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Petrocodon asterostriatus (Gesneriaceae), a new species from Guangxi, China

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Petrocodon asterostriatus F. Wen, Y.G. Wei & W.C. Chou *sp. nova* (Gesneriaceae) is described and illustrated. It is a lithophytic species that grows in the limestone area of southwestern Guangxi, China. According to the maximum likelihood (ML) analysis, it nests in a strongly supported clade (BP = 92%) that includes *P. lui*, *P. tiandengensis*, *P. integrifolius*, *P. albinervius* and *P. ionophyllus*. Of these, *P. asteriostriatus* is morphologically closest to *P. integrifolius*, but can be distinguished from it by the shape and size of bracts, the indumentum of the pedicel, the shape and indumentum of the corolla tube, the shape of the lip lobes, and the indumentum and glands of filaments.

Introduction

Petrocodon (Gesneriaceae subfam. Didymocarpoideae) comprises at least 47 species (Möller 2019, http://gccc.gxib.cn, https://padme.rbge.org.uk/GRC) that are mainly distributed from eastern and southwestern China to the northern parts of the Indo-China Peninsula. Based on a recent phylogenetic analysis, the karst areas of southwest and south China are the centers of origin, differentiation and diversity of *Petrocodon* (Chen *et al*. 2014). Indeed, the genus is morphologically most diverse in China, which hosts at least 44 species, with 24 of them in Guangxi (Wei 2018, Wen *et al*. 2022). Most of the species are narrow endemics, isolated in unique habitats, for example, entrances to and in karst caves and gorges. This is also true for *P. flavus* (Middleton *et al*. 2015) in north Thailand, *P. vietnamensis* (Xin *et al*. 2021) in north Vietnam and *P. jiangxiensis* (Su *et al*. 2019) in Jiangxi Province in east China.

As part of an ongoing National Gesneriaceae Germplasm Bank (NGGRB) project to understand the biodiversity, systematics and evolution of gesneriads in southwestern China, we

conducted extensive fieldwork throughout the karst regions of Guangxi. The karst caves and gorges in SE Guangxi provide many ideal habitats for *Petrocodon* and some other genera such as *Primulina* and *Paraboea* comprising mainly lithophytic species. In a karst cave in Tiandeng County, we collected specimens of an unknown species of Gesneriaceae. It had straight filaments, a divided stigma with two lobes, and its leaf blades appeared similar to those of *Petrocodon lui* (Xu *et al.* 2010, Weber *et al.* 2011), *P. ionophyllus* (Li *et al.* 2020a) and *P. albinervius* (Nong *et al.* 2021). We suspected it might be an undescribed species of *Petrocodon*. To ascertain generic placement of this species in *Petrocodon*, we amplified its ITS and *trn*L-F sequences which were subsequently used in phylogenetic analyses.

Material and methods

We randomly selected three mature and flowering individuals as voucher specimens. Macromorphological characters were observed by the naked eye, while micromorphological characters were studied using a dissecting microscope. The morphological characters of the studied specimens were compared with the protologues and descriptions of all previously described relevant *Petrocodon* species (*viz*. Wang *et al.* 1998, Li & Wang 2007, Xu *et al.* 2008, 2010, Lu *et al.* 2017, Wei *et al*. 2010, Wei 2018, Li *et al.* 2020a, 2020b, Zhang *et al.* 2020, Nong *et al.* 2021), and actual type specimens and digital images of recently published species from the herbaria AHU, IBK and PE. Those similar species of *Petrocodon*, as well as the undescribed one, were introduced from their type localities for cultivation in the gardens of the GCCC for further observation. The terminology used in the description follows Harris and Harris (2001) and Wang *et al.* (1998).

