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***Rhodiola yushuensis*, a new species of *Rhodiola* (Crassulaceae) from Qinghai, China**

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Abstract

A new species from the Qinghai-Tibetan Plateau is described and illustrated. *Rhodiola yushuensis* S.Y. Meng et J. Zhang *sp. nov.* is similar to *Rhodiola smithii* (Hamet) S. H. Fu, but it differs in its inner caudex leaves with obvious petioles and the leaf blades are obovate-triangular (vs. inner caudex leaves with strongly descends to the base of the petiole, and the leaves are long linear) and the outer caudex leaves are scalelike, triangular-suborbicular (vs. scalelike, narrowly triangular). Principal component analysis (PCA) analysis of the leaf traits and seed coat architecture also showed that *R. yushuensis* can be separated from *R. smithii*. The nrDNA internal transcribed spacer (ITS) sequences also support the claim that this plant is a new species, and it is grouped with *R. smithii*.

Keywords: *Rhodiola*, Principal component analysis (PCA), Scanning electron microscopy (SEM), New species, Internal transcribed spacers (ITS)

Introduction

Rhodiola Linnaeus (1753: 1035) (Crassulaceae) is a highly radiated genus within Crassulaceae with abundant morphological diversities that distributed at high altitudes and frigid zones of the northern hemisphere (Thiede & Eggli 2007, Mayuzumi & Ohba 2004). There are 55 (16 endemics) out of a total of 90 species in China (Fu & Ohba 2001). The majority of the taxa are found on the Qinghai-Tibetan Plateau (QTP) and its adjacent areas, which are the centre of diversity. Species of *Rhodiola* have been used as important adaptogens, sources of hemostasis, and tonics in traditional Tibetan medicine for thousands of years (Li *et al.* 2016, Rohloff 2002, Sharma & Misra 2018). The development and utilization of *Rhodiola* have been subjected to intense study in recent years.

In our phylogenetic study of *Rhodiola* (Zhang *et al.* 2014a, Zhang *et al.* 2014b, Wang *et al.* 2019), we collected specimens on a large scale for many years and discovered some new species of *Rhodiola*, some of which have been published (Zhang *et al.* 2015). In this study, we describe a new species which endemic to Yushu, northeast of the QTP. It was first discovered in 2010 with only fruit, similar to *Rhodiola smithii* (Hamet 1913: 8) S. H. Fu (1965: 122). We collected *R. smithii* at sandy grasslands, gravelly places on sandy beaches or rock crevices all over the interior of QTP and found significant morphological differences that distinguish the two species. In 2019, we also found it during a survey of Chinese herbal medicine resources in Yushu, and a flowering specimen was collected in June 2020. The discovery of this new species expands our understanding of *Rhodiola* plants and will help us to understand the rapid radiation of *Rhodiola* in the QTP.

Materials and methods

Morphometric analysis

We collected two populations of *R. yushuensis* S.Y. Meng et J. Zhang from Qinghai Province (Table 1). We sampled 10 individuals from each population for morphological and molecular research and compared the morphological characters of *R. yushuensis* with those of species that are closely related to it. We deposited voucher specimens in the Herbarium of Peking University (PEY), China.

We observed and measured the inner caudex leaf traits and conducted a principal component analysis (PCA) using Origin 2020 (Origin Lab Corporation, Northampton, MA, USA). We measured five traits, including the length of leaf (L), the width of leaf (W), shape of the blade (S=W/L), distance between the widest part of the blade and the base of the petiole (D) and relative length of the distance between the widest part of the blade and the base of the petiole (B=D/L) (Figure S1). All of the data is quantitative.

Scanning electron microscopy (SEM) observations

We collected pollen and seeds from *R. yushuensis* and *R. smithii*. The samples were directly fixed on a sample stage and shadowed with gold using DV-502B. We performed scanning electron microscopy using a Helios Nanolab G3 UC at 5 kV and measured the length and width of samples using ImageJ2 (Rueden *et al.* 2017).

TABLE 1. Information on the collection of *Rhodiola yushuensis* and *Rhodiola smithii*.

Taxon	Location	Altitude/m	Latitude North	Longitude East	Voucher
<i>R. yushuensis</i> 1	Tongtianhe, Yushu	3,536	N33° 7' 35.73"	E97°7' 55.21"	C037
<i>R. yushuensis</i> 2	Sanjiangyuan	3,478	N33° 1' 34.20"	E97°12' 54.48"	C046
<i>R. smithii</i>	Namling	4,723	N89°5'47.13"	E 29°41'9.95"	MH071

TABLE 2. Accession information relating to the internal transcribed spacer (ITS) sequences downloaded from GenBank.

