



Primulina mabaensis (Gesneriaceae), a new species from a limestone cave of northern Guangdong, China

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Abstract

We describe and illustrate the new species *Primulina mabaensis* from northern Guangdong, China. Its generic placement in the recently recircumscribed *Primulina* is confirmed by phylogenetic analyses of ITS and *trnL-F* intron-spacer DNA sequences. *Primulina mabaensis* is similar to *P. repanda* var. *guilinensis*, differing from the latter by its much larger flowers with corollas 2.5–3.0 cm (vs. 8–10 mm) long and corolla tubes 2.0–2.5 cm (vs. 6–8 mm) long and ca. 7 mm (vs. ca. 3 mm) in diameter at the corolla mouth. *Primulina mabaensis* is currently only known from limestone caves and rock surfaces of a karst tower in the Maba Man Archeology Site.

Key words: *Chirita*, *Chiritopsis*, ITS, molecular taxonomy, Old World Didymocaroid Gesneriaceae, rare plant, Sino-Vietnamese limestone karst, *trnL-F* intron-spacer

Introduction

The region stretching across the border areas of southern China and northern Vietnam is renowned for its spectacular limestone karst terrains and rich biodiversity (Xu 1995, Hou *et al.* 2010, López-Pujol *et al.* 2011). As karst ecosystems elsewhere in Southeast Asia (Clements *et al.* 2006), the Sino-Vietnamese limestone karst flora is also distinctive in harboring a myriad of species-rich plant genera with exceptionally high endemism. *Aspidistra* Ker Gawler (1822: p628; Asparagaceae; e.g., Lin *et al.* 2010), *Begonia* Linnaeus (1753: 1056; Begoniaceae; Peng *et al.* 2012), *Elatostema* Forster & Forster (1775: 53; Urticaceae; e.g., Wei *et al.* 2011), *Impatiens* Linnaeus (1753: 937; Balsaminaceae; e.g., Yu *et al.* 2009), *Polystichum* Roth (1800: 31; Dryopteridaceae; He & Zhang 2011) and several genera of Gesneriaceae (Wei 2010, Weber *et al.* 2011a, Liu *et al.* 2012, Wen *et al.* 2012a, Xu *et al.* 2012a) are but a few of the many species-rich and highly diversified genera, represented by species often confined to a few caves and crevices of limestone formations in the Sino-Vietnamese karsts.

During the course of botanical inventory of limestone karst caves in northern Guangdong in 2010, we collected an unknown Gesneriaceae whose gross morphology appears to be intermediate between *Chirita* sect. *Gibbosaccus* Clarke (1883: 130) and *Chiritopsis* Wang (1981: 21), especially resembling that of *Chiritopsis repanda* var. *guilinensis* (Wang 1992: 299) Möller & Weber in Weber *et al.* (2011b: 784). After consulting relevant literature (Wang *et al.* 1998, Ye & Peng 2006, Wei 2010, Wang *et al.* 2011, Weber *et al.* 2011b, Liu *et al.* 2012, Wen *et al.* 2012b, Wu *et al.* 2012a, Wu *et al.* 2012b, Xu *et al.* 2012a, Xu *et al.* 2012b) and performing molecular phylogenetic analyses (Xu *et al.* 2012a), we confirm it is an undescribed species of *Primulina* Hance (1883: 169; Gesneriaceae), a recently recircumscribed genus (now including *Chirita* Buch.-Ham. ex Don (1825: 89) and *Chiritopsis* Wang (1981: 21)) comprising ca. 150 species of predominately calciphilous herbs distributed mainly in southern China and northern Vietnam (Wei 2010, Wang *et al.* 2011, Weber *et al.* 2011b, Xu *et al.* 2012a).

