

MOLECULAR PHYLOGENETICS OF MELIACEAE (SAPINDALES) BASED ON NUCLEAR AND PLASTID DNA SEQUENCES¹

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Phylogenetic analyses of Meliaceae, including representatives of all four currently recognized subfamilies and all but two tribes (32 genera and 35 species, respectively), were carried out using DNA sequence data from three regions: plastid genes *rbcl*, *matK* (partial), and nuclear 26S rDNA (partial). Individual and combined phylogenetic analyses were performed for the *rbcl*, *matK*, and 26S rDNA data sets. Although the percentage of informative characters is highest in the segment of *matK* sequenced, *rbcl* provides the greatest number of informative characters of the three regions, resulting in the best resolved trees. Results of parsimony analyses support the recognition of only two subfamilies (Melioidae and Swietenioideae), which are sister groups. Meliaceae are the only tribe recognized previously that are strongly supported as monophyletic. The members of the two small monogeneric subfamilies, *Quivisianthe* and *Capuronianthus*, fall within Melioidae and Swietenioideae, respectively, supporting their taxonomic inclusion in these groups. Furthermore, the data indicate a close relationship between Aglaieae and Guareae and a possible monophyletic origin of Cedreleae of Swietenioideae. For Trichilieae (Melioidae) and Swietenieae (Swietenioideae) lack of monophyly is indicated.

Key words: *matK*; Meliaceae; nuclear and plastid DNA; phylogeny; *rbcl*; 26S rDNA; Sapindales.

Meliaceae are a woody family widely distributed throughout the tropics and subtropics, with only slight penetration into temperate zones; they occur in a variety of habitats from rain forests and mangrove swamps to semi-deserts. The timbers of certain Meliaceae are some of the most sought after in the world, such that natural stands have been much depleted (Keay, 1996; Plumtree, 1996; Rodan and Campbell, 1996; Snook 1996; Verissimo et al., 1998; Laurance, 1999; O'Neill et al., 2001). Other uses of Meliaceae comprise shade and street trees, fruit trees, and, last but not least, sources of biologically active compounds (reviewed in Mabberley et al., 1995). Among other secondary metabolites, Meliaceae synthesize and accumulate bitter and biologically active nortriterpenoids, which are also known as limonoids and meliacins. These and other compounds have aroused considerable commercial interest due to their insect-antifeedant (e.g., Abdelgaleil et al., 2001; Simmonds et al., 2001), insect-repellent (e.g., Shukla, et al., 1997; Valladares et al., 1999) or/and insecticidal (e.g., Schneider et al., 2000; Greger et al., 2001), molluscicidal (e.g., Pina et al., 1998; Singh et al., 1998), antifungal (e.g., Govindachari et al., 1999; Engelmeier et al., 2000), bactericidal (e.g., Kumar and Gopal, 1999; Aboutabl et al., 2000), and plant-antiviral (e.g., Singh et al., 1988) activities as well as their numerous medicinal effects in humans and animals (e.g., Bam-

ba et al., 1999; Benencia et al., 2000; Benosman et al., 2000). The limonoids (or meliacins) might offer possibilities for infrafamiliar taxonomy (e.g., Taylor in Pennington et al., 1981; Mulholland et al., 1998), but our knowledge is still extremely fragmentary (Mabberley et al., 1995) so that comprehensive phylogenetic conclusions (Da Silva et al., 1984, 1999; Agostinho et al., 1994; Neto et al., 1998) have been premature and often conflicting.

Compared to other groups of similar size, Meliaceae contain a relatively wide range of floral, fruit, and seed morphologies. For example, within *Aglaia* alone inflorescences can vary from one-third to two-thirds of a meter long with profuse branching and abundant flowers to a much reduced few-flowered inflorescence 1–2 cm long. Seeds of Meliaceae are some of the most diverse and intricate in structure so far investigated (Mabberley et al., 1995). They are usually pendulous and epitropous (but apotropous in the Australian *Synoum*, Guareae) in relation to the placenta. They are usually anatropous (but hemi-anatropous in most Turraeae and *Cipadessa*), occasionally orthotropous as in all *Chisocheiton* and some *Guarea* species, and campylotropous in *Nymania* (Turraeae), for example. In general, a diversity of primitive morphological characters can be observed side-by-side with an array of derived ones, but these are typically connected by intermediates. For this reason, the family has been a source of systematic difficulty, as the taxonomic history of the group clearly shows (summary in Pennington and Styles, 1975). Until the generic monograph of the family by Pennington and Styles (1975), there was persistent disagreement as to the number of genera and their circumscriptions and the best way to accommodate them in tribes and subfamilies. This uncertainty may have been due to the diffuse and often reticulate nature of variation that seems to have been based on several parallel evolutionary trends occurring in flowers and fruits independently (Penning-

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TABLE 1. Characters used to distinguish the subfamilies of Meliaceae sensu Pennington and Styles (1975).

Character	Melioidae	Quivisianthoideae	Swietenioideae	Capuronianthoideae
Buds	naked (not all)	naked	with scale leaves	naked
Fruits	loculicidal capsule, berry, drupe, nut	loculicidal capsule	septifragal capsule	septifragal capsule
Seeds	not corky, not winged	winged	winged or corky	corky

ton and Styles, 1975). Thus, some genera and most tribes can only be diagnosed by using a combination of several characters. Each character taken separately may occasionally be unreliable as a diagnostic tool.

