

An evaluation of tribes and generic relationships in Melioideae (Meliaceae) based on nuclear ITS ribosomal DNA

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Phylogenetic analyses of Melioideae, including representatives of all currently recognized tribes, were carried out using nuclear ITS ribosomal DNA sequence data. The secondary structure models employed for ITS1 and ITS2 allowed optimization of the alignment across Meliaceae genera of both subfamilies, yielding a maximum amount of information without the exclusion of some highly variable sites. This study is the first to assess the current circumscription of Melioideae and its tribes in detail, with data independent of morphology. Maximum parsimony, maximum likelihood and Bayesian analyses of nuclear ITS, in contrast to analyses based on plastid *rbcL*, confirm monophyly for Aglaieae, Sandoriceae and Melieae, an isolated position for Vavaeae, the position of *Pterorhachis* and *Quivisianthe* in Melioideae, and a close relationship between Turraeae and Trichilieae. Trichilieae are morphologically and genetically the most complex tribe. Trichilieae cannot be separated from Turraeae, Vavaeae and Sandoriceae. *Anthocarapa* and “*Pseudocarapa*” form a clade but exhibit a high number of autapomorphies, which needs further investigation. We propose to keep *Naregamia* separate from *Turraea*, and to reconsider the present circumscription of Trichilieae.

KEYWORDS: internal transcribed spacer (ITS), Meliaceae, Melioideae, molecular phylogenetics, *rbcL*

INTRODUCTION

Meliaceae are a widely distributed subtropical and tropical angiosperm family occurring in a variety of habitats, from rain forests and mangrove swamps to semi-deserts (Pennington & Styles, 1975; Pennington & al., 1981; Pannell, 1992; Mabberley & al., 1995). Together with the contributions on Meliaceae in *Flora Neotropica* by Pennington & al. (1981) and in *Flora Malesiana* by Mabberley & al. (1995), the most authoritative work on the family is the generic monograph by Pennington & Styles (1975). Currently recognized are 49 to 51 genera with about 565 species (Pennington & Styles, 1975; Mabberley & al., 1995; Cheek, 1996; Chase & al., 1999; Mabberley, 2000). Pennington & Styles (1975) recognized four subfamilies, of which Melioideae and Swietenioideae consist of seven tribes with 34 to 36 genera and three tribes with 13 genera, respectively. *Quivisianthoideae* and *Capuronianthoideae* each contain a single monotypic genus (*Quivisianthe* Baill. and *Capuronianthus* Leroy) and were newly recognized by Pennington & Styles (1975). A re-

cent reassessment of the circumscription of the four subfamilies by means of phylogenetic analyses of sequence data from three regions (plastid *rbcL*, *matK*, nuclear 26S rDNA) showed that the members of the two small monogeneric subfamilies, *Quivisianthe* and *Capuronianthus*, fall within Melioideae and Swietenioideae, respectively, supporting their taxonomic inclusion in these groups (Muellner & al., 2003).

Pennington & Styles (1975) found a wide range of morphological variation especially in the subfamily Melioideae. To obtain an improved tribal scheme compared to that of Harms (1940), Pennington & Styles (1975) subordinated the supposed evolutionary significance of individual characters in favour of groupings based on correlations between the maximum number of characters of use at this level of classification and on detection of discontinuities in variation of these characters. Pennington & Styles (1975) argued that the most natural grouping of genera was obtained by basing classification on a large number of characters; thus, artificial assemblages resulting from the weighting of a few characters were

avoided. Using these principles, Pennington & Styles (1975) recognized seven tribes within subfamily Melioideae but stated that limits of Trichilieae, Aglaiaeeae and Guareeae could only be defined by overlapping morphological, anatomical and palynological characters. All tribes of Melioideae are represented in Malesia, but only two (Guareeae, Trichilieae) are pantropical and two other ones are restricted to the Old World (Turraeeae, Melieae); the remainder are restricted to Indomalesia and the western Pacific (Vavaeeae, Aglaiaeeae, Sandoriceae; Mabblerley & al., 1995).

The internal transcribed spacers (ITS) of nuclear ribosomal DNA (nrDNA), defined as the unit containing the ITS1 spacer, 5.8S rRNA gene and ITS2 spacer, are not only useful in assessing relationships at the infrageneric, but also at higher taxonomic levels (Hershkovitz & Zimmer, 1996; Soltis & Soltis, 1998). Secondary structure models of RNA transcripts, employed in the taxonomic group under investigation, allow for optimizing alignment of variable and putatively phylogenetically informative regions of ITS even across more distantly related taxa. This is due to the fact that the secondary structure of ITS is more conserved than the primary sequence (Mai & Coleman, 1997; Coleman & al., 1998).

In this study we performed maximum parsimony, maximum likelihood and Bayesian analyses of sequence data from nuclear ITS to estimate phylogenetic relationships within subfamily Melioideae for which former analyses of plastid *rbcL*, *matK* and nuclear 26S rDNA did not provide sufficient information (Muellner & al., 2003). Based on 51 species, including representatives of all currently recognized tribes, this study thus provides the first detailed reassessment of tribal and generic relationships in Melioideae. The ITS data are compared to *rbcL* data recently collected in the course of a survey on the biogeographic history of Meliaceae (Muellner & al., 2006).

MATERIALS AND METHODS

Plant material. — We analysed ITS sequences of 51 species of subfamily Melioideae (ingroup) and one species each of genera *Swietenia* Jacq., *Khaya* A. Jussieu, *Toona* (Endl.) M. Roem. and *Cedrela* P. Browne of subfamily Swietenioideae as outgroups (Appendix). The justification for the inclusion of the ingroup taxa in Melioideae and *Swietenia*, *Khaya*, *Toona* and *Cedrela* in Swietenioideae was based on a previous evaluation of the higher-level classification of Meliaceae (Muellner & al., 2003).

Plant material was collected during excursions to Thailand, Malaysia, Sri Lanka and Australia and from the living collections of Forestry Research Institute Malaysia (FRIM), Kebun Raya (Bogor Botanic Garden), Indonesia, and the Royal Botanic Gardens, Kew, U.K. Herbarium

specimens are deposited at FHO, FR, K, NBG, NCU and WU.

Isolation of DNA and amplification. — Field-collected material was dried and stored in silica gel prior to DNA extraction (Chase & Hills, 1991). DNA extraction and PCR amplification followed Muellner & al. (2005). The fragment size amplified was between 627 and 664 bp for the entire ITS region. After amplification, samples were gel purified using the QIAquick gel extraction kit (QIAGEN, Margarettella, Vienna, Austria).

Sequencing. — PCR primers were also used for sequencing. Cycle-sequencing followed Muellner & al. (2005). Sequencing reactions were run on an ABI PRISM 377 DNA Sequencer and on an ABI 3100 capillary sequencer following the manufacturer's protocols.

