Systematics and biogeography of the non-viny grape relative *Leea* (Vitaceae)

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Leea, sometimes treated as the monogeneric family Leeaceae, is sister to the rest of the grape family, Vitaceae, but its systematics is poorly known. Phylogenetic relationships in *Leea* were reconstructed with parsimony and Bayesian methods using nuclear ribosomal sequences to assess species circumscriptions, morphological evolution and biogeography. The internal transcribed spacer secondary structure model for *Leea* facilitated homology assessments during sequence alignment. Nine morphological characters were mapped onto the phylogenetic tree. Four major clades in *Leea* were supported, with *L. asiatica s.l.* (=clade I) as the earliest diverging clade and having plesiomorphic free stamens. Clade II, which includes the prickle-bearing species, is sister to clade III, which includes species with comparatively large flowers. Clade IV, sister to clade II + III, was resolved into four subclades. Each subclade included accessions of *L. indica* and *L. guineensis* intermixed with six other morphologically distinct species, showing the polyphyly of these two species as currently circumscribed. Flower colour, previously used to characterize species, was shown to be unreliable for species identification. Dating analyses estimated that *Leea* originated in Indochina in the Late Cretaceous (65–86.19 Mya, 95% highest posterior density). The members of the major clades later spread to India, Africa, Madagascar, South-East (SE) Asia and tropical Australasia. Major species diversification occurred in the Neogene, when dynamic environmental and geological changes in SE Asia presented new ecological niches. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, **171**, 354–375.

ADDITIONAL KEYWORDS: 5S-NTS – cryptic species – internal transcribed spacer – Leeaceae – morphological evolution – Old World tropics – phylogeny – secondary structure – species complex – taxonomy – Vitales.

INTRODUCTION

Leea D.Royen ex L. is sister to the remainder of the grape family, Vitaceae, which includes one of the most economically important fruit crops in the world, *Vitis vinifera* L. (Chase *et al*., 1993; Ingrouille *et al*., 2002), but the systematics and evolutionary history of Leea are poorly known. This is especially unfortunate as this tropical genus has been used ethnobotanically for its cardiac, analgesic and tuberculostatic properties, areas that need further research (Op de Beck *et al*., 1998, 2003). *Leea* was formally described by Linnaeus (1767), with the type species as *L. aequata* L. designated by Ridsdale (1974).

Leea has previously been associated with Rhamnales (Cronquist, 1981), but this has been refuted based on molecular evidence, which showed it to be closest to Vitaceae *s.s.* (Chase *et al*., 1993; Ingrouille *et al*., 2002). The familial assignment of Leea has been contentious, being included in Vitaceae (APG, 1998; APG II, 2003; APG III, 2009; Ingrouille *et al*., 2002) or placed into its own monogeneric family, Leeaceae (Planchon, 1887; Nair, 1968; Ridsdale, 1974,

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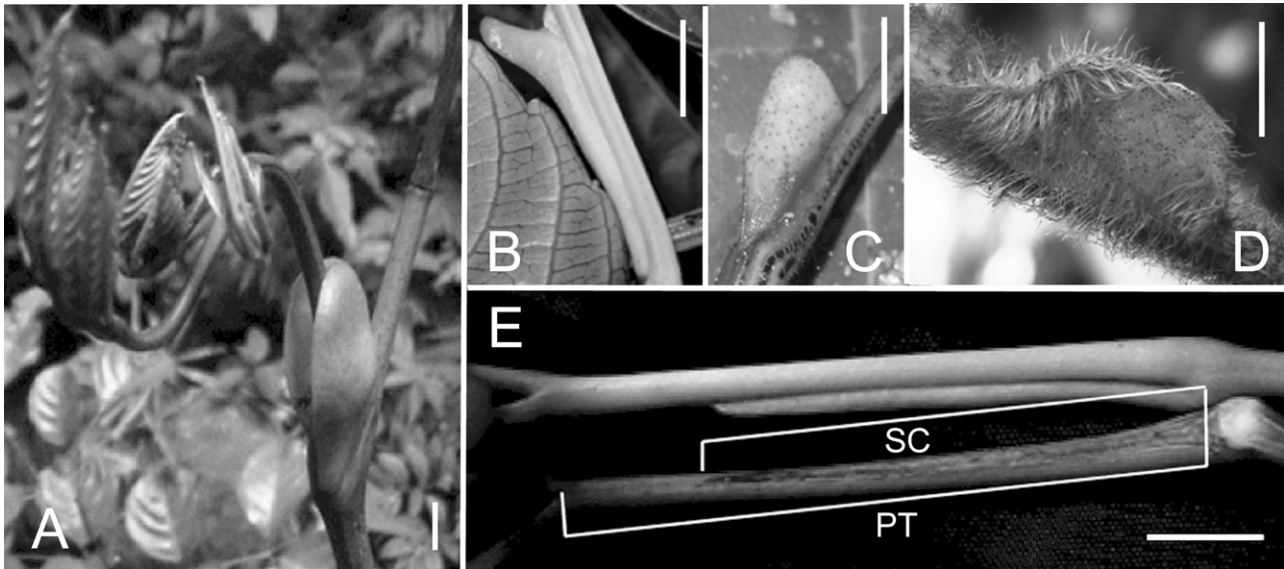
Figure 1. Variations in stipule morphology of Philippine *Leea* (scale, 1 cm). A, stipule of *L. manillensis* enclosing developing leaves. B, *Leea aculeata*, wing-type. C, *Leea* sp. 2, obovate type. D, *Leea cumingii*, obovate type. E, *Leea philippinensis*, wing-type (top); stipule scar length to petiole length ratio (SC/PT, bottom). Photo credit: J.M.

1976; Latiff, 2001; Wen, 2007a, b). In the Angiosperm Phylogeny Group (APG) system (APG, 1998; APG II, 2003; APG III, 2009) it was treated as the sole genus in subfamily Leeoideae Burmeister of Vitaceae, with the rest of the 14 genera of Vitaceae placed in subfamily Viticoideae Eaton. *Leea* and Vitaceae *s.s.* form the order Vitales, which until now has had an ambivalent position in the tree of life, switching alliances between Caryophyllales (Chase *et al.*, 1993), Saxifragaceae (Savolainen *et al.*, 2000), Dilleniaceae (Hilu *et al.*, 2003) and the rosids (Soltis *et al.*, 2003; Jansen *et al.*, 2006).

The inclusion of *Leea* in Vitaceae has been justified based on its possession of pearl glands, raphides, shared corolla-stamen primordia and phloem plastids similar to other Vitaceae (APG, 1998; APG II, 2003; APG III, 2009). However, unlike members of Vitaceae *s.s.*, *Leea* spp. do not form tendrils and are erect herbs, shrubs and trees (not climbing vines) with terminal inflorescences and characteristically large stipular structures (Fig. 1; Ridsdale, 1974, 1976). Flowers of *Leea* also possess ovaries with secondary septa and a distinct elaborate floral disc (i.e. floral tube not derived from corolla lobes) capped by connate stamens (Ridsdale, 1976; Wen, 2007a, b). The anthers detach as a coherent unit along with the filaments during anthesis (Gerrath, Lacroix & Posluszny, 1990; Molina, 2009). On the basis of these morphological differences, we prefer the continued segregation of *Leea* into its own family, Leeaceae, as originally described by Dumortier (1829).

Leea spp. grow in dry deciduous forests, open grasslands, and montane or lowland rainforests throughout the Old World tropics from Africa to Asia, northeastern Australia, New Guinea and islands of the Pacific (Fiji, Solomon Islands, Caroline Islands), but are most diverse in Indomalaya, including India, Indochina (i.e. Cambodia, Laos, Myanmar, Thailand and Vietnam), tropical China (i.e. Guangdong, Guangxi, Yunnan and Hainan provinces) and Malesia (i.e. Brunei, Indonesia, East Timor, Malaysia, New Guinea, Philippines, Singapore). Ridsdale's (1974, 1976) revisions listed 34 *Leea* spp. due to his broad species concept, but Li (1998) reported as many as 153 species. Clarke (1881) recognized 29 species from India. However, Clarke himself admitted that he had 'little confidence in the limits of any (species), except the Bengal ones' (Clarke, 1881: 100). He also split the genus into two series: the red-flowered Rubriflorae and the green-flowered Viridiflorae. This subgeneric classification was not adopted by Ridsdale (1976: 756) in the most comprehensive monograph of the genus, as he found it 'unreliable'. In his revision of Malesian species, Ridsdale (1976) combined overlapping and polymorphic morphologies, ranging from glabrous, small-leaved (*c*. 30 mm long) forms to pubescent, large-leaved morphs (*c*. 300 mm long) into species complexes that encompassed vast geographical distributions, e.g. the red-flowered *Leea guineensis* G.Don and the usually white-flowered *L. indica* (Burm.f.) Merr.

Morphological characters that have been used in the past for taxonomic diagnosis need to be evaluated

based on the molecular phylogenetic data to understand character evolution in *Leea*. Ridsdale (1974, 1976) adopted broad species concepts for both *L. indica* and *L. guineensis*, considering overlapping vegetative forms in each species complex defined solely by flower colour. This difficulty in delimiting morphospecies may be due to either true cryptic evolution (i.e. speciation not accompanied by morphological change, Bickford *et al.*, 2007) or failure to identify subtle interspecific morphological differences. As Ridsdale based his revisionary work largely on herbarium specimens, which are often incomplete relative to the live source plant, it is not unlikely that he missed pertinent taxonomic features that would have otherwise helped distinguish *Leea* spp. However, morphostasis has also been reported in many other Palaeotropical plant taxa such as *Aglaia* Lour. (Meliaceae), *Diospyros* L. (Ebenaceae, Pannell & White, 1988), and *Macaranga* Thouars and *Mallotus* Lour. (Euphorbiaceae, Kulju *et al.*, 2007), so some *Leea* spp. may just be truly cryptic. An overwhelming amount of taxonomically frustrating transitional forms exist in plants from the geologically complex and spatially fragmented Malesian region (Hall, 1998; Morley, 1998, 2000; Woodruff, 2003), which has provided not only a wide range of opportunities for speciation, but also the breakdown of incipient speciation, potentially giving rise to hybrids with intermediate morphologies.

Until now, no phylogenetic study has focused on *Leea*. The aim of this project was to elucidate the evolutionary relationships in *Leea* using molecular markers [internal transcribed spacer (ITS), 5S-nontranscribed spacer region (NTS)]. The phylogenetic framework and sampling allow for the evaluation of taxon delimitations, even more so when supplemented with morphological data, and also help to develop biogeographical hypotheses on the diversification of *Leea* across the Old World tropics.

MATERIALS AND METHODS

TAXON SAMPLING

Ninety accessions from 22 *Leea* spp. representing the morphological and geographical diversity of the genus were sampled (Table 1). Five accessions from Vitaceae *s.s.* (five species), one from Dilleniaceae (one species) and one from Saxifragaceae (one species) were used as outgroups (Table 1). Leaf material for DNA extraction was either obtained from herbarium specimens when permission was granted by the lending institution or from silica-dried material collected by us or donated by colleagues (U. Ferreras, L. Co and S. Yap) and botanic gardens (Ecology and Evolutionary Biology Plant Growth Facilities, University of Con-

necticut; Botanical Garden, University of Copenhagen; Denver Botanic Garden; and Singapore Botanic Garden). Repeated DNA extraction efforts on herbarium specimens of *L. alata* Edgew., *L. grandifolia* Kurz, *L. simplicifolia* Zoll. & Moritzi, *L. tetramera* Burtt and *L. thorelii* Gagnep. were unsuccessful and sequences were not obtained from these species. Destructive sampling from the few available specimens of *L. tinctoria* Baker and *L. unifoliata* Merr. was prohibited by their source institutions. *Leea curtisii* King, *L. krukoffiana* Ridsdale, *L. saxatilis* Ridl. and *L. smithii* Koord. were not sampled due to unavailability of material.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA was extracted following a modified CTAB protocol used for Vitaceae (Soejima & Wen, 2006). DNA samples were then air-dried and maintained in $1 \times$ TE buffer at $-20\,°C$ for short-term storage. As the entire ITS region, which is > 700 bp long, could not be successfully amplified, primer pairs for each of its shorter spacers (< 300 bp) were designed: P79 (forward: AAGGATCATTGTCGARCCYGCA) and P80 (reverse: AGATATCCGTTGCCGAGAGTC) for ITS1, and P81 (forward: ACGACTCTCGGCAACGGATATCT) and P82 (reverse: ATGCTTAAAC-TCAGCGGGTGTTCC) for ITS2. 5S-NTS was amplified using a nested PCR approach, initially with the forward primer CACCGGATCCCATCAGAACT and the reverse primer TTAGTGCTGGTATGATCGCA (Udovicic, McFadden & Ladiges, 1995) and then with the internal primers TTGGGAAGTYYCYTGTGTTGCA (forward) and TGGTATGATCGCACCCRTCATG (reverse) designed specifically for *Leea* by J.E.M.

Amplification reactions were performed in a 25-μL volume containing Choice Taq Mastermix (1.5 mM MgCl$_2$, 10 mM Tris-HCl at pH 9.0, 10 mM KCl, 8 mM (NH$_4$)$_2$SO$_4$, 0.05% Triton X-100, dNTP mix; Denville cat. no. CB4070-7), 0.7 μM primer, 0.05 μg μL^{-1} bovine serum albumin, 5% dimethylsulphoxide, 0.8 M betaine, additional MgCl$_2$ (to 2.5 mM final concentration) and 3 μL template DNA, which was prepared by diluting DNA extracts 1:50 with water. PCR reactions were conducted using an Applied Biosystems GeneAmp System 9700 using the following programme for ITS: $97\,°C$ for 1 min, followed by 35 cycles of $95\,°C$ for 1 min, $53\,°C$ for 1 min and $68\,°C$ for 2 min, ending with a final extension of $72\,°C$ for 4 min. For 5S-NTS, the programme was $94\,°C$ for 2 min, followed by 27 cycles of $94\,°C$ for 1 min, $60\,°C$ for 1 min and $72\,°C$ for 1 min, ending with a final extension of $72\,°C$ for 4 min.

