Phylogenetics and biogeography of the parasitic genus *Thesium* L. (Santalaceae), with an emphasis on the Cape of South Africa

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Thesium is a large genus of parasitic shrubs belonging to tribe Thesieae of Santalaceae. It has a principally Old World distribution, with the greatest diversity being found in southern Africa. Little is known about the relationships within *Thesium* or its relationships with its closest relatives. In this article, we present a first estimate of species-level phylogenetic relationships in *Thesium* based on internal transcribed spacer (ITS) and *trnL–trnF* sequence data, and use this to explore the biogeographical history of the group. One hundred and four samples representing 72 *Thesium* spp. were included in a phylogenetic analysis. Plastid and combined data resolve *Thesium* as paraphyletic relative to *Thesidium* and *Austroamericium* with high posterior probability and bootstrap support. ITS sequence data place *Thesidium* as sister to a large *Thesium* clade, but with weak support. Ancestral range reconstruction and dating analysis suggest a southern African origin for the group, with a crown age of 39.1 ± 11.9 Mya, followed by dispersal into Europe and South America. A large clade of Cape species split in the Miocene from a clade comprising tropical species (25.5 ± 7.3 Mya) with the diversification of extant species beginning at 16.7 ± 6.3 Mya. © 2010 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2010, **162**, 435–452.

ADDITIONAL KEYWORDS: hemiparasite – parasite – southern Africa – trans-Atlantic dispersal.

INTRODUCTION

Thesium L. is a large genus of predominantly perennial root-parasitic shrubs (sometimes referred to as hemiparasites as they possess chlorophyll) belonging to tribe Thesieae Reichenb. of Santalaceae. *Thesium* spp. obtain some of their nutritional needs from other plants. Species in the genus are often unattractive, having a yellowish colour and lacking leaves. The flowers are frequently small and creamy-white, and most species are probably pollinated by small bees and flies (Hendrych, 1972).

Thesium has a principally Old World distribution, with the greatest diversity being found in southern Africa. Of the *c*. 300 species of *Thesium* (Mabberley,

2008), approximately 150 are native to southern Africa, a further 60 species occur in tropical and northern Africa, and the rest of the genus is primarily distributed in Europe and Asia. Only three species occur in South America, with two species centred in Brazil and a third native to Venezuela. *Thesium* is widely distributed in South Africa, with high densities of species in the Cape (or Cape Floristic Region; Manning & Goldblatt, 2000). With 81 currently recognized species native to the Cape, including 35 endemics, *Thesium* is one of the largest genera in the region (Goldblatt & Manning, 2002). Despite its species' richness, *Thesium* has received little explicit systematic attention, either locally or globally. Such systematic understanding is important for the understanding of the evolution of biotic communities. An increasing grasp of the phylogenetic relationships of Cape taxa and their non-Cape relatives has been vital

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for furthering our understanding of the patterns and processes that have driven the evolution of the Cape region as a whole (Linder, 2003; Galley & Linder, 2006; Verboom *et al.*, 2009).

For decades, the origin and evolution of the Cape flora has been the subject of some debate (see Levyns, 1964; Axelrod & Raven, 1978; Galley & Linder, 2006; Galley, Linder & Zimmermann, 2009). Levyns (1964) hypothesized a tropical African origin for many Cape lineages, with subsequent migration southwards into the Cape. Other authors have suggested a vicariance scenario, in which the Cape flora represents a relic of a previously widespread African flora (Wild, 1968; King, 1978). Recently, Galley *et al.* (2007) have shown that there is a general trend for unidirectional migration from the Cape into the Drakensberg (in the eastern part of South Africa) and thence northwards into tropical Africa. With strong affinities to other floristic centres outside of the Cape region, *Thesium* is an ideal candidate for investigating the relationships of the Cape with other floras. Hendrych (1972) hypothesized that *Thesium* originated in southern Africa, subsequently migrating north into the rest of Africa and further into Europe and Asia. This hypothesis has not yet been evaluated in a phylogenetic context against the alternatives that: (1) *Thesium* biogeography is the result of vicariance, following isolation caused by climatic fluctuations, or (2) *Thesium* originated in the north, migrating south with subsequent diversification in southern Africa.

Most systematic work on the genus has been based primarily on the morphological and geographical attributes of the species (Fig. 1). Originally described by Linnaeus (1753), *Thesium* received limited atten-tion until the 1800s, early treatments being hampered by poor species' sampling and often focusing on particular geographical regions (Hendrych, 1972). Throughout the first half of the 19th century, three infrageneric groups were recognized at sectional level, namely *Frisea* Endl., *Thesiosyris* Endl. and *Thesium* R.Br. (= *Euthesium* Benth.). These sections were broadly distinguished by floral morphology, especially flower shape and perianth structure, and geographical distribution, with *Thesium* being predominantly European and *Frisea* and *Thesiosyris* predominantly occurring in southern Africa.

The first comprehensive treatment of the genus was conducted by De Candolle (1857a, b). He based his subgeneric classifications on more material than had been examined by previous authors, examining all 112 species known at the time. Within a single year, De Candolle produced two treatments, first (1857a) dividing *Thesium* into five sections, but soon modifying this scheme and splitting the genus into six sections (Table 1). In his second scheme, De Candolle accepted the separation of the genus *Thesidium* from *Thesium*, as proposed by Sonder (1857). *Thesidium* is a small genus (approximately eight species) of parasitic shrubs endemic to the southern part of Africa, where its range overlaps completely with that of *Thesium*. It differs from *Thesium* in being monoecious and having unisexual, tetramerous flowers. Recent molecular work (Der & Nickrent, 2008) has identified *Thesidium* as sister to *Thesium*, but limited sampling hampered the ability of these authors to evaluate the monophyly of the two genera.

In his treatment of the genus, Hill (1915) created four new sections (Table 1, see also Fig. 1). His treatment, however, was based on a limited geographical

Table 1. Broad level infrageneric classifications of *Thesium*, and geographical locality of each major grouping

De Candolle (1857b)	Hill (1915)	Pilger (1935)	Hendrych (1972)	Region
Section *Frisea*	Section *Annulata*	Section *Frisea**	Subgenus *Frisea*†	Southern Africa
	Section *Penicillata*			Southern Africa
	Section *Barbata*			Southern Africa
Section *Euthesium* [= *Thesium*]	Section *Imberbia* -Subsection *Fimbriata*	Section *Euthesium* [= *Thesium*]	Subgenus *Thesium* [= *Thesium*]	Africa/Eurasia
Section *Discothesium*	-Subsection *Subglabra*			Africa/Eurasia
Section *Aetheothesium*	-Subsection *Subglabra*			Africa/Eurasia
Section *Chrysothesium*		Section *Chrysothesium*	Subgenus *Chrysothesium*	Eurasia
Section *Psilothesium*		Section *Psilothesium*	Genus *Austroamericum* (Hendrych, 1963)	South America
Genus *Thesidium* (Sonder, 1857)		Genus *Thesidium*	Genus *Thesidium*	Southern Africa

