



## *Diospyros daweishanensis* (Ebenaceae), a new species from Southwest China

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**ABSTRACT:** A new species of Ebenaceae, *Diospyros daweishanensis* Z. X. Zhang, Gui L. Zhang & C. Y. Zou, from Southwest China, is described and illustrated. Colour figures, distribution map and photographs of type specimens are provided. The similarities and differences between this species and others are discussed. Morphologically, its lanceolate leathery leaf with unobtrusive venation, 5(-6)-lobed calyx and the reflexed fruiting calyx are clearly distinct from those of other species. Molecular phylogeny also supports its classification as a distinct species.

**KEY WORDS:** *Diospyros*, Ebenaceae, morphology, taxonomy, phylogenetics.

### INTRODUCTION

*Diospyros* Linnaeus (1753) in the family Ebenaceae is a genus of more than 800 species found throughout the tropics and subtropics (Wallnöfer, 2001; Duangjai *et al.*, 2006, 2009; POWO, 2023). The greatest species diversity occurs in the Asia-Pacific region where several species are used as timber or for their edible fruits (Bakhuizen van den Brink, 1936–1955; Singh, 2005). In China, 72 species and five varieties of the genus have been recorded, with 47 being endemic (Lee *et al.*, 1996; Li *et al.*, 2006; Tian *et al.*, 2014; Huang *et al.*, 2015; Nong *et al.*, 2017; Zhao, *et al.*, 2018a,b; Tong and Xia, 2019; Sima *et al.*, 2021; Liu *et al.*, 2024). Recently, 10 species have been discovered and reported from southwestern China alone. These findings underscore the importance of further botanical exploration in the remote Southwest China.

Several interesting *Diospyros* species were collected during a field survey in the tropical evergreen forests of Yunnan Province, China. Subsequent observations of these species were conducted across different seasons, and male and female flowers of one of the species were collected. In-depth studies of the flowers and fruits confirmed that this species is morphologically distinct from any known species. Additionally, phylogenetic analyses using multiple plastid DNA sequences supported its classification as a distinct species, not closely related to other morphologically similar species. Therefore, it is described here as a new species.

### MATERIALS AND METHODS

The herbarium specimens of *Diospyros* preserved at IBK, PE, IBCS, KUM, HITBC, and SYS were studied,

along with digital images of the Southwest Asian type specimens from the herbaria of the Royal Botanic Garden Edinburgh (E), the Arnold Arboretum (A), the Royal Botanic Gardens, Kew (K), the Muséum National d'Histoire Naturelle (P), and the New York Botanical Garden (NY). The material of the new species was compared with the relevant literature (Hiern, 1873; Lecomte, 1928, 1929, 1930; Bakhuizen van den Brink, 1936–1955; Phengkklai, 1981, 1987, 2005; Lee *et al.*, 1996; Huang *et al.*, 2015; Nong *et al.*, 2017; Zhao, *et al.*, 2018a,b; Tong and Xia, 2019; Duangjai *et al.*, 2018, 2020; Sima *et al.*, 2021). The morphological description of the new species is based on its female and one of the male specimens.

The phylogenetic analyses of Duangjai *et al.* (2006, 2009) indicated that the genus *Diospyros* is monophyletic, based on DNA sequences from eight plastid regions (*rbcL*, *atpB*, *matK*, *ndhF*, *trnK* intron, *trnL* intron, *trnL-trnF* spacer, and *trnS-trnG* spacer), forming 11 clades with 119 *Diospyros* species. To clarify the taxonomic status and phylogenetic placement of the new species, we conducted phylogenetic analyses of the genus *Diospyros* based on sequence data of the eight plastid regions. In total, 181 DNA accessions representing 25 species 1 form were compiled, incorporating 24 newly generated and previously published datasets from GenBank (Duangjai *et al.*, 2009, 2018). These sequence data include two new accessions from the new species and one *D. hexamera* C. Y. Wu. *D. maingayi* (Hiern) Bakh. The source of the materials and GenBank accession numbers are provided as supplementary material in Table S1.

