

PHYLOGENY OF MALPIGHIACEAE: EVIDENCE FROM CHLOROPLAST *NDHF* AND *TRNL-F* NUCLEOTIDE SEQUENCES¹

CHARLES C. DAVIS,^{2,5} WILLIAM R. ANDERSON,³ AND
MICHAEL J. DONOGHUE⁴

²Department of Organismic and Evolutionary Biology, Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts 02138 USA; ³University of Michigan Herbarium, North University Building, Ann Arbor, Michigan 48109-1057 USA; and

⁴Department of Ecology and Evolutionary Biology, Yale University, P.O. Box 208106, New Haven, Connecticut 06520 USA

The Malpighiaceae are a family of ~1250 species of predominantly New World tropical flowering plants. Intrafamilial classification has long been based on fruit characters. Phylogenetic analyses of chloroplast DNA nucleotide sequences were analyzed to help resolve the phylogeny of Malpighiaceae. A total of 79 species, representing 58 of the 65 currently recognized genera, were studied. The 3' region of the gene *ndhF* was sequenced for 77 species and the noncoding intergenic spacer region *trnL-F* was sequenced for 65 species; both sequences were obtained for the outgroup, *Humiria* (Humiriaceae). Phylogenetic relationships inferred from these data sets are largely congruent with one another and with results from combined analyses. The family is divided into two major clades, recognized here as the subfamilies Byrsonimoideae (New World only) and Malpighioideae (New World and Old World). Niedenzu's tribes are all polyphyletic, suggesting extensive convergence on similar fruit types; only de Jussieu's tribe Gaudichaudieae and Anderson's tribes Acmanthereae and Galphimieae are monophyletic. Fleshy fruits evolved three times in the family and bristly fruits at least three times. Among the wing-fruited vines, which constitute more than half the diversity in the family, genera with dorsal-winged samaras are fairly well resolved, while the resolution of taxa with lateral-winged samaras is poor. The trees suggest a shift from radially symmetrical pollen arrangement to globally symmetrical pollen at the base of one of the clades within the Malpighioideae. The Old World taxa fall into at least six and as many as nine clades.

Key words: biogeography; fruit evolution; Malpighiaceae; molecular; *ndhF*; phylogeny; systematics; *trnL-F*.

The Malpighiaceae are an angiosperm family of trees, shrubs, and vines in the tropical and subtropical forests and savannas of both Old and New Worlds. They comprise ~1250 species in 65 genera, with ~100 species belonging to the 15 Old World genera (W. Anderson, unpublished data). The only overlap between the Malpighiaceae in the two hemispheres consists of two species of predominantly New World genera that also occur in west Africa (Anderson, 1990a).

The monophyly of the Malpighiaceae has recently been confirmed using molecular data (Chase et al., 1993; Cameron, Chase, and Anderson, 1995; Wurdack and Chase, 1996) and is also supported by morphological characters (Anderson, 1979a, 1990a). Floral morphology in neotropical Malpighiaceae is distinctive and highly conserved (Anderson, 1979a; see Fig. 1). In contrast, fruits of Malpighiaceae are extremely diverse, providing the most important characters for delimiting genera. Niedenzu (1928) recognized five tribes in two subfamilies, placing all genera with unwinged fruits in his wholly New World subfamily Planitorae, with two tribes separated on

the dehiscence or nondehiscence of the fruits. His other subfamily, Pyramidotora, comprised three tribes, one with bristly fruits (New World), one with lateral-winged samaras (with two subtribes, one New World, the other Old World), and one with dorsally winged samaras (also with two subtribes, one New World, the other Old World). Subsequent classifications of Malpighiaceae have deviated little from Niedenzu's treatment. For example, Hutchinson (1967) recognized five tribes, three identical to Niedenzu's, one equivalent to Niedenzu's Planitorae (Malpighieae), and a fifth (Gaudichaudieae) resurrected from de Jussieu's 1843 monograph. Takhtajan's (1997) classification was nearly identical to the one proposed by Hutchinson aside from changes in taxonomic rank.

In 1978 W. Anderson dismembered Niedenzu's subfamily Planitorae, arguing that Niedenzu and other authors used too few characters and, thus, produced artificial groupings. Anderson recognized a wingless New World subfamily, Byrsonimoideae, but excluded several genera with unwinged fruits that he felt were more closely related to wing-fruited genera. He did not place the excluded genera in Niedenzu's tribes, nor did he publish any formal reclassification for the rest of the family. The study presented here is an attempt to apply molecular data to the problem of the phylogeny of the Malpighiaceae and compare the results of that phylogenetic analysis to available data on the morphology and biogeography of the family. Our study parallels and complements an investigation of Malpighiaceae phylogeny using *matK* and *rbcL* sequences obtained for a similar set of accessions (Cameron et al., 2001).

The phylogenetic analyses of Malpighiaceae reported here were based on nucleotide sequences of the chloroplast gene *ndhF* and the noncoding *trnL-F* region. *ndhF* encodes a subunit of the nicotinamide dehydrogenase complex and shows

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⁵ Author for correspondence (e-mail: cdavis@oeb.harvard.edu).

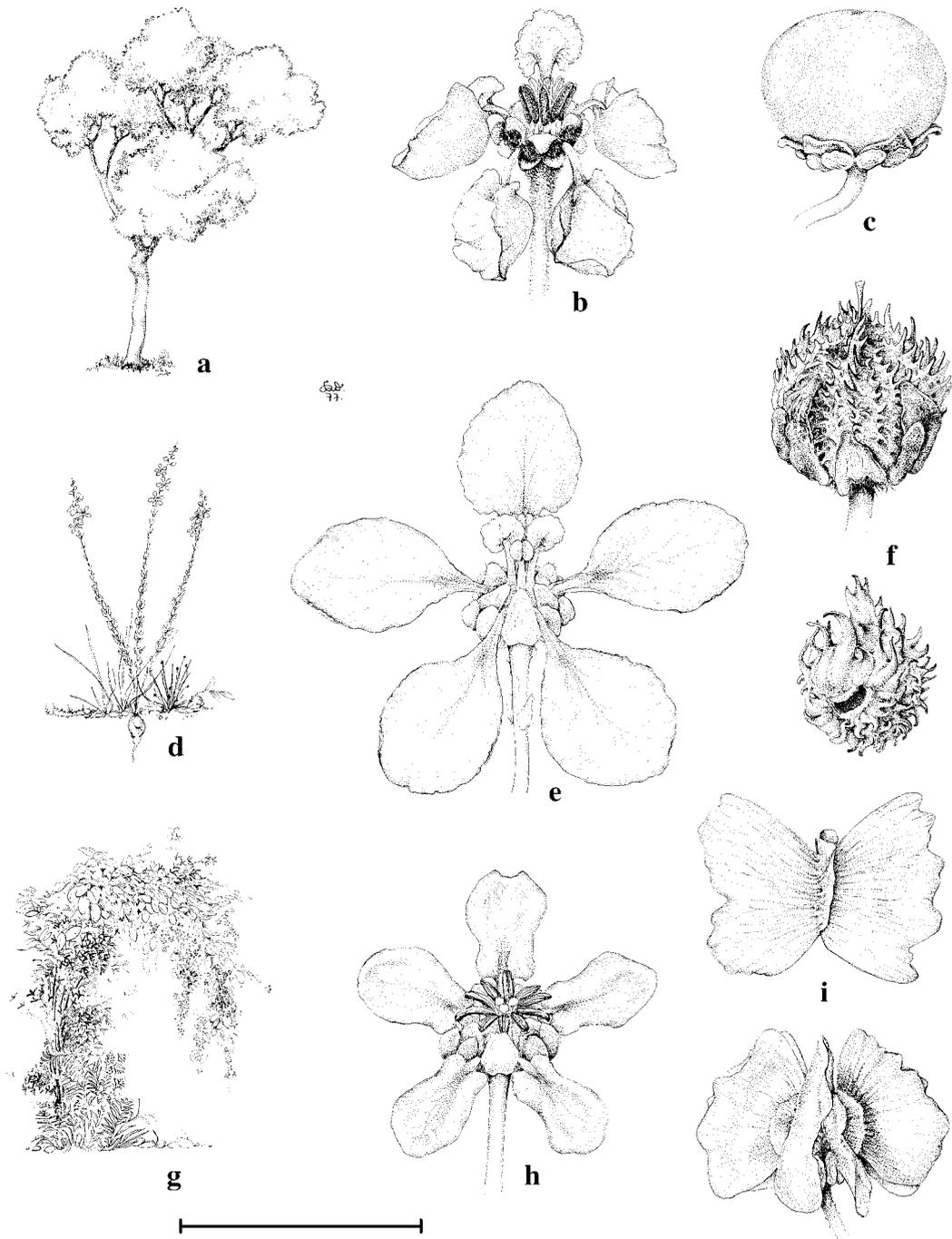


Fig. 1. Comparison of three genera of Malpighiaceae. (a–c) *Byrsonima*: (a) tree 12 m tall; (b) flower; (c) drupaceous fruit. (d–f) *Camarea*: (d) perennial herb 40 cm tall; (e) flower; (f) dry fruit breaking apart into three aculeate nutlets. (g–i) *Mascagnia*: (g) vine in shrub 2 m high; (h) flower; (i) dry fruit breaking apart into three samaras. Length of scale: 10 mm in (b), (c), (e), and (h); 5 mm in (f); 17 mm in (i). From Anderson (1979a).

approximately twice the average mutation rate of *rbcl* (Sugiura, 1989; Olmstead and Sweere, 1994). It has been used successfully to infer phylogenetic relationships within and among angiosperm families (Olmstead et al., 1992; Olmstead and Sweere, 1994; Clark, Zhang, and Wendel, 1995; Kim and Jansen, 1995; Neyland and Urbatsch, 1995, 1996; Olmstead and Reeves, 1995; Scotland et al., 1995; Wagstaff et al., 1998; Alverson et al., 1999; Ferguson, 1999). The noncoding *trnL-F* region displays a high frequency of mutations, but has also

been used in assessing phylogenetic relationships within traditional angiosperm families (Palmer et al., 1988; Clegg, Learn, and Golenberg, 1991; Soltis and Soltis, 1998).

MATERIALS AND METHODS

Taxon sampling—We generated 77 new *ndhF* sequences from the more variable 3' end of the gene (see Catalán, Kellogg, and Olmstead, 1997; Ferguson, 1999) and 65 new *trnL-F* sequences for 79 accessions of Malpighi-

aceae (Table 1) representing 58 of the 65 genera currently recognized by W. Anderson. Broader phylogenetic analyses based on several genes (Wurdack and Chase, 1996; K. Wurdack, University of North Carolina, personal communication) indicate that the New World genus *Humiria* (Humiriaceae) is relatively closely related to Malpighiaceae; *Humiria* was, therefore, included in our analyses for rooting purposes.

Molecular methods—Genomic DNAs for 74 species of Malpighiaceae were provided by Mark W. Chase (Royal Botanic Garden, Kew); additional extractions were made at Harvard. Most specimens were collected in the field, preserved in silica gel, and vouchered as herbarium specimens. Otherwise, DNA was extracted from previously collected herbarium material. Genomic DNA was isolated from dried leaves using the procedure outlined by Palmer et al. (1988), with a hot cetyltrimethylammonium bromide (CTAB) extraction method (Doyle and Doyle, 1987) used most frequently. All extractions were purified on ethidium bromide/CsCl gradients. The *ndhF* primers were designed from Malvales by R. Nyffeler (Harvard University, unpublished data, personal communication), and *trnL-F* intergenic spacer (IGS) primers were designed from tobacco, rice, and *Marchantia* by Taberlet et al. (1991). 800 base pairs (bp) of the 3' end of *ndhF* was amplified using primers 5.5F and 10.2R, which reside between primers 5 and 6 and 10 and 11 (Olmstead and Sweere, 1994; Table 2), respectively. The *trnL-F* region was amplified using primers trnC and trnF (Table 2). The *trnL-F* region we amplified corresponds to two of the three noncoding spacer regions examined by Taberlet et al. (1991): the *trnL* intron and an intergenic spacer between the *trnL* 3' exon and *trnF*.

