A New Species of Pedicularis from Sichuan Wolong National Nature Reserve: Panda Pedicularis (Postprint)

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Date: 2021-06-02T00:00:00+00:00

Abstract

Panda Pedicularis is a new species of *Pedicularis* discovered in the Wolong National Nature Reserve, Sichuan. This new species belongs to the alternate-leaf group, with basal leaves forming clusters. Its corolla is of the short-tubed beaked type, with the lower lip enclosing the beak. The floral tube undergoes an approximately 180-degree twist near the calyx end, positioning the corolla' s lower lip laterally upward—a feature that clearly distinguishes it from other native *Pedicularis* species. Molecular phylogenetic analysis indicates that Panda Pedicularis is a member of Clade 7, forming sister relationships with members of the flame series, false European series, beak-tooth series, fern-like series, and long-beak series, yet its corolla morphological characteristics are distinctly different from those of its close relatives.

Full Text

Preamble

Pedicularis pandania (Orobanchaceae), a New Species of *Pedicularis* from the Wolong National Nature Reserve, Sichuan Province, China

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Abstract

Pedicularis pandania (Orobanchaceae) is a new species discovered in the Wolong National Nature Reserve, Sichuan, China. This species belongs to the alternate-leaved group, characterized by abundant basal leaves and a short-tubed, beaked corolla in which the lower lip completely envelops the beak. A distinctive feature is the approximately 180-degree torsion of the corolla tube near the calyx, which positions the lower lip laterally above the flower—clearly distinguishing it from other Chinese *Pedicularis* species. Molecular phylogenetic analysis indicates that *P. pandania* is a member of Clade 7, forming a sister group to members of Series Flammeae, Pseudo-oederianae, Rhynchodontae, Filiculae, and Macrorhynchae. However, its corolla morphology clearly differs from these related species.

Keywords: *Pedicularis*, Orobanchaceae, Sichuan, Wenchuan, Wolong

Introduction

Pedicularis L. is the largest genus in the family Orobanchaceae, comprising approximately 600 species widely distributed across the northern temperate zone. More than two-thirds of these species are concentrated in the mountainous regions from southwestern China to the western Himalayas (Tsoong, 1963; Yang et al., 1998; Yu & Wang, 2020). According to existing floristic records, Yunnan Province has the richest *Pedicularis* flora in China, followed by Sichuan Province and the Tibet Autonomous Region (Tsoong, 1963; Yang et al., 1998; Wang, 2006). In recent years, intensive field investigations have led to the discovery and description of several new *Pedicularis* species, primarily from the Himalayan-Hengduan Mountain region of southwestern China (Yang et al., 2003; Yu et al., 2010; Liu & Yu, 2015; Yu et al., 2018; Li et al., 2019).

In August 2019, during a biodiversity survey in the Sichuan Wolong National Nature Reserve, the authors encountered an unknown *Pedicularis* species. In July 2020, further investigations were conducted, and plant specimens along with DNA materials were collected (Fig. 1 [Figure 1: see original paper]: A-F). Through comparative morphological analysis and phylogenetic investigation, we confirmed that this unknown taxon represents a new species. Because it was discovered in the giant panda' s habitat of Sichuan Wolong National Nature Reserve, we name this new species *Pedicularis pandania* W.B. Yu, H.Q. Lin & Y.Hong Cheng.

1. Materials and Methods

Voucher specimens and DNA materials of the new *Pedicularis* species were collected from the Sichuan Wolong National Nature Reserve in Wenchuan County, Sichuan Province. Fresh leaves were collected and preserved in silica gel. Total genomic DNA was extracted using a modified CTAB method (Doyle & Doyle, 1987), followed by high-throughput sequencing library construction. The selected fragment size for sequencing libraries was approximately 350 bp, with paired-end 150 bp sequencing performed on the NovaSeq 6000 System (Illumina) (Zeng et al., 2018; Li et al., 2021). The sequencing data were assembled using the GetOrganelle software package to reconstruct the chloroplast genome and ribosomal DNA (Jin et al., 2020). The nrITS, *matK*, *rbcL*, and *trnL-F* regions were then extracted and integrated into the phylogenetic matrix of Yu et al. (2015). A maximum likelihood tree was constructed using RAxML software (Stamatakis et al., 2008), with bootstrap support values estimated from 1,000 replicates. Specific parameter settings followed Yu et al. (2015).

For pollen electron microscopy, materials were obtained from dried herbarium specimens. Three mature flowers were randomly selected from the specimen, and anthers were extracted from the galea using forceps. Pollen grains were transferred to SEM stubs, sputter-coated with gold, and observed under a scanning electron microscope (ZEISS EVO LS10, Germany) for imaging.