Sequence editing and alignment. — For editing and assembly of the complementary strands, 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. ITS sequences were explored for the presence of several structural motifs. Thus, in the ITS1 region we searched for the presence of the conserved angiosperm motif GGCRY-(4 to 7 n)-GYGYCAAGGAA (Liu & Schardl, 1994), which was also found in several gymnosperms (Maggini & al., 1998). We also looked for the presence of the conserved (C1–C6) and variable (V1–V6) domains determined for plant ITS2 sequences (Hershkovitz & Zimmer, 1996), as well as for the conserved angiosperm motif 5'-GAATTGCAGAATCC-3' within the 5.8S rDNA gene, which can be used to differentiate between flowering plants, fungi and algae (Jobes & Thien, 1997). Folding predictions of secondary structures of the ITS1 and ITS2 RNA transcripts were made at the M. Zuker web server (<http://www.bioinfo.rpi.edu/~zukerm/>) by use of the mfold program version 3.1 (Mathews & al., 1999; Zuker & al., 1999). Foldings were conducted at 37°C. After a first rough alignment with CLUSTAL version X (Thompson & al., 1997), corrections were made manually by using secondary structure predictions of ITS1 and ITS2 RNA transcripts as a guide for alignment across genera. Secondary structure predictions were confirmed by hemi-compensatory base changes (hemi-CBCs) and full compensatory base changes (CBCs) that preserved the predicted folding pattern. First, the secondary structure used was not always the energetically most favourable, but rather the folding that was common to all genera and species and supported by CBCs and hemi-CBCs. Second, the structural motifs common to all eukaryote ITS2 (Coleman, 2007) were present there, exactly in their expected positions in the secondary structure. These were the most conserved sequences, as also expected. A total of 794 aligned positions were included in the matrices for phylogenetic analyses for ITS (including ITS1, 5.8S rDNA and ITS2). Gaps were coded

as missing data. All sequences are deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>).

Phylogenetic analysis. — Individual maximum parsimony (MP) analyses of the ITS and the *rbcL* dataset (data for the latter region were obtained from Muellner & al., 2006) were performed using PAUP*4.0b10 (Swofford, 2002). Visual inspection of the individual bootstrap consensus trees was used for determining congruence of datasets (Whitten & al., 2000). Although there were strongly supported (> 85% bootstrap), incongruent patterns among the individual analyses, direct combination was carried out to confirm observations based on the separate analyses (trees not shown). Substitutions at each nucleotide position were treated as independent, unordered, multi-state characters of equal weight (Fitch parsimony; Fitch, 1971). Heuristic searches were performed using 1,000 random additions of taxa, tree bisection-reconnection (TBR) branch swapping and MulTrees on (keeping multiple, shortest trees). Robustness of clades was estimated using the bootstrap (Felsenstein, 1985) with 5,000 replicates with simple sequence addition, TBR branch swapping and MulTrees on.

Bayesian analyses were conducted with MrBayes version 3.01 (Huelsenbeck & Ronquist, 2001) using four Markov chains simultaneously started from random trees. Modeltest 3.06 (Posada & Crandall, 1998, 2001) was used to select the optimal substitution model (GTR, general time reversible model). One million cycles were performed, 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 majority rule consensus in PAUP (version 4.0b10; Swofford, 2002). The percentages on this tree are the Bayesian posterior probabilities.

Maximum likelihood (ML) analyses were performed with PAUP*4.0b10 (Swofford, 2002). The substitution model employed in the analyses was the same as for the Bayesian analyses.

RESULTS

Structure, size and composition of ITS. — Length of the entire ITS region, including ITS1, 5.8S rDNA and ITS2, varied among Melioideae accessions from 627 to

Table 1. Statistics for the maximum parsimony analyses of the internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA), defined as the unit containing the ITS1 spacer, 5.8S rRNA gene and ITS2 spacer, and of plastid *rbcL*.

Dataset	ITS	<i>rbcL</i>
No. of all accessions/of Melioideae accessions	55/51	37/33
No. of characters included	794	1,387
No. of variable sites	499	186
No. of informative characters	403	97
Length of shortest tree (no. of steps)	2,421	293
Number of shortest trees	3	7,199
Consistency index	0.38	0.59
Retention index	0.54	0.82

664 bp. ITS1 ranged in length from 233 to 273 bp, 5.8S rDNA from 156 to 172 bp, and ITS2 from 214 to 238 bp. The mean GC ratios of Melioideae taxa for the sequences of ITS1, 5.8S and ITS2 were 66%, 55% and 66%, respectively. The complete set of statistics for all datasets is summarized in Tables 1 and 2.

Phylogeny estimation based on ITS. — The aligned ITS matrix consisted of 794 characters (Table 1). For the entire ITS matrix, 499 (63 %) positions were variable and 403 (51 %) were potentially parsimony informative. The parsimony search produced three most parsimonious trees of 2,421 steps with consistency index (CI) = 0.38 and retention index (RI) = 0.54 for the entire ITS matrix (Fig. 1). Bayesian results derived from the entire ITS matrix are shown in Figure 2. The broad phylogenetic patterns are similar to the MP analysis: Aglaieae are monophyletic (51% bootstrap percentage, BP; 97% posterior probability, PP), Guareae are paraphyletic (Figs. 1, 2). Turraeeae are paraphyletic and appear in a clade with representatives of Trichilieae (53% BP; 94 PP; Fig. 2). Members of the latter also appear in other parts of the tree. Sandoriceae are monophyletic (100 BP; 100 PP; Figs. 1, 2), as are Melieae (87 BB; 100 PP; Figs. 1, 2). Maximum likelihood results reflect the same broad patterns (tree not shown).

Phylogeny estimation based on *rbcL*. — The aligned *rbcL* matrix consisted of 1,387 characters (Table 1). For the *rbcL* matrix, 186 (13%) positions were variable and 97 (7%) were potentially parsimony informative. The parsimony search produced 7,199 most parsimonious trees of 293 steps with CI = 0.59 and RI = 0.82 (Table 1). Bayesian tree topology derived from the *rbcL* matrix is

Table 2. Characterization of ITS in Melioideae and outgroup taxa.

