

Glabrella bogaoi, a new species of Gesneriaceae from southern China

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ABSTRACT: Based on morphological and molecular data, *Glabrella bogaoi*, a new species from limestone areas in southern China, is described and illustrated here. *G. bogaoi* is morphologically distinct from the other species of *Glabrella* by its stems, which can grow up to 5–30 cm, calyx segments that are adpressed to the corolla base, and corolla outside white. It is morphologically most similar to *G. mihieri*. Furthermore, a phylogenetic analysis of the combined matrix of *trnL-F* and ITS sequences, including 4 taxa of *Glabrella*, has been provided. The phylogenetic analysis indicates that *G. bogaoi* is an independent new species. A table and a key to distinguish the new species from all other *Glabrella* species are also provided.

KEY WORDS: Glabrella mihieri, Glabrella longipes, Glabrella leiophylla, limestone flora, molecular taxonomy, morphology.

INTRODUCTION

Based on molecular evidence and morphological characteristics (combination of indistinct short stems, 5–6 cm long, glabrous petiole and leaf blade), the genus *Glabrella* Mich.Möller & W.H.Chen was established in 2014 and initially contained only two species, *viz. G. longipes* (Hemsl.) Mich.Möller & W.H.Chen and *G. mihieri* (Franch.) Mich.Möller & W.H.Chen, two species that were formerly placed in *Briggsia* Craib (Möller *et al.*, 2014). In 2015, one more species was added to the genus: *G. leiophylla* (F.Wen & Y.G.Wei) F.Wen, Y.G.Wei & Mich.Möller (Wen *et al.*, 2015a,b). These species are currently found only in limestone areas of southern Chinese provinces: NW Guangxi, SE Yunnan, Guizhou, S Sichuan, S Chongqing, SW Hubei (Fig. 1) (Wang *et al.*, 1998; Wen *et al.*, 2015a,b).

During the fieldwork in Guangxi and Guizhou, China, in July and October 2014, and April 2020, the authors discovered an unidentified flowering plant of Gesneriaceae and suspected that it might be a new species of *Glabrella*, based on its vegetative and floral characteristics. After consulting the *Flora of China* (Wang *et al.*, 1998) and other relevant literature (Möller *et al.*, 2014; Wen *et al.*, 2015a,b), as well as comparing this unknown species with its morphologically similar species and performing molecular phylogenetic analyses, the authors concluded that this species was clearly different from the currently known *Glabrella* species (Fig. S1). Here, the authors describe and illustrate this new species and discuss the morphological and molecular phylogenetic evidence upon which taxonomic conclusions are based.

MATERALS AND METHODS

Morphological characters of the presumed new species were observed and measured in the field and from

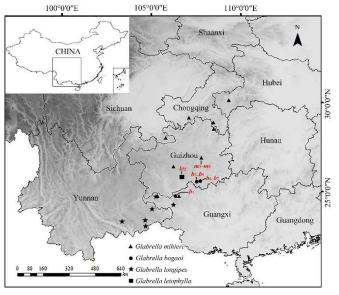


Fig. 1. Geographic distributions of *Glabrella bogaoi* (circle), *G. leiophylla* (square), *G. longipes* (star) and *G. mihieri* (triangle) in China. The red font represent the molecular samples for this study (e.g. *b*1 for *Glabrella bogaoi*-1, etc).

herbarium specimens. For molecular work, silica-gel dried leaves were collected from diverse localities including the type locality of *Glabrella leiophylla* (Table 1). Voucher specimens of the newly collected material of the species are deposited in IBK and PE.

The authors sampled seven individuals of *Glabrella bogaoi* from three populations and collected three individuals of *G. mihieri*, one individual of *G. longipes*, and one individual of *G. leiophylla* for molecular phylogenetic analysis (Table 1). Total genomic DNA was extracted from silica-gel dried leaves using the CTAB method according to Doyle and Doyle (1987). Primers for the ITS (White *et al.*, 1990) and *trnL-F* (Taberlet *et al.*, 1991) regions were chosen for phylogenetic analyses as



Table 1. Taxon, DNA samples collection sites and GenBank accession numbers of the sequences in this study.

