

BAYESIAN ANALYSIS OF COMBINED CHLOROPLAST LOCI, USING MULTIPLE CALIBRATIONS, SUPPORTS THE RECENT ARRIVAL OF MELASTOMATACEAE IN AFRICA AND MADAGASCAR¹

SUSANNE S. RENNER

Systematische Botanik, Ludwig-Maximilians University, D-80638 Munich, Germany

A new biogeographic scenario for Melastomataceae (Morley and Dick, American Journal of Botany 90(11) pp. 1638–1645, 2003) accepts an *ndhF*-based phylogeny for the family by Renner et al. (American Journal of Botany 88(7): 1290–1300, 2001), but rejects those authors' divergence time estimates. Morley and Dick concluded that Gondwanan vicariance, rather than the more recent long dispersal proposed by Renner et al. explains the presence of the family in Africa and Madagascar. To assess the strength of this conclusion, a Bayesian analysis was conducted on three times the amount of sequence data used before (*ndhF*, *rbcL*, *rpl16*; 3100 base pairs [bp], excluding all gaps). The Bayesian approach to divergence time estimation does not rely on a strict molecular clock and employs multiple simultaneous minimal or maximal bounds on node ages. Reliance on northern mid-latitude fossils of Melastomataceae for calibrations was avoided or reduced by using alternative fossil and tectonic calibrations, including all those suggested by Morley and Dick. Results reaffirm the relatively recent spread of melastome lineages among the southern continents and refute the breakup of Gondwana as a plausible explanation for the presence of Dissochaeteae/Sonerileae in Madagascar and Africa and the presence of Melastomeae in Africa and Southeast Asia. Melastomeae appear to have reached Africa around 17–15 million years (my) ago, while Dissochaeteae and Sonerileae apparently reached Madagascar at 17–15 and 20–18 my ago. I also explored the effects of constraining Melastomeae to minimally 76 my old (to have reached Africa by island hopping as postulated by Morley and Dick). This resulted in an estimate for their arrival in Africa of 35 my ago and for Dissochaeteae and Sonerileae in Madagascar of 28 and 33 my ago, still implying long-distance dispersal. The Bayesian 95% credibility ranges around these dates, however, are large. Regardless of the increasing sophistication of molecular estimates of divergence time, Gondwanan scenarios will remain untestable as long as biases in the fossil record can justifiably be invoked to explain away the absence of fossils.

Key words: Bayesian divergence time estimation; biogeography; chloroplast DNA; multiple simultaneous calibrations; Melastomataceae fossils; relaxed molecular clock.

The use of local molecular clocks calibrated with fossils or tectonic events is rapidly becoming an accepted biogeographic tool for dating the divergences of clades (Herendeen and Crane, 2003; Renner and Givnish, 2004). Analytical approaches now allow relaxing the molecular clock assumption as well as multiple simultaneous calibrations and combined data partitions, each with its own model of evolution if necessary (Sanderson, 2002; Thorne and Kishino, 2002). Calibrations can take the form of fixed points, minimal ages, maximal ages, or time windows. At the same time, it is becoming clear that large data sets are needed to narrow confidence intervals on model estimation and, by implication, age estimates (Sanderson, 2003).

These advances, however, have not overcome the problems that suitable fossils for calibration are scarce and that even reliably assigned fossils place only minimal ages on the nodes just above the stem lineages along which their morphology is assumed to have evolved. Also, a multivariate analysis of relationships between various clade attributes and earliest appearance in the fossil record (considering both pollen and macrofossils) found a significant bias in earliest appearance with respect to breadth of geographic distribution, widespread families having a much higher chance of being represented as

fossils than more narrowly distributed families, whether tropical or temperate (Ricklefs and Renner, 1994). Surprisingly, there was no bias in early fossil presence towards temperate over tropical or woody over herbaceous families. Overall, earliest fossil appearances appeared to bear little relation to the ages of most families of flowering plants inferred from fossils and sister group relationships.

Melastomataceae is a diverse (4500 species in 150–166 genera) rain forest and tropical savanna clade with a limited macrofossil record at low latitudes and a reasonable one at high latitudes. The earliest fossils of Melastomataceae (leaves) are from the Eocene of northern North America (Hickey, 1977; Wehr and Hopkins, 1994) and the Upper Oligocene of Colombia (Huertas, 1977 [Upper Oligocene, not Eocene as stated by Morley and Dick, 2003; L. N. Parra, University of Medellín, personal communication in Renner et al., 2001]). Currently unresolved is the age of potentially mid-Eocene leaves from the Fonseca formation in Minas Gerais, Brazil (Duarte, 1956; not Panama as stated by Morley and Dick, 2003). These leaves were attributed to *Tibouchina* (tribe Melastomeae) and originally were thought to be Pliocene or Upper Miocene, but re-assigned to the Middle or Upper Eocene based on pollen stratigraphy (Lima and Salard-Chebaldoeff, 1981). There is so far no radiometric date for the Fonseca strata (H. Schorscher, Mineralogy and Petrology, University of São Paulo, personal communication, 2004; L. Bergqvist, Departamento de Geologia, Federal University of Rio de Janeiro, personal communication, 2004).

The Miocene has yielded melastome seeds from throughout

¹ Manuscript received 20 November 2003; revision accepted 11 May 2004.

The author thanks Bob Ricklefs, Gudrun Kadereit (née Clausing), Peter Fritsch, Fabian Michelangeli, and Frank Rutschmann for helpful comments, Doug Stone and Fabian Michelangeli for information about unpublished results, and Robyn Burnham, Lucy Gomes Sant'Anna, Lilian Bergqvist, Keith Richards, and Mark Bush for information about Melastomataceae fossils.

Eurasia (e.g., Dorofeev, 1960, 1963, 1988; Collinson and Pin-gen, 1992; Dyjor et al., 1992; Fairon-Demaret, 1994; Mai, 1995, 2000), leaves from North America (Spokane; 18–16 million years (my) ago; J. Wolfe, University of Arizona, Tucson, personal communication, 2000), leaves from Ecuador and Bolivia (R. Burnham, Museum of Paleontology, University of Michigan, personal communication), and leaves from Sumatra (Geyler, 1887 [Geyler's "Eocene" sites were reassigned to the Upper Miocene by Kräusel, 1929]).

The sister clade of Melastomataceae, Memecylaceae, is first represented by fossil wood from the Upper Eocene/Lower Oligocene of northern Germany (Gottwald, 1992).

A fossil-calibrated molecular clock analysis that used *ndhF* sequences obtained for 91 species (from 59 genera) of Melastomataceae, Memecylaceae, Crypteroniaceae, Alzateaceae, Rhynchoalycaceae, Oliniaceae, and Peneaceae (the last five families are hereafter referred to as the CAROP clade), Myrtaceae, Onagraceae, and Lythraceae placed the initial diversification of crown group Melastomataceae at the Paleocene/Eocene boundary and attributed the pantropical distribution of the tribes Melastomeae and Dissochaeteae/Sonerileae to long-distance dispersal during the Miocene or Oligocene across the Atlantic and Indian Oceans, respectively (Renner et al., 2001). A new biogeographic scenario for Melastomataceae (Morley and Dick, 2003) accepts the *ndhF*-based phylogeny for the family by Renner et al. (2001), but rejects those authors' divergence time estimates, stressing instead the breakup of Gondwana as explaining much of the deeper history of Melastomataceae. To fully understand this reevaluation, it is necessary to briefly summarize current views on the major clades of Melastomataceae, their geographic ranges, and the extent to which they have been sampled.

