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Article *in* Annales Botanici Fennici · October 2019

DOI: 10.5735/085.056.0413

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Source: Annales Botanici Fennici, 56(4-6) : 277-284

Published By: Finnish Zoological and Botanical Publishing Board

URL: https://doi.org/10.5735/085.056.0413

Ann. Bot. Fennici 56: 277–284 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 23 May 2019

Petrocodon jiangxiensis (Gesneriaceae), a new species from Jiangxi, China

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Received 2 Jan. 2019, final version received 2 May 2019, accepted 3 May 2019

Su L.Y., Pan B., Hong X., Zhao Z.G., Fu L.F., Wen F. & Maciejewski S. 2019: *Petrocodon jiangxiensis* (Gesneriaceae), a new species from Jiangxi, China. — *Ann. Bot. Fennici* 56: 277–284.

Based on morphological, cytological and molecular data, a new species of Gesneriaceae, *Petrocodon jiangxiensis* F. Wen, L.F. Fu & L.Y. Su from Jiangxi Province, China, is described and illustrated. Morphologically, it resembles the most *P. asterocalyx*. Both species have axillary cymes, an actinomorphic calyx and similarly shaped corolla, but *P. jiangxiensis* can be easily distinguished by ovate-elliptic to broadly ovate leaf blades, three bracts with a crenulate to serrate margin, smaller calyx lobes 5–6 × ca. 1 mm, smaller corolla 1.7–2.3 cm long, and glabrous anthers. *Petrocodon jiangxiensis* has a 2n = 36 somatic chromosome number.

Introduction

Traditionally, *Petrocodon* was a small genus of three species of lithophytic perennial herbs belonging to the tribe Didymocarpeae of Gesneriaceae. The flowers in *Petrocodon* have a small, white, bell-shaped corolla (Hance 1883, Wang *et al*. 1998, Li & Wang 2004, Wei 2007, Wei *et al*. 2010, Jiang *et al*. 2011, Wen *et al*. 2012). After a thorough study based on molecular data, *Petrocodon* was redefined and now comprises at least 36 species and one variety (Weber *et al*. 2011, Möller *et al*. 2016, Möller 2019, Wen *et al*. 2019). *Petrocodon* is most diverse in limestone areas, and many new taxa were discovered and published from there, e.g., *P. tongziensis* (Zhang *et al*. 2019), *P. pulchriflorus* (Lu *et al*. 2017b) and *P. retroflexus* (Guo *et al*. 2016). In fact, 32 species are strictly limited to limestone areas, while *P. hancei* grows on a variety of substrates and *P. asterocalyx* occurs only in the Danxia landform (Zhang *et al*. 2018), a vast area formed from red-coloured sandstones and conglomerates of largely Cretaceous age. Cytological data on this genus are scarce, and chromosome numbers are known for four species of *Petrocodon* only.

There are nine genera of Gesneriaceae recorded for Jiangxi Province, east China (Xu *et al*. 2017). Before the present report, only one species of *Petrocodon* (*P. hancei*) was reported from there. During our fieldwork in Leping County (Jiangxi Province) in 2017, we found an unknown species of Gesneriaceae with a small, pale purple to white corolla and exsert pistil. We examined specimens deposited at IBK, KUN, ANU, PE, consulted local and national floras, and relevant literature including recently published papers describing new species with small flowers, purple corolla and exsert pistil (e.g., Wang *et al*. 1990, 1998, Li & Wang 2004, Wei *et al*. 2010, Xu *et al*. 2014, 2017, Zhang *et al*. 2018). As a result, we were able to confirm we had a new species at hand, which is closely related to *P. asterocalyx*.

Material and methods

Chromosome preparations

Plants were collected from the wild from the type locality in Leping County, Jiangxi Province, China. Later we cultivated some of these plants in a greenhouse at the Gesneriad Conservation Center of China (GCCC). Leaf cuttings yielded new root tips when grown hydroponically for 2–3 weeks. The new root tips were pretreated with 0.002 mol l^{-1} 8-hydroxyquioline at 13 °C for 4–5 h. After fixing for 24 h in Carnoy solution (3:1 ethanol:acid) at 4 °C, dissociating, staining and squashing followed (cf. Jong & Möller 2000). Chromosome numbers were determined in at least 20 cells from 10 different root tips with well-spread chromosomes in metaphase. Pictures were taken using a light microscope (LEICA DM 2500) equipped with a camera (Leica DFC420).

