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Molecular phylogeny of *Acacia* subgenus *Phyllodineae* (Mimosoideae : Leguminosae) based on DNA sequences of the internal transcribed spacer region

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Abstract. The largest monophyletic group within *Acacia* is subgenus *Phyllodineae*, with more than 950 predominately Australian species, the majority characterised by adult foliage consisting of phyllodes. Molecular sequence data from the internal transcribed spacers (ITS) of the nuclear ribosomal DNA repeat were used to investigate the monophyly of seven sections within the subgenus. A nested PCR approach was used to amplify the ITS region. Fifty-one species representative of all sections were sequenced together with one outgroup taxon *Lysiloma divaricata* (Ingeae).

Phylogenetic parsimony analysis suggested that there are two main clades within *Phyllodineae* but that only one section, *Lycopodiifoliae*, is apparently monophyletic. In one of the main clades, *Lycopodifoliae* is related to some taxa in sections *Alatae* and *Pulchellae* and some members of section *Phyllodineae*. In the second main clade, sections *Juliflorae*, *Plurinerves* and *Botrycephalae* cluster with other members of section *Phyllodineae*. The two sections that are characterised by bipinnate foliage, *Botrycephalae* and *Pulchellae*, are nested within phyllodinous clades, indicating that at least two separate reversals to bipinnate leaves have occurred. *Botrycephalae* is paraphyletic with respect to taxa from section *Phyllodineae* that have uninerved phyllodes and racemose inflorescences.

Introduction

The genus *Acacia* Miller currently includes about 1300 species and forms the second-most species-rich genus in the family Leguminosae (Mabberley 1997; Maslin 2001). *Acacia* is widespread with species in Africa, the Americas, Asia and Australia. In Australia, there are approximately 960 species, which makes *Acacia* the largest genus of vascular plants in that region (Maslin 2001). Despite generic revision by Pedley (1986), the currently accepted classification of *Acacia* divides the genus into the following three subgenera (Vassal 1972): *Acacia*, *Aculeiferum* and *Phyllodineae*. There is growing molecular and morphological evidence that *Acacia* is not monophyletic (Chappill and Maslin 1995; Grimes 1999; Robinson and Harris 2000; Miller and Bayer 2000, 2001). For a comprehensive review of the taxonomic history of *Acacia* see Maslin *et al.* (2003).

Acacia subgenus *Phyllodineae* has been described as the 'Australian group' (Guinet 1969; Ross 1981). Of the 950 species of *Phyllodineae*, only 18 occur outside the Australian continent (Pedley 1975). Recent molecular studies have demonstrated that *Phyllodineae* is monophyletic and is sister to members of the tribe Ingeae (Miller and Bayer 2000, 2001; Robinson and Harris 2000). The majority of taxa in *Phyllodineae* have adult foliage that is phyllodinous, although 69 taxa have adult foliage that is bipinnate.

Sectional rankings within *Phyllodineae* are somewhat confused, although the classification of Pedley (1978) is most commonly accepted (Maslin 1995*a*) and is the classification used in this study (Table 1). Of the seven sections recognised by Pedley (1978), three are large and widespread (*Phyllodineae*, *Juliflores* and *Plurinerves*), while the other four (*Botrycephalae*, *Pulchellae*, *Alatae* and *Lycopodiifoliae*) are smaller and generally have more restricted distributions (Table 2). The sections are characterised by combinations of macro-morphological characters (for a simplified key see Maslin 1995*a*). These characters include the presence of phyllodes or compound leaves; phyllode nervature (plurineved or uninerved); and

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Table 1. A comparison of the classification schemes of *Acacia* subgenus *Phyllodineae* by Bentham (1875), Vassal (1972), Pedley (1978), Pedley (1986) and Maslin and Stirton (1997), modified from Chappill and Maslin (1995)

Where possible equivalent groups are aligned. G. = genus; Ser. = series; S.ser. = subseries; S.g. = subgenus; Sect. = section; S.sect. = subsection

