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Phylogeny, biogeography, and the evolution of life-history traits in *Leucadendron* (Proteaceae)

Nigel P. Barker[a,*], Alain Vanderpoorten[b], Cynthia M. Morton[c], John P. Rourke[d]

[a] *Molecular Ecology and Systematics Group, Department of Botany, Rhodes University, P.O. Box 94, Grahamstown 6140, South Africa*
[b] *Department of Life Sciences, University of Liège, Sart Tilman, B-4000 Liège, Belgium*
[c] *Associate Curator, Head of Botany, Carnegie Museum of Natural History, 4400 Forbes Avenue, Pittsburgh, PA 15213, USA*
[d] *Compton Herbarium, National Botanical Institute, Kirstenbosch, P. Bag X7, Claremont 7735, South Africa*

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Abstract

Leucadendron is a moderately large genus of Proteaceae almost entirely restricted to the Cape Floristic Region of southern Africa. The genus is unusual in being dioecious and sexually dimorphic. ITS sequence data were obtained from 62 of the 96 currently recognized taxa (85 species and 11 subspecies). Phylogenetic analyses were conducted under Maximum Likelihood and parsimony and resolved nine groups of species with varying degrees of bootstrap support, but relationships between these groups are largely unsupported. The phylogeny conflicts with the current taxonomic arrangement, which is based mainly on fruit morphology. The two sections of the genus, Alatosperma and Leucadendron, and several subsections within these sections, are resolved as non-monophyletic. This means that taxonomically important characters (such as fruit shape) have evolved multiple times, as the species with nut-like fruit (resolved into two of the nine groups) appear to have evolved independently from ancestors with winged fruit. Based on the topology obtained, the life history traits of anemophily, myrmechochory, and re-sprouting have also originated multiple times. Dispersal–Vicariance (DIVA) analysis suggests that the genus had an ancestral area in the Karoo Mountain and Southeastern phytogeographic centres of endemism in the southwestern Cape.

Keywords: *Leucadendron*; Dispersal–Vicariance analysis; ITS; Phylogeny; Proteaceae; Life history traits; Serotiny

1. Introduction

The Proteaceae are an ancient plant family, with a fossil record that dates back to the Cretaceous (Dettmann and Jarzen, 1996; Drinnan et al., 1994; Hill et al., 1995 and references therein), predating the break-up of Gondwanaland. According to Douglas (1995), 79 genera are recognized, distributed over seven subfamilies and 12 tribes. Past classifications of the family (Johnson and Briggs, 1963, 1975, Venkata Rao, 1971) have been found to be inconsistent with molecular studies (Hoot and Douglas, 1998). Several genera are speciose (eg *Grevillea* and *Hakea* in Australia, *Protea* and *Leucadendron* in southern Africa), and there are approximately 1700 species in the family, making it one of the most prominent flowering plant families in the southern hemisphere (Douglas, 1995; Johnson and Briggs, 1975). In Africa, the Proteaceae comprises about 400 taxa (Rebelo, 1995), 83% of which are found in the Cape Floristic Region (CFR) in South Africa (Tansley and Brown, 2000). Many of these taxa are rare and under threat (Tansley, 1998).

A previous study on the African taxa of Proteaceae using ITS sequence data showed that 10 of the 13 African proteoid genera form a well-supported clade, centred in the south-western Cape of South Africa (Barker et al., 2002). This clade, called the "Cape clade"

* Corresponding author. Fax: +27 46 622 5524.
E-mail address: n.barker@ru.ac.za (N.P. Barker).

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by Barker et al. (2002), contains a subset of taxa included in the tribe Proteae *sensu* Johnson and Briggs (1975), and is most closely related to a paraphyletic group of two Australian genera: *Adenanthos* (tribe Franklandieae) and *Isopogon* (tribe Conospermeae), all of subfamily Proteoideae. The basal-most genus in the "Cape clade" is *Leucadendron* R.Br. (Barker et al., 2002), a position that supports Johnson and Briggs's (1975) and Midgley's (1987) earlier contentions that the genus is basal, retaining "primitive" characters.

Leucadendron is a fairly large genus comprising 96 taxa (85 species and 11 subspecies). It is one of four dioecious genera of the Proteaceae (*Aulax*, *Dilobeia*, and *Heliciopsis* are the others). Morphological and cytological synapomorphies for the genus include a haploid chromosome number of 13 (Johnson and Briggs, 1975; Midgley, 1987) and a cone-like inflorescence. Species in this genus show a variety of reproductive and other survival strategies, such as serotiny, that have been interpreted as adaptations to life in the fynbos ecosystem, which is fire prone and comprises generally nutrient poor soils (Cowling and Holmes, 1992). *Leucadendron* species have been the focus of several studies examining these adaptations (Bond, 1985; Hattingh and Gillomee, 1989; Le Maitre, 1988a,b; Midgley, 1987).

The current taxonomy of *Leucadendron* is provided by Williams (1972), who revised the genus following the early principles of "phylogenetic advancement" as espoused by Davis and Heywood (1963). Midgley (1987) used a divergence index approach to elucidate relationships between the 12 subsections recognized by Williams (1972). Thus, within a biogeographic, ecological and taxonomic context, resolving the species-level relationships would be essential to address a number of phylogenetic and evolutionary issues. Here we report on the first of our attempts to produce a species-level phylogeny of *Leucadendron* based on DNA sequence data.

The Internal Transcribed Spacer (ITS) regions have been widely used in plant systematic studies at the species level. The ITS regions are, despite their hundreds to thousands of copies in the eukaryote genome, often remarkably homogeneous within the same species (Baldwin et al., 1995). This homogeneity is attributed to concerted evolution of the entire repeat (Hillis and Dixon, 1991), a process based on gene conversion and recombination that maintains great similarity among repeat units within a species (see Elder and Turner, 1995 for review). In addition, although the ITS appear to play a role in the maturation of nuclear rRNAs, they are cleaved or otherwise digested during the assembly of the ribosomal subunits (Hershkovitz et al., 1999). Selective constraints are thus much lower on the spacers than on the coding regions, leading to higher inter- and even intrataxon variability, a factor that has been the main cause of their wide ranging application in plant and animal studies. However, it is now increasingly acknowledged that ITS alone may provide misleading phylogenies due to incomplete lineage sorting, gene duplication (and problems of orthology that may occur when concerted evolution is disrupted), presence of pseudogenes and hybridization (Alvarez and Wendel, 2003; Bailey et al., 2003). Some of the problems associated with the use of ITS may be circumvented by the parallel use of low-copy nuclear regions, such as the introns of the nitrate–reductase gene (Howarth and Baum, 2002), LEAFY (Hoot and Taylor, 2001; Oh and Potter, 2003) and ncpGS (Emshwiller and Doyle, 1999; Yockteng and Nadot, 2004). However, the widespread use of low-copy nuclear genes, while on the increase, remains somewhat limited by the non-universality of the primers, and the costs and difficulties associated with primer development.

Despite these problems (especially that of paralogy) ITS is still a valid tool in generating species-level phylogenies (Razafimandimbison et al., 2004), but it is strongly advisable that phylogenetic evidence from ITS sequence data is complemented by other sources of cytoplasmic data, such as the mitochondrial or chloroplast genomes and morphology. However, our initial attempts to use cpDNA sequence data to obtain a plastid phylogeny of *Leucadendron* indicated that levels of variation are low, and we thus report on the results of an ITS sequencing study. Although we acknowledge the issues associated with the use of ITS, we argue that the results presented here constitute a first attempt, and, to date, our best estimate of the phylogeny of *Leucadendron*. This first phylogenetic inference, together with the hypotheses that we provide on the evolution of phytogeographic patterns and the evolution of life history traits in the genus, will form a basis from which other data will be added in order to test those hypotheses.

