyses, indicating any conflicting signal in the cpDNA data set was weak.

4.1. Phylogenetic relationships and taxonomic implications

Correspondence between relationships suggested by morphological and molecular data in Arctotidinae has been discussed previously (McKenzie et al., 2005, 2006a). Therefore the following discussion is focused on findings from the present study that build on those of previous studies.

The present study confirms the finding of McKenzie et al. (2006a) that *H. nervosa* and *H. rueppellii* are closely related and belong to the earliest-diverging extant clade ('Landtia'), and provides evidence that *H. parvifolia*, which was not included in the previous study, is a member of the same clade. This supports Beauverd's (1915) observation that *H. parvifolia* possesses similarities in mor-

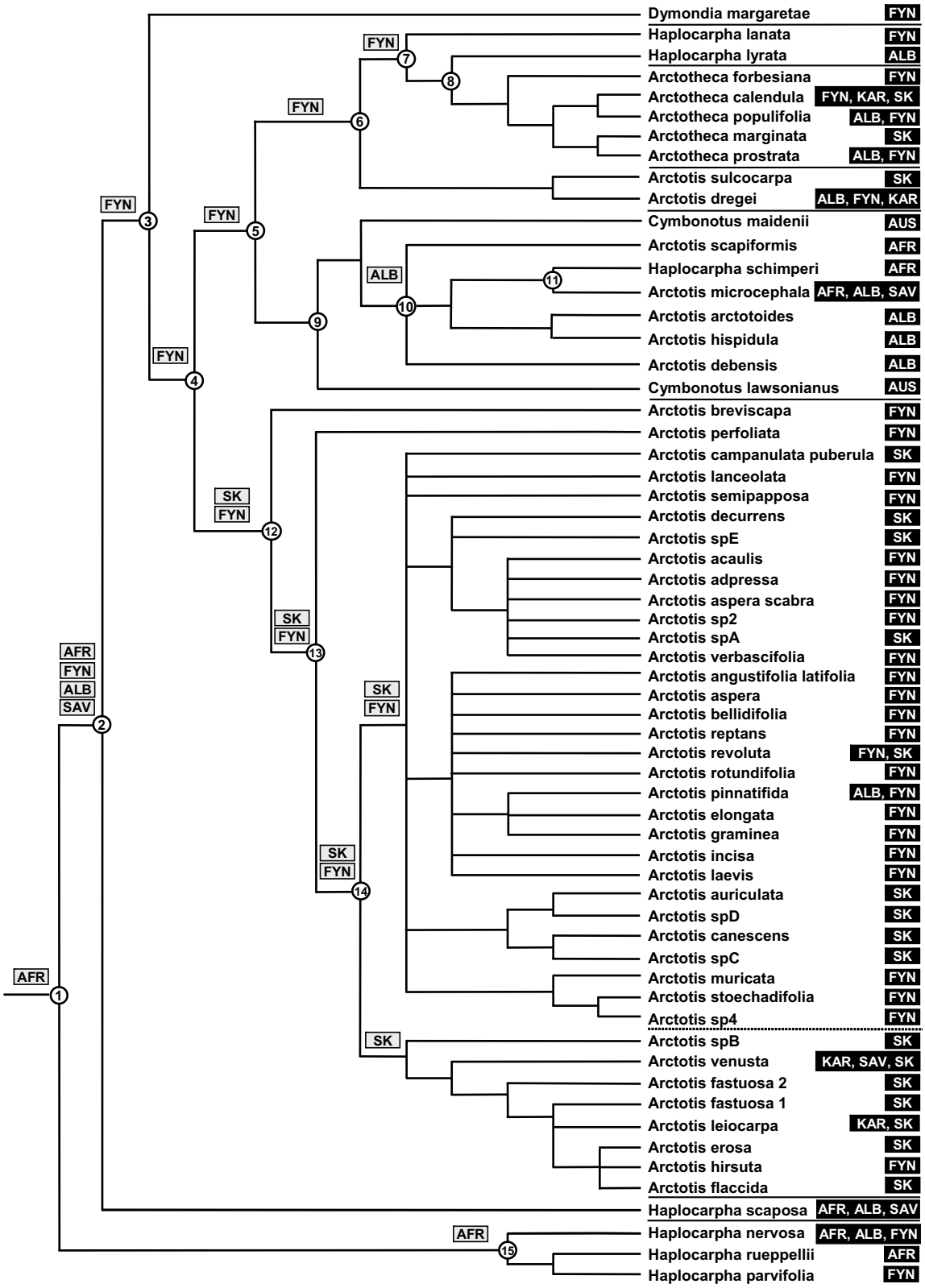

Fig. 4. Ancestral areas in Arctotidinae indicated by DIVA analysis, mapped on a simplified cladogram from Bayesian-inference analysis of the total evidence data set. Key to area codes: AFR, afromontane biome; ALB, Albany centre of endemism; AUS, Australia; FYN, fynbos biome; KAR, Nama-Karoo biome, SAV, savanna biome; SK, Succulent Karoo biome. These regions in black boxes represent extant distributions, those in grey represent ancestral areas. Numbers in circles and squares (calibration points) represent node numbers associated with node ages listed in Table 4.

phology to *H.* subg. *Landtia*, and specifically *H. rueppellii*. *Haplocarpha nervosa*, *H. rueppellii* and species now placed in their synonymy have been segregated as *Landtia* in the past (e.g. Lessing, 1831, 1832; Harvey, 1865; Bentham, 1873; Phillips, 1951). The morphological characters previously used to diagnose *Landtia* have been deemed untenable (e.g. Hedberg, 1957) and, especially focusing

on fruit morphology (McKenzie et al., 2005), the placement of *H. parvifolia* in the same clade further complicates morphological characterization of the clade. *Haplocarpha rueppellii* and *H. scaposa* possess a cypsela anatomy distinct from the other Arctotidinae sampled by Reese (1989), which thus might offer a possible synapomorphy for the 