



Iris calcicola (Iridaceae), a new species from limestone areas of northern Guangxi, China based on morphological and molecular evidence

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ABSTRACT: *Iris calcicola* Z.C.Lu, Z.P.Huang & Yan Liu, a new species of *Iris* sect. *Lophiris* was found from limestone areas of Guangxi, China. *Iris calcicola* is similar to *Iris japonica* Thunb., but differs by its inflorescence simple; flowering stems ascending, with 2–5 branches; spathes 2, narrowly lanceolate, 2–3.8 cm long, 1–2 (3)-flowered, apex acuminate; flower segments obliquely ascending, not spreading when blooming; pedicel enveloped by spathes or subequal to spathes; outer segments elliptic, with prominent, irregular, yellow crest.

KEY WORDS: *Iris japonica*, *Iris formosana*, *Iris* sect. *Lophiris*, ITS, molecular phylogeny, taxonomy, *trnL-F*.

INTRODUCTION

Iris L. (Linnean, 1753) belongs to Iridaceae, and distributed in temperate regions of the northern hemisphere. There are about 300 species of *Iris* in the world and about 60 species in China. *Iris* is mainly distributed in southwest, northwest and northeast China (Zhao, 1985; Zhao *et al.*, 2000).

During the investigation of medicinal plant resource in April 2019, we collected a flowering species of *Iris* from limestone shrubs in Rong'an County, northern Guangxi, China. After carefully checked the morphological characters of the flowers outer segments, we confirmed it is an unknown species of *Iris* sect. *Lophiris* Tausch (Tausch, 1823) in *Iris* subgen. *Limniris* (Tausch)Spach (Spach, 1846). There are eight species of *Iris* sect. *Lophiris* in China (Zhao *et al.*, 2000). After that, we consulted relevant literature (Zhao, 1980; Zhao, 1985; Ying, 2000; Zhao *et al.*, 2000; Dong and Li, 2008; Crespo *et al.*, 2015; Wilson, 2020; Xiao *et al.*, 2021) and analyzed the molecular phylogenetic relationships, we finally confirmed it is a new species, and described below.

MATERIALS AND METHODS

We sampled three individuals of *Iris calcicola* from the four currently known populations for molecular phylogenetic analyses. The nuclear ribosomal internal transcribed spacer (ITS) and *trnL-F* intron-spacer were selected for phylogenetic analyses. Total genomic DNA was extracted from silica-gel dried leaves using the CTAB method according to Doyle and Doyle (1987). The polymerase chain reaction (PCR) procedures described in Guo *et al.* (2016) were followed. The sequences were manually checked and edited according to the corresponding chromatograms generated by sequencing

from both directions. All the samples were successfully amplified and sequenced and a total of six sequences (three ITS sequences and three *trnL-F* sequences, respectively) were newly obtained. The newly acquired sequences have been submitted to GenBank.

In addition, 41 sequences (18 ITS sequences and 23 *trnL-F* sequences) from species of *Iris* and *Gladiolus* were downloaded from GenBank and used in the following phylogenetic reconstruction. *Gladiolus imbricatus* L. (Linnean, 1753) and *Gladiolus palustris* Gaudin (Gaudin, 1828) were selected as outgroups. In total, 47 accessions of 24 species were included in the analyses. The GenBank accession numbers of the downloaded sequences are listed in Table 1.

DNA sequences were aligned using the program MUSCLE 3.8.31 (Edgar, 2004) and adjusted manually in Bioedit 5.0.9 (Hall, 1999). A few sites which were ambiguously aligned even after the manual adjustment were pruned before phylogenetic analyses. We reconstructed the phylogeny using maximum likelihood (ML) and Bayesian inference (BI). Firstly, we reconstructed the maximum likelihood trees based on the ITS and *trnL-F* data, respectively, and compared the ML trees to check whether any phylogenetic conflict existed between the plastid and nuclear data. Although there were some phylogenetic conflicts found among other species, the positions of the *Iris calcicola* were relatively stable between the plastid and ITS trees, therefore, we reconstructed phylogeny based on the concatenated data to further test the affinity of the new species. ML analyses were performed using RAXML-VI-HPC (Stamatakis, 2006) with the substitution model GTR+G and 1000 rapid bootstrap searches (BS). Bayesian analyses (BI) were conducted in MrBayes 3.2.6 (Fredrik *et al.*, 2012) with the optimal substitution model K81uf+I+G selected by ModelTest (Posada and Crandall, 1998) according to the

**Table 1.** The information of the taxa included in molecular phylogenetic analyses. Newly generated sequences of taxa are highlighted in bold.