Double-stranded polymerase chain reaction (PCR) products were cleaned using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and PCR products were sequenced in both directions using dye-terminators and cycle sequencing protocols (Perkin Elmer, Wellesley, Massachusetts, USA). Sequencing for each region used the PCR primers noted above in addition to two internal primers for *trnL-F*, trnD and trnE (Taberlet et al., 1991; Table 2). Sequences were obtained using a Model 377 automated sequencer (Perkin Elmer) and chromatograms were assembled into contiguous sequences and checked for accuracy using the software program Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Both strands were sequenced with a minimum overlap of 80% of the total sequence length. All newly generated sequences were submitted to GenBank (Table 1).

Phylogenetic analyses—Nucleotide and amino acid sequences were aligned by eye, and primer sites were trimmed from the data sets. Individual and combined parsimony analyses were conducted using PAUP* 4.0 (Swofford, 1999). An initial heuristic search of 100 random taxon addition replicates was conducted with tree-bisection-reconnection (TBR) branch swapping and MULPARS in effect, but retaining only ten trees after each replicate. The resulting consensus tree was then used as a backbone constraint to search for trees not consistent with the initial trees. This method was employed due to the excessive number of trees generated for unconstrained heuristic searches. This search strategy should detect that there are no shorter trees and that the strict consensus tree reflects all of the most parsimonious trees (Catalán, Kellogg, and Olmstead, 1997). Searches using the combined *trnL-F* and *ndhF* data were conducted the same as above using both a reduced taxon data set, which included only those taxa sampled for both gene sequences, and an expanded taxon data set, including all taxa sampled for either gene. As an additional assessment of our search strategy we implemented the parsimony ratchet (Nixon, 1999) using PAUPRat (Sikes and Lewis, University of Connecticut, unpublished computer program). For each of the four data sets we implemented five searches using 200 iterations for each search and reweighting was conducted for 15% of the available characters. Bootstrap support (Felsenstein, 1985) was estimated based on 1000 replicates using simple taxon addition, TBR, MULPARS, and holding ten trees at each replicate.

The "Templeton test" as implemented in PAUP* (Swofford, 1999) was used to assess support for clades appearing in the most parsimonious trees (Templeton, 1983; see also Larson, 1994). The shortest trees in which the monophyly of a clade of interest was not supported were used as a constraint and compared to the shortest trees in which the clade of interest was present.

For each constraint search we used only the reduced taxon sampling. Monophyly constraints of interest were a monophyletic Byrsonimoideae, an Old World hiraeoid clade, an Old World banisterioid clade, and three independent constraints for the monophyly of three traditionally recognized genera: *Aspicarpa*, *Janusia*, and *Mascagnia*. In order to assess congruence between the two independent data sets we conducted the incongruence length difference (ILD) test (Farris et al., 1994), implemented as the partition homogeneity test in PAUP* 4.0 (see Mason-Gamer and Kellogg, 1996). We used the heuristic search strategy presented above with 999 random repetitions to generate the distribution. As a conservative estimate of significance we present only the highest *P* values for each assessment of congruence.

We have overlaid Hutchinson's 1967 tribal classification of Malpighiaceae onto the consensus tree based on the combined expanded analysis using parsimony. Fruit type for each species was scored and treated as an unordered multistate character (Table 1) and mapped onto one of the most parsimonious trees from the combined expanded data matrix using MacClade version 3.0 (Maddison and Maddison, 1992). The tree was selected at random among those trees that were most parsimonious with respect to the character of interest. Similarly, we recorded the geographic distribution of each taxon as New World or Old World (Table 1) and mapped that information onto one of the most parsimonious trees from the combined expanded data matrix.

RESULTS

Sequences/matrices—The *ndhF* region we sequenced had an aligned length of 768 bp. The longest pairwise distance within the ingroup was between *Gaudichaudia albida* and *Galphimia gracilis* (70 steps; 9.11%). The largest overall distance was 101 steps (13.15%) between *Dinemandra ericoides* and *Humiria balsamifera* (the outgroup). Of the 315 variable positions within the ingroup, 172 were parsimony informative.

Individual *trnL-F* sequences within Malpighiaceae ranged in length from 1028 bp (*Blepharandra heteropetala*) to 709 bp (*Ptilochaeta bahiensis*), and the outgroup sequence (*Humiria*) was 943 bp. Length variation was mainly attributable to several A-T rich regions that could not be aligned readily due to repeated motifs and numerous indels. These hyper-variable regions, totaling 180 bp, were excluded from the analyses. Over the entire alignment we identified 113 indels located in the *trnL-F* region that were scored and added to the matrix as unordered gap characters. This coding was done using a program developed and distributed by R. Ree (Harvard University, personal communication). After exclusion of the hypervariable regions and inclusion of the gap characters, the aligned matrix was 1219 bp in length. The longest ingroup distance was between *Caucanthus auriculatus* and *Diacidia ferruginea* (88 steps; 7.22%). The longest overall distance was 150 steps (12.3%) between *Caucanthus auriculatus* and *Humiria balsamifera* (the outgroup). Of the 481 variable nucleotide positions within the ingroup, 198 were parsimony informative.

The combined data matrix was 1987 bp in length. The reduced data set contained 63 ingroup species from which complete sequences were obtained from both gene regions (see Table 1). Of the 777 variable nucleotide positions within the ingroup, 345 were parsimony informative. The expanded data set contained 79 ingroup species for which each species was sequenced for at least *ndhF* or *trnL-F* (Table 1). Of the 796 variable nucleotide positions within the ingroup, 370 were parsimony informative.

Phylogenetic analyses—Parsimony searches on the *ndhF* data resulted in 990 trees of 652 steps (Fig. 2). Searches using the *trnL-F* data resulted in 840 trees of 750 steps (Fig. 3).

Searches on the combined reduced taxon data set including *trnL-F* gap coding resulted in 760 trees of 1328 steps (Fig. 4). Searches on the combined expanded taxon data set resulted in 1000 trees of 1408 steps (Fig. 5). In all cases, use of the consensus backbone constraint trees failed to find any trees of equal or less length that contradicted the respective consensus trees. Similarly, trees generated from PAUPRat were identical to those presented here.

Trees generated from the individual data sets are highly congruent. Bootstrap support for many clades was quite high in the separate analyses and was, in general, increased in the combined analyses. The results of the IILD test ($P = 0.810$) suggest that patterns of character state variation between *ndhF* and *trnL-F* do not differ significantly. Given the similar topologies obtained in all of our analyses, we will discuss in detail only the topologies generated from the combined expanded data matrix (Fig. 5), noting any well-supported conflicts between data sets. The bootstrap values for these trees were generally less than those from the reduced taxon data set due to the inclusion of taxa with missing data.

Malpighiaceae are split into two major clades (Fig. 5), which we will refer to by the subfamilial names, Byrsonimoideae and Malpighioideae (100 and <50% bootstrap values, respectively). The Byrsonimoideae include the byrsonimoid (99%) and acmantheroid (100%) clades. The Malpighioideae comprise the bulk of diversity within Malpighiaceae and include the galphimioid (100%), acridocarpoid (100%), mcvaughoid (100%), *Barnebya*, and the banisterioid (100%) clades. The galphimioids are weakly placed (<50%) as sister to the rest of the Malpighioideae in the combined analyses. In *trnL-F* trees, the galphimioids form a weakly supported clade with Byrsonimoideae, whereas their position is unresolved by *ndhF*. The remainder of the Malpighioideae form a strongly supported clade (100%) with the acridocarps basal in this clade. The *mcvaughoids* are placed with moderate support (70%) as sister to the remaining Malpighioideae. The enigmatic South American genus *Barnebya* is weakly placed (58%) as sister to the banisterioids. This last result is also found with *ndhF* (53%), but is contradicted in the *trnL-F* trees, which find *Barnebya* sister (71%) to the *mcvaughoids*, that clade forming a clade with the acridocarps (<50%). Sister to the remainder of the banisterioids is a well-supported (85%) ptilochaetoid clade with two subclades, one of the Mexican genus *Lasiocarpus* and the southern South American genus *Ptilochaeta* (98%), the other containing the Chilean endemics *Dinemagonum* and *Dinemandra* (100%). The tristellateioids (<50%) are placed as sister to a large clade (83%; not supported by *ndhF*) containing most of the wing-fruited genera. The tristellateioid clade is poorly supported (i.e., <50%) in the *ndhF* trees and is not detected in the *trnL-F* trees. Included in the tristellateioids is a clade containing *Tristellateia* and *Heladena* (100%), which is sister to a poorly supported (<50%) clade containing *Bunchosia*, *Thryallis*, and *Echinopterys*. The latter clade is not present in the *trnL-F* trees and is poorly (<50%) supported in the *ndhF* trees. The rest of the banisterioid clade contains six major lineages: malpighioids, stigmaphylloids, tetrapteroids, hiraecoids, and the genera *Ectopopterys* and *Lophopterys*. The tetrapteroids are weakly supported in all analyses and are not detected in the *trnL-F* trees. In contrast, the stigmaphylloids form a strongly (100%) supported clade that contains the gaudichaudioid (99%) and cordoboid (100%) clades. The hiraecoid clade is not recovered in the *trnL-F* trees, but is moderately to poorly supported (<50%

ndhF, 75% reduced, and 55% expanded) in the *ndhF* and combined analyses. *Lophopterys* is very weakly (<50%) placed as sister to the malpighioid clade, and *Ectopopterys* is placed but with little support in a tetrachotomy with the hiraecoids, the tetrapteroids, and the clade containing the malpighioids plus the stigmaphylloids.

DISCUSSION

Byrsonimoideae and Galphimioids—Our data show that Hutchinson's tribe Malpighieae (1967), which is identical to Niedenzu's subfamily Planitorae, is nonmonophyletic (Fig. 6). When Anderson (1978) described the subfamily Byrsonimoideae, he subjected the unwinged fruit types of the Planitorae/Malpighieae to critical evaluation and broadened the taxonomic base to include characters other than those of the fruit. He justified the exclusion of several taxa from the Planitorae on the grounds that some smooth-walled fruit types represented examples of convergence and were likely derived from wing-fruited ancestors. For example, although the fruits of *Byrsonima*, *Bunchosia*, and *Malpighia* are all indehiscent and have a fleshy exocarp, they are only superficially similar. Fruits of *Byrsonima* contain a single bony stone, which results from fusion of the three endocarps. In *Bunchosia* each of the two or three carpels contributes a smooth, cartilaginous, seed-containing endocarp, but these are free from each other. *Malpighia* fruits possess fibrous stones that are connate only along a central axis, and the individual stones bear ridges or crests in the position of the wings seen in wing-fruited Malpighiaceae. Aside from *Bunchosia* and *Malpighia*, Anderson (1978) also argued that *Dicella*, *Heladena*, and *Thryallis* should be excluded from Byrsonimoideae. In our analyses all five of these genera are clearly removed from the Byrsonimoideae. Anderson's tribes Acmanthereae (*Acmanthera*, *Coleostachys*, and *Pterandra*) and Galphimieae (*Galphimia*, *Lophanthera*, and *Spachea*) are monophyletic in our results. However, his tribe Byrsonimeae (including *Blepharandra*, *Byrsonima*, *Diacidia*, *Burdachia*, *Glandonia*, and the subsequently described *Mcvaughia*) is not monophyletic and the genera are divided between the byrsonimoid and mcvaughoid clades. The monophyly of Anderson's Byrsonimoideae is strongly rejected (Templeton test $P = 0.0012$). One perplexing outcome from our study is the phylogenetic placement of the galphimioids. Our *trnL-F* data detected a Byrsonimoideae that included the galphimioids (Fig. 3), and these results were duplicated with better support using *matK* and *rbcL* (Cameron et al., 2001), but our combined analyses placed the galphimioids (without strong support) as sister to the rest of the Malpighioideae. Given the weak support for this relationship, and the evidence from chromosome numbers (see below), we expect that in future analyses the galphimioids will group with the Byrsonimoideae.

