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Petrocodon zhonglii (Gesneriaceae), a new species from South China

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ABSTRACT: *Petrocodon zhonglii*, a new species of Gesneriaceae from the Danxia landform in Jiangxi, South China, is described and illustrated. The new species is morphologically similar to *P. coriaceifolius* and *P. hancei*, but differs from the latter two by its ventricose corolla tube and stipitate capsule. The new species can further be distinguished from *P. coriaceifolius* by its longer pedicel, and from *P. hancei* by its sparsely strigillose leaf blade with entire to slightly serrate margins and two conspicuous stigma lobes. In addition, the new species exhibits obvious phylogenetic distance, different phenology and ecological niche to its morphologically similar species. According to current information, the conservation status of the new species should be assessed as Data Deficient (DD) based on the IUCN Red List Categories and Criteria.

KEY WORDS: flora of Danxia landform, Jiangxi, morphology, *Petrocodon coriaceifolius*, *Petrocodon hancei*, taxonomy.

INTRODUCTION

The redefined *Petrocodon* Hance in Weber *et al*. (2011) is becoming a large genus in Gesneriaceae. The genus currently possesses around 55 species (GRC, 2024), with the center of species diversity in South and Southwest China (Chen *et al.*, 2014). The re-circumscribed *Petrocodon* also displays a great diversity in floral morphology after the incorporation of species from nine different genera, including *Calcareoboea* C.Y. Wu ex H.W. Li, *Tengia* Chun, *Dolicholoma* D. Fang & W.T. Wang, *Paralagarosolen* Y.G. Wei, *Lagarosolen* W.T. Wang, *Didymorcarpus* Wallich, *Wentsaiboea* D. Fang & D.H. Qin, *Primulina* Hance and *Allocheilos* W.T. Wang (Wang *et al.*, 2011; Weber *et al*., 2011; Xu *et al*. 2014; Liu *et al*., 2024). In addition, the genus shows highly edaphic specialization, with the majority of species endemic to karst limestone and only five species discovered and confirmed from the Danxia landform. Among these five species found in the Danxia landform, three, i.e., *P. asterocalyx* F. Wen, Y.G. Wei & R.L. Zhang (Zhang *et al*., 2018), *P. chishuiensis* Z.B. Xin, F. Wen & S.B. Zhou (Xin *et al*., 2020) and *P. wui* F. Wen & R.B. Zhang (Zhang *et al*., 2023), are endemic here, while *P. hancei* (Hemsl.) A. Weber & Mich. Möller (Weber *et al*., 2011) and *P. chongqingensis* F. Wen, B. Pan & L.Y. Su (Su *et al.*, 2019a) are to be found in both karst and Danxia habitats. Both of the karst and Danxia landforms exhibit high heterogeneity of micro-habitats (Hao *et al*., 2015), which may harbor more narrowly distributed species yet to be discovered.

During our field work in 2017, we found a putatively new species in a Danxia landform in Jiangxi, South China. The plants showed leaf blades similar to *P. coriaceifolius* (Y.G. Wei) Y.G. Wei & Mich. Möller, but the flowers were not observed at this time. To reveal the identity of the newly found plants, we carried out additional field work in August 2023. Fortunately, the plants were flowering then. We found that the rosette leaves with decussate arrangement, coriaceous leaf blade, bilobed stigma and stipitate capsule places the plants in the redefined *Petrocodon* (Wang *et al.*, 2011; Weber *et al.*, 2011, 2020). Specifically, the gross floral morphology of the newly collected plants resembles those of *P. hancei*, while it can be mainly distinguished from the latter by its ventricose corolla tube and stipitate capsule. To further confirm its taxonomic status, we carried out more detailed morphological comparisons and molecular phylogenetic analyses. All results from these analyses show that the plants found in Jiangxi represent a new species of the redefined *Petrocodon*, which is described here.

