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Paraphlomis nana (Lamiaceae), a new species from Chongqing, China

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Abstract: Most species of *Paraphlomis* are distributed in the south of the Yangtze River in China. In this study, a new species, *P. nana*, from northeast Chongqing in the north of the Yangtze River is described and illustrated. Bayesian inference and maximum likelihood analyses based on two nuclear ribosomal DNA regions (ITS and ETS) and three plastid DNA markers (*rpl32-trnL*, *rps16*, and *trnL-trnF*) were carried out to explore the phylogenetic position of the new species within *Paraphlomis*. Though the nuclear and plastid trees reveal incongruent placements of *P. nana* in *Paraphlomis*, which may be caused by chloroplast capture, a close relationship between the new species and *P. albiflora* is supported by molecular phylogenetic, morphological, and geographic evidence. However, the two species can be clearly distinguished from each other by the plant height and lamina and calyx morphology.

Key words: Chloroplast capture, *Matsumurella,* Paraphlomideae, the Yangtze River

1. Introduction

With approximately 30 species, *Paraphlomis* (Prain) Prain (Lamiaceae) are mostly accustomed to the subtropical evergreen broad-leaved forests or limestone areas in southern China (Wu and Li, 1977; Li and Hedge, 1994; Harley et al., 2004). Diagnostic characteristics of *Paraphlomis* include: perennial herbs with simple hairs; axillary verticillasters; actinomorphic calyx tubular to obconical, 5-lobbed; 2-lipped (1/3) corolla with upper lip hairy outside but not bearded along the margin; and truncate apex of ovary (Wu and Li, 1977; Li and Hedge, 1994; Harley et al., 2004; Bendiksby et al., 2011). The genus was poorly studied since its description, but has received increasing attention in recent decade: (1) it was assigned to a newly established tribe Paraphlomideae of subfamily Lamioideae together with *Matsumurella* Makino and *Ajugoides* Makino (Bendiksby et al., 2011); (2) a preliminary molecular phylogenetic study of *Paraphlomis* has been carried out with infrageneric relationships being discussed (Chen et al., 2021); and (3) an increasing number of new species of the genus have been continuously discovered and reported (e.g., Ding et al., 2019; Zhang et al., 2020; Chen et al., 2021, 2022).

During our recent field investigations in northeast Chongqing, China, a putative new species of *Paraphomis* was found in two separate localities. Based on molecular phylogenetic analyses and morphological comparisons, we confirmed its specific status and taxonomic placement within *Paraphomis*, and named it as *P. nana* Y.P. Chen, C. Xiong & C.L. Xiang.

2. Materials and methods

2.1. Molecular phylogenetic analyses

The ingroups included 37 accessions of 18 species and four varieties/subspecies of *Paraphlomis* and two species of *Matsumurella*, while the outgroups were represented by two species each of *Phlomis* L. and *Phlomoides* Moench of tribe Phlomideae. Except for the two accessions of the new species and one accession of *P. foliata* subsp. *montigena* X.H. Guo & S.B. Zhou that were newly sampled here, sequences of the remaining accessions were all retrieved from previous studies (Chen et al., 2021, 2022).

Since we failed to amplify the *rpl16* sequences for the new species, only five of the six DNA regions used in Chen et al. (2021, 2022), i.e. the nuclear ribosomal internal and external transcribed spacers (ITS and ETS) and three plastid DNA markers (*rpl32-trnL*, *rps16*, and *trnL-trnF*), were selected for the molecular phylogenetic analyses. Total genomic DNA was extracted from silica-gel-dried leaf material using the

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Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Primers used for the polymerase chain reaction (PCR) amplification and sequencing of the five regions were the same as that of Chen et al. (2021), while PCR mixtures and procedures followed those described in Chen et al. (2016). Voucher information and GenBank accession numbers for all sequences are listed in Appendix 1.

Partitioned Bayesian inference (BI) and partitioned maximum likelihood (ML) analyses as implemented using MrBayes (Ronquist et al., 2012) and RAxML-HPC2 (Stamatakis, 2014), respectively, on the Cyberinfrastructure for Phylogenetic Research Science (CIPRES) Gateway (http://www.phylo.org/; Miller et al., 2010) were employed to reconstruct the phylogenetic trees. Detailed settings for the BI and ML analyses followed those described in Chen et al. (2019). The resulting trees with posterior probabilities (PP) and Bootstrap support (BS) values were visualized and annotated in TreeGraph 2 (Stover and Müller, 2010).