Molecular methods

To test the systematic placement of the new species, the nuclear ribosome internal transcribed spacers (ITS) region and chloroplast *trn*L-F intron spacer (*trn*L-F) were chosen to reconstruct the phylogenetic tree of *Petrocodon*, since these two regions were frequently used in previous taxonomic and phylogenetic works of (e.g. Möller *et al*. 2009, 2011, Weber *et al*. 2011, Chen *et al*. 2014, Xu *et al*. 2014, Yu *et al*. 2015, Lu *et al*. 2017a, 2017b, Zhang *et al*. 2018, 2019). We extracted total genomic DNA of the new species and *Petrocodon hunanensis* from silica-dried leaves collected from the field using a CTAB method (cf. Doyle 1987). PCR amplification and sequencing followed Chen *et al*. (2014). The ingroups comprised 37 *Petrocodon* samples representing 24 species including the presumed new species. Outgroups (*Primulina pinnata* and *P. dryas*) were sampled based on previous phylogenetic analyses of Gesneriaceae (Möller *et al*. 2009, 2011, Weber *et al*. 2011). We acquired molecular data for this study from GenBank , except for the new species and *P. hunanensis* whose molecular data were not available before the completion of this study (cf. Appendix 1).

Sequence alignment and phylogenetic analysis

DNA sequences were edited and assembled using Lasergene Navigator 7.1 (DNAstar, Madison, WI, USA), aligned using the program MUSCLE implemented in the software MEGA5 (Tamura *et al*. 2011), and adjusted manually in Bioedit 5.0.9 (Hall 1999). The incongruence length difference (ILD) test was perform in PAUP* 4.0b10 (Swofford 2002) to verify whether any phylogenetic conflict between ITS and *trn*L-F existed. The test outcome ($p = 0.118$) indicated that the nuclear ribosome internal transcribed spacers (ITS) region and chloroplast *trn*L-F intron spacer (*trn*L-F) can be used in the analysis. Reconstruction of phylogenetic tree by maximum parsimony (MP) method was also carried out in PAUP* 4.0b10 following Zhang *et al*. (2018).

Results

Chromosome cytology

We captured the somatic chromosomes of *Petrocodon jiangxiensis* at the metaphase (Fig. 1). The species' chromosomes are small (0.7–2.1 μm), and the somatic chromosome number (2n) is 36, with two relatively small satellites. The position

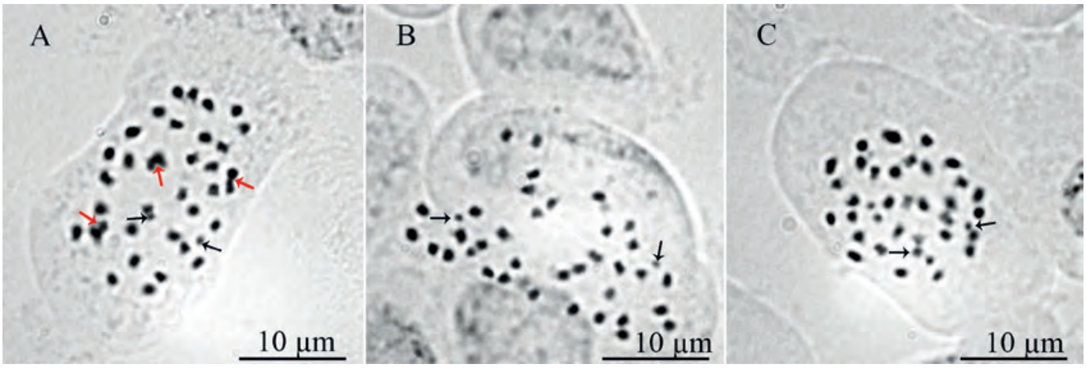

Fig. 1. Somatic chromosomes at metaphase of *Petrocodon jiangxiensis* (2n = 36). — **A**, **B** and **C** are from different cells. Red and black arrows indicate overlapping/touching chromosomes and satellites, respectively.

of centromere could not be determined so that a detailed karyotype analysis was not possible.