Leaf material of the undescribed species was collected from the type locality in Tiandeng County (Guangxi, China), and immediately dried in silica gel for DNA extraction (Chase & Hills 1991). The plastid *trn*L-F intron spacer region (*trn*L-F) and the nuclear ribosomal internal transcribed spacer (ITS) region were used in the study. The primers of *trn*L-F were 5′-CGAAATCGGTaGACGCTACG-3′ and 5′-TCTGCTCTACCAGCTGAGCTAT-3′, while those for ITS were 5′-GGAAGGAGAAGTCGTAACAAGG-3′ and 5′-CACGCTTCTCCAGACTACA-3′ (Weber *et al*. 2011). DNA extraction, PCR amplification, and sequencing followed Möller *et al*. (2009, 2011). To elucidate the phylogenetic affinities of the undescribed species within the genus, we assembled a matrix including further 39 samples representing 26 species downloaded from GenBank (Appendix 1). The ingroup contained 38 samples from 23 species of *Petrocodon*, and *Primulina dryas* and *P. pinnata* were chosen to represent an outgroup based on previous phylogenetic analyses (Möller *et al*. 2009, 2011, Weber *et al*. 2011, Zhang *et al*. 2018).

We performed phylogenetic analyses of the included *Petrocodon* species based on the combined data set of *trn*L-F and ITS sequences using maximum likelihood (ML). For ML analysis, we employed IQ-TREE ver. 2.0.6 (Nguyen *et al*. 2015) with 1000 bootstrap replicates (Hoang *et al.* 2018) and default ModelFinder (Kalyaanamoorthy *et al*. 2017), and found K3P + G4 as the best-fit substitution model. Tree visualization was carried out in FigTree ver. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Visual comparison of optimal tree topologies of *trn*L-F and ITS data sets was used to compare topological inconsistencies. Conflicts between tree topologies were considered significant when the inconsistent topologies were ≥ 80% of the bootstrap values (Fu *et al.* 2022). Visual inspection showed no significant topological contradictions for bootstrap support consistency between the *trn*L-F and ITS data sets (results not shown). Thus, these regions were combined in our analyses. We selected data with support values above 50 for retention (Xu *et al.* 2014).

Results and discussion

Upon close examination of the flowering specimens and careful observation of vegetative and reproductive organs of living plants cultivated in the gardens of the National Gesneriaceae Germplasm Resources Bank (NGGB) of the Guangxi Institute of Botany (GXIB) and the Gesneriad Conservation Center of China (GCCC), we

