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RESEARCH ARTICLE

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Genetic diversity and geographic structure in *Aglaia elaeagnoidea* (*Meliaceae*, *Sapindales*), a morphologically complex tree species, near the two extremes of its distribution

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Key words

Aglaia
biogeography
dispersal
internal transcribed spacer (ITS)
Meliaceae
molecular clock
Sapindales

Abstract *Aglaia elaeagnoidea* is the most widespread and one of the more morphologically diverse complex species in the largest genus of the mahogany family (*Meliaceae*, *Sapindales*). We performed maximum parsimony, maximum likelihood and Bayesian analyses (nuclear ITS rDNA) to estimate genetic relations among samples of *Aglaia elaeagnoidea*, and their phylogenetic position within *Aglaia* (more than 120 species in Indomalesia, Australasia, and the Pacific islands). Based on 90 accessions of *Melioideae* (ingroup) and four taxa of *Cedreloideae* (outgroup), this study 1) provides a first assessment of the genetic diversity of *Aglaia elaeagnoidea*; 2) investigates the geographic structure of the data in selected eastern and western regions of its distribution; and 3) suggests that Australia has been colonized only recently by *A. elaeagnoidea* and other species within the genus (Miocene/Pliocene boundary to Pliocene). Based on DNA data, morphology and additional evidence derived from biogenetic trends (secondary metabolites), the name *Aglaia roxburghiana* could be reinstated for specimens from the western end (India, Sri Lanka), but we have no data yet to indicate definitely where *A. roxburghiana* ends and *A. elaeagnoidea* begins either morphologically or geographically. Viewed in a more general context, *Aglaieae* are an ideal model group for obtaining more insights into the origin and evolution of Indomalesian and Australian biotas.

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INTRODUCTION

The genus *Aglaia* Lour. presents an example for a group of plants in which species delimitation has been a major challenge to botanists (Pannell 1992). *Aglaia* is the largest genus of the predominantly subtropical and tropical angiosperm family *Meliaceae* (mahogany family, order *Sapindales*). It comprises at least 120 arborescent species and presents more taxonomic problems in species delimitation than any other genus of the family (Pannell 1992, 1995, 1998a, b, 2004, Muellner 2008, Muellner et al. 2005, 2008a). *Aglaia* forms an important component of the moist tropical forest in the Indomalesian region. The total distribution range 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 Mariana (Saipan, Roti, and Guam) and Caroline Islands (Palau and Ponape) in Micronesia (Pannell 1992).

In the most recent taxonomic treatment of the whole genus, Pannell (1992, 1998a, b) adopted a wide species concept. For many species even the most indicative morphological characters, such as indumentum, fruit, and floral morphology, show considerable variation. Pannell (1992) recognized different types of species. 'Isolated species' are morphologically distinct and with either a small (e.g., *A. coriacea* Korth. ex Miq.) or extensive (e.g., *A. cucullata* (Roxb.) Pellegr.) geographical distribution. In contrast, members of morphologically closely resembling pairs or larger groups of species are often separable only by using the combined variation of several overlapping characters (Pannell 1992). Members of these groups may be allopatric (e.g., *A. elliptica* Blume and *A. cinnamomea* Baker f.; 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).

Muellner et al. (2005) provided the first evaluation of these taxonomic concepts with data independent of morphology. Their study based on maximum parsimony and Bayesian analyses of nuclear ITS and plastid *rps16* intron, as well as comparison of chemical profiles observed by means of high performance liquid chromatography (HPLC) and gas chromatography coupled to mass spectrometry (GC-MS), indicated that variable and complex species were more heterogeneous, i.e., probably containing more than one taxon each, than taxonomically isolated species (Muellner et al. 2005).

We performed maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses of sequence data from 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, to estimate genetic relationships within one of the morphologically complex and geographically most widely distributed species of the genus, *Aglaia elaeagnoidea* (A.Juss.) Benth. The aims of our study were to 1) provide a first assessment of the genetic diversity of *A. elaeagnoidea* by sampling near the two extremes of its distribution range; 2) investigate the geographic structure of the data over the sampled geographic range; and 3) infer the geographic and temporal origin of selected clades within *Aglaia*. The DNA data are compared to chemical profiles of the respective specimens to investigate if phylogenetic relationships are reflected by biogenetic trends.

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Table 1 Voucher information and GenBank accession numbers for samples used in this study (in alphabetical order). Voucher specimens are deposited in the following herbaria: FHO = Daubeny Herbarium, University of Oxford; FR = Herbarium Senckenbergianum, Senckenberg Research Institute; K = Royal Botanic Gardens Kew; KEP FRI = Forestry Institute Malaysia; KYGH = Kyoto Takeda Herbal Garden; NCU = University of North Carolina; WU = University of Vienna.

