



Primulina guizhongensis (Gesneriaceae), a new species from Guangxi, China

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Abstract

A new species of *Primulina* is described and illustrated from southern China (Guangxi Zhuang Autonomous Region) as *P. guizhongensis*. This new species is similar to *P. spadiciformis* based on morphological characters. Sequences of nuclear ribosomal internal transcribed spacer (ITS) region and the plastid *trnL-F* intronspacer (*trnL-F*) from the new species and its 22 relatives are used to resolve generic placement of the new species in *Primulina*. In spite of three species are vastly different in morphology, the molecular evidences showed that the closest relatives of *P. guizhongensis* are *P. mollifolia* and *P. luochengensis*. The conservation threat analysis is summarized according to the IUCN Red List Categories and Criteria.

Introduction

The genera *Chirita* Buch.-Ham. ex Don (1822: 83) and *Chiritopsis* Wang (1981: 21) were always considered to be closely related (Li & Wang, 2007). Recently molecular studies (Weber *et al.* 2011, Wang *et al.* 2011) has shown that the originally monotypic genus *Primulina* Hance (1883) belongs to this group, and that genus was enlarged to include *Chirita* and *Chiritopsis*. Meanwhile new species in this group were reported, for example *P. sinovietnamica* Wu & Zhang (2012: 13), which hinted to morphological characters of similar species not being consistent with molecular evidence (Wu *et al.* 2012). Guangxi is the distribution and diversity centre of *Primulina* in China and worldwide (Wei *et al.* 2010) and numerous new taxa have been recently discovered in this genus (e.g. Huang *et al.* 2012, Wen *et al.* 2012, Wen *et al.* 2012, Wu *et al.* 2012).

One of the authors of this paper, Bo Pan, found a population of *Primulina* at Liujiang County, Liuzhou, Guangxi, China in 2007. He collected some flowering specimens, and after carefully consulting the relevant literature (e.g. Li & Wang 2004, Wei *et al.* 2010), the *Flora of China* (Wang *et al.* 1990, Wang *et al.* 1998) and herbarium specimens, we concluded that it is a new species. The new species is described and illustrated here and its conservation status is evaluated.

Material and methods

We collected leaf material of our possible new species, using silica gel to dry it in the field (Chase & Hills 1991) for DNA extraction. The nuclear ribosomal internal transcribed spacer (ITS) region and the plastid *trnL-F* intronspacer (*trnL-F*) were used as molecular markers. The molecular methods and protocols followed Möller *et al.* (2009, 2011). Genbank accession numbers for ITS and *trnL-F* of our new species are JN644337 and JN644340, respectively (Table 1).

DNA sequences of the new species were found to be similar to the sequences of the recently circumscribed *Primulina* (Wang *et al.* 2011, Weber *et al.* 2011b) based on comparison with Blast N in NCBI

(<http://blast.ncbi.nlm.nih.gov/>). To elucidate the phylogenetic affinities of the new species, ingroup (22 species of *Primulina*) and outgroup (*Ornithoboea wildeana* Craib, *Paraboea rufescens* (Franch.) B.L.Burt) selection were chosen based on recent phylogenetic analyses (Li *et al.* 2007, Möller *et al.* 2011, Weber *et al.* 2011), and from which sequences were available from GenBank (Table 1).

TABLE 1. Species names, voucher numbers and accession numbers of ITS and *trnL-F* sequences used in this study. ? indicates the new species.