A conservative classification that preserved tribe names in Melastomataceae to the extent possible, given current understanding of relationships, recognized the following nine groups as probably monophyletic (Clausing and Renner, 2001a, b): Astronieae with four genera (150 spp.) in Southeast Asia; Bertolonieae with at least the genera *Bertolonia*, *Monolena*, and *Triolena*, all in the neotropics; Blakeeae with two genera (160 spp.) in the neotropics (only two species have been sequenced and only for *ndhF*; Renner et al., 2001); Dissochaeteae/Sonerileae with a mix of genera formerly assigned to these two tribes, ranging from Southeast Asia to Madagascar and tropical Africa (Clausing and Renner, 2001b); Kibessieae with 15 species in the genus *Pternandra* in Southeast Asia; Melastomeae with 48 genera and some 300 species in the neotropics, 185 in mainland Africa, 48 in Madagascar, and 50 in Southeast Asia (Renner and Meyer, 2001); Merianieae, with an unknown number of genera in the neotropics; Miconieae, with 30 genera and approximately 2200 species in the neotropics (Michelangeli et al., 2004); and Microlicieae with six genera in the neotropics (Almeda and Martins, 2001; Fritsch et al., 2004). Of these nine major clades, one, Melastomeae, is pantropical and one other, Dissochaeteae/Sonerileae, occurs in both Asia and Africa/Madagascar. A neotropical clade of cauliflorous genera related to *Bellucia* should probably also be ranked as a tribe since it is highly distinct from Miconieae where it has traditionally been placed (Penneys et al., 2004; also Results presented here).

Although a few morphologically problematic genera have not yet been sequenced (discussed in Clausing and Renner, 2001a, b; Renner and Meyer, 2001; Fritsch et al., 2004; Michelangeli et al., 2004), major lineages of Melastomataceae are

reasonably well supported (70–100% bootstrap support) by data presented in the cited studies and below. Their precise relationships, however, are poorly understood. It is clear that Kibessieae are sister to all other Melastomataceae and that the next branching lineage probably is Astronieae. Beyond that, we only know that Merianieae are sister to Miconieae and that Melastomeae are sister to Microlicieae (Clausing and Renner, 2001a; Fritsch et al., 2004; the present study). Additional solidly (>80% bootstrap) supported relationships are at shallower levels and include the nesting of African Melastomeae within neotropical Melastomeae, the nesting of Asian Melastomeae among African ones, and the nesting of Madagascan and African Dissochaeteae/Sonerileae among Southeast Asian ones.

The new biogeographic scenario for the family and its relatives (Morley and Dick, 2003) puts forward several hypotheses that can be tested against published and new data. These authors' main hypotheses about Melastomataceae are as follows: (1) Basal Melastomataceae radiated in South America in the latest Cretaceous. (2) Melastomeae originated in South America early enough to reach Africa and India overland or by island hopping, and with India serving as a Noah's Ark, they then continued on to Southeast Asia. (3) Merianieae/Miconieae entered North America from South America sometime during the Eocene. (4) Astronieae, Kibessieae, and Dissochaeteae/Sonerileae reached Southeast Asia from Africa via the India Ark.

In order to evaluate the relative merits of Morley and Dick's hypotheses compared to hypotheses that stress long-distance dispersal as proposed by Renner et al. (2001). I present the results of a Bayesian analysis of combined *ndhF*, *rbcL*, and *rpl16* data, followed by Bayesian divergence time estimation under a relaxed clock assumption and with different combinations of multiple simultaneous minimal or maximal calibrations. Calibrations using outgroups and/or tectonic events, such as the breakup of Gondwana, follow suggestions by Morley and Dick (2003).

MATERIALS AND METHODS

Taxon sampling and sequence alignment—Complete *rbcL*, *ndhF*, and *rpl16* sequences of 52 species of Melastomataceae and outgroups were selected from the data sets of Clausing and Renner (2001a, b) and Renner and Meyer (2001). To this were added sequences of *Castratella piloselloides* and *Eriocnema fulva* from Fritsch et al. (2004). New *rbcL* sequences were generated for *Bellucia pentamera* (AF215534), *Dissotis fruticosa* (GenBank accession AY456133), *Heterocentron elegans* (GB accession AY456135), and *Medinilla rubrifrons* (GB accession AY456134) from DNA aliquots already used in the earlier studies; for *Bellucia pentamera*, also *ndhF* and *rpl16* (AF215578, AF215615). As in Clausing and Renner (2001a), a few missing sequences were complemented with those of close relatives, viz. *Astronia macrophylla* and *A. smilacifolia*, *Gravesia guttata* and *G. viscosa*, *Melastoma malabathricum* and *M. sanguineum*, *Memecylon bakerianum* and *M. edule*, and *Tibouchina longifolia* and *T. urvilleana*. Authors of taxonomic names, voucher information, and GenBank accession numbers are listed in the cited publications. Trees were rooted with *Ludwigia*, a representative of Onagraceae (Clausing and Renner, 2001a).

Sequence alignment was done manually and was unproblematic for the *ndhF* and *rbcL* genes. For the *rpl16* intron, information on folding was incorporated based on the secondary structure proposed in Kelchner (2002). *Rpl16* is a group II intron (Kelchner, 2002), the group of introns capable of self-splicing, which involves open reading frames. Group II introns are characterized by a uniform structure of six major domains radiating from a central wheel, and their secondary and tertiary structure are under strong stabilizing selection, with only a few sites free to mutate frequently. To locate and align

stem regions, I compared the Melastomataceae *rpl16* intron sequences to homologous sequences aligned in Kelchner (2002).

Phylogenetic analyses—Parsimony analyses were conducted with version 4.0b.10 of PAUP* (Swofford, 2002) and used branch-and-bound searching with 10 random taxon addition replicates and tree bisection-reconnection swapping, holding 100 trees in memory, with the multiple trees and collapse options in effect, but not the steepest descent option. Clade support was assessed via nonparametric bootstrapping (implemented in PAUP), using 100 replicates and the same search strategies, except that only one tree was held in memory. The three individual data partitions yielded topologies that did not differ significantly (as judged from bootstrap support for differently resolved nodes), and the data were therefore combined. Subsequent parsimony analyses and all Bayesian analyses were performed on the combined data (54 taxa, 3100 base pairs [bp] after exclusion of all DNA insertions or deletions that involved the majority of positions in a character row).

Bayesian analyses for topology estimation relied on MrBayes version 3.0b4 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003); Bayesian analysis for time estimation relied on Thorne and Kishino's (2002) programs *estbranches* and *multidivtime* (freely available from J. Thorne's web page: <http://statgen.ncsu.edu/thorne/multidivtime.html>). Models for the Bayesian analyses were selected based on simultaneous evaluation of 56 models of sequence evolution in DT-ModSel (Minin et al., 2003). The latter uses a Bayesian information criterion approach based on decision theory to gauge the different models' performance in terms of branch-length error and degree of over-fitting. For the concatenated data, DT-ModSel preferred the transversion model, which accommodates variable base frequencies and variable transversions, plus a parameter to accommodate variable substitution rates among sites (modeled as a gamma [G] distribution with shape parameter alpha) and a proportion of sites modeled as invariable (I). This model is not implemented in MrBayes, but differs in only one parameter from the Hasegawa-Kishino-Yano model, which assumes variable base frequencies and variable transition and transversion frequencies. Parameter values for the HKY + G + I model were estimated simultaneously in MrBayes, using a random starting tree and four rate categories.

