

## RESEARCH ARTICLE

# Home at last: Molecular phylogenetic analyses support subsuming *Metastachyidium* within *Phlomoides* (Lamiaceae)

Yue Zhao,<sup>1\*</sup> Fei Zhao,<sup>2\*</sup> Yasaman Salmaki,<sup>3</sup> Alan Paton,<sup>4</sup> Jie Cai,<sup>5</sup> Bryan T. Drew,<sup>6</sup> Chonour Mahmoudi,<sup>7</sup> Petr Efimov,<sup>8</sup> Orzimat T. Turginov,<sup>9</sup> Cun-Zhu Liang,<sup>1</sup> Ya-Ping Chen<sup>2</sup> & Chun-Lei Xiang<sup>2</sup>

1 Ministry of Education Key Laboratory of Ecology and Resource Use of the Mongolian Plateau & Inner Mongolia Key Laboratory of Grassland Ecology, School of Ecology and Environment, Inner Mongolia University, Hohhot 010000, P.R. China

2 CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P.R. China

3 Center of Excellence in Phylogeny of Living Organisms, Department of Plant Science, College of Science, University of Tehran, Tehran 14155-6455, Iran

4 Royal Botanic Gardens, Kew, Richmond TW9 3AB, United Kingdom

5 Germplasm Bank of Wild Species in Southwest China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, P.R. China

6 Department of Biology, University of Nebraska-Kearney, Kearney, Nebraska 68849, U.S.A.

7 Department of Botany, Faculty of Science, Charles University, Prague, 128 01, Czech Republic

8 Komarov Botanical Institute of the Russia Academy of Sciences, Saint-Petersburg 197376, Russian Federation

9 Institute of Botany, Academy of Sciences of the Republic of Uzbekistan, 100047 Tashkent, Uzbekistan

\* Yue Zhao and Fei Zhao contributed equally to this work.

Addresses for correspondence: Ya-Ping Chen, [chenyaping@mail.kib.ac.cn](mailto:chenyaping@mail.kib.ac.cn); Chun-Lei Xiang, [xiangchunlei@mail.kib.ac.cn](mailto:xiangchunlei@mail.kib.ac.cn)

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**Abstract** Distributed in Central Asia, *Metastachyidium* (Lamiaceae) is a poorly understood and rare monotypic genus, with few collections known. The systematic position of this enigmatic genus within Lamiaceae has remained unresolved due to its poor representation in herbaria and coincident lack of available materials for molecular phylogenetic analysis. Facilitated by some recent collections, we performed Bayesian inference and maximum likelihood analyses, using an 80-protein-coding plastid-gene dataset of Lamiaceae, to infer the systematic placement of *Metastachyidium* at the tribal level within Lamiaceae. In addition, we used an 8-plastid-DNA-region dataset as well as the nuclear ribosomal internal transcribed spacer to determine the phylogenetic affinities of *Metastachyidium*. All phylogenetic analyses agree that *Metastachyidium* is a member of Phlomoideae and deeply nested within the genus *Phlomoides*, suggesting the need to expand the latter to include *Metastachyidium*. Hence, a new combination, *Phlomoides sagittata* comb. nov., is proposed, and we present the first available photographs and an amended morphological description of *P. sagittata*. In addition, the infrageneric circumscription of *Phlomoides* is not supported, as most sections and subsections are not monophyletic. Hybridization and incomplete lineage sorting, following rapid diversification within *Phlomoides*, seem to be the source of incongruence between the nuclear and plastid tree topologies.

**Keywords** Central Asia; Lamiaceae; nrITS; Phlomoideae; *Phlomis*; plastome phylogenomics

**Supporting Information** may be found online in the Supporting Information section at the end of the article.

## ■ INTRODUCTION

Lamiaceae is the sixth-largest angiosperm family, comprising more than 230 genera and over 7000 species (Harley & al., 2004; Li & al., 2016; F. Zhao & al., 2021a). In the most recent generic-level treatment of Lamiaceae (Harley & al., 2004), the systematic positions of 10 genera were considered as incertae sedis, most of which have now been resolved (Chen & al., 2014, 2016; Li & al., 2016). The most recently updated classification divided Lamiaceae into 12 subfamilies and 22 tribes (F. Zhao & al., 2021a), of which Lamiaceae is

the second-largest subfamily (next to Nepetoideae), with nearly cosmopolitan distribution and containing about 1260 species in 62 genera (F. Zhao & al., 2021a).

During the past two decades, major advances have been made towards clarifying phylogenetic relationships and taxonomy of Lamiaceae at various taxonomic levels (Wink & Kaufmann, 1996; Lindqvist & Albert, 2002; Scheen & Albert, 2007, 2009; Scheen & al., 2008, 2010; Bendiksby & al., 2011, 2014; Salmaki & al., 2012, 2013; Xiang & al., 2013; Chen & al., 2014; Roy & Lindqvist, 2015; Li & al., 2016; Yao & al., 2016; Siadati & al., 2018). Among these

studies, the most influential ones were carried out by Scheen & al. (2010) and Bendiksby & al. (2011), which reconstructed the backbone phylogeny of Lamioideae, proposed new tribes, and redefined and/or resurrected some genera. Since the aforementioned studies, only four genera (*Betonica* L., *Colquhounia* Wall., *Galeopsis* L., *Roylea* Wall. ex Benth.) remained unclassified at the tribal level within Lamioideae, while three genera (*Metastachydium* Airy Shaw ex C.Y.Wu & H.W.Li, *Paralamium* Dunn., *Pseudomarrubium* Popov) have not been included in any published molecular phylogenetic study. Later, using low-copy nuclear pentatricopeptide repeat (PPR) sequences, Roy & Lindqvist (2015) demonstrated that *Roylea* is a member of Marrubieae. Subsequently, F. Zhao & al. (2021a) established two new tribes (Colquhounieae, Betoniceae) and resurrected Galeopseae to accommodate *Colquhounia*, *Betonica*, and *Galeopsis*. The monotypic genus *Paralamium* was later resolved as sister to the monotypic genus *Craniotome* Rechb. and a member of tribe Pogostemoneae (F. Zhao & al., 2021b). Currently, only *Metastachydium* and *Pseudomarrubium* have not been included in a molecular phylogenetic publication and the systematic positions of the two genera within Lamioideae remain unknown.

