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Saxifraga sunhangiana (Saxifragaceae), a new species from Gansu, China

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ABSTRACT: *Saxifraga sunhangiana*, a new species of the genus *Saxifraga* sect. *Irregulares* (Saxifragaceae) from Gansu, China, is described and illustrated. This species is distinguishable by its abaxial leaf surface with singularly long white villous hairs and rather long rhizomes. Morphological and molecular data indicate that *S. sunhangiana* belongs to *S.* sect. *Irregulares*, and closely related to *S. rufescens*. The new species differs from *S. rufescens* by its terminal flower with two elongated, fusiform-lanceolate petals and leaves with glabrous petiole.

KEY WORDS: *Irregulares,* ITS sequences, *Saxifraga fortune*, *S. rufescens*, taxonomy, phylogeny.

INTRODUCTION

Saxifraga L., the largest genus of Saxifragaceae, comprises more than 440 species mainly distributed in alpine areas of Asia, Europe, North America, Andean South America (Pan *et al*., 2001; Tkach *et al*., 2015a, b). *Saxifraga* is taxonomically difficult, as it displays remarkable morphological variation. Based on Engler & Irmscher's (1913) system, Gornall (1987) presented a revised classification with 15 sections, 19 subsections and 34 series. Previous molecular phylogenetic studies suggested that *Saxifraga* is monophyletic if *Saxifraga* sect. *Micranthes* (Haw.) D. Don is excluded (Soltis *et al*., 1996; Prieto *et al*., 2013; Deng *et al*., 2015; Tkach *et al*., 2015a). *Saxifraga* sect. *Irregulares* Haw., currently comprises 16 species and grows in moist temperate forests in East Asia (Magota *et al*., 2021). They are characterized by zygomorphic flowers (Tkach *et al*., 2015b). This section comprises one of the basal clades of *Saxifraga* first described by Haworth (Haworth, 1803; Soltis *et al*., 2001; Zhang *et al*., 2015; Tkach *et al*., 2015b; Zhang *et al*., 2020).

China has a vast territory with a wide range of complex and diverse topographies and soils and covering several climate types, which contribute to the wealth of Chinese botanical diversity (Sun *et al*., 2017; Chen *et al*., 2018). In China, *Saxifraga* comprises about 222 species, among which 13 species belongs to *S.* sect. *Irregulares*, which includes the recently reported new species, *S. daqiaoensis* F.G.Wang & F.W.Xing (Wang *et al*., 2008), *S. kegangii* D.G.Zhang, Y.Meng & M.H.Zhang (Zhang *et al*., 2017), *S. luoxiaoensis* W.B.Liao, L.Wang & X.J.Zhang (Zhang *et al*., 2018), *S. shennongii* L.Wang, W.B.Liao & J.J.Zhang (Zhang *et al*., 2019), *S. damingshanensis* W.B.Liao, W.Y.Zhao & J.H.Jin (Zhao *et al*., 2019), and *S. viridiflora* X.J.Zhang, T.Deng, J.T.Chen & H. Sun (Zhang *et al*., 2021).

During a recent field survey, we collected an unidentified specimen of *Saxifraga* in Gansu province, China. This specimen resembles *S. rufescens* Balf.f. in its unspotted abaxial leaf surface, but is distinct from the latter in that its abaxial leaf surface has singularly long white villous hairs, glabrous petioles and terminal flower with two elongated, fusiform-lanceolate petals. Subsequent morphological comparisons and phylogenetic analysis support the status of the taxon as a new species of *Saxifraga* sect. *Irregulares*, which is described herein.

MATERIALS AND METHODS

***Morphological observation*:** Morphological data for description of this new species were collected from observation of the specimens both in the wild and herbarium, and compared to *Saxifraga rufescens* and *S. fortunei* Hook., as the leaf abaxial surfaces of these three species are unspotted, which can differ them from other species in sect. *Irregulares*. The voucher specimens of our collections were deposited in the herbarium of Kunming Institute of Botany (KUN), Kunming, China. Herbarium specimens of the *S.* sect. *Irregulares* were examined from CDBI, KUN, NAS, PE, SM and WUK (acronyms follow Thiers, 2018), either by examining the specimens directly, or electronically through the National Plant Specimen Resource Center (http://www.cvh.ac.cn/index.php), and JSTOR Global Plants web portal (https://plants.jstor.org/).

