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Molecular phylogeny and taxonomic notes on *Premna* (Lamiaceae) in Thailand

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The genus *Premna*, one of the largest woody genera of Lamiaceae, comprises approximately 130 species distributed across the tropics and subtropics of the Old World. Previous classification and identification of the genus has mainly relied on morphological studies, resulting in significant taxonomic ambiguity and misidentification. In Thailand, over 20 *Premna* species have been recorded but their phylogenetic relationships have never been well investigated, particularly within the *P. serratifolia* complex which is the most taxonomically complicated species with the most extensive synonymy within the genus, leading to uncertainty regarding species recognition. This study presents the first comprehensive molecular phylogeny of *Premna* in Thailand by utilizing four chloroplast regions (*ndhF*, *rbcL*, *rps16*, and *trnL-F*), and focuses on resolving the intricate relationships within the *P. serratifolia* complex while providing taxonomic notes for Thai species. The results confirm the monophyly of *Premna* and reveal two primary clades which are discussed in the context of both morphological and molecular evidence. We successfully disentangled the taxonomic confusion surrounding the *P. serratifolia* complex with reinstatement of *P. paniculata*, and *P. punctulata* while *P. cordifolia* was confirmed as a distinct species and occurs in Thailand. We also treated *P. octonervia* under *P. serratifolia*. Additionally, *P. interrupta* var. *smitinandii* was reduced to a synonym of *P. interrupta*, *P. coriacea* was excluded from the Thai flora, and *P. coriacea* var. *villosa* was lectotypified. As a result, 21 species are now recognized in Thailand. To facilitate future identification efforts, we provide an updated diagnostic key for all species, detailed morphological descriptions, a comprehensive list of examined specimens within the *P. serratifolia* complex, and field photographs of several species. These findings enhance our understanding of the taxonomy and phylogeny of *Premna* in Thailand, offering a robust framework for further research on this taxonomically challenging genus.

Keywords cpDNA, Mint family, *Premna serratifolia*, Synonymy, Systematics

Premna L. consists of about 130 species distributed in the tropics and subtropics of Asia, Africa, and Australia[1–6]. The genus was first described by Linnaeus[7] based on the two species: *P. serratifolia* L. and *P. integrifolia* L. (now synonymized under the former one). The generic name was derived from the Greek word '*Premnon*' which means 'stump of the tree', likely alluding to the short and twisted tree trunks of the type species. The genus could be distinguished from other Lamiaceae genera by its terminal inflorescences, mostly small zygomorphic to subactinomorphic flowers, a two-lipped calyx with 4–5 lobes or unlobed, a typically two-lipped, five-lobed corolla with dense white hairs on the inner surface, and drupaceous fruits[3,4,8].

Several species of *Premna* are widely recognized in Asia for their medicinal applications, attributed to their active phytochemical constituents and diverse bioactivities[9]. For example, local people in northern Thailand boil the stems and leaves of *P. tomentosa* Willd. to cure lumbago[10]. This species also holds significance in traditional Indian medicine, as it contains flavonoids, tannins, alkaloids, and terpenoids, compounds associated with antihyperlipidemic, hepatoprotective, anticancer, antioxidant, and antibacterial properties[11]. In western India, various vegetative parts of *P. barbata* Wall. ex Voigt are employed by local populations to treat a wide range of conditions, including joint and back pain, diarrhea, wound healing, throat infections, bacterial diseases, fever, herpes simplex infections, and arthritic pain[12]. Several studies have explored the antibacterial and mosquito

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larvicidal activities of essential oil extracts from various species, including *P. acutata* W. W. Sm., *P. cambodiana* Dop, *P. mollissima* Roth, *P. odorata* Blanco, *P. puberula* Pamp., *P. serratifolia*, and *P. tomentosa*[11,13,14]. Among these, the essential oils derived from the leaves of *P. odorata* have demonstrated notable anti-aging properties[15].

After the transfer from Verbenaceae to Lamiaceae[2,16], *Premna* has been shown to be monophyletic[5,6,17,18] and its phylogenetic position within Lamiaceae has been well explored as a member of the subfamily Premnoideae in previous large-scale molecular phylogenetic studies[5,19,20]. Recently, several new species of *Premna* have been reported from China, Myanmar, and Vietnam, such as *P. bhamoensis* Y. T. Tan & B. Li, *P. caridantha* Y. H. Tan & B. Li, *P. vietnamensis* B. Li, and *P. grandipaniculata* Y. H. Tan & B. Li[17,21–23]. The nomenclatural changes have been also made with consideration of on Chinese and Vietnamese species[24–27]. Nevertheless, classification and identification within the genus *Premna* are considered highly complex within the Lamiaceae and require further clarifications[5].

Premna serratifolia, a species widely distributed across southern and southeastern Asia, Northern Australia, and western Africa, has been traditionally utilized by people in Malesian regions as a vegetable and for its medicinal properties. Various parts of the plant are used to treat ailments such as fever, stomach-ache, diarrhea, shortness of breath, and breast milk production[3]. Furthermore, this species produces diverse essential oils with bioactive compounds that demonstrate potential therapeutic applications[14]. Despite its ecological and pharmacological importance, the delimitation of *P. serratifolia* has faced significant taxonomic challenges due to morphological variation across its extensive geographical range, particularly in the shape and size of leaves and inflorescences[3]. More than 50 taxa have been placed in synonymy with *P. serratifolia*[4], reflecting confusion in its taxonomic history. However, some synonymized species such as *P. paniculata* H. R. Fletcher, and *P. punctulata* C. B. Clarke, exhibit apparently distinct morphological and ecological characteristics that differentiate them from *P. serratifolia*, warranting further investigation for their taxonomic identities. The taxonomic status of *P. cordifolia* Roxb. is also uncertain, as this accepted species was previously regarded as invalidly published and later synonymized under *P. serratifolia* in a recent taxonomic treatment[3]. This ambiguity highlights the need for a comprehensive re-evaluation to clarify its taxonomic identity. The taxonomic complexity surrounding *P. serratifolia* underscores the need for comprehensive studies to clarify its species boundaries and phylogenetic relationships. Such efforts will not only enhance our understanding of this genus but also contribute to the preservation and sustainable utilization of its valuable resources.

In Thailand, the first comprehensive taxonomic work of *Premna* was carried out by Fletcher[28], recognizing 24 species (30 taxa). Subsequently, Moldenke[29] listed 28 species (36 taxa) without detailed morphological description. However, there were marked differences in species recognition between these two taxonomic treatments, highlighting the need for further clarification and revision of the genus in Thailand. For example, Moldenke[29] notably excluded *P. coriacea* C. B. Clarke, *P. herbacea* Roxb., *P. nana* Collett & Hemsl., and *P. racemosa* Wall. ex Schauer [= *P. interrupta* Wall. ex Schauer in this study] in his work, while Fletcher's taxonomic treatment was lacking *P. benglaensis* C. B. Clarke, *P. chevalieri* Dop, and *P. esculenta* Roxb. After reporting three new records for Thailand: *P. punctulata*, *P. rabakensis* Moldenke, and *P. stenobotrys* Merr.[30], Leeratiwong et al.[8] published the first synopsis of *Premna* in Thailand based on the above-mentioned works, recognizing 23 species. However, several following studies have introduced nomenclatural changes that significantly impact species recognition within the genus in Thailand. For example, when revising *Premna* for the *Flora Malesiana*, de Kok[3,4] treated four species listed in Thailand as synonyms of *P. serratifolia*, including *P. cordifolia*, *P. octonervia* Merr. & F. P. Metcalf, *P. paniculata*, and *P. punctulata*, and synonymized *P. hamiltonii* J. L. Ellis and *P. pyramidata* Wall. ex Schauer under *P. odorata*, and *P. tomentosa*, respectively. These treatments have contributed to taxonomic uncertainty regarding total number of species in Thailand, complicating species identification. Furthermore, our preliminary findings further suggest the taxonomic status of some taxa in Thailand, including *P. cordifolia*, *P. coriacea*, *P. interrupta* var. *smitinandii* Moldenke, and *P. octonervia*, requires reassessment. Therefore, these discrepancies highlight the challenges in achieving consensus on *Premna* taxonomy and underscore the need for a comprehensive taxonomic revision to clarify both the *P. serratifolia* complex and the status of these taxa in Thailand.

Phylogenetic studies on Asian Lamiaceae have utilized chloroplast regions as molecular markers to delimit species complexes and to infer interspecific relationships, such as genera *Coleus* Lour., *Chelonopsis* Miq., *Clerodendrum* L., *Isodon* (Benth.) Schrad. ex Spach, *Paraphlomis* Prain, *Phlomoides* Moench, *Plectranthus* L'Hér., *Pogostemon* Desf., and *Vitex* L.[19,31–38]. Previous taxonomic ambiguities within these genera have been clarified through morphological characteristics and molecular analyses based on multi-locus approach of chloroplast regions, such as *matK*, *ndhF*, *rbcL*, *rpl32-trnL*, *rps16*, *rpsF*, *rpsR2*, *trnL*, *trnL-F*, *trnS-G*[5,19,31,34,35,39,40]. In *Premna*, only few phylogenetic analyses have demonstrated that three to five chloroplast regions allow the resolution of phylogenetic placements and constructing the relationships across both regional and larger scales[5,17]. However, most of these analyses have been based on species primarily sampled from China and Vietnam. Integrating molecular and morphological evidence is essential for resolving species delimitation within the *P. serratifolia* complex and clarifying the overall phylogenetic relationships of *Premna* in Thailand.

The present study aims to: (1) establish a comprehensive phylogenetic framework with a focus on *Premna* in Thailand using four chloroplast markers (*ndhF*, *rbcL*, *rps16*, and *trnL-F*); (2) revise the species delimitation within the *P. serratifolia* complex in Thailand based on morphological, ecological investigations and molecular analyses; (3) clarify taxonomic status of *P. cordifolia* in Thailand (4) reassess the taxonomic status and typification of certain species in Thailand; and (5) determine the number of *Premna* occurring in Thailand and provide a revised identification key.