2. Materials and methods

2.1. Taxon sampling

Owing to the highly restricted and inaccessible distribution of many of the species of *Leucadendron*, leaf material of most of the species was obtained from plants in cultivation in Kirstenbosch Botanic Gardens, Cape Town, South Africa. Additional species were obtained from regional flower shows or from the DNA bank at Kirstenbosch. Only a few species were collected from the field. Table 1 lists all the taxa sampled and provides sectional and subsectional classification and voucher details and GenBank accession numbers. Leaf material was dried using silica gel (Chase and Hills, 1991), and material of two species was obtained from herbarium material housed in the Compton Herbarium, Kirstenbosch. The ingroup included 62 of the 96 currently

Table 1
Voucher, Genbank, and geographic distribution details of species of *Leucadendron* sampled in this study, including infrageneric taxonomy sensu Williams (1972)

Leucadendron species	Section	Subsection	Voucher	GenBank No.	Geographic subdivision of CFR
album(Thunb.) Fourcade	Leuc.	Leuc.	GFS	AY692167	L/Kar/SE
arcuatum(Lam.) Williams	Leuc.	Memb.	K 517/70	AY692208	LW
argenteum(L.) R.Br.	Leuc.	Leuc.	K 396/76	AY692184	Pen/SW
*barkerae*Williams	Leuc.	Nuc.	GFS	AY692180	LW/Kar
brunioidaes Meisn.	Leuc.	Vill.	K 279/70	AY692175	LW/(SW)
*burchellii*Williams	Leuc.	Nuc.	LHMS 421	AY692216	SW
chamalaea(Lam.) Williams	Leuc.	Vent.	K 200/70	AF508858	SW/LW
comosum (Thunb.) R.Br.	Alat.	Comp.	K 787/71	AY692217	SW/Lan/Kar/SE
conicum (Lam.)Williams	Alat.	Trig.	GFS	AY692195	SE/(Lan)
coniferum L. Meisn.	Alat.	Alat.	K 832/75	AY692194	Ag/SW/Pen
*corymbosum*Berg.	Leuc.	Cun.	NPB 1673	AY692210	SW
daphnoides(Thunb.) Meisn.	Leuc.	Nuc.	K 649/74	AY692177	SW
*discolor*Philips & Hutch.	Alat.	Alat.	K 265/77	AY692202	LW
*dregei*E.Mey. ex Meisn.	Leuc.	Leuc.	GFS	AY692166	Kar
dubium (Buek ex Meisn) Phillips & Hutch.	Leuc.	Vill.	LHMS 475	AY692211	LW
*elimense*Philips ssp. *elimense*	Leuc.	Vent.	LHMS 425	AY692222	Ag
*elimense*Philips ssp. *salteri* Williams	Leuc.	Vent.	LHMS 630	AY692220	SW
*elimense*Philips ssp. *vyeboomense*Williams	Leuc.	Vent.	LHMS 415	AY692219	SW
ericifolium R.Br.	Leuc.	Uni.	K 1286/83	AF508855	Lan
*eucalyptifolium*Buek ex Meisn.	Alat.	Alat.	K 843/75	AY692197	Lan/Kar/SE/(SW)
*flexuosum*Williams	Alat.	Alat.	K 276/70	AY692169	SW
*floridium*R.Br.	Alat.	Trig.	K 32/67	AY692188	Pen
*galpinii*Philips & Hutch	Leuc.	Vill.	Williams 557 (NBG)	AY692213	Ag
*gandogeri*Schinz ex Gandoger	Alat.	Alat.	K 6/79	AY692193	SW/(Ag)
glaberrimum (Schltr.) Compton	Leuc.	Nuc.	LHMS 480	AY692218	LW
immoderatum Rourke MS	Alat.	Comp.	Rourke s.n.	AY692206	SW
lanigerum Buek. ex Meisn. var. laevigatum	Alat.	Alat.	K 964/70	AY692170	SW
laureolum (Lam.) Fourcade	Alat.	Alat.	K 631/75	AY692190	SW/Ag/Pen
*laxum*Williams	Leuc.	Cun.	K 1012/75	AY692185	Ag
levisanus(L.) Berg.	Leuc.	Vill.	LHMS 559	AY692174	SW/Pen
linifolium (Jacq.)R.Br.	Leuc.	Vill.	K 845/75	AY692176	SW/Ag
loeriensis Williams	Alat.	Trig.	K 11/79	AY692191	SE
loranthifolium(Salisb. ex Knight) Williams	Leuc.	Nuc.	K 817/97	AF508857	LW
*macowanii*Philips	Alat.	Trig.	K 186/72	AY692189	Pen
meridianum Williams	Alat.	Alat.	LHMS 427	AY692199	Ag
meyerianum H. Buek ex Philips & Hutch	Leuc.	Nuc.	K 600/74	AY692179	LW
microcephalum (Gandoger) Gandoger & Schinz.	Alat.	Brun.	K 988/75	AY692196	SW
*modestum*Williams	Alat.	Alat.	K 837/75	AY692221	SW/Ag
*muirii*Philips	Alat.	Comp.	GFS	AY692212	Ag
*nervosum*Philips & Hutch	Leuc.	Nerv.	Kirstenbosch (s.n.)	AY692171	SW
*nitidum*H. Buek ex Meisn.	Leuc.	Car.	NPB 1426	AY692183	LW
*nobile*Williams	Alat.	Comp.	Rourke 619 (NBG)	AF508856	SE/Kar
*osbournei*Rourke	Alat.	Comp.	Rourke s.n.	AY692168	Kar
*platyspermum*R. Br.	Alat.	Comp.	K 888/69	AY692205	SW/Ag
*pondoense*Van Wyk	Alat.	Trig.	K 103/92	AY692187	Pondo
roodii Phillips	Leuc.	Nuc.	K 1508/69	AY692215	LW
*rubrum*Burm. f.	Leuc.	Leuc.	Williams 181 (NBG)	AY692186	Pen/SW/LW/Lan/Kar/SE
salicifolium(Salisb.) Williams	Alat.	Trig.	K 68/80	AY692203	SW/(LW)/(Lan)
*salignum*Berg.	Alat.	Alat.	NPB1421	AY692172	Pen/SW/LW/Lan/Kar/SE /Ag
sericeum(Thunb.) R.Br.	Leuc.	Car.	LHMS 596	AY692182	LW
*sessile*R.Br.	Leuc.	Nuc.	K 642/74	AY692178	SW
singulare Williams	Leuc.	Alien.	Bond 1715 (NBG)	AY692209	Kar
spissifolium(Salisb. ex Knight) Williams ssp. *fragrans*Williams	Alat.	Alat.	K 1518/70	AY692198	Lan/SE/Kar
spissifolium (Salisb. ex Knight) Williams ssp. *spissifolium*	Alat.	Alat.	K 7/79	AY692192	Pen/SW/LW/Lan
stellare(Sims) Sweet	Leuc.	Vill.	K 11/76	AY692173	SW
strobilinum(L.) Druce	Alat.	Alat.	K 739/70	AF508859	Pen
teretifolium(Andr.) Williams	Alat.	Comp.	K 1268/69	AY692207	Ag/SW/Lan/Kar
thymifolium(Andr.) Williams	Leuc.	Vill.	LHMS 387	AY692214	SW
*tinctum*Williams	Leuc.	Nuc.	K 12/79	AY692181	Lan/Kar

(*continued on next page*)

Table 1 (*continued*)

Leucadendron species	Section	Subsection	Voucher	GenBank No.	Geographic subdivision of CFR
*uliginosum*R.Br. ssp. *uliginosum*	Alat.	Trig.	K 9/79	AY692201	SE/Lan
verticillatum(Thunb.) Meisn.	Leuc.	Cun.	K 1312/98	AY692204	SW
xanthoconus(O.Ktze.) K.Schum.	Alat.	Alat.	K 1275/69	AY692200	SW/Pen

Section codes. Alat., = Alatosperma, Leuc. = Leucandendron.
Subsection codes. Alat. = Alata, Alien = Aliena, Brun. = Brunneobracteata, Car = Carinata, Comp. = Compressa, Cun. = Cuneata, Leuc. = Leucadendron, Memb. = Membranacea, Nerv. = Nervosa, Nuc. = Nucifera, Trig. = Trigona, Uni. = Uniflora, Vent. = Ventricosa, Vill. = Villosa.
Voucher details and geographic distribution. Voucher number preceded with a "K" are Kirstenbosch Botanic Garden numbers, and numbers preceded by NPB are collections made by the senior author, and are housed in the Selmar Schonland Herbarium (GRA). Numbers preceded by LHMS are from the Leslie Hill Molecular Systematics laboratory, Compton Herbarium, Kirstenbosch. The herbarium abbreviation NBG (Compton Herbarium) indicates herbarium specimens that were used to obtain DNA. GFS = samples collected (unvouchered) from the George Flower Show in 1997. The geographic distribution is given according to the phytogeographical subdivisions (centres) of the Cape Floristic Region as presented by Linder (2003), based on the distribution data and maps provided by Williams (1972).
Key to geographic subdivisions: SW = Southwestern Centre, NW = Northwestern Centre, Pen = Cape Peninsula, Ag = Agulhas Plain, Lan = Langeberg Centre, Kar = Karoo Mountain Centre, SE = Southeastern Centre, Pondo = Pondoland endemic. Codes in parentheses indicate minor extensions into that centre.

