

MELASTOMEAE COME FULL CIRCLE: BIOGEOGRAPHIC RECONSTRUCTION AND MOLECULAR CLOCK DATING

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Abstract.—*Rhexia*, with 11 species in the Coastal Plain province of North America, is the only temperate zone endemic of the tropical eudicot family Melastomataceae. It is a member of the only pantropical tribe of that family, Melastomeae. Based on the chloroplast gene *ndhF*, we use a fossil-calibrated molecular clock to address the question of the geographic origin and age of *Rhexia*. Sequences from 37 species in 21 genera representing the tribe's geographical range were analyzed together with five outgroups. To obtain better clade support, another chloroplast region, the *rpl16* intron, was added for 24 of the species. Parsimony analysis of the combined data and maximum-likelihood analysis of *ndhF* alone indicate that the deepest split is between *Rhexia* plus its sister group, a small Central American genus, and all other Melastomeae. Old World Melastomeae are monophyletic and nested within New World Melastomeae. Although likelihood-ratio tests of clock and nonclock substitution models for the full or moderately pruned datasets rejected the clock, these models yielded identical topologies (for 30 taxa) with few significantly different branch lengths as assessed by a Student's *t*-test. Age estimates obtained were 22 million years ago (Mya) for the divergence of *Rhexia* from its sister group, 12 Mya for the dispersal of Melastomeae from the New World to West Africa, and 1 Mya for the diversification of *Melastoma* in Southeast Asia. The only other genus of Melastomeae to have reached Southeast Asia from Africa or Madagascar is *Osbeckia*. The age and geographic distribution of fossils, which come from Miocene sites throughout Eurasia, suggest that Melastomeae once ranged from Eurasia across Beringia to North America from whence they reached South America and subsequently Africa and Southeast Asia. Climate deterioration led to their extinction in the Northern Hemisphere, with *Rhexia* possibly surviving in Coastal Plain refugia.

Key words.—Biogeography, fossil calibration of molecular clocks, maximum-likelihood phylogeny, *Melastoma*, *ndhF* gene, *Rhexia*, *rpl16* intron.

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The North American Coastal Plain phytogeographic province, which includes Virginia, the Carolinas, southern Georgia, and most of Florida, is thought to have acquired its endemic taxa relatively recently because it was repeatedly flooded during the Pleistocene. A few presumed older lineages are thought to have persisted in noninundated refugia (Thorne 1993). One of the endemics is *Rhexia*, a genus of Melastomataceae with 11 species of which 10 are restricted to the southeastern part of the Coastal Plain and one ranges north to Ontario (Kral and Bostik 1969). Except for *Rhexia*, Melastomataceae are restricted to the tropics, where they comprise approximately 4500 species in 150–166 genera (Renner 1993). *Rhexia* species are perennial herbs that usually occur in bogs; their common name, meadow beauties, refers to their striking purple, white, or yellow flowers. The seeds are wind dispersed. Because the subtropical tip of Florida harbors a number of tropical elements, including one species of Melastomataceae introduced from Cuba or the Bahamas (*Tetrazygia bicolor* [Mill.] Cogn.), a likely explanation for the range of *Rhexia* is that it derives from a West Indian, Mexican, or Central American ancestor. To test this hypothesis and to explain the occurrence of an endemic genus in North America of an otherwise entirely tropical family, we here use a chloroplast gene phylogeny and a molecular clock approach.

Traditionally, *Rhexia*, which has unusual flowers for the family, was placed in a tribe by itself (Renner 1993) or together with *Monochaetum* and *Pachyloma* as Rhexieae (Cogniaux 1891). *Monochaetum* (45 species) ranges from Mexico to the Andes and *Pachyloma* (six species) from the Andes to

the Guayana shield. A molecular phylogeny for the family (Clausing and Renner 2001) does not support a relationship between *Rhexia* and *Monochaetum*. (*Pachyloma* has not yet been sequenced.) Rather, *Rhexia* appears closest to *Arthrostemma*, a genus of seven species in Mexico, Central America, and Colombia that belongs in Melastomeae (as does *Monochaetum*). Melastomeae, as well as *Arthrostemma* and *Rhexia*, are characterized by strongly curved (cochleate) seeds (Fig. 1) not found in any other group of Melastomataceae.

The earliest known Melastomeae are from seeds that come from a series of Eurasian Miocene deposits (Dorofeev 1960, 1963, 1988; Collinson and Pinggen 1992; Dyjor et al. 1992; Fairon-Demaret 1994; Mai 1995, 2000). The seeds (Fig. 1), which are 0.8–1.5 mm long, exhibit large testa tubercles arranged in rows, similar to what is seen in *Rhexia*, *Arthrostemma*, and *Pachyloma* (cf. Whiffin and Tomb's [1972] figs. 15–21 with our Fig. 1). This type of testa ornamentation is synapomorphic for the *Rhexia*-*Arthrostemma*-*Pachyloma* subclade of Melastomeae, showing that these fossils postdate the initial radiation of Melastomeae. The seeds therefore constrain the minimum age of the tribe itself.

Melastomeae (including Rhexieae) contains about 550 species in 48 genera and is the only tribe of Melastomataceae that is pantropically distributed. Based on morphology and geography, we selected representatives from throughout the tribe's range to reliably place *Rhexia* and ensure that the phylogeny would include the first-diverging Melastomeae and thus the basalmost node to which the fossils would be assigned for calibration purposes. Unexpectedly, *Rhexia* and *Arthrostemma* themselves were found to be the first-branch-