Species	GenBank accession numbers	
	ITS	<i>trnL-F</i>
<i>Gladiolus imbricatus</i>	MK005907	KM887321
<i>Gladiolus palustris</i>	MK005919	KM887312
<i>Iris calcicola-1</i>	MW520180	MW523023
<i>Iris calcicola-2</i>	MW520181	MW523024
<i>Iris calcicola-3</i>	MW520182	MW523025
<i>Iris confusa</i>	-	KC510943
<i>Iris delavayi</i>	AF488751	LT984477
<i>Iris dichotoma</i>	DQ277638	LT984483
<i>Iris ensata</i>	DQ277637	LT628002
<i>Iris formosana</i>	-	KC510945
<i>Iris forrestii</i>	AF488752	LT984478
<i>Iris japonica</i>	MH703374	KC510948
<i>Iris laevigata</i>	DQ277643	EU939481
<i>Iris mandshurica</i>	DQ277642	DQ286795
<i>Iris milesii</i>	-	KC510951
<i>Iris proantha</i>	-	KC510952
<i>Iris pseudacorus</i>	DQ277646	MN481446
<i>Iris rossii</i>	KT595305	KF170877
<i>Iris ruthenica</i>	DQ277640	KF170873
<i>Iris sanguinea</i>	DQ277636	LC373221
<i>Iris setosa</i>	KC118894	KF170886
<i>Iris sibirica</i>	MF543721	EU939499
<i>Iris tectorum</i>	MH711021	KY319459
<i>Iris uniflora</i>	DQ277641	LT628009
<i>Iris versicolor</i>	MW040469	EU939518
<i>Iris watti</i>	-	KY319460

Akaike Information Criterion (AIC). All BI analyses were run for 100,000,000 generations with four chains in two parallel runs and sampled every 5000 generations by a burn-in of the first 5000 trees. The convergence of the two parallel runs was guaranteed by the splitting frequency less than 0.005. All other parameters were set as default.

RESULTS

The combined matrix used for phylogenetic reconstruction had a length of 1767 characters (*trnL-F*: 886 characters, ITS: 881 characters) including 530 parsimony informative sites (*trnL-F*: 81 characters, ITS: 449 characters), 686 variable but parsimony uninformative sites (*trnL-F*: 140 characters, ITS: 546 characters) and 1081 constant sites (*trnL-F*: 746 characters, ITS: 335 characters). The parameter of consistency index (CI), retention index (RI) and homoplasy index (HI) were 0.743, 0.792 and 0.257 for the combined data (0.832, 0.895, and 0.168, and 0.765, 0.812 and 0.235 for the *trnL-F* and ITS data, respectively).

All *Iris* taxa formed a monophyletic clade in the combined data trees (BS = 99%; PP = 1.00) (Fig. 1). In the tree, *Iris calcicola* formed as a monophyletic lineage

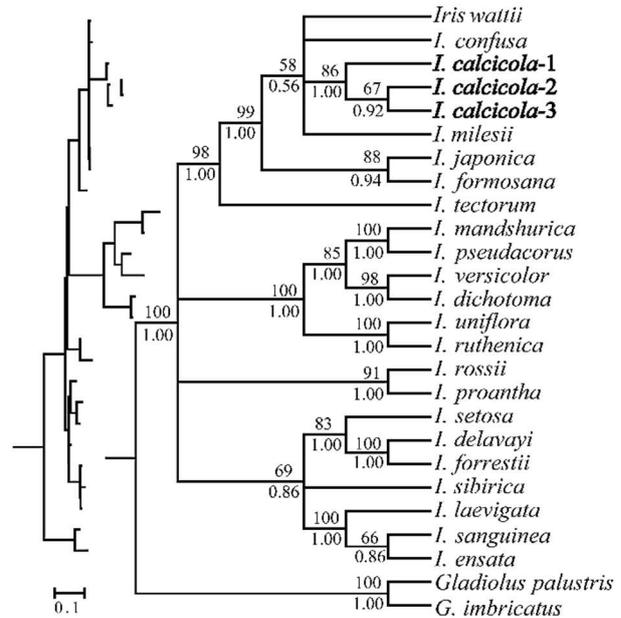


Fig. 1. The best ML tree from the analyses of combined ITS and chloroplast *trnL-F* region. BI posterior probability/ML bootstrap support values (>0.5 or 50%) are shown below and above the branch around the corresponding node. *Iris calcicola* is highlighted in bold.

