

CAMELLIA HAINANICA (THEACEAE) A NEW SPECIES FROM HAINAN, SUPPORTED FROM MORPHOLOGICAL CHARACTERS AND PHYLOGENETIC ANALYSIS

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Abstract

An unusual *Camellia* specimen was noticed during field investigations in Hainan Island. In order to identify the phylogenetic position, the complete chloroplast genome sequences and the DNA barcode gene, *matk*, of the new species and closely related species were employed. The results confirmed that the new species belonged to *Camellia* section and had close genetic relationship with *C. gauchowensis*, *C. crapnelliana*, *C. vietnamensis*. The general morphology characters of the new plant species, *Camellia hainanica* YL Zhao et ZG Xu, sp. nov. were described. The pollen microstructure was studied by scanning electron microscope, then above features were compared with closely phylogenetic related *Camellia* species. Although morphological details were most similar to *C. oleifera*, the new species have larger fruit and brown rough pericarp. At the same time, the new species can be differentiated by its morphological characters, such as branchlets, bracteoles, stamens and fruit from other related species. All evidences from the characters of morphology, pollen and molecular phylogeny indicated that the plant was a new species and belonged to *Camellia* sect. *Oleifera*.

Key words: *Camellia hainanica*; Morphology; Pollen micromorphology; Phylogenetic analysis.

Introduction

Camellia L. is the largest genus in the family Theaceae. It is predominantly distributed in eastern and southern Asia, from the Himalayas east to Japan and Indonesia (Chang & Ren, 1998, Ming & Bartholomew, 2007, Van The *et al.*, 2019). China is the center of diversity of *Camellia* with more than 80% of the known species occurring there (Chang 1961, Chang & Ren, 1998). The plants of *Camellia* are shrubs or small trees and generally occur in subtropical and tropical mountain evergreen broad-leaved forests. Furthermore, many species of *Camellia* are economically important for supplying tea, oil and ornamental camellia (Gao *et al.*, 2005, Vijayan *et al.*, 2009).

Hainan Island, as the region forms part of the Indo-Burma biodiversity hot-spot, is one of the largest Island in China. (Myers *et al.*, 2000). Biodiversity of the suite is ecologically significant and a lot of new species were found in past years (Guo *et al.*, 2018). At the same time, due to an invasion of alien species, urbanization, tourism, deforestation, and the pollution, many plant species are under threat. It's very important to survey biological resources and evaluate survival state, especially for the new species. It is the high time for new species finding in Hainan Island.

In 2016, during field investigations in the Chengmai County of the Hainan Province, we noticed an unusual *Camellia* specimen. The specimen was similar to *Camellia oleifera* Abel., however, it had larger and brown fruits, so they could be distinguished from each other immediately in the wild. Later, more materials including a range of specimens with flowers and fruits were collected when we revisited the locality in 2017. After a critical morphological comparison, we confirmed that the plant represents an undescribed new species belonging to *Camellia* sect. *Oleifera*. This study aimed to confirm the

new taxon based on evidences from morphological characters and phylogenetic analysis.

Material and Methods

Complete chloroplast genome sequencing and phylogenetic analysis: Chloroplast genome can supply important information during the plant classification process (Dogan *et al.*, 2017). The seeds of *C. hainanica* YL Zhao et ZG Xu, sp. nov. were collected from object species in Chengmai, Hainan Province, and fresh leaves were obtained from the plants grown through seed germination. Methods for extraction of whole genomic DNA, sequencing, filtration and annotation followed Zhang *et al.*, (2017). Then the complete chloroplast sequence of *C. hainanica* with annotation has been submitted to NCBI (<https://www.ncbi.nlm.nih.gov/>) and was deposited with the accession number of MF541730.

Complete chloroplast genome sequence of *C. hainanica* was uploaded onto the NCBI website and the similar sequences species were identified (Zhang *et al.*, 2000). Complete sequences of 42 species, which all belonged to Theaceae with 24 *Camellia* species, were employed during the process of phylogenetic analysis with maximum likelihood method in MEGA7 to identify phylogenetic position of the new species (Kumar *et al.*, 2016). At the same time, the recommend plant DNA barcode gene, *matK*, of 30 *Camellia* species were collected and phylogenetic analysis were done to further confirm whether the species was different from others.

