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## *Dillenia nagalim* (Dilleniaceae): a new species based on morphological and molecular characters from Manipur, Northeast India

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### Abstract

*Dillenia nagalim* (Dilleniaceae), here described, has been collected from Manipur, Northeast India, from where it is endemic and known only from the type specimen. The new species shows close morphological similarity to *D. pentagyna* and *D. hookeri* but differs from the latter in having a short habit of 0.6–1.2 m tall, an amplexicaul short petiole, leaves with double serrate margins, an acuminate leaf apex, showy flowers, numerous stamens, and 7–8 carpels. We have highlighted the diagnostic morphological characters, colour photographs, phenology, conservation status, and artificial keys for easy identification. Molecular phylogenetic analysis based on the *rbcL* gene indicates the possibility of these new species. The discovery of this new species highlights the phytogeographical importance of the region situated within the Indo-Burma biodiversity hotspot, where anthropogenic activities are intense and considered “Critically Endangered” (CR) under the IUCN Red List Categories and Criteria.

**Key words:** Phylogenetic tree, Indo-Burma hotspot, conservation, Critically Endangered

### Introduction

*Dillenia* Linnaeus (1753: 535), an honour of a German-born botanist, Johann Jacob Dillenius (1684–1747), belongs to the Dilleniaceae family and has ca. 60 accepted species distributed across Madagascar, India, Southeast Asia, and Australia and introduced to Caroline Island, the Caribbean Sea, the Gulf of Guinea Islands, and Ecuador (Hoogland 1952; POWO 2025), and grows primarily in the tropical and subtropical regions. Linnaeus, while studying Dillenius’s colossal and significant works on this plant having showy flowers and fruits, dedicated “*Critica Botanica*” to Dillenius in 1736. Later, in his “*Species Plantarum*,” confirm the genus and described *Dillenia indica* Linnaeus (1753: 535) under the class “Polygynia”.

In India, the genus is reported to have eight species, namely *D. andamanica* (C. E. Parkinson 1935:452), *D. aurea* (Smith 1806:65), *D. bracteata* (Wight 1840:358), *D. indica* (Linnaeus 1753: 535), *D. pentagyna* (Roxburgh 1795:21), *D. retusa* (Thunberg 1791:200), *D. scabrella* (D. Don 1825: 226) (Roxburgh 1814: 105) (Wallich 1830:20), and *D. tirupatiensis* (J. Swamy & Rasingam 2020:16636) (Jain *et al.* 1979; Sharma *et al.* 1993; Swamy *et al.* 2020). In Manipur only *D. indica* and *D. pentagyna* were reported (Agrawala *et al.* 2023), the state is a distinctive geographical location and harbours a rich biological flora and fauna. During a botanical expedition to an extremely remote region, Choro village, Kamjong district, Manipur, on the Indo-Myanmar border, we found a noteworthy species of *Dillenia*,

a shrub plant characterized by yellow flowers, which were collected for further examination, study, and now here described as a new species.

The plants of the genus *Dillenia* are an evergreen or semi-evergreen tree or shrub, and characterized by dense hirsuteness in the stem and leaves with serrate margins, and the leaf tips are acute, acuminate, obtuse, or rounded. The inflorescence is either showy in clusters or solitary flowers with petals white or yellow colour, and the fruits are indehiscent and enclosed by a persistent calyx.

Combining molecular data with classical taxonomy provides a comprehensive classification as well as robust and reliable evolutionary data (Will *et al.* 2005). Standardized DNA barcoding of the chloroplast gene, ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*), is used for the identification and discrimination of *Dillenia* species. (CBOL Plant Working Group 2009; Braukmann *et al.* 2017). Thus, after critical analysis and comparison with all morphological and molecular characters of known species, herbarium specimens, and available literature (Hoogland 1952; Jain *et al.* 1979; Sharma *et al.* 1993; Choudhary *et al.* 2012; Tan and Latiff 2014; Thooptianrat *et al.* 2017; Swamy *et al.* 2020; Agrawala *et al.* 2023) we concluded that the plant is different from all known species of *Dillenia*, described here as *Dillenia nagalim*. The new species is classified as “Critically Endangered (CR)” in accordance with the standards and guidelines given by the International Union for Conservation of Nature (IUCN) (IUCN 2012; 2024), based on a very restricted distribution range and intense anthropogenic activities.