Species	Accession no.	Species	Accession no.
<i>Phedimus aizoon</i> (L.) 't Hart	KF113682.1	<i>Rhodiola rhodantha</i> (A.Gray) H.Jacobsen	KF879828.1
<i>Phedimus kamschaticus</i> (Fischer & C. A. Meyer) 't Hart	AB088612.1	<i>Rhodiola sacra</i> (Prain ex Hamet) S. H. Fu	KF113719.1
<i>Rhodiola alterna</i> S. H. Fu	KF113684.1	<i>Rhodiola semenovii</i> (Regel et Herd.) A. Br.	KJ569952.1
<i>Rhodiola chrysanthemifolia</i> (Lévl.) S. H. Fu	KP114711.1	<i>Rhodiola sexifolia</i> S. H. Fu	KF113722.1
<i>Rhodiola hobsonii</i> (Prain ex Hamet) S. H. Fu	KP114741.1	<i>Rhodiola sinuata</i> (Royle ex Edgew.) S. H. Fu	KF113723.1
<i>Rhodiola humilis</i> (Hk. f. et Thoms.) S. H. Fu	KF113702.1	<i>Rhodiola smithii</i> (Hamet) S. H. Fu	KF113725.1
<i>Rhodiola integrifolia</i> Raf.	KF879827.1	<i>Rhodiola stapfii</i> (Hamet) S. H. Fu	KF113726.1
<i>Rhodiola liciae</i> (Hamet) S. H. Fu	KF113706.1	<i>Rhodiola tieghemii</i> (Hamet) S. H. Fu	KF113729.1
<i>Rhodiola ovatisepala</i> (Hamet) S. H. Fu	KP114762.1	<i>Rhodiola wallichiana</i> (Hk.) S. H. Fu	KF113730.1
<i>Rhodiola prainii</i> (Hamet) H. Ohba	KF113711.1	<i>Rhodiola yushuensis</i> S.Y. Meng et J. Zhang	MT981706.1

DNA extraction, sequencing and phylogenetic analysis

We extracted DNA from leaves that had been dried with silica gel using a Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) using the nuclear ribosomal internal transcribed spacer (ITS) regions as molecular markers and following the manufacturer's instructions. The PCR amplification and sequencing primers were ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') for ITS (White *et al.* 1990). We downloaded 17 ITS sequences of other species of *Rhodiola* and two accessions of *Phedimus* as an outgroup

from the NCBI. The GenBank accession numbers of all the sequences analysed are shown in Table 2. We aligned the sequences using MEGA7.0.26 followed by manual adjustments (Kumar *et al.* 2016).

Nucleotide substitution model parameters were determined for Bayesian inference (BI) and maximum likelihood analyses in jModelTest 2.1.6 using the Bayesian information criterion (BIC) and Akaike information criterion (AIC), respectively (Guindon & Gascuel 2003, Darriba *et al.* 2012). The software was integrated into Cyberinfrastructure for Phylogenetic Research (CIPRES, <http://www.phylo.org>), which enabled it to operate online (Miller *et al.* 2010). The Bayesian inference was implemented using MrBayes 3.2.6 under the TIM2ef+G model (Ronquist *et al.* 2012). We ran four Markov Chain Monte Carlo (MCMC) chains, sampling one tree every 1,000 generations for 10,000,000 generations starting with a random tree. We discarded the first 25% of generations as burn-in trees and used the remaining trees to construct a consensus tree presented as posterior probabilities (PP) to estimate the robustness of BI trees. Maximum likelihood analyses were performed by iqtrees 1.6.12 with 1000 bootstraps under the TIM2ef+G model (Nguyen *et al.* 2015, Hoang *et al.* 2018). The BI tree and ML tree were merged by sumtrees, which was integrated into DendroPy (Sukumaran & Mark 2010).

TABLE 3. Morphological comparison of the diagnostics of *Rhodiola yushuensis* with the related species *R. smithii*.