Molecular analysis

We reconstructed the maximum parsimony (MP) trees using combined ITS and *trn*L-F sequence data. The length of the molecular matrix containing 39 samples representing 26 species was 1379 characters (650 characters for ITS and 729 characters for *trn*L-F). It included 123 (8.8%) variable and 213 (15.2%) parsimony-informative characters. The maximum parsimony analysis on the combined data generated 108 trees, with tree length = 553 steps, consistency index (CI) = 0.741 and retention index (RI) = 0.805. The strict consensus tree (Fig. 2) was highly resolved and tree topology was consistent with that in the previous phylogenetic studies (Möller *et al*. 2011, Chen *et al*. 2014, Zhang *et al*. 2018, 2019). The species described here and *P. asterocalyx* form a clade with a strong statistical support (BS = 100).

Taxonomic treatment

Petrocodon jiangxiensis F. Wen, L.F. Fu & L.Y. Su, *sp. nova* (Fig. 3)

Type: China. Jiangxi Province: Leping County, Wenshan Town, growing in rocky crevices at the foot of a limestone hill, 28°44´N, 117°15´E, ca. 280 m a.s.l., in flower, 2 May 2017 Wen Fang & Hong Xin, *WF170502-01* (holotype IBK, isotype AHU).

Etymology: The type locality, Jiangxi Province, China was the source for the specific epithet.

Herbs, perennial, acaulescent. Leaves 8–12 or more, basal, leaf blade ovate-elliptic to broadly ovate, 3–6.5 × 2–5 cm, apex acute to acuminate, base often oblique, cuneate to broadly cuneate, margin denticulate to serrate, adaxial surface with appressed pubescence, abaxial surface brown hispid along veins; lateral veins 5–6 on each side of midrib, adaxially concave, abaxially prominent. Petiole, 1.5–3.8 cm long or longer, terete, ca. 2.5 mm in diameter, sparsely brown hispid. Cymes 1–5, axillary, 1 to 2 branched, 6–20 flowered; peduncle brown, 9–13 cm long, ca. 2.5 mm in diameter, sparsely brown hispid; pedicel green 8–20 mm long, ca. 0.8 mm in diameter, densely white hispid. Bracts 3, free, green, lateral ones opposite, oblong or oblanceolate, occasionally oblong-elliptic, apex subacute, 4.5–7 × 2–3.5 mm, middle one linear to narrowly oblanceolate, 2.5–3 × 2–2.5 mm, all margins crenulate to serrate, outside densely white hispid, inside glabrous, bracteoles linear, 2.4–2.8 × 0.5–0.6 mm, outside densely brown hispid, inside glabrous; pedicel 9–13 mm long. Calyx green, actinomorphic, 5-parted to base, lobes narrowly lanceolate to linear 5–6 × ca. 1 mm, outside densely hispid, inside glabrous. Corolla pale purple to white, one dark-purple longitudinal stripe on each one of lower lip lobe, 17–23 mm long, outside with appressed pubescence, inside glabrescent, tube campanulate-tubular, 8.5–11.5 mm long, 3–4 mm in diam. at mouth; limb distinctly