Bayesian topology estimation used one cold and three incrementally heated Markov chain Monte Carlo (mcmc) chains run for between 100 000 and 1 million cycles, with trees sampled every 100th generation, each using a random tree as a starting point and a temperature parameter value of 0.2 (the default in MrBayes). For each data set, mcmc runs were repeated twice as a safeguard against spurious results. The first 1000 to 10 000 trees were discarded as burn-in because chains clearly became stationary after this many cycles; the remaining trees were used to construct Bayesian consensus trees. Examination of the log-likelihoods and the observed consistency between runs suggests that these burn-in periods were sufficiently long.

Divergence time estimation—As assessed by earlier likelihood ratio tests (Renner et al., 2001), substitutions in the 54-taxon cpDNA data sets (individually or combined) could not be modeled as clocklike, mainly because of large differences between the ingroup and the outgroups. I therefore adopted a Bayesian approach that does not assume a strict clock and that allows multiple calibration as well as the simultaneous use of different models for data partitions where appropriate (Thorne et al., 1998; Thorne and Kishino, 2002). The approach is based on the assumption that simultaneous analysis of several gene loci (when these can safely be assumed to share a common set of divergence times) with multiple calibrations will detect the frequently weak signal in single data sets in spite of violations of the clock in each of the individual partitions (Thorne and Kishino, 2002; Yang and Yoder, 2003). Thorne's *multidivtime* program requires a Unix environment and uses an mcmc approach to approximate prior and posterior probabilities. As recommended in Thorne's manual, PAML's *baseml* program (version 3.14; Yang, 1997) and the F84 + G model (with five rate categories) were used to estimate nucleotide substitutions in each of the three data partitions as well as for the concatenated data. The topology used for *baseml* and all following steps was the first of six equally parsimonious trees found in the branch-and-bound search for the concatenated data. The F84 + G model is the only model so

far implemented in *estbranches*, which is the component of Thorne's program package that estimates branch lengths. This model accommodates variable base frequencies, transition/transversion bias, and rate heterogeneity among sites. To convert the PAML output into model files acceptable for *estbranches*, I used Thorne's *paml2modelinf* program. Besides estimating branch lengths, *estbranches* also calculates the variance-covariance structure of the branch length estimates, which provides an important means to judge the information content of the data (Results).

The output from *estbranches* forms the input for *multidivtime*, which calculates node divergence times, given user-specified constraints (such as fossil-based calibrations, next paragraph). I used the following data-dependent settings in the *multidivtime* control file: Length, sampling frequency, and burn-in period of the Markov chain were set to 100 000 trees sampled every 10th generation, with a burn-in of 1000 trees. The a priori expected number of time units between tip and root was set to 1 because I set the time unit to 100 my. The standard deviation of the prior for the time between tips and root is recommended to equal the number of time units between tips and root and so was set to 1. The a priori rate at the root node was set to 0.002, based on Thorne's recommendation that it be calculated by dividing the median distance between the ingroup root and the ingroup tips obtained from *estbranches* by the time unit. The prior for the Brownian motion parameter ν , which determines the permitted rate change between ancestral and descendant nodes, was set to 1, following the manual's recommendation that the time units between root and tips to the power of ν be about 1. The standard deviation on ν was also set to 1.

The *multidivtime* control file also requires setting number and kind (maximal or minimal) of constraints on node times. I explored combinations of the following constraints provided by fossils and geological events: (1) A maximal age of 130 my was set for the CAROP families, Melastomataceae, plus Memecylaceae (node 1 in Fig. 2), based on an assumed age of the angiosperms of 141–132 my as suggested by their earliest fossils (Hughes, 1994; Brenner, 1996). (2) The split between *Eugenia* and *Myrtus* (node 2 in Fig. 2) was set to minimally 88 my, following Morley and Dick's (2003) use of the earliest Myrtaceae pollen to constrain this node. Possibly this overestimates the node's age because the subfamily (Myrtoideae) to which *Myrtus* and *Eugenia* belong appears not much older than 77 my (Sytsma et al., 2004). (3) The breakup of South America and Africa provided a minimal age of 90 my for node 3 in Fig. 2, the divergence between neotropical Alzateaceae and the South African Rhynchocalycaceae/Peneaceae (Conti et al., 2002). (4) The same tectonic event served to set a minimal age of 90 my for node 4, the divergence between neotropical *Mouriri* and African/Southeast Asian *Memecylon* (Morley and Dick, 2003; but see Discussion). (5) In one of two alternative placements, the oldest (53-my-old) fossil leaves showing the characteristic venation of Melastomataceae (Hickey, 1953) were used to set a minimal age of crown Melastomataceae, node 5 in Fig. 2. (6) The alternative placement of these leaves is at node 6, the stem lineage of Meranieae/Miconieae (Morley and Dick, 2003). (7) In some analyses, the divergence of Melastomeae (node 7 in Fig. 2) was constrained to a minimal age of 25 my, based on 23–26-my-old seeds (Renner and Meyer, 2001). (8) I also explored the effects of constraining Melastomeae (node 7) to minimally 76 my old to have reached Africa overland or by island hopping as postulated by Morley and Dick (2003, p. 1640). This constraint also covers the possibility that *Tibouchina*-like leaves from the Fonseca formation in Minas Gerais (Duarte, 1956) could be mid-Eocene (48–37 my; Lima and Salard-Chebaldaff, 1981), which would make the tribe Melastomeae almost twice as old as its earliest seeds (although not 76 my old as assumed by Morley and Dick).

RESULTS

A chi-square test of homogeneity of base frequencies across taxa was run, excluding missing or ambiguous sites and using just the informative sites ($\chi^2 = 52.36$, $df = 159$; $P = 1$) and revealed no nucleotide bias among taxa. Parsimony analysis of the individual data sets revealed no topological contradictions that had significant bootstrap support, and the three partitions were therefore combined. After exclusion of most

