

Two new species, *Primulina multifida* and *P. pseudomollifolia* (Gesneriaceae), from karst caves in Guangxi, China

Wei-Bin XU¹, Bo PAN¹, Yan LIU¹, Ching-I PENG^{2,*}, and Kuo-Fang CHUNG^{3,*}

¹Guangxi Institute of Botany, Guangxi Zhuangzu Autonomous Region and the Chinese Academy of Sciences, Guilin 541006, China

²Herbarium (HAST), Biodiversity Research Center, Academia Sinica, Nangang, Taipei 115, Taiwan

³School of Forestry and Resource Conservation, National Taiwan University, Daan, Taipei 106, Taiwan

(Received June 27, 2011; Accepted December 6, 2011)

ABSTRACT. *Primulina pseudomollifolia* W.B. Xu & Yan Liu and *P. multifida* B. Pan & K.F. Chung from karst caves in Guangxi Zhuangzu Autonomous Region, China are described and illustrated as two new species. Their generic placement in the recently recircumscribed *Primulina* is supported by phylogenetic analyses of DNA sequence nuclear ITS and chloroplast *trnF-L* intron spacer regions. *Primulina pseudomollifolia* is similar to *P. mollifolia* (D. Fang & W. T. Wang) J.M. Li & Y.Z. Wang in being densely pubescent on both blade surfaces, but differs in the base of its blades being cuneate to broadly cuneate, its 12-14 mm long white to pink corolla 8-9 mm long tubular corolla tube, 5-6 mm long filaments, 3 staminodes, and flowering from July to September. *Primulina multifida* resembles *P. renifolia* (D. Fang & D. H. Qin) J. M. Li & Y. Z. Wang, but differs in the blade margins being pinnatifid; its ovate to broadly ovate bracts, 3-4 × 1.5-2 mm, 5-7 mm long tubular corolla tube, 3-5 mm long filaments, 3 staminodes, linguiform stigma, and flowering from June to August.

Keywords: Gesneriaceae; Guangxi; Karst cave; Limestone flora; New species; *Phylogenetic analysis*; *Primulina mollifolia*; *Primulina multifida*; *Primulina pseudomollifolia*; *Primulina renifolia*.

INTRODUCTION

Guangxi Zhuangzu Autonomous Region of southern China resides in the heart of an immense limestone terrain stretching across the Sino-Vietnamese border (Xu, 1995). Renowned for its picturesque karst landscapes, Guangxi is also one of the major biodiversity hotspots in China (López-Pujol et al., 2011), distinguished for its extraordinarily high diversity and endemism of vascular plants associated with limestone ecosystems (Xu, 1995; Hou et al., 2010). *Aspidistra* Ker Gawl. (e.g., Hou et al., 2009; Lin et al., 2010; Liu et al., 2011), *Polystichum* Roth (e.g., He and Zhang, 2011; Zhang and He, 2009a,b; Zhang et al., 2010), *Begonia* L. (e.g., Ku et al., 2008; Peng et al., 2008, 2010), *Impatiens* L. (e.g., Yu et al., 2009), and several genera of Gesneriaceae (e.g., Xu et al. 2010; Pan et al., 2010; Wu et al., 2011) are but a few of the many species-rich and highly diversified plant taxa that are restricted to caves and crevices of various limestone formations. However, the majority of these limestone species are extremely rare, known only from a handful of localities.

As limestone caves and crevices are fragile microhabitats that are highly vulnerable to the increasing economic activities in the region (Clements et al., 2006), their effective conservation depends on urgent and accurate biodiversity updates.

In the course of karst cave floristic surveys in Guangxi in 2004-2009, we collected two interesting species of the genus *Chiritopsis*. After consulting national floras and other relevant literature, we were unable to identify these as any known species in the region (e.g., Wang et al., 1998; Li and Wang, 2004; Shen et al., 2010). *Chiritopsis* species are perennial herbs with stout rhizomes and basal rosette leaves (Li and Wang, 2007) found exclusively in limestone caves and crevices in Guangxi and Guangdong (Shen et al., 2010). *Chiritopsis* differs from the closely related *Chirita* sect. *Gibbosaccus* C.B. Clarke in its smaller corolla and overall size and its ovaries being shorter than its styles (Li and Wang, 2007). Containing more than 100 described species (Möller et al., 2011), *Chirita* sect. *Gibbosaccus* is also one of the most characteristic taxa of the remarkably diverse limestone flora in the Sino-Vietnamese border. Recent molecular phylogenetic studies have surprisingly challenged the monophyly of *Chiritopsis* as well as its allied *Chirita* sect. *Gibbosaccus* (Li and Wang, 2007; Möller et al., 2009;

*Corresponding author: E-mail: bopeng@sinica.edu.tw (Ching-I PENG); E-mail: kuofangchung@ntu.edu.tw (Kuo-Fang CHUNG).

Wang et al., 2011; Weber et al., 2011b). Instead, these two taxa, along with the Guangxi endemic *Wentsaiboea* D. Fang & D.H. Qin (Fang and Qin, 2004), form a highly supported clade with the monotypic *Primulina* Hance nested within it (Wang et al., 2011; Weber et al., 2011b). Because of the taxonomic priority, the definition of *Primulina* was expanded to accommodate *Chirita* sect. *Gibbosaccus*, *Wentsaiboea* (*W. renifolia*) and *Chiritopsis* (Wang et al., 2011; Weber et al., 2011b).

