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Molecular phylogenetics of the Australian acacias of subg. *Phyllodineae* (Fabaceae: Mimosoideae) based on the *trn*K intron

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Abstract. With over 960 species, *Acacia* is the largest genus of plants in Australia with all but nine of these species classified as subgenus *Phyllodineae*. DNA sequences for the chloroplast *trn*K region were sequenced for over 100 species to test sectional classification and survey species relationships within this subgenus. Only one of the seven recognised sections was found to be monophyletic; however, the close relationship of sect. *Botrycephalae* to certain racemose, uninerved species of sect. *Phyllodineae* is confirmed. Support is found for an expanded version of Vassal's *Pulchelloidea*, with the addition of sect. *Lycopodiifoliae* and several members of sect. *Phyllodineae*. These species, while morphologically distinct in adult foliage, possess similar seedling characteristics. The multinerved species are unresolved, indicating a rapid morphological radiation with little chloroplast sequence divergence among these species. The low levels of sequence divergence, large numbers of morphological species groups and the adaptive radiation of the group are discussed.

Introduction

Acacia Mill. contains more than 1300 species subdivided into three subgenera: subg. *Acacia*, subg. *Aculeiferum* and subg. *Phyllodineae*. With over 960 species in Australia, *Acacia* is the largest genus of plants in Australia and all but nine of these species are classified to subg. *Phyllodineae*. Most Australian *Acacia* species are endemic with a few species whose range includes the islands of the Indian and Pacific Oceans from Madagascar to Hawaii (Maslin 2001).

Acacia subg. *Acacia* and subg. *Aculeiferum* grow in temperate to subtropical areas of the Americas, Africa and Asia and are represented in Australia by seven and two species, respectively, which are confined to the north of the contintent. Morphological (Chappill and Maslin 1995; Grimes 2000) and molecular datasets (Miller and Bayer 2000, 2001; Robinson and Harris 2000; Clarke *et al.* 2001) have shown that *Acacia* is non-monophyletic. Chloroplast DNA restriction site data (Robinson and Harris 2000) and nuclear and cpDNA sequence data (Miller and Bayer 2000, 2001) place the tribe *Ingeae* as the sister taxon to the 'Australian acacias' (namely *Acacia* subg. *Phyllodineae*).

The *Phyllodineae* is divided into seven sections based on foliage and inflorescence characters (Pedley 1978). While these sections may not be considered as natural groups (Pedley 1986; Chappill and Maslin 1995; Brain and Maslin 1996; Maslin and Stirton 1997), they form a useful framework for investigation (Table 1). Two sections, *Botrycephalae* (42 species) and *Pulchellae* (27 species), representing 7% of the species, have bipinnate leaves that are common to the rest of the *Mimosoideae*. The 17 species of sect. *Lycopodiifoliae* are small shrubs with whorled phyllodes. Section *Alatae* (21 species) is considered an unnatural assemblage and contains species with decurrent phyllodes or are aphyllodinous.

The three largest sections of the subgenus contain over 88% of the species. Section *Phyllodineae* (408 species) contains species that have uninerved phyllodes, while species of sections *Juliflorae* (235 species; spicate inflorescence) and *Plurinerves* (212 species; globose inflorescence) have multinerved phyllodes (Maslin 2001). The relationships within and among these three sections are unclear. It is clear, however, that some species from these three sections (sect. *Phyllodineae*, *Plurinerves* and *Juliflorae*) are more closely related to species of other sections. For example, some racemose species of sect. *Phyllodineae* are more closely related to sect. *Botrycephalae* species than they are to other phyllodinous species (Tindale and Roux 1969, 1974; Vassal 1972; Pettigrew and Watson 1975; Chappill and Maslin 1995; Miller and Bayer 2000, 2001; Murphy *et al.* 2000). Also natural groups exist that contain taxa that could be ascribed to both sect. *Phyllodineae* and sect. *Plurinerves*, such as the *A. wilhelmiana* group

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Table 1. Sectional classification of *Acacia* subg. *Phyllodineae* based on Pedley (1978)
The number of species sampled in present study is given in parentheses

Section	Approximate number of species	Foliage type	Inflorescence type
Botrycephalae	42 (17)	Bipinnate	Globose, mostly racemose
Pulchellae	27 (7)	Bipinnate	Globose or spicate, non-racemose, or rudimentary racemes
Lycopodifolieae	17 (3)	Whorled phyllodes	Globose, non-racemose
'*Alatae*'	21 (6)	Decurrent or aphyllodinous	Globose, non-racemose
Phyllodineae	387 (30)	Uninerved phyllodes (4-nerved when not flat)	Mostly globose, racemose (22) or non-racemose (8)
Juliflorae	235 (29)	Multinerved phyllodes	Spicate, racemose or non-racemose
Plurinerves	212 (14)	Multinerved phyllodes	Globose, racemose or non-racemose

(Maslin 1990). These exceptions clearly indicate the need for investigating evolutionary relationships within *Acacia* subg. *Phyllodineae*.

The aims of the current study are to test the monophyly of the seven sections within subg. *Phyllodineae* and to survey overall evolutionary trends in the subgenus by sampling as widely as possible among the multitude of morphological species groups. To accomplish this goal, the cpDNA intron of the transfer RNA gene for lysine (*trn*K) was sequenced in over 100 species. This region includes the about 900 bp of non-coding intron sequence and about 200 bp of the maturase encoding gene (*mat*K).

Materials and methods

Maslin and Stirton (1997) in a recent generic and infrageneric classification outlined 'a list of critical species on which to build a comparative data set'. This list describes morphological groups within each section of subg. *Phyllodineae* that could be used to systematically sample the large number of species in the genus.

