



Paraphlomis youyangensis (Lamiaceae), a new species from southeastern Chongqing, China

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ABSTRACT: The newly discovered *Paraphlomis youyangensis*, native to southeastern Chongqing, China, is introduced to the scientific community. This species exhibits similarities in floral structure to *P. kuankuoshuiensis* but is distinguishable by several discrete features, such as less pronounced rhizomes (<0.5 vs. 2–4 cm), elongated stolons (present vs. absent), smaller leaf blades (1.8–5.5 × 1.2–3.1 vs. 10–37 × 3–8 cm), leaf margins (shallowly repand and mucronulate vs. serrulate), and shorter bracteoles (1.2–4 vs. ca. 5 mm). Phylogenetic assessments based on two nuclear genes and three chloroplast genes indicated that *P. youyangensis* has a closer evolutionary relationship with *P. yingdeensis* but is differentiated from the latter by long stolons, smaller leaf blades (vs. 6.2–16.5 × 4–11.5 cm), leaf margins (vs. crenate-serrate), and notably shorter bracteoles (vs. 7–8 mm).

KEY WORDS: cpDNA, limestone flora, nrDNA, *Paraphlomis jiangyongensis*, *P. kuankuoshuiensis*, *P. nana*, *P. yingdeensis*.

INTRODUCTION

Paraphlomis was first treated as a section of *Phlomis* Linn. (Prain, 1901) and was later raised to a genus (Chen *et al.*, 2021; Prain, 1908). *Paraphlomis* was then treated as a member of the tribe Paraphlomideae Benth. within the subfamily Lamiaceae in the Lamiaceae family (Wu and Li, 1977; Bendiksby *et al.*, 2011). *Paraphlomis* species are characterized by erect galeate corollas with disproportionately elongated upper lips, rounded and bearded upper corolla lips, a five-toothed calyx, and bifurcated styles that divide into nearly equal lengths at the end (Bendiksby *et al.*, 2011; Ko *et al.*, 2014). This genus is comprised of two sections, i.e., *Paraphlomis* and *Obconicocalyx*; the former's calyces are tubular or tubular-campanulate while the latter's are obconical (Wu and Li, 1977). However, recent molecular phylogenetic studies have challenged the monophyletic status of the genus, revealing that it is non-monophyletic because *Matsumurella chinensis* (Benth.) Bendiksby has been placed deep within *Paraphlomis* (Chen *et al.*, 2021).

Approximately 24 *Paraphlomis* species are listed in the *Flora Reipublicae Popularis Sinicae* (Wu and Li, 1977). These species have a broad distribution, spanning China, India, Indonesia, Laos, Malaysia, Myanmar, Thailand, and Vietnam, as noted in the *Flora of China* (Li and Hedge, 1994). The northernmost distribution limit of *Paraphlomis* species is 31°N (Wu and Li, 1977) and *P. koreana* found in Korea (Ko *et al.*, 2014) extends its distribution range northward. The species number increased to about 30 in 2021 (Chen *et al.*, 2021). Since 2022, seven new species of *Paraphlomis* have been reported in Southern China: *P. hsiwenii* Y.P.Chen & XiongLi (Chen *et al.*, 2022b), *P. jinggangshanensis*

Boufford, W.B. Liao & W.Y. Zhao (Zhao *et al.*, 2022), *P. longicalyx* Y.P.Chen & C.L.Xiang (Chen *et al.*, 2022a), *P. nana* Y.P. Chen, C. Xiong & C.L. Xiang (Chen *et al.*, 2022c), *P. strictiflora* J.C. Yuan, B. Chen & C.L. Xiang (Yuan *et al.*, 2022), *P. caloneura* K.J. Yan, Y.P. Chen & Y.F. Huang (Yan *et al.*, 2023), and *P. yingdeensis* W.Y.Zhao, Y.Q.Li & Q.Fan (Guo *et al.*, 2023).