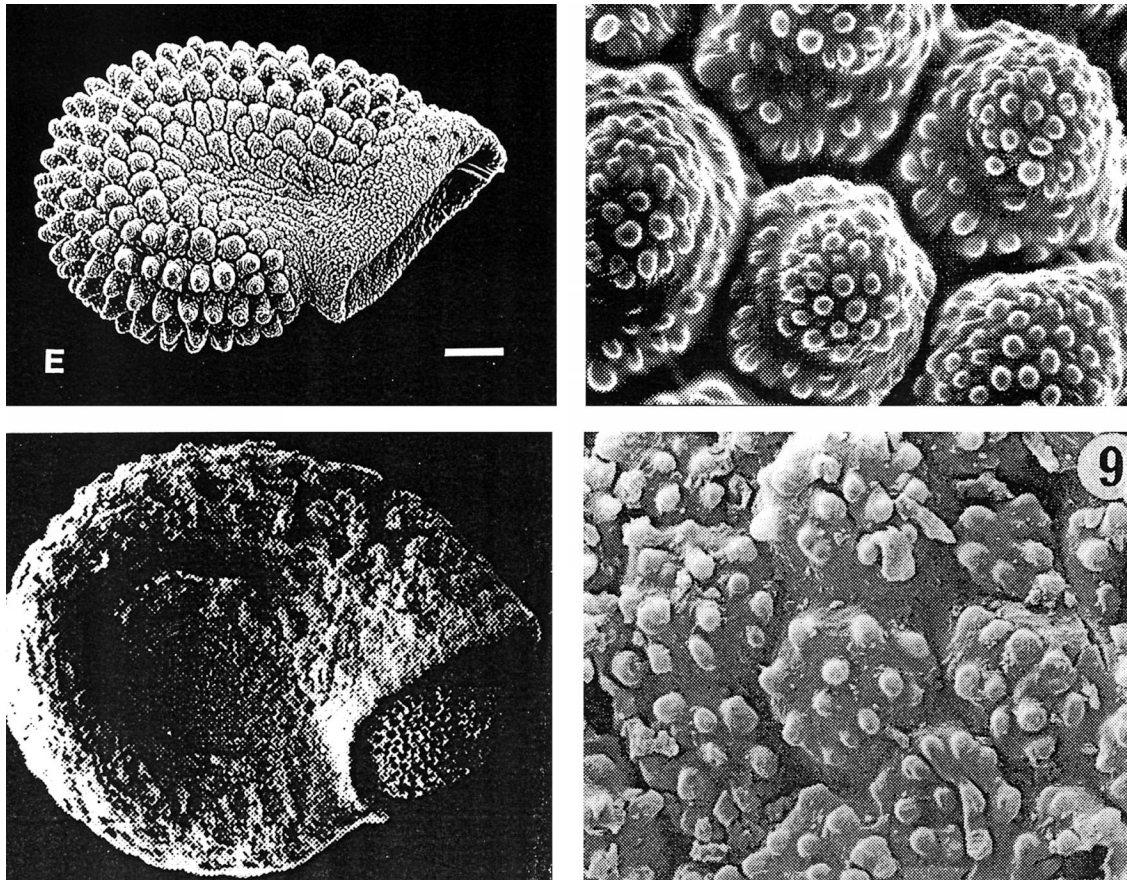


FIG. 1. Extant and fossil seeds of Melastomeae. Top left: *Pterolepis alpestris* from Brazil. Bar = 100 μ m. Top right: *Pterolepis riedeliana* from Brazil, testa detail (both from Renner 1994). Lower left: Early Mid-Miocene seed from Kreuzau, Germany (approximately same magnification as extant seed). Lower right: Testa detail (both reproduced with permission from Collinson and Pinggen 1992).

ing lineage of Melastomeae, whereas South American, African, and Southeast Asian Melastomeae represent much younger lineage divergences. The Eurasian range of the fossils, the derived placement of African and Asian taxa in the phylogenetic tree, and the age estimates obtained suggest a highly dynamic biogeographic history of Melastomeae.

MATERIALS AND METHODS

Taxon Sampling and Sequencing

We sequenced the *ndhF* gene in 37 species of Melastomeae, representing 21 genera (Table 1). All Asian genera of Melastomeae are represented. Of the 17 African and Madagascan genera, eight are not represented: the monotypic *Cailliella*, *Dinophora*, *Dionychastrum*, *Guyonia*, *Nerophila*, and *Pseudosbeckia* from Africa, *Amphorocalyx* (five spp.) from Madagascar, and *Antherotoma* (15 spp.) from Africa and Madagascar. Of neotropical Melastomeae, we lack 21 genera, all of them small. Five representatives of Microlicieae, Blakeeae, and Bertolonieae (Table 1) were used as outgroups based on the results of Clausing and Renner (2001). For a subset of 24 taxa, *ndhF* sequences were combined with *rpl16* intron sequences obtained from the same DNA samples to achieve better clade support.

DNA samples were isolated from silica-gel dried or fresh

leaf material using DNeasy plant mini kits (Qiagen, Valencia, CA) or NucleoSpin plant DNA extraction kits (Macherey-Nagel, Dören, Germany) according to the manufacturers' instructions. Polymerase chain reaction (PCR) amplification followed standard protocols. The *rpl16* intron was amplified as described in Clausing and Renner (2001). The *ndhF* gene was amplified with primers developed by Olmstead and Sweere (1994). We amplified the region between positions 972 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). PCR products were purified either by running the entire product on a low-melting-point agarose gel and then recovering the DNA with 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 used the ABI Prism Dye Terminator ready reaction kit (Perkin Elmer, Norwalk, CT), using 2.5 ng of primer in a 5- μ L reaction volume. The dye was removed by ethanol precipitation and samples then run on ABI 373 or ABI 377 automated sequencers at the universities of Mainz (*ndhF*) or Missouri–St. Louis (*rpl16*). Consensus sequences were constructed using Sequencher software (ver. 3.1; GeneCodes Corp., Ann Arbor, MI). Alignment was done manually. A single region of 51 base pairs (bp) in the *rpl16*

matrix and one region of 4 bp in the *ndhF* matrix were excluded from all analyses because of doubtful alignment or ambiguous base calls, respectively.

Inference of Phylogeny

Phylogenetic analyses of the aligned sequences were conducted with PAUP* (ver. 4.0b.4a, Swofford 2000). For parsimony analyses, characters were unordered and equally weighted, gaps were treated as missing data. Heuristic searches were run using 10 random taxon addition sequence replicates with one tree held at each step, followed by tree-bisection-reconnection (TBR) swapping. The steepest-descent and multiple-trees settings were in effect during all searches. Separate analyses were performed on the *rpl16* matrix and the corresponding *ndhF* matrix to test for conflict among well-supported nodes (as assessed by bootstrap frequencies $\geq 50\%$). Bootstrap frequencies were estimated based on 1000 replications with the same settings as used in searching, except that closest taxon addition was used. In the absence of conflict, the two matrices were concatenated. The concatenated matrix was analyzed under parsimony, using the same settings as before.

