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Implications of Chloroplast DNA Restriction Site Variation for Systematics of *Acacia* (Fabaceae: Mimosoideae)

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ABSTRACT. Comparative restriction site mapping of the chloroplast genome of 26 accessions of *Acacia* plus outgroups (*Caesalpinia*, *Albizia*, and *Ebenopsis*) was carried out to analyze phylogenetic relationships among the subgenera of *Acacia* and, in particular, within *Acacia* subgenus *Acacia*. One or more taxa from each of seven New World species-groups plus two African species of *Acacia* subgenus *Acacia* were included in the analysis to generate hypotheses of the evolution and radiation of this group. Restriction maps constructed from data from 11 restriction enzymes yielded 212 informative restriction sites out of a total of 900. Parsimony analysis resulted in a total of 12 most parsimonious trees of 663 steps each. The strict consensus tree and bootstrap and decay indices indicate strong support for monophyly of *Acacia* subgenus *Acacia* and provisional support for the paraphyly of *Acacia* overall. Moderately to strongly supported clades within *Acacia* subgenus *Acacia* indicate that the mesically adapted *A. macracantha* species-group is polyphyletic and may represent lineages with sister group relationships with both the ant-acacias and the more xerically adapted *A. farnesiana* species-group. A group of Caribbean species was found to be ancestral in *Acacia* subgenus *Acacia* and African and South American species were found to be relatively derived with respect to North American species, lending support to a Boreotropical, rather than a Gondwanan, hypothesis of the historical biogeography of *Acacia* subgenus *Acacia*.

The systematics of the legume subfamily Mimosoideae in general, and its largest genus, *Acacia* Mill., in particular, has been in a state of flux. At a higher taxonomic level, however, legume systematists generally agree that subfamily Mimosoideae is monophyletic and diverged from within subfamily Caesalpinioideae, most likely having a sister group relationship with a member of tribe Caesalpinieae such as *Dimorphandra* Schott (Doyle 1995; Elias 1981). Confirmation of this relationship is provided by phylogenetic analyses of *rbcL* sequence (Doyle et al. 1997) and morphological, anatomical, and chemical (Chappill 1995) data. Various taxonomic schemes have been proposed for subfamily Mimosoideae (Bentham 1875; Elias 1981; Hutchinson 1964; Taubert 1891; and Schulze-Menz 1964), with Elias' (1981) recognition of five tribes the most widely used. Two of these tribes are assemblages of three anomalous genera of uncertain affinities (*Parkia* R. Br. and *Pentaclethra* Benth. in tribe Parkieae, and *Mimozyanthus* Burkart in tribe Mimozyantheae); they are distinguished on the basis of calyces with imbricate aestivation and being either synsepalous (Parkieae) or aposepalous (Mimozyantheae). The remaining three tribes comprise the bulk of the genera and species (ca. 3000 spp., Nielsen 1992) of the subfamily and all have calyces with

valvate aestivation. Tribe Mimoseae is distinguished on the basis of having ten free stamens; tribe Acacieae has many free stamens; and tribe Ingeae has many stamens with at least partially fused filaments. While very few explicitly cladistic hypotheses have been generated for the Mimosoideae, a general hypothesis exists for the derivation of tribes Ingeae and Acacieae relative to Mimoseae. This is supported by floral morphology: Mimoseae and outgroups in the Caesalpinieae share reduced stamen number and Ingeae and Acacieae share the apomorphy of indefinite stamens. Further, shifts in pollen and anther morphology, from numerous tetrads or octads per locule in Mimoseae to few polyads per locule in Ingeae and Acacieae, may, along with increase in stamen number, represent a trend toward increased specialization in pollen dispersal (Polhill 1994).

Although it may be reasonable to conclude that the Ingeae and Acacieae are derived with respect to the Mimoseae, there is little certainty about the relationships among members of the Ingeae and Acacieae. Recent phylogenetic analyses have shown that the tribe Ingeae is probably paraphyletic with respect to the Acacieae (Chappill and Maslin 1995; Grimes 1995, 1999). Further, generic delimitations within these two tribes are also in question. The

core genus of the Ingeae, *Pithecellobium* Mart., has been split into a number of segregate genera (Barneby and Grimes 1996, 1997; Nielsen 1981, 1992). At present, the Acacieae consist of the monotypic genus *Faidherbia* A. Chev. and the very large genus *Acacia* having 1200 species, 900 of these in Australia (Mabberley 1987; Raven and Polhill 1981). The genus *Acacia* has been the subject of several major taxonomic revisions. Bentham (1875) divided the genus into six series (*Gummiferae*, *Vulgares*, *Filicinae*, *Botrycephalae*, *Pulchellae*, and *Phyllodineae*) while Vassal (1969–1972) created a largely concordant taxonomy of three subgenera: *Acacia* (= series *Gummiferae*), *Aculeiferum* (= series *Vulgares* and *Filicinae*), and *Heterophyllum* (= series *Botrycephalae*, *Pulchellae*, and *Phyllodineae*). Bentham's and Vassal's treatments differ primarily with regard to the placement of several species, and Vassal's emphasis on seedling morphology contrasts with Bentham's emphasis on more readily observable vegetative characters. Britton and Rose (1928), in a partial revision of the North American and West Indian members, subdivided the genus into many separate genera. None of these genera are accepted by modern researchers, but they are often useful in delimiting groups of species within *Acacia*. Most recently, researchers have debated the appropriateness of continued recognition of *Acacia* as a single genus, the morphological diversity of Vassal's subgenera (= Bentham's series), united by force of the single character of having free stamens despite the diversity of their floral, fruit, and vegetative morphologies, and questions regarding the relationship of *Acacia* to genera of the tribe Ingeae being cited as evidence that *Acacia* may not constitute a monophyletic group (Maslin 1987, 1988, 1989; Pedley 1986, 1987a, 1987b).