*Hill's sections *Annulata*, *Penicillata*, *Barbata* retained as series of Pilger's section *Frisea*.
†Hill's sections *Annulata*, *Penicillata*, *Barbata* retained as sections of Hendrych's subgenus *Frisea*.
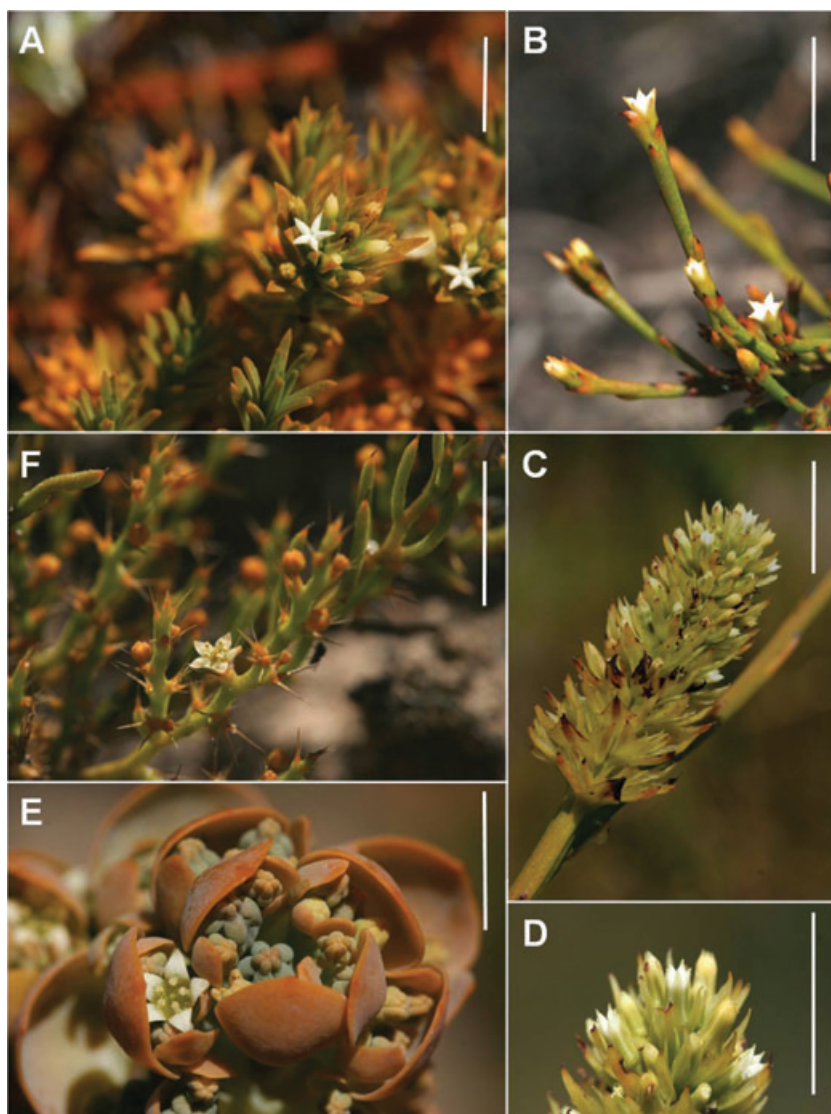
Figure 1. Species representing three of Hill's (1915) infrageneric groupings. Species shown are as follows: A, *Thesium carinatum* (section *Barbata*); B, *T. euphrasioides* (section *Barbata*); C, D, *T. spicatum* (section *Annulata*); E; *T. euphorbioides* (section *Imberbia*); F, *T. spinulosum* (section *Imberbia*). All scale bars, 1 cm. All photographs taken at Baardskeerdersbos, Western Cape, South Africa by GAV.

sample, as he focused on species from South Africa, paying little attention to species found outside southern Africa. As a result of this sampling bias, Hill's treatment was not readily reconcilable with that of De Candolle. It was Pilger (1935) who first attempted to address the mismatch between these two treatments by examining a large number of *Thesium* spp. from across the distribution of the genus. In his treatment, *Thesium* was split into four sections (Table 1) which broadly agree with De Candolle's and earlier authors' works, but incorporate the groupings that Hill had devised for the South African *Thesium* spp. The majority of species surveyed by Hill fall into Pilger's section *Frisea*, whereas some (from section *Imberbia*) fit into *Thesium* [as *Euthesium*] (Table 1).

The most recent comprehensive study of the genus is that of Hendrych (1972), who attempted to provide a broad outline of the group and revise the existing subgeneric Northern Hemisphere classifications. His study was, however, limited because of its focus on Northern Hemisphere species, paying little attention to southern African species. He offered little insight into the origin and evolution of the Cape members of *Thesium* or into the phylogenetic relationships within the genus. In addition, Hendrych focused

solely on morphology and geography, not taking molecular data into account. Hendrych's treatment included three subgenera within *Thesium*: *Frisea*, *Thesium* and *Chrysothesium* DC. (Table 1). Hendrych (1963, 1972) also removed the three South American species from *Thesium* to a new genus, *Austroamericium*. This was based on their disjunct distribution relative to the rest of *Thesium* and their divergent morphological attributes, particularly fruit and floral morphology.

Phylogenetic data have gained increasing importance in both the delimitation of higher taxa (Schrire & Lewis, 1996; De Queiroz, 2007) and in tracing the evolutionary histories of lineages, including their biogeographical origins (Avise, 1994). In order to enhance the utility and predictive power of higher taxa, taxonomists set out to reclassify taxa that are found to be paraphyletic into monophyletic groupings (Backlund & Bremer, 1998). Phylogenetic studies of *Thesium* are few, and those that exist generally focus at a higher taxonomic level (e.g. Der & Nickrent, 2008), and sample a limited number of *Thesium* spp. As a result, relationships amongst *Thesium* spp. remain poorly understood (Hendrych, 1972), as does the relationship between *Thesium* and its closest relatives.

In this article, we present a first estimate of species-level phylogenetic relationships in *Thesium* using plastid and nuclear molecular data with a particular focus on Cape species, and use this to explore the taxonomy and biogeographical history of the group, employing Bayesian- and parsimony-based phylogenetic analyses. Specifically, we aim to: (1) evaluate the relationships between *Thesium* and its segregate genera *Thesidium* and *Austroamericium* (*sensu* Hendrych, 1963); (2) assess relationships among *Thesium* spp., especially in relation to existing infrageneric classifications; and (3) evaluate, from a molecular phylogenetic perspective, Hendrych's southern origin hypothesis for the genus *Thesium*.

MATERIAL AND METHODS

TAXON SAMPLING

One hundred and four accessions (Appendix, Table 2), representing 72 *Thesium* spp., were sampled for the phylogenetic analysis. Fifty-seven of the sampled species were from South Africa, with a further 14 species sampled from tropical Africa, Europe and Asia. Nine *Thesium* (seven Eurasian, two tropical African) and two *Austroamericium* species were sampled from herbarium specimens housed at the Royal Botanic Gardens, Kew (K; see Appendix). Three *Thesidium* spp. were also sampled. *Buckleya lanceolata* (Thesiae), *Exocarpus spartens* (Anthoboleae)

Table 2. Number of species sampled according to region

Region	Number of species	Species sampled
Southern Africa	160	57
Tropical Africa	82	16
Eurasia	79	7
South America	3	2
North Africa and Madagascar	13	0

and *Leptomeria cunninghamii* (Osyrideae) were sampled as outgroup taxa.

DNA ISOLATION, AMPLIFICATION AND SEQUENCING

Total DNA was extracted from silica-dried, field-collected samples or herbarium material using a modified version of the cetyltrimethylammonium bromide (CTAB) extraction protocol outlined in Doyle & Doyle (1987). Samples were ground using a small amount of fine silica in mortars preheated to 65 °C to facilitate grinding. DNA was precipitated in ethanol (for silica-dried samples) or isopropanol (for herbarium samples) at −20 °C for up to 10 days. Additional purification was performed using a caesium chloride/ethidium bromide gradient (Csiba & Powell, 2006) and a dialysis procedure. All DNA samples were stored in the DNA bank at the Royal Botanic Gardens, Kew (http://data.kew.org/dnabank/homepage.html).

One plastid region and one nuclear region were selected. The *trnL–trnF* region of the plastid genome (spacer and intron) was amplified in one reaction using the primers c and f designed by Taberlet *et al.* (1991). In some cases, amplification also required the use of the internal primers d and e. Polymerase chain reactions (PCRs) were performed in 25 μL volumes, containing 22.5 μL of ReddyMix PCR Master Mix (containing 2.5 mM $MgCl_2$; ABgene, Epsom, Surrey, UK), 0.5 μL of bovine serum albumin (BSA; 0.04%), 0.5 μL of each primer and 1 μL of DNA template. The ribosomal internal transcribed spacer (ITS) from the nuclear genome was generally amplified using primers 17SE and 26SE of Sun *et al.* (1994), but, when these reactions failed, the ITS region was amplified using the ITS4 and ITS5 primers (White *et al.*, 1990). Each 25 μL reaction contained 21.5 μL of ReddyMix PCR Master Mix (containing 1.5 mM $MgCl_2$), 1 μL of dimethyl sulphoxide (DMSO), 0.5 μL of BSA, 0.5 μL of each primer and 1 μL of DNA template. Amplification of both regions employed the same thermal profile: an initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 60 s, 45 °C for 60 s and 72 °C for 90 s, and completed with a final

extension of 72 °C for 4 min. PCR products were then purified on columns (Nucleospin® Extract II minicolumn kit; Macherey-Nagel, Düren, Germany). Cycle sequencing was performed using the BigDye Terminator Cycle Sequencing kit (version 3.1; Applied Biosystems, Warrington, Cheshire, UK) following the manufacturer's protocol, and the same primers as used for amplification. Products from the cycle sequencing reactions were cleaned on a Biomek NXS8 automated workstation (Beckman Coulter, High Wycombe, Buckinghamshire, UK) and visualized on a 3730 DNA Analyser (Applied Biosystems).