'Landtia' clade. Given the similarity in external vegetative morphology in the 'Landtia' clade, stem or leaf anatomy might also yield informative characters. Similarities in the morphology of *H. parvifolia* and *H. oocephala* have been noted previously (McKenzie et al., 2005, 2006a), but Funk et al. (2007) found that a sample of *H. oocephala* (misidentified as *H. lanata* in their paper) was placed on a monotypic lineage diverging after the 'Landtia' clade.

Placement of *H. scaposa* and *Dymondia margaretae* on monotypic branches in all analyses confirmed previous findings (McKenzie et al., 2006a). Segregation of *H. scaposa* into a monotypic genus is strongly supported by the molecular data, but the species shares certain distinctive morphological features (e.g. papillose filaments, pappus scales with long acute apices) with species in the 'core Haplocarpha' clade.

Sampling of all five *Arctotheca* species in the present study resolved a well-supported *Arctotheca* clade and confirmed a sister relationship with 'core Haplocarpha' (McKenzie et al., 2006a), but with the inclusion of *H. lyrata* in the *Arctotheca* clade in cpDNA-derived phylogenies. In the total-evidence analyses, 'core Haplocarpha' formed a basal grade sister to *Arctotheca*. As discussed earlier, sampling of additional DNA regions and multiple accessions is needed to resolve the evolutionary history of *H. lyrata*.

The two species of *Arctotis* sect. *Anomalae* sampled (*A. dregei* and *A. sulcocarpa*) formed a highly supported clade distinct from 'core Arctotis' in all analyses, in agreement with previous results (McKenzie et al., 2006a). Lewin (1922) distinguished sect. *Anomalae* from other sections of *Arctotis* by the possession of neuter ray florets (female in all other *Arctotis* sections), a trait also found in *Arctotheca* and *H. parvifolia*. The species of *A.* sect. *Anomalae* share some distinctive characteristics with 'core Arctotis', such as possession of cypselae with well-developed abaxial wings (McKenzie et al., 2005), but other features (e.g. possession of floral scent, papillose filaments) supports a sister relationship with 'core Haplocarpha'–*Arctotheca*.