For visualization, PCR products were run on 1% agarose gels stained with 0.5 μg mL^{-1} ethidium bromide. If double bands were observed, the desired

Table 1. Accessions and sequences used in phylogenetic analyses including voucher information (collector, number, provenance, and herbarium source)

Species *sensu* Ridsdale	New classifications	Collector, collector number	Provenance	Sequence used
Cayratia acris (F.Muell.) Domin (Vitaceae)	NA	See Rossetto *et al.* (2002)	–	ITS1
Cissus tweediana Planch. (Vitaceae)	NA	See Rossetto *et al.* (2002)	–	ITS1
Clematicissus angustissima (F.Muell.) Planch. (Vitaceae)	NA	See Rossetto *et al.* (2002)	–	ITS1
Dillenia sp. (Dilleniaceae)	NA	*Molina s.n.* (PUH)	Isabela, Philippines	ITS1
Liquidambar orientalis Mill. (Saxifragaceae)	NA	Shi *et al.*, unpublished	–	ITS1
Tetrastigma sp. (Miq.) Planch. (Vitaceae)	NA	*Molina 4* (CHRB)	Isabela, Philippines	ITS
Vitis vinifera L.	NA	See Velasco *et al.* (2007)	–	ITS
Leea aculeata Blume	*Leea aculeata* Blume	*Coode et al. 5449* (L)	Mindoro, Philippines	ITS1, 5S-NTS
Leea aculeata Blume	*Leea aculeata* Blume	*Molina 19* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea aculeata Blume	*Leea aculeata* Blume	*Yap s.n.*, July 2007 (PUH)	Laguna, Philippines	ITS1, 5S-NTS
Leea acuminatissima Merr.	*Leea acuminatissima* Merr.	*Ferreras s.n.*, Oct. 2006 (PUH)	Aurora, Philippines	ITS, 5S-NTS
Leea acuminatissima Merr.	*Leea acuminatissima* Merr.	*Wen 8242* (US)	Laguna, Philippines	ITS, 5S-NTS
Leea aequata L.	*Leea aequata* L.	*Burley et al., 2803* (L)	West Kalimantan, Indonesia	ITS1, 5S-NTS
Leea aequata L.	*Leea aequata* L.	*Chow & Wan 80030* (MO)	Yunnan, China	ITS1, 5S-NTS
Leea aequata L.	*Leea aequata* L.	*Coode 6243* (A)	Sulawesi, Indonesia	ITS1, 5S-NTS
Leea aequata L.	*Leea aequata* L.	*Davies 99069* (A)	Borneo	ITS, 5S-NTS
Leea aequata L.	*Leea aequata* L.	*Kessler 3080* (L)	Sulawesi, Indonesia	ITS, 5S-NTS
Leea aequata L.	*Leea aequata* L.	*Wen 10172* (US)	Sulawesi, Indonesia	5S-NTS
Leea aequata L.	*Leea aequata* L.	*Wen 7494* (US)	Thailand	ITS, 5S-NTS
Leea aequata L.	*Leea aequata* L.	*Yap 4* (CHRB)	Bohol, Philippines	ITS, 5S-NTS
Leea amabilis Hort.Veitch. ex Mast	*Leea amabilis* Hort.Veitch. ex Mast	*Argent & Wilkie 9415* (A)	Central Kalimantan, Indonesia	ITS, 5S-NTS
Leea angulata Korth. ex Miq.	*Leea angulata* Korth. ex Miq.	*Mitchell 5* (CANB)	Christmas Island, Australia	ITS1, 5S-NTS
Leea angulata Korth. ex Miq.	*Leea angulata* Korth. ex Miq.	*Wen 10230* (US)	Sulawesi, Indonesia	ITS, 5S-NTS
Leea asiatica (L.) Ridsdale	*Leea asiatica* (L.) Ridsdale	*Averyanov et al., 2190* (MO)	Kon Tum, Vietnam	ITS1, 5S-NTS
Leea asiatica (L.) Ridsdale	*Leea asiatica* (L.) Ridsdale	*Chand 8276* (MICH)	Assam, India	ITS
Leea asiatica (L.) Ridsdale	*Leea asiatica* (L.) Ridsdale	*Maxwell 90718* (A)	Thailand	ITS1
Leea aspera Wall. ex Roxb.	*Leea aspera* Wall. ex Roxb.	*Ram 576* (A)	West Nepal	ITS1
Leea aspera Wall. ex Roxb.	*Leea aspera* Wall. ex Roxb.	*Suzuki et al., 9480014* (A)	Central Nepal	ITS
Leea asiatica (L.) Ridsdale	*Leea asiatica* (L.) Ridsdale	*Wen 9036* (US)	China	ITS
Leea compactiflora Kurz	*Leea compactiflora* Kurz	*Hui 55103* (US)	China	ITS
Leea compactiflora Kurz	*Leea compactiflora* Kurz	*Averyanov et al., 1602* (MO)	Kon Tum, Vietnam	ITS, 5S-NTS
Leea congesta Elmer	*Leea congesta* Elmer	*Molina 3* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea congesta Elmer	*Leea congesta* Elmer	*Molina s.n.*, July 2006 (CHRB)	Isabela, Philippines	ITS, 5S-NTS

Table 1. *Continued*

Species *sensu* Ridsdale	New classifications	Collector, collector number	Provenance	Sequence used
Leea coryphantha Lauterb.	*Leea coryphantha* Lauterb.	*Hoogland and Craven 10688* (CANB)	Papua New Guinea	ITS2, 5S-NTS
Leea coryphantha Lauterb.	*Leea coryphantha* Lauterb.	*Takeuchi et al., 13581* (A)	Papua New Guinea	ITS2
Leea gonioptera Lauterb.	*Leea gonioptera* Lauterb.	*Wen s.n., Aug. 2008* (US)	Papua New Guinea	ITS, 5S-NTS
Leea guineensis G.Don	*Leea maculata* Desf.	*Alcool 7506* (NY)	Kivu, Congo	ITS, 5S-NTS
Leea guineensis G.Don	*Leea* sp. 1	*Kress 97–5877*	Myanmar	ITS, 5S-NTS
Leea guineensis G.Don	*Leea manillensis* Walp.	*Wen 8303* (US)	Mountain Province, Philippines	ITS
Leea guineensis G.Don	*Leea manillensis* Walp.	*Yap s.n.,* (PUH)	Palawan, Philippines	ITS1, 5S-NTS
Leea guineensis G.Don	*Leea manillensis* Walp.	*Bartlett 15924* (MICH)	Lanao, Philippines	ITS1
Leea guineensis G.Don	*Leea aff. setuligera* C.B.Clarke	*Kress 37301* (US)	Myanmar	ITS, 5S-NTS
Leea guineensis G.Don	*Leea cf. arborea* Telf. ex Wight & Arn.	*Lorence & Lecordier 2647* (MO)	Mauritius	ITS
Leea guineensis G.Don.	*Leea dentata* Craib	*Maxwell 90692* (A)	Chiang Mai, Thailand	ITS1, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Canfield 696* (US)	Palau, Caroline Islands	ITS
Leea guineensis G.Don.	*Leea cuspidifera* Baker	*Gentry and Schatz 62078* (MO)	Madagascar	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea maculata* Desf.	*Gereau et al., 5851* (MO)	Tanzania	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea maculata* Desf.	*Gobbo 119* (MO)	Tanzania	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Liao 1206* (A)	Lanyu, Taiwan	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Molina 13* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Molina 18* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Molina 31* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Molina 32* (CHRB)	Negros, Philippines	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Molina 37* (CHRB)	Negros, Philippines	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea coccinea* Planch.	*Richards and von Bargen 264* (DBG)	Cultivated (Denver Botanic Garden, geographic source not indicated)	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Wagner 6727* (F)	Lanyu, Taiwan	ITS1, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Yap 6* (CHRB)	Leyte, Philippines	ITS1, 5S-NTS
Leea guineensis G.Don.	*Leea manillensis* Walp.	*Yap 7* (CHRB)	Surigao del Sur, Philippines	ITS, 5S-NTS
Leea guineensis G.Don.	*Leea monticola* Desc.	*Wen 9569* (US)	Madagascar	ITS, 5S-NTS
Leea heterodoxa K.Schum. & Lauterb.	*Leea heterodoxa* K.Schum. & Lauterb.	*Heyligers 1583* (CANB)	Papua New Guinea	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea javanica* Blume	*Bourell 2438* (A)	Palawan, Philippines	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea longifoliola* Merr.	*Chow 78319* (A)	Hainan, China	ITS
Leea indica (Burm.f.) Merr.	*Leea guineensis* G.Don	*Fell and Stanton 3177* (CANB)	Queensland, Australia	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea indica* (Burm.f.) Merr.	*Fernandes 2031* (A)	Mumbai, India	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea guineensis* G.Don	*Jackes 2622* (JCT)	Queensland, Australia	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea javanica* Blume	*Lee 126* (SING)	Singapore	ITS, 5S-NTS

Taxon (Ridsdale)	Taxon (re-named)	Voucher	Locality	Markers
Leea indica (Burm.f.) Merr.	*Leea* sp. 2	*Molina 6* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea* sp. 2	*Molina 7* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea cumingii* C.B.Clarke	*Molina 8* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea indica* (Burm.f.) Merr.	*Nicolson 2995* (US)	Mysore, India	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea javanica* Blume	*Ramadhanil & Schultze 818* (CANB)	Sulawesi, Indonesia	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea novoguineensis* Val.	*Regalado et al., 705* (CANB)	Solomon Islands	ITS
Leea indica (Burm.f.) Merr.	*Leea novoguineensis* Val.	*Schodde 2483* (A)	Papua New Guinea	ITS1
Leea indica (Burm.f.) Merr.	*Leea novoguineensis* Val.	*Smith 6286* (US)	Viti Levu, Fiji	ITS1
Leea indica (Burm.f.) Merr.	*Leea novoguineensis* Val.	*Smith 7773* (US)	Ngau, Fiji	ITS, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea novoguineensis* Val.	*Takeuchi & Ama 16543* (A)	Papua New Guinea	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea novoguineensis* Val.	*Takeuchi & Wiakabu 4320* (CANB)	Papua New Guinea	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea novoguineensis* Val.	*Takeuchi 4316* (F)	Papua New Guinea	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea javanica* Blume	*Wen 10237* (US)	Sulawesi, Indonesia	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea dentata* Craib	*Wen 7498* (US)	Thailand	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea javanica* Blume	*Wen 8341* (US)	Malaysia	ITS1, 5S-NTS
Leea indica (Burm.f.) Merr.	*Leea dentata* Craib	*Anderson 5149* (A)	Chiang Mai, Thailand	ITS1, 5S-NTS
Leea macrophylla Roxb. ex Hornem.	*Leea macrophylla* Roxb. ex Hornem.	*Wen 7415* (US)	Thailand	ITS1, 5S-NTS
Leea macrophylla Roxb. ex Hornem.	*Leea macrophylla* Roxb. ex Hornem.	*Wen 7417* (US)	Thailand	ITS, 5S-NTS
Leea macropus K.Schum. & Lauterb.	*Leea macropus* K.Schum. & Lauterb.	*Takeuchi 16698* (A)	Papua New Guinea	ITS
Leea macropus K.Schum. & Lauterb.	*Leea macropus* K.Schum. & Lauterb.	*Takeuchi 9048* (A)	Papua New Guinea	ITS1, 5S-NTS
Leea magnifolia Merr.	*Leea magnifolia* Merr.	*Edano 3509* (MICH)	Mindoro, Philippines	ITS1
Leea papuana Merr. & L.M. Perry	*Leea papuana* Merr. & L.M. Perry	*Kanis 1339* (CANB)	Papua New Guinea	ITS, 5S-NTS
Leea philippinensis Merr.	*Leea philippinensis* Merr.	*Molina 17* (CHRB)	Isabela, Philippines	ITS, 5S-NTS
Leea philippinensis Merr.	*Leea philippinensis* Merr.	*Yap s.n., July 2007* (PUH)	Samar, Philippines	ITS1, 5S-NTS
Leea quadrifida Merr.	*Leea quadrifida* Merr.	*University of San Carlos 821* (L)	Surigao del Sur, Philippines	ITS1
Leea rubra Blume	*Leea rubra* Blume	*Lee 127* (SING)	Singapore	ITS, 5S-NTS
Leea rubra Blume	*Leea rubra* Blume	*Martensz 718* (CANB)	Northern Territory, Australia	ITS1, 5S-NTS
Leea rubra Blume	*Leea rubra* Blume	*Pullen 6703* (A)	Papua New Guinea	ITS1, 5S-NTS
Leea setuligera C.B.Clarke	*Leea setuligera* C.B.Clarke	*Chand 3311* (MICH)	Assam, India	5S-NTS
Leea spinea Desc.	*Leea spinea* Desc.	*Barthelat 646* (MO)	Mayotte	ITS, 5S-NTS
Leea zippeliana Miq.	*Leea zippeliana* Miq.	*Pullen 7351* (CANB)	Papua New Guinea	ITS1
Leea zippeliana Miq.	*Leea zippeliana* Miq.	*Schodde & Craven 4387* (CANB)	Papua New Guinea	ITS1, 5S-NTS

Taxa were first identified following Ridsdale's treatments (1974, 1976; first column, compare with Fig. 4), with some re-named in the second column except for the outgroups (*Cayratia acris*, *Cissus tweediana*, *Clematicissus angustissima*, *Dillenia* sp., *Liquidambar styraciflua*, *Tetrastigma* sp., *Vitis vinifera*). NA, not applicable.

fragment was cut out of the gel and treated with QIAEX II Gel Extraction Kit (Qiagen cat. No. 20051) to yield cleaned DNA that was used for a second PCR reaction. PCR products of the desired size were cleaned using ExoSAP-IT (USB cat. No. 78201) following the manufacturer's specifications and then submitted to Genewiz Inc. for sequencing. Each DNA fragment (ITS1, ITS2, 5S-NTS) was sequenced in both directions.