Results

A total of 3797 nucleotides were analyzed from four chloroplast markers (1015 for *ndhF*, 1279 for *rbcL*, 795 for *rps16*, and 708 for *trnL-F*) after being concatenated and aligned. The topologies generated from the ML and BI

analyses were slightly different (Fig. 1, Supplementary Fig. S1). In the BI analysis, *P. menglaensis* B. Li was placed in different position in comparison to the ML analysis within subclade I. Minor conflicts were also observed in the different analyses: *P. ligustroides* Hemsl. and *P. vietnamensis* within subclade III (Fig. 1, Supplementary Fig. S1). However, these minor conflicts were only observed in otherwise similar subclades. Given the overall branch support and robustness of the topology, the BI tree was selected here for interpreting relationships within *Premna* in Thailand with the posterior probability (PP) inferred from BI analysis and the bootstrap value (BS) from ML analysis marked above branches, respectively (Fig. 1).

All species of *Premna* in this study formed a monophyletic clade with full supports [1.00/100 – 1.00 from BI posterior probabilities (PP) and 100% from ML bootstrap (BS), the values are listed in the same order below] (Fig. 1). Within *Premna*, two primary clades were formed. Clade I (1.00/97) consists of *P. mekongensis* W. W. Sm., *P. punicea* C. Y. Wu, and *P. tenii* C. Pei with unresolved relationships. Clade II contains the remaining species with *P. tomentosa* and *P. serrata* H. R. Fletcher unresolved with respect to a large clade (1.00/99) which

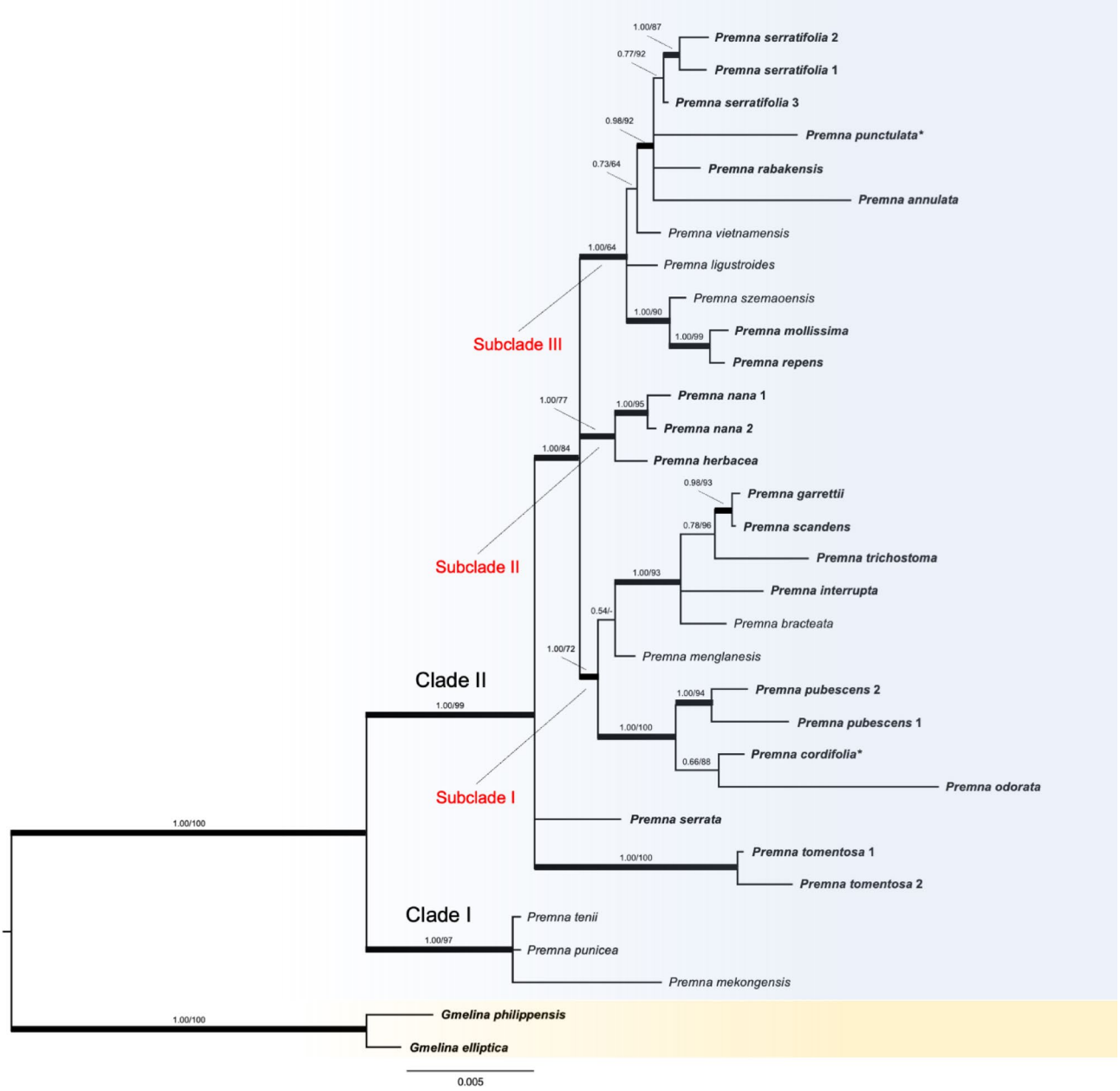

Fig. 1. Bayesian 50% majority-rule consensus tree of *Premna* with emphasis on Thai species based on concatenated four chloroplast regions dataset: *ndhF*, *rbcL*, *rps16*, and *trnL-F*. Bayesian inference posterior probability (PP) and maximum likelihood bootstrap support (BS) and are shown in order above the branches, respectively. The bold lines indicate more than 0.95 PP and bold font refers to species presented in Thailand. A dash (–) indicates a different clade obtained from maximum likelihood analysis. Labelled clades (I and II) and subclade (I to III) are recognized in this study.

could be divided into three subclades. Though relationships of the three subclades were unresolved, each of them obtained moderate to high support: subclades I (1.00/72) and III (1.00/64) accommodates seven and six species found in Thailand, respectively, while subclade II (1.00/77) consists of two species: *P. herbacea* and *P. nana*.

A taxonomic note of *Premna* in Thailand was achieved through the investigation of herbarium specimens, morphological and ecological characters, as well as examinations on type specimens. These findings, combined with molecular analyses, have successfully resolved the *P. serratifolia* complex in Thailand, as summarized in Table 1 and shown in Figs. 1–5 (see Taxonomic note of species within the *P. serratifolia* complex in Thailand and Supplementary Table S1). Additionally, our study has validated the taxonomic status of *P. coriacea*, *P. interrupta* var. *smitinandii*, and *P. octonervia* in Thailand (see Taxonomic notes below).

Discussion

Phylogenetic reconstruction

By using multi-locus approach of four chloroplast markers, this study provided the first phylogenetic relationships with emphasis on species of *Premna* in Thailand (bolded species, Fig. 1). Our findings are strongly consistent with previous studies based on chloroplast regions and plastome analyses, confirming that the genus *Premna* is a monophyletic within the subfamily Premnoideae and forms a sister relationship with *Gmelina* L.[5,6,17,20,32,41,42]. However, the phylogenetic positions of some species remain unresolved due to polytomy, such as *P. ligustroides*, *P. serrata*, and *P. tomentosa*. These unresolved relationships have been commonly observed in previous phylogenetic analyses within *Premna*[5,17] and other taxa in many Lamiaceae genera, such as *Leucas* R.Br., *Otostegia* Benth., *Phlomis* L., *Phlomoides*, *Salvia* L., *Stachys* L.[37,43–45]. This may be the result of insufficient data, as few chloroplast markers have been used. Therefore, further studies employing more comprehensive approaches, such as plastid genome, a target enrichment or whole genome analyses, are necessary to explore these relationships (*e.g.* Zhao et al.[6,46], Satthaphorn et al.[47]). In this study, phylogenetic relationships based on four concatenated chloroplast markers resolved in two clades (Fig. 1). In following, phylogenetic positions and morphological characteristics are discussed.

CLADE I—This highly supported clade includes species distributed in China and Vietnam, namely *P. mekongensis*, *P. punicea*, and *P. tenii*, which are characterized by morphological synapomorphies such as cymes in a dense capitate inflorescence and calyces distinctly 5-lobed. This phylogenetic placement is consistent with morphology-based classification[48]. Further molecular study is required to test whether all species with the similar traits as recorded in *Flora of China*[49] and adjacent regions form a monophyly.

CLADE II —The well-supported clade consists of a clade comprising subclades I to III (red labelled in Fig. 1), with the unresolved relationships among *P. serrata* and *P. tomentosa*. These two latter species possess distinct morphological characteristics from all species in Thailand. *Premna serrata* has serrate leaf margin and distinct calyx lobes (1–1.8 mm long)[8,28]. *Premna tomentosa*, previously recognized as *P. pyramidata* by Chen and Gilbert[49] and Leeratiwong et al.[8], exhibits stellate or dendritic hairs on both leaf surfaces, a unique trait distinguishing from other *Premna* (vs. non-stellate or dendritic in other species). The presence of this characteristic has been documented in several morphological studies[3,4,8,22,49]. In addition, this clade incorporated several species that have been not included in previous phylogenetic studies, such as *P. annulata* H. R. Fletcher, *P. cordifolia*, *P. repens* H. R. Fletcher, *P. scandens* Roxb., *P. serrata*, *P. tenii*, *P. tomentosa*, and *P. trichostoma* Miq.

Three unresolved subclades (subclades I to III) within Clade II were identified based on phylogenetic positions (with high ML and full BS supports, Fig. 1) and morphological characteristics. These subclades include several species in Thailand, as well as members of the *P. serratifolia* complex in Thailand. Although clear morphological characteristics have been not identified to support the assignment of infrageneric ranks across all subclades, we observed certain habits and morphological affinities among particular species to discuss with their phylogenetic positions.

