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Petrocodon retroflexus sp. nov. (Gesneriaceae) from a karst cave in Guizhou, China

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Petrocodon retroflexus Q. Zhang & J. Guo, a new species of Gesneriaceae from Guizhou, China, is described and illustrated. The new species is morphologically similar to *Petrocodon viridescens* W. H. Chen, Mich. Möller & Y. M. Shui, but differs by its leaf blade being ovate, broadly ovate to elliptic, 8–20 × 5–15 cm and densely pilose on both surfaces, narrowly triangular, 9 mm long, densely pilose bracts, white corolla that is decurved near the middle, with adaxial lip shallowly 4-partitioned and retroflexed, and pistil ca 1.3 cm long and densely pilose and glandular-pilose.

Petrocodon Hance is a small genus of lithophytic perennial herbs of Gesneriaceae, distributed mainly in the limestone regions of southern China and Chinese neighboring areas. The genus is one of the most taxonomically puzzling groups of the Old World Gesneriaceae. According to a recent re-circumscription based on molecular phylogenetic studies as well as morphology, *Petrocodon* has been expanded to accommodate all or some species of seven other genera, including the monotypic *Calcareoboea*, *Paralagarosolen*, *Tengia* and *Dolicholoma*, and one or a few species of *Primulina*, *Lagarosolen* and *Didymocarpus*, yielding a total of 29 recognized species (Weber et al. 2011).

During the course of a floristic survey in the karst caves of Guizhou, China, in August 2012, we discovered a rare plant of Gesneriaceae in Changshun County. After consulting the 'Flora of China', other relevant literature (Wang et al. 1990, 1998, Li and Wang 2004, Wei 2010, Wang et al. 2011, Weber et al. 2011, Wen et al. 2012, Chen et al. 2014, Xu et al. 2014), and herbarium specimens, we found that the plant differs from all the known species of *Petrocodon* s.l., but is similar to *Petrocodon viridescens* W. H. Chen, Mich. Möller & Y. M. Shui with respect to its 4-lobed adaxial lip. Based on morphological and molecular data, we confirmed that it is a significant new species and hence we describe it below as *Petrocodon retroflexus*.

Methods

As the plastid *trnL-F* and nuclear ribosome internal transcribed spacer (ITS) sequences have been most frequently used for phylogenetic analyses of Gesneriaceae, (Denduangboripant et al. 2007, Wang et al. 2010, Möller et al. 2011a, 2011b, Weber et al. 2011, Chen et al. 2014), these two DNA fragments were selected and obtained from the new species to test its position in the phylogeny. Total DNA was extracted from silica-gel dried leaves using the CTAB method (Doyle and Doyle 1987). The polymerous chain reaction (PCR) amplification procedures started with an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and then extension at 72°C for 1 min, with a final extension at 72°C for 10 min after the cycles. Primers for amplification and sequencing were adopted from previous studies (trnXL: GGATATGGCGAAATTGGTAG and trnXF: AGGAACCAGATTTGAACTGG for trnL-F region; ITS1: AGAAGTCGTAACAAGGTTTCCGTAGG and ITS4: TCCTCCGCTTATTGATATGC for ITS region; Baldwin 1992, Zhang et al. 2011).

As expected based on morphological observations, in our initial BLAST search through GenBank using DNA sequences of the new species, the closest relatives with the highest hit scores were species in the recently recircumscribed *Petrocodon*. According to the previous phylogenetic studies, the generic closest relative of *Petrocodon* is *Primulina* Hance s.l., a recently redefined and drastically expanded genus containing more than 160 species (Möller et al. 2011a, Wang et al. 2011). Therefore, all the *trnL-F* and ITS sequences of *Petrocodon* taxa and a few *Primulina* representatives available from GenBank were selected, downloaded and included in this study. *Lysionotus petelotii* and *Lysionotus pauciflorus* were included as outgroups. The downloaded sequences included

27 species for the ITS sequence and 28 species for the *trnL-F* sequence. The GenBank accessions of all the downloaded sequences and the newly obtained sequence are as listed in Appendix 1. Sequences were aligned using MUSCLE 3.8.31 (Edgar 2004) and adjusted manually in Bioedit 5.0.9 (Hall 1999).