The monotypic genus *Metastachydium*, represented by *M. sagittatum* (Regel) C.Y.Wu & H.W.Li, is sporadically distributed in northwest China and southwest Kazakhstan (Li & Hedge, 1994; Harley & al., 2004; Mukhtubaeva & al., 2017). *Metastachydium sagittatum* is a rarely collected species and was originally described as *Phlomis sagittata* Regel (Regel, 1880) based on plants collected by Regel in 1877 in Kuldscha (near Yining City, Ili Kazak Autonomous Prefecture of Xinjiang, China) (*Regel s.n.*). The species was later moved to *Ballota* L. by Regel (1886). Due to its morphological resemblance to *Stachys* L., Knorring (1954) established the monotypic genus *Metastachys* Knorr. in the *Flora of the U.S.S.R.* Considering that *Metastachys* Knorr. is a later homonym of *Metastachys* (Benth. & Hook.f.) Tiegh. (Van Tieghem, 1895), Airy Shaw (1966) therefore suggested to use the name “*Metastachydium* Airy Shaw”. Unfortunately, the name was not validly published because the literature regarding the basionym was not cited. Li (1975) later validated the name and *Metastachydium* Airy Shaw ex C.Y.Wu & H.W.Li was accepted as the correct name.

Cantino & Sanders (1986) placed *Metastachydium* in subfamily Lamioideae based on pollen morphology (i.e., the presence of tricolpate and 2-celled pollen), and this treatment was adopted by subsequent taxonomists (Abu-Asab & Cantino, 1994; Li & Hedge, 1994; Harley & al., 2004; Olmstead, 2016). Although major progress has been made towards elucidating phylogenetic relationships within Lamioideae, the placement of *Metastachydium* has remained elusive and the genus has not been assigned to any of the tribes updated by recent phylogenetic studies (Scheen & al., 2010; Bendiksby & al., 2011; F. Zhao & al., 2021b). Some morphological characters (i.e., bracts subulate and calyx teeth with apical spines) of *Metastachydium* point to a kinship with *Phlomooides* Moench, but this relationship has not been explored using molecular

data. A major hindrance to clarifying the systematic position of *Metastachydium* has been a lack of material for DNA extraction and morphological examination. The genus was only known from a few old collections until recently. Except for general descriptions of external morphology (Li, 1977; Li & Hedge, 1994; Harley & al., 2004), only micromorphological features of pollen grains have been reported thus far (Cantino & Sanders, 1986).

China is one of the seven diversity centers of Lamiaceae (Hedge, 1992; Harley & al., 2004; Rose & al., 2022), with at least six endemic genera (Xiang & al., 2017). During the past decade, an increasing number of studies have been carried out to resolve the placements of several incertae sedis genera endemic to China (Li & al., 2012; Chen & al., 2014, 2016; F. Zhao & al., 2021b). Recently, three additional populations of *Metastachydium sagittatum* were found in Xinjiang Province of northwest China following our repeated search efforts. The availability of fresh material enabled DNA extraction and offered an opportunity to investigate the phylogenetic position of *Metastachydium* based on molecular data.

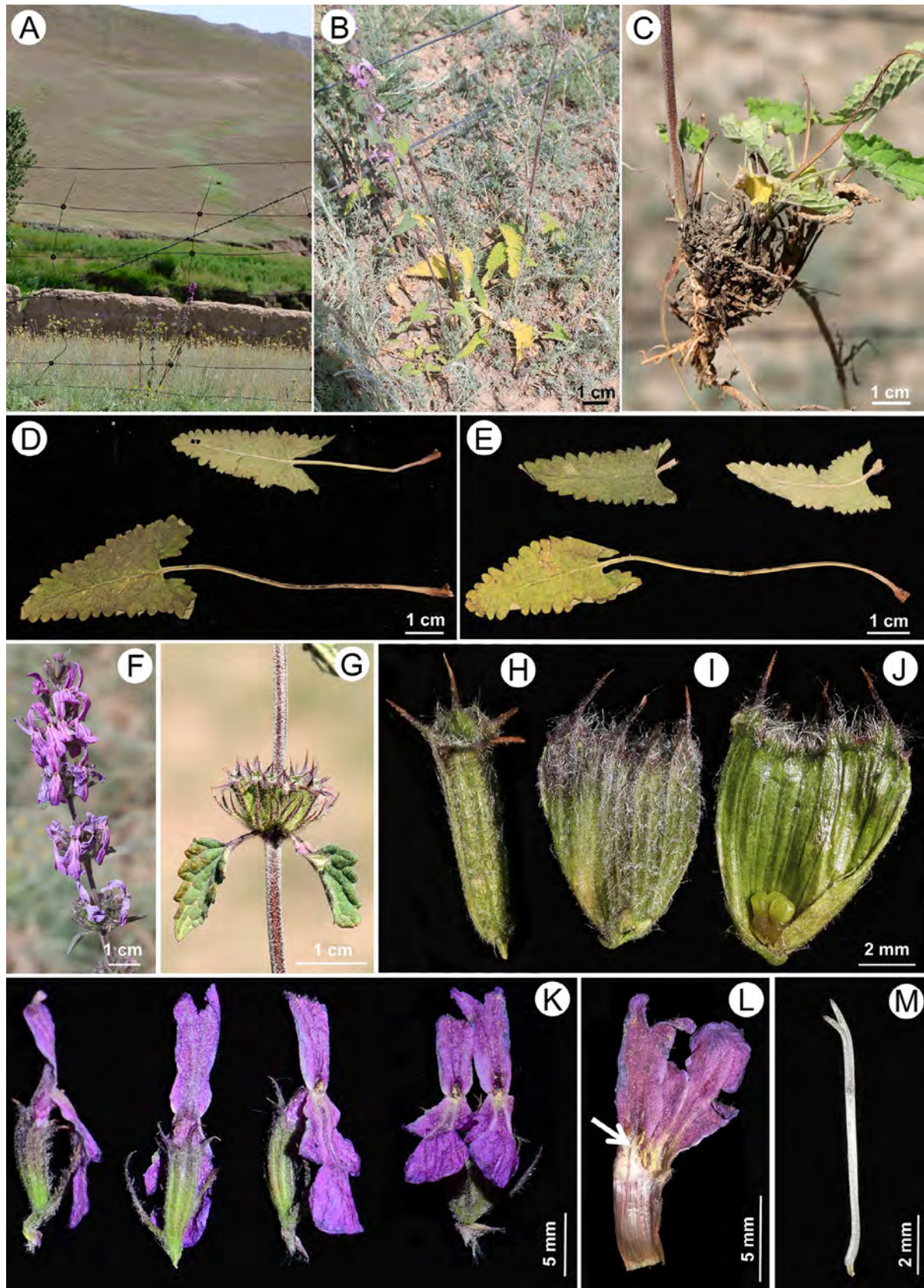
Here, using a combination of plastid genome-scale data, and both an 8-plastid-DNA (*atpB-rbcL*, *psbA-trnH*, *rpl16*, *rpl32-trnL*, *rps16*, *trnL-trnF*, *trnT-trnL*, *trnK*) and nuclear ribosomal internal transcribed spacer (nrITS) region dataset, we present the first molecular phylogenetic study of *Metastachydium* and provide compelling evidence for its phylogenetic position within Lamioideae. The objectives of this study are to: (1) investigate the phylogenetic placement of *Metastachydium* within Lamioideae, (2) identify which lineage(s) within Lamioideae is most closely related to *Metastachydium*, and (3) further contribute to a comprehensive phylogenetic framework for Lamioideae. In addition, the procurement and examination of living materials of *M. sagittatum* enabled a revision of the morphological description of this rare species.

