CLEYERA LIPINGENSIS VAR. *XIUSHANENSIS* (PENTAPHYLACACEAE): A NEW VARIETY FROM CHONGQING, CHINA

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Abstract

Cleyera lipingensis var. *xiushanensis* Zhe Zhang, Xin-Yun Lv & H.P. Deng var. nov. from Chongqing, China, is described and illustrated. This new variety is similar to *C. lipingensis* var. *lipingensis* and *C. lipingensis* var. *taipinensis* in leaves and fruits shape, as well as in the presence of serrate leaf margins. However, it can be distinguished by several key morphological characteristics: larger leaves (ca. 7 – 12 × 3 – 4.5 cm), more than 13 (–17) secondary veins on each side of the midrib, a usually 3 (–4) lobed style with pubescence, and a 3 (–4) loculed ovary with 8 – 10 ovules per locule. Phylogenetic analyses based on the nuclear ribosomal internal transcribed spacer (nrITS) region confirm that *C. lipingensis* var. *xiushanensis* is closely related to *C. lipingensis* var. *lipingensis*.

Key words: Morphology; Phylogeny; New variety; Taxonomy.

Introduction

Pentaphylacaceae, traditionally recognized as a monogeneric family within Theales (Cronquist, 1981). It is now placed in Ericales, comprising 12 genera according to Weitzman *et al*., (2004), APG III (2009), and APG IV (2016), or 14 genera according to Schönenberger *et al*., (2005). The genus *Cleyera* Thunb. (Pentaphylacaceae), consisting of 24 species, are distributed in East, South, and Southeast Asia, as well as tropical America (Newman *et al*., 2007; Ming & Bartholomew 2007; POWO, 2023). In China, nine species (7 endemic) have been recorded (Nakai *et al*., 2007). *Cleyera* is sister to *Adinandra* Jack, but is distinguished by the lower number of ovules per locule and the seeds are 1-2 mm long and situated on a central axis (Keng, 1972; Ming & Bartholomew, 2007; Tsou *et al*., 2016; Nguyen, 2017).

In 2023, during a biodiversity survey in Taiyangshan Municipal Nature Reserve, Xiushan, Chongqing, we encountered a distinctive *Cleyera* species at an altitude of 630 m. This species exhibited unique secondary vein counts, ovary chambers, and styles that distinguished it from all known *Cleyera* species. After an extensive literature review and examination of specimens in the Chinese Virtual Herbarium (CVH), we concluded that the *Cleyera* population in Taiyangshan Municipal Nature Reserve represents an undescribed variety of *C. lipingensis* var. *lipingensis* (Hand.-Mazz.) T.L. Ming (2007:444) [synonym: *C. japonica* var. *lipingensis* (Hand.-Mazz.) Kobuski (1937:127)], which is generally similar to *C. lipingensis* var. *taipinensis* (H. Keng) T. L. Ming (2007:444) (Nakai *et al*., 2007). Consequently, we compared the new variety with these two varieties based on treatments in the literature (Keng, 1972; Nagamasu, 2006; Nguyen *et al*., 2017; Ming & Bartholomew, 2007). In this study, we describe this new variety based on morphological characteristics and investigate its phylogenetic position using DNA sequences.

Materials and Methods of Phylogenetic Study

To clarify the phylogenetic position of the new variety, we included 15 representative species of *Adinandra* and *Cleyera*, following Tsou *et al*., (2016). *Norantea guianensis* Aubl. (1969:563) was selected as the outgroup. One nuclear region (nrITS) was used in the phylogenetic analyses. We generated sequences for 20 accessions representing 14 species of *Adinandra* and *Cleyera*. Table 1 provides voucher information and GenBank accession numbers for each taxon.

Table 1. GenBank accession numbers of the sequence data generated in this study.