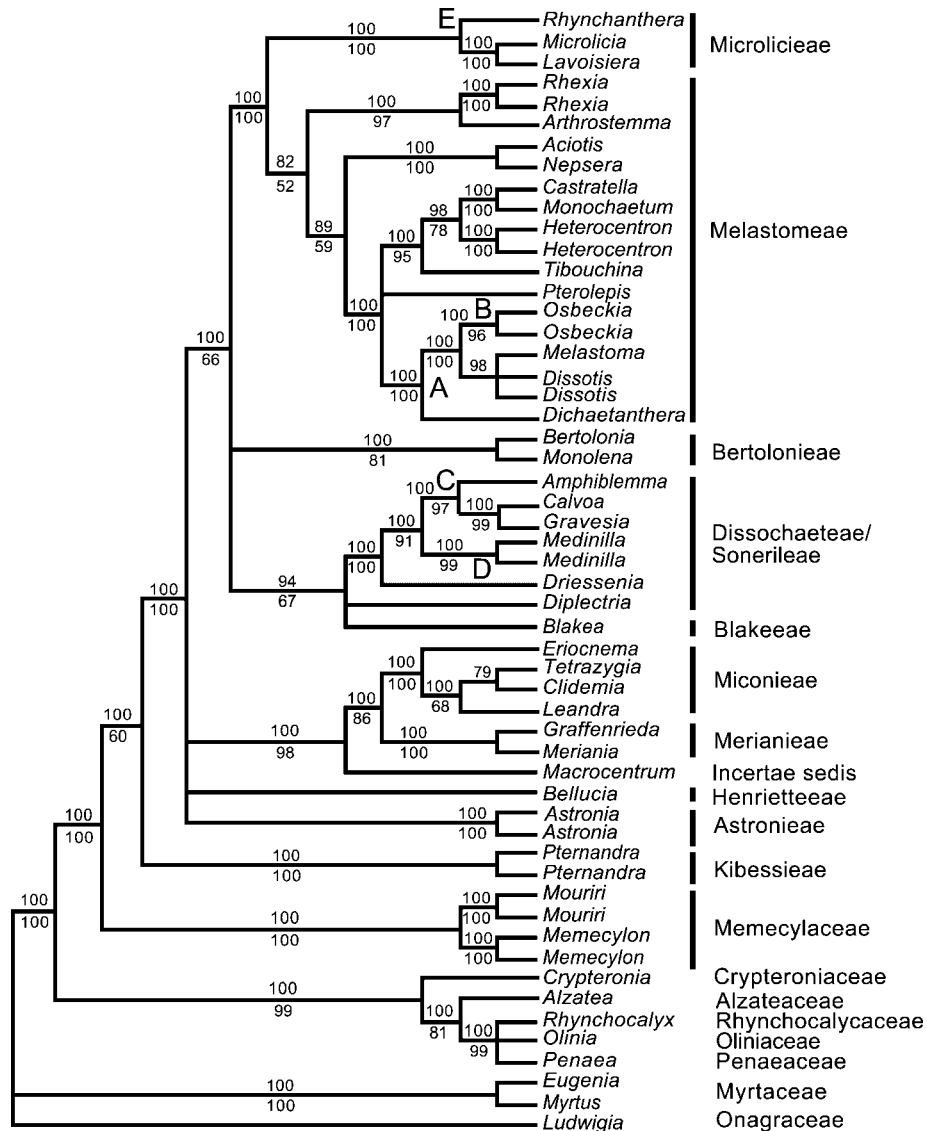


Fig. 1. Phylogeny of Melastomataceae and closest relatives obtained from combined cpDNA *rbcL*, *ndhF*, and *rpl16* sequences under the HKY + G + I model using Bayesian inference. Above branches: posterior probabilities from a 70% majority rule consensus tree of 5000 trees; below branches: bootstrap support >50% under parsimony from 100 pseudo-replicates. Tribes are those of Clausen and Renner (2001a) and Penneys et al. (2004, Henrietteae), and lettered nodes A–E refer to nodes in Fig. 2.

gapped characters (Material and Methods), analysis of the concatenated data resulted in six equally parsimonious trees, all on one island (consistency index [CI] = 0.62, rescaled consistency index [RC] = 0.51, retention index [RI] = 0.82). A 70% majority rule consensus of 5000 trees obtained from a Bayesian analysis under the Hasegawa-Kishino-Yano + G + I model is shown in Fig. 1. Most of the tree's backbone has only limited statistical support from bootstrapping and posterior probabilities.

Divergence time estimation relied on the concatenated cpDNA data set, rather than the individual ones, to reduce the variance on branch length estimates. Results from different combinations of simultaneous calibrations are listed in Table 1, and Fig. 2 is a representative chronogram (its topology is that of the first of six equally parsimonious trees, used as input for the Bayesian time estimation) showing the calibration points and nodes of biogeographic interest.

Whether or not northern mid-latitude fossils of Melastomataceae were used in addition to outgroup-derived and tectonic calibrations made little difference to the estimates for the arrival of Melastomeae in Africa and Southeast Asia and for Dissochaeteae/Sonerileae in Madagascar/Africa (Table 1). The arrival and diversification of Melastomeae in Africa (Fig. 2, node A) appears to have occurred around 17–15 my ago, that of Sonerileae in Madagascar at about 20–18 my ago, and that of Dissochaeteae in Madagascar at 17–15 my ago (Fig. 2, nodes C and D; for 95% confidence intervals see Table 1).

Because Eocene North American leaves may represent Merianieae/Miconieae (Renner et al., 2001; Morley and Dick, 2003), the analysis was repeated with the stem lineage of this clade constrained to minimally 53 my old (Fig. 2, node 6). This made little difference for the remaining intra-Melastomataceae ages (Table 1, columns 2 vs. 3).

Constraining crown Melastomeae to minimally 76 my old

TABLE 1. Bayesian estimates of divergence times in millions of years plus their 95% credibility intervals. Results in the different columns were obtained by placing minimal or maximal constraints on nodes 1–7 as listed in Materials and Methods and in the legend for Fig. 2. Results from a run in which Melastomeae were constrained to at least 76 my old (following Morley and Dick, 2003) are reported in the text.

Nodes in Fig. 2	Constraints on nodes 1–4 ^a	Constraints on nodes 1–5 ^b	Constraints on nodes 1–4,6 ^c	Constraints on nodes 1–4,6,7 ^d	Constraints on nodes 1,2,6,7 ^e
A = Arrival of Melastomeae in Africa	15 (9, 25)	15 (8, 25)	17 (9, 27)	17 (10, 27)	16 (9, 24)
B = Crown group <i>Osbeckia</i> in Southeast Asia	7 (3, 13)	7 (3, 13)	8 (4, 14)	8 (4, 15)	7 (4, 13)
C = Arrival of Sonerileae in Africa/Madagascar	18 (9, 31)	18 (9, 31)	20 (11, 32)	20 (10, 32)	18 (10, 30)
D = Divergence of Asian and Madagascan <i>Medinilla</i>	15 (8, 27)	15 (6, 27)	17 (8, 28)	17 (8, 28)	15 (7, 26)
E = Crown Microlicieae	16 (9, 28)	16 (8, 28)	19 (10, 30)	19 (10, 31)	16 (9, 26)

^a No melastome fossils.

^b No Melastomeae seed fossils; “early” melastome leaf.

^c No Melastomeae seed fossils; “late” melastome leaf.

^d Melastomeae seeds plus “late” leaf fossil.

^e No geological constraints.

(to have reached Africa by island hopping as postulated by Morley and Dick) resulted in an estimate for their arrival in Africa of 35 my and of Dissochaeteae and Sonerileae in Madagascar of 28 and 33 my.

Finally, because tectonic calibrations assume vicariance explanations, rather than test vicariance hypotheses, the analysis was repeated yet again with just fossil-based calibrations (Table 1, column 5).

DISCUSSION

Basal Melastomataceae and sister taxa—Much of the study of Morley and Dick (2003) is concerned with the biogeography of the sister clades of Melastomataceae (see also Conti et al., 2002). These eight families were represented by 15 species in the *ndhF* data set of Renner et al. (2001). While this ensured proper rooting, it was not considered sufficient to draw biogeographic conclusions, and none were put forward. (Note that the cladogram in Renner et al. [2001] lacks bootstrap support for most outgroup relationships.) By contrast, Morley and Dick (2003, p. 1641) felt, “Whether we look at its closest relatives or Melastomataceae itself, the cladogram of Renner et al. (2001) provides strong support for a Late Cretaceous West Gondwana center of radiation for the Melastomataceae.”

