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 Vavaeeae, the position of Pterorhachis and Quivisianthe in Melioideae, and a close relationship between Turraeeae and Trichilieae. Trichilieae are morphologically and genetically the most complex tribe. Trichilieae cannot be separated from Turraeeae, Vavaeeae 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 semideserts (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 recent reassessment of the circumscription of the four subfamilies by means of phylogenetic analyses of sequence data from three regions (plastid rbcL, matK, nuclear 26SrDNA) showed that the members of the two small monogenic 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 Melioidae but stated that limits of Trichilieae, Aglaiaeae and Guareeae could only be defined by overlapping morphological, anatomical and palynological characters. All tribes of Melioidae are represented in Malesia, but only two (Guareeae, Trichilieae) are pantropical and two other ones are restricted to the Old World (Turraeae, Melieae); the remainder are restricted to Indomalesia and the western Pacific (Vavavaeae, Aglaiaeae, Sandoiriaea; Mabberley & 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; Soltsis & Soltsis, 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 Melioidae for which former analyses of plastid \textit{rbcL}, \textit{matK} and nuclear 26S rDNA did not provide sufficient information (Mueellner & 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 Melioidae. The ITS data are compared to \textit{rbcL} data recently collected in the course of a survey on the biogeographic history of Meliaceae (Mueellner & al., 2006).

### MATERIALS AND METHODS

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

**Sequencing.** — PCR primers were also used for sequencing. Cycle-sequencing followed Mueellner & 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 GCCRY-(4 to 7 n)-GYGCAAGGAA (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′-GAAATGCAGGATCC-3′ within the 5.8S rDNA gene, which can be used to differentiate between flowering plants, fungi and algae (Joves & 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) with 5,000 replicates with simple sequence addition, TBR branch swapping and MulTrees on.

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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 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 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 rDNA gene and ITS2 spacer, and of plastid rbcL.

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

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

<table>
<thead>
<tr>
<th>Region</th>
<th>Length (no. characters)</th>
<th>Length (bp)</th>
<th>Mean GC ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire ITS</td>
<td>794</td>
<td>627–664</td>
<td>63</td>
</tr>
<tr>
<td>ITS1</td>
<td>338</td>
<td>233–273</td>
<td>66</td>
</tr>
<tr>
<td>5.8S</td>
<td>214</td>
<td>156–172</td>
<td>55</td>
</tr>
<tr>
<td>ITS2</td>
<td>242</td>
<td>214–238</td>
<td>66</td>
</tr>
</tbody>
</table>

Outgroup | 636–650 | 247–257 | 164 | 225–228 | 69 | 73 | 55 | 74 |
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 Turraeeae 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; Turraeeae 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.](image-url)

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Aphanamixis borneensis (5)
Aphanamixis polystachya (5)
Sphaerosacme decandra (5)
Cabralea canjerana (6)
Guarea glabra (6)
Ruagea pubescens (6)
Heckeldora stautili (6)
Anthocarapa nitidula (6)
"Pseudocarapa" nitidula (6)
Synoum glandulosum (6)
Vavaea amicorum (3)
Lepidotrichilia volkensii (4)
*Trichilia prieursana* (4)
*Trichilia emetica* (4)
Owenia vernicosa (4)
Malleastrum mandenense (4)
Pterorhachis zenkeri (4)
Nymania capensis (1)
Naregania alata (1)
Turraea sericea (1)
Turraea heterophylla (1)
Calodecaryia crassifolia (1)
Humbertioturraea sp. (1)
Ekebergia capensis (4)
Cipadessa baccifera (4)
Pseudoclausena chrysogyne (4)
Munronia pinnata (1)
Sandoricum koetjape (7)
Sandoricum borneense (7)
Quivisianthe papiae
Walsura tubulata (4)
*Melia azedarach* (2)
Azadirachta indica (2)
Toona sp.
Khaya anthothea
Swietenia macrophylla

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Aphanamixis polystachya (5)
Sphaerosacme decandra (5)
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Tribe affiliation within Meliaceae. — 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 Pterorhachis 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 Meliaceae. 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...
Guareae are always monophyletic. Second, Sandoricae, Turraeeae and Trichilieae are closely interrelated (Figs. 1–5). Vavaea is sister to the clade formed by Guareae and Aglaiae in the MP ITS tree (Fig. 1) and is sister to the clade uniting Aglaiae/Guareae and most Turraeeae/Trichilieae in the rbcL tree (Fig. 3).

Tribe Aglaiae. — Aglaiae, 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 Malasia.

