Molecular phylogenetics of *Acacia* (Fabaceae: Mimosoideae) based on the chloroplast *matK* coding sequence and flanking *trnK* intron spacer regions[1]

Joseph T. Miller[2] and Randall J. Bayer

Centre for Plant Biodiversity Research, CSIRO Plant Industry, GPO Box 1600, Canberra, Australia 2601

The tribe Acacieae (Fabaceae: Mimosoideae) contains two genera, the monotypic African *Faidherbia* and the pantropical *Acacia*, which comprise about 1200 species with over 950 confined to Australia. As currently recognized, the genus *Acacia* is subdivided into three subgenera: subg. *Acacia*, subg. *Aculeiferum*, and the predominantly Australian subg. *Phyllodineae*. Morphological studies have suggested the tribe Acacieae and genus *Acacia* are artificial and have a close affinity to the tribe Ingeae. Based on available data there is no consensus on whether *Acacia* should be subdivided. Sequence analysis of the chloroplast *trnK* intron, including the *matK* coding region and flanking noncoding regions, indicate that neither the tribe Acacieae nor the genus *Acacia* are monophyletic. Two subgenera are monophyletic; section Filicinae of subgenus *Aculeiferum* does not group with taxa of the subgenus. Section Filicinae, eight Ingeae genera, and *Faidherbia* form a weakly supported paraphyletic grade with respect to subg. *Phyllodineae*. *Acacia* subg. *Aculeiferum* (*s. s.*) is sister to the grade. These data suggest that characters currently used to differentiate taxa at the tribal, generic, and subgeneric levels are polymorphic and homoplasious in cladistic analyses.

Key words: *Acacia*; chloroplast DNA; Ingeae; *matK*; phylogeny.

Bentham (1842) described the tribe Acacieae Benth. as one of three tribes comprising the subfamily Mimosoideae and included within it many genera that are today classified in tribe Ingeae Benth. Later Bentham (1875) restricted his definition of tribe Acacieae to include only a single genus *Acacia* Mill. As currently defined, tribe Acacieae contains only two taxa, the large cosmopolitan genus *Acacia* and the monotypic African genus *Faidherbia* A. Chev. (Vassal, 1972, 1981).

The main character that distinguishes the Acacieae from the Ingeae, free filaments of the stamens while the Ingeae has united filaments, is not maintained in all taxa with some having filaments shortly united at base (Vassal, 1981). Other characters shared between the tribes are: numerous stamens and eight polyads per anther (Chappill and Maslin, 1995) and the close relationship of the Ingeae and Acacieae has been noted (Guinet, 1981; Vassal, 1981). The relationship of *Faidherbia* is troublesome as it has stamens that are shortly united at base and has pollen similar to some taxa of the Ingeae, but was placed in the Acacieae (Guinet, 1981). The tribe Mimoseae Bronn shares the character state of free stamens with the Acacieae, but the Mimoseae has as many or twice as many stamens as petals while the Acacieae has numerous stamens (Vassal, 1981). Guinet (1990) noted pollen structural symmetry shared among some Mimoseae and *Acacia* subg. *Acacia*. These conflicting character states make a classification, based solely on morphological characters, difficult.

Within *Acacia*, Bentham (1875) recognized six series, but recent authors have amalgamated these into three major groups (Table 1) either at the generic or subgeneric level (Vassal,

1972; Pedley, 1986; Maslin and Stirton, 1997). Subgenus *Acacia* and subg. *Aculeiferum* Vassal, with over 120 and 180 species, respectively, are pantropical while subg. *Phyllodineae* (DC.) Seringe, with over 950 species, is largely confined to Australia (Ross, 1981; Maslin and Stirton, 1997).

Subgenus *Acacia* has bipinnate leaves, stipular spines, and colporate pollen with a smooth exine with columellae, whereas subgenus Aculeiferum has bipinnate leaves, no stipular spines, but may have two to three prickles near the stipules, and porate pollen with a smooth exine but without columellae (Vassal, 1981). Subgenus *Phyllodineae* is the more diverse and variable of the subgenera. Most species have leaves reduced to vertically flattened phyllodes in a diverse range of sizes and shapes, but others have bipinnately compound leaves. They do not have prickles, but can be spinescent and have extraporate or porate pollen with the exine reticulate without columellae (Vassal, 1981).

Sections among the phyllodinous taxa have been derived based on phyllode nervature and inflorescence structure (Pedley, 1978). While the sections may not be considered as natural groups (Pedley, 1986; Brain and Maslin, 1996; Chappill and Maslin, 1995) they form a useful framework for investigation (Table 1). Section Phyllodineae contains species with one-nerved phyllodes while sects. Juliflorae (flowers in spikes) and Plurinerves (Benth.) Maiden & Betche (flowers in heads) taxa have multinerved phyllodes. Within these plurinerved taxa differences can be noted between microneurous phyllodes (numerous, fine longitudinal nerves) and oligoneurous phyllodes (few, distant longitudinal nerves; Maslin and Stirton, 1997).

There is growing agreement among researchers that the genus needs to be divided, but there is uncertainty regarding the number of terminal taxa involved, their interrelationships, and their taxonomic rank (Maslin and Stirton, 1997). In particular, there is disagreement on interrelationships of the three subgenera. Pedley (1986) hypothesized that subg. *Phyllodineae* arose from within subg. *Aculeiferum* and that subg. *Aculeiferum* and subg. *Acacia* had separate origins from within the tribe

[2] Author for correspondence.

TABLE 1. Classification of the Acacieae as adopted in present paper. This is a synaptic scheme based on Vassal (1972) and Pedley (1978, 1986). Under subgenus, the parenthetical generic names are those adopted by Pedley (1986).

Genus	Subgenus	Section[a]
Faidherbia		
Acacia	subg. *Acacia* (genus *Acacia*)	
	subg. *Aculeiferum* (genus *Senegalia*)	sect. Aculeiferum
		sect. Monacanthea
		sect. Filicinae
	subg. *Phyllodineae* (genus *Racosperma*)	sect. Alatae (21)
		sect. Botrycephalae (42)
		sect. Juliflorae[b] (235)
		sect. Lycopodifoliae (17)
		sect. Phyllodineae[c] (387)
		sect. Plurinerve[b] (212)
		sect. Pulchellae (27)

[a] The number of species for the sections of subg. *Phyllodineae* is indicated in the parentheses.

[b] For the purpose of this paper, this large section has been divided into microneurous vs. oligoneurous groups following Maslin and Stirton (1997).

[c] For the purpose of this paper, this large section has been divided into racemose vs. nonracemose groups following Maslin and Stirton (1997).

