RESEARCH ARTICLE



# Paraphlomis jinggangshanensis (Lamiaceae), a new species from Jiangxi, China

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Academic editor: Eberhard Fischer | Received 11 June 2022 | Accepted 14 July 2022 | Published 2 August 2022

Citation: Zhao W-Y, Zhang Z, Fan Q, Chen C-Q, Liao W-B, Boufford DE (2022) Paraphlomis jinggangshanensis (Lamiaceae), a new species from Jiangxi, China. PhytoKeys 204: 1–8. https://doi.org/10.3897/phytokeys.204.87654

### Abstract

*Paraphlomis jinggangshanensis* (Lamiaceae), a new species from Jiangxi Province, China, is described and illustrated. The new species is morphologically similar to *P. intermedia*, but can be easily distinguished from the latter by its cordate leaf base (*vs.* cuneate, decurrent), stem and calyx tube with glandular hairs (*vs.* short pubescent), and glabrous anthers (*vs.* ciliate anthers). A phylogenetic analysis, based on ITS regions, suggests that *P. jinggangshanensis* represents a separate branch in *Paraphlomis* and is closely related to Clade II. It is currently known only from Jinggangshan National Natural Reserve. Because of its limited distribution and small population size, the species was assessed as Near Threatened (NT) according to the IUCN Red List Categories and Criteria.

### **Keywords**

IUCN, Jinggangshan, Paraphlomideae, phylogenetic

# Introduction

*Paraphlomis* (Prain) Prain, a member of the tribe Paraphlomideae Bendiksby (Lamiaceae: Lamioideae) (Bendiksby et al. 2011; Li et al. 2016; Zhao et al. 2021), is characterized by its herbaceous habit, actinomorphic calyx with five lobes less than half as long

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as the tube, corolla 2-lipped (1/3) with hairy upper lip but hardly bearded along the margin, included stamens and an apically truncate ovary (Wu and Li 1977; Bendiksby et al. 2011; Ko et al. 2014; Chen et al. 2021). *Paraphlomis* is endemic to eastern and southeastern Asia, including China, India, Indonesia, Korea, Laos, Myanmar, Thailand, and Vietnam (Li and Hedge 1994; Ko et al. 2014; Zhang et al. 2020; Chen et al. 2021).

China, with 23 species documented in the *Flora of China* (Li and Hedge 1994), is the distribution center of *Paraphlomis*. Recently, a number of new species and infraspecies of *Paraphlomis* were described in China, including *P. javanica* var. *pteropoda* D. Fang & K.J. Yan and *P. javanica* var. *angustifolia* f. *albinervia* D. Fang & K.J. Yan (Yan and Fang 2009); *P. breviflora* B.Y. Ding, Y.L. Xu & Z.H. Chen (Ding et al. 2019); *P. kuankuoshuiensis* R.B. Zhang, D. Tan & C.B. Ma (Zhang et al. 2020); *P. jiangyongensis* X.L. Yu & A. Liu and *P. coronata* (Vaniot) Y.P. Chen & C.L. Xiang (Chen et al. 2022a); *P. longicalyx* Y.P. Chen & C.L. Xiang (Chen et al. 2022b).

During a botanical expedition to Jinggangshan National Nature Reserve, western Jiangxi Province in June 2013, David Boufford and Wen-Bo Liao discovered an unknown species of *Paraphlomis* in Xiangzhou village. Its stem and leaves were densely covered with glandular trichomes and the base of leaves was clearly cordate. Based on its morphological characteristics, which differed from other species of *Paraphlomis*, we suspected that it represented an undescribed species. After carefully comparing it with congeneric specimens, consulting the literature, observing its morphology over two years of additional field investigations (in 2020 and 2021), as well as conducting molecular studies, we confirmed that the species is new to science and formally describe it below.

# Materials and methods

### Morphological study

The flowering and fruiting plants of the putative new species were examined in the field and compared with herbarium specimens deposited in A, GH and SYS (herbarium acronyms as in Thiers 2022). All morphological characteristics were measured using dissecting microscopes. Morphological characteristics of similar species of *Paraphlomis* were further observed in digital images of specimens available online at A, GH, KUN, NAS, PE and SYS. Five main characters (habit, leaf shape, calyx, anthers and trichomes) of the putative new species and its most similar species, *Paraphlomis intermedia*, were thoroughly compared.