One of the character states used to circumscribe the Byrsonimoideae was a chromosome number of $x = 6$, as opposed to $x = 10$ in the rest of the family (Anderson, 1978). When he summarized those and additional chromosome numbers (Anderson, 1993), all of the counts for the genera retained in the Byrsonimoideae as recognized here, and for the galphimioids, were still $n = 6, 12, \text{ or } 24$. Therefore, $x = 6$ does appear to be a consistent character of the subfamily in our restricted sense, but given the structure of the tree (Fig. 5), we cannot say whether that is an ancestral or a derived condition. *Mcvaughia*, described after 1978 but assigned to Byrsonim-

TABLE 1. Taxa sequenced.

Taxon	Fruit type ^a	Distribution ^b	Voucher	Chloroplast region ^c	
				<i>ndhF</i>	<i>rnlL-F</i>
Humiriaceae					
<i>Humiria balsamifera</i> Aubl.	UIF	NW	Anderson 13654 (MICH)	GBAN-AF351007	GBAN-AF350941
Malpighiaceae					
<i>Acmanthera latifolia</i> (Adr. Juss.) Griseb. in Mart.	UDeDr	NW	Anderson 13771 (MICH)	GBAN-AF351013	GBAN-AF350947
<i>Acridocarpus macrocalyx</i> Engl.	WDeDr	OW	C. C. Davis 99-9 (A)	GBAN-AF351017	GBAN-AF350951
<i>Acridocarpus natalitius</i> Adr. Juss.	WDeDr	OW	P. Goldblatt s.n. (PRE)	GBAN-AF351016	GBAN-AF350950
<i>Aspicarpa brevipes</i> (DC.) W. R. Anderson	WDeDr	NW	Cházaro 6295 (MICH)	GBAN-AF351082	GBAN-AF350967
<i>Aspicarpa hirtella</i> Rich.	WDeDr	NW	Anderson 13525 (MICH)	GBAN-AF351033	GBAN-AF350960
<i>Aspicarpa pulchella</i> (Griseb. in Mart.) O'Donell & Lourteig	WDeDr	NW	Anderson 13614 (MICH)	GBAN-AF351026	GBAN-AF350953
<i>Aspidopterys elliptica</i> (Blume) Adr. Juss.	WDeDr	OW	XVI.G.110 (BO)	GBAN-AF351019	
<i>Banisteriopsis hypericifolia</i> (Adr. Juss. in A. St.-Hil.) W. R. Anderson & B. Gates	WDeDr	NW	Anderson 13638 (MICH)	GBAN-AF351037	GBAN-AF350971
<i>Barnebya dispar</i> (Griseb. in Mart.) W. R. Anderson & B. Gates	WDeDr	NW	Leoni 1473 (MICH)	GBAN-AF351073	GBAN-AF351006
<i>Blepharandra heteropetala</i> W. R. Anderson	UIDr	NW	Anderson 13782 (MICH)	GBAN-AF351008	GBAN-AF350942
<i>Brachylophon curtisii</i> Oliv.	WDeDr	OW	Middleton et al. 387 (A)	GBAN-AF351018	GBAN-AF350952
<i>Burchardia armeniaca</i> (Cav.) DC.	UIF	NW	FTG 77-999C (FTG)	GBAN-AF351045	GBAN-AF350978
<i>Burchardia sphaerocarpa</i> Adr. Juss.	UIDr	NW	Anderson 13774 (MICH)	GBAN-AF351071	GBAN-AF351004
<i>Byrsonima crassifolia</i> (L.) H.B.K.	UIF	NW	FTG 81-680A (MICH)	GBAN-AF351011	GBAN-AF350945
<i>Callaeum septentrionale</i> (Adr. Juss.) D. M. Johnson	WDeDr	NW	Anderson 13491 (MICH)	GBAN-AF351058	GBAN-AF350992
<i>Caucanthus auriculatus</i> (Radlk.) Nied.	WIDr	OW	E. Knox 2128 (MICH)	GBAN-AF351020	GBAN-AF350954
<i>Coleostachys genipifolia</i> Adr. Juss.	UDeDr	NW	Anderson 13752 (MICH)	GBAN-AF351012	GBAN-AF350946
<i>Cordobia argentea</i> (Griseb.) Nied.	WDeDr	NW	Anderson 13583 (MICH)	GBAN-AF351081	
<i>Diacidia ferruginea</i> (Maguire & K. D. Phelps) W. R. Anderson	UIDr	NW	Berry et al. 4987 (MICH)	GBAN-AF351010	GBAN-AF350944
<i>Dicella nucifera</i> Chodat	UIDr	NW	Anderson 13607 (MICH)	GBAN-AF351048	GBAN-AF350981
<i>Dinemagonum gayanum</i> Adr. Juss.	WDeDr	NW	Simpson 83-10-8-5 (MICH)	GBAN-AF351084	
<i>Dinemandra ericoides</i> Adr. Juss.	WDeDr	NW	Dillon & Teillier 5103 (MICH)	GBAN-AF351069	GBAN-AF351002
<i>Diplopterys cabrerana</i> (Cuatrec.) B. Gates	WDeDr	NW	Burnham 1774 (MICH)	GBAN-AF351039	GBAN-AF350973
<i>Echinopterys eglandulosa</i> (Adr. Juss.) Small	BDeDr	NW	Van Devender 98-178 (MICH)	GBAN-AF351047	GBAN-AF350980
<i>Ectopterys soejartoi</i> W. R. Anderson	WDeDr	NW	Callejas 11806 (MICH)	GBAN-AF351064	GBAN-AF350997
<i>Excentradenia propinqua</i> (W. R. Anderson) W. R. Anderson	WDeDr	NW	Lindeman et al. 51 (MICH)	GBAN-AF351062	GBAN-AF350995
<i>Flabellaria paniculata</i> Cav.	WDeDr	OW	Congdon 414 (K)	GBAN-AF351083	
<i>Gallardia fischeri</i> Hicken	WDeDr	OW	Luke & Robertson 2683 (K)	GBAN-AF351035	GBAN-AF350991
<i>Galphimia gracilis</i> Bartl.	UDeDr	NW	Anderson 13580 (MICH)	GBAN-AF351015	GBAN-AF350969
<i>Gaudichaudia albida</i> Schlechtend. & Cham.	WDeDr	NW	FTG 79-235 (FTG)	GBAN-AF351034	GBAN-AF350949
<i>Gaudichaudia mcvaughii</i> W. R. Anderson	WDeDr	NW	Anderson 13309A (MICH)	GBAN-AF351032	GBAN-AF350968
<i>Glandonia macrocarpa</i> Griseb. in Mart.	UIDr	NW	Anderson 12699C (MICH)	GBAN-AF351072	GBAN-AF351005
<i>Heladena multiflora</i> (Hook. & Arn.) Nied.	WDeDr	NW	Anderson 13776 (MICH)	GBAN-AF351044	GBAN-AF350977
<i>Heteropterys ciliata</i> Nied.	WDeDr	NW	Anderson 13778 (MICH)	GBAN-AF351049	GBAN-AF350982
<i>Heteropterys leona</i> (Cav.) Exell	WDeDr	NW, OW	Anderson 13599 (MICH)	GBAN-AF351050	GBAN-AF350983
<i>Hiptage benghalensis</i> (L.) Kurz	WDeDr	OW	Herbst 9221 (MICH)	GBAN-AF351057	GBAN-AF350990
<i>Hiraea fagifolia</i> (DC.) Adr. Juss.	WDeDr	NW	Anderson 13593 (MICH)	GBAN-AF351060	GBAN-AF350994
<i>Hiraea smilacina</i> Standl.	WDeDr	NW	Anderson 13794 (MICH)	GBAN-AF351061	GBAN-AF350962
<i>Janusia anisandra</i> (Adr. Juss.) Griseb. in Mart.	WDeDr	NW	Anderson 13694 (MICH)	GBAN-AF351028	GBAN-AF350965
<i>Janusia californica</i> Benth.	WDeDr	NW	Anderson 12539 (MICH)	GBAN-AF351031	GBAN-AF350964
<i>Janusia linearis</i> Wiggins	WDeDr	NW	Anderson 12540 (MICH)	GBAN-AF351030	GBAN-AF350963
<i>Janusia mediterranea</i> (Vell.) W. R. Anderson	WDeDr	NW	Anderson 13686 (MICH)	GBAN-AF351029	
<i>Jubelina rosea</i> (Miq.) Nied. in Pulle	WDeDr	NW	Mori 23855 (MICH)	GBAN-AF351079	
<i>Lasiocarpus</i> sp.	BDeDr	NW	Anderson 13834 (MICH)	GBAN-AF351066	GBAN-AF350999

TABLE 1. Continued.

