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Molecular phylogenetics of *Acacia* subgenera *Acacia* and *Aculeiferum* (Fabaceae : Mimosoideae), based on the chloroplast *matK* coding sequence and flanking *trnK* intron spacer regions

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Abstract. The genus *Acacia* is subdivided into the following three subgenera: subg. *Acacia*, subg. *Aculeiferum* and the predominantly Australian subg. *Phyllodineae*. Morphological and molecular studies have suggested that the tribe *Acacieae* and genus *Acacia* are artificial and have a close affinity to the tribe *Ingeae*. Sequence analysis of the chloroplast *trnK* intron, including the *matK* coding region and flanking non-coding regions, were undertaken to examine taxon relationships within *Acacia* subgenera *Acacia* and *Aculeiferum*. Subgenus *Acacia* is monophyletic while subgenus *Aculeiferum* is paraphyletic. Within the subgenera, major divisions are found based on biogeography, New World *versus* African–Asian taxa. These data suggest that characters such as inflorescence and prickle and/or stipule type are polymorphic and homoplasious in cladistic analyses within the subgenera.

Introduction

The tribe *Acacieae* Benth. was originally described as one of three tribes comprising the subfamily Mimosoideae (Bentham 1842). Bentham included in the *Acacieae* many genera that are today classified in tribe *Ingeae* Benth., but he later (Bentham 1875) restricted his definition of tribe *Acacieae* to include the single genus *Acacia* Mill., with the other genera transferred to tribe *Ingeae*. Other than the genus *Acacia* itself, the tribe currently includes the monotypic African genus *Faidherbia*. The pantropical *Acacia* is distributed from the Americas, Africa, Asia and has its largest diversity in Australia.

Bentham (1875) recognised six series within *Acacia*, but recent authors have amalgamated these into three major groups, either at the generic or subgeneric level (Vassal 1972; Pedley 1986; Maslin and Stirton 1997; see Maslin *et al.* 2003, for a review of *Acacia* nomenclatural history). 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).

Acacia has been distinguished by free filaments of the stamens while the stamens of *Ingeae* have filaments partway united; however, this is not true in all taxa, as some species of *Acacia* have filaments shortly united at base (Vassal 1981). There are no definitive single character synapomorphies holding the genus together. Subgenus *Acacia* has bipinnate leaves, stipular spines and colporate pollen with a smooth exine with columellae, whereas subg. *Aculeiferum* has bipinnate leaves, no stipular spines, but may have two or 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 have spinescent stipules and have extraporate or porate pollen with the exine reticulate without columellae (Vassal 1981). In comparison, most taxa of the tribe *Ingeae* have porate pollen without columnae, and with areolate or smooth exines.

Acacia is non-monophyletic, based on chloroplast DNA evidence (Miller and Bayer 2000, 2001; Robinson and Harris 2000; Clarke *et al.* 2001; Luckow *et al.* 2003), nuclear DNA (Miller and Bayer 2000) and morphology (Chappill and Maslin 1995; Grimes 2000). A recent subfamily analysis of chloroplast DNA places *Acacia* subg. *Acacia* deep within the tribe *Mimoseae*, while subg. *Aculeiferum*, the tribe *Ingeae* and *Acacia* subg. *Phyllodineae* form a derived clade in this