### Phylogenetic analyses

The nuclear DNA Internal Transcribed Spacers (ITS) was used for reconstructing the phylogeny of the suspected new species and related taxa based on previous study (Chen et al. 2021; Chen et al. 2022a). Most sequences were downloaded from GenBank, except for the new species, which was newly sequenced in the present study. Genomic DNA of the suspected new species was extracted from silica-gel-dried leaves using the

modified 2 × CTAB procedure of Doyle and Doyle (1987). The ITS sequences were amplified with primer pairs ITS4/ITSA, with PCR amplification and sequencing following Chen et al. (2016). A total of 18 accessions, representing 17 species of *Paraphlomis* and one species (*Phlomoides bracteosa* (Royle ex Benth.) Kamelin & Makhm.) of a related genus were sampled in the phylogenetic study. *Phlomoides bracteosa* was selected as an outgroup. The GenBank accession numbers are listed in Table 1. Nucleotide sequences were aligned and cleaned using MAFFT 7 (Katoh and Standley 2013). The phylogenetic relationships were assessed using the Maximum Likelihood (ML) method, which was constructed using the program IQ-TREE (Nguyen et al. 2015) with the best-fitting models (TIM+F+G4) chosen according to Bayesian Information Criterion (BIC).

Species	Voucher	ITS
Paraphlomis albida	A. Liu et al. LK0841 (CSFI); Ningyuan, Hunan, China	MW602124
Paraphlomis brevifolia	L. Wu & W.B. Xu 10965 (IBK); Yangshuo, Guangxi, China	MW602142
Paraphlomis coronata	C.L. Xiang 358 (KUN); Jiangkou, Guizhou, China	MW602123
Paraphlomis formosana	Zhong 3676 (E); Taiwan, China	JN680356
Paraphlomis gracilis	A. Liu LK0931 (CSFI); Changsha, Hunan, China	MW602134
Paraphlomis hirsutissima	Fang091060 (KUN); Yunnan, China	EU827096
Paraphlomis hispida	X. Li LX200702 (GXF); Napo, Guangxi, China	MW602132
Paraphlomis intermedia	X. Zhong et al. ZX16823 (CSH); Suichang, Zhejiang, China	MW602135
Paraphlomis javanica var. pteropoda	X. Li 2020090501 (GXF); Jingxi, Guangxi, China	MW602140
Paraphlomis javanica	L.B. Jia et al. JLB0029 (KUN); Maguan, Yunnan, China	MW602143
Paraphlomis jiangyongensis	A. Liu et al. LK1104 (CSFI); Jiangyong, Hunan, China	MW602129
Paraphlomis jinggangshanensis	W.Y. Zhao, Z.C. Liu, Z. Zhang, X.J. Li, ZWY-2060(SYS);	ON960152
	Jinggangshan, Jiangxi, China	
Paraphlomis kwangtungensis	Y.P. Chen & Y. Zhao EM1391 (KUN); Huaiji, Guangdong, China	MW602126
Paraphlomis lanceolata	C.Z. Huang s.n. (KUN); Guidong, Hunan, China	MW602145
Paraphlomis lancidentata	X. Zhong et al. ZX16824 (CSH); Suichang, Zhejiang, China	MW602136
Paraphlomis membranacea	Fang091057 (KUN); Yunnan, China	EU827094
Paraphlomis paucisetosa	X.X. Zhu s.n. (KUN); Malipo, Yunnan, China	MW602125
Paraphlomis paucisetosa	X. Li LX200704 (GXF); Napo, Guangxi, China	MW602133
Paraphlomis seticalyx	A. Liu et al. LK1088 (CSFI); Daoxian, Hunan, China	MW602127
Phlomoides bracteosa	Anders 11464 (M); Afghanistan, Kunar, Chapadarrah	JN680373

Table 1. GenBank accession numbers of the sampled species used in this study.