ALIGNMENT AND ITS SECONDARY STRUCTURE PREDICTION

The ITS and the faster-evolving 5S-NTS, derived from the nuclear ribosomal RNA (nrRNA), were used to resolve phylogenetic relationships in *Leea*. To alleviate alignment ambiguities and improve homology assessments, *Leea* ITS was aligned following secondary structural information inferred from the method of free energy minimization (FEM), which assumes that the most optimal RNA conformation has the lowest folding free energy (Mathews & Turner, 2006). Previous studies have affirmed the phylogenetic utility of the ITS region (Baldwin *et al*., 1995), but only a few studies have employed its secondary structure as the guide for alignment in plant taxa, which may considerably improve phylogenetic estimation [Gottschling *et al*. (2001) for Boraginales; Goertzen *et al*. (2003) for Asteraceae; Bellarosa *et al*. (2005) for *Quercus* L., Fagaceae; Campbell *et al*. (2005) for *Picea* A.Dietr., Pinaceae; Muellner *et al*. (2008) for tribe Aglaieae, Meliaceae; Molina & Struwe (2009) for tribe Potalieae, Gentianaceae]. Secondary structure models include stems or helices, which form contiguous base pairs that may be interrupted by bulges or loops (unpaired nucleotides on each strand).

Individual ITS and 5S-NTS sequences were assembled and trimmed in Sequencher ver. 4.6 (Gene Codes Corp.) and initially aligned with ClustalW (European Bioinformatics Inst.) using default parameters, then manually adjusted in Microsoft Word. ITS1 and ITS2 were adjusted to follow secondary structure information generated for *L. aequata* by the software RNAstructure v. 5.3 (Reuter & Mathews, 2010) using the command 'Fold RNA single strand'. Twenty structures are automatically stored in each output file, with the first one having the lowest calculated free energy (the most probable structure), and the other 19 are alternative hypotheses sampled heuristically (D. H. Mathews, pers. comm.).

Base-pairing probabilities for helices (alternatively called stems) in the secondary structure predictions were calculated using the partition function tool of RNAstructure (Mathews & Turner, 2006). Detailed methodology is available in Molina & Struwe (2009). On average, 91% of base pairs with a probability of 0.99 or greater of pairing ($P_{BP} \geq 0.99$) are correctly predicted based on comparative sequence analysis (Mathews, 2004), whereas only 83% of base pairs with $P_{BP} \geq 0.90$ may be correctly predicted. The generated helix files were modified to retain only highly probable base pairs ($P_{BP} \geq 0.90$). These were exported into XRNA (B. Weiser & H. Noller, University of California, Santa Cruz) to draw secondary structure models of ITS1 and ITS2 for *L. aequata* (*Kessler 3080*; GenBank no. JN160932) and *Vitis vinifera* (AM423427).

The ITS (ITS1, ITS2) and 5S-NTS sequence data were concatenated to produce a combined data matrix which included coded gaps. Gaps were coded with Gapcoder (Young & Healy, 2003), which implements the simple indel coding of Simmons & Ochoterena (2000).

PHYLOGENETIC ANALYSES OF MOLECULAR DATA

Phylogenetic inference was conducted in PAUP* v 4.0 (Swofford, 2003) and MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001) for the concatenated ITS + 5S-NTS dataset, with and without coded gaps. An equally weighted parsimony analysis was implemented using a heuristic search with 500 addition sequence replicates, imposing a rearrangement limit of 5000 000 per replicate (MULTREES = on, random stepwise addition sequence with TBR branch swapping). Bootstrap support for clades were generated from 500 nonparametric replicates, each with one random addition-sequence replicate (rearrlimit = 5000 000, limitperrep = yes, MULTREES on). To select the best-fitting model under Akaike's information criterion, MrModeltest 2.2 (Nylander, 2004) was used, which called for a GTR model with equal nucleotide frequencies and with gamma-distributed rate variation across sites. Coded gaps were treated as binary characters (i.e. restriction data) as suggested in the MrBayes manual (Ronquist, Huelsenbeck & Teslenko, 2011). Four independent runs of 2.5 million iterations each were performed (nchains = 4) resampling trees every 500 generations. Twenty-five per cent of the samples, as suggested in the manual, were discarded as burnin and the sumt command was used to summarize the trees and generate the consensus tree with clade posterior probabilities. Tracer v1.4 (Rambaut & Drummond, 2007) was used to analyse trace files generated by Bayesian MCMC runs (e.g. MrBayes and BEAST) to assess convergence to the desired posterior distribution.

MORPHOLOGICAL CHARACTER ANALYSIS

About 900 herbarium specimens from A, CANB, CHRB, DBG, F, K, L, MICH, MO, NY, PUH, UC and

US (abbreviations according to *Index Herbariorum*) from 30 *Leea* spp. (*sensu* Ridsdale, 1974, 1976) were surveyed and of these about 200 had intact reproductive and vegetative structures that were examined for the coding of morphological characters. These characters, which were chosen because they showed variation across species (Ridsdale, 1974, 1976), were coded into a data matrix as six discrete and three continuous traits (Appendices 1, 2). To avoid erroneous homology assumptions due to the subjective nuances for flower colour, only corolla lobe colour was coded (excluding colour of floral tube, which is not derived from the corolla lobes, and is white to cream in most species; J. Molina, pers. observ.). The program Mesquite (Maddison & Maddison, 2010) was used to infer ancestral states for each morphological character using the 50% majority rule consensus tree from the Bayesian analyses as backbone. Mesquite does not choose between the resolution that maximizes reversals (ACCTRAN) or parallelisms (DELTRAN), but shows an ambiguous state reconstruction at a node if there are multiple possible most-parsimonious resolutions that disagree at that node.

Ancestral states were reconstructed using the parsimony criterion as likelihood reconstruction in Mesquite cannot handle polymorphic characters. To account for phylogenetic uncertainty, discrete ancestral states were summarized over the 95% credible set of trees generated by the Bayesian analysis. This feature calculates the proportion of trees that contains a particular node in the set and the fraction of the node-containing trees that exhibit the trait. Accounting for phylogenetic uncertainty is currently unavailable for continuous characters. Thus, ancestral states were reconstructed on the consensus topology (not a set of trees) using the option 'Trace character history' in Mesquite, which automatically partitioned continuous characters into discrete ranges. We applied the squared change assumption for the parsimony model. The output reconstructions were summarized with the ancestral states mapped on the Bayesian consensus tree.

MOLECULAR DATING AND BIOGEOGRAPHICAL ANALYSES

A smaller data matrix (one accession per species) was used as the input file in BEAST v.1.6.1 (Drummond & Rambaut, 2007) to estimate species divergence times. We implemented an uncorrelated relaxed lognormal clock as the posterior density for the parameters ucld.stdev [95% highest posterior density (HPD): 0.40–0.74] and coefficient of variation (95% HPD: 0.41–0.81) did not encompass zero, meaning that the data are not quite clock-like and indicate some degree of rate heterogeneity (Drummond & Rambaut, 2007;

Smith, Beaulieu & Donoghue, 2010). The Akaike information criterion in MrModeltest (Nylander, 2004) recommended GTR + G for the concatenated ITS and 5S-NTS dataset (without gap coding). The Yule process, which assumes a constant speciation rate per lineage, was specified for the tree prior and is recommended for species-level phylogenies (Drummond *et al.*, 2007).

Some taxa were constrained to be monophyletic based on phylogenetic results from our parsimony and Bayesian analyses (Figs 3, 4), and their clades were calibrated with fossils or known geological evidence. Both the Vitales crown group and the *Leea* crown group were assigned a uniform distribution with 65 Mya as the lower bound corresponding to the estimated age for the Deccan Traps (Allègre *et al.*, 1999), where the oldest fossil, *Leeoxylon multiseriatum* Prakash & Dayal was collected, and 119 Mya as the upper bound representing the Vitales/rosid split (Wang *et al.*, 2009). We think that applying the same uniform prior is justifiable because both the Vitales and *Leea* crown groups could not be any older than the Vitales/rosid split (maximum age of 119 Mya) or younger than the minimum age of 65 Mya based on fossil evidence.

The *L. angulata* Korth ex. Miq. – *L. spinea* Desc. clade was specified a uniform distribution, namely 5–23 Mya corresponding to the Miocene epoch, which is the estimated age for *L. eojaponica* Watari, the fossil of which closely resembled the wood of *L. angulata* (Watari, 1951).The Philippine subsets of clade III and clade IV were each assigned a uniform prior of 0–35 Mya, the maximum age corresponding to the first emergence of some land in the Philippines (Steppan, Zawadzki & Heaney, 2003).

Two independent MCMC runs of 10 000 000 generations, sampling every 1000 steps, were sufficient to achieve effective sample size (ESS) values > 200. The tree output files were combined in LogCombiner (included in the BEAST package) after removing 25% of the trees as burnin. The maximum clade credibility tree, which represents the sampled tree with the highest clade posterior probabilities, was visualized using TreeAnnotator (included in the BEAST package) with divergence times corresponding to mean ages taken from the entire sample of trees for that clade.

Both Lagrange-20110117 (Ree & Smith, 2008) and S-DIVA (Yu, Harris & He, 2010) were used to reconstruct ancestral geographical range. Lagrange is a likelihood method of estimating ancestral areas based on dispersal, extinction and cladogenesis (DEC; Ree & Smith, 2008), which incorporates a time component by specifying an ultrametric tree, whereas S-DIVA applies the parsimony-based method of DIVA (Ronquist, 1997) while accounting for phylogenetic

uncertainty using the methods of Nylander *et al.* (2008). *Leea* ancestral areas were inferred by coding species as belonging to any of these six areas of endemism: (A) tropical Africa/Madagascar and adjacent islands (Mauritius, Mayotte, Comoros); (I) India; (D) Indochina including tropical China; (W) West and Central Malesia, including Malay Peninsula, Sumatra, Java, Borneo, Sulawesi and Palawan (the last now politically part of the Philippines, but connected to Borneo prior to the Pleistocene); (G) northeast Australia including New Guinea, Bismarck Archipelago, Fiji and the Solomon Islands, hereafter referred to as Australasia; (P) the rest of the Philippine islands plus Taiwan and Palau.

Areas were optimized on the BEAST maximum clade credibility tree in Lagrange including only one outgroup, *Cayratia acris* (F.Muell.) Domin. of Vitaceae. Three dispersal matrices corresponding to different time intervals were implemented: 89–119 Mya, allowing only intra-Gondwana (A, I, G) and intra-Laurasia (D, W) dispersals (Ali & Aitchison, 2008); 35–89 Mya, allowing free dispersal among all areas except the Philippines (P), islands of which had not emerged at this time; and 0–35 Mya, when free dispersal among all included areas was already possible. When dispersal between areas is allowed, the dispersal probability was set to 1. In S-DIVA, 1000 random trees from the posterior distribution of trees generated by the BEAST analysis were used as input. In both Lagrange and S-DIVA the maximum number of areas was set to two because only two *Leea* spp. in our analyses, *L. aequata* and *L. rubra* Blume, occur in more than two areas, and their current distributions are presumably due to secondary range expansion as they are each deeply embedded in clades whose early diverging lineages have restricted ancestral ranges (i.e. Indochina).

RESULTS

ALIGNMENT AND ITS SECONDARY STRUCTURE

Multiple peaks corresponding to intragenomic polymorphisms for individual nucleotides were seldom detected in both the ITS and the 5S-NTS sequencing profiles, suggesting that ITS sequences obtained have been sufficiently homogenized. Polymorphic nucleotides were assigned IUPAC ambiguity codes. The length of ITS1 and ITS2 in *Leea* is 279–301 and 253–262 bp, respectively. The G+C content (mol%) is 67.8%. 5S-NTS length is 186–374 bp with a G+C content (mol%) of 65.0%. In general it was difficult to amplify the two ITS markers from herbarium specimens despite repeated attempts and troubleshooting. Table 1 lists the sequences available for each accession. Sequences generated from this study were deposited under GenBank accession numbers

JN160885–JN161046. The combined ITS and 5S-NTS dataset consisted of 1375 characters including coded gaps. There was a total of 83 gap characters, of which 22 sites were from ITS1, 43 from 5S-NTS and 18 from ITS2.

The ITS alignment was refined based on the secondary structural models predicted by free energy minimization in RNAstructure v. 5.3. These models are here illustrated for ITS1 and ITS2 of the type species of *Leea*, *Leea aequata*, and for *Vitis vinifera* (Fig. 2). FEM identified five helices (1A, 1B, 1C, 1D, 1e*; Fig. 2A) in ITS1 of *L. aequata* that are well supported (i.e. base pairing probability at least 90%) by the partition function calculation of RNAstructure v. 5.3. The first four helices found in *Leea* correspond to stems found in ITS1 of the outgroups *Dillenia* L. (GenBank no. JN160885) and *Liquidambar* L. (AF304524; not shown but available upon request). The sequences in stems 1A, 1B and 1D could not be aligned between the outgroups and the *Leea* ingroup unless the stems were marked in the primary sequence. Thus, the stems provided anchor points by which sequences may be effectively aligned despite nucleotide differences.

Stem 1C was readily alignable for all taxa as this pertains to the Universal Core Motif in angiosperms (Liu & Schardl, 1994). *Leea* stem 1e* was not identified in the asterid models mentioned above or in the outgroups *Dillenia* and *Liquidambar*. However, 1e* is found in *Tetrastigma* (Miq.) Planch. (GenBank no. JN160886; not shown) and *Vitis* (Fig. 2B) of Vitaceae. Stem 1e* may also be present in the other genera of Vitaceae (*Cayratia* Juss., *Cissus* L. and *Clematicissus* Planch.), but the sequences obtained from GenBank (AF365985, AY998779 and AY037913, respectively) lack the last few bases necessary to determine the occurrence of stem 1e*. Stem 1e* was also not found in three other randomly chosen rosid taxa, complete ITS sequences of which were obtained from GenBank [*Aronia* Medik. sp. (EF127043, Rosaceae), *Elaeocarpus williamsianus* Guymer (DQ448691, Elaeocarpaceae) and *Quercus petraea* (Matt.) Liebl. (EU628558, Fagaceae)] and were folded using FEM in RNAstructure (secondary structure models available from the authors upon request).