Material and Methods

For molecular phylogenetic analyses, DNA sequences of nuclear ITS and chloroplast *trnL-F* intron-spacer (Li & Wang 2007, Wang *et al.* 2011, Weber *et al.* 2011b, Xu *et al.* 2012a) were collected from the type locality in the field and a specimen vouchered to become the type (*Chung & Chang 1744*, HAST). The experimental protocols outlined in Xu *et al.* (2012a), including DNA extraction, conditions for PCR and sequencing and multiple sequence alignment, were followed. The aligned matrix included 25 species of *Primulina*, with *Didymocarpus podocarpus* Clarke (1883: 76) and two species of *Petrocodon* Hance (1883: 167) selected as outgroups based on recent phylogenetic studies (Möller *et al.* 2009, Möller *et al.* 2011, Weber *et al.* 2011b; Appendix 1). The aligned matrix was analyzed using maximum parsimony (MP) and maximum likelihood (ML) optimality criteria and the Bayesian Inference of phylogeny (BI). MP analyses were conducted with all sites used in MEGA5.10 (Tamura *et al.* 2011) using the min-mini heuristic method, with 1000 bootstrap replicates searched using the default options of the Tree-Bisection-Reconnection (TBR). Ten ML analyses were conducted using RAxML (Stamatakis *et al.* 2008) via RAxML BlackBox (<http://phylobench.vital-it.ch/raxml-bb/>). For ML analysis, the matrix was partitioned (e.g., ITS vs. *trnL-F*) with gamma model of rate heterogeneity and proportion of invariable sites estimated by the program. Prior to BI analyses, DNA substitution models for each partition were selected by MrModeltest (Nylander 2004). MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003) was employed to perform BI analysis with all parameters and procedures following those detailed in Xu *et al.* (2012a).

Results and Discussion

The DNA matrix includes 1364 aligned positions (ITS: 673 bp; *trnL-F*: 691 bp) of which 406 (ITS: 332; *trnL-F*: 74) are variable and 215 (ITS: 191; *trnL-F*: 24) are parsimoniously informative. For each data partition, the Akaike Information Criterion (AIC) in MrModeltest selected the symmetrical model with gamma distributed (SYM+G) for ITS and the general time reversible model with gamma distributed (GTR+G) for *trnL-F*. The best ML tree (log likelihood score = -5810.074153) of the ten runs of RAxML analyses is depicted in Fig. 1, with summaries of node supports of ML, MP, and BI analyses. MP analyses resulted in 108 equally most parsimonious trees of 725 steps (CI = 0.713103, RI = 0.662338, RCI = 0.472315). The 50% majority consensus cladogram is basically compatible with the best ML tree (Fig. 1) except for the clades highlighted by the dash-lines. Results of BI analysis were also highly congruent with ML analysis except for the two clades highlighted by “X” in Fig. 1.

Based on all three analyses, the new species was placed sister to the type species of the genus *Primulina tabacum* Hance (1883: 169) with high support (Fig. 1), confirming the generic placement of the new species. Although morphologically the species seems most similar to *P. repanda* var. *guilinensis*, our phylogenetic analyses indicates that the new species is only distantly related to that species. Interestingly our data also indicates that *P. repanda* var. *guilinensis* is not sister to the typical variety *P. repanda* (Wang 1981: 23) Wang in Wang *et al.* (2011: 62) var. *repanda*. Further studies with increased sampling are currently underway to address the numerous remaining questions in *Primulina*.

Taxonomic Treatment

Primulina mabaensis K.F.Chung & W.B.Xu, *sp. nov.* (Fig. 2 & 3)

Primulina mabaensis is similar to *P. repanda* var. *guilinensis* (Fig. 4) in its leaf shape, differing from the latter by its larger flowers with corolla 2.5–3.0 cm (vs. 8–10 mm) long and corolla tube 2.0–2.5 cm (vs. 6–8 mm) long, and ca. 7 mm (vs. ca. 3 mm) in diam at the corolla mouth.