# Results

# Morphological comparison

In morphology, the putative new species was most similar to *Paraphlomis intermedia* C.Y. Wu & H.W. Li. A comparison of their morphological features is presented in Table 2. These two species share such features as rhizomes with dense fibrous roots, calyx tube obconical, calyx teeth broadly triangular to broadly ovoid triangular and corolla white. The new species, however, differs from *P. intermedia* by its cordate leaf base (*vs.* cuneate, decurrent), stem and calyx tube with glandular trichomes (*vs.* short

Characters	Paraphlomis jinggangshanensis	Paraphlomis intermedia
Habit	erect, stem solitary, unbranched	erect, stem with branches in upper part
Rhizome	transverse, internodes 1.5–4 cm	inconspicuous, not transverse
Trichomes on stem	puberulent, trichomes retrorse	glandular trichomes erect
Leaf base	Cordate	broadly cuneate, abruptly decurrent
Calyx	obconical, sparsely pubescent outside	tubular or obconical, with dense glandular trichomes
		outside
Anthers	ovoid, ciliate	ovoid, glabrous
Nutlets	sparsely pubescent	glabrous

Table 2. Morphological comparison of Paraphlomis jinggangshanensis and Paraphlomis intermedia.

pubescence), anthers glabrous (*vs.* ciliate). Furthermore, the rhizome of *P. intermedia* has internodes about 1.5–4 cm long (observed in the type specimen), while the rhizome of the putative new species is rather shorter.

### Phylogenetic placement of the putative new species

The aligned sequences of ITS were 627 bp in length. The resulting phylogenetic tree of *Paraphlomis* in this study was similar to that in a previous study (Chen et al. 2021). Our putative new species formed a separate branch (Fig. 1: ML = 62) that was sister to the previously suggested clades II, and IV by Chen et al. (2021). Fruit morphology is the main factor to distinguish subordinate grades of *Paraphlomis*. Specifically, species of Clade II have glabrous nutlets included in the fruiting calyces, species of Clade III have hairy nutlets, and species of Clade IV share glabrous nutlets that are obviously inflated and exserted from the calyx (Chen et al. 2021). The putative new species was closest to Clade II since its glabrous nutlets were included within the fruiting calyx. However, the putative new species was easily distinguishable from other species in Clade II by being densely covered with glandular trichomes and by the cordate leaf base.

### Taxonomic treatment

Paraphlomis jinggangshanensis Boufford, W.B. Liao & W.Y. Zhao, sp. nov. urn:lsid:ipni.org:names:77302738-1 Fig. 2 井冈山假糙苏

**Type.** CHINA. Jiangxi Province, Jinggangshan City, Jinggangshan National Natural Reserve, roadsides, 26°38'N, 114°15'E, 740 m alt., 10 September 2021, *Wan-Yi Zhao, Zhong-Cheng Liu, Zhong Zhang, XU-Jie Li, ZWY-2060* (holotype: SYS!; isotypes: A!, SYS!)

**Diagnosis.** *Paraphlomis jinggangshanensis* is morphologically similar to *P. intermedia*, but differs by its pubescence of glandular trichomes, cordate leaf base, manybranched stems and glabrous anthers.

**Description. Herbs,** perennial, 0.4–1.0 m tall. **Rhizomes** short (not transverse), taproot obscure; roots fibrous. **Stems** erect, simple or much branched above middle,



**Figure 1.** Phylogenetic relationships among 17 species of *Paraphlomis* and *Phlomoides bracteosa* based on ITS sequences. Numbers above branches indicate Maximum Likelihood bootstraps (ML). The new species described in this study is shown in bold.