Research by the authors and collaborators has concentrated on the systematics of the approximately 60 species of New World *Acacia* subgenus *Acacia* Vassal (Ebinger and Seigler 1987a, 1987b, 1992; Clarke et al. 1989, 1990; Lee et al. 1989; Seigler and Ebinger 1988, 1995; Ebinger et al. in mss.). Subgenus *Acacia* (=series *Gummiferae* Benth.) is distinguished on the basis of its stipular spines and absence of prickles. The 62 African species of *Acacia* subgenus *Acacia* comprise the bulk of the Old World species, since only ten species occur in Asia, Australia, and the Pacific, and have been the subject of a recent revision (Ross 1979). New World members of *Acacia* subgenus *Acacia* are thought to have resulted from a radiation separate from that which produced Old World members of the subgenus

(Guinet and Vassal 1978), as evidenced by there being no species native to both the New and Old Worlds and the presence of ecologically vicariant groups of species, e.g. the New and Old World ant-acacias (Ross 1981). Although the New World and African members are more than likely a result of separate radiations, a more refined understanding of how these two groups are related (i.e., sister group or derived-ancestral relationship) is one of the goals of our present research.

For the purpose of making revision of New World *Acacia* subgenus *Acacia* more manageable, the species have been divided into seven informal species-groups on the basis of inferred relatedness as judged by morphological similarity. No formal infrasubgeneric classification will be offered for New World members of *Acacia* subgenus *Acacia* until one can be made using the results of a revision of the entire series for the New World, now near completion, and a modern, stable taxonomy for the genus *Acacia* overall that utilizes both molecular and morphological data from a worldwide perspective of both the Ingeae and Acacieae.

Five of the seven groups of New World species are diagnosed by a number of synapomorphies while two of the species-groups (*A. farnesiana* and *A. macracantha*) are defined by combinations of synapomorphies of more inclusive groups (Table 1). The seven species-groups are the *A. rigidula* and *A. constricta* species-groups both occurring in northern Mexico and the southwestern U.S., the *A. farnesiana* species-group, which is distributed throughout the neotropics and has been introduced into the paleotropics, the American ant-acacias of Mexico and Central America, the monotypic *A. choriophylla* species-group of the Bahamas and Cuba, the *A. macracantha* species-group, which is distributed throughout the neotropics, and the *A. aculeifera* species-group of Cuba, Hispaniola, the Bahamas and Turks and Caicos Islands, and Anegada, British Virgin Islands. The African species, though greater in number, are more homogeneous in their vegetative and reproductive morphologies, all sharing with the New World *Acacia constricta* and *Acacia rigidula* species-groups features such as medibracteate peduncles, seeds flattened in cross-section, and a pericarpic strip lining the valves of the fruit. Mexico is the center of diversity of the subgenus in the New World as evidenced by the fact that most of the species of five of the six species-groups occur there. Species with distributions in South America are of four classes: 1. *A. macracantha* of the *A. macracantha* species-group and *A. tortuosa* of the *A. farnesiana*

TABLE 1. New World species-groups of *Acacia* subgenus *Acacia* with diagnostic synapomorphies, general distributions, and habitat preferences.

Species-group	Synapomorphies	Distribution	Habitat preference
<i>A. rigidula</i> species-group	white flowers, loosely spicate inflorescences, 4 sepals and petals	Mexico, sw U.S.	xeric
<i>A. constricta</i> species-group	aciculate spines, subopposite leaflets, mottled seeds	Mexico, sw U.S.	very xeric
<i>A. farnesiana</i> species-group	involucral bract positioned at apex of peduncle (shared with <i>A. choriophylla</i> and <i>A. macracantha</i> species-groups), leaf production from short shoots (shared with <i>A. rigidula</i> , <i>A. constricta</i> and <i>A. acuiifera</i> species-groups), pulpy endocarp (shared with <i>A. macracantha</i> , <i>A. choriophylla</i> , and <i>A. acuiifera</i> species-groups and American ant-acacias)	Mexico, Central and South America, West Indies	xeric
American ant-acacias	enlarged spines, <i>Pseudomyrmex</i> symbiont, beltian bodies, seed arils	Mexico, Central America	mesic
<i>A. choriophylla</i> species-group	reduced stipular spines (unique), inflorescence borne on elongated shoots in leaf axils, floral bracts covering flowers in bud, corollas 1-1.3 times longer than calyces (all three shared with American ant-acacias); lustrous, coriaceous leaflets (shared with <i>A. acuiifera</i> species-group)	West Indies	xeric
<i>A. macracantha</i> species-group	large leaves and production of leaves primarily in long shoots (shared with <i>A. choriophylla</i> and American ant-acacias); woody fruit valve texture (shared with American ant-acacias and <i>A. farnesiana</i> and <i>A. choriophylla</i> species-groups)	Mexico, Central and South America, West Indies	mesic
<i>A. acuiifera</i> species-group	stipular spines borne in short shoots on older growth, chartaceous fruit valve texture, low stamen number	West Indies	xeric

species-group that have distributions bounded on the south by Caribbean Coastal areas of Colombia and Venezuela. 2. Three species belonging to the *A. farnesiana* species-group that occur in cis- and trans-Andean subtropical dry forests in Chile, Argentina, and Bolivia. 3. Three species belonging to the *A. macracantha* species-group with nearly allopatric distributions along the Andes from Colombia to Ecuador, through Peru and into Bolivia. And 4. Two species, *A. rorudiana* Christoph. of the *A. macracantha* species-group, and *A. insulae-jacobae* Riley of the *A. farnesiana* species-group, which are endemic to the Galápagos Islands. Whether the South American-endemic species are derived or ancestral with respect to their North American relatives is an important question that has implications for the biogeographic portion of this study.

The only New World species of *Acacia* subgenus *Acacia* for which a revision has not been published or submitted for publication are those of the Caribbean. A total of 17 species of *Acacia* subgenus *Acacia* has been described from the Caribbean (Acevedo-Rodríguez 1996; Barneby and Zaroni 1989; Bässler 1998; Correll and Correll 1982; Howard 1988; Liogier 1985, 1988). Three of the Caribbean species also have distributions on the mainland and are clearly members of mainland species-groups (*A. farnesiana* and *A. tortuosa*, belonging to the *A. farnesiana* species-group and *A. macracantha*, belonging to the *A. macracantha* species-group).