Unlike some other tropical woody groups, Meliaceae also exhibit a considerable range of chromosome numbers (Styles and Vosa, 1971; review in Styles and Bennett, 1992). A base number of $n = 14$ or multiples such as 28, 42, or even higher ploidy represent, according to Khosla and Styles (1975), the vestiges of an archaic stock of $n = 7$, which has been considered by many workers in the field as one of the original base numbers of angiosperms (Stebbins, 1971). Differences in number have been observed between even putatively closely related species, e.g., within *Swietenia*, *Cedrela*, and *Toona*. At the subfamilial and tribal levels, chromosome numbers would appear to have no obvious pattern (Styles and Vosa, 1971). Polyploid series are by no means a rare phenomenon in the group, being apparent in *Aphanamixis*, *Aglaiia*, *Chisocheton*, *Dysoxylum*, and *Swietenia*, for example, including the occurrence of intraspecific chromosome races in some genera (Khosla and Styles, 1975; Mabberley, et al., 1995). Extensive hybridization accompanied by introgression may have occurred in these groups, perhaps leading to the reticulate patterns of morphological traits and the complex interrelationships of the various taxa that are now encountered (Khosla and Styles, 1975). Karyological data give a rather confusing picture of the relationships between taxa, and although some conclusions have been drawn, they have remained mostly speculative (e.g., Mehra et al., 1972; Datta and Samanta, 1977).

The most authoritative work on Meliaceae is at present the generic monograph by Pennington and Styles (1975); currently recognized are 50 genera with about 575 species (Pennington and Styles, 1975; Mabberley et al., 1995; Chase et al., 1999). Pennington and Styles (1975) recognized four subfamilies; Melioidae and Swietenioideae⁶ consist of seven tribes with 35 genera and of three tribes with 13 genera, respectively.

⁶ Antedated by the obscure name Cedreloideae (D. J. Mabberley, personal communication, Royal Botanic Gardens Sydney) which is in the course of being proposed for formal rejection to the committee for spermatophytes of IAPT.

Quivisianthoideae and *Capuronianthoideae* contain a single monotypic genus each (*Quivisianthe* and *Capuronianthus*, respectively). The last two subfamilies were newly recognized by Pennington and Styles (1975). The following characters, among others, were particularly important for the delimitation of subfamilies (Table 1): bud scales (presence/absence), fruits (fleshy/leathery or dry, loculicidal or septifragal dehiscence, capsules, berries, drupes or nuts), and seeds (winged/not winged, corky/not corky). Pennington and Styles (1975) stated that only Swietenioideae have bud scales. Later studies have shown that the presence of bud scales is not restricted to Swietenioideae but can be also found in several representatives of Melioidae (e.g., in some species of *Turraea*, *Trichilia*, and *Ruagea*; Pennington et al., 1981; Cheek, 1990b). Melioidae and *Quivisianthe* share loculicidal dehiscence of their capsules, although in Melioidae capsules are only one out of four fruit types and include also berries, drupes, and nuts. In Melioidae, fruits are fleshy and seeds are never winged with a woody or corky testa, but they usually have a fleshy arillode or sarcotesta. In *Quivisianthoideae*, fruits are dry, and seeds are winged with a dry, non-fleshy testa. Swietenioideae and *Capuronianthus* share capsules with septifragal dehiscence. In *Capuronianthus*, seeds are not fleshy or winged but have a corky sarcotesta. In Swietenioideae, seeds are variously winged but are not fleshy, and they have a woody or corky sarcotesta.

To assess circumscription of the subfamilies and tribes proposed by Pennington and Styles (1975) and infer phylogenetic relationships among them, we performed parsimony analyses of sequence data from three regions: plastid *rbcL*, *matK*, and nuclear 26S rDNA. We included representatives of all four currently recognized subfamilies and all but two tribes (32 genera and 35 species altogether). The phylogenetic utility of 26S rDNA sequence data in the angiosperms has recently been demonstrated (e.g., Kuzoff et al., 1998; Asmussen et al., 2000). Larson (1991) suggested that the difference in rates of base substitution between conserved core regions and expansion segments of 26S rDNA could be exploited for phylogenetic inference at different taxonomic levels; conserved core regions could be used at higher taxonomic levels, and expansion segments could be employed among more closely related

TABLE 2. Statistics for each of the maximum parsimony analyses (CI = consistency index; RI = retention index; GC = guanine and cytosine).

Data set	<i>matK</i>	<i>rbcL</i>	26S rDNA	<i>matK/rbcL</i>	<i>matK/rbcL/26S rDNA</i>
No. of taxa	32	44	33	47	47
No. of Meliaceae	28	38	30	41	41
No. of characters	893	1387	880	2280	3160
No. of variable sites	297	247	130	544	674
No. of informative characters	148	170	71	318	389
Length of shortest tree (no. of steps)	460	474	289	934	1251
No. of shortest trees	9700	612	46	9840	3559
CI	0.69	0.58	0.48	0.63	0.57
RI	0.84	0.84	0.64	0.84	0.79
GC content	36.20	43.86	60.52	41.48	46.15

TABLE 3. Statistics for transitions (ts) and transversions (tv) based on optimizations on one of the trees from the combined maximum parsimony analysis of all three genes (CI = consistency index, RI = retention index).

Data set	<i>matK</i>	<i>rbcL</i>	26S rDNA
No. of steps (ts/tv)	461	480	310
No. of ts	255	277	230
No. of tv	206	203	80
Ts/tv ratio	1.24	1.36	2.88
CI	0.69	0.57	0.44
RI	0.84	0.84	0.57
CI of ts	0.77	0.68	0.45
RI of ts	0.85	0.84	0.53
CI of tv	0.62	0.45	0.43
RI of tv	0.83	0.83	0.61

taxa. We sequenced in this study a roughly 1200 base-pair fragment from the 5' end of the gene, which includes four of the expansion segments. The *matK* gene has been shown to have one of the highest nucleotide substitution rates among 20 plastid genes greater than 1 kilobase (kb) in length, and it is currently being used for inferring phylogenetic relationships at the intrafamilial level (e.g., Koch et al., 2001; Mort et al., 2001). Because of the large data base of *rbcL* sequences available (nearly every family and nearly complete sets of genera for some families) and because patterns of relationships and support derived from *rbcL* data sets are not significantly worse than commonly sequenced noncoding regions (Chase et al., 2000), it makes sense to include *rbcL* as one of the regions when sequencing more than one region (Chase and Albert, 1998).