## ■ MATERIALS AND METHODS

**Morphological study.** — Specimens of *Metastachydium* and *Phlomooides* from 28 herbaria (B, BM, C, CDBI, E, FI, GH, HIB, IBSC, K, KUN, KYO, LE, M, MA, MAO, MO, MW, NAS, P, PE, S, SG, TI, US, W, WUK, XJBI) and our own collections from the field were examined.

**Taxon sampling and dataset construction.** — Specimens of *Metastachydium sagittatum* were collected from Nieleke County (Zhao Y. & Xiao J.F. ZY 24) (Fig. 1), Xinyuan County (Ya J.D. & al. 16CSI2989), and Tekes County (Ya J.D. & al. 16CSI2196) of the Xinjiang Uygur Autonomous Region, China.

For the first set of analyses, we aimed to resolve the tribal assignment of *Metastachydium* within Lamioideae. The in-group sampling comprised a total of 61 accessions from 57 species and 24 genera, covering all 13 tribes of Lamioideae recognized by F. Zhao & al. (2021a). *Cymaria dichotoma* Benth. from subfamily Cymarioideae, the sister group of Lamioideae (Li & al., 2016; F. Zhao & al., 2021a, 2021b),



**Fig. 1.** Morphology of *Metastachydium sagittatum*. **A**, Habitat; **B**, Plant; **C**, Roots; **D**, Basal leaves; **E**, Stem leaves; **F**, Inflorescence; **G**, Fruiting calyx; **H–J**, Calyx; **K**, Different views of flowers; **L**, Dissected flower, showing the short stamens (arrow); **M**, Pistil. — Photos by Yue Zhao and Jin-Fei Xiao.

was also included as an ingroup. Six species from subfamily Scutellarioideae were selected as the outgroup based on the results from previous phylogenetic analyses (Li & al., 2016; F. Zhao & al., 2021a, 2021b). We reconstructed the backbone phylogeny of Lamioideae using 80 plastid-encoded protein genes (dataset CP80), with plastomes from 2 individuals of *M. sagittatum*, 12 species of *Phlomis*, 1 species of *Phlomis* L., 2 species of *Paraphlomis* (Prain) Prain, and 1 species of *Betonica* newly sequenced for this study, and the remaining 50 accessions downloaded from GenBank (suppl. Table S1).

The first set of analyses indicated that *Metastachydium* is deeply nested within genus *Phlomis* of Phlomisaceae, which guided a second set of analyses to clarify the position of *Metastachydium* within *Phlomis*. Two datasets were constructed for this second step. The first dataset included eight plastid DNA regions (*atpB-rbcL*, *psbA-trnH*, *rpl16*, *rpl32-trnL*, *rps16*, *trnL-trnF*, *trnT-trnL*, *trnK*; dataset CP8), while the second dataset consisted of nrITS. A total of 39 species of *Phlomis*, covering the major distribution areas and most sections and subsections of the genus, were selected as the ingroup. Three taxa from the other genus within Phlomisaceae, *Phlomis* (Scheen & al., 2010; Bendiksby & al., 2011; Mathiesen & al., 2011; Salmaki & al., 2012; Li & al., 2016; F. Zhao & al., 2021a), were sampled as the outgroup. In total, 425 sequences were newly generated in this study, and 32 sequences were gleaned from previous studies (Li & al., 2019; Y. Zhao & al., 2020; F. Zhao & al., 2021a; Min & al., 2021) or GenBank (Appendix 1).

#### DNA extraction, amplification, and sequencing. —

Total genomic DNA (gDNA) was obtained using the modified CTAB method (Doyle & Doyle, 1987) for both silica gel dried leaves and herbarium material. Polymerase chain reaction (PCR) primers, mixtures, and procedures of nrITS followed those described in Xiang & al. (2013), *atpB-rbcL*, *psbA-trnH*, and *trnT-trnL* followed Albaladejo & al. (2005), *trnK* and *rpl32-trnL* followed Salmaki & al. (2012), and *rpl16*, *rps16*, and *trnL-trnF* followed Chen & al. (2021). Amplified PCR products were sequenced on an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, California, U.S.A.) after purification with a QIAquick PCR purification Kit (BioTek, Beijing, China).

For material used for next-generation sequencing, gDNA was sheared into ca. 300 bp fragments. Libraries were constructed from fragmented gDNA using the NEBNext Ultra II DNA Library Prep Kit for Illumina and sequenced from both ends of 150 bp on the Illumina HiSeq 2000 platform (Illumina, San Diego, California, U.S.A.) at BGI Genomics (BGI-Shenzhen, China). Approximately 1–8 GB of raw data was generated for each sample.

Voucher information for newly sequenced taxa and GenBank accession numbers for all sequences used in this study are provided in Appendices 1 and 2 and suppl. Table S1.

**Plastome assembly and annotation.** — Adapter sequences were trimmed, and low-quality reads were removed using Fastq-mcf v.1.04.636 in the ea-utils package (<http://github.com/ExpressionAnalysis/ea-utils>; Aronesty, 2013).

The quality of raw paired-end reads was evaluated using the FastQC v.0.11.8 software (Andrews, 2018) with the parameter set as  $Q \geq 25$  to acquire high-quality reads for *de novo* assembling, which was implemented in the GetOrganelle v.1.7.5 pipeline (<http://github.com/Kinggerm/GetOrganelle>; Jin & al., 2020). Resulting contigs were visualized using Bandage v.0.8.1 (Wick & al., 2015) and manually corrected when necessary. Raw reads were mapped to the assembled plastome sequences using the Bowtie2 (Langmead & Salzberg, 2012) plugin in Geneious v.11.0.3 (Kearse & al., 2012) to validate the assembly error.

