



Gelsemiaceae (Gentianales) expanded to include the enigmatic Asian genus *Pteleocarpa*

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The enigmatic South-East Asian monotypic genus *Pteleocarpa* has been considered as a genus *incertae sedis* among the eudicots for a long time. Molecular data (plastid and nuclear ribosomal regions) from 44 widely sampled species across Lamiidae and phylogenetic analyses have finally clarified its familial relationships, and it is here included in Gelsemiaceae (order Gentianales). Its morphological characteristics support a placement in this family and order as a result of the presence of potential synapomorphies, such as imbricate and commonly yellow corollas, latrorse anther dehiscence, divided styles and compressed seeds. Unique characters for *Pteleocarpa* in Gelsemiaceae are alternate leaves and indehiscent samaras. © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, 175, 482–496.

ADDITIONAL KEYWORDS: Boraginaceae – chloroplast DNA – classification – morphology – phylogeny – taxonomy – South-East Asia.

INTRODUCTION

Pteleocarpa Oliv. occurs in lowland and lower slope forests of South-East Asia (see Appendix 1 for full taxonomy and distribution). The only species in this enigmatic genus has long intrigued botanists after it was first described as *Dodonaea lamponga* by Miquel (1861: 511). Since then, this species and its synonyms have been associated with several families, but have never really quite fitted in morphologically, because of the unusual combination of macromorphological characters. The species is a large tropical tree (to >37 m tall) with alternately arranged, simple and non-stipular leaves with entire margins (Ng, 1989; Riedl, 1997; Fig. 1D, F). The leaves are up to 10 cm × 3 cm and glabrous, but the branches in the inflorescences are pubescent. Large terminal inflorescences bear

numerous perfect, hypogynous and pentamerous flowers with basally fused calyx lobes, and sympetalous yellow corollas (Fig. 1A–C). The calyx is small, with calyx lobes only 2 mm long, and the corolla is about 8 mm long. The single pistil has two distinct styles (Figs 1C, 2B), and the fruit is a wide-winged samara up to 5 cm in diameter (Figs 1E, 2E), with one to two seeds (Fig. 2F).

Miquel (1861) placed this species in Sapindaceae (Sapindales: Rosidae), but with uncertainty. Independently of Miquel, Oliver (1873) described the genus *Pteleocarpa* based on his new species *Pteleocarpa malaccensis* Oliv., placed in Olacaceae (Santalales: Core Eudicots). Not long after this, a third species was described by Beccari (1877), *Pteleocarpa longistyla* Becc., who remarked on its unusual features and classified it with some hesitation in Boraginaceae (or Ehretiaceae, a segregate of Boraginaceae that is sometimes classified as its own family, e.g. Weigend *et al.*,

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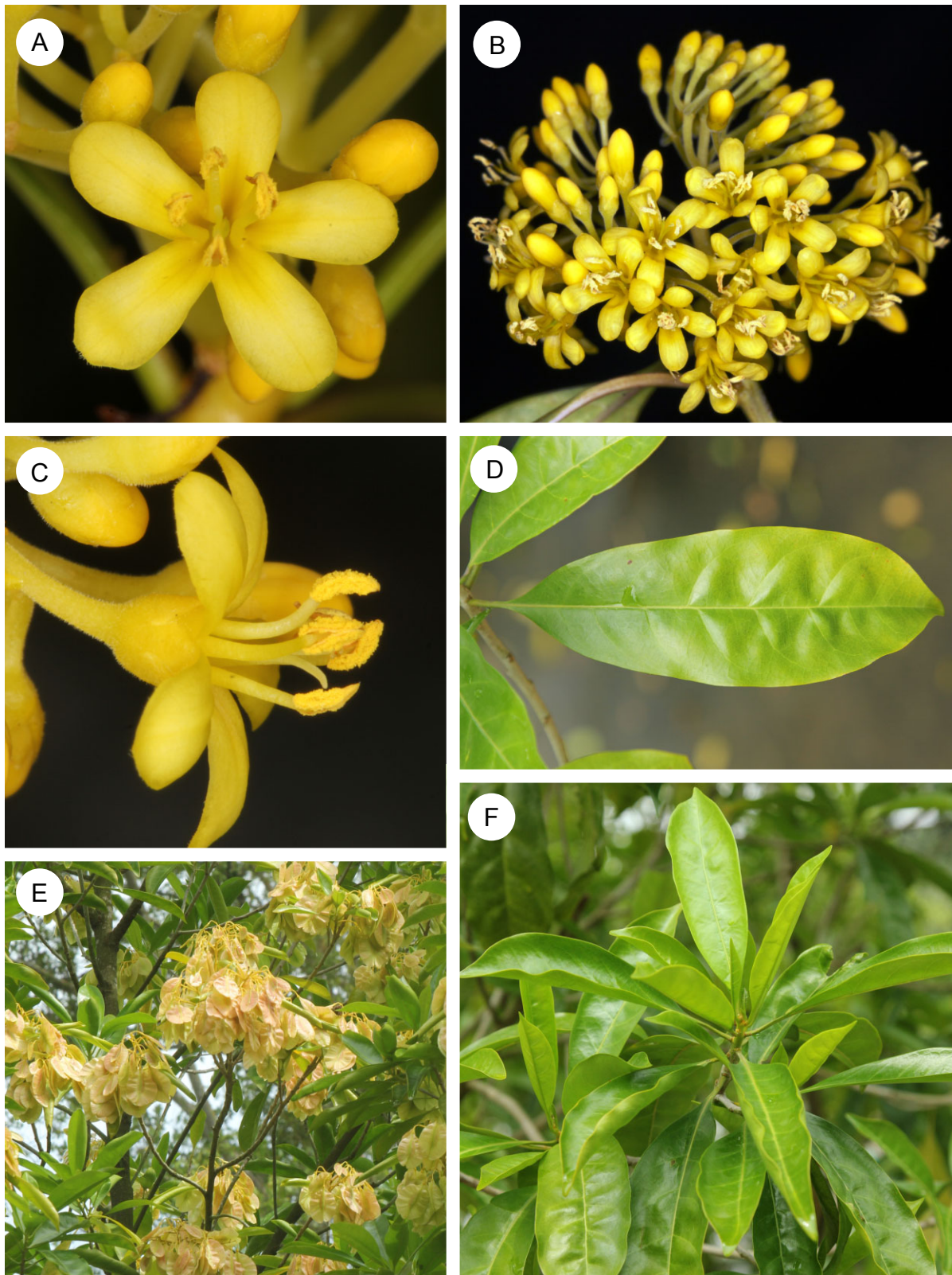


Figure 1. Morphology of *Pteleocarpa lamponga*. A, Flower from above. B, Inflorescence. C, Flower from the side showing latrorse anthers. D, Entire and simple leaf. E, Fruiting branches on tree. F, Vegetative shoot showing alternate leaf arrangement. Photographs © Wee Foong Ang, used with permission. Photographs from cultivated specimens in Singapore.

