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Rubus tianmuensis (Rosaceae), a new species from southeastern Anhui Province, China

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Abstract

Rubus tianmuensis, a newly discovered species belonging to the genus *Rubus* and Rosaceae family, is native to the Tianmu Mountains in southeastern Anhui Province. Genetically, it is related closely to *R. chingii*; however, several key morphological features position *R. tianmuensis* apart from *R. chingii*. Morphological analysis indicated that *R. tianmuensis* is characterized by persistently pedate leaves (vegetative and reproductive) with differentiated terminal leaflets on distinct petiolules, a trait consistently observed in both wild populations and cultivated specimens under under standard growth conditions. Additionally, the pedicels exhibit dense pubescence comprising short glandular trichomes and recurved prickles. Phylogenetic analysis based on ITS sequences confirmed the distinctiveness of this new species from *R. chingii*.

Key words: Rosales, systematics, taxonomy, *Rubus*

Introduction

The genus *Rubus* Linnaeus (1753:492) is one of the largest genera in the Rosaceae family, comprising approximately 750 species worldwide (Lu 1983; Thompson 1997). According to the *Flora of China*, 208 species of *Rubus* are distributed in China, and 139 of them are endemic (Lu & Boufford 2003). Although *Rubus* species are predominantly distributed across the temperate regions of the Northern Hemisphere, a few of them extend into tropical areas and the Southern Hemisphere. Most of these species are distributed in East Asia and North America, and China is among the regions with the greatest *Rubus* diversity (Focke 1910, 1911, 1914; Huang *et al.* 2015; Xu *et al.* 2024). The extensive distribution and species diversity within this genus have made its classification particularly challenging. Recently, several new *Rubus* species, such as *Rubus kaznowskii* Kosiński & Ziel. in Kosiński *et al.* (2021: 27), *Rubus longistipularis* D.A. Espinel-Ortiz & Romol. in Espinel-Ortiz *et al.* (2021: 141), *Rubus atroroseus* M. Lepší, P. Lepší & Velebil in Lepší *et al.* (2022: 213), and *Rubus puyumaensis* J.Y. Huang, P.H. Chen & A.C. Chung in Chen *et al.* (2024: e115431), have been discovered and named. Chinese scholars have made significant research endeavors aimed at species classification and its revisions (Gu *et al.* 2000; Li *et al.* 2001). In recent years, a series of new distribution records have been reported (Zhang *et al.* 2017, 2021; Mao *et al.* 2012).

During a survey of traditional Chinese medicinal resources conducted in Xuancheng City, Anhui Province, China, from 2014 to 2018, an unknown species of the genus *Rubus* was discovered. After transplantation and prolonged comparative cultivation under controlled conditions, the diagnostic characters of this taxon remained stable, with no significant variations observed between wild populations and cultivated specimens. Morphological observations, comparisons, and phylogenetic analysis confirmed that this unknown population represents a new species.

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Material and methods

Population sampling:—Voucher specimens and DNA materials of the new *Rubus* species were collected from the Qingliangfeng National Nature Reserve in Jixi County, Anhui Province, China. Fresh leaves were dried and preserved using silica gel immediately after collection. Sampling was conducted three times during key developmental stages in 2021: growth initiation (February to March), flowering (March to April), and fruiting (May to June). A total of 40 individuals representing 35 *Rubus* species were selected, including three individuals each from the new species and *Rubus chingii* Hu (1925: 141).

For phylogenetic analysis, nuclear ribosomal internal transcribed spacer (nrITS) markers were selected for each species. Additionally, GenBank sequences (MN577912) of *Malus doumeri* (Bois) A. Chev. in Camus (1920: 1129) and (FJ899098) of *Prunus domestica* L. (1753:475) were used as outgroup references. Accordingly, new sequences for *Rubus* were generated in this study (Table 1).

TABLE 1. Specimens and GenBank accession numbers.