Type:—CHINA. Guangdong Province: Shaoguan City, Quijiang District, Maba Man Archeology Site, Lion Rock, on moist surface of a limestone cave, 24.675°N, 113.580°, ca. 65 m, 24 April 2010, *Chung & Chang 1744* (holotype HAST!; isotype IBK!).

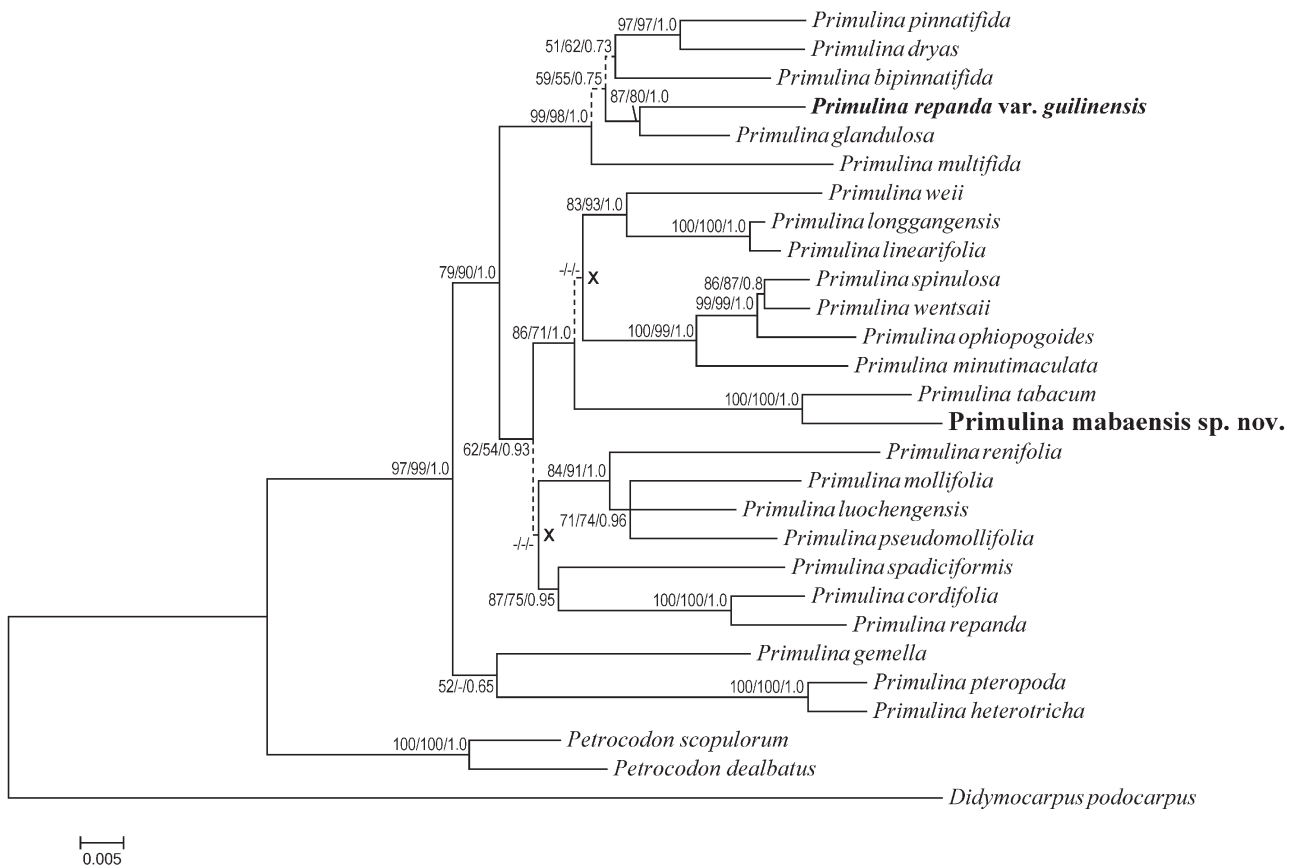


FIGURE 1. The best ML tree from the analysis of ITS and chloroplast *trnL-F* intron-spacer region. Branch support is shown at each node as ML bootstrap/MP bootstrap/Bayesian PP. Dash-line denotes clades collapsed in the 50% majority-rule consensus tree of the 108 most parsimonious trees of MP analyses. The character “x” indicates clades also collapsed in the Bayesian consensus cladogram.