4-angled, grooved, densely covered with short glandular trichomes. Leaves opposite; petiole to 9 cm long, with dense short glandular trichomes, green or purplish green; lamina ovate to ovate-oblong, papery,  $4-10.2 \times 2.5-6.5$  cm, base cordate, margin crenate, apex acuminate; abaxially light green, covered with glandular trichomes (more densely so on veins), with glandular spots; adaxially green, densely covered with glandular trichomes, with glandular spots; lateral veins 4 or 5 pairs. Verticillasters 10-12 flowered, globose, 2.5-3.0 cm in diam; bracteoles few, ovate-triangular, apex obtuse, ca. 1 mm long, with short glandular trichomes, deciduous; pedicels 1.0-1.5 mm long, or obsolete. Calyx green, tubular-obconical, slight curving, ca. 7 mm long, with dense glandular trichomes outside, glabrous except for glandular trichomes on teeth inside, conspicuously 5-veined; teeth 5, subequal, triangular, ca. 1 mm long, apex acute. Corolla white, 1.2-1.6 cm long, with dense glandular trichomes outside, pilose annulate in throat inside; tube 8–10 mm long, straight, slightly dilated toward throat, obvious longer than calyx tube; corolla 2-lipped, upper lip oblong, margin entire, ca. 4 mm long, ca. 2.5 mm wide; lower lip 3-lobed, 4-5 mm long, dotted with red spots inside, middle lobe ovate to suborbicular, apex obtuse or retuse, lateral lobes obliquely oblong, apex obtuse. Stamens 4, inserted above middle of corolla tube, straight, included, filaments flat, sparsely puberulent-villous; anther cells 2, divergent, ovoid, glabrous. Style filiform, included, glabrous, apex subequally 2-lobed. Ovary 4-loculed, glabrous. Nutlets 4, triquetrous-obovoid, brown at maturity, ca. 2.2 mm long, apex rounded, glabrous. (Fig. 2)

**Distribution and habitat.** Based on our field observations, *Paraphlomis jinggangshanensis* is located only in Xiangzhou, in the Jinggangshan National Natural Reserve, Jiangxi Province.



**Figure 2.** *Paraphlomis jinggangshanensis* **A** habit, growing on gravelly hillside **B** plant, stems much branched **C** flowering branch **D** leaves with long petiole, base cordate **E** both surfaces of leaf blade with dense glandular trichomes **F** stem, petiole, and calyx tube with dense glandular trichomes **G** front view of corolla, lower lip dotted with purplish red spots, throat villous annulate **H** inner view of corolla, filaments borne in middle of corolla tube; red arrow indicates glabrous style; anthers glabrous **I** lateral view of flower **J** inflorescence **K** fresh nutlets (glabrous) and inner view of calyx tube (**A–D** by Zhong Zhang **E–K** by Wan-Yi Zhao).

This area has been considered to be in the subtropical monsoon climate region. *Paraphlomis jinggangshanensis* often occurs in evergreen broadleaved forests along roads above valleys.

**Conservation status.** This species is currently known to occur only in the Jinggangshan National Natural Reserve in three populations numbering more than two thousand individuals. A road divides the distribution range of *P. jinggangshanensis*. Human activity (such as roadside weed removal) and exotic species have a negative effect on population regeneration. *Paraphlomis jinggangshanensis* is here suggested to be Near Threatened (NT) according to IUCN categories guidelines 10.1 (IUCN Standards and Petitions Subcommittee 2022).

**Phenology.** Flowering was observed from May to October, and fruiting from July to November.

**Etymology.** The specific epithet "jinggangshanensis" is derived from the type locality, Jinggangshan National Natural Reserve, Jiangxi Province, China.

Additional specimens examined (paratypes). CHINA. Jiangxi: Jinggangshan City, Jinggangshan National Natural Nature Reserve; NE of the town of Ciping; vicinity of Xiangzhou, roadside, above valley, 26°37'49"N, 114°15'49"E, 545–575 m, 6 June 2013, *David E. Boufford, Wen-Bo Liao, Bao-Huan Wu, Hui-Min Xu & Tian-Tian Yuan 43074* (A); Jinggangshan National Natural Reserve, roadsides, 26°38'N, 114°15'E, 740 m alt., 18 June 2021, *Zhong Zhang Luofu-01* (A, SYS); *ibid.*, 15 July 2021, *Zhong Zhang Luofu-06* (A, SYS).

## Acknowledgements

We thank Zhong-Cheng Liu, Xu-Jie Li for help with the field work and Xin-Jian Zhang, Qian-Yi Yin for providing images of herbarium specimen of *Paraphlomis* in KUN and PE. We thank Kai-Kai Meng and Yan-Shuang Huang for help with the phylogenetic analysis. This work was supported by the Basic Work Special Project of the National Ministry of Science and Technology of China (2013FY111500), and the National Natural Science Foundation of China (NSFC31970016).