Species name	Collection site	Voucher number	ITS GenBank no.
Rubus tianmuensis	Jixi County, Xuancheng City, Anhui Province, China	R2021037	PQ215005
R. tianmuensis	Jixi County, Xuancheng City, Anhui Province, China	R2021016	PQ215006
R. tianmuensis	Jixi County, Xuancheng City, Anhui Province, China	R2021036	PQ215004
R. chingii	Shangrao City, Jiangxi Province, China	R2022035	PQ214991
R. chingii	Panan County, Jinhua City, Zhejiang Province, China	R2021020	PQ214990
R. chingii	Jixi County, Xuancheng City, Anhui Province, China	R2021014	PQ214989
R. grayanus var. *trilobatus*	Jixi County, Xuancheng City, Anhui Province, China	R2021015	PQ214995
R. corchorifolius	-	-	LC624294
R. corchorifolius	Huangshan City, Anhui Province, China	R2021003	PQ214992
R. columellaris	-	-	KP093156
R. peltatus	Jixi County, Xuancheng City, Anhui Province, China	R2021019	PQ215001
R. longisepalus	-	-	MW474728
R. longisepalus var. *tozawae*	-	-	MWA74727
R. sumatranus	Jixi County, Xuancheng City, Anhui Province, China	R2015001	PQ215007
R. hirsutus	Huangshan City, Anhui Province, China	R2021002	PQ214996
R. crataegifolius	-	-	GU980786
R. trianthus	Jixi County, Xuancheng City, Anhui Province, China	R2021012	PQ215010
R. ellipticus	-	-	HG004841
R. ellipticus var. *obcordatus*	Liupanshui City, Guizhou Province, China	R2021029	PQ215010
R. transvaaliensis	-	-	OL899079
R. rigidus	-	-	OL899123
R. innominatus	Huangshan City, Anhui Province, China	R2021007	PQ214997
R. chiliadenus	Liupanshui City, Guizhou Province, China	R2021022	PQ214988
R. parvifolius	Hefei City, Anhui Province, China	R2021017	PQ215000
R. coreanus	Huangshan City, Anhui Province, China	R2021005	PQ214993
R. thibetanus	-	-	MH711174
R. nessensis	-	-	KM037442
R. pruinosus	-	-	KMO37496
R. inopertus	Bijie City, Guizhou Province, China	R2021026	PQ214998
R. swinhoei	Huangshan City, Anhui Province, China	R2021006	PQ215008
R. buergeri	Huangshan City, Anhui Province, China	R2021021	PQ214987
R. tephrodes	Huangshan City, Anhui Province, China	R2021001	PQ215007
R. setchuenensis	Bijie City, Guizhou Province, China	R2021023	PQ215003

......continued on the next page

TABLE 1. (Continued)

Species name	Collection site	Voucher number	ITS GenBank no.
R. pirifolius	Liupanshui City, Guizhou Province, China	R2021028	PQ215002
R. lambertianus	Liupanshui City, Guizhou Province, China	R2021027	PQ214999
R. reflexus	-	-	JN407519
R. conothyrsoides	-	-	KM037283
R. bifrons	-	-	KM037212
R. armeniacus	-	-	OL899216
R. dollnensis	-	-	KMO37306
Malus doumeri	-	-	MN577912
Prunus domestica	-	-	F1899098

DNA extraction, amplification and sequencing:—Approximately 0.1 g of fresh leaves from the newly discovered *Rubus* species were placed in a mortar precooled with liquid nitrogen. Genomic DNA was extracted from each sample using the Plant Genomic DNA Extraction Kit (SoLarbio ,China). The nrITS region was amplified using universal primers (ITS1: 5’-TCCGTAGGTGAACCTGCGG-3’, ITS4: 5’-TCCTCCGCTTATTGATATGC-3’). The PCR amplification system comprised 10 μL of 2x Taq Plus Master Mix II, 0.5 μL of each primer (10 μM ITS1 and ITS4), 7 μL of ddH_2O, and 2 μL of DNA template. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. The amplification products were purified using a DNA Purification Kit (Beijing Solarbio Science & Technology Co., Ltd., China) and sent to General Biosystems (Anhui) Co., Ltd. for sequencing.

Phylogenetic analysis:—All ITS sequence data from this study (Table 1) have been submitted to the NCBI GenBank. Among them, there are 17 sequences were downloaded from NCBI GenBank. ITS sequences were aligned using MAFFT v. 7.450 (Katoh *et al.* 2013), and any unaligned ends were removed. Phylogenetic analysis was conducted using both Bayesian inference (BI) and maximum likelihood (ML) methods. For BI, MrBayes v. 3.2.7 (Ronquist *et al.* 2012) was used to generate the phylogenetic tree based on Markov Chain Monte Carlo (MCMC) runs for 1,000,000 generations, applying the GTR + GAMMA model of nucleotide substitution as determined by ModelTest-NG (Darriba *et al.* 2020). The ML tree was constructed using FastTree software with the GTR + GAMMA model, and the Shimodaira-Hasegawa (SH) test (Price *et al.* 2010) was applied. Phylogenetic tree figures were created using Figtree v. 1.4.3 (Rambaut 2009). All sample sequence information used for the evolutionary analysis can be found in Supplementary file 1.