The remaining 14 species are restricted to the Greater and Lesser Antilles and the Bahamas. Three of these species, *Acacia polyphyrgenes* Greenm., *A. cupeyensis* Léon, and *A. seifriziana* Léon, are very restricted in distribution in Cuba, known only from the type collections, and belong to the *A. farnesiana* species-group. One species from the Bahamas and Cuba, *A. choriophylla*, shares several features with the remaining ten species, but is otherwise quite distinctive and cannot comfortably be placed with any other New World species-group. The remaining ten species form a cohesive, natural group and share a number of features not found in any mainland species-group; these species are informally designated the *A. acuífera* species-group. All members of this species-group are edaphically specialized, being restricted to serpentine areas or calcareous outcrops, and all, except *A. acuífera*, which is widely distributed in the Bahamas, are narrowly endemic, being restricted to one or at most several localities. With the exception of *A. anegadensis* Britton from Anegada, British Virgin Islands, all of the

narrowly endemic species are known only from either Hispaniola or Cuba.

With revisions published or submitted for publication of five of the seven New World species-groups (Seigler and Ebinger 1988, 1995; Clarke et al. 1989, 1990; Lee et al. 1989; Ebinger et al. in mss.) and the revision of the West Indian species in manuscript form, the groundwork has been laid for asking phylogenetic questions about the evolution and radiation of these species. In this context, these revisions serve several main functions: first, they establish and delimit the nomenclatural, taxonomic, and evolutionary units through detailed analysis of morphological data and the delineation of synonymies; second, they provide a sound basis for subsequent study because they insure that characters for cladistic analysis are selected from a comprehensive set of morphological characters that have been studied in view of the entire organism and across the range of variation for the entire taxon; and third, they provide detailed knowledge of the ecology, natural history, and distribution of the taxa that can be integrated into models of the evolution and biogeography of the group.

Restriction site mapping of chloroplast DNA was chosen for phylogenetic analysis because of its ability to provide many informative characters, even in comparison to DNA sequence from any particular gene (Jansen et al. 1998). The study was undertaken with the following main objectives: 1. To test for monophyly of *Acacia* subgenus *Acacia*. 2. To test for monophyly of certain New World species-groups within *Acacia* subgenus *Acacia* and the relationships of these groups relative to each other and to African representatives. 3. To evaluate hypotheses of character evolution relevant to radiation in response to the ant-plant symbioses and progressive xeric adaptations. And 4. To evaluate competing biogeographical hypotheses that have been proposed for *Acacia*.

MATERIALS AND METHODS

The cpDNA restriction site study included one to four taxa from each of the New World species-groups of *Acacia* subgenus *Acacia* and two African members of *Acacia* subgenus *Acacia* or a total of 14 of the 125 species (11.2%) of the subgenus. Other taxa included one African and six New World members of *Acacia* subgenus *Aculeiferum* sections *Monacantha* and *Aculeiferum*, one member of *Acacia* subgenus *Aculeiferum* section *Filicinae*, two members of tribe Ingeae (*Ebenopsis ebano* and *Albizia julibris-*

sin), and one member of subfamily Caesalpinioideae (*Caesalpinia pulcherrima*; Table 2). Two accessions of each of three ingroup taxa (*A. choriophylla*, *A. acuífera*, and *A. farnesiana* var. *guanacastensis*) were included to document the extent of cpDNA restriction site variation within species or varieties. In total, 29 taxa were considered.

Leaf material was collected in the field and dried using calcium sulfate desiccant or fresh leaf material was obtained from plants grown in the University of Illinois greenhouse. Total DNA was extracted using a modified CTAB procedure (Doyle and Doyle 1987). Initial extraction with standard 2% (w/v) CTAB provided virtually no high molecular weight DNA following ultracentrifugation, but addition of 2% (w/v) PVP 40 and increasing the CTAB concentration to 6% (w/v) resulted in adequate yields of high molecular weight DNA. The DNA was initially digested using four restriction enzymes (*Hind*III, *Bam*HI, *Eco*RV, and *Bgl*II) that were known to recognize 40–80 sequences in the tobacco chloroplast genome (Shinozaki et al. 1986). A pilot study revealed little variation among the species using these four enzymes and seven enzymes expected to recognize more sequences (up to 150) in the tobacco chloroplast genome were used in addition to the original four enzymes in the expanded study (*Dra*I, *Hinc*II, *Ava*I, *Eco*RI, *Nci*I, *Sty*I, and *Cla*I). The DNA fragments were separated by electrophoresis in 1% agarose gels and bidirectionally transferred to MagnaCharge (MSI, Inc.) nylon filters. The filters were hybridized with probes generated by the random priming technique (USB Biochemicals) from a set of 43 clones representing virtually the entire tobacco chloroplast genome (provided courtesy of Jeff Palmer, Indiana University). Hybridized fragments were visualized by autoradiography and the size of these fragments and their order with respect to a tobacco DNA standard were used to construct restriction maps for all of the taxa for each of the 11 enzymes.

The eleven maps contained 900 restriction sites, which were scored as either present or absent for each taxon, and the resulting data matrix was analyzed using PAUP 3.1.1 (Swofford 1993). For the initial analysis, characters were analyzed unordered, with Wagner parsimony, a heuristic search, random addition sequence, and tree bisection-reconnection branch swapping. The trees were rooted using *Caesalpinia pulcherrima*. The strength of support for the cladistic relationships found in the initial analysis was assessed using both bootstrap (Felsenstein 1985) and decay (Bremer 1988; Dono-

ghue et al. 1992) analyses. A bootstrap analysis was performed with 1000 replicates, a heuristic search, random addition sequence, and tree bisection-reconnection branch swapping. PAUP was used to search for trees up to 7 steps longer than the most parsimonious trees found in the initial analyses before the memory capabilities of the Power Macintosh 8500 used to perform these analyses were exceeded. The distribution of lengths of random trees generated from the original data set were analyzed for skewness (Sokal and Rohlf 1981). A high degree of left-skewness has been suggested as an indication of significant phylogenetic information content in a data set (Archie 1989; Faith and Cranston 1991; Hillis 1991; Hillis and Huelsenbeck 1992). Weighted parsimony analyses were also carried out using the STEPMATRIX option of PAUP in an effort to perform an analysis that takes into account the greater likelihood of the independent losses of restriction sites than their independent gains without being limited by the overly restrictive limitations of Dollo parsimony (Albert et al. 1992). The weighted parsimony analyses were run with the use of a constraint tree that constrained all taxa except *Caesalpinia pulcherrima* to monophyly. Successive analyses were conducted starting with a ratio of weights of gains:losses of 1.6:1.0 and descending by values of 0.1 to 1.1:1.0; these analyses were conducted twice, with ancestral states designated as first unknown and then all zero (restriction site absent) resulting in 12 individual weighted parsimony analyses. Both types of designations for ancestral states in a weighted parsimony analysis are subject to criticism (for review see Olmstead and Palmer 1994).

