



Morphological and molecular identification for *Saxifraga yangshuoensis* (sect. *Irregulares*, Saxifragaceae), a new species from Guangxi, China

Hai-Ling CHEN¹, Wei-Bin XU¹, Wan-Yi ZHAO², Yan LIU^{1,*}

1. Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, Guangxi, China. 2. State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China. *Corresponding author's email: gxibly@163.com

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ABSTRACT: *Saxifraga yangshuoensis* (Saxifragaceae), a new species from Yangshuo County in northeast Guangxi, China, is described and illustrated. It closely resembles *S. damingshanensis* and *S. shennongii* in stolons absent, leaves abaxially usually with spots, smallest petals base with yellow spotted, but differs from its leaves thickly papery, slightly revolute when dried, margin subentire, adaxially densely covered glandular hispid, abaxially densely covered with pale green spots, petiole base margin nearly glabrous and petals apex greenish. Combine morphological comparisons and phylogenetic analysis based on *psbA-trnH* and *matK*, confirmed that the new species differed from those similar species of *S. sect. Irregulares*.

KEY WORDS: Chloroplast genome, *Irregulares*, *Saxifraga damingshanensis*, *Saxifraga shennongii*.

INTRODUCTION

Encompassing over 440 species, *Saxifraga* Linnaeus (1753) is the largest and taxonomically complex genus of Saxifragaceae, and widely distributes throughout the Northern Hemisphere (Tkach *et al.*, 2015). Recent molecular studies suggested that *Saxifraga* is monophyletic, on condition that *Saxifraga* sect. *Micranthes* (Haw.) D. Don (1822) is excluded (Soltis *et al.*, 1996, 2001, 2013; Xiang *et al.*, 2012; Deng *et al.*, 2015; Tkach *et al.*, 2015). This genus was recognized at least 13 sections and 9 subsections (Tkach *et al.*, 2015). *Saxifraga* sect. *Irregulares* Haw. (1803) is characterized by long petiolate leaves and flowering stems that are leafless or have small bracts, zygomorphic flowers with two elongated petals, and the stamens with club-shaped filaments (Tkach *et al.*, 2015; Zhang *et al.*, 2021). This section contained only 15–20 species are restricted distributed in eastern Asian, mainly in China and Japan (Zhang *et al.*, 2020; Zhang *et al.*, 2022). In China, *S. sect. Irregulares* comprises 14 species, including seven new species reported in recent years (Wang *et al.*, 2008; Zhang *et al.*, 2017; Zhang *et al.*, 2018, 2021, 2022; Zhang *et al.*, 2019; Zhao *et al.*, 2019).

In mid-September 2018, during floristic surveys in Yangshuo County of northeast Guangxi, China, a flowering *Saxifraga* sect. *Irregulares* species were collected. Checking morphological characters in February 2019, showed that the leaves of this species are thickly papery, margin subentire, slightly revolute when dried, abaxially densely covered with pale green spots and petals apex greenish. These morphological characters could not matched known species of *Saxifraga* sect. *Irregulares*. Unfortunately, it wasn't flowering in the wild at that time, we were unable to gather any more

information. Until early October 2021, the first author made specially surveys to collect materials of this unknown species again. After carefully consulting the relevant literature and examining the herbarium specimens of similar species, as well as morphological and molecular phylogenetic analysis. We finally confirmed that it is an undescribed species, which is described below as *S. yangshuoensis*.

MATERIALS AND METHODS

At least 10 living individuals of the presumed new species for field observations were carried out in Yangshuo. Specimens have been collected for examination and comparison to those of kindred species deposited at the herbaria IBK, IBSC, JIU, LBG, PE and SYS (acronyms follow Thiers, 2022). Fresh materials were collected from the field and then dried with silica gel for molecular phylogenetic analyses. Sequences of other species of *Saxifraga* were obtained from GenBank (Appendix 1). The specimen vouchers were deposited in IBK, PE and SYS.