The *Acacia* subg. *Phyllodineae* ingroup sampling of the present study was based on these morphological groups (Table 2). Species were sampled from all seven sections. *Paraserianthes lopantha* and *Pararchidendron pruinosum* were sampled as outgroups based on previous results (Miller and Bayer 2000, 2001).

Seeds were scarified and allowed to imbibe and planted in sterile potting soil in a glasshouse. The first true leaf was detached and pulverised in liquid nitrogen. DNA was extracted either with a Plant DNAzol Reagent kit (GibcoBRL Inc., Grand Island, New York, USA) or by the CTAB method (Doyle and Doyle 1987). Initial DNA amplification used the *trn*K-3914 (Johnson and Soltis 1994) and Ac283R primers (Miller and Bayer 2000). These primers amplified the 5′ intron and approximately 250 bp of the *mat*K coding region. The *trn*K intron region was amplified by PCR with *Taq*-DNA polymerase and sequenced at CSIRO Plant Industry as outlined in Miller and Bayer (2000).

Chromatographic traces and contiguous alignments were edited using Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). All sequences were deposited in Genbank (Table 2). Sequences were aligned manually with minimal gaps and base substitutions. Indels were scored as separate characters. The data were analysed with all characters unweighted. Maximum parsimony analyses were performed on the aligned sequences by using the heuristic search option (excluding uninformative characters) in PAUP 4.02 (Swofford 1999). A four-step search method for multiple islands was performed with 10 000 random replicates (Olmstead and Palmer 1994). The data were reweighted according to the rescaled consistency index from these 10 000 trees (Farris 1969). The analysis was repeated and reweighted until consensus tree topology did not change with further reweighting (Richardson *et al.* 2000). This procedure effectively downweights homoplasious characters. Support for internal branches was evaluated by using the fast bootstrap method with 10 000 replicates (Felsenstein 1985).

Results

Sequence characteristics

The aligned length of the sequenced portion of the *trn*K intron was 1061 bp with 253 bp from the 5′ coding region of the *mat*K gene and 808 nucleotides sequenced in the upstream intron region (Table 3). The *trn*K intron sequence contained 123 potentially informative base substitutions and six indels were scored. All scored indels were in the 5′ non-coding region. The highest sequence divergence (4.8%) was between *A. incurva* and *A. continua*, with a mean divergence among all species of 1.6%.

Of the six scored indels, two were found mainly in species of sect. *Botrycephalae*. One indel was found in all *Botrycephalae*, except *A. elata*, *A. jonesii*, *A. mitchellii*, *A. parvipinnula*, *A. decurrens* and *A. latisepala*, and in *A. pruinocarpa*, *A. podalyriifolia* (both sect. *Phyllodineae*) and *A. platycarpa* of sect. *Plurinerves*. The other indel was found in all species of *Botrycephalae*, in most species of sect. *Phyllodineae* and in three species of sect. *Pulchellae*.

Topology of the major clades

A maximum parsimony analysis of the entire unweighted data set was not able to run to completion due to computer memory constraints. The analysis was stopped with 10 000 fully swapped trees in memory. These equally parsimonious trees of 320 steps were found with a CI of 0.62 and a RI of 0.83.

The topology of the 50% majority rule consensus tree (Fig. 1) has several basic components: (i) a clade (A)

Table 2. Species used in study
All vouchers are deposited at the Australian National Herbarium (CANB)