Complementary sequences were assembled and edited using Seqman (DNASTAR Inc., Madison, WI, USA) and aligned in MegAlign (DNASTAR Inc.) using the CLUSTALW (Thompson, Higgins and Gibson, 1994) alignment algorithm. Automatically aligned sequences were then exported in BioEdit v7.0 (Hall, 1999) and the alignments were checked and edited by eye. Simple gap coding was implemented in GapCoder (Young & Healy, 2003) to incorporate any insertion/deletion (indel) information present. Indels for which homology could not be confidently inferred (especially indels adjacent to homopolymers), and stretches of DNA sequence that could not be confidently aligned, were excluded from subsequent phylogenetic analyses. Stretches of sequence for which outgroup taxa could not be aligned to ingroup taxa with any confidence were treated as unknown for the outgroup taxa. All sequences generated as part of this study have been submitted to GenBank (Appendix), and aligned matrices to TreeBase (www.treebase.org: matrix number SN4795-25231).

PHYLOGENETIC ANALYSES AND MOLECULAR DATING

Phylogenetic relationships were inferred using both parsimony and Bayesian inference. Prior to combined analysis of the plastid and nuclear gene regions, separate parsimony analyses, comprising only terminals for which both regions were available, were conducted to allow for an evaluation of incongruence. These separate analyses included only those accessions for which both plastid and nuclear sequences were available (72 species). Combined analyses, however, included all available accessions. The incongruence length difference (ILD) test (Farris *et al.*, 1994), as implemented in PAUP 4.0b10 (Swofford, 2002), was conducted to test for incongruence. Owing to the propensity of the ILD test to type I error (Cunningham, 1997), conflict was considered to be significant if the P value was below $\alpha = 0.01$. Separate trees for each locus were also visually compared, with conflict considered to be supported when both conflicting nodes had bootstrap (BS; Felsenstein, 1985) support greater than 75%. Parsimony searches were conducted heuristically in PAUP 4.0b10 (Swofford, 2002), with 10 000 random addition replicates, tree bisection–reconnection (TBR) branch swapping and MULTREES in effect. BS values were calculated on the basis of 500 replicates, each involving a heuristic search set-up as follows: simple addition sequence, TBR branch swapping and MAXTREES set to 500. Bayesian searches were conducted in MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001), with different DNA substitution models implemented for the different data partitions (i.e. ITS, *trnL–trnF* and indels). A GTR + I + Γ model (Yang, 1994) was selected using ModelTest 3.7 (Posada & Crandall, 1998) applied to both sequence partitions, and a Markov-k model (Lewis, 2001) was applied to the coded indel data. The analyses were performed using two independent MCMCMC runs, each comprising four chains (one cold and three heated), and a random starting tree. Each chain was run for 10^6 generations, sampling every 100th generation, giving a total of 10 000 samples per run. Plots of the log-likelihood scores against generation time were generated in Tracer v1.3 (Rambaut & Drummond, 2005) to determine when stationarity was achieved, and thus to estimate the 'burn-in' period. At the same time, the effective sample size (ESS) for each parameter of the models used was also employed to confirm sampling adequacy. Trees from the 'burn-in' were discarded prior to the calculation of posterior probabilities (PPs).

Molecular dating was conducted in BEAST v1.4.2 (Drummond & Rambaut, 2007), using a log-normal relaxed clock. Dating was conducted on both nuclear and plastid data and on the combined data (see Pfeil, 2009). Because the dataset comprised two loci, a mixed model was used, with separate models applied to nuclear and plastid datasets (GTR + I + Γ). Molecular evolution model parameters were assigned flat priors, whereas tree priors were modelled according to a Yule speciation process. The analysis was calibrated by setting a single age prior estimated for the divergence between *Leptomeira + Exocarpus* and *Buckleya + Thesium*. A normal calibration prior with a mean of 73.65 and standard deviation of 7.00 was used, based on a separate dating of a higher level phylogenetic analysis (Der & Nickrent, 2008). Calibration for that analysis was based on fossil evidence for the divergence between Santalaceae and Misodendraceae + Loranthaceae (see Vidal-Russell & Nickrent, 2008). Again, log-likelihood scores were plotted against generation time, together with ESS values, and visualized in Tracer, with 'burn-in' trees discarded prior to generating the consensus tree.

Distributions of South African *Thesium* species were scored using Jordaan (2003), whereas non-South African species were characterized for broad geo-

Table 3. Tree statistics for internal transcribed spacer (ITS), *trnL–trnF* and the combined dataset. Consistency indices (CI) were measured excluding parsimony-uninformative characters.

Marker	Number of sites	Potentially parsimony informative	CI	RI	Tree length
ITS	937	295 (31.5%)	0.54	0.79	1033
trnL–trnF	1272	353 (21.8%)	0.65	0.83	833
Combined	2209	624 (28.3%)	0.54	0.80	2108

RI, retention index.

graphical distribution (Steyermark, 1951; Hendrych, 1964; Polhill, 2005). Ancestral character state reconstruction was conducted in Mesquite v2.5 (Maddison & Maddison, 2008), using likelihood optimizations according to Galley *et al.* (2009). Reconstruction was conducted on the tree with maximum PP obtained from the BEAST search. Nodes were reconstructed for a given area, if they were significantly present for that area and significantly absent for all other areas. Nodes that were significantly absent from all areas were treated as equivocal.

RESULTS

Aligned matrices of ITS and *trnL–trnF* were 937 and 1272 bases long, respectively (Table 3). Separate analyses consisted of 72 ITS and *trnL–trnF* sequences, and combined analyses consisted of 91 ITS and 86 *trnL–trnF* sequences. ITS had higher ingroup sequence divergence (0–17.5% vs. 0–9.0%) and a higher percentage of potentially parsimony-informative characters (31.5% vs. 21.8%) than *trnL–trnF*. The *trnL–trnF* region, however, displayed the greatest variation in length and, consequently, had more indels (158 vs. 64). A summary of the tree statistics is shown in Table 3.

SEPARATE ANALYSES

The trees derived from the separate plastid and ITS analyses are presented in Figure 2. The plastid data resolve *Thesium* as paraphyletic relative to *Thesidium* and *Austroamericium* with high PP and BS support (PP = 1.00; BS = 100). In contrast, ITS sequence data place *Thesidium* as sister to a large clade consisting of all *Thesium* spp. sampled, but with weak support (PP = 0.53; BS < 50) for the monophyly of the latter. In general, the ITS tree is better supported, especially at deeper nodes, than the plastid tree, which is consistent with its higher proportion of potentially informative characters (Table 3). Results of the ILD test suggest that nuclear and plastid data are not significantly discordant (*P* > 0.01), although supported conflict (BS > 75) occurs in two areas.

Firstly, the plastid data place *Thesium ericaefolium* A.DC. in a clade of species mostly drawn from Hill's section *Annulata* (BS = 88), whereas ITS places it as sister to a second accession of *T. ericaefolium* (BS = 96), both of these being resolved as sister to *T. glomeruliflorum* Sond. (BS = 97). Secondly, within a Cape-dominated clade which is, in general, poorly resolved, the plastid data place *T. hispidulum* Lam. as sister to *T. polycephallum* Schltr. (BS = 79), *T. paniculatum* L. as sister to one sample of *T. capituliflorum* Sond. (BS = 79) and *T. carinatum* A.DC. as sister to another sample of *T. capituliflorum* (BS = 99). In contrast, ITS places *T. hispidulum* as sister to *T. carinatum* (BS = 100) and *T. capituliflorum* as sister to *T. capituliflorum* (BS = 97). We consider this incongruence as minor and, as only terminal branches are involved, a plastid/nuclear combined analysis was conducted.