Many recent studies have indicated that combined molecular data using regions with different numbers of variable sites provide resolution at different taxonomic levels, and phylogenetic resolution and levels of support are improved by directly combining independent molecular data sets (e.g., Chase and Cox, 1998; Soltis et al., 1998, 1999, 2000; Qiu et al., 1999; Savolainen et al., 2000a). Our approach here is to assess patterns of support for each gene separately and, if no evidence of incongruence is present, to combine all three in a single analysis as the basis for further discussion.

MATERIALS AND METHODS

Plant material—The taxa used in this study, their sample designation, and GenBank accession numbers have been archived at the Botanical Society of America website (<http://ajbsupp.botany.org/v90/>). We included 32 (out of the 50) genera and 35 species, comprising representatives of all four subfamilies and all but two tribes.

Isolation of DNA and amplification—Total genomic DNA was isolated using the procedure described by Doyle and Doyle (1987) except that most DNA samples were purified by cesium chloride/ethidium bromide gradients (1.55 g/mL). Polymerase chain reaction (PCR) amplification was carried out in a PTC-100 Programmable Thermal Controller (MJ Research, Margaritella, Bio-Trade, Vienna, Austria) using the following primers: 26S1>, 26S2>, and 1229REV< for 26S rDNA (Kuzoff et al., 1998), 390F> and 1326R< for *matK* (Cuénoud et al., 2002) and 1F> and 1460R< for *rbcL* (Fay et al., 1998). The fragment size amplified was between 870 and 1210 bp for 26S, 870 and 910 bp for *matK*, and 1436 and 1460 bp for *rbcL* (this variation in length is due to the variable downstream position of the ribosomal control site for which 1460R< was designed). A 100- μ L reaction mix contained 62 μ L ddH₂O, 10 μ L 10 \times reaction buffer, 8 μ L 15 mmol MgCl₂, 8 μ L 10 mmol dNTPs, 2 μ L of the primers each (20 pmol), 2 μ L template DNA (100–2000

ng/ μ L), 1 μ L 1 unit/ μ L DNA polymerase (Promega, Mannheim, Germany), as well as 5 μ L dimethyl sulfoxide (DMSO) for nuclear 26S rDNA and 5 μ L bovine serum albumin (BSA; 0.4%) for plastid genes. These additives are thought to stabilize the enzyme, reduce secondary structure problems, or favor precise annealing (Palumbi, 1996). Amplifications were carried out using the following program (with slight modifications for some accessions): initial denaturation for 3 min at 95°C, followed by one cycle of denaturation for 1.5 min at 95°C, annealing for 1 min at 45°C and extension for 1 min at 72°C, followed by 36 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 48°C, and extension for 1 min at 72°C. The amplification was completed by holding the reaction mixture for 10 min at 72°C to allow complete extension of the PCR products. After amplification, samples were gel purified using a QIAquick gel extraction kit (Qiagen, Margaritella, Vienna, Austria).

Sequencing—The same primers as cited above were also used for sequencing. For *rbcL*, four additional internal sequencing primers were used: 636F>, 724R< (Fay et al., 1998), F2N> (5'-CCAAGTTGAGAGAGATAAATTGAACAAG-3') and F2NN> (5'-GCAAATACTAGCTTGGCTCATTATTGCCG-3'), the last two designed for this study. A 10- μ L cycle sequencing reaction mix contained 7 μ L purified template DNA (100–400 ng/ μ L), 2 μ L BigDye Terminator RR Mix (Applied Biosystems, Vienna, Austria) and 1 μ L 10-pmol primer. The following program was used (GeneAmp PCR System 9700, Applied Biosystems): denaturation for 10 s at 96°C, annealing for 5 s at 50°C, extension for 4 min at 60°C (25 cycles). To produce better quality 26S rDNA sequences, we added 2% DMSO to the cycle sequencing reactions; DMSO breaks down secondary structure formed by guanine- and cytosine-rich regions and thereby produces longer lengths of readable sequence. The templates were sequenced on an ABI PRISM 377 DNA Sequencer (ABI) using dye terminators following protocols provided by the manufacturer (ABI).

Sequence editing and alignment—For editing, the software programs Autoassembler version 1.4.0 (Applied Biosystems) and DNA STRIDER version 1.2 (Christian Marck, CEA—Commissariat à l'Énergie Atomique/Saclay, France) were used. Alignment of sequences was done by eye following the recommendation of Kelchner (2000). A total of 893, 1387, and 880 nucleotides were included in the matrices for phylogenetic analyses for *matK*, *rbcL*, and 26S rDNA, respectively. Gaps were coded as missing data (there were not many gaps within these taxa, so alignment was a simple matter). These sequences have been deposited in GenBank under the accession numbers AY128144–AY128252 (<http://www.ncbi.nlm.nih.gov/>); the combined matrix is available from A. N. Muellner (alexandra.muellner@univie.ac.at) and M. W. Chase (m.chase@rbgkew.org.uk).

Phylogenetic analysis—Individual and combined parsimony analyses of the 26S, *matK*, and *rbcL* data sets were performed using PAUP* 4.0b8 (Swofford, 2001) on a Power Macintosh G4. Measures of incongruence like the incongruence length difference (ILD) test have recently been demonstrated not to be useful as indicators of data partition combinability (e.g., Yoder et al., 2001; Reeves et al., 2001). Moreover, hypotheses of conflict based on inspection of trees have been largely supported by subsequent statistical comparisons in several studies (e.g., Mason-Gamer and Kellogg, 1996). Therefore visual inspection of the individual bootstrap consensus trees was used for determining combinability of the three data sets (Whitten et al., 2000). In case of not strongly supported (<85% BP) and incongruent patterns between the individual trees, direct combination was regarded as appropriate. Each included nucleotide position was treated as an independent, unordered, multistate character of equal weight (Fitch parsimony; Fitch, 1971). Heuristic searches were performed using addition sequence set at 1000 random additions of taxa, tree bisection-reconnection (TBR) branch swapping, and MULTTREES on (keeping multiple shortest trees) but holding only 10 trees per replicate to reduce time spent in swapping on large numbers of suboptimal trees. After these 1000 replicates, we then used the shortest trees found as starting trees for a search with a tree limit of 15000, which was reached in some cases; this procedure will often find more trees at this shortest length than were found in the 1000 replicates of random taxon entry. Robustness of clades was estimated using the bootstrap (Felsenstein, 1985) with 1000 replicates with simple sequence