Taxon sampling and morphological studies: The new taxon, *C. hainanica* YL Zhao et ZG Xu, sp. nov., was found during the *Camellia* species survey process in Chengmai County. Several populations of this species were found near the finding sites in Chengmai. Specimens have been deposited at SYS and IBSC.

The related species were identified based on the above phylogenetic analysis and ten morphological characters, branchlets, leaf shape, leaf size, flower size, bracteoles and sepals, petals, stamens, ovary, styles and fruit were obtained and compared with each other. Pollen were collected from the flowers of above specimen and were studied by scanning electron microscope. The pretreatment and device parameters referenced Xie et al's method at 25 kV (Xie *et al.*, 2016). The morphological details were compared with the related species.

Results and Discussion

Relationships between *C. hainanica* and closely related taxa: Phylogenetic analysis is a useful tool to confirm the new species and evolutionary position (Pedrazapeñalosa *et al.*, 2015). The phylogenetic result

based on complete chloroplast genome sequence of 42 species also proves the credibility of the analysis and the near source species are almost clustered in same clade (Fig. 1). *C. hainanica* was grouped *Camellia* and located in an ancient evolutionary position according with Hainan Island's separation geographical location. Genetic relationship between *C. hainanica* and *C. crapnelliana* is close, however *C. hainanica* is a different species. *matK* gene is always recommended as the reference DNA barcoding for distinct genetic loci (Jiang *et al.*, 2018). Based on the *matK* with more rigorous phylogenetic analysis, the plant was further confirmed as a new species and could not be clustered into one group with other species (Fig. 2). The result also indicated that the new species had close relative relationship with *C. oleifera*, *C. gauchowensis*, *C. vietnamensis*.