Species	GenBank accession no.
Adinandra hainanensis	AF456255
A. latifolia	AY096023
A. elegans	AY626845
A. formosana	AY626846
A. lasiostyla	AY626847
A. millettii	AY626848
A. glischroloma	HM061547
A. bockiana	HM061548
A. lasiostyla	HM061550
A. millettii	KP092617
A. millettii	KP092618
A. nitida	KP092619
A. nitida	KP092620
A. bockiana	MN579515
A. glischroloma	OQ832760
Cleyera lipingensis* var. *xiushanensis	**PV132331**
C. lipingensis	AF456257
C. pachyphylla	AY096025
C. japonica var. *morii*	HM061545
C. japonica var. *japonica*	HM061546
Norantea guianensis	FJ037835

DNA extraction, PCR amplification, sequencing, sequence editing, and sequence assembly were conducted following the protocols of Zhang *et al*., (2013). The primers utilized for PCR amplification were as specified by Li *et al*., (2016) and Taberlet *et al*., (1991). The nrITS datasets were analyzed using Bayesian inference (BI) in PhyloSuite (Zhang *et al*., 2020). The optimal substitution model (GTR+G) was selected using the Model Finder option based on the Bayesian Information Criterion (BIC) (Kalyaanamoorthy *et al*., 2017). The Markov chain Monte Carlo (MCMC) algorithm was run for 2.0 × 107 generations with four incrementally heated chains, starting from random trees and sampling one out of every 1,000 generations.

Results

C. lipingensis var. *xiushanensis* was similar to *C. lipingensis* var. *lipingensis* and *C. lipingensis* var. *taipinensi*, but differs in its having more than13 (–17) secondary veins on each side of middle vein, usually 3 (–4)-lobed and pubescent style , and 3 (–4)-loculed ovary with 8–10 ovules per locule. Detailed comparison of morphological characters among this taxon and the morphologically close relatives was provided (Table 2).

The results indicated that the nrITS region of *C. lipingensis* var. *xiushanensis* is 704 bp in length. A Bayesian inference (BI) phylogeny, including BI posterior probability (BP) values, is presented in Fig. 1. As shown in Fig. 1, *C. lipingensis* var. *xiushanensis* was resolved as sister to *C. lipingensis* var. *lipingensis* (BP=0.954) and subsequently formed a sister relationship with *C. pachyphylla* (BP=1).

Thus, integrating both morphological and molecular evidence, we recognize *C. lipingensis* var. *xiushanensis* as a new variety of *C. lipingensis* var. *lipingensis*. The formal description is provided below:

Taxonomic Treatment

Cleyera lipingensis (Hand.-Mazz.) T.L. Ming **var. *xiushanensis*** Zhe Zhang, Xin-Yun Lv & H.P. Deng, *var. nov.* (秀山红淡比) (Figs. 2-4).

Table 2. Morphological comparisons of *Cleyera lipingensis* var. *xiushanensis*, *C. lipingensis* var. *lipingensis* and *C. lipingensis* var. *taipinensi*.