Region	Length (no. characters)	Length (bp)		Mean GC ratio (%)	
		Melioideae	Outgroup	Melioideae	Outgroup
Entire ITS	794	627–664	636–650	63	69
ITS1	338	233–273	247–257	66	73
5.8S	214	156–172	164	55	55
ITS2	242	214–238	225–228	66	74

identical to the MP results (Fig. 3), with one exception: *Aglaia* and *Lansium* formed a clade supported by 51% in the Bayesian majority rule consensus tree. Posterior probabilities are plotted on the MP tree (Fig. 3). Again,

Aglaieae are monophyletic (74 PP; Fig. 3) and Guareae paraphyletic (Fig. 3). With the exception of *Munronia*, all other representatives of Turraeeae are members of a monophyletic group (98 BP; 71 PP; Fig. 3). All members

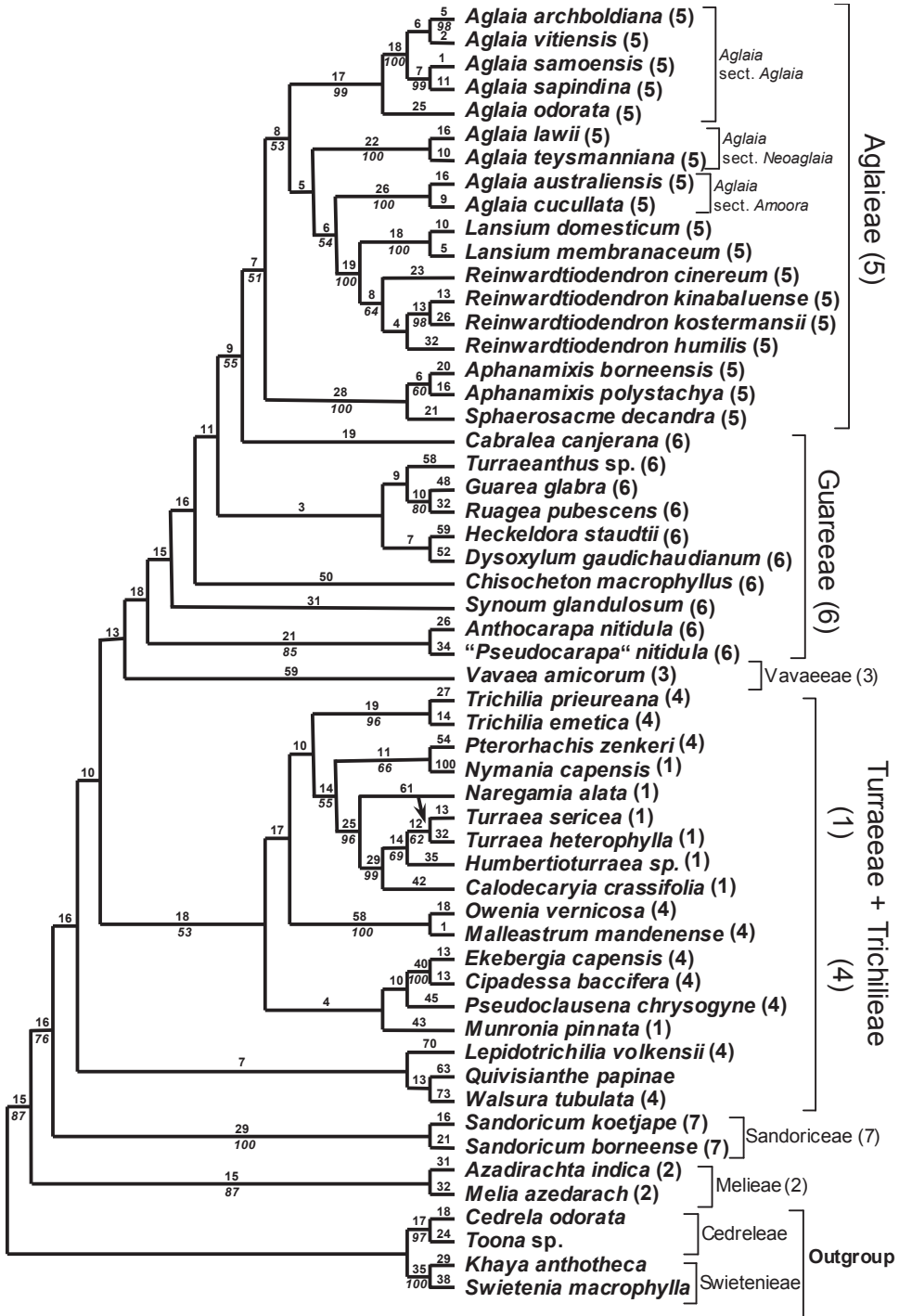


Fig. 1. One of the three most parsimonious trees obtained from the maximum parsimony analysis of the ITS nrDNA dataset of 55 Meliaceae accessions. Tribal names and numbers after Pennington & Styles (1975). Numbers above branches are estimated branch lengths (DELTRAN optimization), numbers below branches are bootstrap percentages (5,000 replicates); in *italics*. The arrow indicates a group not present in the strict consensus tree.

of Turraeae appear in a clade with representatives of Trichilieae (67 BP; 100 PP; Fig. 3). Again, members of the latter appear in other parts of the tree. *Vavaea*, *Quivisianthe* and *Sandoricum* are interdigitated with Trichilieae. Maximum likelihood results are almost identical to the MP and Bayesian topologies (Fig. 4).

Maximum likelihood results based on the combined ITS/*rbcL* matrix support Aglaieae as monophyletic; Guareae as paraphyletic; Turraeae as paraphyletic, appearing in a clade with Trichilieae (Fig. 5). As for the single ITS and *rbcL* analyses, members of the latter also appear in other parts of the tree (Fig. 5).