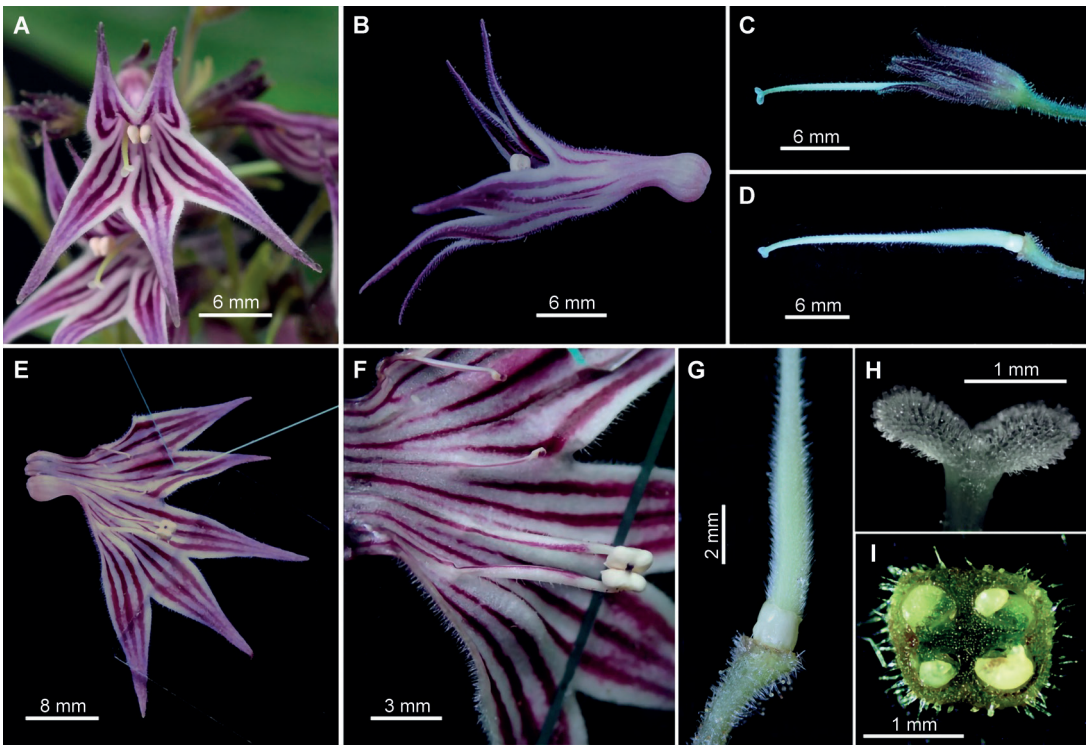

Fig. 1. *Petrocodon asterostriatus* in cultivation. — **A**: Frontal view of corolla. — **B**: Lateral view of corolla. — **C**: Pistil with calyx and pedicel. — **D**: Pistil without calyx. — **E**: Opened corolla. — **F**: Close-up of corolla showing stamens (filaments, anthers and staminodes). — **G**: Ovary and disc. — **H**: Stigma. — **I**: Cross section of ovary.

found several morphological peculiarities. The corolla tube was broadly funnelform with an almost spherical inflated base, the corolla lip lobes were narrowly lanceolate to narrowly triangular or acute triangular, the filament was sparsely glandular puberulent, the stigma had two lobes that were inconspicuously round to oval (Fig. 1).

Petrocodon asterostriatus F. Wen, Y.G. Wei & W.C. Chou, *sp. nova* (Figs. 1–4)

Type: China. Guangxi Zhuang Autonomous Region, Chongzuo City, Tiandeng County, Xiaoshan township, Xiaoshan village, 107°7′N, 22°59′E, ca. 620 m a.s.l., growing on shaded and moist rock surfaces at the entrance to a limestone cave behind the village, 7 July 2020 *Fei-Cheng Zhao* ZFC200720-01 (holotype: IBK, isotype: IBK, PE).

Etymology. The epithet *asterostriatus* refers to the dark maroon stripes on the corolla lobes and throat, giving the corolla a star-shaped colour pattern.

Perennial herb, rhizomes subterete, 6–13 cm long, 4.5–8.5 mm in diam. Leaves 6–15, basal, opposite, petiole 6–13 × ca. 0.5 cm, densely white pubescent; leaf blade yellowish-green to green, papyraceous, often almost symmetrical, broadly ovate to subcordate, 8–12 × 6–10 cm, base cordate, apex obtuse to subacute, margins entire, densely pubescent on both surfaces; lateral veins 4–7 on each side of mid-rib, prominent abaxially, slightly sunken adaxially. Cymes 4–8, axillary, 1–3-branched, 6–21-flowered; peduncle 6–10 cm long, densely puberulent. Bracts 2, opposite, broadly lanceolate or ovate, 10–25 × 3–6 mm, bracteoles 2, opposite, 9–18 × 2–4 mm, linear-lanceolate, all bract and bracteole margins entire, apex acuminate, densely white pubescent on both surfaces, occasionally with tertiary bracteoles 2, opposite, linear, margin entire, both sides pubescent; pedicels 1–2 cm long, densely white puberulent, but sparsely glandular puberulent at top of pedicel close to calyx. Calyx 5-lobed to base, lobes lanceolate to linear-lanceolate, pale purplish-

Fig. 2. *Petrocodon asterostriatus*. — **A**: Habitat. — **B**: Habit. — **C**: Flowers.

