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Phanera mekongensis (*Fabaceae*: *Cercidoideae*), a new species from Thailand as supported by morphological and molecular evidence

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Key words

Bauhinia
ITS
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Phanera
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trnL-F

Abstract *Phanera mekongensis*, a new species from Phu Wua Wildlife Sanctuary, Northeastern Thailand, is described and illustrated. The morphological description of the new species is provided and discussed in comparison with closely related species. The molecular data from nuclear and chloroplast markers show that the new taxon is robustly positioned in a well-supported *Phanera* clade that includes the type species of the genus, *P. coccinea*.

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INTRODUCTION

Phanera Lour. was first described by De Loureiro (1790), who established the genus from a single species, *P. coccinea* Lour. It was later recognised as a subgenus within a broadly circumscribed *Bauhinia* s.lat. (Larsen et al. 1980, 1984, Wunderlin et al. 1981, Chen 1988, Wunderlin & Eilers 2009, Chen et al. 2010). However, recent molecular studies (Lai et al. 1997, Bruneau et al. 2001, 2008, Hao et al. 2003, Sinou et al. 2009, 2020) showed *Bauhinia* to be polyphyletic. Phylogenetic studies allowed Mackinder & Clark (2014) to reinstate *Phanera* as a genus distinct from *Bauhinia* s.lat., different by a combination of characters, like the liana habit, presence of tendrils, calyx lobes that are markedly distinct and 2–3 fertile stamens per flower. However, the circumscription of the genus *Phanera* remains loosely defined. Subsequently, Clark et al. (2017) transferred ten taxa within *Phanera* subg. *Phanera* subsect. *Corymbosae* de Wit to a new genus *Cheniella* R.Clark & Mackinder, supported by the character combination of having simple leaves that are emarginate or bilobed, elongate hypanthia that are as long as or much longer than the calyx lobes, indehiscent or tardily dehiscent fruits that are laterally compressed and oblong, and seeds that have a distinct funicle. With these recent changes, the genus *Phanera* is now understood to be narrow in its definition and composed of fewer than 80 species, but relationships amongst all these genera within the *Phanera* clade remain unresolved in all recent phylogenetic analyses. Lastly, regarding the limits of these genera, Sinou et al. (2020) included *Lasiobema* (Korth.) Miq. in *Phanera* to broaden the generic circumscription, and suggested that *Cheniella* should be re-evaluated as an infrageneric group of *Phanera* s.str. rather than as a distinct genus.

After the account of the genus *Bauhinia* was published in the Flora of Thailand by Larsen et al. (1984), several species were discovered as new to science. Recently, for example, three new species were described namely: *Bauhinia siamensis* K.Larsen & S.S.Larsen (Larsen & Larsen 2002; presently *Phanera siamensis* (K.Larsen & S.S.Larsen) Mackinder & R.Clark), discovered in Northern Thailand; *Bauhinia nakhonphanomensis* Chatan (Chatan 2013; presently *Phanera nakhonphanomensis* (Chatan) Mackinder & R.Clark) and *Phanera larseniana* Chantar., Mattapha & Wangwasit (Chantaranothai et al. 2017) discovered from areas adjacent to Phu Wua Wildlife Sanctuary, Bueng Kan Province, Northeastern Thailand. In this paper, we describe an additional new species of *Phanera*, which was discovered in the area near to where the latter two species were found.

The new species, *Phanera mekongensis* Mattapha, Suddee & Duangjai, is discovered from the cultivated collection in Phu Wua Wildlife Sanctuary medicinal garden, headquarters office, Bueng Kan Province. To help determine the affinities of this new species, nuclear and chloroplast sequences are analysed phylogenetically.

Morphologically, the new species has affinities to a Chinese species, *Phanera hekouensis* (T.Y.Tu & D.X.Zhang) Krishnaraj (Krishnaraj 2014); formerly *Bauhinia hekouensis* T.Y.Tu & D.X.Zhang (Tu & Zhang 2013), but it is clearly distinct by several characteristics, justifying its recognition as new to science. The molecular evidence from nuclear and plastid sequences indicates that the new species belongs to the major *Phanera*

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clade, which also includes the type species of the genus, *P. coccinea*. We provide a comparison of the morphology of these species along with phenological and other relevant information for the new species.

MATERIALS AND METHODS

Molecular work

Taxon and DNA regions sampling

To analyse the phylogenetic position of the putative new species, we used a total of 110 taxa representing 13 genera of the subfamily *Cercidoideae*. Of these, ten species of the genus *Phanera* that are mostly not available in GenBank (http://www.ncbi.nlm.nih.gov/genbank/), including a putative new species and the type species of the genus (*P. coccinea*), were newly sequenced. Nuclear ribosomal ITS sequences and the *trnL* intron and *trnL-F* spacer (hereafter *trnLF* region) of *Cercidoideae* were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/). Voucher information and GenBank accession numbers are provided in the Appendix. Species of the genus *Cercis* were chosen as outgroup, thereby following previous studies (Lai et al. 1997, Bruneau et al. 2001, 2008, Hao et al. 2003, Sinou et al. 2009, 2020).

DNA extraction, PCR amplification and sequencing

Leaf samples of the putatively new species and *P. kockiana* (Korth.) Benth. were collected from cultivated plants at Kasetsart University, Bangkok, and ten other species of *Phanera* and two species of *Cheniella* were collected in the field and preserved in silica gel. Total DNA was extracted following the 2× cetyltrimethylammonium bromide (CTAB) procedure of Doyle & Doyle (1987) with minor modifications. We washed ground plant material with sorbitol buffer (Tel-zur et al. 1999) followed by centrifugation before incubating with CTAB buffer. The *trnLF* region of our samples was amplified using the universal primers c and f (Taberlet et al. 1991). PCR was performed in a total reaction mixture of 50 µL containing 25 µL of 2× DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 21 µL of nuclease-free water, 1 µL of bovine serum albumin (BSA (20 mg/mL), 1 µL of each primer (20 mmol/L) and 1 µL of template DNA. The PCR programme consisted of an initial 3 min pre-melt at 94 °C and 35 cycles of 45 sec denaturation at 94 °C, 45 sec annealing at 50 °C, and a 1 min extension at 65 °C, followed by final extension of 6 min at 65 °C. The internal transcribed spacers (ITS) of nuclear ribosomal DNA were amplified using the 5F (Möller & Cronk 2001) and 4R (White et al. 1990) primers. As above, the PCR was performed in a total reaction mixture of 50 µL, but the volume of nuclease-free water was reduced to 20 µL and 1 µL DMSO was added. The PCR programme was the same as above, except that the extension step was conducted at 72 °C. All of the successfully amplified products were cleaned using FastAP Thermosensitive Alkaline Phosphatase and Exonuclease I (Thermo Fisher Scientific, Waltham, MA, USA). Ultimately, cleaned PCR products were sent to Macrogen (Seoul, Korea) for Sanger sequencing using the same primers as in the initial amplification. Raw sequences were edited and assembled using AutoAssembler v. 1.4.0 (Applied Biosystems 1995). All newly generated sequences have been deposited in GenBank (Appendix).

Sequence alignment and phylogenetic analyses

The sequences were aligned for each marker separately using ClustalX (Larkin et al. 2007) and later manually adjusted using MacClade 4.08 (Maddison & Maddison 2005). The aligned sequences of these two regions, ITS and *trnLF*, were analysed separately and also combined in a concatenated matrix. In the latter case, ITS and *trnLF* sequence data lacking in some taxa were treated as missing. These three datasets were analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) methods.

