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Petrocodon pulchriflorus sp. nov. (Gesneriaceae) from Guangxi, China

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Abstract

Petrocodon pulchriflorus, a new species of Gesneriaceae is described and illustrated from the limestone area of southwestern Guangxi, China. It is similar to *Petrocodon guangxiensis*, but can be distinguished by several characters, such as the size and shape of leaf blades, inflorescences and corolla, and the position of stamens. Also the phylogenetic affinity of *Petrocodon pulchriflorus* is presented based on the plastid *trnL-F* and nuclear ribosomal ITS sequences.

Introduction

Petrocodon Hance (1883: 167) is a genus of lithophytic perennial herbs in the family Gesneriaceae, mainly distributed in the limestone regions of southwestern China. As a consequence of recent taxonomic revision based on a molecular phylogenetic study, all species of *Petrocodon*, *Paralagarosolen* Wei (2004: 528), *Calcareoboea* Wu ex Li (1982: 241), *Tengia* Chun (1946: 279), *Dolicholoma* Fang & Wang (1983: 18), *Lagarosolen* Wang (1984: 11), a few species of *Didymocarpus* Wallich (1819: 378) and *Wentsaiboea tiandengensis* Liu & Pan (2010: 739) were incorporated into the expanded *Petrocodon* Hance (Weber *et al.* 2011b). To date, the genus comprises ca. 30 species after the recircumscription and recent description of new species (Wang *et al.* 2011, Weber *et al.* 2011b, Wen *et al.* 2012, Chen *et al.* 2014, Xu *et al.* 2014, Guo *et al.* 2016).

During an expedition for investigating karst cave plants in March 2016, we discovered some plants in flower from three localities in Tiandeng County, southwestern Guangxi, China, which was assumed that belonged to *Petrocodon s.l.* After comparing the species to all other congeners described (Wei 2010, Liu *et al.* 2011, Weber *et al.* 2011b, Chen *et al.* 2014, Xu *et al.* 2014, Guo *et al.* 2016) and specimens of Gesneriaceae deposited at IBK, KUN and PE Herbaria, we confirmed that it is a new species, and hence we describe and illustrate it below as *Petrocodon pulchriflorus.*

Methods

Fresh leaf materials were collected and preserved in silica gel for quick drying. Total genomic DNA was extracted using CTAB method (Doyle 1987). The nuclear ribosome internal transcribed spacers (ITS) and partial chloroplast *trnL* and *trnL-F* intergenic spacer (*trnL-F*) were amplified and sequenced according to the procedures and conditions as described in Guo *et al.* (2016). We queried the highly similar sequences in GenBank using BLAST search and found that the most similar sequences were those of *Petrocodon s.l.* Then we downloaded a total of 36 ITS and *trnL-F* sequences representing 18 species of *Petrocodon s.l.* to test the phylogenetic affinity of the new species in the genus. According to the previous research, *Primulina* Hance (1883: 169) is the sister genus of *Petrocodon s.l.* (Weber *et al.* 2011a, 2011b). Thus we selected *Primulina ningmingensis* (Liu & Wu 2011: 422) Xu & Chung (2012: 4) and *Pri. swinglei* (Merr. 1918: 156) Möller & Weber (2011: 785) as the outgroups. The GenBank accession numbers of the downloaded sequences are listed in Appendix 1.

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DNA sequences were aligned using the program MUSCLE 3.8.31 (Edgar 2004) and adjusted manually in Bioedit 5.0.9 (Hall 1999). We reconstructed the phylogeny using maximum likelihood (ML) and Bayesian inference (BI). First, we reconstructed the maximum likelihood trees based on ITS and *trnL-F* data, respectively, and compared the ML trees to check whether there existed any phylogenetic conflict between the chloroplast and nuclear data. Although there were some phylogenetic conflicts found among other species, the positions of the new species were relatively stable between the plastid and ITS trees. Therefore, we reconstructed the phylogenetic trees based on concatenated data to further test the affinity of the new species. ML analyses were performed using RAxML-VI-HPC (Stamatakis 2006) with the substitution model GTR+G and 1000 rapid bootstrap searches (BS). For the Bayesian analyses, the optimal substitution model K81uf+I+G was selected by ModelTest (Posada & Crandall 1998) according to the Akaike Information Criterion (AIC). BI analyses were conducted in MrBayes 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 by a burn-in of the first 5000 trees. The convergence of the two parallel runs was guaranteed by the splitting frequency less than 0.005. All other parameters were set as default.