Taxon	Voucher (Herbarium)	Origin	GenBank accessions ITS
Aglaia archboldiana A.C. Smith	*Greger 696* (WU)	Fiji	AY695524
A. argentea Blume	*Greger 532* (WU)	Thailand	AY695525
A. australiensis Pannell	*Greger 662* (WU)	Australia	AY695571
A. basiphylla A. Gray	*Greger 692* (WU)	Fiji	AY695527
A. chittagonga Miq.	*Greger 756* (WU)	Bangladesh	AY695528
A. coriacea Korth. ex Miq.	*Greger 822* (WU)	Thailand	AY695529
A. crassinervia Kurz ex Hirn	*Greger 523* (WU)	Thailand	AY695530
A. cucullata (Roxb.) Pellegrin	*Brunei Museum s.n.* (K)	Brunei	AY695572
A. edulis (Roxb.) Wall.	*Greger 626* (WU)	Thailand	AY695533
A. elaeagnoidea Benth. (1)	*Pacher HG 922* (WU)	Sri Lanka	EU310243
(2)	*Greger 753* (WU)	Bangladesh, Chittagong	EU340990
(3)	*Greger 658* (WU)	Southeastern Thailand, Chonburi	EU340988
(4)	*Greger 494* (WU)	Thailand, Rayong	EU340986
(5)	*Greger 504* (WU)	Southeastern Thailand, Chanthaburi	EU340987
(6)	*Greger 882* (WU)	South Vietnam, Bien Hoa	EU340991
(7)	*Greger 800* (WU)	Southern Thailand, Hat Yai	EU340989
(8)	*Greger 645* (WU)	Australia, Qld., Port Douglas	EU340981
(9)	*Greger 646* (WU)	Australia, Qld., Port Douglas	EU340982
(10)	*Greger 647* (WU)	Australia, Qld., Port Douglas	EU340983
(11)	*Greger 649* (WU)	Australia, Qld., Port Douglas	EU340985
(12)	*Greger 648* (WU)	Australia, Qld., Port Douglas	EU340984
(13)	*Greger 650* (WU)	Australia, Qld., Cairns	AY695536
A. elliptica Blume	*Greger MEL36* (WU)	Indonesia	AY695540
A. eximia Miq.	*Greger 540* (WU)	Thailand	AY695541
A. exstipulata (Griffith) Theobald	*Greger 717* (WU)	Thailand	AY695543
A. forbesii King	*Greger 538* (WU)	Thailand	AY695546
A. glabrata Teijsm. & Binn.	*Wilkie 93349* (K)	Indonesia	AY695547
A. grandis Korth. in Miq.	*Greger 571* (WU)	Thailand	AY695548
A. korthalsii Miq.	*Greger 807* (WU)	Thailand	AY695549
A. lawii (Wight) C.J. Saldanha	*Greger 573* (WU)	Thailand	AY695573
A. leucophylla King	*Greger 561* (WU)	Thailand	AY695550
A. macrocarpa (Miq.) C.M. Pannell	*Church* et al. *775* (K)	Indonesia	AY695576
A. meridionalis C.M. Pannell	*Greger MEL22* (WU)	Australia	AY695577
A. odorata Lour.	*Greger 903* (WU)	Thailand	AY695552
A. oligophylla Miq.	*Greger 706* (WU)	Thailand	AY695554
A. pachyphylla Miq.	*Greger 812* (WU)	Thailand	AY695555
A. perviridis Hiern	*Greger 13* (WU)	Thailand	AY695556
A. rugulosa C.M. Pannell	*Mat Asri KEP FRI 25591* (K)	Malaysia	AY695578
A. samoensis A. Gray	*Greger 752* (WU)	Samoa	AY695557
A. sapindina (F. von Muell.) Harms	*Greger 669* (WU)	Australia	AY695558
A. sexipetala Griffith	*Greger 524* (WU)	Thailand	AY695531
A. silvestris (M. Roemer) Merrill	*Greger 679* (WU)	Thailand	AY695561
A. simplicifolia (Bedd.) Harms	*Greger 484* (WU)	Thailand	AY695560
A. spectabilis (Miq.) Jain & Bennet	*Greger 660* (WU)	Thailand	AY695579
A. tenuicaulis Hiern	*Greger 901* (WU)	Thailand	AY695564
A. teysmanniana (Miq.) Miq.	*Greger 704* (WU)	Thailand	AY695539
A. tomentosa Teijsm. & Binn.	*Greger 818* (WU)	Thailand	AY695566
A. vitiensis A.C. Smith	*Greger 691* (WU)	Fiji	AY695569
Anthocarapa nitidula (Benth.) T.D. Penn.	*Chanel 1110* (K)	Melanesia	DQ861615
Aphanamixis borneensis Harms	*Beaman 8208* (K)	Malaysia (Borneo)	AY695583
A. polystachya (Wall.) R.N. Parker	*Samuel 14* (WU)	Sri Lanka	AY695584
Astrotrichilia sp.	*Richard 25* (K)	Madagascar	DQ2388060
Azadirachta indica A.Juss.	*Samuel 5* (WU)	Sri Lanka	AY695594
Cabralea canjerana (Vell.) Mart.	*Pennington 17067* (K)	Peru	DQ861617
Calodecaryia crassifolia Leroy	*Croat 31521* (K)	Madagascar	DQ861631
Cedrela odorata L.	*Chase 2112* (K)	Indonesia (Bogor III.B.2)	DQ861606
Chisocheton macrophyllus King	*Chase 1309* (K)	Indonesia (Bogor III.F.30a)	DQ861613
Cipadessa baccifera Miq.	*Chase 1310* (K)	Indonesia (Bogor III.B.90)	DQ861627
Dysoxylum gaudichaudianum (A.Juss.) Miq.	*Chase 1312* (K)	Indonesia (Bogor III.F.90)	DQ861619
Ekebergia capensis Sparrm.	*MG 246* (Cynthia Morton)	South Africa	DQ861623
Guarea glabra Vahl	*Chase 336* (NCU)	USA	AY695591
Heckeldora staudtii (Harms) Staner	*Chase 3311* (K)	Cameroon	AY695592
Humbertioturraea sp. (*H. labatii* Lescot ined.)	*Bardot-Vaucoulon 160* (K)	Madagascar	DQ861632
Khaya anthotheca C.DC.	*Chase 2859* (K)	K Living Collection 1967-35601 (source plant: Amherst College, Massachusetts)	DQ861608
Lansium domesticum Correa	*Chase 2113* (K)	Indonesia (Bogor III.B.100)	AY695586
Lansium cf. *membranaceum* (Kosterm.) Mabb.	*Pannell 1934* (FHO)	Sumatra	DQ861611
Lepidotrichilia volkensii (Gürke) J.-F.Leroy ex B.T.Styles & F.White	*Hughes 189* (K)	Tanzania	DQ861620
Malleastrum mandenense Leroy	*Cheek* et al. *3-17-5* (K)	Madagascar	DQ861626
Melia azedarach L.	*Chase 2867* (K)	K Living Collection 1953-37801 [Donation from KYGH]	AY695595
Munronia pinnata (Wall.) Theob.	*Samuel 6* (WU)	Sri Lanka	DQ861604
Naregamia alata Wight & Arn.	*Kanodia 89603* (K)	India	DQ861629
Nymania capensis Lindb.	*Chase 270* (NCU)	South Africa	DQ861633

Table 1 (cont.)