Table 2
Sampling coverage of the sections ad subsections of *Leucadendron*

Section	Subsection	Sampling
Alatosperma	Alata	14 (25)
	Brunneobracteata	1 (1)
	Compressa	7 (9)
	Trigona	7 (9)
Leucadendron	Aliena	1 (2)
	Carinata	2 (2)
	Cuneata	3 (3)
	Leucadendron	4 (4)
	Membranacea	1 (4)
	Nervosa	1 (1)
	Nucifera	9 (17)
	Uniflora	1 (1)
	Ventricosa	4 (5)
	Villosa	7 (11)
Total		62 (96)

The first number in the sampling column indicates the number of taxa for which ITS sequence data was obtained, and the number in parentheses is the total number of taxa (species and subspecies) in each subsection.

recognized taxa of *Leucadendron*. All of Williams's (1972) subsections were sampled (see Table 2 for a summary of sectional and subsectional coverage). The closely related genus *Serruria*, represented by *S. aemula* and *S. adscendens*, was chosen as outgroup (Barker et al., 2002).

2.2. Molecular protocols

DNA was extracted from these samples using the hot CTAB method of Doyle and Doyle (1987). The nuclear ITS was amplified by PCR and sequenced according to the protocol described in Barker et al. (2002) for all 64 species. The sequences from each PCR product were assembled, checked, and corrected where necessary using Sequencher version 3.01 (Gene Codes Corporation, 1995).

2.3. Data analysis

2.3.1. Phylogeny reconstruction

The ITS sequences were imported into the alignment package DAPSA (DNA And Protein Sequence Alignment; written by E.H. Harley, Department of Chemical Pathology, University of Cape Town Medical School, Observatory, 7935, South Africa) and aligned by eye, with gaps inserted where necessary to preserve positional homology. Two methods of phylogenetic analysis were used: parsimony and maximum likelihood.

2.3.1.1. Maximum likelihood. An adequately parameter-rich model was chosen using hierarchical likelihood ratio tests as implemented by Modeltest 3.04 (Posada and Crandall, 1998). The parameters of the selected model were estimated by Maximum Likelihood on the basis of a Neighbor Joining topology and then fixed in heuristic searches employing 100 random replications. The trees were swapped using the TBR algorithm implemented by PAUP 4.0b10 and branches of zero length were collapsed during the search. Support for clades was assessed by means of a bootstrap analysis that was conducted, due to time constraints, with 1000 replicates using simple taxon addition.

2.3.1.2. Parsimony analysis. A parsimony analysis was conducted under equally weighted maximum-parsimony using heuristic searches with 1000 random addition replicates saving a maximum of 20,000 trees and TBR branch swapping. All shortest trees obtained from the random addition procedure were then used as starting trees for a heuristic search using TBR branch swapping. Support for clades was assessed using the full bootstrap method, with 1000 replicates, but MAXTREES was restricted to 1000 in order to limit the time this analysis took. Analyses were conducted using PAUP 4.0b10.

2.3.2. Dispersal–vicariance analysis

The geographic distribution of each species sampled was examined using the maps provided by Williams (1972). On the basis of these maps each species was allocated to one or more of the phytogeographical regions of the Cape Floristic Region. These floristic centres of endemism were first noted by Weimark (1941) and have since been reassessed by, among others, Oliver et al. (1983), Goldblatt and Manning (2002), and Linder (2001, 2003). The boundaries as outlined by Linder (2003, Fig. 12) are followed here (Fig. 1). A taxon-area matrix was analysed using DIVA (Ronquist, 1997). Because DIVA requires a fully dichotomised tree, a fully resolved tree based on the likelihood topology was created using MacClade version 3 (Maddison and Maddison, 1992). Polychotomous clades in this tree were dichotomized randomly to obtain full resolution. Two analyses using DIVA were conducted: one using the defaults, and one where the maximum number of ancestral areas was constrained to three ("MAXAREAS = 3"). Because of the size of the data set, each analysis had to be done in two segments; the first comprising a data set of Groups IV to VI (see Fig. 2 for details on these groups), and then the ancestor of this clade was added as a terminal to the second data set containing the remainder of the basal clades. Because of the variety of possible resolutions of polychotomous nodes, this process was repeated using three different fully resolved topologies.

2.4. Life history information and assessment

Information on the life history traits of serotiny, prost-fire survival strategies (reseeding or resprouting), pollination syndrome, and myrmechochory was mainly obtained from Williams (1972) and Midgley (1987), but other sources as indicated in Table 3 were also used. These character states for each species were mapped onto the topology obtained from the ML analysis.

3. Results

The substitution model that best fitted the NJ tree of the whole ITS region was a General Time-Reversible model (Rodriguez et al., 1990) with a γ distribution to model rate heterogeneity among sites and the following settings: rate matrix $R(\mathrm{AG}) = 2.836$, $R(\mathrm{AT}) = 0.3583$, $R(\mathrm{CG}) = 0.3583$, $R(\mathrm{CT}) = 5.8095$, proportion of invariable sites = 0.4616, γ distribution shape parameter = 0.8032. Sequence divergence among ITS sequences in *Leucadendron* ranged between 0.0% and 8.0% with an average of 2.7%. The ML analysis of the ITS data set using these model parameters resulted in two trees ($-\ln L = 2298.894$) involving the same topology, one of which (chosen randomly) is presented in Fig. 2.

The parsimony analysis was based on 101 parsimony-informative characters (61 when outgroups are

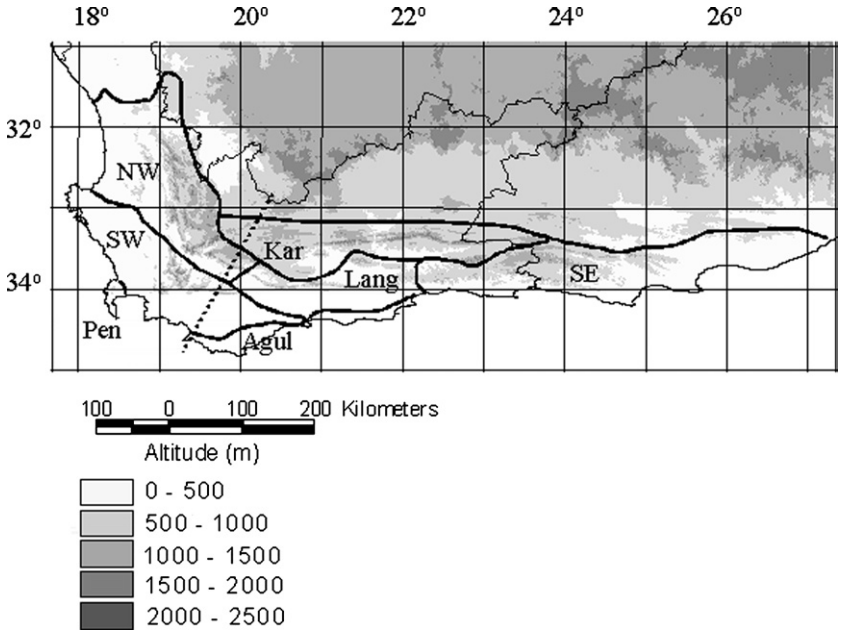

Fig. 1. Topographic map of southern Africa showing the phytogeographical centres of endemism as presented by Linder (2003). Key: SW = Southwestern Centre, NW = Northwestern Centre, Pen = Cape Peninsula, Agul = Agulhas Plain, Lang = Langeberg Centre, Kar = Karoo Mountain Centre, SE = Southeastern Centre. The dashed line demarcates the winter rainfall region (west of line) and the non-seasonal rainfall region (east of line), as noted by Williams (1972).