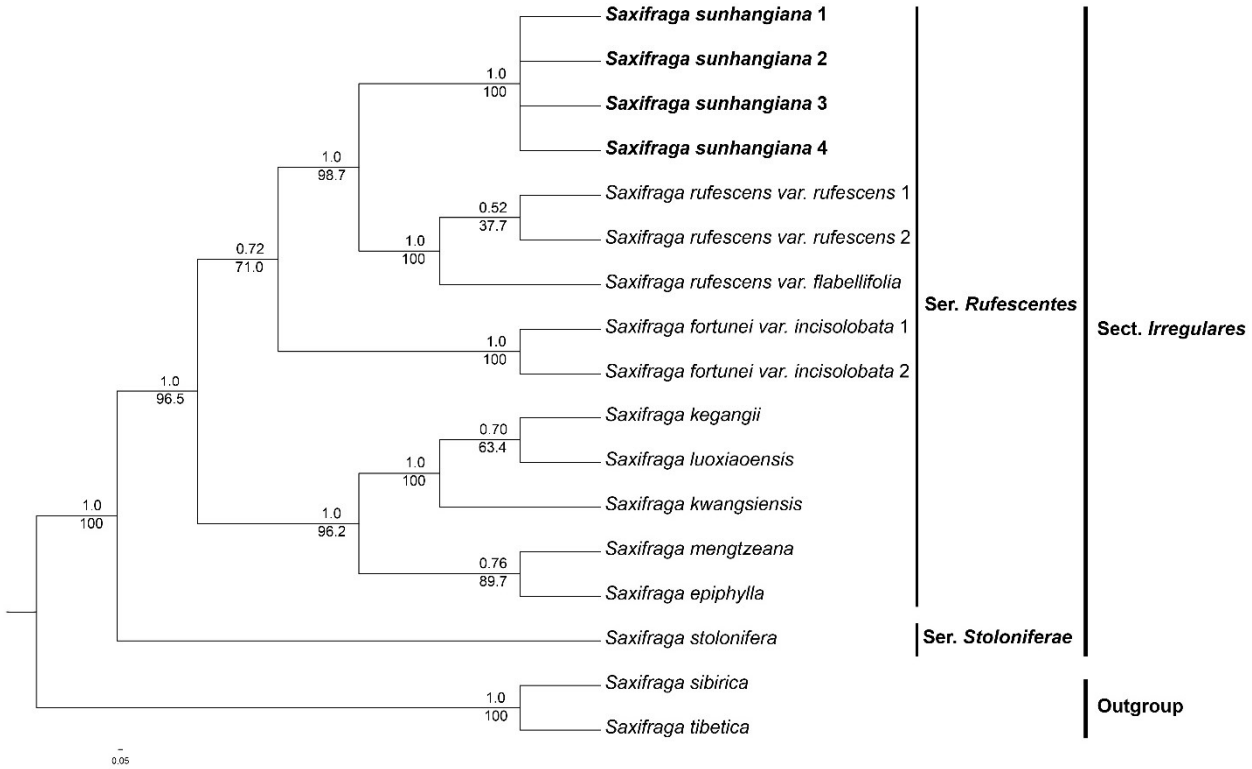

Fig. 1. Bayesian consensus tree of 11 species of ***Saxifraga*** based on their ITS sequences, with ***S. sibirica*** and ***S. tibetica*** as the outgroup. Numbers above branches indicate Bayesian posterior probability, numbers below branches are ML bootstraps.

Table 1. Voucher information and GenBank accessions for phylogenetic analysis.

Taxon	Voucher	GenBank number
S. sunhangiana 1	*Deng11386* (KUN)	OM720002
S. sunhangiana 2	*Zhangxj100* (KUN)	OM720003
S. sunhangiana 3	*Zhangxj101* (KUN)	OM720004
S. sunhangiana 4	*Zhangxj102* (KUN)	OM720005
Sequences downloaded from NCBI		
Taxon	GenBank number	
S. rufescens var. *flabellifolia*	LC534836	
S. rufescens var. *rufescens*	MK685996 & MK685998	
S. fortunei var. *incisolobata*	LC534837 & LC534841	
S. kegangii	MF074217	
S. luoxiaoensis	MK092512	
S. kwangsiensis	MK685989	
S. mengtzeana	LC534835	
S. epiphylla	MK685991	
S. stolonifera	MK092500	
S. tibetica	LN812551	
S. sibirica	MH711180	

***Molecular analyses*:** We sampled 15 collections representing 9 species of *Saxifraga* sect. *Irregulares*, including the new species. *Saxifraga sibirica* L. from *S.* sect. *Mesogyne* and *S. tibetica* Losinsk. from *S.* sect. *Ciliatae* were selected as outgroup based on previous molecular phylogenetic relationships (Zhang *et al*., 2017). Leaf materials were collected from field works and dried herbarium specimens. Sequences for other taxa were obtained from GenBank. Voucher information and GenBank accession numbers are also provided in Table 1.