Holland and Funk (2006) broadened the concept of *Cymbonotus* to encompass the three Australian-endemic species of Arctotidinae by formally transferring *Arctotis maidenii*. Our results demonstrate that the 'Cymbonotus' clade has an Afro-Australian distribution and also includes the East African *Haplocarpha schimperi* and southern African species currently placed in *Arctotis* sect. *Austro-orientales* (McKenzie et al., 2006a; this study). Our data resolved a well-supported 'Cymbonotus' clade, but whether it is sister to 'core Arctotis' or the *Arctotis* sect. *Anomalae* —'core Haplocarpha'— *Arctotheca* lineage remains uncertain, seemingly reflecting a rapid divergence of the three lineages. *Cymbonotus maidenii* was sister to *C. lawsonianus* and *C. preissianus* in the study of Funk et al. (2007), which utilized both cpDNA and ITS data, and was not monophyletic with *C. lawsonianus* in the present study, a finding consistent with disparities in the morphology of these species (McKenzie et al., 2005; R.J. McKenzie, unpublished data). A relationship between *C. maidenii* and *Arctotis perfoliata*, as indicated by cypsela morphology (McKenzie et al., 2005), is not supported by the molecular data.

A sister relationship between *Haplocarpha schimperi* and *Arctotis microcephala* within the 'Cymbonotus clade' is indicated. Despite the limited divergence in non-coding DNA sequences between the two species, *H. schimperi* has undergone greater evolution in phenotypic traits, especially in cypsela morphology (McKenzie et al., 2005), and possesses at least four autapomorphic character states absent in other members of the 'Cymbonotus' clade (geotropic capitula, woolly tomentum on the ray limb abaxial surface, a coronate pappus, and cypsela abaxial ribs not developed into wings).

The present study, in which 45 of the approximately 60 species of *Arctotis* were sampled, reinforces the conclusion of McKenzie et al. (2006a) that the sections *Anomalae* and *Austro-orientales* should be excluded from *Arctotis* in order to render the genus monophyletic. *Arctotis* sect. *Austro-orientales* (excluding *A. erosa* and *A. perfoliata*) has a close phylogenetic relationship with *Cymbonotus*, rather than with 'core Arctotis'. *Arctotis erosa*, an annual species placed in sect. *Austro-orientales* by Lewin's (1922), belongs in the 'annual Arctotis' clade in 'core Arctotis'. As discussed earlier, further investigations are needed to resolve the phylogenetic affinities of *Arctotis perfoliata*. In all analyses *Arctotis breviscapa*, an annual species endemic to sandveld in the southwestern Cape, was placed sister to the rest of the 'core Arctotis' clade and is indicated to be an early divergence from the 'core Arctotis' lineage. This agrees with Lewin's (1922) hypothesis of evolutionary relationships among his sections in *Arctotis*. *Arctotis breviscapa* is anomalous in possessing cypselae with two well-developed adaxial wings in addition to three abaxial wings (McKenzie et al., 2005), but is otherwise similar in morphology to 'core Arctotis'. The resolution of annual and perennial 'core Arctotis' clades by the ITS data conflicts with Lewin's (1922) infrageneric classification of *Arctotis*. These clades were not retrieved in the cpDNA phylogenies, which might reflect ancestral polymorphisms, hybridization and introgression, or insufficient informative sites in the cpDNA data.

4.2. Biogeography and timing of diversification of Arctotidinae

The estimated divergence dates presented in this paper are a first attempt at dating Arctotidinae diversification and must be interpreted with caution. Resolution of the timing of divergence events preferentially requires either a complete and reliably dated fossil record or reliable molecular mutation rates, neither of which are presently available for Arctotideae. Owing to the poor fossil record, obtaining reliable calibration points to date phylogenetic reconstructions within Asteraceae is a widespread problem. Rather than using an indirect calibration point derived from a higher-level molecular-dating analysis, which may potentially compound the error in estimated divergence dates (Graur and Martin, 2004), we utilized ITS mutation rates, representing the fastest and slowest extremes and an average rate, published for other Asteraceae taxa. It is acknowledged that the divergence dates estimate the evolution of the ITS region and not necessarily speciation or cladogenetic events. Natural selection is likely to act differentially on mutations (and thus evolution rates will vary) between loci and lineages. The strength of concerted evolution (Hillis et al., 1991) on the ITS region may vary between lineages and in lineages of ancestral hybrid origin. In addition, the similarity of the rates used to the overall ITS mutation rate in Arctotidinae is presently unknown.