COMBINED ANALYSES

Combining ITS and plastid data generally strengthened nodal support values (Fig. 3). As in the separate plastid analysis, both *Thesidium* and *Austroamericium* are nested within *Thesium*, the monophyly of each being strongly supported (Fig. 3: PP = 1.00, BS = 100). Within *Thesium*, two principal clades are resolved with varying degrees of support (Fig. 3). Clade 1 (PP = 1.00, BS = 100) comprises two well-supported clades: a Eurasian clade (PP = 1.00, BS = 100) and the *Thesidium* clade (PP = 1.00, BS = 100). Clade 2, with high posterior and low BS support (PP = 1.00, BS < 75), is dominated by two principal clades: a Cape clade (PP = 1.00, BS < 75) and a strongly supported Tropical clade (PP = 1.00, BS = 100), including the two sampled South American species of *Austroamericium*. Completing Clade 2 is a South African-centred grade comprising *T. pungens* + *T. spinulosum* (PP = 1.00, BS < 50) and *T. triflorum* + *T. squarrosum* (PP = 1.00, BS < 50). Deep relationships within the Cape clade are poorly resolved and unsupported, possibly reflecting rapid diversification. Some assemblages are, however, resolved, although support is weak to moderate.
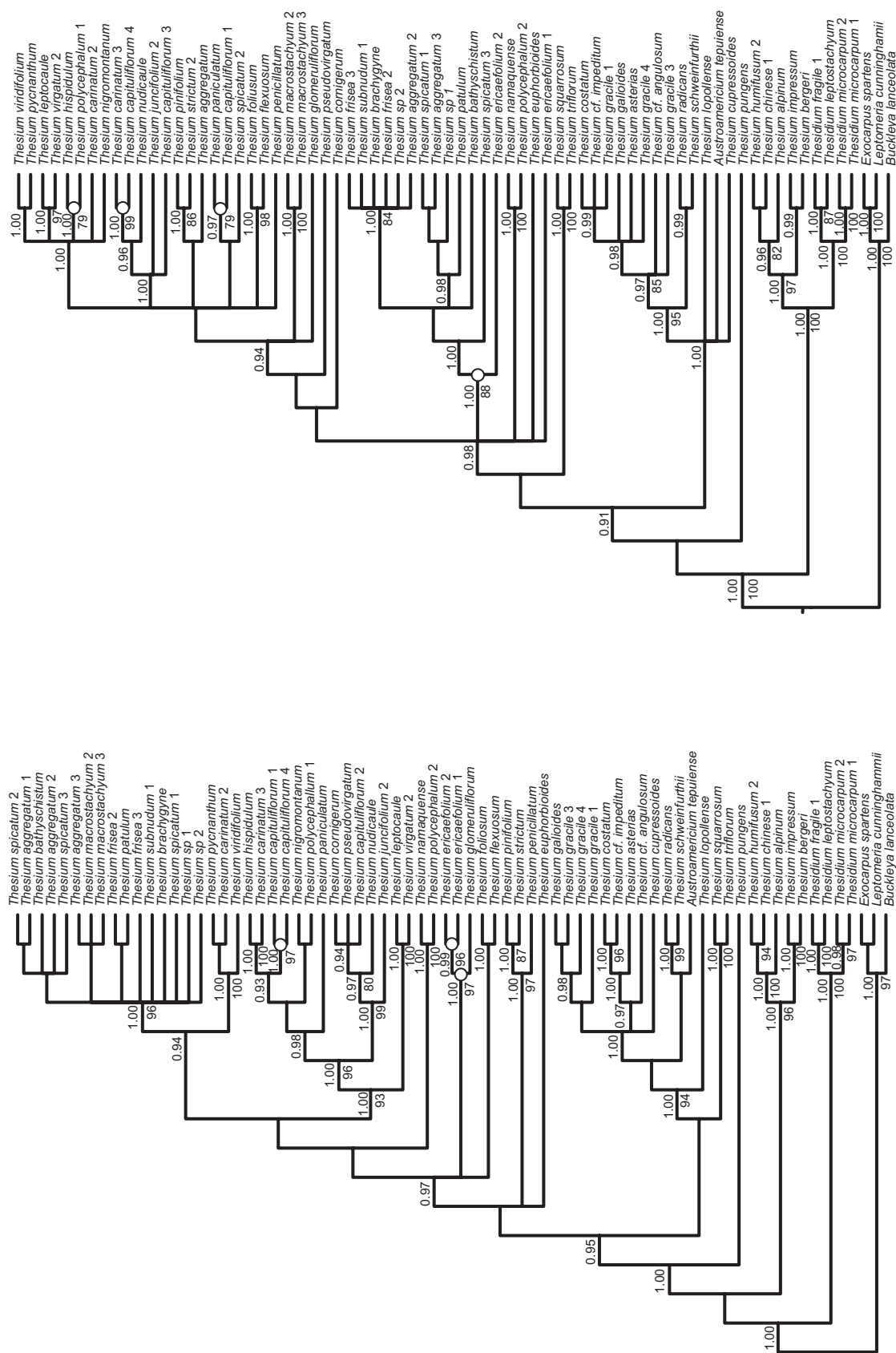
Figure 2. Consensus trees resulting from the separate Bayesian analyses of internal transcribed spacer (ITS) and *trnL–trnF* datasets. Only taxa with both plastid and ITS data available were included in these separate analyses. Posterior probabilities above 0.90 and bootstrap values above 75 are shown above and below the branches, respectively. Open circles indicate incongruent clades discussed in the text.
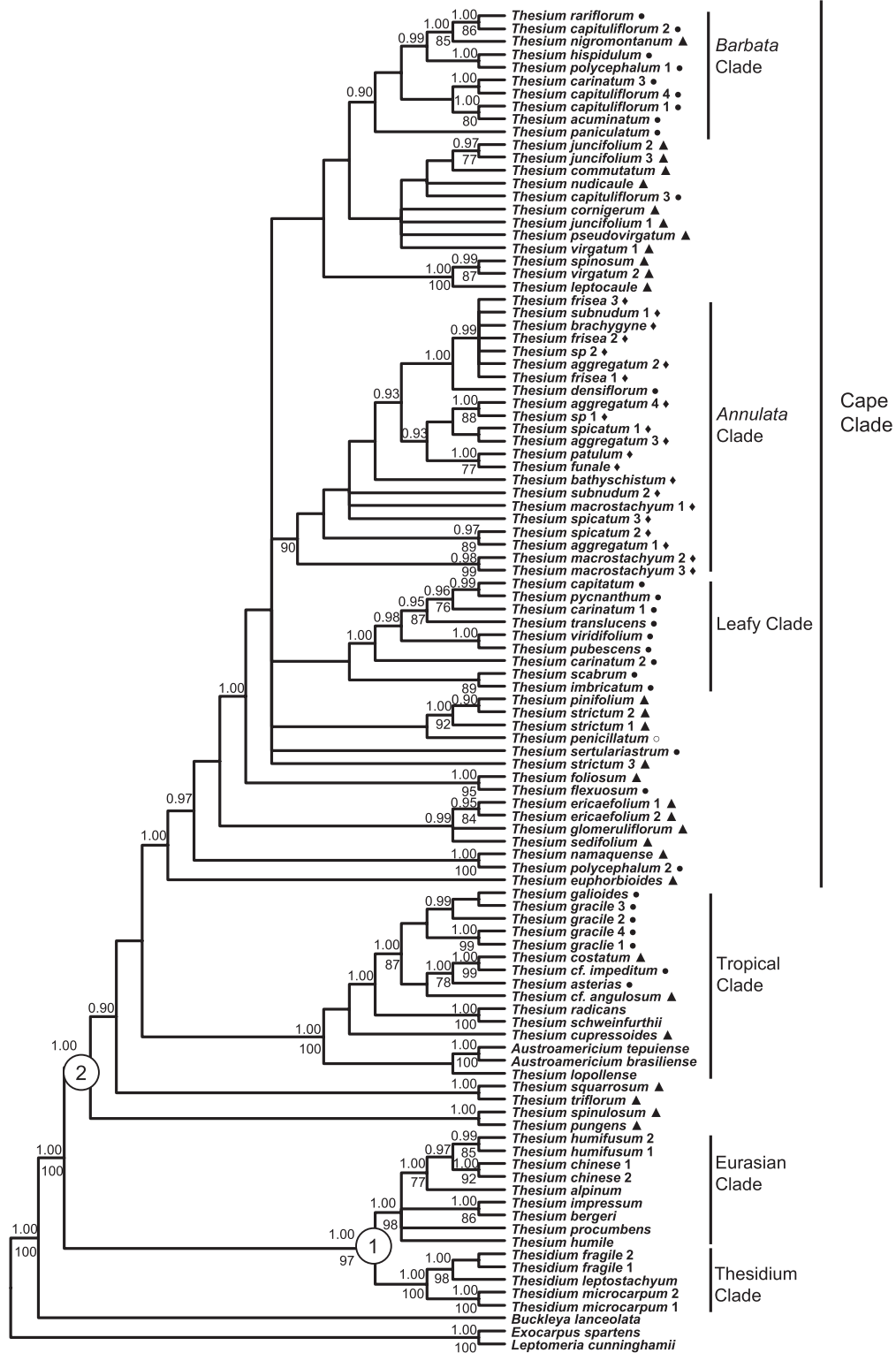
Figure 3. Consensus tree of the combined internal transcribed spacer (ITS) and *trnL–trnF* Bayesian analysis. Posterior probabilities above 0.90 and bootstrap values above 75 are indicated above and below the branches, respectively. Clades referred to in the text are labelled. Symbols next to South African species' names indicate the subgeneric classification based on Hill (1915) and are coded as follows: ●, section *Barbata*; ▲, section *Imberbia*; ○, section *Penicillata*; ◆, section, *Annulata*.

