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Research

***Primulina fangdingii* (Gesneriaceae), a new species from Guangxi, China**

Bo Pan, Bing-Mou Wang, Li-Hua Yang, Bi-Dan Lai and Peng-Wei Li

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Primulina fangdingii, a species of Gesneriaceae new to science from Guangxi, China, is corroborated by both morphological and molecular evidence. The new species is similar to *P. longzhouensis* in leaf shape and size, but is easily distinguished from the latter by its adaxially glabrous leaf blades, yellow corolla with reddish inner surface and yellow filaments. It is also similar to *P. repanda* in corolla color and corolla shape, but can be diagnosed by its fleshy small leaves with glabrous adaxial surface, glandular and eglandular pubescent pedicels, yellow filaments and annular disc. In addition, *Primulina fangdingii* is somewhat similar to nine other species of *Primulina*, including *P. cordifolia*, *P. danxiaensis*, *P. xiuningensis*, *P. chingipengii*, *P. cordistigma*, *P. diffusa*, *P. jingxiensis*, *P. petrocosmeoides* and *P. sinovietnamica*, in either floral or vegetative morphology. However, all these species differ from *P. fangdingii* by a series of morphological characters. Phylogenetic analyses with ITS and two chloroplast DNA regions (*rPL32-trnL*, *trnL-F*) revealed that *P. fangdingii* is sister to *P. luochengensis* and isolated from its morphological relatives. *Primulina fangdingii* is described and illustrated here, and a key to the new species and its morphological allies is provided.

Keywords: *Chiritopsis*, morphology, phylogeny, *Primulina longzhouensis*, taxonomy

Introduction

The genus *Primulina* Hance was originally monotypic and contained only *P. tabacum* Hance (Hance 1883). However, molecular phylogenetic studies revealed that *P. tabacum*, two species of *Wentsaiboea* D.Fang & D.H.Qin, and all species of *Chiritopsis* W.T.Wang were imbedded in *Chirita* sect. *Gibbosaccus* Clarke, and together they form a monophyletic group with high support diagnosed by unique character combinations (Li and Wang 2007, Möller et al. 2009, 2011, Wang et al. 2010, 2011, Weber et al. 2011). Consequently, the genus *Chirita* was disassembled and most species of *Chirita* sect. *Gibbosaccus*, two species of *Wentsaiboea* and all species of



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Chiritopsis were combined into *Primulina* (Wang et al. 2011, Weber et al. 2011, Xu et al. 2012). *Primulina* as currently delimited has become the most speciose genus of the family Gesneriaceae in China, including more than 220 species (GRC 2022, Wen et al. 2022), with most species distributed in the limestone areas of southern China (Wei et al. 2010, Xu 2017, Wei 2019, Li et al. 2021). The species of *Primulina* are characterized by being perennial herbs with more or less well-developed rhizomatous stems, somewhat or distinctly fleshy and usually opposite leaves (sometimes in whorls), zygomorphic corollas, usually broadly funnel-shaped corolla tubes, two stamens with coherent anthers and a characteristic chiritoid stigma (Weber 2004, Wang et al. 2011, Weber et al. 2011). They usually inhabit shaded and humid environment, such as caves or cave-like microhabitats, in karst formations (Xu et al. 2021).

In June 2016, during field investigations in the limestone areas of Guangxi, we discovered an unknown *Primulina* species with small flowers (ca 1 cm long) and conical tube, with an only slightly shorter adaxial than abaxial lip, with sub-rounded lobes, as well as an ovoid ovary, which are typical characteristics of species of the former genus *Chiritopsis* (Wang 1981) and now synonymized under the genus *Primulina* (Weber et al. 2011, 2013). There are more than 20 *Chiritopsis*-like *Primulina* species and three varieties described (Ning et al. 2014, Wen et al. 2014, Xu et al. 2019, 2020, Qin et al. 2020). Four of the *Chiritopsis*-like species, i.e. *P. cordifolia* (D.Fang & W.T.Wang) Yin Z.Wang, *P. danxiaensis* (W.B.Liao, S.S.Lin & R.J.Shen) W. B. Liao & K. F. Chung, *P. repanda* (W.T.Wang) Yin Z.Wang and *P. xiuningensis* (X.L.Liu & X.H.Guo) Mich.Möller & A.Weber, possess similar yellow or pale-yellow corollas as our unknown species (Wang 1981, 1982, Liu and Guo 1989, Shen et al. 2010). However, the distinctly fleshy leaves and yellow filaments of our unknown species differentiates it from those four *Chiritopsis*-like *Primulina* since they all have herbaceous leaves and white filaments. Additionally, three *Chiritopsis*-like species with fleshy leaves, i.e. *Primulina chingipengii* W.B.Xu & K.F.Chung, *P. jingxiensis* (Yan Liu, W.B.Xu & H.S.Gao) W.B.Xu & K.F.Chung and *P. longzhouensis* (B.Pan & W.H.Wu) W.B.Xu & K.F.Chung, resemble the unknown species in the vegetative parts (Xu et al. 2009, 2019, Pan et al. 2010, Wen et al. 2014). However, the unknown species can be unambiguously distinguished from these three species by a series of floral characters. Besides, several non-*Chiritopsis*-like *Primulina* species, such as *P. cordistigma* F.Wen, B.D.Lai & B.M.Wang, *P. diffusa* Xin Hong, F.Wen & S.B.Zhou, *P. petrocosmeoides* B.Pan & F.Wen and *P. sinovietnamica* W.H.Wu & Qiang Zhang, are somewhat similar to the unknown species in vegetative morphology (Wu et al. 2012, Pan and Wen 2014, Zhou et al. 2014, Lai et al. 2016), but they are very different from the undescribed species in floral features (for details, see the key below).

