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Primulina rufipes, a new species of Gesneriaceae from Guangxi, China

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ABSTRACT: *Primulina rufipes* Y.L. Su, P. Yang & Yan Liu (Gesneriaceae), a new species from Guangxi, China, is described and illustrated. Molecular phylogenetic analyses unambiguously put this species in *Primulina*. Morphological observations and comparisons showed that *P. rufipes* is similar to *P. huaijiensis* Z.L. Ning & J. Wang, differing by all over the plant densely pubescent and glandular-puberulent, leaf blade elliptic to rhomboid-ovate, and obliquely campanulate corolla without appendages. *P. rufipes* is also similar to *P. yulinensis* Ying Qin & Yan Liu and *P. gongchengensis* Y.S. Huang & Yan Liu in vegetative characters, but its obliquely campanulate corolla is obviously different from the latter two species.

KEY WORDS: Karst cave, limestone flora, morphology, molecular phylogeny, *Primulina gongchengensis*, *P. huijiensis*, *P. yulinensis*.

INTRODUCTION

Recent progress in molecular phylogenetic showed that the formerly monotypic *Primulina* Hance (Hance, 1883) was recircumscribed and expanded to include *Chirita* sect. *Gibbosaccus* C.B. Clarke, *Chiritopsis* W.T. Wang, *Wentsaiboea* D. Fang & D.H. Qin (excluding *W. tiandengensis* Yan Liu & B. Pan in Liu *et al*., 2010) (Wang *et al*., 2011; Weber *et al*., 2011), becoming the largest genera of Gesneriaceae in China. The nuclear internal transcribed spacer (ITS) and the chloroplast intergenic spacer *trnL-F*, are useful in addressing the taxonomical problems of *Primulina* (Guo *et al*., 2015; Wang *et al*., 2011; Weber *et al*., 2011; Xu *et al*., 2012; Xu *et al*., 2019). Until 2020, there are about 234 species of *Primulina* were recorded, which are mainly distributed in southern China and northern Vietnam (IPNI, 2020; Wen *et al*., 2020). In China, about 216 species have been discovered and described from the south and southwest areas (Guo *et al*., 2015; Wen *et al*., 2020). In the past decade, the number of new species in *Primulina* has steadily increased by about 10 per year (Xu *et al*., 2019). As this trend continues, the numbers of *Primulina* will continue to grow. However, traditional generic circumscription in Gesneriaceae is based largely on floral morphology and many of the newly described species are differed only by trivial morphological differences (Guo *et al*., 2015; Xu *et al*., 2012). Therefore, it is essential to combine morphological observation with molecular data for generic placement and species description, taking into account of both morphological and genetic divergences (Guo *et al*., 2015; Xu *et al*., 2012).

During a field work in Yanshan District, Guilin City, Guangxi, one species of *Primulina* was discovered on June 2019. This species is similar to *P. gongchengensis* in vegetative characters, but phenology of efflorescence and fructescence is distinctly different from *P. gongchengensis* has attracted the attention of the authors. Therefore, we went thrice to observe flowers during April to May 2020, and collected specimens and took photographs. The obliquely campanulate corolla showed that it's obviously different from *P. gongchengensis*. By consulting the relevant literatures (Fang and Qin, 2004; Liu *et al*., 2010; Ning *et al*., 2013; Wang, 1986; Weber *et al*., 2011; Wu *et al*., 2017; Xu *et al*., 2019), we found that the species is similar to *P. huaijiensis* Z.L. Ning & J. Wang in flower, but differs in the plant densely pubescent and glandular-puberulent, leaf blade elliptic to rhomboid-ovate, and obliquely campanulate corolla without appendages. To further assure generic placements of this species in *Primulina*, ITS sequences and *trnL-F* sequences of this species were amplified and included for phylogenetic analyses.

MATERIALS AND METHODS

Morphological observation

All the morphological characteristics, such as rhizomes, leaves, inflorescences, flowers and capsules were observed and measured in the field. The measurements, shape, color and other details given in this description are based on living plants.