MATERIALS AND METHODS

Phylogenetic analysis

To confirm the taxonomic identity of the newly found plants, we further carried out molecular phylogenetic analyses. We sequenced two commonly used molecular marker regions (i.e., ITS and *trn*L-F) from two samples (YLH426 and SXZ257, both collected from the type locality) of the new species and three samples (SXZ030 form Pingle, Guangxi; SXZ171 form Renhua,

Guangdong; SXZ305 form Jianghua, Hunan) of *P. hancei*. We also downloaded sequences of the two marker regions of 39 other *Petrocodon* species and two *Primulina* species from NCBI GenBank. We used the two *Primulina* species as outgroup species based on previous nuclear and plastid phylogenies (Yang *et al.*, 2023). The methods of DNA extraction, PCR amplification and sequence alignment followed our previous studies (Yang *et al*., 2020; Shi *et al*., 2024). To assess the combinability of the ITS and *trn*L-F sequences, we conducted an incongruence length difference (ILD) test (Farris *et al.*, 1995) using PAUP* 4.0a (Swofford, 2003). Thereafter, a Maximum Likelihood (ML) phylogeny was inferred based on the ITS, *trn*L-F and concatenated matrix using IQ-TREE v2.1.4 (Nguyen *et al*., 2015), respectively. In IQ-TREE, the parameter '-m MFP' was set to determine the best-fit substitution model (Kalyaanamoorthy *et al*., 2017), and 1000 ultrafast bootstrap approximation (UFBoot) replicates were used to provide unbiased support values (Hoang *et al*., 2018). The newly sequenced DNA sequences were deposited into NCBI GenBank, and all sequences used in this study are listed in Table S1.

Morphological observations

The morphological observations and measurements of the new species were conducted in the field. We also checked specimens of other *Petrocodon* species deposited in IBSC, PE, KUN, IBK and GXMI, as well as digital images available at the Chinese Virtual Herbarium (http://www.cvh.ac.cn). In addition, for a more detailed morphological comparison, we carried out a comprehensive literature study, including all relevant monographs (Wang *et al*., 1998; Li and Wang 2004; Wei *et al*., 2010) and recently published literature (e.g., Wang *et al.*, 2011; Weber *et al*., 2011, 2020; Chen *et al.*, 2014; Xu *et al*., 2014; Zhang *et al*., 2018; Su *et al*., 2019a, b; Xin *et al*., 2020; Li *et al*., 2023; Tan *et al*., 2023; Zhang *et al*., 2023; Liu *et al*., 2024; Tang *et al*., 2024; Xiong *et al*., 2024). The description of the new species follows the terminology used by Wang *et al.* (1998) and Harris and Harris (1994).

RESULTS AND DISCUSSION

Phylogenetic relationships

The combined matrix possessed a total length of 1555 nucleotide bases (ITS: 713, *trn*L-F: 842), of which 293 (19.3%; ITS: 118, *trn*L-F: 51) were putatively parsimony-informative sites. The ILD test yielded a *p*-value of 0.5, indicating that there was no significant incongruence between the sequences of ITS and *trn*L-F. The ML phylogeny inferred using the combined matrix and current taxa sampling revealed that the two individuals (collected from two different subpopulations at the type locality) of the newly collected species clustered together with full support (UFBoot = 100%; Fig. 1), and were sister to *P. hancei* with moderate support (UFBoot = 84%; Fig. 1). It is worth noting that *P. hancei*, for which six samples from six different geographic locations were analyzed, including Pingle (Guangxi), Hezhou (Guangxi), Lechang (Guangdong), Renhua (Guangdong), Jianghua (Hunan), and an unknown location in Guangdong, formed a monophyletic clade with maximum support. The result that both samples of the new species and those of *P. hancei*, each formed a maximally supported clade, while their relationship included uncertainties (see individual gene tree topologies Figs. S3–4), suggests that the two species might represent two independent evolutionary lineages (de Queiroz, 2007). The phylogenetic distances between the newly found species and other morphologically similar species are more distant than it between *P. hancei*. Our ML phylogeny clearly shows that *P. coriaceifolius* together with *P. asterocalyx*, *P. multiflorus*, *P. ferrugineus*, *P. scopulorum*, *P. lancifolius*, and the allies of *P. dealbatus* formed a well-supported clade (UFBoot = 95%; Fig. 1), and *P. pseudocoriaceifolius* is sister to *P. longgangensis* W.H. Wu & W.B. Xu (Xu *et al.*, 2014) with full support. The phylogeny (Fig. S3) estimated from the ITS sequence was generally consistent with the result of the combined matrix, while the phylogeny (Fig. S4) estimated from the *trn*L-F sequence cannot provide valuable information due to numerous nodes possess UFBoot support less than 50%. Despite the phylogenies inferred from the combined matrix and ITS sequence resolved most morphological species (except for *P. dealbatus*), that includes two or more samples in our study, as monophyly, respectively, with full or high supports (UFBoot ≥ 95%), we noticed that the relationships among these species are far from been fully clarified. The poor resolution of the backbone of phylogenies estimated in our study and also other studies (e.g., Weber *et al*., 2011; Chen *et al*., 2014; Xu *et al.*, 2014) might result from insufficient information stored in the two used molecular markers. The high-throughput sequencing, such as whole genome resequencing, can generate hundreds of loci with relatively low-cost, and hence might provide an opportunity to resolve the phylogenetic relationships within the genus *Petrocodon* with high support. However, this cannot be guaranteed by just adding more sequence data because that the genus, together with other core Didymocarpinae members, has undergone a recent allopolyplodization event and rapid radiation (Yang *et al.*, 2023), making the phylogenetic reconstruction extremely difficult (Whitfield and Lockhart, 2007; Oxelman *et al.*, 2017). In addition, although the poor resolution of the plastid gene (*trn*L-F) tree, there are several well-supported topological conflicts between the plastid gene tree and the nuclear gene (ITS) tree. For example, the *P. albinervis* is sister to *P. asterostriatus* in the nuclear tree with full support (Fig. S2), but is sister to both *P. ionophyllus* and *P. integrifolius* in the plastid tree with high support (UFBoot = 99%; Fig. S3). These conflicts