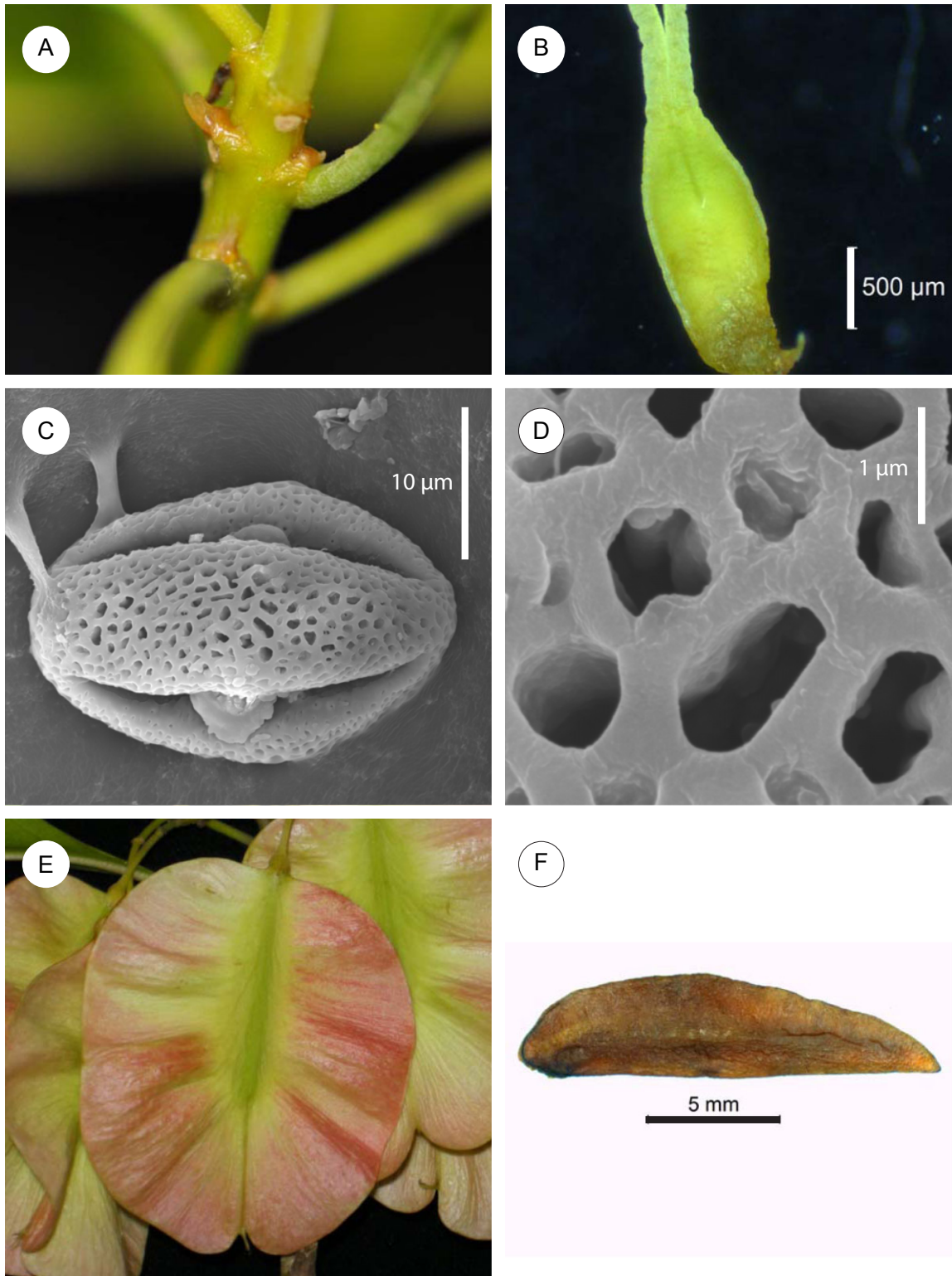


Figure 2. Morphology and anatomy of *Pteleocarpa lamponga*. A, Leaf nodes with resin. B, Immature syncarpous ovary, showing separated styles. C, Pollen grain, equatorial view, showing tricolporate pollen with lateral extensions at the pores. D, Reticulate pollen exine. E, Mature winged fruit, showing truncate apex with two small styles. F, Seed, flattened and unwinged. Photographs © Sugumaran Manickam, from cultivated material at the Forest Research Institute of Malaysia (FRIM).

2013). Icacinaceae and Cardiopteridaceae have also been mentioned as possible families for *Pteleocarpa* (reviewed in Veldkamp, 1988). Since then, botanists have argued for its exclusion from Boraginaceae, Cardiopteridaceae and Icacinaceae (Brummitt, 2011) and Olacaceae (Gottwald, 1982), but a suitable home for this genus has yet to be found.

Gottwald (1982) investigated the wood characteristics of *Pteleocarpa* and concluded that there was little similarity to the wood of Boraginaceae. However, he said, 'an equal or even better match for *Pteleocarpa* can be observed in a number of other families, as for instance in genera of Apocynaceae or Rubiaceae, but also Humiriaceae and Linaceae' (Gottwald, 1982: 165), but this was noted by Veldkamp (1988: 48) as, 'unlikely relations to me in first instance', and was never implemented in any formal classifications.

Veldkamp (1988) wrote in his review, 'Its taxonomic position thus remains as perplexing as it was to Beccari. Creating a distinct family for it, by want of anything better, is hardly useful, for although it would then have a box of its own, we still don't know where to file it.' In most 20th century classifications, *Pteleocarpa* has been treated, albeit reluctantly, as a member of Boraginaceae (World Agroforestry Centre, 2013) or as a genus *incertae sedis* (Veldkamp, 1988; Riedl, 1997). Most recently, Brummitt (2011) described the monotypic family Pteleocarpaceae, but did not indicate in which order he placed this new family.

This study revisits the morphology of this curious Asian genus and, for the first time, uses substantial molecular data and phylogenetic analyses to place it finally in its correct evolutionary context. Preliminary molecular data indicated a relationship to Gelsemiaceae (Olmstead & Ferguson, 2001; Refulio & Olmstead, 2014), and the classification of *Pteleocarpa* in Gelsemiaceae was accepted in APG II (2003), based on unpublished data. Our morphological and molecular work presented here shows that *Pteleocarpa* is a unique and well-supported member of Gelsemiaceae (Gentianales: Lamiidae: Asteridae).

MATERIAL AND METHODS

TAXON SAMPLING

Material of *Pteleocarpa lamponga* (Miq.) Bakh. ex K. Heyne for DNA analysis was obtained from a cultivated specimen on the grounds of the Forest Research Institute of Malaysia (FRIM), Kepong, Malaysia (voucher *N. S. Mat Asri #FRI 26732*; deposited at KEP). We obtained plastid data from 35 species across Lamiidae, including *Pteleocarpa*, and representatives from Apocynaceae, Boraginaceae, Convolvulaceae, Gelsemiaceae, Gentianaceae, Loga-

niaceae, Phrymaceae, Rubiaceae, Scrophulariaceae and Solanaceae, and from three members of Garryales as outgroups, from GenBank for phylogenetic analyses (Table 1). We selected species for which DNA sequence data were available for three plastid regions (*matK*, *ndhF*, *rbcL*), with the exception of *Gentianopsis virgata* (Raf.) Holub (*ndhF*, *rbcL* only), *Mostuea brunonis* Didr. (*ndhF*, *rbcL* only), *Sabatia angularis* Pursh (*matK*, *rbcL* only), *Saccifolium bandeirae* Maguire & J.M. Pires (*matK*, *rbcL* only) and *Strychnos spinosa* Lam. (*matK*, *rbcL* only). In addition, we used nuclear ribosomal (nr) data for the external transcribed spacer (ETS) from six species of Gelsemiaceae and *Pteleocarpa*, and five representatives of Apocynaceae and Loganiaceae as outgroups (Table 1).