Sampling and DNA sequencing

We randomly selected three plants from the same population to collect their leaves for DNA experiment. Fresh leaf materials were preserved in silica gel for quick drying. Total genomic DNA was extracted from dried leaves using modified cetyl trimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). ITS and *trnL-F* were amplified and sequenced following the methods of Möller *et al*. (2009) and Smissen *et al*. (2004), respectively. Besides, we downloaded the ITS and *trnL-F* sequences from GenBank for 188 *Primulina* species and

Table 1. Morphological comparison of ***Primulina rufipes***, ***P. huaijiensis***, ***P. gongchengensis*** and ***P. yulinensis***.

Character	***Primulina rufipes***	***P. huaijiensis***	***P. gongchengensis***	***P. yulinensis***
Leaf	4–8 × 4–7.5 cm, rhomboid-ovate, elliptic, cordate, densely pubescent and glandular-puberulent abaxially	2.5–4.0 × 2.8–4.5 cm, reniform, sparsely glandular-puberulent	6–20(30) × 2–10(15) cm, rhomboid-ovate or elliptic, densely glandular-pubescent	3.3–7 × 2.8–7.7 cm, cordate, densely pilose and glandular-puberulent
Corolla	8–10 mm long, obliquely campanulate, corolla lobes white to pale violet inside	7–9 mm long, white, gibbous, with lamellar appendages in the throat	22–28 mm long, tubular, purple	10 mm long, urceolate, white
Pistil	7–9 mm long	4–5 mm long	15–18 mm long	ca. 6 mm long
Style	5–7 mm long	2–3 mm long	12–14 mm long	4 mm long

two *Petrocodon* species. Species and GenBank accession numbers employed in this study are listed in the Supplementary materials (Table S1).

Phylogenetic analysis

The DNA sequences were aligned using the default parameters in MUSCLE 3.8.31 (Edgar, 2004) and further adjusted manually in BioEdit ver. 5.09 (Hall, 1999). Due to the limited phylogenetic signals in each of the two DNA segments (ITS and *trnL-F*), we performed the phylogenetic analyses only with the concatenated sequence matrixes using maximum likelihood (ML) method and Bayesian inference (BI). ML analyses were performed using RAxML-VI-HPC (Stamatakis, 2006) with the substitution model GTRGAMMA and 1000 rapid bootstrap searches (BS). For the Bayesian analyses, the best-fitting substitution model K81uf + I + G was determined using the corrected Akaike information criterion (AIC) in Modeltest ver. 3.06 (Posada and Crandall 1998). BI analyses were conducted in MrBayes ver. 3.2.6 (Ronquist *et al*., 2012). All BI analyses were run for 100 000 000 generations with four chains in two parallel runs and sampled every 5000 generations after a burn-in of the first 5000 trees. An average standard deviation below 0.01 was assumed to show that the two runs converged to a stationary distribution. All other parameters were set as default.

RESULTS AND DISCUSSION

Morphological analysis

Primulina rufipes is similar to *P. huijiensis*, from which it differs by its densely pubescent and glandular-puberulent all over the plant, elliptic to rhomboid-ovate leaf blade, and obliquely campanulate corolla without appendages. It is also close to *Primulina yulinensis* and *P. gongchengensis* in vegetative characters, but it can be easily distinguished from the obliquely campanulate corolla. The morphological comparison between the new species and another similar species is shown in Table 1.