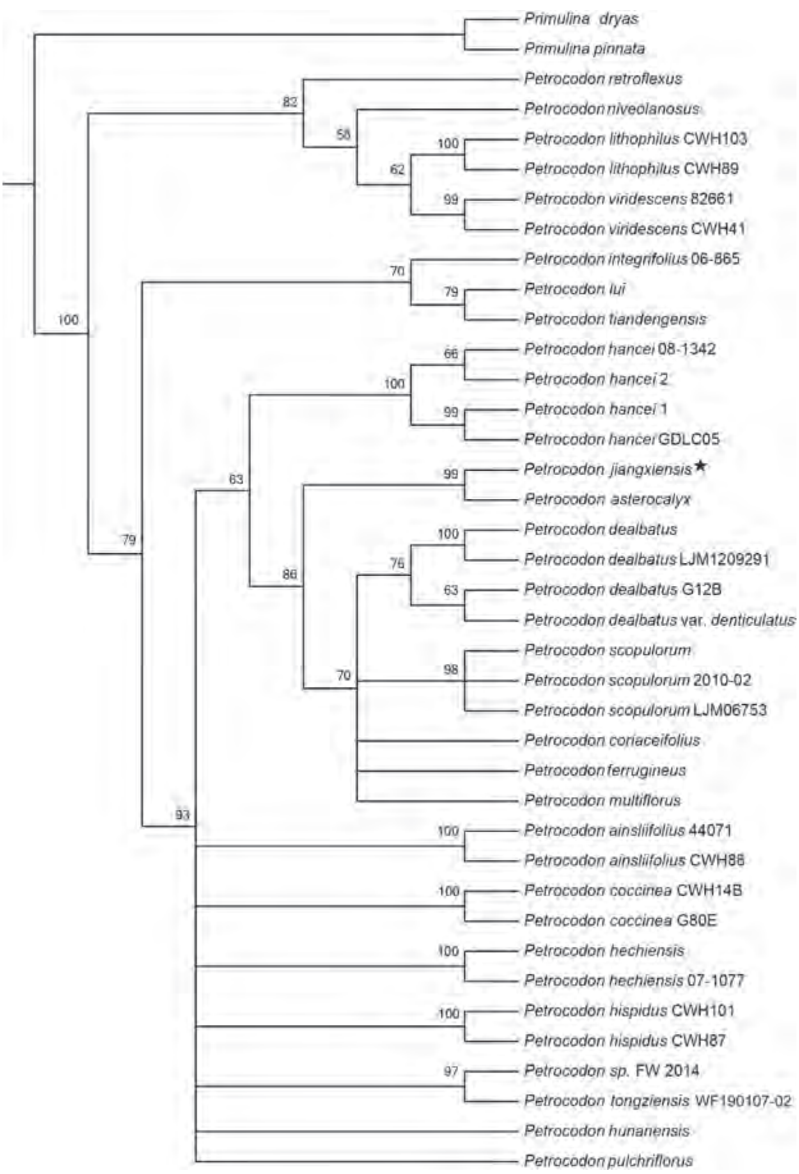

Fig. 2. Strict consensus tree resulted from a Maximum-parsimony (MP) analysis based on combined *trn*L-F and ITS sequences of 26 species. Bootstrap values > 50% by MP analysis are shown around the corresponding nodes. The star indicates the new species.

2-lipped, adaxial lip 2-lobed, lobes triangular, ca. 6 mm long, abaxial lip 3-lobed, lateral lobes obliquely triangular, central one triangular, ca. 12 mm long. Stamens 2, adnate to ca. 8 mm above base of corolla tube; filaments dark purple, linear, 4–5 mm long, sparsely erectly puberulent, anthers glabrous, dark brown, 2.3–2.5 mm long. Staminode 3, glabrous, lateral ones ca. 0.7 mm long, 3.5–4 mm above base of corolla tube respectively, median one ca. 3.7 mm above base of corolla tube, tiny, ca. 0.4 mm long, barely visible. Disc about 1.0 mm high, margin entire. Pistil ca. 18 mm long; ovary linear, nearly glabrous ca. 9.5 mm long; style dark brown, sparsely white pubescent, ca. 8.5 mm long, exsert from corolla. Stigma 2, bifid, broadly ovate, ca. 0.2 mm long for each one. Capsule glabrous, 1.8–3.5 cm long, ca. 1.5 mm in diameter. Flowering from April to May, fruiting in July.

Distribution and habitat. *Petrocodon jiangxiensis* was found growing mostly in crevices and on surfaces of rocks in an evergreen broad-