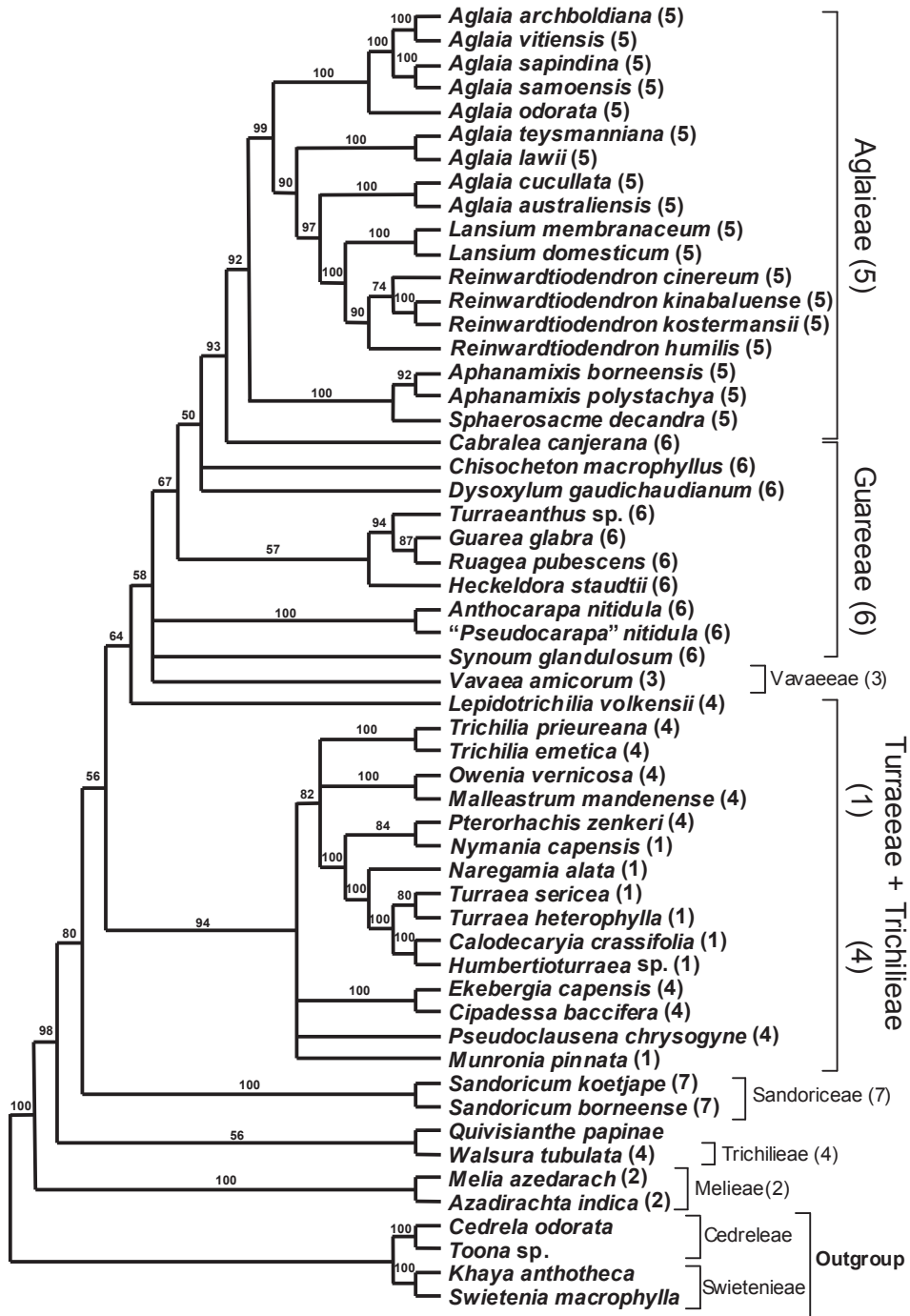


Fig. 2. Bayesian tree (10,000 total trees, burn-in of 500 trees) of the ITS nrDNA dataset of 55 Meliaceae accessions. Tribes after Pennington & Styles (1975). Numbers above branches are Bayesian posterior probabilities.

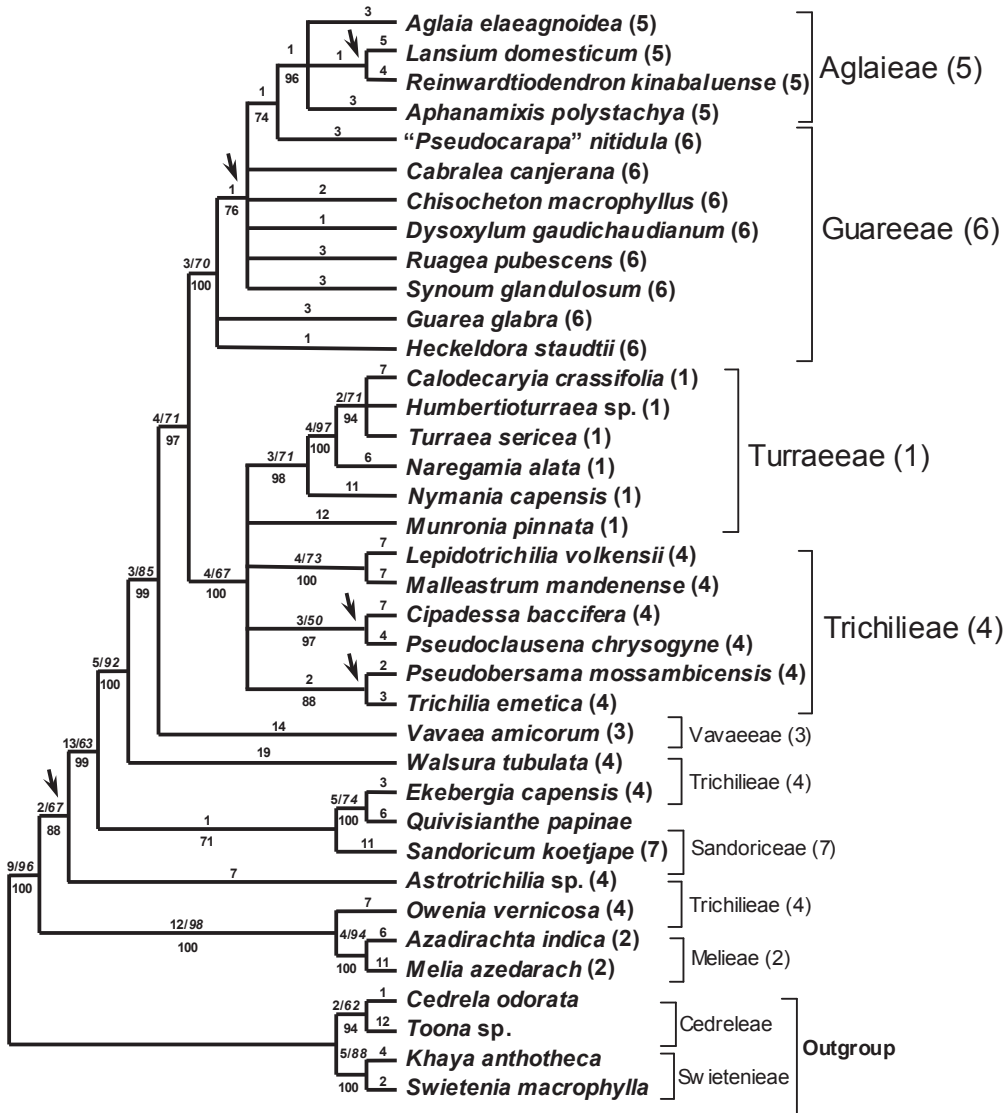


Fig. 3. One of the 7,199 most parsimonious trees obtained from the maximum parsimony analysis of the plastid *rbcL* dataset of 37 Meliaceae accessions. Tribes after Pennington & Styles (1975). Numbers above branches are estimated branch lengths (DELTRAN optimization), and bootstrap percentages (1,000 replicates; in *italics*). Numbers below branches are Bayesian posterior probabilities (10,000 total trees, burn-in of 1,100 trees). Arrows indicate groups not present in the strict consensus tree.

DISCUSSION

Tribal affiliation within Melioideae. — At a glance, *Pterorhachis* Harms is distinct from all other Meliaceae on morphological grounds and resembles instead some members of Sapindaceae (Pennington & Styles, 1975). Placed in Meliaceae tribe Turraeeae by Harms (1940), a critical examination of morphology, wood and pollen showed that it definitely belongs in Meliaceae and is related to *Trichilia* L. (Pennington & Styles, 1975). This study confirms the position of *Pter-*

orhachis in subfamily Melioideae and a close relationship to tribes Trichilieae and Turraeeae (Figs. 1, 2).