Section affiliation	Species	Voucher	Genbank accession number
Juliflorae	*A. acradenia* F.Muell.	CANB 615659	AF523118
Juliflorae	*A. acuminata* Benth.	CANB 615660	AF523170
Lycopodifoliiae	*A. adoxa* var. *adoxa* Pedley	CANB 615681	AF523076
Botrycephalae	*A. adunca* A.Cunn. ex Don.	CANB 615646	AF523136
Alatae	*A. alata* R.Br.	CANB 615641	AF523084
Phyllodineae	*A. ampliceps* Maslin	CANB 615684	AF523074
Juliflorae	*A. aneura* F.Muell. ex Benth.	CANB 615661	AF523171
Phyllodineae	*A. angusta* Maiden & Blakely	CANB 615685	AF523153
Phyllodineae	*A. anthochaera* Maslin	CANB 615686	AF523160
Phyllodineae	*A. aphanoclada* Maslin	CANB 615687	AF523154
Alatae	*A. aphylla* Maslin	CANB 615642	AF523139
Plurinerves	*A. assimilis* subsp. *assimilis* S.Moore	CANB 615706	AF523166
Juliflorae	*A. atkinsiana* Maslin	CANB 615662	AF523181
Juliflorae	*A. aulcocarpa* A.Cunn. ex Benth.	CANB 615563	AF274214
Juliflorae	*A. auriculiformis* A.Cunn. ex Benth.	CANB 615663	AF523169
Juliflorae	*A. ayersiana* Maconochie	CANB 615665	AF523172
Phyllodineae	*A. bancroftiorum* Maiden	CANB 615574	AF274156
Phyllodineae	*A. binervata* DC.	CANB 615570	AF274218
Phyllodineae	*A. blakelyi* Maiden	CANB 615688	AF523151
Juliflorae	*A. brachystachya* Benth.	CANB 615666	AF523173
Juliflorae	*A. bulgagensis* Tindale & Stuart J.Davies	CANB 615667	AF523183
Phyllodineae	*A. calamifolia* Sweet ex Lindl.	CANB 615689	AF523148
Plurinerves	*A. calcicola* Forde & Ising	CANB 615577	AF274220
Phyllodineae	*A. camptoclada* C.R.P.Andrews	CANB 615690	AF523161
Juliflorae	*A. catenulata* C.T.White	CANB 615668	AF523174
Botrycephalae	*A. chinchillensis* Tindale	CANB 615647	AF523127
Phyllodineae	*A. chrysocephala* Maslin	CANB 615701	AF523157
Plurinerves	*A. cochlearis* (Labill.) H.L.Wendl.	CANB 615707	AF523156
Plurinerves	*A. cognata* Domin	CANB 615708	AF523167
Juliflorae	*A. colei* var. *cloei* Maslin & L.A.J.Thomson	CANB 615564	AF274215
Phyllodineae	*A. conferta* A.Cunn. ex Benth	CANB 615702	AF523158
Alatae	*A. continua* Benth.	CANB 615643	AF523138
Plurinerves	*A. coriacea* var. *coriacea* DC.	CANB 615709	AY180923
Juliflorae	*A. craspedocarpa* F.Muell.	CANB 615669	AF523175
Juliflorae	*A. cretata* Pedley	CANB 615670	AF523119
Phyllodineae	*A. cultriformis* A.Cunn. ex G.Don	CANB 615571	AF274219
Juliflorae	*A. curranii* Maiden	CANB 615671	AF523179
Juliflorae	*A. cyperophylla* F.Muell. ex Benth.	CANB 615672	AF523178
Botrycephalae	*A. dangarensis* Tindale & Kodela	CANB 615648	AF523130
Botrycephalae	*A. dealbata* subsp. *dealbata* Link	CANB 615649	AF523135
Botrycephalae	*A. deanei* subsp. *deanei* (R.T.Baker) Welch, Coombs & McGlynn	CANB 615650	AF523128
Phyllodineae	*A. declinata* R.S.Cowan & Maslin	CANB 615691	AF523122
Botrycephalae	*A. decurrens* Willd.	CANB 615651	AF523132
Juliflorae	*A. denticulosa* F.Muell.	CANB 615673	AF523182
Pulchellae	*A. drummondi* var. *drummondi* Lindl.	CANB 615714	AF523106
Botrycephalae	*A. elata* A.Cunn. ex Benth.	CANB 615558	AF274149
Pulchellae	*A. empelioclada* Maslin	CANB 615715	AF523143
Phyllodineae	*A. fasciculifera* F.Muell. ex Benth.	CANB 615692	AF523159
Botrycephalae	*A. fulva* Tindale	CANB 615652	AF523129
Phyllodineae	*A. gentisifolia* Link	CANB 615703	AF523163
Alatae	*A. glaucoptera* Benth.	CANB 615559	AF274217
Juliflorae	*A. gonoclada* F.Muell.	CANB 615680	AF523120
Pulchellae	*A. guinetii* Maslin	CANB 615716	AF523137
Alatae	*A. incurva* Benth.	CANB 615644	AF523146

Table 2. (*continued*)

Section affiliation	Species	Voucher	Genbank accession number
Juliflorae	*A. jibberdingensis* Maiden & Blakely	CANB 615674	AF523180
Botrycephalae	*A. jonesii* F.Muell. & Maiden	CANB 615653	AF523124
Pulchellae	*A. lateriticola* Maslin	CANB 615717	AF523144
Botrycephalae	*A. latisepala* Pedley	CANB 615654	AF523125
Juliflorae	*A. leiocalyx* (Domin) Pedley	CANB 615565	AF274216
Phyllodineae	*A. leiophylla* Benth.	CANB 615693	AF523149
Botrycephalae	*A. leucoclada* Tindale	CANB 615560	AF274212
Phyllodineae	*A. ligulata* A.Cunn. ex Benth.	CANB 615573	AF274155
Phyllodineae	*A. lineata* A.Cunn. ex G.Don.	CANB 615704	AF523162
Juliflorae	*A. longifolia* (Andrews) Willd.	CANB 615675	AF523086
Lycopodifoliiae	*A. lycopodifolia* A.Cunn. ex Hook.	CANB 615682	AF523077
Juliflorae	*A. lysiphloia* F.Muell. ex Benth.	CANB 615566	AF274151
Plurinerves	*A. mackeyana* Ewart & Jean White	CANB 615710	AY180922
Botrycephalae	*A. mearnsii* De Wild.	CANB 615655	AF523110
Plurinerves	*A. melanoxylon* R.Br.	CANB 615580	AF274166
Juliflorae	*A. merinthophora* E.Pritz.	CANB 615563	AF274214
Phyllodineae	*A. merrickiae* Maiden & Blakely	CANB 615694	AF523150
Phyllodineae	*A. microbotrya* Benth.	CANB 615575	AF274157
Botrycephalae	*A. mitchellii* Benth.	CANB 615656	AF523126
Juliflorae	*A. monticola* J.M.Black	CANB 615567	AF274152
Juliflorae	*A. mucronata* Willd. ex H.L.Wendl.	CANB 615743	AY180921
Phyllodineae	*A. myrtifolia* (Sm.) Willd.	CANB 615695	AF523147
Phyllodineae	*A. neriifolia* A.Cunn. ex Benth.	CANB 615696	AF523152
Pulchellae	*A. nigricans* (Labill.) R.Br.	CANB 615718	AF523141
Phyllodineae	*A. notabilis* F.Muell.	CANB 615576	AF274158
Plurinerves	*A. nuperrima* Baker f.	CANB 615578	AF274164
Plurinerves	*A. nyssophylla* F.Muell.	CANB 615711	AF523165
Juliflorae	*A. olgana* Maconochie	CANB 615676	AF523177
Phyllodineae	*A. pachyacra* Maiden & Blakely	CANB 615697	AF523121
Juliflorae	*A. pachycarpa* F.Muell. ex Benth.	CANB 615568	AF274153
Botrycephalae	*A. parramattensis* Tindale	CANB 615561	AF274150
Botrycephalae	*A. parvipinnula* Tindale	CANB 615657	AF523123
Phyllodineae	*A. penninervis* var. *penninervis* Sieber ex DC	CANB 615698	AF523155
Pulchellae	*A. pentadenia* Lindl.	CANB 615719	AF523142
Plurinerves	*A. platycarpa* F.Muell.	CANB 615581	AF274223
Phyllodineae	*A. podalyriifolia* A.Cunn. ex G.Don	CANB 615699	AF523134
Phyllodineae	*A. pruinocarpa* Tindale	CANB 615700	AF523133
Pulchellae	*A. pulchella* var. *pulchella* R.Br.	CANB 615720	AF523100
Juliflorae	*A. ramulosa* var. *ramulosa* W.Fitzg.	CANB 615677	AF523176
Plurinerves	*A. redolens* Maslin	CANB 615712	AF523168
Plurinerves	*A. retivenea* F.Muell.	CANB 615582	AF274224
Phyllodineae	*A. rossei* F.Muell.	CANB 615569	AF274162
Phyllodineae	*A. siculiformis* A.Cunn. ex Benth.	CANB 615705	AF523164
Botrycephalae	*A. silvestris* Tindale	CANB 615658	AF523131
Botrycephalae	*A. spectabilis* A.Cunn. ex Benth.	CANB 615562	AF274213
Alatae	*A. spinescens* Benth.	CANB 615645	AF523082
Lycopodifoliiae	*A. spondylophylla* F.Muell.	CANB 615683	AF523140
Plurinerves	*A. suaveolens* (Sm.) Willd.	CANB 615579	AF274221
Plurinerves	*A. translucens* A.Cunn. ex Hook.	CANB 615713	AF523087
Juliflorae	*A. tumida* F.Muell. ex Benth.	CANB 615678	AF523111
Phyllodineae	*A. victoriae* Benth.	CANB 615572	AF274226
Juliflorae	*A. wanyu* Tindale	CANB 615679	AF523145
Ingeae	*Pararchidendron pruinosum* (Benth.) I.C.Nielsen	CANB 615549	AF274127
Ingeae	*Paraserianthes lophantha* subsp. *lopantha* (Willd.) I.C.Nielsen	CANB 615550	AF274128