MOLECULAR TECHNIQUES AND PHYLOGENETIC ANALYSIS

We amplified ETS from *P. lamponga* following published protocols (Li, Zhang & Alexander, 2001; Jiao & Li, 2007) and cloned the polymerase chain reaction (PCR) product using the TA cloning kit (Invitrogen Corporation, Carlsbad, CA, USA). We screened 16 clones by colony PCR, resulting in five positive clones for sequencing by GENEWIZ (Seattle, WA, USA) using the T7 and M13R primers (Invitrogen Corporation).

Sequences were edited in Sequencher version 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA), aligned initially by MUSCLE (Edgar, 2004) and manually adjusted for each DNA region using MacClade version 4.08 (Maddison & Maddison, 2005). We reconstructed phylogenetic trees for all plastid regions combined and separately for ETS using Bayesian, maximum likelihood (ML) and maximum parsimony (MP) analyses. For Bayesian and ML analyses, models of evolution for each dataset were determined separately by jModelTest version 2.1 (Guindon & Gascuel, 2003; Posada, 2008). The model selected under the Akaike information criterion (Akaike, 1974) for each plastid region and ETS was GTR + I + Γ .

Bayesian analyses were conducted separately in MrBayes version 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) via the CIPRES Science Gateway version 3.3 (Miller, Pfeiffer & Schwartz, 2010) for ETS and the combined plastid dataset, with data partitioned under the selected model for each plastid region. We used default priors of no prior knowledge for the parameters of these models. Parameters for nucleotide frequencies, substitution rates and gamma shape were unlinked across data partitions. All partitions were allowed to evolve under different rates, and site-specific rates were allowed to vary under a flat Dirichlet prior across partitions. Bayesian analyses were conducted with three independent Markov chain Monte Carlo

Table 1. Taxa and associated molecular data (GenBank accession numbers) used in phylogenetic analyses for this study

Taxon	Family	<i>matK</i>	<i>ndhF</i>	<i>rbcL</i>	ETS
<i>Acokanthera oblongifolia</i> (Hochst.) Benth. & Hook.f.	Apocynaceae	HQ384553	HQ384847	X91758	–
<i>Alstonia scholaris</i> (L.) R.Br.	Apocynaceae	AJ429321	AJ011982	X91760	–
<i>Anthocleista grandiflora</i> Gilg	Gentianaceae	JF270645	AJ235829	JF265290	–
<i>Antonia ovata</i> Pohl	Loganiaceae	AJ388200	AJ235832	AJ235817	–
		AJ388270			
<i>Apocynum cannabinum</i> L.	Apocynaceae	HQ384552	HQ384846	HQ384907	–
<i>Aucuba japonica</i> Thunb.	Garryaceae	GQ997060	GQ997066	GQ997098	–
<i>Borago officinalis</i> L.	Boraginaceae	AJ429308	L36393	HQ384927	–
<i>Buddleja davidii</i> Franch.	Scrophulariaceae	HQ384530	HQ384835	L14392	–
<i>Cephalanthus occidentalis</i> L.	Rubiaceae	AY538377	AJ236288	X83629	–
<i>Cinchona pubescens</i> Vahl	Rubiaceae	Z70197	AJ235843	X83630	–
<i>Coffea arabica</i> L.	Rubiaceae	EF044213	EF044213	EF044213	–
<i>Convolvulus cneorum</i> L.	Convolvulaceae	HQ384565	HQ384852	HQ384916	–
<i>Cordia nevillei</i> Alston	Boraginaceae	HQ384571	HQ384860	HQ384923	–
<i>Eucommia ulmoides</i> Oliv.	Eucommiaceae	AJ429317	AJ429113	L01917	–
<i>Exacum affine</i> Balf.f.	Gentianaceae	AJ010515	AJ011983	L11684	–
		AJ011444			
<i>Gardenia thunbergia</i> Thunb.	Rubiaceae	Z70198	AJ235844	X83637	–
<i>Garrya elliptica</i> Douglas	Garryaceae	AJ429319	HQ384864	L01919	–
<i>Gelsemium elegans</i> Benth.	Gelsemiaceae	EF077196	EF077205	EF077212	EF077187 EF077188
<i>Gelsemium rankinii</i> Small	Gelsemiaceae	EF077195	EF077206	EF077211	EF077189 EF077190
					EF077191 EF077192
					EF077193
<i>Gelsemium sempervirens</i> (L.) J.St.-Hil.	Gelsemiaceae	Z70195	EF077202	L14397	EF077185 EF077186
<i>Gentianopsis virgata</i> (Raf.) Holub	Gentianaceae	–	HQ384845	L14398	–
<i>Geniostoma antherotrichum</i> Gilg & Gilg-Ben.	Loganiaceae	–	–	–	JX029190
<i>Kopsia fruticosa</i> (Roxb.) A.DC.	Apocynaceae	Z70178	AJ235824	X91763	–
<i>Logania serpyllifolia</i> R.Br.	Loganiaceae	–	–	–	JX029170
<i>Luculia gratissima</i> (Wall.) Sweet	Rubiaceae	AJ429325	AJ011987	HQ384914	–
<i>Mimulus guttatus</i> DC.	Phrymaceae				
<i>Mitreola petiolata</i> (J.F.Gmel) Torr. & A.Gray	Loganiaceae	–	–	–	JX029177
<i>Mostuea batesii</i> Baker	Gelsemiaceae	–	–	–	EF077182
<i>Mostuea brunonis</i> Didr.	Gelsemiaceae	HQ384557	AJ235828	L14404	–
<i>Mostuea hirsuta</i> (T.Anderson ex Benth. & Hook.f.) Baill.	Gelsemiaceae	EF077194	EF077201	–	EF077184
<i>Mostuea surinamensis</i> Benth.	Gelsemiaceae	–	–	–	EF077183
<i>Nerium oleander</i> L.	Apocynaceae	GQ997641	GQ997647	GQ997679	–
<i>Ophiorrhiza mungos</i> L.	Rubiaceae	AY538408	AJ130838	X83656	–
<i>Periploca graeca</i> L.	Apocynaceae	Z98178	AJ235825	AJ002889	–
<i>Periploca sepium</i> Bunge	Apocynaceae	–	–	–	EF077180
<i>Psychotria hispidula</i> Standl.	Rubiaceae	HQ384561	HQ384851	HQ384913	–
<i>Pteleocarpa lamponga</i> (Miq.) Bakh. ex K.Heyne	Gelsemiaceae	HQ384558	HQ384849	HQ384911	KJ000643
<i>Sabatia angularis</i> (L.) Pursh	Gentianaceae	HQ384549	–	HQ384905	–
<i>Saccifolium bandeirae</i> Maguire & J.M.Pires	Gentianaceae	HQ384550	–	AJ242609	–
<i>Solanum lycopersicum</i> Lam.	Solanaceae	AM087200	AM087200	AM087200	–
<i>Spigelia scabra</i> Cham. & Schltdl.	Loganiaceae	HQ384559	HQ384850	HQ384912	–
<i>Strychnos nux-vomica</i> L.	Loganiaceae	Z70193	–	L14410	–
<i>Strychnos spinosa</i> Lam.	Loganiaceae	JF270955	–	JF265613	EF077181
<i>Vinca minor</i> L.	Apocynaceae	HQ384554	HQ384848	HQ384908	–

(MCMC; Yang & Rannala, 1997) analyses of one million generations for ETS and ten million generations for the combined plastid dataset. Metropolis coupling for each analysis was conducted under the default settings. Convergence was determined when the average standard deviation of split frequencies remained at < 0.01 . For ETS and the combined plastid dataset, the first 14% and 1% of trees, respectively, were discarded before convergence. For each analysis, the remaining trees from each run were pooled to construct a 50% majority rule consensus tree and to obtain posterior probabilities (PP).