Traditional generic circumscription in Gesneriaceae is based largely on floral morphology (e.g., Wang et al., 1998). These recent phylogenetic studies and taxonomic remodeling thus highlight their complicated evolutionary histories, cautioning application of the highly homoplasious floral traits when assigning generic position to Sino-Vietnamese Gesneriaceae (Möller et al., 2011). For instance, of the two recently described species *Wentsaiboea luochengensis* Y. Liu & W.B. Xu and *W. tiandengensis* Y. Liu & B. Pan published prior to the taxonomic changes (Liu et al., 2010), only *W. luochengensis* was placed within *Primulina* (Möller et al., 2011), while *W. tiandengensis* was phylogenetically allied

to *Petrocodon* Hance (Weber et al., 2011a). To assure the phylogenetic relationships and generic placement of the two new “*Chiritopsis*”-like species, DNA sequence data were collected and analyzed. Our results (Figure 1) confirmed that the two new species were indeed members of the recircumscribed *Primulina* (Wang et al., 2011; Weber et al., 2011b), adding two new species to this remarkably rich and diverse genus.

MATERIAL AND METHODS

Total genomic DNA was extracted from silica-gel dried leaves using the CTAB protocol (Doyle and Doyle, 1987). DNA sequences of nuclear internal transcribed spacers (ITS) and the chloroplast *trnL-F* intron-spacer region (*trnL-F*) were amplified based on the PCR procedures outlined in Möller et al. (2009). PCR products were purified using Exo-Sap and cycle-sequenced using Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, CA).

In our initial search through NCBI GenBank using DNA sequences of the two new species, the closest BLAST hits

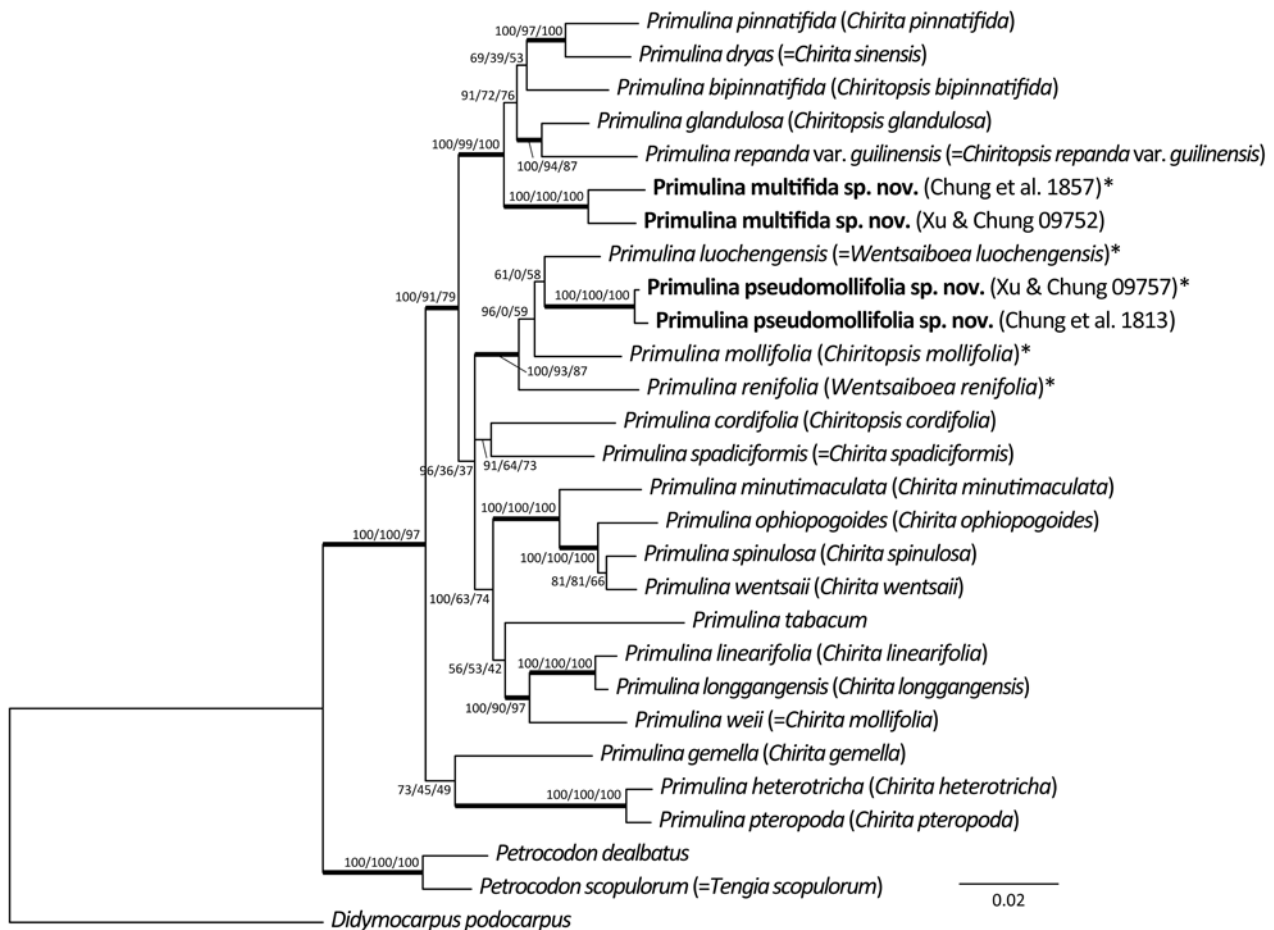


Figure 1. Bayesian consensus cladogram with average branch lengths. Numbers adjacent to nodes are Bayesian posterior clade probabilities (PP), Maximum likelihood bootstrap (MB) values, and Maximum parsimony bootstrap (PB) values (PP/MB/PB). Asterisks (*) denote samples collected from type localities. Highly supported (PP>95 and MB>90) clades are depicted with thick lines.