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Table 1. Source of seeds

Tribe	Subgenus	Section	Species	Voucher	GenBank acc. no.
Acacieae			*Faidherbia albida* (Delile) A.Chev.	CANB 615551	AF274129
Acacieae	*Acacia*		*A. arenaria* Schinz	CANB 615728	AF523184
Acacieae	*Acacia*		*A. bidwillii* Benth.	CANB 615585	AF274130
Acacieae	*Acacia*		*A. caven* (Molina) Molina	CANB 615552	AF274131
Acacieae	*Acacia*		*A. cochliacantha* Humb. & Bonpl. ex Willd.	CANB 615587	AF274133
Acacieae	*Acacia*		*A. constricta* Benth.	CANB 615588	AF274135
Acacieae	*Acacia*		*A. drepanolobium* Harms ex Sjostedt	CANB 615726	AF523192
Acacieae	*Acacia*		*A. erioloba* E.Mey.	CANB 615729	AF523193
Acacieae	*Acacia*		*A. farnesiana* (L.) Willd.	CANB 615722	AF523115
Acacieae	*Acacia*		*A. haematoxylon* Willd.	CANB 615730	AF523189
Acacieae	*Acacia*		*A. karroo* Hayne	CANB 615590	AF274137
Acacieae	*Acacia*		*A. luederitzii* Engl.	CANB 615725	AF523186
Acacieae	*Acacia*		*A. neovernicosa* Isely	CANB 615724	AF523113
Acacieae	*Acacia*		*A. nilotica* (L.) Willd. ex Delile	CANB 615592	AF274139
Acacieae	*Acacia*		*A. pennatula* (Cham. & Sch.) Benth.	CANB 615553	AF274134
Acacieae	*Acacia*		*A. rigidula* Benth.	CANB 615723	AF523188
Acacieae	*Acacia*		*A. schaffneri* (S.Watson) F.J.Herm.	CANB 615586	AF274132
Acacieae	*Acacia*		*A. schottii* Torr.	CANB 615589	AF274136
Acacieae	*Acacia*		*A. seyal* Delile	CANB 615591	AF274138
Acacieae	*Acacia*		*A. tortilis* (Forssk.) Hayne	CANB 615593	AF274140
Acacieae	*Aculeiferum*		*A. galpinii* Burtt Davy	CANB 615736	AF523098
Acacieae	*Aculeiferum*	*Aculeiferum*	*A. catechu* (L.) Willd.	CANB 615594	AF274141
Acacieae	*Aculeiferum*	*Aculeiferum*	*A. erubescens* Welw. ex Oliver	CANB 615731	AF523185
Acacieae	*Aculeiferum*	*Aculeiferum*	*A. laeta* R.Br. & Benth.	CANB 615732	AF523187
Acacieae	*Aculeiferum*	*Aculeiferum*	*A. modesta* Wall	CANB 615595	AF274142
Acacieae	*Aculeiferum*	*Aculeiferum*	*A. senegal* (L.) Willd.	CANB 615553	AF274143
Acacieae	*Aculeiferum*	*Filicinae*	*A. boliviana* Rusby	CANB 615555	AF274144
Acacieae	*Aculeiferum*	*Monacanthea*	*A. berlandieri* Benth.	CANB 615596	AF274145
Acacieae	*Aculeiferum*	*Monacanthea*	*A. bonariensis* Gill. ex Hook. & Arn.	CANB 615597	AF274146
Acacieae	*Aculeiferum*	*Monacanthea*	*A. coulteri* Benth.	CANB 615738	AF523105
Acacieae	*Aculeiferum*	*Monacanthea*	*A. dolichostachya* S.F.Blake	CANB 615737	AF523190
Acacieae	*Aculeiferum*	*Monacanthea*	*A. eriocarpa* Brenan	CANB 615733	AF523191
Acacieae	*Aculeiferum*	*Monacanthea*	*A. glomerosa* Benth.	CANB 615556	AF274147
Acacieae	*Aculeiferum*	*Monacanthea*	*A. roemeriana* Scheele	CANB 615734	AF523099
Acacieae	*Aculeiferum*	*Monacanthea*	*A. schweinfurthii* Brenan & Exell	CANB 615735	AF523101
Acacieae	*Aculeiferum*	*Monacanthea*	*A. wrightii* Benth. ex A.Gray	CANB 615598	AF274148
Acacieae	*Phyllodineae*	*Juliflorae*	*A. acuminata* Benth.	CANB 615660	AF523170
Acacieae	*Phyllodineae*	*Pulchellae*	*A. pulchella* R.Br. in W.T.Aiton	CANB 615720	AF523100
Ingeae			*Pararchidendron pruinosum* (Benth.) I.C.Nielsen	CANB 615549	AF274127
Ingeae			*Paraserianthes lophantha* (Willd.) I.C.Nielsen.	CANB 615550	AF274128
Mimoseae			*Mimosa tenuiflora* (Willd.) Poir.	CANB 615541	AF274120