ML analyses were conducted separately in GARLI version 2.0 (Zwickl, 2006) via the CIPRES Science Gateway for ETS and the combined plastid dataset, with data partitioned under the selected models for each plastid region. All analyses were run under the default settings, but included ten search replicates, model parameters unlinked across subsets and different subset rates allowed. MP analyses were conducted using PAUP* (version 4.0b10; Swofford, 2002) with 1000 replicates using random-order-entry starting trees, tree bisection–reconnection (TBR) swapping and Multrees in effect.

To assess the reliability of clades in the resulting ML trees for each dataset, we conducted 1000 and 500 non-parametric bootstrap (BS) replicates (Felsenstein, 1985) for ETS and plastid datasets, respectively, in GARLI. BS replicates were conducted under the above settings, but included one search replicate and 10 000 generations as the first part of the termination condition. BS trees were summarized using NCLconverter version 2.1 (Lewis & Holder, 2010) and CONSENSE version 3.66 (Felsenstein, 2006) via the CIPRES Science Gateway. BS analyses for the parsimony analysis were conducted in PAUP* (version 4.0b10; Swofford, 2002), with 1000 replicates and settings as above, except for only 100 replicate starts per BS replicate.

MORPHOLOGY AND ANATOMY

Morphological characteristics of *Pteleocarpa* were observed from herbarium sheets, alcohol-preserved material and in the field at Singapore Botanical Garden (Singapore), FRIM and Rimba Ilmu Botanic Garden, Kuala Lumpur (Malaysia). Fifty-one herbarium sheets and two fruit collections preserved in alcohol (E. J. H. Corner 6068 and E. J. H. Corner 6069) of *P. lamponga* were provided as a loan by Singapore herbarium (SING). *Mostuea* and *Gelsemium* data gathered for previous studies (e.g. Struwe, Albert & Bremer, 1994; Struwe, in press) from specimens at NY and UPS provided comparative materials. Flowers and fruits of *Pteleocarpa* were rehydrated in water and dissected using Zeiss Stemi-2000 and Leica EZ4 com-

pound microscopes, under 5–50× total magnification. Images of the micromorphological characters were taken with a Leica MZ 9.5 (LAS version 3.8.0). For SEM work, pollen grains were fixed in 8% glutaraldehyde and Sorensen's phosphate buffer (1 : 1) for 1 h. The grains were then fixed overnight in osmium tetroxide and passed through a series of ethanol dehydration steps from 10% to 100%, before placing in pure acetone (20 min). Subsequently, critical point drying was carried out in a Leica EM CPD030. The pollen grains were then placed onto aluminium stubs with double-sided tape and a thin layer of gold was applied over the samples for 1 min using an automated sputter coater. The electron micrographs were viewed on a FESEM-JSM-7001F. In addition, morphological and anatomical data from the literature were compiled from the following sources: *Gelsemium* (Leenhouts, 1963; Duncan & DeJong, 1964; Ornduff, 1970; Wyatt *et al.*, 1993; Ping-tao & Leeuwenberg, 1996), *Mostuea* (Leeuwenberg, 1961) and *Pteleocarpa* (Oliver, 1873; Veldkamp, 1988; Ng, 1989; Jarvie & Ermayanti, 1996; Riedl, 1997; Cheksum, 1998; Brummitt, 2011; World Agroforestry Centre, 2013). See also Rueangsawang & Chantaranonthai (2014) for a morphological and palynological description of *Pteleocarpa*.

RESULTS

MOLECULAR

Based on combined plastid data from *matK*, *ndhF* and *rbcl* (Fig. 3) and ETS data (Fig. 4), Gelsemiaceae, including *Pteleocarpa*, is strongly supported as monophyletic (plastid: PP = 1.00, ML BS = 100%, MP BS = 100%; ETS: PP = 1.00, ML BS = 98%, MP BS = 96%). Based on combined plastid data, *Mostuea* is sister to a clade comprising *Gelsemium* and *Pteleocarpa* with variable support (PP = 0.89, ML BS = 73%, MP BS = 66%), whereas *Pteleocarpa* is sister to a clade comprising *Gelsemium* and *Mostuea* with variable support in ETS analyses (PP = 0.87, ML BS = 75%, MP BS = 46%). Therefore, our molecular data show *Pteleocarpa* as excluded from other families of Lamiidae sampled (Apocynaceae, Boraginaceae, Convolvulaceae, Gentianaceae, Loganiaceae, Phrymaceae, Rubiaceae, Scrophulariaceae and Solanaceae) and strongly supported as included in Gelsemiaceae. However, relationships among the three genera in Gelsemiaceae are unclear.

MORPHOLOGY

Our observations confirm that the fruits of *P. lamponga* are large, strongly two-winged (rarely three-winged) samaras (Figs 1E, 2E), with only one to two seeds developing within each fruit. The remnants of the styles and stigmas are persistent, and are