2. Results

2.1 Phylogenetic Analysis

The aligned sequence matrices for nrITS, *matK*, *rbcL*, and *trnL-F* were 680 bp (variable sites/phylogenetically informative sites: 460/377), 768 bp (429/294), 654 bp (169/99), and 1,511 bp (700/443), respectively. The combined matrix comprised 3,613 bp, including 1,758 variable sites and 1,213 informative sites.

Phylogenetic analysis revealed that *Pedicularis pandania* belongs to Clade 7 (Fig. 2 [Figure 2: see original paper]), which primarily consists of alternate-leaved species but also includes some opposite/whorled-leaved groups (Tkach et al., 2014; Yu et al., 2015). Within this clade, *P. bella* Hook.f. occupies the most basal position, followed by four independent subclades separated by opposite/whorled-leaved or alternate-leaved groupings. *Pedicularis pandania* is a member of the small alternate-leaved subclade Clade 7E (BS=100). Its close relatives include members of Series Flammeae Prain, Pseudo-oederianae Limpr., Rhynchodontae Prain, Filiculae Li, and Macrorhynchae Li from Tsoong' s subgenus *Rhizophyllum* (Tsoong, 1955, 1963). However, the exact sister group of *P. pandania* remains unclear, as it forms a highly isolated lineage, similar to *P. filicula* Franch. ex Maxim.

2.2 Morphological Analysis

Pedicularis pandania is an alternate-leaved species with clustered basal leaves and petioles up to 10 cm long. The base of the rhizome bears numerous persistent petioles of old leaves or scaly, lignified leaves. The most distinctive characteristic is the corolla lower lip that envelops the beak (Fig. 1: B, E). This feature is also observed in Series Franchetranae Prain, Pseudomacranthae P.C. Tsoong, Wilsoniae L., *P. bella*, *P. tricolor* Hand.-Mazz., *P. megalantha* D.Don, and *P. insignis* Bonati. However, the corolla tube of *P. pandania* is twisted, causing the entire flower to droop with the lower lip positioned laterally above (Fig. 1: E), similar to *P. megalantha*, whereas in other lip-enveloping species, the lower lip is raised upward.

The pollen grains of *P. pandania* are subspherical, radially symmetrical, and medium-sized (polar axis 22.46–25.56 m × equatorial axis 19.66–23.24 m), with tricolpate apertures and granular exine ornamentation (Fig. 1: G-I).

2.3 Taxonomic Treatment

Pedicularis pandania W.B. Yu, H.Q. Lin & Y.Hong Cheng, sp. nov. Fig. 1: A-F, Fig. 3 [Figure 3: see original paper]

Herbs perennial, 10–40 cm tall, drying black. Roots fascicled, up to 25 cm long. Stem erect, 1 to several, unbranched, cylindrical pubescent, with woody marcescent leaves and petioles of preceding years and lanceolate scales persistent at base. Leaves almost all basal, sometimes 1 to 3 alternate cauline leaves; leaf petiole 3–9 cm long, leaf blade lanceolate-oblong ca. 2–7 cm long × 1.4–2.6 cm wide, abaxially persistent and furfuraceous, adaxially glabrescent or sparsely pubescent, pinnatipartite to pinnatisect; segments 8–13 pairs, opposite or subopposite, regularly pinnatifid or dentate. Inflorescences racemose, 5–25 cm long, 8–20-flowered; bracts leaf-like, shorter than flowers. Pedicel 4–31 mm long. Calyx campanulate, up to 10 mm long, sparsely pubescent, $±$1/4 cleft anteriorly; lobes 5, unequal, abaxially lateral pair larger, leaf-like and toothed, adaxially lateral pair small and toothed, and posterior one acicular. Corolla rose, ca. 20 mm long; tube ca. 12 mm long, equal to calyx, twisted near the calyx making the corolla upside down; beak ca. 10–12 mm, slender, slightly twisted, galea crested; corolla lower-lip enclosed the beak, ca. 12–15 mm long × 20–25 mm wide, middle lobe rounded, smaller than lateral lobes; four filaments pubescent. Capsule ovoid-lanceolate to long ovoid, ca. 20 mm long.

Type: China. Sichuan Province, Wenchuan County, Sichuan Wolong National Natural Reserve, Yeniugou, alt. 3,700–4,100 m, alpine meadows or shrubs, 28 Jul. 2020, Hong-Qiang Lin & Yue-Hong Cheng 20200728257 (holotype: HITBC; isotypes: HITBC, KUN).