The combined nuclear data set and the combined plastid data set were analyzed separately. Topological incongruence between the reconstructions inferred from the two data sets was visually inspected based on the thresholds of PP ≥ 0.95 and/or BS ≥ 70%.

2.2. Taxonomic studies

Morphological comparisons of the new species with other taxa of *Paraphlomis* were carried out based on specimens deposited in 20 public herbaria (BM, CDBI, E, GNNU, GXMI, HAST, HIB, IBK, IBSC, JIU, JJF, K, KUN, KYO, NAS, PE, SM, SZ, TI, and WUK; abbreviations follow Thiers, 2021), as well as our own observations and collections during field investigations. Taxonomic and related floristic literature, especially protologues of all published names of *Paraphlomis* were reviewed. The terminology used by Li and Hedge (1994) was adopted here for the morphological description of the new species.

3. Results and discussion

A total of 15 sequences were newly generated in present study. The length of the final combined nuclear data set and combined plastid data set was 1255 bp (812 bp for ITS, 443 bp for ETS) and 2484 bp (855 bp for *rpl32-trnL*, 812 bp for *rps16*, 817 bp for *trnL-trnF*), respectively. The topologies of the BI and ML trees were largely congruent with each other; therefore, the BI trees are selected for the present discussion (Figures 1 and 2), with BS support values of the ML trees being superimposed on the Bayesian nodes together with the corresponding PP value.

Consistent with previous studies (Chen et al., 2021, 2022), *Paraphlomis* is shown to be paraphyletic with the two species of *Matsumurella* nested within it (Figure 1: PP = 1.00/BS = 100%; Figure 2: PP = 1.00/BS = 83%). Both the nuclear and plastid data sets yielded poorly resolved topologies, but the relationships within the *Paraphlomis-Matsumurella* clade in the nuclear tree (Figure 1) are relatively better resolved and more in accordance with morphology than that of the plastid tree (Figure 2). Several hard incongruences between the topologies of the two data sets can be recognized, especially at the placements of *P. javanica* var. *pteropoda* D. Fang & K.J. Yan, *P. albiflora* (Hemsl.) Hand.-Mazz., as well as the new species. *Paraphlomis nana* is shown to be sister to *P. albiflora* in both trees (Figures 1 and 2: PP = 1.00/BS = 99%), however, the two species is recovered in a well-supported clade (Clade II in Chen et al., 2021) in the nuclear tree (Figure 1: PP = 0.99/BS = 95%), but group with *M. chinensis* (Benth.) Bendiksby and *P. seticalyx* C.Y. Wu in the plastid tree (Figure 2: PP = 0.98/BS = 64%). Possible causes for the above cytonuclear discrepancies may involve chloroplast capture and/or incomplete lineage sorting. Considering that incomplete lineage sorting much often occurs in taxa associated with rapid radiations (Whitfield and Lockhart, 2007; Oliver, 2013), chloroplast capture, i.e. the introgression of a chloroplast genome from one species to another (Rieseberg and Soltis, 1991; Tsitrone et al., 2003), may explain most of the incongruences in present study.

The new species share with other members of Clade II sensu Chen et al. (2021) the glabrous and not significantly inflated nutlets, however, it can be distinguished from all other species of the clade by its apex of calyx teeth bristle-like-acuminate (vs. acute or acuminate) (Figures 3 and 4). *Paraphlomis nana* is similar to *P. albiflora* in the indumentum of plants and morphology of corolla; the two species are also geographically close to each other that both of them are distributed in central China along or at the north of the Yangtze River (Figure 5), whereas the remaining species from the same clade (and even most species of *Paraphlomis*) are distributed at the south of the Yangtze River in southeast China (Wu and Li, 1977; Li and Hedge, 1994). Thus, the discovery of the new species promotes our understanding of the geographic distribution of *Paraphlomis*.

Despite the close relationship between *P. nana* and *P. albiflora*, the two species can be distinguished from each other by the plant height and lamina and calyx morphology (Table 1). Plants of *P. nana* are dwarf herbs less than 5 cm tall and with short internodes, while the height of *P. albiflora* plants varies between 30 cm and 60 cm. In contrast with the ovate to broadly ovate laminae of *P. albiflora* with acute apices and broadly cuneate or truncate bases, *P. nana* has ovate laminae with obtuse apices and cuneate to broadly cuneate bases. Moreover, the calyx teeth of *P. nana* are 3 mm long with bristle-like-acuminate apices, differing from that of *P. albiflora* which are 1 mm long with acute apices. A more comprehensive morphological comparison of the two species is summarized in Table 1.