Table 3. Nucleotide character statistics for the *trn*K/*mat*K region

Outgroup species not included in theses calculations. Characters 1–3 contain data from the entire sequence. The rest of the statistics are calculated without 73 characters excluded from the analyses. The characters were excluded because of difficulty in alignment. Most *mat*K characters are mononucleotide repeats; this refers to the sequenced 5′ portion of the *mat*K coding region

Character	5′ *trn*K intron region	*mat*K partial	Total
Aligned length (bp)	808	253	1061
Length, range (bp)	599–702	248–253	802–955
G+C content mean (%)	33	30	32
Mean sequence divergence (%)	1.7	1.6	1.6
Variable sites (%)	31	27.5	30
Potentially informative sites (%)	13	11	12
Constant sites (%)	69	72.5	70
Autapomorphic sites (%)	18	16.5	18
Synapomorphic indels	6	0	6
Synapomorphic indel size range (bp)	3–8	—	3–8
Base substitutions	95	28	123
Total informative characters	101	28	129

containing all sect. *Botrycephalae* species sampled, most species of sect. *Phyllodineae* and one species of section *Alatae* (*A. continua*) and two species of sect. *Plurinerves* (*A. declinata* and *A. cochlearis*), (ii) a clade (B) containing all species sampled of sections *Lycopodiifoliae* and *Pulchellae*, three species of sect. Alatae (*A. alata*, *A. aphylla* and *A. spinescens*) and five species of sect. *Phyllodineae* (*A. ampliceps*, *A. blakelyi*, *A. chrysocephala*, *A. ligulata* and *A. myrtifolia*), (3) two smaller clades (Clades F and G) are included in an unresolved polytomy containing mostly species with multinerved phyllodes (referable to sect. *Juliflorae* and sect. *Plurinerves*), 10 species of sect. *Phyllodineae* and one taxon of sect. *Alatae* (*A. incurva*).

Clade A (Fig. 1) contains all sampled species of sect. *Botrycephalae*; however, these species do not form a monophyletic group. Most of the species fall in two major subclades (Clades A1 and A2), but allied with them are four species of sect. *Phyllodineae* (*A. binervata*, *A. conferta*, *A. penninervis* and *A. podalyriifolia*), and *A. continua* of sect. *Alatae*. Two other sect. *Botrycephalae* species (*A. mitchellii* and *A. parvipinnula*) are separate from the two major subclades and are mixed with sect. *Phyllodineae* species. The species of sect. *Phyllodineae* in Clade A (except *A. conferta*) have racemose inflorescences.

All species of sections *Lycopodiifoliae* and *Pulchellae* are located in Clade B, along with three species of sect. *Alatae* and five species of sect. *Phyllodineae*. Section *Lycopodiifoliae* is the only section found to be monophyletic in this study (Fig. 1, Clade C), supported by a bootstrap value of 68%. Section *Pulchellae* comprises two clades (D and E), both with bootstrap support of over 50%.

The major unresolved portion of the cladogram consists mainly of multinerved species (sections *Juliflorae* and *Plurinerves*), with 10 species of sect. *Phyllodineae* and two species of sect. *Alatae*. Clade G is comprised only of species from sect. *Juliflorae*, while Clade F contains species from all three sections.

After three rounds of reweighting the dataset by the rescaled consistency index, the tree topology stabilised and the parsimony analysis found over 10 000 trees (Fig. 2). All major clades (A–F) found in the majority rule tree are found in the reweighted tree. The major difference is that the *Lycopodifoliiae–Pulchellae* clade (Clade B) is now basal to the rest of the ingroup species. A large clade (Clade H) appears in the reweighted tree that is not found in the unweighted analysis. This clade, comprised of sect. *Juliflorae* species, is found in 96% of the trees and combines three species pairs and several unresolved species from the unweighted tree into a single clade. Six of these species are part of the closely related *A. aneura* (mulga) complex, a group of closely related species of sect. *Juliflorae* that are found in arid and semi-arid Australia (Maslin and Stirton 1997).