To further validate the specific status of the undescribed species, we conducted a molecular phylogenetic reconstruction to examine the relationship between the unknown species and its allies.

Material and methods

Taxon sampling

We sampled 63 accessions of *Primulina* (Supporting information), including 23 species and two varieties of the *Chiritopsis*-like *Primulina* species, and four non-*Chiritopsis*-like *Primulina* species representing the main clades recovered by Xu et al. (2021). Sequences of the undescribed species, i.e. *Primulina fangdingii* (see the taxonomic treatment), *P. maciejewskii* F.Wen, R.L.Zhang & A.Q.Dong and *P. sinovietnamica* were newly acquired while those of the remaining species were retrieved from previous studies (Kang et al. 2014, Kong et al. 2017, Xu et al. 2019, 2021). *Petrocodon dealbatus* Hance and *Petrocodon hancei* (Hemsl.) A.Weber & Mich.Möller were treated as outgroups (Wang et al. 2011, Weber et al. 2011).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from silica-dried leaves using a modified CTAB protocol (Doyle and Doyle 1987) and used as the template in the polymerase chain reaction (PCR). The internal transcribed spacer (ITS, including ITS1, 5.8S subunit and ITS2), and two chloroplast DNA regions (*rpl32-trnL*, *trnL-F*) were amplified using the primers ITS-p5/u4 (Cheng et al. 2016), *rpL32-trnL* (Shaw et al. 2007) and *trnL-F-c/f* (Taberlet et al. 1991), respectively. The PCR conditions for all DNA regions included initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 54°C for 30 s and 72°C for 50 s, with a final extension at 72°C for 5 min. PCR products were purified using Tian quick Midi Purification Kit following the manufacturer's protocol, and then directly sequenced with the same primers as used for PCR amplification. All the sequences generated in the present study have been deposited in GenBank (Supporting information).

Sequence alignment and phylogenetic analysis

Contig assembly, sequence alignment and manual adjustment were performed in Geneious ver. 7.1.8 (Kearse et al. 2012). We analyzed the ITS, chloroplast (*rpL32-trnL* and *trnL-F*), and the combined datasets using maximum parsimony (MP) and Bayesian inference (BI) methods on CIPRES Science Gateway Portal (Miller et al. 2010). The combinability of nuclear and chloroplast regions was checked by the incongruence length difference (ILD) test (Farris et al. 1994, 1995) as implemented in PAUP*4.0b10 (Swofford 2003).

MP analysis was implemented in PAUP*4.0b10 (Swofford 2003) with all characters equally weighted and unordered. Starting trees were obtained via stepwise addition with 1000 replicates of random addition. A heuristic search with tree-bisection–reconnection (TBR) branch-swapping algorithm, MulTrees on and steepest descent off was conducted. Descriptive tree statistics, including consistency index (CI;

Kluge and Farris 1969), retention index (RI; Farris 1989a) and rescaled consistency index (RC; Farris 1989b), were obtained using PAUP*. Branch support was assessed by 10 000 bootstrap (BS; Felsenstein 1985) replicates using heuristic searches as described above except that the number of random addition was set to 10.

Substitution models were selected for each dataset using the Akaike information criterion (Akaike 1974) implemented in MrModeltest ver. 2.3 (Nylander 2004). BI analysis was conducted in MrBayes ver. 3.2.7 (Ronquist et al. 2012). Data was partitioned based on gene regions in order to accommodate differing evolutionary rates for the chloroplast and combined datasets. For each dataset, two runs each with four Markov chain Monte Carlo (MCMC) chains were performed for 10 million generations with trees sampled every 1000th generation. Convergence between both runs was confirmed by examining whether the values for potential scale reduction factor (PSRF) were close to 1 and standard deviation of the split frequencies fell below 0.01. Additional convergence diagnostics was performed using Tracer ver. 1.7.1 (Rambaut et al. 2018) to check if each parameter had an effective sample size (ESS) > 200. To estimate clade posterior probabilities (PP), a 50% majority-rule consensus was built from the remaining sampled trees after discarding the first 25% as burn-in. The phylogenetic trees were visualized in FigTree ver. 1.4.4 (A. Rambaut, Univ. of Edinburgh, <http://tree.bio.ed.ac.uk/software/figtree>) and modified in Adobe Illustrator.