The MP analyses were performed using PAUP* v. 4.0b10 (Swofford 2002) with a heuristic search option using the tree-bisection-reconnection (TBR) branch swapping algorithm, 10 starting trees were obtained by random addition with 1 000 replicates and all gaps treated as 'missing'. All characters were designated as equally weighted and unordered. Bootstrap support was calculated with 1 000 replications with the stepwise addition option set as simple (Felsenstein 1985).

MrModeltest v. 2.2 (Nylander 2004) was used to select the appropriate substitution model for ML and BI analyses. The models of molecular evolution were selected using the Akaike Information Criterion (AIC). Bayesian analysis was undertaken using MrBayes v. 3.2.7a (Ronquist et al. 2012) through the CIPRES Science Gateway (http://www.phylo.org/; Miller et al. 2015). The chosen models were SYM+G for all three data matrices. The MrBayes analysis used two runs of four chains for 10 000 000 generations, sampling every 1 000 generations. The first 25 % of trees was discarded as burn-in. The remaining trees were imported into PAUP* v. 4.0b10 (Swofford 2002) and a 50 % majority rule consensus tree was produced to visualise the posterior probabilities (PP).

For ML analysis the individual and the concatenated dataset files were saved in Phylip format and uploaded to the CIPRES site. The analysis was performed using RAxML HPC2 Workflow on XSEDE (v. 8.2.12) on the CIPRES Science Gateway (Stamatakis 2014). The search for the best-scoring ML tree in a single program run under the GAMMA Model of rate heterogeneity was conducted. All generated trees of the analyses with branch lengths and bootstrap values were viewed with FigTree v. 4.0 (http://tree.bio.ed.ac.uk/software/figtree/; Rambaut 2010).

Morphological observation, identification and nomenclature

Specimens of the putative new species were compared with descriptions of species in the genus *Bauhinia* s.lat. in previously published regional floras and related comprehensive accounts, namely the Flora of Thailand (Larsen et al. 1984), Flore du Cambodge, du Lao et du Viet-Nam (Larsen et al. 1980), Flora Malesiana (Hou et al. 1996) and Flora of China (Chen et al. 2010). We also consulted specimens in herbaria, namely BK, BKF, K, KKU, NLS (National University of Laos, Faculty of Natural Science), P and QBG (herbarium abbreviations follow Thiers 2016). Specimens of closely related species were also consulted from Kew (https://apps.kew.org/herbcat/navigator.do) and from Paris (https://science.mnhn.fr/institution/mnhn/collection/p/item/search/form?lang=en_US) using online searches. For the nomenclature and circumscription of most *Phanera* species we followed Mackinder & Clark (2014), Sinou et al. (2020), Jiang (2020) and the Plants of the World online (http://www.plantsoftheworldonline.org/). The detailed morphological study was based on the living material and herbarium specimens kept at BKF. Morphological characters were described mainly on living specimens. Flowers were dissected and measured under a stereomicroscope. Photographs were taken and detailed illustrations drawn. The conservation status of the new species was assessed following the IUCN Red List Categories and Criteria (IUCN 2019). A geographical distribution point map was created using QGIS v. 2.14.1-Essen (QGIS Development Team 2016).

Table 1 Data of the aligned sequences used in the phylogenetic analyses.

	ITS	*trnL-F*	ITS + *trnL-F*
Taxa (total accessions)	88	84	108
Length (aligned)	709	1299	2008
No. variable characters (%)	451 (63.61)	281 (21.63)	716 (35.66)
No. potentially parsimony informative characters (%)	361 (50.92)	164 (12.62)	485 (24.15)
Substitution model	SYM+G	SYM+G	SYM+G

RESULTS

Phylogenetic analyses

The number of taxa and characteristics of the sequences for the three data matrices are summarised in Table 1. The phylogenetic analyses, maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI), for the individual and concatenated datasets are generally congruent. Consequently, we present the relationships in the 50 % majority-rule consensus BI trees from the individual markers (Fig. 1) and from the concatenated dataset (ITS+*trnL-F*), with the associated PP and the BP values from MP and ML analyses in Fig. 2. The phylogenetic position of the new species is shown in bold dark red, written as *Phanera mekongensis sp. nov.* in the trees.

Individual dataset (ITS, trnL-F)

In the BI analysis of the nuclear dataset *Phanera* is divided into multiple clades. The new species was recovered as a sister to a subclade that includes *Phanera coccinea* and *P. nervosa*, with a high support value (BI = 0.97; Fig. 1). The BI analysis of *trnL-F* also showed similar relationships for the new species with strong support (PP = 1) as forming a clade with *P. coccinea* and *P. bassacensis* (Pierre ex Gagnep.) de Wit (Fig. 1 right).