Plastome sequences were initially annotated using the Plastid Genome Annotator (PGA) (Qu & al., 2019). The putative start/stop codons and intron positions were manually adjusted according to comparisons with the published plastome of *Phlomis betonicoides* (Diels) Kamelin & Makhm. (GenBank accession number: MN617020; Y. Zhao & al., 2020) in Geneious (Kearse & al., 2012). The tRNA boundaries were further verified using the online tRNAscan-SE service (Lowe & Chan, 2016). The coding regions (CR) and eight plastid DNA regions used in dataset CP8 were extracted from the annotated complete plastome sequences for subsequent alignment.

#### Sequence alignment and phylogenetic analyses. —

Raw sequences resulting from PCR amplification were assembled and edited with Geneious (Kearse & al., 2012). All sequences were initially aligned using MAFFT v.7.221 (Katoh & Standley, 2013) and then manually adjusted in PhyDE v.0.9971 (Müller & al., 2010).

For the three datasets (CP80, CP8, nrITS), maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out for phylogenetic reconstruction. Both ML and BI analyses were performed on the Cyberinfrastructure for Phylogenetic Research Science (CIPRES) Gateway (<http://www.phylo.org/>; Miller & al., 2010), using RAXML-HPC2 v.8.2.12 (Stamatakis, 2014) and MrBayes v.3.2.7a (Ronquist & al., 2012) on XSEDE, respectively.

For the second set of analyses, the nrITS dataset and the combined plastid dataset (dataset 8CP) were initially analyzed separately. Prior to the BI analyses, the best-fit substitution model for each dataset was selected using jModelTest v.2.1.7 (Darriba & al., 2012) based on the Akaike information criterion (AIC), other detailed parameters for BI and ML analyses followed those described in Chen & al. (2022a). All resulting trees with nodal support values were visualized and edited in FigTree v.1.4.2 (Rambaut, 2014). We followed F. Zhao & al. (2021b) and defined branches with posterior probabilities (PP) > 0.95 and bootstrap values (BS) > 80% as strongly supported, PP = 0.90–0.95 and BS = 70%–80% as moderately supported, while PP < 0.90 and BS < 70% as weakly supported.

## RESULTS

**Plastome features and gene content.** — We failed to assemble the complete plastomes for *Stenogyne sessilis* Benth.

and *Phlomis purpurea* L. The remaining 66 plastomes displayed a typical quadripartite structure consisting of a pair of inverted repeat (IR) regions (25,180–25,739 bp) separated by the large single copy (LSC; 81,266–84,807 bp) and small single copy (SSC; 16,768–17,715 bp) regions, and the genome size ranged from 149,736 bp (*Stenogyne haliakalae* Wawra) to 153,272 bp (*Holmskioldia sanguinea* Retz.). Nucleotide length, number of genes and GC content were summarized in suppl. Table S1. The complete plastome of most species contained 114 unique genes when duplicated genes were counted only once, and included 80 protein-coding genes, 30 tRNAs and 4 rRNAs. All these genes and RNAs were duplicated in the IR regions. The newly sequenced and annotated plastomes were submitted to the National Center for Biotechnology Information (NCBI) database with the accession numbers ON815615–ON815632. The plastome maps of newly sequenced species are provided in suppl. Fig. S1.

**Sequence characterization.** — Properties for the different datasets are summarized in Table 1. The original aligned length of the dataset CP80 was 69,593 bp and the final length used in the dataset was 69,292 bp (after removing ambiguously aligned sites), of which 11,973 (17.28%) were variable. The aligned length of the combined CP8 dataset was 7988 bp (2379 positions for *atpB-rbcL*, 421 for *psbA-trnH*, 933 for *rpl16*, 719 for *rpl32-trnL*, 921 for *rps16*, 957 for *trnK*, 875 for *trnL-trnF*, 783 for *trnT-trnL*). In the *psbA-trnH* spacer, five species were found to have inversions, and those inversions were reverse-complemented prior to phylogenetic analyses to reduce the effect of homoplasy, as suggested by Whitlock & al. (2010) and Degtjareva & al. (2013). After removing ambiguously aligned sites (470–484 bp), the aligned length of the nrITS dataset included 720 bp, of which 182 bp (25.28%) were variable.

The final alignments and all phylogenetic trees were deposited in figshare (<https://doi.org/10.6084/m9.figshare.20177417.v1>).

**Phylogenetic reconstruction.** — For each of the three datasets (CP80, CP8, nrITS), ML and BI analyses yielded identical topologies (suppl. Figs. S2–S7). Therefore, only the ML trees (Figs. 2–4) are presented here for discussion of phylogenetic relationships, with BS and PP indicated.