To select a suitable substitution model for the *ndhF* data, we compared the likelihood scores of the single best trees found for 41 sequences (*Comolia* had been excluded, see Results) under the Hasegawa-Kishino-Yano model (HKY85; Hasegawa et al. 1985) and under the general time-reversible model (GTR; Yang 1994). Both models included a discrete approximation of a gamma (Γ) distribution with four rates to model rate heterogeneity among sites (Yang 1996) and used empirically observed base frequencies. Both also assumed some fraction of sites to remain invariant (P_{inv}). Starting trees for maximum-likelihood (ML) analyses were minimum-evolution trees (Rzhetsky and Nei 1992; implemented in PAUP) generated using log-determinant genetic distances (Lockhart et al. 1994). The swapping strategy employed was TBR swapping. For both models, HKY85 and GTR, all model parameters were estimated simultaneously with tree searching. Because the interdependence of model parameters and tree topology is weak as long as strongly supported groups are maintained, parameters can be estimated without a complete search (Yang and Kumar 1996; Sullivan et al. 1999; Sanderson and Kim 2000). We interrupted parameter estimation for the variously pruned *ndhF* datasets (see Molecular Clock Analyses) after 500 to 733 TBR swaps on the single tree in memory at the time and then swapped to completion under the estimated parameters. After complete swapping, a likelihood-ratio test (LRT) was used to evaluate whether the HKY85 model or the GTR model better explained the data.

Subsequent ML analyses all used the GTR + Γ + P_{inv} model and the model parameters found in step 1. Several searches under this model were allowed to swap to completion. Bootstrap frequencies were obtained under the minimum evolution (ME) criterion, using ML distances under the same model and parameters as used to obtain the respective tree, TBR swapping, and 1000 replicates.

Molecular Clock Analyses and Calibrations

LRTs were used to assess whether substitutions in *ndhF* could be modeled as clocklike. Because searches in which

the clock assumption was added to the model could not be swapped to completion, LRTs may have been biased against clock-enforced models. When the full 42 taxa dataset rejected the clock assumption (see Results), we sequentially took out fastest- and slowest-changing sequences (determined by visual inspection) and repeated the LRT. Pruned datasets still rejected the clock (Results). We next excluded the 120 missing or ambiguous characters and repeated the LRT. The data still rejected the clock, albeit barely.

Using PAUP's tabulation of terminal branch and internode lengths for the clock and nonclock trees, we performed a Student's *t*-test on all matchable segments to assess which and how many were significantly affected by the clock assumption. A matchable tree segment was one that linked the same two nodes or node and terminal taxon in the clock tree as it did in the nonclock tree. A few internodes in the nonclock trees were so short that standard errors on them were not calculated by PAUP* and they could therefore not be included in the test (Results). For the matchable branches and internodes, the difference in their length under the two models was divided by the standard error (taken from the nonclock model) and the result was evaluated using a two-tailed *t*-distribution. Being uncertain about the appropriate degrees of freedom, we conservatively used two standard errors as indicating significance.

To convert branch lengths in the clock tree into absolute ages, the oldest Melastomeae fossils were used to constrain the age of the basalmost node. To allow for dating uncertainties, two dates, 26 Mya and 23 Mya, were used that bracket the likely age of the oldest seeds (Discussion). The million-year chronology of Tertiary epochs used was that of Berggren et al. (1995).

Potential underestimation of the tribe's age could result from placing the oldest fossils at the basal node when they may represent a later stage during the clade's history. For Melastomeae, this problem is minimized by circumstantial evidence. Thus, the existence of Melastomeae seeds from throughout the Lower, Middle, and Upper Miocene and the Pliocene suggests that the fossil record of these seeds is quite complete. Also, the finds have been critically assessed by different workers and thus are likely correctly identified. Second, background knowledge about the derived phylogenetic position of Melastomeae within Melastomataceae (Clausing and Renner 2001) and about the Eocene (53 Mya) age of the earliest fossils of the family Melastomataceae (reviewed in Renner et al. 2001) places an upper boundary on the age of Melastomeae.

We used binomial probability theory to estimate the standard deviation (SD) of the distance from a fixed calibration node to the tips and then used this value to obtain the SDs of the estimated ages. The binomial distribution describes the number of successes for independent trials. This distribution does not account for multiple substitutions or unequal probability of change among sites. However, the model of nucleotide divergence used in these analyses takes multiple substitutions into account and thus estimates the number of independent nucleotide changes, for which a binomial distribution is therefore appropriate. The number of invariant sites affects the probability of substitution at variable sites, but has little effect on the SD of the number of substitutions.

TABLE 1. Sources of material used for the Melastomeae molecular phylogeny. Herbaria are abbreviated as follows: BONN, Bonn University; CAS, California Academy of Sciences; CAY, Cayenne University; HNU, Hanoi University; K, Kew, London; MJG, Mainz University; MO, Missouri Botanical Garden; P, Paris; and QCNE, Universidad Catolica de Quito. The 33 sequences newly generated for this study are marked with an asterisk.

