

AGLAIA (MELIACEAE): AN EVALUATION OF TAXONOMIC CONCEPTS BASED ON DNA DATA AND SECONDARY METABOLITES¹

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We performed maximum parsimony and Bayesian analyses (nuclear ITS rDNA, plastid *rps16* intron) to estimate phylogenetic relationships within *Aglaia* (over 100 species in Southeast Asia, the Pacific, and Australia) and its relations among Aglaieae (Meliaceae). Based on 67 accessions of Aglaieae, three taxa of Guareae, and two taxa of Melieae (outgroup), this study provides the first assessment of the current circumscription of Aglaieae, *Aglaia*, and its sections and to a more limited extent of species concepts in *Aglaia*. DNA data are compared to recently collected data on chemical profiles. Our analyses indicate (1) the monophyly of Aglaieae; (2) the polyphyly of *Aphanamixis*; (3) the paraphyly of *Aglaia*; (4) the existence of at least three entities with respect to *Aglaia*: (a) the core group of *Aglaia* section *Amoora* (dehiscent fruits) with close relationships to *Lansium* and *Reinwardtiadendron*, (b) a group comprising morphological intermediates between the two sections, and (c) the core group of *Aglaia* section *Aglaia* (indehiscent fruits). Macro- and micromolecular data indicate that complex species are more heterogeneous, i.e., probably containing more than one taxon each, than taxonomically isolated species. A third section in *Aglaia* is recognized to accommodate *A. lawii*, *A. teysmanniana*, and *A. beccarii*.

Key words: *Aglaia*; chemotaxonomy; internal transcribed spacer (ITS); Meliaceae; molecular phylogenetics; plastid ribosomal protein gene intron (*rps16*); Sapindales.

Aglaia Lour., the largest genus of the subtropical and tropical angiosperm family Meliaceae (order Sapindales), contains at least 106 (115) arborescent species and presents more taxonomic problems in species delimitation than any other genus of the family (Pannell, 1992, 1998a, b; Mabberley et al., 1995; Pannell, 2004). *Aglaia* forms an important component of the moist tropical forest in the Indomalaysian region. The total range of the genus comprises the tropics of Southeast Asia from Sri Lanka and India to Australia (Queensland, Northern Territory, and Western Australia) and as far east as the island of Samoa in Polynesia and north to the Marianne (Saipan, Roti, and Guam) and Caroline Islands (Palau and Ponape) in Micronesia (Pannell, 1992).

During the past few years, the genus has received increasing scientific focus due to its bioactivity potential. Flavaglines, especially cyclopenta[*b*]benzofurans, were shown to be potent insecticides (Brader et al., 1998; Bacher et al., 1999; Nugroho et al., 1999; Dreyer et al., 2001; Greger et al., 2001). In addition to that, cytotoxic (Cui et al., 1997) and antifungal (Engelmeier et al., 2000) effects were found for these compounds, so far only known from *Aglaia* species. Other classes of natural products occurring in *Aglaia* include lignans (Brader et

al., 1998; Greger, 2000; Wang et al., 2001), flavonoids, and bisamides (Greger et al., 2000, 2001), the last also only known from *Aglaia*; some of these exhibit cytotoxic and antiviral properties (Saifah et al., 1993, 1999). Furthermore, many terpenoids are reported (Fuzzati et al., 1996; Brader et al., 1998; Mohamad et al., 1999; Puripattanavong et al., 2000; Weber et al., 2000; Greger et al., 2001). However, so far no chemotaxonomic framework exists for *Aglaia*. In contrast, at higher taxonomic levels first attempts have been made to use limonoids (bitter tetranortriterpenoids) as marker compounds that might offer possibilities for assessing intergeneric taxonomy in Meliaceae (e.g., Taylor, 1981; Mulholland et al., 1998), but knowledge is still so fragmentary (Mabberley et al., 1995) that comprehensive phylogenetic conclusions (Da Silva et al., 1984, 1999; Agostinho et al., 1994; Neto et al., 1998) have either been premature or conflicting.

Besides its unique biogenetic trend to produce biologically highly active flavaglines, *Aglaia* is morphologically distinguished from most other genera in Meliaceae by its characteristic indumentum of peltate scales or stellate hairs; simple hairs are never found on vegetative parts of the plant (Pannell, 1992). *Lepidotrichilia* (Harms) J. F. Leroy, *Astrotrichilia* (Harms) J. F. Leroy, *Pterorhachis* Harms, and *Chisocheton* section *Rhetinosperma* (Radlk.) Mabberley also possess a stellate indumentum, and *Trichilia* rarely has stellate or peltate scales. These genera might therefore be confused with *Aglaia* on indumentum alone, but they are easily separated on floral characters. When stellate hairs or scales occur in genera other than these, their structure is different; they are usually either forked hairs or clumps of simple hairs [e.g., *Melia*, *Aphanamixis polystachya* (Wall.) R. N. Parker] and nearly always interspersed with simple hairs. The shoot apices in these taxa

¹ Manuscript received 30 April 2004; revision accepted 16 November 2004.

The authors thank Tod F. Stuessy, Head of the Dept. of Higher Plant Systematics and Evolution, University of Vienna, for the facilities provided; Annette W. Coleman, Brown University, USA, for her advice on alignment of ITS sequences based on secondary structure; and Brigitte Brem, University of Vienna, Austria, for allowing us to use data on secondary metabolites in *Aglaia* from her Ph.D. thesis. Financial support for this study was provided by the FWF to Rosabelle Samuel (grant no. P14150-BOT).

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are densely covered with simple hairs, not stellate hairs or peltate scales (Pannell, 1992). However, well-defined sets of morphological characters that can be used to define genera are clearly absent in Aglaieae, which is reflected by the complicated taxonomic history of *Aglaia*.

Tribe Aglaieae, including *Aglaia*, *Aphanamixis* Blume (three species), *Lansium* Correa (three species), *Reinwardtiidendron* Koord. (seven species), and *Sphaerosacme* Wall. ex Royle (one species), was first described by Blume (1825) and owes its present circumscription to the work of Pennington and Styles (1975). In the past, *Amoora* had been separated from *Aglaia* because of differences in the number of anthers (de Candolle, 1878) or confined to either those species with three petals (King, 1895) or those with dehiscent fruits (Harms, 1940). The inclusion of *Aphanamixis* and *Sphaerosacme decandra* (Wall.) Pennington in *Amoora* by some authors (e.g., de Candolle, 1878) added to the confusion. Pellegrin (1911) also considered that *Lansium* should be included in *Aglaia*, and Kostermans (1966) combined the two genera. His *Aglaia* section *Lansium* contained 7–8 species of *Lansium*, six of *Reinwardtiidendron*, and one or two of *Lepisanthes* Blume (Sapindaceae; see Pennington and Styles, 1975; Mabblerley, 1985). In the most recent account of *Aglaia*, Pannell (1992) followed Pellegrin (1911) and Pennington and Styles (1975), who reduced *Amoora* to *Aglaia*. Pennington and Styles (1975) united *Amoora* and *Aglaia* because the sets of character state pairs previously used to separate them were not sufficiently correlated in all species, and there was a gradation in the structure of their secondary xylem. Pannell (1992) recognized two sections in *Aglaia*, *Aglaia* and *Amoora*, separated by fruit dehiscence. Two species, *Aglaia lawii* and *A. teysmanniana*, have flower characters intermediate between the two sections, but they were placed in section *Amoora* because of their dehiscent fruits (Pannell, 1992).