RESULTS

The unweighted PAUP analysis found 12 most parsimonious trees of 663 steps each. The strict consensus tree of those 12 trees, with bootstrap values and decay indices, is shown in Fig. 1. Figure 2 shows one of the 12 most parsimonious trees as a phylogram that illustrates relative branch lengths as an indication of the number of mutations associated with each clade. Of 900 restriction site characters in the original data matrix, 212 (23.6%) were deemed to be parsimony informative, 297 (33%) were autapomorphic, and 391 (43.4%) were invariant. The consistency index excluding uninformative characters for each of the 12 most parsimonious trees was 0.575, the retention index was 0.854 and the rescaled consistency index was 0.651. No variation in restriction site maps was found within the two spe-

cies and one variety for which two accessions were included in this study (*A. choriophylla*, *A. acuífera*, and *A. farnesiana* var. *guanacastensis*).

The distribution of lengths of random trees generated from the data set was found to have a g_1 (skewness) value of -0.614285 . The very negative g_1 statistic is a measure of a highly left-skewed distribution ($P \ll 0.01$, Hillis and Huelsenbeck 1992) and is an indication of a high degree of phylogenetic information in the data set. The weighted parsimony analyses generated trees whose topologies were a subset of those found in the unweighted parsimony analysis. More heavily weighted analyses resulted in fewer most parsimonious trees than less heavily weighted analyses.

The strict consensus tree shows two major clades, one comprising *Acacia* subgenus *Acacia* and one comprising *Acacia* subgenus *Aculeiferum* and the two genera of the tribe Ingeae (*Albizia julibrissin* and *Ebenopsis ebano*). The very strong support for these two major clades (decay indices greater than seven and bootstrap values of 99 or 100 %) and the fact that *Acacia* subgenus *Aculeiferum* is more closely related to *Albizia* and *Ebenopsis* than to *Acacia* subgenus *Acacia* are strong indications that *Acacia* is a paraphyletic group despite the fact that *Acacia* subgenus *Heterophyllum* was not sampled in this study. *Albizia* and *Ebenopsis* were originally included as outgroup taxa; the fact that they appear to be sister taxa to *Acacia* section *Filicinae* and derived with respect to *Acacia* sections *Monacantha* and *Aculeiferum* must be considered as *Acacia* and tribes Ingeae and Acacieae are revised.

There is also strong support for the clade defining *Acacia* subgenus *Aculeiferum* sections *Monacantha* and *Aculeiferum* as a monophyletic group and for clades defining groups of species within these sections. Although only weakly supported by the bootstrap analysis, *Acacia galpinii*, an African species, was found to be sister to all other members within this series. *Acacia willardiana*, a representative of a group that has indurate, but very small stipules occupies the next most basal clade above *A. galpinii*. *Acacia acapulcensis* is a representative of a group that is armed with prickles and often has a liana-like growth habit; it occupies a clade sister to the most highly derived group of *Acacia* subgenus *Aculeiferum*, *A. berlandieri*, *A. roemeriana*, *A. parviflora*, and *A. greggii*, that are armed with prickles and occur in northern Mexico and the southwestern U.S.

Within *Acacia* subgenus *Acacia*, moderately to strongly supported clades include the one defining

the African species (*A. sieberiana* and *A. horrida*); the clade defining *A. macracantha* plus five representatives of the *A. farnesiana* species-group; and the clade defining *A. cornigera*, an ant-acacia, *A. choriophylla*, and three members of the *A. macracantha* species-group (*A. pennatula*, *A. cochliacantha*, and *A. aroma* from South America). *Acacia acuífera*, chosen as the sole representative of the *Acacia acuífera* species-group, is sister to all other examined members of *Acacia* subgenus *Acacia*, although this relationship is weakly supported. *Acacia rigidula* and *A. constricta*, representatives of their respective species-groups, are sister taxa, but low bootstrap and decay values leave this conclusion tenuous. *Acacia choriophylla* was found to be a sister to *A. cornigera* and not closely related to *A. acuífera*.

DISCUSSION

The results of this study provide information that helps answer questions concerning the phylogeny, character evolution, and biogeography of *Acacia* subgenus *Acacia* as well as outgroup taxa. With regard to the genus overall, while *Acacia* subgenus *Acacia* is likely monophyletic, *Acacia* as a whole appears to be paraphyletic with respect to members of the Ingeae. Prior concepts of the Acacieae and Ingeae being lineages derived independently from the Mimoseae (Elias 1981) need to be reexamined in the light of these new data, which are in general agreement with other recent phylogenetic analyses of morphological and molecular data from *Acacia* and the Mimosoideae. Chappill and Maslin (1995) presented results of phylogenetic analyses of morphological data from a broad sampling of genera of the Ingeae and Acacieae with outgroup Mimoseae and Parkieae and found *Acacia* subgenera *Aculeiferum* and *Heterophyllum* to share a clade derived with respect to Mimoseae and Parkieae and at the basal position of a grade containing the genera of the Ingeae and *Acacia* subgenus *Acacia*, which shares a clade with *Calliandra* Benth. and *Pithecellobium*. Support values for clades in this analysis were not given, but the analysis does allow for the conclusion that *Acacia* subgenus *Aculeiferum* and *Heterophyllum* share a close relationship while subgenus *Acacia* is a distinct lineage not closely related to the other two subgenera. Grimes (1995), in a phylogenetic analysis of morphological data of the Ingeae and several Acacieae using characters related to inflorescence morphology and heterochronic development patterns and in a subsequent analysis using similar data and taxon selection (Grimes 1999),

TABLE 2. Taxa included in cpDNA restriction site mapping study. All vouchers are deposited at the Herbarium, Department of Plant Biology, University of Illinois (ILL). Infrageneric classification of *Acacia* follows Vassal (1969–1972). "PSL" = Department of Plant Biology Greenhouse, Plant Sciences Laboratory, University of Illinois.