Species name (according to Weber A. <i>et al.</i> 2011)	Species name (before)	Voucher number	<i>trnL-F</i>	ITS
<i>Primulina bipinnatifida</i> (W.T. Wang) Y.Z. Wang	<i>Chiritopsis bipinnatifida</i> W.T. Wang		DQ872806	DQ872842
<i>Primulina cordifolia</i> (D. Fang & W.T. Wang) Y.Z. Wang	<i>Chiritopsis cordifolia</i> D. Fang & W.T. Wang		DQ872803	DQ872845
<i>Primulina dryas</i> (Dunn) Mich. Möller & A. Weber	<i>Chirita sinensis</i> Lindl.		FJ501524	FJ501348
<i>Primulina gemella</i> (D. Wood) Y.Z. Wang	<i>Chirita gemella</i> D. Wood		FJ501523	FJ501345
<i>Primulina glandulosa</i> (D. Fang, L. Zeng & D.H. Qin) Y.Z. Wang	<i>Chiritopsis glandulosa</i> D. Fang, L. Zeng & D.H. Qin		DQ872804	DQ872841
<i>Primulina glandulosa</i> var. <i>yangshuoensis</i> (Fang Wen, Yue Wang & Q.X. Zhang) Mich. Möller & A. Weber	<i>Chiritopsis glandulosa</i> D. Fang, L. Zeng & D.H. Qin var. <i>yangshuoensis</i> Fang Wen, Q.X. Zhang & Yue Wang		HQ632948	HQ633045
<i>Primulina guizhongensis</i> ?		WF071001	JN644340	JN644337
<i>Primulina heterotricha</i> (Merr.) Y.Z. Wang	<i>Chirita heterotricha</i> Merr.		DQ872816	DQ872826
<i>Primulina linearifolia</i> (W.T. Wang) Y.Z. Wang	<i>Chirita linearifolia</i> W.T. Wang		DQ872810	DQ872834
<i>Primulina longgangensis</i> (W.T. Wang) Y.Z. Wang	<i>Chirita longgangensis</i> W.T. Wang		AJ492290	FJ501347
<i>Primulina luochengensis</i> (Yan Liu & W.B. Xu) Mich. Möller & A. Weber	<i>Wentsaiboea luochengensis</i> Yan Liu & W.B. Xu		HQ632949	HQ633046
<i>Primulina minutimaculata</i> (D. Fang & W.T. Wang) Y.Z. Wang	<i>Chirita minutimaculata</i> D. Fang & W.T. Wang		DQ872815	DQ872828
<i>Primulina mollifolia</i> (D. Fang & W.T. Wang) Y.Z. Wang	<i>Chiritopsis mollifolia</i> D. Fang & W.T. Wang		DQ872802	DQ872847
<i>Primulina ophiopogoides</i> (D. Fang & W.T. Wang) Y.Z. Wang	<i>Chirita ophiopogoides</i> D. Fang & W.T. Wang		DQ872814	DQ872829
<i>Primulina pinnata</i> (W.T. Wang) Y.Z. Wang	<i>Chirita pinnata</i> W.T. Wang		FJ501526	FJ501349
<i>Primulina pinnatifida</i> (Hand.-Mazz.) Y.Z. Wang	<i>Chirita pinnatifida</i> (Hand.-Mazz.) B.L. Burt		FJ501527	FJ501350
<i>Primulina pteropoda</i> (W.T. Wang) Y.Z. Wang	<i>Chirita pteropoda</i> W.T. Wang		DQ872817	DQ872827
<i>Primulina repanda</i> var. <i>guilinensis</i> (W.T. Wang) Mich. Möller & A. Weber	<i>Chiritopsis repanda</i> W.T. Wang var. <i>guilinensis</i> W.T. Wang		AJ492292	FJ501351
<i>Primulina spadiciformis</i> (W.T. Wang) Mich. Möller & A. Weber	<i>Chirita spadiciformis</i> W.T. Wang		AJ492291	FJ501346
<i>Primulina spinulosa</i> (D. Fang & W.T. Wang) Y.Z. Wang	<i>Chirita spinulosa</i> D. Fang & W.T. Wang		DQ872813	DQ872830
<i>Primulina tabacum</i> Hance	<i>Primulina tabacum</i> Hance		AJ492300	FJ501352
<i>Primulina weii</i> Mich. Möller & A. Weber	<i>Chirita mollifolia</i> D. Fang, Y.G. Wei & J. Murata		DQ872811	DQ872832
<i>Primulina wentsaii</i> (D. Fang & L. Zeng) Y.Z. Wang	<i>Chirita wentsaii</i> D. Fang & L. Zeng		DQ872812	DQ872831
	<i>Paraboea rufescens</i> (Franchet) B. L. Burt		DQ872825	DQ865196
	<i>Ornithoboea wildeana</i> Craib		DQ872824	DQ865197

Nucleotide sequences were edited and aligned with the CLUSTAL W ver. 1.83 (Thompson *et al.* 1997) and MEGA 4.0 (Tamura *et al.* 2007). The ITS and *trnL-F* sequences were analyzed together after assessing their character congruence by the incongruence length difference (ILD; $P = 0.14$) test in PAUP* 4.0b10 (Swofford 2002). Phylogenetic analyses were performed using maximum parsimony (MP) method implemented in PAUP* 4.0b10. Alignment gaps were treated as missing data. Heuristic searches were performed using a starting tree built from stepwise addition with TBR branch swapping and 1000 random addition replicates. To assess confidence in clades, bootstrap analyses based on 1000 replicates with 10 random additions per replicate were used.