Taxon	Voucher (Herbarium)	Origin	GenBank accessions ITS
Owenia vernicosa F. Muell.	*Evans M3071*	Australia	DQ861622
Pseudobersama mosambicensis (Sim) Verdc.	*Bidgood, Abdallah & Vollesen 1426* (K)	Tanzania	DQ238064
'*Pseudocarapa nitidula*' (Benth.) T.D.Penn.	*Chase 3313* (K)	Australia	DQ861616
Pseudoclausena chrysogyne (Miq.) T.P.Clark	*Muellner 2052* (FR)	Malaysia (FRIM Arboretum)	DQ861602
Pterorhachis zenkeri Harms	*Breteler 2741* (K)	Cameroon	DQ861628
Quivisianthe papinae Baill.	*Phillipson 1650* (K)	Madagascar	DQ861605
Reinwardtiodendron cinereum (Hiern) Mabb.	*KEP FRI 26877* (K)	Malaysia (Perak)	AY695588
R. humile (Hassk.) Mabb.	*Trichon VT 641* (FHO)	Sumatra	DQ861612
R. kinabaluense (Kosterm.) Mabb.	*ALFB 112/87* (K)	Malaysia (Borneo)	AY695589
R. kostermansii (Prijanto) Mabb.	*Kostermans 19215* (K)	Indonesia (W Sumbawa)	DQ861634
Ruagea pubescens Karst.	*Pennington & Frere 13761* (K)	Ecuador	AY695593
Sandoricum borneense Miq.	*Chase 1313* (K)	Indonesia (Bogor III.B.92)	DQ861601
Sphaerosacme decandra (Wal.) T.D.Penn.	*Williams & Stainton 8533* (K)	Ecuador	AY695590
Swietenia macrophylla King	*Chase 250* (NCU)	USA	DQ861609
Synoum glandulosum (Sm.) A.Juss.	*Schodde 5101* (K)	Australia	DQ861618
Toona sp.	*Terrazas* s.n. (K)	Australia	DQ861607
Trichilia emetica Vahl	*Sieglstetter 15* (FR)	West Africa	EF136577
T. prieureana A.Juss.	*Neumann 1518* (FR)	West Africa	EF136576
Turraea sericea Sm.	*Civeyrel 1336* (K)	Madagascar	DQ861630
T. heterophylla Sm.	*Küppers 2212* (FR)	West Africa	EF136578
Turraeanthus sp.	*Carvalho 4348-1* (K)	Equat. Guinea	DQ861614
Vavaea amicorum Benth.	*Katik* et al. *74722* (K)	Papua New Guinea	DQ861610
Walsura tubulata Hiern	*Chase 1314* (K)	Indonesia (Bogor VIII.B.127)	DQ861625.

The ITS region was the DNA marker of choice since previous analyses of plastid *rbc*L, *mat*K, and nuclear 26S rDNA did not provide sufficient phylogenetic information for *Melioideae* (compare Muellner et al. 2003, 2006), and screening with other plastid markers (e.g. *rps*16 intron, IGS *atp*B-*rbc*L, *trn*L intron and IGS *trn*L-*trn*F) did not exhibit a sufficient amount of informative sites. In addition, single copy nuclear genes, in contrast to ITS, are at present technically demanding to sequence and generally not retrievable from herbarium samples, because their amplification is highly subject to DNA quality (Cowan et al. 2006). This is especially true for tropical plants with high amounts of bioactive and/or PCR-inhibitory compounds, like the *Meliaceae* (A.N. Muellner pers. obs.).

MATERIAL AND METHODS

We analyzed ITS data of 90 accessions of *Melioideae*, including representatives and type species of all three currently recognized sections within *Aglaia* (*Amoora*, *Neoaglaia,* and *Aglaia*), 13 accessions of *A. elaeagnoidea* from different locations of its distribution range (Fig. 1), and four accessions of *Cedreloideae* as outgroup taxa (Table 1). Broadscale intrafamilial phylogenetic relationships of *Meliaceae* were previously assessed using DNA sequence data from three regions: plastid genes *rbc*L, *mat*K, and nuclear 26S rDNA (Muellner et al. 2003, 2006). Phylogenetic relationships within *Melioideae* have recently been assessed using nuclear ribosomal DNA sequence data (Muellner et al. 2008b).

Plant material

Material of *Aglaia elaeagnoidea* was collected during excursions to Sri Lanka, Bangladesh, Thailand, Vietnam and Australia. Herbarium vouchers of this species are deposited at WU; herbarium specimens of the other *Meliaceae* are deposited at FHO, FR, FRIM, K, NCU, and WU (Table 1). ITS sequences of 35 taxa of *Aglaia* (other than *A. elaeagnoidea*) and 46 other taxa were available from the first authors' previous work on *Meliaceae* (Muellner et al. 2005, 2008b). Besides 48 accessions of *Aglaia*, our ITS matrices also include representatives of all other genera of tribe *Aglaieae* (*Lansium* Corrêa, *Reinwardtiodendron* Koord., *Aphanamixis* Blume, *Sphaerosacme* Wall. ex Royle), plus representatives of all other tribes of *Melioideae*.

Isolation of DNA, amplification, and sequencing

Total DNA from leaf fragments was extracted using a NucleoSpin Plant kit (Macherey-Nagel, Dueren, Germany). PCR amplification was carried out using the primers in Muellner et al. (2005). A 50-µL reaction mix contained 24.6 µl sterile ddH_20, 5 µl 10× reaction buffer Y (peqlab, Erlangen, Germany), 2.4 µl $MgCl_2$ (25 mM), 1 µl peqGold Taq Polymerase (5 U/µl), 0.5 µl of the primers each (10 pmol; biomers.net, Ulm, Germany), 8 µl dNTP mix (2.5 mM; Carl Roth, Karlsruhe, Germany), 5 µl template DNA (c. 25–50 ng/µl), 1 µl BSA (1×; New England Biolabs, Frankfurt, Germany), as well as 2 µL DMSO (dimethyl sulfoxide; Stratagene Europe, Amsterdam, Netherlands) to stabilize the enzyme, reduce secondary structure problems, favour precise annealing, and prevent preferential amplification of pseudogenes. Amplifications were carried out using the programme of Muellner et al. (2005). PCR products were cleaned using a NucleoSpin Extract II kit (Macherey-Nagel, Dueren, Germany). The same primers as cited above were used for sequencing. Sequencing reactions were run on a CEQTM 8800 Genetic Analysis System (Beckman Coulter, Krefeld, Germany), following the manufacturer's protocols.