Both maximum likelihood (ML) and maximum parsimony (MP) were employed to reconstruct the phylogeny. ML analyses of each datasets were performed using RAxML (Stamatakis 2006), with the settings of 1000 rapid bootstrap searches and thereafter a thorough ML search with the substitution model GTR + G selected by Modeltest (Posada and Crandall 1998). For the MP analyses executed in PAUP 4.0b10 (Swofford 2003) of each datasets, 100 replicates of random stepwise addition with tree bisection–reconnection (TBR) branch swapping were performed using heuristic searches, with all most-parsimonious trees saved at each replicate (MulTree on). Support for each branch was assessed using bootstrap analyses with 100 bootstrap replicates, each with 10 stepwise additions. All other parameters were set as default. Homoplasy levels were assessed by consistency index (CI), retention index (RI) and rescaled consistency index (RC). Because results of the individual analyses revealed no well-supported topological conflicts, i.e. incongruence with BS > 70% among each datasets, the two DNA regions were combined into a concatenated matrix and analyzed using ML and MP methods with the same settings as above.

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.3fj52>.

Results

The aligned positions of ITS and *trnL-F* datasets were 700 and 872 base pairs, respectively. The combined matrix of the two markers thus consisted of 1572 characters, of which

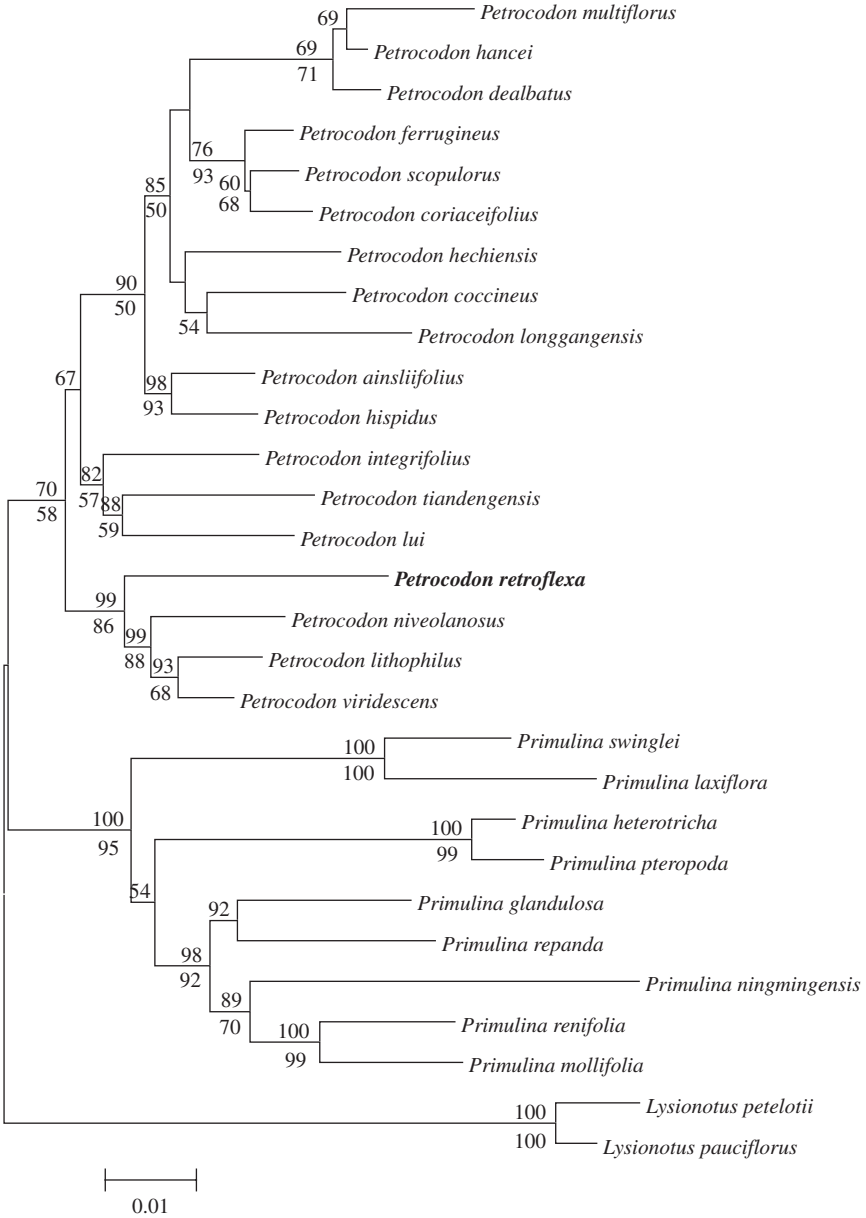

Figure 1. The best ML tree from an analyses of the combined ITS and chloroplast *trnL–F* region. ML/MP bootstrap support values (> 50%) are shown above and below the branch around the corresponding node. The new species is highlighted in bold.

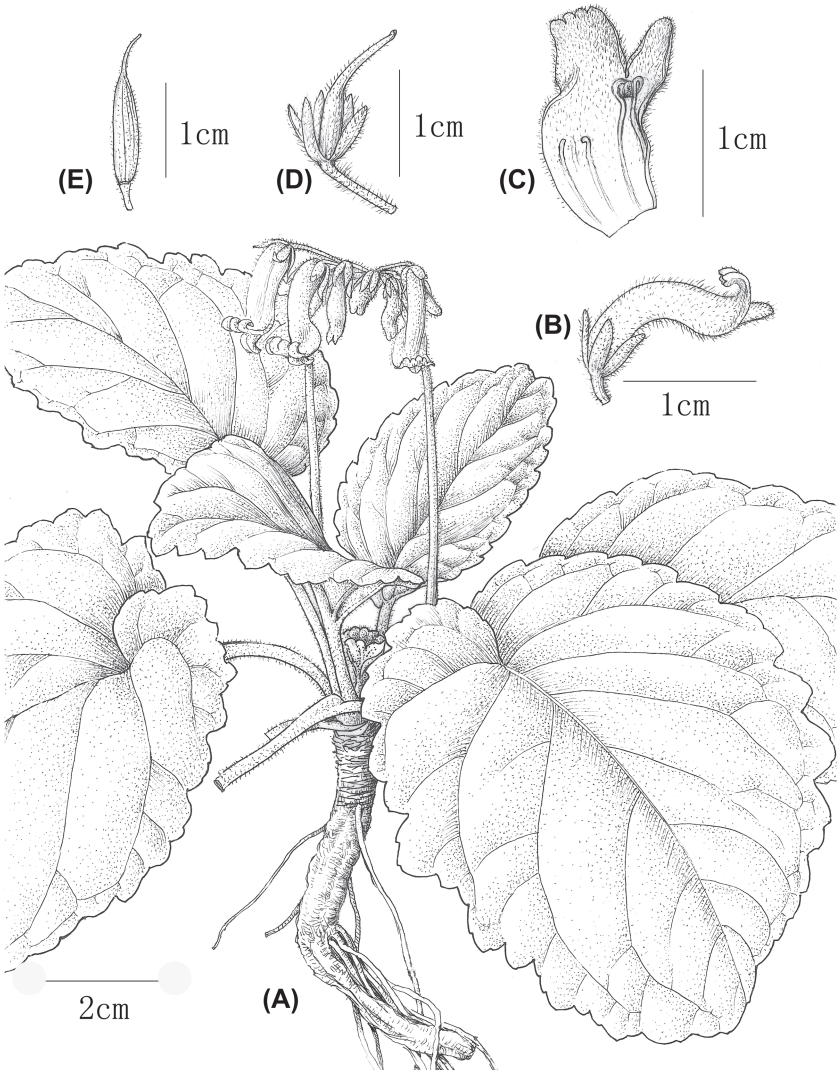

Figure 2. *Petrocodon retroflexus* sp. nov. (A) habit, (B) side view of flower, (C) opened corolla with stamens and staminodes, (D) pistil and calyx, (E) mature fruit. Drawn by W.-H. Lin from the holotype.

