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MOLECULAR PHYLOGENETICS OF MELASTOMATACEAE AND MEMECYLACEAE: IMPLICATIONS FOR CHARACTER EVOLUTION[1]

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Melastomataceae are among the most abundant and diversified groups of plants throughout the tropics, but their intrafamily relationships and morphological evolution are poorly understood. Here we report the results of parsimony and maximum likelihood (ML) analyses of cpDNA sequences from the *rbcL* and *ndhF* genes and the *rpl16* intron, generated for eight outgroups (Crypteroniaceae, Alzateaceae, Rhynchocalycaceae, Oliniaceae, Penaeaceae, Myrtaceae, and Onagraceae) and 54 species of melastomes. The sample represents 42 of the family's currently recognized ~150 genera, the 13 traditional tribes, and the three subfamilies, Astronioideae, Melastomatoideae, and Memecyloideae (= Memecylaceae DC.). Parsimony and ML yield congruent topologies that place Memecylaceae as sister to Melastomataceae. *Pternandra*, a Southeast Asian genus of 15 species of which five were sampled, is the first-branching Melastomataceae. This placement has low bootstrap support (72%), but agrees with morphological treatments that placed *Pternandra* in Melastomatacaeae because of its acrodromal leaf venation, usually ranked as a tribe or subfamily. The interxylary phloem islands found in Memecylaceae and *Pternandra*, but not most other Melastomataceae, likely evolved in parallel because *Pternandra* resembles Melastomataceae in its other wood characters. A newly discovered plesiomorphic character in *Pternandra*, also present in Memecylaceae, is a fibrous anther endothecium. Higher Melastomataceae lack an endothecium as do the closest relatives of Melastomataceae and Memecylaceae. The next deepest split is between Astronieae, with anthers opening by slits, and all remaining Melastomataceae, which have anthers opening by pores. Within the latter, several generic groups, corresponding to traditional tribes, receive solid statistical support, but relationships among them, with one exception, are different from anything predicted on the basis of morphological data. Thus, Miconieae and Merianieae are sister groups, and both are sister to a trichotomy of Bertolonieae, Microlicieae + Melastomeae, and Dissochaeteae + Blakeeae. Sonerileae/Oxysporeae are nested within Dissochaeteae, Rhexieae within Melastomeae, and African and Asian Melastomeae within neotropical Melastomeae. These findings have profound implications for our understanding of melastome morphological evolution (and biogeography), implying, for example, that berries evolved from capsules minimally four times, stamen connectives went from dorsally enlarged to basal/ventrally enlarged, and loss of an endothecium preceded poricidal dehiscence.

Key words: endothecium; Melastomataceae; Memecylaceae; Myrtales; *ndhF*; phylogeny; *rbcL*; *rpl16*.

Melastomataceae Juss. comprise shrubs, woody climbers, herbs, or trees and occur throughout the tropics in montane to lowland forests, savannas, and disturbed vegetation. Circumscribed narrowly to exclude Memecylaceae DC., Melastomataceae comprise ~4570 species in 150–166 genera (Renner, 1993; this includes a list of all Melastomataceae and Memecylaceae genera, with species number and geographic distribution; several genera have been combined since then [Michelangeli, in press; Meyer, in press; Clausing, in press]). Memecylaceae, or Memecyloideae when placed as a subfamily in Melastomataceae, are a pantropical lineage of primary forest trees or more rarely shrubs that includes six genera and ~430 species, mostly in Southeast Asia. Melastomataceae, in contrast, are more species rich in the New World, although, as is true of Memecylaceae, most of their structural diversity resides in the paleotropics. Throughout this paper, we refer to Melastomataceae and Memecylaceae as families, using the circumscription given them by de Candolle (1828a, b), to avoid repeated use of Melastomataceae sensu lato and Melastomataceae sensu stricto (our data and discusssion will address the topic of melastome circumscription).

Melastomataceae can usually be recognized by their acrodromally veined leaves in which one or more pairs of strongly developed lateral primary veins run in convergent arches from the base to the leaf apex. Flowers are bisexual, radially symmetric, and diplostemonous, and stamens often have enlarged and/or appendaged connectives. About 2150–2350 species in 38 genera have berries and 2000–2200 species in 112 genera have capsules.

Whether Melastomataceae should be circumscribed widely to include Memecylaceae (Naudin, 1849–1853; Triana, 1871; Cogniaux, 1891) or narrowly to exclude that group (de Candolle, 1828a, b; Dahlgren in Dahlgren and Thorne, 1984; Johnson and Briggs, 1984; APG, 1998) was discussed in detail, and answered in favor of the second option, by Renner (1993). Unable to find a morphological synapomorphy that

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would unite Melastomataceae and Memecylaceae to the exclusion of related Myrtalean families and in view of similarities between Memecylaceae and Myrtaceae, such as the presence of stamen glands, she suggested that Memecylaceae might be closest to Myrtaceae, and Melastomataceae to Crypteroniaceae (Renner, 1993). These hypotheses were contradicted by Conti, Litt, and Sytsma's (1996) *rbcL* data, which showed 100% bootstrap support for a Memecylaceae + Melastomataceae clade.

Conti, Litt, and Sytsma's (1996) finding of a Memecylaceae/Melastomataceae sister-group relationship was based on sequences from one Memecylaceae and four Melastomataceae, representing two of the family's tribes (Melastomeae and Rhexieae). It was therefore important to increase DNA and taxon sampling, especially of basalmost Melastomataceae, to evaluate the robustness of Conti, Litt, and Sytsma's results as well as the possibility that Memecylaceae might be nested in Melastomataceae, as suggested by Bremer (1988).

We also wanted to test hypotheses concerning within-Melastomataceae relationships derived from a morphological cladistic analysis (Renner, 1993). These hypotheses addressed the evolution of seed shape, stamen appendages, and fruit type (capsules vs. berries). Renner's cladistic results had led to a proposed new classification of Melastomataceae that circumscribed three tribes more broadly than done by her immediate predecessors.

Until 1993, the family's classification had essentially been that of Triana (1866, and slightly modified, 1871; Renner, 1993, includes a table contrasting the major classification systems of Melastomataceae). Triana had extensive knowledge of the family in the field and, working in London and Paris, had access to all important collections then available. One of his main contributions was to separate Old and New World genera into relatively homogeneous groups, which he recognized as tribes. This resulted in 13 tribes placed in three subfamilies. Memecylaceae were one of the three subfamilies. In order to key out the many tribes, Triana relied on characters such as connective appendages, number of floral parts, and geography. For example, Dissochaeteae are distinguished from Miconieae, and Osbeckieae from Tibouchineae, by the first of each pair being paleotropical, the second neotropical. Cogniaux (1891, p. 9) distinguished the problematic pairs by degree of ovary–hypanthium fusion, hypanthium pubescence, floral merosity, and connective prolongations. Following Triana's work, one additional tribe was proposed, the Cyphostyleae (Gleason, 1929), which includes three little-known Andean genera with ten species (details in Renner, 1993). From herbarium material, we were able to amplify one-third of the *ndhF* gene for *Cyphostyla*, but this proved insufficient for secure placement of this apparently highly divergent taxon.

To assess the monophyly of Renner's broadly defined tribes and the different views on Melastomataceae/Memecylaceae relationships, we generated sequences from two cpDNA genes and one intron for 54 ingroup species, of which 45 were used in analyses of combined data, representing the 13 traditional tribes and three subfamilies. Because long-branch effects can be introduced into a data set by the inclusion of too-distant or fast-evolving outgroups, as observed in empirical and theoretical studies (Chase et al., 1993; Lyons-Weiler, Hoelzer, and Tausch, 1998; Takezaki and Gojobori, 1999), we sampled eight outgroup taxa, including the sister clade of Melastomataceae/Memecylaceae and three genera from more distant families. The resultant phylogenetic reconstruction for Melastomataceae is used to study stamen, fruit, seed, and leaf venation evolution.

MATERIALS AND METHODS

Taxon sampling, DNA isolation and amplification, and sequence alignment—Table 1 lists all species newly sequenced for this study, with sources and GenBank accession numbers. The species represent 42 of ~150 currently recognized genera. Trees were rooted with species of Crypteroniaceae, Alzateaceae, Rhynchocalycaceae, Oliniaceae, Penaeaceae, and Myrtaceae, with *Ludwigia* (Onagraceae) added to represent more distant Myrtales (Conti, Litt, and Systma, 1996).

Total DNA was isolated from silica gel-dried, herbarium, or fresh leaves using a modified CTAB procedure (Smith et al., 1991), DNeasy plant mini kits (QIAGEN Inc., Valencia, California, USA), or NucleoSpin plant DNA extraction kits (Macherey-Nagel GmbH & CoKG, Dören, Germany) according to manufacturers' instructions. Standard polymerase chain reaction (PCR) protocols were used, but since Melastomataceae DNA generally works poorly, amplifications often had to be repeated several times to obtain enough product.