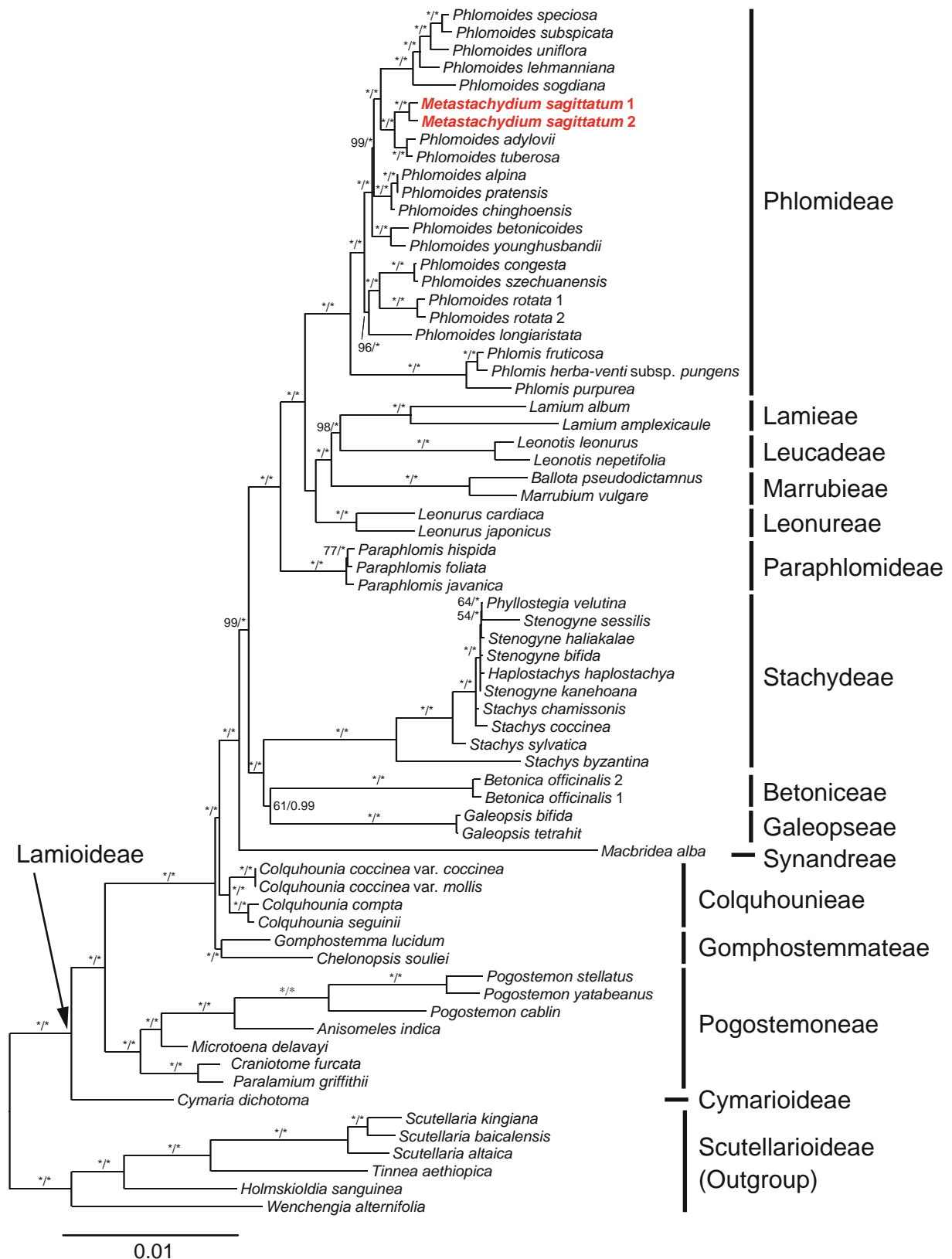
In our phylogenetic analyses based on the dataset CP80, Lamioideae was robustly supported as monophyletic (Fig. 2, ML-BS = 100%/BI-PP = 1.00; all support values follow this order hereafter) and 13 tribes were recognized. Phylogenetic relationships among these tribes were as reported by F. Zhao & al. (2021a). The two individuals of *Metastachyidium sagittatum* formed a clade deeply nested within *Phlomoidea* of Phlomoideae and sister to the *P. adylovii* Lazkov and *P. tuberosa* (L.) Moench clade (Fig. 2, 100%, 1.00).

In the second step of analyses based on datasets CP8 and nrITS, strongly supported conflicts were found between the plastid and nuclear trees (Figs. 3, 4). The main incongruence was found in the placement of the clade comprising *Phlomoidea hamosa* (Benth.) Mathiesen and *P. longiaristata* (C.Y. Wu & H.W. Li) Salmaki (“Notochaete” clade, characterized by having hooked floral bracts and calyx spines). In the CP8 tree, “Notochaete” clade is sister to a clade composed of a part of *P. sect. Phlomoidea* Kamelin & Makhm. (mainly distributed in forests or on plateau meadows of southern China, i.e., *P. rotata* (Benth. ex Hook.f.) Mathiesen and *P. umbrosa* (Turcz.) Kamelin & Makhm.) with convincing support values (Fig. 3, 95%, 1.00), but is sister to *Metastachyidium* + another part of *P. sect. Phlomoidea* (mainly distributed in steppes of northern China and neighboring countries, i.e., *P. tuberosa* and *P. mongolica* (Turcz.) Kamelin & A.L. Budantzev) with moderate support values in the nrITS tree (Fig. 4, 72%, 0.91).

With an expanded sampling of *Phlomoidea*, *M. sagittatum* was shown to be sister to a clade comprising *P. adylovii*, *P. mongolica*, *P. tuberosa*, *P. puberula* (Krylov & Serg.) Adylov & al., and *P. hybrida* (Zelen.) Kamelin & Makhm. (Fig. 3, 100%, 1.00; Fig. 4, 61%, –).

**Table 1.** The statistics of all datasets for phylogenetic analysis.

Dataset	No. of taxa	Nucleotides (ambiguous sites excluded) [bp]	GC content (%)	No. of constant sites [bp]	No. of variable sites [bp]	No. of parsimony-informative sites [bp]
CP80	68	69,292	38.4	57,319 (82.72%)	11,973 (17.28%)	7,770 (11.21%)
<i>atpB-rbcL</i>	51	2,379	39.3	2,266 (95.25%)	113 (4.75%)	73 (3.07%)
<i>psbA-trnH</i>	51	421	32.6	370 (87.89%)	51 (12.11%)	35 (8.31%)
<i>rpl16</i>	51	933	33.2	843 (90.35%)	90 (9.65%)	58 (6.22%)
<i>rpl32-trnL</i>	51	719	31.6	630 (87.62%)	89 (12.38%)	67 (9.32%)
<i>rps16</i>	51	921	35.3	849 (92.18%)	72 (7.82%)	44 (4.78%)
<i>trnK</i>	51	957	34.2	864 (90.28%)	93 (9.72%)	71 (7.42%)
<i>trnL-trnF</i>	51	875	36.1	816 (93.26%)	59 (6.74%)	41 (4.69%)
<i>trnT-trnL</i>	51	783	29.5	712 (90.93%)	71 (9.07%)	51 (6.51%)
CP8	51	7,988	35.3	7,350 (92.01%)	638 (7.99%)	440 (5.51%)
nrITS	51	720	64.5	538 (74.72%)	182 (25.28%)	125 (17.36%)