In the structural model of ITS2 for *L. aequata* (Fig. 2C), six stems (2A, 2B, 2c*, 2c**, 2d*, 2D) were determined to be well supported (BP ≥ 90%). 2A, 2B and 2D were also identified in other eudicot taxa examined and may be homologous. 2c*, 2c** and 2d* do not correspond to stems found in outgroup taxa including *Tetrastigma* (not shown) and *Vitis* (Fig. 2D). However, when the ITS2 sequence of *L. asiatica* (L.) Ridsdale (*Suzuki et al.*, *9480014*) was folded using the same methodology, a stem allegedly corresponding to 2C of the other taxa was identified (not shown).
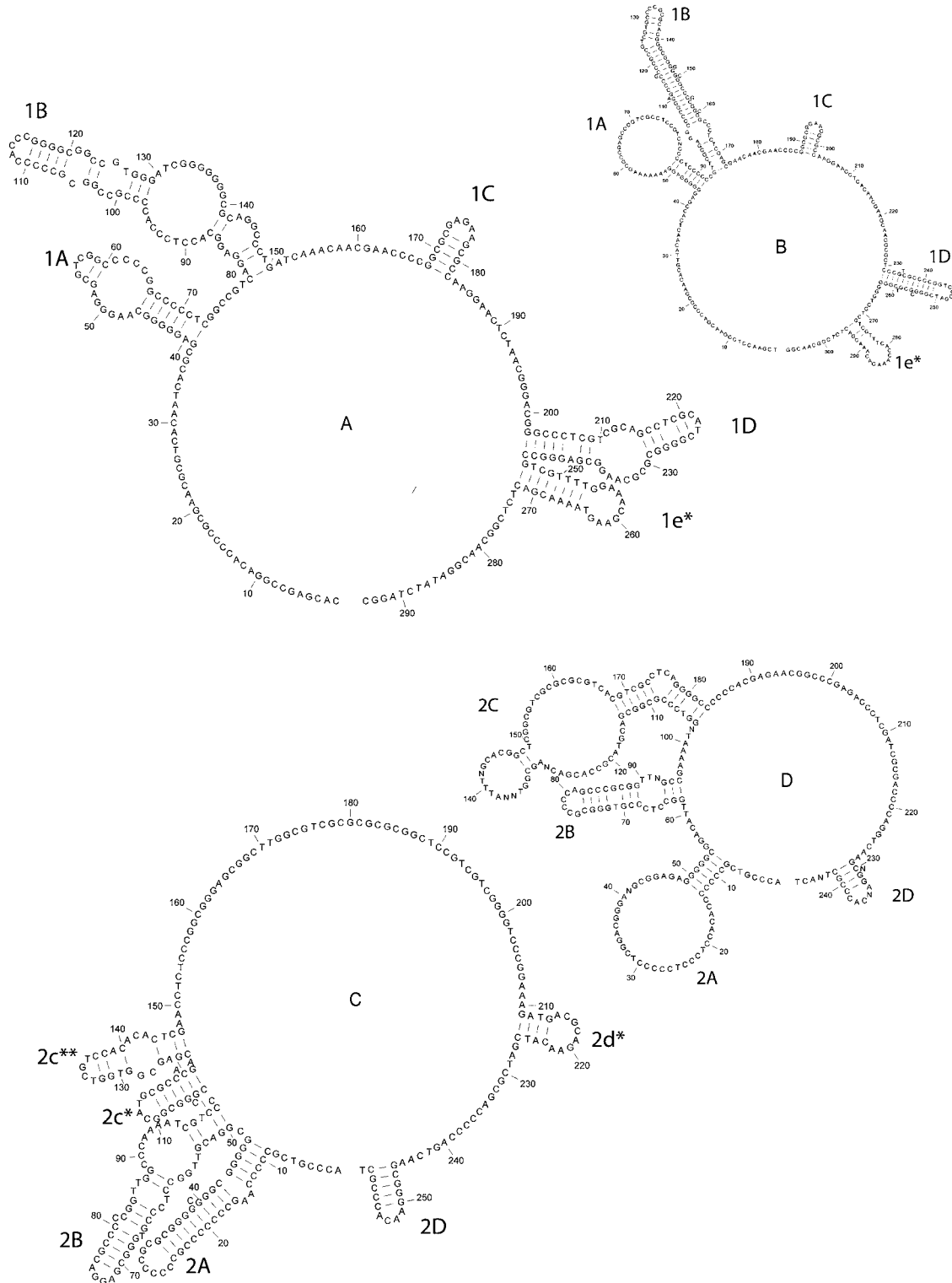
Figure 2. Structural models of ITS in *Leea aequata* and *Vitis vinifera*. Motifs homologous to stems in other eudicots are labelled following the annotations of Goertzen *et al*. (2003). Only base pairs with pairing probabilities ≥ 90% are shown. A, secondary structure model of *Leea aequata* ITS1. *Leea* stems 1A–1D are homologous to stems in other eudicots examined, except for stem 1e*, which was only present in Vitaceae. B, model of *Vitis* ITS1. C, model of *Leea* ITS2. *Leea* stems 2A, 2B and 2D are homologous to stems in other eudicots. *Leea aequata* 2c* and 2c** represent stems not homologous to stem 2C of *Anvillea* (Goertzen *et al*., 2003). D, model of *Vitis* ITS2.

Nonetheless, stem 2C was a hypervariable region that was difficult to align among the taxa examined, in spite of the comparatively similar positioning of this motif.

The structure of stem 2B is almost invariant across taxa, and is a helix interrupted by a universally conserved pyrimidine bulge (Mai & Coleman, 1997). In *L. aequata* (Fig. 2C), this bulge is made of unpaired TT-CT on opposite strands. The same nucleotides make up the bulge in *Tetrastigma*, *Vitis* L. (Fig. 2D) and *Liquidambar*, but in *Dillenia* the nucleotides are CC-CT. Stem 2D is relatively variable in nucleotide sequence compared with stem 2B, but was also easy to align after compensatory mutations were identified.

PHYLOGENETIC ANALYSES

The data matrix as well as all the phylogenetic trees in this study may be downloaded from Treebase (study ID 12167). Parsimony analysis in PAUP was conducted with coded gaps (Fig. 3) and without (results not shown). Gap coding slightly improved bootstrap support (BS) for the major clades and subclades. In the alignment that included coded gaps, 383 sites belonged to ITS1, of which 26.6% was missing data and 226 were potentially parsimony-informative characters (PIC). ITS2 consisted of 396 sites, of which 45.2% was missing data and 96 PIC. In total, 513 sites of the alignment belonged to 5S-NTS with 51.8% as missing data and 264 PIC.

The concatenated alignment of 1375 sites had 454 sites (33%) that were constant and 623 (45%) PIC. The most-parsimonious trees from the concatenated ITS + 5S-NTS dataset were 2491 steps in length, with a consistency index (CI) of 0.58 and a retention index (RI) of 0.80. Convergence to the desired posterior distribution in the Bayesian analyses was achieved after 2.5 million generations, with the standard deviation of split frequencies < 0.01. Moreover, ESS for all sampled parameter values in MrBayes was > 300, suggesting reasonable sampling of independent data points. Trace plots for all sampled parameters also did not show sharp fluctuations indicative of good mixing or low autocorrelation among samples.

Leea is monophyletic with four major clades (I–IV) in the parsimony (Fig. 3) and Bayesian (Fig. 4) results. However, the topologies differ in the placement of major clades with Clade II recovered as sister to Clade III in the parsimony result with relatively strong support (87% BS), but shown as part of a trichotomy with clades III and IV in the Bayesian consensus topology (Fig. 4).

MORPHOLOGICAL CHARACTER ANALYSES

Reconstructed ancestral states are only presented for well-supported nodes [posterior probability (PP)

≥ 0.95] and if all the node-containing trees possessed this ancestral state at those nodes (Fig. 4). Similar flower colour, which Ridsdale (1974, 1976) considered a salient taxonomic feature, is homoplastic, except for the yellow corolla lobe color of African species (character 10, Fig. 4). Green corolla lobe colour (G) is plesiomorphic (Fig. 4). White corolla colour (W) evolved twice in clades III and IV, whereas red corolla colour (R) evolved twice in clade IV. Ridsdale (1974, 1976) emphasized the diagnostic value of stipule shape in species identifications and provided detailed illustrations of these in his revisions, with those of *L. indica* and *L. guineensis* encompassing a variety of stipule morphologies. However, these cannot be directly assessed on herbarium specimens as most stipules are deciduous, but the ratio of stipule scar length to petiole length (SC/PT, Fig. 1E) was measurable. A stipule scar measuring ≥ 0.8 of the entire length of the petiole is a synapomorphy for clade I (Fig. 4, character 1), as is an SC/PT < 0.5 for Clade IV (character 9).

In both parsimony (Fig. 3) and Bayesian (Fig. 4) results, Clade I is represented by *L. asiatica sensu* Ridsdale (1974; including *L. aspera* Wall. ex Roxb.). It is the sister to the rest of the genus, a well-supported position in both analyses. Of the morphological characters examined, SC/PT ≥ 0.8 is unique to this group. Clades II + III + IV have fused stamens (Fig. 4, inset, character 2) in contrast to Clade I, which has the plesiomorphic feature of free stamens from the common ancestor of Vitales. Clade II includes *Leea* spp. that bear prickles, which is a distinct synapomorphy (character 3 in Fig. 4).

Clade III includes *Leea* spp. that possess longer, thicker flowers (> 4 mm long in dried material; Fig. 4, character 4), > 30% longer than the average in non-clade III members, and relatively larger fruits (fruit width > 14 mm, character 5). In clade III, the New Guinea species and the Philippine species each form monophyletic groups and are sisters. The lack of seeds and fruits in most specimens precluded detailed studies of these, but based on mapping Ridsdale's (1974, 1976) descriptions of the endosperm, complex endosperm rumination [i.e. highly uneven endosperm surface due to ingrowths of surrounding tissues (Bayer & Appel, 1996)] evolved in the ancestor of the New Guinea and Philippine species of Clade III (character 6 in Fig. 4). Of the New Guinea species, *L. gonioptera* Lauterb. and *L. zippeliana* Miq. have unifoliolate leaves; the other species are strictly pinnate. The Philippine species are distinctive in having tetramerous flowers (character 7 in Fig. 4) as elsewhere in the genus, flowers are pentamerous.