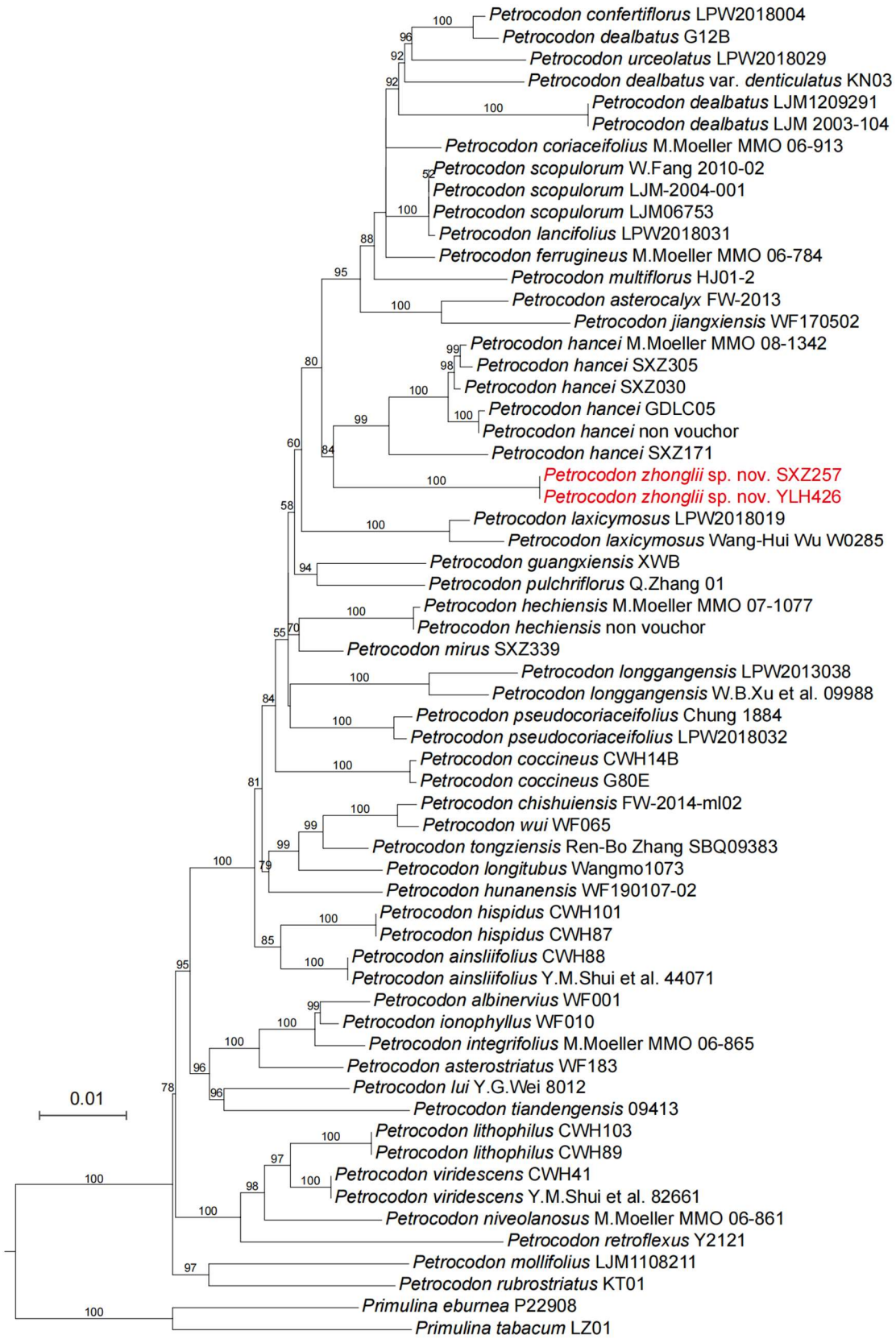

Fig. 1. Maximum likelihood (ML) tree inferred from the combined matrix of ITS and *trn*L-F. Number above each branch is the ultrafast bootstrap approximation (UFBoot) value of the corresponding node. UFBoot values less than 50% are not shown.

TABLE 1. Morphological comparison between ***Petrocodon zhonlii***, ***P. coriaceifolius*** and ***P. hancei***.

Characters	*P. zhonglii*	*P. coriaceifolius*	*P. hancei*
Leaf blade	sparsely strigillose, margin entire to slightly serrate	sparsely strigillose, margin entire	densely strigillose, margin obviously denticulate
Pedicel length	1.2–2.5 cm	3–5 mm	0.5–1.2 cm
Corolla tube	ventricose on abaxial side	flat on abaxial side	flat on abaxial side
Stigma	with two conspicuous lobes	with two conspicuous lobes	with two compressed and inconspicuous lobes
Capsule	stipitate	not stipitate	not stipitate
Phenology	flowering from August to September, fruiting from October to December	flowering May to June, fruiting from July to August	flowering April to May, fruiting from June to July
Habitat	Danxia landform	Karst landform	both Karst and Danxia landform

Fig. 2. ***Petrocodon zhonglii*** sp. nov. **A-B.** habitat, **C.** habit, **D.** flowering cyme, **E.** flower in oblique front view. Photos by Fu-Tao Zhuang (A) and Xi-Zuo Shi (B–E).

might result from reticulate evolution in the genus which need further studies.

Morphological affinities

The newly collected material is morphologically most similar to *P. coriaceifolius* and *P. hancei*, differing mainly by its leaf blade, pedicel length, ventricose corolla tube and stipitate capsule (Figs. 2–3, S1). Detailed morphological comparisons among these three species are provided in Table 1. In addition, the new species is