(BS = 86%; PP = 1.00), forming a complete polytomy with *Iris watti* Baker ex Hook.f. (Hooker, 1892), *Iris milesii* Baker ex Foster (Baker, 1883) and *Iris confusa* Sealy (Sealy, 1937) with moderate support values (BS = 58%; PP = 0.56). Although *I. calcicola* with *I. japonica* Thunb. (Thunberg, 1794) and *I. formosana* Ohwi (Ohwi, 1934) are morphologically similar, but they do not cluster together in the phylogenetic tree. Thus, the genetic isolation and diagnostic morphological traits strongly support the separation of *I. calcicola* from its two closely-related species, verifying the validity as the independent new species.

TAXONOMIC TREATMENT

Iris calcicola Z.C.Lu, Z.P.Huang & Yan Liu, *sp. nov.*

岩生鳶尾 Figs. 2–4

Type: CHINA. Guangxi: Liuzhou City, Rong'an County, Siding Town, Siding Village, in the near foot of limestone forests or shrubs, elevation ca. 350 m, 6 April 2019, flowering, *Rong'an Exped. 450224190406055LY* (holotype: IBK00430865; isotype: IBK00430866 and CSH0190464).

Diagnosis: *Iris calcicola* is similar to *Iris japonica* Thunb., but differs from the latter species in its inflorescence simple; flowering stems ascendent, with 2–5 branches; spathes 2, narrowly lanceolate, 2–3.8 cm long, 1–2 (3)-flowered, apex acuminate; flower segments obliquely ascending, not spreading when blooming; pedicel enveloped by spathes or subequal to spathes; outer