## Materials and Methods

### Fieldwork and morphological studies

Botanical field surveys were conducted from May 2024 to May 2025 in Manipur, Northeast India, and the location map was created using geographic coordinates. Standard taxonomic methods have been followed for the collecting, desiccation, and subsequent processing of the herbarium specimens (Jain and Rao 1977; Bridson and Forman 1989; Paul *et al.* 2020). Photographs of the habitat and plant parts were taken with a Canon EOS 1200D and OM System TG—7 digital camera. Flowers were dissected, examined, and recorded under the Hoverlabs stereozoom microscope (HV-ZOOM-IV TR) along with image viewing and processing software called “ImageView” to measure the object sizes.

Type specimens of related species were examined online via JSTOR Global Plants (<https://plants.jstor.org/>), Plants of the World Online (<https://powo.science.kew.org/>), and World Flora Online (<https://www.worldfloraonline.org/>). Additionally, protologues and pertinent taxonomic literature were examined to make sure that the species is new. The holotype specimen was deposited at the Central National Herbarium, Howrah (CAL), and the isotypes specimens were deposited at the Dhanamanjuria Herbarium (DMH) and Delhi University Herbarium (DUH). With the objective of enabling the identification of the new species, detailed morphological descriptions, colour photographic slides, and an emended key for all species of this genus from India are provided. We also assess the ecology and conservation status in accordance with the standards and guidelines set forth by the International Union for Conservation of Nature (IUCN) (IUCN 2012; 2024).

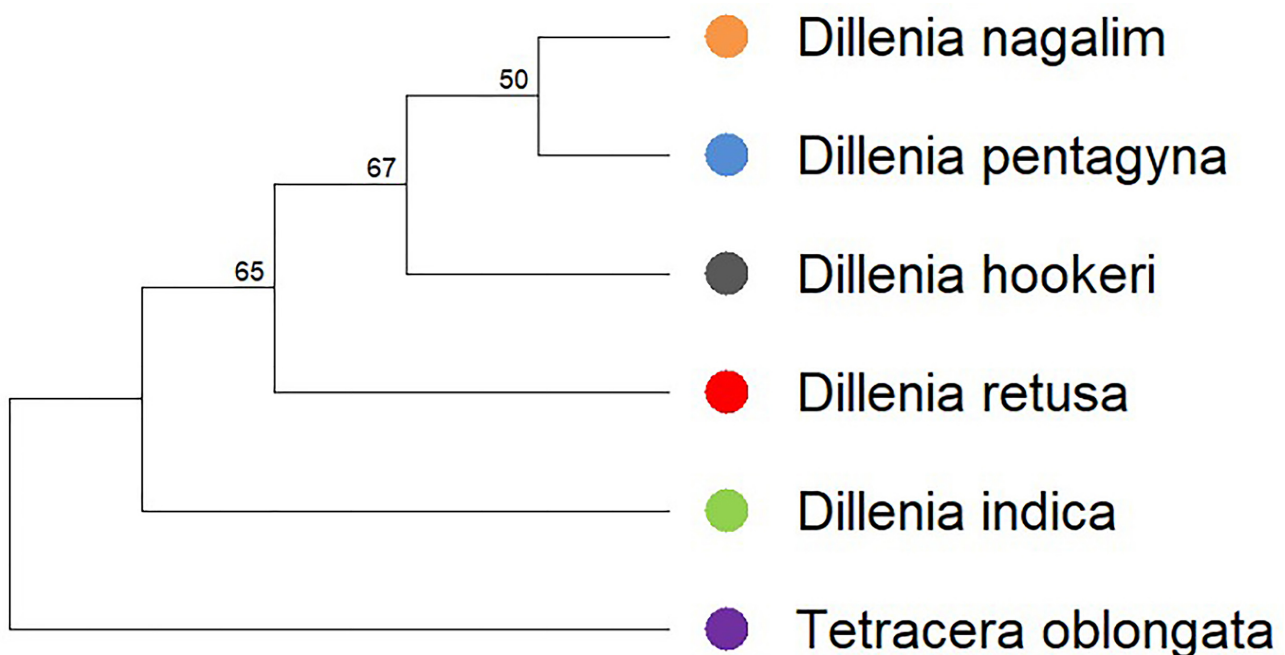
### Isolation of genomic DNA, sequencing and molecular analysis