Sequence editing and alignment

Editing and assembly of the complementary strands were carried out with SeqManTM II v5.07 (Lasergene, DNASTAR, Inc., Madison, WI, USA). ITS sequences were explored for the presence of several structural motifs: the conserved angiosperm motif GGCRY – (4 to 7 n) – GYGYCAAGGAA (Liu & Schardl 1994); the conserved (C1-C6) and variable (V1-V6) domains determined for plant ITS2 sequences (Hershkovitz & Zimmer 1996); and the conserved angiosperm motif 5´-GAATTGCA-GAATCC-3´ within the 5.8S rDNA gene (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 v3.1 (Mathews et al. 1999, Zuker et al. 1999). Foldings were conducted at 37 °C. After a first rough alignment with CLUSTAL version X (Thompson et 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 and full compensatory base

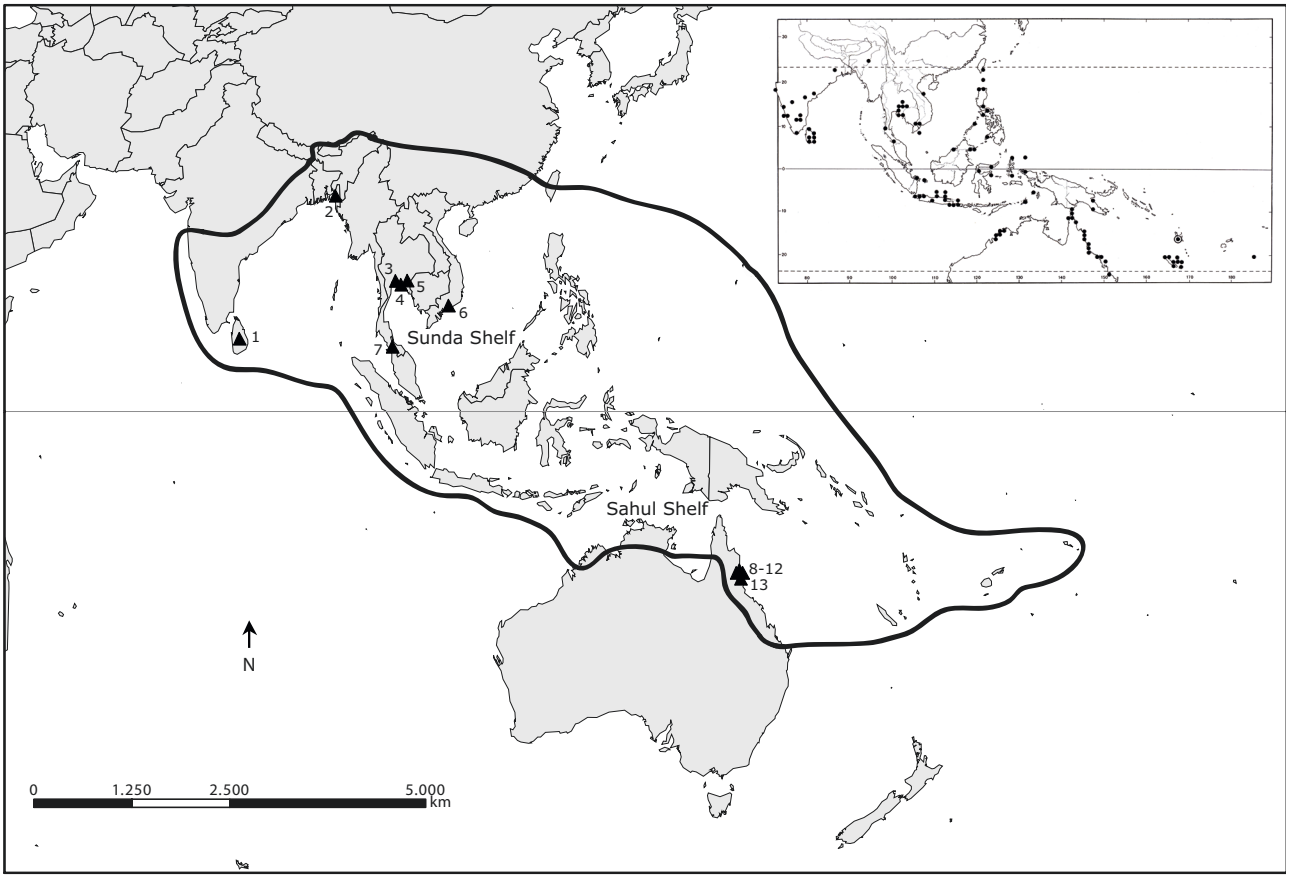

Fig. 1 Locations and number designation of specimens sampled, and total distribution of *Aglaia elaeagnoidea* (small map on the right: after Pannell 1992). Outer line delimits the geographical distribution of the tribe *Aglaieae*.

changes that preserved the predicted folding pattern. A total of 803 aligned positions were included in the matrices for phylogenetic analyses of ITS (including ITS1, 5.8S rDNA, and ITS2). Gaps were coded as missing data. Aligned matrices are available from the first author (alexandra.muellner@senckenberg.de); new sequences have been deposited in GenBank under the accession numbers EU340981–EU340991 (http://www.ncbi.nlm.nih.gov/).

Phylogenetic analyses

MP analyses of the ITS dataset were performed using PAUP* v4.0b10 (Swofford 2002). 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 the option MulTrees (keeping multiple, shortest trees), but holding only 10 trees per replicate to reduce time spent in swapping on large numbers of trees. After 1 000 replicates, we then used the shortest trees found as starting trees for a swapping-to-completion search (but with a tree limit of 15 000). Robustness of clades was estimated using bootstrapping (Felsenstein 1985) with 1 000 replicates, using simple sequence addition, TBR branch swapping, and MulTrees, again holding 10 trees per replicate. We consider 75–84 % bootstrap values moderate support and 85–100 % strong support.

ML analyses were performed with RAxML v2.2.1 (Stamatakis 2006, http://icwww.epfl.ch/~stamatak/index-Dateien/Page443.htm), and Bayesian analyses with MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003, http://mrbayes.csit.fsu.edu/). The substitution models employed in these analyses were found using Modeltest v3.06 (Posada & Crandall 1998, http://darwin.uvigo.es/software/modeltest.html), which indicated the general time reversible model as best fitting our data with a proportion of invariable sites and a gamma shape parameter alpha to model rate heterogeneity (GTR + I + G). For the Bayesian analyses, model parameters were estimated directly during two parallel runs, using four simultaneous chains and two million cycles each, sampling one tree every 100 generations. Trees that preceded stabilisation of the likelihood value were excluded, and the remaining trees were used to calculate posterior probabilities via the construction of a majority rule consensus tree in PAUP. For the ML searches with RAxML we employed the GTR + G model, using 25 rate categories (instead of four as used in the Bayesian analyses).

Divergence time estimation

A likelihood-ratio test (LRT) rejected the null hypothesis of rate constancy for ITS. We therefore employed non-parametric rate smoothing (NPRS; Sanderson 1997) as implemented in TreeEdit v1.0-a4.61 (Rambaut & Charleston 2000) and a relaxed Bayesian clock approach as implemented in the 'multidivtime' program of Thorne & Kishino (2002, http://statgen.ncsu.edu/thorne/).