Clade IV contains the species complexes *L. indica* and *L. guineensis*, neither of which is monophyletic, and the distinct morphospecies *L. aequata*,
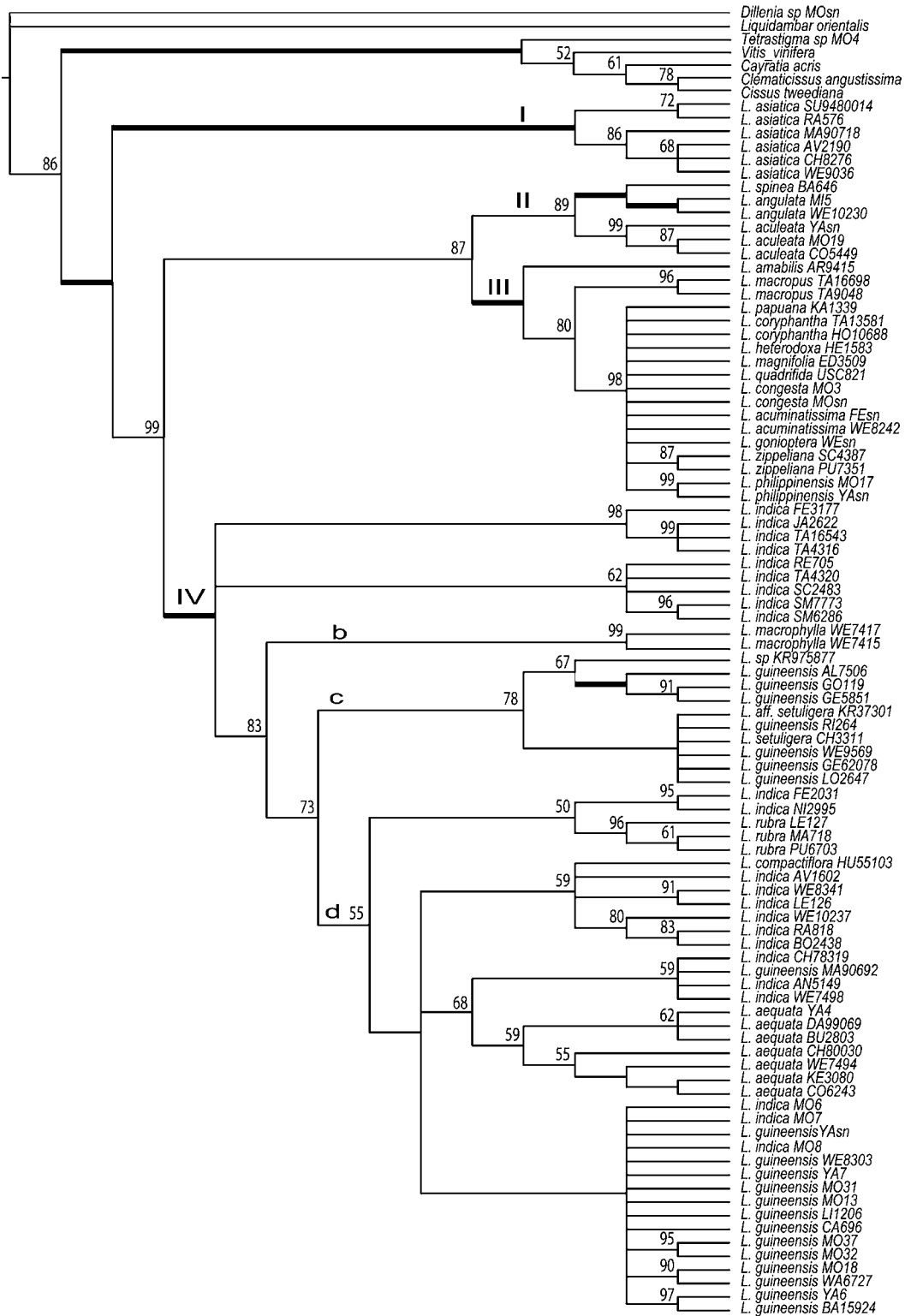
Figure 3. Strict consensus tree from parsimony analysis. Terminals are labelled with names according to Ridsdale (1974, 1976) and include collection information for the accessions (first two letters of the first collector's last name and collection number; see Table 1). Bold lines represent nodes with 100% bootstrap support (BS). Numbers above branches include only BS values 50% or higher. Roman numerals indicate major clades of *Leea* (I–IV), and b, c and d indicate subclades in Clade IV. Subclade IVa (compare with Fig. 4) is not recovered as monophyletic and is not labelled.
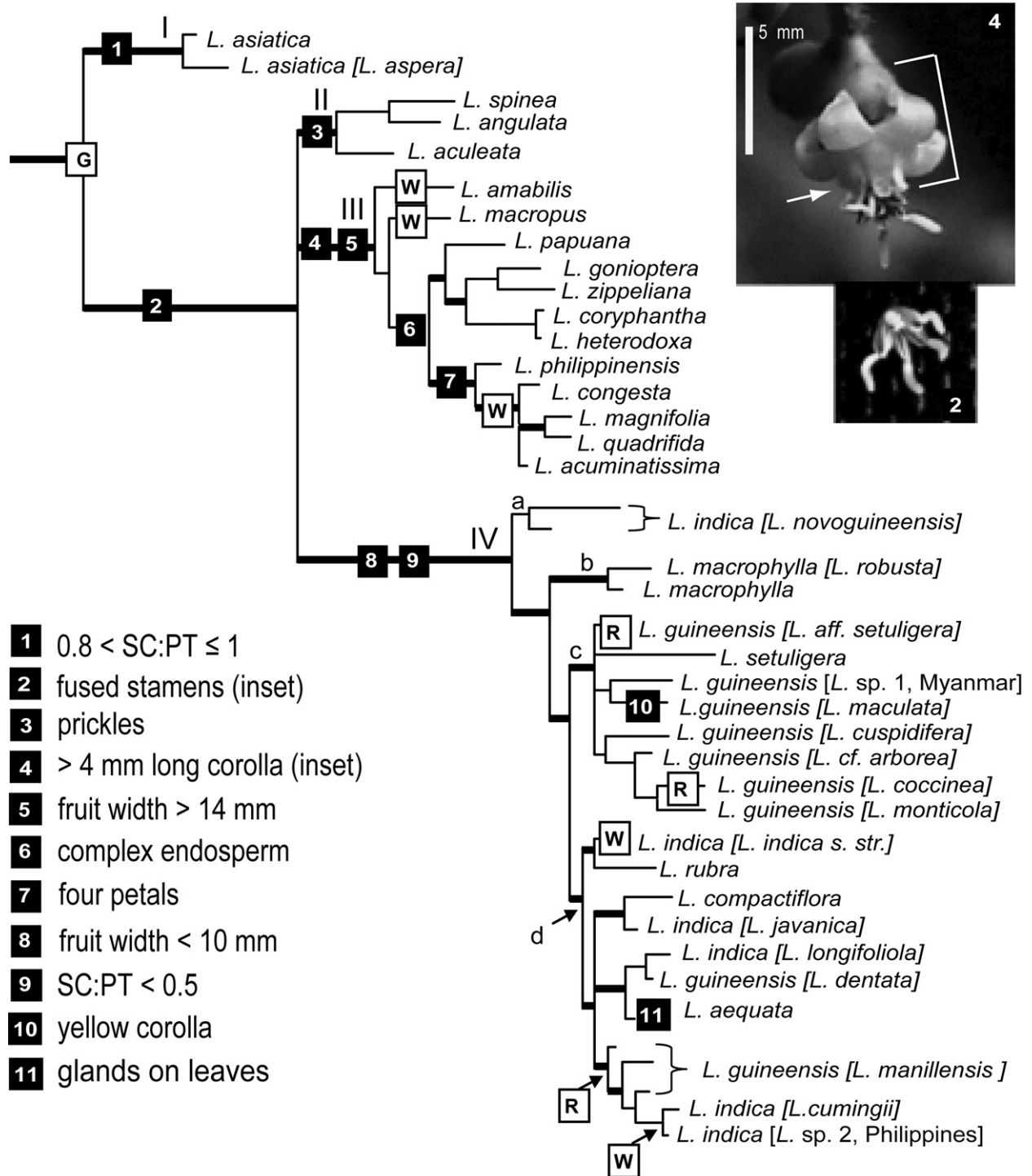
Figure 4. Majority rule consensus tree from Bayesian analysis (MrBayes). Accessions belonging to the same species were collapsed such that each species is represented by one terminal. Terminals are labelled with names according to Ridsdale (1974, 1976) followed by resurrected species names and putative new species in brackets (compare with Table 1). Bold lines represent nodes with ≥ 0.95 posterior probability (PP). Ancestral states of morphological characters reconstructed using Mesquite are mapped. Only ancestral states for well-supported nodes (PP ≥ 0.95) are presented, and in the case of discrete traits, only unequivocal states are presented (i.e. all node-containing trees that have this ancestral trait at those nodes). Corolla colour (G, green; R, red; W, white) was also mapped to illustrate how homoplastic this trait is. Insets: 4, how corolla length was measured (in bracket), with the floral tube marked with an arrow; 2, staminal fusion in *Leea* spp.

L. compactiflora Kurz, *L. macrophylla* Roxb. ex Hornem., *L. rubra* and *L. setuligera* C.B.Clarke. In the Bayesian consensus tree, four subclades (a–d) occur in Clade IV (Fig. 4), of which only subclade IVa was not recovered in the parsimony analysis (Fig. 3). Species in clade IV share relatively smaller fruits (< 10 mm wide in dried specimens; character 8 in Fig. 4). *Leea aequata* is distinguishable from *L. indica s.l.* in possessing large pearl glands on the underside of the leaf surface (character 11 in Fig. 4). *Leea compactiflora* is similar vegetatively to both *L. indica* and *L. guineensis*, but differs in having large conspicuous bracts on its flowers. The reddish-flowered *L. rubra* is separated from *L. guineensis* by its distinctive wing-like stipules that occupy the entire petiole. Stipules in *Leea guineensis s.l.*, in contrast, are obovate in shape and do not span the whole petiole length.

MOLECULAR DATING AND BIOGEOGRAPHICAL ANALYSES

Ancestral area reconstruction using parsimony and likelihood methods yielded similar results. For certain nodes where there is a conflict between methods in the inferred ancestral range, the area with the highest probability from the alternative method is shown. The ancestor of Vitales originated during the Early Cretaceous but its distribution at that time is unclear. The origin of *Leea* was placed in Indochina (D) by S-DIVA in the Late Cretaceous (72.05 Mya, 95% HPD: 65.00–86.19 Mya, Fig. 5, node 1). Clade I originated from Indochina in the Miocene (node 2). Clades II, III and IV originated in the Oligocene (nodes 3, 4 and 6). Clade II + III shared a West/Central Malesian (W) ancestor (node 5) in the Eocene that diversified in Malesia (W, G and P). A long-distance dispersal across the Indian Ocean to Madagascar (A) also occurred in Clade II during the Miocene (node 7). The ancestor of Clade III encompassed West/Central Malesia and Australasia (node 4). Clade IV (node 6) has an ancestral range encompassing the disjunct Indochina (D) and Australasia (G). Of the four subclades of Clade IV, three originated in Indochina (nodes 9, 10 and 11) then dispersed independently to Africa/Madagascar (A) and parts of Malesia (W, P). One subclade was inferred to have originated in Australasia (node 8).

DISCUSSION

PHYLOGENETIC UTILITY OF ITS SECONDARY STRUCTURE

The ubiquitous presence of stems 1A–1D and 2A–2D in phylogenetically distant rosid plant groups and in *Leea* suggests that these secondary structures have been evolutionarily constrained across many plant groups. These conserved motifs facilitate homology assessments despite nucleotide differences during sequence alignment. These stems were also identified in asterid taxa from Gentianaceae (Molina & Struwe, 2009) and Asteraceae (Goertzen *et al.*, 2003).

However, among the rosid taxa examined, stem 1e* was not present but occurs in Vitaceae, including *Leea*, suggesting that this may be a conserved motif unique to Vitales. Further comparative sequence analyses are necessary to confirm this. The *Leea* pyrimidine bulge in stem 2B was also found in the phylogenetically distant Gentianaceae (Molina & Struwe, 2009), suggesting its universality in angiosperms. Stem 2C *sensu* Goertzen *et al.* (2003) was not supported in *L. aequata*. This particular stem may not be under the same selective pressure as the other more conserved motifs such as stems 1C, 1D, 2B and 2D. Nonetheless, the presence of more evolutionarily conserved stems provided useful anchor points to guide the alignment, which was difficult based on sequence identity alone. This was also shown by Molina & Struwe (2009) in Gentianaceae, in which the secondary structure-guided alignment produced a comparatively more accurate phylogenetic tree compared with alignments produced by ClustalX (Thompson *et al.*, 1997) and MAFFT (Katoh *et al.*, 2002). This underlies the utility of ITS secondary structures in resolving phylogenies, not only in closely related plant taxa, but even across all eukaryotes (Coleman, 2009).

MOLECULAR PHYLOGENY AND TAXONOMY

Parsimony and Bayesian analyses resolved the phylogeny into four major clades, and were concordant in the groupings of taxa. Clade I is composed of two subclades (Figs 3, 4). One subclade morphologically corresponds to *L. crispa* L. (leaves coriaceous, glabrous above), and the other corresponds to *L. aspera* (leaves membranous, scabrous with white appressed hairs). These two species were both synonymized with *L. asiatica* by Ridsdale (1980) because of intermediate forms. The combination of greenish flowers, a narrow wing-like stipule traversing the length of the petiole (SC/PT > 0.8), moderately large fruits (*c.* 12 mm wide) and extremely serrate leaflets with strongly pinnate venation taxonomically distinguishes members of Clade I from other species.

The relationships among clades II, III and IV are ambiguous based on the Bayesian (MrBayes) analysis, although the monophyly of clades II and III is moderately supported with 87% BS (Fig. 3). Clade II includes three prickle-bearing species from Malesia (*L. aculeata* Blume, *L. angulata*), Madagascar and Mayotte (*L. spinea*) (Fig. 4). Species of Clade III (Fig. 4) possess generally longer and more coriaceous
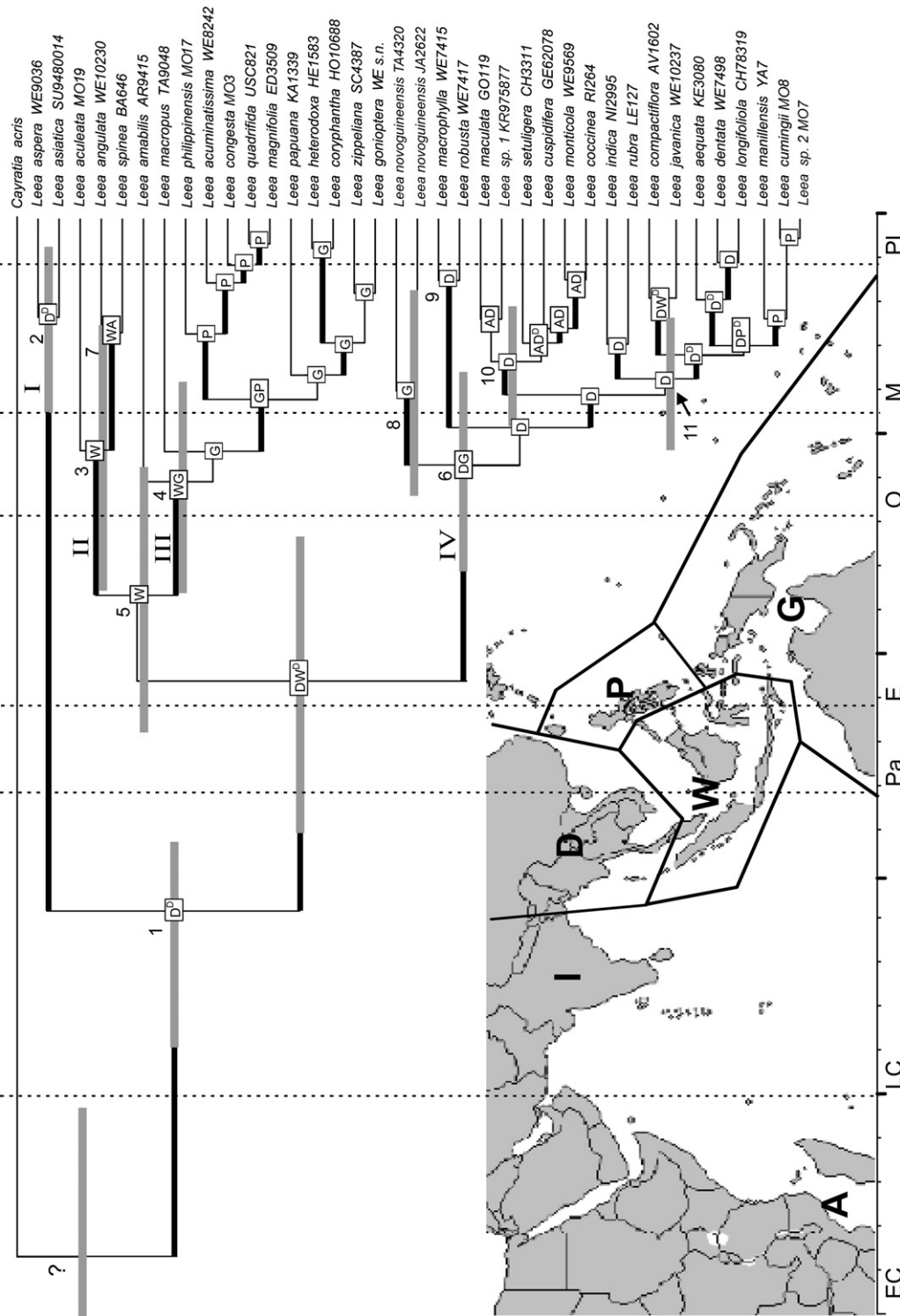
Figure 5. Dated maximum clade credibility tree from BEAST using one accession per species. Taxon names follow new classifications and include collection information for the accessions (first two letters of the first collector's last name and collection number; see Table 1). Bold lines represent nodes with 0.95 posterior probability or higher. Ancestral areas inferred by S-DIVA and/or Lagrange are shown in boxes and are superimposed on nodes: (A) tropical Africa/Madagascar and adjacent islands (Mauritius, Mayotte, Comoros); (I) India; (D) Indochina including tropical China; (W) West/Central Malesia (G) Australasia; (P) Philippines. Only the most supported ancestral areas from both methods are presented. In the case of conflicting ancestral ranges between methods, the inferred area with the higher probability from the alternative method is shown and indicated with a superscript (D, DIVA; L, Lagrange). Numbered nodes are discussed in the text. Geological periods are delineated by dashed lines (EC, Early Cretaceous; LC, Late Cretaceous; Pa, Palaeocene; E, Eocene; O, Oligocene; M, Miocene; Pl, Pliocene). Grey bars below inferred areas represent the 95% highest posterior density (HPD) for divergence dates of major nodes.