Character	*C. lipingensis* var. *xiushanensis*	*C. lipingensis* var. *lipingensis*	*C. lipingensis* var. *taipinensi*
Shape and size of leaf	oblong to oblong-lanceolate, ca. 7–12 × 3–4.5 cm	oblong, oblong-elliptic, obovate, or obovate-elliptic, ca. 6–9 × 2.5–3.5 cm	oblong-lanceolate, ovate, or obovate-spatulate, ca. 3–5.5 × 1–2 cm
Secondary veins	over 13 (–17) on each side of midvein and visible on adaxial surfaces	6–8 on each side of midvein and visible on both surfaces or abaxially obscure	6–8 on each side of midvein and visible on both surfaces
Inflorescence	Flowers axillary, (–2) 4–5 in a cluster	Flowers axillary, 3 (–5) in a cluster	Flowers axillary, 2–4 in a cluster
Pedicel	ca. 1–1.5 cm	ca. 1–2 cm	ca. 1-2 cm
Sepals	ovate to orbicular, 2–2.5 × ca. 3 mm, margin ciliolate, apex retuse	ovate to orbicular, 2.5 × ca. 2.5 mm, margin ciliolate, apex rounded	ovate to orbicular, 2–3 × ca. 2–2.5 mm, margin ciliolate, apex rounded, tip retuse
Petals	white, obovate to oblong, ca. 13 × 9 mm	white, obovate-oblong, ca. 8 mm	white, obovate-oblong, ca. 8 mm
Stamens	26–28, ca. 2–4 mm	25–30, ca. 4–6 mm	25–30, ca. 5 mm
anthers	fusiformis, ca. 1.5–2 mm, with filiform trichomes on the periphery.	ovate to long ovate, ca. 1.5 mm, with filiform trichomes, connective exserted	ovate, ca. 1.5 mm, with filiform trichomes, connective exserted
Style	ca. 5 mm, stigma usually 3 (–4) lobed with pubescent	ca. 6 mm, apically 2 lobed	ca. 5 mm, glabrous, apically 2-lobed
Ovary	ovoid, glabrous, 3 (–4)-loculed, 8–10 ovules per locule	globose, glabrous, 2-loculed, more than 10 ovules per locule	ovoid, glabrous, 2-loculed, more than 10 ovules per locule

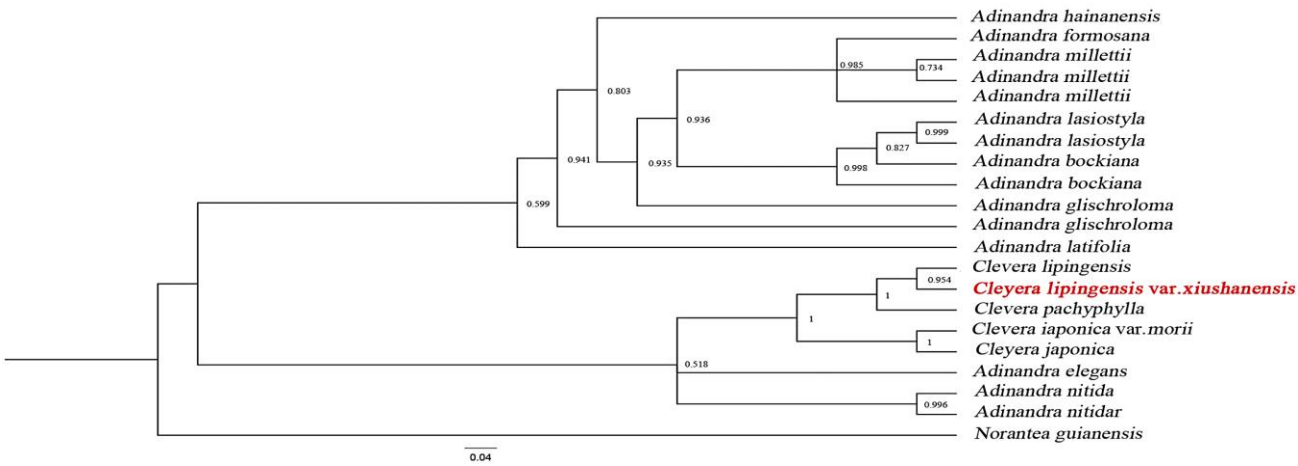

Fig. 1. Phylogenetic relationships between the genera of *Cleyera* and *Adinandra* inferred from ITS sequences. The species of *Norantea* was used as outgroup. Numbers above the branches represent Bayesian posterior probability(BP), respectively. *C. lipingensis* var. *xiushanensis* is noted in bold.