Phylogenetic analysis

The combined matrix used for phylogenetic reconstruction had a length of 1871 characters (ITS: 949 bp; *trnL-F*: 922 bp), including 531 parsimony informative sites (ITS: 422 bp; *trnL-F*: 109 bp), 923 variable but parsimony uninformative sites (ITS: 634 bp; *trnL-F*: 289 bp) and 948 constant sites (ITS: 315 bp; *trnL-F*: 633 bp). The Bayesian majority-rule consensus tree is depicted in Fig. 1, annotated with bootstrap values (BS) of the ML analysis and posterior probabilities (PP) of BI analysis. The phylogenetic result presented here is roughly consisted with previous studies (Guo *et al*., 2015; Xu *et al*., 2019). All *Primulina* species including the assumed new one (*P. rufipes*) are clustered together in a lineage (BS = 100 %; PP = 1.00). Three samples of *P. rufipes* formed a clade with maximum support (BS = 100 %; PP = 1.00), but the definite systematic position of *P. rufipes* is still unclear considering the poorly resolved phylogenetic relationships recovered here. Although this specie unique morphological characters and we samples three accession that formed a clade with strong supports, the three samples were collected from one population, the interrelationships within the genus are not well resolved. Further study based on a broader sampling and more markers is still needed. According to its obliquely campanulate corolla, it can be distinguished from most species of *Primulina*. There are only 32 species with small corollas in *Primulina*, then only five of them have obliquely campanulate corolla, respectively *P. mollifolia* (D. Fang & W.T. Wang) J.M. Li & Yin Z. Wang, *P. renifolia* (D. Fang & D.H. Qin) J.M. Li & Yin Z. Wang, *P. luochengensis* (Yan Liu & W.B. Xu) Mich. Möller & A. Weber, *P. huaijiensis* Z.L. Ning & J. Wang, and *P. dichroantha* F. Wen, Y.G. Wei & S.B. Zhou. The morphological comparison showed that the plant indumentum, leaf blade shape, corolla color or stigma of these five species were different from the new species. The specific details are shown in the taxonomic key to *Primulina rufipes* and allies.

TAXONOMIC TREATMENT

Primulina rufipes Y.L. Su, P. Yang & Yan Liu, ***sp. nov.*** **红柄小花苣苔 Fig. 2 & 3**

***Type*:** CHINA. Guangxi, Guilin City, Yanshan District, Qifeng Town, elev. 182 m, 1 May 2020, *Yanshan Exped., 450311200501001LY* (holotype: IBK!, isotypes: IBK! and PE!).