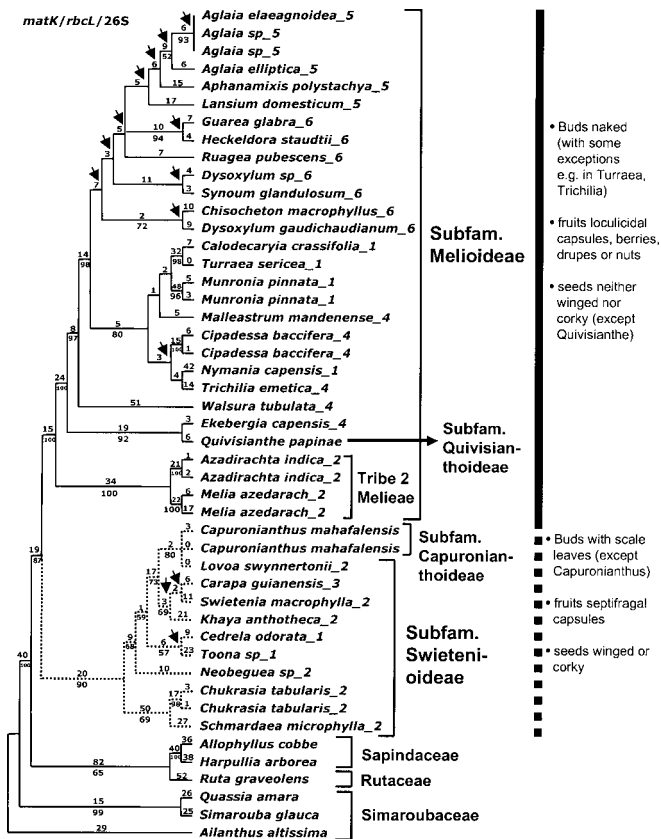


Fig. 1. One of the 3559 most parsimonious trees obtained from the maximum parsimony (MP) analysis of the combined data set (26S rDNA, *matK*, *rbcL*) of 41 Meliaceae accessions. Subfamilies and tribes after Pennington and Styles (1975). Numbers above branches are estimated branch lengths (ACCTRAN optimization), numbers below branches are bootstrap percentages (1000 replicates). Arrowheads indicate groups not present in the strict consensus tree.

addition, TBR branch swapping, and MULTREES on but holding 10 trees per replicate to reduce time spent on each replicate. As outgroup, representatives of the closely related families Rutaceae, Sapindaceae, and Simaroubaceae were used (Gadek et al., 1996; Savolainen et al., 2000b). Patterns of DNA sequence evolution for each gene was assessed on one of the shortest combined trees. To calculate the number of transitions (ts) and transversions (tv) and their collective consistency indices (CI) and retention indices (RI), a step matrix was used to calculate the number of tv by weighting ts to zero. From tv values, those of the ts were calculated (Whitten et al., 2000).

Bayesian analysis was conducted in MrBayes (Huelsenbeck and Ronquist, in press) on the three-gene matrix using four Markov chains simultaneously started from random trees. A simple evolutionary model was used because more complicated models ran too slowly: a simple two-parameter (transition-transversion) substitution model (nst = 2, rate = equal and basefreq = equal). One million cycles were performed with each model, sampling a tree at every 100 generations. Trees that preceded the stabilization of the likelihood value (the burn in) were excluded, and the remaining trees were used to construct a consensus in PAUP (version 4.0; Swofford, 2001).

RESULTS

Parsimony analysis—The mean GC ratios for the sequences of *matK*, *rbcL*, and 26S rDNA were 36%, 44%, and 61%, respectively. Whereas for the *rbcL* matrix 170 characters (out of 1387, i.e., 12.3%) were parsimony informative, 148 characters (out of 893, i.e., 16.6%) were parsimony informative

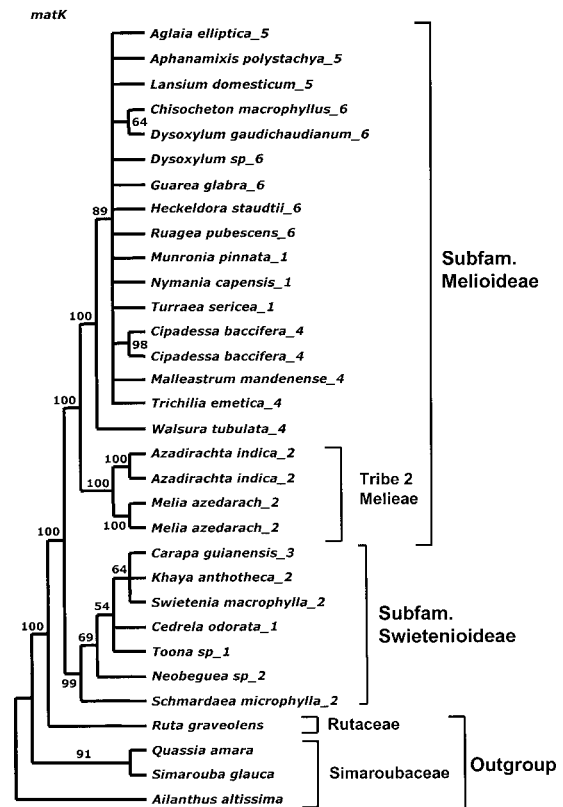


Fig. 2. Bootstrap consensus tree (MP) of the *matK* sequence data set of 28 Meliaceae accessions. Subfamilies and tribes after Pennington and Styles (1975). Numbers are bootstrap percentages (1000 replicates).

for *matK*, and 71 characters (out of 880, i.e., 8.1%) for 26S rDNA. On the shortest combined tree shown (Fig. 1), evolution of each gene was assessed. The ts/tv ratio for *matK* was 1.24, CI and RI for ts were 0.77 and 0.85, for tv 0.62 and 0.83, respectively. The ts/tv ratio for *rbcL* was 1.36, CI and RI for ts were 0.68 and 0.84, respectively, and for tv 0.45 and 0.83, respectively. The ts/tv ratio for 26S was 2.88, CI and RI for ts were 0.45 and 0.53, respectively, and for tv 0.43 and 0.61, respectively.