The closest relatives of Melastomataceae are Memecylaceae. The phylogeny and biogeography of this clade of six genera (approximately 450 spp.) is the focus of ongoing research (D. Stone and B. Baldwin, Department of Integrative Biology, University of California, Berkeley, personal communication). Memecylaceae have their greatest morphological diversity in tropical East Africa, where four genera occur. Two of the African genera, *Memecylon* (300 spp.) and *Lijndenia* (15 spp.), extend to India, Sri Lanka, and Malaysia. The remaining two genera occur in the neotropics. Based on data available in 2000, it appeared that “more data are needed to resolve whether the neotropical genera of Memecylaceae are nested among paleotropical memecylons, which is indicated by preliminary combined gene and intron data” (Renner et al., 2001, p. 1297), a conclusion that fit the available data and still seems appropriate.

The data of Stone and Baldwin eventually may resolve whether the initial split between Memecylaceae and Melastomataceae occurred in Africa, on the India Ark, or in Southeast Asia, where first-branching Melastomataceae are endemic today (below). Both families were present in Early, Middle, and

Upper Eocene Laurasia, as documented by fossils (Hickey, 1977; Gottwald, 1992; Wehr and Hopkins, 1994). If Melastomataceae/Memecylaceae fossils were also discovered in Early Eocene (or older) African or Indian strata, a Gondwanan origin of melastomes, minimally of their stem lineage, could be inferred. Unfortunately, Late Eocene to Oligocene heterocolpate pollen from Cameroon, West Africa (Salard-Cheboldaeff, 1978, 1981), which Morley and Dick (2003) adduce a sign for the Eocene presence of melastomes in Africa, does not settle the matter because, as they point out, pollen of Melastomataceae cannot be distinguished from that of Combretaceae. Salard-Cheboldaeff indeed described three heterocolpate pollen types of which she compared the oldest, Upper Eocene *H. laevigatus*, to *Terminalia* (Combretaceae; also Muller, 1981; Coetzee and Muller, 1984); Upper Eocene to Oligocene *H. verrucatus* to Lythraceae or Melastomataceae; and the relatively youngest, Oligocene *H. pseudostriatum*, to *Dissotis*, a genus of Melastomeae. The indistinctly finely striate exine that distinguishes *H. pseudostriatum*, however, is unusual in extant Melastomataceae (Patel et al., 1984).

Note that Early Eocene African melastome fossils would only refute Renner et al.’s (2001) hypothesis that the initial *diversification* of Melastomataceae occurred north of the Tethys if they could be assigned to Kibessieae and/or Astronieae. This is because *diversification* refers to the crown group—here the splits between Kibessieae, Astronieae, and remaining Melastomataceae—not the stem lineage.

Biogeography of Melastomataceae—The credibility intervals I obtained are very broad (Table 1). The following interpretation of the family’s history tries to accommodate these wide ranges, in some cases resulting in vague or multiple possible scenarios.

A non-controversial idea at this stage is that Kibessieae (15 spp.), which are endemic in Southeast Asia, with most of their species in Borneo, may have originated on the Indian Plate after separation from Madagascar (68–45 my ago) or in Southeast Asia (Renner et al., 2001; Morley and Dick, 2003). By contrast it is untenable that “. . . most of the basal groups [of Melastomataceae] are South American with subsequent branching relating to differentiation in Africa, Madagascar, and Southeast Asia. This suggests that the primary radiation of Melastomataceae may have been in South America during the latest Cretaceous.” (Morley and Dick, 2003, p. 1642). Given the lack of statistically supported resolution of much of the family phylogeny (Renner et al., 2001; Fig. 1 in the current

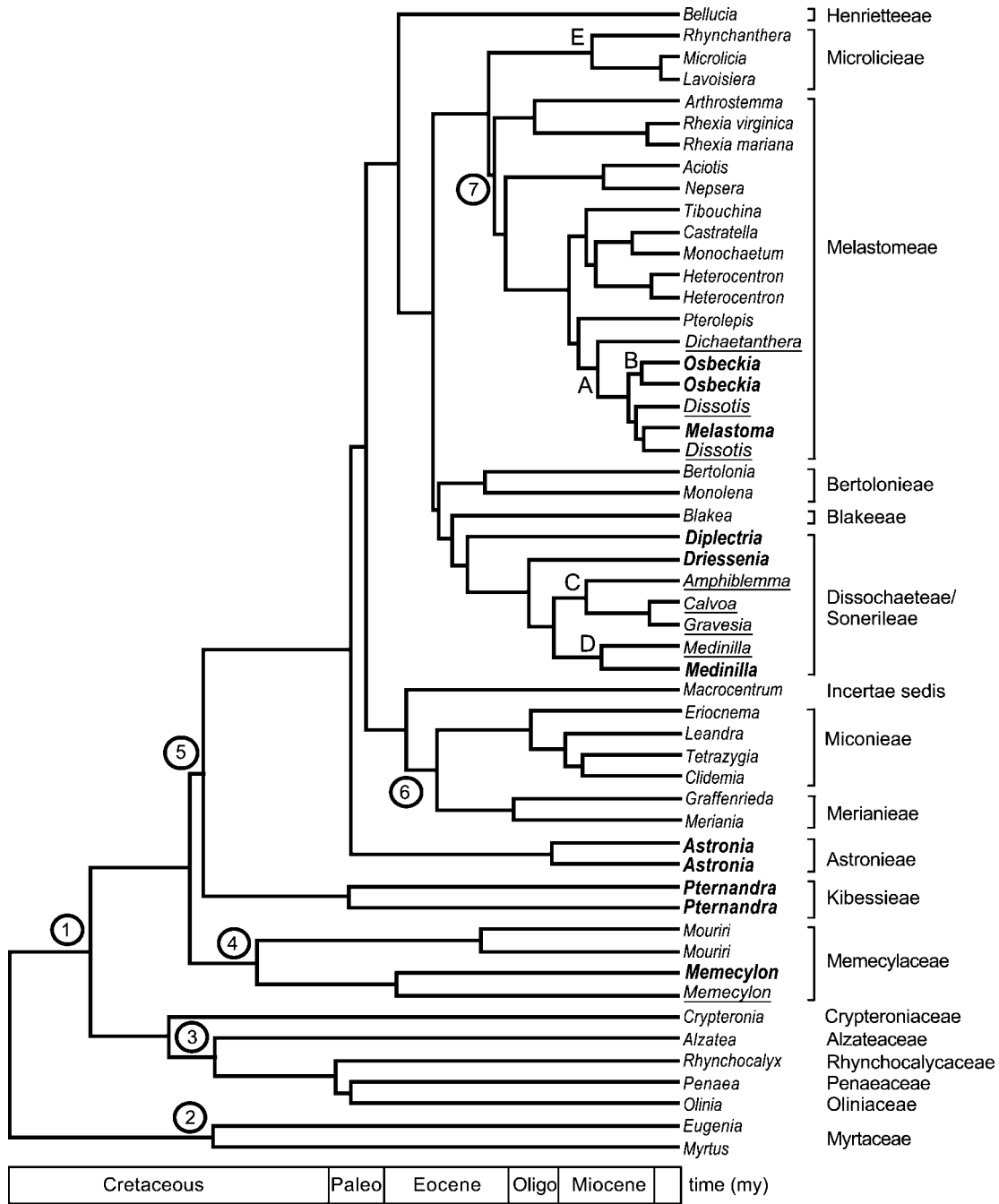


Fig. 2. Chronogram for Melastomataceae with branch lengths obtained by Bayesian time estimation, using one of the six equally parsimonious topologies obtained from the data in Fig. 1. The tree is rooted with an Onagraceae (see text). The particular combination of minimal (Mi) and maximal (Ma) constraints used in this run is shown in Table 1, column 4 (i.e., node 5 was not constrained in this run). Numbered nodes refer to the following Mi and Ma constraints (not all used in all analyses; see Table 1): (1) Crown group CAROP families, Memecylaceae, plus Melastomataceae: Ma = 130 my; (2) Myrtaceae crown group: Mi = 88 my; (3) split between South African and neotropical CAROP members: Mi = 90 my; (4) split between paleotropical and neotropical Memecylaceae: Mi = 90 my; (5) crown group Melastomataceae: Mi = 53 my; (6) stem lineage of Merianieae/Miconieae: Mi = 53 my; (7) crown group Melastomeae: Mi = 26–23 my. Estimated divergence times for nodes A–E, with 95% credibility intervals, are given in Table 1. Paleo = Paleocene, Oligo = Oligocene. Melastomes from India/Asia are shown in boldface type while those from Africa/Madagascar are underlined. The remaining Melastomataceae genera are from the neotropics.

