

Multiple Miocene Melastomataceae dispersal between Madagascar, Africa and India

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Melastomataceae *sensu stricto* (excluding Memecylaceae) comprise some 3000 species in the neotropics, 1000 in Asia, 240 in Africa, and 230 in Madagascar. Previous family-wide morphological and DNA analyses have shown that the Madagascan species belong to at least three unrelated lineages, which were hypothesized to have arrived by trans-oceanic dispersal. An alternative hypothesis posits that the ancestors of Madagascan, as well as Indian, Melastomataceae arrived from Africa in the Late Cretaceous. This study tests these hypotheses in a Bayesian framework, using three combined sequence datasets analysed under a relaxed clock and simultaneously calibrated with fossils, some not previously used. The new fossil calibration comes from a re-dated possibly Middle or Upper Eocene Brazilian fossil of Melastomeae. Tectonic events were also tentatively used as constraints because of concerns that some of the family's fossils are difficult to assign to nodes in the phylogeny. Regardless of how the data were calibrated, the estimated divergence times of Madagascan and Indian lineages were too young for Cretaceous explanations to hold. This was true even of the oldest ages within the 95% credibility interval around each estimate. Madagascar's Melastomeae appear to have arrived from Africa during the Miocene. *Medinilla*, with some 70 species in Madagascar and two in Africa, too, arrived during the Miocene, but from Asia. *Gravesia*, with 100 species in Madagascar and four in east and west Africa, also appears to date to the Miocene, but its monophyly has not been tested. The study afforded an opportunity to compare divergence time estimates obtained earlier with strict clocks and single calibrations, with estimates based on relaxed clocks and different multiple calibrations and taxon sampling.

Keywords: Bayesian divergence time estimation; *Gravesia*; long-distance dispersal; Madagascar; *Medinilla*; Melastomeae

1. INTRODUCTION

The goal of the present analysis is to test the recent out-of-India hypothesis for the arrival of three tribes of Melastomataceae, namely Dissochaeteae, Melastomeae and Sonerileae in Southeast Asia (Morley & Dick 2003). This hypothesis goes back to the view that Melastomataceae (here used in the narrow sense, excluding Memecylaceae) date to the Upper Cretaceous, with the fragmentation of Gondwana playing a major role in the establishment of tribes within the family (Raven & Axelrod 1974; Gentry 1982 (see table 5 therein), 1993; Renner 1993; Jacques-Félix 1994). Although these earlier studies did not elaborate on a possible role of India as a northward conveyor of Melastomataceae, they certainly implied that major lineages had diverged from each other in Gondwanan times and were separated further by plate fragmentation. Molecular phylogenies allow testing of these hypotheses in two ways: first, by providing information on the geographical direction of sequential events; and second, by providing information on relative and absolute timing based on molecular clocks. Based on molecular

sequence data, northward transport via India has been inferred, for example, for the plant families Crypteroniaceae (Conti *et al.* 2002; Rutschmann *et al.* 2004) and Dipterocarpaceae (Ashton & Gunatilleke 1987; Ducousso *et al.* 2004) and the animal lineages acrodont lizards (Rastegar-Pouyani & Papenfuss 2000), ranid frogs (Biju & Bossuyt 2003), caecilian amphibians (Wilkinson *et al.* 2002) and ratite birds (Cooper *et al.* 2001). For Melastomataceae, however, molecular phylogenies have revealed that divergence events thought to date back to well-understood Gondwanan events, for example the break-up of South America and Africa, occur at very different distances from the phylogenetic trees' roots, some being placed near the root, others close to the tips (Renner & Meyer 2001; Renner *et al.* 2001). Accordingly, hypotheses of trans-oceanic long-distance dispersal were put forward to explain the shallowest geographical disjunctions. Explaining them other than by different absolute ages would have required assuming tremendous rate heterogeneity in the data. The possibility of strong rate heterogeneity, of course, is at the heart of widespread distrust of molecular clock-based estimates.

Over the past 5 years, approaches that attempt to deal with rate heterogeneity between lineages have proliferated (reviewed in Magallón 2004), affording an opportunity to

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Table 1. Species diversity of Melastomataceae genera native around the Indian Ocean. (The assignment of genera to Dissochaeteae and Sonerileae follows tradition (Renner 1993), but the monophyly of these tribes is doubtful (Clausing & Renner 2001a,b).)