Morphological observations:—We conducted field investigations and performed observational studies to obtain accurate morphological traits and phenological characteristics of the newly described taxon. Specimens were collected at peak flowering stages to enable comprehensive morphometric analyses of leaves, inflorescence and fruit structures, including their dimensions, features and colours. High-resolution photographs document key diagnostic features, and minute morphological details including floral structures and indumentum were captured using a ZEISS Stemi 508 stereomicroscope.

Source of Data:—Comparative morphological data for *R. chingii* , *R. quinquefoliolatus* T. T. Yu & L. T. Lu (1982: 306) , and *R. zhaogoshanensis* T. T. Yu & L. T. Lu (1982: 303) were obtained from the *Flora of China* (Lu & Boufford 2003), *Flora Reipublicae Popularis Sinicae* (Yu & Lu 1985), type specimens (PE, KUN herbaria), and field observations (2018-2022). The distribution data of *R. chingii* were obtained through field surveys, while the records for *R. quinquefoliolatus* and *R. zhaogoshanensis* were sourced from the Chinese Virtual Herbarium (CVH, https://www.cvh.ac.cn/index.php) and the Global Biodiversity Information Facility (GBIF, https://www.gbif.org). Using ArcGIS software, we generated species distribution maps based on these data to compare spatial distribution differences and overlaps among these closely related species.

Results

Molecular phylogenetics:—For the 40 individuals representing 35 *Rubus* species, along with *Malus doumeri* and *Prunus domestica*, 24 sequences were obtained in this study, and 18 sequences were downloaded from NCBI GenBank. Molecular analysis using both BI (Figure 1) and ML methods revealed a similar topology referred to the methods of predecessors (Wang *et al.* 2016 and Huang *et al.* 2023). The three samples from the new species clustered together with high support (posterior probability [PP] = 0.99; SH support values [SS] = 79). The new species and *R. chingii* formed a sister group (PP = 0.98; SS = 89), which, in turn, was identified as a sister to the pair *R. corchorifolius* Linnaeus f. (1781:263)/*R. columellaris* Tutcher (1914:31) (PP = 0.87; SS = 80) and *R. grayanus* var. *trilobatus* T. T. Yu & L. T. Lu (1982:307) (PP = 0.94; SS = 76). These results indicated that, based on the ITS locus, the locus grouped the new species into a clade with consistent morphological attributes; this ITS-based clustering, coupled with its distinct morphological traits, showed the new species was morphologically distinct from other congeners, with ITS data further supporting this separation.

Morphological comparison:—Morphological analyses of the new taxon identified its closest affinity to *R. chingii*, *R. quinquefoliolatus*, and *R. zhaogoshanensis*, supporting its classification within section *Idaeobatus* Focke (1874: 143). This taxonomic placement aligns with the diagnostic criteria of the *Flora of China* (Lu & Boufford, 2003), which defines the section by the following characters: pinnately compound leaves bearing (3–)5–11 leaflets; stipules linear-lanceolate to narrowly triangular; inflorescences solitary or comprising few-flowered cymes; and mature aggregate fruits (etaerios) detaching cleanly from the torus. Nevertheless, the new taxon can be readily distinguished from its congeners by distinct features of the leaves, stipules, flowers, calyx, and pedicel (Table 2). Notable differences in leaf shape, as well as distinct stipule and indumentum characteristics, strongly support the recognition of *R. tianmuensis* as a new species.