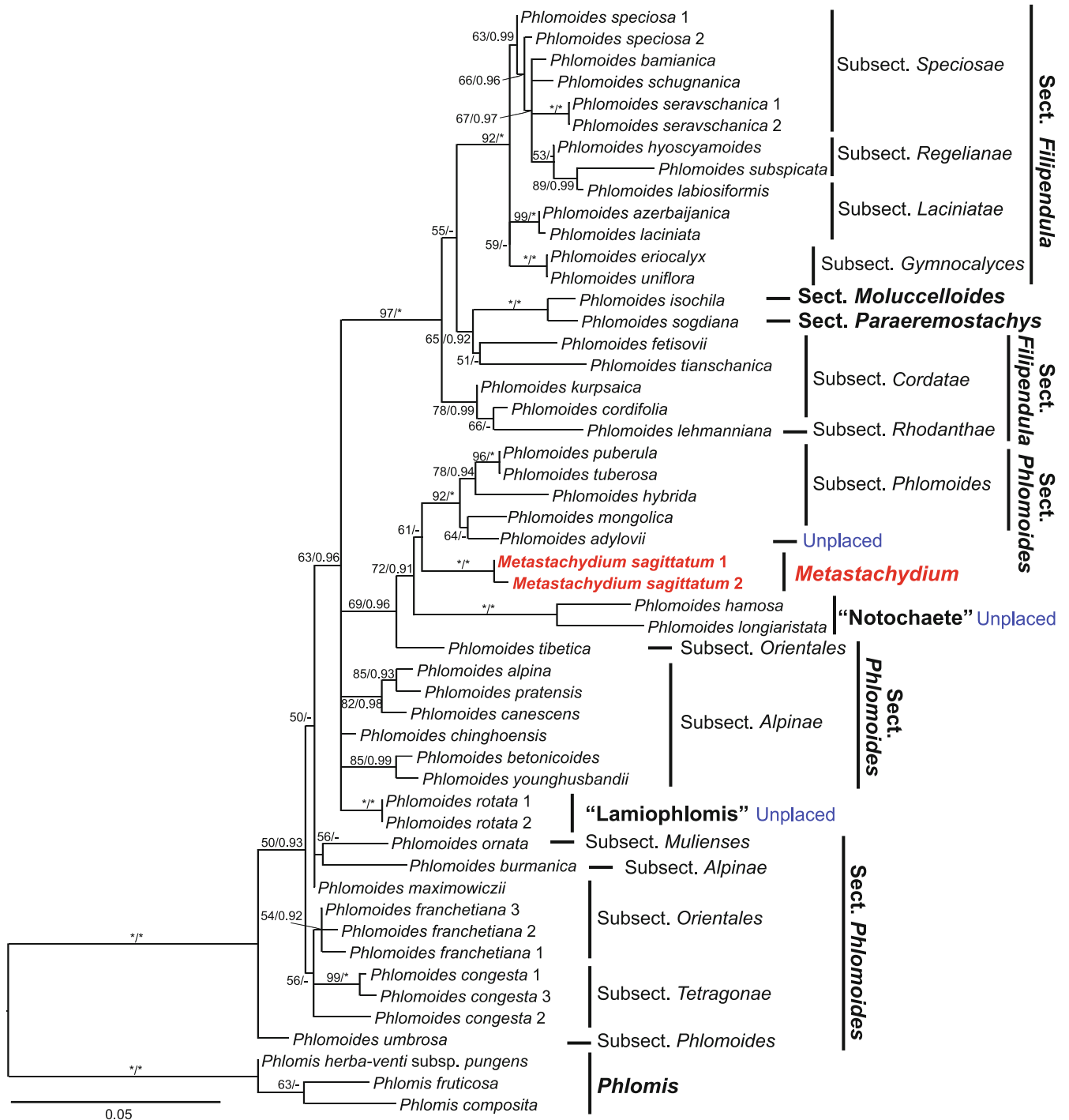
**Fig. 2.** Phylogeny of Lamioideae inferred by maximum likelihood (ML) based on the combined plastid dataset CP80 with ambiguously aligned sites excluded for analysis. Support values displayed on the branches follow the order ML-BS/BI-PP (“\*” indicates BS = 100% or PP = 1.00). Tribal classification of Lamioideae is based on F. Zhao & al. (2021a). Scale bar denotes the expected number of substitutions per site in ML analysis. Multiple accessions of the same species are numbered according to suppl. Table S1.



■ DISCUSSION

***Metastachyidium* is a member of *Phlomoidea* within *Phlomoidea*.** — Although phylogenetic studies have greatly promoted our understanding of the phylogenetic relationships

and evolution within Lamiioideae (Wink & Kaufmann, 1996; Lindqvist & Albert, 2002; Scheen & Albert, 2007, 2009; Scheen & al., 2008, 2010; Bendiksby & al., 2011, 2014; Salmaki & al., 2012, 2013; Xiang & al., 2013; Chen & al., 2014; Roy & Lindqvist, 2015; Li & al., 2016; Yao & al., 2016;



**Fig. 4.** Phylogeny of *Phlomoidea* inferred by maximum likelihood (ML) based on the nrITS dataset with ambiguously aligned sites excluded for analysis. All clades with BS < 50% have been collapsed. Support values displayed on the branches follow the order ML-BS/BI-PP. (“-” indicates PP < 0.9 and “\*” indicates BS = 100% or PP = 1.00). Sectional and subsectional classification of *Phlomoidea* is based on Kamelin & Makhmedov (1990) and Sennikov & Lazkov (2013). Scale bar denotes the expected number of substitutions per site in ML analysis. Multiple accessions of the same species are numbered according to Appendix 1.

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Siadati & al., 2018), the systematic placement of *Metastachydium* and *Pseudomarrubium* has never been tested using molecular data. Here, we present the first molecular phylogenetic study of *Metastachydium* and clarify its position within Lamioideae based on a sampling of all tribes of the subfamily, using a plastome-scale dataset. The topology and support values of major clades of Lamioideae in the resulting tree (Fig. 2) are generally consistent with our prior study (F. Zhao & al., 2021b), and *Metastachydium* is strongly supported as a member of *Phlomoides* within Phlomideae. Based on an expanded sampling of *Phlomoides*, our results further reveal that *M. sagittatum* is sister to a clade consisting of *P. adylovii*, *P. mongolica*, *P. tuberosa*, *P. puberula*, and *P. hybrida* in both plastid and nrITS trees (Figs. 3, 4), although with poor support values in the nrITS tree.

Initially, *Metastachydium sagittatum* was described as *Phlomis sagittata* and considered closely related to *Phlomis tuberosa* L. (= *Phlomoides tuberosa*, the type of *Phlomoides*) since they share a similar leaf shape and 2-lobed posterior corolla lips (Regel, 1880). The epithet “sagittata” refers to the sagittate leaves of *Phlomis sagittata* (= *M. sagittatum*), a character that Regel (1880) considered distinct and used to differentiate *M. sagittatum* from other *Phlomis* species. However, the circumscription of *Phlomis* has since changed dramatically, with most of its species being transferred to the resurrected *Phlomoides* (Kamelin & Makhmedov, 1990).