Discussion

Monophyly of sections

While most sections within subg. *Phyllodineae* are non-monophyletic, some robust relationships are apparent. The present study agrees with previous work that species of sect. *Botrycephalae* are closely related to certain species of sect. *Phyllodineae* with racemose inflorescences (Tindale and Roux 1969, 1974; Vassal 1972; Pettigrew and Watson 1975; Chappill and Maslin 1995; Miller and Bayer 2000, 2001; Murphy *et al.* 2000). Previous chloroplast sequence results (Miller and Bayer 2000, 2001; Murphy *et al.* 2000) have suggested that the *Botrycephalae* is non-monophyletic. The present study with its larger sampling strategy continues to suggest the *Botrycephalae* as non-monophyletic; however, support for these relationships is weak.

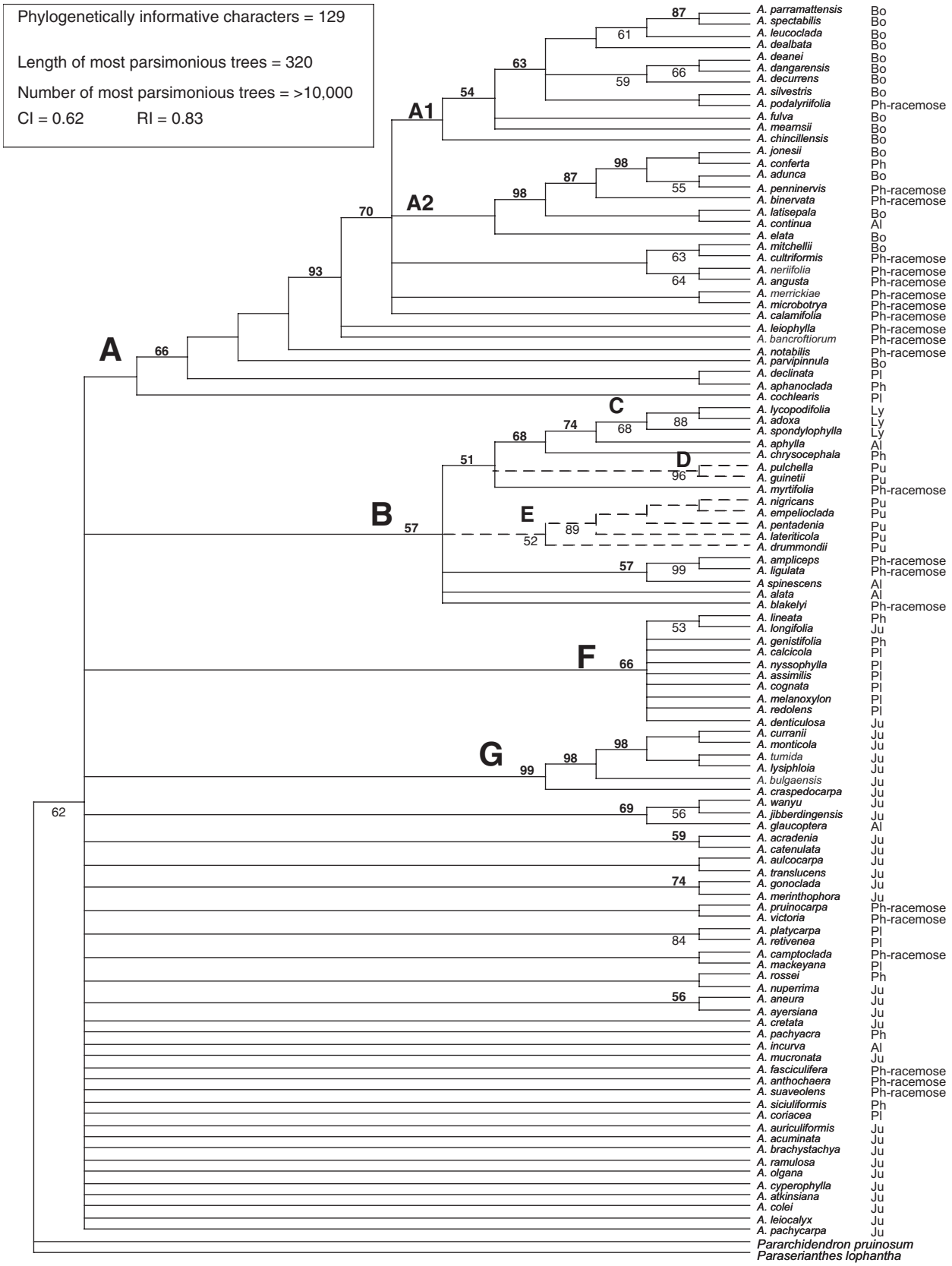

Fig. 1. Majority rule consensus tree of 10 000 most parsimonious trees. Clades marked A–G are discussed in text. Numbers above lines (bold) are the percentage of the equally most parsimonious trees with this node. Nodes found in all trees are unlabeled. Numbers below lines are bootstrap values. See text for discussion of labeled clades. Branches with dotted lines (Clades D and E) are species in sect. *Pulchellae*. Second column indicates section affiliation: Al = *Alatae*, Bo = *Botrycephalae*, Ju = *Juliflorae*, Ly = *Lycopodifoliiae*, Ph = *Phyllodineae*, Pl = *Plurinerves* and Pu = *Pulchellae*.