In her recent taxonomic treatment of the genus, Pannell (1992, 1998a, b) adopted a wide species concept, and thus for many species even the most indicative morphological characters, such as indumentum, fruit, and floral morphology, vary considerably. Pannell (1992) recognized different types of species in her monograph of the genus. “Isolated species” are morphologically distinct species without any close relatives and with either small (e.g., *A. coriacea* Korth. ex Miq.) or extensive (e.g., *A. cucullata* (Roxb.) Pellegrin) geographical distribution. In contrast, members of closely related pairs or larger groups of species are often separable only by using the combined variation of several overlapping characters. Members of these groups may be allopatric (e.g., *A. elliptica* Blume and *A. cinnamomea* Baker fil.; Pannell, 1993) or sympatric (e.g., *A. korthalsii* Miq. and *A. speciosa* Blume). In “variable species,” variation is relatively simple, usually involving two variants linked by intermediates. “Complex species” have a more extensive, complicated, and putatively reticulate pattern of variation, for which extremes appear at first sight to belong to distinct species (Pannell, 1992, 1998a, b).

The controversial taxonomic history of Aglaieae and *Aglaia* and the lack of consensus about taxon delimitation at all taxonomic levels based on morphology make it clear that there is need for additional research. Molecular techniques can be used to investigate genetic diversity and relationships among species. These data provide helpful tools for taxon delimitation especially in plant groups in which the number of diagnostic morphological characters is limited and parallel evolutionary trends might obscure phylogenetic relations (Muellner et al.,

2003). Moreover, during the past few decades biodiversity within *Aglaia*, constituting one of the most important sources of biologically active compounds within Meliaceae, has become severely threatened due to habitat loss. Currently, 95 species of *Aglaia* are included in The World Conservation Union Red List (IUCN, 2003). However, measures for conserving biodiversity are only effective if species are genetic entities, i.e., taxonomic species reflect evolutionarily distinct units. This emphasizes the importance of reevaluation of the taxonomic framework for *Aglaia*.

Phylogenetic studies have demonstrated that 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 useful in assessing relationships at infrageneric levels (e.g., Baldwin and Markos, 1998; Whitten et al., 2000; Vaasen et al., 2002; Chase et al., 2003). Plastid noncoding regions like the *rps16* intron have also been shown to be suitable for inferring phylogenetic relationships at lower taxonomic levels (Edwards and Gadek, 2001; Popp and Oxelman, 2001; Wanntorp et al., 2001; Clarkson et al., 2002). Combined analyses of *rps16* intron and ITS sequences have been shown to provide a useful tool for resolving difficult taxonomic issues (Oxelman et al., 1997).

In this study, we performed maximum parsimony and Bayesian analyses of sequence data from these two regions (nuclear ITS and plastid *rps16* intron) to estimate phylogenetic relationships within tribe Aglaieae and within its largest genus *Aglaia*. The aim of our investigation was to assess the current circumscription of (1) tribe Aglaieae, (2) *Aglaia*, and (3) sections, and (4) the wide species concept proposed for *Aglaia* by Pannell (1992). The DNA data are compared to recently collected data on chemical profiles of the respective taxa of *Aglaia* to investigate if phylogenetic relationships between species and within complex species are reflected by biogenetic trends.

MATERIALS AND METHODS

We analyzed ITS and *rps16* intron data of 67 accessions of Aglaieae, including representatives and type species of both currently recognized sections within *Aglaia* (*Amoora* and *Aglaia*), three accessions of Guareae, and two accessions of Melieae (outgroup), all being members of Melioideae (Appendix, see Supplementary Data accompanying the online version of this article). Intrafamilial phylogenetic relationships of Meliaceae were previously assessed by an evaluation of the higher-level classification using DNA sequence data from three regions: plastid genes *rbcL*, *matK*, and nuclear 26S rDNA (Muellner et al., 2003). Guareae are the tribe genetically closest to Aglaieae (Muellner et al., 2003; A. N. Muellner, R. Samuel, M. W. Chase, T. F. Stuessy, unpublished manuscript). For most of the *Aglaia* species referred to as “variable” or “complex” (Pannell, 1992), 2–5 individuals were included in this study.

Plant material—Plant material of ingroup taxa was collected during excursions to Bangladesh, Thailand, Fiji, Samoa, and Australia or taken from herbarium specimens. Outgroup taxa *Azadirachta* and *Melia* were collected during excursions to Sri Lanka and from the living collections of the Royal Botanic Gardens, Kew, UK. Herbarium specimens, including the newly collected material, are deposited at the University of Vienna Herbarium (WU), Austria, or the Herbarium of the Royal Botanic Gardens Kew (K), UK (Appendix, see Supplementary Data accompanying the online version of this article).

Isolation of DNA, amplification, and sequencing—Field-collected material was dried in silica gel prior to DNA extraction (Chase and Hills, 1991). Total

TABLE 1. Characterization of ITS in genus *Aglaia* and outgroup taxa.

Region	Length (no. characters)	<i>Aglaia</i> length (bp)	Outgroup length (bp)	<i>Aglaia</i>		
				GC ratio (range in %)	Mean GC ratio (%)	Outgroup mean GC ratio (%)
Entire ITS	741	650–662	639–645	58–66	62	67
ITS1	313	263–274	247–252	60–72	65	71
5.8S	172	163–172	164	53–55	54	55
ITS2	256	221–227	228–229	59–67	64	70

DNA was extracted from similar amounts of silica-gel-dried tissue as well as from herbarium specimens following the cetyltrimethyl-ammonium bromide (CTAB) procedure of Doyle and Doyle (1987) with the following modifications: after precipitation with isopropanol and subsequent centrifugation, the DNA pellet was washed with 70% ethanol, dried at 37°C, and resuspended in TRIS-EDTA (TE) buffer. 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: 17SE and 26SE (Sun et al., 1994), ITS4 (White et al., 1990), as well as the newly created primer pair F1-ITS (5'-GATCGCGGCGACTTGGGCGGTTTC-3') and R1-ITS (5'-GGTAGTCCCGCTGACCTGGG-3') for the fragment comprising part of 18S rDNA, the entire ITS region and part of 26S rDNA; rpsF, rpsR2 (Oxelman et al., 1997) as well as the newly created primer pair F-rps (5'-ATCCGCTATGGATTCTTTACATC-3') and R-rps (5'-CTCTCA-TAACTCAAGTTGGATAAC-3') for the *rps16* intron (partial). A 50- μ L reaction mix contained 45 μ L 1.1 \times ReddyMix PCR Master Mix (2.5 mmol MgCl₂; Advanced Bioenzymes, Surrey, UK), 1 μ L of the primers each (20 pmol), 1 μ L template DNA (100–2000 ng/ μ L), as well as 2 μ L dimethyl sulfoxide (DMSO) for ITS and 5 μ L bovine serum albumin (BSA; 0.4%) for the *rps16* intron. These additives are thought to stabilize the enzyme (BSA), reduce secondary structure problems (DMSO; e.g., within-strand Watson-Crick pairing of ITS; Baldwin et al., 1995), or favor precise annealing (DMSO; Palumbi, 1996). Amplification and gel purification of amplification products were carried out according to Muellner et al. (2003), with slight modifications for some taxa. The same primers as described earlier were used for sequencing. Sequencing was carried out according to Muellner et al. (2003).