were species in the recently recircumscribed *Primulina* (Wang et al., 2011; Weber et al., 2011b) as expected based on morphological characteristics. To further elucidate the phylogenetic affinities of these new taxa, we included species of *Primulina* (Wang et al., 2011; Weber et al., 2011b) with both ITS and *trnL-F* sequences available in Genbank in our analyses. In total, the phylogenetic analyses consisted of 16 species of *Primulina* with negligible ambiguous nucleotides (Appendix 1), including 13 species of *Chirita* sect. *Gibbosaccus*, 4 species of *Chiritopsis*, 1 species of *Wentsaiboaea* (Liu et al., 2010; Möller et al., 2011), and the type species of *Primulina* (*P. tabacum* Hance). To test phylogenetic affinities of *Primulina mollifolia* (*Chiritopsis mollifolia*) and *P. renifolia* (*W. renifolia*) that are morphologically similar to *P. pseudomollifolia* and *P. multifida*, respectively, their ITS and *trnL-F* sequences were also gathered from plants collected in their type localities. *Petrocodon dealbatus* Hance, *Pet. scopulorum* (= *Tengia scopulorum* (Chun) Yin Z. Wang), and *Didymocarpus podocarpus* C.B. Clarke were chosen as outgroups based on recent phylogenetic analyses (Möller et al., 2011; Weber et al., 2011b).

DNA sequences were aligned using the program MUSCLE implemented in the software MEGA5 (Tamura et al., 2011) with minor manual adjustments. The aligned sequence matrix is available upon request to the corresponding author. Phylogenetic analyses were conducted based on Maximum Parsimony (MP) and Maximum Likelihood (ML) using MEGA5 (Tamura et al., 2011) and Bayesian Inference (BI) using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). We used the min-mini heuristic search option to partially delete indels and search MP trees for MP analysis. Clade supports were calculated based on 100 bootstrap resamplings. In ML analysis and based on results of mrModeltest (Nylander, 2004; see below), we selected the General Time Reversible model with gamma distributed (GTR+G) rate among site and 6 discrete gamma categories. We chose the Nearest-Neighbor-Interchange (NNI) inference method to search the ML tree with all sites used and the initial tree automatically selected. Clade supports were evaluated based on 100 bootstrap resamplings. We used mrModeltest (Nylander, 2004) to select DNA substitution models based on the Akaike Information Criterion (AIC) for BI analysis. For ITS, AIC selected the Symmetrical model with gamma distributed (SYM+G) and the model GTR+G was selected for *trnL-F*. BI analyses were conducted using mixed models specified to each data partition. We performed one run of Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analyses, with a random starting tree and four chains for each run (one 3 cold and three heated). The MCMCMC length was two million generations, and the chain was sampled every 100th generation from the cold chain. Bayesian clade posterior probabilities and average branch lengths were calculated based on the sampled trees after the first 10% of the sampled trees were discarded as burn-in. The burn-in for each run was determined by plotting the likelihood values against the generations.

RESULTS AND TAXONOMIC TREATMENT

1. *Primulina pseudomollifolia* W. B. Xu & Yan Liu, sp. nov.—TYPE: CHINA. Guangxi Zhuangzu Autonomous Region: Rongshui Xian (County), Rongshui Zhen (Township), alt. 110 m, on moist limestone rock face in a karst cave, 19 July 2009, *Wei-Bin Xu & Kuo-Fang Chung 09757* (holotype: IBK; isotypes: HAST, PE). 假密毛小花苣苔 Figures 2, 3

Similis P. mollifoliae (D. Fang & W. T. Wang) J. M. Li & Y. Z. Wang, *sed foliis basi cuneatis usque late cuneatis, corolla alba usque rosea, 12-14 mm longa, tubo corollae tubulari, 8-9 mm longo, filamentis 5-6 mm longis, staminodiis 3 differt.*

Herbs perennial. Rhizome subterete, 10-30 mm long, 5-10 mm wide. Leaves 10-15, basal, long petiolate, subcarinate, papery when dry; petiole flattened, 4-10 cm long, 2-4 mm wide, densely pubescent; blades ovate to broadly ovate or elliptic to suborbicular, 2.5 × 1.5-3 cm, densely pubescent on both surfaces, base cuneate to broadly cuneate, sometimes inequilateral, margin repand, apex obtuse to rounded; lateral veins inconspicuous, 2-3 on each side. Cymes 5-10, axillary, 1-4-branched, 4-30-flowered; peduncle 6-15 cm long, 1-2 mm in diam.,

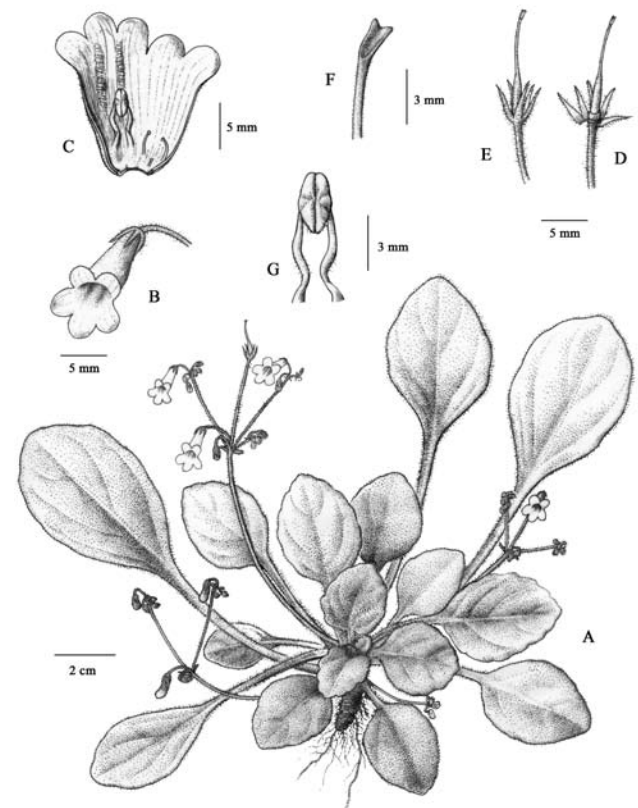


Figure 2. *Primulina pseudomollifolia* W. B. Xu & Yan Liu. A, habit; B, flower; C, corolla opened showing stamens and staminodes; D, calyx opened showing disc and pistil; E, pistil and calyx; F, stigma; G, stamens. (Drawn by S. Q. He from the holotype).