Plant genomic DNA was isolated from the collected sample using the CTAB method (Doyle & Doyle 1987). Polymerase Chain Reaction (PCR) amplifications (Mullis and Faloona 1987) of isolated gDNA with the *rbcL* gene were performed with primer pairs F-GTTGGCTTCACAGCTCGTCT, R-GAAACGGTCTCTCCAACGCATA. The PCR product was purified, and DNA sequencing was carried out at Barcode BioSciences Pvt. Ltd., Bangalore, India. The sequence of the *rbcL* gene was analyzed in BLAST homology searches in the non-redundant NCBI-nr database available at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov) (Altschul *et al.* 1990; Boratyn *et al.* 2012). A phylogenetic tree was constructed using MEGA 12 (Kumar *et al.* 2024) with bootstrap values of 1000 replications (Felsenstein 1985) to depict sequence relatedness of the *D. nagalim* with other members of Dilleniaceae. Sequences of *D. hookeri* (FJ860349), *D. indica* (FJ860350), *D. pentagyna* (KX344680), and *D. retusa* (FJ860353) were obtained from NCBI GenBank databases, and the sequence of *Tetracera oblongata* (FJ860392.1) was used as an outgroup species. The evolutionary history was inferred by using the maximum likelihood (ML) method based on the Tamura-Nei model (Tamura and Nei 1993).

## Results and discussion

### Molecular data

Molecular studies strengthen the degree of precision in morphological studies, which are now essential to contemporary taxonomy. After successful PCR amplification and sequencing, produced 627 base pairs. The phylogenetic tree (Fig. 1) was constructed from sequences supported by bootstrap ( $\geq 50$ ) and demonstrates effective identification and molecular discrimination capabilities that corroborate *D. nagalim* as a new species. The ML phylogenetic tree provides evidence for the unique taxonomic classification of *D. nagalim* as a new species, closely aligning with *D. pentagyna* and *D. hookeri*, having satisfactory bootstrap support. Studies conducted by Fouad *et al.* (2022) and Bell *et al.* (2017) demonstrate that *rbcL* is highly effective for the authentication and discrimination of plant species, thereby reinforcing its taxonomic utility.



**FIGURE 1.** A maximum likelihood (ML) tree drawn using MEGA 12 (Kumar *et al.* 2024) from *rbcL* gene sequences. Bootstrap support values for major nodes at  $\geq 50$  are shown. The *rbcL* gene sequence of *Tetracera oblongata* (GenBank accession FJ860392.1) was used as an outgroup.