Species	Voucher (Herbarium); GenBank accession numbers for <i>ndhF</i> and <i>rp116</i>	Species range
<i>Aciotis indecora</i> (Bonpl.) Triana	Sothers 347 (MO); <i>rp116</i> : AF215604*	South America
<i>Aciotis purpurascens</i> (Aubl.) Triana	Renner 2154b (QCNE); AF215561/AF322231*	South America
<i>Arthrostemma ciliatum</i> Pav. ex D. Don	Cult. BG Mainz; AF215562/ AF215605	Mexico to Andes; Greater Antilles; natu- ralized in Hawaii
<i>Bertolonia maculata</i> DC.	Cult. BG Mainz; AF215550/ AF215597	Brazil
<i>Blakea trinervia</i> L.	Cult. BG Mainz; AF215555/ AF215600	Jamaica
<i>Centradenia inaequilateralis</i> (Schlecht. & Cham.) G. Don	Cult. BG Mainz; AF215563/ AF215606	Central America
<i>Comolia coriacea</i> Gleason	Cult. BG Munich; <i>ndhF</i> : AF272799*	Venezuela
<i>Dichaetanthera arborea</i> Baker	Clausing 281 (MJG); AF272800*/AF294470*	Madagascar, genus also in Africa
<i>Dichaetanthera asperrima</i> Cogn.	Clausing 280 (MJG); AF215564/AF215607	Madagascar, genus also in Africa
<i>Dionycha bojerii</i> Naudin	Clausing 300 (MJG); <i>ndhF</i> : AF272801*	Madagascar
<i>Dissotis fruticosa</i> (Brenan) Brenan & Keay	Cult. BG Mainz; AF272802*/ AF210377*	Nigeria
<i>Dissotis grandiflora</i> (Sm.) Benth. (type of <i>Dissotis</i> subgen. <i>Dissotis</i>)	Porembski 41 (BONN); <i>ndhF</i> : AF272803*	widespread in Africa
<i>Dissotis rotundifolia</i> (Sm.) Triana See under <i>Heterotis</i>		
<i>Heterocentron elegans</i> (Schldl.) Kuntze	Cult. BG Mainz; AF272804*/ AF325926*	Central America
<i>Heterocentron subtriplinervium</i> (Link & Otto) A. Brown & Bouché	Cult. BG Mainz; AF215566/ AF210374	Central America, naturalized in Hawaii
<i>Heterotis rotundifolia</i> (Sm.) Jacq.-Félix (= <i>Dissotis rotundifolia</i> (Sm.) Triana)	Cult. BG Mainz; AF215565/ AF270745	widespread in Africa, naturalized in Bra- zil, Indonesia, and Hawaii
<i>Lavoisiera cordata</i> Cogn.	Almeda 7798 (CAS); AF215582/AF210371	Brazil
<i>Melastoma beccarianum</i> Cogn.	Clausing 249 (MJG); <i>ndhF</i> : AF272805*	Borneo
<i>Melastoma crinitum</i> Naudin	Meyer 9535 (MJG); <i>ndhF</i> : AF272806*	Indonesia, Malaysia, Philippines
<i>Melastoma cyanoides</i> Sm. See under <i>Otanthera</i>		
<i>Melastoma dodecandrum</i> Lour.	Yao 9267 (P); <i>ndhF</i> : AF272808*	South China, Vietnam, Hong Kong
<i>Melastoma imbricatum</i> C. B. Clarke	Cult. BG Mainz; <i>ndhF</i> : AF272809*	SE Asia, Philippines, Australia
<i>Melastoma malabathricum</i> L.	Meyer 9641 (MJG); <i>ndhF</i> : AF272810*	Mauritius, Seychelles, SE Asia, Austra- lia, S. Pacific Ocean; naturalized in Hawaii
<i>Melastoma orientale</i> Guillaumin	Meyer 9617 (MJG), cult. BG Mainz; <i>ndhF</i> : AF272811*	Thailand, Laos, Vietnam
<i>Melastoma pellegrinianum</i> (H. Boissieu) K. Meyer	Meyer 9619 (MJG); <i>ndhF</i> : AF272812*	Thailand, Vietnam, Philippines
<i>Melastoma sanguineum</i> Sims	Tu 0033 (HNU), cult. BG Mainz; AF270754/AF270751	Indochina, Sumatra, Borneo, and Moluc- cas
<i>Melastoma</i> × <i>sanguineum</i> Sims	Cult. BG Mainz; AF215567*/ AF215608*	Cultivated BG Mainz
<i>Melastoma septemnervium</i> Lour.	Meyer 9601 (MJG); <i>ndhF</i> : AF272813*	Japan, South China, Taiwan, Vietnam, naturalized in Hawaii
<i>Melastomastrum capitatum</i> (Vahl) A. & R. Fern. (= <i>Dissotis capitata</i> (Vahl) Hook.)	Kayombo 1158 (MO); <i>ndhF</i> : AF272814*	Centered in West Africa
<i>Microlepis oleaefolia</i> (DC.) Triana	Almeda 7746 (CAS); <i>ndhF</i> : AF272815*	Brazil
<i>Microlicia fasciculata</i> Cogn.	Almeda 7717 (CAS); AF215583/AF210370	Brazil
<i>Monochaetum calcaratum</i> (DC.) Triana	Cult. BG Munich; AF215568/ AF210372	Mexico to Central America

TABLE 1. Continued.

Species	Voucher (Herbarium); GenBank accession numbers for <i>ndhF</i> and <i>rpl16</i>	Species range
<i>Nepsera aquatica</i> (Aubl.) Naud.	Miller & Morello 8853 (MO); AF215569/AF210373	Central and South America, Antilles
<i>Osbeckia aurata</i> H. Perr. See under <i>Rousseauxia</i>		
<i>Osbeckia chinensis</i> L.	Meyer 9643 (MJG); AF215570/ AF210378	India, Indochina to Australia and Japan
<i>Osbeckia nepalensis</i> Hook.	Cult. BG Mainz; <i>ndhF</i> : AF272817*	India to Indochina
<i>Osbeckia stellata</i> Ham. ex Ker-Gawl.	Meyer 9602 (MJG); <i>ndhF</i> : AF272818*	India to Indochina
<i>Otanthra cyanoides</i> (Sm.) Triana (= <i>Melastoma cyanoides</i> Sm.)	Halford Q786 (K); <i>ndhF</i> : AF272807*	SE Asia, Philippines, Australia
<i>Pterolepis glomerata</i> (Rottb.) Miq.	Miller & Morello 8845 (MO); AF215571/AF210376	South America, naturalized in Hawaii
<i>Rhexia mariana</i> L.	Cult. J. J. Wurdack; AF272819*/AF323723*	Coastal plains province of North Amer- ica
<i>Rhexia virginica</i> L.	Cult. J. J. Wurdack; AF215587/ AF215623	Coastal plains province of North Amer- ica
<i>Rhynchanthera grandiflora</i> (Aubl.) DC.	Prévost 3281 (CAY), cult. BG Mainz; AF215584/AF210369	Central and South America
<i>Rousseauxia aurata</i> (H. Perr.) Jacq.-Félix (= <i>Osbeckia auro-</i> <i>ta</i> H. Perr.)	Clausing 324 (MJG); <i>ndhF</i> : AF272816*	Madagascar
<i>Tibouchina longifolia</i> (Vahl) Baillon ex Cogn.	Cult. BG Bonn; AF215572/ AF210375	Central and South America
<i>Tibouchina urvilleana</i> (DC.) Cogn.	Cult. BG Mainz; AF272820*/ AF322234*	Brazil, naturalized in Hawaii
<i>Tristemma mauritianum</i> J.-F. Gmelin	Clausing 292 (MJG); AF272821*/AF322233*	Genus centered in West Africa, this spe- cies in Madagascar and São Tomé

According to the binomial distribution, the number of nucleotide substitutions (S) is equal to the product of the total number of nucleotides in a sequence (N) times the proportion of nucleotides substituted (p). Thus, $S = Np$. The SD of this value is the square root of $Np(1 - p)$, or $SD(S) = \sqrt{Np(1 - p)}$. The SD of the number of nucleotides substituted divided by the total number of nucleotides is the SD of the proportion of nucleotide substitutions. Thus, $SD(p) = \sqrt{p(1 - p)/N}$.