analysis by Luckow *et al.* (in press). The *Ingeae* separates the more derived subg. *Phyllodineae* from the more basal subg. *Aculeiferum*. While all molecular studies show *Acacia* subgenera *Acacia* and *Phyllodineae* forming separate monophyletic clades (Miller and Bayer 2000, 2001; Robinson and Harris 2000; Clarke *et al.* 2001; Luckow *et al.* 2003), doubts remain about the relationships within *Acacia* subg. *Aculeiferum*. Recent work (Luckow *et al.* 2003), involving the entire subfamily Mimosoideae, has shown at least the following three evolutionary lineages within subgenus *Aculeiferum* s.lat.: (i) subg. *Aucleiferum* s.str., (ii) sect. *Filicinae* and (iii) *Acacia coulteri* and relatives. The genus needs to be divided (Maslin and Stirton 1997; Maslin *et al.* 2003). Maslin *et al.* (2003) recommend that five genera should be recognised from *Acacia*, but suggested that deeper sampling is needed, especially in subg. *Aculeiferum*, to determine whether further independent evolutionary lines worthy of generic status are present.

The aim of this study was to investigate the relationships of taxa within two separate monophyletic lineages of *Acacia*, subg. *Acacia* and subg. *Aculeiferum* s.str., with increased taxonomic sampling. In addition, an analysis of exemplar taxa is used to place the two subgenera in a larger context of relationships in the Mimosoideae. To accomplish this goal, the cpDNA intron of the transfer RNA gene for lysine (*trnK*) was sequenced, including the maturase encoding gene

Table 2. Nucleotide character statistics for the *trnK*/*matK* region
Outgroup taxa not included in theses calculations

Character	
Aligned length (bp)	2457
Mean sequence divergence (%)	2.7
Variable sites (%)	20.2
Potentially informative sites (%)	8.9
Constant sites (%)	79.8
Autapomorphic sites (%)	11.3
Synapomorphic indels	7
Synapomorphic indel size range (bp)	4–30
Base substitutions	221
Total informative characters	228

(*matK*) as well as flanking non-coding regions. *MatK* evolves 2–3-fold faster than *rbcL* (Johnson and Soltis 1994; Plunkett *et al.* 1997), and was shown to be informative within these subgenera (Miller and Bayer 2001).

Materials and methods

A 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 groups based on subgeneric morphological characters that could be used to systematically sample the large number of species in the genus. The ingroup sampling of the present study was based on these morphological groups. *Mimosa*, of the *Mimoseae*, was included as the outgroup taxon.

Seeds were acquired from various seed banks (Table 1), 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 pulverised in liquid nitrogen. DNA was extracted with a Plant DNAzol Reagent kit (GibcoBRL Inc. 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, Ac1707R (Miller and Bayer 2000) was created internal to *trnK*-2R and was used in all subsequent polymerase chain reactions (PCR). The region was amplified by PCR using *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 1). 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 analysed together. The data were analysed with all characters unweighted. Maximum parsimony analyses were performed on the aligned sequences, with the heuristic search option (excluding uninformative characters), in PAUP 4.02 (Swofford 1999). A four-step search method for multiple islands was performed with 10000 random replicates (Olmstead and Palmer 1994). Support for internal branches was evaluated by the fast bootstrap method with 10000 replicates (Felsenstein 1985).