maroon, 8–16 × 2–5 mm, puberulent on both sides. Corolla pale purplish-maroon, 3–4 cm long, base near-spherical, outside densely pubescent and sparsely glandular, inside glabrous, tube infundibuliform, ca. 15 mm long, ca. 4 mm in diameter at base, ca. 7.5 mm in diameter at tube opening, limb 2-lipped, adaxial lip 2-lobed to near base, narrowly triangular, lobes ca. 10 mm long, ca. 4 mm wide at base, abaxial lip 3-lobed to more than middle, narrowly lanceolate to narrowly triangular or acute triangular, lobes ca. 13 mm long, ca. 4 mm wide at base, apex attenuate-acuminate, with three purplish-maroon longitudinal stripes. Stamens 2, adnate to ca. 8 mm above corolla tube base; filaments linear, straight, ca. 7 mm long, white with purplish-maroon longitudinal stripes, sparsely glandular puberulent, distally covered with dark purple glands, anthers dorsifixed, reniform, white, ca. 2 mm long, coherent in pairs, thecae confluent at middle, glabrous; staminodes 3, glabrous, adnate to ca. 11 mm above corolla tube base, lateral ones ca. 5 mm long, middle one ca. 8.5 mm long, all glabrous. Disc annular, glabrous, ca. 1.5 mm high, margin entire. Pistil ca. 22 mm long; ovary cylindrical, 7–8 mm long, ca. 1.5 mm in diam., densely white puberulent; style ca. 15 mm long, ca. 0.9 mm wide, densely white puberulent; stigma bilobed, lobes ovate, ca. 1 mm long, ca. 0.6 mm wide. Flowering from May to June, fruiting from July to August.

Distribution and habitat. *Petrocodon asterostriatus* is endemic to Guangxi and known only from the type locality. It grows on moist, shaded