Bentham (1875)	Vassal (1972)	Pedley (1978)	Pedley (1986)	Maslin and Stirton (1997)
	S.g. *Heterophyllum* (Syn. *Phyllodineae*)	S.g. *Phyllodineae*	G. *Racosperma*	S.g. *Phyllodineae*
Ser. *Botrycephalae*		Sect. *Botrycephalae*	Sect. *Racosperma*	Sect. *Botrycephalae*
Ser. *Phyllodineae*		Sect. *Alatae*		Sect. *Alatae*
S.ser. *Alatae*				
S.ser. *Continuae*				
S.ser. *Uninerves*				
	Sect. *Uninervea*	Sect. *Phyllodineae*		Sect. *Phyllodineae*
				a. 'Racemose species'
S.ser. *Plurinerves*				b. 'Non-racemose species'
S.ser. *Pungentes*	Sect. *Heterophyllum*	Sect. *Plurinerves*	Sect. *Plurinervia*	Sect. *Plurinerves*
S.ser. *Calamiformes*	S.sect. *Globuliforae*			a. 'Microneurous species'
S.ser. *Juliflorae*				b. 'Oligoneurous species'
	S.sect. *Spiciferae*	Sect. *Juliflorae*		Sect. *Juliflorae*
				a. 'Microneurous species'
				b. 'Oligoneurous species'
S.ser. *Brunioideae*				
	(rank not used)	Sect. *Lycopodiifolia*	Sect. *Lycopodiifoliae*	Sect. *Lycopodiifolia*
Ser. *Pulchellae*	Sect. *Pulchelloidea*	Sect. *Pulchellae*	Sect. *Pulchellae*	Sect. *Pulchellae*
	S.sect. *Parviscutellae*			
	S.sect. *Magniscutellae*			

Table 2. Sections within subgenus *Phyllodineae*

Total number of species shown and major geographic regions (Maslin and Hopper 1982; Maslin 1995*a*, 2001)

Subgenus *Phyllodineae*	Distribution in Australia	No. of species
Section *Botrycephalae*	Temperate eastern–south-eastern Australia	42
Section *Pulchellae*	Temperate south-western Australia	27
Section *Alatae*	Temperate south-western Australia	21
Section *Lycopodiifoliae*	Tropical and subtropical Australia	17
Section *Phyllodineae*	Temperate southern Australia (W & E)	408
Section *Plurinerves*	South-western and eastern Australia	212
Section *Juliflorae*	Tropical, subtropical and south-western Australia; few eastern and southern	235

inflorescence structure (axillary capitula, racemes or spikes). It has been recognised that groupings of taxa on the basis of these characters may be pragmatic rather than natural groups, with some apparently closely related species classified into different sections (Maslin 1988; Chappill and Maslin 1995; Maslin and Stirton 1997). There is a critical need for a phylogentically based classification within subgenus *Phyllodineae* (Maslin 2001).

In this study, taxa spanning much of the morphological diversity in subgenus *Phyllodineae* have been sampled for phylogenetic analysis to test the monophyly of the sections erected by Pedley (1978). Our phylogenetic analysis is based on sequencing the internal transcribed spacer regions (ITS) of nuclear ribosomal DNA (Baldwin 1992).

Materials and methods

Ingroup taxa were selected from *Acacia* subgenus *Phyllodineae*, with reference to a 'critical list' of species (Maslin and Stirton 1997). The outgroup, *Lysiloma divaricata* (Jacq.) Macbr., was chosen on the basis of results of recent studies, which showed members of the Ingeae as sister to subgenus *Phyllodineae* (Miller and Bayer 2000, 2001). The ingroup comprised 51 taxa and included species sampled from all seven sections of *Acacia* subgenus *Phyllodineae* (Table 3).

Genomic DNA was isolated with Dneasy Plant (Qiagen) according to manufacturer's or CTAB protocol (Doyle and Doyle 1987) and further purified with Qiagen tip20 (Qiagen) following the manufacturer's protocol for genomic DNA purification. The internal transcribed spacer region (ITS) was amplified from purified DNA via the polymerase chain reaction (PCR). An *Acacia*-specific primer, ACF, was designed and used in conjunction with the primer 26SE (Sun *et al.* 1994) to amplify the complete ITS region. A nested PCR approach with the primers listed in Fig. 1 was then used to further amplify the ITS region.