taxon	Africa	Madagascar	India and Sri Lanka	Southeast Asia
Melastomataceae				
<i>Amphorocalyx</i>	0	4	0	0
<i>Antherotoma</i>	15	1 (invasive)	0	0
<i>Dichaetanthera</i>	7	ca. 25	0	0
<i>Dionycha</i>	0	3	0	0
<i>Melastoma</i>	0	0	3	10
<i>Osbeckia (Rousseauxia)</i>	5	10–13	29–37	10 (Laos, Thailand)
<i>Tristemma</i>	15	1 (invasive)	0	0
Dissochaeteae spp./genera	2/1 (<i>Medinilla</i>)	70/1 (<i>Medinilla</i>)	8–10/4 (<i>Kendrickia</i> , <i>Medinilla</i> , <i>Oxyspora</i> , <i>Pseudodissochaeta</i>)	300/9
Sonerileae spp./genera	53/6 (including four spp. of <i>Gravesia</i>)	100/1 (<i>Gravesia</i>)	10–20/3 (<i>Ochthocharis</i> , <i>Sarcopyramis</i> , <i>Sonerila</i>)	ca. 490/22

compare earlier divergence time estimates derived from strict, i.e. globally enforced, molecular clocks (Renner & Meyer 2001; Renner *et al.* 2001) with estimates obtained from relaxed clocks (Renner 2004a), as well as to explore the effects of particular taxa and multiple simultaneous calibration points. Progress in fossil dating, including Melastomataceae fossils, also adds new aspects to be considered when testing the out-of-India hypothesis for Melastomataceae. The data analysed here come from three chloroplast loci and are calibrated with multiple tectonic and fossil constraints. The geographic focus is on Africa, Madagascar and India, not only because of the strong hypothesis that three lineages of Melastomataceae reached Asia on an Indian raft (Morley & Dick 2003), but also because Madagascar provides a particularly well-delimited and tractable study system, because it has only six native genera of Melastomataceae (table 1). (Two additional genera are each represented by an invasive species.) India, too, harbours few native genera, namely *Kendrickia*, *Medinilla*, *Melastoma*, *Ochthocharis*, *Osbeckia*, *Oxyspora*, *Pseudodissochaeta*, *Sarcopyramis* and *Sonerila*, of which all but *Ochthocharis* and *Sarcopyramis* have had at least one species sequenced (Clausing & Renner 2001a; Renner *et al.* 2001); sequences of *Pseudodissochaeta* are incomplete, and none of the sequenced accessions came from India. Sri Lanka has four genera, all also found in India.

2. MATERIAL AND METHODS

(a) *Sampling of Madagascan Melastomataceae and their outgroups*

Some 230 species of Melastomataceae are known from Madagascar, and they belong to six genera in the morphologically defined tribes Dissochaeteae, Melastomeae and Sonerileae (Clausing & Renner 2001b; Almada 2003). Molecular data support the monophyly of Melastomeae, but Dissochaeteae and Sonerileae are too poorly sampled for proper assessment of their circumscription. Global relationships of the six native Madagascan genera have been assessed in family-wide analyses that included 59 genera of Melastomataceae and Memecylaceae, and the outgroup families Alzateaceae, Crypteroniaceae, Oliniaceae, Penaeaceae, Rhynchoalycaceae, Myrtaceae, Onagraceae and

Lythraceae (Renner *et al.* 2001). Datasets of aligned nucleotide sequences are available from TREEBASE, and species names with authors and voucher numbers are listed in Renner *et al.* (2001) and Renner (2004b), except for *Calvoa grandifolia* Cogn. (leg. E. Figueiredo 202, voucher in herbarium LISC, GenBank accession numbers AY660632 and AY667151) and *Tristemma littorale* Benth. (leg. E. Figueiredo 212, voucher at LISC, GenBank accession numbers AY660631 and AY667150), both newly sequenced for this study. Both are endemic to São Tomé and Príncipe, oceanic islands in the Gulf of Guinea, off the coast of Cameroon. As in Clausing & Renner (2001a), missing sequences were either coded as question marks (part of *ndhF* of *Bellucia pentamera*, *ndhF* for *Calvoa grandifolia* and *Tristemma littorale*; *rbcl* for *Aciotis purpurascens*, *Nepsera aquatica*) or complemented with those of close relatives, namely *Astronia macrophylla* and *A. smilacifolia*, *Gravesia guttata* and *G. viscosa*, *Melastoma malabathricum* and *M. sanguineum*, *Memecylon bakerianum* and *Mem. edule*, and *Tibouchina longifolia* and *T. urvilleana*.

Table 1 shows the species richness of the genera occurring on Madagascar. Except for *Amphorocalyx* and the invasive species of *Antherotoma*, all genera have been sampled for *ndhF* and most also for *rbcl* and/or *rpl16*. *Amphorocalyx* is morphologically close to *Dichaetanthera*, with at least one species recently transferred to that genus.