Taxon	Fruit type ^a	Distribution ^b	Voucher	<i>rnhF</i>	<i>trnL-F</i>	Chloroplast region ^c
<i>Lophanthera lactescens</i> Ducke	UDeDr	NW	Anderson 13781 (MICH)	GBAN-AF351009	GBAN-AF350943	
<i>Lophopterys floribunda</i> W. R. Anderson & C. C. Davis	WDeDr	NW	Anderson 13754 (MICH)	GBAN-AF351078	GBAN-AF350957	
<i>Malpighia emarginata</i> DC.	UIF	NW	Anderson 13621 (MICH)	GBAN-AF351023	GBAN-AF350956	
<i>Malpighia stenvisii</i> W. R. Anderson	UIF	NW	C. C. Davis 1019 (MICH)	GBAN-AF351022		
<i>Mascagnia anisopetalata</i> (Adr. Juss. in A. St.-Hil.) Griseb. in Mart.	WDeDr	NW	Anderson 13598 (MICH)	GBAN-AF351053	GBAN-AF350986	
<i>Mascagnia bracteosa</i> Griseb. in Mart.	WDeDr	NW	Anderson 13777 (MICH)	GBAN-AF351055	GBAN-AF350988	
<i>Mascagnia chaseti</i> W. R. Anderson	WDeDr	NW	Anderson 13710 (MICH)	GBAN-AF351054	GBAN-AF350987	
<i>Mascagnia dipholophylla</i> (Small) Bullock	WDeDr	NW	Anderson 13816 (MICH)	GBAN-AF351063	GBAN-AF350996	
<i>Mascagnia hippocrateoides</i> (Triana & Planch.) Nied.	WDeDr	NW	Anderson 13802 (MICH)	GBAN-AF351059	GBAN-AF350993	
<i>Mascagnia sepium</i> (Adr. Juss. in A. St.-Hil.) Griseb. in Mart.	WDeDr	NW	Chase 90160 (MICH)	GBAN-AF351025	GBAN-AF350959	
<i>Mascagnia stannea</i> (Griseb.) Nied.	WDeDr	NW	Anderson 13804 (MICH)	GBAN-AF351056	GBAN-AF350989	
<i>Mcvanahia bahiana</i> W. R. Anderson	UIDr	NW	Anderson 13690 (MICH)	GBAN-AF351070	GBAN-AF351003	
<i>Mezium araujoi</i> Schwacke ex Nied. in Engl. & Prantl	WDeDr	NW	Anderson 13672 (MICH)	GBAN-AF351051	GBAN-AF350984	
<i>Mionandra camaroides</i> Griseb.	WDeDr	NW	Anderson 13585 (MICH)	GBAN-AF351080		
<i>Peixotoa glabra</i> Adr. Juss. in A. St.-Hil.	WDeDr	NW	Anderson 13636 (MICH)	GBAN-AF351036	GBAN-AF350970	
<i>Peregrina linearifolia</i> (A. St.-Hil.) W. R. Anderson	WDeDr	NW	Krapovickas & Cristóbal 46451 (MICH)	GBAN-AF351027	GBAN-AF350961	
<i>Pterandra arborea</i> Ducke	BDeDr	NW	Anderson 13766 (MICH)	GBAN-AF351014	GBAN-AF350948	
<i>Ptilochaeta bahiensis</i> Turcz.	BDeDr	NW	Anderson 13725 (MICH)	GBAN-AF351068	GBAN-AF351001	
<i>Ptilochaeta nudipes</i> Griseb.	WIDr	OW	Phillipson 3451 (MICH)	GBAN-AF351067	GBAN-AF351000	
<i>Rhynchochloa philippsonii</i> W. R. Anderson	WDeDr	OW	XVIII.F.172 (BO)	GBAN-AF351040	GBAN-AF350958	
<i>Rysopterys timoriensis</i> (DC.) Adr. Juss. in Deless.	WDeDr	OW	Hammel 17746 (MO)	GBAN-AF351074	GBAN-AF350974	
<i>Spaethea correae</i> Cuatrec. & Croat	WDeDr	NW	Steiner 2275 (MICH)	GBAN-AF351041	GBAN-AF350975	
<i>Sphedammocarpus pruriens</i> (Adr. Juss.) Szyszyl.	WDeDr	OW	Anderson 13693 (MICH)	GBAN-AF351065	GBAN-AF350998	
<i>Stigmaphyllon paralias</i> Adr. Juss. in A. St.-Hil.	WDeDr	NW	Anderson 13793 (MICH)	GBAN-AF351038	GBAN-AF350972	
<i>Stigmaphyllon puberum</i> (Rich.) Adr. Juss.	WDeDr	NW	FTG X-1-6 (FTG)	GBAN-AF351077		
<i>Stigmaphyllon sagraeanum</i> Adr. Juss.	WDeDr	NW	Chase 90158 (MICH)	GBAN-AF351076		
<i>Tetrapterys discolor</i> (G. Mey.) DC.	WDeDr	NW	Anderson 13644 (MICH)	GBAN-AF351052	GBAN-AF350985	
<i>Tetrapterys microphylla</i> (Adr. Juss. in A. St.-Hil.) Nied.	WDeDr	NW	Anderson 13657 (MICH)	GBAN-AF351046	GBAN-AF350979	
<i>Thryallis longifolia</i> Mart.	WDeDr	NW	Immelman 650 (PRE)	GBAN-AF351021	GBAN-AF350955	
<i>Triaspis hypericoides</i> Burch.	BIDr	NW	Anderson 13581 (MICH)	GBAN-AF351075		
<i>Tricomaria usilloi</i> Hook. & Arn.	WDeDr	NW	Hammel 17816 (MICH)	GBAN-AF351024		
<i>Triopteris rigida</i> Sw.	WDeDr	NW	C. C. Davis 99-25 (A)	GBAN-AF351043		
<i>Tristellateia africana</i> S. Moore	WDeDr	OW	McPherson 14398 (MICH)	GBAN-AF351042	GBAN-AF350976	
<i>Tristellateia madagascariensis</i> Poir. in Lam.	WDeDr	OW				

^a Fruit types: U = unwinged, W = winged, B = bristly, De = dehiscent, I = indehiscent, Dr = dry, F = fleshy; fruits with reduced wings are scored as winged.

^b Distribution: NW = New World, OW = Old World.

^c The prefix GBAN- has been added to link the online version of *American Journal of Botany* to GenBank but it is not part of the actual accession number.

TABLE 2. Primers used in polymerase chain reaction and sequencing (from 5' to 3').

Region	Primer name	Sequence
<i>ndhF</i>	5.5F	TTAGTRAYAGTYGGTTGTATTACCC
	10.2R	CCATCRATNACTCGYCYATCAAAAA
<i>trnL-F</i>	trnC	CGAAATCGGTAGACGCTACG
	trnD	GGGGATAGAGGGACTTGAAC
	trnE	GGTTCAAGTCCCTCTATCCC
	trnF	ATTTGAACTGGTGACACGAG

oideae close to *Burdachia* by Anderson (1979b), has $n = 10$, which is consistent with its placement here in the Malpighioideae. It would be especially interesting to know the chromosome numbers of *Burdachia* and *Glandonia*; we would expect them to have numbers based on $x = 10$. *Barnebya* has $n = 30$, a number that could be a multiple of either 6 or 10 (Anderson, 1993). Given its phylogenetic placement with other Malpighioideae it most likely represents a multiple of 10. The counts known for *Acridocarpus* (Mangenot and Mangenot, 1958, 1962; Carr and McPherson, 1986; Paiva and Leitão, 1987) indicate that its base number is $x = 9$, readily derived from $n = 10$ through aneuploidy. Thus, the available counts and the results of this study suggest that $x = 10$ may well be

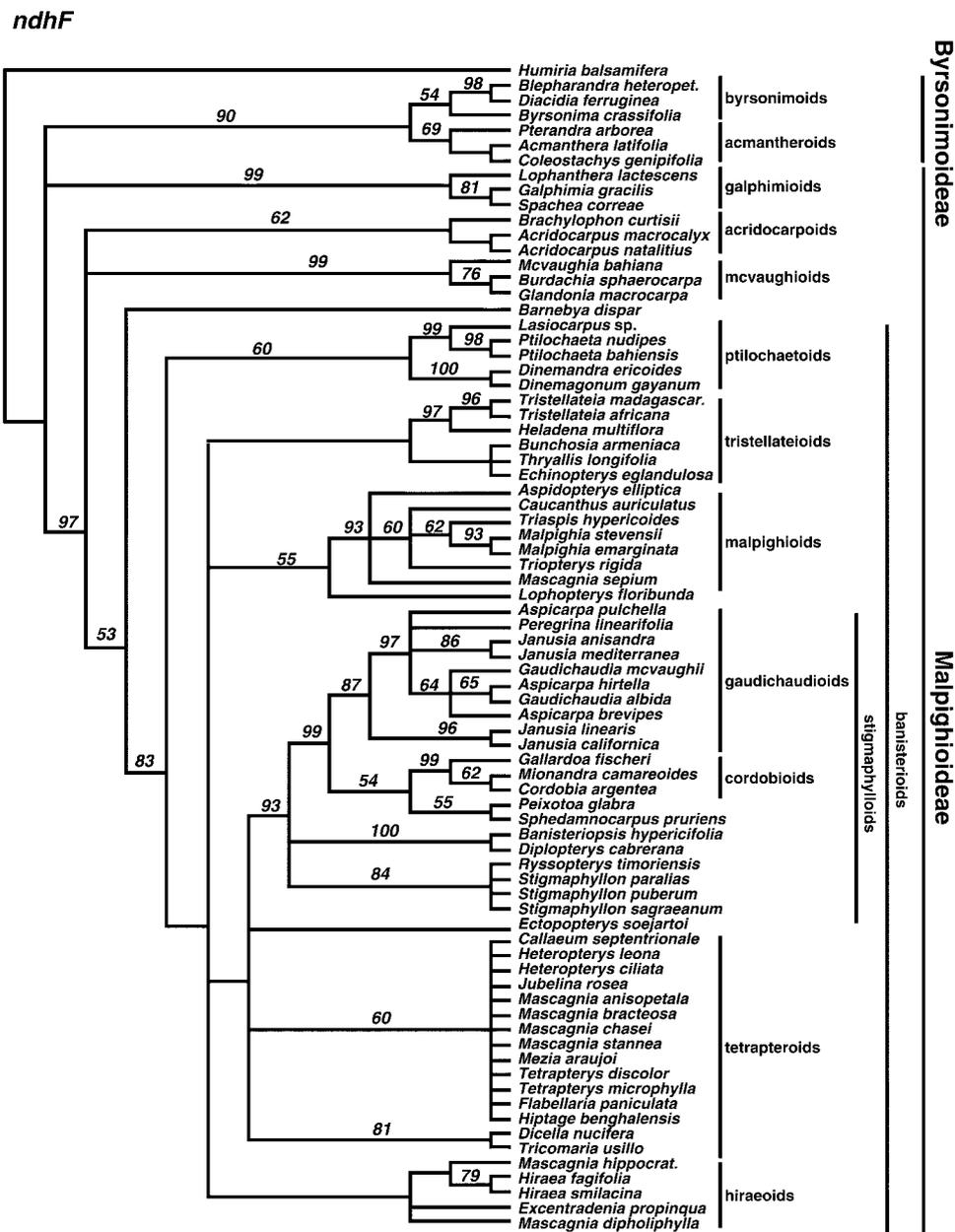


Fig. 2. Strict consensus of 990 equally parsimonious trees based on *ndhF* sequence data. Bootstrap values are given for those clades supported at >50%. Length (L) = 652; consistency index (CI) = 0.6457; retention index (RI) = 0.7674. The designated informal names correspond to moderately to well-supported clades discovered in the analysis of the expanded taxon data set (see Fig. 5).