The total DNA of presumed new species was extracted with the modified CTAB method (Doyle and Doyle, 1987). The *psbA-trnH2* intergenic regions and *matK* gene of chloroplast genome were amplified and sequenced following previously described method (Tate and Simpson, 2003; Zhang *et al.*, 2019; Zhao *et al.*, 2019). Sequences were aligned using the program MUSCLE 3.8.31 (Edgar, 2004) and adjusted manually with Bioedit 7.1.3.0 (Hall, 1999). The concatenated data of *psbA-trnH2* and *matK* sequences was conducted using The "Concatenate Sequence" function in PhyloSuite v1.2.2 (Zhang *et al.*, 2020a). Phylogenetic reconstruction was carried out using maximum likelihood (ML) and Bayesian

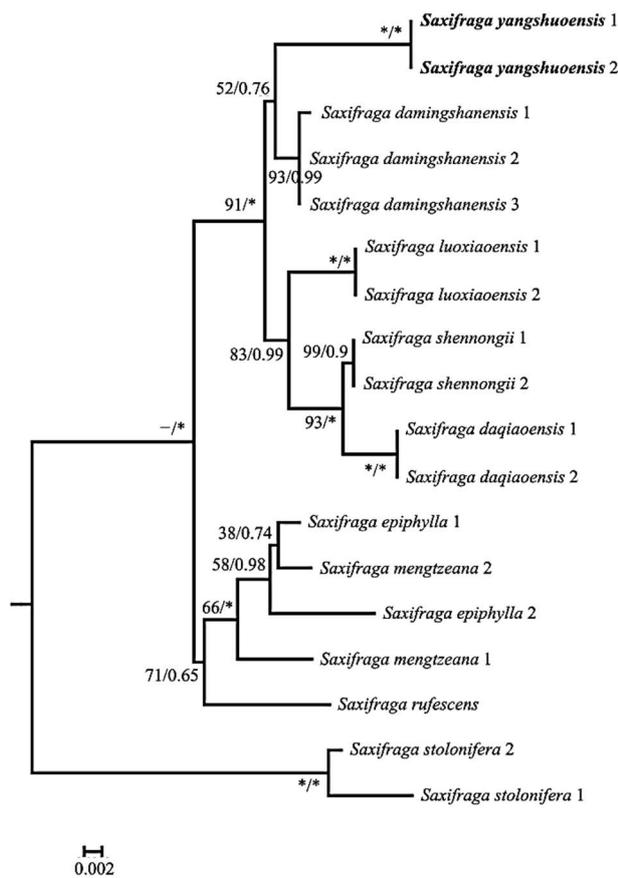


Fig 1. Phylogenetic inference of *Saxifraga yangshuoensis* and related species derived from two chloroplast regions. Numbers above branches are bootstrap value of the Maximum Likelihood (LP) and Bayesian posterior probability (PP), in which LP=100 and PP=1.00 are indicated with an asterisk (*). The new species is shown in bold. Dash above branches indicates no bootstrap value.

inference (BI) methods. ML analysis were performed using RAxML 7.0.3 (Stamatakis *et al.*, 2008), and GTR+G model detected by the jModeltest 2.1.7 was the best-fit model (Darriba *et al.*, 2012). BI analysis was inferred using MrBayes 3.2 implemented in PhyloSuite v1.2.2 (Ronquist *et al.*, 2012; Zhang *et al.*, 2020a). The analysis was under GTR+G model, running for 2,000,000 generations, in which the initial 25% of sampled data were discarded as burn-in.