(b) *DNA data generation, phylogeny estimation and evolutionary rate analyses*

Details of DNA extraction, sequencing, alignment and tree-searching strategies for the data used in this study are provided in Renner (2004b). The particular tree topology that served as the input data for the Bayesian divergence time analysis was the first of six equally parsimonious trees found in a branch-and-bound search using PAUP* v. 4.0b.10 (Swofford 2002) from the concatenated *ndhF*, *rbcl* and *rpl16* sequences of 57 Melastomataceae (including the newly sequenced species of *Tristemma* and *Calvoa*) and outgroups, rooted with the Onagraceae *Ludwigia suffruticosa*. DNA insertions or deletions that involved most positions in a character row were excluded from the analysis, leaving 3100 bp. Statistical support was assessed by non-parametric bootstrapping in PAUP*.

Likelihood ratio tests revealed that substitution rates in the *rbcl* + *ndhF* + *rpl16* dataset could not justifiably be modelled as clock-like. Variation in the rate of substitutions along the tree was



Figure 1. Chronogram for Melastomataceae with branch lengths obtained from Bayesian time estimation, employing six maximal (Ma) and minimal (Mi) constraints and a rooted parsimony topology obtained from combined *rbcL* + *ndhF* + *rpl16* cpDNA sequences. The Onagraceae *Ludwigia* served to root the tree, but is not shown here. The absolute age estimates for nodes A–D with 95% credibility intervals are listed in table 2, row 5. The numbered nodes refer to the following Mi and Ma constraints: (1) root node: Ma = 130 Myr ago; (2) Myrtaceae crown group: Mi = 88 Myr ago; (3) split between neotropical Alzateaceae and its South African relatives: Mi = 90 Myr ago; (4) split between palaeotropical and neotropical Memecylaceae: Mi = 90 Myr ago; (5) stem lineage of Merianieae/Miconieae: Mi = 55 Myr ago; (6) crown group Melastomeae: Mi = 45 Myr ago. Names in black, neotropics; names in green, Africa; names in red, Madagascar; names in pink, Asia.

therefore modelled explicitly. This is feasible by using an MCMC approach and employing an autocorrelation parameter that

minimizes the amount of permitted rate change along branches, thereby constraining the almost infinite number of possible rate

changes along branches. The software package used was that of Thorne & Kishino (2002) (freely available at <http://statgen.ncsu.edu/thorne/>), the key programs being ESTBRANCHES and MULTIDIVTIME. As recommended in Thorne's manual, PAML's BASEML program, v. 3.14 (Yang 1997) and the F84 + G model (with five rate categories) were used to estimate nucleotide substitutions in the concatenated data. The F84 + G model is the only model so far implemented in ESTBRANCHES, and Thorne's package includes software that parses the output from BASEML for ESTBRANCHES, which then calculates a variance/covariance matrix for the branch length estimates, given the sequence data and specified rooted topology. The output branch lengths from ESTBRANCHES formed the priors for an MCMC search in MULTIDIVTIME that sought to find the most likely model of rate change, given the topology, estimated prior branch lengths, and a Brownian motion parameter, ν , that determines the permitted rate change between ancestral and descendant nodes (below). I used the following settings in the MULTIDIVTIME control file: Markov chains sampled 10 000 trees every 100th generation with a burn-in of 100 000 trees. This burn-in had been found sufficient in earlier Bayesian analyses of the same data, which showed that chains became stationary after this many cycles. I repeated the MCMC procedure several times from different starting points and with different numbers of cycles to determine whether the approximations obtained were similar to one another. The *a priori* expected number of time units between root and tips was set to 1 because the chosen unit of time was 100 Myr. The standard deviation of this prior was set to 1, following Thorne's manual, and the *a priori* rate at the root was set to 0.001, based on his recommendation that it be calculated by dividing the median of the distances between the ingroup root and the tips by the time unit. To explore the effects of the *a priori* root rate, I reran one analysis with a root rate 20 times faster, namely 0.029. The prior for ν was set to 1, following the manual's recommendation that the time units between root and tips to the power of ν be *ca.* 1. The standard deviation on ν was also set to 1.

(c) Calibrations

Calibration is the prerequisite for obtaining absolute ages from genetic distances. In Bayesian divergence time estimation, one uses either upper or lower bounds (set by fossils or tectonic events), and estimates then can move freely towards the unconstrained side, until encountering another calibration boundary or the present time. Morley & Dick (2003) were concerned that Laurasian Miocene seed fossils (used for calibration by Renner & Meyer (2001) and Renner *et al.* (2001); see § 4) may underestimate the true age of Melastomataceae. The present study circumvents this problem by no longer employing these fossils for calibration and instead relying on Eocene melastome leaf fossils, outgroup fossils, and the tectonic calibrations of Morley & Dick (2003). Node 1 in figure 1 was assigned a maximal age of 130 Myr ago based on an assumed onset of angiosperm divergence at 141–132 Myr ago as suggested by their earliest fossils (Brenner 1996). Node 2 in figure 1 was assigned a minimal age of 88 Myr ago based on earliest Myrtaceae pollen and following Morley & Dick (2003); this probably overestimates the age of node 2 (Sytsma *et al.* 2004). Node 3 was assigned a minimal age of 90 Myr ago following Morley & Dick (2003), and based on the estimates of Conti *et al.* (2002) and Rutschmann *et al.* (2004) who, however, used Melastomataceae fossils for calibration, which introduces an element of circularity. However, their convincing biogeographic scenario also employs other evidence, not related to Melastomataceae fossils. Node 4 was constrained to minimally 90 Myr