Results

Sequence characteristics

The aligned length of the sequenced portion of the *trnK* intron was 2457 bp and contained 221 informative base substitutions and seven indels (Table 2). The highest divergence (6.8%) in the 5 region was between *A. laeta* and *A. pulchella*, while the lowest divergence was between *A. constricta* and *A. schottii* (0.4%). Maximum parsimony analysis of the entire unweighted data set found 720 trees of 516 steps with a CI of 0.572 and a RI of 0.81.

The overall analysis indicates a monophyletic *Acacia* subg. *Acacia* (Fig. 1); however, *Acacia* subg. *Aculeiferum* is polyphyletic. Five species of subg. *Aculeiferum* are grouped in a clade including the *Ingeae*, *Acacia* subg. *Phyllodineae* and *Faidherbia albida*. *Acacia* subg. *Phyllodineae* and the *Ingeae* are sister taxa, whereas the rest of the clade is unresolved, except for the pairing of two taxa in *Acacia* subg. *Aculeiferum* section *Monacanthea*, *A. coulteri* and *A. dolichostachya*. *Acacia boliviana* of sect. *Filicinae* and two *Acacia* taxa unassigned to section are also in this polytomy.

Acacia *subg.* Acacia

Parsimony analysis defines the three major clades, Clade A of taxa from the Americas and Clades B and C, with taxa from Africa and Australia (Fig. 2). The '*A. farnesiana* group', '*A. constricta* group' and '*A. macracantha* group' were defined by Clarke *et al.* (1989, 1990) and Seigler and Ebinger (1988), respectively. Clade A comprises four subclades that are related to recognised species groups. The New World '*A. farnesiana* group' is sister to the '*A. macracantha* group'. Sister to the combined clades 'farnesiana/macracantha' clade is *Acacia rigidula* of the '*A. rigidula* group'. The remaining clade is the New World '*A. constricta* group.'

The African taxa fall into two clades. One clade (Clade B) contains only the African taxa of the '*A. farnesiana* group' (*A. erioloba* and *A. haematoxylon*). The second clade (Clade C) also contains taxa from Australia and *A. nilotica*, which has an Indian–African distribution. Clade C is less resolved than Clade A. *A. karroo* and *A. seyal* are sister taxa, with a bootstrap value of 87%. Two species with ant galls are sister to *A. tortilis*, with bootstrap support of 78%.

Acacia *subg.* Aculeiferum

The clade of *Acacia* subgenus *Aculeiferum* (Fig. 3) is divided into two well-defined clades (Clades A and B) that, with one exception, each correlate with sectional and geographic groups. Clade A (BV = 99%) comprises species of sect. *Aculeiferum* and these are native to Africa and India, whereas Clade B (BV = 99%) is composed of taxa from section *Monacanthea* that are from the Americas. The only incongruence with the sectional classification is *Acacia eriocarpa*, an African member of section *Monacanthea* that groups with the other African taxa in section *Aculeiferum* clade. *Acacia schweinfurthii*, an African species, is incongruent with the Old