433 bp were variable and 231 bp parsimony informative. The MP 50% majority-rule consensus tree (CI = 0.7208, RI = 0.7172, RC = 0.5169) is largely compatible with the best ML tree (log likelihood score = –6756.3), with bootstrap supports greater than 50% from each analyses on the branches (BS_{MP}, BS_{ML}) (Fig. 1).

The phylogenetic relationships reconstructed from the concatenated matrix were congruent with those reported in Weber et al. (2011) and our new species was resolved in *Petrocodon* (BS_{ML} = 58%, BS_{MP} = 70%). The new species was sister to the group including *P. niveolanosus*, *P. lithophilus* and *P. viridescens* with high support value (BS_{ML} = 99%, BS_{MP} = 86%) in the ML analyses. However, DNA sequences of the new species shows substantial differences from other species of *Petrocodon* (Fig. 1), ascertaining its recognition as distinct species.

Petrocodon retroflexus Q. Zhang & J. Guo sp. nov. (Fig. 2–3)

Petrocodon retroflexus is similar to *P. viridescens* W. H. Chen, Mich. Mőller & Y. M. Shui, but differs by its leaf blade being ovate, broadly ovate to elliptic, 8–20 × 5–15 cm, densely pilose on both surfaces (vs rounded, 3.0–9.5 × 2.5–8.5 cm, hispid on both surfaces), the bracts being narrowly triangular, 9 mm long, densely pilose (lanceolate, 6 mm long, hispid), the corolla being white, decurved near the middle (vs yellow–greenish, ventricose on abaxial side of tube), the adaxial lip being shallowly 4-partitioned, retroflexed, (vs 4-lobed, lobes triangular, spreading), and the pistil ca 1.3 cm long, densely pilose and glandular-pilose (ca 0.8 cm long, pubescent).

Type: China. Guizhou: Changshun County, Duncao Township, 106°27′E, 25°42′N, 1050 m a.s.l., on rock faces of a limestone cave, 20 Aug 2012, Y. S. Huang et al. Y2121 (holotype: IBK, isotypes: PE and IBK).

Etymology

This new species is named after its retroflexed adaxial lip.

Description

Perennial herb. Rhizomes up to 18 cm long, 1.3–2.0 cm across. Leaves 5–10, basal, long petiolate; leaf blade papery,