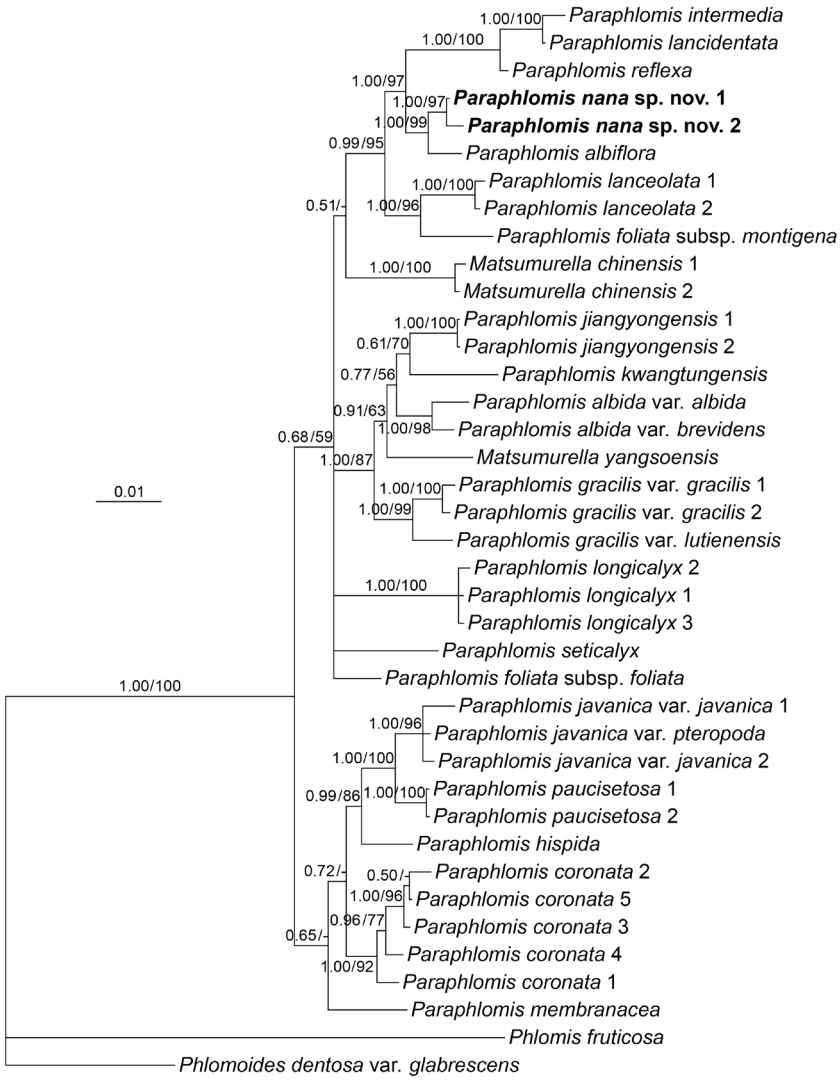

Figure 1. Bayesian 50% majority-rule consensus tree of *Paraphlomis* based on combined nuclear (ITS and ETS) data set. Support values ≥ 0.50 PP or 50% BS are displayed above the branches ("-" indicates a support value < 50% BS). Multiple accessions of the same species are numbered according to Appendix 1.

4. Taxonomic treatment

Paraphlomis nana Y.P. Chen, C. Xiong & C.L. Xiang **sp. nov.** (Figures 3 and 4)

Type: China. Chongqing: Chengkou County, Mingzhong Town, Jinchi Village, Longmenxi, Dabashan National Natural Reserve, on the moist cliff, 108°46′9.24″E, 31°43′55.49″N, alt. 996 m, 7 Jul. 2021, C. Xiong XC21097 (holotype: KUN!; isotypes: CQNM!, IBK!).

Diagnosis: *Paraphlomis nana* is morphologically most similar to *P. albiflora*, but differs by its 1–5 cm tall (vs. 30–60 cm tall) plants, ovate laminae (vs. ovate to broadly ovate) with obtuse apices (vs. acute), and ca. 3 mm long calyx teeth (vs. ca. 1 mm long) with bristle-like-acuminate apices (vs. acute).