Results and discussion

The ITS matrix contained 65 sequences with an aligned length of 890 base pairs (bp), of which 291 were parsimony informative. The chloroplast DNA matrix comprised 65 sequences with a length of 1872 bp (*rpL32-trnL*: 1058, *trnL-F*: 814), of which 133 were parsimony informative. The combined matrix had a length of 2762 characters for analysis. Of these, 424 were parsimony informative. The detailed statistics of individual and combined datasets are summarized in Supporting information.

The phylogenetic trees inferred from ITS and chloroplast datasets were generally congruent except that the phylogenetic position of *Primulina mollifolia* was strongly conflicting (Supporting information). The ILD test indicated a high level of congruence between ITS and chloroplast datasets ($p=0.24$) after removing chloroplast sequences of *P. mollifolia*. Phylogenetic analysis of the combined dataset using the MP method produced 216 most parsimonious trees of 1492 steps with the CI, RI and RC of 0.665, 0.789 and 0.524, respectively. The strict consensus MP tree was generally highly resolved (Fig. 1) and basically congruent with the BI tree (Supporting information). The two accessions of *P. fangdingii* clustered together with high support (BS = 99, PP = 1) and they further formed a pair of sister species with *P. luochengensis* with strong support (BS = 99, PP = 1; Fig. 1).

However, the 11 more or less morphologically allied species of *P. fangdingii* were scattered in different positions of the phylogenetic tree (Fig. 1) and isolated from *P. fangdingii*, indicating that they are distantly related to the new species.

To have a full understanding of the morphological variation of *P. fangdingii*, we conducted thorough field investigations during the past years, from 2012 to 2018, in Liucheng county and adjacent areas, but we did not find any other population. The phenotype of *Primulina fangdingii* is generally very uniform, which is possibly due to the consanguineous mating in a karst cave with relatively stable environment and the occurrence of vegetative propagation by the rhizomatous stems: the main stem may die off and the plants survive and spread with the rooted side branches. Comparative morphological analyses suggest that the character combinations observed in *Primulina fangdingii*, i.e. fleshy leaves with glabrous adaxial surface, yellowish corolla with reddish inner surface and yellowish honey guides, yellow glandular pubescent filaments and annular disc (Figs. 2, 3), are unique in *Primulina*. As the sister species of *P. fangdingii*, *P. luochengensis* can be easily diagnosed by pubescent leaves, obliquely campanulate corolla with purple stripes and glabrous filaments (Fig. 4A; Liu et al. 2010). The most similar species of *P. fangdingii* is *P. longzhouensis*, which is distributed in Longzhou county, southwest of Guangxi, China (Fig. 5). To our knowledge, *Primulina longzhouensis* is known from two small populations in a nature reserve and show little morphological variation. This species is characterized by purple corolla tube, white limb, white inner surface of corolla and white filaments (Fig. 4B–D) and thus differs from *P. fangdingii*. *Primulina repanda* is the species that is most similar to *P. fangdingii* in the general facies of flowers, however, its densely villous herbaceous leaves, reddish honey guides, white filaments, included stigma and unilateral disc (Fig. 4E–G) easily distinguishes it from *P. fangdingii*.

The species of *Primulina* are mainly concentrated in southern China (Xu et al. 2017), a region which has undergone a slow epeirogenic uplift as a result of the upthrust of the Himalayas after the collision between the Indian subcontinent and the mainland of Asia (Axelrod et al. 1998). The consequent subtropical monsoon climate characterized by sharply contrasting dry and rainy seasons may account for the fleshy leaves observed in *P. fangdingii* and its allied species. Since the water and nutrition reserved in the fleshy leaves can help those plants survive periods with adverse conditions, many *Primulina* species have developed diverse fleshy leaves. On the other hand, the reduction of corolla tube length and corolla size that has occurred in the *Chiritopsis*-like *Primulina* species might be accompanied by decreasing taxonomic diversity of pollinators and increased specificity of pollen placement on pollinator's bodies (Tang et al. 2009, Wen et al. 2012, Huang et al. 2016). Both the fleshy leaves and the small flowers are the results of convergent or parallel evolution and have originated independently multiple times.