Pennington & Styles (1975) demonstrated that secondary xylem provides good characters for subfamilial delimitation in Meliaceae, as well as for delimitation of tribal groups within Melioideae. They recognized two groups of tribes within the latter: (1) *Sandoriceae*, *Turraeeae*, *Trichilieae* (except *Cipadessa*) and *Melieae*, and (2) *Aglaieae*, *Guareeae* (except *Turraeanthus*) and *Vavaeeae*. This pattern of relationship among tribes is broadly confirmed by our study (Figs. 1–5). First, Aglaieae plus

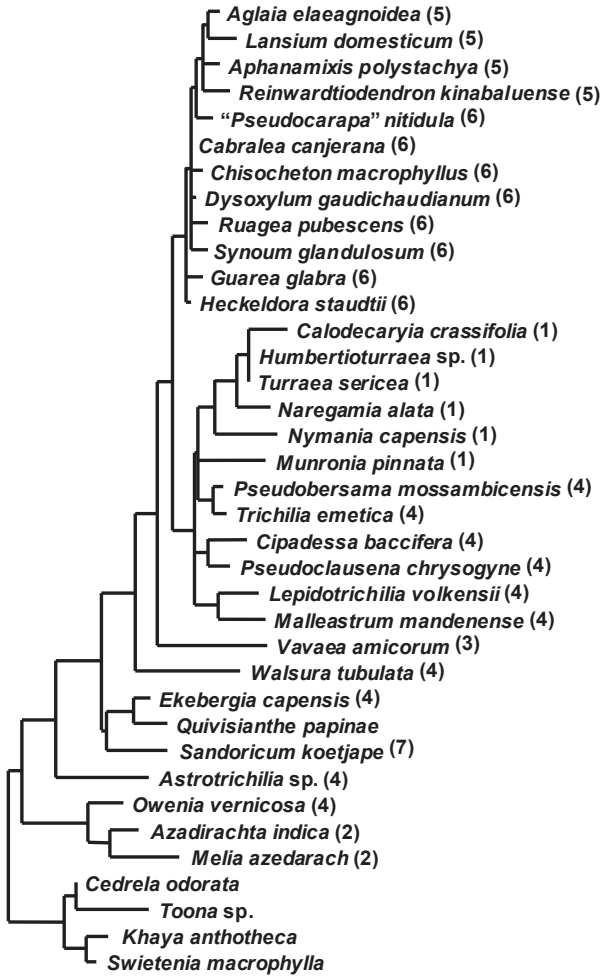


Fig. 4. Tree obtained from the maximum likelihood analysis of the plastid *rbcL* dataset of 37 Meliaceae accessions. Tribal numbers in brackets after species names.

Guareae are always monophyletic. Second, *Sandoriceae*, *Turraeae* and *Trichilieae* are closely interrelated (Figs. 1–5). *Vavaea* is sister to the clade formed by *Guareae* and *Aglaieae* in the MP ITS tree (Fig. 1) and is sister to the clade uniting *Aglaieae*/*Guareae* and most *Turraeae*/*Trichilieae* in the *rbcL* tree (Fig. 3).

Tribe Aglaieae. — *Aglaieae*, currently including *Aglaia* Lour., *Aphanamixis* Blume, *Lansium* Correa, *Reinwardtiodendron* Koord. and *Sphaerosacme* Wall. ex Royle, owe their current circumscription to the work of Pennington & Styles (1975). These five genera are restricted to the Asian tropics and extend into the western Pacific. All except *Sphaerosacme*, of which there is one species, *S. decandra*, restricted to the Himalayas, are represented in Malesia.

The close morphological relationships of *Aglaia*, *Lansium* and *Reinwardtiodendron* collectively with *Aphana-*

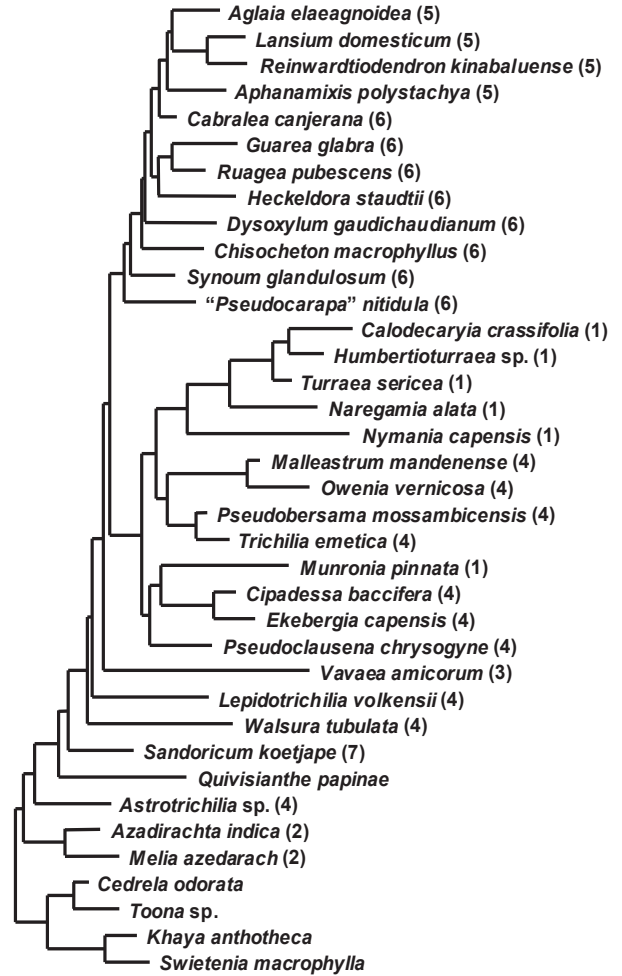


Fig. 5. Tree obtained from the maximum likelihood analysis of the combined ITS/*rbcL* dataset of 37 Meliaceae accessions. Tribal numbers in brackets after species names.

mixis and *Sphaerosacme* are reflected by our phylogenetic trees (Figs. 1–5; for a detailed taxonomic history see Pennington & Styles, 1975; compare Mabberley & al., 1995 and Muellner & al., 2005). A detailed account on the evaluation of taxonomic concepts in the morphologically variable genus *Aglaia* based on DNA data and secondary metabolites was recently published by Muellner & al. (2005).