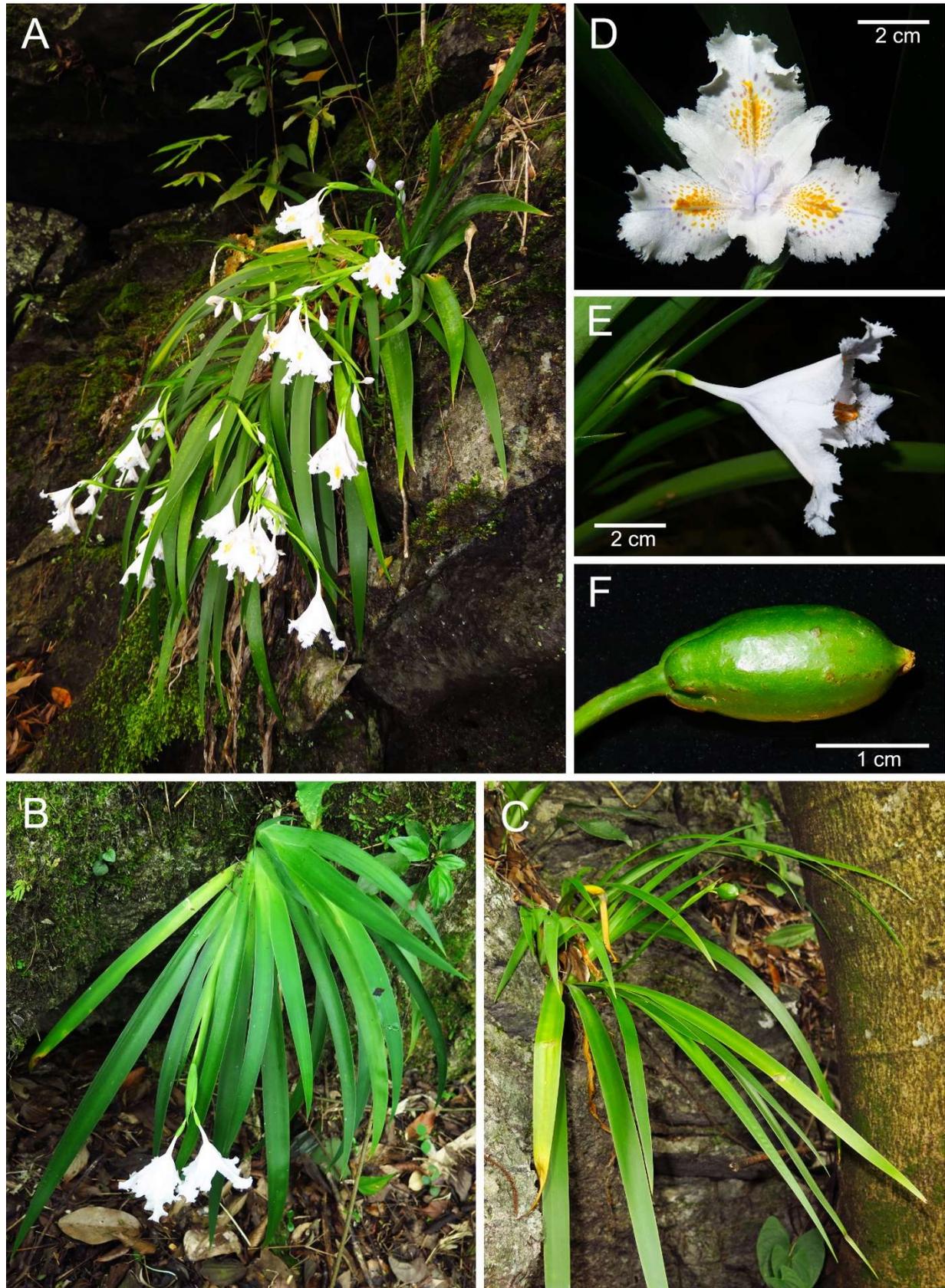


Fig. 2. *Iris calcicola*: A. Habitat, B. Habit with flowers, C. Habit with fruits. D. Flower frontal view, E. Flower lateral view, F. Fruit.



Fig. 3. *Iris calcicola*: A1. Outer segment, A2. Inner segment, A3. Style branch, A4. Perianth tube and stamens, B. Stamens frontal view and dorsal view, C. Opened fruit show seeds, D. Fresh seeds, E. Dry seeds.

segments elliptic, with prominent, irregular, yellow crest.

Description: Perennial herbs. The brown or grayish brown residual fiber of old leaves is surrounding at the base of the plant. **Rhizomes** dimorphic: suberect, inconspicuous, oblate, with many shorter internodes, brown; creeping, internodes long, yellowish white,

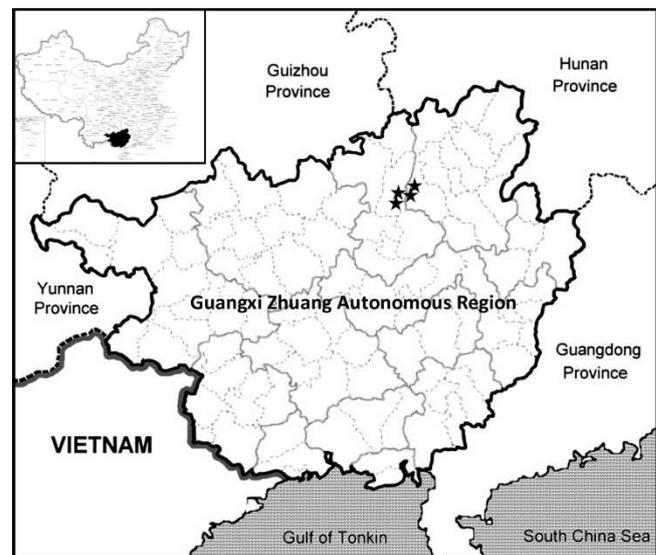
fibrous roots are attached to the nodes of the rhizomes. **Leaves** basal, upper surface bright green, lower surface grayish green, glabrous on both surfaces, sword-shaped, $20\text{--}50 \times 0.7\text{--}2.5$ cm, midvein absent, apex acuminate. **Inflorescence** racemose to racemoso-paniculate; flowering stems ascendent, with 2–5 branches, 15–35 cm