Taxon	Distribution	Collection information
Subfamily Caesalpinoideae		
1. <i>Caesalpinia pulcherrima</i> Sw.	New World Tropics	Cult., PSL; <i>Clarke s.n.</i>
Subfamily Mimosoideae		
Tribe Ingeae		
2. <i>Albizia julibrissin</i> Durazz.	Warm Temperate Asia	Cult., PSL; <i>Clarke s.n.</i>
3. <i>Ebenopsis ebano</i> (Berland.) Barneby & J.W. Grimes	Mexico	Mexico; <i>Seigler 13379</i>
Tribe Acaciaeae		
<i>Acacia</i> subgenus <i>Aculeiferum</i> section <i>Filicinae</i>		
4. <i>A. angustissima</i> Kuntze	Mexico, s.w. U.S.	Cult., PSL; <i>Clarke s.n.</i>
<i>Acacia</i> subgenus <i>Aculeiferum</i> sections <i>Monacantha</i> and <i>Aculeiferum</i>		
<i>A. acatensis</i> Benth. species-group		
5. <i>A. willardiana</i> Rose	Sonora, Baja Calif., Mexico	Cult., PSL; <i>Clarke s.n.</i>
<i>A. greggii</i> species-group		
6. <i>A. berlandieri</i> Benth.	Mexico, U.S.	Cult., PSL; <i>Clarke s.n.</i>
7. <i>A. roemeriana</i> Scheele	Texas	Texas; <i>Seigler 13258</i>
8. <i>A. parviflora</i> Little	Mexico	Mexico; <i>Seigler 13367</i>
9. <i>A. greggii</i> A. Gray	Mexico, U.S.	Cult., PSL; <i>Clarke s.n.</i>
<i>A. riparia</i> Bertero ex Spreng. species-group		
10. <i>A. acapulcensis</i> Kunth	Mexico, Central America	Mexico; <i>Seigler 13594</i>
African species		
11. <i>A. galpinii</i> Burt Davy	Africa	Cult., PSL; <i>Clarke s.n.</i>
<i>Acacia</i> subgenus <i>Acacia</i> African species		
12. <i>A. sieberiana</i> DC.	Africa	Cult., PSL; <i>Clarke s.n.</i>
13. <i>A. horrida</i> (L.) Willd.	Africa	Cult., PSL; <i>Clarke s.n.</i>
<i>A. rigidula</i> species-group		
14. <i>A. rigidula</i> Benth.	Mexico, Texas	Cult., PSL; <i>Clarke s.n.</i>
<i>A. constricta</i> species-group		
15. <i>A. constricta</i> Benth.	Mexico, U.S.	Cult., PSL; <i>Clarke s.n.</i>
<i>A. farnesiana</i> species-group		
16. <i>A. farnesiana</i> (L.) Willd. var. <i>farnesiana</i>	Pantropical, native to New World	Cult., PSL; <i>Clarke s.n.</i>
17. <i>A. farnesiana</i> (L.) Willd. var. <i>guanacastensis</i> Clarke, Seigler & Ebinger	Mexico, Central America	Costa Rica, Guanacaste Prov., Palo Verde National Park; <i>Clarke 86</i>
18. <i>A. farnesiana</i> (L.) Willd. var. <i>guanacastensis</i> Clarke, Seigler & Ebinger	Mexico, Central America	Costa Rica, Guanacaste Prov., Palo Verde National Park; <i>Clarke 90</i>
19. <i>A. tortuosa</i> (L.) Willd.	West Indies	Netherlands Antilles, Aruba; <i>Ebinger 52891</i>
20. <i>A. caven</i> Molina	Southern South America	Cult., Los Angeles Bot. Gard. 52-5-588; <i>Clarke s.n.</i>
American ant-acacias		
21. <i>A. cornigera</i> (L.) Willd.	Mexico, Central America	Cult., PSL; <i>Clarke s.n.</i>

TABLE 2. Continued.

Taxon	Distribution	Collection information
<i>A. choriophylla</i> species-group		
22. <i>A. choriophylla</i> Benth.	Bahamas, Cuba	Bahamas, L. McKee "A"
23. <i>A. choriophylla</i> Benth.	Bahamas, Cuba	Bahamas, L. McKee "B"
<i>A. macracantha</i> species-group		
24. <i>A. macracantha</i> Humb. & Bonpl. ex Willd.	New World Tropics	Cult., Fairchild Tropical Gardens; J. Horn and V. Funk 1754
25. <i>A. aroma</i> Gillies ex Hook. & Arn.	South America	Cult., Fairchild Tropical Gardens, F-6-73-2604; J. Horn and V. Funk 1755
26. <i>A. pennatula</i> (Schldl. & Cham.) Benth.	Mexico, Central America	Mexico; Seigler 13553
27. <i>A. cochliacantha</i> Humb. & Bonpl. ex Willd.	Mexico	Mexico; Seigler 13898
<i>A. acuífera</i> species-group		
28. <i>A. acuífera</i> Benth.	Bahamas, Turks and Caicos Islands	Bahamas; L. McKee "C"
29. <i>A. acuífera</i> Benth.	Bahamas, Turks and Caicos Islands	Cult., Fairchild Tropical Gardens; Seigler s.n.

found *Acacia* subgenus *Acacia* to be derived with respect to other Acaciae sampled (*Faidherbia albida* and *Acacia* subgenera *Heterophyllum* and *Aculeiferum*). Grimes (1995 and 1999) differed in that the single species of subgenus *Heterophyllum* sampled (*A. stenophylla* A. Cunn. ex Benth.) was found to be basal to all other Ingeae sampled in the later study whereas the earlier study placed it in a clade shared by *Faidherbia albida* that is unresolved with respect to the single species of subgenus *Aculeiferum* sampled (*A. senegal* (L.) Willd.) and basal to the two species of subgenus *Acacia* sampled and several New World genera of the Ingeae with spinescent stipules (*Hawardia* Small, *Painteria* Britton and Rose, *Sphinga* Barneby and J. W. Grimes, *Ebenopsis* Britton and Rose, and *Pithecellobium*). These prior studies support the conclusion of this study that *Acacia* is paraphyletic with respect to tribe Ingeae, although the work presented here does not sufficiently sample taxa of the Ingeae or subgenus *Heterophyllum* and the prior studies neither had sufficient taxon sampling of *Acacia* nor showed support values for hypothesized relationships, leaving equivocal such details as whether or not *Acacia* subgenus *Aculeiferum* and *Heterophyllum* constitute a monophyletic group or which genera of the Ingeae are most closely related to each of the three subgenera of *Acacia*. Bukhari et al. (1999) analyzed chloroplast restriction fragment length polymorphism (RFLP) data from 10 species of subgenus *Acacia*, six species of subgenus *Heterophyllum*, five species of subgenus *Aculeiferum*, and *Faidherbia albida*. Wagner parsimo-