The *rbcL* gene was amplified using primers developed by Fay, Swensen, and Chase (1997) and the *ndhF* gene with primers developed by Olmstead and Sweere (1994). We amplified the exon between positions 972 (i.e., codon 305 of solanaceous sequences; Olmstead and Sweere, 1994) and 1955, using forward primer *ndhF*-972F, reverse primer *ndhF*-1955R, and one or two pairs of internal primers (*ndhF*-1318F, *ndhF*-1318R, *ndhF*-1603F, and *ndhF*-1603R). The large intron that interrupts the *rpl16* gene was amplified using primers 1067F and 18R (Asmussen, 1999). PCR products were purified either by running the entire product on a low-melting point agarose gel and then recovering the amplified DNA with the help of QIAquick gel extraction kits (QIAGEN) or by using QIAquick PCR purification columns directly, without a prior gel purification step. Cycle sequencing of the amplified double-stranded products was conducted with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit (Perkin Elmer, Norwalk, Connecticut, USA), using 2.5 ng of primer in a 5-μL reaction volume. Sequencing reactions were purified by ethanol precipitation and run on ABI 373 or ABI 377 automated sequencers at the universities of Mainz (*ndhF, rbcL* p.p.) or Missouri-St. Louis (*rpl16, rbcL* p.p.). Usually, both strands of DNA were sequenced and used to generate a consensus sequence using Sequencher software (version 3.1; GeneCodes Corp., Ann Arbor, Michigan, USA). Alignment was done manually.

For the combined 3-genome region-53-taxon analysis, sequences from the same species and usually from the same total DNA extract were spliced together, with the following exceptions (compare Table 1): *Gravesia viscosa rbcL* and *rpl16* were combined with *Gravesia guttata ndhF*; *Melastoma malabathricum rbcL* was combined with *M. sanguineum ndhF* and *rpl16. Memecylon bakerianum rbcL* and *ndhF* were combined with *M. edule rpl16*; *Tibouchina urvilleana rbcL* was combined with *T. longifolia rpl16* and *ndhF*; and *Triolena obliqua ndhF* and *rbcL* were combined with *T. pustulata rpl16.* In one case, sequences to be spliced came from different genera; *rbcL* and *rpl16* of *Tococa* were supplemented by *ndhF* from *Maieta.* In a few other cases where *rbcL* or *ndhF* could not be obtained for a species (Table 1), missing data symbols (''nnnn'') were entered for that region.

Phylogenetic analyses—Phylogenetic analyses of the aligned sequences were conducted with test version 4.0b.2 of PAUP* (Swofford, 1998). Parsimony analyses were performed using heuristic searches, ten random-taxon-addition replicates and tree bisection-reconnection (TBR) swapping. All minimal trees were saved. The COLLAPSE, but not the STEEPEST DESCENT, options of PAUP were in effect during all searches, and character changes were interpreted under ACCTRAN optimization. Characters were unweighted and unordered, and gaps were treated as missing data. Under parsimony, nonparametric bootstrap support (Felsenstein, 1985) for each clade was estimated based on 1000 replications, using closest taxon addition and TBR swapping. Most-parsimonious trees were generated independently for the three data sets, followed by bootstrap analyses, to assess whether there was statistically sup-

TABLE 1. Species sequenced for the phylogeny of Melastomataceae, with voucher information and GenBank accession numbers. Tribal assignments follow Cogniaux (1891), except for Kibessieae and Melastomeae where Renner's (1993) circumscriptions are followed: AS = Astronieae, BE = Bertolonieae, BL = Blakeeae, DI = Dissochaeteae, KI = Kibessieae, ME = Melastomeae, MC = Microlicieae, MI = Miconieae, MM = Memecyleae, MR = Merianieae, OX = Oxysporeae, RH = Rhexieae, SO = Sonerileae. Outgroup taxa are listed at the end. Herbarium acronyms stand for: AAU = Aarhus University; BH = Bailey Hortorium; CAS = California Academy of Sciences; CAY = ORSTOM, Cayenne; COL = Colombian National Herbarium; MICH = Michigan University; MJG = University of Mainz; MO = Missouri Botanical Garden; QCNE = Ecuadorean National Herbarium, Quito; TURKU = University of Turku. Positions marked with an asterisk indicate where sequences from related species were combined (cf. Materials and Methods).

Species	Tribe	Voucher	Provenance	GenBank accession no.[a]		
				rbcL	*ndhF*	*rpl16*
Aciotis indecora (Bonpl.) Triana	ME	Sothers 347, MO	S America	—	*	GBAN-AF215604
Aciotis purpurascens (Aubl.) Triana	ME	Renner 2154b, QCNE	S America	partial sequence	GBAN-AF215561	GBAN-AF322231
Adelobotrys boissieriana Cogn.	MR	Ruokolainen 10464, TURKU	S America	GBAN-AF215530	partial sequence	GBAN-AF215612
Amphiblemma cymosum (Schr. & Wendl.) Naudin	SO	cult. BG Mainz	West Africa	GBAN-AF215543	GBAN-AF215588	GBAN-AF215624
Arthrostemma ciliatum Pavón ex D. Don	ME	cult. BG Mainz	S America	GBAN-AF215522	GBAN-AF215562	GBAN-AF215605
Astronia macrophylla Bl.	AS	Clausing 208, MJG	SE Asia	GBAN-AF215510	GBAN-AF215548	*
Astronia smilacifolia Triana ex C.B. Clarke	AS	Clausing 189, MJG	SE Asia	GBAN-AF215511	GBAN-AF215549	GBAN-AF215596
Bertolonia maculata DC.	BE	cult. BG Mainz	S America	GBAN-AF215512	GBAN-AF215550	GBAN-AF215597
Blakea trinervia L.	BL	cult. BG Mainz	Jamaica	GBAN-AF215516	GBAN-AF215555	GBAN-AF215600
Blastus borneensis Cogn.	OX	Clausing 163, MJG	SE Asia	—	GBAN-AF215585	GBAN-AF215621
Calvoa orientalis Taub.	SO	cult. Amani BG, Tanzania, voucher C. Orava 1, MJG	East Africa	GBAN-AF215544	GBAN-AF215589	partial sequence
Centradenia inaequilateralis (Schlechtd. & Cham.) G. Don	MC	cult. BG Mainz	Central America	—	GBAN-AF215563	GBAN-AF215606
Clidemia rubra (Aubl.) Mart.	MI	cult. BG Bonn	S America	GBAN-AF215535	GBAN-AF215579	GBAN-AF215616
Dichaetanthera asperrima Cogn.	ME	Clausing 280, MJG	Madagascar	GBAN-AF215523	GBAN-AF215564	GBAN-AF215607
Diplectria divaricata (Willd.) O. Ktze.	DI	Clausing 236, MJG	SE Asia	GBAN-AF270746	GBAN-AF215556	GBAN-AF215601
Dissotis rotundifolia (Sm.) Triana	ME	*rbcL*: Conti, Litt, and Sytsma, 1996; *ndhF* and *rpl16*: cult. BG Mainz	Tropical Africa	GBAN-U26323	GBAN-AF215565	GBAN-AF270745
Driessenia glanduligera Stapf	OX	Clausing 254, MJG	SE Asia	GBAN-AF270749	GBAN-AF215586	GBAN-AF215622
Graffenrieda rotundifolia (Bonpl.) DC.	MR	cult. BG Munich, acc. no. 94/3113	S America	GBAN-AF215532	GBAN-AF215576	GBAN-AF215613
Gravesia guttata (Hook.) Triana	SO	cult. BG Mainz	Madagascar	—	GBAN-AF270755	—
Gravesia viscosa H. Perrier	SO	Clausing 304, MJG	Madagascar	GBAN-AF270750	*	GBAN-AF215625
Heterocentron subtriplinervium (Link & Otto) A. Brown & Bouché	ME	cult. BG Mainz	Central America	GBAN-AF270747	GBAN-AF215566	GBAN-AF210374
Lavoisiera cordata Cogn.	MC	Almeda 7798, CAS	S America	GBAN-AF215540	GBAN-AF215582	GBAN-AF210371
Leandra mexicana (Naud.) Cogn.	MI	cult. BG Bonn	Central America	GBAN-AF215536	GBAN-AF215580	GBAN-AF215617
Macrocentrum repens (Gleason) Wurdack	BE	Berry et al. 6587, MO	S America	GBAN-AF215513	GBAN-AF324498	GBAN-AF215598
Maieta guianensis Aubl.	MI	cult. BG Mainz	S America	GBAN-AF215537	GBAN-AF215581	GBAN-AF215618
Medinilla humbertiana H. Perrier	DI	cult. BG Mainz, voucher Clausing 289, MJG	Madagascar	GBAN-AF215517	GBAN-AF215557	GBAN-AF215602
Melastoma malabathricum L.	ME	Meyer 9641, MJG	SE Asia	GBAN-AF270748	—	—
Melastoma sanguineum Sims	ME	cult. BG Mainz	SE Asia	*	GBAN-AF270754	GBAN-AF270751
Memecylon bakerianum Cogn.	MM	Clausing 318, MJG	Madagascar	GBAN-AF215527	GBAN-AF215573	*
Memecylon edule Roxb.	MM	cult. BG Munich	SE Asia	GBAN-AF215528	GBAN-AF215574	GBAN-AF215609
Meriania nobilis Triana	MR	Barriga 21192, COL	S America	GBAN-AF215533	GBAN-AF215577	GBAN-AF215614
Microlicia fasciculata Cogn.	MC	Almeda 7717, CAS	S America	GBAN-AF215541	GBAN-AF215583	GBAN-AF210370
Monochaetum calcaratum (DC.) Triana	RH	cult. BG Munich	Central America	GBAN-AF215524	GBAN-AF215568	GBAN-AF210372
Monolena primuliflora J.D. Hooker	BE	cult. BG Mainz	S America	GBAN-AF215514	GBAN-AF215553	GBAN-AF270743
Mouriri guianensis Aubl.	MM	cult. BG Munich	S America	GBAN-AF215529	GBAN-AF215575	GBAN-AF215610
Mouriri helleri Britton	MM	Skean 3809, MICH	Puerto Rico	GBAN-sAF270752	GBAN-AF322230	GBAN-AF215611
Nepsera aquatica (Aubl.) Naud.	ME	Miller & Morello 8853, MO	Tropical America	—	GBAN-AF215569	GBAN-AF210373
Osbeckia chinensis L.	ME	Meyer 9643, MJG	SE Asia	GBAN-AF215525	GBAN-AF215570	GBAN-AF210378
Phyllagathis gymnantha Korth.	SO	Clausing 209, MJG	SE Asia	—	GBAN-AF215590	GBAN-AF215626
Pternandra caerulescens Jack	KI	Clausing 175, MJG	SE Asia	GBAN-AF215518	GBAN-AF215558	GBAN-AF322232
Pternandra cogniauxii Nayar	KI	Clausing 47, MJG	SE Asia	GBAN-AF215519	—	—
Pternandra echinata Jack	KI	Clausing 75, MJG	SE Asia	GBAN-AF215520	GBAN-AF215559	GBAN-AF270744