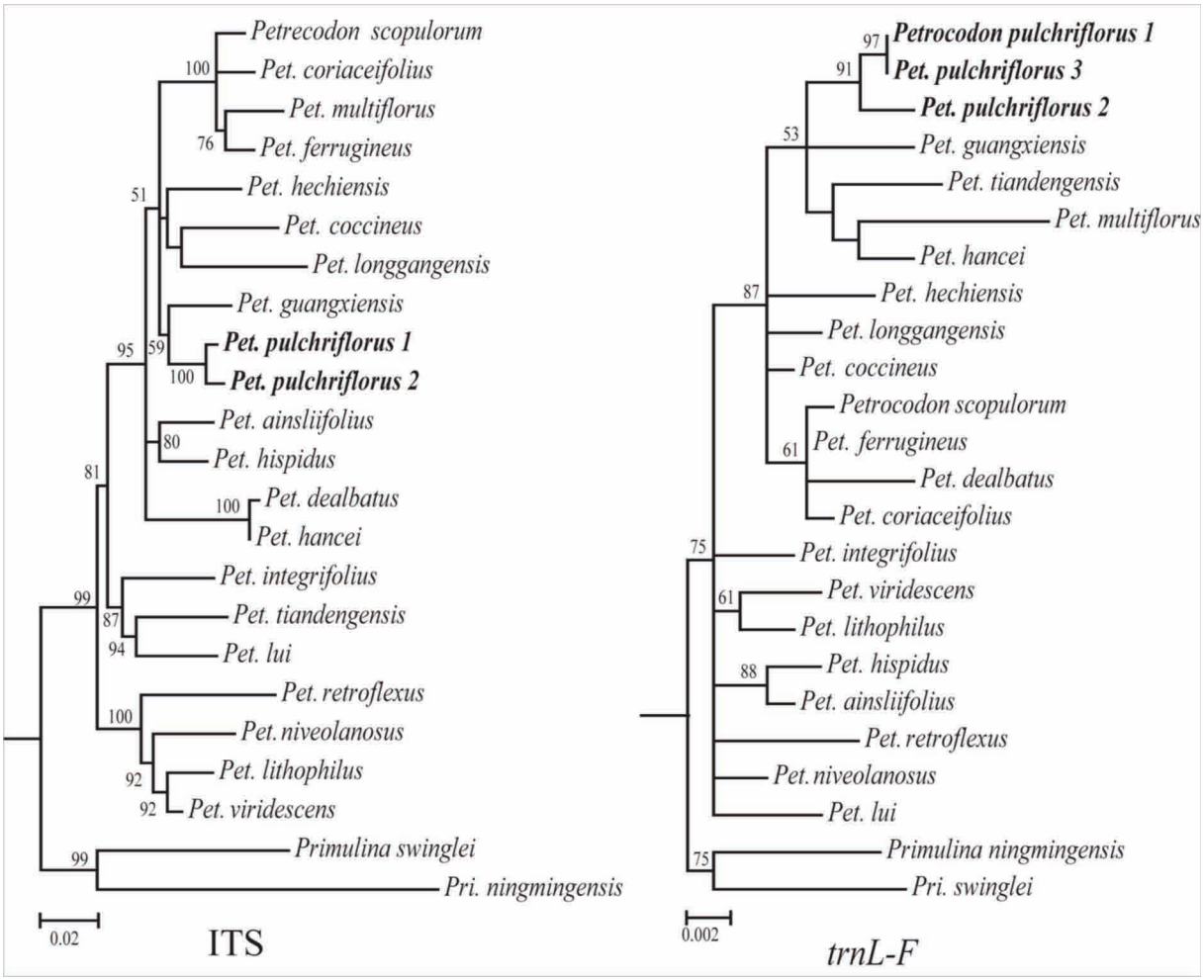

FIGURE 1. The best ITS and *trnL-F* maximum likelihood phylogenetic trees. BS values (>50%) are shown around the corresponding nodes. The new species is highlighted in bold.