Nevertheless, the results obtained permit the formulation of hypotheses regarding the biogeography and timing of diversification of Arctotidinae. Bearing in mind the likely Calyceraceae–Asteraceae divergence within the last 60 Mya based on the oldest fossil pollen evidence (Zavada and de Villiers, 2000), and that Arctotidinae comprise primarily annuals and fast-growing perennials that may flower in their first year after germination, divergence dates estimated with the 'fast' ITS mutation rate are considered the most plausible of the three mutation rates used in this study. Thus the following discussion pertains to the dates estimated with a 'fast' mutation rate.

Furthermore, the dating of Arctotidinae diversification with a 'fast' ITS mutation rate is compatible with the results of a recent molecular-dating study across all major clades of Asteraceae by Kim et al. (2005). Their study focused on the basal nodes and in-

cluded only one representative of Arctotideae (*Gazania krebsiana*), which was indicated to have diverged within the Liabeae–Arctotideae–Vernonieae clade approximately 25 Mya. However, the estimated divergence dates reported by Kim et al. (2005) may be slight underestimates, as the oldest microfossil evidence (Zavada and de Villiers, 2000) currently available for Asteraceae was not considered. Nevertheless, the divergence and early radiation in Arctotidinae may have followed expansion of the Antarctic ice cap, which covered most of that continent by about 17 Mya and had far-reaching global climatic impacts. In southwestern Africa a variety of new arid and semi-arid environments arose at this time (Pickford, 2004).

The ancestral area of Arctotidinae was indicated to be in our afromontane area. The present distribution of the two earliest-diverging extant lineages, the 'Landtia' clade and *Haplocarpha oocephala* (the latter based on Funk et al., 2007), comprises a chain of disjunct populations along the southern and eastern African mountains extending from the northern Cederberg area in the Western Cape province, South Africa to the Ethiopian highlands (Hedberg, 1957; Hilliard, 1977; Pope, 1992; Beyers, 2000; Mesfin Tadesse, 2004). The next lineage to diverge (*Haplocarpha scaposa*) is widespread in montane parts of southeastern Africa and the Zambezi River catchment area (Hilliard, 1977; Pope, 1992). These distributions roughly coincide with what has been termed the Afrotemperate Phytogeographical Region, which comprises the Cape Floral Region, the greater Drakensberg mountains, and the afromontane Centre (Linder, 1990; Galley et al., 2007). The more recent derivation of Arctotidinae lineages in presently semi-arid and arid areas is consistent with trends in rainfall regimes in southwestern Africa since the Oligocene (e.g. see Linder, 2003) and phylogenetic analyses of a diverse range of plant lineages endemic to the winter-rainfall region (Verboom et al., in press).

The estimated divergence of the 'Landtia' clade in the late Miocene follows the change to a more humid climate in the early mid-Miocene about 16 Mya (Dingle and Hendey, 1984). Mesic conditions persisted in southern Africa for about 7–8 Mya until the late Miocene (Partridge, 1997). The extant taxa in the 'Landtia' clade, as well as *H. oocephala* and *H. scaposa*, prefer mesic or perennially/seasonally wet habitats, including bogs, streambanks and seepages. *Dymondia margaretae*, the next-diverging extant lineage within Arctotidinae, is a component of the seasonally inundated vlei vegetation that is a significant component of the Agulhas Plain flora (Cowling et al., 1988). Thus a preference for mesic or hydric habitats might be plesiomorphic in Arctotidinae and reflect an ancestral niche. This pattern exhibits similarities to the radiations of *Ehrharta* (Verboom et al., 2003) and *Thamnocortus* (Linder and Hardy, 2005), in that the earliest-diverging extant species are found in mesic habitats and that xerophytic adaptations or invasion of semi-arid regions are indicated to be derived.