Characters	<i>R. yushuensis</i>	<i>R. smithii</i>
Caudex leaves	outer scalelike, Inner leaflike	outer scalelike, Inner leaflike
Shape	long obovate-triangular	linear to oblong
Long (L)	16.6±4.8 mm	24.8±4.7 mm
Wide (W)	3.1±0.67 mm	1.6±0.16 mm
Shape of Leaflike caudex leaves (S=W/L)	0.20±0.045	0.07±0.018
Leaflike caudex leaves	The distance between the widest part of blade and base of petiole (B)	12.9±2.8 mm
	Relative length of the distance between the widest part of blade and base of petiole (D=B/L)	0.52±0.061
Stem and pedicel	caudex simple, erect	caudex simple, erect
Stem Leaves	alternate, short petiole, long obovate, 6-11 × 2-5 mm	alternate, ovate-linear, 7-14 × 1.3-2.2 mm,
Inflorescences	terminal, 1-5 flower buds form cymes	corymbiform, 3-7 flower buds form cymes
Pollen	elliptic, 25-30 µm long, 12-16 µm wide	oblong-elliptic, 20-25 µm long, 12-18 µm wide
Fruit	follicles 6–8 mm long, erect, usually with outcurved styles, 4–8 seeds each carpel	follicles erect, 6–10 seeds each carpel
Seed	oblong-lanceolate, 0.4-1.2 mm long, 0.2-0.4 mm wide	subobovoid-oblong, ca. 1.0 mm long, 0.4 mm wide

Results

Owing to its two types of caudex leaves, inflorescences corymbiform with more than 3 flowers and similar flower structure, *R. yushuensis* is similar to *R. smithii*. However, a morphological comparison between these two species shows an obvious difference (Table 3, Fig 1). First, the inner caudex leaves of *R. yushuensis* are 16mm long with obvious petioles, and its leaf blades are obovate-triangular. Outer caudex leaves are scalelike, wide triangle. However, the inner caudex leaf blade of *R. smithii* strongly descends to the base of the petiole, and the leaves are long linear. Outer caudex

leaves are scalelike, narrowly triangular. A principal component analysis (PCA) based on the 5 quantitative leaf traits showed that *R. yushuensis* can be separated from *R. smithii* (Fig 2). The PC1 scores, which accounted for 69.9% of the total variation, showed very high correlation with the width of the leaf (W) and distance between the widest part of the blade and the base of the petiole (D). The scores of PC1 were also highly correlated with other traits, such as the length of leaves (L) and relative length of the distance between the widest part of the blade and the base of the petiole ($B=D/L$). The PC2 scores, which explained 21.4% of the total variation, were highly correlated with the shape of the blade ($S=W/L$).