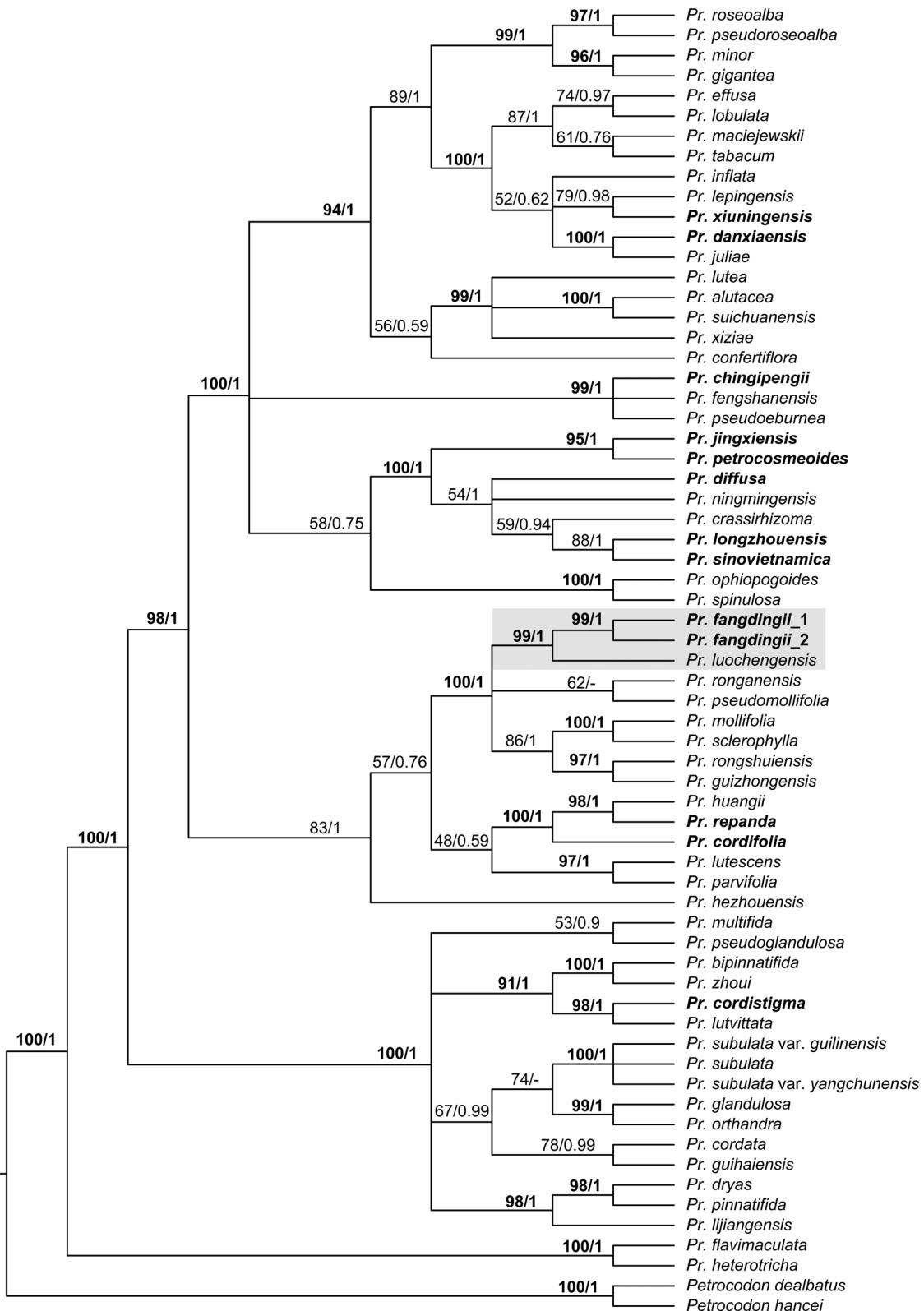


Figure 1. Strict consensus tree of 216 most parsimonious trees based on combined nuclear and chloroplast (*rpL32-trnL*, *trnL-trnF*) DNA sequences. Numbers along branches are bootstrap values and posterior probabilities, respectively. Hyphens indicate no support. Numbers in bold denote branches receiving > 90% branch support. Tips in bold indicate species that are more or less morphologically similar to *Primulina fangdingii*. Pr., *Primulina*.