Most of the extant diversity in Arctotidinae was resolved into three well-supported lineages—'core Arctotis', 'Cymbonotus' and *Arctotis* sect. *Anomalae*–'core Haplocarpha'–*Arctotheca* clades—that were estimated to have diverged during a rapid radiation centred in southern Africa during the late Miocene or around the Miocene–Pliocene boundary. The present-day centre of Arctotidinae diversity is in the winter-rainfall region, but almost all of the species in this region were indicated to be derived. Based on our dating estimates, the extensive radiation in the winter-rainfall region coincided with the trend towards increasing rainfall seasonality and intensified aridification following increased glaciation in Antarctica from 14 Mya (Zachos et al., 2001), associated with strengthening of the South Atlantic high-pressure cell (Linder, 2005). In addition, the development of the Benguela Current about 11–14 Mya and summer drought in southwestern Africa are closely associated (Linder, 2003). The lower species diversity in the summer-rainfall region might reflect either a shorter time period available for diversification, less environmental heterogeneity compared to the winter-rainfall region, or the extant diversity might be relictual and that many species radiated but have gone extinct.

Our estimated dates for the radiation of Arctotidinae in the winter-rainfall region are consistent with estimates obtained for numerous other plant clades endemic or near-endemic to the region (see Linder, 2003, 2005; Verboom et al., in press). However, molecular-dating analyses indicate that the onset of radiations in the winter-rainfall region in different families has not been coordinated but has occurred over the course of at least the last 20 Mya (e.g. Linder et al., 2003, 2005; Schrire et al., 2003; Klak et al., 2003; Bakker et al., 2004; Mummenhoff et al., 2005; Verboom et al. in press).

The 'core Arctotis' clade, in which most of the extant diversity occurs in the Fynbos and Succulent Karoo biomes, was estimated to have diverged around the Miocene–Pliocene boundary. This is consistent with the appearance of fynbos vegetation, and a change to a cooler and drier climate in southwestern Africa, around the Miocene–Pliocene boundary (Linder, 2003). Divergence of the annual and perennial clades within 'core Arctotis' was estimated to have occurred during the late Pliocene. This followed closure of the Panamanian seaway and abrupt strengthening of the Benguela Current about 3.2 Mya, probably related to increased Antarctic glaciation, which further enhanced the seasonal aridity in the northwestern Cape during the past 3 Mya (Marlow et al., 2000). A hypothesized Pliocene origin of the annual life history in *Arctotis* is coincident with estimates for development of annualness in southern African *Nemesia* (Datson et al., 2008).

The 'annual Arctotis' clade is centreed in the semi-arid Succulent Karoo. The 'perennial Arctotis' clade is centred in the Fynbos with outlying species occurring in Namaqualand and the Albany hotspot, and DIVA resolved the ancestral area as being either or both the Fynbos or Succulent Karoo. It is evident that migration in both directions between the Fynbos and Succulent Karoo has occurred in both clades. This might reflect range expansion, contraction and refugial phases during Pleistocene climatic oscillations, which Midgley et al. (2001, 2005) suggested have promoted vicariance and allopatric speciation in the contiguous Fynbos and Succulent Karoo biomes. The Pleistocene climate is characterized by alternating cycles of short interglacial periods (10,000–20,000 years) and longer glacial periods (about 100,000 years; Petit et al., 1999). Expansion and contraction of the winter-rainfall zone during glacial and interglacial periods, respectively, is indicated (Chase and Meadows, 2007). During glacial cycles, much of the western coast of southern Africa and its adjacent interior probably experienced an increase in rainfall concentrated in the winter months (Tankard and Rogers, 1978). This would have facilitated expansion of the mesic and fire-adapted CFR taxa. Conversely, expansion of the semi-arid and drought-adapted Succulent Karoo taxa is hypothesized during the drier interglacial periods.