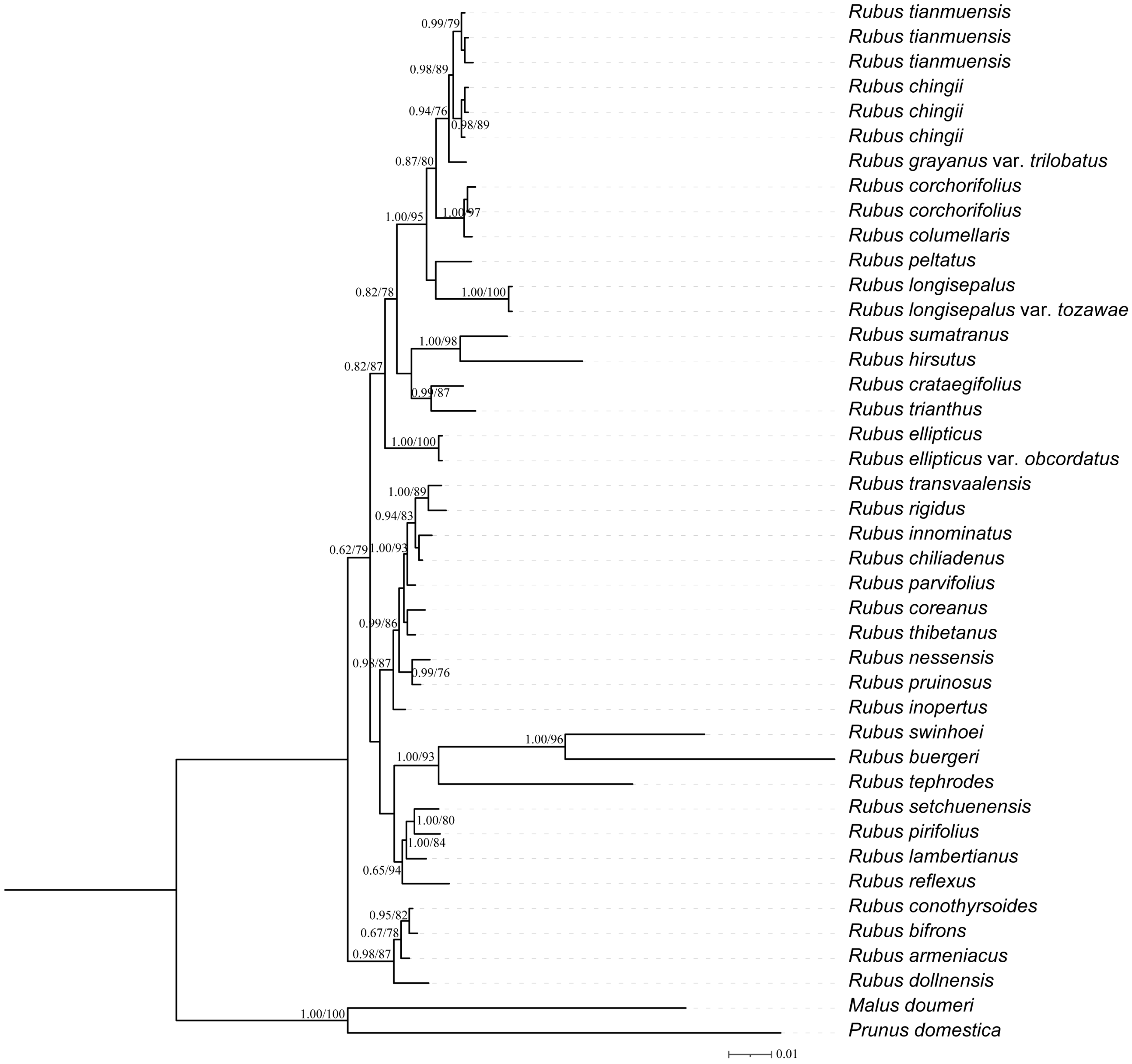

FIGURE 1. Bayesian phylogenetic tree derived from the analysis of combined nuclear ribosomal ITS data. Numbers above the branches are Bayesian posterior probabilities (PP) and ML SH support values (SS).

TABLE 2. Comparison of morphological features among *Rubus tianmuensis*, *R. chingii*, *R. quinquefoliolatus*, and *R. zhaogoshanensis*.

Characters	*R. tianmuensis*	*R. chingii*	*R. quinquefoliolatus*	*R. zhaogoshanensis*
Leaves	Fully lobed, compound leaves, resembling pedately, terminal leaflets with stalks. Short pubescence is present on both sides of the leaves.	Single leaf, palmately deeply lobed, with only pubescence or few hairs along the leaf veins on both sides.	Palmate leaves, with five leaflets and both sides exhibiting only pilose along leaf veins.	Palmate compound leaves, with 3–5 leaflets, and both sides lacking hairs.
Stipules	Lanceolate, up to 2.5 mm wide, with soft fur.	Linear lanceolate.	Linear lanceolate, slightly hairy.	Lanceolate or linear lanceolate, hairless or slightly fluffy.
Flowers	Solitary, with a diameter of 3–4 cm.	Solitary, with a diameter of 3–4 cm.	Solitary, with a diameter of 1.5–2 cm.	Terminal or single axillary, and 2–3 in number.
Calyx	Convex tip, with hairs on both sides, and sepals shorter than petals.	Convex tip, with dense pubescence of short hairs on the outside, and sepals shorter than petals.	Top and tail tips, outside the hairless calyx; fuzzy inner sepal edges, with petals shorter than sepals.	Long tail tip, hairless on the outside, slightly hairy on the edge of inner sepals, opening or folding in fruit, with petals shorter than sepals.
Pedicel	Obvious pubescence, comprising short glandular hairs and hooked spines.	Hairless	Hairless	Hairless
Reference	This article	Yu & Lu 1985; Lu & Boufford 2003	Yu & Lu 1985; Lu & Boufford 2003	Yu & Lu 1985; Lu & Boufford 2003

Taxonomy

Rubus tianmuensis K. Zhang & Yong F. Yin, *sp. nov.* (天目悬钩子) (Figures 2 and 3).