TABLE 1. Continued.

Species	Tribe	Voucher	Provenance	GenBank accession no.[a]		
				rbcL	*ndhF*	*rpl16*
Pternandra hirtella (Cogn.) Nayar	KI	Clausing 180, MJG	SE Asia	GBAN-AF215521	—	—
Pternandra multiflora Cogn.	KI	Clasuing 142, MJG	SE Asia	—	GBAN-AF215560	GBAN-AF215603
Pterolepis glomerata (Rottb.) Miq.	ME	Miller & Morello 8845, MO	Tropical America	GBAN-AF215526	GBAN-AF215571	GBAN-AF210376
Rhexia virginica L.	RH	*rbcL*: Conti, Litt, and Sytsma, 1996; *ndhF* and *rpl16*: cult. BG Mainz	N America	GBAN-U26334	GBAN-AF215587	GBAN-AF215623
Rhynchanthera grandiflora (Aubl.) DC.	MC	cult. BG Mainz, voucher Prévost 3281, CAY	S America	GBAN-AF215542	GBAN-AF215584	GBAN-AF210369
Tetrazygia urbanii Cogn.	MI	Skean 3799, MICH	Puerto Rico	GBAN-AF215538	GBAN-AF270753	GBAN-AF215619
Tibouchina longifolia (Vahl) Baillon	ME	cult. BG Bonn	S America	*	GBAN-AF215572	GBAN-AF210375
Tibouchina urvilleana Cogn.	ME	*rbcL*: Conti, Litt, and Sytsma, 1996; *ndhF*: cult. BG Mainz	S America	GBAN-U26339	—	—
Tococa rotundifolia (Triana) Wurdack	MI	Michelangeli 422, BH	S America	GBAN-AF215539	—	GBAN-AF215620
Topobea brenesii Standley	BL	Almeda & Daniel 7185 (MO)	Central America	—	GBAN-AF271665	—
Triolena obliqua (Triana) Wurdack	BE	Renner 2173, QCNE	S America	GBAN-AF215515	GBAN-AF215554	*
Triolena pustulata Triana	BE	cult. BH Munich	S America	—	—	GBAN-AF215599
OUTGROUP TAXA						
Alzateaceae						
Alzatea verticillata Ruiz & Pavón		*rbcL*: Conti, Litt, and Sytsma, 1996; *ndhF* and *rpl16*: Jiménez 1111, MO	S America	GBAN-U26316	GBAN-AF215591	GBAN-AF222780
Crypteroniaceae						
Crypteronia paniculata Bl.		Tange s.n., AAU	SE Asia	GBAN-AF215545	partial sequence	GBAN-AF215629
Myrtaceae						
Eugenia uniflora L.		cult. BG Mainz	pantropical	GBAN-AF294255	GBAN-AF215592	GBAN-AF215627
Myrtus communis L.		cult. BG Mainz	Meditterran.	GBAN-AF294254	GBAN-AF215593	GBAN-AF215628
Oliniaceae						
Olinia ventosa (L.) Cufod. (=*O. cymosa* Thunb.)		*rbcL* & *ndhF*: Phillipson 3680, MO; *rpl16*: J. Manning s.n.	East and South Africa	GBAN-AF215546	GBAN-AF215594	GBAN-AF222781
Onagraceae						
Ludwigia suffruticosa Walter		cult. BG Mainz	S America	*	GBAN-AF215595	GBAN-AF215630
Ludwigia peruviana (L.) Hara		Conti et al., 1993	S America	GBAN-L10222	—	—
Penaeaceae						
Penaea mucronata L.		*rbcL*: Conti, Fischbach, and Sytsma, 1996; *rpl16*: J. Manning s.n.	South Africa	GBAN-U26331	GBAN-AF270756	GBAN-AF222782
Rhynchocalycaceae						
Rhynchocalyx lawsonioides Oliv.		cult. BG Sydney	South Africa	GBAN-AF215547	GBAN-AF270757	GBAN-AF215631

[a] The prefix GBAN- has been added to each GenBank accession to link the online version of *American Jounal of Botany* to GenBank but is not part of the actual accession number.

ported conflict (i.e., with >50% bootstrap support) among data sets. In the absence of such conflict, the data were combined in a global analysis.

Maximum likelihood (ML) analyses were performed using the general time-reversible model (GTR; Yang, 1994), which estimates independent probabilities for all possible substitutions types in addition to accounting for unequal base frequencies. Rate heterogeneity among sites affects the performance of different tree reconstruction methods, and its estimation has received considerable recent attention (Yang, 1996; Sullivan, Swofford, and Naylor, 1999; Takezaki and Gojobori, 1999). A method for explicitly dealing with this kind of rate variation is the combination of an invariable-sites model, in which some proportion of sites (P_{inv}) is assumed to be completely resistant to change, with a gamma (Γ)-distributed-rates model in which the distribution of relative rates over sites is assumed to follow a Γ distribution whose shape parameter (α) determines rate heterogeneity. The dependence of P_{inv} and α on tree topology is minor as long as strongly supported groups are maintained (Yang and Kumar, 1996; Sullivan, Swofford, and Naylor, 1999). Both parameters can therefore be estimated for distance trees from the same data without complete branch swapping, which greatly reduces the computational demands of maximum likelihood searches. We estimated P_{inv} and α simultaneously, using the discrete gamma approximation of Yang (1994; implemented in PAUP*) with four rate categories to approximate the continuous gamma distribution. Base frequencies were the empirically observed ones.

Starting trees for ML searches were minimum-evolution trees, using LogDet distances, and the swapping strategy was nearest-neighbor-interchange swapping. We used quartet puzzling (Strimmer and von Haeseler, 1996; implemented in PAUP*), a fast tree search algorithm that allows analysis of large data sets, to obtain estimations of support for internal branches in the ML trees. These values are thought to have the same practical meaning as bootstrap values (Strimmer and von Haeseler, 1996).

We also calculated the likelihood score for a tree obtained under the Hasegawa-Kishino-Yano + P_{inv} + Γ model (HKY; Hasegawa, Kishino, and Yano, 1985) to assess whether the more parameter-rich GTR model fit the data significantly better, as judged by a likelihood ratio test, using four degrees of freedom (cf. Sullivan, Swofford, and Naylor, 1999). Both models yielded a single best trees that differed only in the placements of *Heterocentron, Monochaetum, Pterolepis,* and *Tibouchina.* However, the placement of these genera relative to each other was not well supported in any of the reconstructions. The likelihood ratio test rejected the HKY model in favor of the GTR model ($\chi^2 = 2(16427.95 - 16363.10) = 129.7$; $P < 0.001$; 4 df). We therefore used the GTR + P_{inv} + Γ model as the most appropriate for our data.