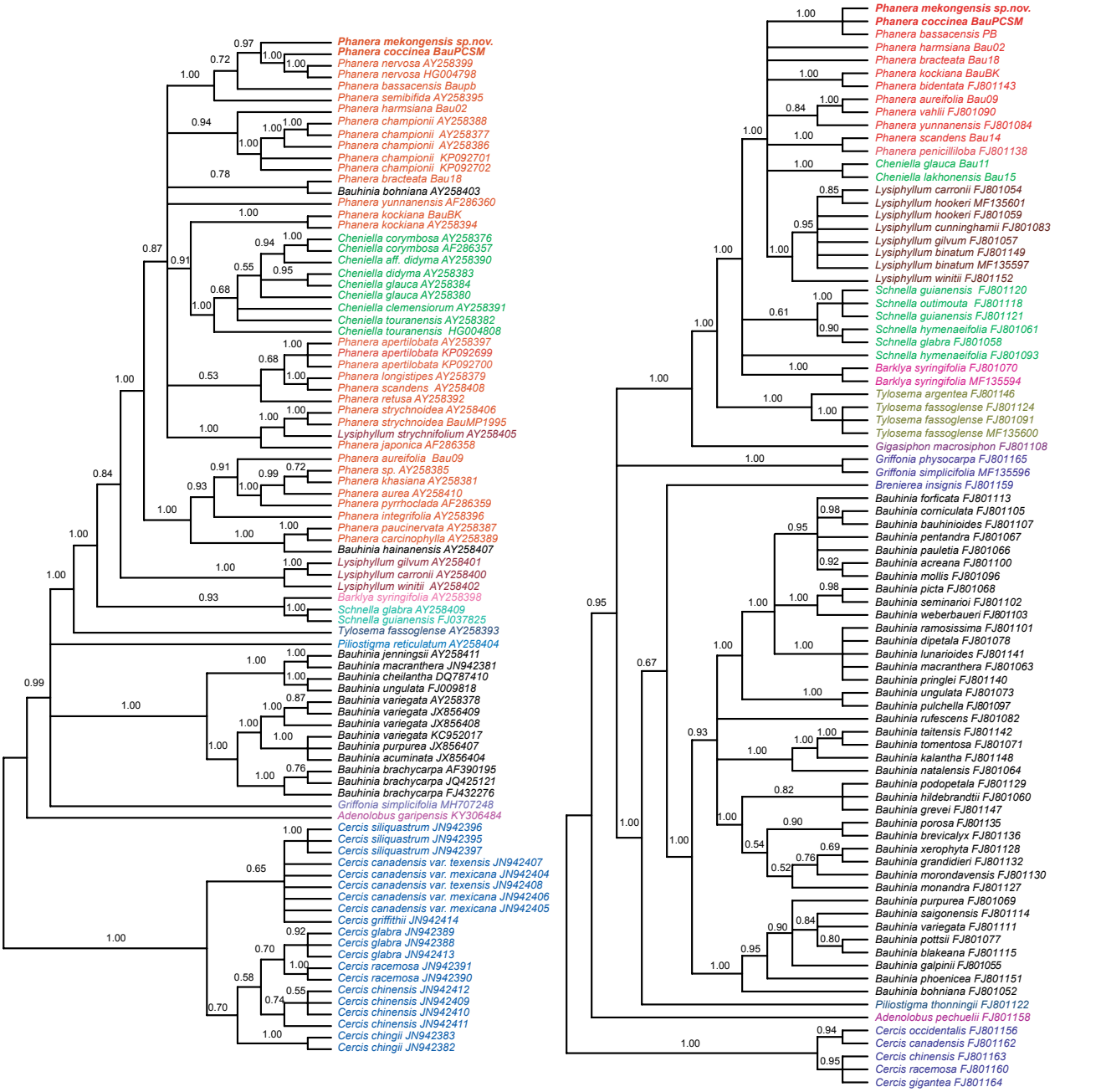

Fig. 1 The 50 % majority-rule consensus tree from Bayesian analyses of the ITS (left) and *trnL-F* (right) of subfamily *Cercidoideae*. The new species, *Phanera mekongensis*, is sister to a subclade that includes *Phanera nervosa* and the type species, *Phanera coccinea*. Numbers above branches are posterior probabilities; values < 0.50 are not shown.

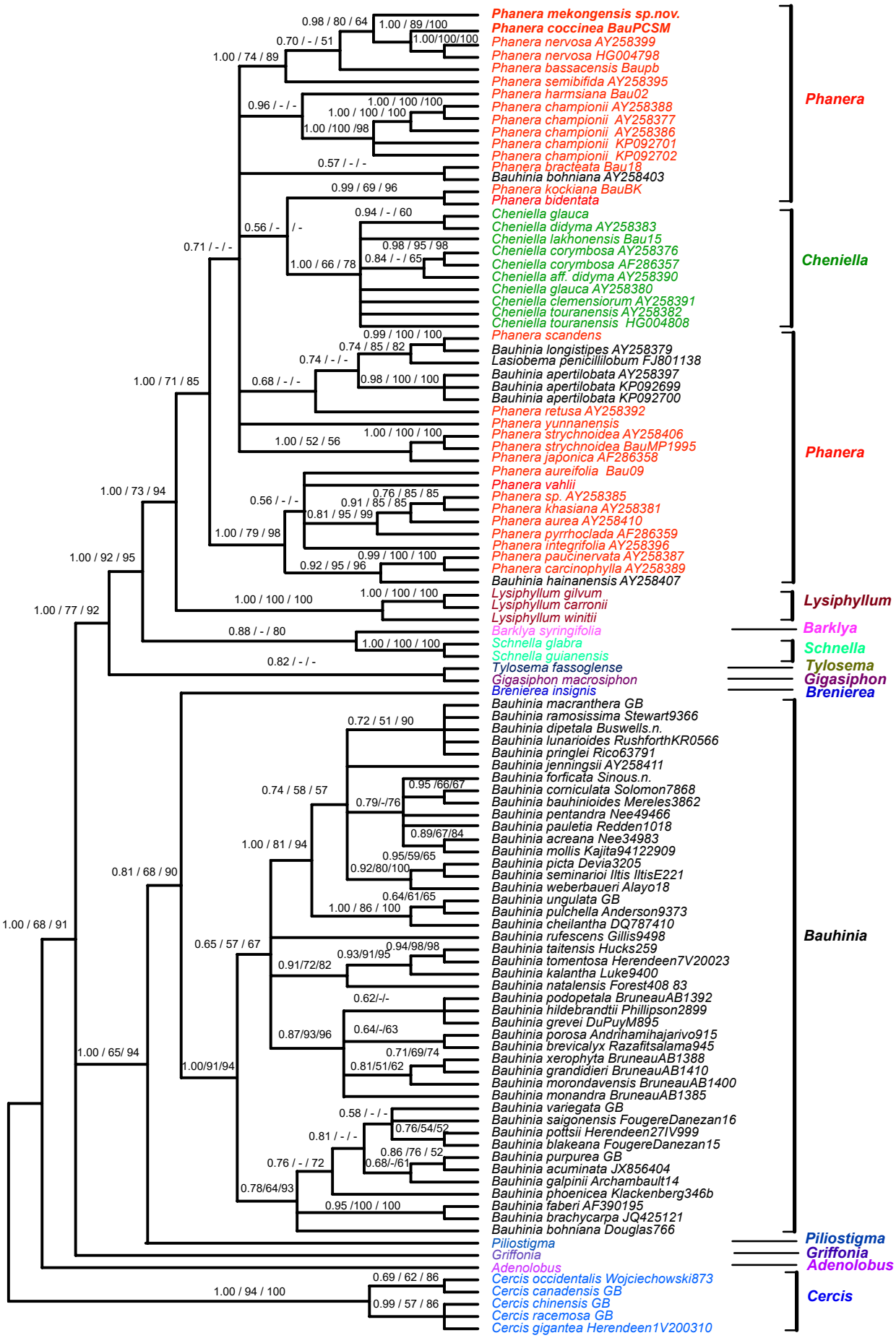

Fig. 2 Phylogram of the 50 % majority-rule consensus tree from Bayesian, maximum parsimony and maximum likelihood analyses of the combined dataset (ITS+*trnL-F*) of subfamily *Cercidoideae*, with the associated PP values and the BP values from MP and ML analyses. The new species, *Phanera mekongensis* is indicated in dark red and occurs as sister to a subclade that includes *P. nervosa* and the type species, *P. coccinea*. Numbers above branches are posterior probabilities (PP), < 0.50 not shown, and BS values of MP and of ML analyses, < 50 not shown, respectively.

Combined dataset

Analyses of the combined dataset generated results are similar to those of the ITS and *trnL-F* analyses individually. The majority-rule consensus BI tree of the concatenated dataset showed that *Phanera mekongensis* was recovered as a subclade with high support values (PP = 0.98, MP = 80 % and ML = 64 %). However, this subclade together with *P. bassacensis* and *P. semibifida* formed a polytomy with the rest of *Phanera*, which includes some species of *Bauhinia*, *Cheniella* and *Lysiphyllum* (Benth.) de Wit (Fig. 2). Therefore, those intermixed species can be recognised as species of *Phanera*, but further sampling is needed to re-evaluate their taxonomic status.

Our results robustly support the recognition of the new species, *Phanera mekongensis*, as a species in the genus *Phanera*. The new species shares morphological characteristics of the genus *Phanera* (see Introduction).

TAXONOMY

Collections of the new species were made in the Phu Wua Wildlife Sanctuary from a cultivated specimen. Plants from these seeds were then grown at the headquarters for several years until flowering. Surprisingly, these plants could not be identified to species and after a thorough morphological investigation of the specimen in comparison to other taxa we concluded that the species is new to science. Therefore, we describe and illustrate herein *Phanera mekongensis*.