Inferred phylogenetic trees resulting from five separate analyses are shown in Figs. 1–5: all three regions combined, *matK* alone, *rbcL* alone, 26S rDNA alone, and a combined plastid analysis. For all but the first, we illustrate only the bootstrap majority-rule consensus tree because our purpose in analyzing the data sets separately is simply to demonstrate that no instances of strongly supported and incongruent patterns are present so that direct combination is appropriate. Numbers after genus name in the trees refers to the tribe numbers given in Pennington and Styles (1975; <http://ajbsupp.botany.org/v90/>).

Analysis of *matK*—The aligned *matK* matrix consisted of 893 bp of which 297 (33%) positions were variable and 148 (17%) of these were potentially parsimony informative. Analysis produced more than 15 000 shortest trees of 460 steps with a CI of 0.69 and an RI of 0.84. In the *matK* bootstrap consensus tree (Fig. 2), Melioideae and tribe Melieae within this subfamily are supported by 100 bootstrap percentage (BP). With the exception of *Walsura*, the remaining representatives of Melioideae are members of an unresolved clade. Swieten-

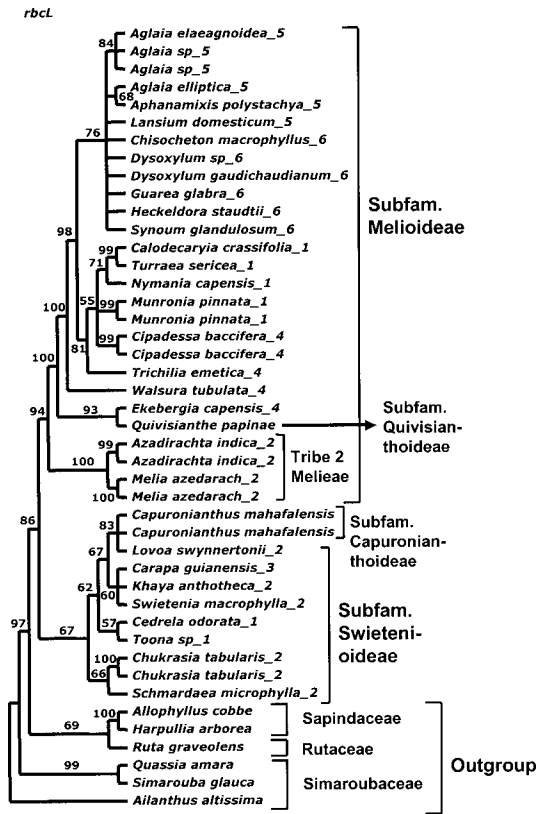


Fig. 3. Bootstrap consensus tree (MP) of the *rbcL* sequence data set of 38 Meliaceae accessions. Subfamilies and tribes after Pennington and Styles (1975). Numbers are bootstrap percentages (1000 replicates).

ioideae are supported by BP 99. There is no confirmation of any tribal circumscription, i.e., representatives of the three tribes within Swietenioideae appear intermixed, but tribal delimitations also cannot be refuted from this evidence.

Analysis of *rbcL*—The aligned *rbcL* matrix consisted of 1387 bp of which 247 (18%) positions were variable and 170 (12%) of these were potentially parsimony informative. Analysis produced 612 shortest trees of 474 steps with a CI of 0.58 and an RI of 0.84. In the *rbcL* bootstrap consensus tree (Fig. 3), Melioideae are monophyletic (BP 94), with BP 100 for Melieae. The representatives of Guareeae and Aglaieae form a common clade (BP 76). Members of Turraeae and Trichilieae are intermixed. Swietenioideae (with *Capuronianthus* included) are monophyletic (BP 67); Swietenieae are paraphyletic/polyphyletic, and the two members of Cedreleae (*Cedrela* and *Toona*) are sister (BP 62) to a clade containing some members of Swietenieae, Xylocarpeae (*Carapa*), and *Capuronianthus*. The members of the two monogeneric subfamilies, *Quivisianthe* and *Capuronianthus*, are embedded within Melioideae and Swietenioideae, respectively.

Analysis of 26S—The aligned 26S rDNA matrix consisted of 880 bp of which 130 (15%) positions were variable and 71 (8%) of these potentially parsimony informative. Analysis produced 46 shortest trees of 289 steps with a CI of 0.48 and an RI of 0.64. The bootstrap consensus tree (Fig. 4) shows little resolution and does not even separate Melioideae from Swietenioideae. It does support the relationship of *Carapa* (Xylo-

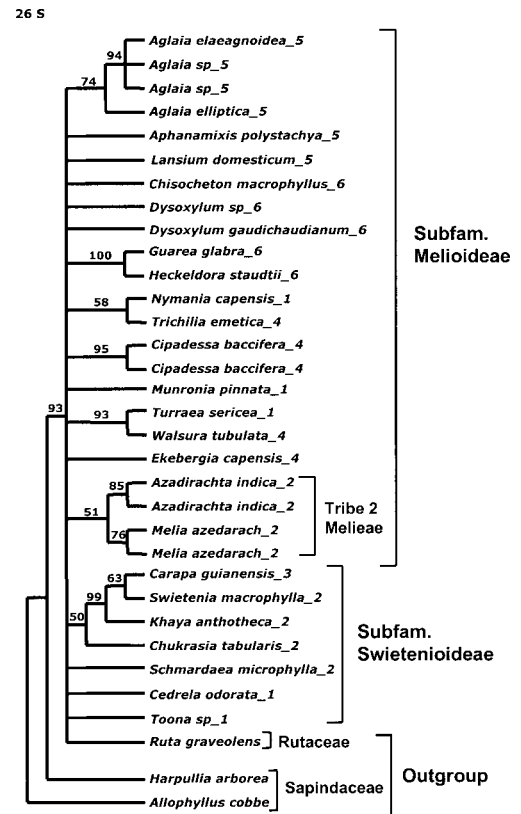


Fig. 4. Bootstrap consensus tree (MP) of the 26S rDNA data set of 30 Meliaceae accessions. Subfamilies and tribes after Pennington and Styles (1975). Numbers are bootstrap percentages (1000 replicates).