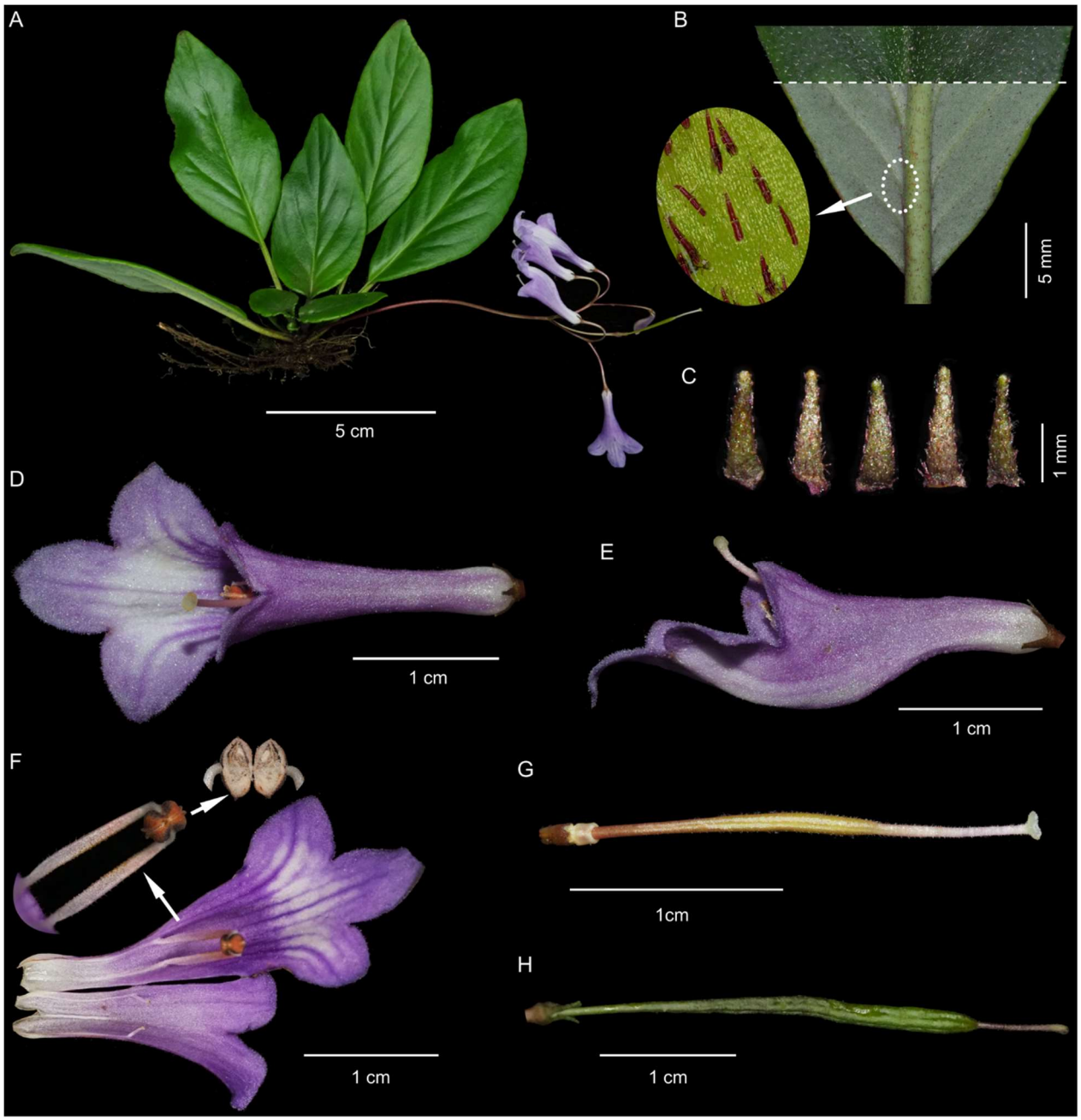

Fig. 3. ***Petrocodon zhonglii*** sp. nov. **A.** habit, **B.** adaxial top. and abaxial bottom (leaf blade surfaces insert showing indumentum), **C.** dissected calyx lobes, **D.** flower top view, **E.** flower side view, **F.** opened flower showing stamens and staminodes inserts showing filaments and dehiscent anthers., **G.** pistil with sepals removed, **H.** young fruit. All photos by Xi-Zuo Shi.

also similar to *P. pseudocoriaceifolius* Yan Liu & W.B. Xu (Xu *et al.*, 2014), *P. jiangxiensis* F. Wen, L.F. Fu & L.Y. Su (Su *et al.*, 2019b) and *P. asterocalyx* in different morphological characteristics, while it can easily be distinguished from all these species by its ventricose corolla tube and stipitate capsule. Despite the low resolution of our phylogenies (see below), they suggest that the morphological similarities between the new species and *P. coriaceifolius*, *P. pseudocoriaceifolius*, *P. jiangxiensis*, and *P. asterocalyx* might represent homoplasies, which appear to be a common evolutionary phenomenon in *Petrocodon* (Lu *et al.*, 2017) and other genus in Gesneriaceae (Wang *et al.*, 2010; Nishii *et al.*, 2015; Puglisi *et al.*, 2016).

TAXONOMIC TREATMENT

Petrocodon zhonglii X.Z. Shi, J.X. Fu & Li H. Yang, ***sp. nov.*** **钟离石山苣苔 2–3, S2**

Type: CHINA. Jiangxi Province: Huichang county,

Junmenling town, Hanxianyan, alt. 352 m, 115.78978238°E, 25.19582789°N, 1 Sept. 2023 (flowering), *X. Z. Shi and J. X. Fu SXZ257* (holotype: IBSC-1025529!, Fig. S2; isotype: IBSC-1025530!).

Diagnosis: *Petrocodon zhonglii* differs from *P. coriaceifolius* and *P. hancei* by its ventricose corolla tube and stipitate capsule. In addition, it can be easily distinguished from *P. coriaceifolius* by its longer pedicel (12–25 mm vs. 3–5 mm), and from *P. hancei* by its sparsely strigillose leaf blade with entire to slightly serrate margin (vs. densely strigillose, obviously denticulate margin) and two conspicuous stigma lobes (vs. compressed and inconspicuous).