Table 3. The classification, voucher details and Genbank accession number for taxa sampled in this study

The classification is after Vassal (1972) and Pedley (1978). MELU = The University of Melbourne, School of Botany Herbarium; MEL = National Herbarium of Victoria; NSW = Royal Botanic Gardens, Sydney Herbarium (NSW); CANB = Australian National Herbarium, Canberra

Taxon	Voucher	Genbank accession
Acacieae Benth.		
Acacia Mill.		
Subgenus *Phyllodineae* (DC.) Ser.		
Section *Phyllodineae* DC.		
A. ampliceps Maslin	MELU DM323	AF360718
A. binervata DC.	CANB 615570	AF487775
A. blakelyi Maiden	CANB 615688	AF487759
A. chrysocephala Maslin	MEL 2080541	AF487760
A. euthycarpa (J.Black) J.Black	MEL 2039729	AF360719
A. falciformis DC.	MELU DM213	AF360720
A. fasciculifera Benth.	CANB 615692	AF487769
A. genistifolia Link	MEL 2033962	AF487770
A. paradoxa DC.	MELU DM203	AF360717
A. penninervis DC.	CANB 615698	AF360721
A. rossei F.Muell.	MEL 2069821	AF487756
A. suaveolens Willd.	CANB 615579	AF487768
A. victoriae Benth.	MEL 2029029	AF487772
Section *Botrycephalae* (Benth.) Taub.		
A. elata Benth.	MELU SRA002	AF360701
A. fulva Tind.	MELU SRA030	AF360702
A. jonesii Maiden	CANB 615653	AF487776
A. latisepala Pedley	MELU IRT537	AF360703
A. leptoclada Cunn. and Benth.	MELU SRA041	AF360704
A. leucoclada Tind.	MELU SRA042	AF487777
A. mearnsii De Wild.	MELU DM200	AF360705
A. mitchellii Benth.	MEL 2018082	AF360706
A. spectabilis Benth.	MEL 2034602	AF487778
A. storyi Tind.	NSW 74766	AF360707
Section *Juliflorae* (Benth.) C.Moore & E.Betche		
A. acradenia F.Muell.	MELU DM312	AF487765
A. acuminata Benth.	CANB 615660	AF360708
A. aulacocarpa A.Cunn. ex Benth.	MEL 283916	AF487766
A. colei Maslin & Thompson	MELU DM326	AF360710
A. curranii Maiden	CANB 615671	AF487764
A. cyperophylla Benth.	CANB 615672	AF487767
A. denticulosa F.Muell.	CANB 615673	AF487763
A. longifolia (Andrews) Willd.	MELU DM201	AF360711
A. lysiphloia F.Muell.	CANB 615566	AF360712
A. multispicata Benth.	CANB 615739	AF487761
A. tumida F.Muell. ex Benth.	MELU DM306	AF360709
A. verticillata (L'Her.) Willd.	MELU DM208	AF360713
A. wanyu Tindale	CANB 615679	AF487762
Section *Alatae* (Benth.) Pedley		
A. alata R.Br.	MELU DM224	AF360699
A. aphylla Maslin	CANB 615642	AF487758
A. spinescens Benth.	MELU DM246	AF360700
Section *Pulchellae* (Benth.) Taubert		
A. drummondii Lindley	MEL 2034627	AF360725
A. guinetii Maslin	CANB 615716	AF487757
A. lateriticola Maslin	MEL 248018	AF487774
A. pentadenia Lindley	MEL 2043540	AF487773
A. pulchella R.Br.	MELU DM268	AF360726
Section *Plurinerves* (Benth.) C.Moore & E.Betche		
A. cognata Maiden and Blakely	CANB 615708	AF487771
A. melanoxylon R.Br.	MELU DM210	AF360723
A. oswaldii F.Muell.	MELU DM250	AF360714
A. platycarpa F.Muell.	MELU DM327	AF360724
A. translucens Cunn. ex Hook.	MELU DM302	AF360722
Section *Lycopodiifoliae* Pedley		
A. adoxa Pedley	MEL 2041667	AF360715
A. lycopodiifolia Hook.	MEL 2044632	AF360716
Ingeae Benth.		
Lysiloma divaricata (Jacq.) Macbr.	CANB 615742	AF487755

PCR reactions were prepared with HotStarTaq DNA Polymerase kits (Qiagen). The total volume of the PCR reactions was 50 μL. Reactions contained 5 μL PCR buffer [containing Tris–HCl, KCl $(NH_4)_2SO_4$, 15 mM $MgCl_2$; pH 8.7], 0.2 mM each dNTP, 3 mM $MgCl_2$, 10 pmol of each primer, 1.25 units HotStarTaq DNA polymerase, 30–100 ng of template DNA and 10 μL Q-solution (Qiagen). Thermal cycling was performed on an Eppendorf Mastercycler gradient thermal cycler with one hold at 95°C for 15 min; 30 cycles of 94°C for 30 s, 63.8°C for 30 s and 72°C for 20 s; and one hold of 72°C for 5 min. PCR products were visualised by agarose gel electrophoresis and stained with ethidium bromide. Products of PCR amplification were purified with CONCERT Rapid PCR Purification System (GibcoBRL) or were extracted from 0.8% agarose, 1 × TBE gel and purified with QIAquick Gel Extraction Kit (Qiagen).