carpeae) to *Swietenia* and *Khaya* (the last two Swietenieae; BP 99) seen with *rbcL*. Melieae are weakly supported (BP 51). *Aglaia* is monophyletic (BP 74), and there are several pairs of genera that are supported: *Guarea/Heckeldora* (BP 100), *Nymanian/Trichilia* (BP 58), and *Turraea/Walsura* (BP 93). The last pair are clearly in conflict with *rbcL*, which placed *Turraea* with *Calodectarya* (not sequenced for 26S rDNA) and this pair with *Nymanian* (BP 99 and 71, respectively).

Combined analysis—Except for the above-noted incongruence related to the relationships of *Turraea*, in none of the three separate analyses did we observe any other occurrences of strongly supported incongruence. We noted this potential case of conflict but proceeded with direct combination in two phases, one for just the two plastid genes (so that the effect of adding 26S rDNA to the plastid results could be better interpreted) and another for all three genes. Analysis of the combined plastid matrix produced 11 564 shortest trees of 934 steps with a CI of 0.63 and an RI of 0.84. In the bootstrap consensus tree (Fig. 5), Melioideae are monophyletic (BP 98), with BP 100 for Melieae. The representatives of Guareeae and Aglaieae form a common clade (BP 60). Members of Turraeae and Trichilieae are intermixed. Swietenioideae are monophyletic (BP 84); Swietenieae are paraphyletic/polyphyletic, and *Capuronianthus* and the members of Cedreleae and Xylocarpeae are nested within them. The members of the two monogeneric subfamilies, *Quivisianthe* and *Capuronianthus*, are embedded within Melioideae and Swietenioideae, respectively.

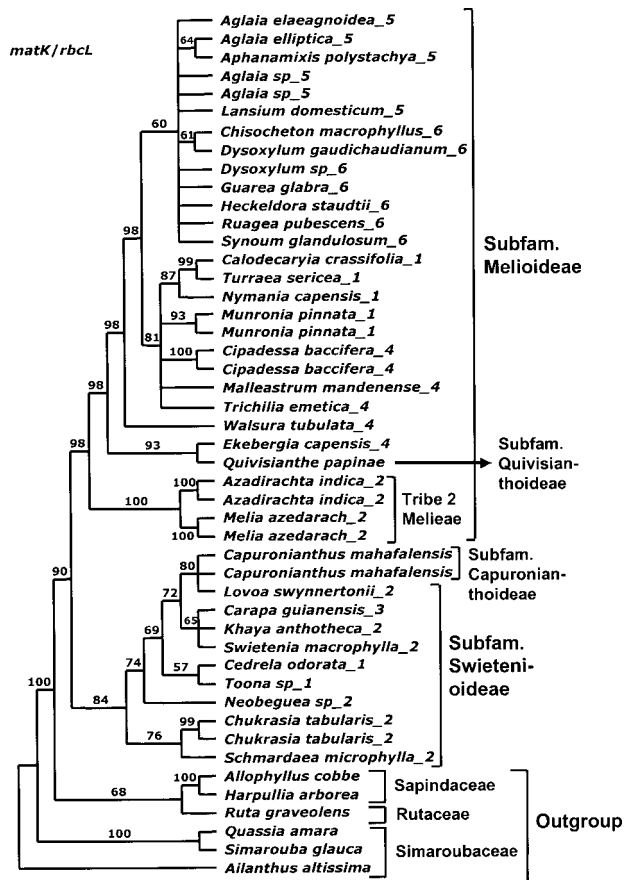


Fig. 5. Bootstrap consensus tree (MP) of the combined plastid data set of 41 Meliaceae accessions. Numbers are bootstrap percentages (1000 replicates).

Analysis of the combined three-gene matrix produced 3559 shortest trees of 1251 steps with a CI of 0.57 and an RI of 0.79. In Fig. 1, we illustrate one of the shortest trees; numbers above branches are estimated numbers of substitution (ACCT-RAN optimization); numbers below branches are bootstrap percentages. Groups that are not present in the strict consensus tree are marked with an arrowhead. This result is more resolved than any of the other analyses, including the combined *matK/rbcL*; the incongruence of *Turraea* noted above appears to have little effect on the combined analysis. Unless otherwise noted, in the following we will make reference to the results of the combined three-gene analysis (i.e., joint matrix *matK/rbcL/26S*; Fig. 1). Relative to just the plastid results, the addition of the 26S rDNA data did not alter patterns noticeably nor were patterns of bootstrap support greatly affected; they definitely did not increase greatly over those obtained with combined *matK/rbcL*, but there appeared to be no negative effect either.

The combined molecular data support the two main lineages that correspond to Melioideae and Swietenioideae (BP 100 and 90, respectively). However, *Quivisianthe* and *Capurionanthus* are embedded in Melioideae and Swietenioideae, respectively. *Quivisianthe* forms a well-supported (92% BP) clade with *Ekebergia*, a member of Trichilidae. *Capurionanthus* forms a clade (80% support) with *Lovoia*, a member of Swietenioideae. Concerning tribal delimitation within Melioideae, the data indicate a close relationship between representatives of Aglaieae

and Guareeae, although this does not receive BP >50. The single tree illustrated shows Guareeae to be paraphyletic to Aglaieae, but the low levels of sequence divergence observed prevent us from concluding that there are definite problems with tribal delimitation. Members of Turraeae and Trichilidae are intermixed, but not as strongly as in the individual plastid results. Due to the low levels of sequence divergence observed, this result cannot be taken as refutation of the monophyly of Turraeae and Trichilidae. Concerning tribal delimitation in Swietenioideae, the data indicate at least the Swietenioideae to be non-monophyletic. The members of Cedreleae (only two genera) form a weakly supported clade (BP 57). Only one genus (*Carapa*) of the two genera of Xylocarpeae was included.