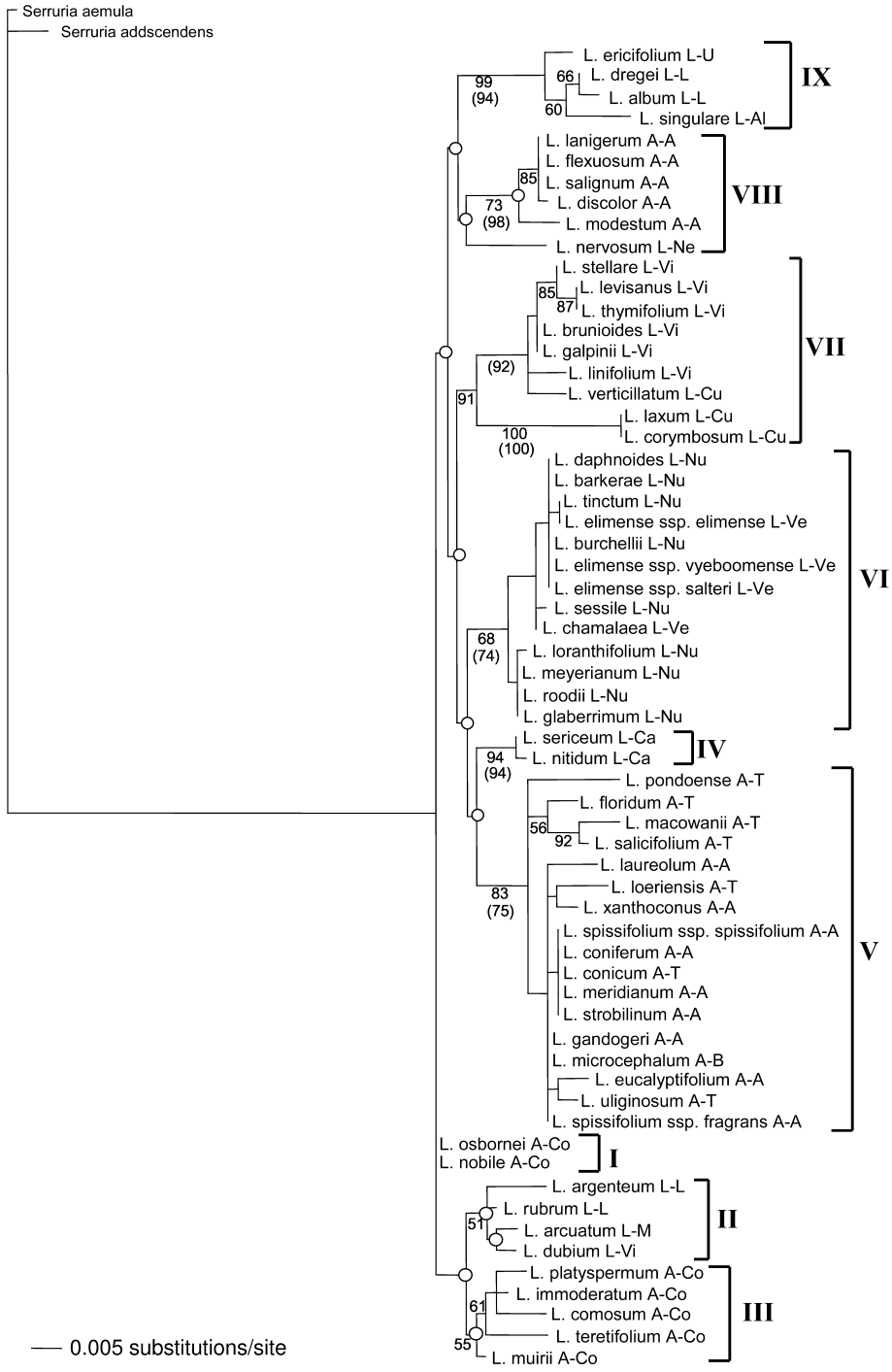

Fig. 2. One of two trees resulting from the ML analysis of the ITS data set using two species of *Serruria* as outgroups. Bootstrap values greater than 50% from the ML bootstrap analysis are indicated under the branches, and those from the parsimony bootstrap analysis appear in parentheses. Note that owing to space constraints in the diagram, parsimony bootstrap values are only given for the nine major groups discussed in the text (indicated by Roman numerals), and not subclades. The first single letter code at the end of the species names refers to the section (A = Alatosperma, L = Leucadendron) and subsection the species was placed in by Williams (1972). The second single or two-letter code refers to the subsections (A = Alata, Al = Aliena, B = Brunneobracteata, Ca = Carinata, Co = Compressa, Cu = Cuneata, L = Leucadendron, M = Membranacea, Ne = Nervosa, Nu = Nucifera, T = Trigona, U = Uniflora, Ve = Ventricosa, Vi = Villosa). The open circles indicate nodes that collapse in the parsimony consensus tree.

excluded), and found in excess of 20,000 equally parsimonious trees ($l = 238$, ci =; 0.571, ri = 0.808, gaps not included in analysis). The consensus tree showed considerable lack of resolution (tree not shown, but nodes that collapse are indicated in Fig. 2).

Table 3
Life history trait data for species of *Leucadendron* sampled in this study

Leucadendron species	Pollination syndrome	Serotiny	Post-fire survival strategy	Myrmechochory[e]
album (Thunb.) Fourcade	Ent.[a]	Yes[a]	Seeder[a]	No
arcuatum (Lam.) Williams	Ent.[a]	No[a]	Sprouter[a]	No
argenteum (L.) R.Br.	Ent.[a]	Yes[a]	Seeder[a]	No
barkerae Williams	Ent.[a]	No[a]	Seeder[a]	No
brunioides Meisn.	Ent.[a]	No[a]	Sprouter[a]	No
burchellii Williams	Ent.[a]	No[a]	Seeder[a]	No
chamalaea (Lam.) Williams	Ent.[a]	No[a]	Seeder[a]	No
comosum (Thunb.) R.Br.	Ent.[a]	Yes[a]	Seeder[a]	No
conicum (Lam.) Williams	Ent.[a]	Yes[a]	Seeder[a]	No
coniferum L. Meisn.	Ent.[a]/Anemo.[b]	Yes[a]	Seeder[a]	No
corymbosum Berg.	Ent.[a]	No[a]	Seeder[a]	No
daphnoides (Thunb.) Meisn.	Ent.[a,b]	No[a]	Seeder[a]	No
discolor Philips and Hutch.	Ent.[a]	Yes[a]	Seeder[a]	No
dregei E.Mey. ex Meisn.	Ent.[a]	Yes[a]	Seeder[a]	No
dubium (Buek ex Meisn) Phillips and Hutch.	Anemo.?[a]	No[a]	Seeder[a]	No
elimense Philips ssp. *elimense*	Ent.[a]	No[a]	Seeder[a]	No
elimense Philips ssp. *salteri* Williams	Ent.[a]	No[a]	Seeder[a]	No
elimense Philips ssp. *vyeboomense* Williams	Ent.[a]	No[a]	Seeder[a]	No
ericifolium R.Br.	Anemo.[a]	No[a]	Seeder[a]	Yes
eucalyptifolium Buek ex Meisn.	Ent.[a]	Yes[a]	Seeder[a]	No
flexuosum Williams	Ent.[a]	Yes[a]	Seeder[a]	No
floridium R.Br.	Ent.[a]	Yes[a] (partial)	Seeder[a]	No
galpinii Philips and Hutch	Ent.[a]	Yes[a]	Seeder[a]	No
gandogeri Schinz ex Gandoger	Ent.[a,b]	Yes[a]	Seeder[a]	No
glaberrimum (Schltr.) Compton	Ent.[a]	No[a]	Seeder[a]	No
immoderatum Rourke MS	Ent	Yes[a]	Seeder	No
lanigerum Buek. ex Meisn. var. laevigatum	Ent.[a]	Yes[a]	Sprouter[a]	No
laureolum (Lam.) Fourcade	Ent.[a,b]	Yes[a]	Seeder[a]	No
laxum Williams	Ent.[a]	No[a]	Seeder[a]	No
levisanus (L.) Berg.	Ent.[a]	Yes[a]	Seeder[a]	No
linifolium (Jacq.) R.Br.	Ent.[a]	Yes[a]	Seeder[a]	No
loeriensis Williams	Ent.[a]	Yes[a]	Seeder[a]	No
loranthifolium (Salisb. ex Knight) Williams	Ent.[a]	No[a]	Seeder[a]	No
macowanii Philips	Anemo.[a]	Yes[a]	Seeder[a]	No
meridianum Williams	Ent.[a]	Yes[a]	Seeder[a]	No
meyerianum H. Buek ex Philips and Hutch	Ent.[a]	No[a]	Seeder[a]	No
microcephalum (Gandoger) Gandoger and Schinz.	Ent.[a,b]	Yes[a]	Seeder[a]	No
modestum Williams	Ent.[a]	Yes[a]	Seeder[a]	No
muirii Philips	Ent.[a]	Yes[a]	Seeder[a]	No
nervosum Philips and Hutch	Ent.[a]	Yes[a]	Seeder[a]	No
nitidum H. Buek ex Meisn.	Ent.[a]	No[a]	Seeder[a]	Yes
nobile Williams	Ent.[a]	Yes[a]	Seeder[a]	No
osbournei Rourke	Ent.[d]	Yes[d]	Seeder	No
platyspermum R.Br.	Ent.[a]	Yes[a]	Seeder[a]	No
pondoense Van Wyk	Ent.[c]	Yes[a]	Seeder[a]	No
roodii Phillips	Ent.[a]	No[a]	Seeder[a]	No
rubrum Burm. f.	Ent.[a]	Yes[a]	Seeder[a]	No
salicifolium (Salisb.) Williams	Anemo.[a,b]	Yes[a]	Seeder[a]	No
salignum Berg.	Ent.[a,b]	Yes[a]	Sprouter[a]	Yes
sericeum (Thunb.) R.Br.	Ent.[a]	No[a]	Seeder[a]	No
sessile R.Br.	Ent.[a,b]	No[a]	Seeder[a]	No
singulare Williams	Ent.[a]	No[a]	Seeder[a]	Yes
spissifolium (Salisb. ex Knight) Williams ssp. *fragrans* Williams	Ent.[a]	Yes[a]	Sprouter[a]	No
spissifolium (Salisb. ex Knight) Williams ssp. *spissifolium*	Ent.[a]	Yes[a]	Sprouter[a]	No
stellare (Sims) Sweet	Ent.[a]	Yes[a]	Seeder[a]	No
strobilinum (L.) Druce	Ent.[a]	Yes[a]	Seeder[a]	No
teretifolium (Andr.) Williams	Anemo.[a]	Yes[a]	Seeder[a]	No
thymifolium (Andr.) Williams	Ent.[a]	Yes[a]	Seeder[a]	No
tinctum Williams	Ent.[a,b]	No[a]	Seeder[a]	No
uliginosum R.Br. ssp. *uliginosum*	Ent.[a]	Yes[a]	Seeder[a]	No