ago based on the breakup of west Gondwana; this again follows Morley & Dick's (2003) use of tectonic calibrations. Node 5, the stem of Meranieae/Miconieae, was constrained to minimally 55 Myr ago, based on one possible placement of Eocene melastome leaves (Morley & Dick 2003). Node 6, the Melastomeae clade, which includes the genus *Tibouchina*, was set to minimally 45 Myr ago based on a possibly Middle or Upper Eocene *Tibouchina*-like leaf from Brazil. This well-preserved material (judging from black and white photographs) comes from a site near Fonseca originally thought to be Pliocene or Upper Miocene (Duarte 1956), but re-assigned to the Middle or Upper Eocene (49–34 Myr ago) based on the associated pollen flora (Lima & Salard-Cheboldaef 1981).

3. RESULTS

(a) Tree topology

The tree resulting from the concatenated *ndhF*, *rbcL* and *rpl16* data matches topologies obtained earlier, at least where the same taxa are included (Clausing & Renner 2001*a,b*; Renner & Meyer 2001; Renner *et al.* 2001; Renner 2004*b*). The newly sequenced *Tristemma littorale*, a coastal endemic from São Tomé re-collected by E. Figueiredo (personal communication) places as a sister to a collection of *Tristemma mauritianum* from Madagascar. The likewise newly sequenced *Calvoa grandifolia*, also from São Tomé, is a sister to an accession of *C. orientalis* from Tanzania.

Three topological findings directly address the out-of-India hypothesis: (i) Madagascar *Gravesia* is in a clade with African Sonerileae (*Amphiblemma* and *Calvoa*) and all three are nested among Asian Sonerileae/Dissochaeteae; (ii) Madagascar *Medimilla* is a sister to Asian *Medimilla* (Dissochaeteae); and (iii) African, Madagascar, Indian and Asian Melastomaeae form a clade nested among neotropical Melastomeae, and Madagascar *Dichaetanthera* (Melastomeae) is a sister to a clade of genera from Africa, India and Indochina (*Dissotis*, *Melastoma*, and *Osbeckia*).

(b) Time estimates

Table 2 summarizes divergence time estimates, standard errors and 95% credibility intervals obtained in earlier strict-clock-one-gene analyses (Renner & Meyer 2001; Renner *et al.* 2001), a relaxed-clock-three-genes analysis (Renner 2004*b*), and the present study, all with somewhat different sets of calibrations and taxa. Studies also differ in their use of single calibrations versus simultaneous multiple calibrations. Estimates, however, largely agree, and whether or not Melastomataceae fossils were used for calibration made little difference (table 2).

4. DISCUSSION

Magnetic anomaly data indicate that India and Madagascar broke away from Africa 165 Myr ago and that the Madagascar section of the Deccan plate reached its present position, *ca.* 400 km from Africa, by 121 Myr ago (Rabinowitz *et al.* 1983; Briggs 2003). India then separated from Madagascar at *ca.* 88 Myr ago and drifted northwards, colliding with Asia some 50 Myr ago (Beck *et al.* 1995; Storey *et al.* 1995; Mahoney *et al.* 2002; Cliff *et al.* 2002). During its northward journey it may have come relatively close to the horn of East Africa, perhaps facilitating trans-oceanic dispersal from East Africa to India (Morley & Dick 2003). The fit perceived by Morley & Dick

Table 2. Divergence time estimates (millions of years ago) obtained from a clock-enforced one-gene analysis that included 91 taxa and estimates obtained from non-parametric three-locus analyses that included 54 and 57 taxa. (Following the estimates are standard errors or 95% credibility intervals (in parentheses). The constraints and results shown in row 5 are the ones illustrated in figure 1, which also shows nodes A–D. An asterisk signifies that a clade was not dated owing to taxon sampling issues.)