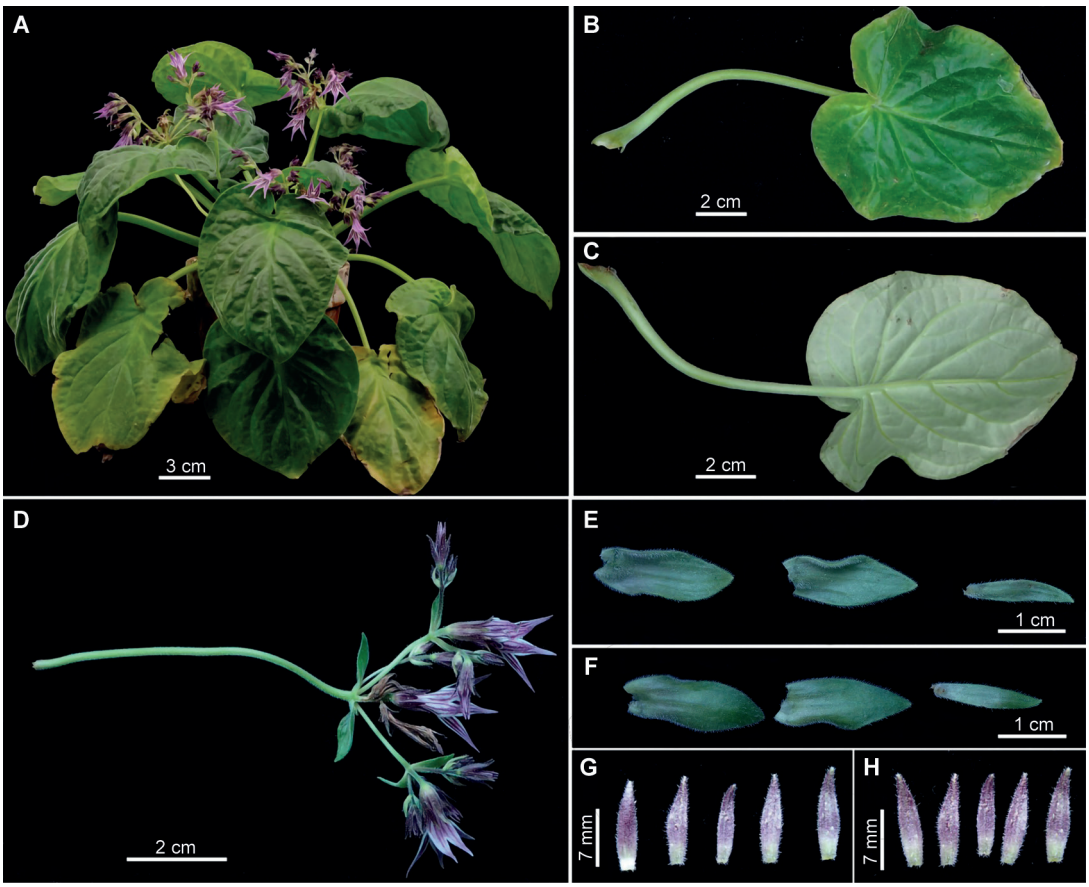

Fig. 3. *Petrocodon asterostriatus* in cultivation. — **A**: Plant in flower. — **B**: Adaxial surface of leaf blade and petiole. — **C**: Abaxial surface of leaf blade and petiole. — **D**: Flowering pair-flowered cyme. — **E**: Adaxial surface of bract, secondary bracteole, and tertiary bracteole (left to right). — **F**: Abaxial surface of bract, secondary bracteole, and tertiary bracteole (left to right). — **G**: Adaxial surface of calyx lobes. — **H**: Abaxial surface of calyx lobes.

limestone rock surfaces at the entrance to karst cave. The cliff slope faces northwest at an angle of up to 60°–80°. The tree canopy is up to 12 m tall, the canopy cover is 75%, the shrub layer cover is 85%, and the herb layer cover is 35%.

Conservation assessment. The type locality of *P. asterostriatus* is close to the village of Xiaoshan. National or local laws or regulations do not protect this area, thus, the population is potentially in danger. In July 2020 at the type locality we found about 80 individuals within about 20 m^2, with half of them being mature individuals and the other half seedlings. In the first half of 2021, about 20 flowering individuals were collected by local people to be sold online because of their attractive flowers. Thus, a severe reduction in the number of mature individuals has already occurred. Although we conducted field surveys in similar habitats many times, no other populations of *P. asterostriatus* were found. Thus, according to the IUCN Red List Categories and Criteria (https://www.iucnredlist.org/resources/redlistguidelines.pdf), *P. asterostriatus* has been provisionally assessed as "Critically Endangered, CR B2ab(v)+C2a(i)" because of its limited distribution, vulnerable habitat, and observed decline in the number of mature individuals.

The new species is morphologically most similar to *P. integrifolius*, differing from it by the shape and size of the bracts, the indumentum of the pedicel, the shape and indumentum of the corolla tube, the shape of lip lobes, and the indumentum and glands of filaments (Appendix 2).