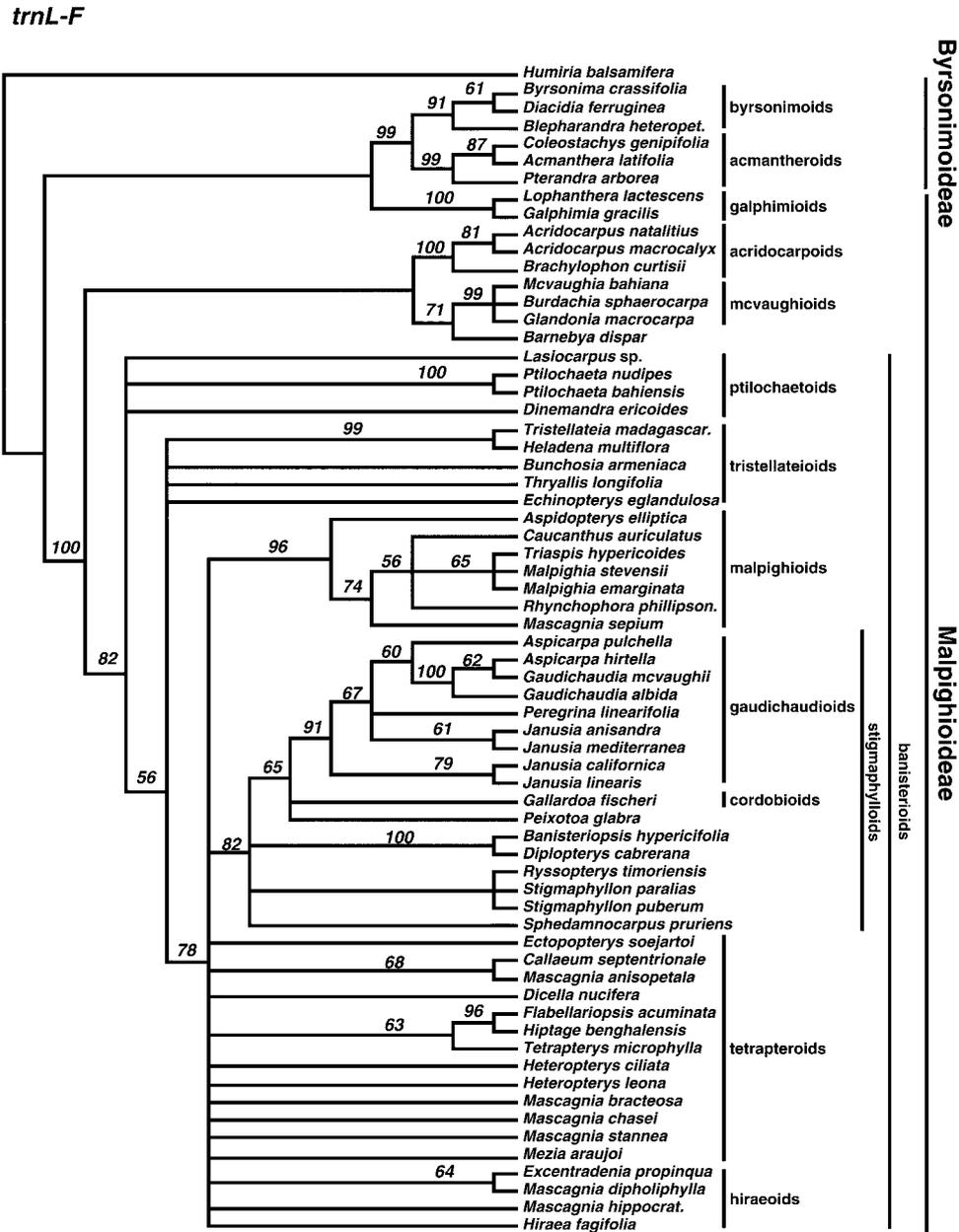


Fig. 3. Strict consensus of 840 equally parsimonious trees based on *trnL-F* sequence data. Bootstrap values are given for those clades supported at >50%. L = 750; CI = 0.7360; RI = 0.8224. The designated informal names correspond to moderately to well-supported clades discovered in the analysis of the expanded taxon data set (see Fig. 5).

ancestral in our subfamily Malpighioideae, above the galphimoids.

Malpighioideae—Acridocarpoids—The acridocarpoids (Fig. 5) comprise *Acridocarpus* and *Brachylophon*. Morphology supports the sister relationship of *Acridocarpus* and *Brachylophon*. *Acridocarpus* has a schizocarpic fruit with a large dorsal wing on each samara and no trace of lateral crests; *Brachylophon* has a similar fruit except that the dorsal wing has been reduced to a crest. In both genera the anthers are poricidal, a rare condition in the family, and both have subulate styles with tiny terminal stigmas, like those found in most Byrsonimoideae and the mcvaughioids (W. Anderson, personal

observation). The pollen in both is tricolporate, but in *Acridocarpus* it is derived in that the colpi fuse at the poles (syn-colporate) (Lobreau, 1967, 1968; Lowrie, 1982).

Mcvaughioids—The mcvaughioid clade is a well-supported (100%) lineage containing *Mcvaughia*, *Burdachia*, and *Glandonia*. These three genera have unwinged, indehiscent fruits. If the ancestor of the Malpighioideae (above the galphimoids) had dry schizocarpic fruits with wings (e.g., *Acridocarpus*), the fruits of *Mcvaughia*, *Burdachia*, and *Glandonia* must be derived in their indehiscent, unwinged fruits. There is little in the structure of most of those fruits to signal derivation from a schizocarpic, wing-fruited ancestor, but it is worth noting

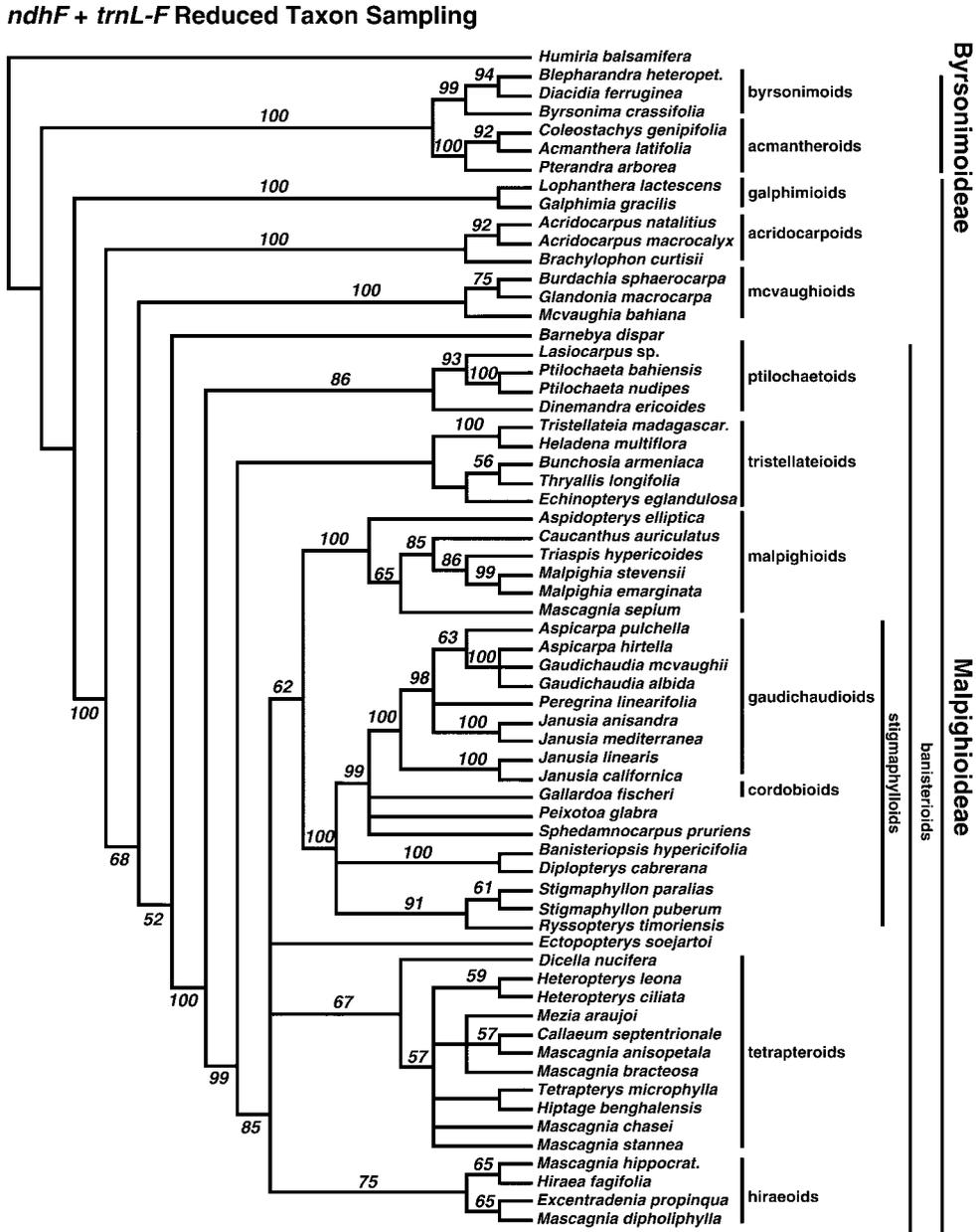


Fig. 4. Strict consensus of 760 equally parsimonious trees based on *trnL-F* and *ndhF* sequence data with the reduced taxon data set (63 ingroup species). Bootstrap values are given for those clades supported at >50%. L = 1328; CI = 0.7101; RI = 0.7950. The designated informal names correspond to moderately to well-supported clades discovered in the analysis of the expanded taxon data set (see Fig. 5).

that the fruit of *Burdachia prismatocarpa* Adr. Juss. bears 8–9 longitudinal aerenchymatous ribs or winglets in the approximate position of the lateral and dorsal wings on many fruits in the banisterioid clade (see Anderson, 1981).

The pollen grains in the Byrsonimoideae, the galphimioids, the acridocarpoids, and the mcvaughioids are mostly tricolporate or readily derived from a tricolporate ancestor (Anderson, 1978, 1979b; Lowrie, 1982). Tricolporate pollen, which is common in other rosoid families, is presumably plesiomorphic in the Malpighiaceae (Anderson, 1990a).

Barnebya—*Barnebya* is sister to the banisterioids and has a schizocarpic fruit, with each mericarp bearing a large dorsal

wing. As Anderson and Gates pointed out when they described *Barnebya* (1981), its samara is very similar to the samara of *Acridocarpus*, and primarily on that basis, they suggested that *Acridocarpus* might be close to the ancestor of *Acridocarpus*, which is relatively derived in many of its characters. Our data do not support that suggestion (Fig. 5). If this study places *Barnebya* correctly, it is clearly derived in the characters emphasized by Anderson and Gates (1981), such as the alternate estipulate leaves, the perigynous hypanthium, and the samaras attached at maturity to the receptacle by two coriaceous marginal ribs. The pollen of *Barnebya* is different from that of most basal malpighs in that it lacks ectoapertures and has other anomalies, such that Lowrie (1982) doubted its placement with