paper), the only basal groups identified so far are Kibessieae and Astronieae, both endemic in tropical Asia, with most their species in Borneo and the Malay Archipelago. For Astronieae, Morley and Dick (2003, p. 1642) suggest that it “appears to have had the same history as Memecylaceae, with differenti-

ation on the Indian Plate (68–45 Ma).” This is an intriguing possibility, and further phylogenetic work on Astronieae and Memecylaceae may be able to decide the matter.

Among the least satisfactory subplots of current scenarios (Renner et al., 2001; Morley and Dick, 2003) is the history of

Merianieae/Miconieae. This is because of the lack of support along the family tree's backbone and poor taxon sampling; Miconieae have 2200 species in 30 genera, of which five are included here. That the circumscription of Miconieae is more problematic than suspected is suggested by two recent findings: the addition of *Eriocnema*, formerly placed in Microliciaceae, to Miconieae (Fritsch et al., 2004), and the exclusion of *Bellucia* and its relatives from Miconieae (hinted at in Clausen et al., 2000; Penneys et al., 2004; the present study, Figs. 1 and 2). The closest relatives of Merianieae/Miconieae also are unknown, and the coarse sampling prevents reliable estimation of initial divergence times. Morley and Dick's (2003, p. 1642) assertion that Merianieae/Miconieae "need to have been well established by the Paleocene, before the closure of the Central American land bridge (55 Ma) in order for members of this group to disperse to North America, thus accounting for North American leaf fossils which compare with this group" therefore cannot yet be tested. Unfortunately, the first available phylogeny of Miconieae (Michelangeli et al., 2004), based on nrITS sequences, provides little support for the deeper splits within core-Miconieae and therefore does not decide whether West Indian and Central American species branch off before South American ones or are nested within them. Such nesting would provide hints about a predominantly northwards or southwards spread during the clade's evolution.

By contrast, the hypotheses concerning Melastomeae and Dissochaeteae/Sonerileae put forward by Morley and Dick (2003, p. 1642) can be addressed with the current data. These hypotheses state that: "subsequent dichotomies within Melastomeae and Dissochaeteae/Sonerileae can be explained . . . by dispersals from South America via Africa and Madagascar to Southeast Asia. Under this scenario, basal groups must have been in place in South America between about 78 and 74 Ma" and "The Dissochaeteae/Sonerileae lineage could be explained in a similar manner to low latitude Melastomeae with dispersal from South America via Africa (between 76 Ma and 70 Ma) and the Indian Plate (68–50 Ma) to Southeast Asia (after 45 Ma) . . ." (Morley and Dick here use "dispersal" to mean overland migration through a Gondwanan connection.) Even disregarding that Dissochaeteae/Sonerileae do not occur in South America, these hypotheses do not match well-supported parts of the family tree. First, basal groups of melastomes are not found in South America, but instead in Southeast Asia (Kibessieae and Astronieae, which are endemic in tropical Asia). Second, the African and Madagascar members of Dissochaeteae and Sonerileae (*Amphiblemma* from West Africa, *Calvoa* from East Africa, a *Gravesia* species from Madagascar, and also *Medinilla* from Madagascar) are nested within Asian Dissochaeteae (*Diplectria/Driessenia*; Figs. 1 and 2). If Dissochaeteae/Sonerileae had rafted from Africa to Asia on an Indian ark, African Dissochaeteae/Sonerileae should be basal to Asian ones. As shown here, the opposite is the case (also Clausen and Renner, 2001b, with denser taxon sampling). Estimated divergence times suggest that Dissochaeteae and Sonerileae had arrived and diversified in Africa (or Madagascar) by the Miocene (Table 1). Taxon sampling here is too small to decide whether Madagascar was reached directly from Asia or whether some lineages first reached Africa and then crossed the Mozambique Channel (a question that is the focus of a related study; Renner, 2004, b). The time estimated for the diversification of *Amphiblemma*, *Calvoa*, and *Gravesia* in Africa/Madagascar in the present study (20–18 my ago) is older than that obtained from just *ndhF* (Renner et al., 2001: 11–10

my ago), but the credibility intervals around the Bayesian estimates encompass the earlier estimates. I do not know whether the older ages are due to gene or taxon sampling effects; the *ndhF* analysis included 29 species of Dissochaeteae/Sonerileae, while the current *ndhF* + *rbcL* + *rpl16* analysis included only seven representatives of this diverse clade.

Besides the non-matching divergence sequences of Neotropical, African, and Asian genera of Melastomeae and Dissochaeteae/Sonerileae, a second weakness of Morley and Dick's (2003) hypothesis about the history of these two clades is that "subsequent dichotomies within Melastomeae and Dissochaeteae/Sonerileae" are attributed to the same Gondwanan events that are assumed to explain more basal splits in the family. Given the widely differing genetic distances from the tree's root to, for instance, the African Melastomeae clade (Fig. 2, node A) and the Miconieae/Merianieae clade (Fig. 2, node 6), the attribution of both to the same vicariance event implies extreme substitution rate heterogeneity within the family. Such extreme rate heterogeneity, if true, would invalidate all attempts to infer time from these data, including those of Morley and Dick.

For Melastomeae, which today comprise 300 species in the neotropics, 185 in mainland Africa, 48 in Madagascar, and 50 in Southeast Asia, Renner et al. (2001) proposed arrival in Africa, and subsequently Madagascar and Asia, via long-distance dispersal, while Morley and Dick (2003) asserted that the clade's disjunct range relates to the breakup of Gondwana. The current Bayesian analysis estimates the arrival of Melastomeae in West Africa as having occurred about 17–15 my ago, closely matching *ndhF*-based estimates of 14–11 my ago with relatively similar taxon sampling (Renner and Meyer, 2001; Renner et al., 2001). Workers familiar with both neotropical and African Melastomeae have found them to be similar morphologically, sometimes to the point where species could easily be placed in the same genus (e.g., African *Nerophila*, West Indian and South American *Chaetolepis*). An even stronger morphological argument could be made for African-Indian-Asian Melastomeae and Sonerileae, with at least two genera, *Osbeckia* and *Ochthocharis*, spanning these regions (e.g., Hansen and Wickens, 1981). However, the monophyly of *Osbeckia* is not supported by preliminary molecular data (Renner and Meyer, 2001) and that of *Ochthocharis* has not been tested.