In June 2023, a species similar to those in the genus *Paraphlomis* was discovered in Youyang County, Chongqing, China. This newly discovered species shared similarities in height and foliage size with *P. jiangyongensis* X.L.Yu & A.Liu and *P. nana*, while its floral features bear a resemblance to those of *P. kuankuoshuiensis* R.B.Zhang, T.Deng & C.B.Ma. Significant differences from the aforementioned species were revealed detailed after comparing vegetative and reproductive characteristics. This species was further distinguished through a phylogenetic analysis employing two nuclear and three chloroplast genes, reinforcing its unique status. As a result, this plant has been recognized as a new species within the *Paraphlomis* genus.

MATERIAL AND METHODS

Morphological comparison

A comparative morphological study was conducted by examining living plants and dried specimens. The terminology utilized to describe the morphological characteristics conformed to Li and Hedge (1994) and Hickey and King (2021). Type specimens of the new species were deposited in the herbaria of ZY, KUN, and SCFI (Thiers, 2023). Related samples were examined either firsthand or through high-resolution photographs obtained from various herbaria (Table S1), such as AU, BH,

**Table 1.** Morphological and geographical comparison of *Paraphlomis youyangensis*, *P. kuankuoshuiensis*, and *P. yingdeensis*.

Characters	<i>P. youyangensis</i>	<i>P. kuankuoshuiensis</i>	<i>P. yingdeensis</i>
Long stolons	present	absent	absent
Indumentum on stems	villous	strigose	villous
Lamina shape	oblong, orbicular or obovate	long elliptic or long obovate	obovate
Lamina size (cm)	1.8–5.5 × 1.2–3.1	10–37 × 3–8	6.2–16.5 × 4–11.5
Lamina margin	shallowly repand and mucronulate	serrulate	crenate-serrate
Lateral veins (pair)	4–6	8–12	5–7
Bracteole shape	linear-lanceolate or subulate	linear-lanceolate	lanceolate to linear
Bracteole length (mm)	1.2–4	ca. 5	7–8
calyx color	red or dyed white	red	light green
Calyx tooth length (mm)	ca. 3	1–2	3–4
Calyx tooth shape	triangular	triangular-lanceolate	triangular lanceolate
Corolla color	white	white	yellow
Distribution area in China	Chongqing	Guizhou	Guangdong

BNU, FJFC, GXMG, IBK, IBSC, JIU, JJF, KUN, LBG, NAS, PE, SCFI, SM, and ZY. This research was additionally bolstered by digital resources provided by the National Specimen Information Infrastructure platform (Ministry of Science and Technology of China, 2013), and the Plant Photo Bank of China website (Institute of Botany of Chinese Academy of Sciences, 2008).

Phylogenetic analysis

Five DNA markers were utilized for the phylogenetic reconstruction, comprised of two nuclear ribosomal regions (the internal and external transcribed spacers [ITS and ETS], respectively) and three plastid DNA regions, i.e., *rpl32-trnL*, *rps16*, and *trnL-trnF*. The methodologies for the primers, DNA extraction, PCR amplification, and sequencing followed procedures outlined by Chen *et al.* (2022c) and Yuan *et al.* (2022). These protocols were executed by Sangon Biotech Company (Shanghai, China).

The phylogenetic analyses incorporated five sequences from the newly discovered species, in addition to sequences of related species within the genus, which were obtained from GenBank (Benson *et al.*, 2013). A total of 38 accessions representing 30 *Paraphlomis* species, including varieties and subspecies were used in the analysis (Table S2). Two species of *Matsumurella* Makino were chosen as additional ingroups, while *Phlomis fruticosa* L. and *Phlomoides dentosa* var. *glabrescens* (Danguy) C.L. Xiang & H. Peng from the tribe Phlomideae were selected as the outgroups, following Chen *et al.* (2022b).