Results

The aligned ITS and *trnL-F* datasets were 679 and 837 base pairs including 152 and 56 variable sites with 122 and 16 informative sites, respectively. The combined matrix thus consisted of 1516 characters including 208 variable sites with 138 informative sites. The parameters of consistency index (CI), retention index (RI) and homoplasy index (HI) were 0.773, 0.670, and 0.227, and 0.939, 0.889 and 0.061 for the ITS and *trnL-F* data, respectively. Even when phylogenetic incongruence between the ITS and *trnL-F* was detected, this does not affect the phylogenetic positions of the new

species, which is embedded within *Petrocodon s.l.*; *Pet. guangxiensis* (Liu & Xu 2011: 682) Xu & Chung (2014: 966) was recovered as the sister species in the ITS tree with low support (BS = 59%), and no clear sister relationship was observed in the *trnL-F* tree, but an unresolved polytomy including *Pet. guangxiensis* (Fig.1). In the combined analyses, the monophyly of *Petrocodon s.l.* (BS = 100%, PP = 1.00) and the sister relationships (BS = 75%, PP = 0.99) of the new species and *Pet. guangxiensis* were also moderately to strongly supported (Fig. 2). Further examination indicated that the new species differs from the sister *Pet. guangxiensis* by 27–28 substitutions and 7–8 substitutions in the ITS and *trnL-F* sequences, respectively.