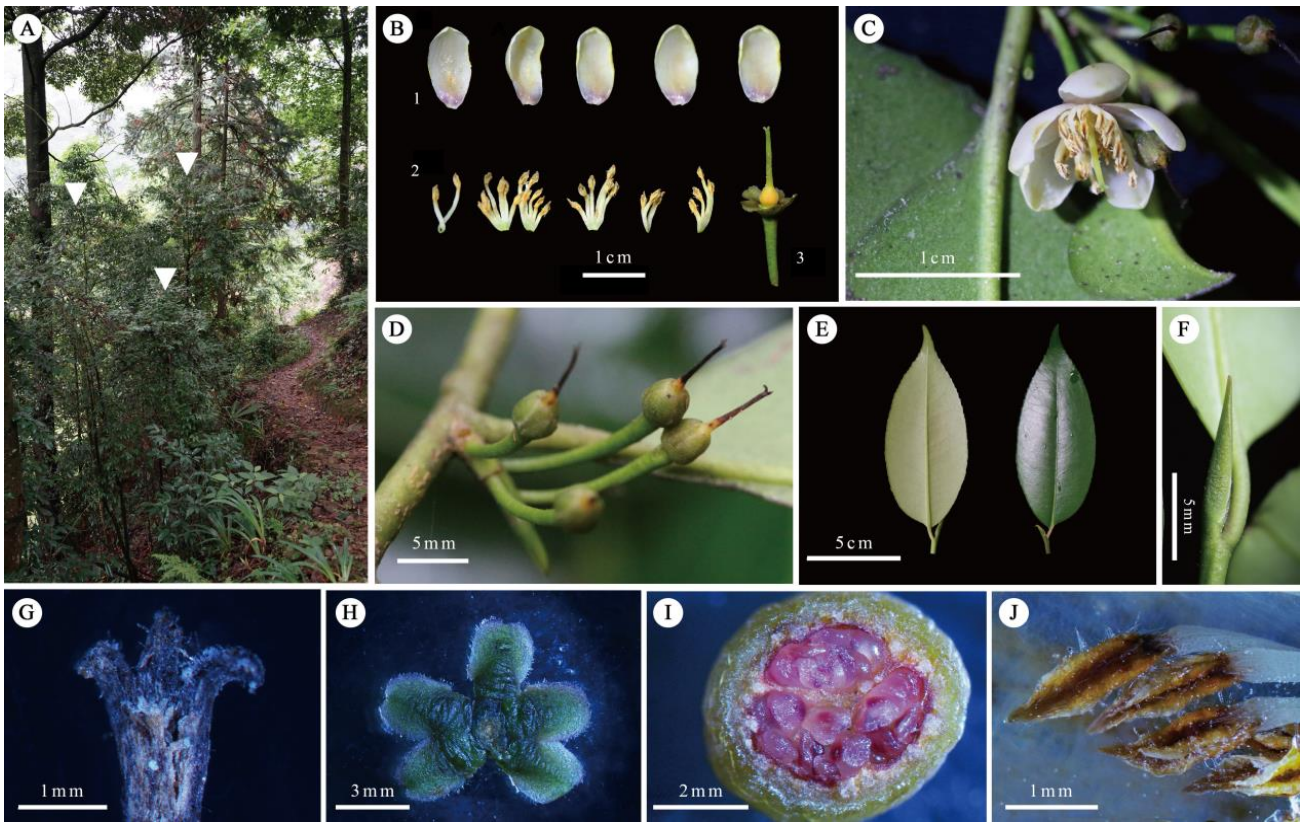

Fig. 2. *C. lipingensis* (Hand.-Mazz.) T. L. Ming var. *xiushanensis* Zhe Zhang, Xin-Yun Lv & H. P. Deng. A. habitat; B. one dissected flower, showing petals (1), stamens (2) and sepals with pistil (3); C. one opening flower; D. young fruits; E. leaves; F. bud; G. stigma; H. sepals; I. transverse section of ovary; J. anthers.