The input topology for the time estimation was the ITS ML tree. Parameter values in 'multidivtime' were estimated with PAML's baseml v3.14 (Yang 1997). The program 'estbranches' (Thorne et al. 1998) was then used to calculate branch lengths and their variance, given the sequence data (94 ITS sequences of a length of 803 nt), the model parameter values from PAML, and the specified rooted topology. Branch lengths from 'estbranches' became the priors for mcmc searches in 'multidivtime' (Thorne & Kishino 2002) that sought to find the most likely model of rate change (with rate change assumed to be log-normally distributed), given the topology, time constraints on nodes (below), and a Brownian motion parameter (ν that

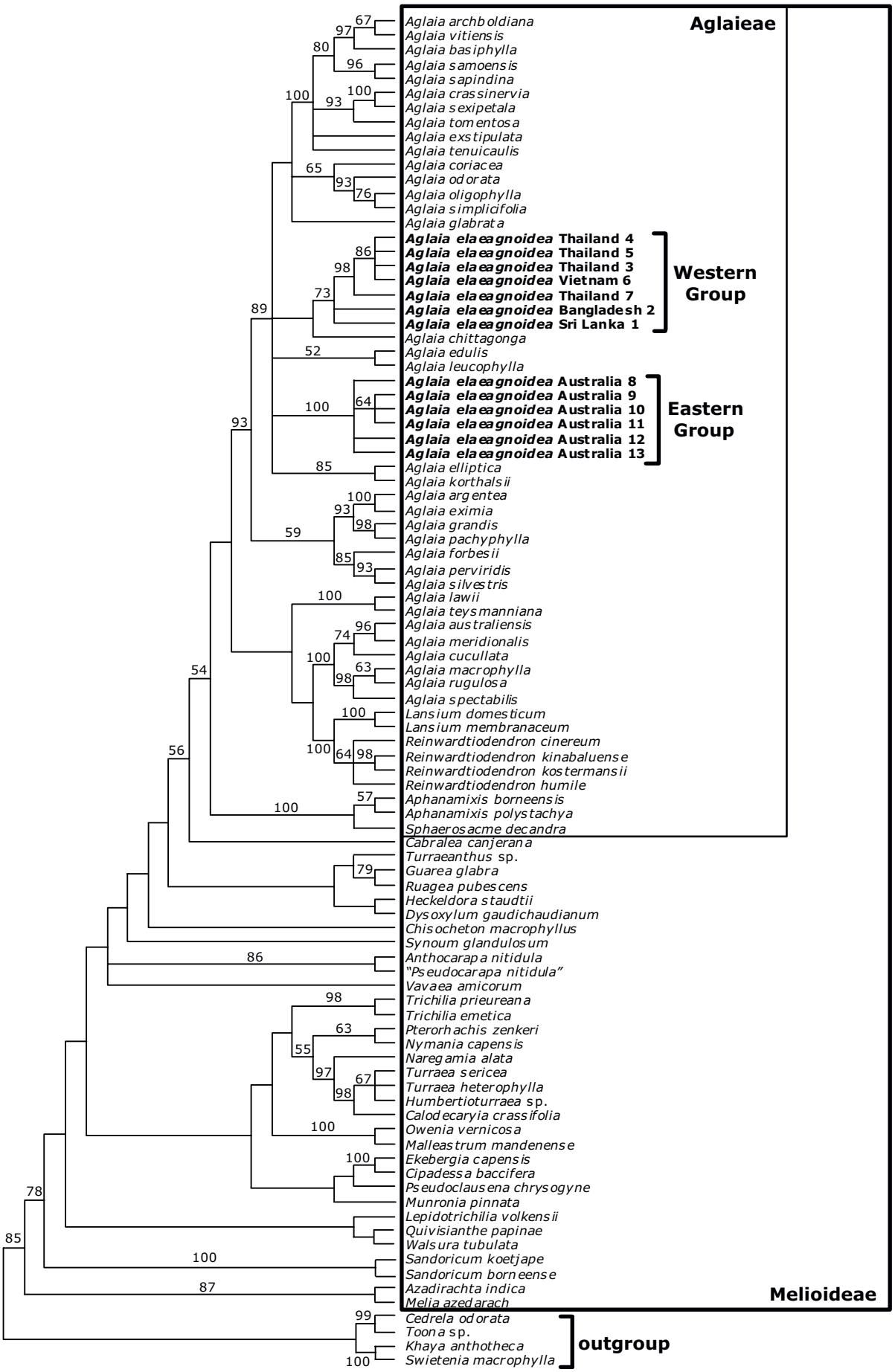

Fig. 2 Strict consensus tree of 288 trees obtained from the maximum parsimony analysis of the ITS dataset of 94 *Meliaceae* accessions. Numbers are bootstrap percentages (1 000 replicates).