Bayesian analysis—Bayesian results (tree not shown) are nearly identical to the parsimony tree (Fig. 1); some weakly supported groups are differently arranged (e.g., the position of *Synoum*), but in neither case does this receive either a high bootstrap or posterior probability. In some cases, the posterior probabilities are higher than the bootstrap percentages, but all clades with high posterior probabilities are also present and receive at least moderate bootstrap support in the parsimony analyses.

DISCUSSION

Molecular evolution—Although the percentage of informative characters is highest in the sequenced *matK* region sequenced here, *rbcL* provides more informative characters, resulting in the best resolved and supported trees obtained in this study. Perhaps if we had developed PCR primers that allowed us to sequence the whole *matK* exon, there would have been more information in *matK*, but these primers would have required a great more time to develop. The results from the analysis of 26S rDNA were disappointing to us due to the low number of variable sites and their poor performance. We would not recommend this region to other researchers for the purposes of examining infrafamilial relationships.

The appearance of incongruence relating to the position of *Turraea* should be noted and investigated further. Although potentially a case of incongruence, direct combination did not seem to produce any of the expected signs of strong disagreement, i.e., less resolution and diminished bootstrap percentages. There is no particular reason based on morphology why *Turraea* (Turraeae) should be allied with *Walsura* (Trichilidae), and in the plastid and combined analyses *Turraea* comes out with other Turraeae. It could be argued that simply because there are more variable sites in the plastid matrices that they overwhelm the pattern obtained with 26S rDNA but in general strongly supported incongruent patterns decrease support and resolution, so we are uncertain about the best perspective from which to view this situation. We note it here so that future workers will investigate it further.

The ts/tv ratios were as expected for plastid and nuclear coding regions; for 26S rDNA they were much higher, but *matK* had a much less skewed pattern than did *rbcL*, a fact noticed by other researchers (Kores et al., 2000; Whitten et al., 2000). Kores et al. argued that these patterns indicate that *matK* might be a pseudogene, but at the least it indicates that *matK* has different functional constraints operating on it than does *rbcL*. We note here that insertions and deletions in *matK*

were all in triplets, indicating that *matK* is now or was recently functional.

Familial relationships of Meliaceae—As outgroups, we included representatives of the putatively closely related families Rutaceae, Sapindaceae, and Simaroubaceae, but sampling was too limited here to say much about interfamilial relationships. Some members of these families have formerly been included in Meliaceae (De Candolle, 1824, 1878; Hooker, 1862; Radlkofer, 1890; Harms, 1896, 1940), but the question of whether *Flindersia* and *Chloroxylon* (usually placed in Rutaceae; e.g., Scott et al., 2000) and *Ptaeroxylon* and *Cedrelopsis* (previously assigned to Ptaeroxylaceae; e.g., Gadek et al., 1996; Chase et al., 1999; but in Rutaceae sensu APG, 1998) should be included in Meliaceae has been debated for some time (e.g., review in Pennington and Styles, 1975; Chase et al., 1999). DNA data (Gadek et al., 1996; Chase et al., 1999; Savolainen et al., 2000b) have shown that the closest relatives of Meliaceae are Simaroubaceae and Rutaceae, but *Flindersia/Chloroxylon* and *Ptaeroxylon/Cedrelopsis* are excluded from Meliaceae (and not included here). Among other families, Meliaceae, Rutaceae, Sapindaceae, and Simaroubaceae form the core group of Sapindales as recognized in Cronquist's broad concept of this order (Cronquist, 1968) and confirmed by DNA studies (Gadek et al., 1996).

Subfamilies—Of the four subfamilies included here, the two largest, Swietenioideae, and Melioideae have been recognized in some form or other since 1789 (de Jussieu). Their rank and circumscription, however, have frequently been debated. Adrien de Jussieu (1830) treated them as families, an opinion that Harms (1940) supported. Nevertheless, Harms treated them as subfamilies, together with a third, based on *Cedrela*. Apart from the inclusion of *Cedrela* and the related taxon *Toona*, Swietenioideae have remained a remarkably stable taxon. There has been only serious dispute over the inclusion of the mangrove genus *Xylocarpus* and the related *Carapa*. Harms (1940) placed them in Melioideae on the basis of seed characters and Kribs (1930) in Swietenioideae on the basis of wood anatomy alone. Pennington and Styles (1975), examining the secondary xylem of a variety of species, presented evidence that *Cedrela* (and *Toona*), *Xylocarpus* and *Carapa* have so much in common with the rest of Swietenioideae that their exclusion was unjustified. Our data (*matK*, *rbcL*, and all combined analyses; Figs. 1, 2, 3, and 5) confirm that these taxa belong to Swietenioideae. Furthermore the data indicate a close relationship between *Cedrela* and *Toona*, forming monophyletic clades in the *rbcL* (Fig. 3) and combined bootstrap analyses (Figs. 1, 3, and 5). Pennington and Styles (1975) stated furthermore that the secondary xylem of Swietenioideae is virtually uniform and consistently different from that of Melioideae. The pollen of most Swietenioideae and most Melioideae, on the other hand, is so similar that it confirms the decision to treat them as subfamilies. Both hypotheses are strongly confirmed by our study. First of all, the *matK* and *rbcL* trees (Figs. 2 and 3) as well as the combined trees (Figs. 1 and 5) support recognition of the two main subfamilies. Only the 26S matrix does not provide support for these two natural groups (Fig. 4). Second, our data (except 26S) support the monophyly of Meliaceae with Melioideae and Swietenioideae appearing as sister groups and thus are also compatible with their taxonomic rank as subfamilies (Figs. 1, 2, 3, and 5).