Ingeae. A recent morphological cladistic analysis also showed a sister relationship between subg. *Aculeiferum* to subg. *Phyllodineae* (Chappill and Maslin, 1995). These two genera were nested within the Ingeae, but separate from subg. *Acacia* that was also nested within the Ingeae. Alternatively, a cladistic analysis of floral development morphology suggests a close relationship of subgenera *Acacia* and *Aculeiferum* nested in the Ingeae separate from subg. *Phyllodineae* (Grimes, 1999).

The character states inconsistencies among the tribes, character polymorphisms within tribes and genera, and disagreement among published analyses indicate that a reevaluation of the tribes based upon an independent, data set is necessary. The aim of this study was to test the monophyly of tribe Acacieae and of the genus *Acacia* and to investigate phylogenetic relationships among the three subgenera within *Acacia* using chloroplast DNA sequences. To accomplish this goal, the cpDNA intron of the transfer RNA gene for lysine (*trnK*) was sequenced. This region includes the maturase encoding gene (*matK*) as well as flanking noncoding regions. The entire intron is ~2300 bp with the coding region of *matK* being ~1500 bp. The *matK* evolves two- to threefold faster than *rbcL* (Johnson and Soltis, 1994; Plunkett, Soltis, and Soltis, 1997), and this sequence has been used mainly at the infrageneric level, where a faster evolving chloroplast gene than *rbcL* is desired.

MATERIALS AND METHODS

A recent generic and infrageneric classification outlined "a list of critical species on which to build a comparative data set" (Maslin and Stirton, 1997). This list describes morphological groups within each subgenus that could be used to systematically sample the large number of species in the genus. The Acacieae ingroup sampling of the present study was based on these morphological groups. Species were sampled evenly from all three subgenera of *Acacia* (Table 2) and the monotypic *Faidherbia albida* was also included.

Eight genera from the Ingeae and a single genus, *Mimosa,* of the Mimoseae were included as outgroup taxa. Preliminary analysis indicated that the Ingeae

taxa were ingroup taxa as they nested within the Acacieae in all analyses. In subsequent analyses, *Mimosa tenuiflora* was used as the outgroup. The selection of these outgroup taxa was based on morphological evidence (Chappill and Maslin, 1995; Grimes, 1999).

Seeds were acquired from various seed banks (Table 2), scarified, placed into a petri dish with Whatman paper, and left to germinate at 25°C with 12 h of light per day. The first true leaf was detached and pulverized in liquid nitrogen. DNA was extracted using a Plant DNAZOL Reagent kit (GIB-COBRL, Grand Island, New York, USA). Initial DNA amplification used the *trnK*-3914 and *trnK*-2R primers made from Saxifragaceae (Johnson and Soltis, 1994). An *Acacia* specific primer (Fig. 1) was created internal to *trnK*-2R and was used in all subsequent polymerase chain reaction (PCR) reactions. The *trnK* intron region was amplified via the PCR using Taq DNA polymerase (Perkin-Elmer Applied Biosystems, Norwalk, Connecticut, USA). The PCR reaction mixture consisted of 5 µL of 20× reaction buffer, 6 µL of 25 mmol/L magnesium chloride solution, 16 µL of a 1.25 mmol/L 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. The PCR samples were heated to 94°C for 3 min prior to the addition of DNA polymerase to denature unwanted proteases and nucleases. The double-stranded PCR products were produced via 30 cycles of denaturation (94°C for 1 min), primer annealing (48°C for 1 min), and extension (72°C for 2 min). A 7-min final extension cycle at 72°C followed the 30th cycle to ensure the completion of all novel strands.

Double-stranded PCR products were cleaned with the QIAquick PCR kit (QIAgen, Hilden, Germany) and were sequenced using the dideoxy chain termination method with the use of the Big Dye Terminator RR Kit® and an ABI automated sequencer (Perkin-Elmer Applied Biosystems, Norwalk, Connecticut, USA) at CSIRO, Plant Industry. An annealing temperature of 57°C was used for sequencing reactions. Initial sequences were generated with primer *trnK*-3914. Four *Acacia* specific internal sequencing primers (Fig. 1) were designed and made, based on initial sequence data. The cycle sequencing protocol followed manufacturer's instructions.

Chromatographic traces and contiguous alignments were edited using Sequencer™ 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). All sequences were deposited in GenBank (Table 2). The coding region was determined by comparison to *Rosa persica* (Genbank number GBAN-AB011974). Sequences were aligned manually with minimal gaps and base substitutions. Indels were scored as separate characters. The *matK* coding region and the flanking spacer region were analyzed separately and the entire sequence analyzed together. The data were analyzed with all characters unweighted. A second analysis double weighted transversions over transitions. For this second analysis, the *matK* coding region was weighted as follows: the second-codon position was weighted twice the third position and all noncoding sequence, while the first position was weighted twice the second position.

Maximum parsimony analyses were performed on the aligned sequences using the heuristic search option (excluding uninformative characters) in PAUP 4.02 (Swofford, 1999). A four-step search method for multiple islands was performed using 10 000 random replicates (Olmstead and Palmer, 1994). 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 *trnK* intron was 2448 bp with 1557 bp forming the *matK* coding region and an additional 891 nucleotides sequenced in the flanking intron region (Table 3). The intron sequence contained 192 informative base substitutions and 11 indels. Most of the indels (8/11) were in the 5′ noncoding region. The mean divergence among taxa was greatest in the small 50-bp section 3′ to the *matK* coding region. The *matK* coding region codes from 502 to 515 amino acids. The highest divergence (7.4%) in the 5′ region was between *A. penninervis* and *A. schaffneri,* while the divergence between

the outgroup *Mimosa* and the Ingeae genus *Pararchidendron* was the highest for the *matK* coding region (4.0%).

Of the 11 indels, four were autapomorphic for *A. boliviana* of *Acacia* subg. *Aculeiferum* sect. Filicinae. Subgenus *Acacia* has two clade specific indels and a homoplasious indel was shared with subg. *Aculeiferum* sect. Filicinae. Indels also supported clades within subg. *Acacia* and subg. *Aculeiferum* sect. *Aculeiferum.*

Topology of the major clades—Topologies of the cladograms derived from the spacer and *matK* regions were congruent with slightly better resolution on the data from the *matK* coding region. Maximum parsimony analysis of the entire unweighted data set found 2236 trees of 398 steps with a CI of 0.62 and an RI of 0.83 (Farris, 1989). The topology of the strict consensus tree (Fig. 2) has four basic components: (1) a clade (A) of *Acacia* subg. *Acacia,* (2) a clade (B) of *Acacia* subg. *Aculeiferum* sects. Aculeiferum and Monacanthea, (3) a grade (C) including *Faidherbia albida,* all Ingeae genera included in the study, and *A. boliviana* of *Acacia* subg. *Aculeiferum* sect. Filicinae, and (4) a clade (D) comprising *Acacia* subg. *Phyllodineae.*

Constraint analyses were conducted to test the monophyly of Acacieae and *Acacia.* When the tribes Acacieae and Ingeae were constrained as monophyletic and the analysis repeated, an additional 12 steps were added to the most parsimonious tree. An additional ten steps were added to the shortest tree when *Acacia* (without *F. albida*) was constrained as monophyletic. Neighbor-joining analysis also placed the Ingeae within the polyphyletic Acacieae.