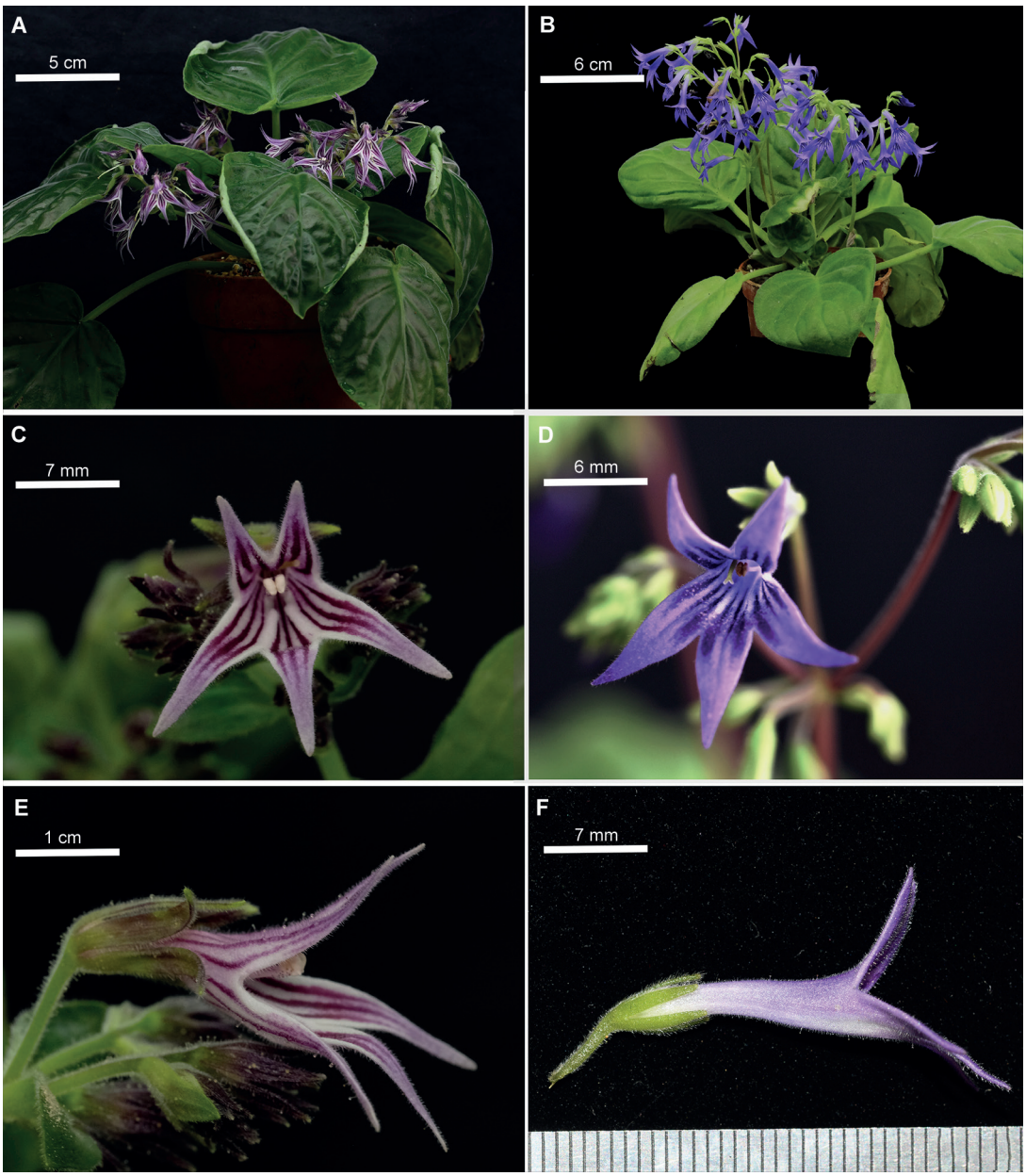

Fig. 4. Comparison of *Petrocodon integrifolius* (**A**, **C**, **E**) and *P. asterostriatus* (**B**, **D**, **F**). — **A** and **B**: Plants flowering in cultivation. — **C** and **D**: Frontal view of corolla and stigma. — **E** and **F**: Lateral view of corolla.

The aligned matrix of *trn*L-F and ITS sequences comprised 1879 characters (*see* Appendix 3). Of the 455 (24.42%) variable characters, 235 (12.51%) were parsimony-informative. The phylogenetic trees collectively indicated that the new species was sister to *P. integrifolius* with strong support (BP = 100%). Thus we focused on the most resolved topology that was generated by the ML analysis based on the combined data (Fig. 5).

All sampled *Petrocodon* taxa clustered together as a monophyletic group (BP = 100%). Three strongly supported clades attributable to *Petrocodon* were recovered. The new species