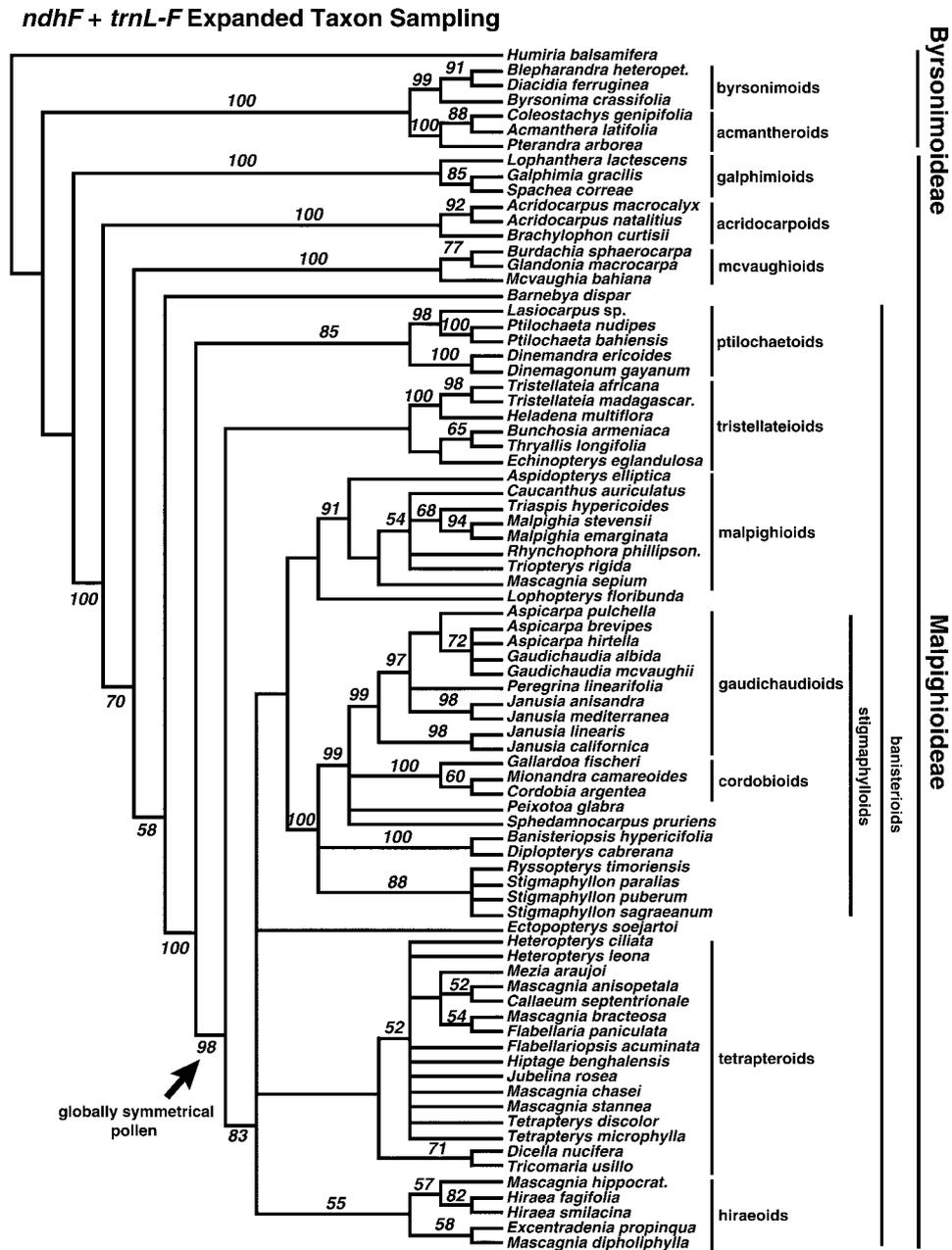


Fig. 5. Strict consensus of 1000 equally parsimonious trees based on *trnL-F* and *ndhF* sequence data with the expanded taxon data set (79 ingroup species). Bootstrap values are given for those clades supported at >50%. L = 1408; CI = 0.6911; RI = 0.7936. The designated informal names correspond to moderately to well-supported clades discovered in this analysis.

the other genera in the mcvaughiioid clade or with *Acridocarpus*.

Banisterioids—**Ptilochaetoids**—Sister to the rest of the banisterioids is the clade we have called the ptilochaetoids, comprising the three South American genera *Dinemandra*, *Dinemagonum*, and *Ptilochaeta* plus the Mexican genus *Lasiocarpus*. Those genera fall into two well-supported clades; each of those clades is internally coherent in its gross morphology, but the two clades together are quite unlike each other in most characters, including their fruits (Niedenzu, 1928; Simpson, 1989; W. Anderson, personal observation). It is especially in-

triguing that Lowrie (1982) found them to share a similar polycolporate pollen morphology, which is so peculiar in the family that he put all four of these genera together in his “*Lasiocarpus* group” and suggested that they were allied phylogenetically to the byrsonimoid groups with tricolporate pollen. The ptilochaetoid clade is a striking example of a grouping supported by both DNA sequences and pollen in spite of other morphological differences.

The rest of the banisterioids are a strongly supported clade that contains most of the genera and species of the Malpighiaceae. Pollen morphology supports the clade—these banisterioids have globally symmetrical pollen grains, with the pores

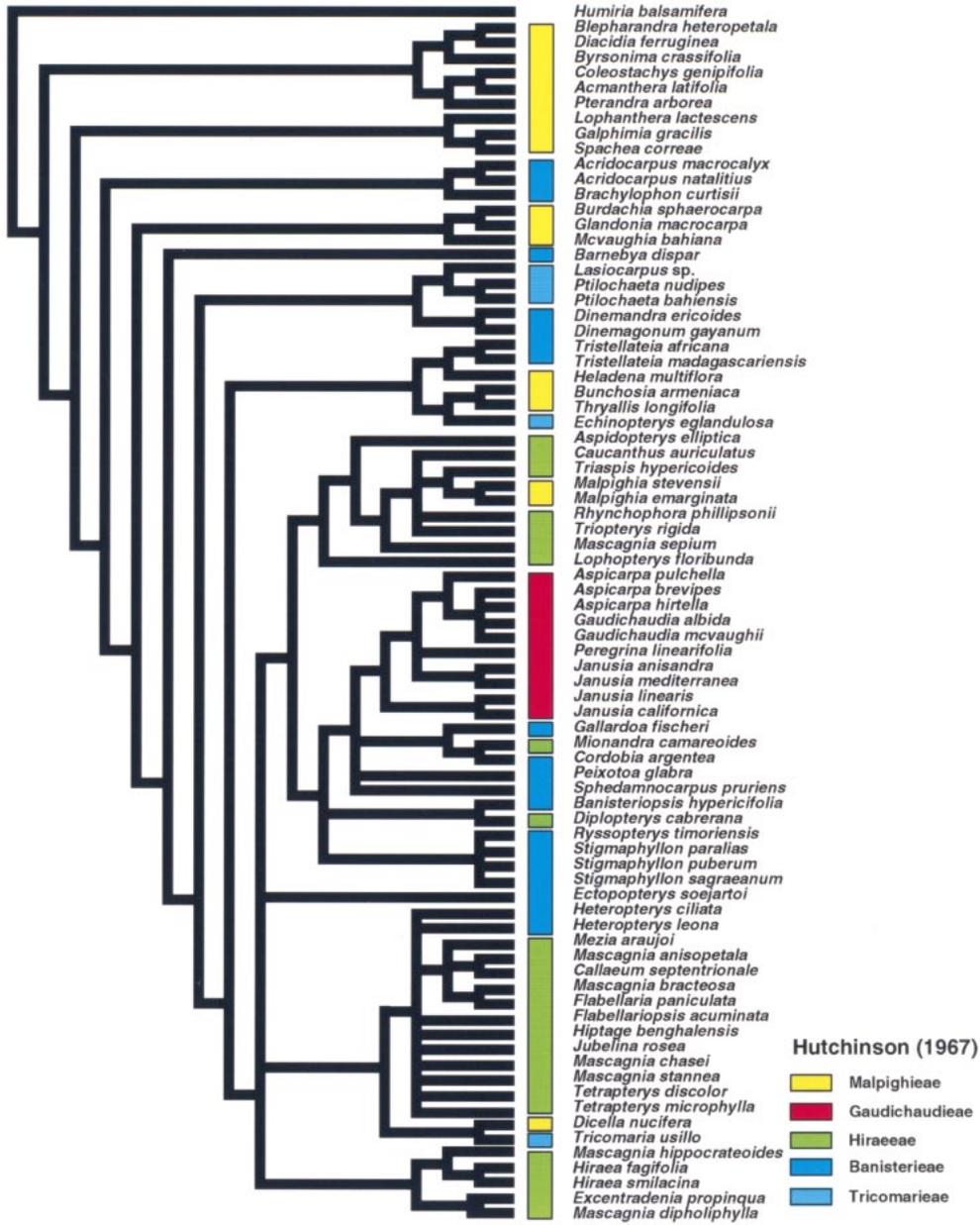


Fig. 6. Optimization of Hutchinson's (1967) tribes onto the strict consensus tree from Fig. 5. The following genera were published after 1967 but are easily assigned to Hutchinson's tribes, which we have done: *Barnebya*, *Ectopopterys*, *Excentradenia*, *Mcvaughia*, and *Peregrina*. In addition, we have reassigned *Lophopterys* from Banisterieae, where Hutchinson placed it, to Hiraeae, where he certainly would have placed it if he had seen the fruit of *L. floribunda*.

not all in the same plane and the ectoapertures, if present, variously oriented (Fig. 5). All nonbanisterioids plus the ptilochaetoids have radially symmetrical pollen, i.e., the pores are equatorial and the colpi, if present, are oriented at right angles to the equator (Lowrie, 1982). Lobreau (1967) described *Rhynchophora humbertii* as having tricolporate pollen, but that is probably not the case; see comments below under *Rhynchophora*.

Tristellateioids—The tristellateioids are a miscellany of morphologically diverse and isolated genera; several of its branches are poorly supported and may not persist in future analyses. However, the very strong support for the association

of *Heladena* and *Tristellateia* is perplexing. *Heladena* is a South American genus that seems quite without morphological synapomorphies with the Old World genus *Tristellateia* (W. Anderson, personal observation). Lowrie (1982) did not consider their pollen grains to be especially similar, although both are polyporate.

Aside from the ptilochaetoids and tristellateioids, the remainder of the banisterioids fall into a well-supported but poorly resolved clade. Because of the distribution of its representatives in that clade the genus *Mascagnia* merits special comment. It has already been noted that *Mascagnia* contains diverse species with lateral-winged samaras that do not fit readily into segregate genera (Anderson, 1981, 1990b). In Fig.

5 the seven species sampled are scattered among at least four clades, showing that *Mascagnia* is indeed nonmonophyletic. The monophyly of *Mascagnia* is rejected by a Templeton test ($P = 0.0253$).

Malpighioids—One of the two well-supported large clades in the banisterioids is the one we are calling malpighioids. That clade comprises the genus *Malpighia*, our only representative of *Mascagnia* section *Mascagnia* (the probably monophyletic part of *Mascagnia* that includes the type of the genus), the West Indian genus *Triopterys*, and the Old World genera *Aspidopterys*, *Caucanthus*, *Rhynchophora*, and *Triaspis*. *Rhynchophora* is a special case that will be discussed separately below. Except for *Malpighia*, which has a fleshy fruit, the other genera have samaras with the lateral wings dominant and continuous at the base (as do several genera in other clades). W. Anderson has suggested (1987, 1990b) that, given its other morphological characteristics and the rudimentary winglets on the endocarp concealed by the fleshy exocarp, *Malpighia* is likely to have had its origin in or near *Mascagnia* sect. *Mascagnia*, so it is not surprising to see those two taxa together in this clade. The distribution of New World and Old World genera within the clade is not what we would have predicted, but those branches are weakly supported. The pollen is informative in this case. Lowrie (1982) considered the pollen of *Malpighia* and *Mascagnia* section *Mascagnia* to be a derived type that he called “mascagnoid,” and he called that of *Triopterys* “near mascagnoid,” saying it could easily be derived from a mascagnoid grain. Those data suggest that we should expect the four American taxa in this clade to come together in future analyses. Lowrie found the pollen grains of the Old World genera *Aspidopterys* and *Triaspis* to be very similar to each other (his “aspidopteroid” type), suggesting a close relationship of these genera. *Caucanthus auriculatus* has problematic pollen. Lowrie found it to fit his tetrapteroid type, quite different from that of *C. albidus* Nied., which has aspidopteroid pollen. While that does not help us to interpret this clade, it is worth noting that *Caucanthus auriculatus* also has an indehiscent fruit (see discussion of fruit evolution); with anomalous fruits and pollen, *C. auriculatus* clearly merits closer study.