The trees derived from the weighted analysis (not shown) differed from the unweighted analysis by switching the positions of the subg. *Acacia* and subg. *Aculeiferum* (*s. s.*) clades. The sister position of subg. *Acacia* to the Filicinae/Faidherbia/Ingeae was weakly supported by a bootstrap value of <50%. The other major difference was that the weighted analysis placed *F. albida* within the Ingeae.

Topology of clades with Acacia—*Subgenus Acacia*—The basal portion of the strict consensus tree (Fig. 2) contains two clades, (1) *Acacia* subg. *Acacia* (Fig. 2A) and (2) the rest of the taxa studied (Fig. 2B, C, D). This indicates a significant divergence of *Acacia* subg. *Acacia* from other ingroup taxa. *Acacia* subg. *Acacia* formed a well-supported clade with 21 synapomorphies (SYN = 21) and 100% bootstrap support (BV = 100%). Two synapomorphic indels and a homoplasious indel, which is shared with *Acacia* subg. *Aculeiferum* sect. Filicinae, support the clade (Fig. 2).

One clade consists of the African species *A. seyal, A. karroo, A. nilotica,* and *A. tortilis* with the Australian species *A. bidwillii* (SYN = 7; BV = 99%). A second clade (SYN = 2; BV = 84%) consists of two New World groups, the *A. farnesiana* group (*A. caven* and *A. schaffneri*; SYN = 9; BV = 100) and the *A. macracantha* group (*A. cochliacantha* and *A. pennatula*; SYN = 7; BV = 100%). The third clade is the New World *A. constricta* group (*A. constricta* and *A. schottii*; SYN = 3; BV = 96%). The *A. farnesiana* group and the *A. constricta* group were defined by Clarke, Seigler, and Ebinger (1989, 1990, respectively).

Subgenus Aculeiferum—*Acacia* subg. *Aculeiferum* forms a monophyletic clade when *A. boliviana* of sect. Filicinae is excluded (Fig. 2B). This species appears as part of a basal grade

(C) to subg. *Phyllodineae.* The rest of the subgenus is supported as monophyletic (SYN = 3; BV = 81%) and is sister to the Ingeae/Filicinae/*Faidherbia* grade. All branches within the clade have bootstrap support of >88%.

Clade B comprises two clades that correlate with sect. Aculeiferum (SYN = 15; BV = 100%) and Monacanthea (SYN = 13; BV = 100%). Section Aculeiferum is confined to Africa and Asia, whereas sect. Monacanthea is pantropical. Two clades appear within the sect. Monacanthea clade, one (SYN = 7; BV = 100%) is the *A. berlandieri* group (*A. berlandieri* and *A. wrightii*; sensu Maslin and Stirton, 1997). The other clade (SYN = 6; BV = 100%) consists of *A. glomerosa* of the *A. glomerosa* group and *A. bonariensis,* which has affinities to the *A. riparia* group (Maslin and Stirton, 1997).

The sect. Aculeiferum clade is further supported by one indel. In the basal position of this clade is *A. catechu* with paired prickles; a more derived element of the clade (*A. senegal*) with prickles in threes (Ross, 1979) grouped with the Indian taxon *A. modesta* (SYN = 4; BV = 88%).

Faidherbia, Ingeae and sect. Filicinae—A grade consisting of *Faidherbia albida, A. boliviana* of *Acacia* subg. *Aculeiferum* sect. Filicinae and the eight Ingeae genera (Fig. 2C) is basal to a clade containing *Acacia* subg. *Phyllodineae.* These taxa are from Australia, the New World, and Africa. This grade is weakly supported with only two branches having a bootstrap value >50%. The sister position of *Faidherbia albida* is supported by a 69% bootstrap value and seven synapomorphies. The Ingeae are not monophyletic. An Australian species of *Pararchidendron* is basal to a trichotomy. The trichotomy consists of (1) six of the eight Ingeae genera, (2) the Australian species *Cathormion umbellatum,* and (3) a monophyletic *Acacia* subg. *Phyllodineae* clade. Relationships within these Ingeae taxa are not well supported, with only high support for the sister relationship of *Ebenopsis* and *Havardia* (SYN = 7; BV = 99%).

Subgenus Phyllodineae—*Acacia* subg. *Phyllodineae* formed a monophyletic clade (Fig. 2D). The support for this clade is lower than for clades representing the other subgenera (SYN = 3; BV = 56%). Because this subgenus contains over 950 species (Maslin and Stirton, 1997), the 18 taxa sampled here cannot represent the complexity of the subgenus. The four largest of the seven sections (Tables 1 and 2) recognized by Pedley (1978) are represented, including uninerved and plurinerved phyllodinous species as well as species with bipinnately compound leaves.

The clade is unresolved with five lineages. (1) Two uninerved taxa with racemose inflorescences referable to section Phyllodineae, *Acacia ligulata* of the *A. bivenosa* group (Chapman and Maslin, 1992) and *A. myrtifolia* of the *A. myrtifolia* group (Maslin, 1995a), group together with four synapomorphies. (2) The uninerved nonracemose *A. siculiformis* does not group with any other taxon. (3) *Acacia lineata,* a uninerved nonracemose species of sect. Phyllodineae, occurs with the plurinerved oligoneurous *A. melanoxylon* of sect. Plurinerves. (4) A clade of five plurinerved species of sects. Plurinerves and Juliflorae are joined with the uninerved nonracemose *A. rossei,* sect. Phyllodineae, basal (SYN = 11). Two closely related species pairs occur within the clade. *Acacia monticola* and *A. lysiphloia* are supported as sister species (SYN = 6; BV = 78%). Also *A. nuperrima* and *A. translucens* are sisters supported by two synapomorphies. These two groups were also

TABLE 2. Sources of DNA used in this study.