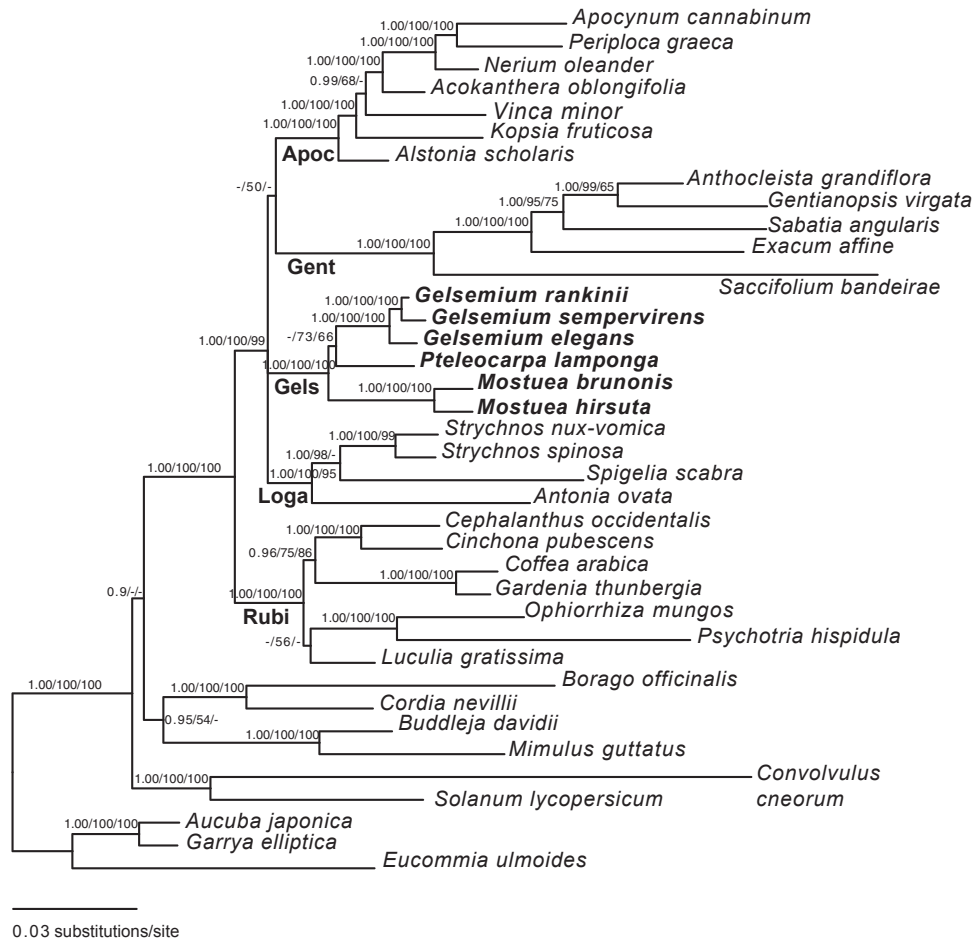


Figure 3. Phylogenetic relationships among Gelsemiaceae and across Lamiidae based on combined plastid regions (*matK*, *ndhF* and *rbcL*). Bayesian 50% majority-rule consensus tree displayed with posterior probabilities ≥ 0.90 , likelihood bootstrap values (BS) $\geq 50\%$ and parsimony BS values $\geq 50\%$, respectively, displayed above the branches. Gelsemiaceae in bold. Gentianales family acronyms shown at nodes: Apoc, Apocynaceae; Gels, Gelsemiaceae; Gent, Gentianaceae; Loga, Loganiaceae; Rubi, Rubiaceae.

retained as two small, separate, stylar parts at the apex of the fruit. Sometimes the apex of the fruit is slightly truncate, with the two styles in a slight depression. The seed is flattened (compressed), but is wingless (Fig. 2F).

Colleters are finger-shaped, multicellular glands common in Gentianales on the inside base of leaves and calyces, and a good synapomorphy for the order even if not uniformly present. Our investigations show some colletter-like structures on the inside of calyx lobes in *Pteleocarpa*, but further microscopic studies are needed to confirm the presence of this character. Colleters are frequently prominent in young leaves and shoot apices, and often deteriorate and fall off later in development. The structures are tiny, rounded, vesiculose-like and appear to be absent from leaf bases in *Pteleocarpa*, but some axillary buds are resinous, a feature often associated with colletters in Gentianales (Fig. 2A).

The ovule position within the carpels of *Pteleocarpa* is a character that has caused considerable discussion (Veldkamp, 1988), with contradicting reports of either one basal ovule in each locule (Oliver, 1873), or one basal and one apical ovule, of which only the basal one develops (Veldkamp, 1988). Our preliminary investigations based on fresh material of young pistils consistently show only one basal ovule in each locule, but this needs to be confirmed through future microscopic studies of sectioned and stained material.

Pollen grains of *Pteleocarpa* are single, prolate, tricolporate, with reticulate exine and pores with distinct lateral extensions, which are characters that fit well with the palynological characteristics of *Gelsemium* and *Mostuea* (Table 2). Scanning electron micrographs of *Pteleocarpa* pollen are presented in Figure 2C, D.

Selected morphological and anatomical characters are summarized for *Gelsemium*, *Mostuea* and

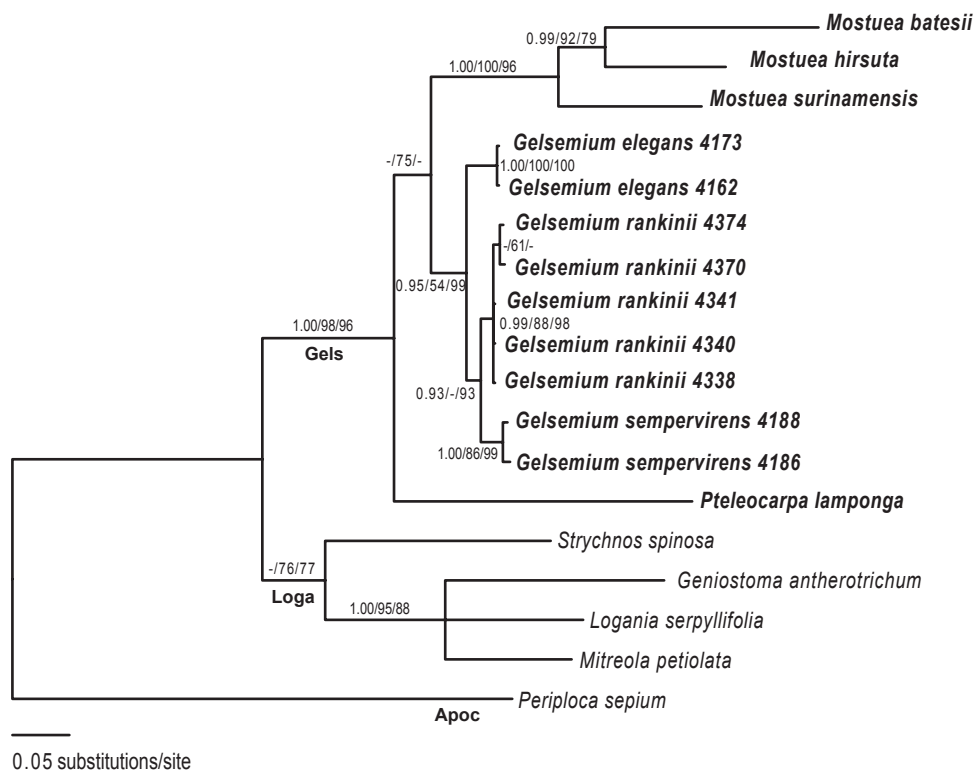


Figure 4. Phylogenetic relationships among Gelsemiaceae and outgroups from Gentianales based on the nuclear ribosomal external transcribed spacer (ETS). Bayesian 50% majority-rule consensus tree displayed with posterior probabilities ≥ 0.90 , likelihood bootstrap values (BS) $\geq 50\%$ and parsimony BS values $\geq 50\%$, respectively, displayed above the branches. Gelsemiaceae in bold. Gentianales family acronyms shown at nodes: Apoc, Apocynaceae; Gels, Gelsemiaceae; Loga, Loganiaceae.