Rhynchophora is a genus of Madagascar with only two species. Its peculiar fruit is indehiscent, and each carpel bears a single horizontal to ascending wing. In *R. phillipsonii* the wings are borne around the apex of the fruit and give the whole fruit the appearance of a helicopter. The homology of that unique wing is not entirely obvious, but it seems most likely to be derived from a displaced lateral wing (W. Anderson, 2001). Lobreau (1967, 1968) initially described the pollen of *R. humbertii* as tricolporate, later as inaperturate. We have not had the opportunity to study pollen of that species, but W. Anderson (2001) reports that in *R. phillipsonii* the pollen of morphologically bisexual flowers is inaperturate (and presumably nonfunctional), while that of staminate flowers is globally symmetrical, with (5) 6 (7) nonequatorial pores, and lacks ectoapertures. That pollen resembles what Lowrie (1982) called his aspidopteroid type, suggesting that *Rhynchophora* is quite at home with the other Old World genera in the malpighioids.

Lophopterys is placed as sister to the malpighioids, but with low bootstrap support. *Lophopterys* is a South American genus with two long narrow lateral wings on each samara and a triangular dorsal winglet (except for the type species, which has lost the lateral wings). Aside from its fruit, the genus is

distinguished by having the paired calyx glands of other neotropical genera replaced by a single large gland, a condition found otherwise only in some species of *Jubelina* and *Meziba* (W. Anderson, personal observation), both of which fall into the unresolved mass of tetrapteroids in our analysis.

Stigmaphylloids—The other large and strongly supported clade in the banisterioids is the stigmaphylloids. The following comments will deal with all the genera except *Gallardoa*, which is discussed separately. Most genera in the clade have apical (terminal) stigmas vs. stigmas on the internal angle of the apex of the style, which is the condition found in *Ectopopterys* and most of the tetrapteroids and hiraeoids, the sisters to the stigmaphylloids. *Cordobia* has internal stigmas; given its other character states and its position in Fig. 5, the condition is probably secondary. The other genus in the stigmaphylloids with internal stigmas is *Stigmaphyllon*. Given its position near the base of this unresolved clade, one cannot help speculating that *Stigmaphyllon* may eventually prove to be sister to the rest of the clade, with terminal stigmas derived in the other genera, including its Old World sister, *Ryssopterys*. It is also worth noting that the enigmatic genus *Ectopopterys* has folioles on the anterior style, similar to those found in *Stigmaphyllon* (and nowhere else in the family). In other characters those two genera are very dissimilar (Anderson, 1980b), but the placement of *Ectopopterys* in a tetrachotomy with the stigmaphylloids in Fig. 5 offers the interesting possibility that they may have shared a common ancestor with folioles.

Most genera of the stigmaphylloids have mericarps with the dorsal wing dominant and thickened along the adaxial edge; such a fruit occurs only in this clade and in the acridocarpoids (Anderson, personal observation). In many cases where the dorsal wing has been reduced (*Aspicarpa*, *Diplopterys*, and *Mionandra*), the derivation from a dorsal wing is obvious. In *Peregrina*, and even more strongly in *Gaudichaudia*, the dorsal wing has been reduced while the lateral wing has become dominant, surely a secondary phenomenon. It is also worth noting that most genera in this clade have mericarps that are suspended from the torus by a carpophore (Hutchinson, 1967), except for *Aspicarpa* and *Diplopterys*, both of which have lost the dorsal wing, so that the presumed adaptive advantage of having a samara dangle from a cartilaginous thread until the wind is strong enough to break it and carry the samara away is no longer relevant. That carpophore is known in no other clade of the family (W. Anderson, personal observation). Most members of this clade, and only those species, have a derived pollen type that Lowrie (1982) called “banisterioid,” and the “Banisteriopsis group” in his classification of the family based on pollen agrees perfectly with the stigmaphylloids in Fig. 5.

Gallardoa is the one somewhat discordant element in the stigmaphylloids. This monotypic genus is endemic to an area in Argentina farther south than that of any other neotropical member of the family. Its samara has narrow dorsal and lateral wings with the lateral wing slightly larger, and lacks a carpophore. Its stigmas are internal. All of these characteristics would suggest an origin in the tetrapteroids or malpighioids. On the other hand, *Gallardoa* has its stipules connate in interpetiolar pairs and the stamens opposite the sepals reduced to staminodes, just as in the other Argentinian endemics *Cordobia* and *Mionandra*, and as in *Peixotoa*, which may be sister to the cordobioids (W. Anderson, personal observation). Lowrie (1982) found its pollen to be unusual but most likely derived from that of *Cordobia* and *Mionandra*. Therefore, in

spite of its anomalies, the morphology of *Gallardoia* is consistent with its placement in Fig. 5.

Gaudichaudioids—Embedded within the stigmaphylloids is the strongly supported clade we are calling the gaudichaudioids. That clade corresponds to Hutchinson's tribe Gaudichaudieae (1967), which is the only monophyletic tribe in his classification of the family (Fig. 6). (As an historical note, Hutchinson was not the first to recognize the tribe Gaudichaudieae. Adrien de Jussieu did so in the first monograph of the family [1843, p. 588, pl. 23]. He used that name and the same characteristics mentioned by Hutchinson [reduced androecium and style number, distinct carpels, carpophore, dimorphic flowers], and his work was undoubtedly the source of Hutchinson's tribe, even though Hutchinson did not cite de Jussieu.) That is also the only tribe in Hutchinson's system that was not based primarily on fruit type, which demonstrates how misleading such overreliance on one set of morphological characters has been in this family. In this case Hutchinson focused mainly on shared floral characteristics, specifically apocarpous gynoecea and the production in many of what he called dimorphic flowers, which were subsequently shown by W. Anderson (1980a) to be chasmogamous and cleistogamous flowers. Additionally, this clade exhibits a trend toward herbaceous habit; its species are among the least woody members of the family (W. Anderson, personal observation). Within the gaudichaudioid clade *Janusia* and *Aspicarpa* are probably not monophyletic as traditionally circumscribed. A monophyletic *Janusia* is not rejected (Templeton test $P = 0.0578$), but in all analyses *Janusia anisandra* and *J. mediterranea* of South America are not directly linked with *Janusia californica* and *J. linearis* of northwestern Mexico. A monophyletic *Aspicarpa* is strongly rejected (Templeton test, $P = 0.0028$). The Mexican species of *Aspicarpa* (*A. brevipes* and *A. hirtella*) are united with the Mexican species of *Gaudichaudia*, rather than with the South American *A. pulchella*. These results call for a re-examination of the generic taxonomy of the gaudichaudioids.

Hiraeoids/Tetrapteroids—The remaining taxa included in our analysis are in the hiraeoid and tetrapteroid "clades," both of which are poorly supported with little or no resolution. Given their weakness, neither group merits detailed comment at this time, but a few points are worth mentioning. The fairly strong support for a clade comprising the representatives of the two major groups in the genus *Hiraea* was to be expected because that genus is coherent in its morphology, with all its species sharing several synapomorphies—elongated stipules borne on the petiole, short-stalked axillary umbels, and butterfly-shaped samaras. Excluding the pair of *Dicella* and *Tricomaria* for the moment, the rest of the tetrapteroids and hiraeoids have the lateral wing(s) of the samara dominant and the dorsal wing more or less reduced, with the exception of *Heteropterys*, one of the two largest genera in the family. In the latter the dorsal wing is dominant and the lateral wings are reduced to crests or often lost. That dorsal wing is thickened on the abaxial edge and bends upward, exactly the opposite of what is found in the stigmaphylloids. That samara type has always made *Heteropterys* inconsistent in its placement with *Stigmaphyllon* and its relatives, where it was placed by Nienzen (1928) and Hutchinson (1967). Its internal stigmas make it difficult to distinguish *Heteropterys* from *Tetrapterys* in flower (W. Anderson, personal observation), and its pollen also favors a tetrapteroid affinity (Lowrie, 1982). The place-

ment of *Heteropterys* with this group of genera is therefore not a surprise, considering all the morphological evidence and setting aside the radical shift in samara type.

The lateral-winged tetrapteroids and hiraeoids are very diverse in their morphology, even more diverse than the number of generic names in Fig. 5 suggests, because those four species of *Mascagnia* probably do not constitute a clade, and *Tetrapterys microphylla* fits poorly in a genus with *Tetrapterys discolor* (W. Anderson, personal observation). In fact, when we consider the weak support for all the clades with lateral-winged samaras (excepting the gaudichaudioid genera *Gaudichaudia* and *Peregrina* and the malpighioids), we can only conclude that more and better data are needed before we can say anything with confidence.

The neotropical genera *Dicella* and *Tricomaria* are paired with fairly strong support in Fig. 5. *Dicella* is a small Central and South American genus of woody vines in which the fruit proper is a large, indehiscent, smooth-walled nut. *Dicella* appears to have transferred the wind dispersal function of wings on the fruit to an accrescent calyx of five winglike sepals, which subtend the fruit proper. *Tricomaria* is a monotypic shrub of very dry areas in Argentina; its fruit (apparently also indehiscent) is covered by long, stiff, vascularized bristles. The two genera have a generally similar pollen type that is widespread among tetrapteroids, although *Tricomaria* is derived in having diorate colpi (Lowrie, 1982). Both genera have the petals abaxially sericeous. Aside from the indehiscent fruit and the hairy petals, there are no obvious morphological synapomorphies uniting these two genera (W. Anderson, personal observation).

Evolution of fruits—We have mapped basic fruit types onto one of the most parsimonious trees used to generate Fig. 5 (Fig. 7). Fruits have long been a major criterion for recognition of genera in the Malpighiaceae, with floral and vegetative characters contributing additional (in some cases primary) characters. With a few exceptions, most notably *Mascagnia*, those genera are supported in our analyses, i.e., where we included two or three species of the same genus, they formed a clade (*Acridocarpus*, *Gaudichaudia*, *Hiraea*, *Malpighia*, *Ptilochaeta*, *Stigmaphyllon*, and *Tristellateia*). It is also the case that similar fruits often characterize clades of two or more genera; examples are *Blepharandra/Diacidia*, the acmantheroids, the galphimiods, *Acridocarpus/Brachylophon*, *Ryssopterys/Stigmaphyllon*, the hiraeoids, *Lasiocarpus/Ptilochaeta*, and *Burdachia/Glandonia*. However, patterns of fruit evolution in the Malpighiaceae are not entirely simple. They have caused problems for past efforts to construct an infrafamilial classification and continue to present challenges today. Several such cases were mentioned above; others will be discussed here in terms of trends in the evolution of fruit types.

Although we included *Humiria* for rooting purposes, the Humiriaceae are morphologically so unlike Malpighiaceae that we are uneasy about comparing fruit types and concluding that unwinged, indehiscent, fleshy fruits such as those of *Humiria* are likely to be ancestral in Malpighiaceae. However, other plausible relatives of Malpighiaceae also lack wings (K. Wurdack, University of North Carolina, personal communication). Furthermore, Byrsonimoideae all have unwinged fruits, without any trace of rudimentary wings to indicate that their common ancestor had wings. The unwinged fruits of most mcvaughnioids are discussed above. In the banisterioids, the nominally unwinged fruits of *Malpighia* show rudimentary

winglets on their endocarps, hidden by the fleshy exocarp, so in that case there seems little doubt that the wingless condition is derived (Figs. 5, 7). The spherical indehiscent nut of *Dicella* is quite without winglets of any kind, but it does bear rounded dorsal ribs that may represent the position of wings in an ancestor; certainly its position in our tree suggests that its un-winged fruit is derived. Among the tristellateoids, both *Heladena* and *Thryallis* have fruits that break apart into nutlets that are unwinged but bear dorsal or apical–dorsal crests that are probably derived from reduced dorsal wings (Anderson, 1995; W. Anderson, personal observation). The most puzzling genus among the banisterioids, with respect to its wingless fruits, is *Bunchosia*. In that genus the cartilaginous endocarps, buried in flesh, are quite smooth and show no sign of a winged ancestry. Given the placement of *Bunchosia* in our tree, embedded in a clade in which most fruits are bristly or winged, it is very probably derived in its winglessness; perhaps developmental-anatomical study of its fruits would shed light on this problem. In summary, the earliest Malpighiaceae may have had unwinged fruits, with winged fruits evolving at or very near the base of the Malpighioideae. Within Malpighioideae wings have apparently been lost in several lineages.