Characters	*P. cordifolia*	*P. paniculata*	*P. punctulata*	*P. serratifolia*
Interpetiolar woody ridges on stem	Present	Absent	Absent	Present
Leaf base shape	Cordate or rounded	Cuneate	Rounded or slightly oblique-rounded	Mostly cuneate
Hairs on nerve axils on abaxial leaf surface	Pubescent	Glabrous	Sparsely pubescent	Densely pubescent
Inflorescence length	4–8 cm	0.7–2 cm	6–20 cm	(0.5–)2–6 cm
Posterior lip of calyx	entire or subentire	apically 3-lobed	entire to subentire	mostly entire to subentire or rarely 2–3-lobed
Corolla length	5–7 mm	5–6 mm	5–6 mm	2–5 mm
Corolla tube surface	Distally sparsely pubescent	Glabrous	Glabrous to distally pubescent	Distally pubescent
Yellow patch on the middle lobe of anterior corolla lip	Present	Absent	Present	Absent
Fruit size	5.32–6.65 × 4.25–5.66 mm	3.22–4.20 × 2.33–3.82 mm	4.37–5.70 × 3.79–5.69 mm	3.33–4.89 × 2.58–2.99 mm
Endocarp protrusion shape	thorn-like	thorn-like	saccate-like	saccate-like
Habitat preference	Dry to moist evergreen forests	Dry to moist evergreen forests	Dry to moist evergreen forests	Coastal areas

Table 1. Morphological comparison between *P. serratifolia* and the taxa previously treated as its synonyms, based on specimen examinations and data from earlier publications[8,28,30,50,58,80].

Subclade I includes nine species of *Premna* which are shrub or woody climber, distributed in Myanmar, China, and Thailand. These species are mostly inhibited in hill evergreen or limestone forests[8]. Of these, five species forming a monophyletic group within this subclade (*P. bracteata* Wall. ex C. B. Clarke, *P. garrettii* H. R. Fletcher, *P. interrupta*, *P. scandens*, and *P. trichostoma*) have shared clavoid fruits and a thin, fleshy pericarp layer, whereas other species exhibit more or less globose fruits with thicker pericarp[3,4,8,49,50]. These fruit characteristics, along with endocarp morphology, have been validated as important diagnostic features for species identification and grouping of *Premna* in Thailand[50] and align with the results of our chloroplast-based analysis. This subclade also involves *P. cordifolia*, a species though to be a synonym under *P. serratifolia* (subclade III) in the Malesian treatment[3]. The presence of *P. cordifolia* in this subclade, integrated with morphological evidence, greatly support the distinction between these taxa, as congruent with earlier treatments[8,50,51]. Additional samples for this subclade exhibiting the synapomorphy is necessary to further establish a robust foundation for a potential morphology-based infrageneric classification. Subclade II consists of two herbaceous species: *P. herbacea* and *P. nana*. Both species display a herbaceous suffrutescent habit with a woody rootstalk and softly woody to herbaceous stems which can be found in dry places, particularly in deciduous forest across southeast Asia[8,49,52]. The close relationships of these two species are consistent with the previous phylogenetic analyses by Li et al.[5] and Hai et al.[17]. *Premna nana* was treated as a synonym of *P. herbacea* in the revision by Chen and Gilbert[49]. However, subsequent studies and our findings have supported the recognition of *P. nana* as a distinct species based on morphological characteristics (see identification key below) and molecular phylogenetic evidence[3–5,8,17].

Subclade III contains *Premna* representatives which are diverse in their habits and morphological characteristics. Most species are shrubs, scandent shrubs, or woody climbers, with the exception of *P. mollissima*, *P. rabakensis*, and *P. szemaoensis* C. Pei, which are tree species. Species belonging to this clade occupy wide range of habitats such as coastal, evergreen, hill evergreen, or limestone forests[8]. Distinct morphological traits among species were observed such as the presence or absence of an interpetiolar ridge on stems, type of hairs on leaf surfaces, the size and shape of the calyx lobes. Some species within this subclade are also endemic, including *P. annulata* (Thailand), *P. szemaoensis* (China), and *P. vietnamensis* (Vietnam)[8,17,49]. Three accessions of *P. serratifolia* sampled from different sides of peninsular Thailand recovered a monophyletic group also present within this subclade (0.77/92) as well as its previously synonymized species, *P. punctulata*[3]. Our results showed that *P. punctulata* was unresolved with respect to *P. serratifolia*, exhibiting a relatively long branch, which reflects a greater degree of genetic change[53]. However, this pattern confirms that these taxa are not fully represented as genetically discrete entities[54]. This result is consistent with previous phylogenetic studies, which likewise reported a distinct phylogenetic position for *P. punctulata* separate from *P. serratifolia*[5,17]. Our findings thus reaffirm this separation, further supported by morphological and ecological evidence (see taxonomic note below).

Taxonomic notes

Note on P. serratifolia complex in Thailand

As part of the preparation of *Premna* for the *Flora of Thailand*, the taxonomic status of three names, including *P. cordifolia*, *P. paniculata*, and *P. punctulata*, has remained ambiguous in relation to *P. serratifolia*, which were thought to be synonyms in the previous study[3]. However, our integrated morphological and ecological investigation, and chloroplast-based phylogenetic analyses support the recognition of these as distinct species, prompting a revision of their taxonomic identities (Table1, Fig. 1). Notably, *P. serratifolia* is confined to coastal habitats, whereas the other species are restricted to evergreen forests. The relevant taxonomic literature, comparative morphology and ecological descriptions, and taxonomic notes for each species are provided. A list of examined specimens is included in Supplementary File, with their mapped distributions shown in Fig. 6.

Premna cordifolia Roxb., [Hort. Bengal.: 95. 1814, *nom. nud.*], Fl. Ind. 3: 78. 1832; Schauer in DC., Prodr. 11: 632. 1847; Miq., Fl. Ned. Ind. 2: 895. 1858; C. B. Clarke in Hook. f., Fl. Brit. India 4(12): 572. 1885; King & Gamble, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74: 818. 1909; H. J. Lam, Verbenaceae Malayan Archipel.: 111. 1919; Ridl., Fl. Malay Penins. 2: 621. 1923; Dop in Lecomte et al., Fl. Indo-Chine 4(7): 816. 1935; H. R. Fletcher, Bull. Misc. Inform. Kew 1938: 418. 1938; Kochummen in Ng, Tree Fl. Mal. 3: 307. 1978; Phuong in N.T. Ban et al., Fl. Vietnam 3: 298. 2005; Leerat. et al., Trop. Nat. Hist. 9(2): 119. 2009.—*Gumira cordifolia* (Roxb.) Kuntze, Rev. Gen. Pl., 2: 507. 1891. Type: *Roxburgh illustration no. 1462* (lectotype K!, selected by Daniel & Rajendran[55]; isolectotype CAL!) (Fig. 2).

Description: Scandent shrub or woody climber. *Branches* with an interpetiolar woody ridge, brown to reddish-brown pubescent. *Leaves* decussate, mostly cordate or cordate-ovate, rarely oblong-ovate, 5–15 by 3–7 cm, apex caudate or acuminate, rarely retuse, base cordate or rounded, margins entire; adaxial surface glabrous, shining, midrib pubescent; abaxial surface glabrous, with yellowish-brown or yellow sessile glands, midrib sparsely brown pubescent, nerve axils of lower half of abaxial leaf surface glabrous to sparsely pubescent; petiole 1–4 cm long. *Inflorescence* corymbose, 4–8 cm long; pedicels 0.5–1.5 mm long. *Calyx* pale green or green, (1.5–)2–3 mm long, unequally 2-lipped; tube 1.2–2.7 mm long; posterior lip entire or subentire; anterior lip 2-lobed, lobes 0.1–0.6 mm long. *Corolla* yellowish-white or white, 2-lipped, 5–7 mm long; tube 2–3.5 mm long, distally sparsely pubescent outside, densely white villous distally inside; posterior lip ovate to elliptic, 2.5–3.5 mm long; anterior lip with middle lobe slightly larger, broadly oblong or oblong-ovate, 1.2–1.5 mm long, with yellow patch on middle lobe; lobes with villose hairs on base. *Stamens* long exserted, filaments 1.5–3.5 mm long; anthers 0.3–0.6 mm long. *Ovary* ovoid to obovoid, 1–1.2 mm long, glabrous with sparse sessile glands at apex; stigma branches 0.15–0.25 mm long. *Fruits* black, broadly obovoid, 4–7 mm long, glabrous with sessile glands.

Distribution: Thailand (Songkhla, Yala, Narathiwat), Vietnam.

Ecology: In open areas or partly shaded primary or secondary evergreen forests, dry evergreen forests, hardwood forests, granite bedrock; elevation up to 380 m. Flowering and fruiting March to July.

Fig. 2. *Premna cordifolia*. (**A**, **B**) habit and habitat; (**C**) flowering branch; (**D**) inflorescence; (**E**) flowers; (**F**) fruits. Photos A, C–F by C. Leeratiwong and B by J. Satthaphorn.

Vernacular: ya yo (ยายอ).

Note: *Premna cordifolia* was considered as a '*nomen nudum*' and listed as a synonym under *P. serratifolia* in de Kok's[3] treatment for *Flora Malesiana*. This corresponds to its first publication by Roxburgh[56] in *Hortus Bengalensis*, which was invalid due to the absence of a description and type specimen. However, the name was later validated by Roxburgh[57] in *Flora Indica*, although the origin of the type locality remains uncertain due to Roxburgh's drawing was based on the cultivated specimen[55]. Therefore, *P. cordifolia* is a validly published and accepted name.

Although the previous treatment[3] lacked the explanation for this inclusion, we presumed that specimens initially thought to be *P. cordifolia* (*non* Roxburgh) are referrable to *P. serratifolia* based on their similar morphological characteristics. However, our morphological investigation and ecological observations (Table 1) demonstrate that the original concept of *P. cordifolia* as published by Roxburgh[57] is clearly distinct from *P. serrtifolia*. Specimens of *P. cordifolia* (sensu Roxburgh) examined from Thailand are listed in the Supplementary File, and additional selected specimens from Vietnam were also investigated to revise its distribution range (*Clemens 3147* [K K001083294!], *Clemens 4452* [K K001083319!], *Evrard 44* [K K001083320!], *Poilane 40706* [K K001083318!]).