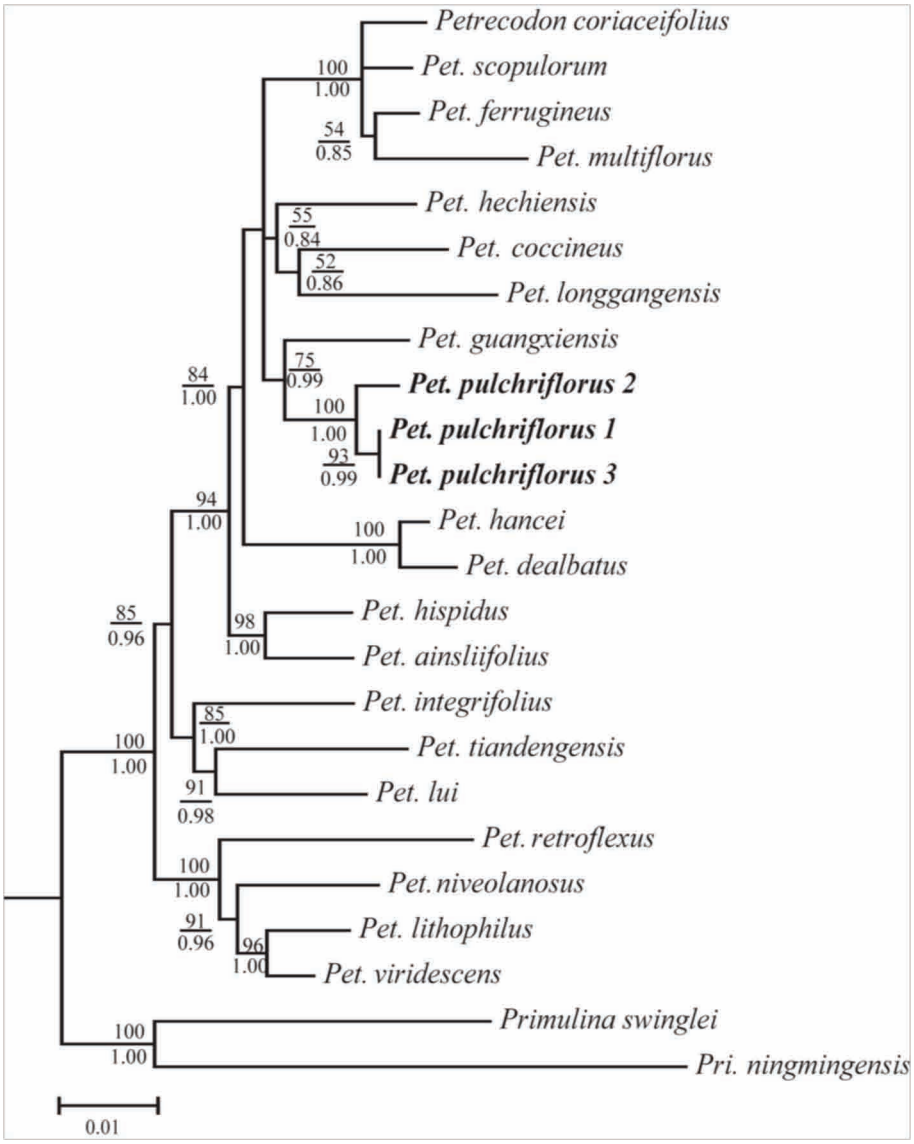

FIGURE 2. The best ML tree based on the combined ITS and *trnL-F* region. BS values (>50%) and PP values (>0.80) are shown above and below the branches. The new species is highlighted in bold.

Taxonomy treatment

Petrocodon pulchriflorus Y.B.Lu & Q.Zhang *sp. nov.* (Figs. 3–4) **Type**:—China, Guangxi Zhuang Autonomous Region: Tiandeng County, Longming Township, 106°56'21.97"E, 22°56'36.18"N, elevation 560–587 m, 30 March 2016 (fl.), *Q.Zhang ZQ160301* (holotype, IBK!; isotype, PE!)

Diagnosis:—Morphologically, *Petrocodon pulchriflorus* is similar to *Pet. guangxiensis*, but can be easily distinguished by the leaf blades, 4–11 cm long (*vs.* 2.5–6.0 cm), leaf margin crenate (*vs.* entire and often repand), 3–10 cm wide (*vs.*1.3–4.3 cm), the corolla orifice with a yellow ring (*vs.*white ring), stamens adnate to 0.8–1.1 cm (vs. 0.5 cm) above the corolla tube base, etc (see more diagnostic characters in Table 1).