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. The ITS was explored for the presence of several structural motifs to check for the potential occurrence of pseudogenes. Thus, in the ITS1 region, we searched for the presence of the conserved angiosperm motif GGCRY—(4 to 7 n)—GYGY-CAAGGAA (Liu and Schardl, 1994). We also looked for the presence of the conserved (C1–C6) and variable (V1–V6) domains determined for plant ITS2 sequences (Hershkovitz and 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 (Jobs and 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/>) using the mfold program version 3.1 (Mathews et al., 1999; Zuker et al., 1999). Folding was conducted at 37°C. After a first rough alignment of sequences using CLUSTAL version X (Thompson et al., 1997), corrections were made manually using secondary structure predictions of ITS1 and ITS2 RNA transcripts as a guide for alignment across genera. A total of 741 and 701 nucleotides were included in the matrices for phylogenetic analyses for ITS (including ITS1, 5.8S rDNA, and ITS2) and for *rps16* (partial intron sequence), respectively. Gaps were coded as missing data. All sequences have been deposited in GenBank (<http://www.ncbi.nlm.nih.gov/>). Aligned matrices are available from A. N. Muellner and M. W. Chase (a.muellner@kew.org; m.chase@kew.org).

TABLE 2. Characterization of ITS in Aglaieae (*Aglaia* excluded) and Guareae.

Region	Aglaieae		Guareae	
	Aglaieae length (bp)	Guareae length (bp)	GC ratio (range in %)	Mean GC ratio (%)
Entire ITS	649–660	639–651	57–64	60
ITS1	265–272	259–264	58–68	63
5.8S	164	156–164	54–55	54
ITS2	219–224	222–224	56–67	62

Maximum parsimony (MP) analysis—Individual and combined maximum parsimony (MP) analyses of the ITS and *rps16* intron data sets were performed using PAUP*4.0b10 (Swofford, 2002). Visual inspection of the individual bootstrap consensus trees was used for determining congruence of the two data sets (Whitten et al., 2000). Because there were no strongly supported (<85% bootstrap), incongruent patterns in the individual trees, direct combination was regarded as appropriate. 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 addition sequence set at 1000 random additions of taxa, tree bisection-reconnection (TBR) branch swapping, and MulTrees on (keeping multiple, shortest trees) but holding only 10 trees per replicate to reduce time spent in swapping on large numbers of trees. After these 1000 replicates, we then used the shortest trees found as starting trees for a swapping-to-completion search (with a tree limit of 15 000 for the *rps16* data set). Robustness of clades was estimated using the bootstrap (Felsenstein, 1985) with 1000 replicates with simple sequence addition, TBR branch swapping, and MulTrees on but holding only 10 trees per replicate to reduce time spent on each replicate (Salamin et al., 2003). We conducted a separate analysis of the *rps16* intron, but the level of variation was so low that the strict consensus tree is highly unresolved, and because of its lack of informativeness we do not illustrate it here. Combining this matrix with that of ITS did produce greater resolution and bootstrap support (results not shown) so collecting these data was worthwhile in spite of their highly unresolved pattern when analyzed alone.

Bayesian analysis—A number of numerical methods are available that allow the posterior probability of a tree to be approximated, the most useful of which is Markov chain Monte Carlo (MCMC). We conducted Bayesian analysis in MrBayes version 2.01 (Huelsenbeck and Ronquist, 2001) on the combined ITS/*rps16* data matrix using four Markov chains simultaneously started from random trees. Modeltest 3.06 (Posada and Crandall, 1998, 2001) was used to select the optimal substitution model (general time reversible model, GTR). One million cycles were performed, sampling a tree at every 100 generations. Trees that preceded the stabilization of the same likelihood value found in all four Markov chains (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.

RESULTS

Structure, size, and composition of ITS—Length of the entire ITS region, including ITS1, 5.8S rDNA, and ITS2, varied among the *Aglaia* DNAs from 650 to 662 base pairs (bp; Table 1). For 5.8S rDNA, most *Aglaia* species exhibited a length of 164 bp (163 bp in *A. silvestris* (M. Roemer) Merrill; 168 bp in the clade formed by *A. odorata* Lour., *A. pachyphylla* Miq., and *A. sapindina* (F. von Muell.) Harms; 172 bp in *A. coriacea*). In Aglaieae (*Aglaia* excluded), the length of the entire ITS region varied from 649 to 660 bp (Table 2). In the three genera of Guareae, the length of the entire ITS region was slightly shorter and varied from 639 to 651 bp (Table 2). For the outgroup taxa (Melieae), the length of the entire ITS region