Morphologically, *P. cordifolia* (sensu Roxburgh) differs from *P. serratifolia* in having cordate or rounded leaf bases (vs. mostly cuneate in *P. serratifolia*), glabrous vein axils on the lower half of the abaxial leaf surface (vs. densely pubescent), a corolla with a large yellow patch on the inside base of the middle lobe of anterior lip (vs. without a yellow patch), and a longer corolla, 5–7 mm long (vs. 2–5 mm long) (Figs. 2, 5). The study of fruit and endocarp morphology by Satthaphorn et al.[50] demonstrated that *P. cordifolia* produces significantly larger fruits (5.32–6.65 × 4.25–5.66 mm) compared to those of *P. serratifolia* (3.33–4.89 × 2.58–2.99 mm). The endocarp protrusion of *P. cordifolia* is type II characterized by sharpened and tapered (thorn-like) protruding structure while *P. serratifolia* bears type I endocarps with rounded, swollen (saccate-like) protruding structure. Ecological observations and herbarium label data further indicate that *P. cordifolia* typically occurs in open or shaded primary or secondary evergreen forests, whereas *P. serratifolia* is usually found in exposed coastal and mangrove habitats (Figs. 5A, 6). Our phylogenetic analyses further support the phylogenetic distinction between *P. cordifolia* and *P. serratifolia*, placing them in well-supported separate subclades (Fig. 1). We therefore confirm that *P. cordifolia* (sensu Roxburgh)[57] occurs in Thailand and clearly treated as a distinct species in the *Flora of Thailand*.

Premna cordifolia can be distinguished from other species regarded in the *P. serratifolia* complex in Thailand, namely *P. paniculata* and *P. punctulata* which are reinstated as distinct species in this study. One key diagnostic feature of *P. cordifolia* is the presence of interpetiolar ridges on the stem, a character absents in both *P. paniculata* and *P. punctulata*. The inflorescence of *P. cordifolia* is longer (4–8 cm) than that of *P. paniculata* (0.7–2 cm), though it overlaps in length with *P. punctulata*. In terms of floral morphology, *P. cordifolia* exhibits both a yellow patch on the corolla and hairs on the corolla tube, while these features are absent in *P. paniculata*. Fruit size also provides a distinguishing character, with *P. cordifolia* bearing larger fruits (5.32–6.65 × 4.25–5.66 mm) compared to *P. paniculata* (3.22–4.20 × 2.33–3.82 mm). Additionally, the shape of the endocarp protrusions differs: *P. cordifolia* possesses thorn-like protrusions, whereas *P. punctulata* exhibits a saccate-like form. Phylogenetic analysis reveals that *P. cordifolia* occupies a distinct position in subclade I, separate from *P. punctulata*, which is placed in subclade III. This integrative evidence, encompassing morphological and molecular data, supports the recognition of *P. cordifolia* (sensu Roxburgh) as a species distinct from both *P. paniculata* and *P. punctulata*.

Premna paniculata H. R. Fletcher, Bull. Misc. Inform. Kew 1938: 201. 1938 & 421. 1938; Leerat. et al., Trop. Nat. Hist. 9(2): 135. 2009 & Thai Forest Bull. (Bot.) 44(2): 122. 2016. Type: Thailand, Prachuap Khiri Khan (Pak Tawan), elev. ca 20 m, 1 August 1931, *A.F.G. Kerr 20,536* (lectotype BK BK257574!, selected by Leeratiwong et al.[58]; isolectotypes BM BM000950181!, E E00284177!, LL LL00375155!, K K001098063!, SING SING0068002!) (Fig. 3).

Description: Scandent shrub to shrub, 2–4 m tall. *Branches* without an interpetiolar woody ridge, glabrous or brown to reddish-brown pubescent. *Leaves* decussate, elliptic or ovate-elliptic, 2–11 by (1–)1–5 cm, apex acute or acuminate, base cuneate, margins mostly entire or rarely slightly serrate distally; adaxial surface mostly glabrous, base and margin with sparse brown appressed hairs; abaxial surface glabrous or sometimes pubescent with sparse brown appressed hairs, with or without sparse yellow to brown sessile glands, nerve axils glabrous; petiole 2–5 mm long. *Inflorescence* corymbose, 0.7–2 cm long; pedicels 0.2–1.5 mm long. *Calyx* campanulate, 2–2.5 mm long, obscurely and equally 2-lipped; tube 1.5–2 mm long; posterior lip 3-lobed, lobes 0.3–0.8 mm long; anterior lip 2-lobed, lobes 0.4–1 mm long; fruiting calyx 2–3 mm long. *Corolla* greenish-white or white, 2-lipped, 3–5 mm long; tube 2–3 mm long, glabrous outside, white villous distally inside; posterior lip 1.2–1.5 mm long; anterior lip with middle lobe larger, 1–1.5 mm long, without yellow patch on middle lobe; lobes glabrous on both sides. *Stamens* long exserted, filaments 1–1.5 mm long; anthers 0.2–0.4 mm long. *Ovary* glabrous, with or without sparse sessile glands at apex; stylar branches 0.1–0.3 mm long. *Fruits* green when young, broadly obovoid, 4–6 mm long, glabrous, sparse sessile glands at apex.

Distribution: Endemic to Thailand (Phetchaburi, Prachuap Khiri Khan).

Ecology: In open areas of secondary dry evergreen forests; elevation 20–150 m. Flowering and fruiting June to August.

Vernacular: sak khi kai prachuap (สักขี้ไก่ประจวบ).

Note: *Premna paniculata* was listed as a synonym of *P. serratifolia* in the treatment of de Kok[3]. This inclusion was not explicitly justified, as the species was merely listed under *P. serratifolia* without further explanation. We presume that de Kok[3] considered the type specimens of *P. paniculata* to represent morphological variation within *P. serratifolia*. Nevertheless, *P. paniculata* is geographically restricted to south-western Thailand. Based on

Fig. 3. *Premna paniculata*. (**A**, **B**) habit with fruiting branch; (**C**) close-up fruits. All photos by C. Leeratiwong.

comprehensive morphological analyses and ecological evidence, we recognize *P. paniculata* as a distinct species and reinstate its taxonomic status in this study. This conclusion is further supported by earlier studies[8,28,50,58].

The type specimen of P. *paniculata* (Kerr 20,536 [K K001098063!]) together with three additional specimens (*Leeratiwong 16–545* [PSU 01683!], *Kerr 20,536* [K K001098063!], *Niyomdham 2978* [BKF SN101085!]) can be

morphologically distinguished from *P. serratifolia* by several diagnostic characters. These include the absence of an interpetiolar woody ridge at the stem nodes (vs. presence of an interpetiolar woody ridge in *P. serratifolia*), glabrous nerve axils on the lower half of the abaxial leaf surface (vs. densely hairy), a glabrous corolla tube externally (vs. distally pubescent), a shorter inflorescence (0.7–2 cm long vs. (0.5–)2–6 cm long), and a posterior calyx lip that is apically 3-lobed (vs. mostly entire to subentire, or rarely 2–3-lobed) (Figs. 3, 5). Endocarp morphology, as documented by Satthaphorn et al.[50], further supports the taxonomic separation of these species: *P. paniculata* exhibits type II endocarps protrusion (thorn-like), whereas *P. serratifolia* bears type I endocarps protrusion (saccate-like). Additionally, these two species occupy distinct ecological niches, with *P. paniculata* confined to secondary dry evergreen forests, endemic to Thailand, in contrast to *P. serratifolia*, which is typically found in coastal or mangrove habitats in wider regions (Fig. 6). Although *P. paniculata* and *P. punctulata* are both found in evergreen forest habitats, they can be readily distinguished based on several morphological characters (Table 1). The leaf base of *P. paniculata* is cuneate, in contrast to the rounded or slightly oblique-rounded base observed in *P. punctulata*. On the abaxial leaf surface, the nerve axils of *P. paniculata* are glabrous, whereas those of *P. punctulata* are sparsely pubescent. Inflorescence length further differentiates the two species, with *P. paniculata* exhibiting notably shorter inflorescences (0.7–2 cm) compared to the longer ones in *P. punctulata* (6–20 cm). The posterior calyx lip of *P. paniculata* is distinctly apically 3-lobed, whereas it is entire to subentire in *P. punctulata*. Additionally, *P. paniculata* is also characterized by the absence of a yellow patch on the middle lobe of the anterior corolla lip, but a feature presents in *P. punctulata*. The endocarp protrusion type also serves as a key diagnostic trait: *P. paniculata* possesses thorn-like (type II) protrusions, while *P. punctulata* exhibits saccate-like (Type I) protrusions, as documented by Satthaphorn et al.[50]. These consistent morphological differences support the recognition of *P. paniculata* and *P. punctulata* as distinct species.

We attempted to include *P. paniculata* in the molecular analyses to clarify its taxonomic placement. However, DNA extraction proved highly challenging due to the limited availability of suitable material. Specimens available for study were mature leaves, and DNA isolated using both CTAB and a commercial extraction kit yielded poor-quality products that consistently failed to amplify. This difficulty is likely attributable to the high levels of polyphenols, polysaccharides, and other secondary metabolites typically accumulated in mature leaf tissues, which are known to interfere with DNA purity and inhibit enzymatic reactions[59–62]. Consequently, future studies should prioritize the use of young leaf material for *P. paniculata* to improve DNA quality and ensure successful molecular analyses.

To clarify the taxonomic status of *P. paniculata*, the combined evidence from morphological characteristics and ecological information as outlined above provides sufficient resolution to support the recognition as a distinct species from both *P. punctulata* and *P. serratifolia*. Accordingly, *P. paniculata*, which was previously treated as a synonym of *P. serratifolia*, is here reinstated.

Premna punctulata C. B. Clarke in Hook.f., Fl. Brit. India 4: 575. 1885; King & Gamble, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74: 817. 1909; H. J. Lam, Verbenaceae Malayan Archipel.: 121. 1919; Ridl., Fl. Malay Penins. 2: 620. 1923; Kochummen in Ng, Tree Fl. Malaya 3: 307. 1978; Keng, Con. Fl. Singapore: 194. 1990; Leerat. et al., Trop. Nat. Hist. 8(1): 9. 2008 & 9(2): 135. 2009.—*Premna serratifolia* sensu de Kok, Fl. Malesiana 23. 327. 2019, *pro parte*. Type: Malaysia, Malacca, 6 June 1865, *Maingay Kew Distribution 1200* (holotype K K000645986!; isotype NY NY00137951!) (Fig. 4).