The key morphological characters of the new species and of its allies are compared in Table 2, along with *Phanera coccinea* subsp. *coccinea*. Morphologically, *P. mekongensis* resembles *P. hekouensis*, a species that is confined to Yunnan, China, as both have a tubular hypanthium, calyx lobes splitting regularly at anthesis and spathulate petals. However, the new species differs significantly in the length of those characters that are much shorter than in *P. hekouensis* and in several characters of the floral part, see the diagnostic description below.

Phanera mekongensis Mattapha, Suddee & Duangjai, *sp. nov.* — Fig. 3, 4; Map 1

Similar to *Phanera hekouensis* (T.Y.Tu & D.X.Zhang) Krishnaraj but differs by having shorter pedicels (8–11 mm in *P. mekongensis* vs 21–34 mm long in *P. hekouensis*), oblong floral buds (vs spindle-shaped in *P. hekouensis*), shorter hypanthium (9–11 mm long in *P. mekongensis* vs 17–21 mm long in *P. hekouensis*), length of petal limb 7–8 mm long (vs 12–22 mm long in *P. hekouensis*), anthers densely silky with hairs on the back (vs glabrous in *P. hekouensis*), stipe of ovary c. 1 mm long (vs 8–15 mm long in *P. hekouensis*) and a short style (less than 1 mm in *P. mekongensis* vs 12–19 mm long in *P. hekouensis*). — Type: *Suddee*, *Puudjaa*, *Hemrat & Kiewbang 5390* (holo BKF; iso BK, BKF, K, KKU, QBG), Thailand, Bueng Kan Province, Bung Khla District, Phu Wua Wildlife Sanctuary, cultivated in a medicinal garden, altitude c. 190 m, 5 Nov. 2018, fl. For paratypes see below.

Etymology. The epithet refers to the Mekong River, which runs close to the Phu Wua Wildlife Sanctuary.

Liana; tendrils opposite, 4–8 cm long, hairy; young twigs densely whitish tomentose when young, becoming ferruginous at maturity. *Leaves* simple, bifid, spirally arranged, coriaceous; petioles 30–35 mm long, pubescent; lamina ovate, 6–13.5 by 5.5–12 cm, apically bifid to 1/4–1/3 of lamina length with a narrow sinus, midrib bristly between the lobes, lobes ovate to lanceolate, tip of lobes triangular-acute, margin tomentose, base deeply cordate, with 2 rounded to oblong alveoles at base of veins, 9–11-veined from base, tertiary veins reticulate-scalariform; upper surface sparsely pubescent; lower surface greyish velvety when young, densely brownish tomentose when fully grown; stipules ovate-lanceolate, 6–20 by 3–9 mm, apex acute, outer surface densely tomentose, inner surface glabrescent, caducous. *Inflorescences* terminal racemes, erect, elongated, slender, lax-flowered, 20–26 cm long, with 20–35 flowers, axis densely tomentose. *Pedicels* 8–11 mm long, densely pubescent; bracts caducous, ovate to lanceolate, 14–15 by 3–4 mm, apex acuminate, outer surface pubescent, inner surface tomentose; bracteoles caducous, linear to lanceolate, 8–9 by 2–3 mm, apex acuminate, outer surface pubescent, inner surface glabrous, inserted in lower part below the middle of pedicels. *Buds* oblong, 7–8 mm long, pubescent, apex acute. *Hypanthium* tubular, 9–11 mm long, densely pubescent. *Calyx* 5-lobed, splitting regularly at anthesis; lobes lanceolate, 7–8 by 2–3 mm, gradually separate from the base to completely separate at apex, equal, apex acute, margin pubescent, outer

Table 2 Morphological comparison of *Phanera mekongensis* with *P. coccinea* subsp. *coccinea*, *P. hekouensis* and *P. nervosa*.

Characters	*P. mekongensis*	*P. coccinea* subsp. *coccinea*	*P. hekouensis*	*P. nervosa*
Stipules	ovate-lanceolate	orbicular	obovate, rotund or orbicular	ovate-auriculate
Leaves	bifid 1/4–1/3 of lamina length	emarginate or bifid about 1/3	bifid to 1/3 of lamina length	bifid c. 1/3
Tip of leaf lobes	triangular-acute	rounded or acute	rounded or acute	rounded
Size of leaves (cm)	6–13.5 by 5.5–12	5–8 by 4–6	c. 12 by 14	6–8 by 7–9
Inflorescences	racemose	corymbose	racemose	racemose-corymbose
Shape of floral buds	oblong	spindle-shaped	spindle-shaped	oblong
Bracteoles	slightly shorter than pedicels, inserted below the middle of pedicels	longer pedicels, inserted to middle of pedicels	shorter than pedicels, inserted to above middle of pedicels	longer pedicels, inserted in middle part of pedicels
Length of hypanthium (mm)	9–11	10–15	17–21	15–20
Length of calyx lobes (mm)	7–8	18–20	18–25	25–30
Petal colour	yellowish to slightly light pink	red	white	white to yellowish
Length of petal limb (mm)	7–8	15–25	12–22	30–32
Insertion of fertile stamens	included in flowers	exserted	exserted	included
Length of stipe of ovary (mm)	c. 1	15–20	8–15	10–12
Length of style (mm)	< 1	20–25	12–19	c. 20
Indumentum of fruits	glabrous	glabrous	pubescent when young, later glabrous	pubescent
Geographic distribution	Thailand	Laos, Vietnam	China (Yunnan)	India, Myanmar, China (Yunnan), Thailand