flowers than species of other clades. However, longer corollas may not be strictly confined to Clade III, as it also occurs in other island taxa such as *L. tinctoria* (São Tomé) and *L. grandifolia* (Nicobar and Andaman Islands), although they may not be closely related to members of Clade III because of their small fruit size (< 10 mm wide) and low SC/PT (< 0.5), but this needs to be phylogenetically tested. The Philippine *L. unifoliata* was not sampled but probably belongs to Clade III by virtue of its tetramerous corolla, which is unique among the Philippine species in this clade (Fig. 4, character 7). Although *L. tetramera* of the Solomon Islands was not included in the analysis, its morphological similarity (> 6 mm corolla, fruit width > 18 mm) to *L. macropus* K.Schum. & Lauterb. of the Bismarck Archipelago suggests a possible evolutionary affinity with the latter (Fig. 4).

Clade IV is predominantly composed of the morphologically homogeneous *L. indica/L. guineensis* complex, but also includes the morphologically distinct *L. aequata*, *L. compactiflora*, *L. macrophylla*, *L. rubra* and *L. setuligera* interdigitated among accessions of *L. indica* and *L. guineensis sensu* Ridsdale (Figs 3, 4). Even *L. macrophylla sensu* Ridsdale (1976) is a species complex, as he combined four morphological entities into this complex because they possess an essentially similar floral structure, in spite of variations in leaf indument and leaf pinnation, from strictly unifoliolate (*Wen 7415*, i.e. *L. macrophylla s.s.*) to pinnate (*Wen 7417*, i.e. *L. robusta* Roxb.).

The apparent polyphyly of *L. indica* and *L. guineensis* calls for a revision of current species circumscriptions. According to Ridsdale (1976), *L. indica* and *L. guineensis* are *only* distinguishable by flower colour with *L. guineensis* possessing red or reddish flowers and *L. indica* having white or cream or even greenish flowers. However, both red and white flowers have originated multiple times in independent lineages (Fig. 4). Ridsdale (1976: 778) admitted that his taxonomic circumscriptions resulted in species complexes that encompass a 'wide range of variability, both geographically and ecologically'. Even Ridsdale was confused in his own identification of non-flowering duplicates of a Philippine specimen by *Fenix 24980* (UC and US), which he annotated independently as representing *L. indica* and *L. guineensis*.

Leea guineensis was originally described by Don (1831) based on a collection from the West African country of Guinea. Although Don did not describe flower colour, Ridsdale (1974, 1976) redefined *L. guineensis* to represent red-flowering morphospecies from a large geographical area spanning tropical Africa to Asia. As our sampling did not include accessions from Guinea, we cannot assign this name for any of the red-flowered accessions included in our analyses identifiable as *L. guineensis s.l.* based on Ridsdale's key, but the other accessions are aptly given resurrected names (Fig. 4).

Descoings (1959) revised *Leea* of Madagascar and came up with seven infraspecific classifications (six forms and one variety) under *L. guineensis*, including f. *monticola* Desc. and var. *cuspidifera* Baker, which we both elevate here to species rank and correspond to *WE9569* and *GE62078*, respectively. One of us (J.W.) has seen *L. cuspidifera* Baker and *L. monticola* Desc. in the field and attests to their species status. *Leea cuspidifera* was treated as a variety of *L. guineensis* by Descoings (1967) and Ridsdale (1974). The taxon is morphologically distinctive by its pilose to pubescent lower leaflet surface, smaller and thinner leaflets, cuspidate leaflet apex and three- to four-pinnate (vs. two- to three-pinnate) leaves in comparison with *L. guineensis s.l.*. *Leea monticola* was considered as a form of *L. guineensis* by Descoings (1967). *Leea monticola* is a slender to scrambling shrub and has relatively small leaflets. The teeth are fewer and finer in comparison with those of *L. guineensis s.l.*.

We also resurrect *L. coccinea* Baker, which is the most popular *Leea* for its use in horticulture (commonly known as West Indian holly in the United States), originally described from a collection in Myanmar. It is distinct in having coriaceous leaves with repand and mildly serrate margins. This appears to be different from the collection from Myanmar (*Leea* sp. 1, *Kress 97–5877*), which we could not identify because of the lack of reproductive structures. We also could not precisely determine the Mauritius specimen *LO2647*, as the BM type of *L. arborea* Telf. ex Wight & Arn., the sole *Leea* from Mauritius, has more of a crenate–serrate margin, whereas *LO2647* has a serrulate margin (i.e. small, sharp, forward-pointing teeth). Discrimination of *LO2647* as different (or identical) from *L. arborea* was prohibited by the scarcity of collections from Mauritius. Thus, we refer to it as *L.* cf. *arborea* in the Bayesian consensus tree (Fig. 4) to draw more attention to this taxon.

Leea indica was described by Burman (1768) as *Staphylea indica* Burm.f., but Merrill (1919) moved it to *Leea*. Ridsdale (1974, 1976) later identified many light-flowered accessions collected from all over Indomalaya and the Pacific Islands as *L. indica*. We redefine *L. indica* to include only accessions with light-coloured flowers from India and Indochina with pinnate glabrous leaflets. We predict that increased sampling of accessions from SE Asia may expand the geographical distribution of *L. indica*.

We assign *L. novoguineensis* Val., included by Ridsdale (1974, 1976) in *L. indica*, to accessions in subclade IVa (Fig. 4), flowers of which are light-coloured (i.e. cream, greenish-white), with multi-pinnate

leaves, small flowers (< 4 mm long), small fruits (< 12 mm wide, dry) and with SC/PT < 0.5, distributed in Australasia. The substantial genetic differences among the accessions of *L. novoguineensis* in this subclade and their wide geographical range could suggest that this may also be a species complex that may be resolved with phylogeographical analysis.

In subclade IVd, based only on herbarium specimens the India-restricted *L. indica* is almost indistinguishable from accessions collected in Vietnam (*AV1602*), Philippines (*BO2438*), Sulawesi (*RA818, WE10237*), Malaysia (*WE8341*) and Singapore (*LE126*), which form a distinct clade, which we refer to here as *L. javanica* Blume. This species only varies from *L. indica s.s.* in fruit size where the latter is 9 mm wide (dried) vs. 6 mm wide (dried) in *L. javanica*. However, we believe that herbarium specimens examined do not capture species differences, and that more field studies are needed. More collections are also needed to ascertain species status of *L. dentata* Craib from Thailand (represented by *MA90692, AN5149*), which is allied to *L. longifoliola* Merr., a species described from the island of Hainan, China. We resurrect *L. longifoliola* here, as it is distinct from other species in having coriaceous leaves disproportionately longer than wide compared with the typical leaf length/width ratio of 2.2–3.8 in *Leea*.

Philippine accessions within subclade IVd form a monophyletic group (Figs 3,4). We have decided to subsume 13 of these accessions, corresponding to previously described species, in *L. manillensis* Walp. from a type collected in Manila, Philippines, in spite being collected from different islands (including Taiwan and Palau), because they all have the same corolla colour (red) with overlapping vegetative morphologies, as seen from herbarium specimens and as observed in the field by one of us (J.M.). This may be an example of cryptic speciation, and additional data may be able to clarify relationships in this group. However, in *L. manillensis*, we recognize two other species, *L. cumingii* C.B.Clarke and a putative new species, *Leea* sp. 2, making *L. manillensis* paraphyletic, which we think is valid due to incomplete lineage sorting from recent speciation. Both species deserve species status for their white flowers (vs. red flowers in *L. manillensis*). In the field they attract a different set of pollinators (Molina, 2009).

Leea cumingii was combined with *L. guineensis s.l.* by Ridsdale (1974, 1976). It is distinct from any other *Leea* in having extremely pubescent leaf and petiole indument ('rufous-shaggy' in protologue). Although *L. cumingii* and *Leea* sp. 2 cluster together in the molecular analyses (Figs 3, 4 in subclade IVd), they differ substantially morphologically. *Leea* sp. 2 has a short stature (< 1 m, suffrutescent herb), glabrous stipules (Fig. 1C) and glabrous leaves, which contrast

with those of the sympatric *L. cumingii*, which is a small pubescent tree (3–6 m) with pubescent stipules (Fig. 1D). The calyx of *Leea* sp. 2 is pale green and does not exhibit the reddish tinge characteristic of *L. cumingii*. Such detailed flower colour, which may represent an important evolutionary trait, is often missing from herbarium notes. One of us (J.M.) believes that these two species possibly hybridize because of their concurrent flowering phenologies and similar pollinator assemblage and the discovery of a population in which individuals are intermediate in morphology (Molina, 2009).

It must be clarified that flower colour is a composite of corolla lobe colour and floral tube colour (see Fig. 4), which are often different. For example, *L. aculeata* has a white floral tube surrounded by light green petals whereas *L. manillensis* (included in *L. guineensis* by Ridsdale, 1976) has red petals enclosing a white floral tube. The colour of the calyx, which can be observed first as it envelops the bud, does not necessarily translate to petal colour, such that in *L. rubra* the bright red calyx subtends the pale-orange petals and whitish floral tube (*Pullen 6703; Specht 1305*). This may not have been realized by Ridsdale (1974, 1976) as some herbarium sheets noted flower colour as 'red buds', which he then assumed to be *L. guineensis*. One morphospecies of *L. guineensis s.l.* (=*L. cumingii*) in the Philippines possesses white petals, but the calyx enclosing the corolla buds is tinged with red (J. Molina, pers. observ.).

Ridsdale's simple dichotomy of corolla colour into red and white becomes untenable as some herbarium sheets have collection notes that describe *Leea* flowers for the same species with a wide range of colours. For example, flower colour in *L. rubra* was independently noted as: 'maroon', 'red calyx, pale orange petals', 'creamy white' and 'red corolla, cream inside, corolla and stamens pale cream'. In *L. philippinensis* Merr., flower colour was reported as: 'green and white', 'white', 'pale yellow', 'cream', 'flowers pink with green petals'. Such variations in flower colour also lend support to the natural variation of this trait and its unreliability in circumscribing *Leea* spp., and this is evident in Figure 4 where the same corolla colour appears multiple times in non-monophyletic lineages, except for yellow corollas, which evolved once in subclade IVc, i.e. only among African species.

Phylogenetic resolution from molecular markers was crucial in exposing the polyphyly of some previous species definitions in *Leea*. Thus, taxonomic circumscriptions should ideally not depend solely on morphology because of some intrinsic problems such as phenotypic plasticity and/or convergent evolution. Some cryptic species, such as *L. guineensis s.s* and *L. indica s.s.*, which are supported by DNA as

distinct, but are not morphologically distinguishable based on herbarium specimens, may in fact be an artefact of inadequate taxonomic investigation, such that the suite of morphological and anatomical characters that can potentially provide taxon-specific characters remain to be discovered. As morphological differences between species are more conspicuous in their natural habitat, revisionary studies of *Leea*, or of any other taxonomically challenging group, must be supplemented with detailed colour studies of reproductive structures and information on habit and ecology.

BIOGEOGRAPHY

Several workers (Wikström, Savolainen & Chase, 2001; Magallón & Castillo, 2009; Wang *et al.*, 2009) have dated the Vitales/rosid split to the Cretaceous (88–119 Mya), which could mean that the *Leea* stem lineage may have already been in place as early as the Late Cretaceous, after the Gondwanan landmasses had already separated (Ali & Aitchison, 2008). Chen (2009: 193) predicted that Vitales (Vitaceae *s.l.*) originated 'from tropical equatorial or southern lands' (vs. northern temperate areas), but our data could not verify this at this time.

The age of the *Leea* crown group based on molecular dating was Late Cretaceous (72.05 Mya, 95% HPD: 65–86.19 Mya). The oldest *Leea* fossils are from the early Eocene of Peru (Berry, 1929; Chen, 2009) and the early Palaeocene Deccan traps of India (Prakash & Dayal, 1964), suggesting that the ancestor of *Leea* may have originated somewhere on Gondwana. Although not currently supported by our analyses, it is possible that the *Leea* crown group or its stem lineage evolved in Gondwana before the Palaeocene, and perhaps reached Laurasia via the Indian plate which collided with Laurasia sometime in the late Eocene (Ali & Aitchison, 2008). This is also known as the out-of-India hypothesis (McKenna, 1973), which has been corroborated by biogeographical patterns of plant taxa such as Crypteroniaceae (Conti *et al.*, 2001; Rutschmann *et al.*, 2004), Dipterocarpaceae (Dayanandan *et al.*, 1999), *Sterculia* L., *Grewia* L., *Polyalthia* Blume, *Gomphandra* Wall. ex Lindl., *Lophopetalum* Wight ex Arn., *Syzygium* P.Browne ex Gaertn. and *Sonneratia* L.f. (Bande, 1992; Morley, 2000). These tropical taxa are now largely confined to the Malesian region, having been extirpated by Neogene aridification in India (Morley, 2000).