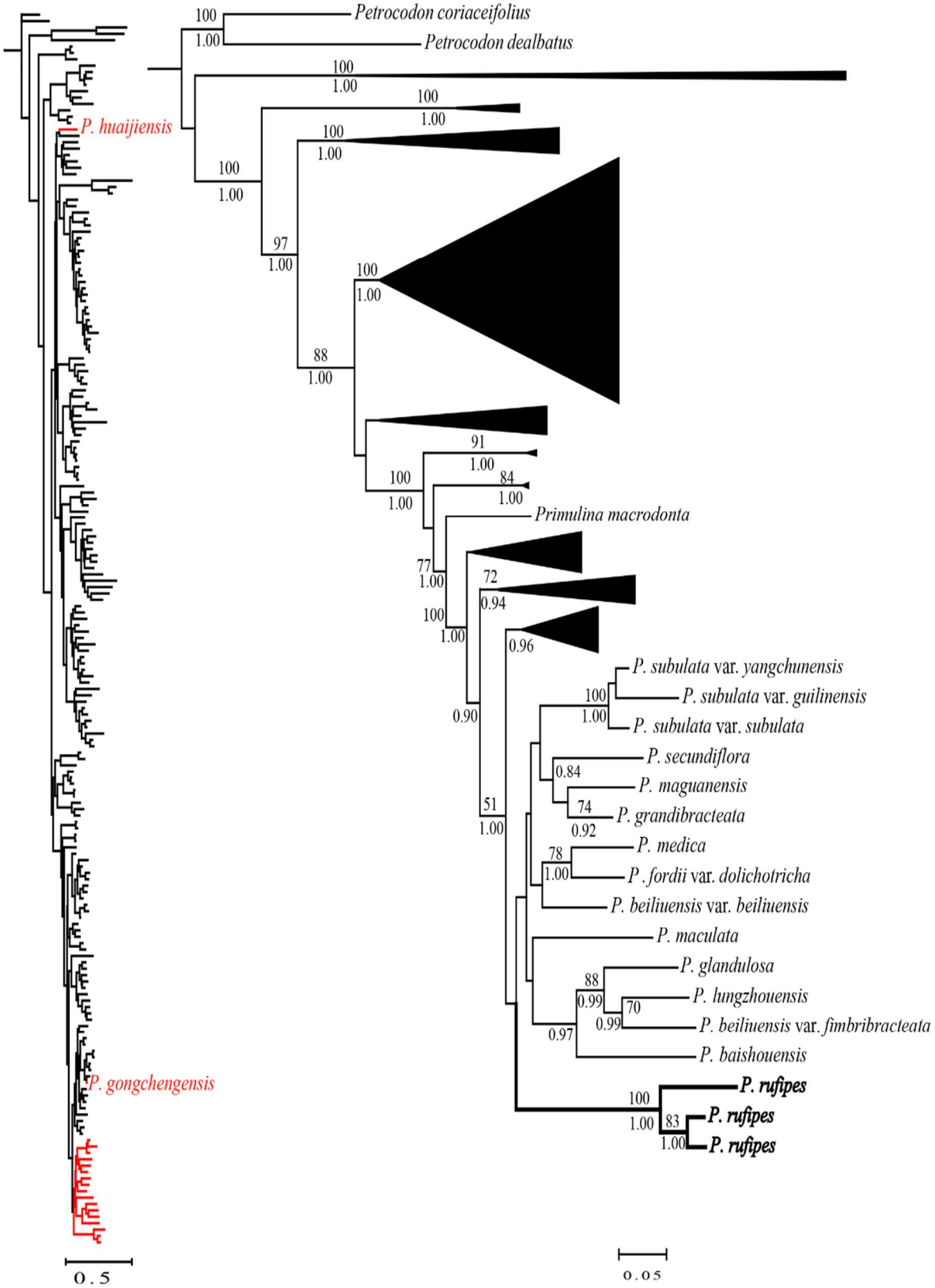

Fig. 1. The Bayesian phylogenetic trees from the analyses of the combined data of the ITS and chloroplast *trnL-F* regions. Bayesian posterior probability (PP > 0.5) and ML bootstrap support values (BS >50) are shown above and below the branch around the corresponding node. The accessions of ***Primulina rufipes*** are highlighted in bold.