The raw sequences were aligned in MEGA7 (Kumar *et al.*, 2016) and the nexus files were transformed into nex files using PAUP 4.0a169 (Swofford, 2017). The nucleotide substitution models were calculated using MrMTgui (Nuin, 2004) and the best-fit Bayes model was GTR+G for ETS, GTR+I+G for ITS, GTR+G for *rpl32-trnL*, GTR+I for *rps16* and F81+I for *trnL-F*, respectively. The sequences were combined in SequenceMatrix 1.8 (Vaidya *et al.*, 2011). Partitioned Bayesian Inference (BI) analyses were employed to generate the phylogeny of

Paraphlomis, using MrBayes (Ronquist *et al.*, 2012). FigTree v1.4.3 (Rambaut, 2016) was employed to visualize and annotate the trees.

RESULTS AND DISCUSSION

Morphological comparison

The leaf shape and leaf size of *P. youyangensis* was similar to those of *P. jiangyongensis* and *P. nana* but these three species can be easily distinguished by many characteristics. *P. youyangensis* and *P. jiangyongensis* are stoloniferous while *P. nana* is not. The stems of *P. youyangensis* are tufted (vs. simple in the other two species) and the indumentum on the stems are villous (vs. retrorse strigose in the other two species). The leaf margins of *P. youyangensis* are shallowly repand and mucronulate while they are crenate in the other two species. The *P. youyangensis* bracteole is longer than that of the other two species (1.5–4 vs. ca. 1 mm). The color of the calyx from *P. youyangensis* is red or dyed white, while it is green in *P. jiangyongensis* and dyed green in *P. nana*. The corollas of *P. jiangyongensis* are yellow, while they are white in the other two species. The upper corolla lips of *P. youyangensis* are shorter than those of the other two species (ca. 3.5 × 2 vs. 7.5–10 × 3 in *P. jiangyongensis* and ca. 5 × 3.5 in *P. nana*). *P. jiangyongensis* is distributed in Hunan Province while the other two species are located in Chongqing Municipality, China.

The flower traits of *P. youyangensis* are similar to those of *P. kuankuoshuiensis*, while the five molecular sequences from nrDNA or cpDNA were close to those of *P. yingdeensis*, and these three species were differentiated by many morphological traits (Table 1). The flower color of *P. yingdeensis* was different from the other two species, i.e., the calyces are light green (vs. red) and the corollas are yellow (vs. white). *P. youyangensis* is stoloniferous, while the other two species are not. The *P. youyangensis* lamina was smaller than that of the other two species (1.8–5.5 × 1.2–3.1 cm vs. 10–37 × 3–8 cm in *P. kuankuoshuiensis* and 6.2–16.5 × 4–11.5 cm in *P. yingdeensis*). The leaf margins



Fig. 1. Bayesian phylogenetic tree related to *Paraphlomis* including *P. youyangensis* based on the ITS, ETS, *rp132-trnL*, *rps16*, and *trnL-trnF* sequences; the lengths of the branches indicate the rates of nucleotide substitution.

are shallowly repand and mucronulate while they are serrulate in *P. kuankuoshuiensis* and crenate-serrate in *P. yingdeensis*. The bracteole of *P. youyangensis* is shorter than that of the other two species (1.2–4 mm vs. ca. 5 mm in *P. kuankuoshuiensis* and 7–8 mm in *P. yingdeensis*). *P. youyangensis* is located in Chongqing City while *P. kuankuoshuiensis* is in Guizhou Province and *P. yingdeensis* is in Guangdong Province, respectively.

Nutlet morphology rather than calyx morphology is of phylogenetic value for the infrageneric classification of

Paraphlomis (Chen *et al.*, 2021). The *P. youyangensis* paratypes were collected during fruiting in 2023 and nutlets were observed in the field with the naked eye and in the laboratory using a stereomicroscope. All of the nutlets and seeds were sterile (Fig. 2) in 2023, which may have occurred due to a pollination failure. Another possible reason may be that this species abandoned sexual reproduction while developing clonal reproduction (stolons and ramets). Nutlet development in this species needs to be further investigated.