Tree scale: 0.0001

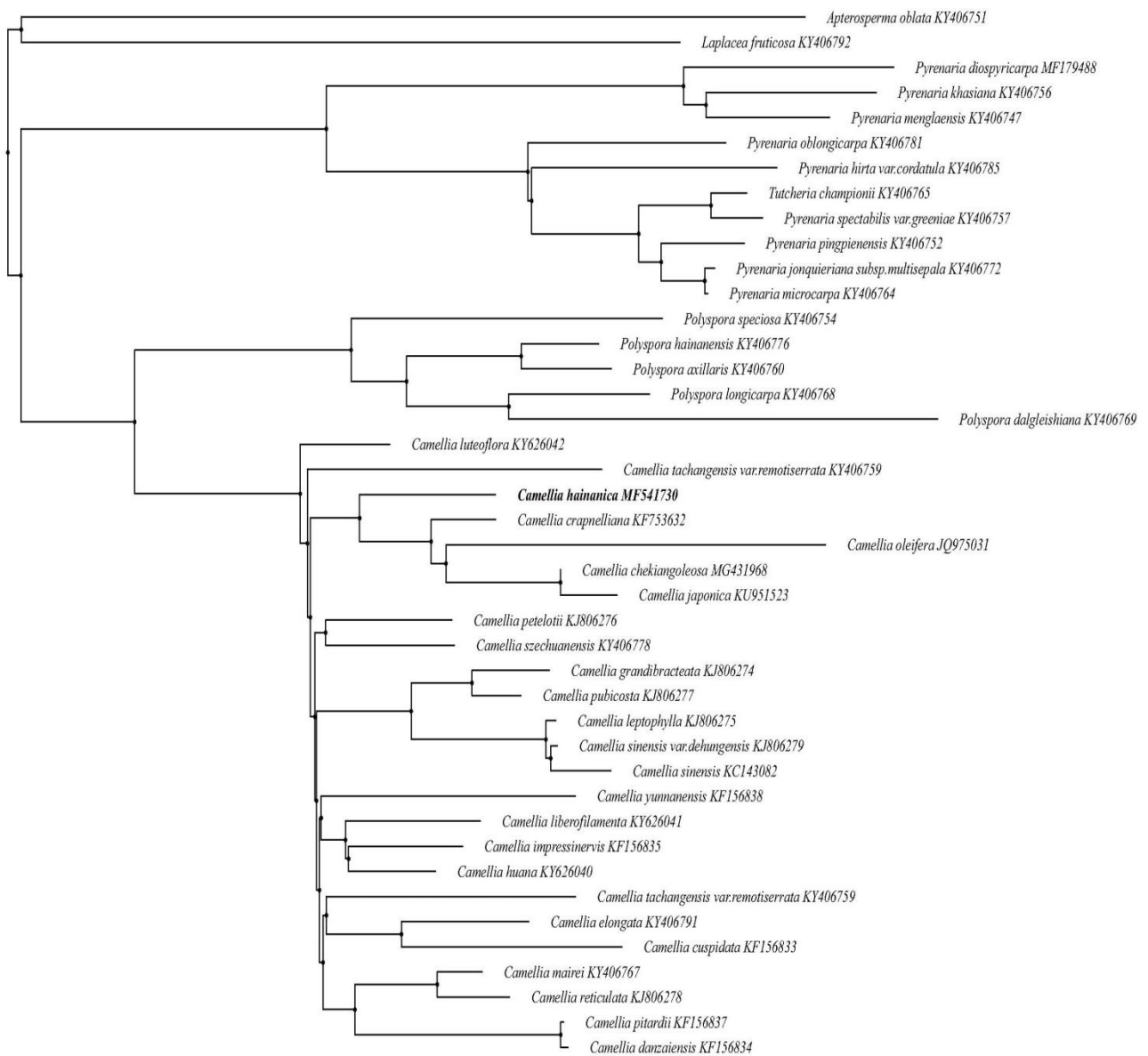


Fig. 1. Phylogenetic relationships between *Camellia hainanica* within other 42 related species resulting from maximum likelihood analysis basing on complete chloroplast genome sequence.