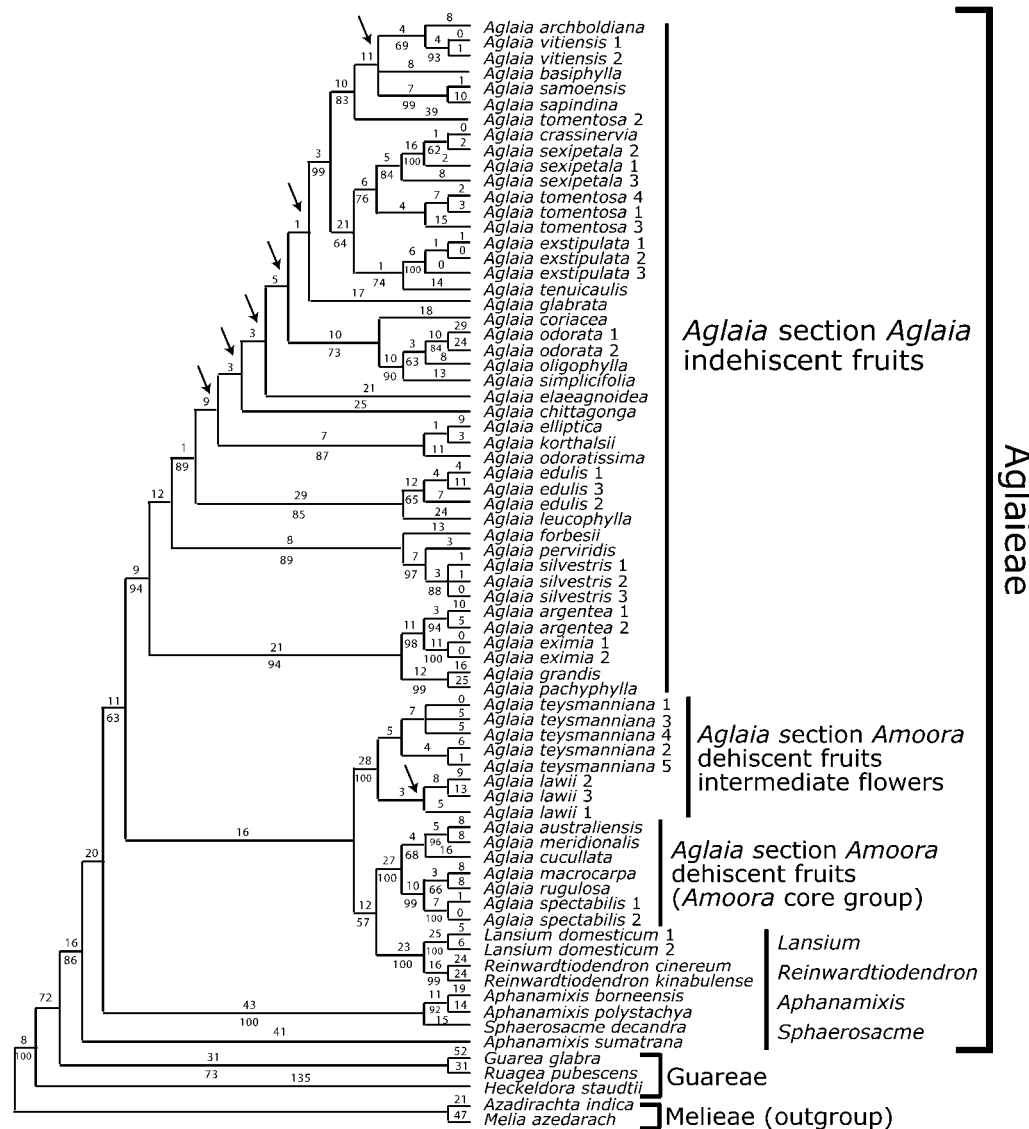


Fig. 1. One of the 234 most parsimonious trees obtained from the maximum-parsimony analysis of the combined data set (ITS nrDNA, *rps16* intron) of 67 *Aglaieae*, three *Guareae*, and two outgroup accessions. Sections of genus *Aglaia* after Pannell (1992). Tribes and genera after Pennington and Styles (1975). Numbers above branches are estimated branch lengths (ACCTRAN optimization), numbers below branches are bootstrap percentages (1000 replicates). Arrows indicate groups not present in the strict consensus tree.

was shorter than in the ingroup and varied from 639 to 645 bp (Table 1). Sequences of all ingroup and outgroup taxa contained the conserved angiosperm motif GGCRY—(4 to 7 n)—GGYCAAGGAA at the ITS1 region (Liu and Schardl, 1994), the conserved (C1–C6) and variable (V1–V6) domains determined for plant ITS2 sequences (Hershkovitz and Zimmer, 1996), and the conserved angiosperm motif 5'-GA-ATTGCAGAATCC-3' within the 5.8S rDNA gene (Jobes and Thien, 1997). Alignment of all ITS region sequence positions resulted in a matrix of 741 characters for the whole set of taxa. The length of the total matrix including 18S and 26S rDNA flanking regions was 947 characters.

Maximum parsimony analysis—The mean ratios of guanine–cytosine base pairs (GC ratios) for the sequences of ITS and *rps16* intron were 62% and 34%, respectively. Whereas for the ITS matrix 289 characters (39%) were potentially par-

simony-informative, 65 characters (9%) were potentially parsimony-informative for the *rps16* intron. Evolution of each region was assessed on the shortest combined tree shown (Fig. 1; Tables 3, 4). The transition/transversion (ts/tv) ratio for ITS was 1.35, consistency index (CI) and retention index (RI) for ts were 0.51 and 0.75, for tv 0.37 and 0.69, respectively (Table 4). The ts/tv ratio for the *rps16* intron was 1.68, CI and RI for ts were 0.63 and 0.75, for tv 0.65 and 0.73, respectively (Table 4).

Combined MP analysis—Because we did not observe any strongly supported (bootstrap percentages, BP < 85) incongruence in either of the two separate MP analyses of ITS and *rps16* intron, we proceeded with direct combination of the ITS and *rps16* intron data sets. The MP analysis of the combined matrix produced 756 shortest trees of 1463 steps, CI of 0.45 and RI of 0.72 (Table 3). Figure 1 shows one of the most

TABLE 3. Statistics for each of the MP analyses.

Data set	ITS	<i>rps16</i>	ITS/ <i>rps16</i>
Number of taxa	72	74	72
Number of Aglaieae	67	67	67
Number of characters included	741	701	1436
Number of variable sites	389	94	481
Number of informative characters	289	65	338
Length of shortest tree (no. of steps)	1336	113	1463
Number of shortest trees	234	>15 000	756
Consistency index	0.44	0.85	0.45
Retention index	0.72	0.93	0.72
GC content (%)	61.72	33.81	48.74

parsimonious trees with branch lengths (ACCTRAN) and bootstrap percentages, the arrowheads indicating groups not present in the strict consensus tree. Tribe Aglaieae are monophyletic (BP 86), and *Aglaia* is paraphyletic. Section *Aglaia* is monophyletic (BP 94). Representatives of the *Amoora* core group form a strongly supported clade (BP 100) that is sister (BP 57) to a clade formed by *Lansium* and *Reinwardtiendron* (BP 100). The two species of *Amoora* with intermediate flower characteristics between the two sections (*Aglaia lawii* (Wight) Saldanha ex Ramamoorthy, *A. teysmanniana* (Miq.) Miq.) form a strongly supported clade (BP 100) that is sister to the clade formed by the *Amoora* core group, *Lansium*, and *Reinwardtiendron*. *Aphanamixis* is potentially polyphyletic. *Aphanamixis borneensis* Harms and *A. polystachya* (Wall.) R. N. Parker are related to *Sphaerosacme decandra* (Wall.) T. D. Penn. (BP 100); the relationship to *A. sumatrana* Harms is unclear.

Analysis of ITS—Maximum parsimony analysis of the entire ITS region, comprising ITS1, 5.8S, and ITS2, produced 234 shortest trees of 1336 steps, CI of 0.44 and RI of 0.72 (Table 3). In the bootstrap consensus tree (tree not shown) tribe Aglaieae are monophyletic (BP 86), and *Aglaia* is paraphyletic as in the combined analysis. Representatives of the *Amoora* core group are strongly supported (BP 100) and sister (BP 56) to *Lansium/Reinwardtiendron* (BP 100). *Aglaia lawii* and *A. teysmanniana* form a strongly supported clade (BP 100). As in the combined analysis, *Aphanamixis borneensis* and *A. polystachya* are related to *Sphaerosacme decandra* (BP 100); the relationship to *A. sumatrana* is unresolved.

Analysis of *rps16* intron—Maximum parsimony analysis of partial *rps16* intron sequences produced more than 15 000 shortest trees of 113 steps, CI of 0.85 and RI of 0.93 (Table

3). Due to the low level of variation and poor resolution in the strict consensus tree, we do not illustrate a tree here. Relative to just the ITS results, the addition of the *rps16* intron data in the combined analyses did not alter topological patterns noticeably.