Description: Woody climber or rarely scandent shrub. *Branches* without an interpetiolar woody ridge, glabrous or brown to reddish-brown pubescent. *Leaves* decussate, ovate to broadly elliptic, 9–15 by 5–9 cm, apex acuminate, acute or rarely retuse, base rounded or slightly oblique-rounded, margins entire; adaxial surface glabrous, midrib sparsely pubescent; abaxial surface brown to reddish-brown pubescent, nerve axils sparsely pubescent, with brown sessile glands; petiole 3–6 cm long. *Inflorescence* corymbose, 6–20 cm long; pedicels 0.5–1.5 mm long. *Calyx* pale green to green, 1.5–2 mm long, equally and obscurely 2-lipped; tube 1.5–2 mm long; posterior lip entire to subentire (rarely 2–3-lobed); anterior lip 2-lobed, lobes 0.1–0.15 mm long; fruiting calyx 1–2.5 mm long. *Corolla* greenish-white or white, 2-lipped, 5–6 mm long; tube 2.7–3.5 mm long, glabrous or sparsely pubescent hairs distally outside, with densely white villous distally inside; posterior lip 1.5–1.7 mm long; anterior lip with middle lobe larger, 1.8–2 mm long, with yellow patch on middle lobe; lobes glabrous on both sides. *Stamens* long exserted, filaments 2–3 mm long; anthers 0.4–0.5 mm long. *Ovary* sparsely pubescent and with sessile glands at apex; stigma branches 0.15–0.3 mm long. *Fruits* black, broadly obovate, 3–7 mm long, glabrous, with or without sessile glands.

Distribution: Thailand (Phatthalung, Songkhla, Narathiwat), Malaysia.

Ecology: In primary to secondary evergreen or dry evergreen forests, along the waterfall; elevation up to 150 m. Flowering March to May, fruiting August.

Vernacular: ยานใบหอม (yan bai hom) (Songkhla).

Note: *Premna punctulata* was treated as a synonym of *P. serratifolia* by de Kok[3]. However, there was no discussion or justification for this synonymization in the previous study. We infer that this decision was likely based on morphological similarities, as the type specimen of *P. punctulata* (*Maingay 1200* [K K000645986!]) had been subsequently determined as *P. serratifolia*. Although the phylogenetic analyses presented here placed *P. punctulata* and *P. serratifolia* in an unresolved clade in both BI and ML trees (Fig. 1), the relatively long branch length separating *P. punctulata* suggests considerable genetic divergence[53]. This molecular distinction aligns with the consistent morphological and ecological difference observed between these two taxa.

Premna punctulata is morphologically distinguished from *P. serratifolia* by the absence of an interpetiolar woody ridge, a longer corolla, the presence of yellow patches on the middle lobe of the anterior lip of the corolla, and a hairy ovary, whereas *P. serratifolia* possesses an interpetiolar woody ridge, has a shorter corolla, lacks yellow corolla patches, and has a glabrous ovary (Figs. 4, 5). This taxonomic uncertainty was previously addressed by Satthaphorn et al.[50], and demonstrated that the fruit width of *P. punctulata* is clearly distinct from

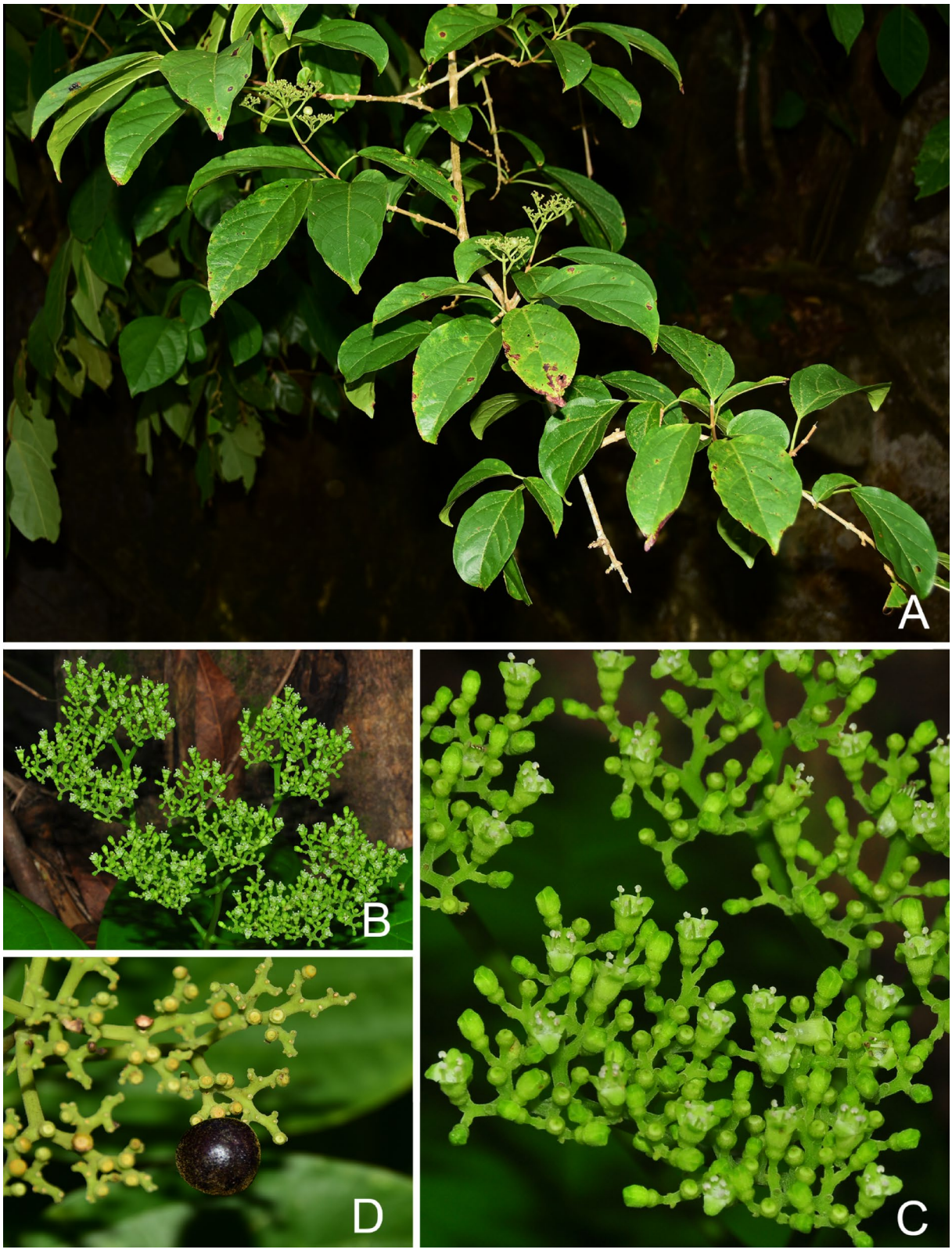

Fig. 4. *Premna punctulata.* (**A**) habit with flowering branch; (**B**) inflorescence; (**C**) closed-up inflorescence; (**D**) fruit. Photos A by J. Satthaphorn and B–D by C. Leeratiwong.

that of *P. serratifolia* (3.79–5.69 mm wide vs 2.58–2.99 mm wide, respectively). Furthermore, the two taxa occupy different habitats: *P. punctulata* occurs in primary to secondary evergreen or dry evergreen forests, whereas *P. serratifolia* is restricted to coastal and mangrove environments.

The separation of these two taxa is further supported by recent phylogenetic studies conducted by Li et al.[5] and Hai et al.[17], as well as morphological evidence provided by Leeratiwong et al.[8,30] and Satthaphorn et al.[50]. Based on the combination of consistent morphological distinctions, ecological differentiation, and the relatively long branch length separating *P. punctulata* from *P. serratifolia* in our phylogenetic analyses, *P. punctulata* is here reinstated as a distinct species.

Premna serratifolia L. Mant. Pl. 2: 253. 1771; Roxb., Fl. Ind. 3: 77. 1832; Schauer in DC., Prodr. 11: 632. 1847; Kurz, Forest Fl. Burma 2: 262. 1877; Munir, J. Adelaide Bot. Gard. 7: 13. 1984; S.L. Chen & M. G. Gilbert in C. Y. Wu & P. H. Raven, Fl. China 17: 26. 1994; A. Rajendran & P. Daniel, Indian Verbenac.: 284. 2002; Leerat. et al., Nat. Hist. J. Chulalongkorn Univ. 9(2): 138. 2009; de Kok, Kew Bull. 68(1): 17. 2013; de Kok in Bramley, Bramley, Fl. Malesiana 23: 327. 2019.—*Premna obtusifolia* var. *serratifolia* (L.) Moldenke, Phytologia 28(4): 403. 1974.—*Premna obtusifolia* f. *serratifolia* (L.) Moldenke, Phytologia 36(5): 438. 1977; Moldenke & Moldenke in Dassanayake & Fosberg, Rev. Handb. Fl. Ceyl. 4: 342. 1983.Type: India, *Hermann Herb. Linn. 782.4* (holotype LINN!) (Fig. 5).

Cornutia corymbosa Burm.f., Fl. Ind.: 132, t. 411, f. 1. 1768, *nom. illeg.* (*non* Lam).—*Premna integrifolia* L., Mant. Pl. Altera: 252. 1771, *nom. superfl.*—*Premna corymbosa* (Burm.f.) Schauer in DC., Prodr. 11: 632. 1847, *nom. illeg.* (*non* Rottler & Willd.).—*Gumira integrifolia* Kuntze, Revis. Gen. Pl. 2: 507. 1891, *nom. superfl.* Type: Sri Lanka [Ceylon], *Hermann 2:1* (lectotype BM-HERM BM000621491!, designated by Verdcourt[63]).