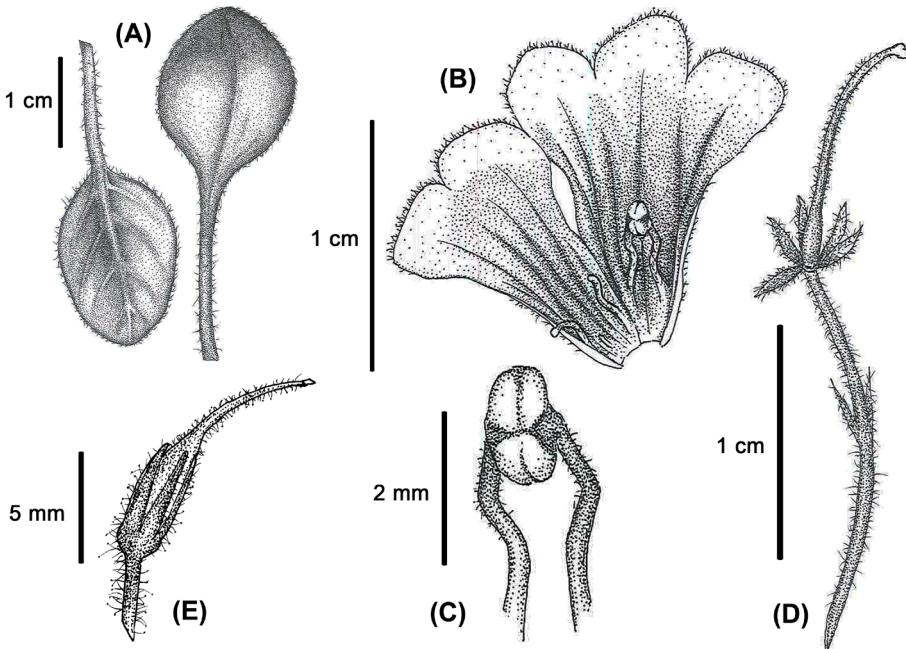


Figure 2. Line drawing of *Primulina fangdingii* sp. nov. (A) Abaxial (left) and adaxial (right) leaf surface, (B) dissected corolla, showing stamens and staminodes, (C) stamens, showing hairy filaments and coherent anthers, (D) divided calyx and hairy pistil, pedicel, bracts and peduncle, (E) capsule. Drawn by Z.-M. Yang based on a living plant introduced from the type locality.

Taxonomic treatment

***Primulina fangdingii* B.M. Wang, B. Pan & B.D. Lai, sp. nov. (Fig. 2, 3)**

A species morphologically most similar to *Primulina longzhouensis* (Fig. 4B–D), but distinguished from the latter by adaxially glabrous leaves (versus appressed pilose leaves), yellowish corolla (versus purplish corolla), reddish lower inner surface of corolla tubes with inconspicuously yellow honey guides (versus white inner surface of corolla tubes without honey guides) and yellow filaments (versus white filaments). It is also similar to *P. repanda* (Fig. 4E–G), but differs by its adaxially glabrous fleshy leaves (versus densely villous herbaceous leaves), yellowish honeyguides (versus reddish honey guides), yellow filaments (versus white filaments), exserted stigma (versus included stigma) and annular disc (versus unilateral disc).

Type: China, Guangxi Zhuang Autonomous Region: Liucheng County, Longtou Town, at the entrance of a moist limestone cave, $24^{\circ}44'36.90''N$, $109^{\circ}12'50.10''E$, elevation 147 m a.s.l., 15 Jun 2016, L.H. Yang & B. Pan YLH353 (holotype: IBSC [0866543]; isotype: IBK [IBK00438702]).

Etymology

The specific epithet is in memory of the Chinese botanist Ding Fang (1926–2017), who was a research fellow of the Guangxi Institute of Traditional Medical and Pharmaceutical Sciences, for his contribution to our knowledge about the medicinal plants in Guangxi (Fang et al. 1985, 1986, Fang 1993, 2012).

Description

Perennial herb. Stems rhizomatous, cylindrical, seldom branched, 0.5–1.0 cm long, 0.3–0.5 cm in diameter; internodes inconspicuous. Leaves 18–35 (–59), opposite in young plants, later phyllotaxy becoming blurred and leaves appearing in a spiral at the apex of the rhizome; petioles flattened, $3.0–20.0 \times 1.5–2.5$ mm, villous; blades elliptic to ovate, $1.0–3.0 \times 0.5–2.2$ cm, fleshy when fresh, thick papery when dry, their adaxial surface glabrous and abaxial surface reddish-purple and villous, obtuse at apex, cuneate to broadly cuneate at base, with margin entire and ciliate; lateral veins inconspicuous. Cymes 6–18, axillary, 1–3-branched, 3–9-flowered; peduncle 3–5 cm long, densely pubescent; bracts 2, opposite, lanceolate, $2.0–3.0 \times 0.5$ mm, densely pubescent, with entire margin. Pedicel 5–8 mm long, densely glandular and eglandular-pubescent. Calyx 5-parted to base; segments equal, narrowly triangular, $3–4 \times 1$ mm, densely pubescent outside, sparsely eglandular-puberulent inside, with entire margin and acute apex. Corolla 1.3–1.5 cm long, glandular and eglandular-puberulent outside, inside sparsely glandular-puberulent; tube tubular, 7.0–10.0 mm long, 2.0–2.6 mm in diameter at the base and 3–5 mm in diameter at the mouth, its outer surface purplish-yellow, upper inner surface and honey guides yellowish, lower inner surface reddish; limb yellow or pale yellow, distinctly 2-lipped; adaxial lip 2-lobed from near base with lobes sub-orbicular, ca $4.0 \times 4.0–4.5$ mm; abaxial lip 3-lobed to the middle with lobes sub-orbicular to broadly ovate, ca $3.0–4.0 \times 4.0–4.5$ mm. Stamens 2, adnate to ca 2.5 mm above the abaxial side of corolla tube base; filaments yellow, ca 3 mm long, geniculate at the middle, glandular