Pteleocarpa (Table 2), showing similarities and dissimilarities in specific characteristics. The placement of *Pteleocarpa* in Gelsemiaceae is supported by several new potential synapomorphies for Gelsemiaceae (Struwe *et al.*, 1994). These include non-unique characters, including imbricate, mostly yellow corollas, latrorse anthers and compressed seeds (Table 2, Figs 1A–C, 2F, 5). More diagnostic characters that unite these three genera in the family Gelsemiaceae include: (1) the presence of at least partially free styles on top of a syncarpous ovary, as two free styles in *Pteleocarpa* (Fig. 2B), and twice branched styles (four arms) in *Gelsemium* and *Mostuea*; and (2) a truncate apex of the fruit in *Pteleocarpa* and *Mostuea*, both of which also have compressed fruits (Figs 1E, 2E), in contrast with ovoid capsules in *Gelsemium*. The samara-like fruit is unique to *Pteleocarpa* (Figs 1E, 2E), but fruit types vary widely in Gentianales. Heterostyly, which is present in *Mostuea* and *Gelsemium* (Duncan & DeJong, 1964; except for some populations of *G. elegans* Benth.), appears to be absent from *Pteleocarpa*, but this should be investigated further. Potential synapomorphies for the place-

ment of *Pteleocarpa* in Gentianales are simple and entire leaves, sympetalous flowers with bicarpellate, syncarpous ovaries, calyx fused at the base and a bilocular fruit.

DISCUSSION

Gelsemiaceae was described when a clade consisting of two genera of Loganiaceae (*Gelsemium* and *Mostuea*) was found to be more closely related to Apocynaceae than to the rest of Loganiaceae based on morphological and molecular data (Struwe *et al.*, 1994). Since then, its distinct position relative to other families of Gentianales has been further supported by additional molecular data (Backlund, Oxelman & Bremer, 2000; Jiao & Li, 2007; Frasier, 2009; Soltis *et al.*, 2011; Refulio & Olmstead, 2014), and it has been widely accepted in classification systems (Takhtajan, 1997; APG, 1998; APG II, 2003; APG III, 2009). Before the reclassification by Struwe *et al.* (1994), Loganiaceae was a family of about 29 genera of widely varying morphologies, generally considered a ‘trash-bag’ family for aberrant members of

Table 2. Overview of morphological and anatomical traits of *Gelsemium*, *Mostuea* and *Pteleocarpa*, based on a literature review and our own observations. General similarities among the three genera are marked in coloured boxes and with numbers: 1, light pink, general similarities among genera of Gelsemiaceae; 2, yellow, potential synapomorphies for Gelsemiaceae; 3, blue, potential plesiomorphies for Gentianales (= present in most taxa); 4, light green, strongly divergent characters for *Pteleocarpa* compared with other members of Gelsemiaceae

Character	<i>Pteleocarpa</i>	<i>Gelsemium</i>	<i>Mostuea</i>
Habit	Trees (up to 40 m tall)	Twining woody vines or half-shrubs	Shrubs or sometimes lianas
Evergreen leaves ³	Evergreen	Evergreen	Evergreen
Shoot indumentum ¹	Young shoot minutely papillose	Glabrous to slightly hairy	Hairy (simple hairs) to nearly glabrous
Branches	Terete	Terete	Terete
Colleters	Colleter-like structures are present on the inside of the calyx lobes, but need to be confirmed with histological sectioning (future work)	Present on inside of sepals, bracts and leaf bases (lacking inside sepals in <i>G. elegans</i>)	Present on inside of sepals, bracts and leaf bases
Leaf arrangement ⁴	Alternate	Opposite (rarely verticillate)	Opposite
Leaf division ³	Simple shape	Simple shape	Simple shape
Leaf shape	Oblong to oblanceolate-elliptic, obovate	Lanceolate, narrowly ovate, elliptic	Variable in shape
Leaf margin ³	Entire	Entire, less often sinuate-dentate	Entire
Stipules ⁴	No stipules	With interpetiolar stipular line	With stipules fused (membranaceous and small, usually triangular), or with interpetiolar line or ochrea
Leaf indumentum	Glabrous	Glabrous	Glabrous or hairy
Inflorescence position	Terminal	Terminal or axillary	Axillary or terminal, mostly on short lateral branches
Inflorescence type	Paniculate	Dichasial, paniculate or thyrsoid	Dichasial (sometimes subcapitate)
Inflorescence, flower numbers	Many-flowered	Many-flowered or few or solitary	One- to many-flowered
Indumentum on peduncles and pedicels	Minutely puberulent to densely pubescent	Glabrous or sparsely pubescent	Hairy or glabrous
Bracts	Present – small and acute, soon detaching	Present (sometimes several pairs) or absent	Small, sepal-like or large and orbicular, one pair only
Flower merosity ³	Pentamerous	Pentamerous	Pentamerous
Ovary position ³	Hypogynous	Hypogynous	Hypogynous
Heterostyly ⁴	Absent	Present (absent in some populations of <i>G. elegans</i>)	Present
Calyx fusion	Free (fused at base)	Free lobes, or fused at base	Nearly free or fused up to one-half of length
Calyx aestivation ³	Imbricate	Imbricate	Imbricate
Calyx indumentum ¹	Glabrous, but ciliate on margin	Glabrous, hairy on the outside, or ciliate on margins only	Sometimes ciliate along margin
Calyx persistence in fruit	Persistent	Deciduous or persistent	Persistent
Corolla fusion ³	Sympetalous	Sympetalous	Sympetalous

Table 2. Continued

Character	<i>Pteleocarpa</i>	<i>Gelsemium</i>	<i>Mostuea</i>
Corolla aestivation ²	Imbricate	Imbricate	Imbricate
Corolla colour ²	Yellow	Yellow, less often orange, sometimes with red markings	White to yellow, rarely cream, orange or red
Stamen position ³	Alternate with petals	Alternate with petals	Alternate with petals
Stamen insertion	In sinuses (tube short)	Inserted in middle to lower half of corolla tube	Inserted 1/4 to 1/3 from base of corolla tube
Stamen size ¹	Equal	Equal	Equal or unequal
Filament shape ¹	Filiform	Filiform	Filiform
Filament fusion ¹	Free	Free	Free
Anther shape ¹	Linear to linear-lanceolate	Ellipsoid	Orbicular to oblong, narrower after anthesis
Anthers base shape ¹	Sagittate	Sagittate	Sagittate
Anther dehiscence ²	Latrorse	Latrorse	Latrorse
Ovary, # carpels ³	Bicarpellate	Bicarpellate	Bicarpellate
Ovary, carpel fusion ³	Syncarpous	Syncarpous	Syncarpous
Ovary, # locules ³	Bilocular	Bilocular	Bilocular
Ovary	No nectary disc (but 'copious nectar' marked on herbarium sheet)	No nectary disc	No nectary disc
Ovary ⁴	On thickened stalk	Not stalked	Not stalked
Style division ²	Two (free)	Twice dichotomously branched (fused at base)	Twice dichotomously branched (fused at base)
Style shape ¹	Erect, linear, long (shorter in fruit)	Erect, linear, long	Erect, linear, long
Style in fruit	Persistent style	Deciduous in fruit, base persistent	Deciduous in fruit
Stigma position ³	Terminal	Terminal	Terminal
Stigma shape ¹	Capitate	Capitate	Capitate
Ovule position	Unclear – basal only or basal + apical positions have been reported; needs to be clarified with histological sectioning (future work)	Axile	Axile to basal
Fruit type ⁴	Samara	Capsule	Capsule
Fruit shape	Orbicular to elliptic, sometimes slightly obcordate	Oblong, elliptic	Bilobed, obcordate, occasionally ellipsoid
Fruit dehiscence ⁴	Indehiscent	Septicidally and/or loculicidally, two or four valves	Septicidally and/or loculicidally
Fruit compression	Compressed	Not compressed	Compressed
Fruit wing ⁴	Present, very large	Absent	Absent
Fruit apex	Truncate	Acute	Bifid to truncate
Fruit ³	Bilocular	Bilocular	Bilocular
# seeds per fruit	One or two	c. 8	One to four (sometimes only one or two seeds develop, resulting in an asymmetric fruit)
Seed shape	Elongate	Orbicular to elongate	Ovate-orbicular
Seed indumentum	Glabrous	Warty, hairy or smooth	Hairy in African species, glabrous in <i>Mostuea surinamensis</i>