Description: Perennial herbs 1–5 cm tall. Stems simple, erect or ascending, 4-angled, densely retrorse strigose. Leaves opposite, 2–3-paired; lamina ovate, papery, 2–7 × 1.5–4 cm, apex obtuse, rarely acute, margin coarsely crenate, base cuneate to broadly cuneate, decurrent, adaxially green, densely to sparsely appressed strigose, abaxially light green, densely to sparsely strigose, lateral veins 3–5-paired; petioles 0.5–3 cm long, densely retrorse strigose. Verticillasters 2–6-flowered, pedicels 1–2 mm long; bracteoles minute, early deciduous, subulate, ca. 1 mm long, densely strigose. Calyx translucent, membranous, campanulate, ca. 8 mm long, appressed strigose outside, glabrous inside; teeth 5, subequal, triangular, straight, ca. 3 mm long, apex bristle-like-acuminate. Corolla white, 1.6–

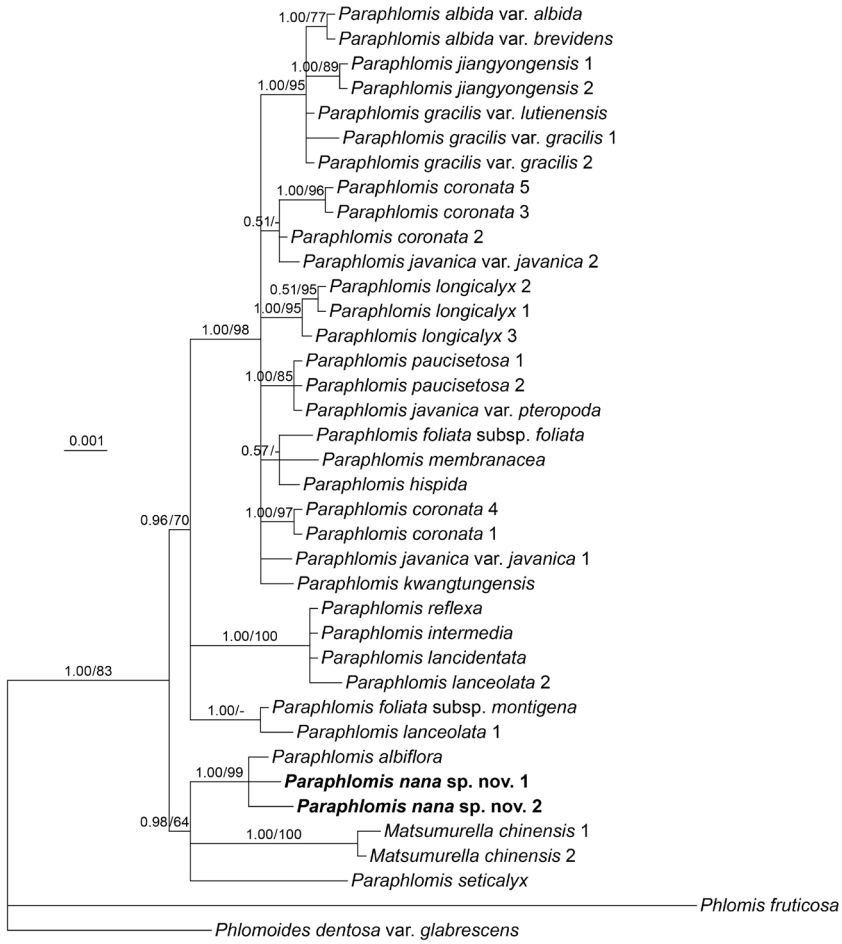

Figure 2. Bayesian 50% majority-rule consensus tree of *Paraphlomis* based on combined plastid (*rpl32-trnL*, *rps16*, and *trnL-trnF*) data set. Support values ≥ 0.50 PP or 50% BS are displayed above the branches ("-" indicates a support value < 50% BS). Multiple accessions of the same species are numbered according to Appendix 1.

2 cm long, strigose outside; tube 1–1.5 cm long, straight, slightly dilated toward throat, villous annulate inside at 1/3 toward base; 2-lipped, upper lip oblong, entire, erect, concave, ca. 5 × 3.5 mm, lower lip spreading, 3-lobed, medium lob largest, subcircular, apex slightly emarginate, ca. 4 mm long, lateral lobes ovate. Stamens 4, straight, included, filaments flat, villous at base, anther cells 2, parallel. Style included, glabrous, apex unequally 2-lobed, lobes subulate. Ovary apex truncate, sparsely glandular. Nutlets dark brown, triquetrous-oblong, 1.8–2 × 1.3–1.5 mm, glabrous.

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

Etymology: The specific epithet refers to the dwarf plants of the new species compared with that of most species of *Paraphlomis*.

Common name (assigned here): Ai Sheng Jia Cao Su (矮生假糙苏; Chinese name).