RESULTS AND DISCUSSION

The concatenated data used for phylogenetic reconstruction had a length of 991 characters (*psbA-trnH2*: 292 characters, *matK*: 699 characters) and 65 parsimony-informative sites were detected. Both methods of maximum likelihood (ML) and Bayesian inference (BI) based on concatenated data generated congruent phylogenetic relationships (Fig. 1). Nine species of *Saxifraga* sect. *Irregulares* were divided into two clades, corresponding to Ser. *Stonoliferae* J.T. Pan (1991) and

Ser. *Rufescentes* Balf. f. (1991). The clade of Ser. *Stonoliferae* includes only one species, *S. stolonifera* Curt. (1774). The clade Ser. *Rufescentes* was divided into two subclades again. One subclade contained *S. epiphylla* Gornall & H. Ohba (2000), *S. mengtzeana* Engl. & Irmsch. (1913), *S. rufescens* Balf.f. (1916), another subclade included *S. damingshanensis* W.B.Liao, W.Y.Zhao & J.H.Jin (2019), *S. shennongii* L. Wang, W.B. Liao et J.J. Zhang (2019), *S. luoxiaoensis* W. B. Liao, L. Wang & X. J. Zhang (2018), *S. daqiaoensis* W. B. Liao, L. Wang & X. J. Zhang (2008) and the unknown species. The phylogenetic tree also indicates that newly named species is most closely related to *S. damingshanensis* (LP: 52; PP: 0.76), and this two species were strongly supported as sister to *S. shennongii*, *S. luoxiaoensis* and *S. daqiaoensis* (LP: 91; PP:1).

Phylogenetic results suggested a close relationship within the new species and *S. damingshanensis* (Fig. 1). Morphologically, these two species share such features as foliar absent, leaf base cordate and mature leaves with glandular trichomes. However, the new species could be distinguished from the latter by its leaves revolute when dried (vs. not revolute), leaf margin usually subentire or slightly undulate (vs. 5–10-lobed, lobes entire), petiole base sheathed and margin sparsely very short glandular trichomes (ca. 0.3 mm) or nearly glabrous (vs. margin with sparsely glandular trichomes (ca. 2.5 mm)), petals apex greenish (vs. white), apex obtuse to subacuminate of two longer petals (vs. acuminate) (Table 1, Fig. 2 & 3). Furthermore, these two species have different phenology and geographical (Table 1). The *S. damingshanensis* is endemic to Damingshan National Nature Reserve, south-central Guangxi, and flowering from August to October (Zhao *et al.*, 2019). While, the new species is only found in Yangshuo Country, northeast Guangxi, and flowering from September to December.

Morphological observation also indicated that *S. yangshuoensis* is similar to *S. shennongii*. They are stolons absent, and leaves abaxially usually concolorous and with spots, smallest petals base with yellow spotted. However, the new species can be easily distinguished from *S. shennongii* by its leaves thickly papery (vs. leathery), slightly revolute when dried (vs. not revolute), margin subentire (vs. 5–10-lobed), abaxially surface of mature leaves with glandular trichomes (vs. glabrous), and the base of petiole sheathed (vs. not sheathed), petals apex greenish (vs. white), shortest petals base with a claw ca. 0.5 mm (vs. ca. 0.7 mm) (Table 1, Fig. 2 & 3). Moreover, the new species flowering from September to December, while *S. shennongii* flowering from March to May (Zhang *et al.*, 2019). More detailed morphological comparisons between the new species and allied species are shown in Table 1.

In conclusion, both the evidences of morphological observation and molecular phylogenetic analysis were supported that the unknown *Saxifraga* founded in Yangshuo

**Table 1.** Diagnostic characters of *Saxifraga yangshuoensis* and comparison with other related species of sect. *Irregulares*.