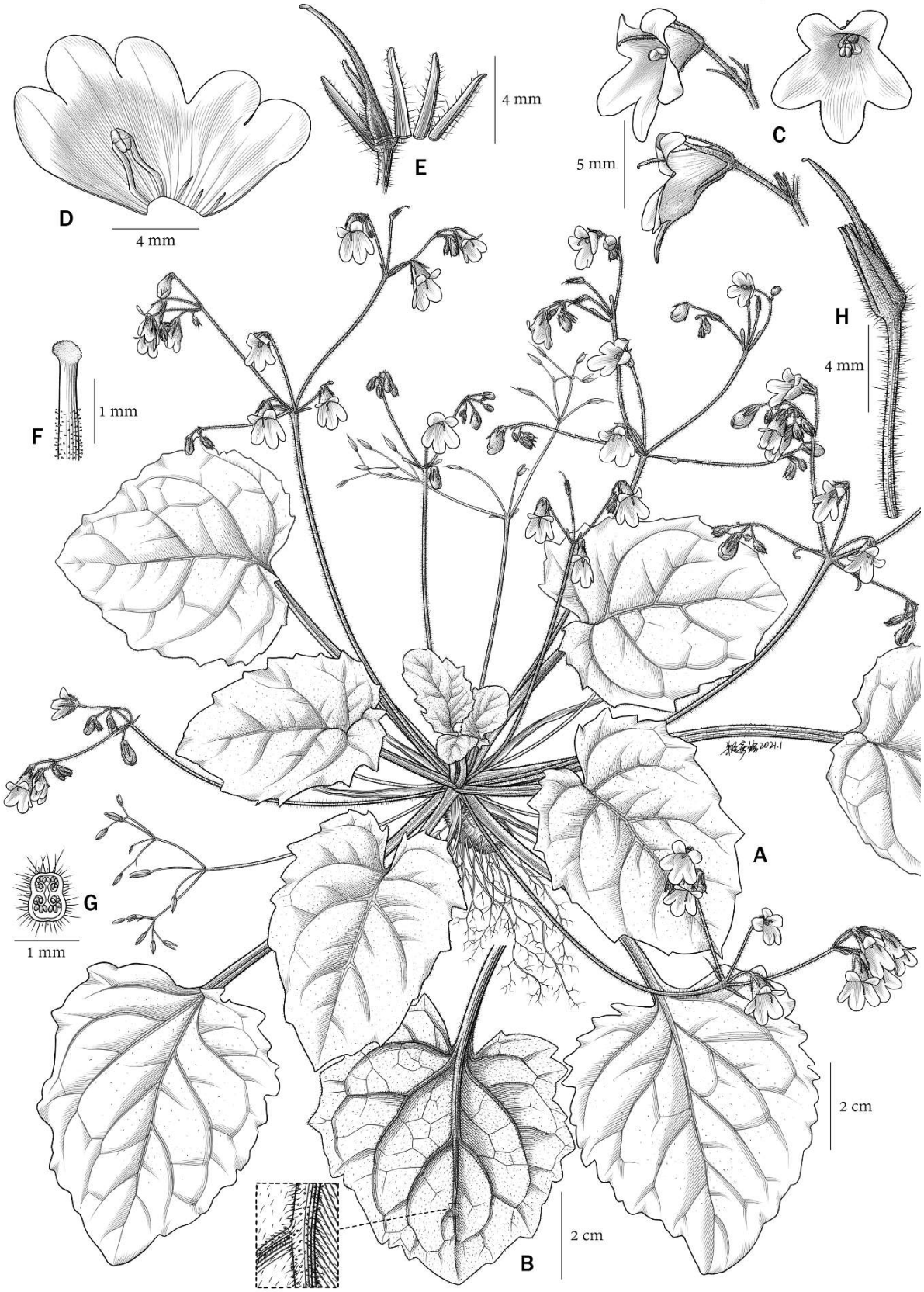

Fig. 2. ***Primulina rufipes*****: A:** Habit. **B:** Abaxial (bottom) view of leaves. **C:** Flower, face view (right), side view (top left and bottom left). **D:** Opened corolla. **E:** Pistil and calyx. **F:** Stigma and style. **G:** Equatorial section of ovule. **H:** Calyx and pistil.