Perennial herbs. Rhizomes subterete, 1–4 cm long, 5–8 mm wide. Leaves 6–13, basal, long petiolate, herbaceous; petioles flattened, 3–6 cm long, 3–5 mm wide, pubescent; blades ovate to broadly ovate or elliptic, 4–9 × 3–6 cm, pubescent on both surfaces, bases cuneate or broadly cuneate to shallowly cordate, margins repand to serrate, apices obtuse to rounded; lateral veins inconspicuous, 3–5 on each side. Cymes 3–7, axillary, 1–2-branched, 4–10-flowered; peduncle 2.5–4.0 cm long, ca. 2 mm in diam, pubescent; bracts 2, opposite, linear-lanceolate, 8–10 × 1.5–2.5 mm, margins entire, pubescent; pedicel 5–15 mm long, pubescent. Calyx 5-parted to near base, lobes lanceolate-linear, 8–10 × 1.0–1.5 mm, apex acuminate, outside pubescent, inside sparsely puberulent, margins entire. Corolla white, 2.5–3.0 cm long, outside puberulent, inside sparsely puberulent; corolla tube 2.0–2.5 cm long, ca. 7 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, 2.5–4.0 × 2.5–3.0 mm; abaxial 3-lobed to over the middle, lobes oblong, 2.5–3.0 × 2–3 mm; stamens 2, adnate to ca. 9 mm above the corolla tube base; filaments linear, 9–10 mm long; anthers 3.5–4.0 mm long, dorsifixed, glabrous; staminodes 3, lateral ones 4–5 mm long, apex capitate, glabrous, adnate to ca. 10 mm above the corolla tube base, middle one ca. 4 mm long, apex capitate, glabrous, adnate to ca. 11 mm above the corolla tube base. Disc annular, ca. 0.6 mm in height, margin repand, glabrous. Pistil 2.0–2.5 cm long, ovary narrowly ovoid, 2.5–3.0 mm long, ca. 1 mm across, puberulent; style 18–20 mm long, puberulent; stigma obtuse-trapeziform, ca. 2 mm long, 1.0–1.5 mm wide, apex 2-lobed. Capsule narrowly ellipsoidal, 8–10 mm long, 3–4 mm across, pubescent.

Distribution, habitat and ecology:—*Primulina mabaensis* is currently only known from the type locality: in caves and crevices on Lion Rock at the Maba Archeological Site, a designated cultural relic site of the Provincial Government of Gungdong. In the type locality, *P. mabaensis* is the most dominant species in the caves and moist limestone rock surfaces.