Distribution and habitat: The new species has only been found in two localities in northeast Chongqing, China (Figure 5). It usually grows on moist cliffs or at streamsides in valleys of evergreen broad-leaved forests at altitudes of 1000–1300 m.

Additional specimens examined: China. Chongqing: Wushan County, Zhuxian Town, Shizhuzi Village, Daguling, Wulipo National Natural Reserve, in the moist valley, 110°6′36.25″E, 31°18′23.79″N, alt. 1310 m, 18 Jul. 2021, C. Xiong & H.L. Zhou XC21126 (KUN); ibid., 11 Sept. 2021, H.L. Zhou s.n. (KUN).

Specimens of *P. albiflora* examined: China. Chongqing: Beibei District, Jinyun Mountain, Beiquan Hydrological Station, alt. 200 m, 6 Jun. 1982, Z.M. Huang

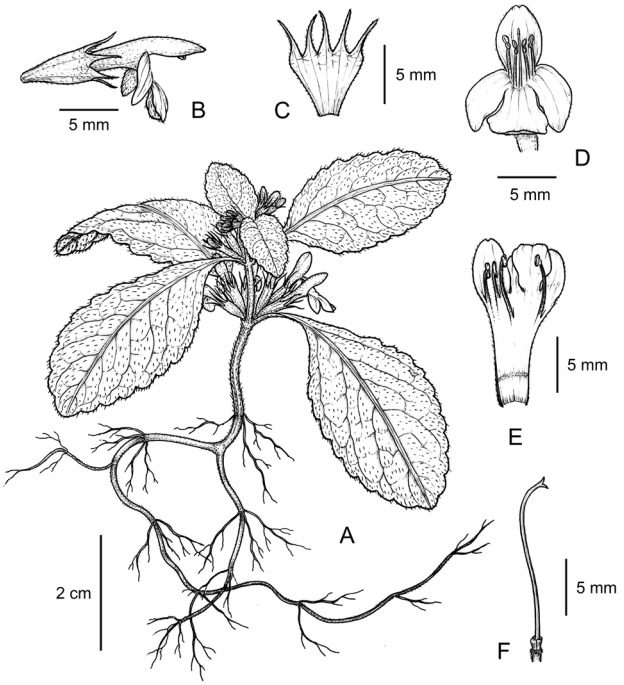

Figure 3. Line drawing of *Paraphlomis nana*. (A) habit (B) lateral view of flower (C) dissected calyx (D) frontal view of corolla (E) dissected corolla (F) pistil. Drawn by J. Tian.

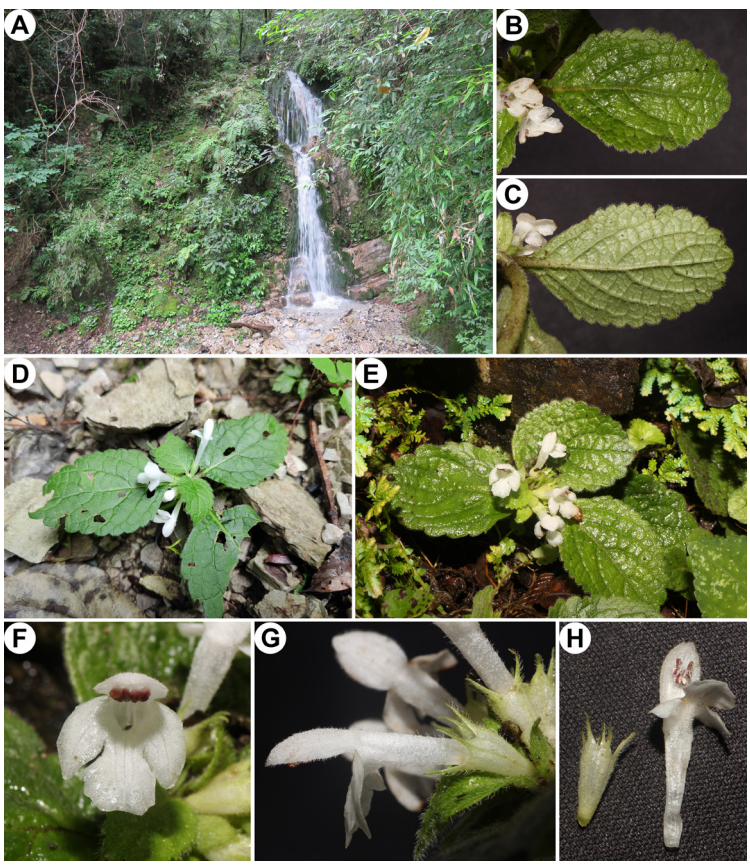

Figure 4. Morphology of *Paraphlomis nana* from the type locality. (A) habitat (B, C) leaves (D, E) plants (F) frontal view of flower (G) lateral view of flower (H) calyx and corolla. Photographed by C. Xiong.