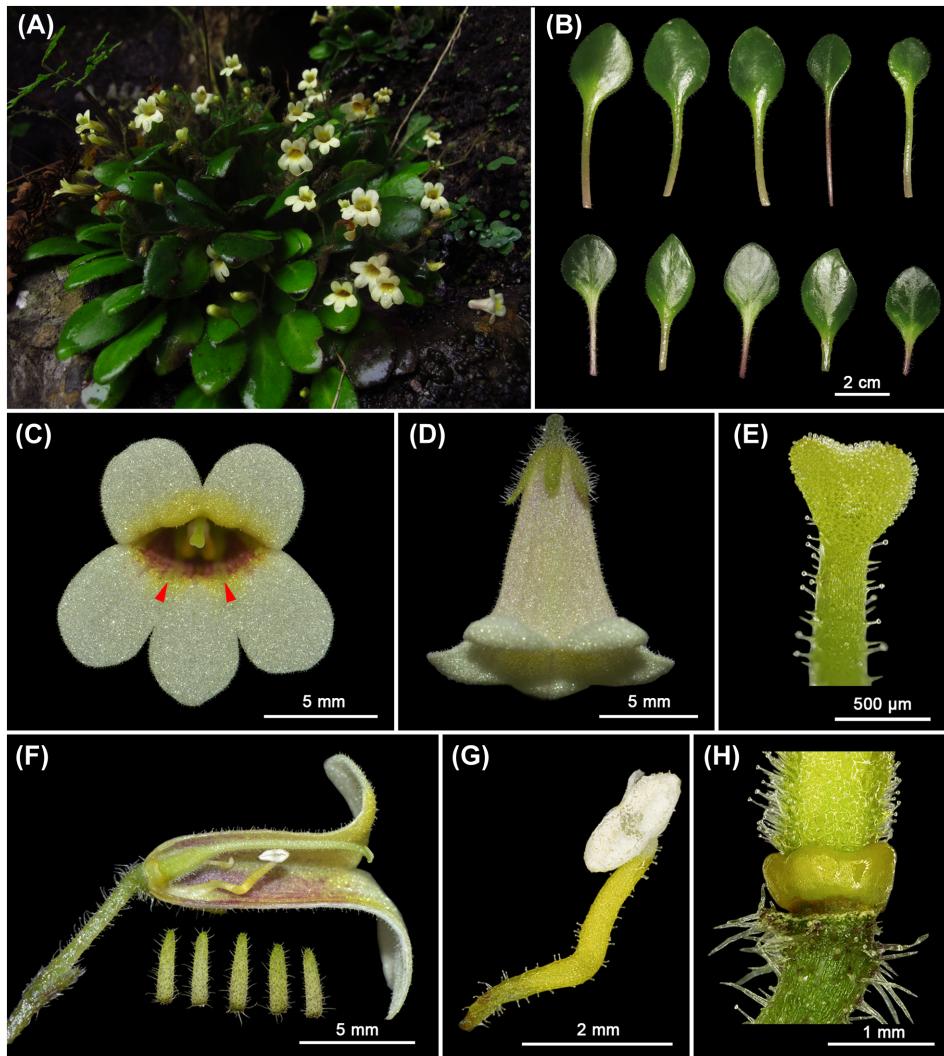


Figure 3. Morphology of *Primulina fangdingii* sp. nov. (A) Habit, (B) variation of leaves from different plants in the type locality, (C) frontal view of corolla, showing the reddish inner lower surface and the inconspicuously yellowish honey guides (red arrows), (D) top view of corolla, (E) stigma shape and glandular and eglandular hairs on the style, (F) peduncle, bracts, pedicel, sepals and longitudinal dissection of corolla, (G) single stamen, showing the glandular and eglandular hairs on the yellow filament and glabrous anther, (H) annular disc and glandular and eglandular hairs on the ovary (upper part) and pedicel (lower part).

and eglandular-puberulent; anthers coherent face to face, divaricate, ca 1.2 mm long, glabrous. Staminodes 3, lateral ones ca 2 mm long, capitate at apex, adnate to 2 mm above the corolla base, the central one inconspicuous, adnate to ca 0.5 mm above the corolla base, all sparsely hairy. Disc annular, slightly repand, ca 0.6 mm high, glabrous. Pistil 8.6–11.5 mm long; ovary narrowly ovoid, 2.1–3.0 mm long, glandular and eglandular-pubescent; style linear, 5.7–7.6 mm long, glandular and eglandular-pubescent; stigma chitoid with upper stigma lobe absent and lower stigma lobe lamellar with slightly bilobed apex, 0.65–0.86 mm long, Capsule narrowly ellipsoid, ca 5 mm long, loculicidally dehiscing. Seed unknown.

Phenology

Flowering from June to July and fruiting from July to August.

Distribution and habitat

Primulina fangdingii is currently only known from one locality in Liucheng County, Guangxi Zhuang Autonomous Region, China (Fig. 5), growing at the entrance of a humid limestone cave shaded by evergreen broad-leaved forest.

Conservation status

Primulina fangdingii is currently known only from the type locality, where we observed ca 200 mature individuals in total. According to IUCN red list criteria (IUCN 2012), the new species is assessed as ‘Critically Endangered’ (CR) based on criterium C (population size estimated to number fewer than 250 mature individuals).