ny analysis was carried out on these data and trees were rooted using *A. senegal*, although no a priori justification is given for this choice. High bootstrap values for clades defining the three subgenera were interpreted as strong support for the monophyly of two of the three subgenera (*Faidherbia* occupied a clade rooted within subgenus *Aculeiferum*). Lack of sampling of Ingeae or Mimoseae leaves untested hypotheses of monophyly of *Acacia* in this study as well as in a study in which Playford et al. (1992) sequenced three clones of 5S DNA from three species of subgenus *Heterophyllum*, two of subgenus *Aculeiferum*, one of subgenus *Acacia*, and *Faidherbia albida*. One of the 5S DNA lineages was evolutionarily informative; clustering on the basis of similarity showed *Faidherbia* to be very dissimilar from the other taxa, the three *Heterophyllum* species to group together and subgenera *Aculeiferum* and *Acacia* to group together separate from subgenus *Heterophyllum*.

In the present study, sampling of taxa belonging to *Acacia* subgenus *Aculeiferum* was sufficient to allow for conclusions regarding the phylogeny of these taxa. While only one African species was included (*A. galpinii*), the fact that it was found to be sister to the New World members of the subgenus is the opposite of what is seen in *Acacia* subgenus *Acacia* (discussed below) and may be an indication that *Acacia* subgenus *Aculeiferum* conforms to a Gondwanan, rather than a Boreotropical hypothesis. With regard to the New World members of *Acacia* subgenus *Aculeiferum* sections *Monacantha* and

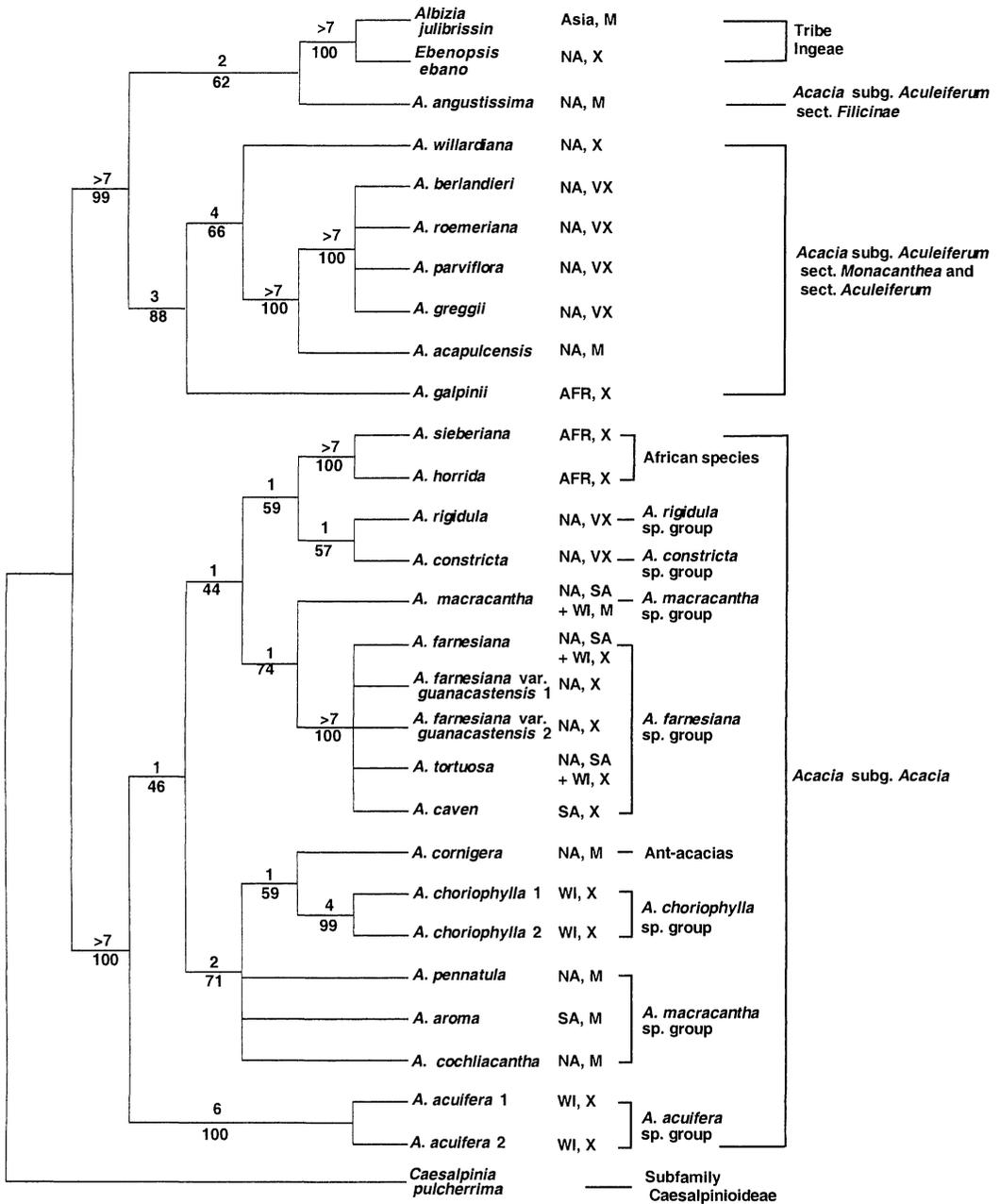


FIG. 1. Strict consensus tree of 12 most parsimonious trees of 663 steps each (consistency index excluding uninformative characters = 0.575, retention index = 0.854, rescaled consistency index = 0.651) based on phylogenetic analysis of cpDNA restriction site data. Bootstrap values as percentages are indicated below clades while decay indices are placed above the clades. *A.* = *Acacia*. Native distributions and habitat preferences for ingroup taxa are listed immediately to the right of species names: AFR = Africa, NA = North America, SA = South America, WI = West Indies, M = mesic, X = xeric, and VX = very xeric.