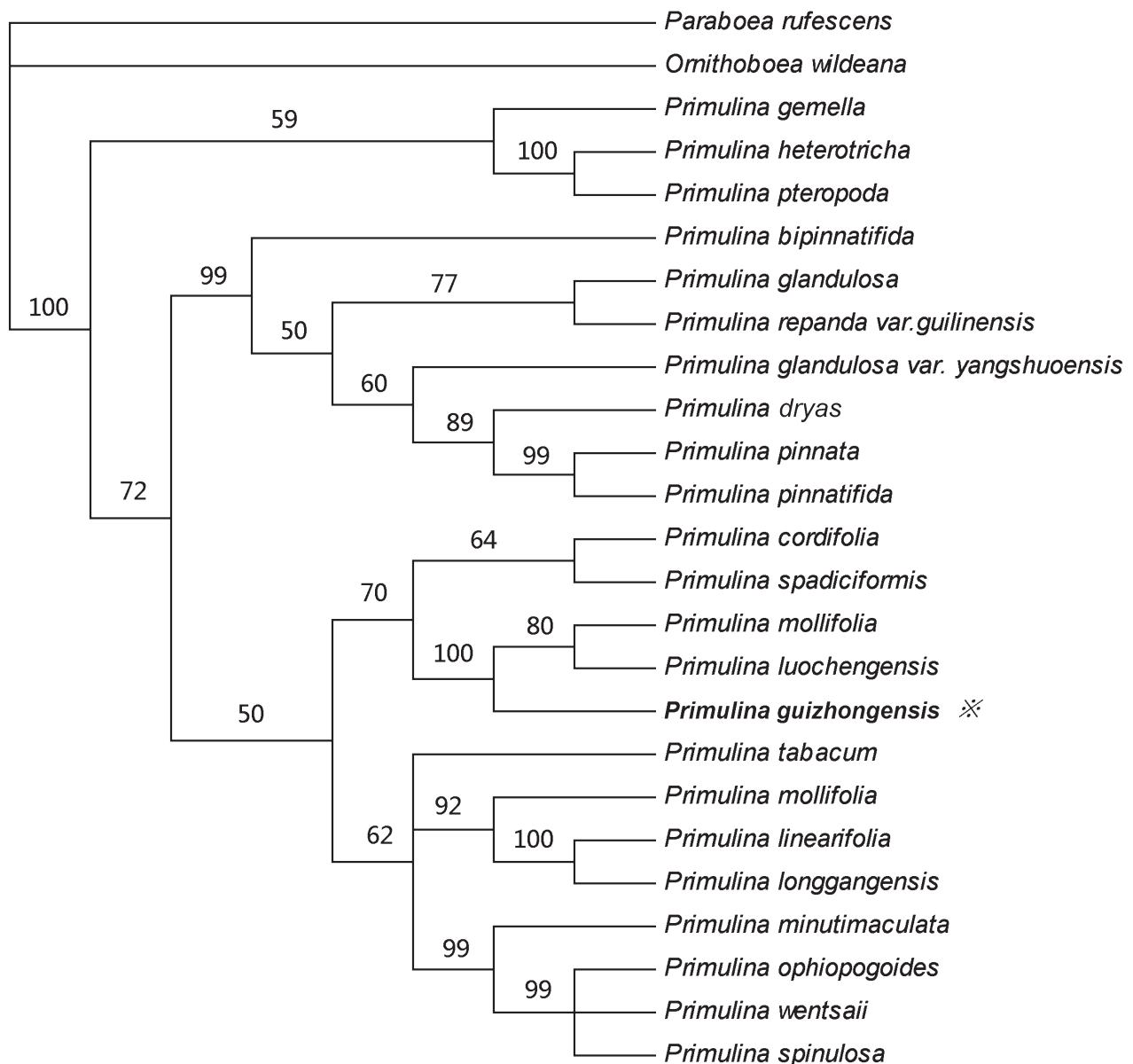


FIGURE 1. The strict consensus tree based on a maximum-parsimony (MP) analysis of combined ITS and *trnL-F* data. Numbers above and below the branches indicate bootstrap values >50% by MP analysis. * indicates the new species.

The MP tree was rooted on *Ornithoboea wildeana* and *Paraboea rufescens*. The samples of *Primulina* formed a monophyletic clade with high bootstrap value (BS = 100%). The new species was strongly supported as sister to a clade (BS = 80%) comprised of *P. luochengensis* (Yan Liu & W.B.Xu) Mich.Möller & A.Weber in Möller *et al.* (2011: 783) and *P. mollifolia* (D.Fang & W.T.Wang) J.M.Li & Yin Z.Wang in Wang *et al.* (2011: 61).