A suggestion by Renner et al. (2001) that puzzled Morley and Dick (2003) was that undiscovered fossils of Melastomeae would need to be nine times older than current oldest fossils to push back estimates to 90–100 my ago for entry into Africa and Madagascar. The logic of this statement is that to push the age of African Melastomeae from the estimated 12 ± 3 my back to 90–100 my, a calibration with fossils nine times older than the approximately 23-my-old seeds would be needed ($100/11 = 9$). I, in turn, was puzzled by Morley and Dick's approach to calibration. They calibrated genetic distances from synonymous nucleotide substitutions in Renner et al.'s (2001) *ndhF* sequences by fixing the age of Melastomeae at 76 my, based on the mere assumption that Melastomeae reached Africa via stepping stones across a still narrow Atlantic. Constraining Melastomeae as 76 my old not surprisingly yields a substitution rate roughly three times slower than constraining them as 23 my old based on their earliest fossils (Renner et al., 2001). The multiple simultaneous calibrations (including ones that do not rely on Melastomeae seeds) in the present study provide a way out of this logical conundrum.

In other words, competing explanations for the occurrence of Miocene Melastomeae seeds from Siberia to Belgium either take the seeds as a close proxy for the group's real age (Renner et al., 2001) or assume that the clade is three times older than its earliest fossil seeds (Morley and Dick, 2003; as explained, Melastomeae are fixed at 76 my for clock calibration by Morley and Dick). According to the Bayesian divergence time estimates obtained *without* relying on calibration with Melastomeae seeds (Table 1, columns 1–3), the initial divergence of Melastomeae is 40–36 my old. Such an age would allow for the clade's arrival in Eurasia either from Beringia (Morley and Dick, 2003, p. 1642) or from Southeast Asia (Renner et al., 2001; Morley and Dick, 2003, in an alternative scenario). However, it would reject arrival via the Late Paleocene/Early Eocene South Greenland land bridge (Morley and Dick's third scenario). In their preferred scenario, however, Morley and Dick see *Rhexia* itself as having arrived in North America toward the end of the Paleocene, followed by dispersal to Eurasia via Beringia. The basis for the conclusion that *Rhexia* itself was the Melastomeae widespread in Miocene Eurasia remains unclear.

In sum, Bayesian analysis of three data partitions under a relaxed clock, calibrated with different sets of simultaneous constraints, suggests that Melastomataceae arrived in Africa and Madagascar during the Miocene, once from the neotropics (Melastomeae) and apparently several times from Asia (Dissochaeteae and Sonerileae). No other clades are currently found in Africa and Madagascar. As discussed in other papers, long-distance dispersal by birds or wind provides plausible explanations (Clausing and Renner, 2001b; Renner et al., 2001; Renner, 2004a).

It is a matter of personal inclination whether one accepts calibrated genetic distances as providing reasonable time estimates or prefers to stress the incompleteness of the fossil record. Age estimates obtained earlier (Renner et al., 2001; Renner and Meyer, 2001) proved robust to the addition of new fossil (from the outgroups) and to different inclusion/exclusion of tectonic events vs. fossils. The idiosyncratic nature of the fossil record is illustrated by a bias in the record of Melastomataceae noted by both Renner et al. (2001) and Morley and Dick (2003). Seeds are known from the Eurasian Miocene, but not from North America. Leaf fossils are known from North America, but not from Eurasia. Morley and Dick attribute these differences to the ecology and taphonomy of melastomes in these regions, whereas Renner et al. (2001, p. 1298) attribute them to the presence of suitable strata and different palaeobotanical traditions. Carpoifloras in North America are limited to a few sites, and with the exception of the Brandon Lignite, are not the same kind of environment as the more thoroughly studied Oligocene/Miocene brown coals of Europe (S. Manchester, Florida Museum of Natural History, Gainesville, personal communication). Conversely, the absence of leaves from Eurasia may have to do with the disintegration during sieving of samples being prepared for carpological investigation (F. Velichkevich, Institute of Geological Sciences, National Academy of Sciences, Belarus, personal communication). It is a pity that few hard data seem to be available on biases in the angiosperm fossil record. Given its inconsistent fossil record, the oldest fossils of Melastomataceae are unlikely to correspond closely to the time of origin of the family, a problem that this study attempts to alleviate via the alternative use of outgroup fossils and even tectonic calibrations (e.g., the

breakup of West Gondwana) as suggested by Morley and Dick (2003).

LITERATURE CITED

- ALMEDA, F., AND A. B. MARTINS. 2001. New combinations and new names in some Brazilian Microlicieae (Melastomataceae), with notes on the delimitation of *Lavoisiera*, *Microlicia*, and *Trembleya*. *Novon* 11: 1–7.
- BRENNER, G. J. 1996. Evidence for the earliest stage of angiosperm pollen evolution: a paleoequatorial section from Israel. In D. W. Taylor and L. J. Kickey [eds.], Flowering plant origin, evolution, and phylogeny, 91–115. Chapman & Hall, New York, New York, USA.
- CLAUSING, G., K. MEYER, AND S. S. RENNER. 2000. Correlations among fruit traits and evolution of different fruits within Melastomataceae. *Botanical Journal of the Linnean Society* 133: 303–326.
- CLAUSING, G., AND S. S. RENNER. 2001a. Molecular phylogenetics of Melastomataceae and Memecylaceae: implications for character evolution. *American Journal of Botany* 88: 486–498.
- CLAUSING, G., AND S. S. RENNER. 2001b. Evolution of growth form in epiphytic Dissochaeteae (Melastomataceae). *Organisms, Diversity and Evolution* 1: 45–60.
- COETZEE, J. A., AND J. MULLER. 1984. The phylogeographic significance of some extinct Gondwana pollen types from the Tertiary of the southwestern Cape (South Africa). *Annals of the Missouri Botanical Garden* 71: 1088–1099.
- COLLINSON, M. E., AND M. PINGEN. 1992. Seeds of the Melastomataceae from the Miocene of Central Europe. In J. Kovar-Eder [ed.], Palaeovegetational development in Europe, 129–139. Museum of Natural History, Vienna, Austria.
- CONTI, E., T. ERIKSSON, J. SCHÖNENBERGER, K. J. SYTSMAN, AND D. BAUM. 2002. Early Tertiary out-of-India dispersal of Crypteroniaceae: evidence from phylogeny and molecular dating. *Evolution* 56: 1931–1942.
- DOROFFEV, P. I. 1960. On the Tertiary flora of Belorussia. *Botanicheskoy Zhurnal SSSR* 45: 1418–1434 (in Russian).
- DOROFFEV, P. I. 1963. The Tertiary floras of western Siberia. *Izdatelstvo Akademii Nauk SSSR, Moskva-Leningrad, Russia* (in Russian).
- DOROFFEV, P. I. 1988. Miocene floras of the Tambov district. *Akademiya Nauk, Leningrad, Russia* (in Russian, posthumous work, F. Y. Velichkevich, ed.).
- DUARTE, L. 1956. Melastomatáceas fósseis da Basia Tertiária de Fonseca, Minas Gerais. *Divisão de Geologia e Mineralogia, Boletim* 161: 8–32.
- DYJOR, S., Z. KVACEK, M. LANUCKA-SRODONIOWA, W. PYSZYNSKI, A. SADOWSKA, AND E. ZASTAWNIK. 1992. The Younger Tertiary deposits in the Gozdnicza region (SW Poland) in the light of recent palaeobotanical research. *Polish Botanical Studies* 3: 1–129.
- FAIRON-DEMARET, M. 1994 [1996]. Les fruits et graines du Miocene de Bioul (Entre-Sambre-et-Meuse, Belgique). Etude qualitative, quantitative et considerations paleoecologiques. *Annales de la Société Géologique de Belgique* 117: 277–309.
- FRITSCH, P. W., F. ALMEDA, S. S. RENNER, A. B. MARTINS, AND B. CRUZ. 2004. Phylogeny and circumscription of the near-endemic Brazilian tribe Microlicieae (Melastomataceae). *American Journal of Botany* 91: 1105–1114.
- GEYLER, H. T. 1887. Über fossile Pflanzen von Labuan. Vega-Expeditionens Vetenskapliga Jakttagelser, Stockholm, vol. IV, 473–507.
- GOTTWALD, H. 1992. Hölzer aus marinen Sanden des Oberen Eozän von Helmstedt (Niedersachsen). *Palaeontographica, Abteilung B* 255: 27–103.
- HANSEN, C., AND G. E. WICKENS. 1981. A revision of *Ochthocharis* (Melastomataceae) including *Phaeoneuron* in Africa. *Kew Bulletin* 36: 13–29. pl. 2, 3.
- HERENDEEN, P. S., AND P. R. CRANE. 2003. Dating in the 21st century: theory and reality of finding a date for your clade. Botanical Society of America meeting abstracts at <http://www.2003.botanyconference.org/>
- HICKEY, J. L. 1977. Stratigraphy and palaeobotany of the Golden Valley formation (Early Tertiary) of western North Dakota. *Memoir* 150. Geological Society of America, Boulder, Colorado, USA.
- HUELSENBECK, J. P., AND F. R. RONQUIST. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.
- HUERTAS, G. 1977. Una Melastomatácea fósil del Terciario carbonífero de Antioquia (Eoceno). *Caldasia* 12: 35–39.
- HUGHES, N. F. 1994. The enigma of angiosperm origins. Cambridge University Press, Cambridge, UK.