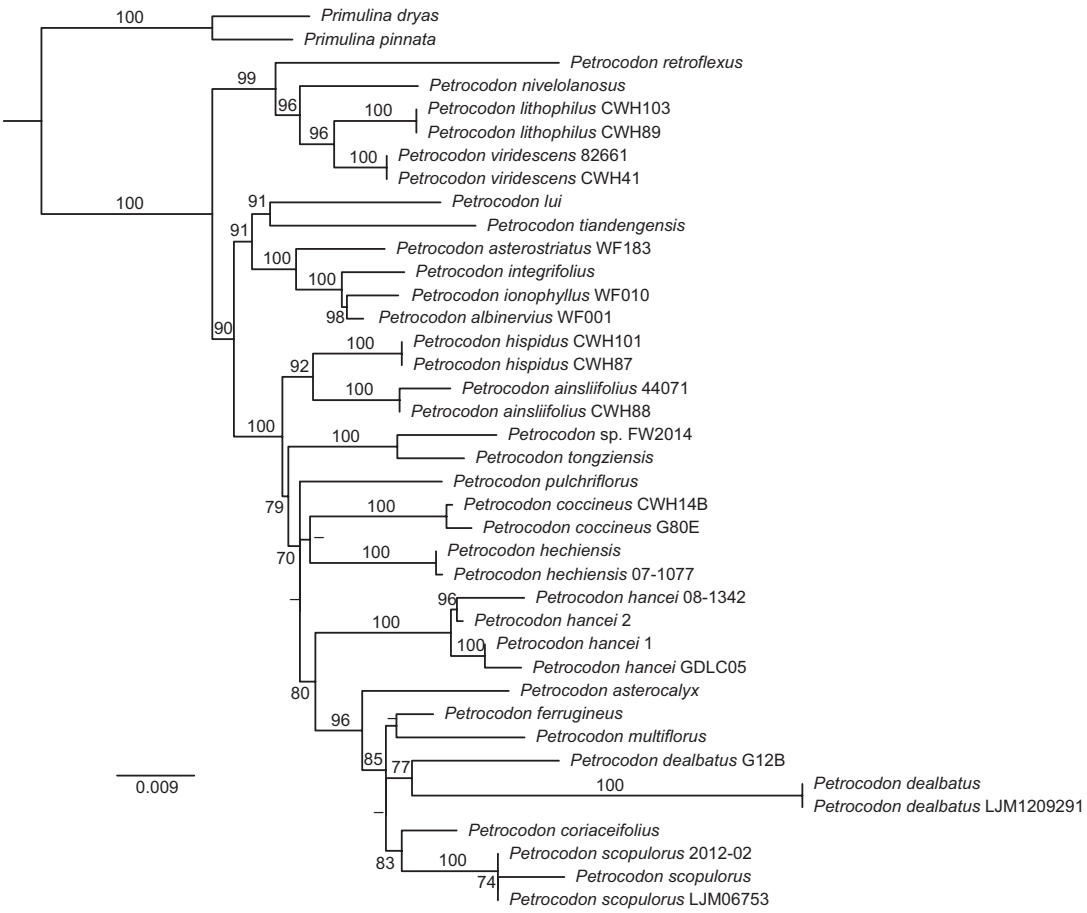

Fig. 5. Phylogenetic tree of *Petrocodon* generated from maximum likelihood (ML) of *trn*L-F and ITS data sets. Numbers on the branches indicate ML bootstrap values (≥ 50%).

belonged in a strongly supported clade (BP = 91%) that also included *P. tiandengensis* (Weber et al. 2011), *P. lui* (Xu *et al.* 2010), *P. integrifolius* (Fang *et al*. 1993, Weber *et al.* 2011), *P. albinervius* (Nong *et al*. 2021) and *P. ionophyllus* (Li *et al*. 2020a) (Fig. 5). Those species, except for *P. tiandengensis*, share many characteristics and are distributed mainly in three counties of Guangxi (Jingxi, Tiandeng, Longzhou). These three counties are located next to each other in the karst regions of southwestern Guangxi.

We found extensive incongruent phylogenies between *trn*L-F and ITS, indicating that reticulate evolution events should be common in this group. Since only one population of the new species has been found we were able to test only one sample, so further research is required based on a broader sampling and more markers.

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Appendix 1. Species, samples (same as in Fig. 5), vouchers, and GenBank accession numbers of ITS and *trn*L-F sequences used in this study.