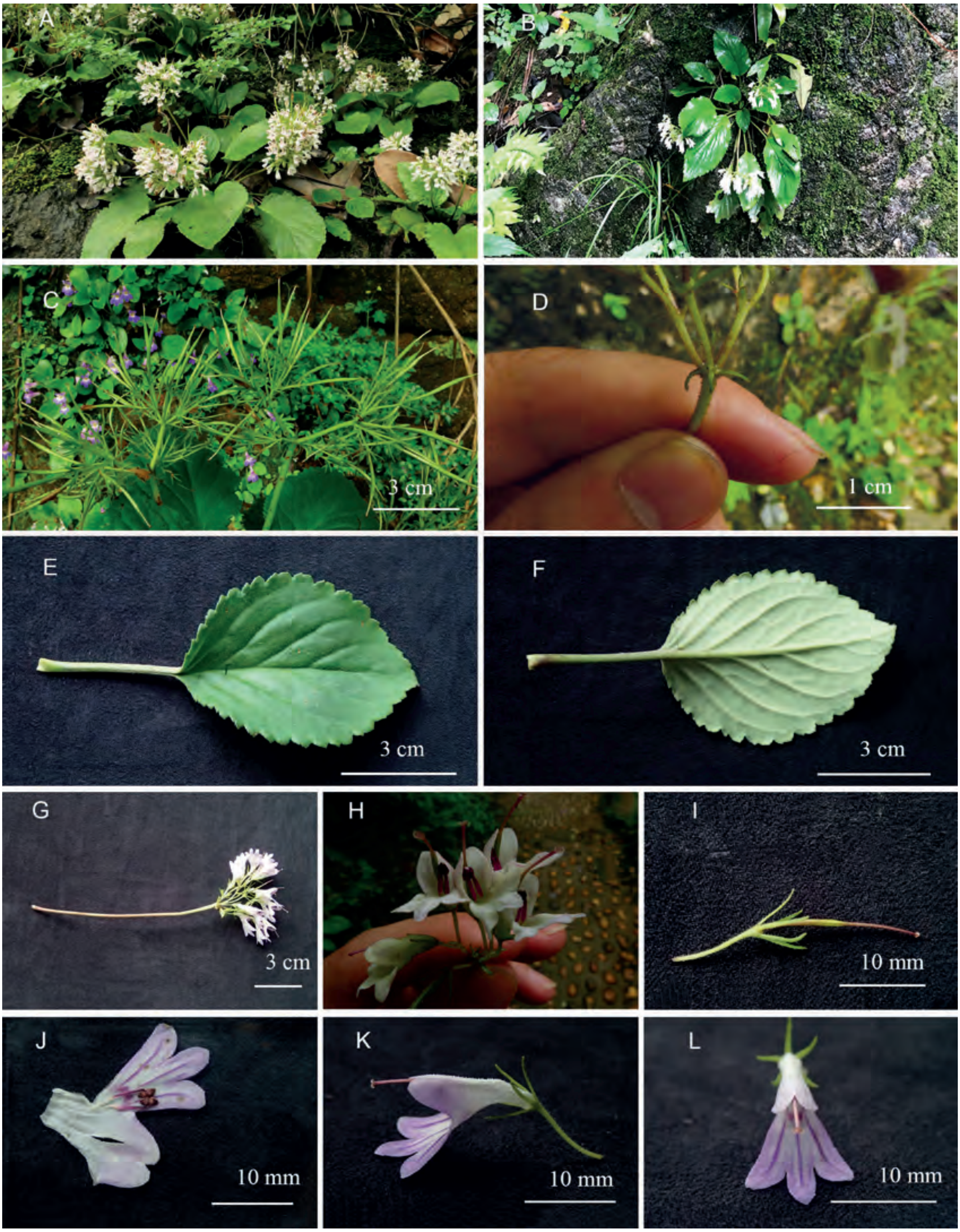

Fig. 3. *Petrocodon jiangxiensis*. — **A** and **B**: Habit of flowering plant in native habitat. — **C**: Fruits. — **D**: Bracts. — **E**: Adaxial leaf surface. — **F**: Abaxial leaf surface. — **G**: Inflorescence. — **H**: Stamens. — **I**: Calyx and pistil. — **J**: Staminode. — **K**: Flower in lateral view. — **L**: Flower in front view.

leaved forest on limestone hills, and on damp and shaded cliffs at the entrance to a limestone cave. Two populations are known to exist in Leping County, Jiangxi Province, China.

Table 1. Morphological comparison of *Petrocodon jiangxiensis* and *P. asterocalyx* (data from Zhang *et al*. 2018).

Characters	*P. jiangxiensis*	*P. asterocalyx*
Leaf blade		
shape	ovate-elliptic to broadly ovate	rhombic-oblong or rhombic
size (cm)	3–6.5 × 2–5	6.5–13.5 × 2–6
Bract		
number	3	2
shape	Oblong to oblanceolate	linear
margin	crenulate to serrate	entire
Corolla length (cm)	1.7–2.3	2.5–3.0
Calyx lobe size (mm)	5–6 × ca. 1	20–40 × 2–3
Stamens	adnate to ca. 8 mm above base of corolla tube	adnate to 1.0–1.1 cm above base of corolla tube
Stamen		
filament length (mm)	4–5	7.8–8.5
anther length (mm)	2.3–2.5	3.5–3.8
anther shape	reniform	elliptical
anther indumentum	glabrous	erectly pubescent
Style		
length (mm)	ca. 8.5	ca. 7
colour	dark purple	white

Discussion

According to the molecular data, *Petrocodon jiangxiensis* is closest to *P. asterocalyx* (BS = 100) which is supported by their having similar morphological characters: both have pale purple to white corolla, actinomorphic calyx and exsert pistil. The two species, however, grow on different types of substrate. *Petrocodon jiangxiensis* occurs in limestone areas, while *P. asterocalyx* is found in Danxia landscape only. Morphological differences between the two species are listed in Table 1.