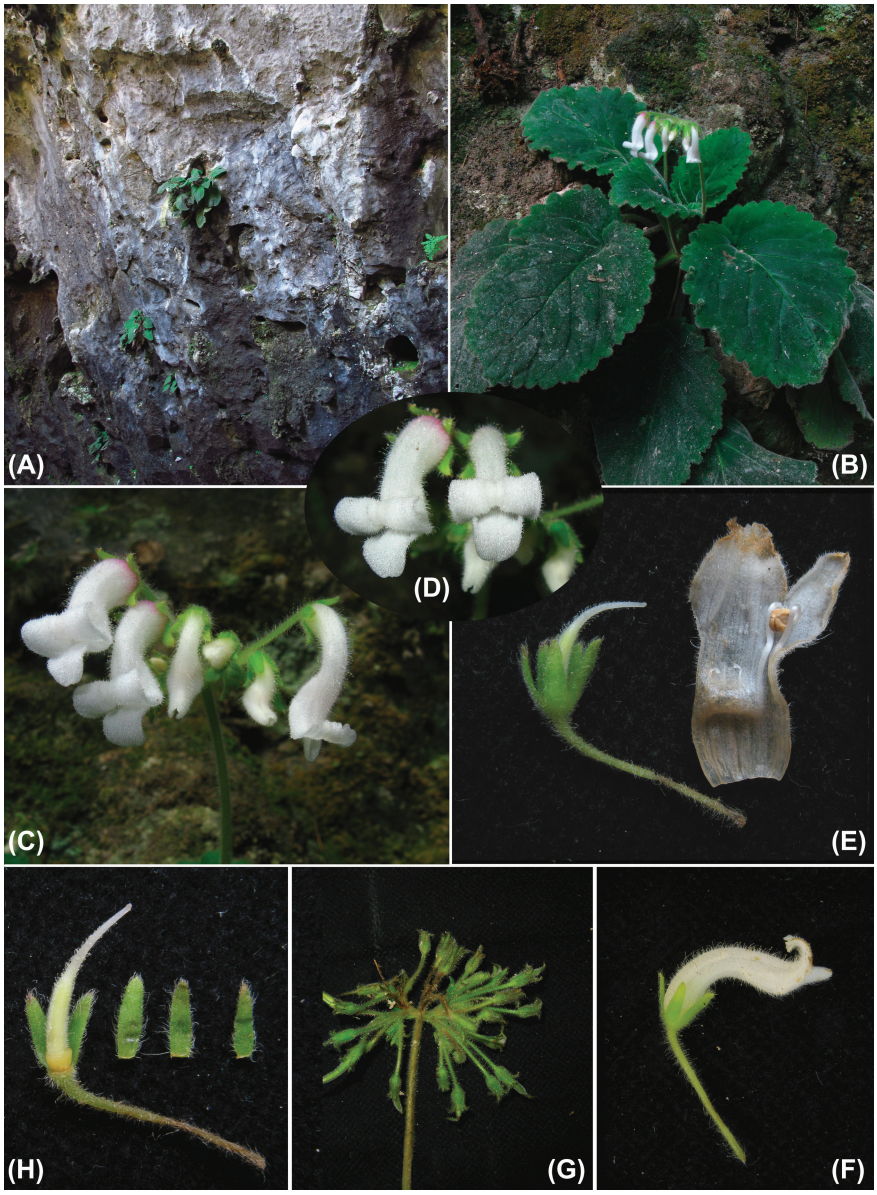

Figure 3. *Petrocodon retroflexus* sp. nov. (A) habitat, (B) habit, (C) inflorescence, (D) front view of flowers, (E) opened corolla with stamens and pistil, (F) side view of flower, (G) infructescence with young fruits, (H) pistil and opened calyx.

ovate, broadly ovate to elliptic, 8–20 cm long, 5–15 cm wide, obtuse at apex, cordate and asymmetrical at base, with crenate margin; adaxial side deeply green, densely pilose; abaxial side green, sometimes pale red to red, densely pilose; lateral veins 5–7; petiole 4–12 cm long, densely pilose. Inflorescences 2 or 3, each with up to 20 flowers; peduncles 12–18 cm long, densely pilose. Bracts narrowly triangular, 9 mm long, densely pilose. Pedicel 1.0–2.5 cm long, densely pilose. Calyx 5-parted to base; lobes equal in size, lanceolate, 6 mm long, 1.5 mm wide, densely pilose outside. Corolla white, occasionally pale pink, decurved near the middle, ca 1.5 cm long, covered with dense pubescence abaxially; tube 1.2 cm long, ca 4 mm in diameter; limb 2-lipped; adaxial lip ca 5 mm long, retroflexed, shallowly 4-partitioned; abaxial lip 1-lobed; abaxial lip lobe ovate-triangular, ca 5 mm long, 2.5 mm wide, round or slightly concave at apex. Stamens 2, ca 5 mm long; filament adnate to the middle of the corolla tube, linear, geniculate near the middle; anthers ovate-triangular, ca 2.5 mm long, glabrous. Staminodes 3, ca 1 mm long, glabrous, adnate to the middle of the tube. Disc ring-like, ca 2.5 mm high. Pistil ca 1.3 cm long; ovary ca 5 mm long, ca 2 mm across, densely brown pilose; style ca 8 mm long, densely pilose and glandular-pilose; stigma 1, apex not capitate. Capsule linear, 1.0–1.5 cm long, ca 2 mm in diameter; persistent stigma ca 6 mm long, shorter than capsule.

Phenology

Flowering occurs in July to September and the fruiting occurs in August to November.

Distribution and habitat

Petrocodon retroflexus is only known from Changshun County, Guizhou, China, growing on rock faces of a single shaded limestone cave, at an elevation of ca 1000 m. The

associated plants are sparse, but include *Cyrtomium hemionitis*, *Ficus tinctoria* subsp. *gibbosa*, *Rhapis excels*, *Oreocnide frutescens*, *Elatostema cuspidatum* et al. Up to date, the new species has only been found in Changshun County, Guizhou, China.