- KELCHNER, S. A. 2002. Group II introns as phylogenetic tools: structure, function, and evolutionary constraints. *American Journal of Botany* 89: 1651–1669.
- KRÄUSEL, B. 1929. Fossile Pflanzen aus dem Tertiär von Süd-Sumatra. *Verhandelingen der Geologie en Mijnbouw Genootschap voor Nederland en Kolonien, Geologie Serie* 8: 329–342.
- LIMA, M. R. DE, AND M. SALARD-CHEBOLDAEFF. 1981. Palynologie des bassins de Gandarela et Fonseca (Eocene de L'Etat de Minas Gerais, Brésil). *Boletim IG, Instituto de Geociencias, USP* 5, 12: 33–54.
- MAI, D. H. 1995. Tertiäre Vegetationsgeschichte Europas. G. Fischer, Jena, Germany.
- MAI, D. H. 2000. Die untermiozänen Floren aus der Spremberger Folge und dem II. Flözhorizont der Lausitz. Teil III. Dialypetalae und Sympetalae. *Palaeontographica Abteilung B* 253: 1–106.
- MICHELANGELI, F. A., D. S. PENNEYS, J. GIZA, D. SOLTIS, M. H. HILLS, AND J. D. SKEAN JR. 2004. A preliminary phylogeny of the tribe Miconieae (Melastomataceae) based on nrITS sequence data and its implications on inflorescence position. *Taxon* 53: 279–290.
- MININ, V., Z. ABDO, P. JOYCE, AND J. SULLIVAN. 2003. Performance-based selection of likelihood models for phylogeny estimation. *Systematic Biology* 52: 674–683.
- MORLEY, R. J., AND C. W. DICK. 2003. Missing fossils, molecular clocks and the origin of the Melastomataceae. *American Journal of Botany* 90: 1638–1645.
- MULLER, J. 1981. Fossil pollen records of extant angiosperms. *Botanical Review* 47: 1–142.
- PATEL, V. C., J. J. SKVARLA, AND P. H. RAVEN. 1984. Pollen characters in relation to the delimitation of Myrtales. *Annals of the Missouri Botanical Garden* 71: 858–969.
- PENNEYS, D. S., F. A. MICHELANGELI, W. S. JUDD, AND J. D. SKEAN. 2004. Henrietteae, a new tribe of neotropical Melastomataceae. Botanical Society of America meeting abstracts at <http://www.botanyconference.org/engine/search/index.php?func=detail&aid=226>
- RENNER, S. S. 2004a. Tropical trans-Atlantic disjunctions, sea surface currents, and wind patterns. *International Journal of Plant Sciences* 165, in press.
- RENNER, S. S. 2004b. Assembly of the Melastomataceae flora of Madagascar inferred from Bayesian divergence time estimation. *Philosophical Transactions of the Royal Society, B*.
- RENNER, S. S., G. CLAUSING, AND K. MEYER. 2001. Historical biogeography of Melastomataceae: the roles of Tertiary migration and long-distance dispersal. *American Journal of Botany* 88: 1290–1300.
- RENNER, S. S., AND T. J. GIVNISH [EDS.]. 2004. Tropical intercontinental disjunctions: Gondwana breakup, immigration from the boreotropics, and transoceanic dispersal. *International Journal of Plant Sciences* 165, in press.
- RENNER, S. S., AND K. MEYER. 2001. Melastomataceae come full circle: biogeographic reconstruction and molecular clock dating. *Evolution* 55: 1315–1324.
- RICKLEFS, R. E., AND S. S. RENNER. 1994. Species richness within families of flowering plants. *Evolution* 48: 1619–1636.
- RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- SALARD-CHEBOLDAEFF, M. 1978 [1979]. Palynologie Maestrichtienne et Tertiaire du bassin sédimentaire littoral du Cameroun. *Pollen & Spores* 20: 215–260.
- SALARD-CHEBOLDAEFF, M. 1981. Palynologie maestrichtienne et tertiaire du Cameroun. *Resultats botaniques. Review of Palaeobotany and Palynology* 32: 401–439.
- SANDERSON, M. J. 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution* 19: 101–109.
- SANDERSON, M. J. 2003. Molecular data from 27 proteins do not support a Precambrian origin of land plants. *American Journal of Botany* 90: 954–956.
- SWOFFORD, D. L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer, Sunderland, Massachusetts.
- SYTSMAN, K. J., A. LITT, M. L. ZHRA, J. C. PIRES, M. NEPOKROEFF, E. CONTI, J. WALKER, AND P. G. WILSON. 2004. Clades, clocks, and continents: historical and biogeographical analysis of Myrtaceae, Vochysiaceae, and relatives in the southern hemisphere. *International Journal of Plant Sciences* 165, in press.
- THORNE, J. L., AND H. KISHINO. 2002. Divergence time estimation and rate evolution with multilocus data sets. *Systematic Biology* 51: 689–702.
- THORNE, J. L., H. KISHINO, AND I. S. PAINTER. 1998. Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution* 15: 1647–1657.
- WEHR, W. C., AND D. Q. HOPKINS. 1994. The Eocene orchards and gardens of Republic Washington. *Washington Geology* 22: 27–34.
- YANG, Z. 1997. PAML: a program package for phylogenetic analysis by maximum likelihood. *CABIOS* 13: 555–556. <http://abacus.gene.ucl.ac.uk/software/paml.html>
- YANG, Z., AND A. D. YODER. 2003. Comparison of likelihood and Bayesian methods for estimating divergence times using multiple gene loci and calibration points, with application to a radiation of cute-looking mouse lemur species. *Systematic Biology* 52: 1–12.