Description: Perennial herb. Rhizome terete, 3–5 cm long, 5–7 mm in diameter; roots fibrous, numerous. Leaves decussate, crowed at apex of rhizome; leaf blade coriaceous, narrowly ovate, 5.2–6.7 × 2.5–3.1 cm, margin entire to slightly serrate, apex acute to obtuse, base cuneate; both surfaces sparsely strigillose; lateral veins 5–6 on each side of midrib, inconspicuous adaxially, prominent abaxially; petiole 0.9–3.5 cm long, 2–3 mm in diameter, with both strigillose and reddish-brown hairs. Cymes 1–3, per cyme 5–9 flowered; peduncle 6–8 cm long, 1–2 mm in diameter, sparsely strigillose; bracts 2, opposite, narrowly triangular, 5–6 × 1–2 mm, acuminate at apex, margin entire, outside with reddish-brown hairs, inside glabrous; pedicel 1.2–2.5 cm long, sparsely strigillose. Calyx 5-parted to base, lobes narrowly triangular, 1.8–2 × 0.4–0.6 mm, acuminate at apex, margin entire, outside reddish-brown hairs, inside glabrous. Corolla purple to reddish-purple, throat white with purple stripes, 2.3–2.5 cm long; outside puberulous, inside glabrous; tube infundibuliform, ventricose on abaxial side, 1.5–1.7 cm long, 6–7 mm in diameter at orifice, 3–4 mm in diameter in middle; limb 2-lipped, both surfaces puberulous; adaxial lip 2-lobed, lobes broadly triangular, 4 × 3 mm, apex rounded to obtuse; abaxial lip 3-lobed, two lateral lobes broadly triangular, 4–5 × 4–5 mm, apex rounded, margin entire; middle lobe broadly elliptic to ovate, 5 × 4 mm, apex obtuse to acute, margin entire. Stamens 2, adnate at 8–9 mm above the corolla tube base; filaments white to brown, 7–8 mm long, straight, sparsely puberulous; anthers coherent face to face, brown, glabrous, elliptic, 2 × 1 mm. Staminodes 3, adnate to 6–7 mm above corolla tube base; two lateral staminodes 2 mm long, middle one ca. 0.5 mm long. Pistil protruded, 1.8–2 cm long; ovary stipitate, brown, 1.2–1.3 cm long, sparsely pubescent; style 6–7 mm long, white to purple, sparsely pubescent; stigma horizontally bilobed, lobes broadly elliptic. Disk annular, brown, margin entire, ca. 1 mm high, glabrous. Capsule liner, stipitate; young fruit ca. 3 cm long, dehisced capsule not seen. Seeds not seen.

Phenology: Flowering from August to September, and fruiting from September to October.

Etymology: The species epithet "zhonglii" is a Latinized form of a personal name, referring to Zhong-Li Han, one of the Eight Immortals (Ba Xian) in Chinese mythology. The type locality of the new species, Hanxianyan, is an important place in memory of Zhong-Li Han. The Chinese name is proposed here as "**钟离石山苣苔**" (**钟离**-refers to zhonglii, **石山苣苔**-refers to *Petrocodon*).

Distribution and habitat: According to our field investigations, *Petrocodon zhonglii* is only known from the type locality with three small populations. In 2023, approximately 600 mature individuals growing on cliffs at elevations of 350–450 m a.s.l. were observed in the field. The three populations cover an area of approximately 2 km^2 in total. However, as have been documented in several other cases (e.g., Yang, 2019), the field investigations of newly found species are always insufficient at the time when it been described. This situation might exist in our study, given that Danxia landforms are widely developed in South China. Further field work is needed to confirm the exact geographical distribution of *Petrocodon zhonglii*. Therefore, following the IUCN Red List Categories and Criteria (IUCN, 2024), the conservation status of this new species should be assessed as Data Deficient (DD) based on current information.

Additional specimen examined (paratypes): CHINA. Jiangxi Province: Huichang county, Junmenling town, Hanxianyan, alt. 419 m, 115.7833806°E, 25.19716389°N, 15 July 2017, sterile, *L. H. Yang & B. Pan YLH426* (IBSC-0882208!); ibid., 2 Aug. 2013, flowering, *X. F. Zeng ZXF13707* (CZH-0017897!).

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