### Taxonomy

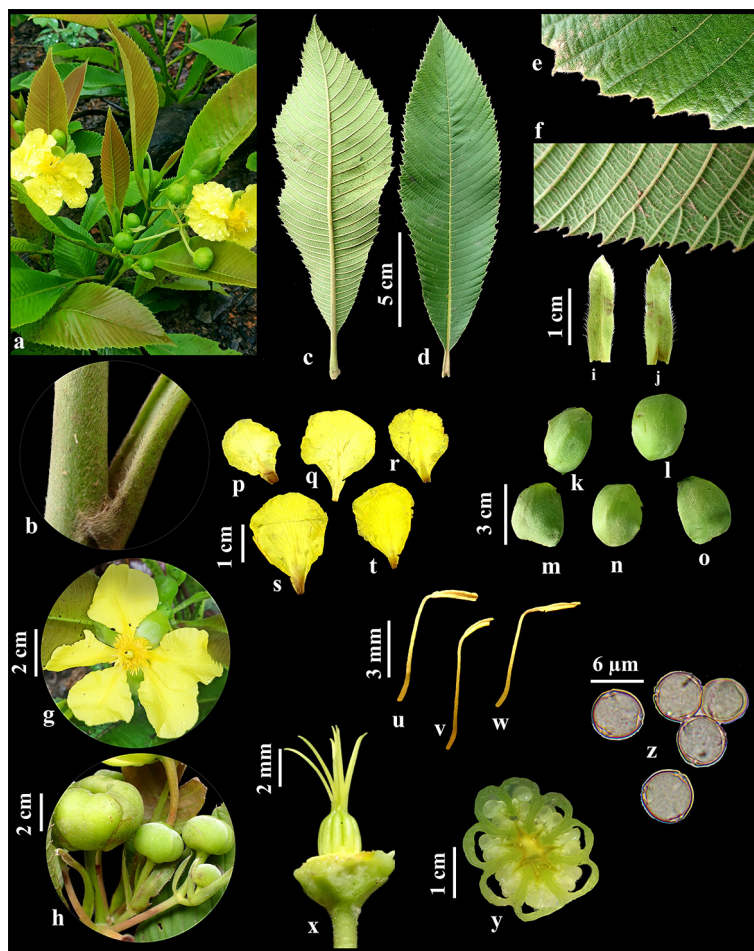
*Dillenia nagalim* S. Kashung, & K. Eshuo *sp. nov.* (Fig. 2)

**Type:**—INDIA. Manipur, Kamjong, Choro village, 24°34'17"N, 94°28'55"E, alt. 300 m, 29 May 2025, Sochanngam Kashung & Kazhuhrii Eshuo SK100015 (holotype, CAL!; isotypes, DMH!, DUH!).

**Diagnosis:**—The newly described species *Dillenia nagalim sp. nov.* shows morphological affinities with *D. pentagyna* and *D. hookeri* but differs from the latter two plants in having a short habit of 2–4 feet tall; shorter petiole (1–2.5 cm long); leaves oblanceolate to lanceolate-elliptic, leaf margin serrate to doubly serrate, apex acuminate, 32–43 nerves; 1–2 bracteoles, apex acute to short acuminate; larger flower, 6–8 cm across, more number of stamens per flower, stamens ca. 270; 7–8 carpels, ovules 14–16; fruit bigger, 3 × 2 cm in diameter and 2–6 seeds per fruit, 4 × 3 mm in diameter (Fig. 2, Table 1).

**Description:**—Shrubs, about 0.6–1.2 m tall, in open tropical deciduous forests. Branched, sympodial, and densely hirsute in young stems, become glabrescent in older branches. Leaf scars clasping about half or more of the branch. Petiolate, petiole 1.2–2.5 cm long, 2–4 mm broad, fibrous brown hairs, slightly amplexicaul; leaves lamina oblanceolate to lanceolate-elliptic, (5.4–)12.5–23.5 cm long, 4.5–7.5 cm wide, margin serrate, doubly serrate, serration ending at