Key to the species in Chongqing

1a. Young branches and terminal buds glabrous, blades margin entire, midrib slightly impressed adaxially ..... *C. japonica*
1b. Young branches and terminal buds pubescent, blades margin serrate, midvein adaxially raised 2
2a. Secondary veins 6-8 on each side of middle vein and visible on both surfaces or abaxially obscure; 2 loculed ..... *C. lipingensis* var. *lipingensis*
2b. Secondary veins over 13 (–17) on each side of middle vein and visible on adaxial surfaces; 3 (–4) loculed ..... ***C. lipingensis* var. *xiushanensis***

Diagnosis: The new variety resembles *C. lipingensis* var. *lipingensis* and *C. lipingensis* var. *taipinensi* but differs in its having bigger leaves ca. 7–12 × 3–4.5 cm (vs. ca. 6–9 × 2.5–3.5 cm in *C. lipingensis* var. *lipingensis* and ca. 3–5.5 × 1–2 cm in *C. lipingensis* var. *taipinensis*), young branchlets, buds, petioles and pedicels pubescent (vs. glabrous in *C. lipingensis* var. *taipinensi*), secondary veins over 13 (–17) on each side of midvein (vs. 6–8 on each side of midvein in *C. lipingensis* var. *lipingensis* and *C. lipingensis* var. *taipinensi*), usually 3 (–4) locule in ovary (vs. 2 locule in ovary), and stigma 3 (–4) (vs. 2 stigma in ovary).

Description: Shrubs or trees, 4–9 m tall. Young branches terete, green, sparsely pubescent, old twigs gray, glabrous; Terminal buds long-conic, 0.8–2 cm long, pubescent. Leaves alternate; blades leathery, oblong, elliptic or oblong-lanceolate, 7–12 × 3–4.5 cm, margin serrate, base cuneate, acute or acuminate at apex, abaxially pale green, both surfaces glabrous, adaxially dark green, glossy, abaxially midvein raised, over 13 secondary veins on either side of the middle vein and visible on adaxial surfaces; petioles 0.5–1 cm long, sparsely pubescent when young, glabrescent. Flowers axillary, (–2) 4–5 in a cluster; pedicels 1–1.5 cm long, glabrous. Sepals ovate to orbicular, 2–2.5 × c. 3 mm, margin ciliolate, apex retuse. Petals white, obovate to oblong, c. 13 × 9 mm, apex rounded. Stamens 26–28, 2–4 mm long; anthers fusiformis, 1.5–2 mm long, with filiform trichomes on the periphery; filament 2–7 mm long, glabrous. Style c. 5 mm long, 3 (–4) -lobed at apex, pubescent. Ovary ovoid, 3–4 mm long, glabrous, 3 (–4)- loculed. Fruits globose, 8–10 mm in diam, 8–10 seeds per locule.

Etymology: The name refers to the locality where this new variety was first found.

Geographical distribution: *C. lipingensis* var. *xiushanensis* is only found in Xiushan county, Chongqing, China.

Habitat and ecology: The new variety *C. lipingensis* var. *xiushanensis* grows in secondary broad-leaved deciduous sparse forests at an elevation of 630 m. Flowering in May-June, and fruiting in October-November.