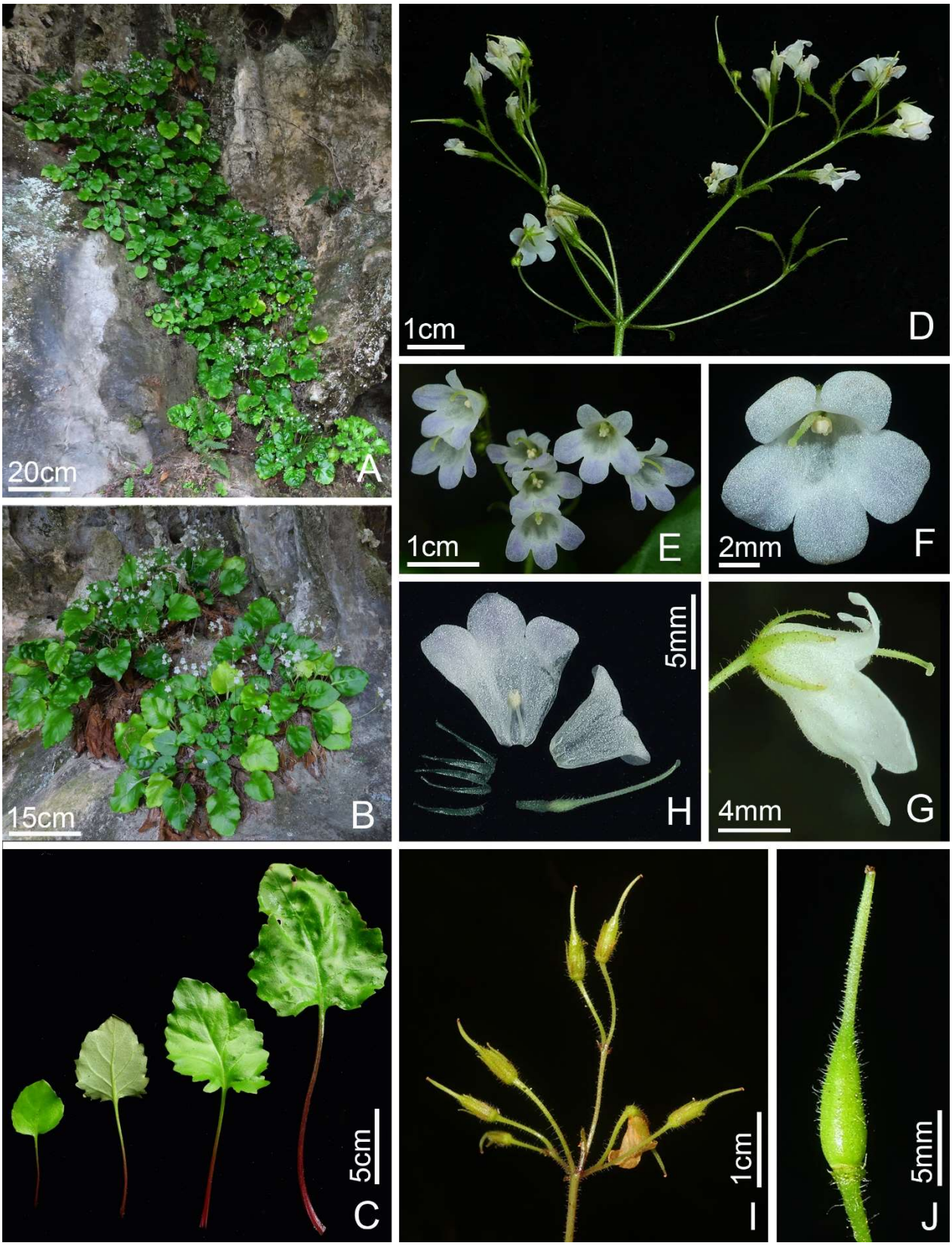

Fig. 3. ***Primulina rufipes*****: A:** Habitat. **B:** Habit. **C:** Leaves. **D, E:** Flowering inflorescences. **F:** Flower in face view. **G:** Flower in side view. **H:** Opened corolla and calyx. **I:** Capsules. **J:** Pistil.

***Diagnosis*:** *Primulina rufipes* is similar to *P. huaijiensis* Z.L. Ning & J. Wang (Fig. S1), but it can be distinguished from the latter by its densely pubescent and glandular-puberulent all over the plant, elliptic to rhomboid-ovate leaf blade leaf blade, and obliquely campanulate corolla without appendages.