Distribution and Conservation Status: *Pedicularis pandania* is currently known only from the Wolong National Nature Reserve in Wenchuan County, Sichuan Province, where it grows in alpine rhododendron scrub or alpine mead-

ows at elevations of 3,700–4,100 m. More than 5,000 flowering individuals have been documented at the known locality, and the species may occur in other areas of the reserve. Since the existing populations are located within a protected area with minimal human disturbance, the threat level is considered low. We recommend assigning this species a conservation status of Least Concern (LC).

Phenology: Flowering occurs from mid-to-late June through late August, with fruiting from July to late September.

Etymology: The specific epithet "pandania" is derived from the English name "panda," as this new species was discovered exclusively in the Wolong National Nature Reserve, a natural habitat of the giant panda, and is currently known only from within this protected area.

Acknowledgments: We thank the Electron Microscopy Laboratory and Supercomputing Center at Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences for technical support, and Dr. Lei Gu from Capital Normal University for valuable discussions.

References

Doyle JJ, Doyle JL, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue [J]. Phytochemistry, 19: 11-15.

Jin JJ, Yu WB, Yang JB, et al., 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes [J]. Genome Biol, 21: 241.

Li X, Wang H, Li DZ, et al., 2019. Taxonomic and nomenclatural notes on *Pedicularis* (Orobanchaceae): I. one new species from northwest Yunnan, China [J]. PhytoKeys, 130: 111-120.

Li RZ, Cai J, Yang JB, et al., 2021. Plastid phylogenomics resolving phylogenetic placement and genera phylogeny of Sterculioideae [J]. Guihaia. 10.11931/guihaia.gxzw202103060.

Liu ML, Yu WB, 2015. *Pedicularis wanghongiae* (Orobanchaceae), a new species from Yunnan, southwestern China [J]. Phytotaxa, 217: 53-62.

Stamatakis A, Hoover P, Rougemont J, 2008. A rapid bootstrap algorithm for the RAxML web servers [J]. Syst Biol, 57: 758-771.

Tkach N, Ree RH, Kuss P, et al., 2014. High mountain origin, phylogenetics, evolution, and niche conservatism of arctic lineages in the hemiparasitic genus *Pedicularis* (Orobanchaceae) [J]. Mol Phylogenet Evol, 76: 75-92.

Tsoong PC, 1955. A new classification system of *Pedicularis* [J]. Acta Phytotax Sin, 4: 71-147.

Tsoong PC, 1963. Flora Reipublicae Popularis Sinacae (Vol. 68): Scrophulariaceae (Pars II) [M]. Beijing: Science Press.

Wang H, 2006. *Pedicularis* L. [M] // Chen SK, Wang H. Flora Yunnnanica (Vol. 16). Beijing: Science Press: 468-611.

Yang FS, Hong DY, Wang XQ, 2003. A new species and a new specific synonym of *Pedicularis* (Scrophulariaceae) from the Hengduan Mountains, China [J]. Novon, 13: 463-467.

Yang HB, Holmgren NH, Mill RR, 1998. *Pedicularis* Linn. [M]// Wu ZY, Raven PH. Flora of China. St. Louis & Beijing: Missouri Botanical Garden Press & Science Press: 97-209.

Yu WB, Huang PH, Li DZ, et al., 2010. A new species of *Pedicularis* (Orobanchaceae) from the Hengduan Mountains, Southwestern China [J]. Novon, 20: 512-518.

Yu WB, Liu ML, Wang H, et al., 2015. Towards a comprehensive phylogeny of the large temperate genus *Pedicularis* (Orobanchaceae), with an emphasis on species from the Himalaya-Hengduan Mountains [J]. BMC Plant Biol, 15: 176.

Yu WB, Wang H, Liu ML, et al., 2018. Phylogenetic approaches resolve taxonomical confusion in *Pedicularis* (Orobanchaceae): reinstatement of *Pedicularis delavayi* and discovering a new species *Pedicularis milliana* [J]. PLoS ONE, 13: e0200372.

Yu WB, Wang H, 2020. Orobanchaceae [M]// Li DZ, Chen ZD, Wang H, et al. The Families and Genera of Chinese Vascular Plants. Beijing: Science Press: 2074-2093.

Zeng CX, Hollingsworth PM, Yang J, et al., 2018. Genome skimming herbarium specimens for DNA barcoding and phylogenomics [J]. Plant Methods, 14: 43.

Note: Figure translations are in progress. See original paper for figures.