Three silica-dried leaf tissue samples were sent to Berrygenomics (Beijing, China) for DNA extraction and whole-genome resequencing. Total genomic DNA was

**Table 1.** Morphological comparison of *Diospyros daweishanensis* with similar species.

Species	Bark	Leaves	Female flowers	Male flowers	Fruits	Fruiting calyx lobes
<i>D. daweishanensis</i>	Black	Lanceolate or elliptic-lanceolate, 5–35 × 3–5.5 cm, base cuneate or rounded, apex acute or acuminate, midrib adaxially impressed, abaxially prominent	Solitary, 5(–6)-merous	Cymose, 5(–6)-merous	Globose, 4–6 cm	Reflexed, not plicate
<i>D. salletii</i>	—	Elliptic or oblong-elliptic, 2–15 × 3.5–5.5 cm, finely reticulated veins on upper surface	Solitary, 4-merous	—	Ovoid or ellipsoid, 2–6 × 2–5 cm	Reflexed, undulate, not plicate
<i>D. toposia</i>	Reddish	Oblong to lanceolate, 15–30 × 4.5–11 cm, base acute, obtuse or rounded, smooth or strongly impressed on upper surface, reticulation prominent on upper surface	Solitary, 4-merous	Cymose, 4-merous	Ovoid or ellipsoid, 2–6 × 2–5 cm	Reflexed, not plicate
<i>D. defectrix</i>	Ash white	Oblong to lanceolate, 6–16 × 2–7 cm, base acute, obtuse or rounded, apex cuspidate with blunt tip, ± impressed on upper surface	Solitary, 4(–5)-merous	Cymose, 4(–5)-merous	Ellipsoid, 1–2.5 × 1–2 cm	Reflexed, not plicate nor undulate
<i>D. cauliflora</i>	Dark brown	Oblong or lanceolate, 11–15 × 3–8 cm, base acute or slightly cuneate, impressed on upper surface, prominent on lower surface	Cymose or solitary (usually on trunk), 4(–5)-merous	Cymose, 4(–5)-merous	Ovoid or ellipsoid, 1.5–3 × 1.5–2.5 cm	Reflexed, undulate, not plicate
<i>D. hexamera</i>	Black	Oblong-lanceolate, 8–18 × 2.7–5.5 cm, base broadly cuneate, apex acute to obtuse	Solitary, 5(–6)-merous	Cymose, 5(–6)-merous	Subglobose, 3–4 × 2.5–3 cm	Incurved, undulate, plicate
<i>D. fleuryana</i>	Dark brown	Lanceolate or oblong-lanceolate, 8–14 × 2–4.5 cm, tapered towards the base and apex, vaguely acuminate to obtuse and short, impressed above	Solitary, 5(–4)-merous	—	Globose, 3–4 cm	Incurved, undulate, plicate