Additional specimens examined (paratypes)

China, Guangxi: Liucheng County, 20 Aug 2012, W.B. Xu & B. Pan 11882 (IBK); ibid., 19 Jun 2016, W.B. Xu & B. Pan

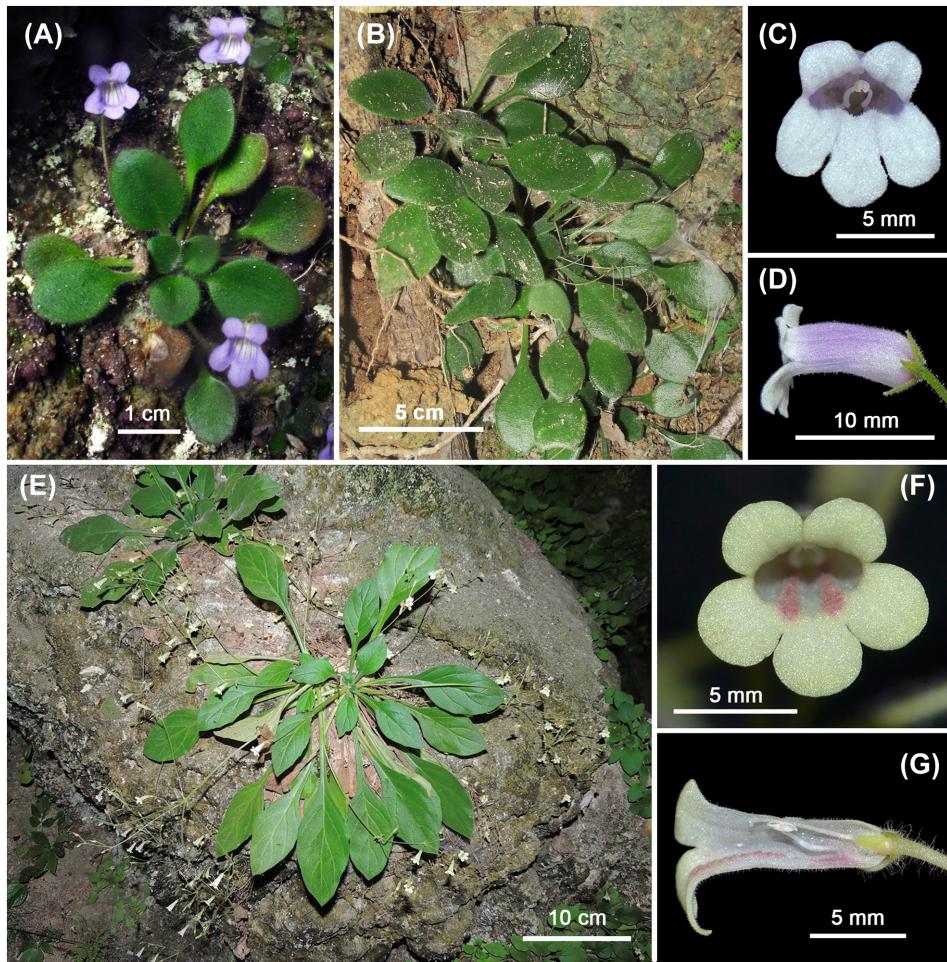


Figure 4. Morphology of the three allied species of *Primulina fangdingii*. (A) Habit of *Primulina luochengensis*. (B–D) *P. longzhouensis*, (B) habit, (C) frontal view of corolla, showing white inner surface and filaments, (D) side view of corolla. (E–G) *P. repanda*, (E) habit, (F) frontal view of corolla, showing yellowish lobes and reddish honey guide, (G) longitudinal section of corolla, showing white inner surface and filament, included pistil and unilateral disc.

12957 (IBK); ibid., 19 Jun 2016, W.B. Xu & B. Pan 12958 (IBK). Cultivated at the South China Botanical Garden, Chinese Academy of Sciences, introduced from Liucheng County, Guangxi Zhuang Autonomous Region, vouchered as P.W. Li LPW2021007 (IBSC, PE).

Similar species

Primulina longzhouensis and *P. repanda* are two species that are most similar to *P. fangdingii* and we have provided detailed comparisons in the diagnosis. In addition, *P. fangdingii* somewhat resembles nine other *Primulina* species as aforementioned. Therefore, we have provided a key including all these morphologically similar species and the new species for their identification.

Key to *Primulina fangdingii* and its allied species

- 1 Leaves fleshy, eglandular-pubescent 2
– Leaves herbaceous, glandular and eglandular-pubescent 9
- 2 Corolla funnelform with the ventral side gibbous or seldom not 3
– Corolla tubular with the ventral side not gibbous 6
- 3 Filaments yellow, geniculate near the base; anthers divergent *P. cordistigma*
– Filaments purple, geniculate at the middle; anthers divaricate 4
- 4 Plants with stolons; corolla with distinct purple stripes *P. diffusa*
– Plants without stolons; corolla with indistinct purple stripes or without stripes 5
- 5 Leaves villous; corolla with distinct honey guides, glandular pubescent at throat *P. petrocosmeoides*
– Leaves appressed villous; corolla without honey guides, glabrous at throat *P. sinovietnamica*
- 6 Corolla purplish 7
– Corolla white or yellowish 8
- 7 Leaves appressed pilose; filaments white, geniculate above base *P. longzhouensis*