Alternatively, the ITS region was amplified without nested PCR, using the primers S3 (Käss and Wink 1997) and 26SE. The PCR reaction mixture (Perkin-Elmer Applied Biosystems) consisted of 5 μL of 20× reaction buffer, 6 μL of 25 mmol L^{-1} $MgCl_2$, 16 μL of a 1.25 mmol L^{-1} dNTP solution in equimolar ratio, 25 pmol of each primer, 10–50 ng of template DNA and 1.0 unit of polymerase in a total volume of 100 μL. PCR samples were heated to 94°C for 3 min prior to the addition of DNA polymerase and thermal cycling was performed by 30 cycles of denaturation (94°C for 1 min), primer annealing (55°C for 1 min) and extension (72°C for 2 min). A 7-min final extension hold at 72°C completed the thermal cycling. PCR products were then purified for sequencing with QIAquick PCR Kit (Qiagen).

Purified DNA was used as a template for direct sequencing with primers S3, S4, S5, S6 (Käss and Wink 1997) and 26SE. Prism Ready Reaction DyeDeoxy Terminator Cycle Sequencing kits or Prism Big Dye Terminator Cycle Sequencing kits (Perkin-Elmer Applied Biosystems) were used for cycle-sequencing reactions, following the manufacturer's protocol. Sequencing gels were run and analysed on ABI automated sequencers at The University of Melbourne, School of Botany Plant Cell Biology Research Centre, and at CSIRO Plant Industry, Canberra.

Contiguous sequences were edited with Sequencher v3.0 (Gene Codes Corporation) and manually aligned in SeqPup v0.6 (Don Gilbert, Indiana University). Sequence alignments and PAUP/Nexus formatted files are available from the authors on request. All sequences are lodged in Genbank (Table 3).

Any uncertain base positions, generally located close to priming sites, and highly variable regions with uncertain sequence homology were excluded from phylogenetic analysis. Individual base positions were coded as unordered multistates and insertions/deletions (indels) were coded as binary or multistate characters. Regions coded as indels were generally excluded from further analysis, unless informative characters of base pair substitutions were present within an indel region. Indel characters were entered into a PAUP/Nexus formatted file and exported for phylogenetic analysis in PAUP v4.0b8 (Swofford 1998). Parsimony analyses were conducted by a four-step heuristic search strategy (Olmstead and Palmer 1994; Miller and Bayer 2000). Uninformative characters were excluded from the analyses and trees were rooted at the outgroup taxon. Branch support values were calculated in PAUP via 1000 heuristic bootstrap replicates, with TBR branch swapping and a tree limit of 10000 trees per replicate.

Results

Features of the internal transcribed spacers and 5.8S gene sequences

Sequencing near the 5′ end of ITS 1, close to the S4 primer, was problematic and a high proportion of the taxa have partial sequences for ITS 1. For those taxa with completely sequenced ITS 1 regions, the length ranged from 214 to 224 base pairs (bp) (Table 4) and the total aligned length was 268 bp. Four informative indel characters, ranging in size