calibration and type of clock	Melastomeae in Africa (node A)	Melastomeae in India/Asia (node B)	Asian Sonerileae in Africa/Madagascar (node C)	Asian <i>Medimilla</i> in Madagascar (node D)
strict clock, single data partition, single calibration points ^{a,b}				
Melastome	12 ± 3 ^a ;	<i>Melastoma</i> crown	10 ± 3 ^a	Madagascar crown
Laurasian	10.7 ± 3 ^b	group: 1 ± 0.9 ^b		group: 1.6 ± 1 ^a
Miocene seeds				
Melastome	14 ± 4 ^a	(*)	11 ± 3 ^a	2 ± 1 ^a
Laurasian				
Eocene leaves				
relaxed clock, three combined data partitions, multiple simultaneous calibrations ^{c,d}				
only outgroup	15 (9, 25) ^c	<i>Osbeckia</i> : 7 (3, 13) ^d	18 (9, 31) ^d	Asian/Madagascar split
fossils plus tectonic events				15 (8, 27) ^d
outgroup fossils, tectonic events, and <i>Meriania</i> -like leaf	17 (9, 27) ^c	<i>Osbeckia</i> : 8 (4, 14) ^d	20 (11, 32) ^d	17 (8, 28) ^d
outgroup fossils, tectonic events, <i>Meriania</i> -like leaf, and <i>Tibouchina</i> -like leaf	34 (25, 44) ^d	<i>Osbeckia</i> : 16 (8, 28) ^d	28 (14, 44) ^d	24 (11, 39) ^d

^a Renner *et al.* (2001); ^b Renner & Meyer (2001); ^c Renner (2004b); ^d This study.

(2003; figure 1) between the ranges of modern tribes, and even genera, with the sequence of fragmentation of west and east Gondwana hinges on the assumption that the divergence between neotropical and African Melastomeae is 74 Myr old 'based on ages of plate tectonic separations' (Morley & Dick 2003, p. 1640). From Africa, the Melastomeae tribe is then thought to rapidly have reached the Deccan plate at *ca.* 72 Myr ago, followed by differentiation 68–45 Myr ago on an Indian raft, and entry into Laurasia after the collision of India and Laurasia at 45 Myr ago. (Morley & Dick place the breakup of India and Madagascar at 68 Myr ago while the studies cited above place it at 88 Myr ago.) In addition to Melastomeae, also Dissochaeteae (*Medimilla*) and Sonerileae (*Gravesia*), tribes of unclear circumscription, are hypothesized to have dispersed by stepping stones from South America to Africa *ca.* 74 Myr ago, followed by diversification and rafting on India, and arrival in Asia after the India–Asia collision at 45 Myr ago. The absence of Dissochaeteae and Sonerileae from South America and their poor representation in mainland Africa are attributed to extinction.

An alternative view, namely that Melastomataceae arrived in Madagascar by long-distance dispersal, came from results of a globally enforced molecular clock calibrated in two ways, once with Miocene Melastomeae seeds, then, as a test, with Eocene Melastomataceae leaves (Renner & Meyer 2001; Renner *et al.* 2001). Melastomeae seeds have been reported from sites throughout Eurasia (Dorofeev 1960, 1963, 1988; Collinson & Pinggen 1992; Dyjor *et al.* 1992; Fairon-Demaret 1994; Mai 2000), whereas Melastomataceae leaves are known from northern

North America, Colombia, Ecuador, Brazil and Sumatra. The oldest of these leaves are from the Early Eocene and Early Middle Eocene of North Dakota and Washington State (Hickey 1977; Wehr & Hopkins 1994), but well-preserved Brazilian leaves may also date to the Middle or Upper Eocene (49–34 Myr ago; Duarte 1956; Lima & Salard-Cheboldaef 1981; cf. § 2c). The family's pollen record is problematic because Melastomataceae pollen resembles that of several other Myrtalean families (Patel *et al.* 1984; details in § 4b(i)). There are no Tertiary macrofossils of Melastomataceae from Africa, Madagascar or tropical Asia, and the out-of-India hypothesis therefore can only be tested by a strict assessment of confidence limits on the relevant molecular clock estimates. Calculation of confidence limits around estimates is particularly feasible in a Bayesian framework because the credibility intervals for node times are part of the standard output and do not need to be calculated by bootstrapping approaches, the application of which to clock analyses is poorly understood.

Of course, fossils only indicate minimal ages, and results based on fossil calibrations may therefore greatly underestimate the true ages of lineages. This study tried to reduce this problem by additionally using several of the tectonic calibrations employed by Morley & Dick (2003), and by using a mix of ingroup and outgroup fossils, including one that may overestimate rather than underestimate the age of a particular node (cf. § 2c). By including as many multiple calibrations as possible and by checking which tectonic events and additional fossils may fall within the confidence limits of the obtained time estimates, one presumably mitigates the effects of poorly assigned fossils. Even fossils

not used in a particular study because of problematic assignment or taxon-sampling issues can provide indirect evidence for, or against, age estimates and biogeographic scenarios (e.g. Plana *et al.* 2004). Another way to improve confidence is a statistical approach that incorporates information on the temporal spread (density) of a taxon's fossil record and, based on this, places confidence intervals around estimates of lineage duration (Foote 1997; Foote & Sepkoski 1999).