Total genomic DNA extracted from leaf materials using DP305 Plant Genomic DNA kits (Tiangen, Beijing, China) following the manufacturer's protocol. The entire ITS region (including internal transcribed spacers ITS1 and ITS2 of nuclear ribosomal DNA and the 5.8S rRNA gene) were amplified using primers ITS4 and ITS5 described in White *et al*. (1990). Parallel chromatograms derived from bi-directional sequencing were checked for accuracy by visual inspection with Chromas v. 2.6.6 (http://www.technelysium.com.au) and integrated into a single sequence. Sequences were then aligned with MEGA version 7.0 and gaps were treated as missing data (Sudhir *et al*., 2016).

Phylogenetic reconstruction was performed using Bayesian inference (BI) and maximum likelihood (ML). The phylogenetic tree based on Bayesian inference was generated using MrBayes version 3.2.6 (Huelsenbeck *et al*., 2001). The phylogenetic analysis based on maximum likelihood was conducted with PhyML version 3.0 (Guindon *et al*., 2010). Detected by the jModeltest 2.1.7, the GTR+G model selected by Akaike information criterion (AIC) was used in BI and ML analyses (Posada, 2008).

Table 2. Diagnostic characters of ***Saxifraga sunhangiana*** and comparison with other related species of sect. *Irregulares*.

Characters	*S. sunhangiana*	*S. rufescens*	*S. fortunei*
Rhizomes	Rather long	Rather long	Short
Leaf	Adaxially glabrous to sparsely hispid, abaxially with long white villous.	Both surfaces strigose, abaxially hairy mainly on the veins.	Adaxially glabrous, abaxially glandular villous
Petiole	Glabrous	Red-brown glandular villous, rarely glabrous.	Glandular villous
Petals	White, with two elongated petals, entire and glabrous, fusiform-lanceolate.	White to pink, only one elongated petals, entire and glabrous, lanceolate to linear.	White, with two elongated petals, margin serrate, glandular ciliate

RESULT

Spots on leaves are inferred to be phylogenetically informative within sect. *Irregulares* ser. *Rufescentes* (Zhang et al., 2020). The new species resembles *S. rufescens* and *S. fortunei* in its unspotted abaxial leaf surface, which distinguish them from other species in sect. *Irregulares*. The morphological characters of the new species, *Saxifraga rufescens*, and *S. fortunei* are presented in Table 2, and a full description is given in the section on Taxonomic Treatment.

A total of 11 taxa were included in phylogenetic analysis (Fig. 1). The resulting multiple alignment of the ITS region, including 5.8S gene, was 647 bp. The 50% majority-rule consensus tree based on Bayesian posterior probability (PP) and maximum likehood bootstraps (ML) of the ITS sequences both showed that four accessions of *S. sunhangiana* grouped together (PP = 1, ML = 100), sister to *S. rufescens* with strong supports (PP = 1, ML = 98.7).

DISCUSSION

The new species *Saxifraga sunhangiana* has zygomorphic flowers and stolons absent, which indicate a position in *S.* sect. *Irregulares* ser. *Rufescentes*. According to our phylogenetic study, *S. sunhangiana*, *S. rufescens* and *S. fortunei* formed a clade. The close relationship of these taxa is also supported by similar morphological features. *Saxifraga sunhangiana* resembles *S. rufescens* and *S. fortunei* in having leaves abaxially unspotted, but it differs from latter two species by its abaxial leaf surface with singularly long white villous hairs, leaf blade adaxially glabrous to sparsely hispid, petiole glabrous, two elongated petals fusiform-lanceolate and margin entire.

Morphological and molecular data both indicate that *S. sunhangiana* is closely related to *S. rufescens*. However, *S. sunhangiana* can easily be distinguished from *S. rufescens* by its flower with two elongated petals fusiform-lanceolate and other morphological features as discussed above. Furthermore, the geographic distribution of this two species is different. *S. rufescens* mainly occurs in Hubei, Sichuan, SE Xizang, and Yunnan province (latitude lower than 30°N), whereas the new species *S. sunhangiana* is endemic to Gansu province (latitude about 32°N), supporting the distinction of our finding as a new species. Here we considered that the latitude gradient plays an important role in the differentiation of the species in *S.* sect. *Irregulares*.