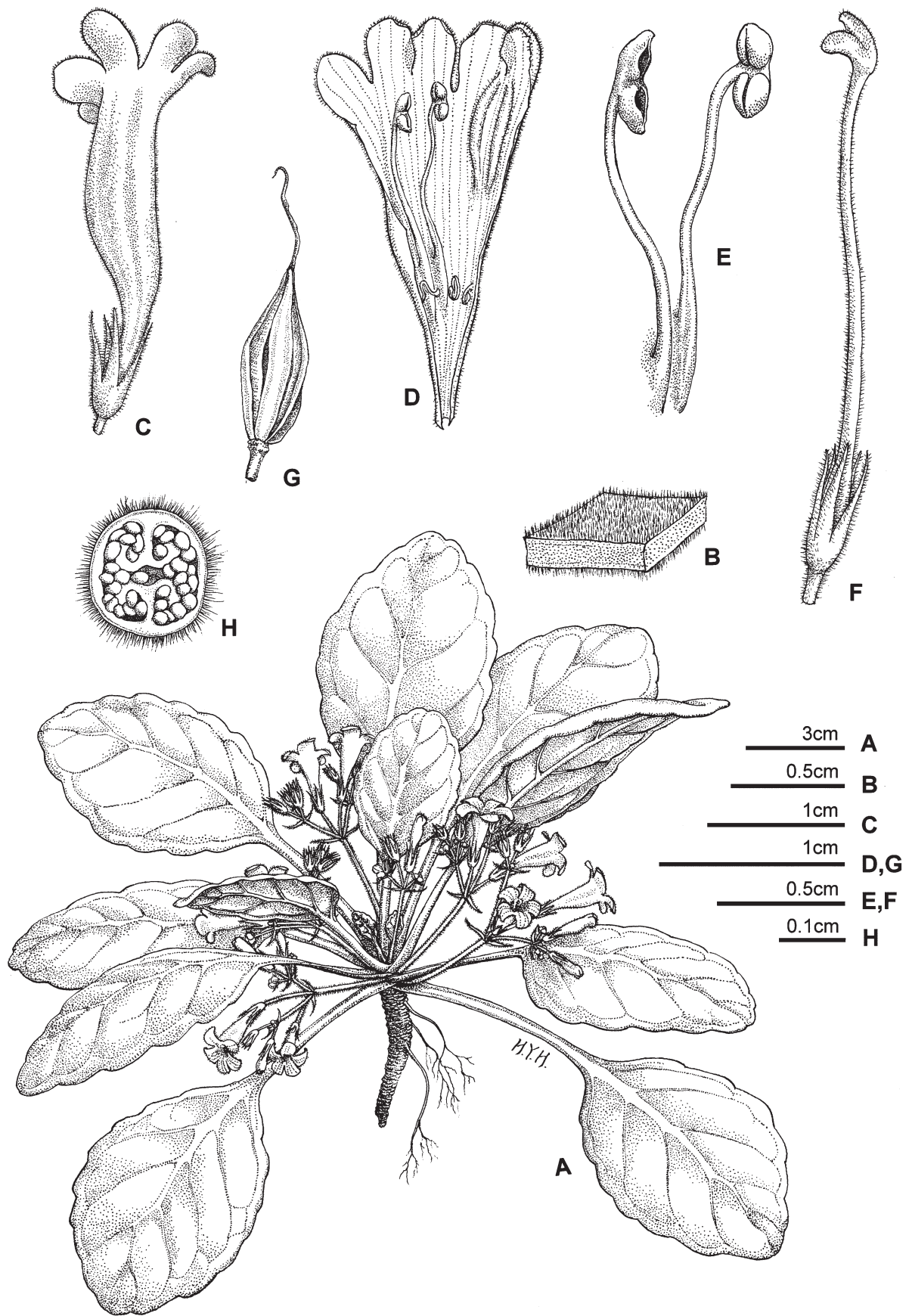


FIGURE 2. *Primulina mabaensis*. A. habit. B. leaf surfaces. C. flower. D. opened corolla, showing stamens and staminodes. E. stamens. F. pistil and calyx. G. capsule. H. cross-section of ovary. Drawn by Han-Yau Huang.