(a) Estimating times from concatenated datasets using multiple simultaneous constraints versus from single genes and single calibrations

Relaxing the clock assumption of a single substitution rate across lineages and using more data for branch length estimation may provide a more solid basis for divergence times than enforcing a global clock on a single locus (at least theoretically, information content can increase with nucleotide number). It is difficult to know though whether the heterogeneity introduced by combining different data partitions, such as genes and introns, offsets the advantages gained by basing estimates on more data, which results in narrower error variances on branch lengths and therefore on times. In this study, I tried to reduce estimation error by concatenating all data partitions. When choosing a molecular evolution model for heterogeneous data partitions, the single most important parameter appears to be the gamma parameter for among-site rate variation (Nylander *et al.* (2004) and references therein). This may justify use of the relatively simple F84 model but with approximation of the gamma shape parameter with five rate categories (the only model so far implemented in Thorne's Bayesian divergence time estimation software). It was surprising how little the estimated divergence times differed between the earlier strict clock + single data partition + single-calibration analysis and the present relaxed clock + multiple data partitions + multiple calibrations analysis (table 2). Even changing the *a priori* rate from 0.001 to 20 times faster, namely 0.029, yielded almost the same age estimates as long as five or six upper or lower bounds had been placed on node ages (data not shown). Melastomataceae appear to have an accelerated rate of nucleotide substitution compared with other Myrtales (Sytsma *et al.* 2004). However, whether substitution rates are high or low is irrelevant for molecular clocks as long as data are internally calibrated and rates internally relatively unchanging.

One of the advantages of the Bayesian approach to divergence time estimation, compared with other relaxed clock approaches (e.g. Sanderson's (2002) penalized likelihood approach) is that credibility intervals are part of the standard output and need not be calculated by bootstrapping. As expected, credibility ranges are influenced by the proximity of nodes in the tree to constraints. Nodes that are constrained or that are near constrained nodes tend to have narrower credibility intervals than other nodes (Nylander *et al.* 2004).

Overall, however, results obtained with the different clock approaches, including the strict clock with a single calibration, judging by the present comparison (cf. table 2), appear not to differ very much. On the one hand, this attests to the consistency of the methods, on the other hand, it warns against a naive belief that relaxed clock approaches truly overcome strong rate heterogeneity

problems (if present) and associated misjudging of true ages.

(b) Implications: exchange of Melastomataceae lineages between Madagascar, Africa and across the Indian Ocean

The tree topologies and estimated divergence times suggest that: (i) Sonerileae dispersed from Asia to Africa, where they apparently reached Madagascar, by trans-oceanic dispersal during the Miocene; (ii) that *Medimilla* also arrived from Asia sometime during the Miocene; and (iii) that the only Asian Melastomeae, *Osbeckia* and *Melastoma*, arrived there from Africa at different times during the Miocene. Earlier sampling of 27 species in 15 genera of Asian and Madagascan Dissochaeteae/Sonerileae for *ndhF* showed even more clearly than the reduced taxon sample used here that the direction of dispersal was from Asia to Madagascar/Africa (Clausing & Renner 2001a; Renner *et al.* 2001).

The following sections place the Miocene dispersal of these lineages in the context of climate, sea surface currents, wind currents, and bird flight paths as well as diaspore morphology and dispersal ecology.

(i) Melastomeae

Most species of *Melastoma* (including *Otanthera* (Meyer 2001)) have been sequenced, and an earlier study found that the apparently very rapid radiation may have began *ca.* 1 Myr ago (Renner & Meyer 2001). Of the 22 species of *Melastoma*, three occur in India, one of them being the invasive *M. malabathricum*, which also occurs on numerous Pacific islands. The genus *Melastoma* differs from most other Melastomeae in possessing fleshy, bird-dispersed fruits. The only other fleshy-fruited Melastomeae are certain species of *Dichaetanthera*, with representatives in East Africa and on Madagascar. Birds that regularly cross the *ca.* 400 km wide Mozambique channel include the Madagascar squacco heron (virtually the entire population 'winters' in Tanzania), the Madagascar cuckoo, a winter migrant from Madagascar to East Africa, the Madagascar pratincole (populations winter along the Kenya/Somali coast, migrating along coastal Tanzania), the broad-billed roller, and the Mascarene martin, the last, however, only an irregular migrant between the mainland and Madagascar (Moreau 1966; J. Reichholf, personal communication; A. Scheuerlein, personal communication).