RESULTS

Sequence data—Each of the sequenced regions is characterized in Table 2. In the case of *rbcL,* a total of 1398 nucleotides, from positions 30 to 1428 of the *rbcL* exon were used in the analyses. For *ndhF,* the aligned sequences, with length variations that introduced gaps, had a length of 1021 nucleotides. The completed alignment of the *rpl16* sequences, with gaps, comprised 1045 nucleotides. We excluded base pairs 217–387 because of alignment ambiguity between the ingroup and the outgroup, mainly due to huge inserts in *Crypteronia* and *Rhynchocalyx.* The concatenated sequences thus comprised 3464 nucleotides, of which 170 were eliminated. This matrix contained 10% autapomorphic variable sites and 23% parsimony-informative sites when all 53 genera were included. Six Melastomataceae lacking *rbcL* sequences (*Aciotis, Blastus, Centradenia, Nepsera, Phyllagathis*) were excluded from most ML searches.

Of 35 sequence-length mutations, most occurred in the *rpl16* intron (Table 2), and several were diagnostic of the ingroup. Nucleotide compositions of the two genes and the intron differ barely (Table 2). Under the HKY model, the average transition-to-transversion ratio across all sequences was 0.77. It was 0.95 and 0.88 for the two genes, and 0.78 for the intron (Table 2).

TABLE 2. Descriptive statistics for separate and combined DNA matrices for 53 Melastomataceae and outgroups.

Data partition	*rbcL* gene	*ndhF* gene	*rpl16* intron	Combined data
Aligned nucleotides	1398	1021	1045/875[a]	3464/3294[a]
Autapomorphic variable sites (% of total sites)	97 (7)	136 (13)	144 (16)	377 (11)
Parsimony-informative sites (% of total sites)	175 (13)	304 (30)	271 (31)	750 (23)
Gaps in ingroup/Gaps between in- and outgroup (gap size range)[b]	0/0	9/2 (3–66)	27/9 (1–223)	35/13
A	0.28	0.30	0.26	0.28
C	0.19	0.15	0.19	0.17
G	0.25	0.16	0.15	0.19
T	0.29	0.39	0.40	0.35
ti/tv[c]	0.95	0.88	0.78	0.77
P_{inv}[d]	0.65	0.34	0.02	0.44
α[d]	0.74	1.23	1.01	1.03

[a] Because of alignment difficulties between the ingroup and the outgroup, a region of 170 bp was excluded from the *rpl16* matrix.

[b] Refers to gap size range in the ingroup only. In *ndhF,* the Microlicieae *Microlicia, Rynchanthera,* and *Lavoisiera* share a deletion of 66 bp; the other gaps are mostly 3-9 bp long. In *rpl16, Amphiblemma* has a deletion of 223 bp; the other gaps are mostly 6-9 bp long.

[c] Maximum likelihood estimates of transition/transversion (ti/tv) ratios were obtained under the Hasegawa-Kishino-Yano (1985) substitution model.

[d] Maximum likelihood estimates of the proportion of invariable sites (P_{inv}) and gamma shape parameter (α) were obtained under the GTR substitution model. An α of >1 means that most sites have intermediate rates, while few sites have very low or very high rates. An α of ≤ 1 means that most sites have very low rates or are almost invariable, while others change at very high rates (Yang and Kumar, 1996).

Rate heterogeneity among sites is measured by α, which is inversely related to the extent of rate variation. Table 2 shows the values for α, estimated under the GTR model. For *rpl16,* α is almost 1 (1.01), indicating a random distribution of the rates at which sites are changing, while for *rbcL,* α is 0.74, indicating that most sites have very low rates while some change at very high rates. For *ndhF,* α is >1, indicating that most sites have intermediate substitution rates, while a few have very high or very low rates.

Phylogenetic analyses—No hard incongruencies were found among strict consensus trees obtained from the individual data sets (not shown), and parsimony analysis of the concatenated sequences showed the same clades as seen in the individual analyses, only with higher bootstrap values. Figure 1 shows the strict consensus of the three equally parsimonious trees (Length = 2443, consistency index [CI] = 0.62, retention index [RI] = 0.80), all in a single tree island. A long branch separates Memecylaceae + Melastomataceae (with 100% bootstrap support) from their closest relatives, a clade of *Crypteronia* (Crypteroniaceae) + *Alzatea* (Alzateaceae) + *Rhynchocalyx* (Rhynchocalycaceae) + *Olinia* (Oliniaceae) + *Penaea* (Penaeaceae), Myrtaceae, and Onagraceae (see the midpoint-rooted trees in Fig. 1). We will subsequently refer to the former group of families as the CAROP clade. If trees are rooted with *Ludwigia* (Onagraceae), a sister-group relationship between the CAROP clade and Memecylaceae/Melastomataceae has 100% bootstrap support. *Pternandra* appears to be

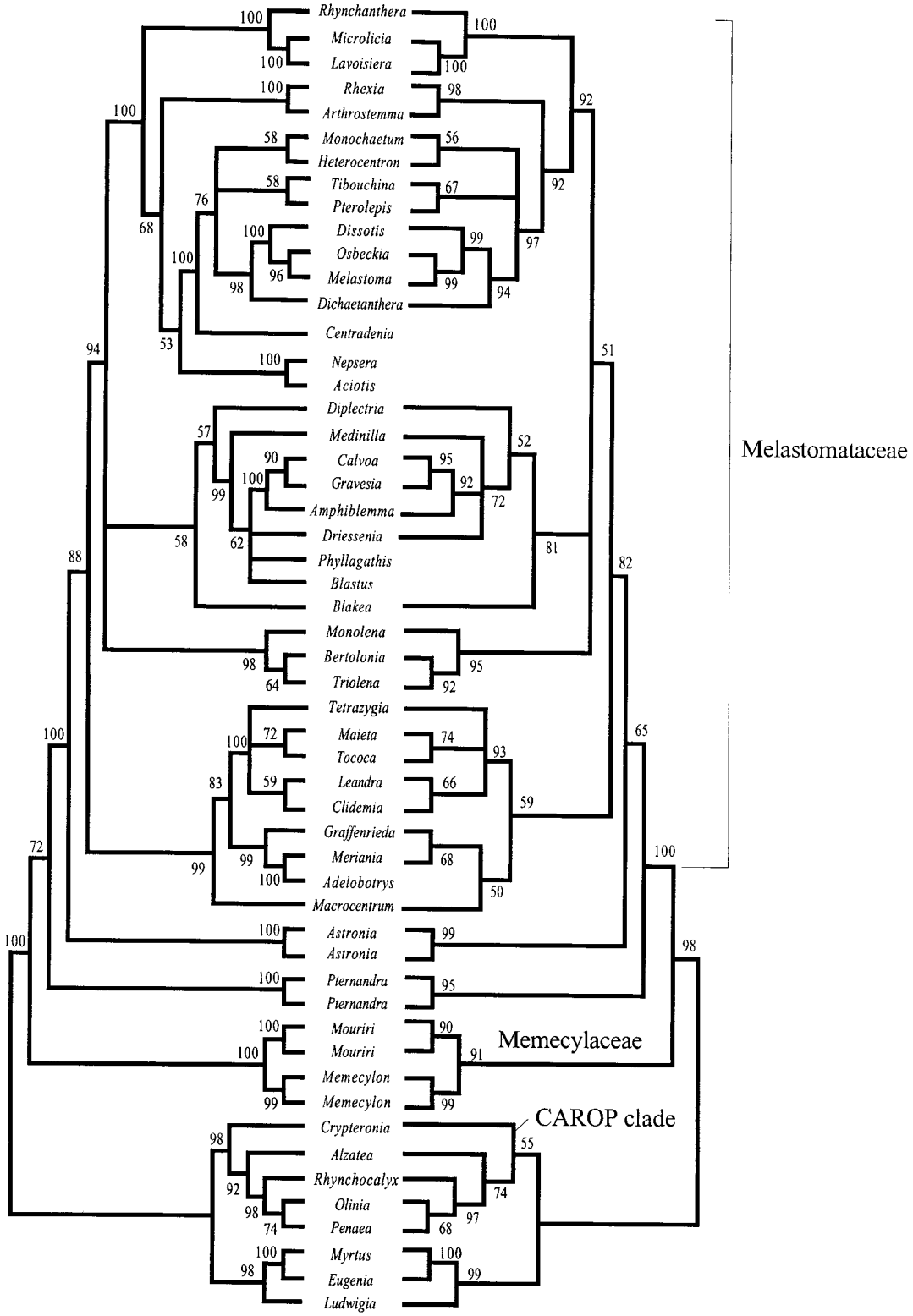

Fig. 1. Left tree: midpoint-rooted strict consensus of three equally parsimonious trees for Melastomataceae and relatives, resulting from combined *rbcL*, *ndhF*, and *rpl16* data (Length = 2443, CI = 0.62, RI = 0.80). Figures at nodes are bootstrap values based on 1000 replicates with TBR swapping. Right tree: midpoint-rooted highest likelihood tree for the same data analyzed under the general time-reversible model with discrete approximation of the gamma distribution to accomodate substitution rate heterogeneity across nucleotide sites. Seven taxa were excluded from maximum likelihood analyses because of incomplete sequences. Support values at nodes result from quartet puzzling and have the same practical meaning as bootstrap values. In both trees, nodes with ≤50% support have been collapsed.

the first-branching Melastomataceae, but support for this placement of *Pternandra* is low (72%). The next-basal branch is *Astronia,* with a bootstrap support of 100%.