Fig. 2. Nutlets of *Paraphlomis youyangensis* A. Sterile nutlets B. Sterile ovules.

Phylogenetic analysis

The aligned lengths of the combined nuclear and combined plastid data sets were 1,146 bp (362 bp for ETS, 784 bp for ITS) and 2,367 bp (766 bp for *rps16*, 790 bp for *trnL-trnF*, 811 bp for *rpl32-trnL*), respectively. The additional ingroups (two *Matsumurella* samples) were deeply nested with the *Paraphlomis* species in the combined nuclear data set (Fig. S1) and in the combined chloroplast data set (Fig. S2), suggesting that these two genera are paraphyletic which was consistent with previous phylogenetic trees (Chen *et al.*, 2021; Chen *et al.*, 2022a). Bendiksby *et al.* (2011) assigned *Ajugoides* Makino, *Matsumurella*, and *Paraphlomis* to tribe Paraphlomideae, and *Paraphlomis* was distinguished from *Matsumurella* by its calyx lobes less than half as long as the tube (Chen *et al.*, 2021).

The nrDNA tree (Fig. S1) supported that *P. youyangensis* was clustered with *P. kuankuoshuiensis* (BI = 0.99) which was consistent with the highly similar flowers between these two species. The relationships between *P. youyangensis* and the other *Paraphlomis* species were not resolved by the plastid tree (Fig. S2). In the combined nuclear and plastid tree (Fig. 1), *P. youyangensis* was sister to *P. yingdeensis* (BI = 0.85) but this clade was paraphyletic. Although the support value (0.99) between *P. youyangensis* and *P. kuankuoshuiensis* in the nuclear tree was higher than the value (0.85) between these two species in the combined nuclear and plastid tree, the resulting phylogeny was better resolved in the combined tree than in the nuclear tree, so the combined tree was utilized in this study. In short, we suggest that *P. yingdeensis* is closely related to *P. youyangensis*.

The morphologically similar species, *P. kuankuoshuiensis*, clustered with *Matsumurella chinensis* (BI = 0.80; Fig. 1) and formed a separate and distinctive clade. *P. youyangensis* and *P. kuankuoshuiensis* fell into different clades and exhibited numerous morphological

differences, as detailed in Table 1. The morphological differences with *P. yingdeensis* are compared in Table 1.

TAXONOMIC TREATMENT

Paraphlomis youyangensis H.Jiang, R.B.Zhang & Tan Deng, *sp. nov.* **Figs. 3–4**

Type: CHINA. Chongqing, Youyang County, Nanmu town, alt. ca. 600 m, 28 July 2023, *Hong Jiang ZRB2531* (fl.) (holotype: ZY 0005001!, isotype: KUN 1589536!, SCFI 3069660!).

Diagnosis: The height and foliage dimensions of *Paraphlomis youyangensis* are similar to those of *P. jiangyongensis* and *P. nana*; however, its floral characteristics are more similar to those of *P. kuankuoshuiensis*. Despite these similarities, *P. youyangensis* is readily distinguishable from *P. kuankuoshuiensis* by several distinct traits: inconspicuous rhizomes (<0.5 vs. 2–4 cm), long stolons (vs. absent), much smaller sized lamina (1.8–5.5 × 1.2–3.1 cm vs. 10–37 × 3–8 cm), shallowly repand and mucronulate lamina margin (vs. serrulate), and shorter bracteoles (1.2–4 mm vs. ca. 5 mm).