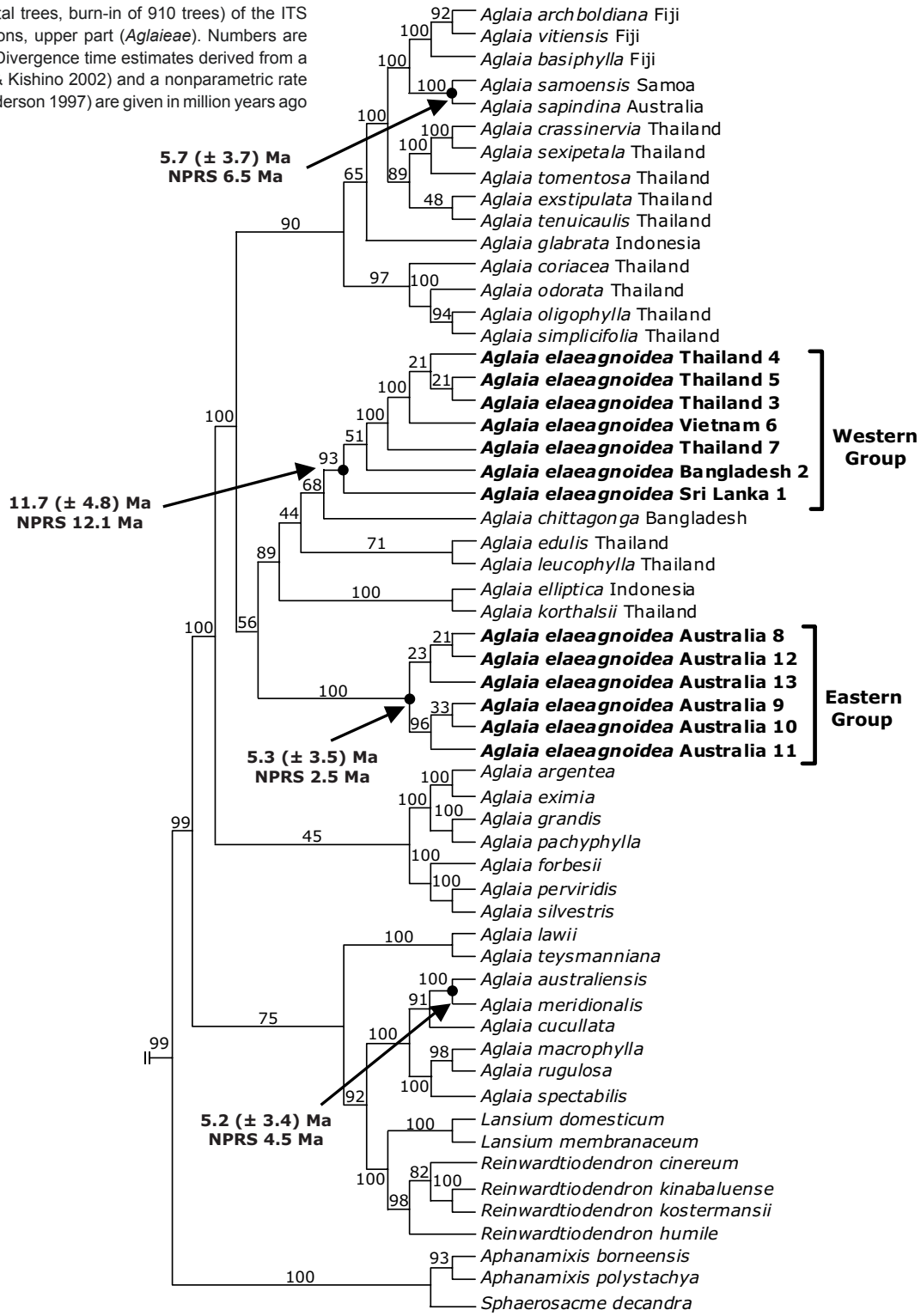

Fig. 3 Bayesian tree (20 000 total trees, burn-in of 910 trees) of the ITS dataset of 94 *Meliaceae* accessions, upper part (*Aglaieae*). Numbers are Bayesian posterior probabilities. Divergence time estimates derived from a Bayesian relaxed clock (Thorne & Kishino 2002) and a nonparametric rate smoothing approach (NPRS: Sanderson 1997) are given in million years ago (Ma) and are marked by arrows.

controls the magnitude of autocorrelation per million years (Myr) along the descending branches of the tree. Prior gamma distributions on parameters of the relaxed clock model were as follows (following the 'multidivtime' manual for setting the mean and SD; http://statgen.ncsu.edu/thorne/multidivtime.html): the mean and SD of the prior distribution for the root age were set to 75 Myr based on fossils (below) and previous studies of Muellner et al. (2006, 2007, 2008a). The mean and SD of the prior distribution for the ingroup root rate were set to 0.0024 substitutions/site/Myr by dividing the median of the distances between the ingroup root and the tips by 75 Myr. The prior and SD for ν were set to 0.013, following the recommendation of Thorne's manual that the time between root and tips multiplied by ν be about 1. Markov chains in 'multidivtime' were run for 1 million generations, sampling every 100th generation for a total of 10 000 trees, with a burn-in of 10 000 generations before the first sampling of the Markov chain.

Constraints and calibrations

Absolute time estimates in the Bayesian approach were obtained by simultaneously constraining four nodes (numbered 1–4 below); for the NPRS clock we constrained one node (numbered 5 below).

1. The root node of our dataset (i.e., the most recent common ancestor of *Melioideae* and *Cedreloideae*) was constrained to maximally 137 million years ago (Ma), based on the onset of angiosperm radiation (Hughes 1994, Brenner 1996).