The single best tree resulting from the ML analysis under the GTR + P_{inv} + Γ model (Fig. 1) shows the same topology as the parsimony tree. The monophyly of Melastomataceae + Memecylaceae again is well supported (98%) and the placement of *Pternandra* as basal in Melastomataceae poorly (65%).

Within core Melastomataceae, several major groups can be discerned (Fig. 1; tribe names for these groups are shown in Fig. 2). They are: (1) a clade comprising the two species of *Pternandra* included in the combined analysis (five species of this genus of 15 species were sequenced [Table 1], but not for all genome regions); (2) a clade comprising the two species of *Astronia* (Astronieae), (3) a clade consisting of Merianieae (*Adelobotrys, Graffenrieda, Meriania*) and (4) their sister group Miconieae (*Clidemia, Leandra, Maieta, Tetrazygia, Tococa*) plus *Macrocentrum,* a genus traditionally placed in Bertolonieae; (5) a clade comprising *Bertolonia, Monolena,* and *Triolena* (Bertolonieae); (6) a clade comprising Dissochaeteae (*Diplectria, Medinilla*) and, nested within them, Sonerileae/Oxysporeae (*Amphiblemma, Blastus, Calvoa, Driessenia, Gravesia, Phyllagathis*) plus *Blakea,* the sole representative of Blakeeae; (7) a clade comprising Melastomeae/Rhexieae; and (8) a Microlicieae clade. The second genus of Blakeeae, *Topobea,* was sequenced for *ndhF* and is sister to *Blakea* in terms of that gene (tree not shown).

The degree of genetic differentiation among Memecylaceae, Melastomataceae, and *Pternandra* becomes apparent when the data are visualized as a phylogram (Fig. 2). The branches leading to these taxa are among the longest in the ingroup.

DISCUSSION

Interfamilial relationships of Melastomataceae—The results of this study support Conti, Litt, and Sytsma's finding (1996) that Melastomataceae and Memecylaceae are sister to a small Southeast Asian/Neotropical/South African clade. This clade consists of Crypteroniaceae (ten spp. in Southeast Asia; long considered a close relative of Melastomataceae on the basis of morphology [cf. Renner, 1993, and references therein]), Alzateaceae (one sp. in South and Central America; Silverstone-Sopkin and Graham, 1986), Rhynchocalycaceae (one sp. in Natal), Oliniaceae (8–10 spp. in South Africa), and Penaeaceae (20 spp. in South Africa). These families share leaf stipules, haplostemonous flowers (Johnson and Briggs, 1984), and ephemeral endothecia (Tobe and Raven, 1983, 1984a, b, 1987a, b). Ephemeral endothecia degenerate early on, and anthers therefore dehisce not via differential shrinking of endothecium cells, but via rupture of walls along their thinnest sections caused by the shrinking of connective cells (H. Tobe, Kyoto University, personal communication). The sister-group relationship between Melastomataceae/Memecylaceae and the CAROP clade is supported by two morphological characters, viz. opposite leaves and stamen connectives that are dorsally enlarged and often massive (Fig. 3). The latter trait may relate to the connectives' role in anther dehiscence or may be correlated with the incurved position of the stamens in bud found in the CAROP families, Melastomataceae, and Memecylaceae. An inflexed bud position may create a tendency for abnormal growth at the points of greatest curvature (Ziegler, 1925; Leinfellner, 1958; Jacques-Félix, 1994).

Monophyly of Melastomataceae—Arguments about the circumscription of Melastomataceae, whether narrowly to exclude Memecylaceae or widely to include that family, have always hinged on the placement of *Pternandra. Pternandra* is a genus of 15 species of trees that is most species rich in Borneo, but extends into peninsular Malaysia (Maxwell [1981] included in *Pternandra* the genus *Kibessia* and two others that traditionally made up Kibessieae [Krasser, 1893]). *Pternandra* is characterized by fleshy capsules with dorsal-median placentas (Maxwell, 1981; Clausing, Meyer, and Renner, 2000) and wood with interxylary phloem islands. The latter trait is also found in Memecylaceae, causing wood anatomists to argue that *Pternandra* was closer to Memecylaceae than to Melastomataceae (van Tieghem, 1891a, b; Janssonius, 1950; van Vliet, 1981; van Vliet, Koek-Noorman, and ter Welle, 1981). This provided an argument for circumscribing the family widely, with *Pternandra* as the ''link'' between two phenetic groups. Similar interxylary phloem, however, is found in at least one species of Melastomataceae, *Dissotis leonensis* (D. Normand in Jacques-Félix, 1994, p. 250; *Dissotis* is nested in Melastomeae; Figs. 1 and 2) and is common in alliances with intraxylary phloem, such as Myrtales. It may be present or absent within single genera or individuals, for example, in the roots, but not stem, of *Lythrum salicaria,* also a myrtalean taxon (van Tieghem, 1891a, b; Metcalfe and Chalk, 1983). Indeed, van Vliet (1981) concluded that ''*Pternandra* is [. . .] nearest to the Melastomatoideae [= Melastomataceae], being similar in the ray type and the coarse vessel-ray and vessel-parenchyma pits and the scanty paratracheal parenchyma.'' Vessel-ray pits in *Pternandra* are simple as in Melastomataceae. In contrast, Memecylaceae have half-bordered vessel-ray pits. These and other anatomical similarities of *Pternandra* to Melastomataceae—for example, *Pternandra* has radially included phloem in addition to its axially included phloem, a trait otherwise only found in the higher Melastomataceae *Medinilla* (van Vliet, 1981)—argue against the possibility that interxylary phloem is plesiomorphic in Memecylaceae and Melastomataceae, and lost in higher Melastomataceae.

A second character possibly linking *Pternandra* and Memecylaceae, discovered during the course of this investigation, is the presence of a fibrous endothecium in both lineages (Fig. 4). The presence or absence of an endothecium appears to be an important phylogenetic marker in the CAROP/Melastomataceae/Memecylaceae alliance, as well as within Melastomataceae, and the character is discussed in detail below (see *Relationships within Melastomataceae*).

Vliet, Koek-Noorman, and ter Welle's (1981) placement of *Pternandra* in Memecylaceae on the basis of the axially included phloem is contradicted by leaf venation. *Pternandra* and Melastomataceae both have acrodromal venation, while Memecylaceae have pinnate or brochidodromal venation (Morley, 1953; Dahlgren and Thorne, 1984; Johnson and Briggs, 1984; G. Clausing and S. S. Renner, personal observations, but see below). Brochidodromal venation is a subtype of pinnate venation in which the secondary veins anastomose close to the leaf margin, which can result in venation patterns that resemble acrodromal venation. Also, leaf clearings by Jacques-Félix, Mouton, and Chalopin (1978) and Klucking (1989) of species from five of the six genera of Memecylaceae—*Memecylon, Mouriri, Lijndenia, Spathandra,* and *Warneckea* (*Votomita* was not studied)—show that Memecylaceae venation can occasionally be truly acrodromal. The thick, opaque leaves of Memecylaceae make observation difficult,