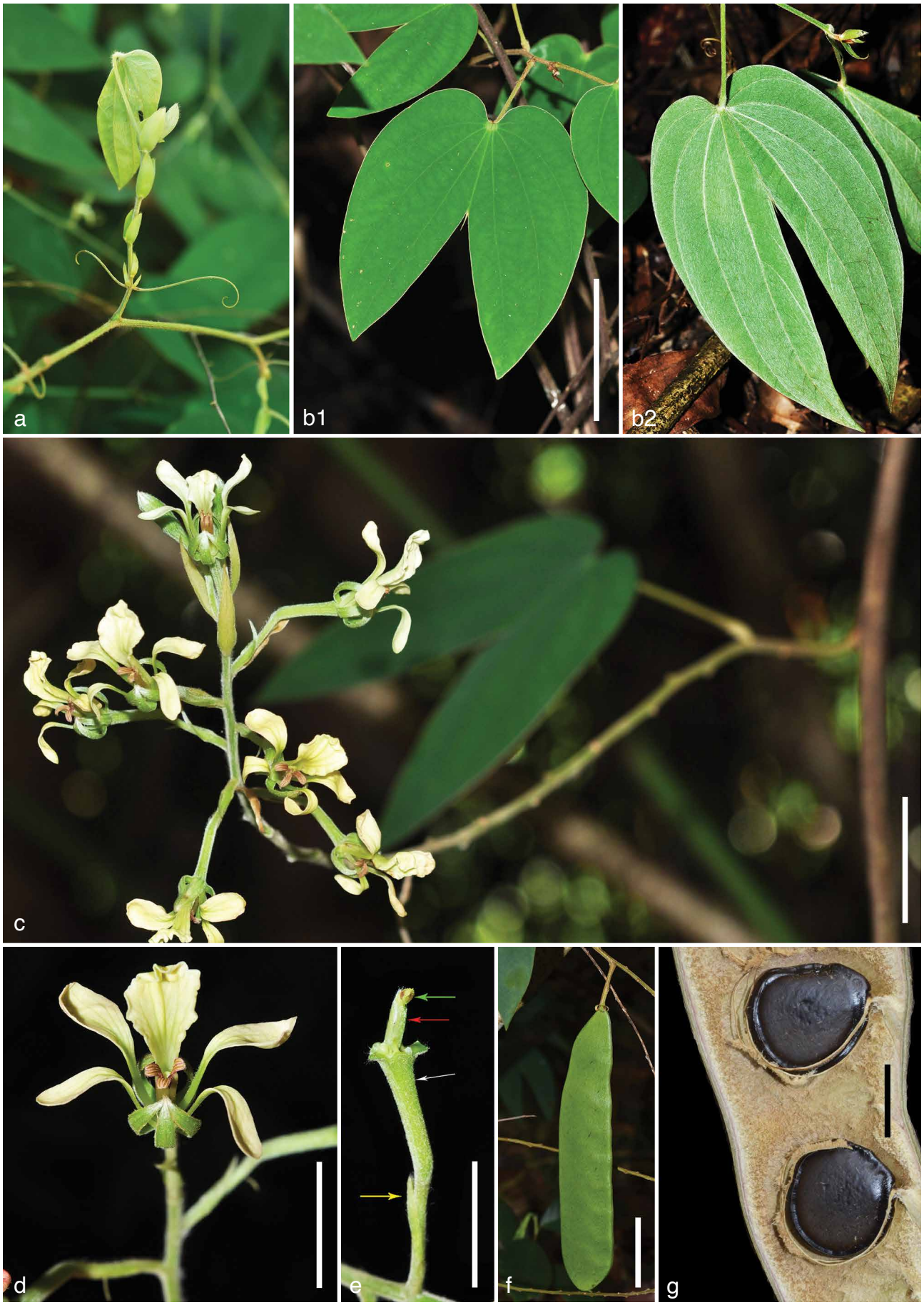

Fig. 3 Photographs of *Phanera mekongensis* Mattapha, Suddee & Duangjai. a. Tendrils and stipules; b1. upper surface of leaves; b2. lower surface of leaves; c. inflorescence; d. flower shown in top view; e. dissected flowers (calyx lobes and petals removed) showing bracteoles (yellow arrow), hypanthium (white arrow), ovary (red arrow) and stigma (green arrow); f. fruit; g. seeds. — Scale bars: b1 = 7 cm; c, g = 1 cm; d = 8 mm; e = 11 mm; f = 8 cm. — Photos by Wittawat Kiewbang.

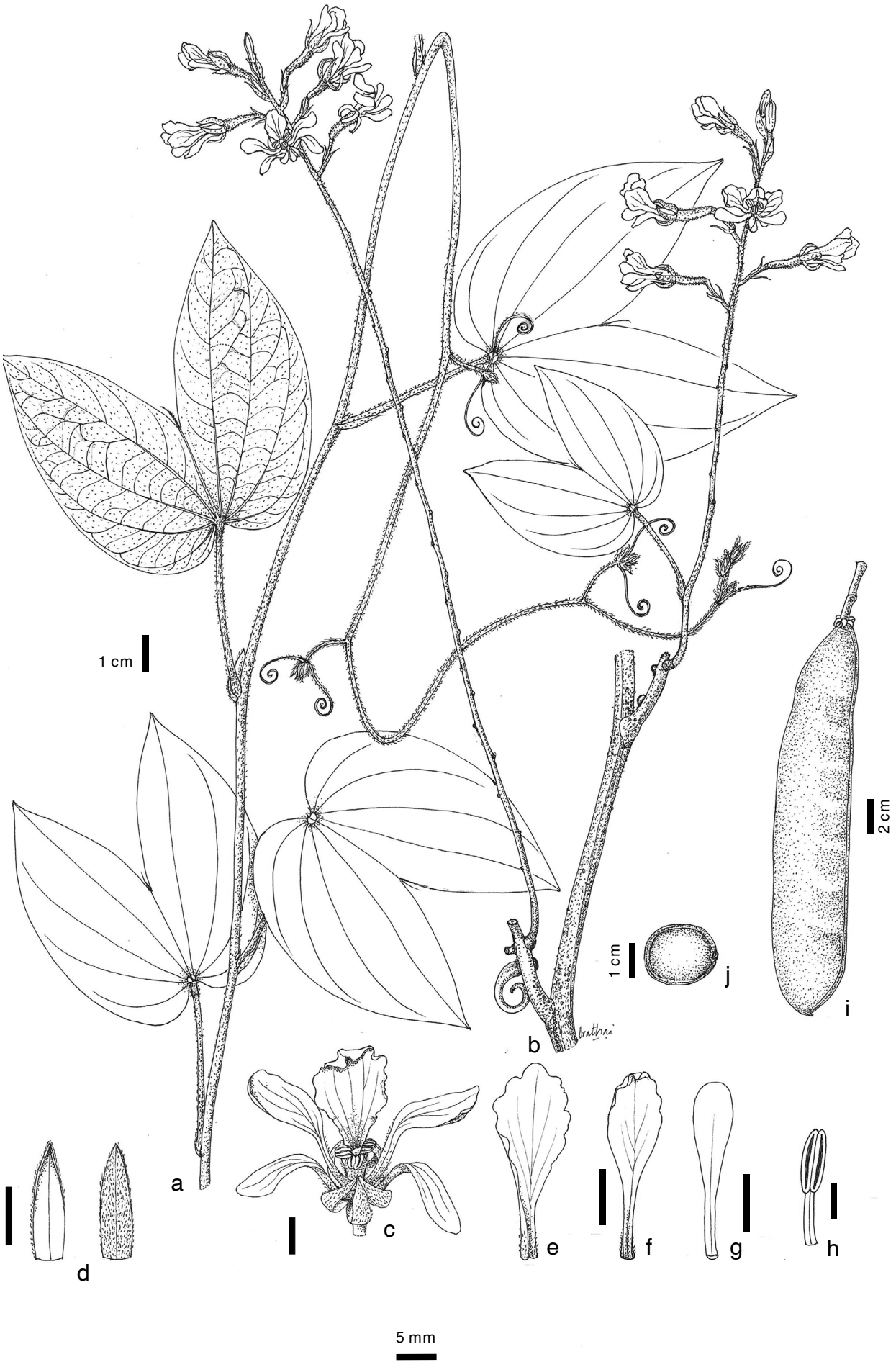

Fig. 4 *Phanera mekongensis* Mattapha, Suddee & Duangjai. a. Leaves and tendrils; b. leaf and inflorescences; c. flower (top view); d. calyx lobes showing inner side (left) and outer side (right); e. posterior petal; f–g. lateral petals; h. stamen, anther shown in front view; i. fruit; j. seed (a–h: *Suddee*, *Puudjaa*, *Hemrat & Kiewbang 5390*; i–j: *Suddee*, *Mattapha*, *Hemrat & Kiewbang 5343*; all BKF). — Drawn by Orathai Kerdkaew.