Taxon	ITS	trnL-F	Sample origin
Anna mollifolia (W.T.Wang) W.T.Wang &	KJ475421	FJ501543	_
K.Y.Pan			
A. ophiorrhizoides (Hemsl.) B.L.Burtt & R.A.Davidson	MK747107	KM062907	_
Glabrella bogaoi sp. nov1	MW029485	MW031096	YC1498, Leve County, Baise, China (holotype locality)
	MW029485	MW031096	
G. bogaoi sp. nov2			ZCY1131, Dushan County, Guizhou, China
G. bogaoi sp. nov3	MW029487	MW031098	ZCY1132, Dushan County, Guizhou, China
G. bogaoi sp. nov4	MW029488	MW031099	ZCY1133, Dushan County, Guizhou, China
G. bogaoi sp. nov5	MW029489	MW031100	ZCY1134, Dushan County, Guizhou, China
G. bogaoi sp. nov6	MW029490	MW031101	ZQ2020043001, Libo County, Guizhou, China
G. bogaoi sp. nov7	MW029491	MW031102	ZQ2020043002, Libo County, Guizhou, China
G. leiophylla (F.Wen & Y.G.Wei) F.Wen, Y.G.Wei & Mich.Möller-1	KY288032		<i>HMQ412</i> , Huishui County, Guizhou, China (holotype locality)
<i>G. leiophylla</i> (F.Wen & Y.G.Wei) F.Wen,	MW029493	MW031104	HZP20200921, Huishui County, Guizhou, China
Y.G.Wei & Mich.Möller-2			(holotype locality)
G. longipes (Hemsl.) Mich.Möller & W.H.Chen-1	GU350653	FJ501545	00403 (GU350653) and <i>MMO 01-122</i> (FJ501545),
			Xichou County, Yunnan, China
G. longipes (Hemsl.) Mich.Möller & W.H.Chen-2	MW029492	MW031103	Cultivated, Locality unknown
G. mihieri (Franch.) Mich.Möller & W.H.Chen-1	GU350646	GU350678	CQ-JFS-03, County unknown, Yunnan, China
G. mihieri (Franch.) Mich.Möller & W.H.Chen-2	FJ501363	FJ501544	Wang 11315B, Chongqing, China
G. mihieri (Franch.) Mich.Möller & W.H.Chen-3	MW029482	MW031105	HZP2020092201, Huangping County, Guizhou, China
G. mihieri (Franch.) Mich.Möller & W.H.Chen-4	MW029483	MW031106	HZP2020092202, Huangping County, Guizhou, China
G. mihieri (Franch.) Mich.Möller & W.H.Chen-5	MW029484	MW031107	HZP2020092203, Huangping County, Guizhou, China
<i>Hemiboea fangii</i> Chun ex Z.Y.Li	KY288033	HQ632882	_
<i>H. rubribracteata</i> Z.Yu Li & Yan Liu	MN334646	HQ632890	_
Lysionotus pauciflorus Maxim.	KY288027	FJ501497	_
L. sangzhiensis W.T.Wang	KJ475422	KM232653	_
Petrocosmea kerrii Craib	JN092441	FJ501502	_
P. nervosa Craib	JN092446	AJ492299	_

Note: A dash (—) indicates missing data; Newly generated sequences of taxa are highlighted in bold; The numbers at the end of the taxon names represent the molecular sample number of the species, corresponding to Fig. 2.

these two loci were the most frequently used in phylogenetic studies of *Glabrella* as well as other Gesneriaceae (Möller *et al.*, 2011). PCR conditions were an initial denaturation at 94 °C for five min, followed by 35 cycles of 30 s at 94 °C, 30 s at 52 °C, and one min at 72 °C, with a final extension at 72 °C for 10 min. Subsequent steps were followed as described in Huang *et al.* (2018). All the samples were successfully amplified and sequenced and a total of 24 sequences (12 ITS sequences and 12 *trnL-F* sequences, respectively), were newly obtained.

The authors queried the newly generated ITS and *trnL-F* sequences in GenBank using BLAST searches and found that the most similar sequences were those of *Glabrella leiophylla* (MW029493 and MW031104), indicating that *G. bogaoi* should indeed be a member of *Glabrella*. And then downloaded a total of 23 sequences (12 ITS sequences and 11 *trnL-F* sequences) from GenBank, of *Glabrella*, *Hemiboea* C.B.Clarke (Clarke, 1888), *Anna* Pellegr. (Pellegrin, 1930), *Lysionotus* D.Don (Don, 1822) and *Petrocosmea* Oliv. (Oliver, 1887). In total, 16 samples of *Glabrella* were included to test the monophyly of the genus, the molecular delineation of the new and existing *Glabrella* species and their phylogenetic