Gumira domestica Hassk., Flora 25, 2, Beibl.: 26. 1842.—*Gumira integrifolia* Hassk., Cat. Hort. Bot. Bogor.: 135. 1844, *nom. superfl.* Type: *Rumphius*, Herb. Amboin. 3: 209, t 134. 1743.

Premna obtusifolia R. Br., Prodr. Fl. Nov. Holland.: 512. 1810.—*Premna integrifolia* L. var. *obtusifolia* (R.Br.) C. Pei, Mem. Sci. Soc. China 1: 75. 1932.—*Premna corymbosa* (Burm.f.) Schauer var. *obtusifolia* (R.Br.) H. R. Fletcher, Notes Roy. Bot. Gard. Edinburgh 19: 178. 1936. Type: Australia, Prince of Wales Island, *Brown s.n.* (= *Bennett 2324*) (holotype BM BM000019556!).

Premna spinosa Roxb., [Hort. Beng.: 46. 1814, *nom. nud.*] Fl. Ind. 3: 77. 1832; Walp., Report. Bot. Syst. 4: 93. 1845. Type: *Roxburgh illustration no. 961* (holotype K!).

Premna foetida Reinw. ex Blume, Bijdr. 14: 816. 1826.—*Gumira foetida* (Reinw. ex Blume) Hassk., Cat. Hort. Bot. Bogor. 1844; H. J. Lam, Verbenaceae Malayan Archipel.: 153. 1919. Syntypes: Indonesia, Java *Reinwardt s.n.* (L L0699518!, L0699519!).

Premna gaudichaudii Schauer in DC., Prodr. 11: 631. 1847.—*P. obtusifolia* var. *gaudichaudii* (Schauer) Moldenke, Phytologia 27: 69. 1973. Type: Archipelago, Mariannae, 1830, *Gaudichuad s.n.* (holotype G-DC, *n.v.*).

Premna sambucina Wall. [Cat. No. 1775, *nom. nud.*] ex Schauer in DC., Prodr. 11: 631. 1847; Kurz, For. Fl. Burma 2: 261. 1877.—*P. corymbosa* var. *sambucina* (Wall. ex Schauer) Moldenke, Known Geogr. Distr. Verbenac. Avicenn.: 68. 1942. Type: India, Moalmyn, 1827, *Wallich, Cat. No. 1775* (holotype G-DC, *n.v.*; isotype K K001114145!).

Premna integrifolia L. var. *angustior* C. B. Clarke in Hook.f., Fl. Brit. India 4: 574. 1885.—*Premna angustior* (C. B. Clarke) Ridl., Fl. Malay Penins. 2: 619. 1923.—*Premna corymbosa* (Burm. f.) Schauer var. *angustior* (C. B. Clarke) H. R. Fletcher, Notes Roy. Bot. Gard. Edinburgh 19: 178. 1936.—*Premna obtusifolia* R.Br. var. *angustior* (C. B. Clarke) Moldenke, Phytologia 5: 87. 1954. Syntypes: Malaysia, Malacca, *Griffith 6030* (K K000645926!, K000645927!).

Premna integrifolia L. subsp. *truncatolabium* H. J. Lam, Verbenaceae Malayan Archipel.: 142. 1919. Type: Papua New Guinea, Kaiser Wilhelmsland, Sepik, *Lederman 6572* (lectotype K K000670846!, designated by De Kok[3]).

Premna integrifolia L. var. *minor* Ridl., Fl. Malay Penins. 2: 619. 1923.—*Premna obtusifolia* R. Br. var. *minor* (Ridl.) Moldenke, Phytologia 5: 88. 1954.—*Premna corymbosa* (Burm. f.) Schauer var. *minor* (Ridl.) H. R. Fletcher, Notes Roy. Bot. Gard. Edinburgh 19: 178. 1936. Type: Malaysia, Pahang, Pekan, *Ridley s.n.* (holotype SING, *n.v.*).

Description: Scandent shrub or shrub, 0.2–5 m high. *Branches* with an interpetiolar woody ridge, glabrous or brown to reddish-brown pubescent. *Leaves* decussate, elliptic, ovate or obovate, (0.5–)2–15 by (0.3–)1.2–10 cm, apex mostly acute or obtuse, rarely acuminate or retuse, leaf base usually cuneate, rarely slightly cordate or rounded, margins entire or serrate distally; adaxial surface glabrous, margins with sparse brown hairs; abaxial surface glabrous or sparse brown pubescent, nerve axils densely pubescent on lower half, with yellow to brown sessile glands; petiole (0.4–)0.8–5.5 cm long. *Inflorescence* corymbose, 1.7–15 cm long; pedicels 0.2–1.5 mm long. *Calyx* green, (1–)1.5–2.5 mm long, unequally 2-lipped; tube 1–1.8 mm long; posterior lip mostly entire to subentire or rarely 2–3-lobed, lobes 0.05–0.1 mm; anterior lip 2-lobed, lobes 0.3–1 mm long; fruiting calyx 2–3 mm long. *Corolla* creamy-white or white, 2-lipped, (2–)3.5–5 mm long; tube (1.8–)2.5–3 mm long, pubescent or sparsely so, with yellow sessile glands at apex outside, densely white villous distally inside; posterior lip 1.2–1.6 mm long; anterior lip with lobes subequal; middle lobe 1.2–2 mm long, without yellow patch on middle lobe; lobes glabrous or sparsely pubescent, with sparse sessile glands outside, glabrous inside. *Stamens* long exserted, filaments 1–3 mm long; anthers 0.3–0.6 mm long. *Ovary* 0.5–1 mm long, glabrous, with or without sessile glands; stigma branches 0.05–0.15 mm long. *Fruits* black, broadly obovoid, 3–8 mm long, glabrous.

Distribution: Thailand (Krungthep Mahanakhon (Bangkok), Samut Sakhon, Chon buri, Rayong, Chantaburi, Trat, Chumphon, Ranong, Suratthani, Phangnga, Phuket, Krabi, Trang, Satun, Songkhla, Narathiwat). Widely distributed throughout the coasts and the islands of tropical and subtropical Asia, Africa, Australia and the Pacific.

Fig. 5. *Premna serratifolia*. (**A**) habit and habitat; (**B**) habit; (**C**) branches with leaves and fruits; (**D**) inflorescence; (**E**) closed-up inflorescence; (**F**) young fruits; (**G**) mature fruits. All photos by J. Satthaphorn.

Ecology: In open, sandy soil or wetland areas of beach forests and mangrove forests, or strand vegetation along the coastal areas and islands, in tidal zone; elevation from sea level up to 80 m. Flowering and fruiting all year round.

Vernacular: Khet nam man ((เค็ดน้ำมัน), cha lueat (ช้าเลือด) (Trat), man kai (มันไก่), sam pra nga (สามประงา)), sam pra nga bai (สามประงาใบ), akkhi thawan thale (อัคคีทวารทะเล).

Note: *Premna serratifolia* is a widely distributed species across Southeast Asia. In recent taxonomic revisions in specific regions, numerous synonyms have been associated with this species, particularly from type specimens originating in the Malay Archipelago[3]. In our study, we focus on defining the species circumscription of *P. serratifolia* in Thailand, providing a comprehensive morphological description while excluding the concepts of the reinstated species *P. paniculata* and *P. punctulata*. The distinguishing morphological features are summarized in Table 1.

Ecologically, *P. serratifolia* typically inhabits beach forests or coastal habitats, clearly differing from the moist evergreen forest habitats associated with the previously synonymized species (Figs. 5, 6). Field observations indicate that the leaves of *P. serratifolia* are notably thick leaves and cuticle, likely as an adaptation to protect against salt spray and strong wind from the sea[64–66]. This species may be actively adapted its seed germination facilitated by monsoon climate, mobile substrates, and salinized[64,67], while other species may require different mode of seed dispersal and germination. In addition, phylogenetic analysis places three accessions of *P. serratifolia*, sampled from the opposite sides of peninsular Thailand, within subclade III of Clade II (Fig. 1) which provide the information of phylogenetic placement with its related species.

Although the species circumscription of *P. serratifolia* in Thailand and its morphologically related species have been clarified in this study, further investigation across a broader geographic range is still required to assess the extent of morphological variation and confirm species delimitation at a monographic scale.

Taxonomic note on *Premna octonervia* in Thailand

Premna octonervia was listed as a distinct species in the synopsis for Thailand by Leeratiwong et al.[8] based on multiple collections from peninsular Thailand, including *Kerr 13309* (K K001084832!), *Puudjaa* et al. *251* (K K00108481!), *Chana* et al. *251* (BKF SN064958!, KKU 15247!), *Leeratiwong 04–53* (BKF SN232004!, KKU 15246!, PSU 0013431!), and *Leeratiwong 05–251* (BKF SN232005!, PSU 0013430!). Our re-investigation showed that these specimens is consistent with the original description of *P. mollissima* rather than *P. octonervia*. Meanwhile, the actual identity of *P. octonervia* can be attributed to *P. serratifolia*, as previously suggested by de Kok[3]. The dried specimens exhibit an ebony color and a lower density of hairs on the leaf surfaces, slightly distinguishing from those found in other regions, where dried specimens are typically yellowish-brown to yellowish-black and have moderately to densely hairy leaf surfaces. This difference may be attributed to environmental influences affecting the secondary compounds within southern Thailand population.

Taxonomic note on *Premna coriacea* in Thailand

The specimens identified as *P. coriacea* (*non* C. B. Clarke) and *P. coriacea* var. *villosa* (*non* Rajendran & Daniel) were included to Thai account by Leeratiwong et al.[8]. Our morphological revision supports these two taxa are *P. scandens*. The actual taxa of *P. coriacea* (lectotype *Law s.n.*, K K000884638!) and *P. coriacea* var. *villosa* (lectotype *s.coll. 2912*; selected here, K K000884633!) are restricted to the Indian subcontinent and do not occur in Thailand.