Description: Perennial herbs, 2–10 cm tall, having numerous fibrous roots and long stolons. **Stems** tufted, erect or ascending, densely villous. **Leaves** opposite, 2–4-paired; petioles 0.8–2 cm long, densely villous; lamina oblong, orbicular or obovate, (thick) papery, 1.8–5.5 × 1.2–3.1 cm, base cuneate to obtuse, margin shallowly repand and mucronulate, ciliate, apex obtuse, rounded or mucronulate, adaxially green, appressed strigose and glandular punctate, abaxially light green, lateral veins 4–6 paired, densely strigose. **Verticillasters** 2–6-flowered, pedicels ca. 1 mm long; bracteoles 2–3, linear-lanceolate or subulate, 1.2–4 mm long, sparsely villous. **Calyx** red or dyed white, tubular-campaniform, tube 4–6 mm long, pilose and glandular outside, glabrous inside; teeth 5, subequal, triangular, straight or reflex, ca. 3 mm long, apex caudate. **Corolla** white, 1.3–2.0 cm long, pilose outside; tube 1–1.2 cm long, straight, slightly dilated toward throat, villous annulate inside at 1/4–1/5 toward base (ca. 2 mm from the base); upper lip oblong, entire, erect, concave, ca. 3.5 × 2 mm, lower lip 3-lobed, purple-red inside, central lobe largest, suborbicular, apex repand, ca. 3 mm long, lateral lobes ovate. **Stamens** 4, straight, ascending below the upper corolla lip; filaments flat, pilose; anther thecae 2, parallel. **Pistils** 1.3–1.5 cm; style included, glabrous; stigma 2-lobed, lobes unequal; ovary 0.4–0.7 mm long, apex truncate, glabrous; disc ring-like. **Nutlets** not seen. **Fl.** Jun–Aug, **FR.** Aug–Sep.

Distribution and habitat: *Paraphlomis youyangensis* was collected in Nanmu Town of Chongqing, China. This species was thriving on limestone formations situated along two neighboring streams and the surrounding hills at an altitude of 600–700 m. It is typically found in moist rock crevices at lower mountain elevations.