Table 4. Results of separate dating analyses. Numbers in parentheses below regions indicate the number of taxa used

Node	Comment	ITS (72)	*trnL–trnF* (72)	Combined (72)	Combined (104)
1	Origin	42.7 ± 13.8	37.2 ± 14.2	35.9 ± 11.5	39.1 ± 11.9
2	Divergence between Eurasian species and *Thesidium* spp.	36.6 ± 12.6	24.1 ± 12.1	28.5 ± 9.9	29.8 ± 10.7
3	Cape origin (maximum age)	25.6 ± 9.9	30.1 ± 13.1	22.2 ± 18.6	25.5 ± 7.3
4	South American divergence	10.1 ± 4.4	8.9 ± 7.7	9.6 ± 4.9	13.4 ± 6
5	Diversification within Cape clade	17.2 ± 6.9	22.9 ± 11.4	13.9 ± 4.9	16.7 ± 6.3

These include a clade (PP = 0.78, BS = 0.90) of species from Hill's section *Annulata* and a clade of 'leafy' *Thesium* species, essentially representing Hill's section *Barbata* (PP = 0.79, BS < 50). Where species were represented by multiple accessions, these generally grouped together in the same major clade, but often failed to form sister relationships with conspecific accessions (Fig. 3).

BIOGEOGRAPHY AND DATING

In general, dates were consistent across analyses based on separate gene regions, and those based on the combined data with full and reduced taxon sampling (Table 4). Broad-scale ancestral range reconstruction (Fig. 4) suggests a southern African origin for the genus, with subsequent dispersal into the rest of Africa and single dispersal events to South America and Europe. The age of the crown node of *Thesium* (Fig. 4: Node 1) is dated to the late Eocene (39.1 ± 11.9 Mya). Within *Thesium*, the divergence of the Eurasian clade from *Thesidium* dates to the Oligocene, 29.8 ± 10.7 Mya (Fig. 4: Node 2), and the origin of the Cape clade to the Miocene (25.5 ± 7.3 Mya; Fig. 4: Node 3). The crown age for the group is 21.3 ± 8.0 Mya. The latter is succeeded by a relatively recent diversification event, beginning at 16.7 ± 6.3 Mya (Fig. 4: Node 5). The South American clade appears to have diverged from its tropical African sister around 13.4 ± 6.0 Mya (Fig. 4: Node 4).

DISCUSSION

In combination, ITS and *trnL–trnF* yielded a well-resolved and, in places, strongly supported phylogenetic hypothesis for the genus *Thesium*. The reciprocal monophyly of *Thesium*, *Thesidium* and *Austroamericium* is contradicted, suggesting a need for generic realignment, although the formalization of this lies outside the scope of this study. Finally, ancestral area reconstruction supports the notion of a southern African origin for the group, with subsequent dispersal out of Africa, and rapid speciation in the Cape.

Concerns have been raised over the use of ITS in phylogenetic studies because of the risk of paralogy associated with the region (Álvarez & Wendel, 2003). A lack of strong and widespread conflict between ITS and plastid data (Fig. 2), however, suggests that paralogy is not a major problem in this study. Where conflict is observed, three possible explanations exist. Firstly, incongruence may be the result of sampling error (Hipp, Hall & Sytsma, 2004). Alternatively, lineage sorting (Avise, 1994; Maddison, 1997) and hybridization (Mckinnon *et al.*, 1999) may cause discordance between datasets, particularly where effective population sizes are large relative to the time since divergence (Maddison & Knowles, 2006). In the current study, the majority of conflict between markers is restricted to geographically distinct clades (Fig. 2), the taxa involved typically having broad distributions. This, coupled with the relatively recent nature of the group (especially in terms of Cape species, see below; Fig. 4), suggests that lineage sorting may account for most of the observed conflict.

Recent divergence also explains the observed pattern of species-level paraphyly (Fig. 3). In addition, many of the species represented here as paraphyletic have broad geographical distributions (Goldblatt & Manning, 2002). Thus, large effective population sizes, coupled with recent divergence, may not have allowed enough time for fixation to occur at the nuclear and plastid loci used in this study (Avise, 2000).

TAXONOMY

Both plastid and combined data reject the reciprocal monophyly of *Thesium* and *Thesidium* (Figs 2, 3), suggesting, instead, that *Thesidium* is embedded within *Thesium*, being sister to the Eurasian *Thesium* clade. Although ITS identifies *Thesidium* as sister to a larger *Thesium* clade, this relationship is unsupported statistically (Fig. 2). Backlund & Bremer (1998) proposed that the primary principle in determining the taxonomic ranking for a set of organisms is monophyly. In order to ensure generic monophyly, two options are available: (1) to sink *Thesidium* into *Thesium*, retaining it as a section in the genus (*Hag-*
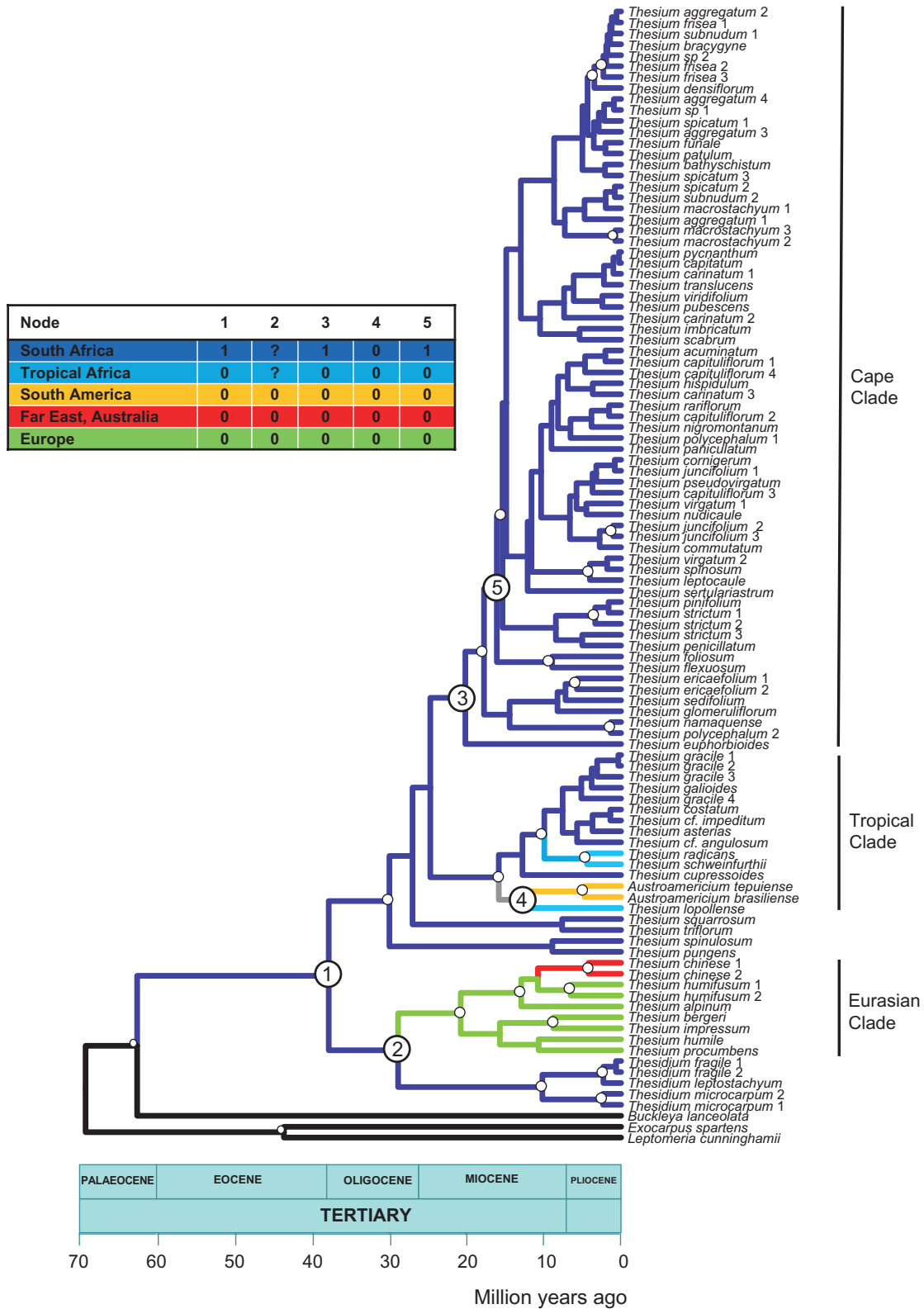
Figure 4. Ancestral area reconstruction (maximum likelihood) on the chronogram produced using a relaxed, log-normal clock (implemented in BEAST; see text for details). Selected clades are numbered for reference. Grey lines correspond to equivocal branches. Open circles indicate nodes with posterior probability (PP) > 0.95.

nothesium sensu De Candolle, 1857a), or (2) to elevate Eurasian *Thesium* species to the genus level.