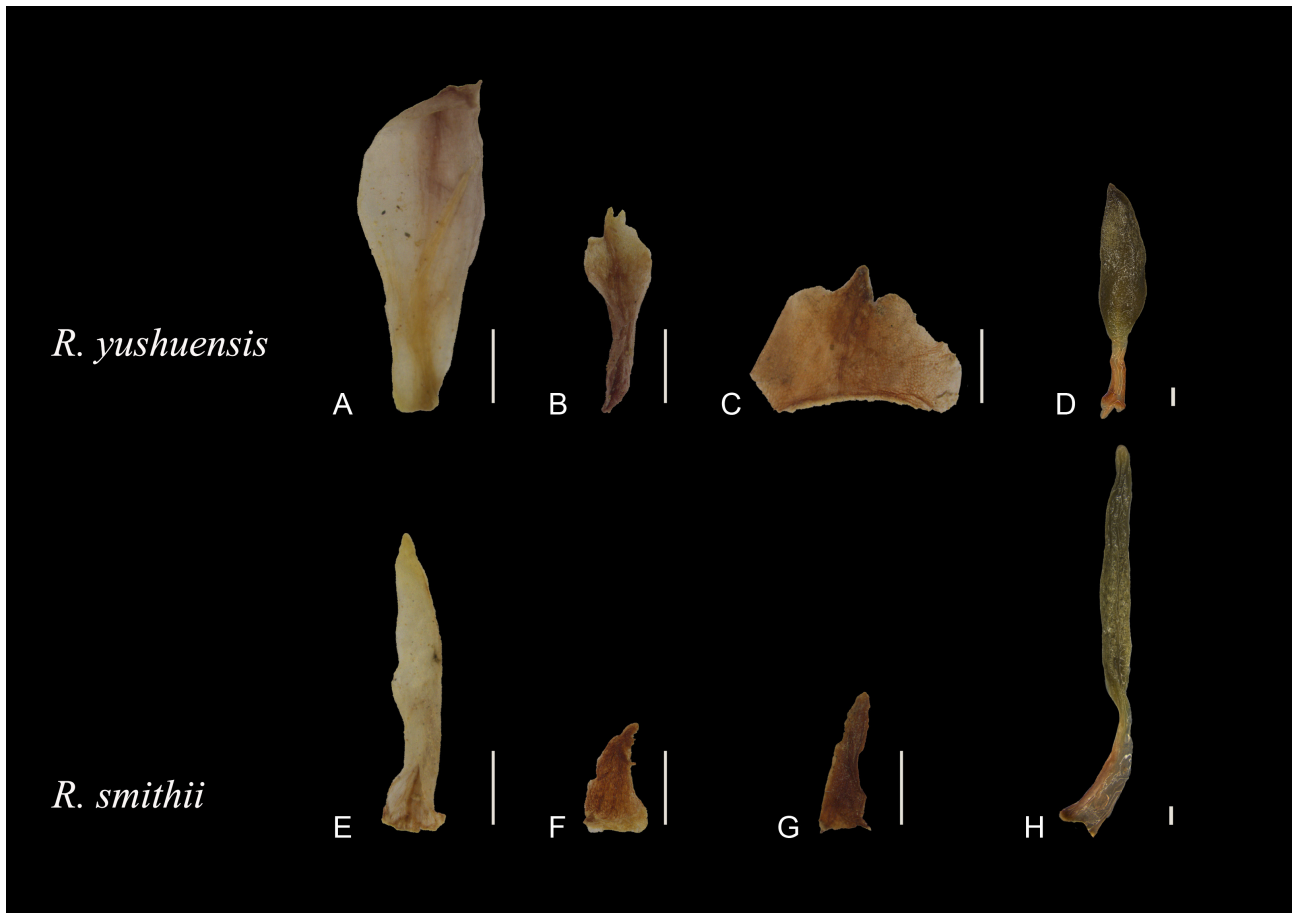


FIGURE 1. Morphological comparison of flowers and leaves between *Rhodiola yushuensis* and *R. smithii*. Scale bars, 1 mm. A, E: Petals; B, F: calyx; C, G: Out caudex leaves (scalelike); D, H: Inner caudex leaves (leaflike).

The scanning electron microscope observation showed that the pollen of *R. yushuensis* was elliptic (length/width=1.53) with relief on the surface, 22.68 μm long and 14.85 μm wide while the pollen of *R. smithii* was longer (length/width=1.93, 28.22 μm long, 14.6 μm width) (Fig 3A, D). The seeds of *R. yushuensis* were oblong-lanceolate with attenuated obtuse ends, while that of *R. smithii* were oblong. And the former was 1166.75 μm long and 388.44 μm wide, slightly larger than the latter (821.34 μm long and 369.7 μm wide), and had a thicker shape (length/width=3 vs. length/width=2.22) (Fig 3B, E). What's more, *R. yushuensis* differed from *R. smithii* owing to its seed coat architecture. Its large fold seed coat ornamentation differed from the small fold on *R. smithii* seed. And it had small wing-like projections which *R. smithii* did not (Fig 3C, F).

We included 20 species in the molecular analyses. The sequence length was 586 bp for the ITS region, of which 352 characters were constant, 82 characters were singleton, and 152 characters were parsimony-informative. The new species was resolved as a sister of *R. smithii* (Bayesian posterior probabilities (PP)/Bootstrap value was 1.00/99), and the two with *R. humilis* formed a well-supported clade (0.93/82) (Fig 4). The BI and ML trees generated coincident topologies and similar support values. The ML tree with its branch length is shown in Fig S2. Consistent with previous studies, these taxa of *Rhodiola* form a comb-like tree form that indicates that rapid radiation had occurred (Zhang *et al.* 2014b).