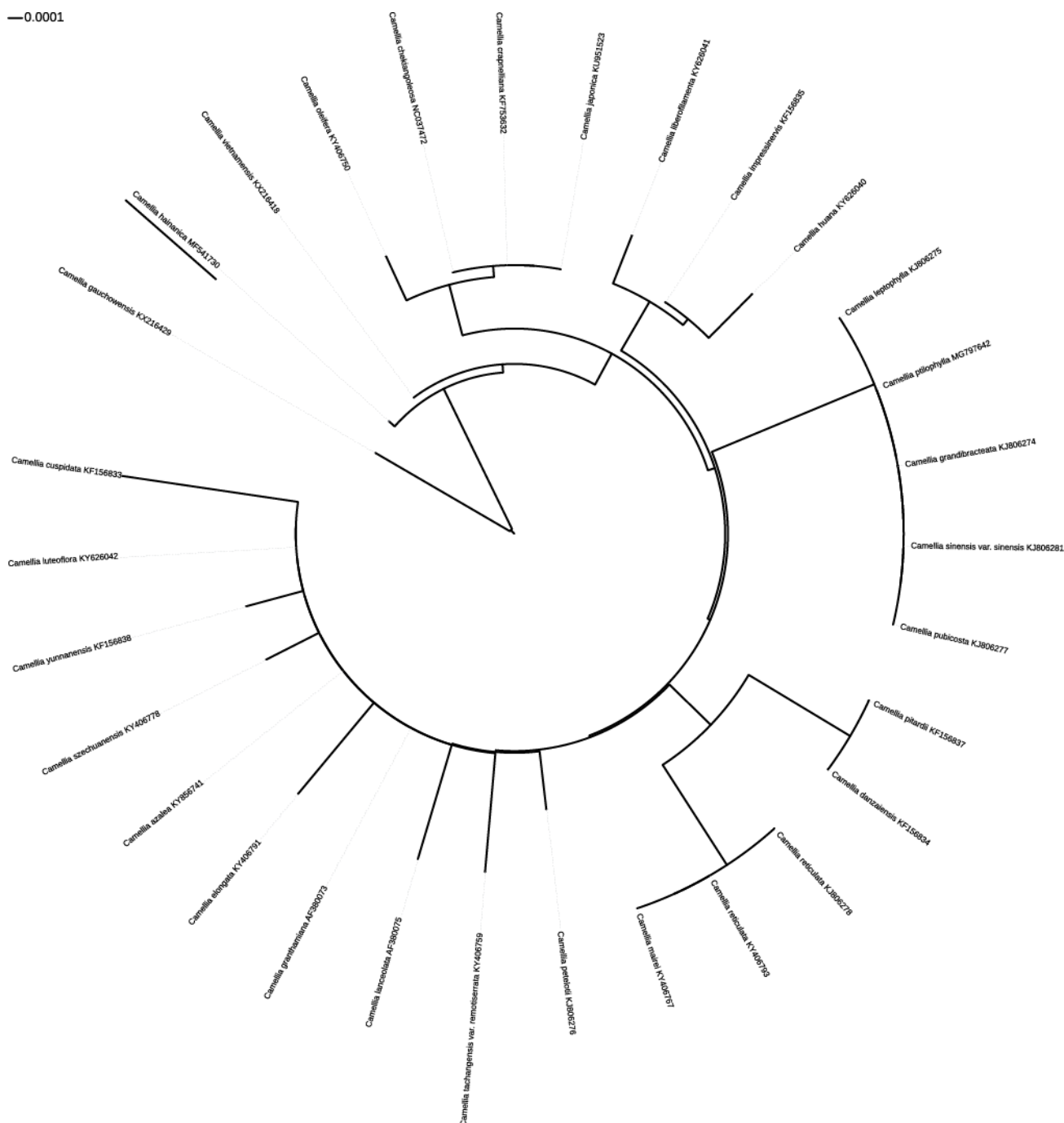


Fig. 2. Phylogenetic relationships between *Camellia hainanica* within 29 *Camellia* species based on *matK* gene using maximum likelihood method.

Based on above phylogenetic analysis and morphology survey, *C. oleifera*, *C. gauchowensis*, *C. crupnelliana*, *C. vietnamensis* were selected to compare with the new species, *C. hainanica*. *Camellia hainanica* is quite similar to *C. oleifera* in gross morphology. However, *C. hainanica* differs from the latter by the following combination of characters: fruit larger ca. 3–6 cm in diameter and pericarp brown and rough. In addition, the new species also resembles *C. gauchowensis* Chang morphologically, but the latter can be distinguished from the new species by the following characters: current year branchlets glabrous, styles 5 distinct, fruit pericarp green and glossy (Chang 1961). A detailed comparison of all the species is summarized in Table 1.

Pollen morphology character is an important feature for plant classification with considerable systematic significance. The difference of pollen between *C. hainanica* and some most closely related species were significant. *Camellia* pollen grains are small, prolate, long-elliptic, trilete rounded three-colpate (Fig. 3). Although the trend of contour is the same, pollen of *C. hainanica*, *C. gauchowensis*, *C. vietnamensis* are bigger than *C. oleifera*. The significantly different irregularly striated exine also showed that *C. hainanica* was a new species.

Thus, the critical morphological comparison suggests that the plant represents an undescribed new species belonging to *C. sect. Oleifera*.

Table 1. Morphological comparison among *Camellia hainanica* and similar species.

Characters	<i>Camellia hainanica</i>	<i>Camellia oleifera</i>	<i>Camellia gauchowensis</i>	<i>Camellia crapnelliana</i>	<i>Camellia vietnamensis</i>
Branchlets	reddish brown, pubescent	reddish brown, pubescent	grayish brown, glabrous	reddish brown, young glabrous, old pubescent	grayish brown, young pubescent, old glabrous
Leaf shape	elliptic or oblong-elliptic	elliptic, oblong-elliptic, or obovate	elliptic	elliptic or oblong-elliptic	elliptic or oblong-elliptic
Leaf size	5–8×2–3 cm	3–10×2–4 cm	5–8×3–4.5 cm	7–13×3–5 cm	5–10×2–5 cm
Flower size	6–9.3 cm in diam.	4–6 cm in diam.	6–7.5 cm in diam.	7–10 cm in diam	6–10 cm in diam
Bracteoles and sepals	10–12 or more	8–11	10–20	3	9
Petals	5–7, 3–4.5×1.5–3 cm	5–7, 2.5–3.5×1.5–2.5 cm	7–8, 3–3.8×1.5–2.6 cm	6–8, 3–5.5–1.5×3.5 cm	9, 4.5–6×3–4.5 cm
Stamens	1.2–1.5 cm, glabrous; outer filament whorl basally connate	1.5 cm, glabrous; outer filament whorl basally connate	1–1.2 cm, glabrous; distinct	1–1.8 cm, glabrous, outer filaments	1.2–1.7 cm, glabrous, outer filament whorl basally connate
Ovary	tomentose, 3–4-loculed	tomentose, 3-loculed	tomentose, 3–5-loculed	tomentose, 3–5-locular	tomentose, 3–5-locular
Styles	3–4, apically 3–4-parted or distinct, nearly to base glabrous or base tomentose	apically 3-lobed to 3-parted, glabrous or base tomentose	5, distinct nearly to base, glabrous	3–4, few 5, free to the base or slight pilose at the base	apically 3-lobed to 3-parted, glabrous or base tomentose
Fruit	3–6 cm in diam., pericarp brown, rough	2–4 cm in diam., pericarp green, glossy	4–7 cm in diam., pericarp green, glossy	8–12 cm in diam, surface rough and brownish	4–6 cm in diam., pericarp green, surface rough and brownish