Combined Bayesian analysis—In the combined ITS/*rps16* intron Bayesian tree (Fig. 2; burn in of 900 trees) Aglaieae are monophyletic (posterior probability, PP, 100), and *Aglaia* is not supported as monophyletic. Section *Aglaia* is monophyletic (PP 100), and section *Amoora* is unresolved with respect to section *Aglaia* and *Lansium/Reinwardtiendron*. The clade formed by the intermediate species *Aglaia lawii* and *A. teysmanniana* is well supported (PP 100), as is that formed by representatives of the core group of section *Amoora* (PP 100) and *Lansium/Reinwardtiendron* (PP 100). Again, *Aphanamixis borneensis* and *A. polystachya* are related to *Sphaerosacme decandra* (PP 100), and the relationship to *A. sumatrana* remains unclear.

DISCUSSION

Relationships of *Aglaia* to other Aglaieae—Comparison of morphological and anatomical features to DNA data—*Lansium* as defined by Pennington and Styles (1975) is distinguished from *Aglaia* by its simple indumentum, pentalocular ovary (rarely to be found in *Aglaia*), and the structure of its style and style head. However, the secondary xylem of *Lansium* was found to be indistinguishable from that of some species of *Aglaia*. Pennington and Styles (1975) also found that *Aglaia* section *Lansium* of Kostermans (1966) was heterogeneous and that those species for which floral characters were known could be confidently placed in either *Lansium* or *Reinwardtiendron*. *Aphanamixis* is morphologically similar to *Aglaia*, *Lansium*, and *Reinwardtiendron* (Pennington and Styles, 1975). The embryo of *Aphanamixis* has cotyledons that are fused throughout their length, a condition unknown elsewhere in the subfamily except in *Sphaerosacme*. Except for the apparent unity of the cotyledons (a feature not investigated in all *Aglaia* species so far), there is no other macroscopic character that reliably separates *Aphanamixis* from *Aglaia*. *Sphaerosacme* has morphological similarities to both *Reinwardtiendron* and *Aphanamixis*; it possesses the floral characters of the first and the fruit and seed characters of the latter (Pennington and Styles, 1975). *Sphaerosacme* [former *L. decandrum* (Wall.) Harms], elevated to generic rank by Pennington and Styles (1975), is morphologically less closely related to *Lansium* and differs from it in floral, fruit, and seed characters. *Sphaerosacme* differs from *Aglaia* further in sepal,

TABLE 4. Statistics for transitions (ts) and transversions (tv) based on optimizations on one of the trees from the combined maximum parsimony analysis (CI = consistency index, RI = retention index).

Data set	ITS	<i>rps16</i>
Number of steps (ts/tv)	1337	126
Number of ts	767	79
Number of tv	570	47
ts/tv ratio	1.35	1.68
CI	0.44	0.64
RI	0.72	0.74
CI of ts	0.51	0.63
RI of ts	0.75	0.75
CI of tv	0.37	0.65
RI of tv	0.69	0.73

nington and Styles, 1975), and (3) most importantly the low support for the clades uniting *Lansium*, *Reinwardtiodendron*, and the *Amoora* core group in the MP analyses (Fig. 1, BP 57).

Sectional classification in *Aglaia*—The type of the formerly recognized genus *Amoora* Roxb., *Amoora cucullata* Roxb., differs from typical *Aglaia* in several morphological characters (e.g., ovary and fruit condition and number of petals and anthers), but since this species was described, other, less extreme species have been discovered that provide morphological intermediates, so that recognition of *Amoora* appeared unacceptable to Pennington and Styles (1975). Finally, Pannell (1992) recognized two sections within *Aglaia*, section *Aglaia* and section *Amoora*, and included species with intermediate characters in the latter. Our study shows that the sections as presently defined (on fruit dehiscence) may be untenable cladistically (Figs. 1, 2). Here we propose to recognize a third section in *Aglaia*: *Neoaglaia* (for a formal description, see end of discussion), comprising *Aglaia teysmanniana*, *A. lawii*, and *A. beccarii* C. de Candolle (a former synonym of *A. lawii*; Pannell, 2004). This decision is supported by several lines of evidence:

1. **DNA data**—The three sections, *Aglaia*, *Neoaglaia*, and *Amoora*, form three strongly supported units among the species of *Aglaia*. The first and largest unit comprises all species of section *Aglaia* that we included in our study (Fig. 1, BP 94; Fig. 2, PP 100). The second unit comprises the two morphologically intermediate species, *Aglaia lawii* and *A. teysmanniana* (Fig. 1, BP 100; Fig. 2, PP 100). The third unit comprises all species of the section *Amoora* core group (Fig. 1, BP 100; Fig. 2, PP 100).
2. **Secondary metabolites**—Species of section *Amoora* can be classified into different groups based on chemical patterns (Brem, 2002; Fig. 2). *Aglaia australiensis* C. M. Pannell, *A. meridionalis* C. M. Pannell, and *A. spectabilis* (Miq.) Jain & Bennet belong to the flavagline chemotype, with *A. spectabilis* clearly differing due to the accumulation of less frequent benzofurans. *Aglaia australiensis* and *A. meridionalis* are the only accessions producing small amounts of unidentified flavonoids (Brem, 2002). *Aglaia lawii* belongs to the bisamide chemotype, characterized by a tendency to accumulate bisamides. Individuals of *Aglaia teysmanniana* form another distinct group marked by exclusive accumulation of terpenoids (terpenoid chemotype; Brem, 2002). Finally, the marker bisamide aglaurubine was found in members of the core group of *Amoora* (*Aglaia australiensis*, *A. meridionalis*, and *A. spectabilis*) but not in former members of *Amoora* with intermediate flowers, *Aglaia teysmanniana* and *A. lawii* (Teichmann, 2002; Fig. 2), here assigned to *Neoaglaia*.
3. **Morphology**—Section *Neoaglaia* differs from section *Amoora* in the variable number of petals and the color of the pericarp. In *Neoaglaia* there are 3–5 petals, and the pericarp is pink; in *Amoora* there are three petals, and the pericarp is yellow, red, orange, or reddish-brown. Fruits in *Neoaglaia* are smaller than in *Amoora*.

Species types in *Aglaia*—Pannell (1992) recognized “taxonomically isolated species,” “variable,” and “complex species.” Of the seven complex species in the genus, one is recognized in section *Amoora* (*Aglaia lawii*) and six in section

Aglaia (*A. edulis* (Roxb.) Wall., *A. elaeagnoidea* (A. Juss.) Benth., *A. elliptica*, *A. korthalsii*, *A. leptantha*, and *A. tomentosa*). We included all but one (*Aglaia leptantha*) currently recognized complex species of both sections in our analyses. For three (*A. lawii*, *A. edulis*, and *A. tomentosa*) of the seven complex species, we included between three and four individuals from different locations. This provides the possibility for a first assessment of the genetic circumscription of these species. For *A. tomentosa* (section *Aglaia*), individuals from different localities appear in different clades (Figs. 1, 2). For *A. lawii* and *A. edulis* (section *Amoora*), individuals from different localities exhibit genetic heterogeneity as well. *Aglaia tomentosa* is polyphyletic (Figs. 1, 2); *A. lawii* and *A. edulis* are either monophyletic (Fig. 1) or paraphyletic (Fig. 2). These findings based on macromolecular evidence of ITS and the *rps16* intron are supported by chemotaxonomic characters. In *Aglaia lawii*, chemical heterogeneity and chemodiversity in individuals from different locations was observed by means of high performance liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC-MS), displaying qualitative variation of major compounds (Brem, 2002). Similar chemical heterogeneity was also found in *A. tomentosa* and *A. edulis*. According to the chemical profiles of *A. tomentosa*, different individuals from several locations can even be assigned to three different chemotypes (flavagline type, lignan type, terpenoid type; Brem, 2002).