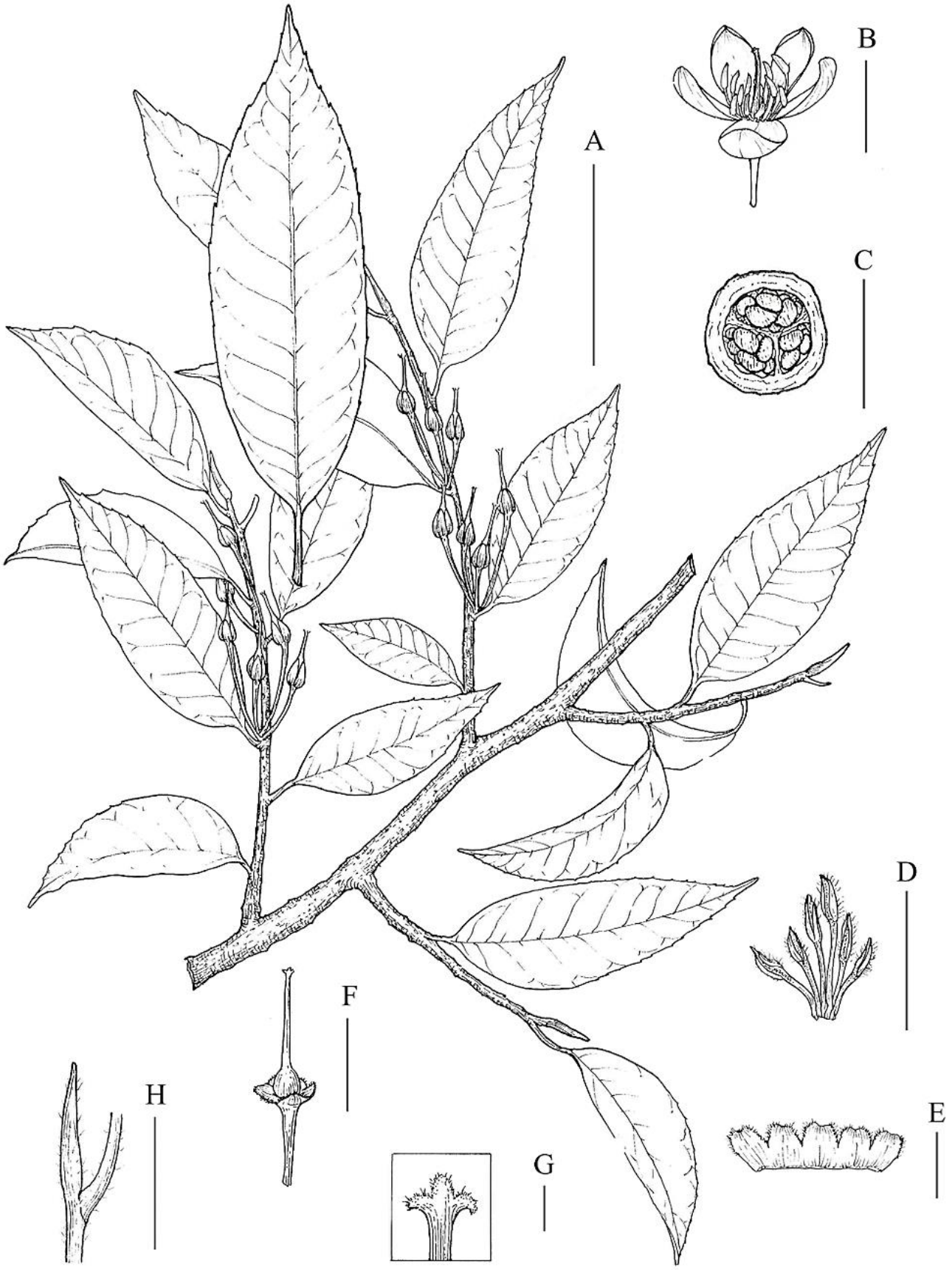

Fig. 3. *C. lipingensis* (Hand.-Mazz.) T. L. Ming var. *xiushanensis* Zhe Zhang, Xin-Yun Lv & H. P. Deng. A. Fruiting branch with young fruits (scale: 5cm); B. Flower (scale: 1cm); C. Transverse section of a young fruit with 3 loculi (scale: 5mm); D. Stamens (scale: 5mm); E. Sepals (scale: 5mm); F. Young fruit (scale: 5mm); G. Stigma (scale: 1mm); H. Bud (scale: 1cm). Drawn by Yi-fan Li.

Type:—CHINA. Chongqing(重庆市): Xiushan County (秀山县), Taiyang Village (太阳村), in secondary broad-leaved evergreen forest, 630 m, 4 June 2023, *Zhe Zhang, Xin-Yun Lv ZZ0421* (holotype: HWA!; isotypes: HWA!, IBSC!, FJFC!).

Fig. 4. Holotype specimen of *C. lipingensis* (Hand.-Mazz.) T. L. Ming var. *xiushanensis* Zhe Zhang, Xin-Yun Lv & H. P. Deng.

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