An alternative scenario, and one that is supported by our results, is the origin of the crown group in Indochina, spreading to India, West/Central Malesia and Australasia, to account for the inferred distributions of Clade I, Clade II + III and Clade IV, respectively. However, the *Leea* fossil exhumed from the Eocene of Peru becomes difficult to account for with this scenario.

Regardless of the origin of *Leea*, it is noteworthy that African/Malagasy *Leea* spp. are embedded in Asian clades, suggesting long-distance dispersal from Asia as the timing of the split (mid Miocene) does not fit a Gondwanan vicariance event. This trend has also been illustrated by Renner, Clausing & Meyer (2001) in some members of Melastomataceae, by Kulju *et al.* (2007) in *Mallotus* and *Macaranga* (Euphorbiaceae) and by Warren *et al.* (2010) in several plant and animal taxa.

Disjunct distributions, as inferred for the ancestor of Clade IV, simultaneously being present in both Indochina and Australasia, but not in the intervening West Malesian region may be an artefact of inadequate taxon and geographical sampling. Inclusion of data from the Malayan–Sumatran *L. simplicifolia*, and from other unsampled cryptic species in the intervening regions of Java, Sulawesi, Lesser Sunda islands and Moluccas, may confirm if this is a real disjunction. Alternatively, extinction of a putative Indochinese or West Malesian ancestor may have also brought about this disjunct ancestral range.

By the mid Miocene, warm and moist conditions throughout Indomalaya allowed a proliferation of mixed warm temperate and paratropical forests as far as Japan (Morley, 1998). This allowed *Leea* to expand its distribution, which would explain the fossil wood of *L. eojaponica* collected in the Miocene deposits of Simane, Japan (Watari, 1951). It was also in the mid Miocene that large areas of the Philippines and New Guinea had been uplifted (Hall, 1998; Steppan *et al.*, 2003), encouraging the diversification of Clade III, which is endemic to these areas. Clade IV had also undergone rapid radiation, perhaps facilitated by the ecological novelties presented by the Indomalayan region during the Neogene (Hall, 1998, 2002, 2009; Morley, 1998, 2000). During this time, populations were repeatedly fragmented by high sea stands (*c.* 100 m above present-day levels), which was often accompanied by warmer, wetter weather that promoted rainforests (Morley, 1998, 2000; Woodruff, 2003). Morphological similarity of cryptic *Leea* spp. may have been a consequence of strong stabilizing selection on optimal phenotypes that were well adapted to fluctuating environments (Sheldon, 1996; Sotuyo *et al.*, 2007). The dynamic geological and environmental changes experienced by Neogene Indomalaya, such as the emergence of many oceanic islands, plate convergence, sea-level fluctuations and alternating wet and dry climates following the Himalayan uplift (Hall, 1998; Woodruff, 2003), may have driven the rapid radiation and the great diversity of *Leea* in this region.

REFERENCES

Ali JR, Aitchison JC. 2008. Gondwana to Asia: plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166–35 Ma). *Earth-Science Reviews* **88:** 145–166.

Allègre CJ, Birck JL, Capmas F, Courtillot V. 1999. Age of the Deccan traps using ^{187}Re–^{187}Os systematics. *Earth and Planetary Science Letters* **170:** 197–204.

APG. 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* **85:** 531–553.

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141:** 399–436.

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161:** 105–121.

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. 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.

Bande MB. 1992. The Paleogene vegetation of peninsular India (megafossil evidences). *Palaeobotanist* **40:** 275–284.

Bayer C, Appel O. 1996. Occurrence and taxonomic significance of ruminate endosperm. *Botanical Review* **62:** 301–310.

Bellarosa R, Simeone MC, Papini A, Schirone B. 2005. Utility of ITS sequence data for phylogenetic reconstruction of Italian *Quercus* spp. *Molecular Phylogenetics and Evolution* **34:** 355–370.

Berry EW. 1929. An Eogene tropical forest in the Peruvian desert. *Proceedings of the National Academy of Sciences USA* **15:** 345–346.

Bickford D, Lohman DJ, Sodhi NS, Ng PK, Meier R, Winker K, Ingram K, Das I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22:** 148–155.

Burman NL. 1768. *Flora Indica*. Amsterdam: Cornelium Haek. & Johannem Schreuderum.

Campbell CS, Wrighta WA, Coxa M, Vining TF, Majorc CS, Arsenault MP. 2005. Nuclear ribosomal DNA internal transcribed spacer 1 (ITS1) in *Picea* (Pinaceae): sequence divergence and structure. *Molecular Phylogenetics and Evolution* **35:** 165–185.

Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price RA, Hills HG, Qiu Y-L, Kron KA, Rettig JH, Conti E, Palmer JD, Manhart JR, Sytsma KJ, Michaels HJ, Kress WJ, Karol KG, Clark WD, Hedrén M, Gaut BS, Jansen RK, Kim K-J, Wimpee CF, Smith JF, Furnier GR, Strauss SH, Xiang Q-Y, Plunkett GM, Soltis PS, Swensen SM, Williams SE, Gadek PA, Quinn CJ, Eguiarte LE, Golenberg E, Learn GH Jr, Graham SW, Barrett SCH, Dayanandan S, Albert VA. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL. Annals of the Missouri Botanical Garden* **80:** 528–580.

Chen I. 2009. History of Vitaceae inferred from morphology-based phylogeny and the fossil record of seeds. PhD Thesis, University of Florida.

Clarke CB. 1881. A revision of the Indian species of *Leea. Journal of Botany* **19:** 100–106. 135–142, 163–167.

Coleman AW. 2009. Is there a molecular key to the level of 'biological species' in eukaryotes? A DNA guide. *Molecular Phylogenetics and Evolution* **50:** 197–203.

Conti E, Eriksson T, Schonenberger J, Sytsma KJ, Baum DA. 2001. Early Tertiary out-of-India dispersal of Crypteroniaceae: evidence from phylogeny and molecular dating. *Evolution* **56:** 1931–1942.

Cronquist A. 1981. *An integrated system of classification of flowering plants*. New York: Columbia University Press.

Dayanandan S, Ashton PS, Williams SM, Primack RB. 1999. Phylogeny of the tropical tree family Dipterocarpaceae based on nucleotide sequences of the chloroplast *rbcL* gene. *American Journal of Botany* **86:** 1182–1190.

Descoings B. 1959. Revision des *Leea* de Madagascar. *Mémoires de l'Institut Scientifique de Madagascar, Série B, Biologie Végétale* **9:** 1–34.

Descoings B. 1967. *Leea*cées. *Flore de Madagascar et des Comores* **124 bis:** 1–7.

Don G. 1831. *Leea guineensis*. In: Don G, ed. *A general history of the dichlamydeous plants*, *Vol. 1*. London: Rivington, 712.

Drummond AJ, Ho S, Rawlence N, Rambaut A. 2007. *A rough guide to BEAST 14*. Available at: http://beast.bio.ed.ac.uk

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7:** 214.

Dumortier BCJ. 1829. *Analyse des familles de plantes*. Tournay: J. Casterman.

Gerrath JM, Lacroix CR, Posluszny U. 1990. The developmental morphology of *Leea guineensis*. II. Floral development. *Botanical Gazette* **151:** 210–220.

Goertzen L, Cannone J, Gutell R, Jasen R. 2003. ITS secondary structure derived from comparative analysis: implications for sequence alignment and phylogeny in the Asteraceae. *Molecular Phylogenetics and Evolution* **29:** 216–234.

Gottschling M, Hilger HH, Wolf M, Diane N. 2001. Secondary structure of the ITS1 transcript and its application in a reconstruction of the phylogeny of Boraginales. *Plant Biology* **3:** 629–636.

Hall R. 1998. The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall R, Holloway JD, eds. *Biogeography and geological evolution of South East Asia*. Leiden: Backhuys Publishers, 99–131.

Hall R. 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions and animations. *Journal of Asian Earth Sciences* **20:** 353–434.

Hall R. 2009. Southeast Asia's changing palaeogeography. *Blumea* **54:** 148–161.

Hilu KW, Borsch T, Müller K, Soltis DE, Soltis PS, Savolainen V, Chase MW, Powell M, Alice LA, Evans R, Sauquet H, Neinhuis C, Slotta TA, Rohwer JG, Campbell CS, Chatrou L. 2003. Angiosperm phylogeny based on *matK* sequence information. *American Journal of Botany* **90:** 1758–1776.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17:** 754–755.

Ingrouille MJ, Chase MW, Fay MF, Bowman D, Van der Bank M, Bruijn A. 2002. Systematics of Vitaceae from the viewpoint of plastid *rbcL* DNA sequence data. *Botanical Journal of the Linnean Society* **138:** 421–432.

Jansen RK, Kaittanis C, Lee SB, Saski C, Tomkins J, Alverson AJ, Daniell H. 2006. Phylogenetic analyses of *Vitis* (Vitaceae) based on complete chloroplast genome sequences: effects of taxon sampling and phylogenetic methods on resolving relationships among rosids. *BMC Evolutionary Biology* **6:** 32.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30:** 3059–3066.

Kulju KKM, Sierra SEC, Draisma SGA, Samuel R, van Welzen PC. 2007. Molecular phylogeny of *Macaranga*, *Mallotus*, and related genera (Euphorbiaceae *s.s.*): insights from plastid and nuclear DNA sequence data. *American Journal of Botany* **94:** 1726–1743.

Latiff A. 2001. Diversity of the Vitaceae in the Malay Archipelago. *Malayan Nature Journal* **55:** 29–42.

Li C. 1998. *Leea*. *Flora Reipublicae Popularis Sinicae* **48:** 3–12.

Linnaeus C. 1767. *Leea* van Royen ex L. *Mantissa Plantarum* **1:** 17.

Liu JS, Schardl CL. 1994. A conserved sequence in internal transcribed spacer 1 of plant nuclear rRNA genes. *Plant Molecular Biology* **26:** 775–778.

Maddison WP, Maddison DR. 2010. *Mesquite: a modular system for evolutionary analysis*. Version 2.73 Available at: http://mesquiteproject.org

Magallón S, Castillo A. 2009. Angiosperm diversification through time. *American Journal of Botany* **96:** 349–365.

Mai JC, Coleman AW. 1997. The internal transcribed spacer 2 exhibits a common secondary structure in green algae and flowering plants. *Journal of Molecular Evolution* **44:** 258–271.

Mathews DH. 2004. Using an RNA secondary structure partition function to determine confidence in base pairs predicted by free energy minimization. *RNA* **10:** 1178–1190.

Mathews DH, Turner DH. 2006. Prediction of RNA secondary structure by free energy minimization. *Current Opinion in Structural Biology* **16:** 270–278.

McKenna MCC. 1973. Sweepstakes, filters, corridors, Noah's Arks, and beached Viking funeral ships in paleogeography. In: Tarling DH, Runcorn SK, eds. *Implications of continental drift to the earth science*. London: Academic Press, 291–304.

Merrill E. 1919. *Leea indica*. *Philippine Journal of Science* **14:** 245.

Molina J. 2009. Floral biology of Philippine morphospecies of the grape relative *Leea* (Leeaceae). *Plant Species Biology* **24:** 53–60.

Molina J, Struwe L. 2009. Utility of secondary structure in phylogenetic reconstructions using nrDNA ITS sequences – an example from Potalieae (Gentianaceae: Asteridae). *Systematic Botany* **34:** 414–428.

Morley RJ. 1998. Palynological evidence for Tertiary plant dispersals in the SE Asian region in relation to plate tectonics and climate. In: Hall R, Holloway JD, eds. *Biogeography and geological evolution of South East Asia*. Leiden: Backhuys Publishers, 211–234.

Morley RJ. 2000. *Origin and evolution of tropical rain forests*. New York: Wiley.

Muellner AN, Pannell CM, Coleman A, Chase MW. 2008. The origin and evolution of Indomalesian, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography* **35:** 1769–1789.

Nair NC. 1968. Contribution to the floral morphology and embryology of two species of *Leea* with a discussion on the taxonomic position of the genus. *Journal of the Indian Botanical Society* **47:** 193–205.

Nylander JAA. 2004. *Mrmodeltest 2.2*. Program distributed by the author. Uppsala University: Evolutionary Biology Centre.

Nylander JAA, Olsson U, Alström P, Sanmartín I. 2008. Accounting for phylogenetic uncertainty in biogeography: a Bayesian approach to dispersal–vicariance analysis of the thrushes (Aves: *Turdus*). *Systematic Biology* **57:** 257–268.

Op de Beck P, Cartier G, David B, Dijoux-Franca MG, Mariotte AM. 2003. Antioxidant flavonoids and phenolic

acids from leaves of *Leea guineense* G.Don (Leeaceae). *Phytotherapy Research* **17:** 345–347.

Op de Beck P, Dijoux-Franca MG, Cartier G, Mariotte AM. 1998. Quercitin 3′-sulphate from leaves of *Leea guineensis*. *Phytochemistry* **47:** 1171–1173.

Pannell CM, White F. 1988. Patterns of speciation in Africa, Madagascar, and the tropical Far East: regional faunas and cryptic evolution in vertebrate–dispersed plants. *Monographs in Systematic Botany from the Missouri Botanical Garden* **25:** 639–659.

Planchon JE. 1887. Monographie des Ampélidées vrais. In: de Candolle C, ed. *Monographiae phanaerogamarum 5, 2*. Paris: Masson, 305–654.

Prakash U, Dayal R. 1964. Fossil wood resembling *Elaeocarpus* and *Leea* from Deccan Intertrappean Beds of Mahurzari near Nagpur. *Palaeobotanist* **12:** 121–127.