***Description*:** Perennial herb. **Rhizome** subterete. **Leaves** basal, 5–14; **petiole** 5–11cm long, red, sparsely pubescent and glandular-puberulent; **leaf blade** herbaceous, rhomboid-ovate, elliptic or cordate, 4–8 × 4–7.5 cm, densely pubescent adaxially, densely pubescent and glandular-puberulent abaxially, apex obtuse or slightly acute, base asymmetric, broadly cuneate to cordate, margin irregularly serrate to denticulate; lateral veins 4–7 on each side of the midrib. **Cymes** axillary, 3–7 inflorescences per plant, 1–4-branched, 7–32-flowered for each inflorescence; **peduncle** 8–16 cm long, sparsely pubescent and glandular-puberulent. **Bracts** opposite, linear to lanceolate or spathulate, 5–10 × 1–3 mm, reflexed, margin entire or lobed, both surfaces sparsely pubescent and glandular-puberulent. **Pedicel** 0.5–1.5 cm long, sparsely pubescent and glandular-puberulent. **Calyx** 5-parted from base, sepals linear-lanceolate, sparsely pubescent and glandular-puberulent, 4–6 × 0.6–0.8 mm. **Corolla** obliquely campanulate, abaxially swollen, 8–10 mm long, sparsely pubescent outside, glabrous inside; tube white, 5–6 mm long, 4–6 mm in diameter at the mouth; corolla lobes white to pale violet, adaxial lip distinctly 2-lobed, lobes ovate to suborbicular, ca. 3 × 4 mm; abaxial lip 3-lobed, lobes ovate, ca. 3 × 4 mm, apex rotund or obtuse. **Stamens** 2, adnate to 1 mm above the corolla tube base; **filaments** linear, glabrous, ca. 3 mm; **anthers** reniform, cream to light apricot, ca. 1.5 mm long, glabrous. **Staminodes** 3, glabrous, slightly swollen at apex, adnate to 0.5 mm above the corolla tube base, lateral ones ca. 1 mm long, middle one ca. 0.7 mm long. **Pistil** 7–9 mm long; **disc** ring-like, ca. 0.2 mm height, glabrous, margin inconspicuously repand; **ovary** narrowly ovoid, ca. 2 × 1.2 mm, densely pubescent; **style** 5–7 mm long, sparsely pubescent; **stigma** sub-hippocrepiform, ca. 0.5 mm long. **Capsule** ovoid, ca. 6 mm long, ca. 2 mm in diameter, sparsely pubescent and glandular-puberulent.

***Distribution, habitat and ecology*:** *Primulina rufipes* was found growing at the entrance of karst cave in Yanshan District, Guilin City, Guangxi, China. The habitat of the new species is located in the subtropical zone, which belongs to subtropical monsoon climate. The main companion species are *Primulina. bipinnatifida* (W.T. Wang) Yin Z. Wang & J.M. Li, *Lindenbergia muraria* (Roxburgh ex D. Don) Bruhl, *Adiantum malesianum* Ghatak, etc.

***Phenology*:** flowering from April to May; fruiting from June to July.

***Etymology*:** The specific epithet refers to petiole red of the new species.

***Additional specimen examined* (paratype):** CHINA. Guangxi, Guilin City, Yanshan District, Qifeng Town, elev. 182 m, 16 June 2019, *Yanshan Exped., 450311190616031LY* (IBK!).

Taxonomic key to *Primulina rufipes* and allies

1a. Corolla white.
  2a. Leaf blade elliptic to rhomboid-ovate, densely pubescent and glandular-puberulent all over the plant; corolla obliquely campanulate, without appendages in the throat ...…….… ***P. rufipes***
  2b. Leaf blade reniform to suborbicular, glabrous or sparsely glandular-puberulent; corolla gibbous, with lamellar appendages in the throat ...…….…..... ***P. huaijiensis***
1b. Corolla purplish red.
  3a. Leaf blade elliptic, base broadly cuneate …..… ***P. luochengensis***
  3b. Leaf blade reniform to suborbicular.
    4a. corolla adaxial lip white and abaxial lip reddish purple; anthers locules slightly divaricate …..……..…. ***P. dichroantha***
    4b. Corolla lip purplish red, anthers locules obviously divaricate
      5a. Leaf blade glabrous or sparsely glandular-puberulent; corolla obliquely campanulate; filaments geniculate ……. ……. ***P. renifolia***
      5b. Leaf blade densely pubescent; corolla nearly tubular; filaments straight ……...……..… ***P. mollifolia***

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LITERATURE CITED

Doyle, J.-J. and J.-L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19:** 11–15.

Edgar, R.-C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. **32(5)**: 1792–1797.

Fang, D. and D.-H. Qin. 2004. *Wentsaiboea* D. Fang et D.H. Qin, a new genes of Gesneriaceae from Guangxi, China. Acta Phytotax. Sin. **42**: 533–536.

Guo, J., B. Pan, J. Liu, W.-B. Xu and K.-F. Chung. 2015. Three new species of *Primulina* (Gesneriaceae) from limestone karsts of China based on morphological and molecular evidence. Bot. Stud. **56(1)**: 34–46.