RESULTS

Inference of Phylogeny

The combined *rpl16* and *ndhF* data for 24 taxa yielded an internally more supported phylogenetic hypothesis than did either dataset alone. These results are reported first, followed by the results of a ML analysis of the 42 *ndhF* sequences subsequently used in molecular clock analyses.

The 24 aligned *rpl16* sequences contributed 991 characters, 144 of them parsimony informative and 114 varying autapomorphically. *ndhF* contributed 1002 characters, 127 of them informative and 112 variable. Consensus trees of the 440 or 36 parsimony trees obtained, respectively, from the 24 *rpl16* sequences or the 24 *ndhF* sequences were similar to each other and to the tree obtained from the combined data. Analysis of the combined *rpl16* and *ndhF* data for 24 taxa yielded a single island of 26 equally parsimonious trees ($L = 763$, $CI = 0.76$, $RI = 0.78$; Fig. 2). The combined data support the monophyly of Melastomeae, albeit weakly (63% bootstrap). Old World Melastomeae form a clade (89%) that

is deeply nested within neotropical Melastomeae. Within Old World Melastomeae, West African and Madagascan groups are basal to a clade that includes the Indochinese and Mallesian taxa (Fig. 2).

The 42 aligned *ndhF* sequences comprised 1012 bp of which four were excluded (Materials and Methods). Mean base composition was similar across taxa ($A = 0.29-0.31$; $C = 0.14-0.16$; $G = 0.15-0.17$; $T = 0.38-0.40$). The LRT comparing the HKY85 and GTR models showed that the latter better explained the data. Likelihood scores were -4372.00 under HKY85 + Γ and -4267.82 under GTR + Γ (for trees with 41 taxa; *Comolia* was excluded). Thus, $\ln L = 2(4372.00 - 4267.82) = \chi^2 = 208.36$ ($P < 0.005$, $df = 4$).

The single highest likelihood tree obtained after complete TBR swapping under the GTR + Γ + P_{inv} model is shown in Figure 3. Melastomeae form a clade (83% bootstrap) that is sister to Microlicieae (73% bootstrap). Old World Melastomeae are nested within New World Melastomeae. As found in the *ndhF* + *rpl16* parsimony analysis, the deepest split within Melastomeae is between *Rhexia* + *Arthrostemma* and all other Melastomeae, but this now has low internal support (55% bootstrap). Among Old World Melastomeae, a West African group (*Melastomastrum*, *Tristemma*) branches off first. The Southeast Asian *Melastoma*, with 10 of its 21 species sampled (the single species of *Otanthra* has been transferred into *Melastoma* based on the molecular data presented here; Meyer 2001), is monophyletic (82%) and falls in a polytomy with African, Madagascan, Indian, and Indochinese taxa (*Dionycha*, *Dissotis*, *Heterotis*, *Osbeckia*, *Rousseauxia*).

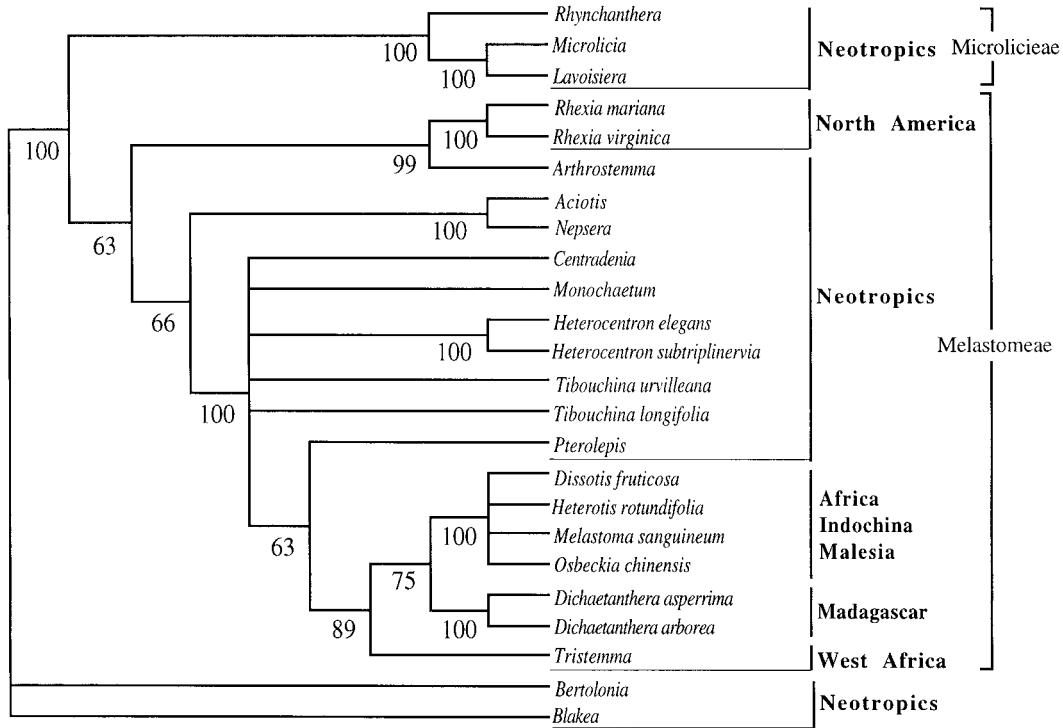


FIG. 2. Strict consensus of 26 equally parsimonious trees (L = 763, CI = 0.76, RI = 0.78) found for combined *ndhF* and *rpl16* cpDNA sequences of 24 Melastomeae and outgroups. Values below branches indicate bootstrap support > 50% based on 1000 replications.