Camellia hainanica YL Zhao et ZG Xu, sp. nov. (Figs. 4 and 5).

Type: China. Hainan Province: Chengmai County, Changshu Village, elev. 80 m, 19°38' 25.56" N, 110°0' 56.02" E, 26 Aug 2017, YL Zhao & ZG Xu 170801(holotype: SYS! isotype: IBSC!).

Diagnosis: *Species affinis C. oleiferae* Abel., *sed capsulis majorius 3–6 cm in diametric, pericarpio brunneo aspero differt.*

Description: Small trees, 4–8 m tall. Young branches grayish brown; current year branchlets reddish brown, pubescent. Petiole 5–7 mm, pubescent; leaf blade elliptic or oblong-elliptic, 5–8× 2–3 cm, leathery, abaxially pale green and glabrous, adaxially dark green and hirtellous along midvein or glabrescent, midvein raised on both surfaces, secondary veins 6–8 on each side of midvein, abaxially slightly raised or invisible, and adaxially slightly impressed, base broadly cuneate or cuneate, margin serrulate, apex acute to shortly acuminate and with an obtuse tip. Flowers solitary or paired, axillary or subterminal, 6–9.3 cm in diam., sessile. Bracteoles and sepals 10–12 or more, caducous, margin ciliolate; outer bracteoles and sepals semiorbicular, 1–3 mm, glabrous; inner bracteoles and sepals sepeloid, ovate to suborbicular, 0.8 to 1 cm, outside tomentose, inside glabrous, margin ciliolate. Petals 5–7, white, distinct, obovate, 3–4.5× 1.5–3 cm, apically 2-parted or emarginate. Stamens 1.2–1.5 cm, glabrous; outer filament whorl basally connate for 2–3 mm. Ovary tomentose, 3–4-loculed; styles 3–4, 1–1.5 cm, glabrous or base tomentose, apically 3–4-parted or distinct nearly to base. Capsule globose or compressed globose, 3–6 cm in diam., 3–4-loculed with 1–3 seeds per locule; pericarp 3–5 mm thick when dry, woody, brown, rough, splitting into 3–4 valves, Seeds brown, semiglobose, 2–2.5 cm in diam.

Phenology: Flowering Nov–Dec; Fruiting Aug–Oct.

Etymology: The specific epithet “*hainanica*” means the species is collected from Hainan Island, China.

Distribution and habitat: So far, *Camellia hainanica* is known from its type locality, Chengmai County in Hainan, China. It occurs in evergreen broadleaved forests edges or along village. According to our field survey, three populations are found from two towns (Jinjiang Town and Honggang Town) of Chengmai County, respectively.

Paratypes: China. Hainan Province: Chengmai County, Changshu Village, elev. 80 m, 19° 38' 25.56" N, 110° 0' 56.02" E, 15 Nov. 2016, YL Zhao & XG Shi 161131 (SYS); Chengmai County, Beifang Village, elev. 83 m, 19°38' 11.20" N, 109°59' 20.40" E, 21 Oct. 2016, YC Tang & L Wu 161021 (ZNL); Chengmai County, Honggang Village, elev. 85 m, 19°34' 00" N, 110°03' 20" E, 30 Oct. 2016, L Wu & W Zhang 161030 (ZNL).