(*continued on next page*)

Table 3 (*continued*)

Leucadendron species	Pollination syndrome	Serotiny	Post-fire survival strategy	Myrmechochory[e]
verticillatum (Thunb.) Meisn.	Ent.[a]	No[a]	Seeder[a]	No
xanthoconus (O. Ktze.) K.Schum.	Ent.[a]	Yes[a]	Seeder[a]	

Data for pollination syndrome (Ent. = Entomophily, Anemo. = Anemophily), Serotiny (presence = Yes), Post-fire survival strategy (seeder = parent killed by fire, population continues from seed bank; sprouter = parents re-sprout after fire), Myrmechochory (based on presence of eliasome, indicated by "Yes"), and degree of sexual dimorphism is provided.

[a] Williams (1972).
[b] Hattingh and Giliomee (1989).
[c] Van Wyk (1990).
[d] Rourke (1997).
[e] Taken from Character 20 in Midgley (1987).

Our ML analysis resolves eight clades and a basal polytomy in the genus, termed here Groups I–IX and labelled as such in the figures. The parsimony analysis did not fully resolve some of these clades. These clades receive a range of bootstrap support (in both parsimony and ML analyses) from poor to very strong, but support for the relationships between these clades in both methods of analysis was generally poor. We base the following discussion on the ML analysis (and its associated bootstrap analysis), but also provide bootstrap values for the major clades from the parsimony analysis in Fig. 2. Some of the groups resolved in the analysis correspond (at least in part) to some of the subsections sensu Williams (1972), and morphological features (some of which have previously been considered taxonomically unimportant) are found to support some of these groupings.

The dispersal–vicariance analysis reconstructed the ancestral distributions of some groups as being restricted to one or a few areas, while for other groups the ancestral distributions could not be unequivocally inferred. The degree of resolution of these areas at deeper levels in the tree depended on the software constraints. Setting MAXAREAS to three limited the number of ancestral areas, but without this limitation, the ancestral areas at deeper nodes were not resolved unequivocally. Fig. 3 (aided by Table 4) indicates the ancestral areas for the nodes.

4. Discussion

As noted in the introduction, a number of workers have recently raised major concerns about the utility of the ITS data (see Alvarez and Wendel, 2003; Bailey et al., 2003 for a review). We are reasonably satisfied that ITS pseudogenes are absent, as the highly conserved 5.8S region shows very little variation, a test used by Razafimandimbison et al. (2004) to aid in the identification of functional copies versus ITS pseudogenes. As the data here was obtained by means of direct PCR-sequencing, we cannot be sure of how many paralagous copies of ITS there are in each sample. In addition, it must be noted that Mast (1998) alludes to the presence of multiple paralogues in an ITS study on *Banksia*, so this might be a problem in other members of the family. Paralogy in itself becomes an issue only if the multiple copies within a genome become more divergent than copies among species (Hershkovitz et al., 1999). Although it is true that the PCR can favour one of the paralogues (Hershkovitz et al., 1999), paralogy is usually readily detected by the impossibility to read the sequences due to the ambiguity in assigning bases at each site due to the presence of multiple, conflicting copies (see, e.g., Forest and Bruneau, 2000; Vanderpoorten et al., 2004).

4.1. Taxonomic implications

Because the topology presented here is merely a gene tree (Doyle, 1992; Brower et al., 1996), it is possible that the ITS data only reveal a partially correct evolutionary history. Hence we make no formal taxonomic changes, but do propose a number of steps that may be considered once additional data has been obtained. A parallel study utilising cpDNA data to test these results is currently underway.

Although the data set did not include every *Leucadendron* species, and although the phylogeny was not fully resolved, it is immediately apparent that the results obtained here do not support the current sectional and subsectional classification (Williams, 1972). Section Leucadendron is paraphyletic and section Alatosperma (those species with flattened winged fruit) is resolved into several lineages, suggesting that the fruit characters as utilised by Williams (1972) are not informative at this level. In many cases, the smaller, well-supported clades are comprised of species from one or two subsections, indicating that most of Williams' subsections are not always monophyletic, and in some instances can be merged.

The non-monophyly of the two sections in the genus implies that the two most common fruit types ("nuts" and winged fruit) have not had simple evolutionary histories. It appears from these results that the flattened winged fruit is ancestral, and the species with nut-like fruit have evolved from a winged-fruited ancestor. However, this scenario is limited by a lack of bootstrap

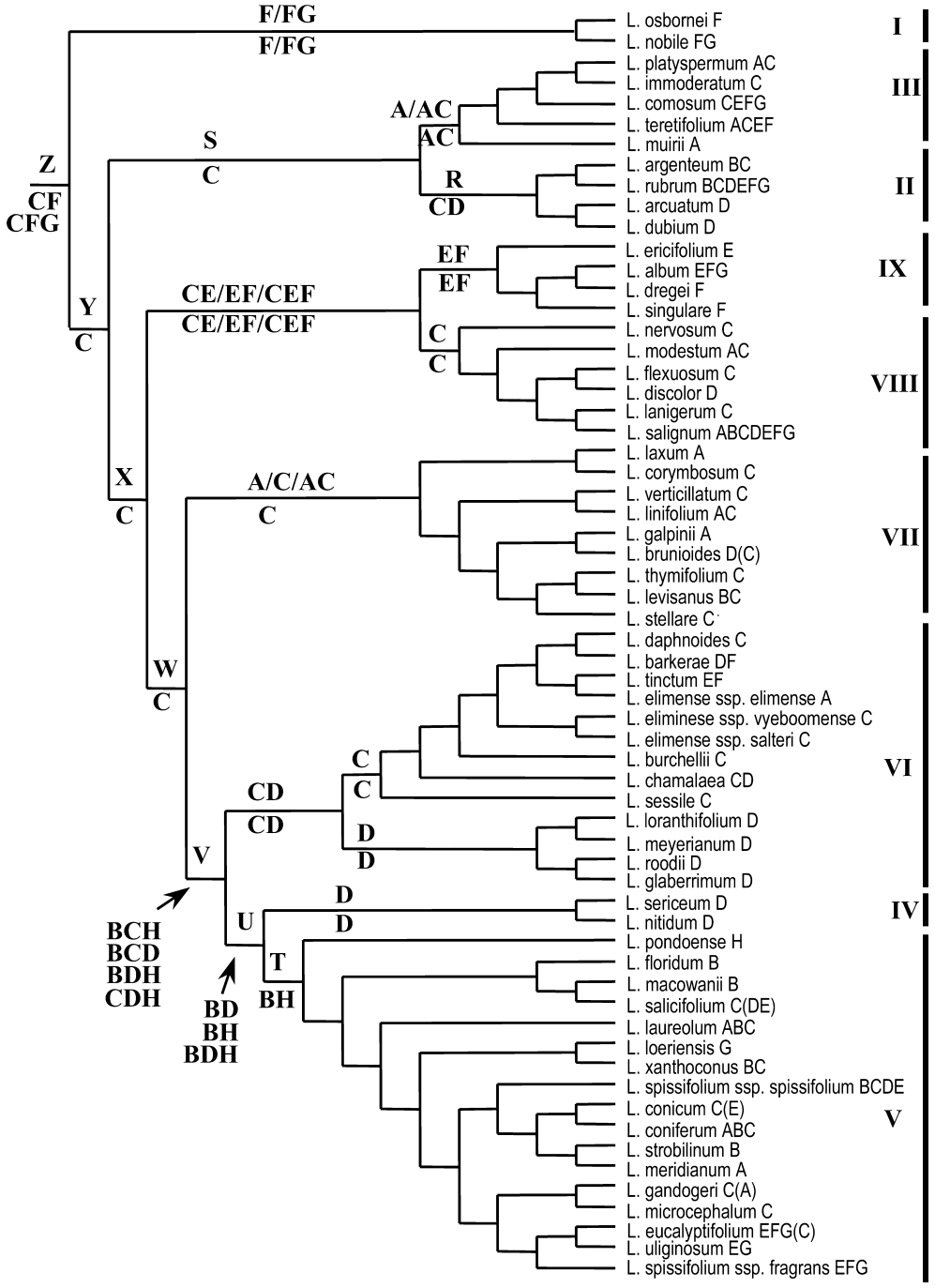

Fig. 3. Fully resolved topology (based on tree shown in Fig. 2) with ancestral areas as obtained from an analysis using DIVA (Ronquist, 1997). Letters next to the species names refer to currently inhabited areas (letters in parentheses indicate marginal occupancy in that area). Letters above the branches are results from an analysis using the defaults in DIVA, those below from the analysis restricting maximum areas to three. Codes A to H are as follows: A = Agulhas Plain, B = Cape Peninsula, C = Southwestern Centre, D = NW = Northwestern Centre, E = Langeberg Centre, F = Karoo Mountain Centre, G = Southeastern Centre, H = Pondoland. Remaining codes are explained in Table 4.