Figure 3. *Primulina pseudomollifolia* W. B. Xu & Yan Liu. A, habitat; B, C, habit; D, flowers face view; E, flower side view; F, young fruit.



Figure 4. *Primulina mollifolia* (A-C) and *P. renifolia* (D, E). A, habit; B, flower side view; C, flowers face view; D, habit; E, flowers.

pubescent; bracts 2, opposite, linear-lanceolate, 4-5 × 0.5-1 mm, margin entire, pubescent; pedicel 10-30 mm long, pubescent. Calyx 5-parted to base, lobes lanceolate-linear, 3-4 × 0.5-1 mm, apex acuminate, outside pubescent, inside sparsely puberulent, margins entire. Corolla white to pink, 12-14 mm long, outside puberulent, inside sparsely puberulent, with 2 pale purple stripes; corolla tube 8-9 mm long, 4-5 mm in diam. at the mouth, ca. 3.5 mm in diam. at the base; limb distinctly 2-lipped, white; adaxial lip 2-parted to over the middle, lobes oblong or rounded, 2.5-4 × 2-3 mm; abaxial 3-lobed to near the middle, lobes oblong, 2.5-3 × 2.5-3 mm; stamens 2, adnate to 2.5 mm above the corolla tube base; filaments linear, 5-6 mm long, geniculate near the middle, sparsely puberulent; anthers 2-3 mm long, dorsifixed, glabrous; staminodes 3, lateral ones 2-3 mm long, apex capitate, glabrous, adnate to ca. 2 mm above the corolla tube base, middle one ca. 0.8 mm long, apex capitate, glabrous, adnate to ca. 0.7 mm above the corolla tube base. Disc annular, ca. 0.7 mm in height, margin repand, glabrous. Pistil 8-12 mm long, ovary narrowly ovoid, 3-5 mm long, ca. 1 mm across, puberulent; style 4-6 mm long, puberulent; stigma obtuse-trapeziform, ca. 2 mm long, 0.7-1 mm wide, apex 2-lobed. Capsule narrowly ellipsoidal, 7-10 mm long, 1.5-2 mm across, pubescent.

Additional specimens examined. CHINA. Guangxi Zhuangzu Autonomous Region, Rongshui Xian, Rongshui Zhen, 4 Oct 2004, *Yan Liu L1116* (IBK); same locality, 29 Sep 2005, *Yan Liu & Wei-Bin Xu 050901* (IBK); same locality, 4 Sep 2008, *Wei-Bin Xu 08125* (IBK); same locality, 19 July 2009, *Wei-Bin Xu & Kuo-Fang Chung 09761* (IBK); same locality, 8 Mar 2011, *Wei-Bin Xu & Yu-Song Huang 11149* (IBK). Rongshui Xian, Rongshui Zhen, Guding Village, 19 July 2009, *Kuo-Fang Chung, Wei-Bin Xu, Tze-Lin Pan, & Yung-Shin Tsai 1813* (HAST).

Ecology and distribution. *Primulina pseudomollifolia* is known only from two localities in Rongshui Xian, Guangxi Zhuangzu Autonomous Region, China (Figure 7). It grows on moist limestone rock faces at karst cave entrances.

Phenology. Flowering from July to September, fruiting September to October.

Etymology. The specific epithet is derived from its resemblance to *Primulina mollifolia* (D. Fang & W. T. Wang) J. M. Li & Y. Z. Wang.

Notes. *Primulina pseudomollifolia* is similar to *Primulina mollifolia* (D. Fang & W. T. Wang) J. M. Li & Y. Z. Wang (Figure 4A-C) in being densely pubescent on both surfaces of blades, differing by the base of blades cuneate to broadly cuneate (vs. rounded to cordate); corolla white to pink, 12-14 mm long (vs. corolla purplish, 7.5-9 mm long); corolla tube tubular, 8-9 mm long (vs. corolla tube campanulate, 5-6 mm long); stamens adnate to 2.5 mm above the corolla tube base (vs. adnate to 0.4-0.6 mm above the corolla tube base); filaments 5-6 mm long (vs. 2-2.3 mm long); staminodes 3, lateral ones 2-3 mm long, middle one ca. 0.8 mm long (vs. 2, 0.5-1 mm long); flowering from July to September (vs. April to May).

2. *Primulina multifida* B. Pan & K. F. Chung, sp. nov.—TYPE: CHINA. Guangxi Zhuangzu Autonomous Region, Lipu Xian (County), Shuangjiang Zhen (Township), alt. 170 m, on moist limestone rock face in a karst cave, 5 July 2009, *Wei-Bin Xu & Bo Pan 09806* (holotype: IBK; isotype: HAST, PE). 多裂小花苣苔

Figures 5, 6

Species similis *P. renifoliae* (D. Fang & D. H. Qin) J. M. Li & Y. Z. Wang, *sed differt foliis margine pinnatifidis, bracteis ovatis usque late ovatis, 3-4 mm longis, 1.5-2 mm latis, tubo corollae tubulari, 5-7 mm longo, filamentis 3-5 mm longis, staminodiis 3, stigmatibus lingulato.*

Herbs perennial. Rhizome subterete, 10-20 mm long, 4-6 mm wide. Leaves 6-15, basal, long petiolate, herbaceous; petiole subterete, 3-15 cm long, 1.5-3 mm wide, pubescent and glandular-pubescent; blades reniform to obovate, 3-5 × 5-8 cm, pubescent and glandular-pubescent on both surfaces, base cordate to deeply cordate, margin pinnatifid, sometimes repand, apex obtuse to rounded; inconspicuously palmately 3-5-nerved on both surfaces. Cymes 5-9, axillary, 1-3-branched, 4-15-flowered; peduncle 7-18 cm long, 1-2 mm in diam., pubescent and glandular puberulent; bracts 2, opposite, ovate to broadly