In addition to the complex species discussed, we included 16 variable species in addition to 13 taxonomically isolated species in our analyses. For eight of the 16 variable species, we included between two and five specimens from different locations. Within two species (*A. sexipetala*, *A. teysmanniana*), we found genetic heterogeneity similar to that in the complex species (Figs. 1, 2). Although variation among accessions of what are considered complex or variable species by Pannell (1992) may mean that there are multiple biological species being lumped together, this is not the only conclusion that could be reached. If the accessions of a complex species do not form a clade (*A. tomentosa*), this could mean that further study may lead to the recognition of additional species, but it could also be a result of hybridization. The ITS is subject to “capture” through gene conversion, and it often is converted in the direction of the maternal parent (Chase et al., 2003), so agreement of phylogenetic patterns obtained from analyses of ITS and the *rps16* intron do not necessarily refute hybridization as the cause of such patterns (Chase et al., 2003). Another possible explanation for the patterns observed could be the independent evolution of morphologically convergent characters on the two sides of Wallace’s line. This could be the reason for morphological similarity, but genetic non-relatedness in *A. tomentosa*, in which the accessions from Thailand (west of Wallace’s line) and the accession from Australia (east of Wallace’s line) appear in different parts of the trees (Figs. 1, 2). We favor the explanation of morphological convergence over hybridization because (1) intermediates are not usually found in the same locality as the two taxa between which they are intermediate (C. M. Pannell, personal observation), and (2) all accessions of section *Aglaia* (except *A. elaeagnoidea*) collected on the eastern side of Wallace line form a moderately to strongly supported clade (compare top clades in Figs. 1, 2; BP 83, PP 100, BP 70), including the Australian accession of *A. tomentosa*. The fact that *Aglaia tomentosa* is one of the most widespread and morphologically diverse complex species in *Aglaia*, covering most of the total distribution range of the

genus, will make this taxon an appropriate model for evaluating the usefulness of currently used diagnostic characters like the indumentum (structure, size, distribution of trichomes on different parts of the plant) for delimitation of species in *Aglaia* and other Meliaceae in our future studies.

Concluding remarks—Investigations of DNA data and secondary metabolites of *Aglaia* and related genera contribute significantly to a better understanding of the intricate systematic relationships of this group of trees that constitute an important component of the moist tropical forest in the Indomalaysian region. This study is the first to assess the current circumscription of Aglaieae, *Aglaia* and its sections, and species concepts with data independent of morphology. Maximum parsimony and Bayesian analyses of nuclear ITS and plastid *rps16* intron, as well as comparison of chemical profiles observed by means of HPLC and GC-MS, confirm close relationships among the genera of tribe Aglaieae and provide helpful tools for infrageneric delimitation of sections in *Aglaia*. Concerning the relations of *Aglaia* to *Lansium* and *Reinwardtiidendron*, we propose to keep *Lansium* and *Reinwardtiidendron* as genera separate from *Aglaia*, at least for the time being. Within *Aglaia* itself, we recognize three different taxonomic units: section *Aglaia*, comprising members of the present section *Aglaia*, a new section *Neoaglaia*, comprising morphological intermediates between *Aglaia* and *Amoora*, and section *Amoora*, comprising the core group of *Amoora*, now excluding morphological intermediates between the former two sections. These taxonomic decisions are based on DNA data, secondary metabolites, as well as morphological variation. Furthermore, macro- and micromolecular data indicate that variable and complex species are more heterogeneous, i.e., probably containing more than one taxon each, than taxonomically isolated species. *Aglaia tomentosa*, one of the most widespread and morphologically diverse complex species in *Aglaia*, will serve as model taxon for evaluating the usefulness of currently used diagnostic characters in Meliaceae in our future studies.

A third section in *Aglaia*—Section *Neoaglaia* Harms in *A. Engler and K. Prantl, Die Natuerlichen Pflanzenfamilien*, III 4: 300 (1896); Harms in H. Harms and J. Matfeld, *Die Natuerlichen Pflanzenfamilien*, ed. 2, 19 bI: 146 (1940). Lectotype species (designated here): *Aglaia teysmanniana* (Miq.) Miq., *Annales Musei Botanici Lugduno-Batavi* 4: 48–49 (1868).

Petals 3–5 (–6), anthers (5 or) 6–10; fruit small 1–2.8 (–6) × 1.2–2.3 (–3.5) cm, dehiscent; pericarp pink (sometimes carmine red or yellow in *Aglaia lawii*).

Additional species—*Aglaia lawii* (Wight) Saldanha ex Ramamoorthy and *A. beccarii* C. de Candolle. *Aglaia beccarii* was recently removed from synonymy with *A. lawii* (Pannell, 2004). *Aglaia beccarii* is almost confined to Borneo, there being one record from the Philippines.

Notes—Section *Neoaglaia* differs from section *Amoora* in the variable number of petals and the color of the pericarp. In *Amoora* there are three petals, or rarely two in *Aglaia meridionalis*, and the pericarp is yellow, red, orange, or reddish-brown. The mature fruit of most species in section *Amoora* is large, 6 cm or more long and 5 cm or more wide (2.5–5.5 cm wide in the long, narrow fruits of *Aglaia flavida* Merrill &

Perry). The fruits of some Australasian species are smaller. In *Aglaia australiensis* (endemic to Australia), *A. lepidopetala* Harms (endemic to New Guinea) and *A. meridionalis* (endemic to Australia), they are 2.5–4 × 2.5–3 cm.