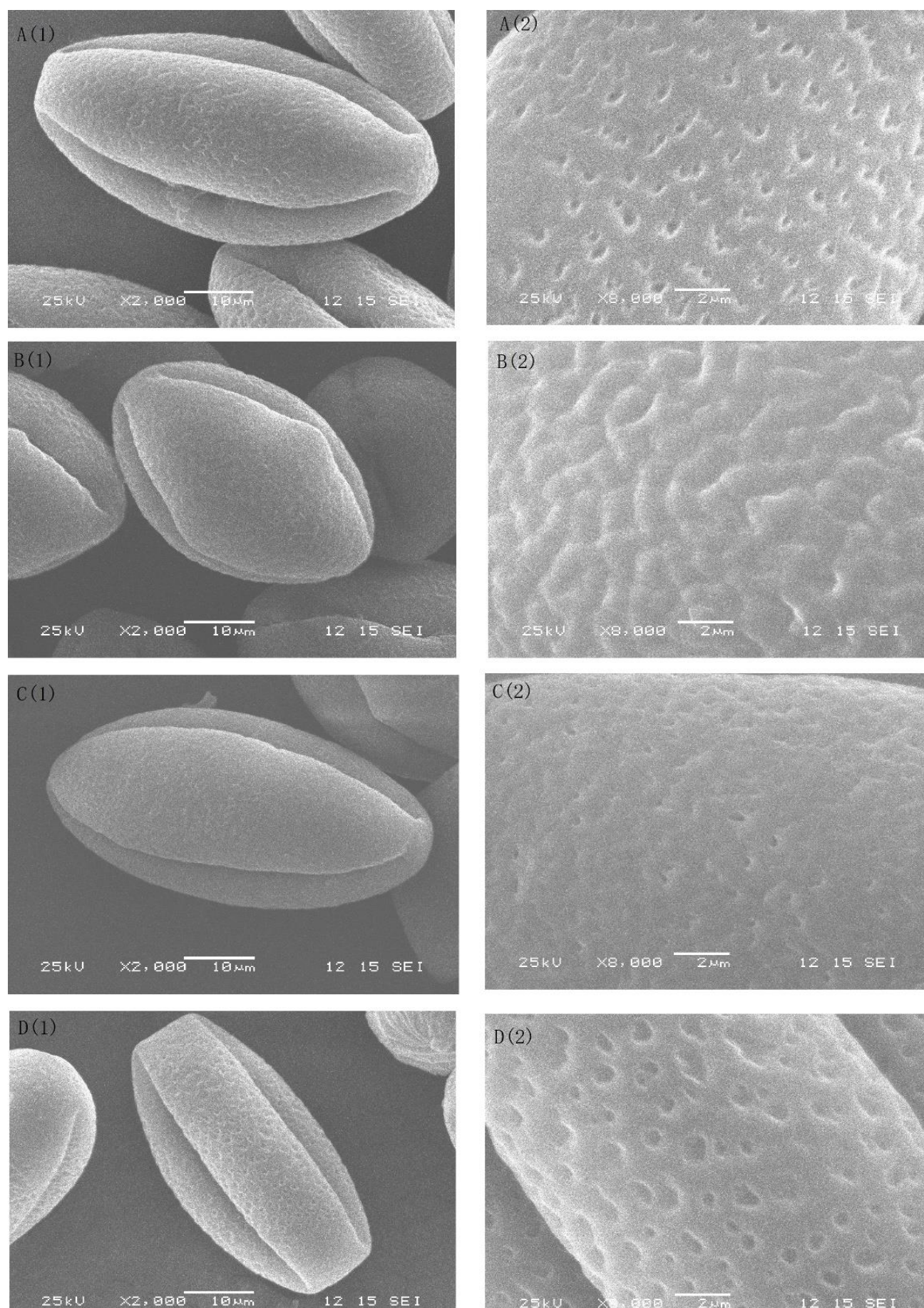


Fig. 3. SEM photographs of pollen grains showing the morphological differences between *Camellia hainanica* (A) and related *Camellia* spp, *Camellia oleifera* (B), *Camellia gauchowensis* (C), *Camellia vietnamensis* (D).