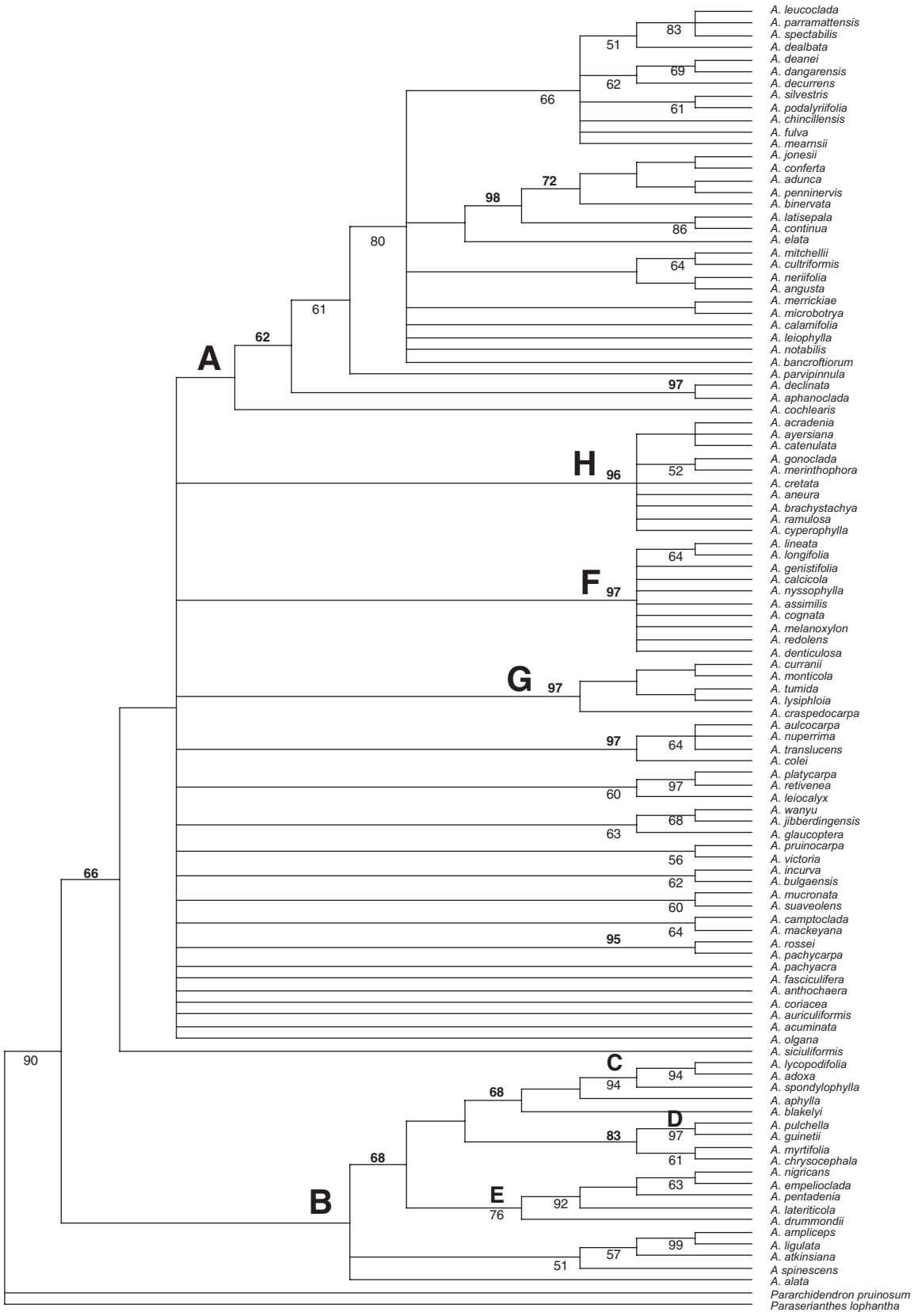

Fig. 2. Majority rule consensus tree of 10 000 most parsimonious trees after three rounds of reweighting the data based on the rescaled consistency index. Notations are as in Fig. 1.

Acacia mitchellii was the only eastern Australian species with bipinnately compound leaves referred to the Western Australian sect. *Pulchellae* by Bentham (1864). It has non-racemose inflorescences, similar to sect. *Pulchellae* and unlike sect. *Botrycephalae*. Guinet *et al.* (1980) and Maslin and Stirton (1997) considered this taxon to be more closely related to the *Botrycephalae*. The present analysis places *A. mitchelli* with the *Botrycephalae*–uninerved racemose species of Clade A (Figs 1, 2), a result not inconsistent with the findings of Guinet *et al.* (1980).

Section *Pulchellae* is endemic in south-western Australia (Maslin 1975, 1979) and Guinet *et al.* (1980) defined a core *Pulchellae* of 21 species comprising four subgroups. Two of these four subgroups concur with those found in the present study (Figs 1, 2). *A. pulchella* and *A. guinetii* form the 'Pulchella group' (Figs 1, 2; Clade D), while *A. nigricans*. *A. empelioclada*, *A. pentadenia* and *A. lateriticola* form the 'Browniana group'(Figs 1, 2; Clade E; Guinet *et al.* 1980). *Acacia drummondii*, the lone representative of the 'Drummondii group' is sister to the Browniana group. The hypothesis of Guinet *et al.* (1980) that the 'Pulchella group' is the closest relative to the 'Browniana group', is not supported.

Guinet *et al.* (1980) also defined sect. *Pulchellae* sens lat., based on seedling, pollen and gross morphology, which included six bipinnate species not sampled in the present study. Guinet *et al.* (1980) further defined the *Pulchelloidea* group, based partly on Vassal's (1972) description, which includes *A. myrtifolia*, of sect. *Phyllodineae* and several species referable to section *Alatae*, including *A. spinescens*, *A. alata* and *A. continua* in the present study. Of these four species all but *A. continua* group with the species of section *Pulchellae* thereby lending support in part for the *Pullchelloidea* group.