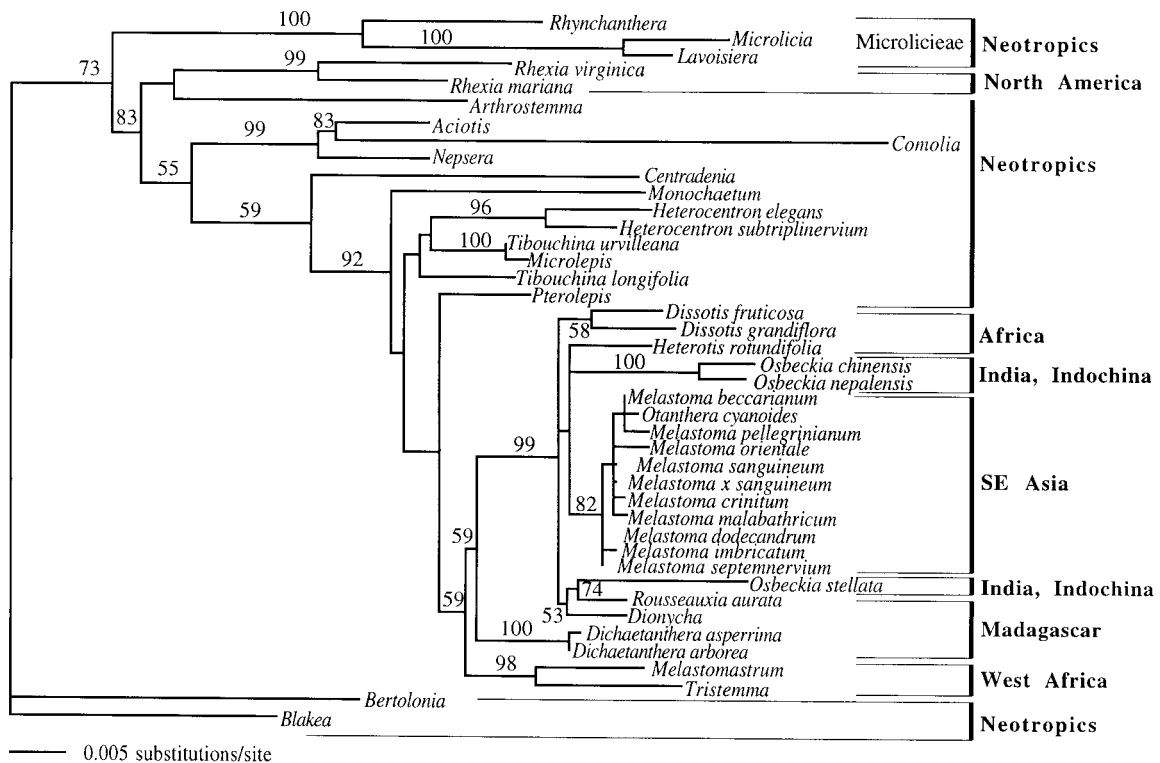


FIG. 3. Maximum likelihood (ML) tree for 42 Melastomeae and outgroup *ndhF* sequences under the GTR + Γ + P_{inv} substitution model. Values at branches indicate bootstrap support based on 1000 replications under minimum evolution criteria using ML distances and TBR swapping.

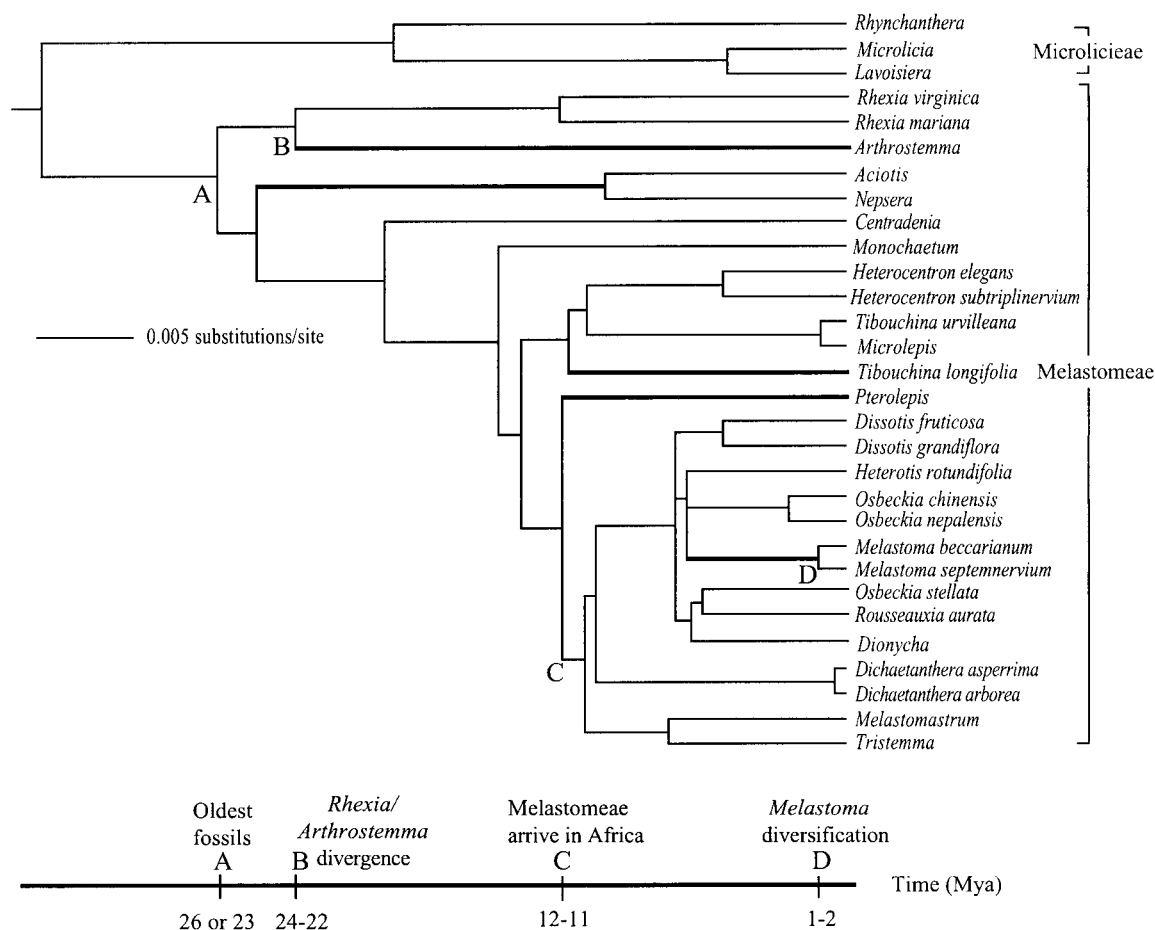


FIG. 4. Clock tree for 30 Melastomeae and outgroup *ndhF* sequences under the GTR + Γ + P_{inv} substitution model. The earliest Melastomeae fossils are between 26 and 23 million years old and constrain the age of node A. Fixing node A at either 26 or 23 million years yields the ages shown on the time axis for nodes B, C, and D. Branches shown in bold change significantly in length between nonclock and clock trees. For standard deviations on estimates, see Table 2.