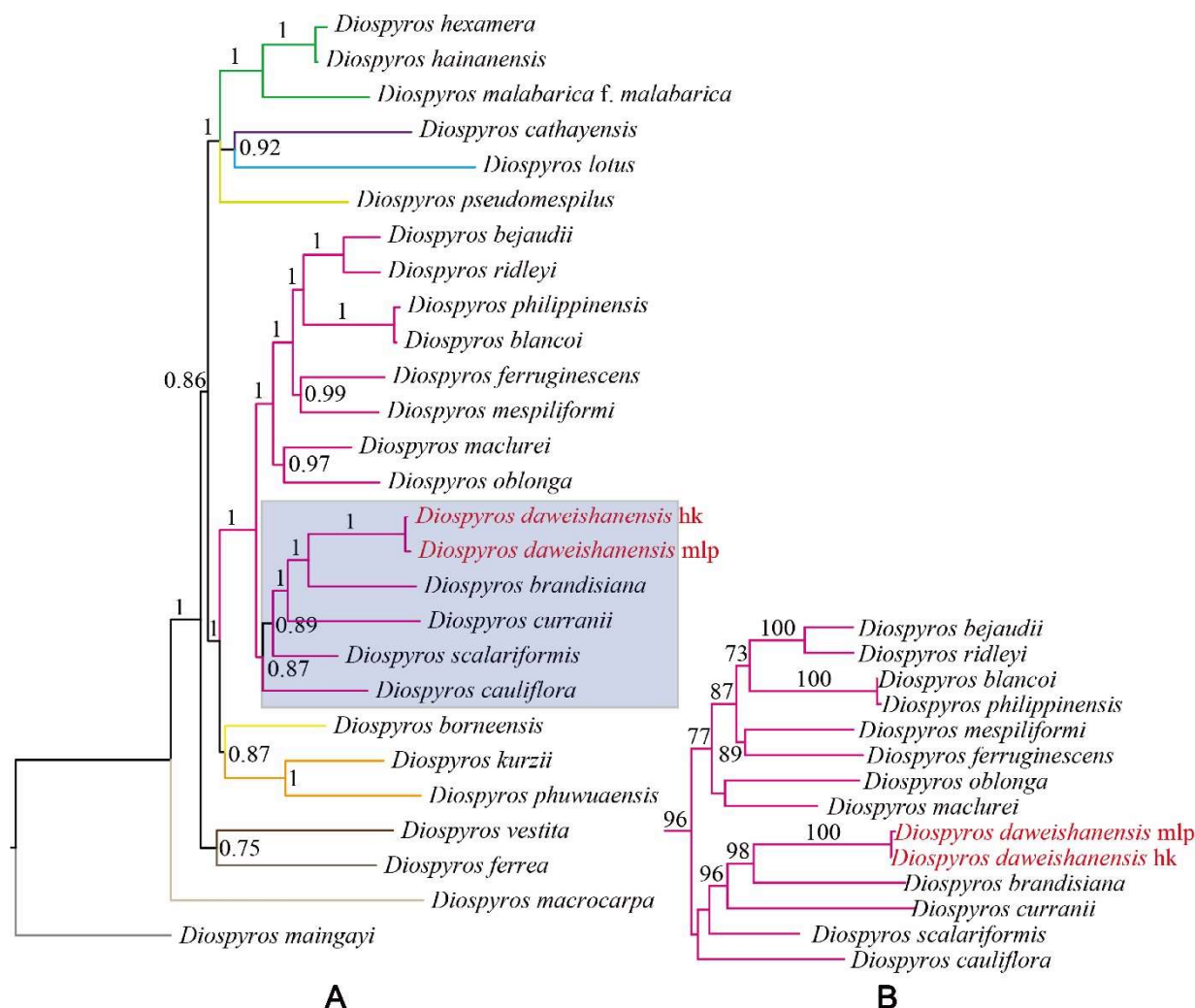
extracted from silica-dried leaf tissue using the mCTAB method (Li *et al.*, 2013). Non-degraded DNA was used to construct paired-end sequencing libraries according to the Illumina library preparation protocol. Sequencing (150 bp pair-end) was performed on the Novaseq6000 system (Beijing, China). This process resulted in three samples with sequenced data amounts of 10.4 GB, 10.0 GB, and 10.7 GB. The whole chloroplast genome was assembled using GetOrganelle v1.7.7.1 (Jin *et al.*, 2020). Extraction of eight plastid regions (*rbcL*, *atpB*, *matK*, *ndhF*, *trnK* intron, *trnL* intron, *trnL-trnF* spacer, and *trnS-trnG* spacer) from the chloroplast genome was performed using Geneious Prime v2020.1.1 (Biomatters Ltd., Auckland, New Zealand). We employed MAFFT v7.490 (Katoh and Standley, 2013) within Geneious Prime for generating alignments and used Geneious Prime to concatenate sequences. Phylogenetic analyses were performed using both Maximum Likelihood (ML) and Bayesian inference (BI). ML was conducted in RAxML v8.2.11 (Stamatakis, 2014) with the substitution model GTRGAMMA and 1,000 rapid bootstrap searches. BI was conducted in MrBayes v3.2.6 (Ronquist *et al.*, 2012) with the optimal substitution model GTR and were run for 1,000,000 generations, with four chains in two parallel runs and one tree sampled every 5,000 generations. The first 25% of sampled trees corresponding to the burn-in period were discarded, and the remaining trees were used to construct a majority rule consensus tree. The resulting trees were visualized using FigTree v1.4.3 (<https://github.com/rambaut/figtree/releases>).

## RESULTS AND DISCUSSION

After conducting a morphological examination and comparing it with the type material and protologues of known *Diospyros* species, it was concluded that the collections of *Diospyros* from Southwest China represent a new species. We describe and illustrate this species below as *D. daweishanensis* Z. X. Zhang, Gui L. Zhang & C. Y. Zou.

The aligned DNA sequence matrix comprised 9136 bp. Both Maximum Likelihood and Bayesian inference analyses were generally congruent. The new species falls within the clade VII. The Bayesian analysis is presented in Fig. 1 A, while Fig. 1 B presents a segment of the maximum likelihood tree showing clade VII (as defined in Duangjai *et al.*, 2009). The clades identified in this study align with those previously reported (Duangjai *et al.* 2009; 2018). Within the *Diospyros* clade VII, two accessions of *D. daweishanensis* are clustered together with robust support (PP 1.00, BS 100), forming a sister relationship with *D. brandisiana* Kurz (PP 1.00, BS 98). The DNA sequences of the two individuals of *D. daweishanensis* are identical and exhibit significant differences from those of other *Diospyros* species (Fig. 1), supporting its recognition as a distinct taxon.