There is strong morphological support for the maintenance of *Thesidium* as a separate genus. Specifically, *Thesidium* species are dioecious plants, with tetramerous flowers, traits not seen among *Thesium* spp. Such morphological differences between the two genera support the principle of maximizing diagnosability (Backlund & Bremer, 1998). All of the Eurasian species sampled in this study come from Hendrych's subgenus *Thesium*, which is characterized by campanulate perigonia, glabrous perianth margins and a pencil of hairs attaching anthers to the perianth lobes (Hendrych, 1972). These morphological characters are not unique to this group, however, with similar floral morphologies being found in South African *Thesium* spp. (in Hill's section *Imberbia*). Given the lack of morphological exclusivity between Eurasian and southern African taxa, coupled with the strength of the support for *Thesidium* being nested within *Thesium*, we feel, on current evidence, that they would be best maintained within *Thesium*. This suggests that *Thesidium* should be reduced to a subgenus within *Thesium*. However, a lack of full sampling of species from the Eurasian subgenus and the absence of a comprehensive comparative morphological study make any final judgement on this potential change premature.

Hendrych (1963) segregated the South American *Thesium* spp. as a new genus, *Austroamericium*, based on their apparent morphological distinctiveness and their geographical disjunction from the rest of the genus (all Old World). Molecular evidence presented here contradicts Hendrych's view, however, suggesting that *Austroamericium* is strongly (PP = 1.00, BS = 100) nested within a predominantly tropical African *Thesium* clade. Hendrych (1972) cited floral structure as a key diagnostic feature of *Austroamericium*, with these species having, among other features, funnel-shaped perianth tubes, with the anthers close to the free lobes of the perigonium. He also suggested that pear-shaped fruits that retain the dried lobes of the perigonium after they have formed and have raised ribs connected by reticulated veins were unique to these South American species. In addition, he cited an annual life history, in conjunction with scale-shaped leaves, as supporting their exclusion from the rest of *Thesium*. Many of the characters identified as characteristic of *Austroamericium* by Hendrych are, however, seen in tropical African *Thesium* spp. For example, many tropical African species are annual, with scale-like leaves, and some species also have ribbed fruit, with vein reticulation (e.g. Group 4, in Polhill, 2005). Although a more thorough morphological investigation is required, such superficial morphological similarity between South American and tropical African taxa, combined with the molecular evidence outlined here, supports the re-inclusion of these species in *Thesium*.

Relationships among South African species generally do not correspond with Hill's (1915) infrageneric groupings, although some patterns are noticeable. Section *Imberbia* forms a paraphyletic grade, which suggests that its morphological characteristics may represent plesiomorphic states. These species have glabrous or fringed perianth margins and hairs attaching the anthers to the perianth segments. Hill's section *Barbata* is represented by two clades within the larger Cape clade. One clade (PP = 0.79, BS < 50) comprises a group of leafy species (Fig. 3: Leafy clade), and the second is sister to a clade (PP = 0.90, BS < 50) of *Imberbia* species. The Cape section *Annulata* receives moderate support (PP = 0.78, BS = 90, Fig. 3) and is distinguished morphologically by having anthers free from the perianth lobes (i.e. without attaching hairs) and a characteristic ring of hairs at the throat of the perianth tube (Hill, 1915). The inclusion of *T. densiflorum* in the *Annulata* clade is exceptional, this species being assigned to section *Barbata* by Hill. Both sections *Barbata* and *Annulata* have a dense apical beard on their perianth lobes, but differ in the presence of attaching hairs behind the anthers in section *Barbata* species. As well as sharing its range with many species from section *Annulata*, some individuals of *T. densiflorum* have been noted to lack attaching hairs behind their anthers (T. Moore, pers. observ.). This species also shares a similar floral arrangement to many species from section *Annulata*, with flowers arranged in 'dense rounded heads at the ends of branches' (Levyns, 1950). The only South African representative of the section *Penicillata* arises as sister to a clade comprising section *Imberbia* species.

Overall, the lack of support for the monophyly of Hill's infrageneric groupings suggests that a new system is required. Increased genetic and taxonomic sampling is required to shed further light on the relationships within *Thesium* (cf. Albach & Chase, 2004).

HISTORICAL BIOGEOGRAPHY

Ancestral area reconstruction (Fig. 4) supports Hendrych's (1972) hypothesis of a southern African origin for the genus. The results presented here mirror a biogeographical pattern shown by several other groups (Galley *et al*., 2007), which exhibit a clear northward dispersal trend out of southern Africa. Based on our limited sampling of the Eurasian species, the occurrence of the genus in Eurasia is apparently the result of a single dispersal event from Africa to Europe and thence to Asia (Fig. 4). Limited

taxon sampling may potentially have a major effect on ancestral reconstruction (Salisbury & Kim, 2001), and here the limited number of Eurasian species sampled (see Table 2) may impact on the reconstruction of the basal node.

The divergence times obtained from the separate dating of each data partition did not differ greatly from those obtained from the combined analysis (cf. Hardy & Linder, 2005; Table 4). South American *Thesium* spp. split from their African relatives approximately 13.4 Mya (Fig. 4). The exact location of the common ancestor is unclear from likelihood optimizations (Node 4: Fig. 4), although a tropical African distribution seems intuitive. Moreover, parsimony optimization suggests a tropical African reconstruction for this node (data not shown). This date is far too recent to be the result of vicariance associated with the break-up of Gondwana (approximately 90 Mya; Sanmartín & Ronquist, 2004), as suggested by Hendrych (1972), but instead indicates the more likely involvement of long-distance dispersal. A large number of plant and animal genera display trans-Atlantic disjunctions (Thorne, 1973) and, in a review of plants displaying trans-Atlantic distributions, Renner (2004) found that, of the 11 genera studied, seven were best explained as recent (< 10 Mya) dispersal events across the Atlantic. She suggested that strong oceanic currents, rather than dispersal by wind or birds, may effectively transport 'rafts' of plant material across the Atlantic. The strongly supported monophyly of the South American *Thesium* spp., nested within a tropical African clade, suggests a single, relatively recent dispersal event from tropical Africa to South America.

High *Thesium* diversity in the Cape is the result of recent diversification within a single lineage (Fig. 4). The relative antiquity of this Cape clade matches that of several other Cape groups, for example Rhamnaceae (Richardson *et al.*, 2001), Irideae (Goldblatt *et al.*, 2002), *Ehrharta* (Verboom, Linder & Stock, 2003), African Restionaceae (Linder, Eldenäs & Briggs, 2003), *Pelargonium* (Bakker *et al.*, 2005) and *Muraltia* (Forest *et al.*, 2007). The radiation of the group, however, only occurred subsequently in the Miocene (16.7 ± 6.7 Mya). A similar pattern was observed in the South African *Pelargonium* (Bakker *et al.*, 2005), which shows recent diversification events in the Miocene and Pliocene. Climate has been recognized as a key driver in the recent radiations of many Cape lineages (Richardson *et al.*, 2001; Verboom *et al.*, 2003; Bakker *et al.*, 2005; McKenzie & Barker, 2008; Verboom *et al.*, 2009) and, in particular, the large climatic fluctuations experienced during the early–late Miocene are suggested to have stimulated speciation in the Cape winter rainfall region at that time (Verboom *et al.*, 2003).