**Table 2.** Comparison among *Iris calcicola*, *I. japonica* and *I. formosana*.

Taxonomic traits	<i>Iris calcicola</i>	<i>I. japonica</i>	<i>I. formosana</i>
Inflorescence	racemose to racemoso-paniculate	racemoso-cymose	racemose to paniculate
Flowering stems	ascendent, with 2–5 branches	erect, with 5–12 branches	erect, with 4 or 5 branches
Spathes	2, narrowly lanceolate, 2–3.8 cm long, 1–2 (3)-flowered, apex acuminate	3–5, broadly lanceolate or oval, 0.8–1.5 cm long, 2–4-flowered, apex obtuse	4–6, 2.5–3 cm long, 3–5-flowered
Flowers	4.5–5 cm in diam., flower segments obliquely ascending, not spreading when blooming	4.5–5.5 cm in diam., flower segments wholly spreading when blooming	7–8 cm in diam, flower segments wholly spreading when blooming
pedicel	enveloped by spathes or subequal to spathes	surpassing spathes	slightly surpassing spathes
outer segments	elliptic, 4–4.5 × 2–2.2 cm	obovate or elliptic, 2.5–3 × 1.4–2 cm	obovate, 4–5 × ca. 2.5 cm
inner segments	elliptic, adaxially glabrous	elliptic or narrowly obovate, adaxially glabrous	oblanceolate to oblong, adaxially dense glandular trichomes

**Fig. 4.** The holotype sheet of *Iris calcicola*.

long, 1 or 2-leaved at the middle and lower part; **spathes** 2, narrowly lanceolate, 2–3.8 cm long, 1–2 (3)-flowered, apex acuminate. **Flowers** white, 4.5–5 cm in diam., segments obliquely ascending, not spreading when blooming; **pedicel** 1.5–3 cm long, enveloped by spathes or subequal to spathes. **Perianth tube** 1.2–2 cm long; **outer segments** elliptic, 4–4.5 × 2–2.2 cm, limb slightly reflexed, with blue-purple lines and yellow or blue-purple spots around central, prominent, irregular, yellow crest, margin denticulate, undulate, base broadly cuneate, apex slightly retuse; **inner segments** elliptic, 3–3.2 × 1.2–1.4 cm, base

**Fig. 5.** The distribution of *Iris calcicola* (★) in Guangxi, China.

broadly cuneate, apex slightly retuse, margin slightly denticulate. **Stamens** ca. 1.5 cm long; anthers linear, white; **filaments** pubescent. **Ovary** fusiform, 6–10 mm long. **Style branches** obovate, 2–2.2 cm × 6–7 mm, middle rib pale blue; terminal lobes fimbriate. **Capsule** ellipsoid-cylindric or ovoid-cylindric, 1–2 × ca. 1.2 cm, apex not beaked. **Seeds** black brown, 2.5–3 × 1.5–2 mm, without aril.

Phenology: Flowering March to April; fruiting May to June.

Etymology: The specific epithet ‘*calcicola*’ refers to the limestone habitats of this new species.

Distribution and habitat: *Iris calcicola* has only been collected from limestone areas of northern Guangxi, China (Fig. 5). It grows in the near foot of limestone forests or shrubs, at an elevation of 300–500 m.

Additional specimen examined (paratypes): CHINA. **Guangxi:** Guilin City, Yongfu County, Sanhuang Town, Qingshui Village, 25 March 2013, fruiting, *Yongfu Exped.* 450326130325050LY (IBK!, GXMG!); Guilin City, Yongfu County, Baishou Town, Jiangxi Village, 11 June 2014, fruiting, *Yongfu Exped.* 450326140611049LY (IBK!, GXMG!); Liuzhou City, Rong’an County, Qiaoban Township,



Gudan Village, 8 April 2019, flowering, *Rong'an Exped. 450224190408023LY* (IBK!); *ibid.*, 22 June 2020, fruiting, *Q. Zhang & Z.P. Huang 20200622001* (IBK!).

Notes: *Iris calcicola* can easily distinguished from the other species of *Iris* sect. *Lophiris* in its leafy stems inconspicuous; inflorescence simple; flowering stems ascendent, with 2–5 branches; flower segments obliquely ascending, not spreading when blooming. Phylogenetic analyses show that *I. calcicola* closely related with *Iris wattii*, *I. milesii* and *Iris confusa* (Fig. 1), but *I. calcicola* differs the latter from the characters of leafy stems inconspicuous; leaves basal. *I. calcicola* is similar to *I. japonica* and *I. formosana* in morphological characters (Fig. S1). The morphological differences of *I. calcicola*, *I. japonica* and *I. formosana* are summarized in Table 2.

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Supplementary materials are available from Journal Website.