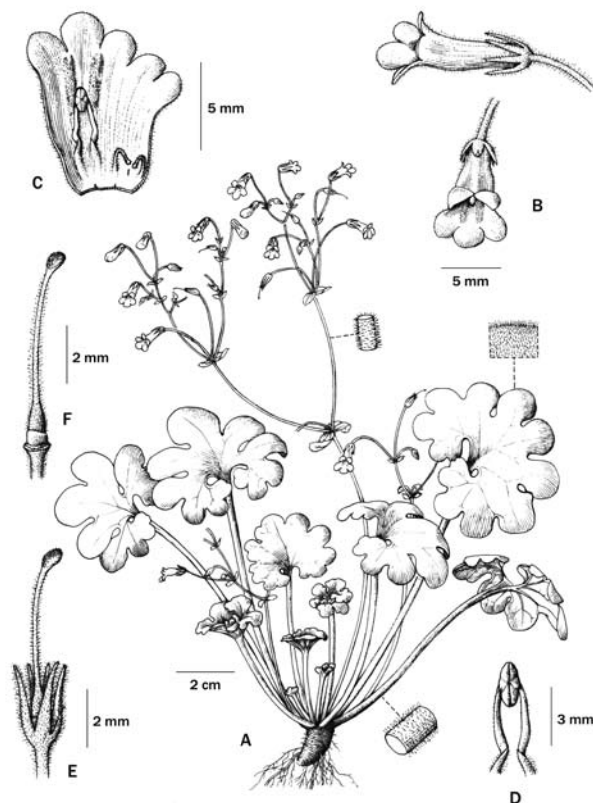


Figure 5. *Primulina multifida* B. Pan & K.F. Chung. A, habit; B, flower, face and side view; C, corolla opened showing stamens and staminodes; D, stamens; E, calyx and pistil; F, pistil and disc. (Drawn by S. Q. He from the holotype).



Figure 6. *Primulina multifida*. A, B, habitat of the population; C, D, Habit; E, Flowers face view; F, flower side view.

ovate, 3-4 × 1.5-2 mm, margin entire, pubescent and glandular-pubescent; pedicel 8-20 mm long, pubescent and glandular-pubescent. Calyx 5-parted to base, lobes lanceolate-linear, 3-6 × 0.5-1 mm, apex acuminate, outside pubescent and glandular-pubescent, inside sparsely puberulent, margins entire. Corolla white, 8-12 mm long, outside puberulent and glandular-puberulent, inside sparsely puberulent, with 2 pale brown stripes; corolla tube 5-7 mm long, 3-4 mm in diam. at the mouth, ca. 2 mm in diam. at the base; limb distinctly 2-lipped, white; adaxial lip 2-parted to over the middle, lobes oblong or rounded, 2-3 × 2-3 mm; abaxial 3-lobed to over the middle, lobes oblong or rounded, 2-3 × 2-3 mm; stamens 2, adnate to 2-2.5 mm above the corolla tube base; filaments linear, 3-5 mm long, geniculate near the base, sparsely puberulent and glandular-puberulent; anthers 1.5-2 mm long, dorsifixed, glabrous; staminodes 3, lateral ones 1.5-2 mm long, apex capitate, sparsely puberulent and glandular-puberulent, adnate to ca. 1.5 mm above corolla tube base, middle one ca. 0.3 mm long, inconspicuously, adnate to ca. 0.8 mm above base of corolla tube. Disc annular, ca. 0.8 mm high, margin repand, glabrous. Pistil 6-9 mm long, ovary narrowly ovoid, 2-3 mm long, ca. 1 mm across, glandular-puberulent; style 3-5 mm long, glandular-puberulent; stigma linguiform, ca. 1 mm long, 0.6-0.8 mm wide. Capsule narrowly ellipsoidal, 4-6 mm long, 1.5-2 mm across, sparsely pubescent.

Additional specimens examined. CHINA. Guangxi Zhuangzu Autonomous Region, Lipu Xian (county), Shuangjiang Zhen (township), 5 July 2009, *Wei-Bin Xu & Bo Pan 09805* (IBK), same locality, 28 July 2009, *Kuo-Fang Chung, Wei-Bin Xu, Bo Pan, Tze-Lin Pan, & Yun-Shin Tsai 1857* (HAST); Yangshuo Xian, Gaotian Zhen, 27 July 2009, *Wei-Bin Xu & Kuo-Fang Chung 09752* (IBK).

Ecology and distribution. *Primulina multifida* is known from the type locality in Lipu Xian and the southern Yangshuo Xian, Guangxi Zhuangzu Autonomous Region, China (Figure 7). It grows on moist limestone rock faces at karst cave entrances.

Phenology. Flowering from June to August, fruiting July to October.

Etymology. The specific epithet is derived from its pinnatifid blade margins.

Notes. *Primulina multifida* resembles *P. renifolia* (D. Fang & D. H. Qin) J. M. Li & Y. Z. Wang (Figure 3D, E), differing by the pinnatifid blade margins (vs. subentire, rarely repand); ovate to broadly ovate bracts, 3-4 × 1.5-2 mm (vs. linear, rarely oblanceolate, 1-3 × ca. 1 mm); tubular corolla tube, 5-7 mm long (vs. campanulate, ca. 4 mm long); adnate stamens to 2-2.5 mm above the corolla tube base (vs. adnate to 0.5 mm above the corolla tube base); 3-5 mm long filaments (vs. 2.5 mm long); 3 staminodes, lateral ones 1.5-2 mm long, middle one ca. 0.3 mm long (vs. 2, ca. 0.75 mm long); linguiform stigma (vs. hippocrepiform); flowering from June to August (vs. April to May).