### References

- Bendiksby M, Thorbek L, Scheen AC, Lindqvist C, Ryding O (2011) An updated phylogeny and classification of Lamiaceae subfamily Lamioideae. Taxon 60(2): 471–484. https://doi. org/10.1002/tax.602015
- Chen YP, Drew BT, Li B, Soltis DE, Soltis PS, Xiang CL (2016) Resolving the phylogenetic position of *Ombrocharis* (Lamiaceae), with reference to the molecular phylogeny of tribe Elsholtzieae. Taxon 65(1): 123–136. https://doi.org/10.12705/651.8
- Chen YP, Liu A, Yu XL, Xiang CL (2021) A preliminary phylogenetic study of *Paraphlomis* (Lamiaceae) based on molecular and morphological evidence. Plant Diversity 43(3): 206– 215. https://doi.org/10.1016/j.pld.2021.03.002

- Chen YP, Xiong C, Zhou HL, Chen F, Xiang CL (2022a) *Paraphlomis nana* (Lamiaceae), a new species from Chongqing, China. Turkish Journal of Botany 46(2): 176–182. https://doi.org/10.55730/1300-008X.2680
- Chen YP, Sun ZP, Xiao JF, Yan KJ, Xiang CL (2022b) *Paraphlomis longicalyx* (Lamiaceae), a new species from the Limestone Area of Guangxi and Guizhou Provinces, Southern China. Systematic Botany 47(1): 251–258. https://doi.org/10.1600/036364422X16442668423572
- Ding BY, Chen ZH, Xu YL, Jin XF, Wu DF, Chen JB, Wu WJ (2019) New species and combination of Lamiaceae from Zhejiang, China. Guangxi Zhi Wu 39(1): 10–15.
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.
- IUCN Standards and Petitions Subcommittee (2022) Guidelines for Using the IUCN Red List Categories and Criteria. Version 15. Prepared by the Standards and Petitions Subcommittee. https://www.iucnredlist.org/resources/redlistguidelines
- Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Ko SC, Lee YM, Chung KS, Son DC, Nam BM, Chung GY (2014) A new species of *Paraphlomis* (Lamiaceae) from Korea: An additional genus to the Korean flora. Phytotaxa 175(1): 51–54. https://doi.org/10.11646/phytotaxa.175.1.6
- Li XW, Hedge IC (1994) Lamiaceae. In: Wu ZY, Raven PH (Eds) Flora of China, Vol. 17. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 50–299. http://www. iplant.cn/info/Lamiaceae?t=foc
- Li B, Cantino PD, Olmstead RG, Bramley GLC, Xiang CL, Ma ZH, Tan YH, Zhang DX (2016) A large-scale chloroplast phylogeny of the Lamiaceae sheds new light on its subfamilial classification. Scientific Reports 6(1): e34343. https://doi.org/10.1038/srep34343
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- Thiers BM (2022) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http:// sweetgum.nybg.org/science/ih/ [accessed 08.06.2022]
- Wu CY, Li HW (1977) Paraphlomis Prain. In: Wu CY, Li HW (Eds) Flora Reipublicae Popularis Sinicae, vol. 65(2). Science Press, Beijing, 545–572.
- Yan KJ, Fang D (2009) A supplement to the *Paraphlomis* (Lamiaceae) from Guangxi, China. Redai Yaredai Zhiwu Xuebao 17(7): 91–92. http://jtsb.ijournals.cn/jtsb\_cn/article/issue/2009\_17\_1
- Zhang RB, Deng T, Dou QL, Wei RX, He L, Ma CB, Zhao S, Hu S (2020) Paraphlomis kuankuoshuiensis (Lamiaceae), a new species from the limestone areas of northern Guizhou, China. PhytoKeys 139: 13–20. https://doi.org/10.3897/phytokeys.139.47055
- Zhao F, Chen P, Salmaki Y, Drew BT, Wilson TC, Scheen AC, Celep F, Bräuchler C, Bendiksby M, Wang Q, Min DZ, Peng H, Olmstead RG, Li B, Xiang CL (2021) An updated tribal classification of Lamiaceae based on plastome phylogenomics. BMC Biology 19(1): e2. https://doi.org/10.1186/s12915-020-00931-z