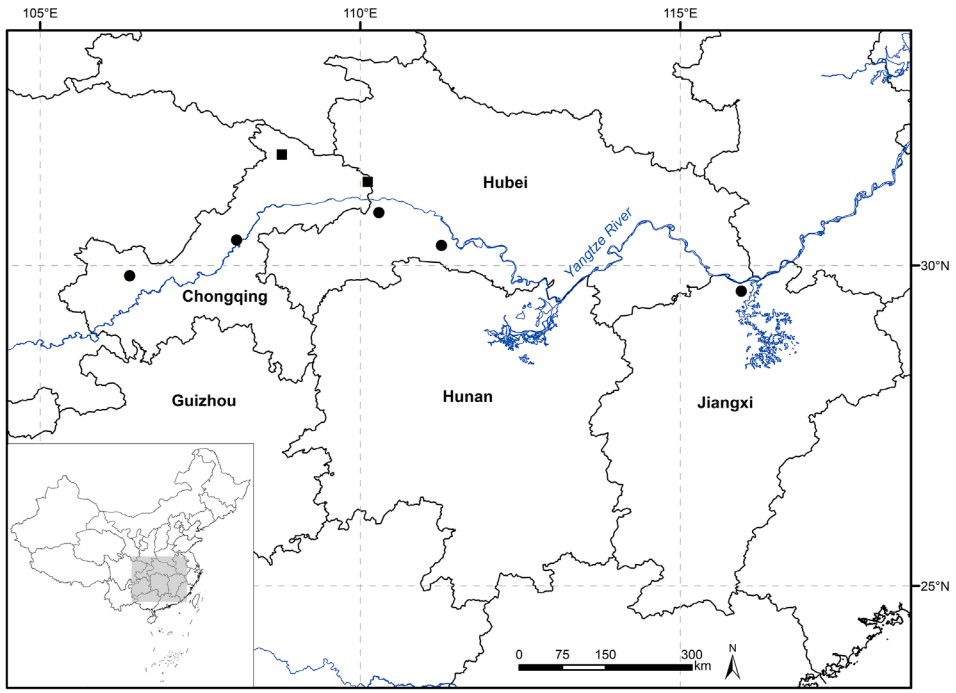

Figure 5. Distribution of *Paraphlomis nana* (square) and *P. albiflora* (circle) in China.

Table 1. Morphological comparisons between *Paraphlomis nana* and *P. albiflora*.

Characters	*P. nana*	*P. albiflora*
Plant	1–5 cm tall	30–60 cm tall
Lamina	Ovate, apex obtuse, rarely acute, base cuneate to broadly cuneate	Ovate to broadly ovate, apex acute, base cuneate to truncate
Verticillaster	2–6-flowered	6–20-flowered
Calyx	Approximately 8 mm long, teeth ca. 3 mm long, apex of teeth bristle-like-acuminate	Approximately 6 mm long, teeth ca. 1 mm long, apex of teeth acute
Corolla	Medium lobe of lower lip without purple spots	Medium lobe of lower lip with purple spots

1461 (PE00992680); Zhongxian County, Mopan Village, alt. 700 m, 16 May 1959, Y.H. Feng 1816 (KUN0228009, SM717305106). Hubei: Badong County, alt. 800 m, 4 Jun. 1939, T.P. Wang 10836 (PE00834687); Badong County, alt. 120 m, 8 Jun. 1939, T.P. Wang 10874 (PE00834688, WUK0026126); Ichang, A. Henry 1575 (syntypes: BM000950526, K000479950), 1910 (syntypes: E00284218, K000479951, P00738064), 3576 (syntypes: GH00001404, K000479952, P00738065), 720 (syntypes: G00424543, K000479953); Yichang City, 10 May 1929, K.K. Chung 3506 (PEY0047059); Yidu City, Jiufenggu Resort, alt. 167 m, 18 Jun. 2017, Y.T. Hou et al. 20170618253 (QFNU0046849). Jiangxi: Jiujiang City, Lushan Mountain, Donglin Temple, 28 May 2013, C.M. Tan & X.G. Wang 13268 (JJF00034347); ibid., 6 Jun. 2018, C.M. Tan et al. 1806393 (JJF00034350).