Figure 7. Distribution of *Primulina pseudomollifolia* (■), *P. mollifolia* (●), *P. multifida* (★) and *P. renifolia* (▲) in Guangxi Zhuangzu Autonomous Region, China.

Molecular Analyses

The ITS matrix contained 28 sequences, including two accessions each for *Primulina multifida* and *P. pseudomollifolia*. The alignment was 675 bp in length. Excluding portions of the *trnL* intron and the *trnL-F* intergenic spacer that were poorly represented in the GenBank sequences, the final aligned *trnL-F* matrix was 695 bp in length.

Results of BI analyses are depicted in Figure 1, showing a 50% majority rule consensus tree of post burn-in sampled trees with posterior clade probabilities (PP) (>50%) and average branch lengths. MP analysis resulted in 17 trees of 713 steps (CI = 0.72, RI = 0.68, RCI = 0.49). Topology of the 50% majority consensus tree was basically concordant with the BI tree with less resolution (not shown). MP bootstrap (PB) values are shown in Figure 1. When subjected to ML analysis, one single tree was uncovered, with the Ln likelihood score = -5947.48. The topology of the single ML tree was identical to the BI tree. ML bootstrap (MB) values are also shown in Figure 1.

In all analyses, the generic placement of the two new species (*Primulina multifida* and *P. pseudomollifolia*) in *Primulina* was strongly supported (PP=100, PB=100, MB=97). The two accessions of *P. multifida* formed a strongly supported clade (PP=100, PB=100, MB=100) that is sister to the clade composed of three species of the former *Chiritopsis* (*P. bipinnatifida* and *P. glandulosa*, and *Chiritopsis repanda* var. *guilinensis*) and two species of former *Chirita* sect. *Gibbosaccus* (*P. pinnatifida* and *P. sinensis*). The two accessions of *P. pseudomollifolia* also formed a strongly supported clade (PP=100, PB=100, MB=100) and were placed within the highly supported clade composed of two former *Wentsaiboea* (*P. luochengensis* and *P. renifolia*) and *P. mollifolia* (*Chiritopsis mollifolia*).

DISCUSSION

Based on DNA sequences of nuclear ITS and a chloroplast *trnL-F* intron spacer, we confirmed the generic placement of the two new Gesneriad species, adding two distinct taxa to the splendidly diverse *Primulina*. *Primulina multifida* and *P. pseudomollifolia* are localized to two limestone caves in Guangxi, as are most plant species confined to limestone caves along the Sino-Vietnamese border. However, these habitats are very fragile, highly vulnerable to ever increasing regional economic activities (Clements et al., 2006). Remarkably, in the past decade, botanists exploring the limestone ecosystems on the Sino-Vietnamese border have uncovered numerous new species from diverse groups ranging from ferns to conifers to various lineages of angiosperms (e.g., Farjon et al., 2002; Peng et al., 2008; Wu et al., 2009; Yu et al., 2009; Lin et al., 2010; Jiang et al., 2011; Pan et al., 2011). The sheer number of these recent discoveries highlights the extraordinary plant diversity in the region, and the urgency of a thorough inventory and conservation assessment of these precious biodiversity hotspots.

Acknowledgments. The authors are grateful to Prof. Fan-Nan Wei (IBK) for the Latin diagnosis and Miss Tze-Lin Pan and Hsuan Chang for collecting DNA sequence data. We also thank Mr. Shun-Qing He (IBK) and Mr. Yun-Xi Zhu (IBK) for the handsome illustrations. We appreciate the helpful comments of the anonymous reviewers. This study was supported by the National Natural Science Foundation of China (Grant no. 41161011) and the Foundation of Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences to Yan Liu (IBK), and National Geographic Society Grant # 8358-07 to Ching-I Peng (HAST), Yan Liu (IBK) and Kuo-Fang Chung (NTUF).