Morphologically, the new species resembles *D. salletii* Lecomte and *D. toposia* Buch.-Ham. group as it has reflexed fruiting calyx. But it looks very different in having calyx lobes (5(–6)-lobed). Another group that is similar to this is the *D. defectrix* H. R. Fletcher and *D. cauliflora* Blume group. However, the former leaves are



**Fig. 1.** Phylogenetic trees of *Diospyros* based on DNA sequence data from eight plastid regions. *D. daweishanensis* is indicated in bold font. **A.** 50 % majority-rule tree from Bayesian inference. Posterior probability values (PP) greater than 0.50 are given above branches. **B.** ML tree with RAXML, displaying only the details of *Diospyros* clade VII. Bootstrap support (BS) values greater than 50 are indicated above branches.

oblong-lanceolate, 11–15 cm long and 3–8 cm wide, with impressions on the upper surface, while the latter are ovate or ovate-oblong, rounded or slightly cordulate at the base, 10–15 cm long and 4–7 cm wide; the leaves of the new species differ significantly from those in the group. The new species is quite similar to *D. hexamera* C. Y. Wu and *D. fleuryana* A. Chev. ex Lecomte group in its bark, leaf shape and venation. However, this species is significantly different from the others due to inconspicuous leaf veins, 5(-6) calyx lobes, the fruiting calyx crown-like and woolly. Detailed morphological comparisons among other taxa are summarized in Table 1.

Phylogenetic data and morphological studies confirmed that *D. daweishanensis* is a distinct species. It has lanceolate leathery leaf and unobtrusive venation, 5(-6) calyx lobes, and reflexed fruiting calyx. In the present phylogenetic analyses (Fig. 1), *D. daweishanensis*

included in the clade VII (Fig. 1, see Figure A with color coding and Figure B) which consists mainly of species from South-East Asia. Nevertheless, further molecular work on the South and Southeast Asian species will be needed to give a deeper understanding of evolution in pantropical *Diospyros*. The shapes and quilts of leaves in *Diospyros* are highly variable and reproductive biology of these species is also poorly studied, due to the fact that they are dioecious, long-lived trees often bearing their flowers at great height. The south-eastern China limestone areas, where four species of *Diospyros* have been recorded, are hotspots of species richness and endemism and have been an important source of vascular plant novelties in the past 20 years (Du et al., 2020; Qian et al., 2020). The remote border regions should be further explored to fully unravel the rich biodiversity there.





Fig. 2. *Diospyros daweshanensis* Z. X. Zhang, Gui L. Zhang & C. Y. Zou. A. Habit. B. Female flowering twigs. C. Male flowering twigs. D. Adaxial view of female flower. E. Immature fruit. F. Ripened fruit. G. Male flowers. H. Longitudinal section of male flower. I. Seeds. J. Longitudinal section of female corolla. K. Transverse sections of the ovary. L. Stamens. Photos by Chun-Yu Zou & Gui-Liang Zhang.



## TAXONOMIC TREATMENT

*Diospyros daweishanensis* Z. X. Zhang, Gui L. Zhang & C. Y. Zou, *sp. nov.* 大围山柿 Figs. 2&3

**Type:** CHINA. Yunnan: Hekou County, Nanxi town, Longbao Village, elevation 650 m, in the tropical rainy forest along a limestone valley, 10 April 2023, female flowers, C. Y. Zou et al. ZCY4317-A (holotype: IBK!, FigS1; isotype: IBK!).

**Diagnosis:** Distinguished from other *Diospyros* species by its lanceolate leathery leaf and unobtrusive leaf veins, with 5(-6) calyx lobes (vs. 3-, 4- and 5-lobed) and crown-like and woolly fruiting calyx (vs. usually spreading or cup-shaped).