Results

Molecular analysis

The combined matrix had a length of 1536 characters, 674 for ITS and 862 for *trnL-F*. Of the 441 (28.7%) varied, 220 (14.3%) were parsimoniously informative, including the indels. The maximum parsimony analysis on the combined matrix resulted in produced a single tree of 744 steps length, a consistency index (CI) of 0.7607, retention index (RI) of 0.6904 and homoplasy index (HI) of 0.2392. The strict consensus tree (Fig. 1) was highly resolved and tree topology was consistent with the previous phylogenetic study by Möller *et al.* (2011).

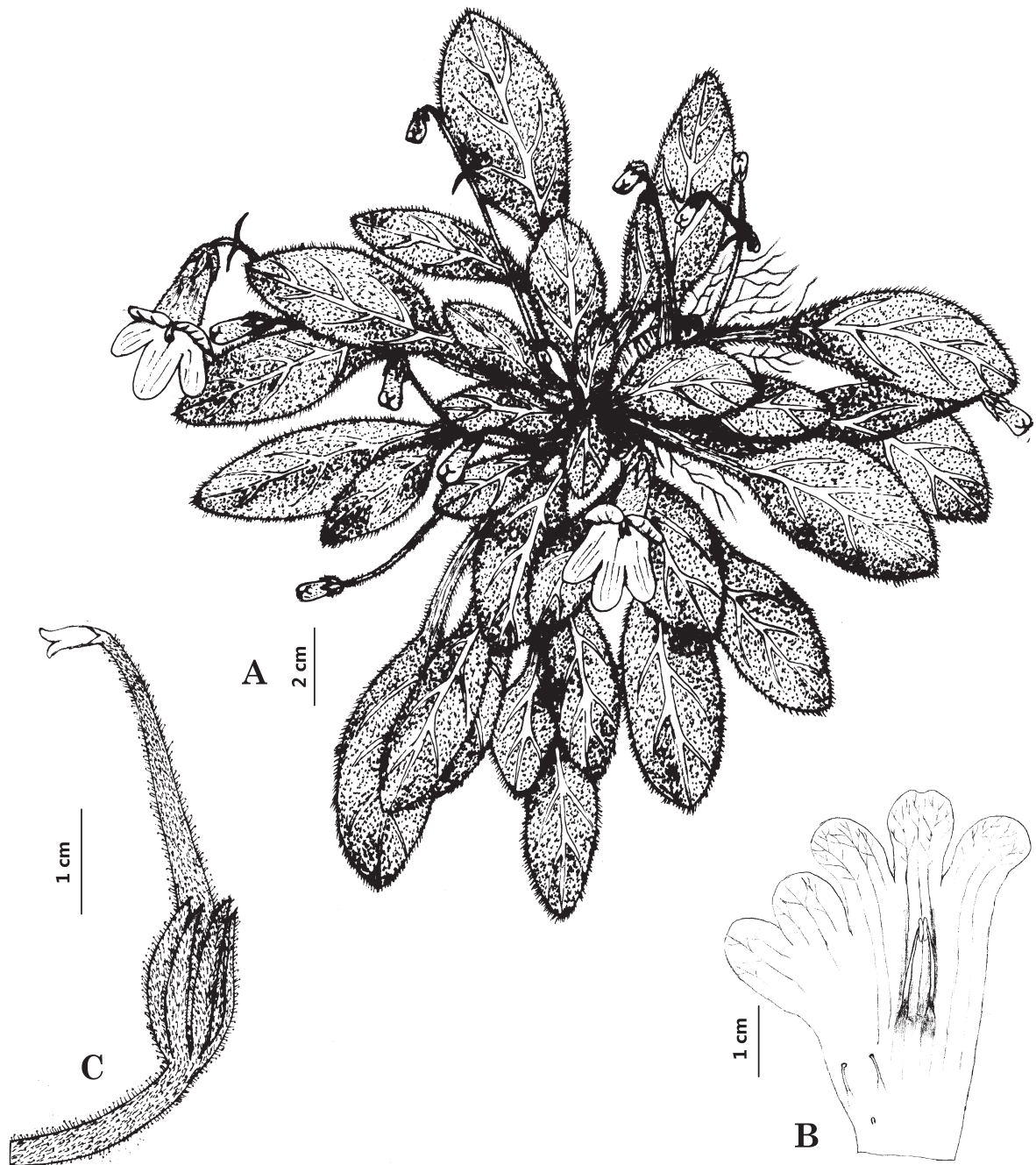


FIGURE 2. *Primulina guizhongensis*. A, habit; B, opened corolla for showing stamens and staminodes; C, calyx, pistil and stigma (Drawn by Qi Wei based on holotype).