In contrast to 'core Arctotis', the extant diversity in the 'Cymbonotus' and *Arctotis* sect. *Anomalae*–'core Haplocarpha'–*Arctotheca* clades is more limited, particularly in the winter-rainfall region, yet they have undergone considerable range expansion in southern Africa and, in the case of 'Cymbonotus', successfully crossed the Indian Ocean to Australia. An ancestral area for the *Arctotis* sect. *Anomalae*–'core Haplocarpha'–*Arctotheca* lineage in the CFR was indicated, implying range expansion followed by speciation in southeastern Africa (*Arctotheca populifolia*, *A. prostrata* and *H. lyrata*), the Nama-Karoo and Succulent Karoo (*Arctotheca calendula*, *A. marginata*, *Arctotis dregei* and *A. sulcocarpa*). Divergence of the winter-annual *Arctotis* sect. *Anomalae* clade possibly during the early Pliocene suggests adaptation to summer-drought conditions.

An origin for the 'Cymbonotus' clade in southeastern Africa is indicated. The dispersal from southern Africa to Australia, possibly

during the Pliocene, occurred long after the severing of a land connection between Africa and Antarctica during the Cretaceous (McLoughlin, 2001). The geologic history of the Indian Ocean Basin since the breakup of Gondwana is well documented. There is no geological evidence for a land mass that might have acted as a 'raft' or continuous bridge between Africa and Australia during the last approximately 20 My. Dispersal could have been either direct or via the few small isolated islands, such as on the Kerguelen Plateau, which could have acted as terrestrial 'stepping stones' for some plants and animals (e.g. Linder et al., 2003; Schwarz et al., 2006). However, the cypselae of all species in the 'Cymbonotus' clade lack adaptations for long-distance dispersal (see McKenzie et al., 2005), so the mode of dispersal is difficult to envisage.

Increasing evidence indicates that many other Southern Hemisphere plant biogeographic patterns are best explained by hypotheses incorporating dispersal rather than Gondwanan vicariance alone (e.g. Baum et al., 1998; Mummenhoff et al., 2004; Cook and Crisp, 2005; Linder and Barker, 2005; Barker et al., 2007). Bidirectional dispersal of plants is implicated between most of the formerly Gondwanan land masses (Sanmartín and Ronquist, 2004). Molecular dating estimates indicate that trans-Indian-Ocean dispersal has occurred within the last 5 Mya in a number of diverse plant groups (e.g. Bakker et al., 1998; Meerow et al., 2003; Linder and Barker, 2005), and trans-oceanic dispersal from Malesia is implicated for elements of Indian Ocean island floras (Renvoize, 1979), indicating that the Indian Ocean is not an insuperable barrier to angiosperm dispersal.

The nesting of *H. schimperi* within the 'Cymbonotus' clade presents another biogeographic puzzle. *Haplocarpha schimperi* is distributed from Eritrea to Tanzania at moderate to high altitudes (Mesfin Tadesse, 2004). Its sister species, *Arctotis microcephala*, is distributed in southern Namibia, southern Botswana, northern South Africa and Lesotho, and occurs at high altitudes in the Eastern Cape and Drakensberg mountains, which thus provide a possible afromontane link between the two species. Northward migration of *A. microcephala* along the eastern African mountain corridor during more mesic Quaternary interglacial periods, followed by extinction in the intervening region in response to increasing aridity, or long-distance migration followed by speciation, are alternative explanations for the present-day distribution. Regardless, divergence of *A. microcephala* and *H. schimperi* is indicated to have been recent, possibly during the Pleistocene.

In conclusion, this study presents a comprehensive molecular phylogenetic investigation into a group of southern African Asteraceae. Our findings correspond with previous studies on the Cape flora in indicating that radiation might be linked to climate changes around the late Miocene and Pliocene. Only one of the clades identified here (the 'core Arctotis' clade) corresponds to a 'Cape clade' *sensu* Linder (2003), and the origins, biogeography, taxonomy and morphology of the subtribe as a whole are rather more complex. The phylogenetic reconstructions resolved well-supported clades and provide the framework for a revised taxonomic classification of Arctotidinae. Future studies focusing on the basalmost lineages and sister taxa are needed to further elucidate the origins of Arctotidinae.

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