Map 1 Collection site of *Phanera mekongensis* Mattapha, Suddee & Duangjai (■).

surface densely pubescent, inner surface glabrous, persistent in fruit. *Petals* 5, light yellow to slightly light pink, posterior one relatively larger than lateral petals, spathulate or obovate; limb of posterior petal 10–11 by 8–9 mm, limps of lateral petals 8–9 by c. 3.5 mm, apex rounded, margin undulate, crisped, inner surface glabrous, outer surface densely whitish pubescent along veins, more dense pubescent on claw, otherwise glabrous, claw of posterior petal 5–6 mm long, claw of lateral petals 6–7 mm long, flattened, pubescent outside and margins. *Fertile stamens* 3, included in the flowers; filaments 5–6 mm long; anthers brown, more or less flattened, oblong, c. 2.5 by 2 mm, dehiscing by longitudinal slits, versatile, densely silky on the back. *Staminodes* 0–2; filaments c. 1 mm long; anthers absent. *Floral disc* absent. *Ovary* shortly stipitate, densely pubescent all over, 7–9-ovuled; stipe c. 1 mm long; style very short, less than 1 mm long, glabrous at upper part; stigma peltate, c. 1 mm diam. *Fruits* oblong, flattened, 20–32 by 2.5–4 cm, dehiscent, woody, glabrous, 6–8-seeded; stipe 2–3 cm long. *Seeds* flattened, elliptic to rounded, 1.4–2 by 1.2–1.7 cm, hilum crescentic, glabrous, black.

Distribution — Seeds were originally collected from Phu Ngoen Village, Bueng Kan and subsequently cultivated in Phu Wua Wildlife Sanctuary medicinal garden (Fig. 4). The precise habitat remains untraceable, and additional investigations are necessary to explore its distribution site in the natural habitat.

Habitat & Ecology — Cultivated in dry evergreen forest, elevation c. 190 m.

Phenology — Flowering: September–October; fruiting: November–February.

Vernacular name (in Thai) — Kam lang chang phueak (กำลังช้างเผือก).

Conservation status — *Phanera mekongensis* is currently known from a single population with a few individuals cultivated in Phu Wua Wildlife Sanctuary. A wild population in the area has not been found. In addition, we have no more information about the occurrence of the species in the areas of Nam Kading National Bio-Diversity Conservation Area, Lao PDR which is rich in endemic species (Tagane et al. 2018). The species can probably be found in the Lao PDR's area, opposite the Phu Wua Wildlife Sanctuary. Therefore, future intensive floristic investigations are necessary to explore the complete distribution range of the species. We assess it here as data deficient (DD) according to the IUCN Red List Categories (IUCN 2019), due to inadequacy of the distribution information.

Additional specimens examined (paratypes). Central, Bangkok, seeds were taken from Bueng Kan Province, but the exact location is unknown, and later cultivated in the Faculty of Forestry, Kasetsart University, 22 Oct. 2018, fl., *Duangjai 2018 s.n.* (BKF). – Northeastern, Bueng Kan, Bung Khla district, Phu Wua Wildlife Sanctuary, cultivated in Medicinal Garden, alt. 190 m, 10 Oct. 2007, fl., *Kaewphoung 01* (BKF); ibid., 8 Feb. 2018, fr., *Suddee*, *Mattapha*, *Hemrat & Kiewbang 5343* (BKF); ibid., 15 Sept. 2018, fl., *Suddee*, *Puudjaa*, *Hemrat & Kiewbang 5378* (BKF); ibid., 15 Oct. 2019, *Suddee*, *Puudjaa*, *Tetsana*, *Thananthaisong*, *Hemrat*, *Kiewbang & Ue-aree 5568* (BKF).

Note — *Phanera mekongensis* is remarkable by its elongate laxly racemose inflorescences, flowers without a floral disc, anthers having a densely silky indumentum on the back and an ovary with a very short style and peltate stigma. Additionally, the morphological differences of the new species with its closely related species are shown in Table 2.

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Appendix List of species, voucher information and NCBI accession numbers of taxa and genera in the subfamily *Cercidoideae* that were used in this study. Sequence: Species, Voucher (Herbarium), *trnL* intron-*trnL-F* spacer NCBI accession number, ITS NCBI accession number. – = absent; newly sequenced species are identified with an *.