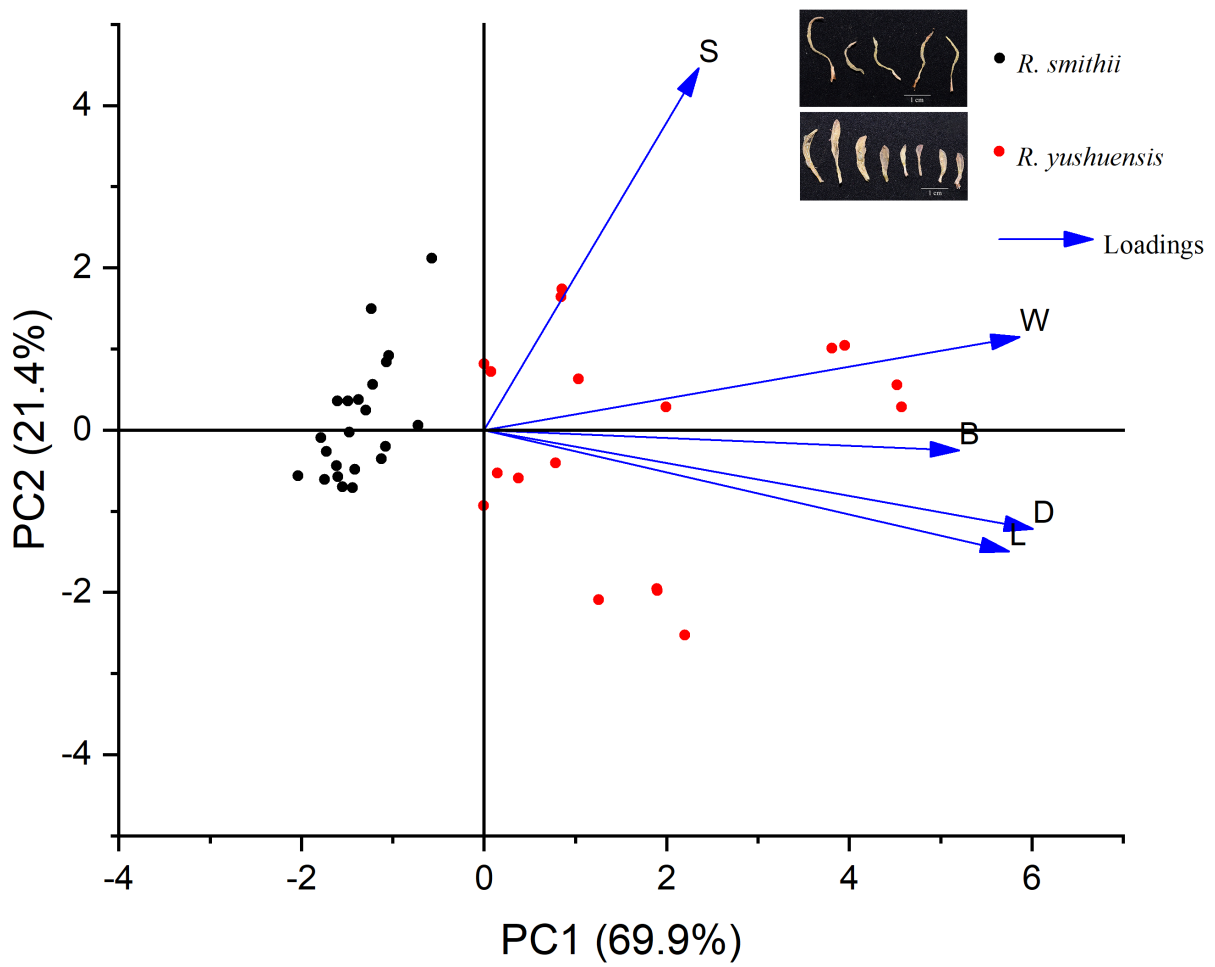


FIGURE 2. Principal component analysis of *Rhodiola yushuensis* and *R. smithii* based on the leaf trait analysis.

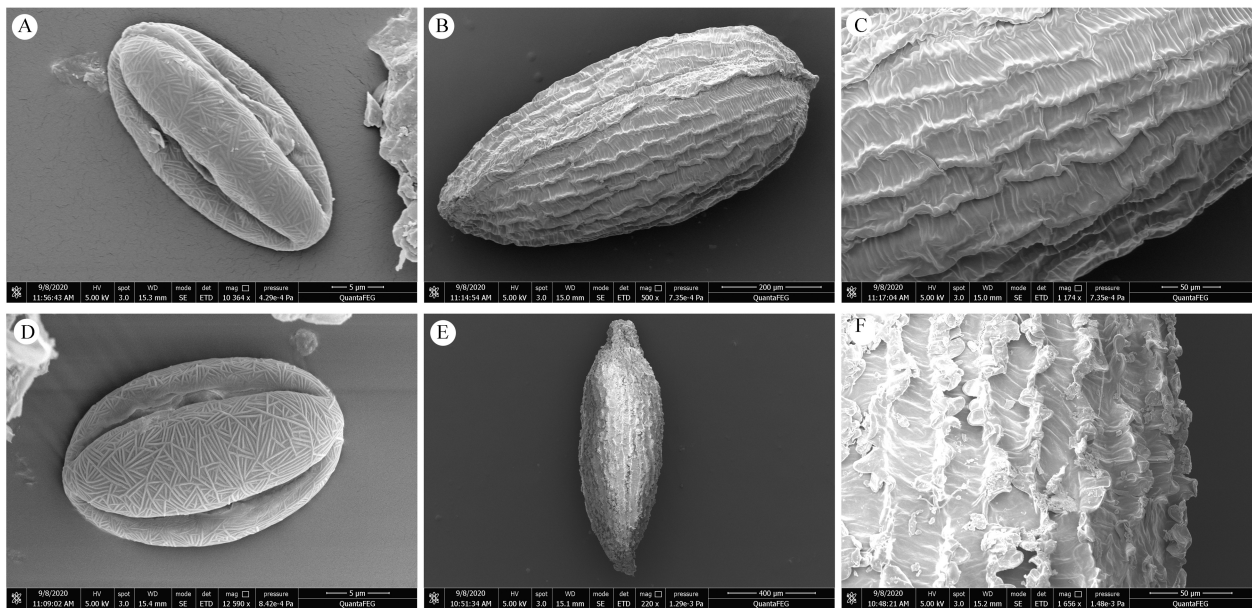


FIGURE 3. SEM microphotographs. A-C: pollen (A) and seed (B, C) of *Rhodiola smithii*; D-F: pollen (D) and seed (E, F) of *R. yushuensis*.