Molecular Clock Analyses and Calibrations

Comolia and the distant outgroups (*Blakea* and *Bertonia*) form long branches in the analysis, whereas *Melastoma* sequences hardly differ from each other (Fig. 3). We therefore sequentially excluded these taxa and reestimated model parameters for the remaining taxa on the tree shown in Figure 3 (Materials and Methods). With just *Comolia* excluded, a LRT rejected the clock model ($\ln L = 2[4317.3515 - 4267.8201] = \chi^2 = 99.06$, $P < 0.001$, $df = 39$), but clock and nonclock topologies (after incomplete searches) were near identical. A Student's *t*-test comparing the 63 matchable branches in the clock and nonclock trees showed that six (10%) differed by more than two standard errors ($P < 0.05$) under the clock model. Five of these are marked in Figure 4, one was the branch leading to the distant outgroups. (Note that 5% of the branches are expected to differ by chance alone.) Seven internodes were too short for standard errors on them to be defined.

After exclusion of *Comolia*, *Blakea*, and *Bertonia*, and all but two *Melastoma* species and parameter reestimation for the remaining 30 taxa, a LRT still rejected the clock ($\ln L = 2[3784.4044 - 3755.4220] = \chi^2 = 57.96$, $P < 0.001$, $df =$

28, $P_{inv} = 0.50$, $\alpha = 1.18$). The topology of the single highest likelihood tree found under the clock (after completed TBR swapping) and that of the single tree found without a clock (when the search was aborted after 13059 swaps) were identical. This suggests that inefficient searching under the clock model was not responsible for the rejection of the clock assumption. Figure 4 shows the clock tree found for the 30 taxa. Branch length comparisons of 55 matched branches in the clock and nonclock tree showed that the lengths of five (9%; marked in Fig. 4) differed by more than two standard errors under the clock. Four internodes were too short for comparison. When all 120 missing and ambiguous characters were excluded from the 30-taxon dataset, TBR searches aborted after 26,726 swaps for the nonclock search and 21,688 swaps for the clock search, both yielded the same topology as found before. The clock assumption remained rejected, albeit barely ($\ln L = 2[3252.5208 - 3225.7931] = \chi^2 = 53.46$, $P < 0.001$, $df = 28$).

To convert branch lengths in the clock tree into times, we fixed the basal Melastomeae node (node A in Fig. 4) at either 26 or 23 Mya. This allows for uncertainty in the dating of the earliest seeds, which come from Early Miocene (23 Mya)

TABLE 2. Age estimates for key events in the history of Melastomeae. Branch lengths in the clock tree (Fig. 4) were calibrated by dividing the distance from node A to the tip (0.03296 substitutions/site) by 26 or 23 million years, alternative ages for the earliest Melastomeae fossils. This yielded rates a (0.0013 substitutions/site/million years) and b (0.0014 substitutions/site/million years), which in turn were used to calculate ages for nodes B, C, and D. Standard deviation (SD) was calculated as explained in Materials and Methods.

Node	GTR + Γ + P_{inv} distance to tip \pm SD	Time (million years) \pm SD using rate a	Time (million years) \pm SD using rate b
B: <i>Rhexia</i> / <i>Arthrostemma</i> divergence	0.03098 \pm 0.0055	24 \pm 4	22 \pm 4
C: Arrival in West Africa	0.01494 \pm 0.0038	11.5 \pm 3	10.7 \pm 3
D: Onset of <i>Melastoma</i> diversification	0.00140 \pm 0.0012	1.1 \pm 0.9	1 \pm 0.9

floras in Belorussia, the Tambov region, and Tomsk in western Siberia (Dorofeev 1960, 1963, 1988). Because the Belorussian strata that contain *Melastomites tertarius* are either Late Oligocene or Early Miocene, we used 26 Mya as an alternative calibration date. Fixing node A (Fig. 4) at 26 or 23 Mya yielded substitution rates of 0.0013 or 0.0014 substitutions/site/million years, respectively. Table 2 shows the age estimates (with their SD) obtained under these rates for the divergence of *Rhexia* from *Arthrostemma*, the arrival of Melastomeae in Africa and the onset of *Melastoma* diversification in Southeast Asia.

If Melastomeae had arrived in Africa overland, they would have to be at least 90 million years old (Pitman et al. 1993). To allow for this possibility, we fixed the age of the African clade (node C in Fig. 4) at 90 Mya. This yielded a substitution rate of 0.00017 substitutions/site/million years, which would make the tribe Melastomeae 194 million years old and the genus *Melastoma* 82 million years old.

DISCUSSION

With the exception of *Rhexia*, Melastomeae are tropical. They are most species rich in South America, where some 550 species are found, followed by continental Africa with 185 species (Jacques-Félix 1994), Madagascar with 48 spe-

cies (Perrier de la Bâthie 1951); and India, Indochina, and Malesia with 50 species (Hansen 1977; Meyer 2001). Twenty-three million years ago, however, their ancestors were widespread in Eurasia, as documented by fossils from Siberia, the Tambov region, Belorussia, Poland, Belgium, and several sites in Germany (Fig. 5). It is unknown when Melastomeae diverged from their sister group, Microlicieae, but the event likely occurred between 53 Mya, the age of the oldest Melastomataceae fossils (Hickey 1977), and 26–23 Mya, the age of the first Melastomeae seeds. The oldest melastome fossils all are from Laurasia. The sister group of Melastomataceae, Memecylaceae, likewise is known from high latitudes (Germany, 40 Mya; Gottwald 1992). It is therefore possible that the divergence of Melastomeae from Microlicieae took place at a time when Melastomataceae were still doing well in subtropical vegetation in Laurasia and that the Miocene seeds of Melastomeae found in Eurasia represent both the time and the place of origin of the clade. Extrapolating from the ecology of today's Microlicieae and Melastomeae, their common ancestor was a shrub of open habitats with bee-pollinated flowers (Renner 1989; Gross 1993; Larson and Barrett 1999) that had stamens with ventrally prolonged connectives (the synapomorphy of the two tribes).