Besides *Melastoma*, Asia harbours only one other member of the Melastomeae, *Osbeckia*, which is most species-rich in northern India and Burma, and also has 10–13 species on Madagascar and five in Africa. *Osbeckia* has capsules with minute wind-dispersed seeds that conceivably may have been transported by monsoon wind systems or occasional storms between Africa, the Seychelles, the Comores, the Chagos archipelago (about half-way between Africa and Indonesia) and India. Alternatively, these seeds could become lodged in floating vegetation. Whatever the means of transport, the Indian species of *Osbeckia* appear to be only 8–7 Myr old (table 2), but denser taxon and gene sampling is needed because Indian, African, Madagascan and Indochinese members of *Osbeckia*, *Dissotis*, *Dionycha*, *Heterotis* and *Rousseauxia* form a poorly resolved plexus (as expected from their very similar morphology; Renner

& Meyer (2001) found *Rousseauxia* embedded among Madagascan *Osbeckia* and *Dionycha*.

The Gondwanan scenario (Morley & Dick 2003), which posits that Madagascan members of Melastomeae, specifically *Dichaetanthera* and *Dionycha*, diversified at *ca.* 72 Myr ago, followed by differentiation of ‘*Osbeckia/Melastoma/Oranthera* on the Indian Plate (68–45 Ma) or in Southeast Asia after 45 Ma’ (Morley & Dick 2003, p. 1642) and ‘differentiation on the Indian Plate prior to collision with Asia in the Middle Eocene’ (Morley & Dick 2003, p. 1642) implies that this group is *ca.* 50 Myr old. This is much older than the oldest molecular estimates for the age of *Osbeckia* that are within the 95% confidence intervals (table 2; *Melastoma* may be as young as 1 Myr old). This level of confidence needs to be compared with the confidence one can place in the fossil record of Melastomataceae from Africa, India, and tropical Asia. This record so far consists of *Astronia*-like leaves (tribe Astronieae) described from the Upper Miocene (12–5 Myr ago) of Sabah (Geyler 1887) and possible pollen records of Melastomeae (Salard-Cheboldaëff 1978, 1981) from Cameroon. The oldest of these, Upper Eocene *Heterocolpites laevigatus* (37–34 Myr), was compared to *Terminalia* (Combretaceae; Salard-Cheboldaëff 1978, 1981; Muller 1981; Coetzee & Muller 1984). The second oldest, Upper Eocene to Oligocene *H. verrucatus*, was comparable to Lythraceae or Melastomataceae, and the youngest, Oligocene *H. pseudostriatus* (34–24 Myr) to *Dissotis*, a genus of Melastomeae. The indistinctly finely striate exine that distinguishes *H. pseudostriatus*, however, is unusual in extant Melastomataceae (Patel *et al.* 1984). If the Oligocene Cameroonian pollen indeed represents Melastomeae, the fossil date would fall inside the 95% confidence intervals around some of the molecular clock estimates for Melastomeae in West Africa (table 2).

(ii) *Sonerileae*

Gravesia has capsules with minute seeds and *ca.* 100 species on Madagascar and four or five in East Africa and Gabon (Perrier de la Bâthie 1951; Almeda 2003). In addition to these species of *Gravesia*, mainland Africa has 53 species of *Sonerileae* in six genera (Jacques-Félix 1994). The hypothesis that African *Sonerileae* are at least 68 Myr old (Morley & Dick 2003) is rejected by molecular topologies and age estimates: the estimated divergence time of the African genus *Calvoa* from the Madagascan genus *Gravesia* is *ca.* 7 Myr ago, with a 95% confidence range of 14 to 0.7 Myr. The divergence of the species *Calvoa orientalis* from Tanzania and *C. grandifolia* from São Tomé, which are separated by some 4000 km, appears to date to the Miocene–Pliocene boundary (figure 1), and the divergence between *Tristemma littorale*, also endemic on São Tomé, and *Tristemma mauritianum* from Madagascar also falls into that same general time period. *Tristemma* has 15 species (Jacques-Félix 1976, 1994) and *Calvoa* 18 (Jacques-Félix 1981), and both are centred in West Africa, with only a few species reaching East Africa or, in the case of *T. mauritianum*, Madagascar. The last is a polymorphic invasive species with fleshy fruits, atypical of *Sonerileae*. Its range shows several disjunctions between difficult to recognize subspecies and varieties, including the three subspecies recognized on São Tomé and the one on Madagascar. (Based on *ndhF*, *Tristemma* was earlier found

to be a sister to the West African *Melastomastrum* (Renner & Meyer 2001).) The Pliocene divergence times obtained here between São Tomé species and their East African or Madagascan relatives fit with those for similar pairs in *Begonia* (Plana *et al.* 2004); however, much denser species sampling in *Calvoa* and *Tristemma* is necessary to assess sister species relationships.