**Description:** Dioecious plants. Tree up to 30 m tall; bark black, smooth, densely lenticellate. Young branchlets green. Twigs pubescent, becoming glabrous with age. Leaves buds covered by bifarious scales, scales ovate to lanceolate, 5–13 × 5–9 mm. Leaves leathery, lanceolate, or elliptic-lanceolate, 5–35 × 3–5.5 cm, apex acute or acuminate, base cuneate or rounded, midrib adaxially impressed, abaxially prominent, glabrous. Petiole 0.5–1.0 cm long, glabrous. Flower buds covered with bifarious scales at base, scales broadly ovate, 5–9 × 4–7 mm, covered by densely black hairs, deciduous after flowering. Male inflorescences in cymes, usually on basal part of current year's branchlets, 0.1–0.5 cm long, each bearing 1–3 flowers. Male flowers 5(-6)-merous; Calyx covered with dense appressed brown hairs outside, with 5 or 6 short triangular lobes, 2–3 × 1.5–2 mm; Corolla white, tubular, 9–13 mm long, pubescent outside, glabrous inside, lobes ovate, ca. 4.0 × 3.0 mm, reflexed; Stamens 16, ca. 5 mm long, often forming 8 pairs, attached at base of corolla tube; anthers oblong, apex apiculate. Female inflorescences consisting of a solitary flower on a ca. 0.2 cm stalk. Female flowers 5(-6)-merous, densely gray-brown hairs; Calyx lobes triangular, 1–1.5 cm long; Corolla white, urceolate, 11–13 mm long, lobes oblong, ca. 5.0 × 4.0 mm, reflexed; Stamens 5(-6), attached at the base of corolla tube, filaments flattened, anthers oblong; Ovary globose, densely gray-brown pubescent; Stigmas 5. Fruits globose, smooth, dia. 4–6 cm, covered with brown hairs, light green when young, turning yellow and orange when ripe, persistent calyx leathery, crown-like, dia. 1.5–2.0 cm. Seeds 2–7, brick red, oblong-elliptic, 1–1.5 × 0.5–0.8 cm.

**Phenology:** Flowering April, fruiting July to August.

**Distribution and habitat:** This new species occurs in Jingping, Hekou, Maguan, and Malipo County, Yunnan Province, expanding to the North of Vietnam (Fig. 4). It is scattered in the tropical rain forests along limestone areas at elevations of 500–800 m in association with *Caryodaphnopsis tonkinensis* (Lecomte) Airy Shaw, *Pometia pinnata* J. R. Forst. & G. Forst., *Brachytome hirtellata* Hu, *Champereia manillana* var. *longistaminea* (W. Z. Li) H. S. Kiu and *Phrynium rheedei* Suresh & Nicolson.

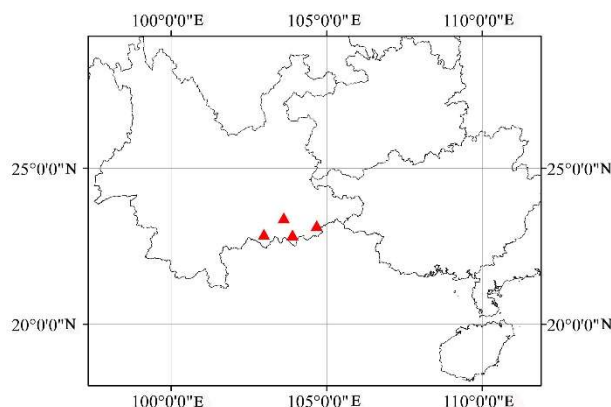


Fig. 4 Distribution of *Diospyros daweishanensis* (triangle).

**Etymology:** The specific epithet refers to the name of the mountain where the type specimens were collected.

**Conservation status:** In our recent surveys, we encountered numerous seedlings, and the habitats are reported to be in good condition. The species is currently distributed within Jingping, Hekou, Maguan and Malipo County in Yunnan Province, as well as in a single location in northern Vietnam, immediately adjacent to Hekou County. Following the Guidelines for Using the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Subcommittee, 2022), this species is considered to be of 'Least Concern' (LC) in China.

**Additional specimens examined (paratypes):** CHINA. Yunnan: Hekou County, Nanxi town, Longbao Village, elevation 650 m, in the tropical rainy forest along a limestone valley, 10 April 2023, male flowers, C. Y. Zou et al. ZCY4317-B (IBK!, BJFC!); Malipo County, Tianbao town, elevation 750 m, 13 April 2023, female flowers, C. Y. Zou, J. Q. Huang & Z. C. Fan ZCY4367 (IBK!, BJFC!); Pingbian County, Wantang town, elevation 887 m, 8 April 2020, male flowers, Y. S. Cheng Pingbian-PB0677 (IBSC!); Maguan County, Gulinjing town, elevation 600 m, 18 May 2022, C. Y. Zou et al. ZCY3518 (IBK!).

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Supplementary materials are available from Journal Website