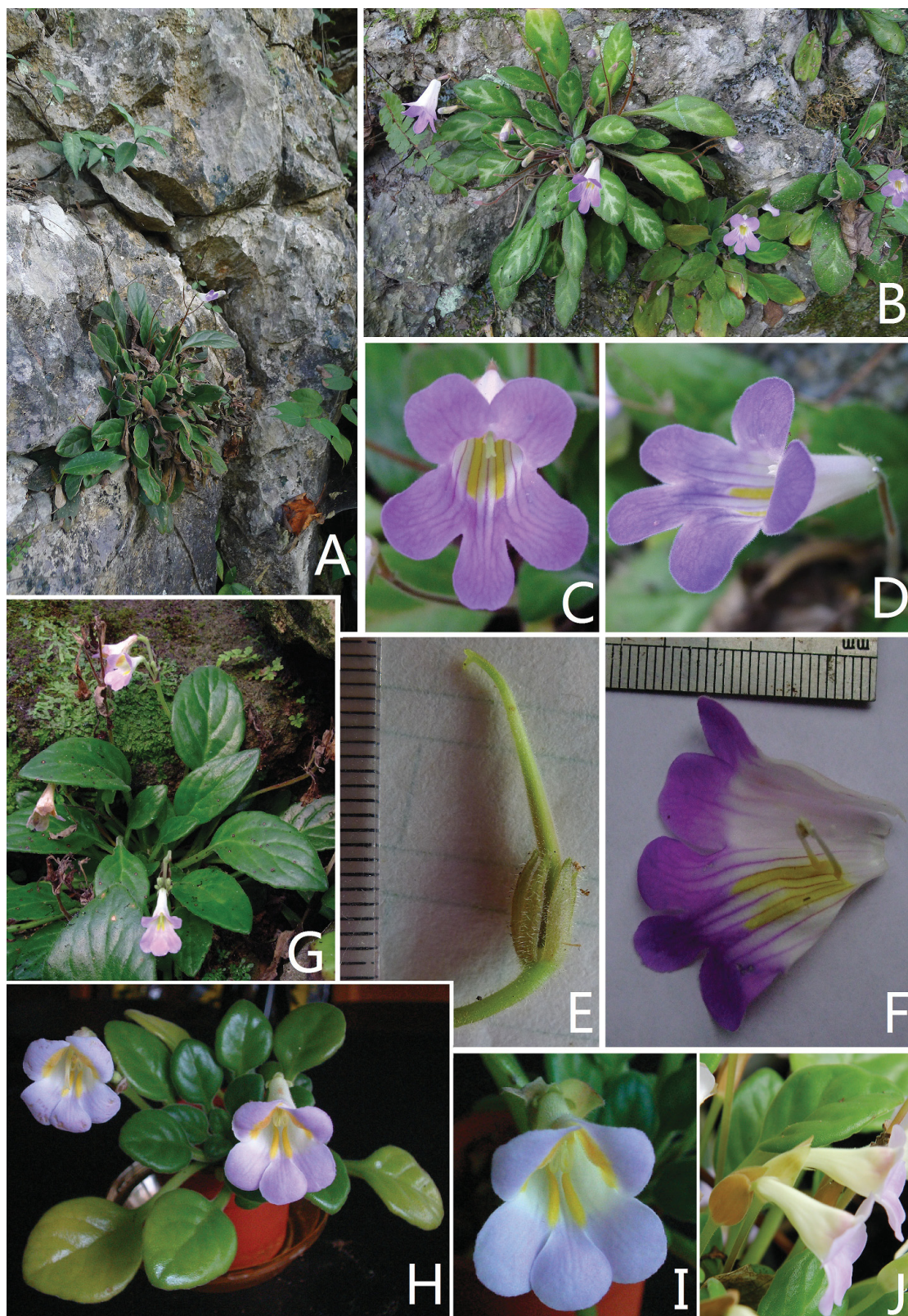


FIGURE 3. The comparison of morphology between two relatives (A–F) *Primulina guizhongensis* (G–J) *P. spadiciformis* A, habitat; B, plants; C, the frontal view of flower; D, the lateral view of flower; E, pistil and calyx; F, opened corolla; G, Habitat; H, plant in flowering time; I, the frontal view of flower; J, the lateral view of flower.

Taxonomic treatment

Primulina guizhongensis Bo Zhao, B. Pan & F. Wen, *sp. nov.* (Figure 2 & Figure 3 A–F)

The new species differs from *P. spadiciformis* by having smaller green linear-lanceolate bracts (0.8–1.2 cm long), longer pedicels (1.8–2.2 cm long), larger calyx lobes (11–15 × 1.5–1.8 mm), corollas outside with glandular pubescence, inside glabrous.

TYPE:—CHINA. Guangxi Zhuang Autonomous Region: Liuzhou, Liujiang County, Futang Town, 24°20'24.81"N, 109°12'33.3036"E, 130 m, 10 October 2007, WF071001 (holotype IBK!, isotype BJFC!)