Parasitic plants may be affected by changes in climate, both directly through impacts on their own physiology and indirectly through impacts on their host (Phoenix & Press, 2005). Thus, range expansions and contractions of both parasites and/or its hosts during periods of climatic fluctuation may have promoted speciation in parasitic lineages. Miocene climatic changes were accompanied by major geomorphic events which significantly altered the substrates available for plant colonization, providing novel habitats (Cowling, Proches & Partridge, 2009). Many studies have shown the effect of nutrient availability on parasite success and fecundity, with parasitic plants grown under high nutrient conditions generally being larger and having higher reproductive outputs (Salonen & Puustinen, 1996). Thus, the exposure of novel, relatively nutrient-rich substrates (Cowling *et al.*, 2009) may have provided opportunities for both *Thesium* spp. and their hosts to diversify into novel, unexploited niches. Some Cape species, particularly from section *Annulata* (e.g. *T. funale* L. and *T. patulum* A.W.Hill) are found almost exclusively on lowland clay-rich soils that were exposed relatively recently during the mid to late Miocene (Cowling *et al.*, 2009). Such large-scale changes in climate and geology may have played an influential role in the evolution of *Thesium*, but little is known about the roles climate and geology play in the evolution of a parasitic lifestyle in plants. This study serves as a preliminary framework for future research, not only on the phylogenetics and biogeography of the genus *Thesium*, but also for the understanding of the evolution of the species-rich Cape region as a whole.

ACKNOWLEDGEMENTS

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APPENDIX

GenBank Accession number and collection details for samples used in phylogenetic analyses.

trnL–trnF	ITS	Species	Distribution	Voucher	Collection locality
GU294680		*Austroamericium brasiliense* Hendrych	South America	Ganev 708, K	Bahia, Brazil
GU294639	GU256824	*Austroamericium tepuiense* Hendrych	South America	Santos *et al.* 236, K	Parana, Brazil
GU294669	GU256863	*Buckleya lanceolata* Mig.	Asia/America	SADO 200, K	Sado-Ga-Sima Niigata Prefecture, Japan
GU294670	GU256864	*Exocarpus spartens* R.Br.	Australia	Verboom 1273, BOL	Western Australia
GU294671	GU256865	*Leptomeria cunninghamii* Mig.	Australia	Verboom 1274, BOL	Western Australia
GU294606	GU256783	*Thesidium fragile 1* Sond.	Southern Africa	Verboom 912, BOL	Kamannassieberg, WC, South Africa
GU294681		*Thesidium fragile 2* Sond.	Southern Africa	Verboom 1305, BOL	Betty's Bay, WC, South Africa
GU294607	GU256784	*Thesidium leptostachyum* Sond.	Southern Africa	Forest 1, NBG	WC, South Africa
GU294609	GU256786	*Thesidium micorcarpum 1* A.DC.	Southern Africa	Verboom 1150, BOL	Kamannassieberg, WC, South Africa
GU294608	GU256785	*Thesidium micorcarpum 2* A.DC.	Southern Africa	Verboom 1149, BOL	Kamannassieberg, WC, South Africa
	GU256845	*Thesium acuminatum* A.W.Hill	Southern Africa	Moore 149, BOL	Red Hill, Cape Town, South Africa
GU294646	GU256832	*Thesium aggregatum 1* A.W.Hill	Southern Africa	Forest 694, NBG	De Hoop, WC, South Africa
GU294659	GU256852	*Thesium aggregatum 2* A.W.Hill	Southern Africa	Louw 12062, BOL	Capricorn Park, WC, South Africa
GU294649	GU256836	*Thesium aggregatum 3* A.W.Hill	Southern Africa	Britton 1904/085, BOL	Jonkershoek, Cape Town, South Africa
	GU256825	*Thesium aggregatum 4* A.W.Hill	Southern Africa	Forest 669, NBG	Table Mountain, WC, South Africa
GU294599	GU256777	*Thesium alpinum* L.	Europe	BK 80311, K	Vitosa Mt., Bulgaria
GU294664	GU256857	*Thesium asterias* A.W.Hill	Southern Africa	Trinder-Smith 432, BOL	Oribi Gorge, KZN, South Africa
GU294654	GU256847	*Thesium bathyshcistum* Schltr.	Southern Africa	Moore 87, BOL	Heuningberg NR, Bredasdorp, South Africa
GU294600	GU256778	*Thesium bergeri* Zucc.	Europe	Brummit&Powell18740	Sterea Ellas Div., Greece
GU294634	GU256819	*Thesium brachygyne* Schltr.	Southern Africa	Louw 11371, BOL	WC, South Africa
	GU256796	*Thesium capitatum* L.	Southern Africa	Moore 111, BOL	Jonkershoek, WC, South Africa
GU294650	GU256840	*Thesium capituliflorum 1* Sond.	Southern Africa	Verboom 1297, BOL	Betty's Bay, WC, South Africa
	GU256843	*Thesium capituliflorum 2* Sond.	Southern Africa	Moore 169, BOL	Grootwinterhoek Mts, WC, South Africa
GU294655	GU256848	*Thesium capituliflorum 3* Sond.	Southern Africa	Moore 165, BOL	Grootwinterhoek Mts, WC, South Africa
GU294652	GU256844	*Thesium capituliflorum 4* Sond.	Southern Africa	Muasya&Striton 4083, BOL	WC, South Africa
	GU256794	*Thesium carinatum 1* A.DC.	Southern Africa	Forest 594, NBG	Cederberg, WC, South Africa
GU294618	GU256798	*Thesium carinatum 2* A.DC.	Southern Africa	Moore 167, BOL	Grootwinterhoek Mts, WC, South Africa
GU294637	GU256822	*Thesium carinatum 3* A.DC.	Southern Africa	Verboom 1311, BOL	Betty's Bay, WC, South Africa
GU294661	GU256854	*Thesium cf. angulosum* DC.	Southern Africa	Verboom 1025, BOL	Garden Castle NR, KZN, South Africa
GU294663	GU256856	*Thesium cf. impeditum* A.W.Hill	Southern Africa	Verboom 1043, BOL	Wakkestroom, MPA, South Africa

APPENDIX *Continued*

trnL–trnF	ITS	Species	Distribution	Voucher	Collection locality
GU294603	GU256781	*Thesium chinese 1* Turcz.	Asia/Australia	Copeland 3482, K	Lake Inverell, NSW, Australia
GU294673		*Thesium chinese 2* Turcz.	Asia/Australia	Kharkevich&Buch s.n., K	Nedezhdensky Dist., eastern Russia
GU294683		*Thesium commutatum* Sond.	Southern Africa	Forest 716, NBG	De Hoop, WC, South Africa
GU294647	GU256833	*Thesium cornigerum* A.W.Hill	Southern Africa	Forest 952, NBG	Landroskop, WC, South Africa
GU294662	GU256855	*Thesium costatum* A.W.Hill	Southern Africa	Verboom 1037, BOL	Garden Castle NR, KZN, South Africa
GU294666	GU256859	*Thesium cupressoides* A. W.Hill	Southern Africa	Verboom 1026, BOL	Garden Castle NR, KZN, South Africa
GU294679		*Thesium densiflorum* A.DC.	Southern Africa	Moore 152, BOL	Camps Bay, Cape Town, South Africa
GU294631	GU256816	*Thesium ericaefolium 1* A.DC.	Southern Africa	Verboom 1296, BOL	Betty's Bay, WC, South Africa
GU294623	GU256803	*Thesium ericaefolium 2* A.DC.	Southern Africa	Moore 89, BOL	Potberg, WC, South Africa
GU294614	GU256791	*Thesium euphorbioides* L.	Southern Africa	Forest 953, NBG	Landroskop, WC, South Africa
GU294630	GU256815	*Thesium flexuosum* A.DC.	Southern Africa	Verboom 1156, BOL	Kamannassieberg, WC, South Africa
GU294619	GU256799	*Thesium foliosum* A.DC.	Southern Africa	Moore 41, BOL	Thumb Peak, WC, South Africa
GU294678		*Thesium frisea 1* L.	Southern Africa	Louw WV14, BOL	WC, South Africa
GU294640	GU256826	*Thesium frisea 2* L.	Southern Africa	Forest CP 3, NBG	Cape Peninsula, WC, South Africa
GU294626	GU256809	*Thesium frisea 3* L.	Southern Africa	NGB 1616, BOL	Drakensberg, KZN, South Africa
	GU256866	*Thesium funale* L.	Southern Africa	Forest 732, NBG	Salmonsdam, WC, South Africa
GU294615	GU256792	*Thesium galioides* A.DC.	Southern Africa	Moore 50, BOL	Prince Alberts Pass, WC, South Africa
GU294621	GU256801	*Thesium glomerulifolium* Sond.	Southern Africa	Moore 46, BOL	Prince Alberts Pass, WC, South Africa
GU294667	GU256860	*Thesium gracile 1* A.W.Hill	Southern Africa	Verboom 1054a, BOL	Barberton, MPA, South Africa
	GU256861	*Thesium gracile 2* A.W.Hill	Southern Africa	Verboom 1054b, BOL	Barberton, MPA, South Africa
GU294665	GU256858	*Thesium gracile 3* A.W.Hill	Southern Africa	Trinder-Smith 424, BOL	Drakensberg, KZN, South Africa
GU294668	GU256862	*Thesium gracile 4* A.W.Hill	Southern Africa	Nowell s.n., BOL	Sani Pass, KZN, South Africa
GU294635	GU256820	*Thesium hispidulum* Lam.	Southern Africa	Louw 9440, BOL	Tulbagh Valley, WC, South Africa
GU294604		*Thesium humifusum 1* DC.	Europe	Fay s.n., K	Hartslock , UK (Kew DNA bank 3687)
GU294602	GU256780	*Thesium humifusum 2* DC.	Europe	Chase 1881, K	Sierra Nevada, Spain (Kew DNA bank 1881)
GU294674		*Thesium humile* Vahl	Europe	Abd el Ghani sn, K	No locality (Kew DNA bank 1858)
	GU256810	*Thesium imbricatum* Thunb.	Southern Africa	Trinder-Smith 423, BOL	Drakensberg, KZN, South Africa
GU294598	GU256776	*Thesium impressum* Steud. ex A.DC.	Asia	Davis 23148, K	Van Dist., Turkey
	GU256834	*Thesium juncifolium 1* DC.	Southern Africa	Muasya&Striton 4081, BOL	WC, South Africa
GU294653	GU256846	*Thesium juncifolium 2* DC.	Southern Africa	Moore 62, BOL	Grootvadersbos, WC, South Africa