Characters	<i>S. yangshuoensis</i>	<i>S. damingshanensis</i>	<i>S. shennongii</i>
Leaf texture	hickly papery, slightly revolute when dried	papery or leathery, not revolute	leathery, not revolute
Petiole of mature leaf	with glandular trichomes, base nearly glabrous	with glandular trichomes	glabrous, only base with glandular hairy
Leaf margin	subentire, slightly undulate	5–10-lobed, lobes entire	5–10-lobed, lobes entire
Adaxially surface of leaves	green, with glandular hispid (2–3.5 mm)	dark green, with glandular trichomes (2.5–4 mm)	green, densely strigose when young, sparsely when old
Abaxially surface of leaves	pale green, with glandular trichomes and pale green or occasionally purple spots	grey, with glandular trichomes and purple spots	white-green, glabrous, with canary yellow spots
Petiole base	petiole base sheathed, margin sparsely very short glandular trichomes (ca. 0.3 mm) or nearly glabrous	petiole base sheathed, margin sparsely glandular trichomes (ca. 2.5 mm)	petiole base not sheathed
Inflorescence	20–40 cm long, 10–40-flowered	15–30 cm long, 10–35-flowered	15–35 cm long, 12–45-flowered
Branches length	4–17 cm	4–5 cm	5–10 cm
Sepals	reflexed	spreading	spreading to reflexed
Petals	apex greenish; shortest 3 petals ovate, 3.5–5 × 1.5–2 mm, base with a claw ca. 0.5 mm; the other two petals lanceolate, apex obtuse to subacuminate, 3-veined; longer petal 10–17 × 1.5–2 mm; longest petal 12–21 × 1.5–2 mm.	apex white; shortest 3 petals ovate, 3–3.5 × 1.5 mm; the other two petals lanceolate, apex acuminate, first longest petal lanceolate, 3-veined; longer petal 13–17 × 2 mm; longest petal 18–22 × 1.5–2.5 mm.	apex white; shortest 3 petals triangular-ovate, 3–3.5 × 1–2 mm, base with a claw ca. 0.7 mm; the other two petals narrowly ovate or lanceolate, apex acuminate, 3–5-veined; longer petal 6–13 × 2 mm; longest ca. 18–23 mm × 2–4 mm.
Flowering period	September to December	August to October	March to May

country is a new species. The new species should be placed in sect. *Irregulares* ser. *Rufescentes* in consideration of its zygomorphic flowers and stolons absent. Until now, *Saxifraga* sect. *Irregulares* has increased to 15 species, provided that the newly described species is counted.

TAXONOMIC TREATMENT

Saxifraga yangshuoensis Hai L. Chen, W.B. Xu & Yan Liu, *sp. nov.* **Figs. 2 & 3A**

Type: CHINA. Guangxi. Guilin City, YangShuo County, JinBao Town, 110.2542°E, 24.8182°N, 566 m alt., 10 Oct 2021, *Hailing Chen, Ying Qin and Jinqian Huang, YS0038* (Holotype: IBK!; Isotypes: IBK!, PE!, SYS!).

Diagnosis: *Saxifraga yangshuoensis* is morphologically similar to *S. damingshanensis* and *S. shennongii*. The main distinctive characters of *S. yangshuoensis* and the latter two is leaves thickly papery, slightly revolute when dried, margin subentire, margin of petiole base sparsely very short glandular trichomes or nearly glabrous, and petals apex greenish.

Description: Perennial herbs, 20–40 cm tall. Stolons absent. **Rhizomes** rather short. **Basal leaves** forming a rosette; petiole 5–10 (15) cm long, fleshy, densely short glandular trichomes (ca. 1 mm long), **petiole** base sheathed, glabrous, margin sparsely very short glandular trichomes (ca. 0.3 mm) or nearly glabrous; **leaf blade** rounded or ovate, apex rounded or obtuse, base deep cordate, thickly papery, 3.0–5.0 (8.3) × 2.5–5.5 (7.5) cm, margin subentire, slightly undulate (occasionally inconspicuous 7–13-lobed), with sparsely glandular trichomes, slightly revolute when dried, adaxially green, densely covered glandular hispid (2–3.5 mm long),