In the protologue of *P. coriacea* var. *villosa* (C. B. Clarke) A. Rajendran & P. Daniel [= *P. villosa* C. B. Clarke in *Flora of British India* by Hooker[68]], two specimens were cited in the original publication without the designation of the holotype. These two specimens (*s.coll. 2912*, [K K000884633!] and *Beddome s.n.* [K K000884632!]) could be considered as syntypes. We therefore selected the specimen of *s.coll. 2912* [K000884633] to be the lectotype of the synonymized taxon due to the specimen being cited in the protologue as well as having complete vegetative and reproductive parts.

Taxonomic note on *Premna interrupta* in Thailand

Premna interrupta Wall. ex Schauer, in A. P. de Candolle, Prodr. 11: 633. 1847.—*Premna interrupta* var. *smitinandii* Moldenke, Phytologia 8: 163. 1962, *syn. nov.*

Leeratiwong et al.[8] recognized *P. interrupta* var. *smitinandii* based on the presence of villose hairs on the abaxial leaf surface (vs. glabrous leaf in typical variety). This variety was established based on the single collection of *Smitinand & Alsterlund* 6783 [holotype LL LL00375145!; isotypes K K001084815!, L L0414245!, L0414256!]. After investigation of various specimens belonging to *P. interrupta* and the type specimen of *P. interrupta* var. *smitinandii*, we found that the density of the indumentum on an abaxial leaf surface of *P. interrupta* var. *interrupta* is variable among different populations from absent to densely hairy, and leaf maturity. Therefore, we do not recognize *P. interrupta* var. *smitinandii* here.

An updated accounted and revised key to species in Thailand

Our taxonomic treatment and notes have resulted in nomenclatural changes for *Premna* in Thailand, as summarized in Table 2. A total of 21 species are recognized in Thailand, and a revised key to the species is provided below.

Key to species of *Premna* in Thailand

1 Leaf surfaces with stellate and dendroid hairs..*P. tomentosa*
1′ Leaf surfaces without stellate and dendroid hairs..2
2 Calyx deeply lobed, with 1– 1.8 mm long..*P. serrata*
2′ Calyx shallowly lobed or entire, with shorter than 1 mm long..3
3 Herb to undershrub with large woody rhizome..4

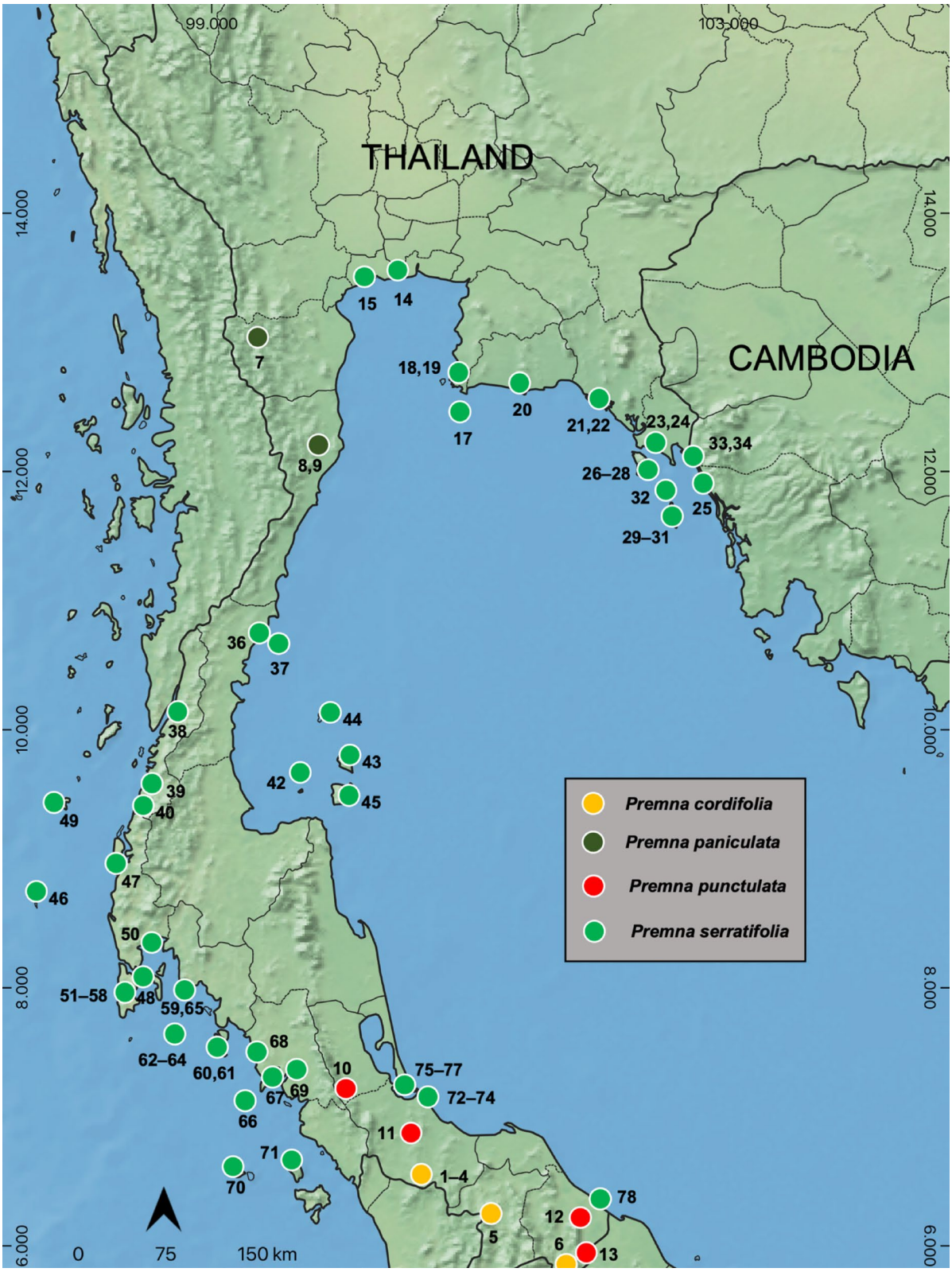

Fig. 6. Distribution map of *P. cordifolia*, *P. paniculata*, *P. punctulata*, and *P. serratifolia* in Thailand. Numbers near locations refer to the collections cited in Supplementary File.

Leeratiwong et al.[8]	This study	Remarks
1. *Premna annulata* H. R. Fletcher	1. *Premna annulata* H. R. Fletcher	
2. *Premna collinsiae* Craib	2. *Premna pubescens* Blume	*P. collinsiae* is synonymized under *P. pubescens* by Leeratiwong et al.[58]
3. *Premna cordifolia* Roxb.	3. *Premna cordifolia* Roxb.	
4. *Premna coriacea* C. B. Clarke (including *P. coriacea* var. *villosa*)		*P. coriacea* (sensu Leeratiwong) is *P. scandens*
5. *Premna fulva* Craib	4. *Premna fulva* Craib	
6. *Premna garrettii* H. R. Fletcher	5. *Premna garrettii* H. R. Fletcher	
7. *Premna hamiltonii* J. L. Ellis	6. *Premna odorata* Blanco	*P. hamiltonii* is synonymized under *P. odorata* (de Kok[3])
8. *Premna herbacea* Roxb.	7. *Premna herbacea* Roxb.	
9. *Premna interrupta* Wall. ex Schauer (including *P. interrupta* var. *smitinandii*	8. *Premna interrupta* Wall. ex Schauer	*P. interrupta* var. *smitinandii* is synonymized under *P. interrupta* (this study)
10. *Premna mollissima* Roth	9. *Premna mollissima* Roth	
11. Premna nana Collett & Hemsl.	10. *Premna nana* Collett & Hemsl.	
12. *Premna octonervia* Merr. & F. P. Metcalf		Recognized as *P. mollissima*
13. *Premna paniculata* H. R. Fletcher	11. *Premna paniculata* H. R. Fletcher	
14. *Premna punctulata* C. B. Clarke	12. *Premna punctulata* C. B. Clarke	
15. *Premna pyramidata* Wall. ex Schauer	13. *Premna tomentosa* Willd.	*P. pyramidata* is synonymized under *P. tomentosa* (de Kok[3])
16. Premna rabakensis Moldenke	14. *Premna rabakensis* Moldenke	
17. *Premna repens* H. R. Fletcher	15. *Premna repens* H. R. Fletcher	
18. *Premna scandens* Roxb.	16. *Premna scandens* Roxb.	
19. *Premna serrata* H. R. Fletcher	17. *Premna serrata* H. R. Fletcher	
20. *Premna serratifolia* L.	18. *Premna serratifolia* L.	
21. *Premna siamensis* H. R. Fletcher	19. *Premna siamensis* H. R. Fletcher	
22. *Premna stenobotrys* Merr.	20. *Premna stenobotrys* Merr.	
23. *Premna trichostoma* Miq.	21. *Premna trichostoma* Miq.	

Table 2. The updated account of *Premna* in Thailand.