LITERATURE CITED

- Clements, R., N.S. Sodhi, M. Schilthuizen, and P.K.L. Ng. 2006. Limestone karsts of southeast Asia: Imperiled arks of biodiversity. *BioScience* **56**: 733-742.
- 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.
- Fang, D. and D.H. Qin. 2004. *Wentsaiboea* D. Fang et D.H. Qin, a new genus of Gesneriaceae from Guangxi, China. *Acta Phytotax. Sin.* **42**: 533-536.
- Farjon, A., N.T. Hiep, D.K. Harder, P.K. Loc, and L. Averyanov. 2002. A new genus and species in Cupressaceae (Coniferales) from northern Vietnam, *Xanthocyparis vietnamensis*. *Novon* **12**: 179-189.
- He, H. and L.B. Zhang. 2011. *Polystichum cavernicola*, sp. nov. (sect. *Haplopolystichum*, Dryopteridaceae) from a karst cave in Guizhou, China and its phylogenetic affinities. *Bot. Stud.* **52**: 121-127.
- Hou, M.F., Y. Liu, Y. Kono, and C.-I Peng. 2009. *Aspidistra daxinensis* (Ruscaceae), a new species from limestone areas in Guangxi, China. *Bot. Stud.* **50**: 371-378.
- Hou, M.F., J. López-Pujol, H.N. Qin, L.S. Wang, and Y. Liu. 2010. Distribution pattern and conservation priorities for vascular plants in southern China: Guangxi Province as a case study. *Bot. Stud.* **51**: 377-386.
- Lin, C.R., C.-I Peng, Y. Kono, and Y. Liu. 2010. *Aspidistra obconica*, Asparagaceae [Ruscaceae], a new species from limestone areas in Guangxi, China. *Bot. Stud.* **51**: 263-268.
- Liu, Y., Y. Kono, C.R. Lin, W.B. Xu, and C.-I Peng. 2011. *Aspidistra erecta* (Asparagaceae), a new species from limestone areas in Guangxi, China. *Bot. Stud.* **52**: 367-373.
- Jiang, R.H., X.C. Zhang, and Y. Liu. 2011. *Asplenium cornutissimum* (Aspleniaceae), a new species from karst caves in Guangxi, China. *Brittonia* **63**: 83-86.
- Ku, S.M., Y. Kono, and Y. Liu. 2008. *Begonia pengii* (sect. *Coelocentrum*, Begoniaceae), a new species from limestone areas in Guangxi, China. *Bot. Stud.* **49**: 167-175.
- Li, J.M. and Y.Z. Wang. 2007. Phylogenetic reconstruction among species of *Chiritopsis* and *Chirita* sect. *Gibbosaccus* (Gesneriaceae) based on nrDNA ITS and cpDNA *trnL-F* sequences. *Syst. Bot.* **32**: 888-898.
- Li, Z.Y. and Y.Z. Wang. 2004. Plants of Gesneriaceae in China. Henan Science and Technology Publishing House, Zhengzhou.
- Lin, C.R., C.-I Peng, Y. Kono, and Y. Liu. 2010. *Aspidistra obconica*, Asparagaceae [Ruscaceae], a new species from limestone areas in Guangxi, China. *Bot. Stud.* **51**: 263-268.
- Liu, Y., 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**: 739-745.
- López-Pujol, J., F.M. Zhang, H.Q. Sun, T.S. Ying, and S. Ge. 2011. Centres of plant endemism in China: places for survival or for speciation? *J. Biogeogr.* **38**: 1267-1280.
- Möller, M., A. Forrest, Y.G. Wei, and A. Weber. 2011. A molecular phylogenetic assessment of the advanced Asiatic and Malesian didymocaroid Gesneriaceae with focus on non-monophyletic and monotypic genera. *Pl. Syst. Evol.* **292**: 223-248.
- 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 'Didymocaroid Gesneriaceae' based on three molecular data sets: incongruence with available tribal classifications. *Amer. J. Bot.* **96**: 989-1010.
- Nylander, J.A.A. 2004. MrModeltest, Version 2.2. [<http://www.abc.se/~nylander/>]
- Pan, B., W.H. Wu, D.X. Nong, and W.B. Xu. 2010. *Chiritopsis longzhouensis*, a new species of Gesneriaceae from limestone areas in Guangxi, China. *Taiwania* **55**: 370-372.
- Peng, C.-I, S.M. Ku, Y. Kono, K.F. Chung, and Y. Liu. 2008. Two new species of *Begonia* (sect. *Coelocentrum*, Begoni-

- aceae) from limestone areas in Guangxi, China: *B. arachnoidea* and *B. subcoriacea*. Bot. Stud. **49**: 405-418.
- Peng, C.-I., Y. Liu, S.M. Ku, Y. Kono, and K.F. Chung. 2010. *Begonia* \times *breviscapa* (Begoniaceae), a new intersectional natural hybrid from limestone areas in Guangxi, China. Bot. Stud. **51**: 107-117.
- Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**: 1572-1574.
- Shen, R.J., S.S. Lin, Y. Yu, D.F. Cui, and W.B. Liao. 2010. *Chiritopsis danxiaensis* sp. nov. (Gesneriaceae) from Mount Danxiashan, south China. Nordic J. Bot. **28**: 728-732.
- Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28**: 2731-2739.
- Wang, W.T., K.Y. Pan, Z.Y. Li, A.L. Weitzman, and L.E. Skog. 1998. Gesneriaceae. In Z.H. Wu and P.H. Raven (eds.), Flora of China, Vol. 18. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, pp. 345-348.
- 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**: 50-64.
- Weber, A., Y.G. Wei, C. Puglisi, F. Wen, V. Mayer, and M. Möller. 2011a. A new definition of the genus *Petrocodon* (Gesneriaceae). Phytotaxa **23**: 49-67.
- Weber, A., D.J. Middleton, A. Forrest, R. Kiew, C.L. Lim, A.R. Rafidah, S. Sontag, P. Triboun, Y.G. Wei, T.L. Yao, and M. Möller. 2011b. Molecular systematics and remodelling of *Chirita* and associated genera (Gesneriaceae). Taxon **60**: 767-790.
- Wu, J.Y., M. Ogisu, H.N. Qin, and S.N. Lu. 2009. A new species of *Mahonia* Nutt. (Berberidaceae) from China. Bot. Stud. **50**: 487-492.
- Wu, W.H., W.B. Xu, and L. Wu. 2011. *Chiritopsis hezhouensis* (Gesneriaceae) from Karst caves in Guangxi, China. Taiwaniana **56**: 132-137.
- Xu, W.B., B. Pan, Y.S. Huang, and Y. Liu. 2010. *Chirita leprosa* sp. nov. (Gesneriaceae) from limestone areas in Guangxi, China. Nordic J. Bot. **28**: 705-708.
- Xu, Z.R. 1995. A study of the vegetation and floristic affinity of the limestone forests in southern and southwestern China. Ann. Missouri Bot. Gard. **82**: 570-580.
- Yu, S.X., Y.T. Hou, Y.L. Chen, and H.N. Qin. 2009. *Impatiens lobulifera* (Balsaminaceae), a new species from limestone areas in Guangxi, China. Bot. Stud. **50**: 365-370.
- Zhang, L.B. and H. He. 2009a. *Polystichum peishanii* (sect. *Haplopolystichum*, Dryopteridaceae): A new fern species from limestone area in Guizhou, China. Bot. Stud. **50**: 101-106.
- Zhang, L.B. and H. He. 2009b. *Polystichum minutissimum*, sp. nov. (sect. *Haplopolystichum*, Dryopteridaceae): The smallest *Polystichum* found in a karst cave in China. Bot. Stud. **50**: 353-358.
- Zhang, L.B., H. He, and Q. Luo. 2010. *Polystichum puteicola*, sp. nov. (sect. *Haplopolystichum*, Dryopteridaceae) from a karst sinkhole in Guizhou, China based on molecular, palynological, and morphological evidence. Bot. Stud. **51**: 127-136.