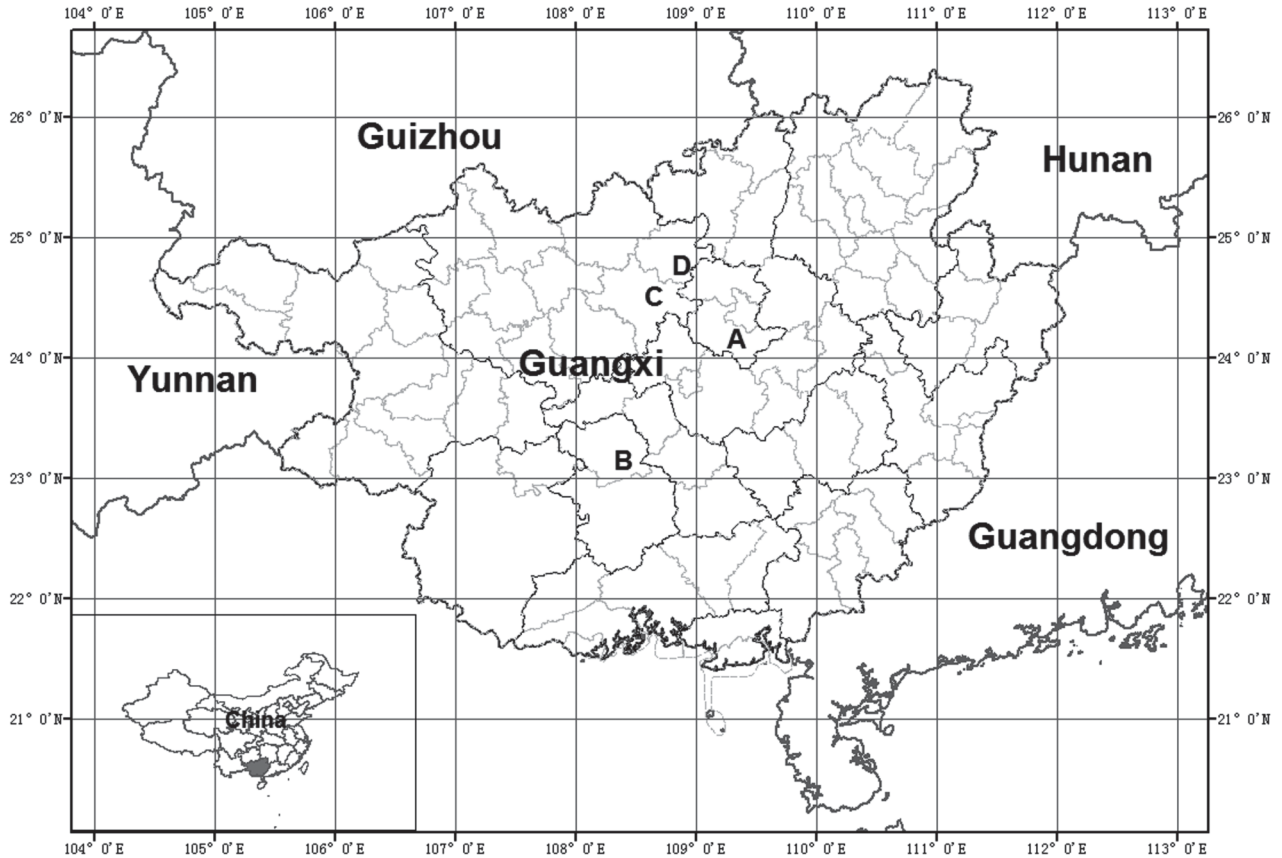
Perennial herbs, acaulescent. Rhizomes cylindrical, ca. 3.5 cm long or longer, 0.6–0.9 cm in diameter, glabrous, internodes inconspicuous. Leaves basal, 20–32 or more; petioles cylindrical, pubescent, 3.5–8.5 cm long; leaf blades slightly oblique, ovate to oblong-ovate, 5.5–10.5 × 2.1–4.5 cm, chartaceous when dry, densely white appressed-pubescent on both sides, bases cuneate or narrowly cuneate, margins subentire, repand or crenate, apices obtuse or rounded; lateral veins 3–4 on each side of midrib, conspicuous. Cymes axillary, 4–12 or more, unbranched, commonly 2–4-flowered, rarely 1; peduncles purplish brown, slender, 5.5–10.5 cm, 0.18–0.25 cm in diameter, densely erect puberulent; bracts 2, opposite, linear-lanceolate, 0.8–1.2 × 0.1–0.15 cm, green, outside puberulent, margins entire, apices acute. Pedicels purplish-brown or greenish-brown, 1.8–2.2 cm long, 1.5–2.0 mm in diameter, puberulent and sparsely assurgent, villose. Calyx lightly purplish brown, 5-sect from base; segments equal, lanceolate, 11–15 × 1.5–1.8 mm, outside densely pubescent, inside glabrous, margins entire, apices acute. Corolla bluish purple to lilac, campanulate, 4.0–4.8 cm long, outside glandular puberulent, inside glabrous; limb distinctly 2-lipped, adaxial lip 2-parted to the middle, lobes slightly oblique broadly ovate, ovate to rounded, ca. 0.95–1.1 × 0.5–1.0 cm, 3-lobed to or over the middle, lobes oblong, the apex of central one emarginate, 12.0–13.3 × 8–9 mm, rounded at apex. Stamens 2, adnate to ca 1.0 cm above the corolla base; anthers reniform, 3.2–3.5 × 0.8–1.1 mm, slightly constricted at the middle, glabrous; filaments geniculate at the middle, ca. 13 mm long, glabrous; staminodes 3, lateral ones short linear, glabrous, 4.0–4.5 mm long, adnate to 0.8–1.0 mm above the corolla base, the central one linear, ca. 0.5 mm long, adnate to ca 4.2 mm above the corolla base; disc annular, margin entire, 0.4–0.5 mm high. Pistil 4.2–4.5 cm long; the transition between ovary and style inconspicuous, densely glandular-puberulent and puberulent. Stigma pale green, obtrapeziform, apex 2-lobed to about one-third of the stigma, ca. 4.5 mm long, lobes triangular. Capsules linear, 3.5–4.0 cm long.