3′ Tree, shrub (0.2–)2–5 m high, scandent shrub or woody climber..5
4 Calyx 1.5–2.5 mm long without glandular hairs; corolla 3–5 mm long; style less than 3.5 mm long, inserted; abaxial surface of leaves glabrous to sparsely scabrid and pubescent with eglandular hairs..*P. herbacea*
4′ Calyx 3–4 mm long with glandular hairs; corolla 5–7 mm long; style 3.5–6 mm long, exserted; abaxial surface of leaves densely villous sometimes mixed with glandular or pubescent hairs..*P. nana*
5 Fruits clavoid (narrowly club-shaped)..6
5′ Fruits narrowly obovoid to broadly obovoid..10
6 Inflorescence a spike-like thyrsi.. *P. interrupta*
6′ Inflorescence a corymbose thyrsi..7
7 Ovary pubescent; abaxial leaf surface densely villous..8
7′ Ovary glabrous; abaxial leaf surface glabrous or sparsely to moderately pubescent..9
8 Calyx lips entire or one lip with two lobes, the other entire or subentire; fruit 4–6 mm long, moderately or sparsely pubescent..*P. garrettii*
8′ Calyx lips with two lobes; fruit 2–4 mm long, glabrous..P. siamensis.9Stigma branches 0.5–1 mm long; flowers usually sessile or rarely pedicellate..*P. trichostoma*
9 Stigma branches 0.5–1 mm long; flowers usually sessile or rarely pedicellate..*P. trichostoma*
9′ Stigma branches 0.2–0.4 mm long; flowers only pedicellate..*P. scandens*
10 Corolla red or pinkish-red..*P. stenobotrys*
10′ Corolla white, greenish-white, yellowish-white, yellow, pale yellow, creamy white or greenish-yellow..11
11 Both leaf surfaces with reddish-brown to orangish-red sessile glands mixed with yellow to brown sessile glands..*P. rabakensis*
11′ Both leaf surfaces with yellow to brown sessile glands, without reddish-brown to orangish-red sessile glands..12
12 Branch nodes with an interpetiolar woody ridge..13
12′ Branch nodes without an interpetiolar woody ridge..17
13 Calyx apically purple, fruits narrowly obovoid..*P. annulata*
13′ Calyx apically green, fruits broadly obovoid..14
14 Posterior lip of calyx mostly 3-lobed, rarely 2-lobed or subentire..15
14′ Posterior lip of calyx mostly entire to rarely 2–3-lobed..16
15 Adaxial leaf surface and midribs with villous hairs; corolla with yellow patch at the inside base of middle lobe of anterior lip..*P. odorata*
15′ Adaxial leaf surface glabrous, midribs pubescent; corolla without yellow patch at the inside base of middle lobe of anterior lip..*P. pubescens*

16 Corolla 5–7 mm long, with yellow patch on inside base of middle lobe of anterior lip; leaf base cordate or rounded; nerve axils of lower half of abaxial leaf surface glabrous to sparsely pubescent..*P. cordifolia*
16′ Corolla 2–5 mm long, without yellow patch on inside base of middle lobe of anterior lip; leaf bases usually cuneate; nerve axils of lower half of abaxial leaf surface densely pubescent..*P. serratifolia*
17 Inside of middle lobe of anterior lip covered with villous hairs along base to apex..*P. mollissima*
17′ Inside of middle lobe of anterior lip glabrous or only covered with villous hairs at the base, other parts glabrous or pubescent..18
18 Leaf margin deeply lobed..*P. repens*
18′ Leaf margin entire or shallowly lobed..19
19 Branches and abaxial leaf surface yellowish-brown villous; fruits apically pubescent, 7–10 mm long..*P. fulva*
19′ Branches and abaxial leaf surface glabrous or brown to reddish-brown pubescent; fruits glabrous, shorter or equal to 7 mm long..20
20 Abaxial leaf surface without hairs on nerve axils; posterior lip of calyx 3-lobed; inflorescences 0.7–2 cm long..*P. paniculata*
20′ Abaxial leaf surface with hairs on nerve axils; posterior lip of calyx entire to subentire or rarely 2–3-lobed; inflorescences 6–20 cm..*P. punctulata*

Conclusion

Species of *Premna* in Thailand included in this study constitute a monophyletic group based on four chloroplast markers (*ndhF*, *rbcL*, *rps16*, and *trnL-F*). Two clades are recognized based on phylogenetic relationships and morphological affinities. Our findings have resolved the taxonomic confusion of previously synonymized species within the *P. serratifolia* complex. *Premna paniculata*, and *P. punctulata* are reinstated to the species level and *P. cordifolia* is validly published as a distinct species, included in the account for Thailand. *Premna coriacea* does not occur in Thailand, and *P. octonervia* and *P. interrupta* var. *smitinandii* are not recognized here. *Premna coriacea* var. *villosa*, a taxon previously thought to be found in Thailand, is lectotypified. Consequently, 21 species have been recognized in the account being prepared for the *Flora of Thailand*. Further work is required to increase samples to construct a more inclusive sample of species *Premna* to provide the basis for an infrageneric classification and biogeographical study for a better understanding the natural history of *Premna*.

Methods

Morphological and ecological investigation

This study is based on field collections from natural habitats during field works in Thailand during 2022 and 2025. All experimental research, field studies involving wild plants, and the collection of plant materials were conducted in strict accordance with relevant institutional, national, and international regulations and guidelines. All plant specimens newly collected for this study were obtained from natural areas located outside officially designated protected zones. Fresh leaves were collected and dried with silica gel, and voucher specimens were deposited at herbarium of Prince of Songkla University (PSU) for further morphological investigations. Herbarium specimens were examined as well as digital image databases from the following herbaria: BKF, BM, CAL, E, K, KKU, L, LL, LINN, NY, PSU, and SING (acronyms follow the Index Herbariorum (Thiers, 2025)). Some type specimens were also examined thorough the Global Plants on JSTOR (https://plants.jstor.org/) and the Global Biodiversity Information Facility (https://www.gbif.org). The ecological information was obtained from the specimen labels and was noted from natural populations during field works. Formal plant identification in this study was performed by J. S., B. L., and C. L.

Taxon sampling and datasets

The samples for phylogenetic analyses of *Premna* in Thailand were based on the taxonomic account of Leeratiwong et al.[8,58]. Eighteen out of 21 species of *Premna* in Thailand were included in this study, covering approximately 86% species diversity of the genus according to Leeratiwong et al.[8]. Of this total number of species in Thailand, we excluded *P. coriacea* [sensu Leeratiwong et al.[8] (*non* C. B. Clarke)] and *P. octonervia* due to this species being treated as a synonym of *P. scandens* and *P. serratifolia*, respectively (see Taxonomic notes). Additional species from neighboring floristic regions were also included in the analyses (*i.e.*, *P. bracteata*, *P. ligustroides*, *P. menglaensis*, *P. mekongensis*, *P. punicea*, *P. szemaoensis*, *P. tenii*, and *P. vietnamensis*). Totally, 30 accessions of *Premna* were sampled from either herbarium specimens or freshly collected silica-dried leaf materials as well as the GenBank database (Supplementary Table S1). Two outgroups from the genus *Gmelina*, belonging to the subfamily Premnoideae, were selected based on the recent classification and previous molecular studies (*e.g.* Li et al.[5], Zhao et al.[6], Rose et al.[20]), consisting of *G. elliptica* Sm. and *G. philippensis* Cham.

Our study selected four chloroplast regions (cpDNA) for constructing phylogenetic reconstruction of *Premna* in Thailand, including *ndhF*, *rbcL*, *rps16*, *trnL-F*. This selection of each marker was based on selected previous phylogenetic analyses widely used across different genera within Lamiaceae, and species of *Premna* which have been sequenced available in GenBank database[5,17,19,34].

DNA extraction, PCR amplification and sequencing

The genomic DNA from silica dried leaf tissue was extracted by the 2 × CTAB method of Doyle and Dolye[69] and DNA from herbarium specimens was isolated by using DNEasy® Plant Mini Kit (QIAGEN®, Valencia, California, USA), following the manufacturer's protocols. The extracted DNA was dissolved in TE buffer and preserved at -20°C for further application and was checked for quality using a spectrophotometer (Thermo Scientific NanoDrop™ 1000, USA) and agarose. The Polymerase Chain Reaction (PCR) was performed to amplify four chloroplast markers, using primers *nhdF*[70], *rbcL*[71], *rps16*[72], and *trnL-F*[73]. The reaction contains 2 µL sample

DNA, 5 µL ExcelTaq™ 5X PCR Master Dye Mix (SMOBIO Technology, Inc., Taiwan), 1 µL of each primer in a final reaction volume of 25 µL (Bio-Rad PCR Thermal Cycler, Model T100, Thailand). The uniform primer conditions were adapted from Li et al.[5] and Hai et al.[17] (an initial denaturation at 94 °C for 5 min, 35 cycles of 30 s denaturation at 94 °C, 1 min primer annealing at 52 °C, 2 min extension at 72 °C, with a final extension of 5 min at 72 °C). PCR products were visualized using agarose gel electrophoresis and were sequenced by Macrogen Inc. (South Korea), using the same primers for the PCR amplification.

Alignment and phylogenetic analyses

Sequences were assembled and edited using Geneious Prime® 2024.0.3[74] (Biomatters Ltd., Auckland, New Zealand [http://www.geneious.com/]) and were verified using the BLAST (Basic Local Alignment Search Tool) algorithm. The sequences were then aligned by MAFFT v7.490[75] to analyze biological data. The aligned dataset was determined as the best-fitting nucleotide substitution model using jModelTest software version 2.16[76]. The concatenated datasets from four chloroplast markers were analyzed using Maximum likelihood (ML) and Bayesian Inference (BI) methods with gaps treated as simple indels. ML analysis was performed using the phylogenetic IQ-TREE 2[77] with 1000 ultrafast bootstraps using the '-B' option. Support for nodes was estimated using 1000 replicates for ultrafast bootstrapping[78]. BI was analyzed using MrBayes version 3.2.2[79]. Four Markov Chain Monte Carlo (MCMC) chains were run for 10 million generations and sampled every 10,000 generations. The first 25% was discarded as burn-in. The phylogenetic trees from both analyses were visualized using FigTree version 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). The consensus tree representing the overall relationships was based on a Bayesian 50% majority-rule consensus tree.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information file.

Received: 31 March 2025; Accepted: 8 September 2025
Published online: 10 October 2025

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Acknowledgements

We would like to thank the staff of herbaria cited in this study for helping with accessibility to the specimens. Thanks to the staff of the Center of Scientific Equipment, Walailak University, for facilitating laboratory work. Special thanks to Dr. Somran Suddee, Dr. Phongsak Phonsena and Assoc. Prof. Dr. Sawai Mattapha for some materials used in this study. Special thanks to Dr. Nattanon Meeprom for the phylogenetic analysis guidance. This work is financially supported by the Individual Research Grant from Walailak University, Contract Number WU66223 and the National Natural Science Foundation of China (Grant No. 32160047).

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Conceptualization, methodology, investigation: J.S., B.L. and C.L. Field collections: J.S. and C.L. Plant identification: J.S., B.L. and C.L. Resource: J.S., A.J.P. and C.L. Analyze the data: J.S. and B.L. Discussion: J.S., A.J.P., B.L. and C.L. Writing – original draft: J.S. Writing – review and editing: A.J.P. and B.L. Funding acquisition: J.S. and B.L.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-19354-3.

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