Although *Phlomoides* was separated from *Phlomis* since as early as 1794 (Moench, 1794), it was consistently treated as a section of *Phlomis* by subsequent taxonomists (Bentham, 1832–1836; Boissier, 1875; Briquet, 1897; Li, 1977; Li & Hedge, 1994). It was not until 1986 that Adylov & al. (1986) resurrected *Phlomis* sect. *Phlomoides* as a genus (*Phlomoides*). Later, Kamelin & Makhmedov (1990) expanded the genus to include 65 species of *Phlomis* and 75 species of *Eremostachys* Ledeb. Subsequently, major changes to the genus were facilitated by recent molecular phylogenetic studies and revealed that the circumscription of *Phlomoides* should be further expanded to include species of *Paraeremostachys* Adylov & al., *Pseuderemostachys* Popov, *Notochaete* Benth., and *Lamiophlomis* Kudô (Pan & al., 2009; Scheen & al., 2010; Bendiksbj & al., 2011; Mathiesen & al., 2011; Salmaki & al., 2012). Based on combined nrITS and plastid DNA data, Salmaki & al. (2012) reconstructed the backbone phylogeny of Phlomideae and showed that only two genera were included in the tribe, *Phlomis* and *Phlomoides*. The two genera can be distinguished from each other by upper corolla lip morphology (compressed and sickle-shaped in *Phlomis*, vs. non-compressed dome-shaped in *Phlomoides*) and life form (shrubs or sub-shrubs in *Phlomis*, vs. herbaceous in *Phlomoides*) (Mathiesen & al., 2011; Salmaki & al., 2012). As currently delimited, *Phlomoides* contains at least 170 species (Salmaki & Joharchi, 2014; Xiang & al., 2014; Y. Zhao & al., 2021a, 2021b) distributed mainly in three regions, Central Asia (59 spp.; Czerepanov, 1995), the Iranian highlands (41 spp.; Salmaki & al., 2012), and China (50 spp.; Xiang & al., 2014).

Morphologically, *Metastachydium* and *Phlomoides* share a similar leaf morphology, toothed and spined calyces, subulate floral bracts, and stellate trichomes. *Metastachydium sagittatum* differs from species of *Phlomoides* mainly in the morphology of the posterior corolla lip and stamens. For most species of *Phlomoides*, the posterior corolla lip is entire and hooded (often deeply concave and dome-shaped), with long simple hairs inside (Salmaki & al., 2012), and the stamens are obviously exerted from the tube. However, as initially postulated by Regel (1880), the sagittate leaves of *Metastachydium* are not a distinguishing feature as they are now known from elsewhere in *Phlomoides* (e.g., *P. tuberosa*, *P. mongolica*). While the posterior corolla lip of *M. sagittatum* is erect and 2-lobed, with stellate trichomes outside and sparsely short simple trichomes inside (denser and longer near margin), stamens are included with extremely short filaments (Fig. 1L). These two characters are rare within *Phlomoides*, and only found in *P. sewerzovii* (Herd.) Mathiesen and *P. boraldaiica* A.L.Ebel. Unfortunately, the latter two species were not included in this study, but presence of the above characters suggests that they are closely related to *M. sagittatum*. In this study, the close relationship among *M. sagittatum*, *P. adylovii*, *P. mongolica*, *P. tuberosa*, *P. puberula*, and *P. hybrida* as recovered in the second set of analyses is also supported by morphological evidence: e.g., the basal leaves of these species are more or less sagittate, and their floral leaves are triangular. There are also biogeographical correlates supporting a close kinship among these species; excepting the widespread *Phlomoides tuberosa* (central Europe to China) and the eastern European *P. hybrida*, all other species are indigenous to Central Asia and the Mongolian Plateau, and usually grow in arid and semiarid habitats.

Based on our above discussion, *Metastachydium* is supported as a member of *Phlomoides* by both molecular phylogenetic and morphological evidence. Therefore, *Metastachydium* should be synonymized with *Phlomoides* and a new combination in *Phlomoides* is needed (see Taxonomic treatment).

**Infrageneric classification and relationships within *Phlomoides*.** — With the inclusion of *Metastachydium*, the circumscription of the traditionally defined genus *Phlomoides* has changed yet again. Although only 40 species of *Phlomoides* (including *Metastachydium*) were sampled, this study clarified relationships within *Phlomoides*. Thus, it is necessary to discuss the infrageneric classification within the genus. The infrageneric classification of *Phlomoides* has been controversial since the resurrection of the genus. Kamelin & Makhmedov (1990) proposed the first infrageneric classification and divided the genus into two sections, *Phlomoides* sect. *Phlomoides* (including most species from traditionally defined *Phlomis* sect. *Phlomoides* (Moench) Briq.) and *Phlomoides* sect. *Filipendula* (Popov) Adylov & al. (including most species from traditionally defined *Eremostachys* sect. *Filipendula* Popov). According to Kamelin & Makhmedov (1990), *Phlomoides* sect. *Phlomoides* is characterized by simple leaves with crenate, toothed, or entire margins, and

unicolor corollas with a fornicated anterior lip. Whereas *Phlomooides* sect. *Filipendula* has pinnatisect or pinnatipartite leaves (rarely entire), and often bicolored or sometimes unicolor corollas with an arched or scythe-shaped anterior lip. The two sections were further subdivided into 9 and 12 subsections, respectively, but most sections and subsections were not supported as monophyletic in recent molecular phylogenetic studies (Mathiesen & al., 2011; Salmaki & al., 2012). Sennikov & Lazkov (2013) subsequently established three new sections based on the phylogenetic framework of *Phlomooides* presented by Salmaki & al. (2012) as well as the shape of calyx, *Phlomooides* sect. *Eremostachys* (Bunge) Sennikov (calyx tubular, corresponding to “*Eremostachys laciniata* core group” sensu Salmaki & al., 2012), *P.* sect. *Moluccelloides* (Bunge) Sennikov (calyx funnel-shaped, corresponding to *Eremostachys* sensu Adylov & al., 1986), and *P.* sect. *Paraeremostachys* (Adylov & al.) Sennikov (calyx campanulate, corresponding to *Paraeremostachys*). Considering that only 38 species of *Phlomooides* (22%) were sampled by Salmaki & al. (2012), the circumscription of these sections and the positions of those unsampled taxa within the genus needs to be further clarified.