Appendix 1. GenBank accession numbers: Species: (ITS/*trnL-F*).

Didymocarpus podocarpus C.B. Clarke: (DQ912688/ FJ501514); *Petrocodon dealbatus* Hance (FJ501358/FJ501537); *Petrocodon scopulorum* (Chun) Y.Z. Wang [= *Tengia scopulorum* Chun]: (GU350637/GU350669); *Primulina bipinnatifida* (W.T. Wang) Yin Z. Wang [= *Chiritopsis bipinnatifida* W.T. Wang]: (DQ872842/DQ872806); *Primulina cordifolia* (D. Fang & W.T. Wang) Yin Z. Wang [= *Chiritopsis cordifolia* D. Fang & W.T. Wang]: (DQ872845/DQ872803); *Primulina dryas* (Dunn) Mich. Möller & A. Weber [= *Chirita sinensis* Lindl.] (FJ501348/ FJ501524); *Primulina gemella* (D. Wood) Yin Z. Wang [= *Chirita gemella* D. Wood]: (FJ501345/FJ501523); *Primulina glandulosa* (D. Fang, L. Zeng & D.H. Qin) Yin Z. Wang [= *Chiritopsis glandulosa* D. Fang, L. Zeng & D.H. Qin]: (DQ872841/DQ872804); *Primulina heterotricha* (Merr.) Yin Z. Wang [= *Chirita heterotricha* Merr.]: (DQ872826/DQ872816); *Primulina linearifolia* (W.T. Wang) Yin Z. Wang [= *Chirita linearifolia* W.T. Wang]: (DQ872834/DQ872810); *Primulina longgangensis* (W.T. Wang) Yin Z. Wang [= *Chirita longgangensis* W.T. Wang]: (FJ501347/AJ492290); *Primulina luochengensis* (Yan Liu & W.B. Xu) Mich. Möller & A. Weber [= *Wentsaiboea luochengensis* Yan Liu & W.B. Xu]: (HQ633046/HQ632949); *Primulina minutimaculata* (D. Fang & W.T. Wang) Yin Z. Wang [= *Chirita minutimaculata* D. Fang & W.T. Wang]: (DQ872828/DQ872815); *Primulina ophiopogoides* (D. Fang & W.T. Wang) Yin Z. Wang [= *Chirita ophiopogoides* D. Fang & W.T. Wang]: (DQ872829/DQ872814); *Primulina pinnatifida* (Hand.-Mazz.) Yin Z. Wang [= *Chirita pinnatifida* (Hand.-Mazz.) B.L. Burt]: (FJ501350/FJ501527); *Primulina pteropoda* (W. T. Wang) Yan Liu [= *Chirita pteropoda* W.T. Wang]: (DQ872827/ DQ872817); *Primulina repanda* var. *guilinensis* (W.T. Wang) Mich. Möller & A. Weber [= *Chiritopsis repanda* var. *guilinensis* W.T. Wang]: (DQ872846/DQ872808); *Primulina spadiciformis* (W.T. Wang) Mich. Möller & A. Weber [= *Chirita spadiciformis* W.T. Wang]: (FJ501346/AJ492291); *Primulina spinulosa* (D. Fang & W.T. Wang) Yin Z. Wang [= *Chirita spinulosa* D. Fang & W.T. Wang]: (DQ872830/DQ872813); *Primulina tabacum* Hance: (FJ501352/AJ492300); *Primulina weii* Mich. Möller & A. Weber [= *Chirita mollifolia* D. Fang, Y.G. Wei & J. Murata]: (DQ872832/ DQ872811); *Primulina wentsaii* (D. Fang & L. Zeng) Yin Z. Wang [= *Chirita wentsaii* D. Fang & L. Zeng]: (DQ872831/DQ872812).

中國廣西岩溶洞穴苦苣苔科報春苣苔屬之兩新種植物

許為斌¹ 盤波¹ 劉演¹ 彭鏡毅² 鍾國芳³

¹ 廣西壯族自治區 中國科學院廣西植物研究所

² 中央研究院 生物多樣性研究中心植物標本館

³ 國立台灣大學 森林環境暨資源學系

本文報導了廣西壯族自治區石灰岩地區苦苣苔科報春苣苔屬 (*Primulina*) 兩新種：假密毛小花苣苔 (*P. pseudomollifolia*) 和多裂小花苣苔 (*P. multifida*)。根據細胞核 ITS 及葉綠體 *trnF-L* 基因間與內插子 DNA 序列，此兩新種植物確定屬於報春苣苔屬。假密毛小花苣苔與密毛小花苣苔 (*P. mollifolia*) 較相近，但不同在於葉基部楔形到闊楔形，花冠白色至粉紅色，12-14 毫米長，花冠筒筒狀，8-9 毫米長，花絲 5-6 毫米長，退化雄蕊 3 枚，花期 7-9 月。多裂小花苣苔與文采苣苔 (*P. renifolia*) 相似，但不同在於葉邊緣羽狀半裂，苞片卵形至闊卵形，長 3-4 毫米，寬 1.5-2 毫米，花冠筒筒狀，5-7 毫米長，花絲 3-5 毫米長，退化雄蕊 3 枚，柱頭舌狀，花期 6-8 月。

關鍵詞：報春苣苔屬；苦苣苔科；新種；岩溶洞穴；石灰岩植物區系；廣西；假密毛小花苣苔；密毛小花苣苔；多裂小花苣苔；文采苣苔。