Our ITS study includes members of all three sections of *Aglaia* (sect. *Aglaia*, sect. *Amoora*, sect. *Neoaglaia*), all but one species of *Aphanamixis*, monospecific *Sphaerosacme* and all but one species each of *Lansium* and *Reinwardtiodendron* (we were unable to amplify these two species). *Aglaia* forms a monophyletic group with *Lansium* and *Reinwardtiodendron* (53 BP, Fig. 1; 99 PP, Fig. 2). *Lansium* and *Reinwardtiodendron* are monophyletic, *Aglaia* is paraphyletic; the three sections of *Aglaia*

each form monophyletic groups (Figs. 1, 2). *Sphaerosacme* is sister to *Aphanamixis*. Altogether, Aglaieae form a monophyletic group (51 BP, Fig. 1; 92 PP, Fig. 2; 96 PP, Fig. 3; Figs. 4–5).

Tribe Guareeae. — Guareeae comprise nine genera, of which two, *Cabrarea* A. Juss. and *Ruagea* Karst., are restricted to tropical America, two, *Heckeldora* Pierre and *Turraeanthus* Baill., to Africa, three, *Anthocarapa* Pierre, *Chisocheiton* Blume and *Dysoxylum* Blume, to Indomalesia and western Pacific and one, *Synoum* A. Juss., to tropical Australia.

Our analysis of ITS includes representatives of all genera of Guareeae and therefore permits a detailed review of relationships within the tribe. As a whole, Guareeae are a paraphyletic group. *Guarea* and *Ruagea* (clade with 80 BP, 87 PP; Figs. 1, 2) are sister to *Turraeanthus* (Figs. 1, 2). The relationship to *Heckeldora*, *Chisocheiton* and *Dysoxylum* lacks strong support; the same applies to *Cabrarea* and *Synoum*. *Anthocarapa nitidula* and a sample collected as “*Pseudocarapa*” *nitidula* (regarded as synonym of the latter; Mabberley & al. 1995) form a clade supported by 85 BP (Fig. 1) and 100 PP (Fig. 2). Although regarded as a single species, the two samples exhibit a high number of autapomorphies (26 and 34, respectively; Fig. 1), which needs further investigation.

Tribe Vavaeae. — Vavaeae are a monogeneric tribe of four species distributed from Sumatra eastwards through Malesia to tropical Australia, Micronesia, Melanesia and Polynesia. *Vavaea* occupies a morphologically isolated position within Melioideae. It possesses most of the individual morphological, anatomical and palynological characters of the subfamily, but in a distinctive combination enabling it to be easily distinguished from all other genera. *Vavaea* has morphological similarities to various tribes and genera: Turraeeae (leaves), Trichilieae (fruit, seed, embryo), Sandoriceae (wood anatomy, pollen), *Aglaia* (pollen). The ambiguous morphological relationships are reflected in our phylogenetic trees: *Vavaea* occupies an isolated position sister to Aglaieae/Guareeae in the MP ITS tree (Fig. 1) and is sister to the clade uniting Aglaieae/Guareeae and most Turraeeae/Trichilieae in the *rbcL* and combined trees (Figs. 3–5).

Tribe Trichilieae. — Trichilieae are a pantropical tribe of twelve genera, *Astrotrichilia* (Harms) J.F. Leroy ex T.D. Pennington & B.T. Styles, *Cipadessa* Blume, *Ekebergia* Sparrm., *Heynea* Roxb. ex Sims, *Lepidotrichilia* (Harms) T.D. Pennington & B.T. Styles, *Malleastrum* (Baill.) Leroy, *Owenia* F. Muell., *Pseudobersama* Verdc., *Pseudoclausena* T.P. Clark, *Pterorhachis* Harms, *Trichilia* L. and *Walsura* Roxb.

The taxonomic history of Trichilieae is complex and closely related to that of Turraeeae (reviewed in Pennington & Styles, 1975). For morphological reasons, Pennington & Styles (1975) concluded that *Pterorhachis*

and *Cipadessa* did not belong in Turraeeae, in which they were placed by Harms (1940). A critical examination of morphology, wood and pollen showed that *Pterorhachis* is closely related to *Trichilia*, from which it differs principally in having more numerous filament appendages and from most species of *Trichilia* in its spheroidal pollen grains (Pennington & Styles, 1975). *Cipadessa* is similar in these same characters to Trichilieae as well, with an hypothesized relationship to *Ekebergia*, and was therefore, like *Pterorhachis*, included in this tribe (Pennington & Styles, 1975). *Pseudobersama* is thought to be closely related to *Trichilia* (Pennington & Styles, 1975).

Our study of ITS reveals *Pterorhachis* as the closest relative of *Nymania*, a member of Turraeeae (66 BP and 84 PP; Figs. 1, 2). A close relationship of *Cipadessa* to *Ekebergia* is confirmed by ITS (100 BP, 100 PP; Figs. 1, 2), though not by *rbcL*. In the analysis of *rbcL*, *Pseudobersama* forms a clade with *Trichilia*, its closest morphological relative. As for the remaining genera of Trichilieae, relationships based on ITS and *rbcL* are incongruent. Based on our results, it is impossible to keep Trichilieae separated from Turraeeae, Vavaeae and Sandoriceae (Figs. 1–5). To reach a robust and well-resolved phylogenetic appreciation of Trichilieae, sampling of additional taxa on species level and the collection of much more data will be necessary.

Tribe Turraeeae. — Turraeeae comprise six or seven genera: *Calodectarya* Leroy, *Humbertioturraea* Leroy, *Munronia* Wight, *Nymania* S.O. Lindb., *Turraea* L. including *Naregamia* Wight & Arn., and perhaps an undescribed genus (“*Turraea breviflora*” Ridley), all restricted to the Old World tropics. The largest is *Turraea*, which is the most widespread; the rest are small genera, one or two restricted to Indomalesia, two to Madagascar, one to southern Africa and one found in both India and southern Africa (Mabberley & al., 1995).

Pennington & Styles (1975) used Turraeeae in the introduction of their generic monograph to illustrate that most tribes in Meliaceae can only be diagnosed by using a combination of several differential characters (as defined by White, 1962). They stated that members of Turraeeae cannot be distinguished from other Meliaceae on the basis of a single diagnostic character and that most character-states typical of the tribe have at least a few exceptions and also occur at least occasionally in other tribes, but always in markedly different combinations. The overall pattern, however, was such that all members of Turraeeae possess many more of the tribal character-states than any excluded species. Thus, Pennington & Styles (1975) claimed Turraeeae, as well as all other tribes in the monograph, to be objectively circumscribed, being based on gaps in the pattern of variation.