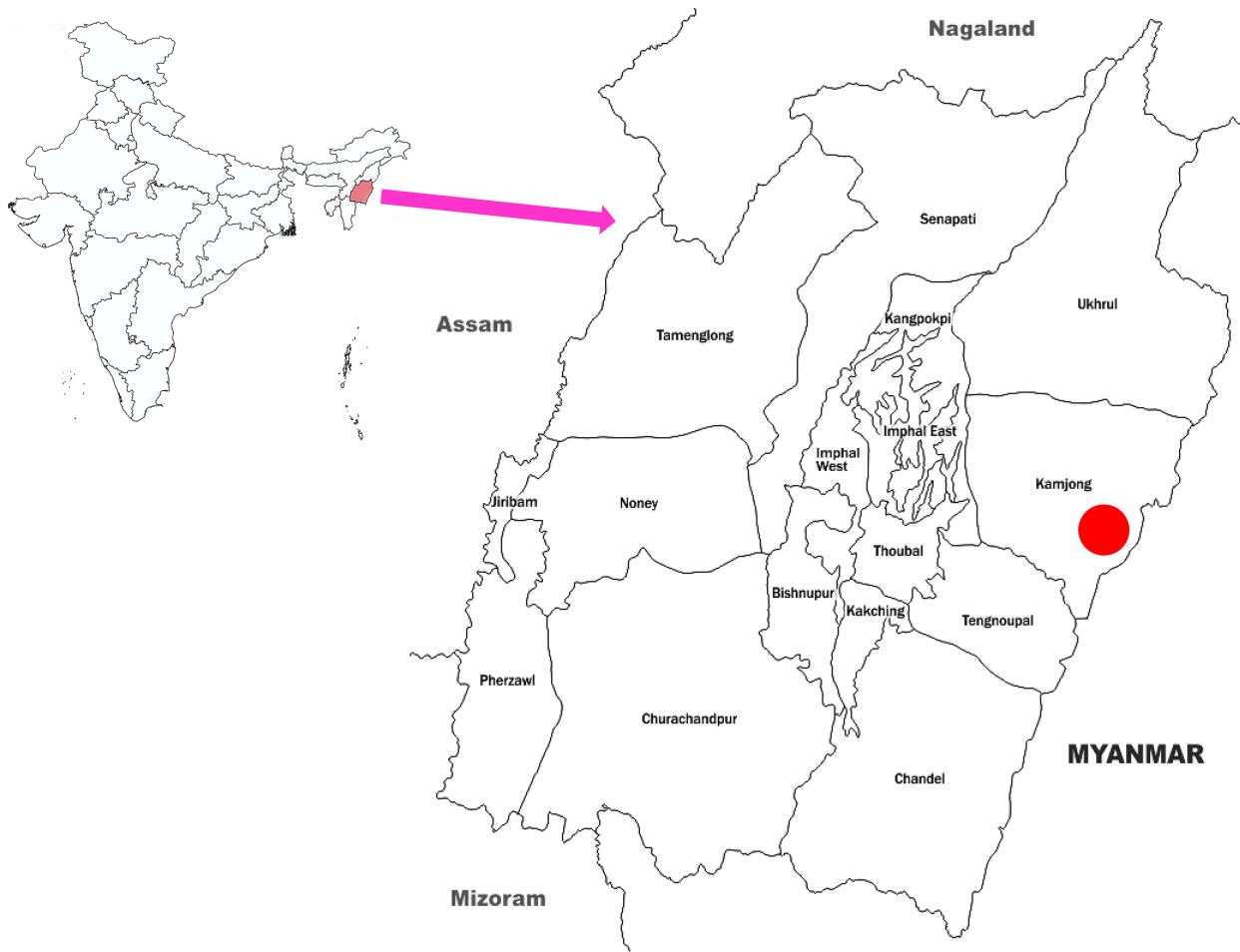
the tip of the teeth and margin, curved; leaf tip acuminate, leaf base cuneate, decurrent, or acute, dorsal surface light green, ventral surface yellowish green, surface densely velvety tomentose, nerves (26–)32–43 (–47) on either side, nerves ending on margin. Flower solitary, or 2–3 together, axillary or terminal, 6–8 cm across; pedicel 2–3 cm long, longer in fruits, up to 4.5, 2–3 mm wide; bracteoles sessile, lanceolate, 2–3.6 cm long, 0.5–0.8 cm wide, apex acute to short acuminate, sericeous, margin with long hairs. Calyx persistent, sepals 5 (–6), green, ovate to oval, imbricate, 2.5–3.6 × 2–2.5 cm in flower, fleshy, densely sericeous outer, inner glabrous. Petals 5, yellow, obovate, 3.5–4.5 (–5.2) × 2–3.5 (–3.8) cm in diameter, wedge-shaped, wavy, apex rounded, margin smooth. Stamens numerous, ca. 270, arranged in 4–5 rows, filament 5–8 mm long, anther lobe 5–6.5 mm long, ca. 0.5–1 mm broad, emarginate, slightly curved in bud, thecae linear, dehiscent by apical pore; pollen grains circular, oval, 5–6.50 µm in diameter, 3 germ pores, smooth exine. Carpels 7–8, style 12 mm long, spreading, curved, cylindrical; ovary 4–6 mm long, glabrous, locule 7–8 lobed, ovules 2 in each locule, fruits/pseudocarps indehiscent, globular, 3 × 2 cm in diameter with enclosing calyx, 2 × 1.4 cm in diameter without enclosing calyx. Seeds are black, 2–6 seeded, obovoid, and 4 × 3 mm in diameter; seeds are surrounded by transparent jelly fluid having a strong pungent of golden berry fruits, each seed being attached by a hilum.