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Appendix 1. Sequence information for all samples used in present study. A "/" indicates a missing sequence. Herbarium abbreviations are listed after the vouchers. The accession numbers marked in bold represent sequences newly generated.

Taxon	Voucher	Country	ITS	ETS	*rpl32-trnL*	*rps16*	*trnL-trnF*
Matsumurella chinensis (Benth.) Bendiksby 1	Y. Yang OYY00316 (KUN)	Pingxiang, Jiangxi, China	MW602147	MW602117	MW602021	MW602053	MW602084
Matsumurella chinensis (Benth.) Bendiksby 2	Y. Yang OYY00131 (KUN)	Guilin, Guangxi, China	MW602148	MW602118	MW602022	MW602054	MW602085
Matsumurella yangsoensis (Y.Z. Sun) Bendiksby	L. Wu & W.B. Xu 10965 (IBK)	Yangshuo, Guangxi, China	MW602142	MW602112	/	/	/
Paraphlomis albida Hand.-Mazz. var. *albida*	A. Liu et al. LK0841 (CSFI)	Ningyuan, Hunan, China	MW602124	MW602091	MW601996	MW602028	MW602060
Paraphlomis albida var. *brevidens* Hand.-Mazz.	Y.P. Chen EM312 (KUN)	Hezhou, Guangxi, China	MW602130	MW602098	MW602003	MW602035	MW602067
Paraphlomis albiflora (Hemsl.) Hand.-Mazz.	C.M. Tan et al. 1806393 (JJF)	Jiujiang, Jiangxi, China	/	MW602101	MW602006	MW602038	MW602069
Paraphlomis coronata (Vaniot) Y.P. Chen & C.L. Xiang 1	E.D. Liu et al. 3043 (KUN)	Emeishan, Sichuan, China	MW602137	MW602107	MW602012	MW602044	MW602075
Paraphlomis coronata (Vaniot) Y.P. Chen & C.L. Xiang 2	Y.P. Chen EM225 (KUN)	Hezhou, Guangxi, China	MW602138	MW602108	MW602013	MW602045	MW602076
Paraphlomis coronata (Vaniot) Y.P. Chen & C.L. Xiang 3	A. Liu s.n. (CSFI)	Jiangyong, Hunan, China	MW602144	MW602114	MW602018	MW602050	MW602081
Paraphlomis coronata (Vaniot) Y.P. Chen & C.L. Xiang 4	C.L. Xiang 358 (KUN)	Jiangkou, Guizhou, China	MW602123	MW602090	MW601995	MW602027	MW602059
Paraphlomis coronata (Vaniot) Y.P. Chen & C.L. Xiang 5	B.Y. Zhang JGS05 (KUN)	Jinggangshan, Jiangxi, China	MW602139	MW602109	MW602014	MW602046	MW602077
Paraphlomis foliata (Dunn) C.Y. Wu & H.W. Li subsp. *foliata*	S.P. Chen s.n. (KUN)	Jiangle, Fujian, China	/	MW602097	MW602002	MW602034	MW602066
Paraphlomis foliata subsp. *montigena* X.H. Guo & S.B. Zhou	Y.C. Dai s.n. (KUN)	Hangzhou, Zhejiang, China	**OM836064**	**OM884453**	**OM884456**	**OM884459**	**OM884462**
Paraphlomis gracilis (Hemsl.) Kudô var. gracilis 1	A. Liu LK0931 (CSFI)	Changsha, Hunan, China	MW602134	MW602104	MW602009	MW602041	MW602072
Paraphlomis gracilis (Hemsl.) Kudô var. *gracilis 2*	C.L. Xiang XCL1315 (KUN)	Chongqing, China	MW602141	MW602111	MW602016	MW602048	MW602079
Paraphlomis gracilis var. *lutienensis* (Y.Z. Sun) C.Y. Wu	C.L. Xiang XCL881 (KUN)	Shibing, Guizhou, China	MW602131	MW602099	MW602004	MW602036	MW602068
Paraphlomis hispida C.Y. Wu	X. Li LX200702 (GXF)	Napo, Guangxi, China	MW602132	MW602102	MW602007	MW602039	MW602070
Paraphlomis intermedia C.Y. Wu & H.W. Li	X. Zhong et al. ZX16823 (CSH)	Suichang, Zhejiang, China	MW602135	MW602105	MW602010	MW602042	MW602073