Our investigation includes representatives of all genera of Turraeeae and therefore allows a detailed review of relationships within the tribe. In our analysis of ITS,

Nyman is sister (as part of a clade with *Pterorhachis*) to the “core group” of Trichilieae, formed by *Turraea*, *Humbertioturraea*, *Calodectarya* and *Naregamia* (Figs. 1, 2). In the *rbcL* tree, *Nyman* is again sister to this core group (Figs. 3–4). *Naregamia* is sister to the clade formed by *Turraea*, *Humbertioturraea* and *Calodectarya* in both the single ITS and *rbcL*, and in the combined trees (Figs. 1–5). The separation of *Naregamia* from these three genera is well supported in ITS and *rbcL* trees (99 BP, Fig. 1; 100 PP, Fig. 2; 71 BP, 94 PP, Fig. 3), emphasizing that *Naregamia* is genetically distinguishable from *Turraea*. *Naregamia* was reduced to synonymy with *Turraea* by Cheek (1996; for a detailed discussion of characters and the status of *Naregamia* and *Turraea* see Cheek, 1990). Cheek (1990) stated that, as far as seed structure was concerned, *Naregamia* could not be separated from *Turraea*. Previously, Pennington & Styles (1975) had claimed *Naregamia* to be easily distinguished from *Turraea* by combined characteristics of leaves, the staminal tube and seed structure. Our data agree with these earlier findings of Pennington & Styles (1975); we propose to keep *Naregamia* separate from *Turraea*. The position of *Munronia* remains ambiguous, as expected by its morphological intermediacy between typical Turraeeae and the remainder of Melioideae (Figs. 1–5). Unfortunately, we were unable to amplify samples of *Turraea breviflora* collected from herbarium specimens located in Kepong (KEP), Malay Peninsula, and in Kew (K), U.K., due to the old age of specimens and resulting poor quality of DNA extracts (high degradation). The species, according to Mabberley & al. (1995) perhaps an undescribed genus, is known only from a few localities in the Malay Peninsula and Singapore. The fruit has never been observed; recent collections are lacking.

As for Trichilieae, an increase of sampling on species level and the collection of additional DNA data will be necessary to make final decisions about a new circumscription of Trichilieae, especially the inclusion/exclusion of *Munronia* in the tribe.

Tribe Sandoriceae. — Sandoriceae are monogeneric with five species, all but one (*S. koetjape*) restricted to western Malesia (Mabberley & al., 1995). Pennington & Styles (1975) claimed *Sandoricum* to be a morphologically distinct genus, without a close relationship to *Dysoxylum* as proposed by Harms (1940) or to Guareeae. *Sandoricum* is at once identifiable by trifoliate leaves, the ribbed staminal tube, characteristic style-head with divided stigma and indehiscent drupaceous fruit, presumably the reason Pennington & Styles (1975) placed the genus in its own tribe.

Our data confirm that *Sandoricum* has no close relationship to either *Dysoxylum* or Guareeae (Figs. 1–5). In our analysis of ITS, the two species of *Sandoricum* form a strongly supported clade (100 BP, Fig. 1; 100 PP, Fig. 2) and are characterized by a relatively high number of

autapomorphies (29, Fig. 1). In the *rbcL* trees, *Sandoricum* is sister to *Ekebergia* and *Quivisianthe* (Figs. 3–4) and again characterized by a relatively high number of autapomorphies (11, Fig. 3).

Tribe Melieae. — Melieae comprise two genera, *Melia* L. (one to possibly three species) and *Azadirachta* A. Juss. (two species), in the wild state restricted to the Old World Tropics. *Melia* and *Azadirachta* are similar morphologically (Pennington & Styles, 1975). Both genera share a number of anatomical characters not recorded elsewhere in Meliaceae (e.g., clusters of minute vessels with spiral wall thickening). Our single and combined analyses of ITS and *rbcL* confirm monophyly of Melieae (Figs. 1–5). In the ITS MP and Bayesian analyses, Melieae are sister to all other Melioideae (Figs. 1, 2). The same is true for the combined analysis (Fig. 5). In the analyses of *rbcL*, Melieae are sister to *Owenia* (98 BP, 100 PP, Fig. 3; Fig. 4), and this clade is sister to all other Melioideae.

Quivisianthe (Quivisianthoideae). — Although treated in a monogeneric subfamily by Pennington & Styles (1975), the authors mentioned in their generic monograph that the genus is similar in its floral structure to some genera in Trichilieae and that the complete staminal tube without appendages and with the anthers or antherodes inserted on the margin is similar to that of *Ekebergia*. Our ITS and *rbcL* data confirm the position of *Quivisianthe* in Melioideae (Figs. 1–5). In the *rbcL* tree, *Quivisianthe* exhibits a close relationship to *Ekebergia* (clade with 74 BP, 100 PP; Fig. 3), whereas for ITS it appears as sister to *Walsura* (Figs. 1, 2). In the combined Bayesian (tree not shown) and ML analyses (Fig. 5), *Quivisianthe* occupies an isolated position, in the MP analysis the genus appears as sister to *Walsura* (tree not shown).

Concluding remarks. — DNA data of Melioideae and related genera contribute to a better understanding of the intricate systematic relationships of this group of trees that constitute an important component of moist tropical forests world-wide. This study is the first to assess circumscription of Melioideae and the component tribes in detail with data independent of morphology. Maximum parsimony, maximum likelihood and Bayesian analyses of nuclear ITS, compared with analyses based on plastid *rbcL*, confirm monophyly for Aglaieae, Sandoriceae and Melieae, an isolated position for Vavaeae, the position of *Pterorhachis* and *Quivisianthe* in Melioideae, and close relations between Turraeeae and Trichilieae. Trichilieae are the most complex clade. *Anthocarapa* and *Pseudocarapa*, regarded as synonym of the latter, form a clade, but exhibit each a high number of autapomorphies, which needs further investigation. We propose to keep *Naregamia* separate from *Turraea* because the two are not exclusively related. These taxonomic decisions are based on DNA data as well as morphological variation.

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Appendix. Vouchers, origin and GenBank accession numbers* of the material used in the study.**SUBFAMILY, Tribe, Species, Collector number and location of herbarium voucher, Origin, GenBank accession numbers**