Table 2. *Continued*

Character	<i>Pteleocarpa</i>	<i>Gelsemium</i>	<i>Mostuea</i>
Seed compression ⁴	Compressed	Compressed	Compressed
Seed wing	Not winged	Winged (not winged in <i>G. rankinii</i>)	Not winged
Vessel pits vested	Vestured	Vestured	Vestured
Fibre-tracheid pits	With numerous distinctly bordered pits	With small bordered pits	With small bordered pits
Pollen apertures ¹	Tricolporate	Tricolporate	Tricolporate
Pollen grain shape ¹	Prolate	Prolate spheroidal to subprolate	Prolate spheroidal to subprolate
Pollen pores with lateral extensions ¹	Small endoapertures with acuminate lateral sides	Pores with lateral extensions	Pores with lateral extensions
Pollen exine	Reticulate	Striato-reticulate	Finely supracriate

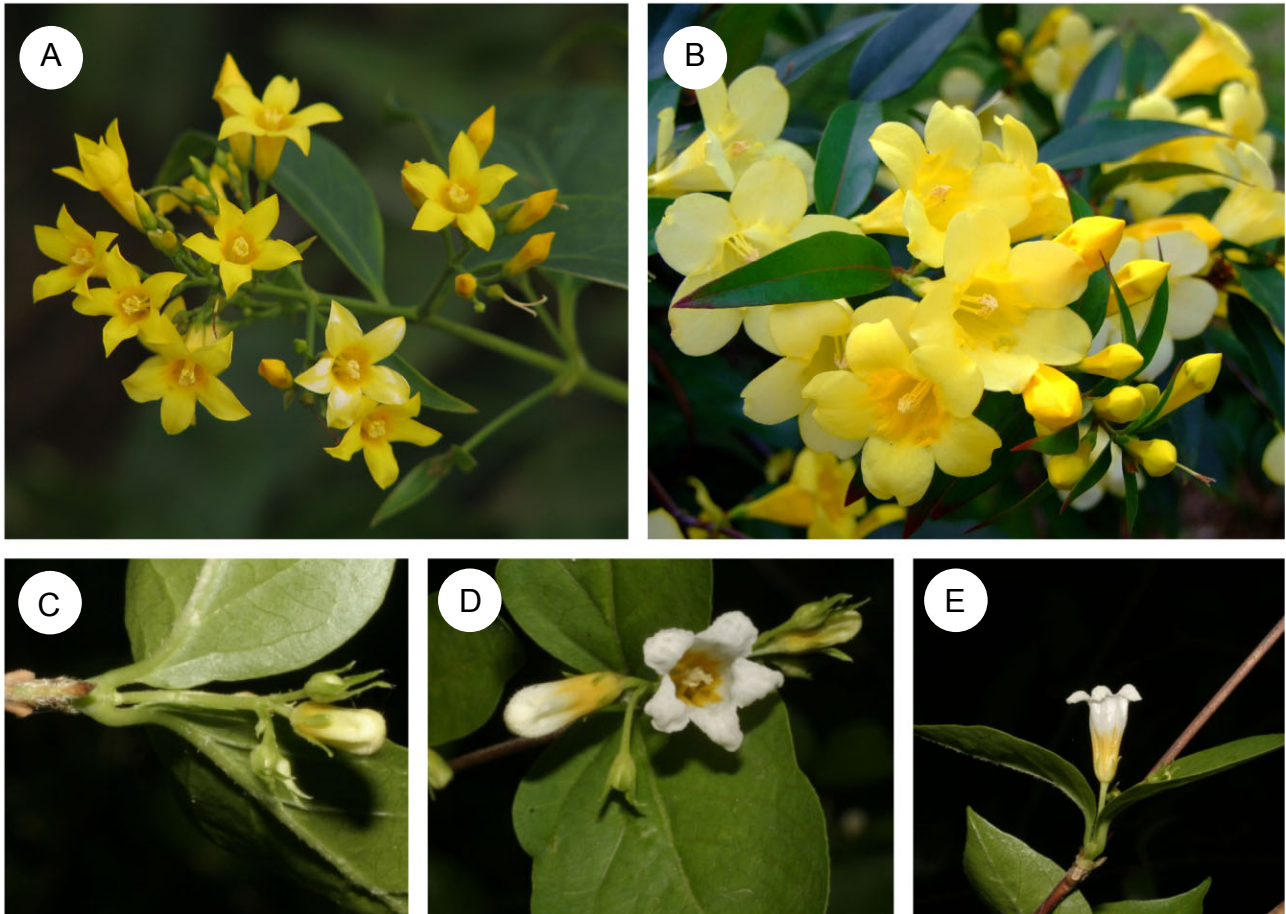


Figure 5. *Gelsemium* and *Mostuea* flower morphology and inflorescences. A, *Gelsemium elegans* inflorescence, from Hong Kong (China). Photograph by Bryan To (CC BY-NC-SA 2.0 license). B, *Gelsemium sempervirens* inflorescence, from plant cultivated at Zoological and Botanical Garden (Hong Kong, China). Photograph by Joseph Kai Yan Wong (CC BY-NC-SA 2.0 license). C–E, Flowers and flowering shoots of *Mostuea hymenocardioides* Hutch. & Dalziel, photographed in the wild in Mali. © Philippe Birnbaum via Brunken *et al.* (2008).

Gentianales. Only 13 genera are left in a now monophyletic family, and the remaining 16 genera have been placed in Desfontainiaceae, Gelsemiaceae, Gentianaceae, Gesneriaceae, Scrophulariaceae and Stilbaceae (Struwe & Motley, in press).

Typical characters of Gentianales are a woody habit (but many herbaceous species are also present in this clade), opposite (rarely alternate or whorled), simple and entire leaves, colleters, and pentamerous, hypogynous, sympetalous flowers. Potential synapomorphies for Gelsemiaceae are twice divided stigmas, mainly yellow corollas (Fig. 5), latrorse anthers and flattened seeds (Struwe *et al.*, 1994; Struwe, in press; Table 2).