Tribe	Subgenus	Section	Species	DNA source[a]	Country	Voucher	GenBank Acc. no.[b]
Mimoseae			*Mimosa tenuiflora* (Willd.) Poir.	OFI	Honduras	CANB 615541	GBAN-AF274120
Ingeae			*Albizia sinaloensis* (Vell.) Morong	DLEG	Mexico	CANB 615583	GBAN-AF274121
Ingeae			*Cathormion umbellatum* (Vahl) Kosterm.	ANBG	Australia	CANB 615544	GBAN-AF274122
Ingeae			*Ebenopsis ebano* (Berland) R.C. Barneby & J.W. Grimes	DLEG	New World cultivated[c]	CANB 615545	GBAN-AF274123
Ingeae			*Enterolobium contorisiliquum* (Vell.) Morong	DLEG	Paraguay	CANB 615546	GBAN-AF274124
Ingeae			*Havardia pallens* Britton & Rose	DLEG	Mexico	CANB 615547	GBAN-AF274125
Ingeae			*Lysiloma acapulcensis* (Kunth) Benth.	OFI	Honduras	CANB 615584	GBAN-AF274126
Ingeae			*Pararchidendron pruinosum* (Benth.) I.C. Nielsen	ANBG	Australia	CANB 615549	GBAN-AF274127
Ingeae			*Paraserianthes lophantha* (Willd.) I.C. Nielsen.	ANBG	Australia	CANB 615550	GBAN-AF274128
Acacieae			*Faidherbia albida* (Delile) A. Chev.	OFI	Ethiopia	CANB 615551	GBAN-AF274129
Acacieae	*Acacia*		*A. bidwillii* Benth.	ATSC	Australia	CANB 615585	GBAN-AF274130
Acacieae	*Acacia*		*A. caven* (Molina) Molina	DLEG	Argentina	CANB 615552	GBAN-AF274131
Acacieae	*Acacia*		*A. schaffneri* (S. Watson) F.J. Herm.	DLEG	USA	CANB 615586	GBAN-AF274132
Acacieae	*Acacia*		*A. cochliacantha* Humb. & Bonpl. ex Willd.	DLEG	Mexico	CANB 615587	GBAN-AF274133
Acacieae	*Acacia*		*A. pennatula* (Cham. & Sch.) Benth.	DLEG	New World cultivated[c]	CANB 615553	GBAN-AF274134
Acacieae	*Acacia*		*A. constricta* Benth.	DLEG	USA	CANB 615588	GBAN-AF274135
Acacieae	*Acacia*		*A. schottii* Torr.	DLEG	USA	CANB 615589	GBAN-AF274136
Acacieae	*Acacia*		*A. karroo* Hayne	DLEG	South Africa	CANB 615590	GBAN-AF274137
Acacieae	*Acacia*		*A. seyal* Delile	OFI	Malawi	CANB 615591	GBAN-AF274138
Acacieae	*Acacia*		*A. nilotica* (L.) Willd ex Delile	DLEG	Kenya	CANB 615592	GBAN-AF274139
Acacieae	*Acacia*		*A. tortilis* (Forssk.) Hayne	DLEG	Israel	CANB 615593	GBAN-AF274140
Acacieae	*Aculeiferum*	Aculeiferum	*A. catechu* (L.) Willd.	DLEG	India	CANB 615594	GBAN-AF274141
Acacieae	*Aculeiferum*	Aculeiferum	*A. modesta* Wall	DLEG	Pakistan	CANB 615595	GBAN-AF274142
Acacieae	*Aculeiferum*	Aculeiferum	*A. senegal* (L.) Willd.	DLEG	Zimbabwe	CANB 615553	GBAN-AF274143
Acacieae	*Aculeiferum*	Filicinae	*A. boliviana* Rusby	QH	New World cultivated[d]	CANB 615555	GBAN-AF274144
Acacieae	*Aculeiferum*	Monacanthea	*A. berlandieri* Benth.	DLEG	New World cultivated[c]	CANB 615596	GBAN-AF274145
Acacieae	*Aculeiferum*	Monacanthea	*A. bonariensis* Gill. ex Hook. & Arn.	DLEG	Argentina	CANB 615597	GBAN-AF274146
Acacieae	*Aculeiferum*	Monacanthea	*A. glomerosa* Benth.	DLEG	Brazil	CANB 615556	GBAN-AF274147
Acacieae	*Aculeiferum*	Monacanthea	*A. wrightii* Benth. ex A. Gray	DLEG	Mexico	CANB 615598	GBAN-AF274148
Acacieae	*Phyllodineae*	Botrycephalae	*A. elata* A. Cunn. ex Benth.	ANBG	Australia	CANB 615558	GBAN-AF274149
Acacieae	*Phyllodineae*	Botrycephalae	*A. parramattensis* Tindale	ATSC	Australia	CANB 615561	GBAN-AF274150
Acacieae	*Phyllodineae*	Juliflorae (oligoneurous)	*A. lysiphloia* F. Muell. ex Benth	ATSC	Australia	CANB 615566	GBAN-AF274151
Acacieae	*Phyllodineae*	Juliflorae (oligoneurous)	*A. monticola* J.M. Black	ATSC	Australia	CANB615567	GBAN-AF274152
Acacieae	*Phyllodineae*	Juliflorae (oligoneurous)	*A. pachycarpa* F. Muell. ex Benth.	ATSC	Australia	CANB 615568	GBAN-AF274153
Acacieae	*Phyllodineae*	Phyllodineae (racemose)	*A. fasciculifera* F. Muell. ex Benth.	ATSC	Australia	CANB 615599	GBAN-AF274154
Acacieae	*Phyllodineae*	Phyllodineae (racemose) "bivenosa group"	*A. ligulata* A. Cunn. ex Benth.	ANBG	Australia	CANB 615573	GBAN-AF274155

TABLE 2.   Continued.

Tribe	Subgenus	Section	Species	DNA source[a]	Country	Voucher	GenBank Acc. no.[b]
Acacieae	*Phyllodineae*	Phyllodineae (racemose) "microbotrya group"	*A. bancrofiatum* Maiden	ATSC	Australia	CANB 615574	GBAN-AF274156
Acacieae	*Phyllodineae*	Phyllodineae (racemose) "microbotrya group"	*A. microbotrya* Benth.	ATSC	Australia	CANB 615575	GBAN-AF274157
Acacieae	*Phyllodineae*	Phyllodineae (racemose) "microbotrya group"	*a. notabilis* F. Muell.	ANBG	Australia	CANB 615576	GBAN-AF274158
Acacieae	*Phyllodineae*	Phyllodineae (racemose) "microbotrya group"	*A. penninervis* Sieberer ex DC.	ATSC	Australia	CANB 615600	GBAN-AF274159
Acacieae	*Phyllodineae*	Phyllodineae (racemose) "myrtifolia group"	*A. myrtifolia* (Sm.) Willd.	ATSC	Australia	CANB 615601	GBAN-AF274160
Acacieae	*Phyllodineae*	Phyllodineae (nonracemose)	*A. lineata* A. Cunn. ex G. Don	DLEG	Australia	CANB 615602	GBAN-AF274161
Acacieae	*Phyllodineae*	Phyllodineae (nonracemose)	*A. rossei* F. Muell.	ANBG	Australia	CANB 615569	GBAN-AF274162
Acacieae	*Phyllodineae*	Phyllodineae (nonracemose)	*A. siculiformis* A. Cunn. ex Benth.	ANBG	Australia	CANB 615603	GBAN-AF274163
Acacieae	*Phyllodineae*	Plurinerves (microneurous)	*A. nuperrima* Baker f.	ATSC	Australia	CANB 615578	GBAN-AF274164
Acacieae	*Phyllodineae*	Plurinerves (microneurous)	*A. translucens* A. Cunn. ex Hook.	ATSC	Australia	CANB 615604	GBAN-AF274165
Acacieae	*Phyllodineae*	Plurinerves (oligoneurous)	*A. melanoxylon* R. Br.	ATSC	Australia	CANB 615580	GBAN-AF274166