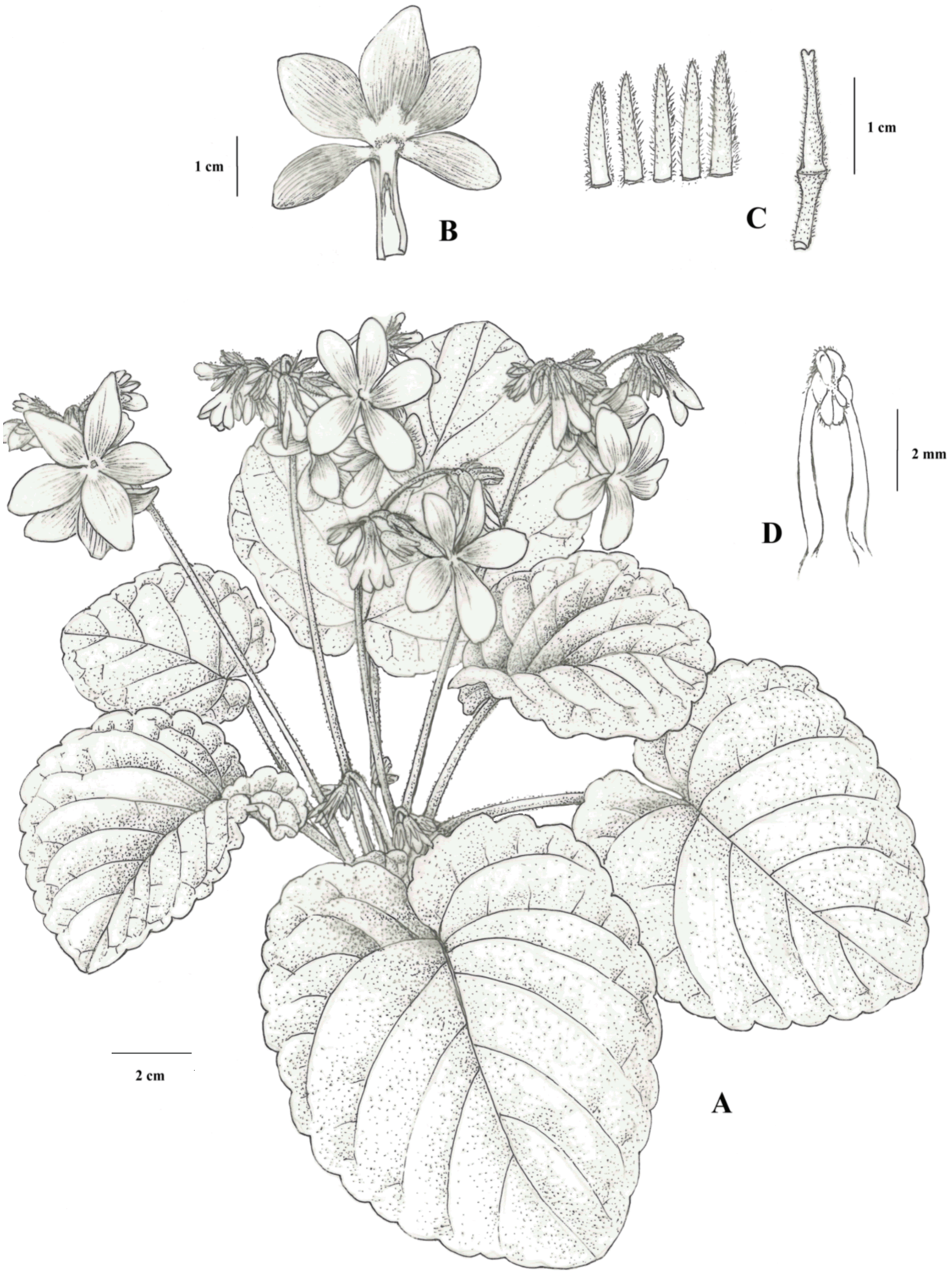

FIGURE 3. *Petrocodon pulchriflorus* (A) habit, (B) opened corolla, (C) pistil and calyx, (D) stamen. Drawn by Wen-Hong Lin from the holotype.