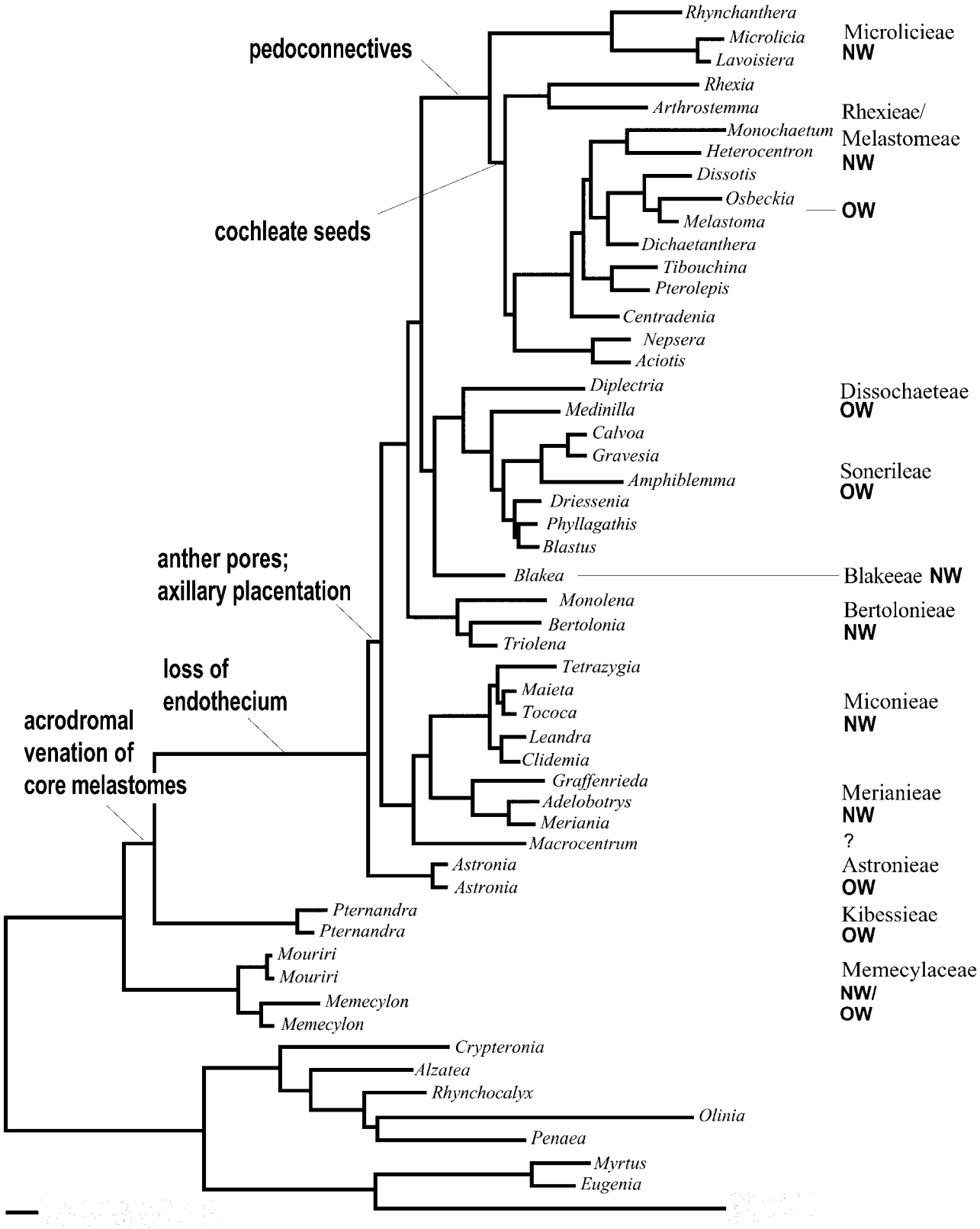

Fig. 2. Midpoint-rooted highest likelihood tree for Melastomataceae and relatives. Major morphological character transitions are shown to the left, tribe names (Cogniaux, 1891) to the right. Melastomeae and Sonerileae are circumscribed widely to include Tibouchineae and Oxysporeae, respectively (Renner, 1993). *Macrocentrum* (with a question mark to its right) is traditionally placed in Bertolonieae. NW = New World, OW = Old World.

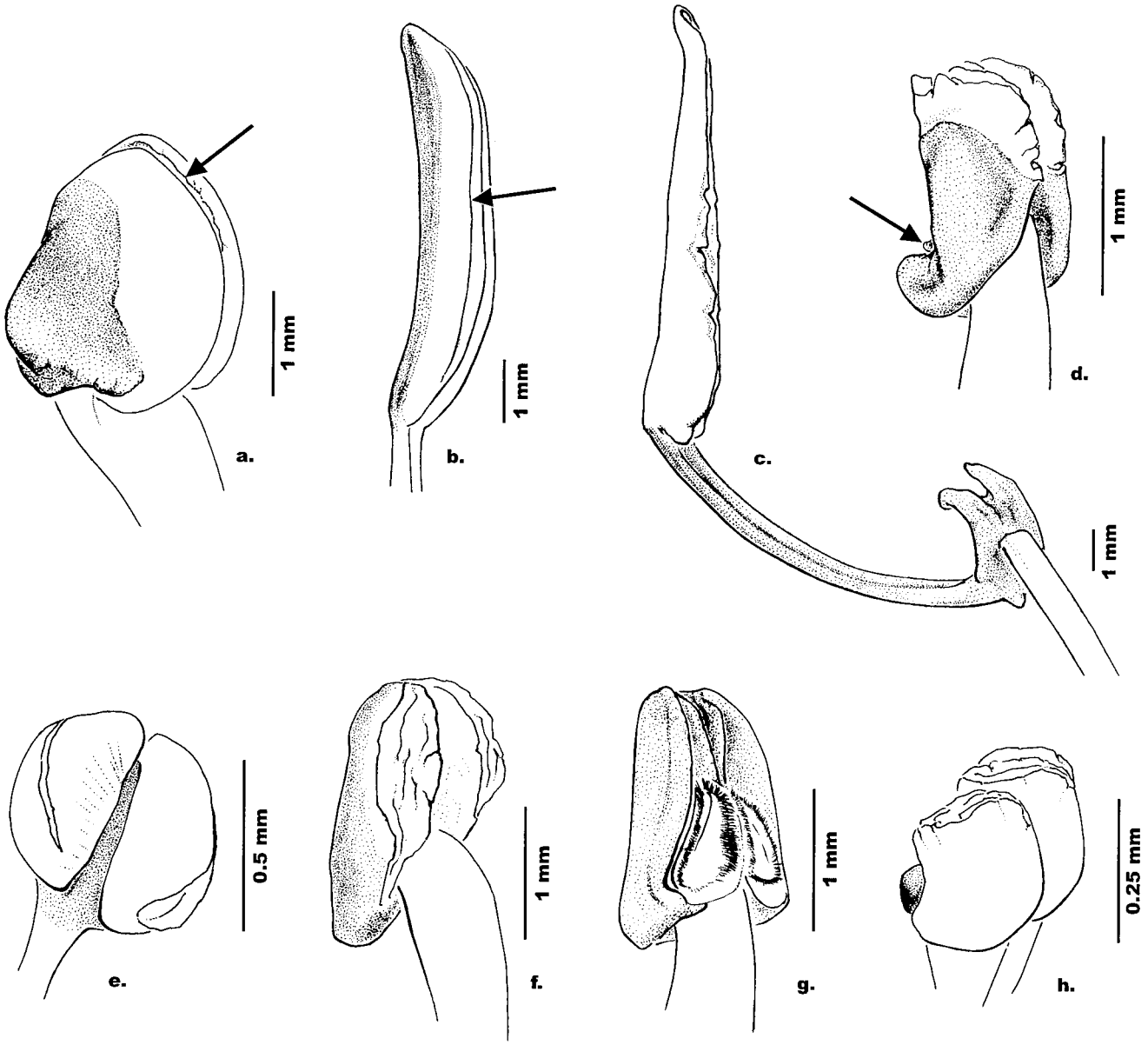

Fig. 3. Stamens of Melastomataceae and their relatives. Pollen sacs white, connective tissue and appendages shaded. (a) *Pternandra caerulescens* (Kibessieae), dorsally with a massive connective, ventrally with short, apical slits (arrow). (b) *Beccarianthus* sp. (Astronieae), the connective barely enlarged, the anthers opening by longitudinal slits (arrow). (c) *Melastoma sanguineum* (Melastomeae), stamen from the outer whorl, showing the basally prolonged connective with its bifid ventral appendage. (d) *Memecylon caeruleum* (Memecylaceae) with a massive connective that carries a dorsal gland (arrow). (e) *Crypteronia paniculata* (Crypteroniaceae) with a shield-like connective carrying two ventral thecae. (f) *Alzatea verticillata* (Alzateaceae), dorsally much enlarged connective with minute ventral thecae. (g) *Penaea mucronata* (Penaeaceae), dorso-apically much enlarged connective with minute ventral thecae. (h) *Olinia ventosa* (Oliniaceae), the connective dorsally only slightly spurred.

and the deeply scalloped courses of the lateral pair of primaries in both brochidodromally and acrodromally veined Memecylaceae obscure the venation's true nature (Klucking, 1989). A detailed phylogeny of Memecylaceae is needed to evaluate whether acrodromal venation is ancestral in this family, and pinnate and brochidodromous venations are secondarily derived as argued by Jacques-Félix, Mouton, and Chalopin (1978; see also Jacques-Félix, 1978, 1994), or whether Memecylaceae are ancestrally pinnate/brochidodromous (Johnson and Briggs, 1984; Renner, 1993). Scalloped primary veins are not seen in Melastomataceae (including *Pternandra*), indicating that there may be family-specific differences between Melastomataceae and Memecylaceae in the timing of lateral leaf expansion relative to the time when secondary veins join the lateral primaries (Klucking, 1989).