Type:—CHINA. Anhui Province: Jixi County, Qingliangfeng National Nature Reserve, at the edge of the hillside forest, 30°09’03”N, 118°50’37”E, alt. 778m, March, 28, 2019, K. Zhang & Yong F. Yin, R190328001 (holotype, ACM! ACM1903280011; isotype, ACM! ACM1903280012).

Diagnosis:—*Rubus tianmuensis* differs from *R. chingii* in several key morphological features. *R. chingii* has simple, palmately lobed leaves with five deep lobes, whereas the new species has compound, pedately divided leaves, with the terminal leaflet having a distinct petiolule. Additionally, the leaves of *R. chingii* are either hairy only along the veins or nearly glabrous on both sides, whereas the new species’ leaves are slightly pubescent on both sides. The stipules of *R. chingii* are linear lanceolate, whereas the stipules of the flowering branches in the new species are lanceolate, wider than those of *R. chingii*, and distinctly hairy. Furthermore, *R. chingii* has glabrous pedicels, whereas the pedicels of the new species are covered with distinct hairs, short glandular hairs, and hooked spines. These distinguishing features clearly delineate the two species.

Description:—Shrubs lianoid, 1.5–3 m tall, deciduous, with prickly branches. Leaves typically pedately 5-foliolate, 7–12 cm long on the vegetative branches; petiole 3–8 cm, with fine prickles; terminal leaflet larger than lateral ones, ovoid, 6–10 cm long, 4–7 cm wide, sometimes 3-lobed; central petiolule 0.5–2 cm, sometimes prickles; lateral petiolules short or nearly sessile; blade apex gradually tapering to a pointed or tail-like shape, margin doubly serrate; both surfaces slightly pubescent, pubescence denser along veins abaxially; central vein abaxially with hooked spines; stipules linear-lanceolate. Leaves pedately divided (sometimes 3-foliolate), 4–10 cm long on the reproductive branches; petiole 2–5 cm, densely pubescent; lateral leaflets often 2-lobed; central leaflet larger than lateral ones, ovoid, 4–7 cm long, 2–3 cm wide; central petiolule 3–5 mm or nearly sessile; apex gradually tapering to pointed; petiole and midrib abaxially with hooked spines; stipules lanceolate, sometimes serrate, 0.7–1.2 cm long, 1–2.5 mm wide, pubescent. Inflorescences terminal on short branchlets, 1-flowered, curved, pendulous, 3–4 cm diameter; pedicel 4–6 cm, with pubescence containing short glandular hairs and hooked spines. Sepals 5, ovate or oval, 6–8 mm long, apex convex, pubescent on both sides. Petals 5, white, alternate with sepals, elliptic or oval, 1–2 cm long, 0.7–1.2 cm wide, apex blunt. Stamens numerous, filaments flattened, anthers dorsifixed. Pistils numerous, ovaries densely gray-pubescent. Aggregate fruit red, subglobose, densely covered with slight pubescence.