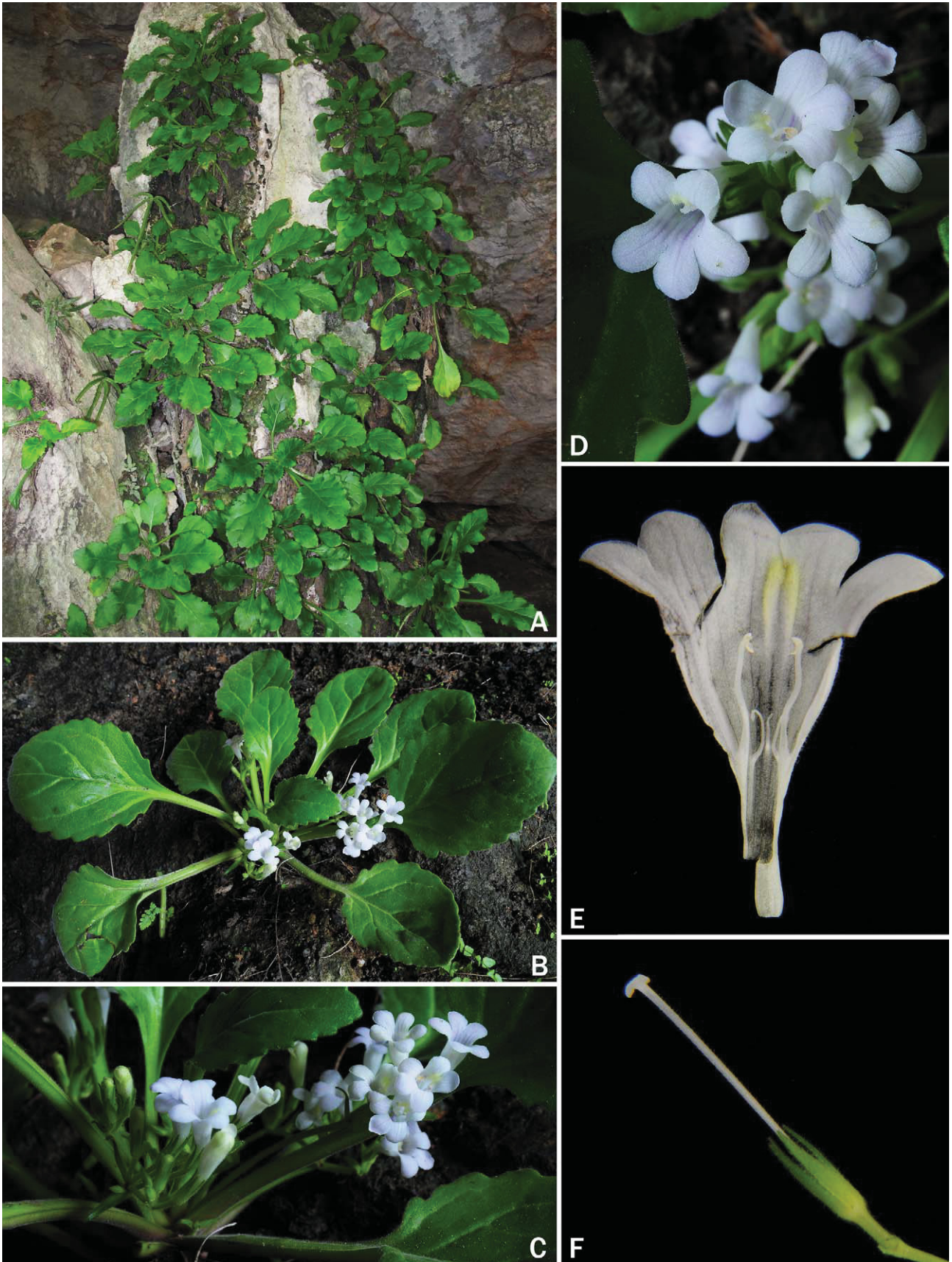


FIGURE 3. *Primulina mabaensis*. A. habitat. B. flowering habit. C. inflorescences. D. flowers. E. opened corolla, showing stamens and staminodes. F. pistil and calyx.

Etymology:—The species epithet is derived from the type locality, Maba Man Archeology Site, Qujiang County, Guangdong Province.

Vernacular name:—Chinese mandarin: ma-ba bao-chun-ju-tai, which translates as ‘Maba Primulina.’