relationships within the genus. Eight species, *Lysionotus* sangzhiensis W.T.Wang (Wang, 1986), *L. pauciflorus* Maxim. (Maximowicz, 1874), *Hemiboea rubribracteata* Z.Y.Li & Yan Liu (Li and Liu, 2004), *H. fangii* Chun ex Z.Y.Li (Li, 1983), *Anna ophiorrhizoides* (Hemsl.) B.L.Burtt & R.A.Davidson (Burtt and Davidson, 1955), *A. mollifolia* (W.T.Wang) W.T.Wang & K.Y.Pan (Wang et al., 1990), *Petrocosmea kerrii* Craib (Craib, 1918) and *P. nervosa* Craib (Craib, 1919), were selected as the outgroup with the *Petrocosmea* species (the most distant outgroup taxa) set to root the tree according to previous studies (Möller et al., 2009, 2011). The GenBank accession numbers of the downloaded and newly generated sequences are listed in Table 1.

The DNA sequences were aligned using the program MUSCLE 3.8.31 (Edgar, 2004) and adjusted manually in Bioedit 5.0.9 (Hall, 1999). Seven sites which were ambiguously aligned after manual adjustment were pruned before phylogenetic analyses. The authors reconstructed phylogenies using maximum likelihood (ML) and Bayesian inference (BI) analyses. First, the authors reconstructed ML trees based on the ITS and *trnL-F* data, respectively, and compared the ML topologies to check whether any phylogenetic conflict



Characters	G. bogaoi	G. mihieri	G. longipes	G. leiophylla
Stem	Stems to 5-30 cm	Stems indistinct or stems to 5 cm	Stems indistinct or stems to 6 cm	Stems indistinct
Leaf blade margin	Irregularly crenate to serrate	Irregularly crenate to serrate	Entire to shallowly serrate	Entire
Leaf blade shape	Narrowly obovate to obovate or oblong; apex subacute; base narrowly cuneate to broadly cuneate, not decurrent	Narrowly obovate to obovate or elliptic; apex rounded to acute; base cuneate, not decurrent	Narrowly obovate to elliptic to broadly ovate; apex obtuse to acute; base peltate or rounded to nearly cuneate, not decurrent	Lanceolate, narrow obovate or obovate, oblong, rounded, occasionally spathulate; apex, acute, subacute, obtuse, rounded, mucronate or occasionally retuse; base cuneate, decurrent
Cymes	1–7-flowered	1–5-flowered	1-5(-7)-flowered	1-flowered, rarely 2
Peduncle Calyx	2.5–8.5 cm Segments usually equal, ovate, 6–8×3–4 mm; adpressed to corolla base	(5–)8–17 cm Segments usually equal, narrowly ovate to narrowly triangular, 4–7×1.5–3 mm; spreading		5–9 cm or longer Segments unequal, lanceolate, the top central one ca. 6 mm long, base ca. 1.5 mm wide, the others ca. 6 mm long, base ca. 2 mm wide; spreading
Corolla (outside)	White	Blue-purple or pale purple to pale yellow		Pale yellow to bright yellow
Filament	Sparsely glandular pubescent	Sparsely glandular pubescent	Glabrous	Glabrous
Style	Glandular pubescent	Glabrescent to sparsely pubescent	Glabrous	Glandular pubescent

Table 2. Morphological comparison of Glabrella bogaoi, G. mihieri, G. longipes and G. leiophylla.

existed between the plastid *trnL-F* and nuclear ITS data. The phylogenetic relationships were essentially congruent between the plastid and ITS ML trees, with slight topological differences outside of Glabrella concerning nodes with weak support. Therefore, the authors reconstructed a phylogeny based on the concatenated trnL-F and ITS data. ML analyses were performed using RAxML-VI-HPC (Stamatakis, 2006) with the substitution model GTR+G detected by ModelTest (Posada and Crandall, 1998) according to the Akaike Information Criterion (AIC) and 1000 rapid bootstrap searches (BS). Bayesian analyses were conducted using MrBayes 3.2.6 implemented in PhyloSuite v1.2.3pre3 (Ronquist et al., 2012; Zhang et al., 2020). And the two regions (ITS and trnL-F) were assigned to its own optimal nucleotide substitution model (HKY+F+I and GTR+F+G4) detected by plug-in function ModelFinder of PhyloSuite v1.2.3pre3. All BI analyses were run for 100,000,000 generations with four chains in two parallel runs and sampled every 5000 generations with a burn-in of the first 5000 trees. The convergence of the two parallel runs was determined by a splitting frequency less than 0.005. All other parameters were set as default.