support at the major nodes along the backbone of the phylogeny. This picture is further complicated by the fact that the fruit of species of subsection Leucadendron have a wind-dispersed nut; dispersal by wind is facilitated by a ''parachute'' structure formed from fused female perianth segments attached to a persistent style. However, this structure is homoplasic given our phylogeny, as this subsection is split in our topology, with two species in Group II and two in Group IX. The lack of congruence between the ITS phylogeny and what appears to be conserved and homologous structures that characterise groups such as subsection Leucadendron is disturbing, as is it difficult to imagine this dispersal adaptation arising twice independently.

Table 4
Table of the possible ancestral areas of nodes marked in Fig. 3, obtained using DIVA (Ronquist, 1997) with default options

Node symbol	Possible ancestral areas
R	BDEG BCDEG BDEFG BCDEFG
S	ABDEG ABCDEG ABDEFG ABCDEFG
T	BH ABGH ABCGH ABEGH ABCEGH ABDEGH ABCDEGH ABEFGH ABCEFGH
U	BDH ABDGH ABCDGH ABDEGH ABCDEGH ABDEFGH ABCDEFGH
V	BDH BCDH ABDGH ABCDGH ABDEGH ABCDEGH ABDEFGH ABCDEFGH
W	ABCDGH ABCDEGH ABCDFGH ABCDEFGH
X	ABDEGH ABCDEGH ABDEFGH ABCDEFGH
Y	ABCDEGH ABCDEFGH
Z	ABCDEFGH

Key. A = Agulhas Plain, B = Cape Peninsula, C = Southwestern Centre, D = NW = Northwestern Centre, E = Langeberg Centre, F = Karoo Mountain Centre, G = Southeastern Centre, H = Pondoland.

As noted above, nine groups of species are resolved. Group I is a basal grade and comprises two species of section Alatosperma, subsection Compressa: *L. nobile* and the recently described *L. osbornei* (Rourke, 1997). Other species from subsection Compressa are placed in other groups, rendering this subsection non-monophyletic. Group II receives 51% bootstrap support and comprises species from three subsections: Membranacea, Villosa and Leucadendron, and is sister to Group III, which comprises five species from subsection Compressa, with 55% bootstrap support.

Group IV is small and well supported group (94% bootstrap) comprising the two species from Subsection Carinata: *L. nitidum* and *L. sericeum.* This is the only group that is congruent with the taxonomic treatment, and this group is distinguished morphologically by dense, silvery pubescence on the foliage, the solid fused perianth tube in the male flowers, and the presence of a double ridge on the adaxial surface of each glabrous nutlet.

Group V receives 83% bootstrap support, and species in this group are distinguished by having flat mature cone scales, flattened leaf laminas and black winged fruit that are flattened (subsection Alata) or slightly trigonal in cross section (subsection Trigona). Subsection Trigona was erected to accommodate those species with fruits that are slightly trigonal in cross section. In retrospect and in the light of these findings, this is a relatively trivial taxonomic character and probably not phylogenetically significant. However, there is a small sub-clade that comprises three species from subsection Trigona (*L. floridum*, *L. macowanii* and *L. salicifolium*), but this receives only 56% bootstrap support. Group V also includes *L. microcephalum* of the monotypic subsection Bruneobracteata, which was erected for its sticky brown involucral bracts, larger than in other species of *Leucadendron.* This taxon has otherwise all the characters of other species in subsection Alata, and we suggest that Group V could be recognized as a single subsection. It is interesting that *L. pondoense*, a geographically disjunct species from the Pondoland Sandstone areas in the Eastern Cape of South Africa, is placed as part of the basal trichotomy within Group V. This suggests that this species might be a relictual descendant of a previously more widespread ancestor with winged fruit. This species has a number of characters that might be considered primitive within this clade, such as small cones with small flattened cone scales in the female cone (i.e. floral bracts) and a complete absence of any flush of yellow pigmentation in the involucral leaves at anthesis such as is typical of all other species in the genus.

Group VI, which receives 68% bootstrap support, includes all sampled species of subsections Nucifera and Ventricosa, and it is suggested that these subsections could be merged and viewed as a single subsection. Subsection Nucifera is characterised by glabrous biconvex nut-like fruits that are produced in non-serotinous cones which open which shed the fruits 3–4 months after pollination, as well as by the presence of a sticky exudate on the young female cones and by very broad leaves. *L. elimense* and *L. chamelaea* of subsection Ventricosa are characterized by a fruit that is a glabrous ventricose nut. However, all these species share adnate female perianth segments (Williams, 1972), which represent a morphological synapomorphy for the clade. The apparent polyphyly of certain species, e.g., the three subspecies of *L. elimense*, results from a lack of resolution of the ITS data. Constraining the three subspecies of *L. elimense* as monophyletic did not result in a significant decrease in log-likelihood, suggesting that no taxonomic conclusion can be made at this level from our data.

There are two sub-clades within Group VI, but neither of these receives more than 50% bootstrap support. However, the smaller clade (*L. loranthifolium*, *L. meyerianum*, *L. roodii*, and *L. glaberrimum*) corresponds to a group of species recognized by Rebelo (1995) that is distinguishable by the presence of hairy male floral bracts. There is thus some morphological support for this dichotomy within Group VI. Furthermore, these two subclades appear to have a vicariant history, with each being restricted to adjoining ancestral areas (see below).

Group VII receives 91% bootstrap support, and comprises species of subsections Cuneata and Villosa. Within this clade there is a sub-clade comprising two species from subsection Cuneata (*L. laxum* and *L. corymbosum*) that receives 100% bootstrap support. A third species of subsection Cuneata (*L. verticillatum*) is resolved in the second, larger, sub-clade that lacks any bootstrap support but includes all but one of the sampled species of subsection Villosa (the remaining species of this section, *L. dubium*, is placed in Group II). All three species of subsection Cuneata are sampled, and it thus appears that there is merit for recognizing a reduced subsection Cuneata that comprises two-species (i.e. excluding *L. verticillatum*). Group VII is a uniform clade characterised by villous mottled ovoid nutlets. Williams (1972) places *L. verticillatum* in subsection Cuneata principally on account of the slightly angular nutlets, and there is no reason why on morphological grounds, *L. verticillatum* should not be placed in subsection Villosa, as the distinction is a rather fine taxonomic one.

Group VIII comprises five species of subsection Alata and *L. nervosum* of the monotypic subsection Nervosa. The inclusion of the latter species as a member of this clade is, however, not supported by bootstrap analyses, while the remaining five species form a clade with 73% bootstrap support. These five species are characterised by flat, black winged glabrous fruits, free female perianth segments, and dimpled mature cone scales bent at right angles in the middle with upper half pubescent and lower half glabrous. The synapomorphy of angled cone scales is a particularly useful morphological character supporting this clade. In addition, SEM studies of the fruit of at least some of the species in this group show that, while flattened like those of species of the larger clade comprising the majority of species from section Alatosperma (Group V), the surface of the fruit possesses raised dome-like bumps, possibly mirroring the dimpled nature of the cone scales (Barker, unpublished data). The unusual nutlet and floral features that unite these five species may have evolved from a *L. nervosa*-like ancestor in a manner paralleling that which led to the similar (but obviously non-homologous) flattened fruit typical of the taxa in section Alatosperma (Group V, discussed above). Furthermore, *L. nervosa* provides a morphological intermediate between the five anomalous species of subsection Alata and Group IX, as both *L. nervosum* and the species of Group IX have conic-acute cones, lanceolate acute straight cone scales, the fruits are obovoid pubescent nutlets and the styles are persistent. Although *L. nervosa* is separated from the five species of subsection Alata by a long branch, and its position does not receive any bootstrap support, it is considered here to be part of this clade. An argument for its identification as a monotypic lineage could carry some merit, but we see little point in naming monotypic lineages at subsection rank.