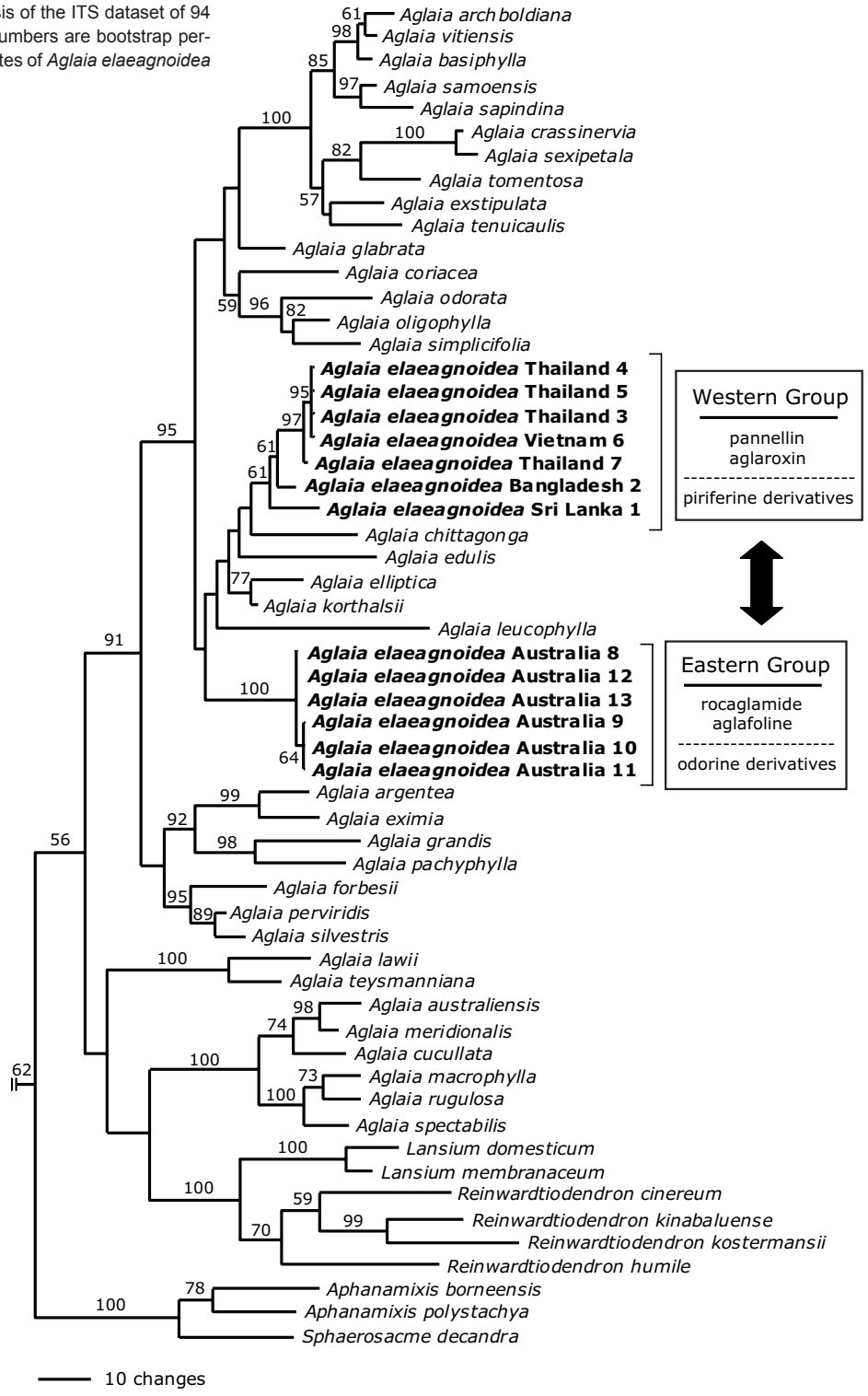

Fig. 4 Maximum likelihood tree from the analysis of the ITS dataset of 94 *Meliaceae* accessions, upper part (*Aglaieae*). Numbers are bootstrap percentages (1 000 replicates). Secondary metabolites of *Aglaia elaeagnoidea* after Brem (2002) and Hofer (2002).

2. The clade comprising *Guarea* and *Ruagea* was constrained to minimally 23.03 Ma (the upper bound of the Late Oligocene), based on fossil pollen of *Guarea* from the Oligocene San Sebastian Formation in northern Puerto Rico (Graham & Jarzen 1969).
3. The crown group of *Melieae* was constrained to minimally 20.43 Ma (the upper bound of the Aquitanian in the Early Miocene), based on fossil pollen of *Melia* (pollen similar to *Melia azedarach*) from the Early Miocene of Cameroon (Salard-Cheboldaeff 1978).
4. The clade comprising *Chisocheton* and the remainder of *Guareae* and *Aglaieae* was constrained to minimally 5.3 Ma (the upper bound of the Late Miocene), based on Miocene fossil wood of *Chisocheton* (*Chisochetonoxylon*) from the Birbhum District in West Bengal (Ghosh & Roy 1979).
5. The stem of *Cedreleae* was constrained to minimally 48.6 Ma (the upper bound of the Early Eocene), based on fruit and seed fossils ascribed to *Toona* from the London Clay (Reid & Chandler 1933, Chandler 1964). These specimens share morphological features of both modern *Toona* and *Cedrela* (T.D. Pennington, RBGK, pers. comm. 2005).

For absolute ages we relied on the geologic time scale of Gradstein et al. (2004).

RESULTS

We checked the ITS sequences for the presence of structural motifs and secondary structure. This enabled us to unambiguously align the entire ITS region across all genera (ingroup and

outgroup), without losing any potentially informative characters for subsequent phylogenetic analyses. There was no indication for multiple copies of ITS in any individual included in our analysis, indicating no need to clone PCR products. Sequences of all ingroup and outgroup taxa contained the conserved angiosperm motifs in ITS1, 5.8S and ITS2 (Liu & Schardl 1994, Hershkovitz & Zimmer 1996, Jobes & Thien 1997).

Phylogeny estimation

Alignment of all ITS region sequence positions resulted in a matrix of 803 characters for the whole set of taxa. The length of the total matrix including 18S and 26S rDNA flanking regions was 1 010 characters. For the entire ITS region, 517 (64 %) positions were variable and 418 (52 %) were potentially parsimony informative. The parsimony search produced 288 most parsimonious trees of 2 863 steps with a consistency index (CI) = 0.34 and a retention index (RI) = 0.61. Fig. 2 shows the strict consensus tree obtained from the MP analysis of 90 ingroup and four outgroup taxa, and bootstrap values. The Bayesian (Fig. 3) and ML (Fig. 4) analyses were all based on the same dataset as used for the MP analysis. The Bayesian tree was based on a total of 20 000 trees and a burn-in of 910 trees (Fig. 3).

Infra-specific polymorphism

We included 13 accessions of the morphologically complex species *Aglaia elaeagnoidea* in our analyses, from two main areas beyond the western and eastern boundaries of Malesia. The samples exhibited high genetic diversity, topologically covering two portions of our phylogenetic trees (Fig. 3, 4). Accessions from western regions showed the following pattern: samples from Thailand and Vietnam formed a strongly supported clade in all trees (98 % bootstrap percentage, BP, Fig. 2; 100 % posterior probability, PP, Fig. 3; 97 % BP, Fig. 4), followed by a grade of samples from Bangladesh and Sri Lanka (Fig. 2–4); all western samples together formed a weakly to moderately supported clade (73 % BP, Fig. 2; 93 % PP, Fig. 3; 61 % BP, Fig. 4; in the following referred to as 'Western Group'). Accessions from eastern regions appeared in a strongly supported clade (100 % BP and PP, respectively, in all trees; in the following referred to as 'Eastern Group').

Divergence time estimation

Divergence time estimates for selected nodes are given in Fig. 3. Results based on NPRS and the Bayesian approach yielded similar age estimates. Age estimates derived from the Bayesian approach, based on several fossils, can be viewed as best approximations. Our Bayesian age estimates suggest that the clade of *Aglaia samoensis* and *A. sapindina* dates back to 5.7 ± 3.7 Ma, the clade uniting samples of the Western Group to 11.7 ± 4.8 Ma, the clade uniting samples of the Eastern Group to 5.3 ± 3.5 Ma, and the clade of *A. australiensis* and *A. meridionalis* to 5.2 ± 3.4 Ma (Fig. 3).

DISCUSSION

Our study 1) provides the first assessment of the genetic diversity of *Aglaia elaeagnoidea* on the basis of a geographic sampling from the two ends of the distribution; 2) investigates the geographic structure of the data over the sampled geographic range; 3) evaluates the two ends of the morphological continuum which led to the wide species concept formerly adopted for *A. elaeagnoidea*; and 4) suggests that Australia has been colonized only recently by *A. elaeagnoidea* and other species. Our conclusions are based on several lines of evidence.