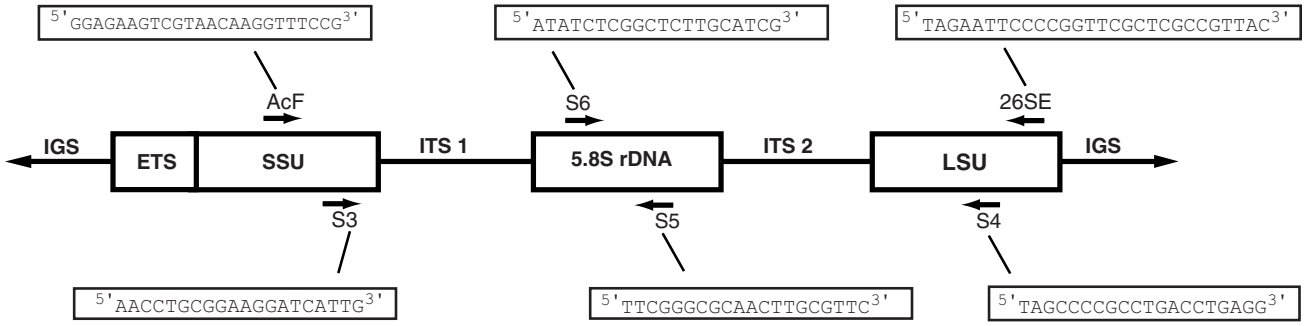

Fig. 1. Diagram of the organisation of the rDNA cistron (not to scale) and primers used for nested and semi-nested PCR to amplify and sequence the ITS region in *Acacia*. The arrows denote the direction of extension of primers. S primers (Käss and Wink 1997) and 26SE (Sun *et al.* 1994). LSU = large-subunit rDNA; IGS = intergenic spacer; ETS = external transcribed spacer; ITS = internal transcribed spacer.

Table 4. Summary of sequence information for completely sequenced internal transcribed spacer DNA regions

DNA region (position in alignment)	ITS 1 (14–281)	5.8S gene (282–440)	ITS 2 (441–703)
Aligned length (bp)	268	159	263
Unaligned length, range (bp)	214–224	159	191–226
Ambiguous sequence and indel regions deleted (bp)	56	0	44
Sequence used (bp)	212	159	219
G+C content (mean%)	69.44	57.2	71.17
Variable sites (%)	42.45	15.09	53.42
Informative sites (%)	19.34	3.14	23.74
Constant sites (%)	52.83	84.91	46.58
Autapomorphic sites (%)	27.83	11.95	29.68
Number of indels	4	0	0
Indel size range (bp)	4–12	—	—
Informative base subsitutions	41	5	52
Total informative characters	45	5	52

from 4 to 12 bp (base positions 65–70, 75–78, 80–91 264–268), and 41 informative base substitutions were scored.

The 5.8S gene region was highly conserved in comparison to the two ITS spacers surrounding it and no length variation was observed in the 52 taxa, all being 159 bp long. However, there was some sequence divergence (15.09% variable sites) and three informative base substitutions. No indel characters were scored and no sequence needed to be excluded from the analysis. It is notable that the G + C content of the 5.8S gene (57.2%) was substantially lower than that found in the two ITS spacers (ITS 1 69.44% and ITS 2 71.17%).

The ITS 2 region ranged in length from 191 to 226 bp and the aligned length was 263 bp. This region had the greatest number of informative characters (52), all of which were base pair substitutions, and the highest proportion of variable sites (53.42%) in the ITS region.

Cladogram topology

The ITS region provided 103 informative characters for the 51 ingroup taxa and the outgroup, *Lysiloma divaricata* (Ingeae). Heuristic parsimony analysis resulted in 116423 equally parsimonious trees (length 420, CI = 0.39, RI = 0.69). Twenty-six resolved nodes were common to all most parsimonious trees, with 13 of these nodes having bootstrap support (bt) >50% (Fig. 2). Only one of the seven sections in subgenus *Phyllodineae* is monophyletic, section *Lycopodifoliae* (Fig. 2, node 20, bt = 100%), although the analysis includes only two exemplars of seventeen species in this group. Section *Phyllodineae* in particular is clearly polyphyletic.

The strict consensus tree (Fig. 2) shows two main clades. Clade A includes, in phyletic sequence, *Acacia victoriae* (Node 1, section *Phyllodineae* with racemose inflorescences), *A. suaveolens* (Node 3, *Phyllodineae* racemose), *A. melanoxylon* (Node 4, *Plurinerves*), *A. fasciculifera*