Group IX is morphologically heterogeneous but receives 99% bootstrap support. This clade includes two species of the polyphyletic subsection Leucadendron, *L. dregei*, and *L. album*. *L. singulare* (the only representative of the two-species subsection Aliena) and *L. ericifolium* of subsection Uniflora form a sister clade to these former two species. The relationship of species of subsection Leucadendron with *L. ericifolium* is novel, as Williams (1972) thought this latter species to be "probably the most advanced species in the genus", as the number of female flowers has been reduced to one, and the male reduced to 12. While such reduction can be considered to be an advancement, it is unique to this species and is thus simply autapomorphic in a phylogenetic context. *L. ericifolium* may be regarded as a highly reduced member of the lineage on account of its conic-acute cones, lanceolate-acute cone scales and pubescent rounded nut-like fruits which are probably a specialised form of the obovoid fruit found in the other members of the clade. *L. ericifolium* also lacks persistent styles, as do other members of subsection *Leucadendron*.

4.2. Evolution of life history traits

As mentioned in the introduction, *Leucadendron* has a number of life history traits, some of which are thought to be adaptations to survival in the fire-prone fynbos biome. Here we briefly discuss the distribution and possible evolution of serotiny and re-seeding versus resprouting as a fire survival strategy, pollination syndrome and myrmechochory (Table 3). These traits have been mapped onto the ML phylogeny (Fig. 4). Owing to the inadequate bootstrap support of the basal and deeper nodes, we felt it unnecessary to reconstruct ancestral states as the bootstrap support, but discuss these data within this limitation.

4.2.1. Wind pollination

The majority of the species in the genus have been recorded as entomophilous, and Williams (1972) reports on the presence of both nectar (in minute quantities, observed in 61 species) and various odours. However, a few taxa are anemophilous, and this pollination syndrome appears to have arisen independently a number of times, being found in some species of Groups II, III, V, and IX. However, detailed field studies on the pollination of the majority of the species are lacking. Corroboration and elaboration of preliminary observations of pollination biology (mostly by Williams, 1972) would be a valuable contribution to the study of the biology of the genus.

4.2.2. Serotiny

Serotiny (the retention of seeds on the maternal plant for an extended period) is found consistently (i.e. in all sampled species) in Groups I, III, V, and VIII. It is also found in some species of Groups II, VII, and IX. Groups

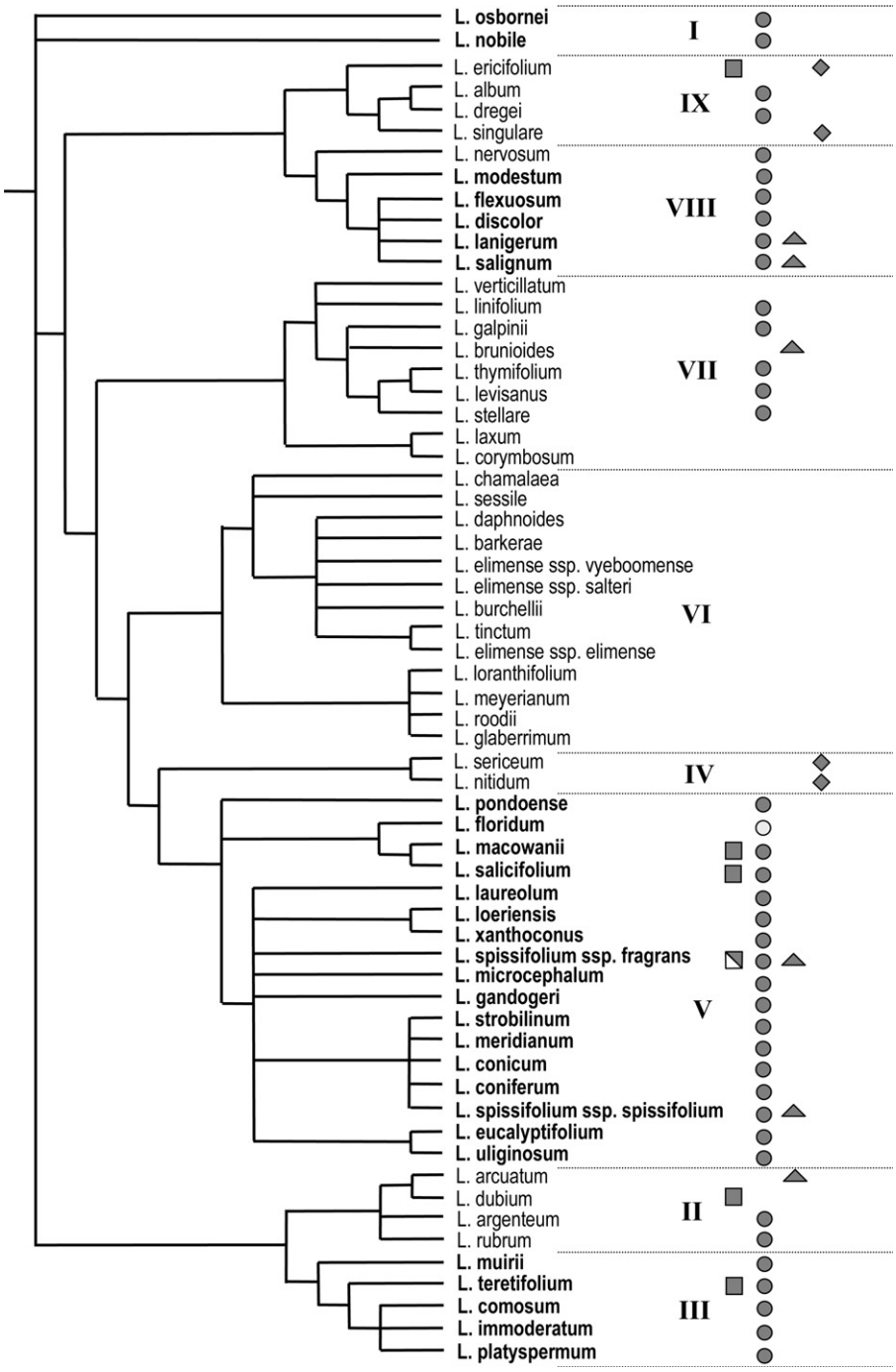

Fig. 4. The maximum likelihood topology with life history traits for each taxon indicated in the columns to the right. Species names in bold possess windged fruit. Key: Square = anemophily; circle indicates serotiny (pale circle indicates partial serotiny); triangle indicates re-sprouting; diamond indicates myrmechochory. See Table 3 for further details.

IV and VI are exclusively non-serotinous, a feature that has been proposed as the primitive condition (Williams, 1972). As the deeper nodes of the phylogeny are not well supported, it is not possible to determine the polarity of this character, but should the existing phylogeny be corroborated by other data, this would suggest that the ancestral condition was one of serotiny, which has been lost completely in some groups (Groups IV and VI), as well as in individual species of other Groups.

4.2.3. Re-seeding and re-sprouting as a fire survival strategy

Bond and Midgley (2001) cite studies in Mediterranean floras (of which the fynbos is one) that indicate that the switch from sprouting to non-sprouting has

occurred repeatedly. This is opposite to the implications from our data, which suggest that non-sprouting taxa (reseeders) are the majority, and re-sprouting as a fire survival mechanism has evolved independently as many as six times (five, if *L. salignum* and *L. lanigerum* are resolved as sister taxa). Re-sprouters are found in Groups II, V, VII, and VIII. As noted by Bond and Midgley (2001), there are no genetic studies of sprouters versus non-sprouters. *Leucadendron* is thus a suitable model for such studies, which are currently underway (Olivieri, personal communication).

4.2.4. Myrmechochory

The data on myrmechochory is taken from Midgley (1987) who scores the presence of an eliasome on the fruit as an indicator of this seed dispersal syndrome. This syndrome is rare in *Leucadendron*, and appears in only four of the sampled taxa. Notably, both species of Group IV (subsection Carinata) are myrmechochorous, suggesting that this trait was common in the ancestor of these species. Myrmechochory has thus arisen independently three times in the phylogeny of sampled taxa.

4.3. Biogeographical implications

The ''Cape clade'' of African Proteaceae (Barker et al., 2002), including *Leucadendron*, fulfills both of Linder's (2003) pre-requisites for consideration as a Cape floral clade: more than 50% of the species occur in the Cape Floristic Region (CFR), and the clade (probably) originated in the CFR, as the basal-most taxa and clades are found exclusively within the CFR. As noted and illustrated by Williams (1972, his Figure 45), the highest species level diversity of *Leucadendron* is found in the Caledon region, a pattern typical of many CFR taxa.