Genetic diversity and geographic structure of Aglaia elaeagnoidea

Aglaia elaeagnoidea is a widespread species, which occurs throughout the range of the genus except for the Solomon Islands and extends beyond the range of any other species in the genus in Western Australia. It appears to have two distinct entities at the eastern and western ends of its range, outside the Malesian area, linked by a series of morphological intermediates through Malesia. It was therefore considered to be a complex species by Pannell (1992). Unlike most species of *Aglaia*, it is not a rain forest tree, apparently adapted to a more seasonal climate. It is usually coastal, especially in the east of the range, but is also found inland and at higher elevations in India, Sri Lanka, Thailand and Borneo, on dry sandy soils and limestone.

On the basis of our geographic sampling strategy, specimens assigned to *A. elaeagnoidea* based on morphological grounds according to the current literature (Pannell 1992, 2004), appear as genetically heterogeneous (Fig. 2–4). Specimens to the far east and west of the range of the species belong to two different genetic entities based on ITS data.

Taxonomic considerations – Aglaia elaeagnoidea and A. roxburghiana

Historically, two main species were recognized in the *Aglaia elaeagnoidea* complex: *A. elaeagnoidea* (basionym *Nemedra elaeagnoidea* A.Juss. 1830) from Australia and *A. roxburghiana* (Wight & Arn.) Miq. (basionym *Milnea roxburghiana* Wight & Arn. 1834) from India and Sri Lanka. Various varieties and species names were applied to both of these to deal with the variation encountered within and between these two extremes in morphology and geographical range. In the monograph of *Aglaia* (Pannell 1992), it was treated as one widespread species, *A. elaeagnoidea*. It is usually a small tree or shrub, 5–10 m tall. The large pale orange or almost white peltate scales are characteristic, but they vary in colour, in the presences and extent of the fimbriate margin and in their density on the plant. In the eastern part of the range, the scales are larger, paler in colour and more frequently entire than in the west, the density is greater on the leaves, but the fruits may have fewer scales than in the west and the aril may be vestigial. In India, *A. elaeagnoidea* is sometimes a larger tree, to 20 m, than in the rest of the range of the species. Large-leaved specimens from the west can also be almost indistinguishable from another complex species, *A. edulis,* unless fruits are present; these are much larger (up to 3.2 × 3.8 cm) in *A. edulis* than are those of *A. elaeagnoidea* (up to 2 × 1.5 cm) and usually 3-locular rather than 2-locular. The molecular data presented here confirm that the two extremes of variation in *A. elaeagnoidea* can be distinguished at the molecular level, just as they can on morphological characters. Interestingly, samples from Australia are not in the clade which separates most eastern species of section *Aglaia* from the rest of the genus (top clade in Fig. 2–4). The material of *A. elaeagnoidea* from Australia was collected from a relatively small area of Queensland and the samples are all similar at the molecular level for the DNA region sequenced. There is more variation in the samples from the west of the range, reflecting the larger area from which they were collected. Specimens from these two widely separated regions are distinct at the morphological and at the molecular level. On morphological and molecular evidence, the name *Aglaia roxburghiana* could be reinstated for specimens from the western end, but we have no data yet to indicate clearly where *A. roxburghiana* ends and *A. elaeagnoidea* begins either morphologically or geographically. This will be subject to further investigations. To reach a detailed appreciation of *A. elaeag-*

noidea in addition to the patterns already elucidated by present evidence, sampling of additional specimens, especially from the Malesian region, will be necessary.

Additional evidence derived from biogenetic trends – patterns of secondary metabolites in the Western versus Eastern Group of Aglaia elaeagnoidea

Broad-based phytochemical comparisons with the 13 samples of *Aglaia elaeagnoidea* collected in various parts of Thailand, Vietnam, Bangladesh, Sri Lanka, and Australia showed chemical differences clearly separating all collections from Australia (Eastern Group) from the others (Western Group). Especially the genus-specific flavaglines of all representatives of the Eastern Group deviated by derivatives with a dimethoxylated aromatic ring A, like rocaglamide and aglafoline, whereas all other collections from the west were characterized by a methylenedioxy and a methoxy group in ring A, like pannellin and aglaroxin A (Brem 2002, Hofer 2002). Additionally, the Australian samples were characterized by the formation of a triterpene derived limonoid, 6-α-acetoxygedunin (Hofer 2002). Although widespread in the *Meliaceae*, limonoids are apparently rare in *Aglaia*, only described so far for another collection of *A. elaeagnoidea* from Sempu Island (Java) also containing the flavagline aglafoline with a dimethoxylated ring A (Fuzzati et al. 1996). The accumulation of different bisamides represents another important chemical character distinguishing the Australian samples from the Western Group. Whereas odorine derivatives are dominating in Australian collections, samples in the Western Group are characterized by piriferine derivatives. Both types of bisamides were also found as characteristic building blocks in the flavaglines (Greger et al. 2008). Different patterns of lignans and flavonoids may also contribute to a taxonomic segregation of the Eastern Group of *A. elaeagnoidea* (Hofer 2002, Greger unpubl.).

Temporal origin of Western and Eastern Groups of Aglaia elaeagnoidea, and relatives

Our results imply that the ancestor of the clade comprising the individuals of the Western Group currently assigned to *Aglaia elaeagnoidea* originated in the western part of the distribution range of the genus and of *A. elaeagnoidea*, respectively, subsequently dispersing eastwards. This pattern from west to east can be observed based on the topologies of the trees within the Western Group (Fig. 3, 4). These topologies suggest that early-branching within *A. elaeagnoidea* occurred in western areas including Sri Lanka and Bangladesh. *Aglaia elaeagnoidea* then seems to have extended its range eastwards to areas including Thailand and Vietnam.

Based on the evidence of the tree topologies suggesting that the Western Group of *A. elaeagnoidea* originated in the very western part of the distribution range of the genus, we were interested in the relative timing of divergence events that had given rise to the western versus eastern clades of *Aglaia* species in our trees. If *Aglaia* had originated in the west, then clades comprising samples from Australia, as representatives of lineages from the east, should be comparably younger. Our divergence time estimates yielded estimates confirming these expectations (Fig. 3). Based on this measure, clades/groups with specimens collected in Australia (from species either restricted in their occurrence to Australia, like *A. australiensis* and *A. meridionalis*, or having a broader range of distribution, like *A. sapindina*; compare Fig. 3) date back to 5.2–5.7 Ma. The Eastern Group of *A. elaeagnoidea* dates back to minimally 5.3 mya. In contrast, the group comprising western *A. elaeagnoidea* dates back to minimally 11.7 mya. This suggests that old Gondwanan land masses like Australia might have been colonized only recently by *A. elaeagnoidea* and other species (compare Muellner et al. 2008a), with divergence events mostly dating back to the Miocene/Pliocene boundary and subsequent Pliocene times.

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