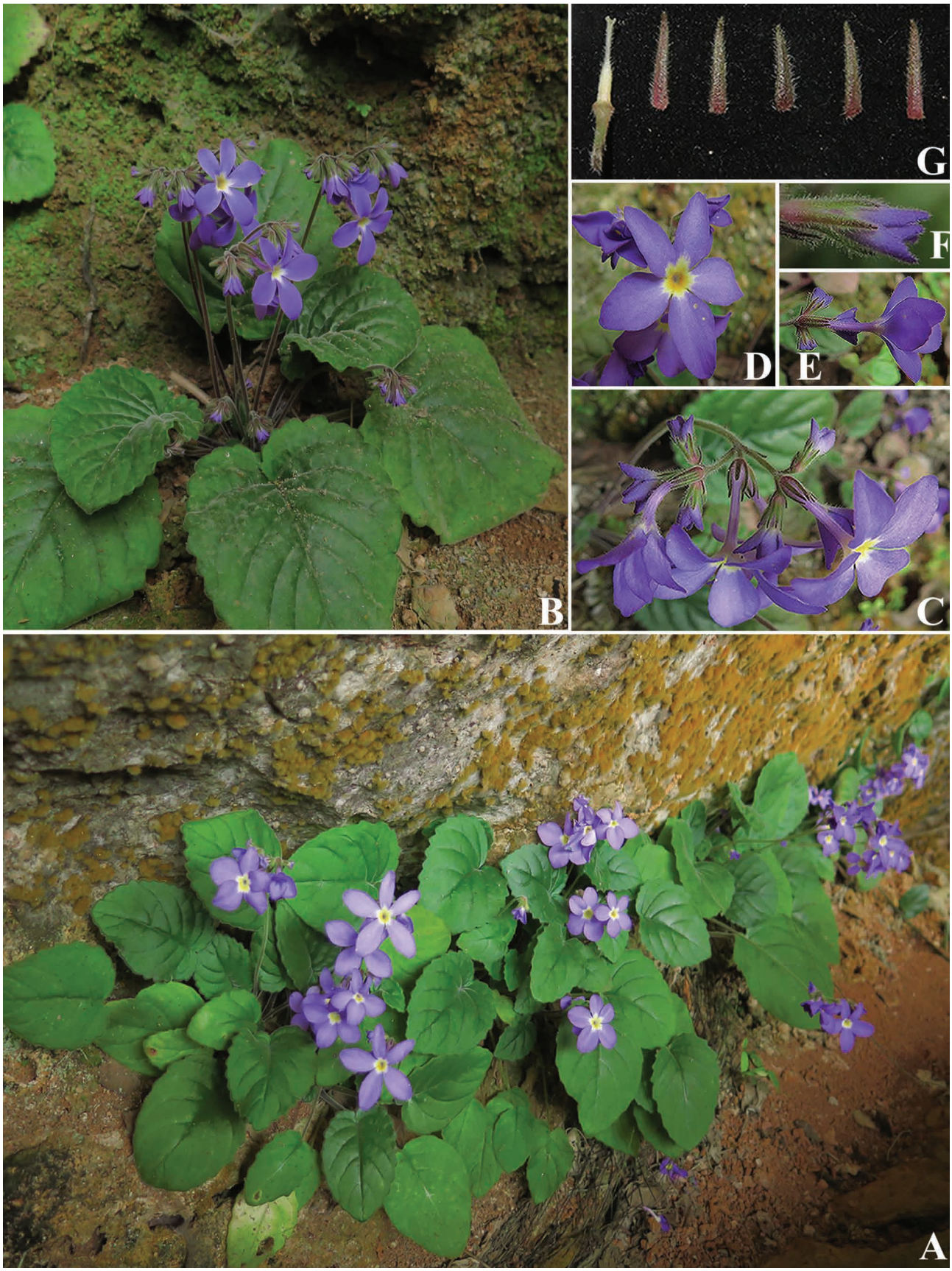

FIGURE 4. *Petrocodon pulchriflorus* (A) habitat, (B) flowering individual, (C) inflorescences, (D) flowers frontal view, (E) flowers side view, (F) early flower, (G) pistil and calyx.

TABLE 1. Diagnostic characters of *Petrocodon pulchriflorus* and the closely related species *Petrocodon guangxiensis*

	Petrocodon pulchriflorus	*Petrocodon guangxiensis*
Leaf blade	ovate to broadly ovate, 4–11 cm long, 3–10 cm wide, margin crenate, base cordate and symmetrical, lateral veins 5–7 on each side.	obliquely ovate to obliquely oblong-ovate, 2.5–6.0 cm long, 1.3–4.3 cm wide, margin entire and often repanding, base inequilateral, lateral veins 3–5 on each side
Inflorescences	inflorescences 5–8, flowers 5–20	inflorescences 3–5, flowers 1–5
Corolla	bluish purple to purple, orifice with a yellow ring	pale purple, orifice with a white ring
Stamens	adnate at 0.8–1.1 cm above the corolla tube base; filament 4 mm long, anthers oblong, 1.1–1.4 mm	adnate at ca. 5 mm above the corolla tube base; filament 2–3 mm long, anthers oblong, ca. 2 mm long
Staminodes	adnate at 7–10 mm above the corolla tube base	adnate at ca. 4 mm above the corolla tube base

Description:—Perennial herb. Rhizomes 5–8 cm long, 4–6 cm across. Leaves 4–8, basal, long petiolate; leaf blade herbaceous, ovate to broadly ovate, 4–11 cm long, 3–10 cm wide, obtuse at apex, cordate and symmetrical at base, with crenate margin; adaxial side deeply green, pubescent; abaxial side pale red to red, sometimes green, densely pubescent; lateral veins 5–7 on each side; petiole 3–11 cm long, densely pilose. Inflorescences 5–8, flowers 5–20; peduncles 5–17 cm long, densely pilose. Bracts 2, lanceolate, opposite, 0.8–1.1 cm long, densely pilose. Pedicel 0.5–7 cm long, densely pilose. Calyx 5-parted almost to the base, lobes linear to lanceolate; lobes equal in size, 0.9–1.2 cm long, 0.3 cm wide, densely pilose outside. Corolla bluish purple to purple, 1.8–2.5 cm long, orifice with a yellow ring, slightly zygomorphic, hypocrateriform. Tube salverform 1.6–2 cm long, orifice 2.8–3.3 mm in diameter, covered with dense pubescence, 1.9–2 mm in diameter at the middle, swollen at base 2.7–3.4 mm in diameter; limb 2-lipped; adaxial lip 2-lobed, 1.9–2.2 cm long, abaxial lip 3-lobed, 1.6–1.9 cm long; adjacent lobes overlapped in early stage of flowering. Stamens 2, included, adnate at 0.8–1.1 cm above the corolla tube base; filament 4 mm long, anthers oblong, 1.1–1.4 mm long, dorsifixed. Staminodes 3, 1.4–1.7 mm long, adnate at 7–10 mm above the tube base. Pistil 1.0–1.2 cm long, densely pilose; ovary 0.3–0.4 cm long, 0.2–0.3 cm across; stigma bifid. Fruit not seen.