Habitat and distribution:—*Primulina guizhongensis* was found growing on the slopes and cliffs of some limestone hills and at the entrance of limestone caves, often at crevices in open evergreen forest near Futang town, Liujiang County, Guangxi, China. The region has a seasonal monsoon climate. The distribution of this new species and its relatives *P. spadiciformis*, *P. mollifolia* and *P. luochengensis* are shown in map 1.

Etymology:—GUI is the abbreviation for Guangxi Zhuang Autonomous Region. The epithet refers to the type locality of this new species being located in the center of Guangxi.

Diagnostic characters:—*Primulina guizhongensis* differs from its *P. spadiciformis* in having smaller green linear-lanceolate bracts, longer pedicel (1.8–2.2 cm long), larger calyx lobes (11–15 × 1.5–1.8 mm), and different corolla indumentum. A detailed comparison of the remarkable characters of two relatives is shown in table 2.

Conservation status:—We carefully investigated the type locality on five visits during four years. *Primulina guizhongensis* appears to be restricted to limestone hills surrounding Futang Town. Although this species is common on local limestone hills, the habitats are too concentrated to those populations are easy to be threatened by local people's activities and this region is not a natural reserve. Using the IUCN Red List categories and criteria version 3.1 (IUCN 2001), a provisional conservation status of Vulnerable VU D1 is assessed for this species.



MAP 1. Distribution of *Primulina guizhongensis* and related taxa. **A**, Known distribution of *P. guizhongensis*; **B**, Probable distribution of *P. spadiciformis*; **C**, Known distribution of *P. mollifolia*; **D**, Known distribution of *P. luochengensis*

TABLE 2. Comparison between *Primulina guizhongensis* and its relative, *P. spadiciformis*.

Characters	<i>P. guizhongensis</i>	<i>P. spadiciformis</i>
Leaf numbers	20–32 or more	ca. 12
Leaf blade shape	slightly oblique, ovate to oblong-ovate	slightly oblique, elliptic
Leaf blade base	cuneate or narrowly cuneate	obliquely cuneate, or one side cuneate and on the other side rounded
Cymes	4–12 or more, 2–4-flowered, rarely 1	ca. 3, 2-flowered
Bracts	linear-lanceolate, 0.8–1.2 cm long, green	spathelike, narrowly boat-like-ovate, 1.5–2 cm long, pale brownish-yellow
Pediceal length (cm)	1.8–2.2	0.1–1.6
Calyx lobes size	11–15 × 1.5–1.8 mm	ca. 5 × 1–1.2 mm
Corolla indumentum	outside with glandular-pubescent and pubescence, inside glabrous	outside with extremely sparse pubescence, inside glandular-pubescent on the yellow spots, stamens base with pubescence
Staminodes	3	2
Flowering time	October	August