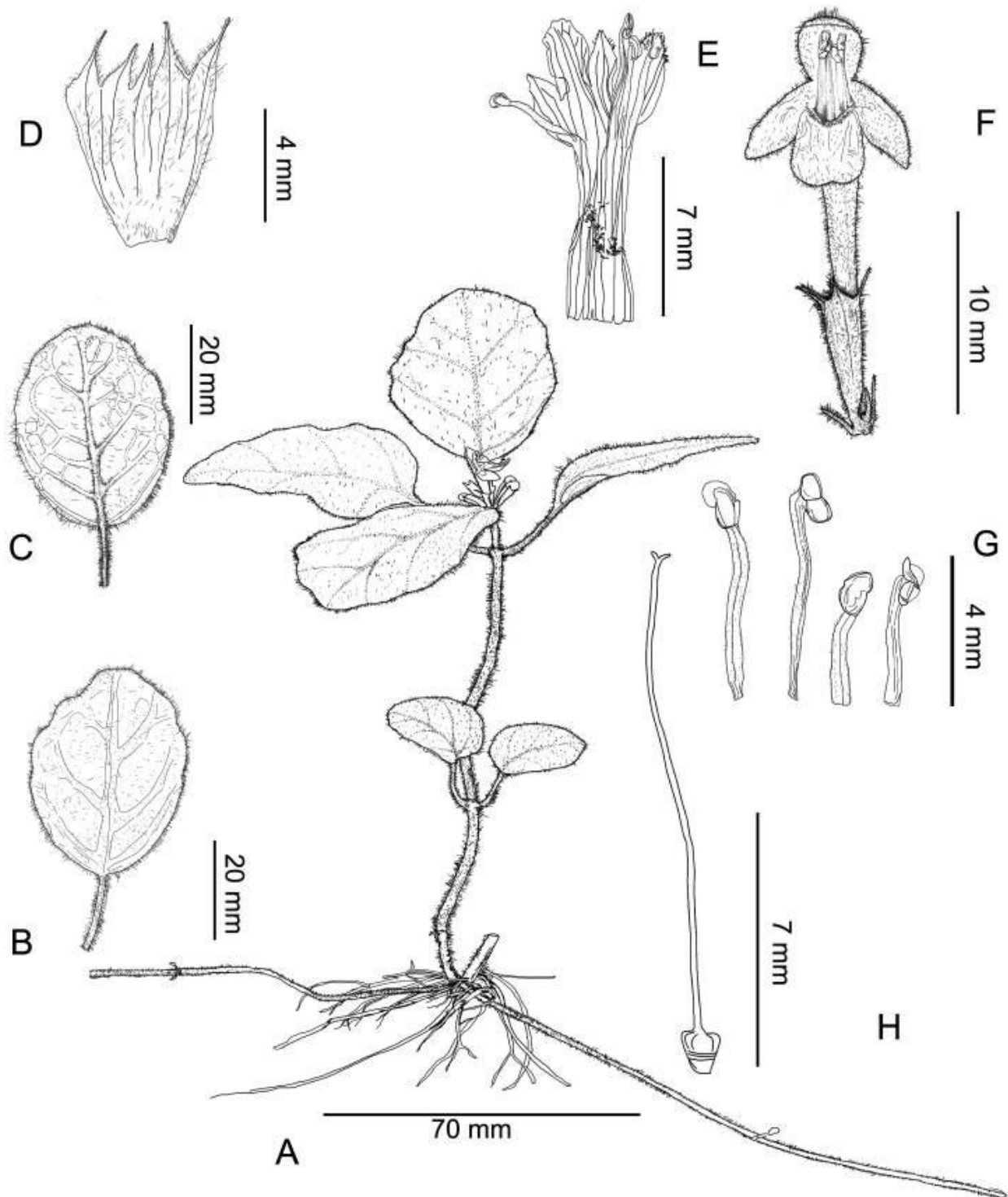


Fig. 3. Line drawing of *Paraphlomis youyangensis* H.Jiang, R.B.Zhang & Tan Deng. **A.** Plant; **B.** Adaxial leaf surface; **C.** Abaxial leaf surface; **D.** Opened calyx; **E.** Opened corolla; **F.** Flower; **G.** Stamens; **H.** Pistil. Drawn by Tan Deng.