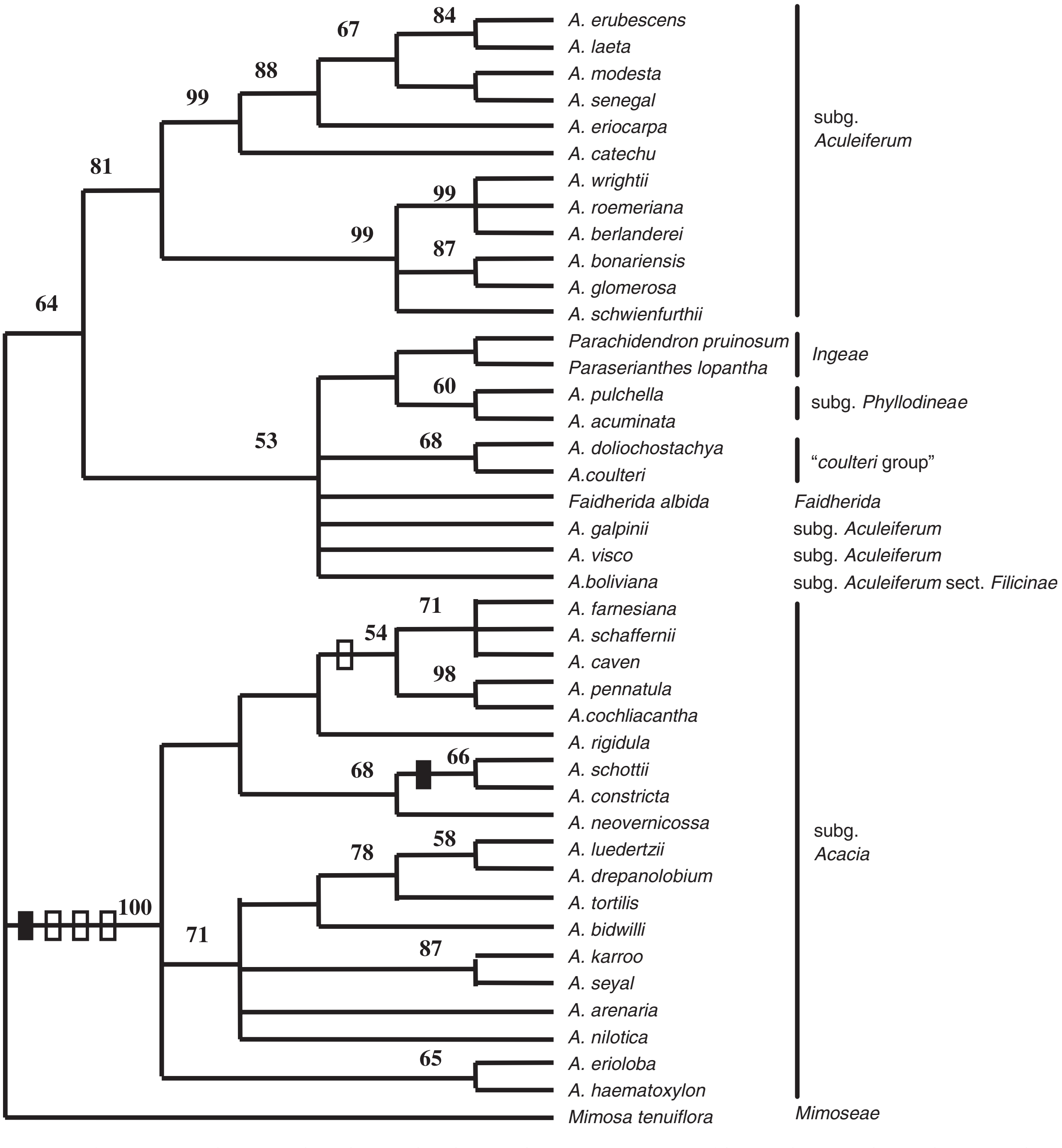

Fig. 1. Strict consensus of 720 trees from the complete dataset. The tree uses 516 steps with a CI of 0.572 and a RI of 0.81. Numbers above line indicate bootstrap support values. Open boxes indicate non-homoplasious indels while shaded boxes indicate a homoplasious indel.

World–New World split as it groups with other members of sect. *Monacanthea*, rather than with the other African species.

The African species *A. erubescens* and *A. laeta* are sister to an Indian–African clade of *A. modesta* and *A. senegal*. The third Indian species, *A. catechu*, is separated from the other Indian taxa by the African species of sect. *Monacanthea*, *A. eriocarpa*.