LITERATURE CITED

- AGOSTINHO, S. M. M., M. F. DAS G. F. DA SILVA, J. B. FERNANDES, P. C. VIEIRA, A. L. PINHEIRO, AND E. F. VILELA. 1994. Limonoids from *Toona ciliata* and speculations on their chemosystematic and ecological significance. *Biochemical Systematics and Ecology* 22: 323–328.
- BACHER, M., O. HOFER, G. BRADER, S. VAJRODAYA, AND H. GREGER. 1999. Thapsakins: possible biogenetic intermediates towards insecticidal cyclopenta[b]benzofurans from *Aglaia edulis*. *Phytochemistry* 52: 253–263.
- BALDWIN, B. G., AND S. MARKOS. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.
- BALDWIN, B. G., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.
- BLUME, C. L. 1825. Bijdragen tot de flora van Nederlandsch Indië, 4, 169–172. Batavia.
- BRADER, G., S. VAJRODAYA, H. GREGER, M. BACHER, H. KALCHHAUSER, AND O. HOFER. 1998. Bisamides, lignans, triterpenes, and insecticidal cyclopenta[b]benzofurans from *Aglaia* species. *Journal of Natural Products* 61: 1482–1490.
- BREM, B. 2002. Distribution and insecticidal properties of characteristic plant constituents from tropical *Aglaia* and *Stemona* species. Ph.D. thesis, University of Vienna, Austria.
- CHASE, M. W., AND H. G. HILLS. 1991. Silica gel: an ideal desiccant for preserving field-collected leaves for use in molecular studies. *Taxon* 40: 215–220.
- CHASE, M. W., S. KNAPP, J. CLARKSON, A. V. COX, J. JOSEPH, I. Y. BUTSKO, J. A. MARSHALL, V. SAVOLAINEN, AND A. S. PAROKONNY. 2003. Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). *Annals of Botany* 92: 107–127.
- CLARKSON, J. J., M. W. CHASE, AND M. M. HARLEY. 2002. Phylogenetic relationships in Burseraceae based on plastid *rps16* intron sequences. *Kew Bulletin* 57: 183–193.
- CUL, B., H. CHAI, T. SANTISUK, V. REUTRAKUL, N. R. FARNSWORTH, G. A. CORDELL, J. M. PEZZUTO, AND A. D. KINGHORN. 1997. Novel cytotoxic 1H-cyclopenta[b]benzofuran lignans from *Aglaia elliptica*. *Tetrahedron* 53: 17625–17632.
- DA SILVA, M. F. DAS G. F., O. R. GOTTLIEB, AND D. L. DREYER. 1984. Evolution of limonoids in the Meliaceae. *Biochemical Systematics and Ecology* 12: 299–310.
- DA SILVA, M. F. DAS G. F., S. M. M. AGOSTINHO, J. R. DE PAULA, J. O. NETO, I. CASTRO-GAMBOA, E. R. FILHO, J. B. FERNANDES, AND P. V. VIEIRA. 1999. Chemistry of *Toona ciliata* and *Cedrela odorata* graft (Meliaceae): chemosystematic and ecological significance. *Pure and Applied Chemistry* 71: 1083–1087.
- DE CANDOLLE, C. 1878. *Amoora & Aglaia*. In A. and C. de Candolle [eds.], *Monographiae Phanerogamarum* I, 579–592, 601–628. Paris, France.
- DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- DREYER, M., B. W. NUGROHO, F. I. BOHNENSTENGEL, R. EBEL, V. WRAY, L. WITTE, G. BRINGMANN, J. MÜHLBACHER, M. HEROLD, P. D. HUNG, L. C. KIET, AND P. PROKSCH. 2001. New insecticidal rocaglamide derivatives and related compounds from *Aglaia oligophylla*. *Journal of Natural Products* 64: 415–420.
- EDWARDS, K. J., AND P. A. GADEK. 2001. Evolution and biogeography of *Alectryon* (Sapindaceae). *Molecular Phylogenetics and Evolution* 20: 14–26.
- ENGELMEIER, D., F. HADACEK, T. PACHER, S. VAJRODAYA, AND H. GREGER. 2000. Cyclopenta[b]benzofurans from *Aglaia* species with pronounced antifungal activity against rice blast fungus (*Pyricularia grisea*). *Journal of Agricultural and Food Chemistry* 48: 1400–1404.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenetics: an approach using the bootstrap. *Evolution* 39: 783–791.