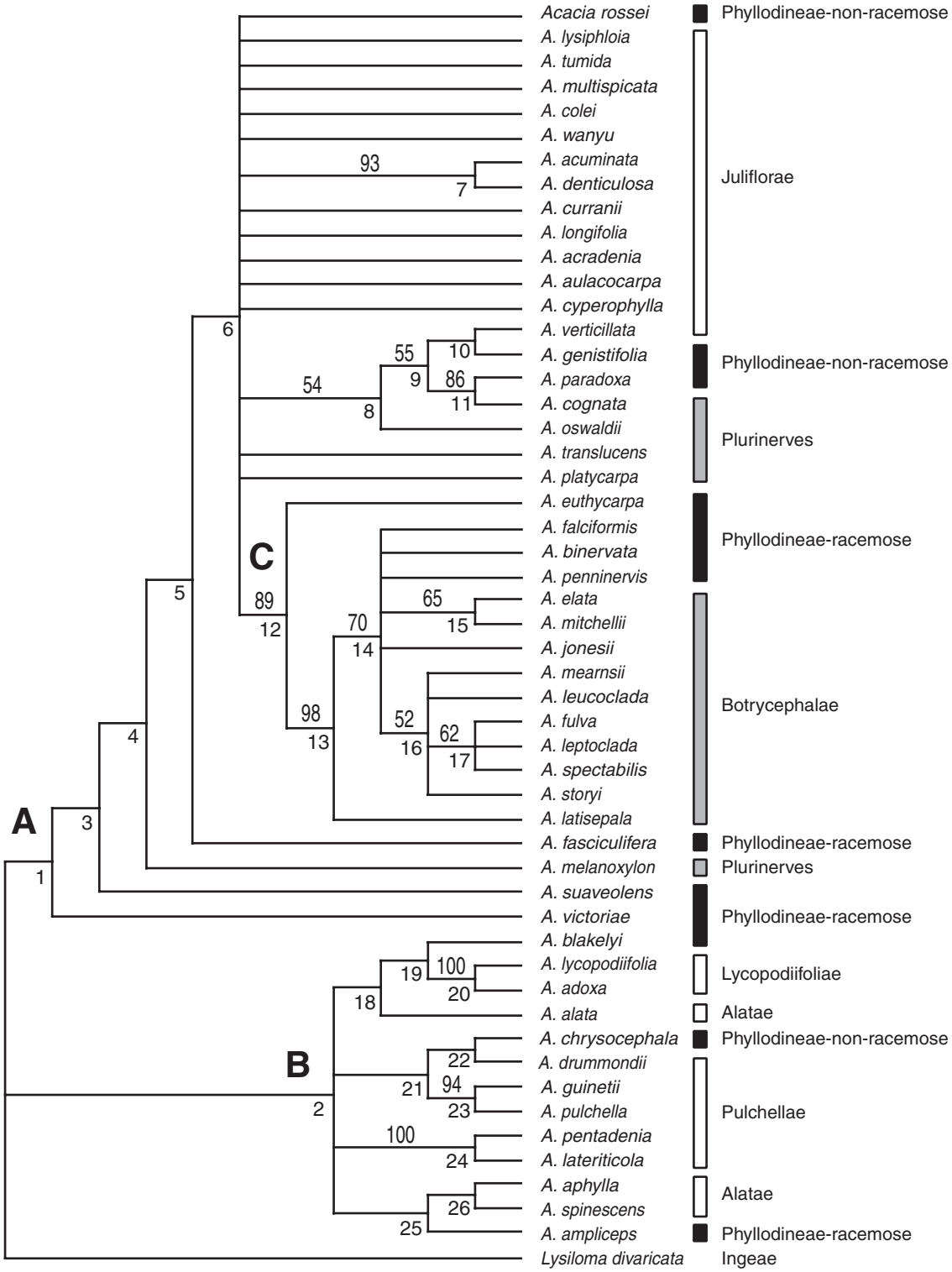

Fig. 2. The strict consensus tree of 116423 equally parsimonious trees (length = 420, CI = 0.39, RI = 0.69) resulting from the ITS sequence data. The numbers above the branches are bootstrap support values. The numbers below the branches are node numbers. The letters A, B and C are clades that are highlighted for discussion. Labels at the right are the sections in subgenus *Phyllodineae sensu* Pedley (1978) and informal groups *sensu* Maslin and Stirton (1997).

(Node 5, *Phyllodineae* racemose) and a clade of 34 other species (Node 6), which includes the remaining members of *Plurinerves*, all members of sections *Juliflorae* and *Botrycephalae* and some taxa of section *Phyllodineae*. At Node 6, three subclades have greater than 50% bootstrap support. The first of these is a species pair of *Juliflorae*, *A. acuminata* and *A. denticulosa* (Node 7, bt = 93%). The second disparate clade (Node 8) includes members of three sections. *Acacia oswaldii* (*Plurinerves*) (Node 8, bt = 54%) is sister to a clade (Node 9, bt = 56%) including *A. verticillata* (*Juliflorae*), *A. genistifolia* (Node 10, *Phyllodineae* non-racemose) and the well-supported sister species (Node 11, bt = 86%) *A. paradoxa* (*Phyllodineae* non-racemose) and *A. cognata* (*Plurinerves*).