Etymology:—This new species is named after its beautiful flowers.

Distribution, habitat and phenology:—*Petrocodon pulchriflorus* is hitherto only known from three close localities at elevational ranges from 450–600 m in Tiandeng County, Guangxi, China, growing on shaded and moist rock surface with tens of individuals at each of the localities. The place is a karst landform with a subtropical montane climate of high temperature, humidity and rainfall and is covered with evergreen forests. Flowering occurs in March–April.

Paratype:—China, Guangxi Zhuang Autonomous Region: Tiandeng County, Longming Township, 106°56’20.06”E, 22°56’34.18”N, elevation 572–593 m, 30 March 2016(fl.), *Q.Zhang ZQ160302* (paratype, IBK!).

Discussion

Both morphological and molecular studies support that *Pet. pulchriflorus* is a new species in *Petrocodon* and it is a close relative to *Pet. guangxiensis*. Morphologically, the new species differs from *Pet. guangxiensis* by numerous discontinuous qualitative and quantitative characters from leaves, inflorescences and flowers. Molecularly, considerable substitutions have been accumulated separating the new species from its closest relative *Pet. guangxiensis*. All these strongly suggest *Pet. guangxiensis* is an independent species and different from all other known species in *Petrocodon*.

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https://doi.org/10.1600/036364414X681437

Appendix 1. GenBank accession numbers of the sequences used in the present study

Species	GenBank accession numbers (ITS/*trnL-F)*
Petrocodon coriaceifolius (Wei) Wei & Möller	HQ633040/HQ632943
Petrocodon scopulorus (Chun) Wang	HQ633044/HQ632947
Petrocodon ferrugineus Wei	HQ633043/HQ632946
Petrocodon multiflorus Wen & Jiang	KJ475411 /KM232660
Petrocodon hechiensis (Wei, Liu & Wen) Wei & Möller	HQ633039/HQ632942
Petrocodon coccineus (Wu ex Li) Wang	KF202292/FJ501516
Petrocodon longgangensis Wu & Xu	KC765114/KC765116
Petrocodon guangxiensis (Liu & Xu) Xu & Chung	JX506899/ JX506791
Petrocodon hancei (Hemsl.) Weber & Möller	KF498051/ KF498253
Petrocodon dealbatus Hance	KF498053/JF697590
Petrocodon hispidus (Wang) Weber & Möller	KF202293/KF202300
Petrocodon ainsliifolius Chen & Shui	KF202291/KF202298
Petrocodon tiandengensis (Liu & Pan) Weber & Möller	JX506960/JX506850
Petrocodon lui (Liu & Xu) Weber & Möller	HQ633035/HQ632938
Petrocodon integrifolius (Fang & Zeng) Weber & Möller	HQ633037/HQ632940
Petrocodon niveolanosus (Fang & Wang) Weber & Möller	JF697576/JF697588
Petrocodon lithophilus Shui, Chen & Möller	KF202296/KF202302
Petrocodon viridescens Chen, Möller & Shui	KF202297/KF202304
Primulina swinglei (Merr.) Möller & Weber	JX506950/JX506841
Primulina ningmingensis (Liu & Wu) Xu & Chung	JX506931/JX506822
Petrocodon retroflexus Zhang & Guo	KX579060/KX579061
Petrocodon pulchiflorus Lu & Zhang	KX579058/KX579059