Petrocodon jiangxiensis has the same chromosome number as *P. niveolanosus*, *P. hechiensis* and *P. jingxiensis*, i.e. 2n = 36 (Liu *et al*. 2014), the basic number thus being x = 18. The same chromosome number in *P. hancei* is, however, 2n = 20 (Cao *et al*. 2003). According to Cao *et al*. (2003) and Liu *et al*. (2014), the chromosome length in the four above-mentioned species of *Petrocodon* is 0.7–3.3 µm, and there are one or two satellites. Also *Primulina s. lato* has 2n = 36 (chromosome length 0.6–2.4 µm), with one or two satellites in some species (Liu *et al*. 2014). This is in accordance with molecular data on the two genera in Möller *et al*. (2009, 2011) and Zhang *et al*. (2018a).

Acknowledgments

This research was financially supported by the Fund of Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain (grant no. 17-259-23), the National Natural Science Foundation of China (grant no. 31860047), the Natural Science Foundation of Guangxi (grant no. 2017GXNSFAA198006), the Science Research Foundation of Guangxi Academy of Sciences (grant no. 2017YJJ23022), and the Key Sci. & Tech. Research and Development Project of Guangxi (grant Guike AB16380053). Special thanks are due to the members of the Gesneriad Society — Bill Price, Betsy Gottshall, Irina Nicholson, Winston Goretsky and Wallace Wells amongst the others — who participated in a field trip led by Hong Xin and Stephen Maciejewski to find gesneriads in the wild. We also thank Michael LoFurno, Temple University, USA for editorial assistance.

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Appendix 1. ITS sequences used in this this study.

Taxon	GenBank accession numbers (ITS, *trn*L-F)
Petrocodon ainsliifolius 44071	HQ633038, HQ632941
Petrocodon ainsliifolius CWH88	KF202291, KF202298
Petrocodon asterocalyx	KC904954, KC904957
Petrocodon coccineus CWH14B	KF202292, KF202299
Petrocodon coccineus G80E	FJ501341, FJ501516
Petrocodon coriaceifolius	HQ633040, HQ632943
Petrocodon dealbatus var. *denticulatus*	JF697578, JF697590
Petrocodon dealbatus	GU350636, GU350668
Petrocodon dealbatus G12B	FJ501358, FJ501537
Petrocodon dealbatus LJM1209291	KR337020, KR476565
Petrocodon ferrugineus	HQ633043, HQ632946
Petrocodon hancei	KC904955, KC904958
Petrocodon hancei	KC904956, KC904959
Petrocodon hancei 08-1314	HQ633041, HQ632944
Petrocodon hancei GDLC05	KF498051, KF49 8253
Petrocodon hechiensis	KR337018, KR476563
Petrocodon hechiensis 07-1077	HQ633039, HQ632942
Petrocodon hispidus CWH87	KF202293, KF202300
Petrocodon hispidus CWH1	KF202294, KF202301
Petrocodon hunanensis WF190107-02	MK941179, MK941180
Petrocodon integrifolius	HQ633037, HQ632940
Petrocodon lithophilus CWH89	KF202295, KF202302
Petrocodon lithophilus CWH103	KF202296, KF202303
Petrocodon lui	HQ633035, HQ632938
Petrocodon multiflorus	KJ475411, KM232660
Petrocodon niveolanosus	JF697576, JF697588
Petrocodon pulchriflorus	KX579058, KX579059
Petrocodon retroflexus	KX579060, KX579061
Petrocodon scopulorus	GU350637, GU350669
Petrocodon scopulorus 2010-02	HQ633044, HQ632947
Petrocodon scopulorus LJM06753	KR337023, KR476567
Petrocodon sp. FW2014	KF680504, KF680503
Petrocodon tiandengensis	JX506960, JX506850
Petrocodon tongziensis	MF872617, MF872618
Petrocodon viridescens 82661	HQ633036, HQ632939
Petrocodon viridescens CWH41	KF202297, KF202304
Primulina dryas	FJ501348, FJ501524
Primulina pinnata	FJ501349, FJ501526