TAXONOMIC TREATMENT

Saxifraga sunhangiana T.Deng, X.J.Zhang & J.T.Chen, ***sp. nov.*** **文縣虎耳草 Fig. 2**

Type**:** CHINA. Gansu. Longnan City, Wenxian county, Baishuijiang National Nature Reserve. 104°45′36″ E, 32°48′36″ N, 1802 m alt., 06 June 2021, J.T. Chen, X.J. Zhang, J.Y. Peng *Deng11386* (Holotype: KUN!; Isotype: JIU!; Isotype: SYS!).

Diagnosis**:** *Saxifraga sunhangiana* is distinguishable by its abaxial leaf surface with singularly long white villous hairs and its rather long rhizomes. It morphologically resembles *S. rufescens*, from which it is easily distinguished by its flower with two elongated petals fusiform-lanceolate (vs. flower with only one elongated petal lanceolate to linear); petiole glabrous (vs. petiole red-brown glandular villous); leaf blade adaxially glabrous to sparsely strigose (vs. leaf blade adaxially strigose).

Description**:** Perennial herbs, 18–40 cm tall. Rhizomes creeping, 10−30 cm long, with persistent sheathed petiole. Stolons absent. Leaves all basal, with petiole 8–15 cm long, glabrous or sparsely short glandular trichomes; petiole base sheathed, margin with sparsely glandular trichomes; leaf blade reniform to cordate, papery, 6.0−8.0 cm long × 6.5−9.6 cm wide, base cordate, margin 7- or 9-lobed, apex acute, lobes irregularly 5−7-dentate at margin, adaxial surface glabrous to sparsely strigose, abaxial surface with long white villous hairs, ca. 10 mm long, unspotted. Bracts 1–4, narrow lanceolate, 3.0–4.0 mm long × 1.2 mm wide, both surfaces glabrous, margin sparsely glandular trichomes. Inflorescence paniculate, 20–35 cm long, 15–20-flowered, branches 5.0−8.0 cm long, glandular pubescent, 4−5-flowered, flowers zygomorphic; pedicels slender, 1.2−2.0 cm long, glandular pubescent. Sepals 5, greenish, spreading to reflexed, triangular lanceolate, 2.0−3.0 mm long × 1.2−1.8 mm wide, abaxially and marginally glandular trichomes, apex obtuse. Petals 5, white, margin entire,