Adenolobus pechuelii (Kuntze) Korcz. & Hillc., *Oliver et aL. 6527* (K), FJ801158, –; ***A. garipensis*** (E.Mey.) Torre & Hillc., *Leistuer 246* (K), –, KY306484; ***Barklya syringifolia*** F.Muell., *Weston 2449* (NSW), FJ801070, –; ***B. syringifolia***, *Yi 14483* (KUN), MF135594, –; ***B. syringifolia***, Taxon:162678, –, AY258398; ***Bauhinia acuminata*** L., –, – JX856404; ***B. acreana*** Harms, *Nee 34983* (USF), FJ801101, –; ***B. bauhinioides*** (Mart.) J.F.Macbr., *Mereles 3862* (USF), FJ801107, –; ***Bauhinia* × *blakeana*** Dunn, *Fougere-Danezan 15* (MT), FJ801115, –; ***B. bohniana*** H.Y.Chen, *Douglas 766* (MEL), FJ801052, –; ***B. bohniana*** H.Y.Chen, *Y.-F. Deng 14097* (IBSC), –, AY258403; ***B. brevicalyx*** Du Puy & R.Rabev. *Razafitsalama 945* (MO), FJ801136, –; ***B. brachycarpa*** WalL. ex Benth., *C. Davis s.n.* (GH), –, AF390195; ***B. brachycarpa*** WalL. ex Benth., *FC 095* (NCU), – FJ432276; ***B. brachycarpa*** WalL. ex Benth., Specimen voucher: 88.1198; Taxon: 183789. The University of California Berkeley Botanical Garden, –, JQ425121; ***B. cheilantha*** (Bong.) Steud., (herbarium not indicated), –, DQ787410; ***B. corniculata*** Benth., *Solomon 7868* (USF), FJ801105, –; ***B. dipetala*** Hemsl., *Buswell s.n.* (BH), FJ801078, –; ***B. forficata*** Link, *Sinou s.n.* (MT), FJ801113, –; ***B. galpinii*** N.E.Br., *Archambault 14* (herbarium not indicated), FJ801055, –; ***B. grandidieri*** Baill., *Bruneau 1410* (MT), FJ801132, –; ***B. grevei*** Drake, *Du Puy M895* (K), FJ801147, –; ***B. hainanensis*** Merr. & Chun ex H.Y.Chen, *P. Zou and R.-L. Han* 16 (IBSC), – AY258407; ***B. hildebrandtii*** Vatke, *Phillipson 2899* (NY), FJ801060, –; ***B. jenningsii*** P.Wilson, Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied, –, AY258411; ***B. kalantha*** Harms, *Luke 9400* (K), FJ801149, –; ***B. lunarioides*** A.Gray ex S. Watson, *Rushforth KR0566* (K), FJ801141, –; ***B. macranthera*** Benth. ex Hemsl., *Bridges 13138* (NY), FJ801063, –; ***B. macranthera***, *C. Schoenfeld & J. Fairey T72M64S* (UC), –, JN942381; ***B. mollis*** (Bong.) D.Dietr., *Kajita 94122909* (TUS), FJ801096, –; ***B. monandra*** Kurz, *Bruneau AB1385* (MT), FJ801127, –; ***B. morondavensis*** Du Puy & R.Rabev., *Bruneau AB1400* (MT), FJ801130, –; ***B. natalensis*** Oliv., *Kirstenboch Botanic Garden 408/83* (herbarium not indicated), FJ801064, –; ***B. pauletia*** Pers., *Redden 1018* (US), FJ801066, –; ***B. pentandra*** (Bong.) Vogel ex D.Dietr., *Nee 49466* (MO), FJ801067, –; ***B. phoenicea*** B.Heyne ex Wight & Arn., *Klackenberg 364b* (K), FJ801151, –; ***B. picta*** (Kunth) DC., *Devia 3205* (NY), FJ801068, –; ***B. podopetala*** Baker, *Bruneau AB1392* (MT), FJ801129, –; ***B. porosa*** Boivin ex Baill., *Andriamihajarivo 915* (MO), FJ801135, –; ***B. pottsii*** G.Don, *Herendeen 27-IV-99-9* (US), FJ801077, –; ***B. pringlei*** S.Watson, *Rico 637-91* (K), FJ801140, –; ***B. pulchella*** Benth., *Anderson 9373* (USF), FJ801097, –; ***B. purpurea*** L.; *Wieringa 4179* (WAG), FJ801069, –; ***B. purpurea***, herbarium not indicated, –, JX856407; ***B. ramosissima*** Benth. ex Hemsl., *Stewart 9366* (USF), FJ801101, KC952017; ***B. rufescens*** Lam., *Gillis 9498* (USF), FJ801082, –; ***B. saigonensis*** Pierre ex Gagnep., *Fougere-Danezan 16* (MT), FJ801114, –; ***B. seminarioi*** Harms ex Eggers, *Iltis & Iltis E221* (USF), FJ801102, –; ***B. taitensis*** Taub., *Hucks 259* (K), FJ801142, –; ***B. tomentosa*** L., *Herendeen 7-V-2002-3* (US), FJ801071, –; ***B. ungulata*** L., *Redden 1017* (US), FJ801073, –; ***B. ungulata***, *Araujo 1569* (HUEFS), –, FJ009818; ***B. variegata*** L., *Bruneau 1303* (MT), FJ801111, –; ***B. variegata*** *D.-X. Zhang s.n.* (IBSC), –, FJ009818; ***B. variegata***, herbarium not indicated, –, AY258378; ***B. variegata***, herbarium not indicated, –, JX856408; ***B. variegata***, herbarium not indicated, –, JX856409; ***B. weberbaueri*** Harms, *Alayo 18* (USF), FJ801103, –; ***B. xerophyta*** Du Puy & R.Rabev., *Bruneau AB1388* (MT), FJ801128, –; ***Brenierea insignis*** Humbert, *Du Puy M430* (K), FJ801159, –; ***Cercis canadensis*** L., *Bruneau 802* (MT), FJ801162, –; ***C. canadensis*** L. var. ***mexicana*** (Rose) M.Hopkins, *R. Nicholson s.n.* (UC), –, JN942404; ***C. canadensis*** var. ***mexicana***, *B.C. Cruz & D. Mahoney 2* (CAS), –, JN942405; ***C. canadensis*** var. ***mexicana***, *P.W. Fritsch 1905* (CAS), –, JN942406; ***C. canadensis*** var. ***texensis*** (S.Watson) M.Hopkins, *D.L. Breedlove & D. Mahoney 72007* (CAS, UC), –, JN942407; ***C. canadensis*** var. ***texensis***, *D.L. Breedlove & D. Mahoney 72007* (CAS, UC), –, JN942408; ***C. chinensis*** Bunge, *Bruneau 1182* (MT), FJ801163, –; ***C. chinensis***, *P.W. Fritsch* 1914 (CAS), –, JN942409; ***C. chinensis***, *P.W. Fritsch* 1916 (CAS), –, JN942410; ***C. chinensis***, *P.W. Fritsch 1918* (CAS), –, JN942411; ***C. chinensis***, *P.W. Fritsch 1919* (CAS), –, JN942412; ***C. chingii*** Chun, *P.R. Fantz 8245* (CAS, NCSU, US), –, JN942383; ***C. chingii***, herbarium not indicated, –, JN942382; ***C. gigantea*** Cheng & Keng f., *Herendeen 1-V-2003-10* (US), FJ801164, –; ***C. glabra*** Pamp., *P.R. Fantz 7236* (CAS, NCSU), –, JN942388; ***C. glabra***, *P.W. Fritsch 1908* (CAS), –, JN942389; ***C. glabra***, *P.W. Fritsch 1915* (CAS), –, JN942413; ***C. griffithii*** Boiss., *P.W. Fritsch xxxx (*CAS), –, JN942414; ***C. occidentalis*** A. Gray, *Wojciechowski 873* (ASU), FJ801156, –; ***C. racemosa*** Oliv., *1980 Sino-American Botanical Expedition s.n* (herbarium not indicated), –, JN942390; ***C. racemosa***, *P.W. Fritsch 1910* (CAS), –, JN942391; ***C. racemosa***, *Herendeen 1-V-2003-07* (US), FJ801160, –; ***C. siliquastrum*** L., *P.W. Fritsch 1911* (CAS), –, JN942395; ***C. siliquastrum***, *W. Roderick*, *s.n.* (UC), –, JN942396; ***C. siliquastrum***, *J. van der Hoek s.n.* (UC), –, JN942397; ***Cheniella clemensiorum*** (Merr.) R.Clark & Mackinder, *D.-X. Zhang s.n.* (IBSC), –, AY258391; ***C. corymbosa*** (Roxb. ex DC.) R.Clark & Mackinder, *D.-X. Zhang s.n.* (IBSC), –, AF286357; ***C. corymbosa***, *D.-X. Zhang s.n. (*IBSC), –, AY258376;***C. didyma*** (H.Y.Chen) R.Clark & Mackinder, Y.