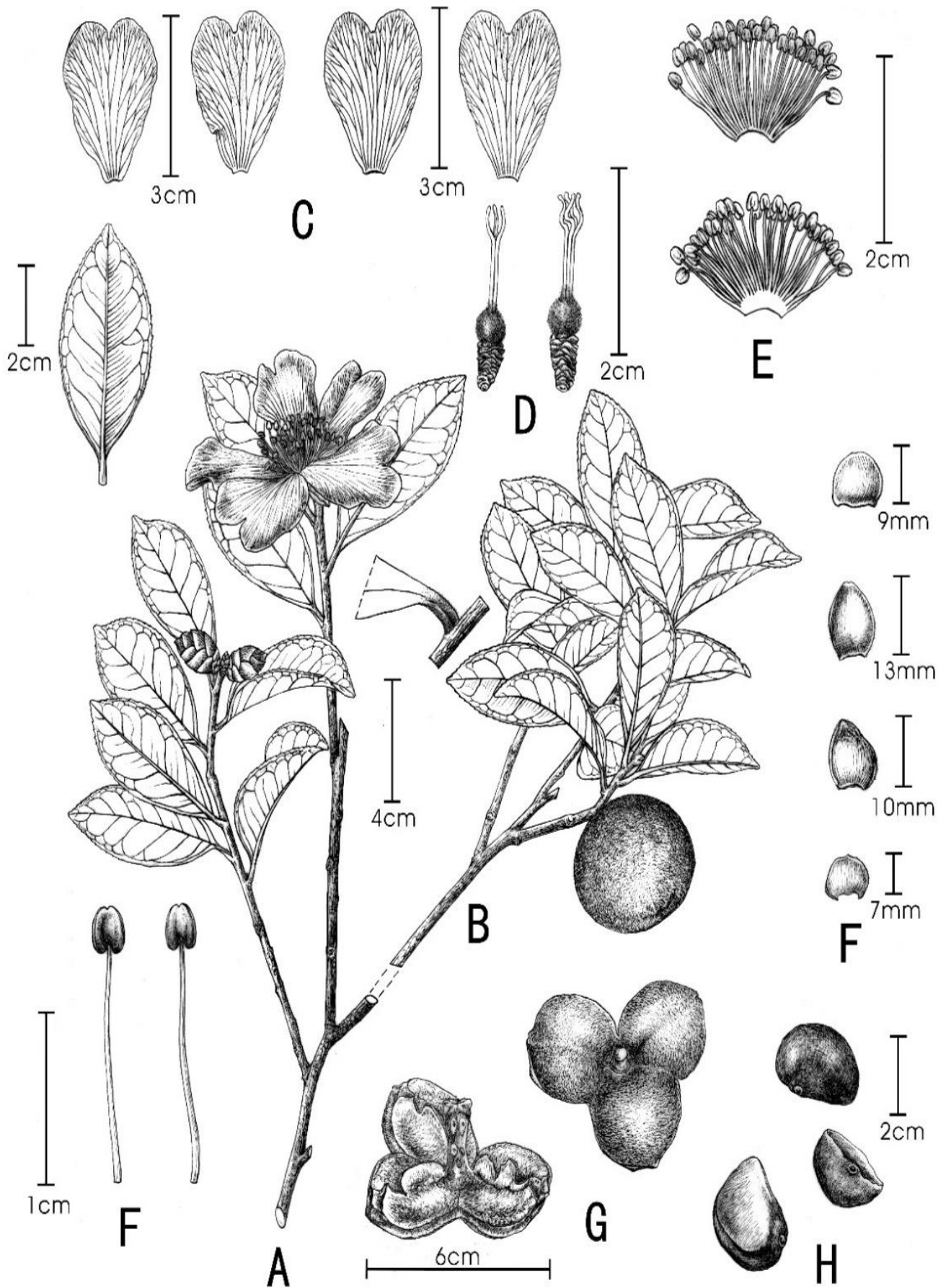


Fig. 4. *Camellia hainanica* Zhao et Shi *sp. nov.* A. Flowering branch; B. Fruiting branch; C. petals; D. pistil, showing tomentose ovary and 3-4 styles; E. Partial stamens, showing outer filament whorl basally connate; F. Partial bracteoles and sepals; G. Loculicidal capsule, apical view(left) and bottom view(right) ; H. Seed; I. Two distinct stamens; J. Leaf. (Drawn by MS Yun-Xiao Liu.).