[a] ANBG = Australian National Botanic Gardens, Canberra; ATSC = CSIRO Australian Tree Seed Centre, Canberra; DLEP = Boyce Thompson Desert Legume Program, Tucson, Arizona, USA; OFI = Oxford Forestry Institute; QH = Queensland Herbarium.

[b] The prefix GBAN- has been added to link the online versions of *American Journal of Botany* to GenBank but is not part of the actual accession number.

[c] Taxa are native to the New World. Seed taken of cultivated plants in United States.

[d] This taxon is native to the New World, but is introduced in Australia.

matK/trnK

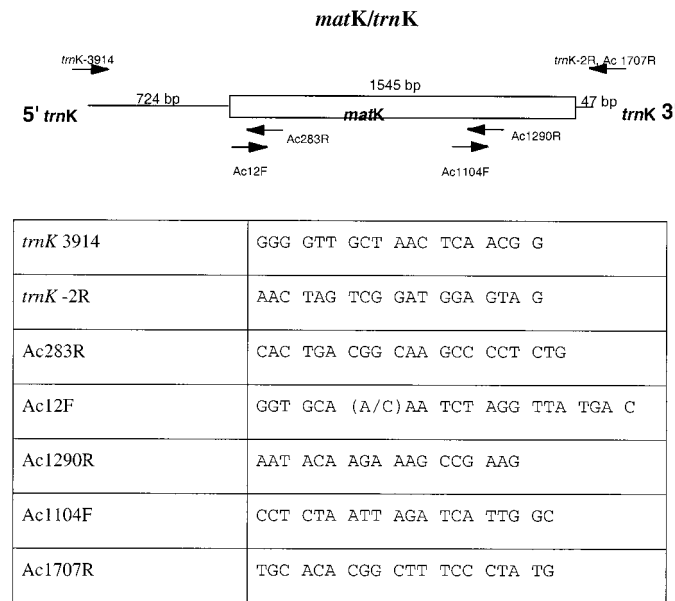| *trnK* 3914 | GGG GTT GCT AAC TCA ACG G |
trnK -2R	AAC TAG TCG GAT GGA GTA G
Ac283R	CAC TGA CGG CAA GCC CCT CTG
Ac12F	GGT GCA (A/C)AA TCT AGG TTA TGA C
Ac1290R	AAT ACA AGA AAG CCG AAG
Ac1104F	CCT CTA ATT AGA TCA TTG GC
Ac1707R	TGC ACA CGG CTT TCC CTA TG

Fig. 1.   Primers used in this study. (A) Structure of the chloroplast *trnK* intron including the *matK* coding sequence. Arrows above figure represent primers used for PCR, and arrows below indicate sequencing primers. Primer *trnK*-3914 was used for both PCR and sequencing reactions. Primer positions are relative to the start codon of *A. penninervis*. (B) Sequences of the primers used in this study. Primers *trnK* 3914 and *trnK*-2R are from Johnson and Soltis (1994).

suggested by Maslin and Stirton (1997). (5) The final clade of the trichotomy is composed of four species with uninerved phyllodes and racemose inflorescences of sect. Phyllodineae and two bipinnate leaved species of sect. Botrycephalae, *A. elata* and *A. parramattensis*. The basal relationship of *A. fasciculifera* is supported by a single synapomorphy. Better supported (SYN = 6; BV = 78%) is the relationship of the uninerved racemose species of the *A. microbotrya* group (Maslin, 1995b) with two species of sect. Botrycephalae. The two bipinnate sect. Botrycephalae species do not form a clade, but individually group with uninerved species *A. bancrofiatum* and *A. penninervis*. The two other species of the *A. microbotrya* group (*A. microbotrya* [SYN = 3; BV = 87%] and *A. notabilis*) are basal taxa to the clade that contains the bipinnate Botrycephalae species.

DISCUSSION

This study begins a series of investigations on the molecular systematics of the genus *Acacia*. The primary objective of the present work was to test the monophyly of *Acacia* and tribe Acacieae using DNA sequence data from the *matK\trnK* chloroplast region. The evidence presented here clearly shows that the genus and tribe are polyphyletic and agree with data from the nuclear Histone H3 data from the same taxa (Miller and Bayer, 2000). These results show eight genera of the tribe Ingeae embedded within *Acacia* and Acacieae.

Based on previous work (Pedley, 1986; Chappill and Maslin, 1995; Grimes, 1999) this study sampled several genera of Ingeae to test the monophyly of *Acacia* and Acacieae. While

TABLE 3. Nucleotide character statistics for the *trnK*/*matK* region. na = not applicable.

Character	5'*trnK* intron region	*matK*	3'*trnK* intron region	Total
Aligned length (bp)	841	1557	50	2448
Length, range (bp)	688–739	1506–1545	50	2254–2331
Amino acids	na	502–515	na	na
G + C content mean %	33	31.5	18	31
Mean sequence divergence %	2.8	1.7	4.5	2.2
Variable (%)	21	16	40	18
Potentially informative sites (%)	10	6.6	20	8
Constant sites (%)	79	84	60	82
Autapomorphic sites (%)	11	9.4	20	10
Indels	8	3	0	11
Indel size range (bp)	1–44	6–30	0	1–44
Ratio of indels to potentially informative sites	1:6.5	1:34	na	1:12.8
Base substitutions	79	103	10	192
Total informative characters	91	106	10	207

this sampling is sufficient to negate the monophyly of the genus and tribe, it is not sufficient to distinguish the interrelationship between the two tribes or their relationship to tribe Mimoseae.