Paraphlomis javanica (Blume) Prain var. *javanica* 1	Y.P. Chen s.n. (KUN)	Kunming, Yunnan, China	MW602121	MW602088	MW601993	MW602025	MW602057
Paraphlomis javanica (Blume) Prain var. *javanica* 2	L.B. Jia et al. JLB0029 (KUN)	Maguan, Yunnan, China	MW602143	MW602113	MW602017	MW602049	MW602080
Paraphlomis javanica var. *pteropoda* D. Fang & K.J. Yan	X. Li 2020090501 (GXF)	Jingxi, Guangxi, China	MW602140	MW602110	MW602015	MW602047	MW602078
Paraphlomis jiangyongensis X.L. Yu & A. Liu 1	A. Liu et al. LK1104 (CSFI)	Jiangyong, Hunan, China	MW602128	MW602095	MW602000	MW602032	MW602064
Paraphlomis jiangyongensis X.L. Yu & A. Liu 2	A. Liu et al. LK1104 (CSFI)	Jiangyong, Hunan, China	MW602129	MW602096	MW602001	MW602033	MW602065
Paraphlomis kwangtungensis C.Y. Wu & H.W. Li	Y.P. Chen & Y. Zhao EM1391 (KUN)	Huaiji, Guangdong, China	MW602126	MW602093	MW601998	MW602030	MW602062
Paraphlomis lanceolata Hand.-Mazz. 1	C.Z. Huang s.n. (KUN)	Guidong, Hunan, China	MW602145	MW602115	MW602019	MW602051	MW602082
Paraphlomis lanceolata Hand.-Mazz. 2	A. Liu et al. LK0825 (CSFI)	Ningyuan, Hunan, China	MW602146	MW602116	MW602020	MW602052	MW602083
Paraphlomis lancidentata Y.Z. Sun	X. Zhong et al. ZX16824 (CSH)	Suichang, Zhejiang, China	MW602136	MW602106	MW602011	MW602043	MW602074
Paraphlomis longicalyx Y.P. Chen & C.L. Xiang 1	G.X. Hu et al. 0279 (KUN)	Huanjiang, Guangxi, China	/	OK104773	OK104779	OK104781	OK104784
Paraphlomis longicalyx Y.P. Chen & C.L. Xiang 2	Y.P. Chen et al. EM583 (KUN)	Huanjiang, Guangxi, China	OK104771	OK104774	OK104778	OK104780	OK104783
Paraphlomis longicalyx Y.P. Chen & C.L. Xiang 3	K.J. Yan & G.Y. Wei 45122120210429002 (GXMI)	Nandan, Guangxi, China	/	OK104772	OK104777	/	OK104782
Paraphlomis membranacea C.Y. Wu & H.W. Li	M.S. Nuraliev 1057 (MW)	Thanh Son, Phu Tho, Vietnam	/	MW602100	MW602005	MW602037	/
Paraphlomis nana Y.P. Chen, C. Xiong & C.L. Xiang 1	C. Xiong XC21097 (KUN)	Chengkou, Chongqing, China	**OM836062**	**OM884451**	**OM884454**	**OM884457**	**OM884460**
Paraphlomis nana Y.P. Chen, C. Xiong & C.L. Xiang 2	C. Xiong & H.L. Zhou XC21126 (KUN)	Wushan, Chongqing, China	**OM836063**	**OM884452**	**OM884455**	**OM884458**	**OM884461**
Paraphlomis paucisetosa C.Y. Wu 1	X.X. Zhu s.n. (KUN)	Malipo, Yunnan, China	MW602125	MW602092	MW601997	MW602029	MW602061
Paraphlomis paucisetosa C.Y. Wu 2	X. Li LX200704 (GXF)	Napo, Guangxi, China	MW602133	MW602103	MW602008	MW602040	MW602071
Paraphlomis reflexa C.Y. Wu & H.W. Li	Z.Z. Yang et al. s.n. (HIB)	Tongshan, Hubei, China	MW602122	MW602089	MW601994	MW602026	MW602058
Paraphlomis seticalyx C.Y. Wu	A. Liu et al. LK1088 (CSFI)	Daoxian, Hunan, China	MW602127	MW602094	MW601999	MW602031	MW602063
Phlomis fruticosa L.	Y. Tong s.n. (KUN)	Shanghai, China (Cultivated)	MW602119	MW602086	MW601991	MW602023	MW602055
Phlomoides dentosa var. *glabrescens* (Danguy) C.L. Xiang & H. Peng	Y.P. Chen EM360 (KUN)	Beijing, China (Cultivated)	MW602120	MW602087	MW601992	MW602024	MW602056