RESULTS AND DISCUSSION

Morphological comparisons

Glabrella bogaoi is morphologically distinct from the other species of Glabrella by its stems, which can grow up to 5-30 cm (vs. indistinct stems up to 5-6 cm in G. mihieri and G. longipes, and indistinct stems in G. leiophylla). Additionally, G. bogaoi has calyx segments that are adpressed to the corolla base (vs. they spread in

G. mihieri, *G. longipes* and *G. leiophylla*), and corolla outside white (*vs.* blue-purple or pale purple to pale yellow in *G. mihieri*, pale purple in *G. longipes*, and pale yellow to bright yellow in *G. leiophylla*) (Table 2).

Phylogenetic analyses

The combined matrix of trnL-F and ITS data used for phylogenetic reconstruction had a length of 1679 characters (ITS: 743 characters; trnL-F: 936 characters) including 273 parsimony informative sites (trnL-F: 48 characters, ITS: 225 characters), 128 variable but parsimony uninformative sites (trnL-F: 49 characters, ITS: 79 characters) and 1278 constant sites (trnL-F: 839 characters, ITS: 439 characters). The consistency index (CI), retention index (RI) and homoplasy index (HI) were 0.798, 0.847 and 0.202 respectively for the combined data (0.778, 0.829, 0.222 and 0.909, 0.936, 0.091 for the ITS and trnL-F data, respectively).

Both, ML and BI analyses on the concatenated ITS and *trnL-F* sequences generated essentially congruent phylogenetic relationships (Fig. 2). In the cladogram, all *Glabrella* individuals formed a single clade [BS (bootstrap value of the Maximum Likelihood): 100; PP (Bayesian posterior probability): 1.00]. Samples of *G. mihieri*, *G. longipes* and *G. leiophylla* fell into one subclade, with *G. mihieri* and *G. leiophylla* as sister species (BS: 83; PP: 1.00) and together as sister to *G. longipes* (BS: 64; PP: 0.88). The seven individuals of *G. bogaoi* were monophyletic (BS: 100; PP: 1.00), and were sister to the clade of the other species. Therefore, the phylogenetic analysis strongly supported the separation of *G. bogaoi* from the other species, verifying the validity as an independent new species (Fig. 2).



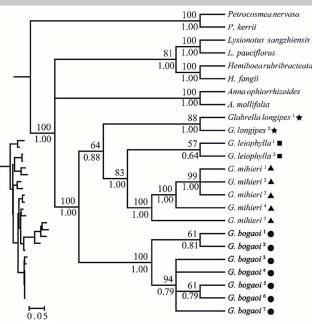


Fig. 2. The Bayesian phylogenetic trees from the analyses of the combined data of the ITS and chloroplast *trnL-F* regions. ML bootstrap support/BI posterior probability values (>0.5 or 50%) are shown below and above the branch respectively around the corresponding node. *Glabrella bogaoi* are highlighted in bold. The superscript at the end of the taxon names represent the molecular sample number of the species, corresponding to Table 1.

TAXONOMIC TREATMENT

Glabrella bogaoi S.W.Li & Yan Liu, sp. nov. 伯高光叶苣苔 Fig. 3

Type: CHINA. Guangxi, Baise City, Leye County, Huaping Town (Yachang Orchid National Nature Reserve), 24°46'N, 106°20'E, 1362 m, 30 October 2014, *Li Shu-Wan et al. YC1498* (holotype: IBK!, isotypes: IBK!, PE!).

Diagnosis: Glabrella bogaoi is morphologically most similar to *G. mihieri* in the shape of the leaf blades, cyme, and filament characteristics, but differs in stems length, which is 5–30 cm long (*vs.* indistinct to 5 cm long), peduncles 2.5–8.5 cm long [*vs.* (5–)8–17 cm long], calyx segments ovate (*vs.* narrowly ovate to narrowly triangular), $6-8\times3-4$ mm long (*vs.* 4–7×1.5–3 mm long), adpressed to corolla base (*vs.* spreading), corolla outside white (*vs.* blue-purple or pale purple to pale yellow), and style glandular pubescent (*vs.* glabrescent to sparsely pubescent).