Conservation status

Based on botanical survey for over two years, *Petrocodon retroflexus* is a very rare species, known only from the type locality, Changshun County, Guizhou. We only found one population in a shaded limestone cave, and the total number of individuals is less than 30. Therefore, it should be classified as 'Critically Endangered' (CR) using the IUCN categories and criteria (IUCN 2001). The plants grow on rock faces of the limestone cave near to a village. The garbage of the village is often dumped into the cave, so that this species is in threatened by disturbance from the villagers.

Additional specimen examined (paratype)

China, Guizhou Province: Changshun County, Duncao Twonship, 106°27′E, 25°42′N, 1010 m a.s.l., on rock faces of a limestone cave, in flower, 20 Aug 2012, Y. S. Huang et al. Y2123 (IBK).

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

Lysionotus pauciflorus Maxim.: AB547215/FJ501497; *Lysionotus petelotii* Pellegr.: HQ632974/FJ501496; *Petrocodon ainsliifolius* W. H. Chen & Y. M. Shui: KF202291/KF202298; *Petrocodon coccineus* (C. Y. Wu ex H. W. Li) Y. Z. Wang: KF202292/FJ501516; *Petrocodon coriaceifolius* (Y. G. Wei) Y. G. Wei & Mich. Mőller: HQ633040/HQ632943; *Petrocodon dealbatus* Hance: KF498053/JF697590; *Petrocodon ferrugineus* Y. G. Wei: HQ633043/HQ632946; *Petrocodon hancei* (Hemsl.) A. Weber & Mich. Mőller: KF498051/KF498253; *Petrocodon hechiensis* (Y. G. Wei, Yan Liu & F. Wen) Y. G. Wei & Mich. Mőller: HQ633039/HQ632942; *Petrocodon hispidus* (W. T. Wang) A. Weber & Mich. Mőller: KF202293/KF202300; *Petrocodon integrifolius* (D. Fang & L. Zeng) A. Weber & Mich. Mőller: HQ633037/HQ632940; *Petrocodon lithophilus* Y. M. Shui: W. H. Chen & Mich. Mőller: KF202296/KF202302; *Petrocodon longgangensis* W. H. Wu &W. B. Xu: KC765114/KC765116; *Petrocodon lui* (Yan Liu & W. B. Xu) A. Weber & Mich. Mőller: HQ633035/HQ632938; *Petrocodon multiflorus* F. Wen & Y. S. Jiang: -/KM232660; *Petrocodon niveolanosus* (D. Fang & W. T. Wang) A. Weber & Mich. Mőller: JF697576/JF697588; *Petrocodon scopulorus* (Chun) Y. Z. Wang: HQ633044/HQ632947; *Petrocodon tiandengensis* (Yan Liu & B. Pan) A. Weber & Mich. Mőller: JX506960/JX506850; *Petrocodon viridescens* W. H. Chen: Mich. Mőller & Y. M. Shui: KF202297/KF202304; *Primulina glandulosa* (D. Fang: L. Zeng & D. H. Qin) Y. Z. Wang: KF498087/JX506789; *Primulina heterotricha* (Merr.) Y. Z. Wang: KF498099/DQ872816; *Primulina laxiflora* (W. T. Wang) Y. Z. Wang: KF498079/JX506801; *Primulina mollifolia* (D. Fang & W. T. Wang) Y. Z. Wang: JQ713839/JX506755; *Primulina ningmingensis* (Yan Liu & W. H. Wu) W. B. Xu & K. F. Chung: JX506931/JX506822; *Primulina pteropoda* (W. T. Wang) Y. Z. Wang: KF498100/DQ872817; *Primulina renifolia* (D. Fang & D. H. Qin) Y. Z. Wang: JQ713840/JX506851; *Primulina repanda* (W. T. Wang) Y. Z. Wang: JX506938/JX506830; *Primulina swinglei* (Merr.) Mich. Mőller & A. Weber: JX506950/JX506841.