With *Pternandra* being the first-branching Melastomataceae, the question whether Memecylaceae should be included in Melastomataceae or ranked as a family, reduces to a matter of ranking and pragmatics of family identification. Among the morphological synapomorphies of Memecylaceae are dorsal glands on the stamen connectives (Fig. 3d), terminal leaf sclereids, paracytic stomates, axially included phloem islands in the secondary wood, fixed epigyny, and one or few large seeds with storage cotyledons (additional differences are listed in

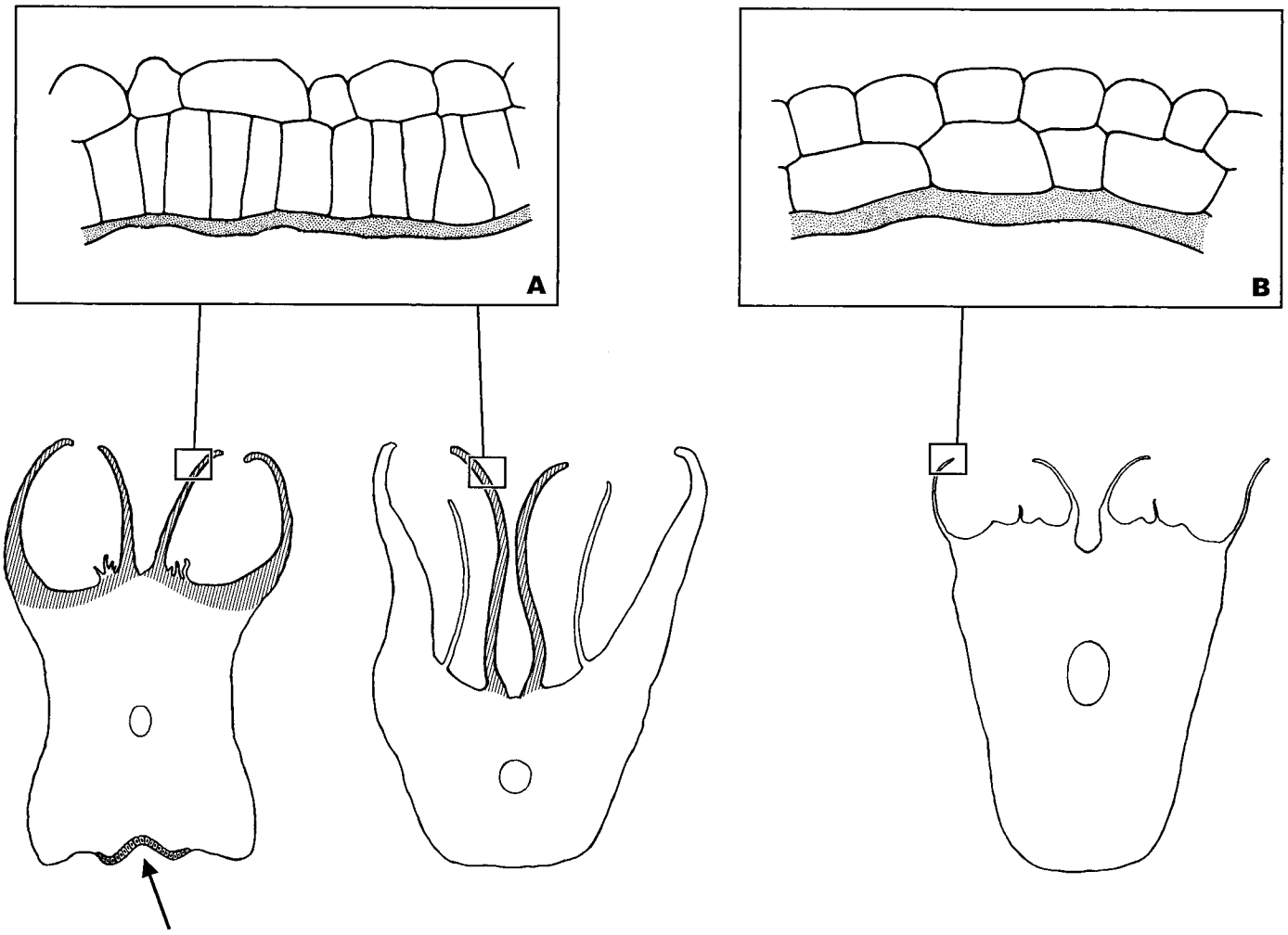

Fig. 4. Cross sections of longicidally dehiscent anthers of, from left to right, *Memecylon caeruleum* (Memecylaceae), *Pternandra caerulescens* (Kibessieae, Melastomataceae), and *Beccarianthus* sp. (Astronieae, Melastomataceae). In *Memecylon* and *Pternandra*, locules open by a fibrous endothecium (hatched). Their walls (inset A, ×400) consist of an epidermis, a fibrous endothecium, and a 1–2-layered tapetum (stippled) that in mature anthers has degenerated. In *Memecylon*, the endothecium encloses the entire locule, while in *Pternandra*, only the ventral half of each locule has an endothecium. The arrow points to the dorsal connective gland that characterizes Memecylaceae stamens. Mature locule walls in *Beccarianthus* (inset B, ×400) lack an endothecium.

Table 3 in Renner, 1993). These traits are not found in the CAROP clade or Melastomataceae (except that *Pternandra* has the phloem islands) and can serve to distinguish Memecylaceae from Melastomataceae in cases where a look at the leaf venation does not suffice.

In the current DNA data, Memecylaceae are represented by two species of *Memecylon*, one from Madagascar and one from Southeast Asia, and two species of *Mouriri*, one from the Amazon basin and the other from Puerto Rico. Two Memecylaceae from Africa (*Warneckea membranifolia, Memecylon cogniauxii*) were sequenced for *ndhF*, and in an *ndhF* tree they group with the other species of *Memecylon.* We are sequencing additional species of Memecylaceae for a low-copy nuclear gene to further test the position of *Pternandra.*

Relationships and major morphological transitions within Melastomataceae—Melastomataceae form a monophyletic clade that is supported morphologically by the fixation of acrodromal venation (Fig. 2). The family appears to be the largest clade of flowering plants characterized by this type of venation; only a few isolated taxa, for example, *Heterocentron, Sonerila, Loreya nigricans,* and *Macairea rufescens,* have pinnate venation (Renner, 1989a, 1993).

The next deepest split in the family is that between *Pternandra* and all other Melastomataceae (compare the phylogram, Fig. 2). Melastomataceae above *Pternandra* are characterized by lack of an endothecium in mature anthers. The absence of endothecia in Melastomataceae has often been noted (Ziegler, 1925; Matthews and Maclachlan, 1929; Subrananyam, 1948; Favarger, 1952; Eyde and Teeri, 1967; G. Clausing and S. S. Renner, personal observations), but *Pternandra* had not been investigated prior to this study. It has a fibrous endothecium resembling that of Memecylaceae (compare illustrations in Venkatesh, 1955), except that in *Pternandra* the endothecium surrounds the ventral half of each locule, whereas in *Memecylon* it encloses the entire locule (Fig. 4). Memecylaceae anthers open by slits in *Memecylon* (Fig. 3d) and by short, drop-shaped slits that function as pores in *Mouriri.* By contrast, most Melastomataceae have anthers that open by pores. Melastomataceae pores develop in a patch at the tip of

the anthers, where the epidermis is reduced and exposed mesophyll dries out and shrivels up. Poricidal dehiscence is an adaptation to pollinators capable of collecting pollen by high-frequency vibration of stamens (Harris, 1905; Buchmann and Buchmann, 1981; Renner, 1989b, 1990a; Gross, 1993; Larson and Barrett, 1999). Poricidally dehiscent Memecylaceae and Melastomataceae are both pollinated by pollen-collecting bees, but they may have acquired this mode of pollination independently. Unfortunately, nothing is known about the mode of pollen collection in *Pternandra* and *Astronia.*

A morphology-based cladistic analysis showed the Southeast Asian Astronieae—*Astronia, Astronidium, Astrocalyx,* and *Beccarianthus* (together 150 spp.)—as sister to all Melastomataceae except *Pternandra,* which had been designated as the functional outgroup (Renner, 1993). This placement of Astronieae was supported by the fixation of poricidal anther dehiscence and axillary placentation in the sister clade to Astronieae (Fig. 2). Astronieae anthers open by longitudinal slits (Fig. 3b), albeit without the help of an endothecium. Their capsules have basal to basal-axile placentas (Maxwell and Veldkamp, 1990a, b). This morphological topology is strongly supported by the molecular data (Fig. 1).

Of the major clades found within the higher Melastomataceae, two had been proposed based on morphology, viz. Melastomeae sensu lato (uniting the neotropical Tibouchineae with the paleotropical Osbeckieae) and a Microlicieae + Melastomeae sister-group relationship (Renner, 1993), but others, such as the Blakeeae + Dissochaeteae (including Sonerileae) clade, contradict morphological hypotheses. Also, although current sampling of Miconieae and Sonerileae, tribes that each comprise 27–30 genera, is sparse, our data refute Renner's (1993) merging of Miconieae with Dissochaeteae and of Sonerileae with Bertolonieae. The former two tribes were thought to uniquely share fleshy berries. However, an anatomical comparison of fruits of Miconieae and Dissochaeteae (Clausing, Meyer, and Renner, 2000) has shown that they are heterogeneous, in agreement with an independent evolution of berries from capsules in the paleotropical Dissochaeteae and neotropical Miconieae.