Current species sampling is also too limited to decide whether the ancestors of the Madagascar-centred genus *Gravesia* reached that island directly from Asia or whether they came from Africa, where *Gravesia* today has four species. Only *G. guttata*, *G. rutenbergiana* and *G. viscosa* have been sequenced, but not for all markers employed here (Clausing & Renner 2001a), and only a single composite ‘*Gravesia*’ is used in the present study (cf. § 2). With only a single *Gravesia* included and the remaining genera represented by one or two species (figure 1), the geographical spread of *Sonerileae* from Madagascar to Africa, or the other way around, remains ambiguous. (The colour-coded tree in Renner *et al.* (2001, figure 2) showed only one of two equally parsimonious scenarios.) Sequencing of the four west and East African species of *Gravesia*, as well as more Madagascan species, is underway.

(iii) *Dissochaeteae*

Medinilla has fleshy, bird-dispersed berries and has some 85 species in tropical Asia, about six in India, 70 in Madagascar, and two in Africa. The last may be vicariant sister taxa: *Medinilla engleri* Gilg is an Eastern Arc endemic and *M. mannii* Hook. filius has a Guineo–Congolian distribution (R. Gereau, personal communication). It would be surprising if the two were not derived from Madagascan or Asian ancestors because all *Medinilla* sequenced so far (eight species, three from Madagascar) are part of a large Madagascan–Asian clade of 14 genera (Clausing & Renner 2001a).

5. CONCLUSIONS

In having their oldest fossils come from Eocene Laurasia, Melastomataceae resemble many other families that now dominate at low latitudes: for example, Leguminosae, Lauraceae, and gingers (Herendeen *et al.* 1992; Manchester & Kress 1993; Burnham & Graham 1999). Whether this reflects a true Eocene scarcity or absence of these lineages at low latitudes or is an artefact of uneven sampling by palaeobotanists is unknown. Efforts are underway to database a vast number of records so that statistical assessment of sampling biases will eventually become possible (H. J. Sims, personal communication). So far, however, our inability to treat the absence-of-evidence problem in a consistent manner has left the door open for speculation and even controversy about lineage ages and co-dependent species accumulation rates. Discerning a signal left by the fragmentation of Gondwana from today’s geographical ranges at low latitudes is especially problematic for Africa and Madagascar, which are known to have undergone severe floristic change during the Miocene. Also, molecular data continuously bring to light new examples of transoceanic long-distance dispersal, even in groups traditionally thought to be poor dispersers, suggesting that even more cases await discovery and throwing doubt on Gondwanan scenarios based only on morphology

and 'normal' dispersal biology (Lavin *et al.* 2004; Renner 2004a). Striking examples of transoceanic long-distance dispersal between Africa, Madagascar and the Seychelles include multiple lineages of frogs (Vences *et al.* 2003, 2004), chameleons (Raxworthy *et al.* 2002), snakes (Nagy *et al.* 2003) and lemurs (Yoder & Yang 2004). Most of these dispersal events were once interpreted as a result of Gondwanan vicariance.

For Melastomataceae, results from molecular clocks, whether strict or relaxed, whether based on a single gene or three combined loci, and whether calibrated with or without Laurasian Melastomataceae fossils, indicate that the family arrived on Madagascar multiple times during the Miocene by transmarine dispersal (Renner & Meyer 2001; Renner *et al.* 2001; Renner 2004b; this study). This remains true even when one accepts the oldest age estimates inside the 95% confidence range. Rafting of Melastomeae, Sonerileae or Dissochaeteae on the Indian plate is not supported by molecular or fossil evidence. Given the many examples of dispersal of animals and plants to Madagascar across the Mozambique channel, facilitated by regularly migrating birds, and the former and present stepping-stone islands in the Mozambique channel and the Indian Ocean, the most parsimonious explanation for the close ties between the region's Melastomataceae is long-distance dispersal. The out-of-India hypothesis, by contrast, is very difficult to reconcile with branching order in the phylogeny and requires assuming tremendous rate heterogeneity in the data: compare the depths of Crypteroniaceae and Asian Melastomeae in figure 1: Crypteroniaceae have been inferred to have rafted northwards via India (Conti *et al.* 2002; Rutschmann *et al.* 2004). However, as discussed above, Melastomataceae do indeed appear to have higher substitution rates than their sister families, which was one of the reasons this and a related study (Renner 2004b) explored a relaxed clock approach that allows for rate heterogeneity among different lineages. In the future, additional melastome fossils may add further constraints or provide direct evidence relevant to the long-distance scenario developed here for Melastomataceae disjunct between Africa, Madagascar, India and Southeast Asia. For example, some genera have characteristic leaves (e.g. *Blakea*, *Topobea*) or inflorescences (some Bertolonieae and Sonerileae). Thus, well-preserved Miocene floras from Ecuador and Bolivia (R. J. Burnham, personal communication), the Latah formation near Spokane (J. Wolfe, personal communication), or Sabah (e.g. Geyler 1887) that include Melastomataceae, may eventually help stabilize the temporal picture of Melastomataceae evolution.

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GLOSSARY

MCMC: Markov chain Monte Carlo