The close morphological relationships of Aglaia, Lansium and Reinwardtiodendron collectively with Aphanamixis 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 Aphananikis. Altogether, Aglaeae form a monophyletic group (51 BP, Fig. 1; 92 PP, Fig. 2; 96 PP, Fig. 3; Figs. 4–5).

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

Our analysis of ITS includes representatives of all genera of Guareae and therefore permits a detailed review of relationships within the tribe. As a whole, Guareae 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, Chisochoten and Dysoxylum lacks strong support; the same applies to Cabralea 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 Vavaeeae.** — Vavaeeae 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 Meliaceae. 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 Aglaeae/Guareae in the MP ITS tree (Fig. 1) and is sister to the clade uniting Aglaeae/Guareae and most Turraeeae/Trichilieae in the rbcL and combined trees (Figs. 3–5).


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 Trichila, 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, Vavaeeae 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.
Nymania is sister (as part of a clade with Pterorhachis) to the “core group” of Trichilieae, formed by Turraea, Humbertioturraea, Calodecaryia and Naregamia (Figs. 1, 2). In the rbcL tree, Nymania is again sister to this core group (Figs. 3–4). Naregamia is sister to the clade formed by Turraea, Humbertioturraea and Calodecaryia 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 Meliodeae (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 Sandoricaceae. — Sandoricaceae are monogenic 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 Guareea. 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 Guareea (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 Meliaceae. — Meliaceae 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 Meliodeae (e.g., clusters of minute vessels with spiral wall thickening). Our single and combined analyses of ITS and rbcL confirm monophyly of Meliaceae (Figs. 1–5). In the ITS MP and Bayesian analyses, Meliaceae are sister to all other Meliodeae (Figs. 1, 2). The same is true for the combined analysis (Fig. 5). In the analyses of rbcL, Meliaceae are sister to Owelia (98 BP, 100 PP, Fig. 3; Fig. 4), and this clade is sister to all other Meliodeae.

Quivisianthe (Quivisianthoideae). — Although treated in a monogenic 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 Meliodeae (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 Meliodeae 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 Meliodeae 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 Aglaeae, Sandoricaceae and Meliaceae, an isolated position for Vavaeeae, the position of Pterorhachis and Quivisianthe in Meliodeae, 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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LITERATURE CITED