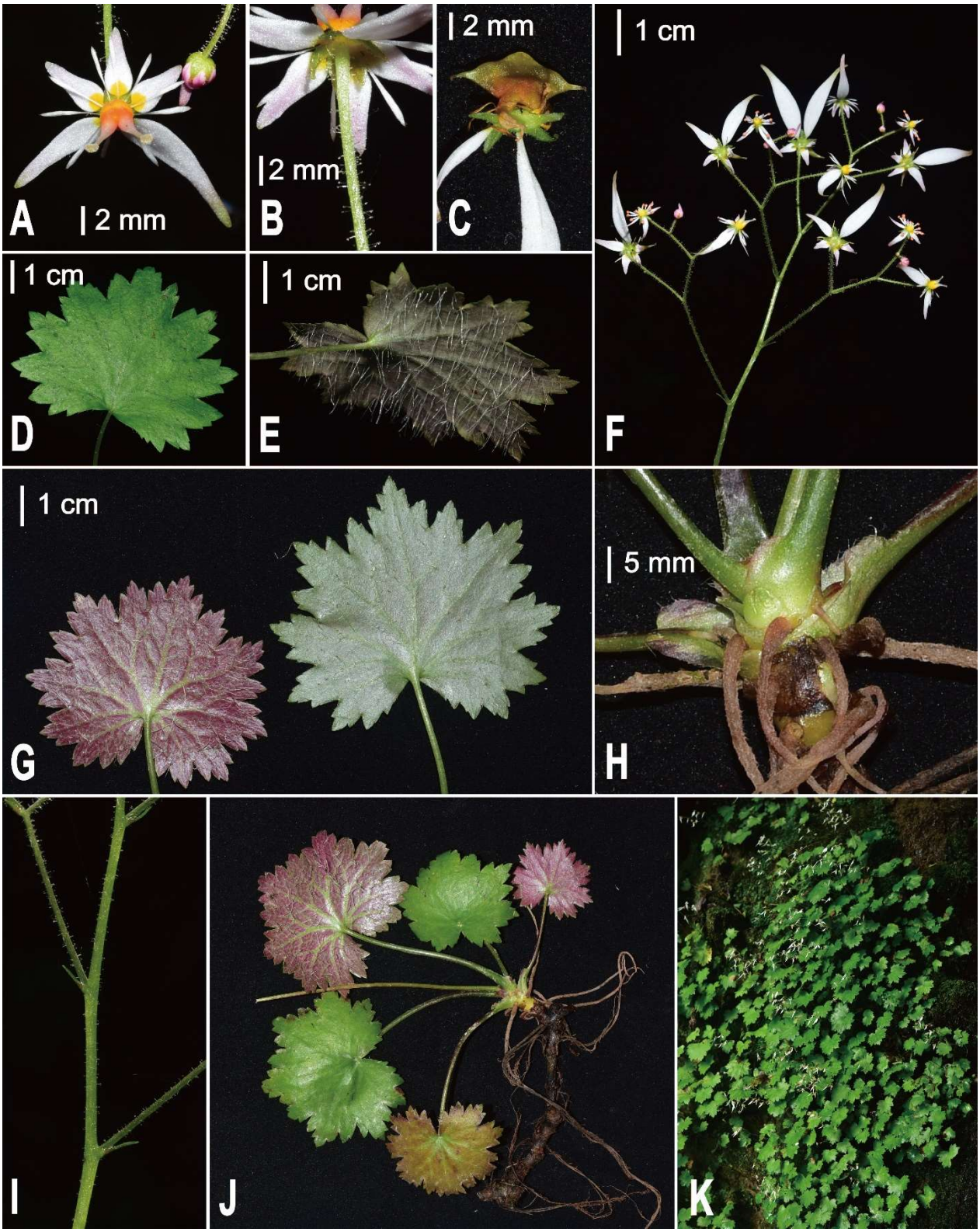

Fig. 2. Illustrations of ***Saxifraga sunhangiana*** T. Deng, X.J. Zhang & J.T. Chen. **A & B.** Flower, petals 5; **C.** Fruit, capsule winged when mature; **D.** Adaxial leaf surface glabrous to sparsely hispid; **E & G.** Abaxial leaf surface with long white villous hairs, ca. 10 mm, unspotted; **F.** Inflorescence; **H.** Rhizomes glabrous, petiole base sheathed; **I.** Scape glabrous to sparsely white glandular piliferous; **J & K.** Plants and habitat. (Photos by Xin-Jian Zhang and Jun-Tong Chen.)

glabrous; the three smallest petals triangular lanceolate, base with a yellow spot, 3.5−5.0 mm long × 2.0−2.4 mm wide, 3-veined; the two elongated petals fusiform-lanceolate, 18−25 mm long × 3.5−5.5 mm wide, 5−7-veined. Stamens 5, filaments club-shaped, 3.2−4.5 mm long. Ovary ovoid, 2.5−4.0 mm long, with an annular nectary disc; styles divergent ca. 1.5−2.0 mm long. Capsule winged when mature, carpels 6.0−8.0 mm long × 3.6−4.0 mm wide. Seeds obround, the two ends slightly bent, ca. 0.6 mm, surface ribbed.

***Etymology*:** *Saxifraga sunhangiana* is named after Prof. Sun Hang (1963–), director of the Kunming Institute of Botany (China) who conducted extensive research in plant taxonomy, biogeography, and evolutionary biology and made outstanding contributions towards understanding the plant diversity of China.

***Distribution and ecology*:** The new species *Saxifraga sunhangiana* is currently known only from Wenxian County, Gansu Province, China. It grows on moist rocks nearby valleys, alt. 1800−2750 m.

***Paratypes*:** CHINA. Gansu. Longnan City, Wenxian county, Baishuijiang National Nature Reserve. 104°45′36″E, 32°48′36″N, 1802 m alt., 06 June 2021, *J.T. Chen, X.J. Zhang, J.Y. Peng Zhangxj 100* (KUN); CHINA. Gansu. Longnan City, Wenxian county, Baishuijiang National Nature Reserve, Qiujaba, ravine along NW branch of Baima River. 104°19′18″E, 32°55′21″N, 2320–2725 m alt., 19 May 2007, *D. E. Boufford 37767* (PE); same locality, 2170–2425 m alt., 21 May 2007, *D. E. Boufford 37818* (PE).

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