The placement of the Ingeae genera within the *Acacia* clade demonstrates the polyphyly of the latter. The polyphyly of Acacieae concurs with the original description of the tribe by Bentham (1842), who described it as containing taxa presently placed in both Acacieae and Ingeae. This result is not surprising since there is not a single morphological character that can separate the tribes (Chappill and Maslin, 1997). The Acacieae has been distinguished from the Ingeae by having free filaments of the stamens while the Ingeae has united filaments, however *Faidherbia albida* and a few species from all three *Acacia* subgenera have shortly united filaments (Vassal, 1981) and some species of Ingeae have filaments that are almost free (Guinet, 1990). Thus without the filament character there are no macromorphological characters that separate the Ingeae and Acacieae, but rather suites of character state changes are needed to separate the tribes. Other characters shared between the tribes are numerous stamens and eight polyads per anther (Chappill and Maslin, 1995).

These data suggest an amalgamation of the Ingeae and Acacieae is needed. However, given that morphological characters previously used to discriminate the tribes are sometimes polymorphic within tribes, further molecular and morphological analyses of the entire subfamily Mimosoideae, especially the tribe Mimoseae, are needed before major realignment can be confidently determined. The Ingeae and Acacieae are thought to be derived from a paraphyletic Mimoseae (Pohill, Raven, and Stirton, 1981), and analyses of the three tribes together will shed light on the phylogeny and morphological character state changes in the Mimosoideae.

As noted above, *Faidherbia albida* contains a unique suite of filament and gland characters which are found individually in *Acacia* species (Ross, 1979). This has led to its placement in the Ingeae and Acacieae, and the present analysis is equivocal, but places it as a unique lineage within the Acacieae/Ingeae. The *matK*/*trnK* intron sequence data weakly ally *Faidherbia* as basal to *Acacia* sect. Filicinae and tribe Ingeae. These results show no clear sister relationship of *Faidherbia* to any particular Acacieae/Ingeae taxa and suggest generic status for *Faidherbia* is appropriate. Increased sampling of Ingeae taxa are needed to address the phylogenetic position of *Faidherbia*.

Bentham (1875) originally proposed six series within *Acacia* with most authors now regarding them as comprising three subgenera or genera (Vassal, 1972; Pedley, 1986; Maslin and Stirton, 1997). The distinction among the three subgenera is supported by the present molecular data except that *Acacia* subg. *Aculeiferum* sect. Filicinae is a separate lineage from other taxa of the subgenera. This supports Bentham's series Filicinae separate from series Vulgares. Series Vulgares has been placed into Vassal's subg. *Aculeiferum* (Vassal, 1972; Pedley, 1986, i.e., *Senegalia*). Guinet and Vassal (1978) placed sect. Filicinae within subgenus *Aculeiferum* based primarily on pollen characters, but noted that the section retains several ancestral morphological character states of the genus.

A high degree of resolution and branch support found within subg. *Aculeiferum* and *Acacia* clades suggests that the *trnK*/*matK* region is well suited to phylogenetic analysis in these group. Two classes of subg. *Acacia* (Fig. 2) correlate to the New World *Pluriseriae*, having seeds in two or three series within the fruit, and the African *Uniseriae*, having seeds in one series in the fruit (Vassal, 1972). Also within subg. *Aculeiferum* two clades are found that correlate to the New World taxa of sect. Monacanthea and the Africa/Asia taxa of sect. Aculeiferum.

Many hypotheses based on morphological characters have been put forward on the relationships among the three *Acacia* subgenera. Disagreement of relationships by workers suggests that the morphological characters used are not sufficient to delimit phylogeny of the group. Several studies have suggested a closer affinity of subg. *Phyllodineae* to subg. *Aculeiferum* than to subg. *Acacia*. This relationship is found in the present study with subg. *Aculeiferum* sister to the clade containing the grade C and the subg. *Phyllodineae* (Fig. 2). A cladistic analysis of morphological data (Chappill and Maslin, 1995; with terminal taxa as genera, subgenera, or sections) indicated a sister relationship of the subg. *Aculeiferum* (including sect. Filicinae) to subg. *Phyllodineae* with these two subgenera placed basal to the Ingeae. This relationship, of subg. *Phyllodineae* and *Aculeiferum*, was also suggested by Pedley (1986) who proposed the origin of the subg. *Phyllodineae* from subg. *Aculeiferum*, which in turn arose from the Ingeae.

The *matK*/*trnK* intron data indicate that subgenus *Acacia* is distinct from the other two subgenera. This is in agreement with Pedley (1986), while the morphological cladistic analysis shows subg. *Acacia* as distinct from the other subgenera and is placed within the Ingeae, sister to *Calliandra* and close to
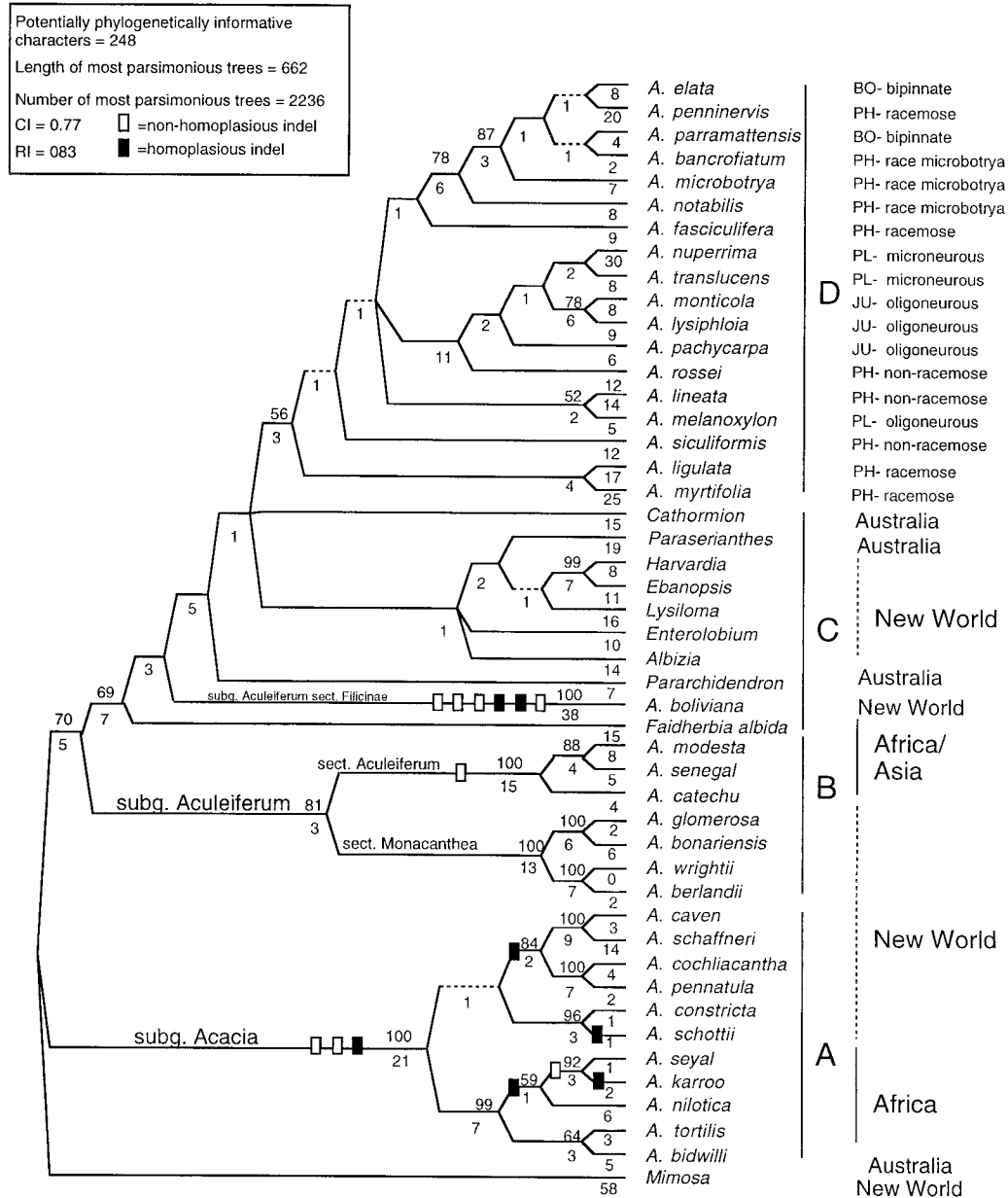
Fig. 2.   One of 2236 most parsimonious trees. A dashed line indicates branches that collapse in the strict consensus tree. Numbers below the lines indicate branch length. Numbers above some lines indicate bootstrap support value. Indels are indicated by boxes. A = *Acacia* subg. *Acacia*, B = *Acacia* subg. *Aculeiferum*, C = *Faidherbia/Acacia* subg. *Aculeiferum* sect. Filicineae/Ingeae, D = *Acacia* subg. *Phyllodineae*. All taxa of clade D are Australian.