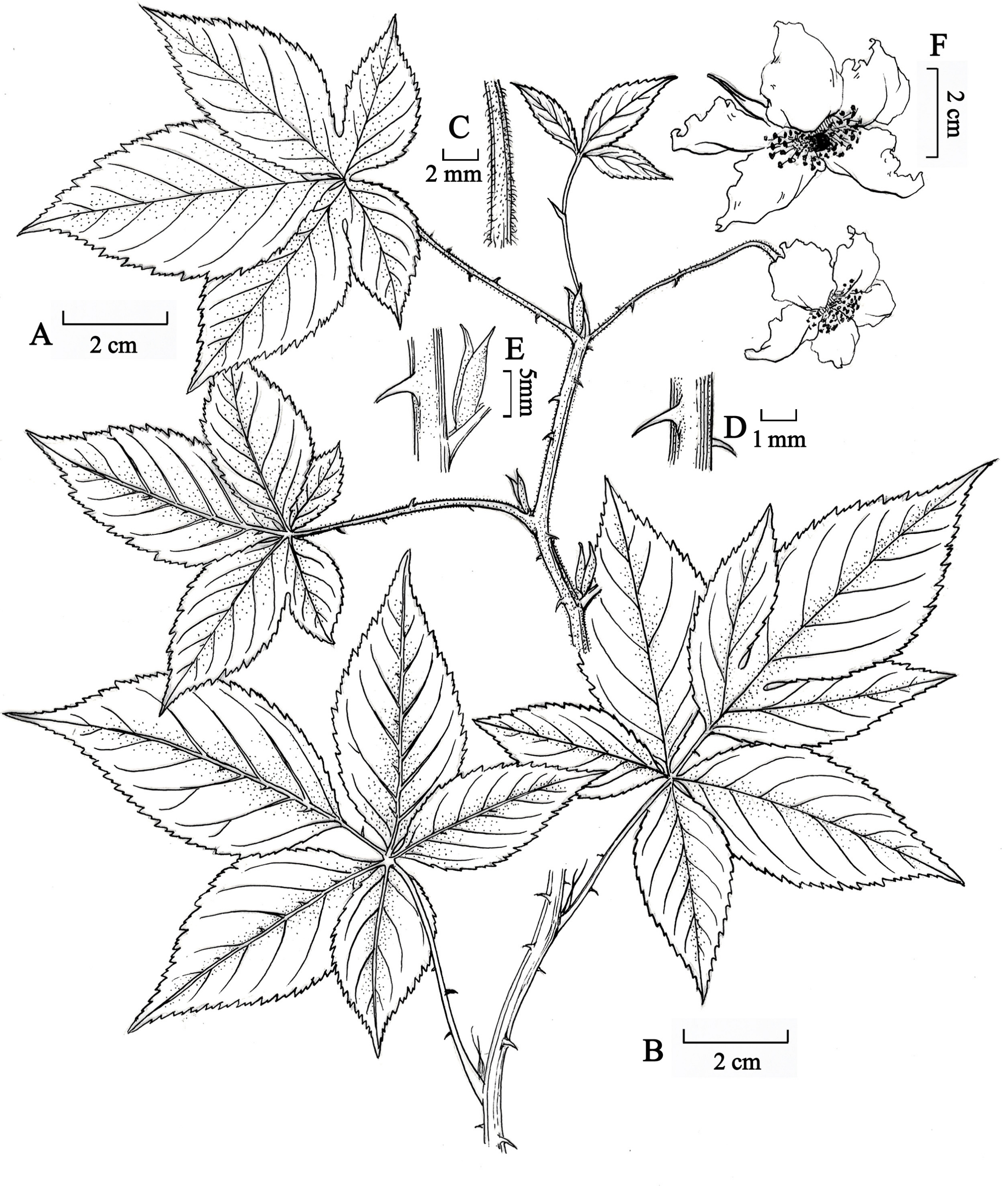

FIGURE 2. *Rubus tianmuensis* K. Zhang & Yong F. Yin. A. Flowering branch (showing the flowering leaf shape). B. Vegetative branch (showing leaf shape during the vegetative period). C. Upper part of the pedicel (showing the glandular and pubescent hairs). D. Lower part of the pedicel (showing the prickle). E. Stipules of flowering branches. F. Flower. Drawn by LiMing Zhang.