Rambaut A, Drummond AJ. 2007. *Tracer v1.4*. Available at: http://beast.bio.ed.ac.uk/Tracer

Ree RH, Smith SA. 2008. Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* **57:** 4–14.

Renner SS, Clausing G, Meyer K. 2001. Historical biogeography of Melastomataceae: the roles of Tertiary migration and long-distance dispersal. *American Journal of Botany* **88:** 1290–1300.

Reuter JS, Mathews DH. 2010. RNAstructure: software for RNA secondary structure prediction and analysis. *BMC Bioinformatics* **11:** 129.

Ridsdale CE. 1974. A revision of the family Leeaceae. *Blumea* **22:** 57–100.

Ridsdale CE. 1976. Leeaceae. *Flora Malesiana* Series I **7:** 755–782.

Ridsdale CE. 1980. *Leea asiatica* (L.) Ridsd., a new name for Nalugu Rheede. In: Manilal KS, ed. *Botany and history of Hortus Malabaricus*. Rotterdam: A. A. Balkema, 189–190.

Ronquist F. 1997. Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46:** 195–203.

Ronquist F, Huelsenbeck J, Teslenko M. 2011. *Draft MrBayes version 3.2 manual: tutorials and model summaries*. Available at: http://mrbayes.sourceforge.net/mb3.2_manual.pdf

Rossetto M, Jackes BR, Scott KD, Henry RJ. 2002. Is the genus *Cissus* (Vitaceae) monophyletic? Evidence from plastid and nuclear ribosomal DNA. *Systematic Botany* **27:** 522–533.

Rutschmann F, Eriksson T, Schönenberger J, Conti E. 2004. Did Crypteroniaceae really disperse out-of-India? Molecular dating evidence from *rbcL*, *ndhF*, and *rpl16* intron sequences. *International Journal of Plant Sciences* **165** (4 Suppl): 69–83.

Savolainen V, Chase M, Hoot S, Morton C, Soltis D, Bayer C, Fay M, de Bruijn A, Sullivan S, Qiu Y-L. 2000. Phylogenetics of flowering plants based upon combined analysis of plastid *atpB* and *rbcL* gene sequences. *Systematic Biology* **49:** 306–362.

Sheldon P. 1996. Plus ça change – a model for stasis and evolution indifferent environments. *Palaeogeography, Palaeoclimatology and Palaeoecology* **127:** 209–227.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49:** 369–381.

Smith SA, Beaulieu JM, Donoghue MJ. 2010. An uncorrelated relaxed-clock analysis suggests an earlier origin for flowering plants. *Proceedings of the National Academy of Sciences USA* **107:** 5897–5902.

Soejima A, Wen J. 2006. Phylogenetic analysis of the grape family (Vitaceae) based on three chloroplast markers. *American Journal of Botany* **93:** 278–287.

Soltis DE, Senters AE, Zanis MJ, Kim S, Thompson JD, Soltis PS, Ronse Decraene LP, Endress PK, Farris JS. 2003. Gunnerales are sister to other core eudicots: implications for the evolution of pentamery. *American Journal of Botany* **90:** 461–470.

Sotuyo S, Delgado-Salinas A, Chase MW, Lewis GP, Oyama K. 2007. Cryptic speciation in the *Caesalpinia hintonii* complex (Leguminosae: Caesalpinioideae) in a seasonally dry Mexican Forest. *Annals of Botany* **100:** 1307–1314.

Steppan SJ, Zawadzki C, Heaney LR. 2003. Molecular phylogeny of the endemic Philippine rodent *Apomys* (Muridae) and the dynamics of diversification in an oceanic archipelago. *Biological Journal of the Linnean Society* **80:** 699–715.

Swofford DL. 2003. *PAUP* phylogenetic analysis using parsimony (*and other methods)*, *Version 4*. Sunderland, MA: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25:** 4876–4882.

Udovicic F, McFadden GI, Ladiges PY. 1995. Phylogeny of *Eucalyptus* and *Angophora* based on 5S rDNA spacer sequence data. *Molecular Phylogenetics and Evolution* **4:** 247–256.

Velasco R, Zharkikh A, Troggio M, Cartwright DA, Cestar, A, Pruss D, Pindo M, Fitzgerald LM, Vezzulli S, Reid J, Malacarne G, Iliev D, Coppola G, Wardell B, Micheletti D, Macalma T, Facci M, Mitchell JT, Perazzolli M, Eldredge G, Gatto P, Oyzerski R, Moretto M, Gutin N, Stefanini M, Chen Y, Segala C, Davenport C, Dematte L, Mraz A, Battilana J, Stormo K, Costa F, Tao Q, Si-Ammour A, Harkins T, Lackey A, Perbost C, Taillon B, Stella A, Solovyev V, Fawcett JA, Sterck L, Vandepoele K, Grando SM, Toppo S, Moser C, Lanchbury J, Bogden R, Skolnick M, Sgaramella V, Bhatnagar SK, Fontana P, Gutin A, Van de Peer Y, Salamini F, Viola R. 2007. A high quality draft consensus sequence of the genome of a heterozygous grapevine variety. *PLoS ONE* **2:** e1326. doi:10.1371/journal.pone.0001326

Wang H, Moore MJ, Soltis PS, Bell CD, Brockington SF, Alexandre R, Davis CC, Latvis M, Manchester SR, Soltis DE. 2009. Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proceedings of the National Academy of Sciences USA* **106:** 3853–3858.

Warren BH, Strasberg D, Bruggemann JH, Prys-Jones RP, Thébaud C. 2010. Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics* **26:** 526–538.

Watari S. 1951. Studies on the fossil woods from the Tertiary of Japan. VII. *Leea* (Vitaceae) from the Miocene of Simane. *Botanical Magazine of Tokyo* **64:** 1–7.

Wen J. 2007a. Leeaceae. In: Kubitzki K, ed. *The families and genera of vascular plants*, *Vol. 9*. Berlin: Springer, 220–224.

Wen J. 2007b. Vitaceae. In: Kubitzki K, ed. *The families and genera of vascular plants*, *Vol. 9*. Berlin: Springer, 466–478.

Wikström N, Savolainen V, Chase MW. 2001. Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London B* **268:** 2211–2220.

Woodruff DS. 2003. Neogene marine transgressions, palaeo-geography and biogeographic transitions on the Thai-Malay Peninsula. *Journal of Biogeography* **30:** 551–567.

Young ND, Healy J. 2003. GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* **4:** 6.

Yu Y, Harris AJ, He X. 2010. S-DIVA (statistical dispersal-vicariance analysis): a tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* **56:** 848–850.

APPENDIX 1

Morphological characters (discrete and continuous) used for ancestral state reconstruction in Mesquite. Characters 1–6 refer to discrete characters; 7–9 to continuous characters, which Mesquite automatically partitioned into discrete ranges. See Appendix 2 for the morphological matrix used as input in Mesquite.

1 Glands – presence of large glands on the underside of the leaf: 0 = absent; 1 = present.
2 Endosperm rumination – degree of unevenness of endosperm surface: 0 = simple rumination [basically with five ingrowths: one along the median plane, two from the raphe, one at each lateral face (Ridsdale, 1976: 759)]; 1 = complex rumination (when lateral ingrowths are more branched/reticulate than in simple rumination).
3 Prickles – presence of prickles on either trunks or branches: 0 = absent; 1 = present.
4 Floral merosity – number of petals: 0 = four petals; 1 = five petals.
5 Corolla lobe colour: 0 = white to cream; 1 = green; 2 = red or reddish; 3 = yellow.
6 Staminal fusion – stamens free or fused: 0 = free; 1 = fused.
7 SC/PT – stipule scar to petiole length ratio (SC/PT) (length of stipule scar divided by petiole length; refer to Fig. 1E).
8 Flower length – length of dry flower from base of flower to tip of floral tube (from dry materials, in mm, see Fig. 4).
9 Fruit width – width of dry fruit measured at the widest point (from dry materials, in mm).

APPENDIX 2

Morphological matrix. Characters 1–6 refer to discrete characters; 7–9 to continuous characters (as they appear in Appendix 1). Polymorphic traits are indicated in parentheses. Taxon names are followed by collection information (first two letters of the first collector's last name and collection number). *Leea* accessions were identified following Ridsdale's treatment (compare with Table 1 for new classifications). '?' indicates no data.

Cayratia acris	?	?	?	?	?	0	?	?	?
Cissus tweediana	?	?	?	?	?	0	?	?	?
Clematicissus angustissima	?	?	?	?	?	0	?	?	?
Dillenia sp. *MOsn*	?	?	?	?	?	?	?	?	?
Liquidambar orientalis	?	?	?	?	?	0	?	?	?
Tetrastigma sp. *MO4*	?	?	?	?	?	1	?	?	?
Leea aculeata MO19	0	0	1	1	1	1	0.76	2.8	11.5
Leea acuminatissima FEsn	0	1	0	0	0	1	1	4.1	15
Leea acuminatissima WE8242	0	1	0	0	0	1	?	?	?
Leea aequata DA99069	1	0	0	1	1	1	0.22	2.4	7
Leea aequata KE3080	1	0	0	1	1	1	0.22	2.4	7
Leea aequata WE7494	1	0	0	1	1	1	0.22	2.4	7
Leea aequata YA4	1	0	0	1	1	1	0.22	2.4	7
Leea amabilis AR9415	0	0	0	1	0	1	0.39	4.6	17.5
Leea angulata WE10230	0	0	1	1	1	1	0.76	2.9	9
Leea asiatica SU9480014	0	0	0	1	1	0	1	2.4	12
Leea aspera AV2190	0	0	0	1	1	0	1	2.4	12

APPENDIX 2. *Continued*

Leea aspera WE9036	0	0	0	1	1	0	1	2.4	12
Leea compactiflora HU55103	0	0	0	1	1	1	0.32	3.25	8.5
Leea congesta MO3	0	1	0	0	0	1	0.17	4.1	13
Leea coryphantha HO10688	0	1	0	1	1	1	0.15	4.1	22.5
Leea guineensis AL7506	0	0	0	1	3	1	0.11	2.4	9
Leea guineensis CA696	0	0	0	1	2	1	0.12	2.7	7
Leea guineensis GE5851	0	0	0	1	3	1	0.11	2.4	9
Leea guineensis GE62078	0	0	0	1	(23)	1	0.23	2.6	9
Leea guineensis GO119	0	0	0	1	3	1	0.11	2.4	9
Leea guineensis MO13	0	0	0	1	2	1	0.12	2.7	7
Leea guineensis MO18	0	0	0	1	2	1	0.06	2.7	7
Leea guineensis MO31	0	0	0	1	2	1	0.12	2.7	7
Leea guineensis MO32	0	0	0	1	2	1	0.06	2.7	7
Leea guineensis MO37	0	0	0	1	2	1	0.06	2.7	7
Leea guineensis LO2647	?	0	?	?	2	1	?	?	?
Leea guineensis RI264	0	0	0	1	2	1	0.17	2.6	?
Leea guineensis WA6727	0	0	0	1	2	1	0.06	2.7	7
Leea guineensis WE9569	?	0	?	?	(23)	1	?	?	?
Leea guineensis YA7	0	0	0	1	2	1	0.06	2.7	7
Leea guineensis YAsn	?	0	?	?	?	1	?	?	?
Leea heterodoxa HE1583	0	1	0	1	1	1	0.18	4.1	25
Leea indica AN5149	0	0	0	1	1	1	0.21	2.6	7
Leea indica AV1602	0	0	0	1	1	1	0.2	2.5	6
Leea indica BO2438	0	0	0	1	1	1	0.2	2.5	6
Leea indica CH78319	?	0	?	?	?	1	?	?	?
Leea indica FE3177	0	0	0	1	1	1	0.19	2.95	7
Leea indica FE2031	0	0	0	1	1	1	0.38	3	9
Leea indica JA2622	0	0	0	1	1	1	0.19	2.95	7
Leea indica MO6	0	0	0	1	0	1	0.17	2.6	7
Leea indica MO7	0	0	0	1	0	1	0.17	2.6	7
Leea indica MO8	0	0	0	1	0	1	0.12	2.7	7
Leea indica LE126	0	0	0	1	1	1	0.2	2.5	6
Leea indica NI2995	0	0	0	1	1	1	0.38	3	9
Leea indica RA818	0	0	0	1	1	1	0.2	2.5	6
Leea indica RE705	0	0	0	1	1	1	0.12	1.8	7
Leea indica SM7773	0	0	0	1	1	1	0.18	2.9	6.5
Leea indica TA4316	0	0	0	1	1	1	0.19	2.95	7
Leea indica TA4320	0	0	0	1	1	1	0.19	2.6	8.5
Leea indica WE10237	?	0	?	?	?	1	?	?	?
Leea indica WE7498	0	0	0	1	1	1	0.21	2.6	7
Leea indica WE8341	?	0	?	?	?	1	?	?	?
Leea macrophylla WE7415	0	0	0	1	1	1	0.12	2.7	7
Leea macrophylla WE7417	0	0	0	1	1	1	0.7	3.5	7
Leea macropus TA16698	0	0	0	1	0	1	0.57	7.9	25
Leea magnifolia ED3509	1	1	0	0	0	1	0.89	4.1	11.5
Leea papuana KA1339	1	1	0	1	3	1	0.89	6.15	24
Leea philippinensis MO17	0	1	0	0	1	1	0.81	4.1	12
Leea philippinensis YAsn	0	1	0	0	1	1	0.81	4.1	12
Leea quadrifida USC821	1	0	0	0	0	1	0.62	4.5	13
Leea rubra LE127	0	0	0	1	(023)	1	0.55	2.9	6
Leea rubra PU6703	0	0	0	1	(023)	1	0.55	2.9	6
Leea setuligera CH3311	0	0	0	1	(23)	1	0.24	2.1	7.5
Leea sp KR37301	0	0	0	1	2	1	0.17	2.6	?
Leea sp KR975877	0	0	0	1	?	1	0.17	2.6	?
Leea spinea BA646	0	1	1	1	1	1	1	3.4	10.5
Leea zippeliana SC4387	1	0	0	1	1	1	1	4.1	12.5