Pithecellobium (Chappill and Maslin, 1995). These two genera have not been sampled in the present study but clearly subg. *Acacia* is not closely related to other Ingeae genera. An alternative result based on inflorescence morphology (Grimes, 1999) and cpRFLP data (Bukhari, Koivu, and Tigerstedt, 1999) did find a close relationship of subgenus *Acacia* to other Acacieae taxa. An inflorescence morphology development study (Grimes, 1999) placed subg. *Acacia* with *Faidherbia* and subg. *Aculeiferum* (excluding sect. Filicinae) in a clade that in turn was nested within the New World *Pithecellobium* complex (*Havardia* and *Ebenopsis* in the present study) of the Ingeae. Likewise the chloroplast restriction fragment-length polymorphism study (Bukhari, Koivu, and Tigerstedt, 1999)

indicates a sister relationship between subg. *Acacia* and subg. *Phyllodineae* (Heterophyllum); however this study only included Acacieae species so that no inferences on the monophyly of the genus can be concluded.

Difference among studies may be due to the questions asked in the research. Chappill and Maslin (1995) focused on the Acacieae with a few Ingeae outgroup taxa, and Grimes (1999) focused on the Ingeae with a few Acacieae outgroup taxa. Thorough sampling of the Acacieae, Ingeae, and Mimoseae is necessary to address these questions.

Three of Bentham's series (Pulchellae, Botrycephalae, and Phyllodineae) have been amalgamated into the Phyllodineae (Vassal, 1972). The separation of sect. Botrycephalae (a group

of Australian bipinnate species) from sect. Phyllodineae is not supported by the *matK/trnK* intron data as it would leave sect. Phyllodineae paraphyletic.

The chloroplast data presented here indicate that the Botrycephalae is derived from the sect. Phyllodineae. These results suggests that the bipinnate condition of the Botrycephalae is a reversal to the ancestral character state of the Mimosoideae (Pedley, 1986). The origin of the Botrycephalae from particular groups of uninerved racemose species, *A. microbotrya* and its allies, is supported by other studies that considered morphology (Chappill and Maslin, 1995), phytochemicals (Tindale and Roux, 1969, 1974), and serological data (Brain and Maslin, 1996). A more detailed analysis of relationships within subg. *Phyllodineae* is in progress by the present authors.

The strict consensus tree (Fig. 2) contains a grade consisting of *Acacia* subg. *Aculeiferum* sect. Filicinae/Faidherbia/Ingeae (Fig. 2C) that is nested within *Acacia*. Within this grade there are few synapomorphies and many terminal autapomorphies (8–15), but, nonetheless, only one branch collapses in the strict consensus tree (Fig. 2). This grade may be due to limited sampling of the Ingeae taxa (Bininda-Emonds, Bryant, and Russell, 1998; Hillis, 1998) since only 8 of ~30 genera of tribe Ingeae have been sampled in the present investigation.

It is possible that long-branch attraction may have artificially affected the grouping of *Faidherbia*/sect. Filicinae/Ingeae/subg. *Phyllodineae*. A subset analysis using 13 placeholders from the major clades was conducted to test this possibility. The essential topology of the strict consensus tree was recovered in this analysis (results not shown); however, *Acacia* subg. *Aculeiferum* sect. Filicinae and *Pararchidendron* were included in the unresolved polytomy that previously contained only *Cathormion*, other Ingeae genera, and subg. *Phyllodineae*. *Faidherbia* remained sister to this polytomy. Consequently, caution must be used when interpreting this portion of the cladogram. Sampling of more taxa and additional sequence data may help resolve this issue.

In conclusion, DNA sequence data from the chloroplast *matK/trnK* region clearly indicates that the *Acacia* and tribe Acacieae are polyphyletic. Subgenera *Acacia* and *Phyllodineae* are monophyletic, while subg. *Aculeiferum* is polyphyletic. The tribe Ingeae is nested within the Acacieae. The nomenclatural changes necessitated by creation of at least two new genera from *Acacia* are enormous as they potentially affect >1000 species worldwide. The 39 species of *Acacia* sampled, representing <5% of the genus, is clearly not enough to elucidate interrelationships within subgenera and sections. More sequence data and increased sampling will be needed before more specific subgeneric interrelationships can be elucidated.

These data suggest a large-scale molecular and morphological reinvestigation of the Ingeae, Mimoseae, and Acacieae is necessary to determine phylogenetic relationships. These data and morphological studies (Chappill and Maslin, 1995; Grimes, 1999) suggest that characters currently used to differentiate taxa are polymorphic within tribes and genera and homoplasious in cladistic analyses. Pollen and anther characters are not sufficient at the tribal level, and within subg. *Phyllodineae* leaf type, venation, and inflorescence structure are not always synapomorphic characters. Continued use of molecular methods is critical to determine phylogenetic relationships and to understand character evolution in the Mimosoideae.