APPENDIX *Continued*

trnL–trnF	ITS	Species	Distribution	Voucher	Collection locality
GU294675		*Thesium juncifolium 3* DC.	Southern Africa	Moore 54, BOL	Grootvadersbos, WC, South Africa
GU294624	GU256804	*Thesium leptocaule* Sond.	Southern Africa	Forest 768, NBG	Baviaanskloof, WC, South Africa
GU294638	GU256823	*Thesium lopollense* Hiern	Tropical Africa	ANGOLA 1959, BOL	Southern Angola
	GU256839	*Thesium macrostacyhum 1* A.DC.	Southern Africa	Moore 140, BOL	Baardskeerdersbosch, WC, South Africa
GU294656	GU256849	*Thesium macrostacyhum 2* A.DC.	Southern Africa	Moore 166, BOL	Grootwinterhoek Mts, WC, South Africa
GU294657	GU256850	*Thesium macrostacyhum 3* A.DC.	Southern Africa	Moore 168, BOL	Grootwinterhoek Mts, WC, South Africa
GU294612	GU256789	*Thesium namaquense* Schltr.	Southern Africa	Forest 896, NBG	Spektakel Pass, NC, South Africa
GU294643	GU256829	*Thesium nigromontanum* Sond.	Southern Africa	Forest 702, NBG	Potberg, WC, South Africa
GU294633	GU256818	*Thesium nudicaule* A.W.Hill	Southern Africa	Louw 12249, BOL	WC, South Africa
GU294636	GU256821	*Thesium panicluatum* L.	Southern Africa	Moore 23, BOL	Pilaarkop, WC, South Africa
GU294641	GU256827	*Thesium patulum* A.W.Hill	Southern Africa	Evans 25/11/08/1, BOL	Stellenbosch, WC, South Africa
GU294629	GU256814	*Thesium penicillatum* A.W.Hill	Southern Africa	Verboom 1140, BOL	Hottentots Hollands NR, WC, South Africa
GU294620	GU256800	*Thesium pinifolium* A.DC.	Southern Africa	Moore 43, BOL	Thumb Peak, WC, South Africa
GU294651	GU256842	*Thesium polycephalum 1* Schltr.	Southern Africa	Verboom 1142, BOL	Springbok, NC, South Africa
GU294613	GU256790	*Thesium polycephalum 2* Schltr.	Southern Africa	Forest 911, NBG	Spektakel Pass, NC, South Africa
GU294672		*Thesium procumbens* C.A.Mey.	Europe	Watson *et al*. 1644, K	Erzurum, Turkey
GU294648	GU256835	*Thesium pseudovirgatum* Levyns	Southern Africa	Britton 1904/084, BOL	Jonkershoek, Cape Town, South Africa
GU294682		*Thesium pubscens* DC.	Southern Africa	Forest CP 4, NBG	Cape Peninsula, WC, South Africa
GU294628	GU256812	*Thesium pungens* A.W.Hill	Southern Africa	Verboom 1340, BOL	Namaqualand, WC, South Africa
GU294617	GU256797	*Thesium pycnanthum* Schltr.	Southern Africa	Britton 1904/082, BOL	Jonkershoek, Cape Town, South Africa
GU294601	GU256779	*Thesium radicans* Hohen. ex A.DC.	Tropical Africa	Collenette 8173, K	Jabal Qahar, Saudi Arabia
	GU256841	*Thesium rariflorum* Sond.	Southern Africa	Moore 100, BOL	Houwhoek, WC, South Africa
	GU256808	*Thesium scabrum* L.	Southern Africa	Moore 155, BOL	Camps Bay, Cape Town, South Africa
GU294605	GU256782	*Thesium schweinfurthii* Engl.	Tropical Africa	Malaisse 13645, K	Kasungami, Zaire
	GU256807	*Thesium sedifolium* A.DC.	Southern Africa	Moore 146, BOL	Baardskeerdersbosch, WC, South Africa
GU294676		*Thesium sertulariastum* A.W.Hill	Southern Africa	Moore 45, BOL	Prince Alberts Pass, WC, South Africa
GU294645	GU256831	*Thesium sp 1*	Southern Africa	Moore 16, BOL	Sir Lowry's Pass, WC, South Africa
GU294658	GU256851	*Thesium sp 2*	Southern Africa	Verboom 1290, BOL	Du Toits Kloof Mountains, WC, South Africa
GU294642	GU256828	*Thesium spicatum 1* L.	Southern Africa	Forest 950, NBG	Landroskop, WC, South Africa
GU294644	GU256830	*Thesium spicatum 2* L.	Southern Africa	Forest 850, NBG	Prince Albert's Pass, WC, South Africa

APPENDIX *Continued*

trnL–trnF	ITS	Species	Distribution	Voucher	Collection locality
GU294660	GU256853	*Thesium spicatum 3* L.	Southern Africa	Verboom 1300, BOL	Betty's Bay, WC, South Africa
	GU256805	*Thesium spinosum* L.f.	Southern Africa	Moore 114, BOL	De Hoop NR, WC, South Africa
GU294627	GU256811	*Thesium spinulosum* A.DC.	Southern Africa	Moore 148, BOL	Baardskeerdersbosch, WC, South Africa
GU294610	GU256787	*Thesium squarrosum* L.f.	Southern Africa	Forest 851, NBG	Uniondale, WC, South Africa
	GU256813	*Thesium strictum 1* P.J.Bergius	Southern Africa	Forest 668, NBG	Table Mountain, WC, South Africa
GU294622	GU256802	*Thesium strictum 2* P.J.Bergius	Southern Africa	Moore 48, BOL	Prince Alberts Pass, WC, South Africa
GU294677		*Thesium strictum 3* P.J.Bergius	Southern Africa	Verboom 1295, BOL	Betty's Bay, WC, South Africa
GU294632	GU256817	*Thesium subnudum 1* Sond.	Southern Africa	Louw 9563, BOL	Tulbagh Valley, WC, South Africa
	GU256837	*Thesium subnudum 2* Sond.	Southern Africa	Moore 96, BOL	Kogelberg, WC, South Africa
	GU256795	*Thesium translucens* Sond.	Southern Africa	Britton 1904/083, BOL	Jonkershoek, Cape Town, South Africa
GU294611	GU256788	*Thesium triflorum* Thunb. ex L.f.	Southern Africa	Moore 128, BOL	Graaf Reinet, EC, South Africa
	GU256838	*Thesium virgatum 1* Lam.	Southern Africa	Moore 14, BOL	Harold Porter BG, WC, South Africa
GU294625	GU256806	*Thesium virgatum 2* Lam.	Southern Africa	Verboom 1153, BOL	Kamannassieberg, WC, South Africa
GU294616	GU256793	*Thesium viridifolium* Levyns	Southern Africa	Forest 680, NBG	Cape Peninsula, WC, South Africa

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