The mature plants of the *Pulchelloidea* group can have varied leaf characters ranging from bipinnate to uninerved phyllodes and some members of the sect. *Alatae* have decurrent phyllodes or do not have phyllodes. However, the seedling ontology of the group is similar. The majority of species from *Acacia* subg. *Phyllodineae* have phyllodes instead of compound leaves at maturity; however; all *Acacia* subg. *Phyllodineae* proceed through several distinct leaf stages, including having a pinnately compound first true leaf. Leaf development can continue with varying numbers of compound leaves (all bipinntely compund after the second leaf) and either maintain the compound leaf at maturity as in sections *Botrycephalae* and *Pullchellae* or develop phyllodes. In the *Pulchelloidea* group the first two true leaves, after the cotyledons, are opposite, appear simultaneously and are both pinnate. The majority of the species of *Acacia* subg. *Phyllodineae* have a single first leaf that is pinnate, followed by a second leaf that is alternate to the first and bipinnate (Vassal 1972).

While the present study supports a version of the *Pulchelloidea* group (Vassal 1972; Guinet *et al.* 1980), it also supports the inclusion of sect. *Lycopodiifoliae* within it. The relationship of the *Pulchelloidea* to the *Lycopodifoliae* was not mentioned by Guinet *et al.* (1980) or Vassal (1972). The species of sect. *Lycopodiifoliae* have phyllodes in whorls and were included in Bentham's (1842) series *Bruinoideae*, which also contained species that have phyllodes subverticillate in groups or crowded around the stem (Pedley 1972). Bentham's *Bruinoideae* is not supported by the present data, as the subverticillate species (*A. conferta* and *A. rossei*) do not ally with the verticillate species (sect. *Lycopodiifoliae*; Figs 1, 2; Clade C). The *Lycopodiifoliae* (*sensu* Maslin and Stirton 1997) do form a monophyletic clade, with the species with ribbed calyx tubes (*A. lycopodifolia* and *A. adoxa*) grouping together.

Vassal's section *Pulchelloidea* was partially defined by seedling ontogeny and Vassal did not include the *Lycopodifoliae* in section *Pulchelloidea*. Analysis of data on seedling ontogeny (Vassal 1972; J. T. Miller, unpubl. data) shows that all species in Clade D (Figs 1, 2) have the section *Pulchelloidea* seedling ontogeny as outlined above. This includes all species of sect. *Pulchella*, sect. *Lycopodifoliae*, several species of sect. *Alatae* and the five species of sect. *Phyllodineae*. This evidence points to an expanded version of Vassal's (1972) *Pulchelloidea* that may be a natural group and shares the characteristic seedling ontogeny. Seedling ontogeny data are not available for all species of *Acacia* subg. *Phyllodineae*; however, most have the more common seedling ontogeny of the first two leaves alternately pinnate and bipinnate. More data are needed to better circumscribe the *Pulchelloidea* group.

The bottom half of the majority rule consensus tree (Fig. 1) is unresolved. The majority of these species are multinerved species of sections *Plurinerves* and *Juliflorae*, with 10 species of sect. *Phyllodineae* and two species of the sect. *Alatae*.

Reweighting of the dataset by the rescaled consistency index, thereby reducing the value of homoplasious characters, only slightly increased resolution. Changes in the strict consensus tree include bringing Clade B (Fig. 2) into a basal position and grouping the *A. aneura* species in Clade H. The *A. aneura* complex is well sampled in this study but only forms a clade when the dataset is reweighted. *A. craspedocarpa* is known to hybridise with *A. aneura* (Miller *et al.* 2002); however, it is not included in Clade H (Fig. 2). Due to the poor resolution in this portion of the cladogram the significance of this result is unknown at this point.

Species sampling, character sampling and adaptive radiation

DNA sequencing work utilising chloroplast and nuclear DNA sequences (Miller and Bayer 2000, 2001) found less

DNA site variation in *Acacia* subg. *Phyllodineae* and the tribe *Ingeae* than in *Acacia* subg. *Acacia* and subg. *Aculeiferum*. This suggests, that in addition to being a more specious group, *Acacia* subg. *Phyllodineae* is a younger evolutionary lineage. The present study confirms this result. The previous work (Miller and Bayer 2001), using the *trn*K–*mat*K region, sampled 18 *Phyllodineae* species in a study focusing on generic relationships. The highest support was found in areas of the cladogram that had the highest sampling density, sect. *Botrycephalae* and certain racemose members of sect. *Phyllodineae*.

Bremer *et al.* (1999) found a positive correlation between the support of a node and the number of characters considered and a negative correlation of support with the increased number of species sampled. These authors suggested that if the goal of a study is to elucidate relationships among major subgroups the best route is to increase the number of characters instead of increasing the taxa sampled. However if the goal is to survey a large group in order to define subgroups, this could be achieved with increased taxon sampling. The present study increased the number of species sampled with a chloroplast gene that has shown sufficient variation to resolve clades within other *Acacia* lineages. The sampling was increased with a goal of surveying a large taxonomic group with high levels of morphological variation in order to define subgroups. Increased taxon sampling can make autapomorphic characters from smaller datasets into synapomorphies when the close relatives are added to the dataset, which leads to more potentially informative sites that can be used to build the phylogenetic tree.

Overall, 12% of the DNA sequence sites are potentially informative, while 18% of the sites are autapomorphic. The addition of more species increased both the number of autapomorphies and the number of potentially informative sites at similar rates (Fig. 3). It appears that autapomorphies from smaller datasets are being turned into synapomorphies when more species are added. Also new autapomorphies are uncovered with denser sampling. The slope of the synapomorphy line (Fig. 3) is less than one (0.87), indicating that additional sampling is not giving the minimal amount of information (slope = 1), in the absence of homoplasy, that is needed to place the species in the phylogeny.