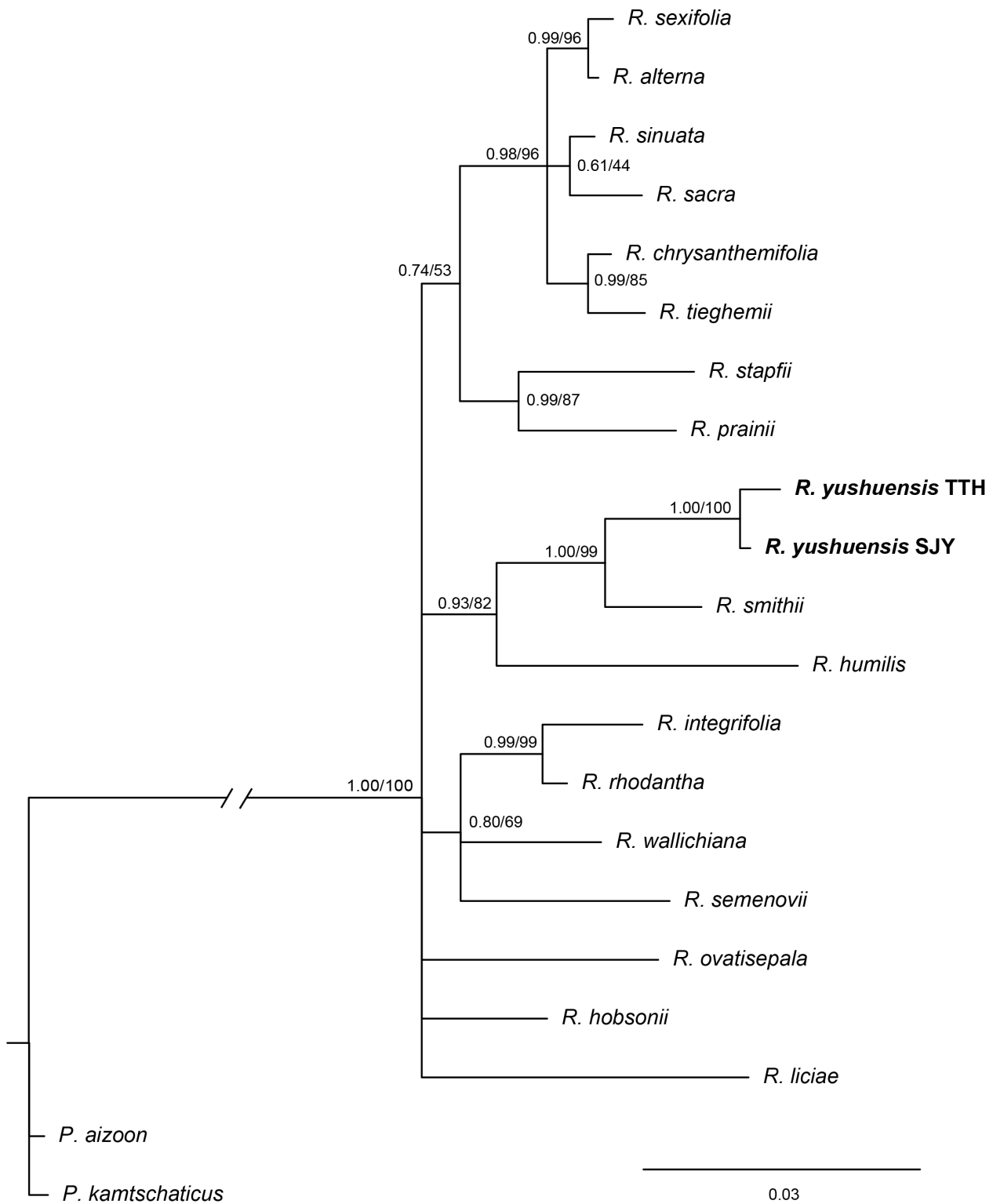


FIGURE 4. Bayesian phylogenetic tree based on ITS sequences for the genus *Rhodiola*. Bayesian posterior probabilities (PP) and bootstrap values (BP) are shown above the branches (PP/BP).