-F. Deng 14470 (IBSC), –, AY258383; ***C***. aff. ***didyma*** (H.Y.Chen) R.Clark & Mackinder, *R.-J. Wang 5* (IBSC), –, AY258390; ***C. glauca*** (Benth.) R.Clark & Mackinder, *R.-J. Wang 1* (IBSC), –, AY258380; ***C. glauca***, *D.-X. Zhang s.n.* (IBSC), –, AY258384; ****C. glauca***, *S. Duangjai 2008 102 (Bau11)* (BKF), MT498358, –; ****C. lakhonensis*** (Gagnep.) R.Clark & Mackinder, *S. Duangjai 2008 30 (Bau15)* (BKF), MT498359, –; ***C. touranensis*** (Gagnep.) R.Clark & Mackinder, *R.-J. Wang 4* (IBSC), –, AY258382; ***C. touranensis*** (Gagnep.) R.Clark & Mackinder, *Liana Mengsong 145_9_8* (HITBC), –, HG004808; ***Gigasiphon macrosiphon*** (Harms) Brenan, *Kew ID1990-1508* (K), FJ801108, –; ***G. physocarpa*** Baill., *Wieringa 4498* (WAG), FJ801165, –; ***G. simplicifolia*** (Vahl ex DC.) Baill., *766* (UIH), MF135596, –; ***G. simplicifolia***, Taxon: 3850, –, MH707248; ***Lysiphyllum binatum*** (Blanco) de Wit, *Hopkins 1729* (K), FJ801149, –; ***L. binatum***, *Yi 4679* (KUN), MF135597, –; ***L. carronii*** (F.Muell.) Pedley, *Weston 2447* (NSW), FJ801054, –; ***L. carronii***, Royal Botanical Garden, Sydney, Australia (living collection); P. Weston 2445 (herbarium not indicated), –, AY258400; ***L. cunninghamii*** (Benth.) de Wit, Fairchild Tropical Garden 6805 (herbarium not indicated), FJ801083, –; ***L. gilvum*** (F.M.Bailey) Pedley, *Weston 2446* (NSW), FJ801057, –; ***L. gilvum*** (F.M.Bailey) Pedley, Royal Botanical Garden, Sydney, Australia (living collection); *P. Weston 2446* (herbarium not indicated), –, AY258401; ***L. hookeri*** (F.Muell.) Pedley, *Weston 2445* (NSW), FJ801059, –; ***L. hookeri***, *Yi 14678* (KUN), MF135601, –; ***L. strychnifolium*** (Craib) A.Schmitz, (Dongphahuan Botanical Garden, Thailand, cultivated), –, AY258405; ***L. winitii*** (Craib) de Wit, *C. Niyomdham s.n.* (IBSC), –, AY258402; ***L. winitii***, 2125 (K), FJ801152, –; ***Phanera apertilobata*** Merr. & F.P.Metcalf, *D.-X Zhang 923* (IBSC), –, AY258397; ***P. apertilobata***, *SCBGP462_1* (IBSC), –, KP092699; ***P. apertilobata***, *SCBGP462_2* (IBSC), –, KP092700; ***P. aurea*** (H.Lév.) Mackinder & R.Clark, *S.-J. Li and X.-X. Huang 4* (IBSC), –, AY258410; ****P. aureifolia*** (K.Larsen & S.S.Larsen) Bandyop., Ghoshal & M.K.Pathak, *S. Duangjai 2009 s.n. (Bau09)* (BKF), MT498356, MT515379; ****P. bassacensis*** (Pierre ex Gagnep.) de Wit, *S. Duangjai20161117 (Baupb)* (BKF), MT498352, MT515375; ****P. bracteata*** Benth., *S. Duangjai s.n. (Bau18)* (BKF), MT498354, MT515377; ***P. bidentata*** (Jack) Benth., *Stone 12643* (K), FJ801143, –; ***P. carcinophylla*** (Merr.) Mackinder & R.Clark, *S.-J. Li and X.-X. Huang 14* (IBSC), –, AY258389; ***P. championii*** Benth., *R.-J. Wang s.n.* (IBSC), –, AY258377; ***P. championii***, *D.-X. Zhang s.n.* (IBSC), –, AY258386; ***P. championii***, *S.-J. Li and X.-X. Huang 24* (IBSC), –, AY258388, ***P. championii***, –, KP092701; ***P. championii***, SCBGP204_2 (herbarium not indicated), –, KP092702; ****P. coccinea*** Lour., Lao PDR: Bolikhamxai, Lanorsavanh et al. 1836 (Biology Herbarium of National University of Laos), MT498351, MT515374; ****P. harmsiana*** (Hosseus) Bandyo P. & Ghoshal, *S. Duangjai 2012 s.n.* (Bau02) (BKF), MT498353, MT515376; ***P. integrifolia*** (Roxb.) Benth., Singapore Botanical Garden (living collection); Ruth Kiew supplied, –, AY258396; ***P. japonica*** (Maxim.) H.Ohashi, South China Botanic Garden (living collection), –, AF286358; ***P. khasiana*** (Baker) Thoth., *R.-J. Wang s.n.* (IBSC), –, AY258381; ****P. kockiana*** (Korth.) Benth., *S. Duangjai 2017 s.n.* (BauBK) (BKF), MT498355, MT515378; ***P. kockiana***, Singapore Botanical Garden (living collection); Ruth Kiew supplied, – , AY258394; ***P. longistipes*** T.C.Chen, *Y.-F. Deng* 14078 (IBSC), –, AY258379; ****P. mekongensis*** Mattapha, Suddee & Duangjai, *S. Duangjai 2018 s.n. (BauWC)* (BKF), MT498350, MT515373; ***P. nervosa*** Benth., *D.-X. Zhang s.n.* (IBSC), –, AY258399; ***P. nervosa***, *Liana Mengsong 145_5_4* (HITBC), –, HG004798; ***P. paucinervata*** (T.C.Chen) X.Y.Zhu, *S.-J. Li and X.-X. Huang 21* (IBSC), –, AY258387; ***P. penicilliloba*** Pierre ex Gagnepain, *Larsen & aL. 31900* (K), FJ801138, –; ***P. pyrrhoclada*** (Drake) de Wit, South China Botanic Garden (living collection), –, AF286359; ***P. retusa*** Benth., Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied, –, AY258392; ****P. scandens*** (L.) Lour. ex Raf., *S. Duangjai 2008 48 (Bau14)* (BKF), MT498357, –; ***P. scandens***, *G.-A. Fu 6052* (IBSC), –, AY258408; ***P. semibifida*** (Roxb.) Benth., Singapore Botanical Garden (living collection), Ruth Kiew supplied, –, AY258395; ***P. strychnoidea*** (Prain) Bandyo P. & Ghoshal, *C. Niyomdham s.n.* (IBSC), –, AY258406; ****P. strychnoidea***, *M.Phoophat 1995* (BKF), –, MT515380; ***P. vahlii*** (Wight & Arn.) Benth., *Gillis 7882* (FTG), FJ801090, –; ***P. yunnanensis*** (Franch.) Wunderlin, *Hart s.n.* (USF), FJ801084, –; ***P. yunnanensis,*** *Y.-F. Deng 14050* (IBSC), –, AF286360; *P.* sp., *S.-J. Li and X.-X. Huang 5* (IBSC), –, AY258385; ***Piliostigma reticulatum*** (DC.) Hochst., Singapore Botanical Garden (living collection); Ruth Kiew supplied, –, AY258404; ***P. thonningii*** (Schumach.) Milne-Redh., *Friis 7184* (K), FJ801122, –; ***Schnella glabra*** (Jacq.) Dugand, *Redden 1038* (US), FJ801058, –; ***S. glabra*** (Jacq.) Dugand, Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied, –, AY258409; ***S. guianensis*** (Aubl.) Wunderlin, *Redden 1024* (US), FJ801120, –; ***S. guianensis***, *Redden 1036* (US), FJ801121, –; ***S. guianensis,*** NL110013, –, FJ037825; ***S. hymenaeifolia*** **(**Triana ex Hemsl.) Britton & Rose, *Nee 6899* (NY), FJ801061, –; ***S. hymenaeifolia***, *Gentry 18078* (USF), FJ801093, –; ***S. outimouta*** (Aubl.) Wunderlin, *Redden 1021* (US), FJ801118, –; ***Tylosema argenteum*** (Chiov.) Brenan, *Alstrup 35* (K), FJ801146, –; ***T. fassoglense*** (Kotschy ex Schweinf.) Torre & Hillc., *Fantz 3715* (FTG), FJ801091, –; ***T. fassoglense***, *Herendeen 21-XII-97-6* (US), FJ801124, –; ***T. fassoglense***, OM 1152 (HJRAU), MF135600, – ***T. fassoglense***, Fairchild Botanic Garden, USA (living collection); Benoit Jonckheere supplied, –, AY258393.