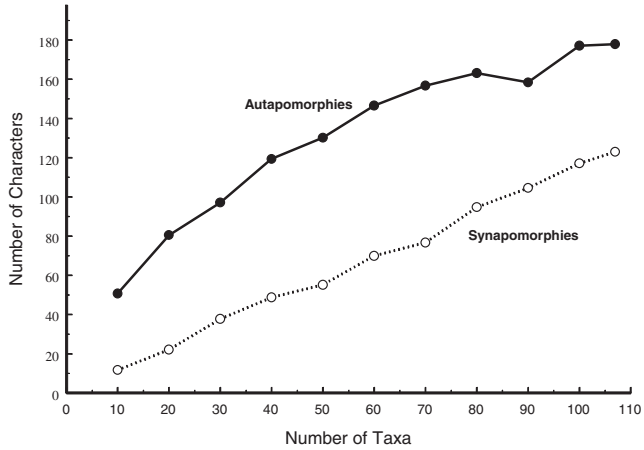

Fig. 3. The number of autapomorphic and synapomorphic characters found when random subsets of species (10–100) were taken from the entire dataset. Each point is an average of five replications.

Increased sampling of species of sections *Pulchellae*, *Lycopodifoliae* and *Alatae*, along with several members of sect. *Phyllodineae*, brings new insights to relationships mentioned in the literature. However, increased sampling did not increase resolution within the *Juliflorae–Plurinerves* group. These species groups could be younger and therefore have less DNA sequence variation or alternatively our lack of resolution could be due to poor sampling within this group. These two sections contain over 450 species, with only 42 included in this study. This sample size of 10% may not be sufficient given the low rate of sequence divergence. However, the rate of addition of informative characters from the *trn*K region does not appear to be sufficient to warrant further taxon sampling.

This result underscores the difficulty in molecular systematic studies of *Acacia*, which has both low levels of sequence polymorphism and a large number of morphologically distinct species and species groups. Therefore, the results of this study indicate that in order to revolve a phylogeny of *Acacia* subg. *Phyllodineae*, a large-scale sequencing effort will be needed. On a smaller scale, the *psb*A–*trn*H intergenic spacer, *trn*L intron and spacer and the entire *mat*K coding region have been sequenced in this group, resulting in 4 kb of chloroplast DNA sequence data for 16 taxa (Luckow *et al.* 2003). This sequence data better resolves the relationships; however, the low taxon sampling is not adequate for a comprehensive survey of the group. Other potential sources of data include the internal transcribed spacers (ITS) and the external transcribed spaces (ets) of the nuclear ribosomal DNA repeat.

These results also emphasise the extent of the large, rapid morphological radiation seen in *Acacia* subg. *Phyllodineae* that is still in progress. This radiation has given rise to over 950 species, but has happened faster than in the other *Acacia* subgenera, as is indicated by the lower level of DNA sequence variation.

This morphological radiation is not a pollinator-driven adaptive radiation as the flower morphology among species shows little variation (Armstrong 1979; Bernhardt *et al.* 1984). The floral variation found within the subgenus is at the level of inflorescence shape, either globose or spicate, the arrangement of the inflorescences on racemes or not, along with colour variation from off white to pale yellow to bright yellow (Maslin 2001). These differences do not appear to greatly affect the pollination systems of the species.

The greatest variation is seen in foliage type (bipinnate leaves *v.* phyllodes) and size, shape and nervature of the

phyllodes. Hopper and Maslin (1978) suggested that Pleistocene climatic fluctuations and the resulting soil diversification due to erosion promoted recent speciation of *Acacia* within south-western Australia. These processes, while allowing for retention of relictual species, provided new ecological niches for the expansion of the *Acacia* flora. The present data can be interpreted in this framework. The *Pulchelloidea* could be an older group that has undergone greater morphological change but has resulted in fewer species. The three large phyllodinous sections (*Phyllodineae*, *Plurinerves* and *Juliflorae*), which predominate in arid and semi-arid Australia, are younger and may have been more susceptible to speciation during the Pleistocene climatic fluctuations.

Conclusions

The present work, along with previous work (Chappill and Maslin 1995; Brain and Maslin 1996; Murphy *et al.* 2000; Miller and Bayer 2001), indicates the complex relationships among the 'Australian acacias'. Morphological data based on phyllodes and inflorescences, while helpful in classifying the immense morphological variation into sections, do not reflect evolutionary relationships. The growing body of evidence shows the non-monophyly of sections such that many species groups are more closely related to species groups in another section than they are to species within their section. This indicates the difficulty in defining characters for elucidation of evolutionary relationships (Chappill and Maslin 1995). Making this problem more difficult is over 99 species groups indicated by Maslin and Stirton (1997) and the lack of polymorphic chloroplast DNA (Murphy *et al.* 2000; Miller and Bayer 2000, 2001). The results from the increased taxon sampling in the present study indicate several important features to the phylogeny of *Acacia* subg. *Phyllodineae*. First, the close relationship of sect. *Botrycephalae* with certain racemose, uninerved species of sect. *Phyllodineae* is confirmed, but doubt is cast on the monophyly of sect. *Botrycephalae*. Second, support is found for an expanded version of Vassal's (1972) *Pulchelloidea* which includes sect. *Lycopodiifoliae* and several members of sect. *Phyllodineae*. Third, the multinerved sections *Plurinerves* and *Juliflorae* are unresolved, indicating a rapid morphological radiation with little chloroplast sequence divergence among species of these groups.

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