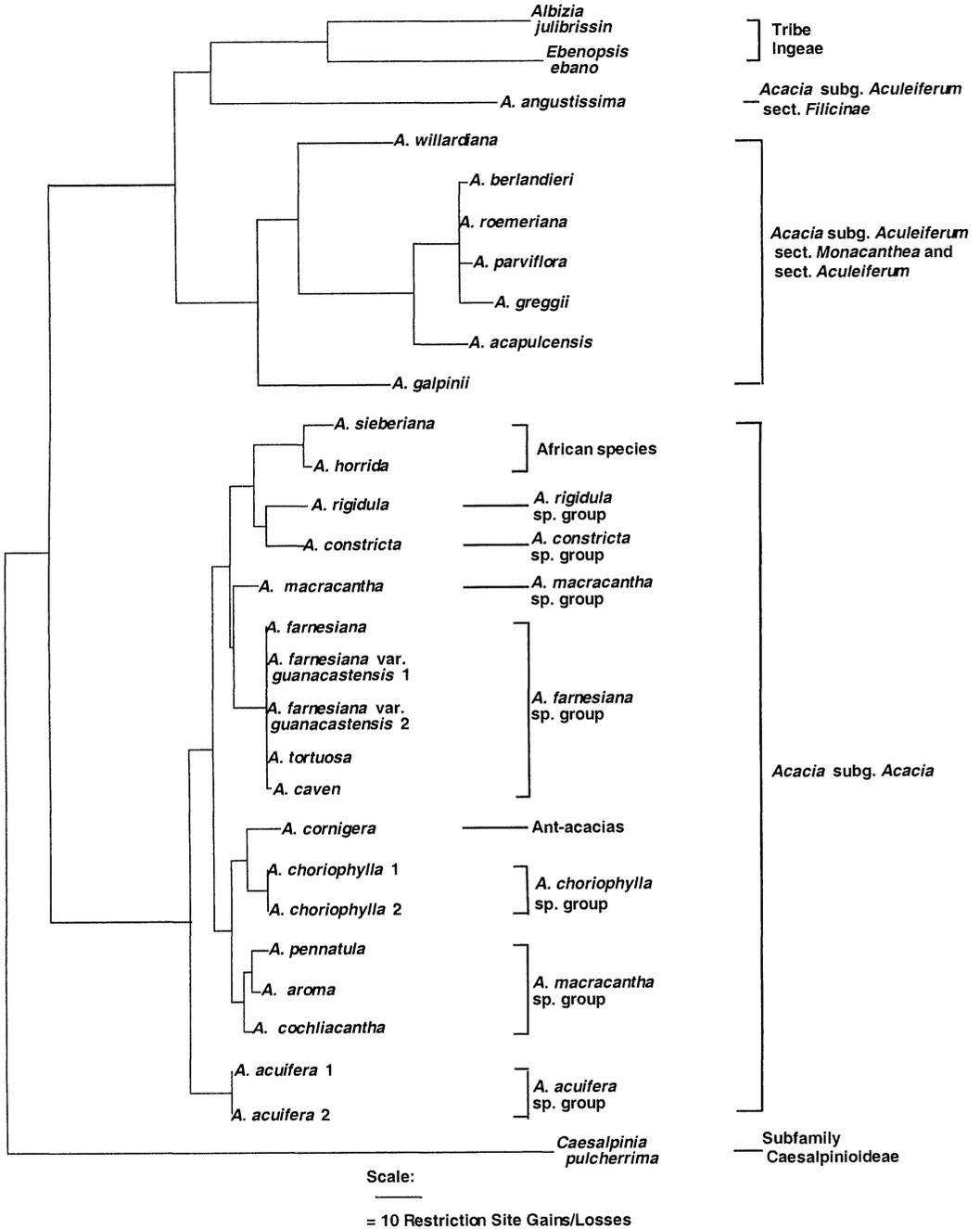


FIG. 2. One of twelve most parsimonious trees of 663 steps each of cpDNA restriction site data. The tree is represented as a phylogram where relative branch lengths are proportional to the number of restriction site mutations associated with each clade.

Aculeiferum, the fact that the more derived members occupy the most xeric habitats of northern Mexico is evidence that this may be a group that conforms to Axelrod's (1950, 1958, 1975, 1979) hypothesis of radiation of plant groups in Mexico in response to increasing aridity.

Two New World species-groups of *Acacia* subgenus *Acacia*, previously delimited on the basis of overall morphological similarities, were sampled sufficiently to test their monophyly. The *A. farnesiana* species-group, represented by *A. farnesiana*, *A. farnesiana* var. *guanacastensis*, *A. caven*, and *A. tortuosa*, is strongly supported as monophyletic and is sister to *A. macracantha*. These four members (5 accessions) of the *A. farnesiana* species-group had identical restriction maps with the exception of *A. caven* of southern South America, which differed from the others by the presence of a single autapomorphic restriction site.

The *A. macracantha* species-group, represented by *A. aroma* from South America plus *A. pennatula*, *A. cochliacantha*, and *A. macracantha*, is polyphyletic with *A. macracantha* occupying a clade sister to the *A. farnesiana* species-group and the other three species sharing a clade with *A. choriophylla* and the ant-acacia, *A. cornigera*. Although this finding controverts phenetic analysis of morphological data that showed the *A. macracantha* species-group to be a very cohesive and well-delimited group (Seigler and Ebinger 1988), the restriction site data confirm this group as sister to several lineages of New World species-groups of *Acacia* subgenus *Acacia*. Thus, although the members of the *A. macracantha* species-group are phenetically similar and share a number of characters (large leaves, leaf production in long rather than short shoots, woody fruit valve texture, involucre bracts positioned at the apex of the peduncle, and pulpy endocarp), all of these characters diagnose the group only in combination with other species-groups and in that sense corroborate the results here, which find the group to form an evolutionary grade having sister group relationships with other species groups. The evolutionary position of members of the *A. macracantha* species-group with respect to other New World species-groups also confirms hypotheses of radiation in response to increasing aridity and radiation of the ant-acacias from an ancestor similar to members of the *A. macracantha* species-group. The ant-acacias, represented by *A. cornigera*, were found to be derived with respect to three members of the *A. macracantha* species-group. This relationship is concordant with predictions based on vegetative mor-