The traditionally recognized Bertolonieae (*Bertolonia, Diplarpea, Macrocentrum, Monolena, Salpinga,* and *Triolena* and the phenetically more isolated *Maguireanthus, Opisthocentra, Tateanthus,* and *Boyania*; Wurdack, 1964) are predominantly herbaceous and share (usually) triquetrous capsules, ovaries with apical scales surrounding the style, and (often) scorpioid inflorescences. These characters were thought to unite them with the paleotropical Sonerileae. That this needs to be reevaluated is suggested by the widely separate placements of *Bertolonia*/*Triolena* and *Monolena* from *Macrocentrum,* and of Bertolonieae from Sonerileae.

Instead of grouping with Bertolonieae, Sonerileae sensu stricto (*Amphiblemma, Calvoa, Gravesia, Phyllagathis*) and Oxysporeae (*Blastus, Driessenia*) were found to be nested within Dissochaeteae (*Diplectria, Medinilla*; Figs. 1 and 2). That Oxysporeae and Sonerileae form a close alliance and should be merged has been pointed out repeatedly (van Vliet, 1981; Renner, 1993). The separation of these tribes was based on whether capsules were more or less round and had a conical apex (Oxysporeae) or strongly 3–5-angled with a concave apex (Sonerileae). Several genera, e.g., *Bredia* and *Driessenia,* have been moved back and forth by different workers, indicating the tribes' problematic distinction (compare Cogniaux, 1891; Diels, 1932; Hansen, 1985). On the other hand, the evolution of the capsular-fruited and partly herbaceous Sonerileae/Oxysporeae from the berry-fruited and often climbing Dissochaeteae implied by molecular topologies is surprising. However, the same nesting is found in a larger *ndhF* analysis that includes species from ten genera of Dissochaeteae and nine of Sonerileae/Oxysporeae (Clausing, 1999; Clausing and Renner, 2001). Also, fruit characters are highly labile in the family and conserve little phylogenetic signal (Clausing, Meyer, and Renner, 2000).

The three sampled genera of Merianieae, on the other hand, form a robust clade, sister to Miconieae. Merianieae have large dorsal connective spurs and elongate cuneate seeds, and these merianoid characters should now be compared to stamens and seeds of Miconieae for clues of derivation from shared ancestral morphologies. The sister-group relationship of the predominantly Andean Merianieae and Miconieae is also seen in a nuclear internal transcribed spacer phylogeny (Clausing, 1999).

Among the groups that agree with earlier morphological hypotheses are the robust clade formed by Microlicieae and Melastomeae (including *Rhexia*). This sister-group relationship is supported by a stamen character, namely basally prolonged connectives (Fig. 3c) that serve as a hinge between pollen sacs and filament and that appear uniquely shared by these two groups (Renner, 1993). Connectives in Melastomataceae, however, are in need of reinvestigation. For example, it is unclear whether there are anatomical or ontogenetic differences between the independently derived basal-ventrally prolonged connectives in Dissochaeteae (*Macrolenes, Dissochaeta*) and the ones of Microlicieae and Melastomeae. These hinges between pollen sacs and filaments, termed pedoconnectives by Jacques-Félix (1953, 1981, 1994), increase the flexibility of anther positioning during anthesis, facilitate the bees' hold on the androecium during vibration, and standardize bee position to ensure stigma contact. Pedoconnectives and their often differentially colored appendages also function to enhance the flowers' visual display (Renner, 1989b; Larson and Barrett, 1999).

Microlicieae sequenced so far share a 66-bp deletion in their *ndhF* sequences. Morphologically, they comprise a cohesive assemblage of genera centered in south-central Brazilian savannas. Potentially synapomorphic are their straight or slightly winged seeds with a foveolate surface (SEMs: Whiffin and Tomb, 1972; Renner, 1990b). Their sister group, Melastomeae sensu lato (i.e., including Tibouchineae, Osbeckieae, and now also *Rhexia*), has two morphological synapomorphies, cochleate seeds and ovaries crowned by persistent trichomes. Cochleate seeds contain curved (campylotropous) embryos, the likely adaptive advantage being that campylotropous seeds contain embryos twice as long as the seed itself, giving better opportunities for early seedling establishment (Bouman and Boesewinkel, 1991). The significance of ovary apex hairs or scales has not been studied, but such emergences may afford protection against insects that oviposit into developing ovaries. These characters are lacking only in a few odd species in the 45–47 genera. Thus, *Rhexia* has glabrous ovaries and lacks ventral connective appendages and so do a few other Melastomeae, which, however, all have the typical cochleate seeds. Because of these and other unusual traits, such as occasionally atropous ovules (Etheridge and Herr, 1968) and mature unilocular anthers, *Rhexia* had been assigned tribal rank, either together with *Monochaetum* and *Pachyloma* (Cogniaux, 1891) or by itself (Renner, 1993). Molecular data now solidly place *Rhexia* and *Monochaetum* in Melastomeae (*Pachyloma* has not been sampled) and indicate that *Rhexia* is sister to the Central

American *Arthrostemma. Arthrostemma* and *Rhexia* share a strongly costate-tuberculate seed testa (Whiffin and Tomb, 1972), four-merous flowers (also found elsewhere in Melastomeae), and hypanthia with sparse glandular pubescence. *Arthrostemma* comprises seven species in Central America, while *Rhexia* consists of 11 species in North America and is the only genus of Melastomataceae endemic in the northern hemisphere.

Another genus placed in Melastomeae by the molecular data, but with ovoid rather than cochleate seeds, is *Centradenia. Centradenia* was treated in Microlicieae by Cogniaux (1891) and in Bertolonieae [sub Sonerileae sensu lato] by Almeda (1977, 1997a) and following him Renner (1993), but it now appears that its seed morphology represents a secondary modification.

Unexpectedly, the African and Asian Melastomeae (*Dichaetanthera, Dissotis, Melastoma,* and *Osbeckia*; Fig. 2) form a clade that is robustly nested within neotropical Melastomeae. A relatively recent derivation of Old World Melastomeae from New World Melastomeae, ~15–12 million years ago judging from molecular clock-based estimates based on a dense sample of *ndhF* sequences from New World and Old World Melastomaeae (Renner and Meyer, in press), agrees with Almeda's (1997b) suggestion that paleotropical Melastomeae retain the same base chromosome number found in many neotropical Melastomeae. However, there is much intrageneric polyploidy and dysploidy. The African-Asian clade also has not yet acquired obvious morphological synapomorphies.

Within Melastomeae, the deepest splits are between *Arthrostemma* + *Rhexia, Nepsera* + *Aciotis,* and the remaining genera (Figs. 1 and 2). *Nepsera* and *Aciotis* both prefer wet habitats, have four-merous flowers, acute white petals, and much-branched fragile inflorescences, traits in which they differ from most Melastomeae, which usually have five-merous flowers, purple petals, and more robust inflorescences than those of *Nepsera* and *Aciotis.* However, denser sampling of neotropical Melastomeae is needed to break up the long branch currently leading to these two genera.

Perspectives—This first molecular phylogenetic assessment of Melastomataceae and Memecylaceae shows that leaf venation, stamen anatomy and morphology, and seed shape and size underwent major transformations early during the families' history, while fruit fleshiness and mode of dehiscence were modified frequently and more recently. The ancestor of Melastomataceae likely had capsular fruits with numerous small seeds, this being the condition in families most closely related to Melastomataceae + Memecylaceae, and in basalmost Melastomataceae (*Pternandra, Astronia*). Within Melastomataceae, berries appear to have evolved from capsules minimally four times, namely in Miconieae, Blakeeae, within Dissochaeteae, and within *Melastoma* (Meyer, 2000; Clausing, Meyer, and Renner, in press). The molecular trees also imply that dorsally massive connectives are ancestral in Memecylaceae and Melastomataceae, characterizing these families and their closest relatives. Future work will have to test the explicit hypotheses that (1) melastome stamen evolution went from dorsally enlarged connectives to basal-ventrally enlarged ones; (2) loss of an endothecium preceded poricidal dehiscence (perhaps as a preadaptation); (3) herbaceous, capsular-fruited Sonerileae/Oxysporeae evolved from woody *Dissochaeta*-like plants with fleshy fruits; and (4) African and Asian Melastomeae are derived from neotropical Melastomeae. Biogeographic implications of a larger *ndhF* phylogeny for the family, especially with regard to the apparently recent diversification of African and Madagascan melastomes as judged by fossil-calibrated genetic distances, are considered elsewhere (Renner, Clausing, and Meyer, in press).

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