abaxially pale green, sparsely covered with glandular trichomes (1–2 mm long), densely covered with pale green or occasionally purple spots; palmate veins 7–9, both surfaces inconspicuous. **Inflorescence** paniculate, 20–40 cm long, 10–40-flowered; **branches** 4–17 cm long, sparsely short glandular trichomes (0.5–1 mm), 2–8-flowered; **pedicels** slender, 1–2 cm long, sparsely short glandular trichomes (0.3–0.5 mm long); **bracts** 4–8, lanceolate to narrowly triangular, 3.0–1.0 × 1.0–2.5 mm, margin with sparsely glandular trichomes. **Flowers** zygomorphic; **sepals** 5, reflexed, narrowly ovate, 2.0–3.0 × 1.0–1.5 mm, adaxially glabrous, abaxial surface and margin with sparsely short glandular trichome, 3 veins inconspicuous. **Petals** 5, white, apex greenish; shortest 3 petals ovate, base with yellow spots, 3.5–5 × 1.5–2 mm, apex acute, base with a claw ca. 0.5 mm long, triplinerved; the other two petals lanceolate, apex gobtuse to subacuminate, margin entire, glabrous, 3-veined; longer petal 10–17 × 1.5–2 mm; longest petal 12–21 × 1.5–2 mm. **Stamens** 10, filaments clavate, ca. 0.4 mm long. **Ovary** ovoid, 1.5–2.5 mm long; **styles** 2, divergent, 1.5–2 mm long. **Capsule** ovoid, ca. 3.5 × 3 mm. **Seeds** elongate-ellipsoid, dark brown, ca. 0.3 mm long.

Distribution and ecology: The new species is currently known to YangShuo County Guilin City, northeastern Guangxi, China, and grows on damp cliffs and rocks nearby valleys in broad-leaved forests, alt. 370–570 m.

Conservation status: *Saxifraga yangshuoensis* is currently known from Yangshuo with approximately 200 mature individuals. Though the population is close to the village, *S. yangshuoensis* was well protected due to its habitat located in the forest of water sources for villagers. Similar habitat were found northeast Guangxi and southern