Characters:—*Primulina mabaensis* is similar to *Primulina repanda* var. *guilinensis* (Fig. 4) in its leaf shape and can be distinguished from the latter by its corolla 2.5–3.0 cm (vs. 8–10 mm) long, corolla tube 2.0–2.5 cm long, ca. 7 mm in diam at the mouth (vs. 8–10 cm long, corolla tube 6–8 cm long, ca. 3 mm in diam at the mouth); filaments linear, 9–10 mm long, adnate to ca. 9 mm above the corolla tube base (vs. subulate, ca. 3 mm long, adnate to ca. 2 mm above the corolla tube base); pistil 2.0–2.5 cm long, ovary 2.5–3.0 mm long, style 18–20 mm long (vs. 7–9 mm long, ovary 1.5–2.0 mm long, style 5–6 mm long).

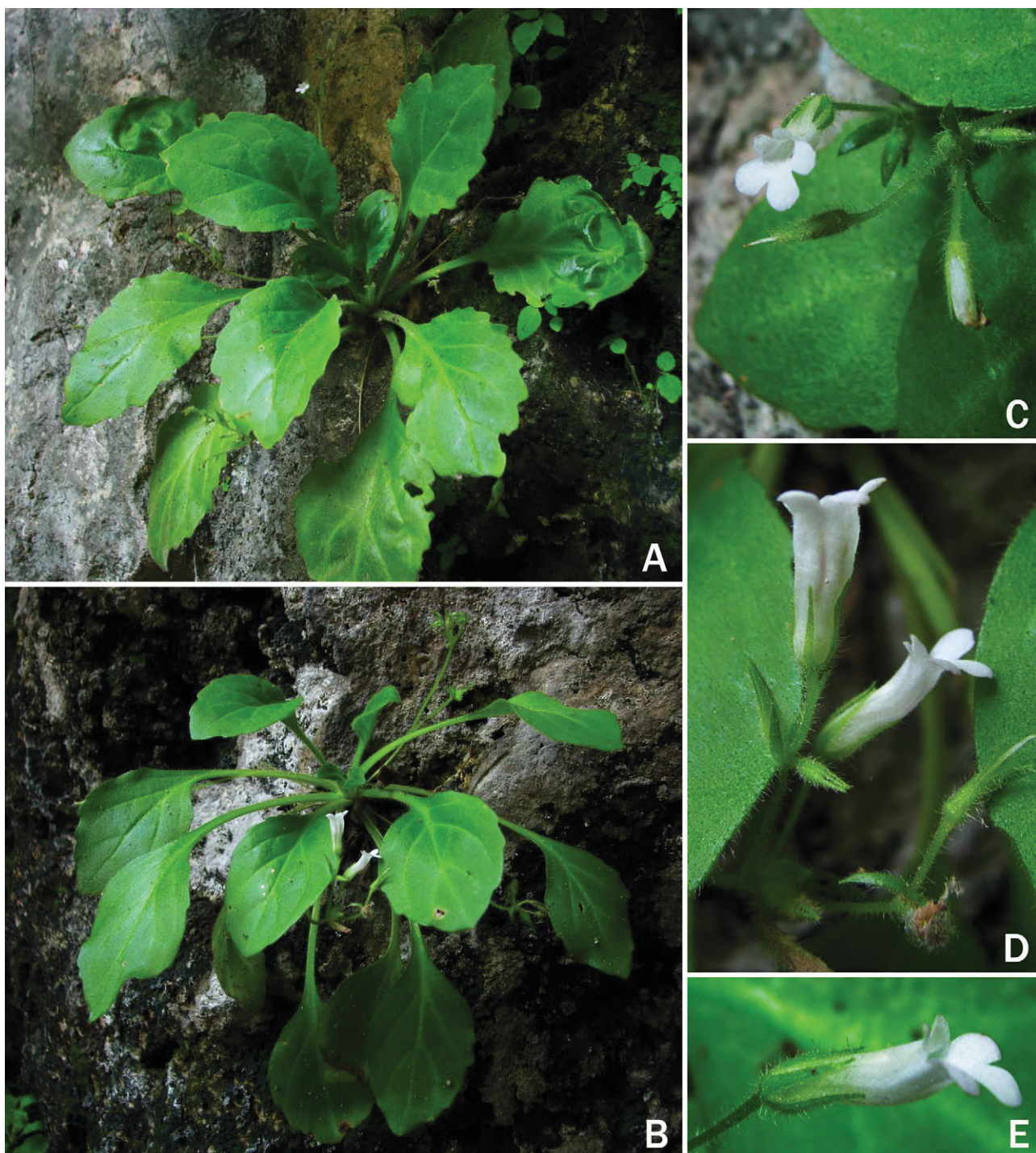


FIGURE 4. *Primulina repanda* var. *guilinensis*. A, B. flowering habit. C. flower, face view. D. Cymes. E. flower, side view.

Additional specimen examined (paratype):—CHINA. Guangdong Province: Shaoguan City, Quijiang District, Maba Man Archeology Site, Lion Rock, 15 April 2011, plant sterile, *Peng, Chung & Xu 22906* (HAST).

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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: GU350637/GU350669; *Primulina bipinnatifida* (W.T. Wang) Yin Z. Wang: DQ872842/DQ872806; *Primulina cordifolia* (D. Fang & W.T. Wang) Yin Z. Wang: DQ872845/DQ872803; *Primulina dryas* (Dunn) Mich. Möller & A. Weber: FJ501348/ FJ501524; *Primulina gemella* (D. Wood) Yin Z. Wang: FJ501345/FJ501523; *Primulina glandulosa* (D. Fang, L. Zeng & D.H. Qin) Yin