- FITCH, W. M. 1971. Toward defining the course of evolution: minimal change for a specific tree topology. *Systematic Zoology* 20: 406–416.
- FUZZATI, N., W. DYATMIKO, A. RAHMAN, F. ACHMAD, AND K. HOSTETT-MANN. 1996. Triterpenoids, lignans and a benzofuran derivative from the bark of *Aglaia elaeagnoides*. *Phytochemistry* 42: 1395–1398.
- GREGER, H., T. PACHER, B. BREM, M. BACHER, AND O. HOFER. 2001. Insecticidal flavaglines and other compounds from Fijian *Aglaia* species. *Phytochemistry* 57: 57–64.
- GREGER, H., T. PACHER, S. VAJRODAYA, M. BACHER, AND O. HOFER. 2000. Intraspecific variation of sulfur-containing bisamides from *Aglaia leptantha*. *Journal of Natural Products* 63: 616–620.
- HARMS, H. 1896. Meliaceae. In A. Engler and K. Prantl [eds.], Die natürlichen Pflanzenfamilien III, vol. 4, 300, 258–308. W. Engelmann, Leipzig, Germany.
- HARMS, H. 1940. Meliaceae. In H. Harms and J. Matfeld [eds.], Die natürlichen Pflanzenfamilien, 2nd ed., 19 bL., 1–172. W. Engelmann, Leipzig, Germany.
- HERSHKOVITZ, M. A., AND E. A. ZIMMER. 1996. Conservation patterns in angiosperm rDNA ITS2 sequences. *Nucleic Acids Research* 24: 2857–2867.
- HUELSENBECK, J. P., AND F. R. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- IUCN [WORLD CONSERVATION UNION]. 2003. 2003 IUCN Red List. Available at website, <http://www.redlist.org/>.
- JOBES, D. V., AND L. B. THIEN. 1997. A conserved motif in the 5.8S ribosomal RNA (rRNA) gene is a useful diagnostic marker for plant internal transcribed spacer (ITS) sequences. *Plant Molecular Biology Reporter* 15: 326–334.
- KING, G. 1895. Materials for a flora of the Malayan Peninsula. *Journal of the Asiatic Society of Bengal* 64: 51–80.
- KOSTERMANS, A. J. G. H. 1966. A monograph of *Aglaia* sect. *Lansium* Koster. (Meliaceae). *Reinwardtia* 7: 221–282.
- LIU, J.-S., AND C. L. SCHARDL. 1994. A conserved sequence in internal transcribed spacer 1 of plant nuclear rRNA genes. *Plant Molecular Biology* 26: 775–778.
- MABBERLEY, D. J. 1985. Florae Malesianae Praecursores LXVII. Meliaceae (diversa genera). *Blumea* 31: 129–152.
- MABBERLEY, D. J., C. M. PANNELL, AND A. M. SING. 1995. Meliaceae. Flora Malesiana series I, vol. 12, part 1. Rijksherbarium/Hortus Botanicus, Leiden University, Leiden, Netherlands.
- MATHEWS, D. H., J. SABINA, M. ZUKER, AND D. H. TURNER. 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *Journal of Molecular Biology* 288: 911–940.
- MIQUEL, F. A. G. 1868. *Aglaia*. In F. A. G. Miquel [ed.], Monographia Meliacearum Archipelagi indici, *Annales Musei Botanici Lugduno-Batavi* 4: 38–58. C. G. Van der Post, Amsterdam, Netherlands.
- MOHAMAD, K., M.-T. MARTIN, H. NAJDAR, C. GASPARD, T. SÉVENET, K. AWANG, H. HADI, AND M. PAÏS. 1999. Cytotoxic 3,4-secoapoptirucallanes from *Aglaia argentea* bark. *Journal of Natural Products* 62: 868–872.
- MUELLNER, A. N., R. SAMUEL, S. A. JOHNSON, M. CHEEK, T. D. PENNINGTON, AND M. W. CHASE. 2003. Molecular phylogenetics of Meliaceae based on nuclear and plastid DNA sequences. *American Journal of Botany* 90: 471–480.
- MULHOLLAND, D. A., M. KOTSOS, H. A. MAHOMED, AND D. A. H. TAYLOR. 1998. Triterpenoids from *Owenia cepiodora*. *Phytochemistry* 49: 2457–2460.
- NETO, J. O., M. F. DAS G. F. DA SILVA, E. R. FO, J. B. FERNANDES, P. C. VIEIRA, AND A. L. PINHEIRO. 1998. Norlimonoids from seeds of *Toona ciliata*. *Phytochemistry* 49: 1369–1373.
- NUGROHO, B. W., R. A. EDRADA, V. WRAY, G. BRINGMANN, M. GEHLING, AND P. PROKSCH. 1999. An insecticidal rocaglamide derivatives and related compounds from *Aglaia odorata* (Meliaceae). *Phytochemistry* 51: 367–376.
- OXELMAN, B., M. LIDÉN, AND D. BERGLUND. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* 206: 393–410.
- PALUMBI, S. R. 1996. Nucleic acids II: the polymerase chain reaction. In D. M. Hillis, C. Moritz, and B. K. Mable [eds.], *Molecular systematics*, 205–247. Sinauer Associates, Sunderland, Massachusetts, USA.
- PANNELL, C. M. 1992. A taxonomic monograph of the genus *Aglaia* Lour. (Meliaceae). Kew Bulletin Additional Series XVI. HMSO, London, UK.
- PANNELL, C. M. 1993. A monograph of *Aglaia* (Meliaceae)—a correction. *Kew Bulletin* 48: 244.
- PANNELL, C. M. 1998a. Taxonomy, ecology and reproductive biology of *Aglaia* (Meliaceae). In H. C. F. Hopkins, C. R. Huxley, C. M. Pannell, G. T. Prance, and F. White [eds.], *The biological monograph: the importance of field studies and functional syndromes for taxonomy and evolution of tropical plants*, a Festschrift for Frank White, 59–77. Royal Botanic Gardens, Kew, London, UK.
- PANNELL, C. M. 1998b. Species delimitation in *Aglaia*. In H. C. F. Hopkins, C. R. Huxley, C. M. Pannell, G. T. Prance, and F. White [eds.], *The biological monograph, the importance of field studies and functional syndromes for taxonomy and evolution of tropical plants*, a Festschrift for Frank White, 124–127. Royal Botanic Gardens, Kew, London, UK.
- PANNELL, C. M. 2004. Three new species, two new subspecies and five new combinations at the subspecific level in *Aglaia* Lour. (Meliaceae). *Kew Bulletin* 59: 87–94.
- PELLEGRIN, F. 1911. Sur les genres *Aglaia*, *Amoora* et *Lansium*. *Notulae Systematicae (Phanerogamie)* 1: 284–290.
- PENNINGTON, T. D., AND B. T. STYLES. 1975. A generic monograph of the Meliaceae. *Blumea* 22: 419–540.
- POPP, M., AND B. OXELMAN. 2001. Inferring the history of the polyploid *Silene aegaeae* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 20: 474–481.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.
- POSADA, D., AND K. A. CRANDALL. 2001. Selecting the best-fit model of nucleotide substitution. *Systematic Biology* 50: 580–601.
- PURIPATTANAVONG, J., S. WEBER, V. BRECHT, AND A. W. FRAHM. 2000. Phytochemical investigation of *Aglaia andamanica*. *Planta Medica* 66: 740–745.
- SAIFAH, E., J. PURIPATTANAVONG, K. LIKHITWITAYAWUID, G. A. CORDELL, H. CHAI, AND J. M. PEZZUTO. 1993. Bisamides from *Aglaia* species: structure analysis and potential to reverse drug resistance with cultured cells. *Journal of Natural Products* 56: 473–477.
- SAIFAH, E., R. SUTTISRI, S. SHAMSUB, T. PENGSUPARP, AND V. LIPUN. 1999. Bisamides from *Aglaia edulis*. *Phytochemistry* 52: 1085–1088.
- SALAMIN, N., M. W. CHASE, T. R. HODKINSON, AND V. SAVOLAINEN. 2003. Assessing internal support with large phylogenetic DNA matrices. *Molecular Phylogenetics and Evolution* 27: 528–539.
- SUN, Y., D. Z. SKINNER, G. H. LIANG, AND S. H. HULBERT. 1994. Phylogenetic analysis of *Sorghum* and related taxa using transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32.
- SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts, USA.
- TAYLOR, D. A. H. 1981. Chemotaxonomy. The occurrence of limonoids in the Meliaceae. In T. D. Pennington, B. T. Styles, and D. A. H. Taylor [eds.], *Meliaceae, Flora Neotropica*, Monograph 28, 450–459. The New York Botanical Garden, Bronx, New York, USA.
- TEICHMANN, K. 2002. Comparative phytochemical analyses of *Aglaia* species from section *Amoora* (Meliaceae). M.Sc. thesis, University of Vienna, Vienna, Austria.
- THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAC, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876–4882.
- VAASEN, A., D. BEGEROW, U. LÜTTGE, AND R. HAMPP. 2002. The genus *Clusia* L.: molecular evidence for independent evolution of photosynthetic flexibility. *Plant Biology* 4: 86–93.
- WANG, B.-G., R. EBEL, B. W. NUGROHO, D. PRIJONO, W. FRANK, K. G. STEUBE, X.-J. HAO, AND P. PROKSCH. 2001. Aglacins A–D, first representatives of a new class of aryltetralin cyclic ether lignans from *Aglaia cordata*. *Journal of Natural Products* 64: 1521–1526.
- WANNTORP, L., H. E. WANNTORP, B. OXELMAN, AND M. KÄLLERSJÖ. 2001. Phylogeny of *Gunnera*. *Plant Systematics and Evolution* 226: 85–107.
- WEBER, S., J. PURIPATTANAVONG, V. BRECHT, AND A. W. FRAHM. 2000. Phytochemical investigation of *Aglaia rubiginosa*. *Journal of Natural Products* 63: 636–642.

- WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], *PCR protocols: a guide to methods and applications*, 315–322. Academic Press, San Diego, California, USA.
- WHITTEN, W. M., N. H. WILLIAMS, AND M. W. CHASE. 2000. Subtribal and generic relationships of Maxillarieae (Orchidaceae) with emphasis on Stanhopeinae: combined molecular evidence. *American Journal of Botany* 87: 1842–1856.
- ZUKER, M., D. H. MATHEWS, AND D. H. TURNER. 1999. Algorithms and thermodynamics for RNA secondary structure prediction: a practical guide. *In* J. Barciszewski and B. F. C. Clark [eds.], *RNA biochemistry and biotechnology*, 11–43. NATO ASI Series. Kluwer Academic Publishers, Dordrecht, Netherlands.