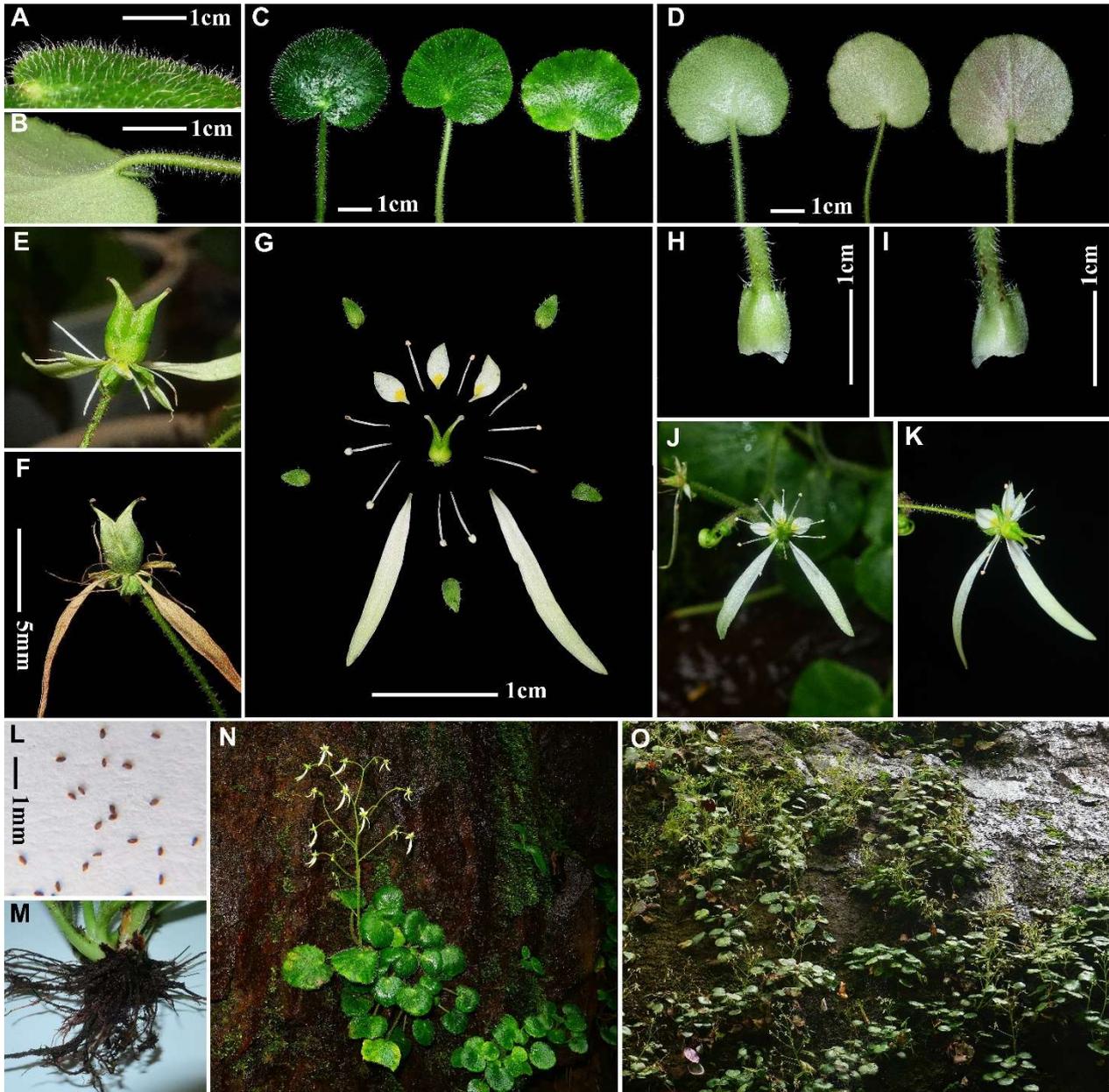


Fig 2. *Saxifraga yangshuoensis* Hai L. Chen, W.B. Xu & Yan Liu. **A.** Adaxially covered glandular hispid; **B.** Abaxially covered with glandular trichomes; **C.** Adaxially surface of leaves; **D.** Abaxially surface of leaves; **E-F.** Fruit; **G.** Detail of flower; **H.** Adaxial surface of sheath; **I.** Abaxial surface of sheath; **J-K.** Flower, front and side view; **L.** Seeds; **M.** Rhizome; **N.** Habit; **O.** Habitat, on moist rocks.

Hunan. Therefore, more field investigations are needed to determine its wild distribution and individuals. We suggest that the new species should be considered as Data Deficient (DD) based on the IUCN Red List Criteria (IUCN, 2012).

Phenology: Flowering was observed from September to December, fruiting from November to the following January.

Etymology: The species epithet is derived from the type locality Yangshuo County, Guilin City, Guangxi, China.

Vernacular name: 陽朔虎耳草 (Chinese pinyin: yáng shuò hǔ ěr cǎo).

Additional specimens examined (paratype): CHINA. Guangxi. Guilin City, Yangshuo County, Jinbao Town, 25 September 2018, *Yangshuo Exped*, 450321180925006LY (IBK!).

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Fig 3. Photograph of the holotype of *Saxifraga yangshuoensis* (A), *S. damingshanensis* (B) and *S. shennongii* (C).

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Appendix 1. GenBank accessions for phylogenetic analysis.

Species	<i>matK</i>	<i>psbA-trnH2</i>
<i>Saxifraga yangshuoensis</i>	ON918633	ON873738
	ON918633	ON873739
<i>Saxifraga damingshanensis</i>	MK976729	MK976727
	MK976732	MK976728
	MK976733	MK976724
<i>Saxifraga mengtzeana</i>	MK092518	MF100607
	LC534874	MF100646
<i>Saxifraga stolonifera</i>	MK092557	MK092599
	MK092551	MK092593
<i>Saxifraga epiphylla</i>	MK092519	MK686010
	MK092520	MK686002
<i>Saxifraga daqiaoensis</i>	MK092533	MK092575
	MK092534	MK092576
<i>Saxifraga luoxiaoensis</i>	MK092539	MK092581
	MK092540	MK092582
<i>Saxifraga shennongii</i>	MK092527	MK092569
	MK092528	MK092570
<i>Saxifraga rufescens</i>	MH116857	MH117313