There are two clades within the sect. *Monacanthea* clade, one (BV = 99%) is the '*A. berlandieri* group' (*A. berlandieri*, *A. wrightii* and *A. roemeriana*; *sensu* Maslin and Stirton 1997). The other clade (BV = 87%) consists of *A. glomerosa* of the '*A. glomerosa* group' and *A. bonariensis*, which has affinities to the '*A. riparia* group' (Maslin and Stirton 1997).

Relationships within *Acacia* subg. *Phyllodineae* are discussed elsewhere (Murphy *et al.* 2003; Miller *et al.* 2003).

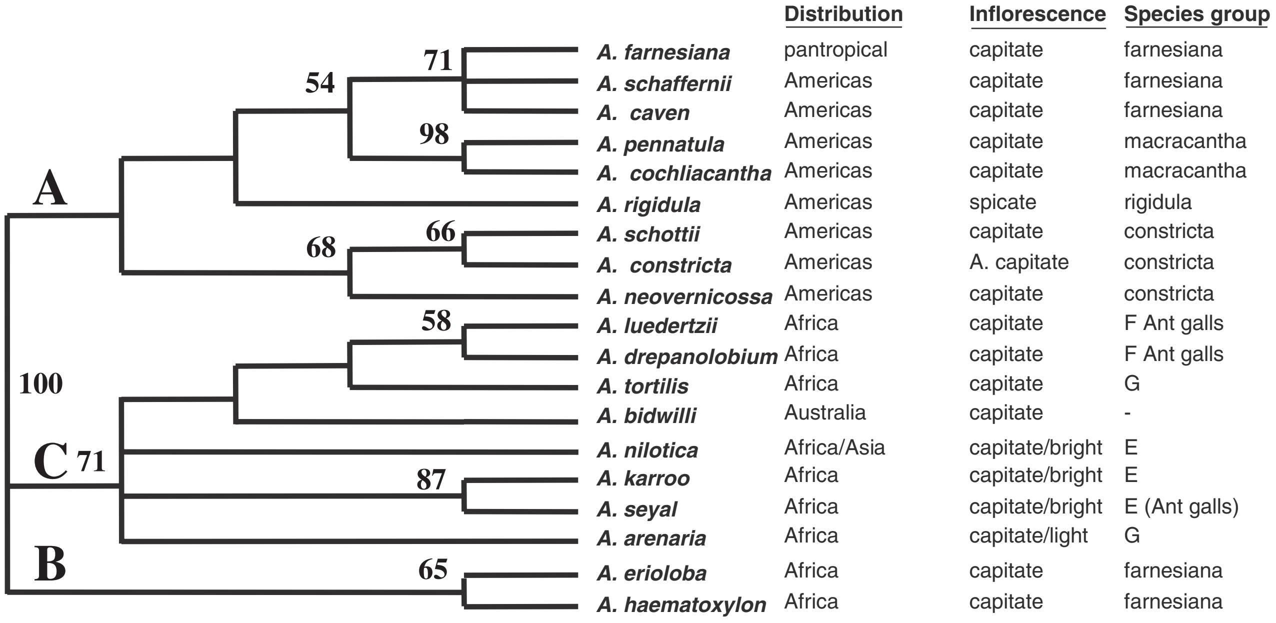

Fig. 2. Strict consensus of the *Acacia* subg. *Acacia* clade from the overall tree shown in Fig. 1. Species Groups E, F and G are from Ross (1979). Other species groups are as explained in text.

Discussion

The macromorphological characters that have been used to separate *Acacia* subg. *Acacia* from subg. *Aculeiferum* are the presence of spinescent stipules in subg. *Acacia* and prickles in subg. *Aculeiferum*. Coupled with that are pollen characters (colporate with columellae in subg. *Acacia v.* porate without columellae in subg. *Aculeiferum*) that clearly differentiate the two groups. The two taxa are not considered closely related, since recent molecular phylogenetic work places subg. *Acacia* deep within the tribe *Mimoseae* (Luckow *et al.* 2003). The ability to demarcate monophyletic lineages on the basis of morphological characters has proven more difficult within the two subgenera.