phology: similarities with respect to leaf morphology between these two groups (large leaves produced only on long shoots with numerous pairs of pinnae and leaflets) and preference for mesic habitats may be interpreted as indications of their common ancestry and specialized features of the ant-acacias (e.g., beltian bodies, enlarged extrafloral nectaries, swollen stipular spines) may be interpreted as synapomorphies that evolved in response to the ant-plant symbiosis as the ant-acacias diverged from an *A. macracantha*-like ancestor. Although the relationships of the most xerically adapted species, the *A. constricta* species-group, to the most mesically adapted species, the *A. macracantha* species-group, is obscured by poorly supported clades with low bootstrap and decay values, there is clearly an overall pattern where more xeric species (e.g., *A. constricta* and *A. farnesiana*) are more highly derived than mesic species, with the exception of the serpentine and karst endemic *A. acuífera* species-group. Moreover, there is good support for the *A. farnesiana* species-group being sister to *A. macracantha* and, at least in this particular instance, an evolutionary grade of progressive xeric adaptation is evident with concomitant transformations in vegetative characters (reduction in size of leaves and leaflets and shift in production of leaves from long shoots to short shoots).

The finding that *Acacia choriophylla* bears a close relationship to the ant-acacias, represented in this study by *A. cornigera*, allows for reevaluation of a number of its morphological characters. Although this relationship is supported by low bootstrap and decay values, there is moderate support for the association of this clade with three members of the *A. macracantha* species-group (*A. pennatula*, *A. aroma*, and *A. cochliacantha*). The results of this study suggest that similarities between the *A. acuífera* species-group and *A. choriophylla* in terms of geographic distribution and their lustrous, coriaceous leaflets may be the result of convergence and that the morphological features that *A. choriophylla* shares with the ant-acacias (peltate floral bracts covering the flowers in bud, corollas 1–1.3 times longer than the calyx, and inflorescences borne on elongated shoots in the axils of the leaves) are synapomorphies indicative of a shared evolutionary history and a recent common ancestor. The molecular evidence is less conclusive for *A. rigidula* and *A. constricta* because their putative sister group status is supported by a decay index of one and a bootstrap value of 57%. Nevertheless, the possession by members of both the *A. constricta* and *A. rigidula* species-groups

of fruits dehiscent along two sutures with a pericarpic strip and without a pulpy endocarp, features not shared by any other New World species-groups of *Acacia* subgenus *Acacia*, is interpreted as evidence of a close relationship on the basis of the findings from the restriction site study. Further, the sister group relationship between the African species and the *A. rigidula* and *A. constricta* species-groups found in the cpDNA study is corroborated by a number of morphological characters uniquely shared by these three groups: seeds flattened in cross-section, fruits dehiscent along two sutures, pericarpic strip lining the valves of the fruit, and medibracteate peduncles.

That both African and South American representatives of *Acacia* subgenus *Acacia* were found to be derived with respect to North American representatives confirms that the biogeographic history of this series conforms to a Boreotropical and not a Gondwanan hypothesis. The Boreotropical hypothesis has been proposed as an alternative to the Gondwanan hypothesis (Wolfe 1975; Tiffney 1985a and 1985b; Lavin and Luckow 1993). These authors propose an alternative to Gondwanan origins of tropical floras, citing abundant paleobotanical evidence for the existence of a more or less continuous tropical flora across North America and Eurasia during the early Tertiary. The dispersal of tropical taxa across northern latitudes was effected by the recession of epicontinental seas that separated eastern from western North America and eastern from western Eurasia and by the existence of warm, equable climates at northern latitudes that allowed for exchange of tropical taxa across the Bering and North Atlantic land bridges. While long recognized as important by animal biogeographers (McKenna 1975), the North Atlantic land bridge has often been ignored by phytogeographers in favor of the more stable Bering land bridge. However, the fact that the North Atlantic had not fully opened in the early Tertiary and that the two routes available across the North Atlantic through England and Scandinavia occurred at latitudes significantly further south than the Bering land bridge implicate this route strongly in the exchange of tropical taxa from North America to Eurasia before cooling at the end of the Eocene and early Oligocene and the widening of the North Atlantic which was ongoing throughout the Tertiary. The cooling at the end of the Eocene epoch displaced tropical elements in North America and Europe south into North American and Eurasian refugia (e.g., tropical dry forests in the Caribbean, low elevation and montane forests

of eastern and southern Mexico, and tropical forests of Southeast Asia) while at the same time the widening of the North Atlantic resulted in isolation of Old and New World groups. Moreover, the geographic isolation of South America during the late Cretaceous and most of the Tertiary implies that only derived elements of a group would be found there. The restriction site mapping study supports a model of the evolution of *Acacia* subgenus *Acacia* wherein the subgenus had an origin in North America and subsequently dispersed to both Africa and South America. Due to lack of variation in restriction maps among closely related ingroup taxa, more explicit confirmation of this model must await documentation of the relationship of the southern South American species (*A. caven* and *A. aroma*) to North American members of their respective species-groups. Moreover, there is only weak support for the African species as being a sister group to the *A. rigidula* and *A. constricta* species-groups (decay index of one and bootstrap value of 59 percent); further clarification of the sister group relationship of the African species will be obtained through the collection of more molecular data and increased sampling of the African species.

Implications of this study for Caribbean biogeography include advocacy for both dispersalist and vicariant models. The fact that *Acacia acuífera* occupies the most basal clade of the entire series is interpreted as evidence of the very long history of this group in the Caribbean. Although cladistic relationships cannot distinguish between the diversification of a lineage resulting from either dispersal or vicariance, comparison with geological or ecological correlates may be used to resolve these differences. Abundant circumstantial evidence for the relictual nature of the *A. acuífera* species-group combined with the molecular evidence presented here point to the diversification of this lineage occurring at a time in the early Tertiary when vicariant events were prevalent. In contrast, *A. tortuosa*, with a mainly Caribbean distribution, was found to have a restriction map identical to two closely related mainland taxa (*A. farnesiana* and *A. farnesiana* var. *guanacastensis*); this lack of molecular divergence combined with numerous morphological similarities (sterile or non-fruiting material of *A. tortuosa* and *A. farnesiana* are often confused) combined with the weediness and widespread distribution of *A. tortuosa* indicate that *A. tortuosa* could, and probably did, disperse to the islands of the Caribbean relatively recently.

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