Species	Sample	Voucher	*trn*L-F	ITS
Primulina dryas	–	C7a	FJ501524	FJ501348
Primulina pinnata	–	G26	FJ501526	FJ501349
Petrocodon ainsliifolius	44071	Y.M. Shui *et al.* 44071	HQ632941	HQ633038
Petrocodon ainsliifolius	CWH88	CWH88	KF202298	KF202291
Petrocodon albinervius	WF001	WF180316-01	ON959495	ON950050
Petrocodon asterocalyx	–	FW-2013	KC904957	KC904954
Petrocodon asterostriatus	WF183	ZFC200720-01	ON959497	ON950052
Petrocodon coccineus	CWH14B	CWH14B	KF202299	KF202292
Petrocodon coccineus	G80E	G80E	FJ501516	FJ501341
Petrocodon coriaceifolius	–	M. Moeller MMO 06-913	HQ632943	HQ633040
Petrocodon dealbatus	–	–	GU350668	GU350636
Petrocodon dealbatus	G12B	G12B	FJ501537	JF697578
Petrocodon dealbatus	LJM1209291	LJM1209291	KR476565	KR337020
Petrocodon ferrugineus	–	M. Moeller MMO 06-784	HQ632946	HQ633043
Petrocodon hancei	08-1342	M. Moeller MMO 08-1342	HQ632944	HQ633041
Petrocodon hancei	1	–	KC904958	KC904955
Petrocodon hancei	2	–	KC904959	KC904956
Petrocodon hancei	GDLC05	GDLC05	KF498253	KF498051
Petrocodon hechiensis	–	–	KR476563	KR337018
Petrocodon hechiensis	07-1077	M. Moeller MMO 07-1077	HQ632942	HQ633039
Petrocodon hispidus	CWH101	CWH101	KF202301	KF202294
Petrocodon hispidus	CWH87	CWH87	KF202300	KF202293
Petrocodon integrifolius	–	M. Moeller MMO 06-865	HQ632940	HQ633037
Petrocodon ionophyllus	WF010	BP P0783	ON959496	ON950051
Petrocodon lithophilus	CWH103	CWH103	KF202303	KF202296
Petrocodon lithophilus	CWH89	CWH89	KF202302	KF202295
Petrocodon lui	–	Y.G. Wei 8012	HQ632938	HQ633035
Petrocodon multiflorus	–	HJ01-2	KM232660	KJ475411
Petrocodon nivelolanosus	–	–	JF697588	JF697576
Petrocodon pulchriflorus	–	–	KX579059	KX579058
Petrocodon retroflexus	–	–	KX579061	KX579060
Petrocodon scopulorus	–	–	GU350669	GU350637
Petrocodon scopulorus	2012-02	W. Fang 2010-02	HQ632947	HQ633044
Petrocodon scopulorus	LJM06753	LJM06753	KR476567	KR337023
Petrocodon sp.	FW2014	FW-2014	KF680503	KF680504
Petrocodon tiandengensis	–	09413	JX506850	JX506960
Petrocodon tongziensis	–	Ren-Bo Zhang SBQ09383	MF872618	MF872617
Petrocodon viridescens	CWH41	CWH41	KF202304	KF202297
Petrocodon viridescens	82661	Y.M. Shui *et al.* 82661	HQ632939	HQ633036

Appendix 2. Morphological comparison between *Petrocodon asterostriatus* and *P. integrifolius*.

	P. asterostriatus	*P. integrifolius*
Leaf blade	broadly ovate to subcordate, yellowish-green to green, often almost symmetrical, densely pubescent on both surfaces	ovate, broadly ovate to orbicular, pale green to green, densely white strigose on both surfaces
Petiole length (cm)	6–13	2–8.5
Cymes	4–8	1–2
Bracts	broadly lanceolate or ovate, 10–25 × 3–6 mm	oblong to lanceolate, 3–8 × 1–3.5 mm
Bracteoles	linear-lanceolate, 9–18 × 2–4 mm	usually lacking, rarely existing and oblong, 1.5–2 × ca. 1 mm
Pedicel	1–2 cm long, densely white puberulent	ca. 0.7 cm long, sparsely glandular puberulent
Calyx lobes	lanceolate to linear-lanceolate, 8–16 × 2–5 mm	narrowly triangular, 7–11 × 1–1.5 mm
Corolla	broadly funnelform with inflated near-spherical base, pale purplish-maroon, 3–4 cm long	thin cylindrical without inflated base, purple, 2.5–2.7 cm long
Filament	ca. 7 mm long, white with purplish maroon longitudinal stripes, sparsely glandular puberulent, distally covered with dark purple glands	ca. 3 mm long, white, stripes absent, pubescent and glandular puberulent, glands absent
Pistil	ca. 22 mm long	ca. 16 mm long

Appendix 3. Molecular data sets used in this study.

	ITS	*trn*L-*trn*F	Overall
Number of sequences (ingroup/outgroup)	37/2	37/2	37/2
Aligned length (bp)	878	1001	1879
Variable characters (bp)	317	138	455
Parsimony-informative characters (bp)	194	51	235
Model selected (ML)	K2P + G4	K3Pu + F + G4	K3P + G4