Acacia *subg.* Acacia

Acacia subg. *Acacia* contains 120–130 species and no meaningful way has been found to subdivide the subgenus on the basis of morphological characters (Maslin and Stirton 1997). Seven groups of American species and two groups of Asian species have been identified although these informal groups do not accommodate all species in these regions (Maslin and Stirton 1997). The four New World species groups analysed in the present study are monophyletic. However, two African species, *A. erioloba* and *A. haematoxylon*, proposed to be close to the '*A. farnesiana* group' (Guinet 1990) form a separate clade outside the New World clade.

Most of the African species of this subgenus have capitate inflorescences, but other characters useful in subdividing the subgenus have remained elusive (Ross 1973, 1979). The African species of subgenus *Acacia* do not resolve well, in congruence with the difficulty of subdividing the group on the basis of morphological characters mentioned by Ross (1973, 1979). Two species that create ant galls, *A. luedertzii* and *A. drepanolobium*, group together. *Acacia seyal* subsp. *fistula*, which does not form ant associations, groups away from the ant-gall clade; however *A. seyal* subsp. *seyal*, not included in the present study, forms ant galls. If the two *A. seyal* subspecies form a monophyletic *A. seyal*, then ant-gall association in the African species has evolved twice or has involved multiple losses of the trait. While the present study did not sample the New World ant acacias, interpolation of the present data to that of Robinson and Harris (2000) and Clarke *et al.* (2001) suggests yet a separate origin of ant relatedness in the New World.

Acacia *subg.* Aculeiferum

According to the present data in subgenus *Aculeiferum*, there is at least one additional lineage separate from the core of subgenus. As this segregate lineage is unresolved, it is possible that it is composed of several lineages. Sufficient differences have been recognised with the American endemic section *Filicinae*, represented here by *Acacia boliviana*, to suggest generic status (Pedley 1978). Likewise, the New World '*Acacia coulteri* group' (Maslin and Stirton 1997; Jawad *et al.* 2000), represented by *A. coulteri* and *A. dolichostachya*, probably represents a lineage separate from the other *Aculeiferum*. Species of the '*A. coulteri*

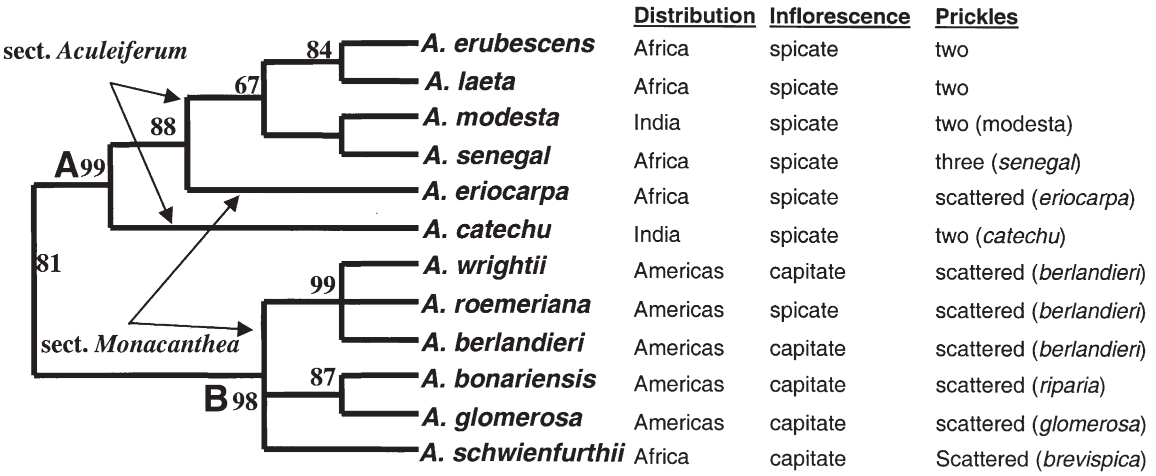

Fig. 3. Strict consensus of the *Acacia* subg. *Aculeiferum* clade from the overall tree shown in Fig. 1.

group' lack prickles, but some species such as *A. coulteri* have spinose horn-like stipules. Otherwise, spinose stipules, a feature of subg. *Acacia*, are rare or absent in subg. *Aculeiferum* (Maslin and Stirton 1997).