LITERATURE CITED

BENTHAM, G. 1842. Notes on Mimoseae, with a synopsis of species. *London Journal of Botany* 1: 318–392, 494–528.

———. 1875. Revision of the suborder Mimoseae. *Transactions of the Linnean Society of London* 30: 335–670.

BININDA-EMONDS, O. R. P., H. N. BRYANT, AND A. P. RUSSELL. 1998. Suprageneric taxa as terminals in cladistic analysis: implicit assumptions of monophyly and a comparison of methods. *Biological Journal of the Linnean Society* 64: 101–133.

BUKHARI, Y. M., K. KOIVU, AND P. M. A. TIGERSTEDT. 1999. Phylogenetic analysis of *Acacia* (Mimosaceae) as revealed from chloroplast RFLP data. *Theoretical and Applied Genetics* 98: 291–298.

BRAIN, P., AND B. R. MASLIN. 1996. A serological investigation of the classification of *Acacia* subg. *Phyllodineae* (Leguminosae: Mimosoideae). *Biochemical Systematics and Ecology* 24: 379–392.

CHAPMAN, A. R., AND B. R. MASLIN. 1992. *Acacia* Miscellany 5. A review of the *A. bivenosa* group (Leguminosae: Mimosoideae: section Phyllodineae). *Nuytsia* 8: 249: 1–283.

CHAPPILL, J. A., AND B. R. MASLIN. 1995. A phylogenetic assessment of tribe Acacieae. *In* M. Crisp and J. J. Doyle [eds.], Advances in legume systematics 7 Phylogeny, 77–99. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

CLARK, H. D., D. S. SEIGLER, AND J. E. EBINGER. 1989. *Acacia farnesiana* (Fabaceae: Mimosoideae) and related species from Mexico, the southwestern U.S. and the Caribbean. *Systematic Botany* 14: 549–564.

———, ———, AND ———. 1990. *Acacia constricta* (Fabaceae: Mimosoideae) and related species from the southwestern U.S. and Mexico. *American Journal of Botany* 77: 305–315.

FARRIS, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417–419.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

GRIMES, J. W. 1999 Inflorescence morphology, heterochrony, and phylogeny in the Mimosoid tribes Ingeae and Acacieae (Leguminosae: Mimosideae). *Botanical Review* 65: 317–347.

GUINET, P. 1981. Mimosoideae: the characters of their pollen grains. *In* R. M. Pohill and P. H. Raven [eds.], Advances in legume systematics, Part 1, 835–858. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

———. 1990. The genus Acacia (Leguminosae, Mimosoideae): its affinities as borne out by its pollen characters. *Plant Systematics and Evolution* 5: 81–90.

———, AND J. VASSAL. 1978. Hypotheses on the differentiation of the major groups in the genus *Acacia* (Leguminosae). *Kew Bulletin* 32: 509–527.

HILLIS, D. M. 1998. Taxonomic: sampling, phylogenetic accuracy, and investigator bias. *Systematic Biology* 47: 3–8.

JOHNSON, L. A., AND D. E. SOLTIS. 1994. *matK* DNA sequences and phylogenetic reconstruction in Saxifragaceae *s. str. Systematic Botany* 19: 143–156.

MASLIN, B. R. 1995a. *Acacia* Miscellany 12. *Acacia myrtifolia* (Leguminosae: Mimosoideae: section Phyllodineae) and its allies in Western Australia. *Nuytsia* 10: 85–101.

———. 1995b. *Acacia* Miscellany 14. Taxonomy of some Western Australian "Uninerves-Racemosae" species (Leguminosae: Mimosoideae: section Phyllodineae). *Nuytsia* 10: 181–203.

———, AND C. H. STIRTON. 1997. Generic and infrageneric classification in *Acacia* (Leguminosae: Mimosoideae): a list of critical species on which to build a comparative data set. *Bulletin of the International Group for the Study of Mimosoideae* 20: 22–44.

MILLER, J. T., AND R. J. BAYER. 2000. Molecular systematics of the Tribe Acacieae (Leguminosae: Mimosoideae). 1995. *In* P. Herendeen and A. Burneau [eds.], Advances in legume systematics 9 Phylogeny. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

OLMSTEAD, R. G., AND J. D. PALMER. 1994. Chloroplast DNA and systematics: a review of methods and data analysis. *American Journal of Botany* 81: 1205–1224.

PEDLEY, L. 1978. A revision of *Acacia* Mill, in Queensland. *Austrobaileya* 1: 75–234.

———. 1986. Derivation and dispersal of *Acacia* (Leguminosae), with particular reference to Australia, and the recognition of *Senegalia* and *Racosperma*. *Botanical Journal of the Royal Linnean Society* 92: 219–254.

PLUNKETT, G. M., D. E. SOLTIS, AND P. S. SOLTIS. 1997. Clarification of the

relationships between Apiaceae and Araliaceae based on *matK* and *rbcL* sequence data. *American Journal of Botany* 84: 565–580.

POHILL, R. M., P. H. RAVEN, AND C. H. STIRTON. 1981. Evolution and systematics of the Leguminosae. *In* R. M. Pohill and P. H. Raven [eds.], Advances in legume systematics, Part 1, 1–26. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.

ROSS, J. H. 1979. A conspectus of the African *Acacia* species. *Memorial Botanical Survey of South Africa* 44: 1–155.

———. 1981. An analysis of the African *Acacia* species: their distribution, possible origins and relationships. *Bothalia* 13: 389–413.

SWOFFORD, D. 1999. PAUP: phylogenetic analysis using parsimony, pre-release version 4.02. Laboratory of Molecular Systematics, Smithsonian Institution, Washington, D.C. and Sinauer, Sunderland, Massachusetts, USA.

TINDALE, M. D., AND D. G. ROUX. 1969. A phytochemical survey of the Australian species of *Acacia. Phytochemistry* 8: 1713–1727.

———, AND ———. 1974. An extended phytochemical survey of Australian species of *Acacia*: chemotaxonomic and phylogenetic aspects. *Phytochemistry* 13: 829–839.

VASSAL, J. 1972. Apport des recherches ontogéniques et séminologiques à l'étude morphologique, taxonomique et phylogénique du genre *Acacia. Bulletin de la Societe d'Histoire Naturelle de Toulouse* 108: 105–247.

———. 1981. Acacieae. *In* R. M. Pohill and P. H. Raven [eds.], Advances in legume systematics, Part 1, 169–171. Royal Botanic Gardens, Kew, Richmond, Surrey, UK.