The study of two little-known Malagasy genera, *Capuronianthus* and *Quivisianthe* by Pennington and Styles (1975) lead to the establishment of two new subfamilies, even though the authors stated that these genera provided connecting links between the two larger subfamilies. They were believed to be so different from each other, as well as from Swietenioideae and Melioideae, that the establishment of two new subfamilies seemed to be justified. Our DNA data show that *Quivisianthe* and *Capuronianthus* are embedded in Melioideae and Swietenioideae, respectively, and that this decision therefore cannot be justified based on phylogenetic grounds (Fig. 3).

Nevertheless it should also be mentioned that this information is solely based on the *rbcL* sequence data because we were unable to amplify either for *matK* or 26S rDNA. However, Pennington and Styles (1975, pp. 445 and 509) stated in their generic monograph that the floral structure of *Quivisianthe* is similar to that of *Ekebergia* (Trichilieae, tribe 4, of Melioideae). However, the dry, loculicidal capsule containing dry winged seeds immediately distinguishes *Quivisianthe* from members of this tribe as well all other members of Melioideae and Swietenioideae. The DNA data confirm a close relationship of *Quivisianthe* to *Ekebergia* (Trichilieae, BP 93; Fig. 3).

Pennington and Styles (1975, p. 511) suggested a distant relationship of *Capuronianthus* with *Carapa* and *Xylocarpus* (Swietenioideae), with which it shares both the partial septifragal dehiscence of the fruit (both genera) and a seed with corky sarcotesta (only *Xylocarpus*). In addition to these, *Carapa* and *Capuronianthus* have the same chromosome number ($2n = 58$). However, in all other characters of subfamilial importance *Capuronianthus* differs from these genera. Moreover, Pennington and Styles (1975) found that in some floral characters, *Capuronianthus* resembles closely genera in Trichilieae (Melioideae). The loculi containing only two fully developed ovules and the capitate style-head are characteristic of many members of the Melioideae, although Pennington and Styles (1975) pointed out that an additional vestigial ovule in the loculus occurs rarely in the latter subfamily. In contrast, loculi with three or more ovules are typical for members of Swietenioideae. In its secondary xylem *Capuronianthus* is intermediate between Swietenioideae and Melioideae. Pennington and Styles (1975) thus concluded that the genus is intermediate between the two subfamilies but nevertheless quite distinct from both, thus justifying the establishment of the subfamily *Capuronianthoideae*. In the molecular trees (Figs. 1, 3, and 5) *Carapa* (as well as *Khaya* and *Swietenia*) are the closest relatives of *Capuronianthus* after *Lovoa*. Bootstrap support for the clade formed by *Capuronianthus* and *Lovoa* is 83% (Fig. 3), and this group is sister to a clade formed by the genera *Carapa*, *Khaya*, and *Swietenia*. Due to the fact that *Capuronianthus* is positioned within Swietenioideae, this genus—like *Quivisianthe*—should not be treated as a subfamily.

Tribes—De Candolle in his *Prodromus* (1824) was the first to attempt to divide the family into tribes, which he based primarily on the number and structure of seeds. A still more detailed account was published in 1830 by Adrien de Jussieu. De Jussieu's classification was an improvement because it was based on a larger number of characters than any previous classification. Hooker in *Genera Plantarum* (Hooker, 1862) followed de Candolle (1824) but differed from de Jussieu (1830) in uniting Cedrelaceae and Meliaceae. All subsequent authors have done the same.

Since the publication of Bentham and Hooker's *Genera*

Plantarum, four comprehensive classifications of the family have been published: one by Casimir de Candolle (1878), two by Harms (1896, 1940), and one based on the anatomy of the secondary xylem by Kribs (1930). As has been already stated, the most authoritative work on generic and tribal delimitation in Meliaceae at present is the generic monograph by Pennington and Styles (1975), although since then a considerable number of new insights and reevaluations with respect to morphological characters has led to slight modifications (e.g., Mabberley, 1979; Cheek, 1989, 1990a, b, 1992, 1996; Cheek and Rakotozafy, 1991). Some genera and most tribes can only be diagnosed by using a combination of several characters. Thus, members of one tribe cannot be distinguished from all other Meliaceae on the basis of single diagnostic characters. Most character states typical of one tribe may have at least a few exceptions in this tribe and also occur at least occasionally in other tribes (Pennington and Styles, 1975). The DNA data collected so far in this study only support the historically stable Melieae (Melioidae; 100% BP in the *matK*, *rbcL*, combined plastid and three-gene trees respectively; Figs. 1, 2, 3, and 5). This tribe comprises the first two species (*Melia azedarach*, *Azadirachta indica*) recognized in the family in the first edition of *Species Plantarum* (1753). All members of this tribe also have a unique six base-pair insertion in *matK* (5'-TTAAGT-3') at site 137–142 (relative to *Melia azedarach*, GenBank no. AY128193). There is also phytochemical evidence for the special position of Melieae (compare Taylor in Pennington et al., 1981; Mulholland et al., 1998). In spite of the thorough investigation of *Melia* and *Azadirachta* in recent years, glabretal-type compounds (triterpenoids) have not been found in these genera in contrast to all other members of Melioidae investigated so far (*Aglaia*, *Guarea*, *Owenia*, *Turraea*, and *Dysoxylum*; Mulholland et al., 1998). For all other tribes, there is not yet enough evidence to evaluate their monophyly. Nevertheless, the preliminary data indicate a close relationship between Aglaieae and Guareeae (Melioidae) and a possibly monophyletic Cedreleae (Swietenioideae; Figs. 1, 3, and 5). Non-monophyletic are at least Trichilieae (Melioidae) and Swietenieae (Swietenioideae; Figs. 1, 3, and 5). To reach a robust and well-resolved phylogenetic appreciation of Meliaceae, sampling of additional taxa and the collection of many more data will be necessary.

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