Although we remain uncertain about the ancestral condition in Malpighiaceae, it seems clear that fleshy, presumably bird-dispersed fruits evolved three times in the family, in *Byrsosima*, *Malpighia*, and *Bunchosia*, all of which are nested in dry-fruited clades. This was suggested by Anderson (1978) on the basis of morphology.

Niedenau (1928) and Hutchinson (1967) recognized the tribe Tricomarieae to accommodate five neotropical genera with fruits that bear many long vascularized bristles scattered over the surface, even though those genera are not especially similar in other aspects of morphology (W. Anderson, personal observation). We were able to include four of those five genera in our study, and those genera (*Tricomaria*, *Echinopterys*, *Lasiocarpus*, and *Ptilochaeta*) fall into three well-separated clades (Fig. 7), suggesting that bristly fruits evolved at least three times in the family. We were not able to obtain *Henleophytum*, a monotypic genus of Cuba. *Henleophytum* shares several morphological synapomorphies with *Heladena*, *Echinopterys*, and *Bunchosia* (W. Anderson, personal observation), so when we are able to add its sequences we expect it to be placed among the tristellateoids, and it may turn out that the bristly fruit evolved only once in that clade.

Indehiscent fruits are scattered through the tree in Fig. 7. We note also that several species of *Malpighia* not included in our study [e.g., *M. albiflora* (Cuatrec.) Cuatrec. and *M. verruculosa* W. R. Anderson] have dehiscent fleshy fruits, and while *Caucanthus auriculatus* has an indehiscent fruit, *C. albidus* has a dehiscent fruit (W. Anderson, personal observation).

The traditional criterion for grouping wing-fruited genera into tribes has been whether the dominant wings were dorsal or lateral, and that difference can be very informative, but overreliance on it led Niedenau (1928) to erect polyphyletic tribes and to misplace some genera. Most genera with the dorsal wing dominant fall into our stigmaphylloids, but dorsally winged samaras are also found in four other clades, those containing *Barnebya*, *Acridocarpus* and *Brachylophon*, *Heteropterys*, and *Dinemagonum*. Niedenau forced them all into his tribe Banisterieae [the species later segregated as *Barnebya dispar* was treated as *Banisteria dispar* (Griseb.) Nied. by Niedenau]. *Dinemagonum* was a particularly egregious case, be-

cause in all characters except the fruit it is very like its Chilean sister genus, *Dinemandra*, as recognized by de Jussieu (1843, p. 585) and Simpson (1989). Niedenau also excluded from his Banisterieae the genera *Diplopterys*, *Gaudichaudia*, and *Mionandra* because they lack a dominant dorsal wing, even though all share morphological synapomorphies with *Banisteriopsis*, *Aspicarpa*, and *Cordobia*, respectively (W. Anderson, personal observation) and are placed in the stigmaphylloids in our analysis. As noted above, de Jussieu (1843) correctly placed *Gaudichaudia* near *Aspicarpa* and *Janusia*. Loss of the dorsal wing and elaboration of the lateral wings likely happened three times in the stigmaphylloids.

Biogeography—Most genera of Malpighiaceae are limited to the New World or the Old World. The only exceptions are two New World genera with one species each also in coastal western Africa; they are *Heteropterys* (*H. leona*; C. Anderson, 2001) and *Stigmaphyllon* [*S. bannisterioides* (L.) C. Anderson; Anderson, 1997]. All of the Old World groups have winged fruits. Niedenau (1928) placed those Old World species with the lateral wings dominant in a subtribe Aspidopteryginae within his lateral-winged tribe Hiraeeae, while those with the dorsal wing dominant were placed in subtribe Sphebamocarpinae within his dorsal-winged tribe Banisterieae. We have plotted geographic distribution on one of the most parsimonious trees from Fig. 5 and added Niedenau's subtribal assignment for each Old World taxon (except *Rhynchophora* Arènes, which was described since 1928 and which we assigned to the subtribe in which he surely would have placed it, and *Heteropterys leona*, which Niedenau classified with the rest of *Heteropterys* in his New World subtribe) (Fig. 8). Niedenau's subtribes are polyphyletic, implying that he relied too heavily on geography in constructing his classification for the family. A monophyletic Old World Banisterieae and Hiraeeae are strongly rejected (Templeton test: $P = 0.0001$ for both).

The Old World genera fall into at least eight clades in Fig. 8. Depending on the ultimate resolution of the malpighioid and tetrapteroid clades in Fig. 5, the Old World genera could eventually be found in as few as six or as many as nine different clades. Except for the acridocarpoid clade, which is discussed below, all of the Old World genera are nested deep within the predominantly New World banisterioid clade. This pattern is consistent with W. Anderson's argument (1990a) that the Malpighiaceae originated and differentiated in the New World at a time when South America was relatively isolated from Africa. Under this scenario only species with winged fruits were able to cross the water barrier and establish lineages that eventually gave rise to the Old World genera. Given Fig. 8, it would strain credulity to suggest an origin and differentiation of the Malpighiaceae in the Old World and radiation from there to the New World (also see Anderson, 1990a). A third possibility is that the radiation of Malpighiaceae predates the separation of South America and Africa (cf. Vogel, 1990). This would require a series of selective extinctions in the two hemispheres, leaving no representatives of any genus from either hemisphere in the other except for the two shared species of *Heteropterys* and *Stigmaphyllon* mentioned above. This is possible, of course, and can eventually be tested with information on the absolute age of splitting events in the tree.

The acridocarpoids consist of *Acridocarpus*, a genus of perhaps 25 species distributed in Africa, Madagascar, the Arabian Peninsula, and New Caledonia, and *Brachylophon*, a genus of one or several species of the Malay Peninsula (Niedenau,

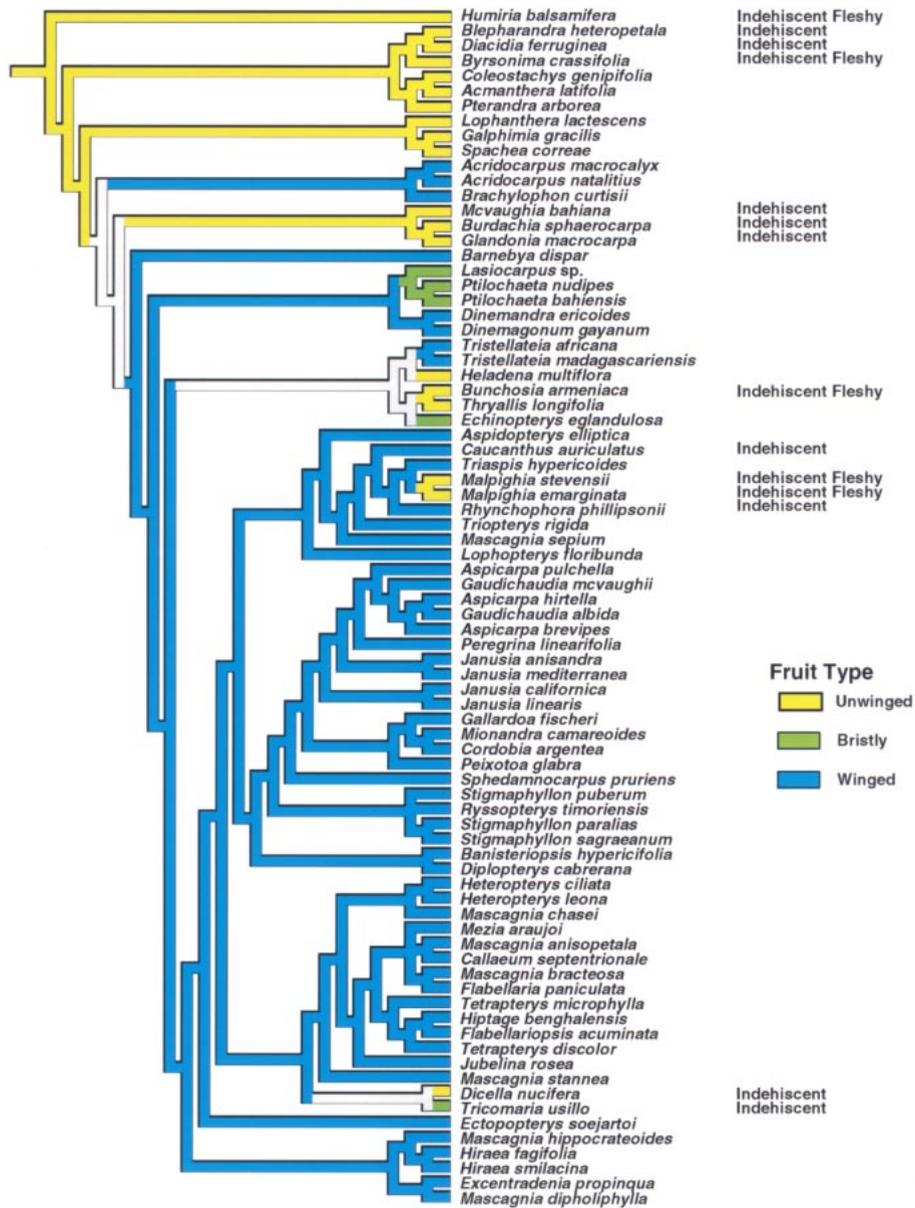


Fig. 7. Optimization of fruit types onto one of the equally most parsimonious trees from the combined data set with expanded taxon sampling. Gold branches indicate unwinged fruits, which are dehiscent and dry except where noted as indehiscent and/or fleshy. Blue branches indicate winged and dry fruits, which are dehiscent except where noted as indehiscent (included here are species with the fruit wings \pm reduced but clearly derived from larger wings). Green branches indicate bristly dry fruits, which are dehiscent except where noted as indehiscent. Equivocal resolutions are indicated by light gray.

1928). These two genera form an especially interesting Old World clade, because their phylogenetic position provides evidence that some Old World–New World disjunctions involved more ancient lineages and perhaps occurred soon after the evolution of winged fruits.

Conclusions—Our phylogenetic results call into question many previous taxonomic conclusions. Too much reliance has been placed on similar fruit characters, which now appear to be homoplastic, and with respect to the Old World taxa, which have been revealed to represent numerous New World–Old World disjunctions, too much emphasis has been placed on geography. In future studies it will be especially desirable to add the seven genera that are not represented in the present

study: from South America, *Camarea*, *Clonodia*, and *Verrucularia*; from Cuba, *Henleophytum*; from Madagascar, *Digoniopterys*, *Philgamia*, and *Microsteira*. In addition, the results reported here should be compared and probably combined with data from other genes and morphological characters. Nuclear genes may be helpful, especially in view of possible conflicts resulting from lineage sorting and “chloroplast capture.” Low-copy-number nuclear genes have provided resolution in several phylogenetic studies (e.g., Mathews and Donoghue, 1999), and we are now assembling Phytochrome C sequences for the accessions used in the present study. Detailed analyses of fruit morphology and development, especially of seemingly homoplastic features, are also clearly needed to improve our understanding of the evolution of Malpighiaceae.

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