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.** Calodendryia crassifolia Leroy, Croat 31521 (K), Madagascar, DQ861631, AY128216; Humberto-turraea sp. (H. Jaffar, Lescot ined.), Madagascar, DQ861632, DQ238058; Munronia pinnata (Wall.) Theob., Samuel 6 (WU), Sri Lanka, DQ861604, AY128236; Naregiania alata Wight & Arn., Kanodia 89603 (K), India, DQ861629, DQ238059; Nymania capensis Lindlb., Chase 270 (NCU), South Africa, DQ861633, AY128238; Turraea sericea Sm., Civeyrél 1336 (K), Madagascar, DQ861630, AY128245; Turraea heterophylla Sm., Kppers 2212 (FR), West Africa, EF136578; *Meliaeae, 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; *Vavaeaeeae, Vavaea amicorum* Benth., Katik & al. 74722 (K), Papua New Guinea, DQ861610, DQ238066/7; *Trichilieae. Astrotrichilia sp.*, Richard 25 (K), Madagascar, DQ238860, 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 volkensii (Gürke) J.-F. Leroy ex B.T. Styles & F. White, Hughes 180 (K), Tanzania, DQ861620, DQ238061; *Malleastrum mandenense* Leroy, Cheek & al. 3-17-5 (K), Madagascar, DQ861626, DQ238062; *Oenienia vernicosa* F. Muell., Evans M3071 (K), Australia, AY695593, DQ238057; *Pseudobrusa mosambicensis* (Sim) Verdc., Bidgood, Abdallah & Vollesen 1426 (K), Tanzania, DQ238064; Pseudoclausea chrysoxyge (Miq.) T.P. Clark, Mueller 2052 (FR), Malaysia (FRIM Arboretum), DQ861602, DQ238065; Pierorhachis zeneri Harms, Breteler 2741 (K), Cameroon, DQ861628; *Trichila emetica* Vahl, Chase 552 (K), K Living Collection 1848-1568, AY128244; *Trichilia emetica* Vahl, Siegletter 15 (FR), West Africa, EF136577; *Trichilia prieureana* A. Juss., Neumann 1518 (FR), West Africa, EF136576; Walsura tulbatula Hiern, Chase 1314 (K), Indonesia (Bogor VIII.B.127), DQ861625, AY128246; *Aglaiaceae, Aglaiia archboldiana* A.C. Smith, Greger 696 (WU), Fiji, AY695524; *Aglaiia elaeagnoides* Benth., Samuel 4 (WU), Sri Lanka, AY128209; Aglaiia odorata Lour, Gregor 903 (K), Thailand, AY695552; *Aglaiia samoensis* A. Gray, Greger 752 (WU), Samoa, AY695571; *Aglaiia sapindina* (F. von MueLL) Harms, Greger 669 (WU), Australia, AY695558; Aglaiia viitiansis A.C. Smith, Greger 691 (WU), Fiji, AY695569; Aglaiia lawii (Wight) C.J. Saldanha, Greger 573 (WU), Thailand, AY695573; *Aglaiia teysmanniana* (Miq.) Miq., Greger 704 (WU), Thailand, AY695539; Aglai australiensis Pannell, Greger 662 (WU), Australia, AY695571; *Aglaiia cuculata* (Roxb.) Pellegrin, Brunet Museum s.n. (K), Brunei, AY695572; *Lansium domesticum* Correa, Chase 2132 (K), Indonesia (Bogor III.B.100), DQ861586, AY128232; *Lansium cf. membranaceum* (Kosterm.) Mabb., Pannell 1934 (FHO), Sumatra, DQ861611; *Reinwardtiiodendron cinereum* (Hiern) Mabb., F.R.I. (Forest Res. Inst.) 26877 (K), Malaysia (Perak), AY695585; *Reinwardtiiodendron humile* (Hassak.) Mabb., Trichon VT 641 (FHO), Sumatra, DQ861612; *Reinwardtiiodendron kinabaluense* (Kosterm.) Mabb., Lamb ALFB 112/87 (K), Malaysia (Borneo), AY695589, DQ238054; *Reinwardtiiodendron kostermanii* (Prijanto) Mabb., Kostermans 19215 (K), Indonesia (W Sumbawa), DQ861634; *Aphananaxis borneensis* Harms, Beamian 8208 (K), Malaysia (Borneo), AY695583; *Aphananaxis polysystachya* (Wall.) R.N. Parker, Samuel 14 (WU), Sri Lanka, AY695584; *Aphananaxis polysystachya* (Wall.) R.N. Parker, Chase 2109 (K), Indonesia (Bogor III.C.68a), AY128213; *Sphaerosacme decandra* (Wal.) T.D. Penn., Williams & Stanton 8533 (K), Ecuador, AY695590; *Guareaceae, Anthocarapa nitidula* (Benth.) T.D. Penn., Chanel 1110 (K), Melanesia, DQ861615; *Pseudocarapa nitidula* (Benth.) T.D. Penn., Tsch. 3313 (K), Australia, DQ861616, DQ238056; *Cabralea canjerana* (Vell.) Mart., Pennington 17067 (K), Peru, DQ861617, DQ238055; *Chisocheton macrophyllus* King, Chase 1309 (K), Indonesia (Bogor III.F.30a), DQ861613, AY128221; *Dioxyxylum gadichaudianum* (A. Juss.) Miq., Chase 312 (K), Indonesia (Bogor III.F.90), DQ861619, AY128227; *Guarea glabra* Vahl, Chase 336 (NCU), U.S.A., AY695591, AY128229; *Heckeldora staubii* (Harms) Staner, Chase 3311 (K), Cameroon, AY695592, AY128230; *Ruegeara pubescens* Karst., Pennington & Frese 13761 (K), Ecuador, AY695593, DQ238057; *Synnum gundulorum* (Sm.) A. Juss., Schodde 5100 (K), Australia, DQ861618, AY128242; *Turraeusanthus sp.*, Carvalho 4348-1 (K), Equat. Guinea, DQ861614; *Sandoricaceae, Sandoricum koetjape* (Burm. f.) Merr., Muller 2050 (FR), Thailand, DQ861600, DQ238068; *Sandoricum borneense* Miq., Chase 1313 (K), Indonesia (Bogor III.B.92), DQ861601; *Quvisianthe papinoides* Baily, Philippson 1650 (K), Madagascar, DQ861605, AY128239; *SWIETENIODEIaeae, Cedreleae, Cedrela odorata* L., Chase 212 (K), Indonesia (Bogor III.B.2), DQ861606, AY128220; *Toona sp.*, Terrazas s.n. (K), Australia, DQ861607, AY128243; *Swieteniaeae, Khaya anthotheca* 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/).