The third subclade within Clade A has strong bootstrap support (Clade C, Node 12, bt = 89%). It includes all members of *Botrycephalae* and four members of section *Phyllodineae* (racemose). *Acacia euthycarpa* (Node 12) is sister to a strongly supported clade (Node 13, bt = 98%) that contains all members of the bipinnate section *Botrycephalae* and some members of section *Phyllodineae* racemose, *A. falciformis*, *A. penninervis* and *A. binervata*. *Botrycephalae* are thus paraphyletic (Node 14), with *A. latisepala*, which has both phyllodes and bipinnate foliage in adult plants, in a basal position. Resolution of relationships between species of *Phyllodineae* racemose and *Botrycephalae* remain unresolved (Node 14). Within the *Botrycephalae*, three nodes have bootstrap support (15, bt = 65%, 16, bt = 52% and 17, bt = 62%). *Acacia elata* and *A. mitchellii* are sister species (Node 15) and *A. mearnsii*, *A. leucoclada*, *A. storyi* group (Node 16) with *A. fulva*, *A. leptoclada* and *A. spectablis* (Node 17).

Clade B (Node 2) contains all exemplar taxa from three sections—*Lycopodiifoliae* (monophyletic), *Pulchellae* and *Alatae*—and three taxa from section *Phyllodineae* (both racemose and non-racemose). *Acacia blakelyi* (*Phyllodineae* racemose) is sister (Node 19) to *Lycopodiifoliae* (Node 20). *Alatae* and the bipinnate section, *Pulchellae*, are not monophyletic, although there are few nodes in Clade B supported by bootstrap values. Sister species *A. guinetii* and *A. pulchella* (Node 23, bt = 94%) and *A. pentadenia* and *A. lateriticola* (Node 24, bt = 100%) are strongly supported, although the relationship of these clades to each other is unresolved (Node 2).

Discussion

Past studies have assumed a natural division between taxa with uninerved phyllodes (in section *Phyllodineae*) and those with plurinerved phyllodes (in sections *Juliflorae* and *Plurinerves*) (Vassal 1972; Pettigrew and Watson 1975; Pedley 1986; Chappill and Maslin 1995; Maslin and Stirton 1997). This division is not supported in the present study. Although Clade A contains all members of *Juliflorae* and *Plurinerves* and 10 taxa from section *Phyllodineae*, these sections were not resolved as monophyletic. Members of the section *Phyllodineae* are in multiple positions in the cladogram, making this section polyphyletic. The current analysis does not provide evidence for the common division of section *Phyllodineae* into racemose or non-racemose subgroups, thereby supporting Maslin and Stirton's (1997) assertion that this is an oversimplified approach.

The ITS data set contains too few informative characters to confirm or refute the existence of a 'plurinerved' group consisting of members of sections *Juliflorae* and *Plurinerves*. Brain and Maslin (1996) found no clear distinction between the uninerved and plurinerved taxa with serological data and Maslin (2001) noted that a number of natural groups in subgenus *Phyllodineae* have both uninerved and plurinerved members. His finding is supported in the current analysis by the sister species relationship of *A. cognata*, in section *Plurinerves*, to *A. paradoxa*, a uninerved taxon in section *Phyllodineae*, indicating that the plurinerved and uninerved conditions are homoplastic.

Ten taxa were sequenced from the *Botrycephalae* to investigate the relationships between this section and the taxa in section *Phyllodineae* that have similar racemose inflorescences. The current analysis suggests that section *Botrycephalae*, a south-eastern Australian group with bipinnate foliage, is paraphyletic with some members of the section *Phyllodineae* with racemose inflorescences nested within it (Node 14) and another racemose species (*A. euthycarpa*) at a basal node (12, Clade C). Although earlier studies have postulated that *Botrycephalae* are related to members of *Phyllodineae* with similar inflorescences (Tindale and Roux 1969, 1974; Vassal 1972; Pettigrew and Watson 1975; Pedley 1986; Chappill and Maslin 1995; Murphy *et al.* 2000; Miller and Bayer 2000, 2001), the ITS data set presented here provides robust evidence for the support of this relationship. *Acacia euthycarpa* is a member of the so-called '*Acacia microbotrya* group' (Maslin 1995*b*), which Tindale and Roux (1969, 1974) and Chappill and Maslin (1995) suggested is related to *Botrycephalae*. However, the analysis of Miller and Bayer (2000) did not resolve the '*A. microbotyra* group' as monophyletic.