Description: Perennial herbs. **Stems** terete, distinct, 5–30 cm tall, 1.5–3.5 mm in diam., distinct internodes but at irregular intervals, 0.8-5.5 cm long. **Leaves** often 3 or 4 clustered at the top of stems; **petiole** 0.5–7 cm long, glabrous; **leaf blade** coriaceous, adaxial surface green, abaxial surface pale green, narrowly obovate to obovate or oblong, $2.5-16\times1-5.4$ cm long, apex subacute, slight bent, base narrowly cuneate to broadly cuneate, margin crenate-serrate or shallowly serrate, both surfaces glabrous, lateral veins 5 or 6 on each side of midrib, on adaxial surface inconspicuous, on abaxial surface slightly

conspicuous. Cymes 1-7-flowered; peduncle 2.5-8.5 cm long, sparsely glandular pubescent; pedicel slender, 3.5-4 cm long, sparsely glandular pubescent. Bracts large 2, wide ovate, ca. 9×7 mm long, margin entire, apex obtuse or subacute, glabrous; bractlets not seen. Calyx 5-lobed, dissected to the middle, segments usually equal, ovate, adpressed to corolla base, $6-8\times3-4$ mm long, apex acute, inside glabrous, outside glabrous to glabrescent, margin entire, obscurely 3-veined. Corolla 4.9-5.5 cm long, outside white, sparsely glandular pubescent, inside glabrous to sparsely puberulent, usually pale yellow or yellow spotted; tube 3.9-4.2×1.3-1.5 cm long; limb distinctly 2-lipped, adaxial lip 2-parted to near base, lobes semi-elliptic, apex rounded, 1.1-1.3×0.8-0.9 cm long, abaxial lip 3-parted to near middle, lobes semiorbicular, apex rounded, 1-1.1×0.9-1 cm long. Stamens 4, adaxial stamens adnate to corolla ca. 1.5 cm above base, ca. 1.4 cm long, abaxial ones adnate to corolla ca. 1.5 cm above base, ca. 1.7 cm long. Filaments sparsely glandular pubescent, 12.5-15.5 mm long, anthers ovoid ca. 1.5 mm long, thecae confluent; staminode 1, ca. 2 mm, adnate to corolla ca. 4 mm above base. Disc annular, lemon-yellow, ca. 2 mm in height, glabrous, margin subentire. Pistil sparsely glandular pubescent, ovary narrowly oblong, lemon-yellow, 1.3-2 cm long, 2-2.5 mm in diameter. Style white, 1.2–1.5 cm long, stigma bilobed, lobes equal, lobes oblate, 2×1.8 mm. Capsule linear, 4.8–5 cm long, 2.5-4 mm in diameter, glabrous to glabrescent.

Distribution and Habitat: At present, *Glabrella bogaoi* is found in the Yachang Orchid National Nature Reserve of northwestern Guangxi, China, and in the Dushan and Libo counties of southern Guizhou, China (Fig. 1). In the Yachang Orchid National Nature Reserve, it grows in a limestone hill gully at an elevation of ca. 1342 m. The tree layer is up to 20 m tall, the canopy cover is 85%, the shrub layer cover is 70% and the herb layer cover is 50%. Its associated species include *Manglietia fordiana* Oliv., *Michelia odora* (Chun) Nooteboom & B.L.Chen, *Acorus gramineus* Soland., *Selaginella delicatula* (Desv.) Alston, *Neolepisorus ovatus* (Bedd.) Ching, *Elatostema sublineare* W.T.Wang, *Glabrella mihieri* (Franch.) Mich.Möller & W.H.Chen, *Laurocerasus phaeosticta* (Hance) Schneid., *Dichroa febrifuga* Lour and others.

Phenology: Flowering (October) and fruiting (November).

Etymology: The specific epithet commemorates Mr. Bo-Gao Huang who was the director of the Yachang Orchid National Nature Reserve and a strong supporter of scientific research.

Additional specimens examined (paratypes): CHINA. Guangxi Zhuang Autonomous Region: Baise City, Leye County, Huaping Town, (Yachang Orchid National Nature Reserve), 1370 m, 29 July 2014, Lu Zhao-Cen, Li Shu-Wan, Fang Zhen-Ming and Chen Hai-Ling YC0861 (IBK); Guizhou Province: Buyi and Miao Autonomous Prefecture of QianNan, Libo County, Jialiang Town, 884 m, 30 April 2020, Huang Zhang-Ping and Pan Bo ZQ2020043001–ZQ2020043002 (IBK); Guizhou Province: Buyi and Miao Autonomous Prefecture of QianNan, Dushan County, Jiali Town, 925 m, 27 April 2020, Zou Chun-Yu, Xu Wei-Bin and Huang Jin-Quan ZCY1131–ZCY1134 (IBK).

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Fig. 3. *Glabrella bogaoi*. A. Habitat. B. Habit. C. Flower front view. D. Flower side view. E. Corolla inside view. F. Pistil. G. Calyx. H. Fertile stamens. I. Bracts. J. Annular disc. K. Stigma. L. Fruit.

Key to the species of Glabrella

- 2a. Stems distinct, 5-30 cm; calyx segments ovate, adpressed to corolla



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