Z. Wang: DQ872841/DQ872804; *Primulina heterotricha* (Merr.) Yin Z. Wang: DQ872826/DQ872816; *Primulina linearifolia* (W.T. Wang) Yin Z. Wang: DQ872834/DQ872810; *Primulina longgangensis* (W.T. Wang) Yin Z. Wang: FJ501347/AJ492290; *Primulina luochengensis* (Yan Liu & W.B. Xu) Mich. Möller & A. Weber: HQ633046/HQ632949; *Primulina mabaensis* K.F. Chung & W.B. Xu: JX506922/JX506814; *Primulina minutimaculata* (D. Fang & W.T. Wang) Yin Z. Wang: DQ872828/DQ872815; *Primulina mollifolia* (D. Fang & W.T. Wang) Yin Z. Wang: JX506866/JX506755; *Primulina multifida* B. Pan & K.F. Chung: JX507031/JX506756; *Primulina ophiopogoides* (D. Fang & W.T. Wang) Yin Z. Wang: DQ872829/ DQ872814; *Primulina pinnatifida* (Hand.-Mazz.) Yin Z. Wang: FJ501350/FJ501527; *Primulina pseudomollifolia* W.B. Xu & Yan Liu: JX506869/JX506759; *Primulina pteropoda* (W.T. Wang) Yan Liu: DQ872827/DQ872817; *Primulina renifolia* (D. Fang & D.H. Qin) J.M. Li & Yin Z. Wang: JX506737/ JX506762; *Primulina repanda* (W.T. Wang) Yin Z. Wang: JX506872/ JX506763; *Primulina repanda* var. *guilinensis* (W.T. Wang) Mich. Möller & A. Weber: DQ872846/DQ872808; *Primulina spadiciiformis* (W.T. Wang) Mich. Möller & A. Weber: FJ501346/AJ492291; *Primulina spinulosa* (D. Fang & W.T. Wang) Yin Z. Wang: DQ872830/DQ872813; *Primulina tabacum* Hance: FJ501352/AJ492300; *Primulina weii* Mich. Möller & A. Weber: DQ872832/ DQ872811; *Primulina wentsaii* (D. Fang & L. Zeng) Yin Z. Wang: DQ872831/DQ872812.