The current study supports the exclusion of the eastern species *A. mitchellii* from the Western Australian bipinnate section *Pulchellae* by Guinet *et al.* (1980). The placement of *A. mitchellii* has been difficult because it is unusual in having free sepals, bipinnate foliage and non-racemose inflorescences, characteristics of section *Pulchellae*. However, unlike some *Pulchellae*, it does not have spinescent stipules. The ITS data show it to be the sister taxon to *A. elata*, a member of the *Botrycephalae* with large leaves and probably a basal taxon in the section (Ariati 2000). An implication of the ITS cladogram in Fig. 2 is that the adult bipinnate condition in subgenus *Phyllodineae* is the result of at least two reversals, in sections *Botrycephalae* and *Pulchellae*. This conclusion is similar to that of Vassal

(1972), Pedley (1986), Guinet *et al.* (1980), Chappill and Maslin (1995) and Maslin and Stirton (1997). The reversal to adult bipinnate foliage may be interpreted as a case of neoteny, since all phyllodinous acacias pass through a pinnate phase during the ontogeny of phyllode development (Pedley 1986).

Section *Pulchellae*, although not resolved as monophyletic within Clade B (Fig. 2), is morphologically distinct and probably monophyletic. Most of the nodes in Clade B lack bootstrap support and the placement of some taxa may be due to limited sampling. Vassal (1972) recognised a section *Pulchelloideae*, based on seedling and other morphological characters (including spinescent stipules), which included members of the *Pulchellae*, *Alatae* and other taxa from section *Phyllodineae*, but Vassal did not include *Lycopodiifoliae* in his study. The finding in the ITS analysis that *Pulchellae*, *Alatae* and some members of section *Phyllodineae* may form a monophyletic group with taxa in the *Lycopodiifoliae* was unexpected, although some evidence for a grouping of taxa in *Alatae*, *Pulchellae* and *Lycopodiifoliae* was resolved in the plastid DNA study of Murphy *et al.* (2000). The current study adds support to such a grouping, but further analysis will be required to determine the morphological characters that are shared by these taxa. Brain and Maslin (1996), using serological data, found 'no strong relationship' between *Pulchellae* and any other group in subgenus *Phyllodineae*, although they did discover a weak association between *Alatae* and taxa in the *Plurinerves*, *Juliflorae* and *Phyllodineae*.

The *Lycopodiifoliae* clade, containing *A. adoxa* and *A. lycopodiifolia*, had 100% bootstrap support and *A. blakelyi* in section *Phyllodineae* is sister to this clade. The *Lycopodiifoliae*, which have phyllodes in whorls, are morphologically distinct from other taxa in subgenus *Phyllodineae*. Rutishauser (1999) showed that the phyllode whorls in *Lycopodiifoliae* are developmentally different from those found in other phyllodinous acacias (*A. verticillata* in section *Juliflorae* and *A. baurei* in section *Phyllodineae* non-racemose). Pedley (1987) suggested that it was likely that section *Lycopodiifoliae* would be segregated from subgenus *Phyllodineae*. However, the results of the present analysis show that the segregation of *Lycopodiifoliae* would leave subgenus *Phyllodineae* paraphyletic. Chappill and Maslin (1995), in their morphological analysis, found that *A. hippuroides* grouped with taxa in section *Plurinerves*. In contrast, Brain and Maslin (1996), with serological data, concluded that *A. hippuroides* was closely related to section *Juliflorae*.

Conclusion

The current study is part of a series on the phylogeny of *Acacia* subgenus *Phyllodineae* to re-assess the infrageneric classification. The need for such a re-assessment has been highlighted in recent years (Chappill and Maslin 1995; Brain and Maslin 1996; Maslin and Stirton 1997; Murphy *et al.* 2000; Miller and Bayer 2001), and the ITS analysis confirms that most sections within the subgenus are not monophyletic.

More comprehensive taxon sampling and more variable markers than those used in the present work are required to resolve all clades. However, it is clear that the relationships resolved here and in previous studies (Chappill and Maslin 1995; Brain and Maslin 1996; Murphy *et al.* 2000; Miller and Bayer 2001) are in many cases unexpected.

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