Discussion

The comparison of morphology showed that flowers were similar between *R. yushuensis* and *R. smithii*, but the caudex leaves were different, which was consistent with the PCA results of leaf traits. In addition, the large fold seed coat architecture of *R. yushuensis* differed from the small fold ornamentation found on the *R. smithii* seeds. Phylogenetic

analyses based on ITS sequences showed that *R. smithii* was the closest relative of *R. yushuensis*. And *R. humilis*, together with these two species, formed a well-supported clade. *R. humilis* distributed in southeast QTP, has similar floral structures, but there are only one type of caudex leaves (leaflike) and one or two flowers on its inflorescences. *R. smithii* is widely distributed in gravelly places on sandy beaches, sandy grasslands and rock crevices in the hinterlands of QTP (Fu & Fu 1984, Fu & Ohba 2001), while *R. yushuensis* is only distributed in rock crevices of the valley northeast of QTP. The differentiation of *R. humilis*, *R. yushuensis* and *R. smithii* may be the result of the adaptation of ancestral species to different habitats with the uplift of QTP. However, the history of differentiation among these species is unclear, and further research is merited.

Taxonomic treatment:

Rhodiola yushuensis S.Y. Meng et J. Zhang *sp. nov.* (Figs 5, 6)

Type:—China. Qinghai, Yushu, Sanjiangyuan, N33° 1' 34.20" E97°12' 54.48", 3,478 m, 28 June 2020, J. Zhang, C046 (holotype: PEY! Isotype: PEY! PE!).

Diagnosis:—Similar to *Rhodiola smithii* (Hamet) S. H. Fu but differing in caudex leaves, outer caudex leaves are wide triangular while the inner caudex leaves with distinctly petiole are obovate-triangular.



FIGURE 5. *Rhodiola yushuensis* S.Y. Meng et J. Zhang, (A) natural habitat, (B) cyme, (C) fruits.

Description:—A perennial low and small herb, 2-4 cm high. Caudex nearly erect, few branches, robust, typically 6-10 mm across; apical part often short, branched and accrescent, crowned by persistent old flowering stems. There are two types of caudex leaves. Outer caudex leaves scalelike, wide triangle, yellow to maroon, long 1.8-2 mm, wide 2.5-3.0 mm. Inner caudex leaves leaflike, long obovate-triangular with the entire margin, acute-obtuse at the apex, 12-25 mm long, 3-8 mm wide, petiole 6-35 mm. Flowering stems 1-3 from the apex of each caudex branch, deciduous, 11-30 mm long, erect, simple, terete and smooth. Leaves alternate, few and scattered, petiole 1-3 mm long, spurless, obovate-triangular, glabrous, 6-11 mm long, 2-5 mm wide, entire; Inflorescences terminal, 4-5 flower buds form cymes, flower buds are pink, pink to red when blooming. Bracts leafy. Flowers 5-merous, calyx 5, base connate 0.5 mm, long triangular, apex acuminate, entire, green, fleshy, 2.5-3 mm long, 0.8-1.0 mm wide; Petals 5, free, oblong, pink to red, apex shortly acuminate, long 6-8 mm, wide 1.5-2.0 mm; Stamens 10, slightly shorter than petals, antepetalous ones inserted ca. 0.5 mm from petal base, long 6-8 mm, antesealous ones 6-8 mm long, anther red; Nectar scales 5, yellow, wide quadrangular, apex emarginate, ca. 0.7 mm long, ca. 0.5 mm wide; Carpel 5, erect, long oblong, long 5-7 mm, base separated; Style long ca. 2.5 mm; Follicles 6-8 mm long, each carpel erect, Styles usually out curved, 4-8 seeds each carpel. Seeds oblong-lanceolate, 0.4-1.2 mm long, 0.2-0.4 mm wide.