As the ITS topology presented here (Fig. 1) has a number of previously mentioned weaknesses, the following discussion is limited. Nonetheless we feel it is pertinent to include this analysis, as it demonstrates some patterns that can be further tested by additional data or parallel studies on other fynbos groups, especially of Proteaceae. By means of DIVA we feel that it is relevant to discuss the ancestral areas of at least some of the (sometimes well supported) groups of species. As noted in the methods section, two analyses were performed using DIVA, differing in the maximum number of areas that the software considered (Fig. 3).

In the selected topology, the ancestral area of Group I is resolved as the Karoo Mountain Centre, or this and the Southeastern Centre. The ancestral area of Group II (51% bootstrap support) is not clearly resolved, unless MAXAREAS is limited to three in which case the ancestral area is given as the Southwestern and Northwestern Centres of endemism. The ancestral area of Group III (55% bootstrap support) is given as either the Agulhas Centre or both this and the Southwestern Centre, despite the fact that some species are found in other centers as well, including the Karoo Mountain Centre. The small Group IV (94% bootstrap support) has the Northwestern Centre as an ancestral area. Group V (83% bootstrap support) has an unresolved ancestral area under the default options, irrespective of different solutions to resolving the polytomies present in the clade. This result is reduced to the Peninsula Centre and one other centre (Pondoland, in the Fig. 3) depending on the topology used when MAXAREAS is limited to three.

The majority of species in Group V are widespread, being found in on average 2.11 phytogeographic centers per species. It is tempting to suggest that this may be, in part, due to the greater dispersal ability of the winged fruit that has confounded the ability of DIVA to clearly resolve an ancestral area under default conditions. However, other factors such as edaphic tolerance may also allow for such widespread dispersion of these species. This is in contrast, for example, with Group VI (68% bootstrap support) that comprises subsections Nucifera and Ventricosa, which have nut-like fruit. These latter taxa are found on average in only 1.3 phytogeographic centers. Once again it is possible to invoke the limited fruit dispersal distances as a cause for this more restricted distribution, and Midgley et al. (2002) note that short-distance dispersal and burial of these fruit by scatter-hoarding rodents occurs in some species with nut-like fruit. The ancestral area for Group VI is resolved as the Southwestern and Northwestern centres. As noted above, Group VI is divided into two subclades (neither with any bootstrap support, but some morphological support). The smaller of the subclades comprises species exclusively from the Northwestern Centre, while the species in the other subclade are mainly found in the Southwestern Centre, and it is thus not unexpected that DIVA resolves the ancestral areas of these subclades as being the Northwestern and Southwestern centers respectively, a result that does not change with the variously resolved polytomies tested here. This suggests that a vicariance event occurred in this group, resulting in this distribution pattern. The ancestral area for Group VII is given as either or both the Southwestern or Agulhas Centres, but restricted to the Southwestern Centre only under the MAXAREAS = 3 restriction. Species within this clade do, however, have extensions into other centers (*L. brunioides*, from the Northwestern Centre with a small presence in the Southwestern Centre, and *L. levisanus* extends into the Peninsula Centre from the Southwestern Centre). Similarly, species of Group VIII have a predominantly Southwestern distribution, with some extension into the Northwestern and Agulhas Centers, and the ancestral area is resolved as being the Southwestern Centre (different solutions to the resolution of polytomies did not affect this result). One species of this clade, *L. salignum*, is the most widespread species sampled here, being found in all

phytogeographic centers of the Cape Floristic Region. Group IX (99% bootstrap support) contains species principally from the Karoo Mountain Centre, with extensions into the Langeberg and Southeastern Centres, but with the Karoo Mountain and Langeberg Centres being shown by DIVA as ancestral.

DIVA is unable to resolve the ancestral area of the genus using the default settings, but when the software is restricted to resolve a maximum of three ancestral areas, it determines this area to be either the Southwestern and Karoo Mountain Centres, or these regions and the Southeastern Centre. It is interesting to note that of the 15 taxa in the more basal groups (I, II, III, and IX), eight are from the Karoo Mountain or Langeberg Centres. However, many of the other groups have an ancestral area in either or both the Southwestern and Northwestern Centres.

Although it is a typical fynbos genus, the radiation of *Leucadendron* probably predates the origin and expansion of the winter rainfall region and associated ''fynbos'' vegetation (Barker et al. in preparation). The fynbos is thought to have originated 6–7Myr ago (Richardson et al., 2001; but see Linder, 2003). Although the age of *Leucadendron* is not known, the Proteaceae fossil record from southern Africa extends well back into the Tertiary (Scholtz, 1985), although there are no known fossils that can be associated with *Leucadendron*. As noted by Linder (2003) it is possible that the Oligocene climate of the Cape was similar to that found in the region today, and that the ancestors of modern day *Leucadendron* could have appeared at this time, survived the more tropical climates of the early Miocene, and subsequently radiated southwards once the more modern climates were re-established in the mid to late Miocene. However, as Linder (2003) notes, there is as yet insufficient evidence to support this scenario, and until a suitable calibration point that will allow for the dating the ages of radiations by means of molecular methods, this interesting scenario remains just that—an interesting scenario. Thus, while the results from the DIVA analysis are not decisive, it appears that *Leucadendron* may have originated from the arid northern fringes of the CFR, possibly well before the establishment of the current climate regime that influences the CFR.

Williams (1972) also notes that there appears to be a strong correlation between species distribution and rainfall. He noted that 59 taxa are found growing only (or almost only) in the winter rainfall zone (to the west of the 20° line of longitude), and 24 taxa are restricted to the non-seasonal rainfall zone, east of the 20° line of longitude (see Williams (1972), his Fig. 48). While this line is parallel to the line of longitude, Williams (1972) also indicates a second line (running from Gansbaai on the coast inland through the region between Robertson and Worcester, shown as a dashed line in Fig. 1). This second line divides the Cape into a western region (that receives its rainfall almost exclusively in winter) and an eastern region. The western region coincides almost exactly with the Peninsula, Southwestern and Northwestern Centres of endemism, while the region to the east of this line comprises the rest of the phytogeographical centres (Agulhas, Langeberg, Southeastern and Karoo Mountain Centres of endemism). When the phylogeny is examined in the context of this broader division, it becomes apparent that the more basal elements (Group I, part of Groups III and IX) contain taxa that are predominantly from the Eastern (non-seasonal) rainfall zone. It thus appears that the more derived groups have radiated from what is currently a non-seasonal rainfall regime into the predominantly winter rainfall regime. However, (as noted above), the lack of support for the spine of the phylogeny limits this interpretation, which also presupposes the current rainfall patterns have been stable over an extended period, which is unlikely.

5. Conclusion

Maximum likelihood and parsimony analyses of ITS sequence data do not fully resolve the phylogeny of *Leucadendron* at the species level, and bootstrap support at all the deeper nodes is lacking. Despite this, the results presented here clearly show that the infrageneric taxonomy of the genus needs a thorough revision. Both sections (Alatosperma and Leucadendron) and many subsections are not monophyletic. Notable among these are subsections Compressa, Alata and Leucadendron. Subsections Nucifera and Ventricosa form a well supported clade that suggests these two subsections ought to be combined into one. At least one species of subsection Cuneata (*L. verticillatum*) ought to be included in subsection Villosa. The relationships of the species from the three monotypic subsections are also resolved. *Leucadendron ericifolium* (subsection Uniflora) is placed basal to species from subsections Leucadendron and Aliena; *L. nervosum* (subsection Nervosa) is associated with elements of a small clade of species that were originally placed in subsection Alata; and *L. membranacea* (subsection Brunneobracteata) is clearly a member of the larger subsection Alata clade, a group that could be considered as section Alatosperma sensu stricto. This means that characters such as fruit shape have evolved independently in different *Leucadendron* lineages.

Given the caveat of limited support at basal nodes, and the fact that this is a single gene tree, an examination of life history traits is limited. However, based on the distribution of these traits, it appears that anemophily, myrmechochory and the ability to re-sprout after fire evolved multiples times. An assessment of serotiny is a little more complex, but it appears that serotiny was ancestral, and has been lost a number of times, most notably in Groups IV and VI.

Dispersal–vicariance analysis suggests that many of the groups identified here have only one or two ancestral areas, but the ancestral area of the more basal nodes is not resolved unequivocally. However it is tentatively proposed that (under analytical constraints) the genus may have radiated from an ancestor in what is now the Karoo Mountain or the Southeastern Centre of endemism, but this is limited by the poorly supported relationships of the species groups.

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