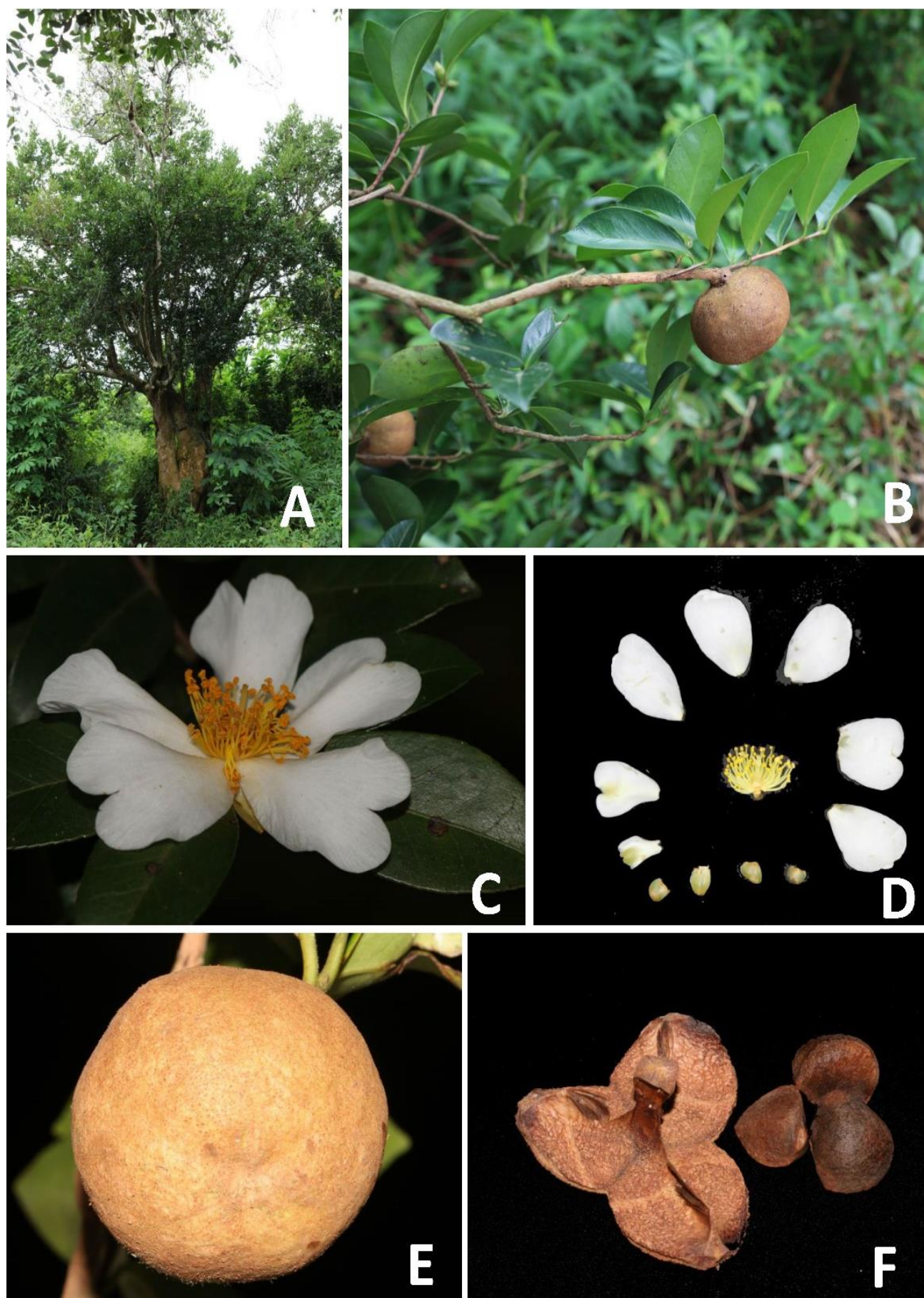


Fig. 5. *Camellia hainanica* Zhao et Shi. A. Habitat, in forest edges; B. Fruiting branch; C. Flowering branch; D. Anatomical structure of a flower, showing petals, stamens, pistil, and partial sepals; E. Capsule; F. Loculicidal capsule and three seeds.

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