Pteleocarpa fits well into the phenotypic traits found in Gentianales, and in Gelsemiaceae in particular, with the exception of a few macromorphological characters. The most obvious difference is the spiral arrangement of the leaves of *Pteleocarpa*; members of Gentianales have opposite leaves (rarely alternate or whorled), often with interpetiolar stipules or ridges between the leaves. *Pteleocarpa* would not be the unique alternate-leaved species in an otherwise mostly opposite-leaved group; in some families (e.g. Apocynaceae, Asteraceae, Scrophulariaceae), the leaf arrangement is not a taxonomically important or consistent character.

Pteleocarpa exhibits several morphological characters that firmly support, or are consistent with, its position in Gentianales based on molecular data, such as simple, entire leaves, pentamerous (mostly) and sympetalous flowers, and bicarpellate, syncarpous ovaries (with apocarpous common in Apocynaceae). Vestured pits are present in the vessels of *Pteleocarpa* and are also characteristic of Gentianales (Gottwald, 1982; Jansen, Baas & Smets, 1998; Jansen & Smets, 2000; Jansen, Baas & Smets, 2001; Rabaey *et al.*, 2010).

If not for the alternate leaves and samaroid fruits, *Pteleocarpa* probably would have been placed in Gentianales long ago. The yellow flowers of *Pteleocarpa* show strong similarities to *Gelsemium* and *Mostuea* of Gelsemiaceae, especially in the division of styles from a syncarpous ovary, the shapes of filaments and stamens, and latrorse stamens (Struwe *et al.*, 1994; Table 2), but also in overall flower colour and *Bauplan* (Figs 1, 5). If, instead of emphasizing its unique samara, the fruit of *Pteleocarpa* were described as 'a compressed, winged fruit with a truncate apex of separated styles and including one to few compressed seeds', then the other genera of Gelsemiaceae would also fit most of these fruit characters. The main difference is the prominent wing on the fruit of *Pteleocarpa*, which gave the genus its name. *Mostuea* has strongly flattened fruits, just like *Pteleocarpa*, but they are capsular and unwinged.

Instead of seeing phenotypic traits as absolute characters that need to fit predefined morphological

concepts (for example, 'samara'), it might be better to see these as states of a transformation series of multiple characters. For example, the separation of the styles in Gelsemiaceae might be a precursor to the often apocarpous ovaries in Apocynaceae, its sister group. An overemphasis on unique characters (samaras and alternate leaves) in the taxonomy of *Pteleocarpa* may have prevented previous taxonomists from recognizing the shared compressed fruits with one to a few compressed seeds, latrorse anthers and yellow to red corollas as traits indicating relationships in this group. Of course, the hindsight permitted by molecular phylogenetic inference is what makes this interpretation clear.

The distribution of *Pteleocarpa* presents no problem for its inclusion in Gelsemiaceae. The South-East Asian distribution of *Pteleocarpa* is partially overlapping on Borneo and Sumatra with the distribution of the only other Asian Gelsemiaceae, *Gelsemium elegans* (Leenhouts, 1963; Ping-Tao & Leeuwenberg, 1996). The other two species of *Gelsemium* are only present in subtropical North America, and *Mostuea* (about a dozen species) is known from eastern South America, Africa and Madagascar. The family is strictly tropical to subtropical.

An intriguing aspect that has not yet been investigated is the phytochemical composition of *Pteleocarpa*. Seeds are reportedly boiled and eaten from this plant, but both *Mostuea* and *Gelsemium* have complex indole alkaloids that can be toxic, especially the compound called gelsemidin. Indeed, *G. elegans* from Asia has supposedly been used for murder and suicide (Leenhouts, 1963). Members of Gentianales are famous for their seco-iridoids (diverse in Gentianaceae), which are often precursors to the alkaloids of many Loganiaceae (such as strychnine), Apocynaceae (e.g. vincristine) and Rubiaceae (quinine, caffeine, etc.). One would presume that *Pteleocarpa* also has some interesting compounds either from the seco-iridoid group or the indole alkaloid groups.

We here include *Pteleocarpa* in Gelsemiaceae (see Appendix 1 for a new synopsis of Gelsemiaceae with *Pteleocarpa*). Molecular support for the clade combining *Pteleocarpa* with *Gelsemium* and *Mostuea* is strong, and evidence for the monophyly of *Gelsemium* and *Mostuea*, exclusive of *Pteleocarpa*, is lacking in our data. However, recent large-scale analyses across the Lamiidae show *Pteleocarpa* as sister to *Gelsemium* + *Mostuea* (Refulio & Olmstead, 2014). Combined with the shared morphological and chemical evidence, this argues against retaining a separate Pteleocarpaceae *sensu* Brummitt (2011). Lateral anther dehiscence is a strong synapomorphy for Gelsemiaceae. Exclusion of *Pteleocarpa* from Gelsemiaceae would eliminate a useful family character for Gelsemiaceae. In addition, the other synapomorphy of

Gelsemiaceae, styles divided into several on top of a syncarpous ovary, would also be affected, as *Pteleocarpa* has two separated styles. We therefore prefer to include *Pteleocarpa* in an expanded Gelsemiaceae, and preserve several good morphological synapomorphies for a clade and family that are also strongly supported by molecular data as a strong monophyletic unit. As for the internal relationships among the three genera of Gelsemiaceae, additional phylogenetic investigations are needed to fully address this issue.

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APPENDIX 1. UPDATED CLASSIFICATION OF GELSEMIACEAE, WITH DETAILS ON PTELEOCARPA

GELSEMIACEAE

Genera: Three; *Gelsemium*, *Mostuea* and *Pteleocarpa*.

Estimated number of species: c. 16 (*Gelsemium*, 3; *Mostuea*, c. 12; *Pteleocarpa*, 1).

Total distribution: South-East Asia (*G. elegans*, *P. lamponga*), eastern Asia (*G. elegans*), Central America (*G. sempervirens*), south-eastern North

America (*G. sempervirens*, *G. rankinii*), north-eastern South America, tropical Africa, Madagascar (*Mostuea*).

Potential synapomorphies: Yellow corolla; latrorse anther dehiscence; syncarpous ovaries with partial or completely separated styles; compressed seeds.

PTELEOCARPA (GELSEMIACEAE)

Pteleocarpa Oliv., Trans. Linn. Soc. London 28: 515 (1873).

Pteleocarpa lamponga (Miq.) Bakh. ex K. Heyne, Nutt. Pl. Ned.-Ind., ed. 2, 2: 1309 (1927).

Dodonaea lamponga Miq., Fl. Ned. Ind., Suppl. 1: 511 (1861).

Pteleocarpa longistyla Becc., Malesia 1: 130 (1877).

Pteleocarpa malaccensis Oliv., Trans. Linn. Soc. 28: 515 (1873).

Distribution: South-East Asia (Brunei, Indonesia, Malaysia, Singapore, Thailand).

Conservation status: Yan Chong, Tan & Corlett (2009) listed this species as extinct in Singapore.

Habitat: Primary and secondary lowland to forests on slopes up to 1500 m altitude, on well-drained soils. It is found in dipterocarp and kerangas forest types.

Fruit dispersal: Wind.

Ethnobotany: The seeds are edible, and commonly boiled and used as a flavouring (Heyne, 1927; World Agroforestry Centre, 2013). The wood is harvested locally and used for buildings and carpentry work (Cheksum, 1998). The species has ornamental value and is used in horticulture and as shade trees (World Agroforestry Centre, 2013).