**FIGURE 2.** *Dillenia nagalim* S.Kashung & K.Eshuo *sp. nov.*, (a) habit, (b) petiole (slightly amplexicaul), (c–d) leaves in dorsal and ventral views, (e–f) leaf margin showing double serration, (g) flower, (h) fruits, (i–j) bracteole, (k–o) calyx, (p–t) petals, (u–w) stamens with filaments, (x) carpel with style, (y) cross-section of ovary showing locules with ovules, and (z) pollen grains with 3 germ pores. Photos by S Kashung, K Eshuo & K Kaini.

**Distribution and ecology:**—The new species described are grown at an altitude of about 250–350 m above sea level, located in Choro village near the Indo-Myanmar border (Fig. 3). The plant population grows in an open tropical deciduous forest along with *Dipterocarpus tuberculatus* Roxb., *Tectona grandis* L.f., *Gluta usitata* (Wall.) Ding Hou, and *Imperata cylindrica* (L.) P. Beauv.

**Phenology:**—The flowering and fruiting of the new species were recorded during the months of May to June.



**FIGURE 3.** Map showing the location of the study area. The red circle indicates the collection site of *Dillenia nagalim* at Choro village, Kamjong District, Manipur. © d-maps.com.

**Etymology:**—The specific epithet “*nagalim*” is a noun in apposition referring to “land of Naga tribes” that is derived from “Naga”—native people, and “*lim*”—land. The plant is collected from the region predominantly inhabited by the native Tangkhul Naga tribe.

**Conservation status:**—The preliminary conservation assessment status of “Critically Endangered,” which can be denoted as “CR B2ab(i,ii,iii,iv,v),” is provisionally recommended for *Dillenia nagalim* in accordance with the guidelines of the IUCN Red List Categories and Criteria (IUCN 2012; 2024). The plant has a very restricted distributional range, making it vulnerable to the impacts of human developmental activities. The place is under the direct threat from anthropogenic disturbances transforming undisturbed open tropical deciduous forests into a new locality or township. The state of Manipur is situated within the Indo-Burma biodiversity hotspot (Myers *et al.* 2000).

**Notes:**—*Dillenia nagalim* exhibits morphological similarities to *D. pentagyna* and *D. hookeri* in its oblanceolate and acuminate leaves, verticillate bracteolate, and yellow corolla. However, notable distinct features of the new species are the leaves with double serrate margins, short habit of 2–4 feet tall, an amplexicaul short petiole, large showy flowers, and many stamens. Table 1 provides a summary of the morphological differences between *D. nagalim*, *D. hookeri*, and *D. pentagyna*.

The *Dillenia* plant is used medicinally and therapeutically against wound healing, cancer, neuroprotection, diabetes, inflammation, abdominal pains, diarrhea, bone fractures, etc. (Hoogland 1952, Gandhi and Mehta 2013; Yazan and Armania 2014; Sabandar *et al.* 2017; Ahmed *et al.* 2025; Nahar *et al.* 2025). During the present investigation, interaction with the local inhabitants revealed that the young, tender leaves were boiled and consumed as vegetables, while the fruits were also eaten for their sweet, pungent aroma similar to golden berry fruits. Our teams have also tasted the fruits in our lab, confirming the local information about their edibility. The discovery of this new species highlights the unique characteristics of the habitats of Manipur, India, that are located within the Indo-Burma biodiversity hotspot, underscoring the critical necessity for their conservation to prevent habitat degradation due to anthropogenic activities.