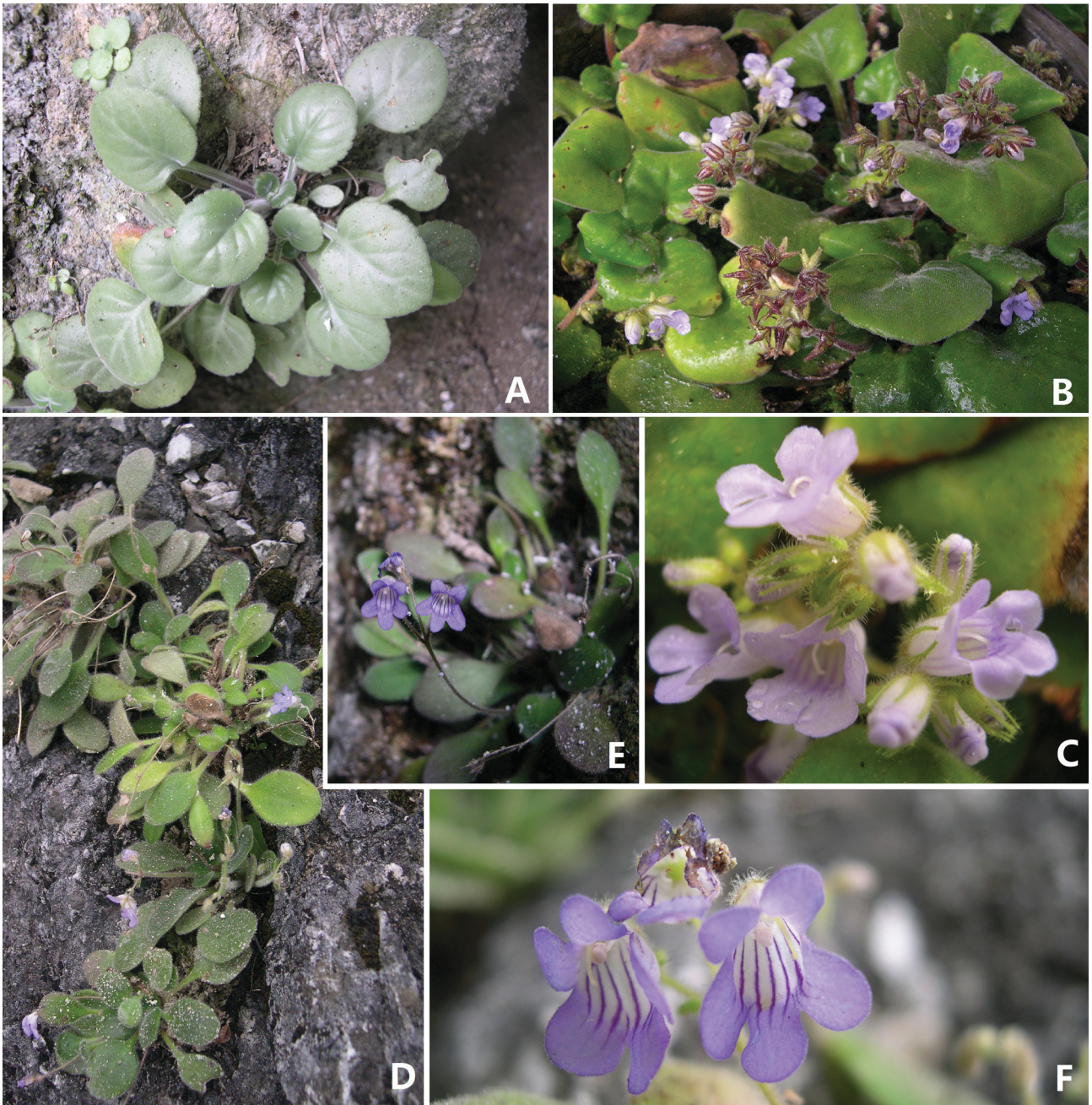


FIGURE 4. The molecular relative, *Primulina mollifolia* and *P. luochengensis*, of *P. guizhongensis*, (A–C) *P. mollifolia*. A, habit; B, cymes; C, cymes and flowers; (D–F) *P. luochengensis*. D, habit; E, cyme; F, flowers.

Discussion

Even though based on DNA evidence we confirmed that our new species is closely related to *P. mollifolia* and *P. luochengensis* (Fig. 1), we not hesitate to describe this new species. The three species look completely different and bear little resemblance to each other (Fig. 4). Recent molecular studies showed that generic placement of species in Gesneriaceae largely differs from the traditional generic classifications using morphological characters (Wang *et al.* 2011, Weber *et al.* 2011b, Wu 2012). Therefore it may be good to combine molecular evidence with morphological data to resolve the taxonomy of Gesneriaceae.

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