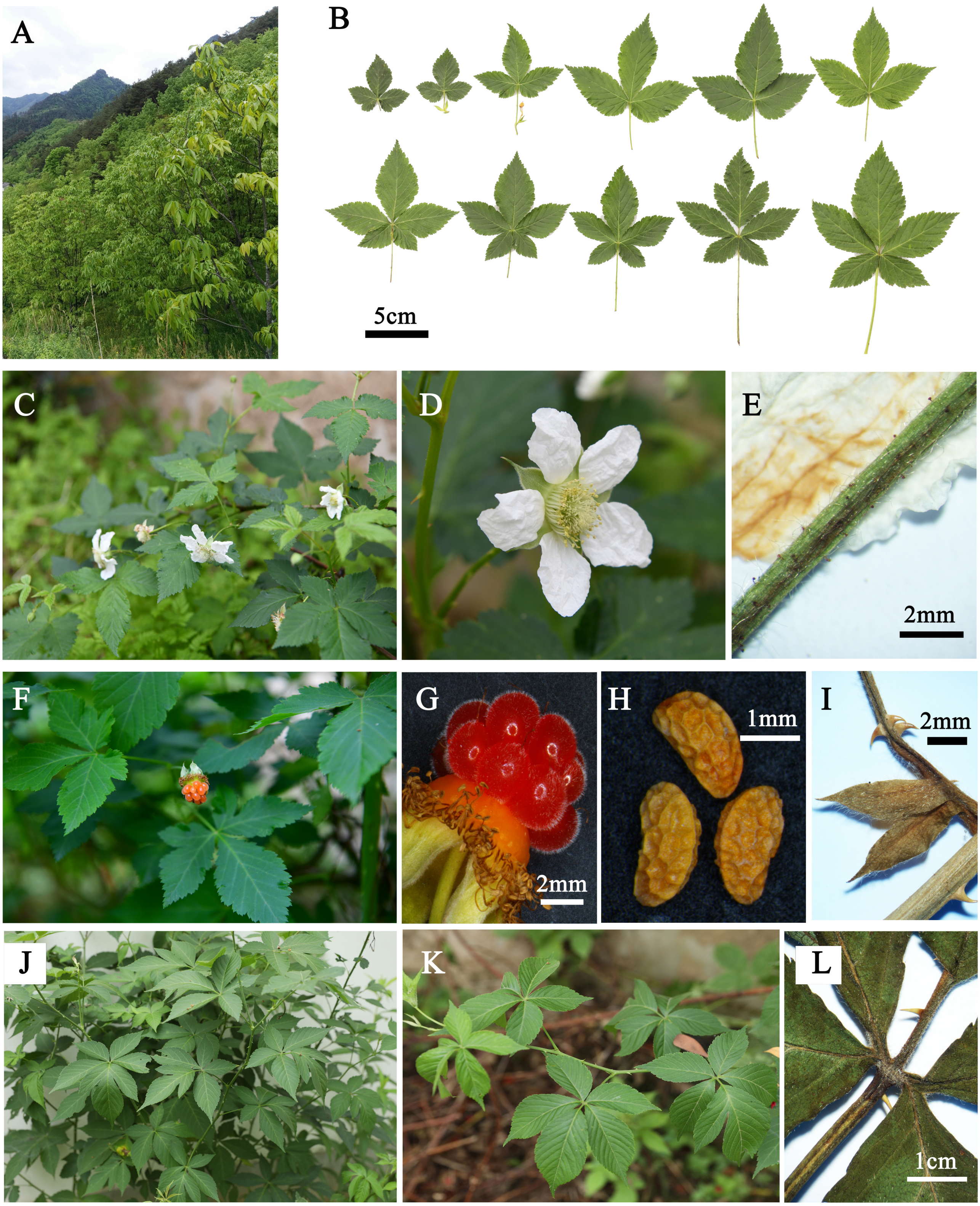

FIGURE 3. *Rubus tianmuensis* K. Zhang & Yong F. Yin. A. Habitat. B. Leaf shape. C. Flowering branch. D. Flower. E. Upper part of the pedicel (showing the glandular and pubescent hairs). F. Fruit branch. G. Fruit. H. Seed. I. Stipules on flowering branches. J. Vegetative plant. K. Vegetative branch (showing leaf shape during the vegetative period). L. Local close-up of a leaf on the vegetative branch (showing the petiolule).

Habitat and distribution:—The new species is primarily distributed in slopes, forest edges, and sunny roadside areas at an altitude of approximately 150–1000 m; their distribution is documented in Guangde County and Qingliangfeng, Jixi County, Anhui Province, China. The distributions of *R. tianmuensis*, *R. chingii*, *R. quinquefoliolatus*, and *R. zhaogoshanensis* were mapped (Figure 4). Based on field survey data, the results indicate that *R. tianmuensis* primarily occurs in southeastern Anhui Province, while *R. chingii* is distributed south of the Yangtze River within the same province. Notably, their distribution ranges exhibit partial spatial overlap in southeastern Anhui.

Phenology:—Flowering occurs from March to April, and the fruiting period ranges from May to June.

Etymology:—Species name is derived from the mountain Tianmu where *Rubus tianmuensis* was discovered (天目悬钩子).