## Key to the species of *Dillenia* from India

- 1a. Flowers white ..... 2
- 1b. Flowers yellow ..... 4
- 2a. Leaf elliptic-obovate; margin crenate, bracteole 3, flowers 6 cm across ..... *D. tirupatiensis*
- 2b. Leaf oblong, obovate; margin dentate, bracteole absent, flowers more than 6 cm across ..... 3
- 3a. Plant ca. 20 m high, leaf obovate, 10–14 nerves, flowers 6–8 cm across ..... *D. retusa*
- 3b. Plant ca. 30 m high, leaf oblong, 30–40 nerves, flowers 15–20 cm across ..... *D. indica*
- 4a. Bracteole absent, flowers in fascicle or rarely solitary ..... 5
- 4b. Bracteole present, flowers in solitary ..... 6
- 5a. Petiole up to 5 cm long, leaf lamina 15–30 × 6–12 cm, flower 2–3 cm across, carpels 5 ..... *D. pentagyna*
- 5b. Petiole up to 2 cm long, leaf lamina 22–45 × 11–17 cm, flower 12 cm across, carpels 8 ..... *D. andamanica*
- 6a. Plant ca. 30 m high, pedicel with caducous bracteoles ..... *D. scabrella*
- 6b. Plant less than 20 m high, pedicel with persistent bracteoles ..... 7
- 7a. Leaf oblanceolate, apex acuminate, silky hirsute ..... *D. hookeri*
- 7b. Leaf elliptic to oblong, apex rounded, obtuse, or truncate, tomentose-hirsute ..... 8
- 8a. Flower in fascicle of 2–3, 4 cm across, carpels 5, seed arillate ..... *D. bracteata*
- 8b. Flower solitary or in two, carpels more than 5, seed exarillate ..... 9
- 9a. Flower solitary or 2–3 together, 6–8 cm across, carpels 7–8, seed obovoid ..... *D. nagalim*
- 9b. Flower solitary, 10–12 cm across, carpels 10–12, seed exarillate ..... *D. aurea*

**TABLE 1.** Diagnostic morphological characters of *Dillenia nagalim* sp. nov., *Dillenia hookeri* and *Dillenia pentagyna*.

Characters	<i>D. nagalim</i>	<i>D. hookeri</i>	<i>D. pentagyna</i>
Habit	Shrubs 0.6–1.2 m tall	Tree 10–12 m or shrubs 2–3 m tall	Tree ca. 25 m tall with 100 cm thick
Leaf	oblanceolate, lanceolate to elliptic, 12–23 × 4–7.5 cm, margin serrate, doubly serrate, base cuneate to acute, apex acuminate	oblong, oblanceolate, 17–22 × 7–9 cm, margin entire to slightly dentate, base acute, apex rounded, slightly acuminate	obovate, 20–50 × 10–20 cm, margin entire to dentate, base acute, apex rounded to obtuse
Petiole	petiole slightly amplexicaul, 1–2.5 cm long, fibrous brown hairs	petiole non- amplexicaul, 1.5–4 cm long, sericeous	petiole non- amplexicaul wing, 1.5–5 cm long, glabrous above, beneath strigose-hirsute
Flower	1(–3) flowers, terminal, lateral/axillary, 6–8 cm across	single, rarely 2, terminal, 4–5 cm across	2–7 flowers, lateral, 2.5–3 cm across
Bracteole	1–2, verticillate, 20–36 × 5–8 mm, apex acute to short acuminate	3, verticillate, 20–35 × 7–10 mm, apex acute or truncate acute	bracteole absent
Corolla	3.5–4.5 × 2.0–3.5 cm in diameter	2.5 × 1.3 mm in diameter	1.5–2.0 × .5–1.0 cm in diameter
Stamens	isomorphic, ca. 270 per flower	isomorphic, ca. 190–200 per flower	2 distinct groups, ca. 60–90 per flower
Carpels	7–8	6–7	5
Ovules	14–16	18	5–20
Fruit	3 × 2 cm in diameter	2 × 2.5 cm in diameter	1.5 × 1.3 cm in diameter
Seed	2–6 seeded, 4 × 3 mm in diameter	1–5 seeded, 3.5 × 3 mm in diameter	1(–2) seeded, 5 × 3.5 mm in diameter.

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