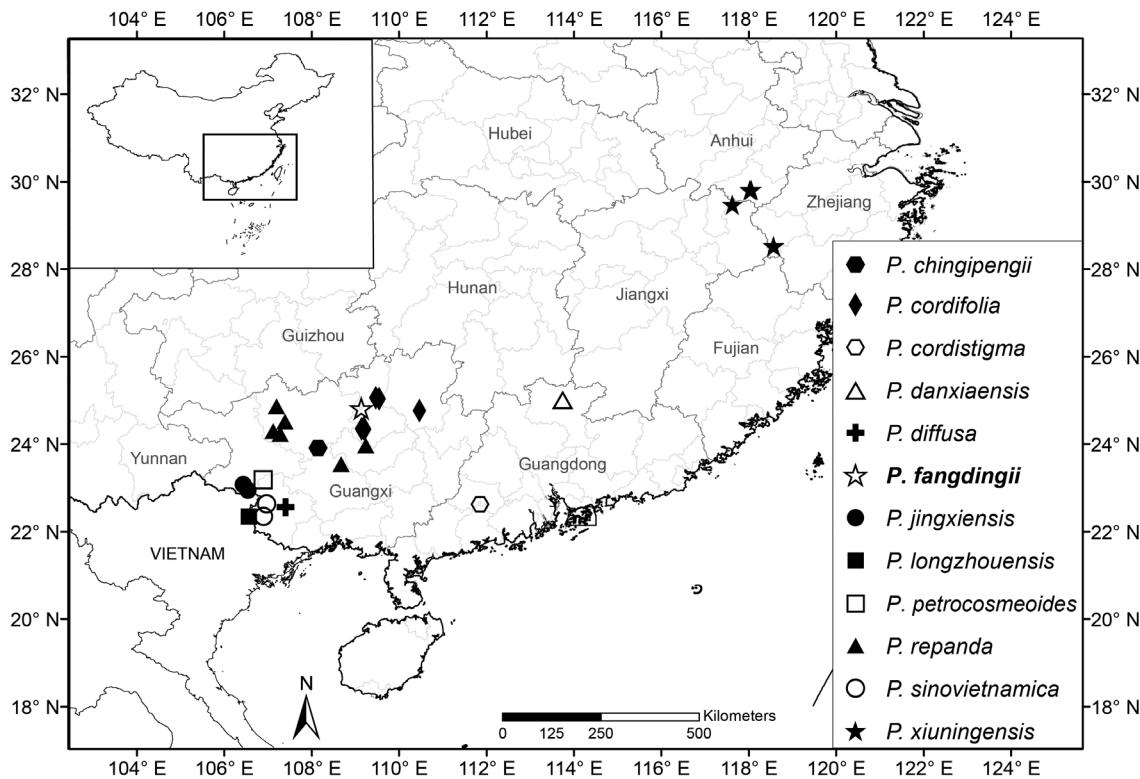


Figure 5. Geographical distribution of *Primulina fangdingii* and its allied species. The map was drawn using ArcGIS ver. 10.6 (ESRI 2017) based on distribution data obtained from the protologue and records in the Chinese Virtual Herbarium (<www.cvh.ac.cn/>).

- Leaves villous; filaments purple, geniculate near middle *P. jingxiensis*
- 8 Blades narrowly elliptic to suborbicular, cuneate at base, almost glabrous at adaxial surface; corolla yellowish with inconspicuous honey guides; filaments yellow *P. fangdingii*
 - Blades broad-ovate to suborbicular, cuneate to cordate at base, densely pubescent at both surfaces; corolla white with conspicuously red-brown honey guides; filaments white *P. chingipengii*
- 9 Leaf blade elliptic to broad-ovate, cuneate at base *P. repanda*
 - Leaf blade ovate to orbicular, cordate at base 10
- 10 Leaf margin palmately lobed *P. danxiaensis*
 - Leaf margin entire or slightly repand 11
- 11 Leaf blade with dense, long, eglandular pubescence and extremely short glandular hairs; corolla with reddish honey guides *P. cordifolia*
 - Leaf blade with long glandular and eglandular hairs; corolla with purplish honey guides *P. xiuningensis*

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Author contributions

Pan Bo: Conceptualization (lead); Funding acquisition (supporting); Investigation (lead); Writing – original draft (supporting). **Bing-Mou Wang:** Investigation (supporting); Resources (lead); Visualization (supporting); Writing – original draft (supporting). **Li-Hua Yang:** Funding acquisition (equal); Investigation (lead); Visualization (supporting); Writing – review and editing (supporting). **Bi-Dan Lai:** Investigation (supporting); Writing – review and editing (supporting). **Peng-Wei Li:** Conceptualization (supporting); Formal analysis (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead).

Data availability statement

There are no additional data for this paper.

Supporting information

The supporting information associated with this article is available from the online version.

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