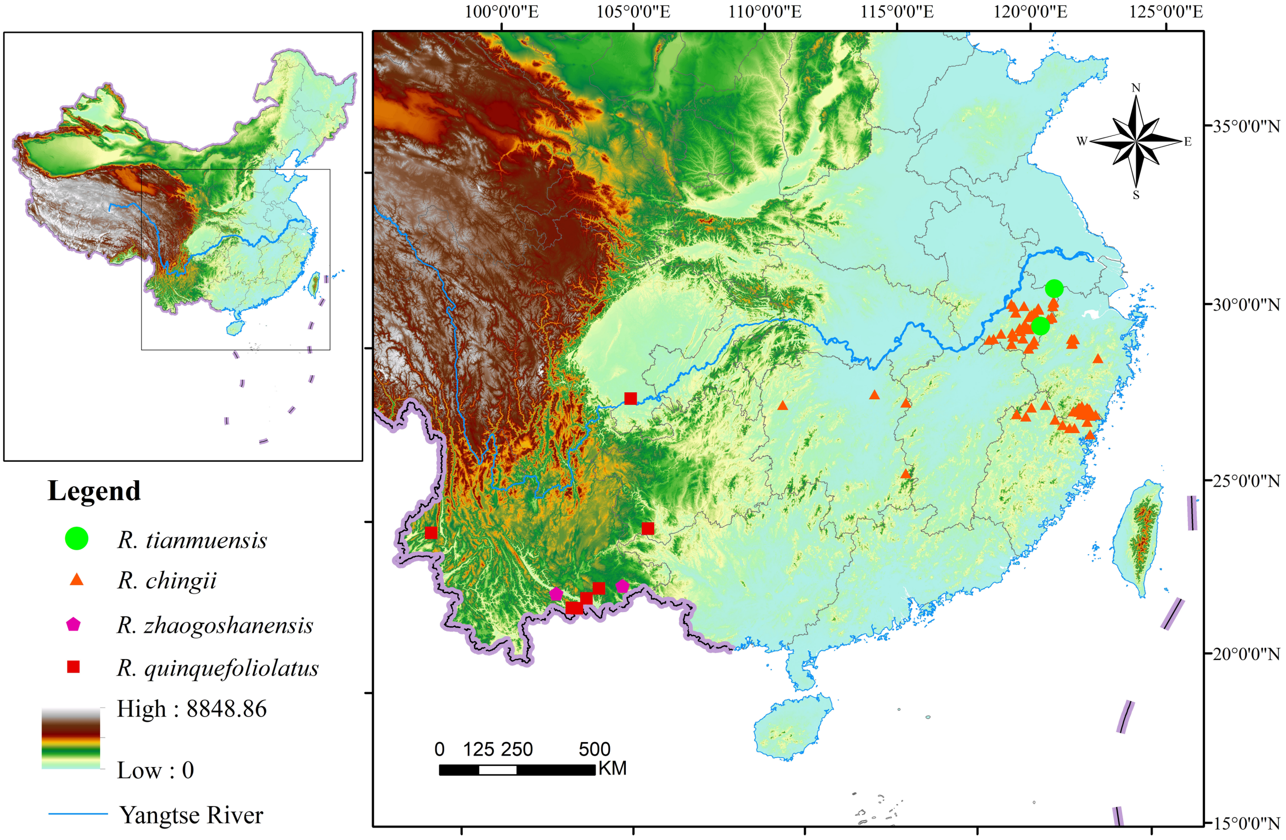

FIGURE 4. Distribution map of *Rubus tianmuensis* (green circle), *R. chingii* (Orange Triangle), *R. quinquefoliolatus* (red square), and *R. zhaogoshanensis* (Purple pentagon).

Discussion

This study validated *R. tianmuensis* as a new Rosaceae species from southeastern Anhui via morphology and ITS-based phylogenetics. Phylogenetically, BI/ML analyses placed it in *Rubus* sect. *Idaeobatus* (consistent with *Flora of China*), forming a well-supported sister group with *R. chingii* (PP=0.98, SS=89) and a monophyletic clade (PP=0.99). This refines East Asian *Rubus* phylogeny, filling gaps in prior work on *R. chingii*-related compound-leaf taxa and aligning with ITS markers' utility for species delimitation.

The unique pedately compound leaves of *R. tianmuensis*—retaining terminal leaflet differentiation through vegetative and reproductive phases—represent an apomorphic trait within section *Idaeobatus*. Across the genus, compound leaves occur in only 12% of species (Thompson 1997), primarily in tropical clades (e.g., *R. cochinchinensis* Trattinnick (1823:97)), suggesting convergent adaptations to high-light environments. Field observations revealed minimal ontogenetic variation in leaf architecture, contrasting with the heteroblastic shifts reported in *R. parvifolius* Linnaeus (1753, 1197) (cf. Xu *et al.* 2019). Notably, the persistent pubescence and recurved prickles on rachises may reflect selective pressures against herbivory, a hypothesis supported by the high insect diversity in Tianmu Mountain forests (Zhao *et al.* 2022).

The recognition of *R. tianmuensis* at species rank, rather than as a variety of *R. chingii*, is justified by multiple diagnostic characters exceeding typical varietal distinctions. While *R. chingii* varieties (*R. chingii* var. *chingii* and *R. chingii* var. *suavissimus* (S.K. Lee) L.T. Lu) differ in the size of flowers, sepal lobes and the sweetness of leaves (Lee 1981; Lu & Boufford 2003), they share synapomorphies including palmately lobed leaves and glabrous pedicels. In contrast, *R. tianmuensis* exhibits: (1) compound vs. simple leaves, (2) glandular-hispid vs. eglandular pedicels, and (3) lanceolate-serrate vs. linear-entire stipules (Table 2). These traits align with Stuessy's (2009) criteria for species delimitation—fixed differences influencing functional morphology. Moreover, the Bayesian posterior probability (PP = 0.99) for reproductive isolation far exceeds thresholds proposed for varietal status (PP < 0.85; Yang & Rannala 2010). This discovery enriched East Asian *Rubus* biodiversity, highlighting molecular-morphological integration's value for complex genus taxonomy.

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