Chloroplast restriction-site data (Clarke *et al.* 2001) support the placement of sect. *Filicinae* with the tribe Ingeae rather than with the *Aculeiferum*. The Clarke *et al.* (2001) study found a core *Aculeiferum* with 100% bootstrap support, with *A. willardiana* ('*A. coulteri* group') as the sister taxon and with *A. galpinii* as the next sister taxon. However, a separate chloroplast restriction-site result (Robinson and Harris 2000) found a weakly supported subg. *Aculeiferum*, with section *Filicinae* sister to the rest of the *Aculeiferum* and *A. galpinii* embedded within the main *Aculeiferum*. A core *Aculeiferum* s.str. can be defined as in Fig. 3; however, these results suggest that *Filicinae* and the '*A. coulteri* group' represent independent evolutionary lineages. Since the '*A. coulteri* group' does not have prickles, such as the core *Aculeiferum* has, it would be best placed outside the *Aculeiferum*. Further sampling and data will be needed to better address the issue of these placements within the Mimosoideae.

This subgenus is considered to have three sections with several taxa not easily placed in any of these groups (Maslin and Stirton 1997). The two larger sections differ in distribution. Section *Aculeiferum* is confined to Africa and Asia, whereas section *Monacanthea* is pantropical. These two larger groups are differentiated on the basis of number and placement of prickles. Section *Aculeiferum* has prickles near the nodes, whereas section *Monacanthea* has scattered prickles along the stem.

The African species of section *Monacanthea* are held together by characters of flower colour, ovaries that are stiptate, climbing or scandent shrubby habit, lack of secondary leaves and some characters of the fruit (Ross 1979). Ross (1979) noted that *A. eriocarpa* was one of four African species of sect. *Monacanthea* with spicate inflorescences and commented that it also differed from the African capitate inflorescent species of sect. *Monacanthea* (*A. schweinfurthii* in the present study) in pollen, seed and seedling characters. Another of these four spicate species was *A. chariessa*, the lone section *Monacanthea* species grouping in an otherwise monophyletic section *Aculeiferum* in the chloroplast restriction-site work of Robinson and Harris (2000). These independent results suggest parallel evolution of prickle and inflorescence type in the subg. *Aculeiferum*, thus negating the use of prickle type of clearly differentiating the sections. These results also indicate two lineages of the African species of subgenus *Aculeiferum*. Within section *Aculeiferum*, the taxa are not grouped on the basis of geography (Indian *v.* African) or whether the prickles are found in pairs or in threes.

The New World taxa fall into two clades. The '*A. riparia* group' (*A. bonariensis*) joins the '*A. glomerosa* group' as suggested by Maslin and Stirton (1997). A separate clade contains the highly supported '*A. berlandieri* group.' All these American taxa have scattered prickles, but do not form subgroups on the basis of inflorescence structure (Fig. 3).

In conclusion, the genus *Acacia* is non-monophyletic and the present study lends insight to relationships among taxa within subgenera *Acacia* and *Aculeiferum*. Subgenus *Aculeiferum* is non-monophyletic and subg. *Acacia* is monophyletic. Both groups can be subdivided into New World *versus* African–Asian species on the basis of the sampling of the present study. It appears that characters such as presence and type of prickle and/or stipular spines as well as inflorescence type are homoplasious but can be useful characters when interpreted in a molecular phylogenetic framework. Further sampling and more polymorphic markers are needed to further investigate these two groups.

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