Hall, T.-A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. **41**: 95–98.

Hance, H.-F. 1883. New Chinese Cyrtandreae. J. Bot. **21**: 165–170.

IPNI. 2020. The International Plant Names Index. [accessed 20 December 2020] https://www.ipni.org/

Liu, Yan, W.-B. Xu and B. Pan. 2010. *Wentsaiboea tiandengensis* sp. nov. and *W. luochengensis* sp. nov. (Gesneriaceae) from Karst caves in Guangxi, southern China. Nordic J. Bot. **28(6):** 739–745.

Möller, M., M. Pfosser, C.-G. Jang, V. Mayer, A. Clark, M.-L. Hollingsworth, M.-H.-J. Barfuss, Y.-Z. Wang, M. Kiehn and A. Weber. 2009. A preliminary phylogeny of the Didymocarpoid Gesneriaceae based on three molecular data sets: incon gruence with available tribal classifications. Amer. J. Bot. **96(5)**: 989–1010.

Ning, Z.-L., G.-F. Li, J. Wang, J.-F. Smith, H. Rasolonjatovo and M. Kang. 2013. *Primulina huaijiensis* (Gesneriaceae), a new species from Guangdong, China. Ann. Bot. Fenn. **50(1-2)**: 119–122.

Posada, D. and K.-A. Crandall. 1998. ModelTest: testing the model of DNA substitution. Bioinformatics **14(9)**: 817–818.

Ronquist, F., M. Teslenko, P.-V.-D. Mark, D.-L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.-A. Suchard and J.-P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice. Syst. Biol. **61(3)**: 539–542.

Smissen, R.-D., I. Breitwieser and J.-M. Ward. 2004. Phylogenetic implications of trans-specific chloroplast DNA sequence polymorphism in New Zealand Gnaphalieae (Asteraceae). Plant Syst. Evol. **249(1-2)**: 37–53.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22(21):** 2688–2690.

Wang, W.-T. 1986. Notulae de Gesneriaceis Sinensibus (VII). Guihaia **6**: 1–15

Wang, Y.-Z., R.-B. Mao, Y. Liu, J.-M. Li, Y. Dong, Z.-Y. Li, and J.-F. Smith. 2011. Phylogenetic reconstruction of *Chirita* and allies (Gesneriaceae) with taxonomic treatments. J. Syst. Evol. **49(1)**: 50–64.

Weber, A., D.-J. Middleton, A. Forrest, R. Kiew, C.-L. Lim, A.-R. Rafdah, S. Sontag, P. Triboun, Y.-G. Wei, T.-L. Yao and M. Möller. 2011. Molecular systematics and remodeling of *Chirita* and associated genera (Gesneriaceae). Taxon **60(3)**: 767–790.

Xu, W.-B., B. Pan, Y. Liu, C.-I. Peng and K.-F. Chung. 2012 Two new species, *Primulina multifida* and *P. pseudomollifolia* (Gesneriaceae), from karst caves in Guangxi, China. Bot. Stud. **53**: 165–175.

Xu, W.-B., H. Chang, J. Huang and K.-F. Chung. 2019. Molecular systematics of Chiritopsis-like *Primulina* (Gesneriaceae): one new species, one new name, two new combinations, and new synonyms. Bot. Stud. **60(1)**: 13–21.

Wen, F., Y.-G. Wei, L.-F. Fu, Z.-B. Xin, S. Li, Z.-J. Huang, Y.-Z. Ge, D.-C. Meng. 2020. The Checklist of Gesneriaceae in China. [accessed 20 December 2020] http://gccc.gxib.cn/cn/about-68.aspx

Wu, H.-T., S.-B. Zhou, W. Ma, O.-W. Wang. 2017. *Primulina dichroantha* (Gesneriaceae), a New Species from a Karst Cave from Guangxi, China. Ann. Bot. Fenn. **54(1-3)**: 95–98.

Supplementary materials are available from Journal Website.