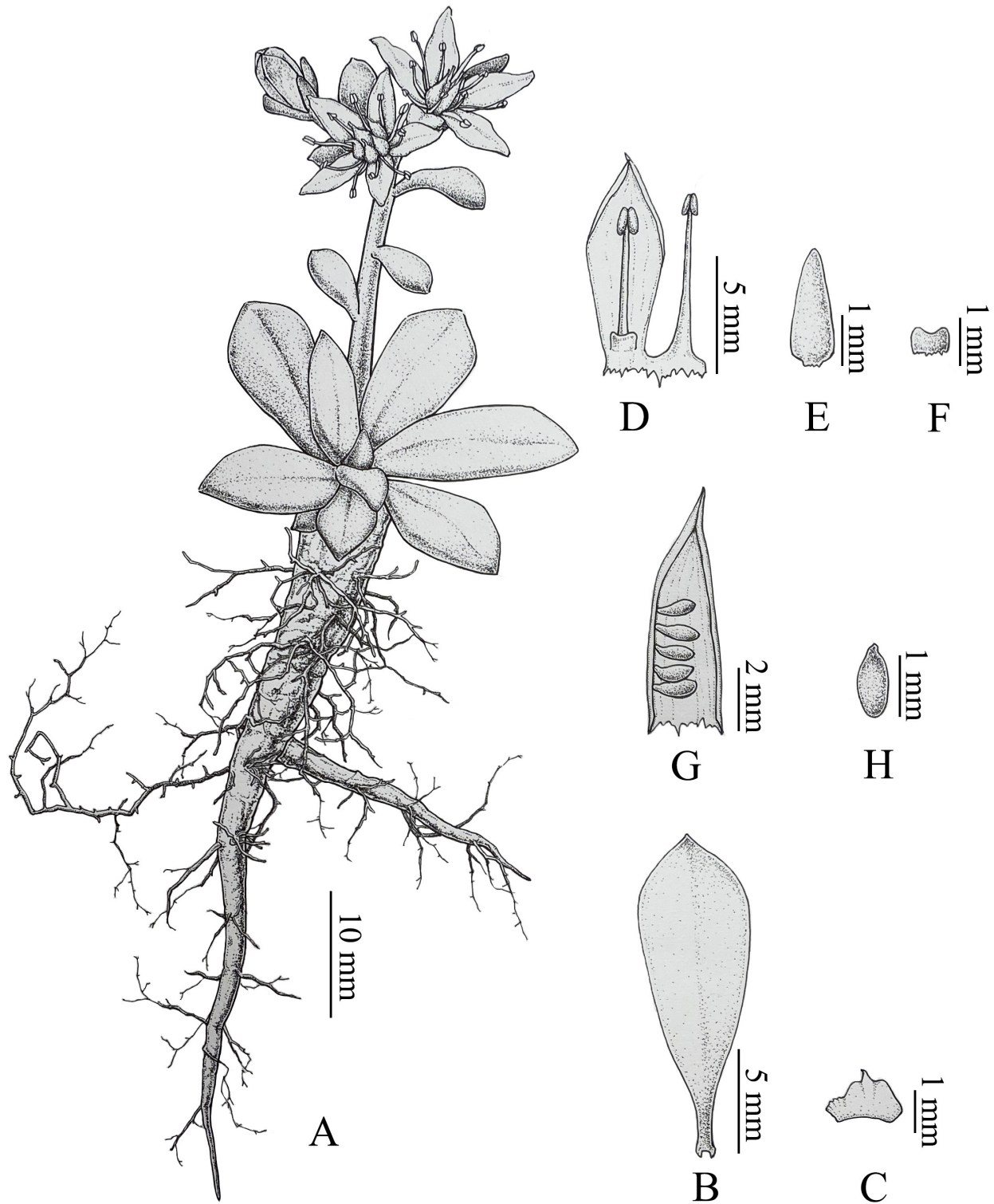


FIGURE 6. Line drawing of *Rhodiola yushuensis* S.Y. Meng et J. Zhang, (A) Habit; (B) Inner caudex leaves (leaflike); (C) Outer caudex leaves (scalelike); (D) Petal and Stamens; (E) Sepal; (F) Nectar scales; (G) Fruit; (H) Seed. Drawing by Ye Lv.

Phenology: Flowers from June to July; fruits from July to October.

Habitat: Rock crevices of the valley, 3800–4000m.

Etymology: The specific epithet of this new species refers to Yushu City.

Common name (assigned here): Yu Shu Hong Jing Tian (玉树红景天; Chinese name).

Proposed IUCN conservation status: The new species has been found in the rock crevices of the Tong-tian River, Zha-qu River and Zi-Qu River valley in Yushu City, Qinghai Province, China. Because there is no medicinal

and economic value, the populations remain relatively stable. However, the habitats are mainly in river valleys and roadsides, and more active economic and construction activities may affect the population. The species is considered to be “Vulnerable” (VUD1) according to the IUCN Red List Criteria (IUCN 2017).

Additional specimens examined (paratypes): CHINA. Qinghai, Yushu, Sanjiangyuan, 3,890 m, 28 August 2010, S. Y. Meng & J.Q. Zhang, zhang02 (PEY); Tongtian River, N33° 7′ 35.73″ E97°7′ 55.21″, 3,536 m, 27 June 2020, J. Zhang, C037(PEY).

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