MELIOIDEAE, Turraeeae, Calodectaria crassifolia Leroy, *Croat 31521* (K), Madagascar, DQ861631, AY128216; *Humbertio-turraea* sp. (*H. labatii* Lescot ined.), *Bardot-Vaucoulon 160* (K), Madagascar, DQ861632, DQ238058; *Munronia pinnata* (Wall.) Theob., *Samuel 6* (WU), Sri Lanka, DQ861604, AY128236; *Naregamia alata* Wight & Arn., *Kanodia 89603* (K), India, DQ861629, DQ238059; *Nymanina capensis* Lindb., *Chase 270* (NCU), South Africa, DQ861633, AY128238; *Turraea sericea* Sm., *Civeyrel 1336* (K), Madagascar, DQ861630, AY128245; *Turraea heterophylla* Sm., *Küppers 2212* (FR), West Africa, EF136578; **Melieae, Azadirachta indica** A. Juss., *Samuel 5* (WU), Sri Lanka, AY695594, AY128215; *Melia azedarach* L., *Chase 2867* (K), K Living Collection 1953-37801 [donation from KYGH], AY695595, AY128234; **Vavaeeae, Vavaea amicornum** Benth., *Katik & al. 74722* (K), Papua New Guinea, DQ861610, DQ238066/67; **Trichilieae, Astrotrichilia** sp., *Richard 25* (K), Madagascar, DQ2388060, *Cipadessa baccifera* Miq., *Chase 1310* (K), Indonesia (Bogor III.B.90), DQ861627, AY128224; *Ekebergia capensis* Sparrm., *MG 246* (Cynthia Morton), South Africa, DQ861623, AY128228; *Lepidotrichilia volkensis* (Gürke) J.-F. Leroy ex B.T. Styles & F. White, *Hughes 189* (K), Tanzania, DQ861620, DQ238061; *Malleastrum mandenense* Leroy, *Cheek & al. 3-17-5* (K), Madagascar, DQ861626, DQ238062; *Owenia vernicosa* F. Muell., *Evans M3071*, Australia, DQ861622, DQ238063; *Pseudobersama mosambicensis* (Sim) Verde., *Bidgood, Abdallah & Vollesen 1426* (K), Tanzania, DQ238064; *Pseudoclausena chrysogyne* (Miq.) T.P. Clark, *Muellner 2052* (FR), Malaysia (FRIM Arboretum), DQ861602, DQ238065; *Pterorhachis zenkeri* Harms, *Breteler 2741* (K), Cameroon, DQ861628; *Trichilia emetica* Vahl, *Chase 552* (K), K Living Collection 1984-1568, AY128244; *Trichilia emetica* Vahl, *Siegelstetter 15* (FR), West Africa, EF136577; *Trichilia prieureana* A. Juss., *Neumann 1518* (FR), West Africa, EF136576; *Walsura tubulata* Hiern, *Chase 1314* (K), Indonesia (Bogor VIII.B.127), DQ861625, AY128246; **Aglaiaceae, Aglaia archboldiana** A.C. Smith, *Greger 696* (WU), Fiji, AY695524; *Aglaia elaeagnoidea* Benth., *Samuel 4* (WU), Sri Lanka, AY128209; *Aglaia odorata* Lour, *Greger 903* (WU), Thailand, AY695552; *Aglaia samoensis* A. Gray, *Greger 752* (WU), Samoa, AY695557; *Aglaia sapindina* (F. von Muell.) Harms, *Greger 669* (WU), Australia, AY695558; *Aglaia vitiensis* A.C. Smith, *Greger 691* (WU), Fiji, AY695569; *Aglaia lawii* (Wight) C.J. Saldanha, *Greger 573* (WU), Thailand, AY695573; *Aglaia teysmanniana* (Miq.) Miq., *Greger 704* (WU), Thailand, AY695539; *Aglaia australiensis* Pannell, *Greger 662* (WU), Australia, AY695571; *Aglaia cucullata* (Roxb.) Pellegrin, *Brunei Museum s.n.* (K), Brunei, AY695572; *Lansium domesticum* Correa, *Chase 2113* (K), Indonesia (Bogor, III.B.100), AY695586, AY128232; *Lansium* cf. *membranaceum* (Kosterm.) Mabb., *Pannell 1934* (FHO), Sumatra, DQ861611; *Reinwardtiodendron cinereum* (Hiern) Mabb., *F.R.I. (Forest Res. Inst.) 26877* (K), Malaysia (Perak), AY695588; *Reinwardtiodendron humile* (Hassk.) Mabb., *Trichon VT 641* (FHO), Sumatra, DQ861612; *Reinwardtiodendron kinabaluense* (Kosterm.) Mabb., *Lamb ALFB 112/87* (K), Malaysia (Borneo), AY695589, DQ238054; *Reinwardtiodendron kostermansii* (Prijanto) Mabb., *Kostermans 19215* (K), Indonesia (W Sumbawa), DQ861634; *Aphanamixis borneensis* Harms, *Beamn 8208* (K), Malaysia (Borneo), AY695583; *Aphanamixis polystachya* (Wall.) R.N. Parker, *Samuel 14* (WU), Sri Lanka, AY695584; *Aphanamixis polystachya* (Wall.) R.N. Parker, *Chase 2109* (K), Indonesia (Bogor III.C.68a), AY128213; *Sphaerosacme decandra* (Wal.) T.D. Penn., *Williams & Stainton 8533* (K), Ecuador, AY695590; **Guareeae, Anthocarapa nitidula** (Benth.) T.D. Penn., *Chanel 1110* (K), Melanesia, DQ861615; “*Pseudocarapa nitidula* (Benth.) T.D. Penn., *Chase 3313* (K), Australia, DQ861616, DQ238056; *Cabrarea canjerana* (Vell.) Mart., *Pennington 17067* (K), Peru, DQ861617, DQ238055; *Chisocheton macrophyllus* King, *Chase 1309* (K), Indonesia (Bogor III.F.30a), DQ861613, AY128221; *Dysoxylum gaudichaudianum* (A. Juss.) Miq., *Chase 1312* (K), Indonesia (Bogor III.F.90), DQ861619, AY128227; *Guarea glabra* Vahl, *Chase 336* (NCU), U.S.A., AY695591, AY128229; *Heckeldora staudtii* (Harms) Staner, *Chase 3311* (K), Cameroon, AY695592, AY128230; *Ruagea pubescens* Karst., *Pennington & Frere 13761* (K), Ecuador, AY695593, DQ238057; *Synoum glandulosum* (Sm.) A. Juss., *Schodde 5101* (K), Australia, DQ861618, AY128242; *Turraeanthus* sp., *Carvalho 4348-1* (K), Equat. Guinea, DQ861614; **Sandoricaceae, Sandoricum koetjape** (Burm. f.) Merr., *Muellner 2050* (FR), Thailand, DQ861600, DQ238068; *Sandoricum borneense* Miq., *Chase 1313* (K), Indonesia (Bogor, III.B.92), DQ861601; *Quivisianthe papinae* Baill., *Phillipson 1650* (K), Madagascar, DQ861605, AY128239; **SWIETENIOIDEAE, Cedreleae, Cedrela odorata** L., *Chase 2112* (K), Indonesia (Bogor III.B.2), DQ861606, AY128220; *Toona* sp., *Terrazas s.n.* (K), Australia, DQ861607, AY128243; **Swietenieae, Khaya anthothea** C. DC., *Chase 2859* (K), K Living Collection 1967-35601 (source plant: Amherst College, Massachusetts), DQ861608, AY128231; *Swietenia macrophylla* King, *Chase 250* (NCU), U.S.A., DQ861609, AY128241.

*All sequences are deposited in GenBank; new sequences are deposited under the accession numbers DQ861600–DQ861602, DQ861604–DQ861620, DQ861622–DQ861623, DQ861625–DQ861634 and EF136576–EF136578 (<http://www.ncbi.nlm.nih.gov/>).