Based on the geographic range of their fossils (Fig. 5) it

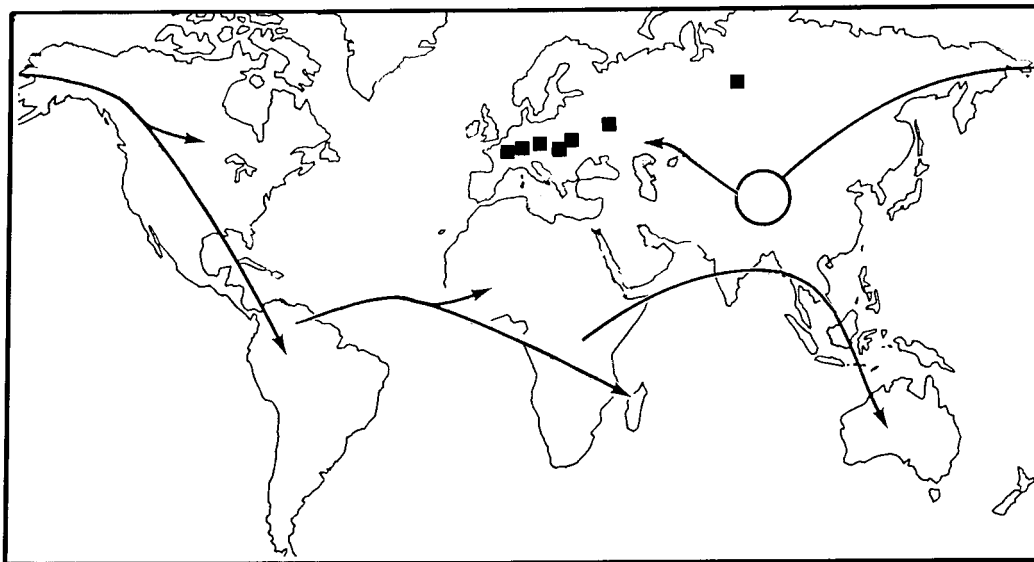


FIG. 5. Major hypothesized dispersal events in the history of Melastomeae. An ancestral area is shown in eastern Laurasia, however, the assumed position of the place of origin does not influence the biogeographic scenario or the time estimates. Fossil localities are marked by black squares.

is possible that Melastomeae entered North America via Beringia as part of the wave of taxa coming across in the Early Miocene, although Beringian crossing was possible into the pre-Middle Miocene, 18 Mya (Manchester 1999). However, the direction of their spread, whether from North America to Eurasia or the other way around, is an open question. Compared to the global high temperatures reached by the end of the Early Eocene, when Melastomataceae as a family spread in Laurasia, Miocene climates were much cooler and favored more mesophytic and more open vegetation. Melastomeae evolved in these cooler climates and were able to persist in Europe into the Upper Miocene (Fairon-Demaret 1994). Lower temperatures present less of a barrier to them than to any other group of Melastomataceae, and many genera grow at high altitudes in the Andes and Himalayas, where they experience occasional night frosts (Hansen 1977; Wurdack 1980). The range of *Osbeckia chinensis* extends as far north as Japan and that of *Rhexia virginica* as far as Ontario. Still, the climate deterioration in the Northern Hemisphere toward the end of the Miocene led to the gradual extinction of Melastomeae, perhaps with the exception of *Rhexia*.

Like other plant groups (Burnham and Graham 1999; Manchester 1999), rodents, and primates (Marshall and Sempere 1993) that used islands of the proto-Antilles arc as stepping stones, Melastomeae may have entered South America in the Mid-Miocene. Their earliest South American fossils, however, date only to the Pliocene (2 Mya; Duarte 1956). Based on the molecular clock, Melastomeae reached Africa 11–12 Mya (Table 2), which must have involved long-distance dispersal from the Caribbean or the South American bulge, probably by wind, because, except for a few paleotropical species (below), Melastomeae are wind dispersed.

Assuming a stepping-stone model of dispersal, the topology agrees with arrival in West Africa because the first-branching paleotropical Melastomeae are the predominantly West African genera *Melastomastrum* (six species) and *Tristemma* (15 species; Jacques-Félix 1994). Arrival in West Africa also agrees with the great morphological similarity between certain Brazilian and West African taxa. Thus, the West African Melastomeae *Nerophila gentianoides* is so similar to Brazilian species in the genus *Chaetolepis* that it was recently transferred into that genus; and the West African *Guyonia tenella* is considered closer to neotropical *Aciotis* than to any African Melastomeae (Jacques-Félix 1994). Both need to be included in future molecular analyses to test the possibility that their presence in Africa results from additional long-distance dispersal events.

The next phylogenetic branches (Fig. 3) consist of *Dichaetanthera*, with 27 species in Madagascar and seven in Africa, followed by a poorly resolved plexus of African, Madagascar, and Southeast Asian taxa (*Dissotis*, *Dionycha*, *Heterotis*, *Osbeckia*, *Rousseauxia*). Morphologically, African and Madagascan *Dissotis*, *Osbeckia*, and *Rousseauxia* are very close to each other (many species have been transferred between these genera) and differences between them and the Indian and Indochinese species of *Osbeckia* are unclear.

The monophyly of the Southeast Asian genus *Melastoma* indicated by *ndhF* is in agreement with a morphological assessment (Meyer 2001). The genus appears to have undergone rapid speciation around one million years ago (judging

from the short branch lengths in Fig. 3 and the calibration in Fig. 4). Lower sea levels during the Pleistocene would have allowed it to spread across narrow channels in the Sundaland plate area and to Australia and various Pacific islands. *Melastoma* is among the few Melastomeae possessing fleshy, bird-dispersed fruits. (The only other fleshy fruited species occur among African *Dichaetanthera*, *Dinophora*, and *Tristemma*.) Cultivated species of *Melastoma* tend to become naturalized and even aggressive.

Contrary to our expectation, current evidence points to *Rhexia* and its closest relative *Arthrostemma* being the sister group to all Melastomeae, not an individual neotropical lineage. A species-level phylogeny for *Rhexia* may resolve whether most species are relatively young or whether some are likely to have survived Pliocene and Pleistocene climate changes in refugia in the southeastern United States as postulated for other groups (Thorne 1993). In any event, our data suggest that the lineage to which *Rhexia* belongs dates to the Miocene and is roughly twice as old as all African and Asian Melastomeae.

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