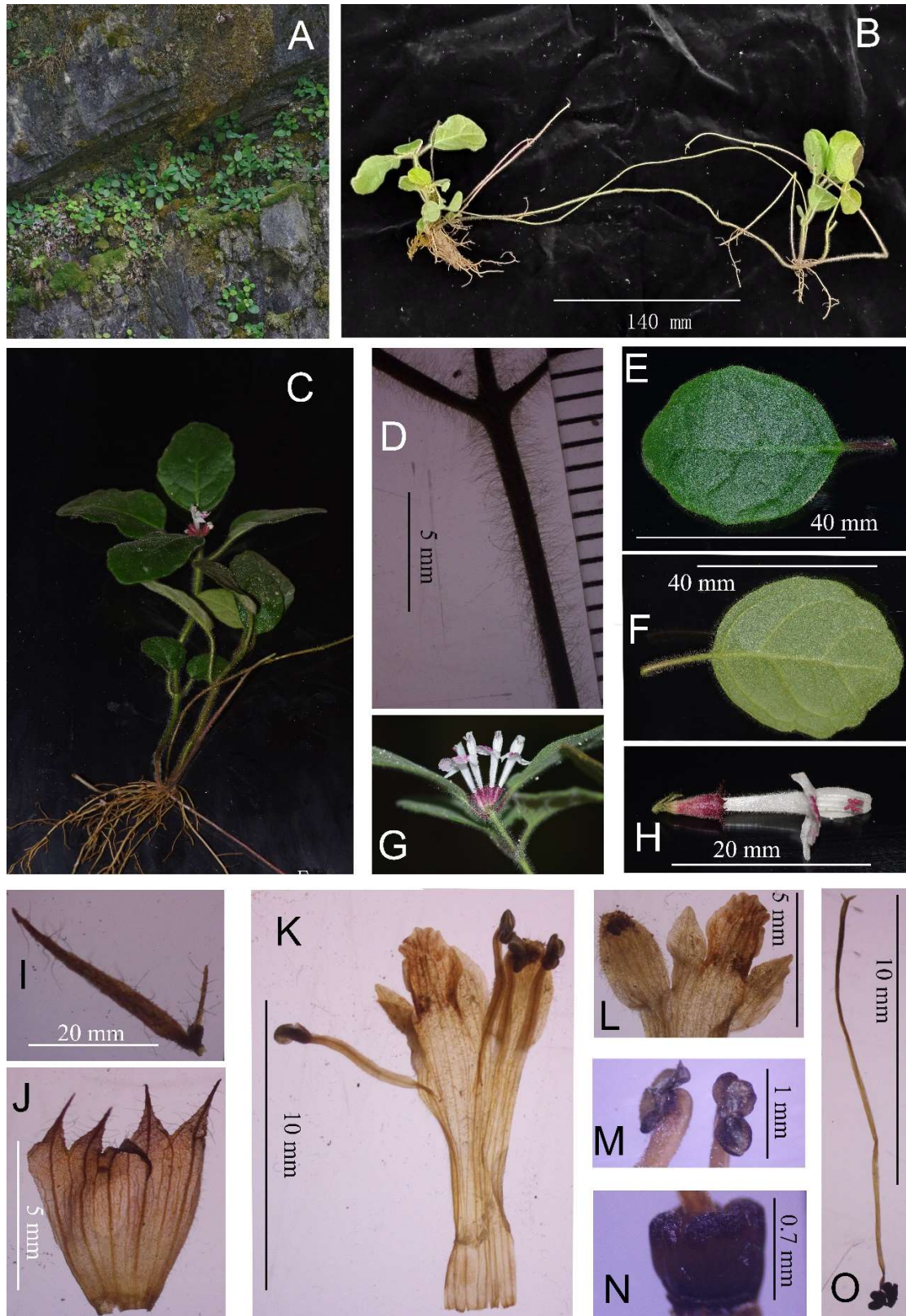


Fig. 4. Photographs of *Paraphlomis youyangensis* H.Jiang, R.B.Zhang & Tan Deng. **A.** Habitat; **B.** One clone, showing long stolons; **C.** Tufted plants; **D.** Stem indumentum; **E.** Adaxial leaf surface; **F.** Abaxial leaf surface; **G.** Inflorescence; **H.** Flower; **I.** Bracteoles; **J.** Opened calyx; **K.** Opened corolla; **L.** Corolla lips; **M.** Thecae; **N.** Ovary; **O.** Pistil. Photographed by Hong Jiang & Ren-Bo Zhang, composed by Tan Deng.



Phenology: Flowering from June to August, fruiting from August to September.

Etymology: The specific epithet “*youyangensis*” is derived from the name of the type locality, Youyang County.

Vernacular name: yǒu yáng jiǎ cǎo sū, 酉阳假糙苏.

Conservation status: *P. youyangensis* was observed exclusively within its type locality, where several thousand individuals were encountered. Given the adequate number of individuals but with a limited distribution range and combined with the observation that all nutlets were sterile in 2023 and that some individuals were situated close to roadsides, we propose classifying its conservation status as "EN" (Endangered) according to the IUCN guidelines (IUCN Standards and Petitions Committee, 2022).

Additional specimens examined (paratypes): CHINA. Chongqing: Youyang County, Nanmu Town, Niqinglu, elev. 600 – 650 m, 2 Sep 2023, *Ren-Bo Zhang ZRB2548* (fr.) (ZY 0005007!, SCFI 3069661!).

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Supplementary materials are available from Journal Website