Excepting *Metastachydium sagittatum*, we also sampled 16 species from *Phlomooides* sect. *Filipendula*, 17 species from *P.* sect. *Phlomooides*, 1 species from *P.* sect. *Paraeremostachys*, and 1 species from *P.* sect. *Moluccelloides* in this study. Our phylogenetic results based on the CP8 and nrITS datasets demonstrate that both *P.* sect. *Filipendula* and *P.* sect. *Phlomooides* sensu Kamelin & Makhmedov (1990) are not monophyletic, as *P. isochila* (Pazij & Vved.) Salmaki of *P.* sect. *Moluccelloides* and *P. sogdiana* (Pazij & Vved.) Salmaki of *P.* sect. *Paraeremostachys* are embedded within the *P.* sect. *Filipendula* clade (Fig. 3, 100%, 1.00; Fig. 4, 97%, 1.00) and species of *P.* sect. *Phlomooides* are recovered in several separate clades (Figs. 3, 4). Since only one species (*P. isochila*) of *P.* sect. *Moluccelloides* and one species (*P. sogdiana*) of *P.* sect. *Paraeremostachys* were sampled here, the monophyly of these two sections is uncertain.

Currently, at least 20 species of *Phlomooides* have not been assigned to a section, of which four species were included here for analyses: *P. adylovii* Lazkov, *P. hamosa*, *P. longiaristata* (formerly known as *Notochaete*), *P. rotata* (formerly known as *Lamiophlomis*). *Phlomooides adylovii* is resolved as a member of *P.* sect. *Phlomooides* and probably has a close relationship with *P. mongolica*. Both species have similar sagittate to triangular basal leaves with crenate to dentate margins. The systematic placement of *P. hamosa*, *P. longiaristata*, and *P. rotata* was unresolved because the positions of these species varied in plastid vs. nuclear trees. Future studies including increased taxon sampling and next-generation sequencing data are needed to clarify their systematic positions within the genus, and a tenable infrageneric classification system is required based on a robust backbone phylogeny.

**Incongruence between data partitions.** — In order to test the phylogenetic placement of *Metastachydium*, we constructed two datasets: a plastid dataset (*atpB-rbcL*, *psbA-trnH*,

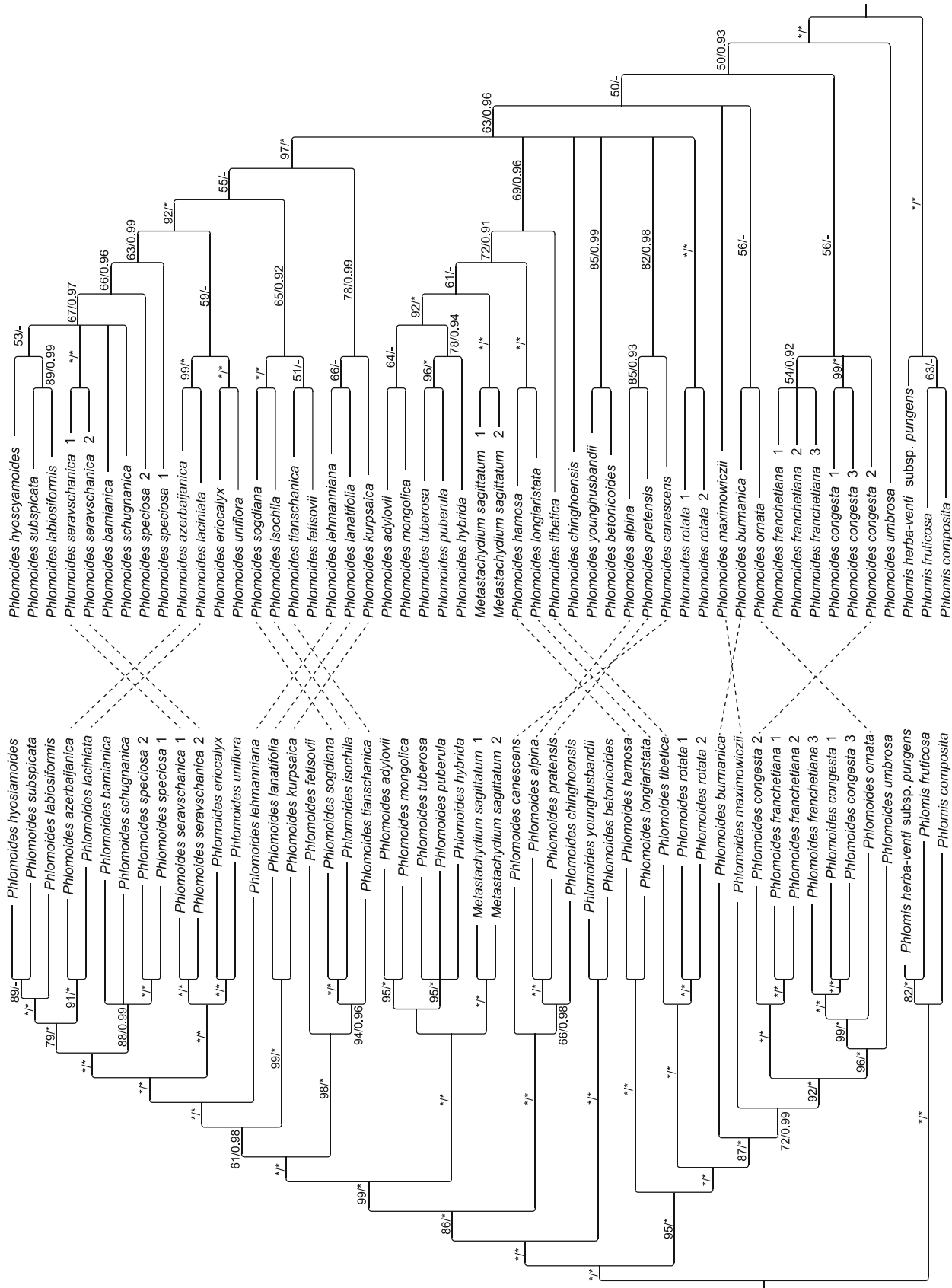
*rpl16*, *rpl32-trnL*, *rps16*, *trnL-trnF*, *trnT-trnL*, *trnK*) and a nuclear dataset (nrITS). All trees derived from the plastid and nuclear data strongly support the monophyly of the expanded circumscription of *Phlomooides* to include *Metastachydium*. However, visual inspection and topological comparison indicate that there are some conflicts between the nuclear and plastid trees (Fig. 5).

Topological incongruence between the nuclear and plastid trees is common in Lamiaceae and has been reported in genera such as *Caryopteris* Bunge (Xiang & al., 2018), *Salvia* L. (Hu & al., 2018; Rose & al., 2021), and *Isodon* (Schrad. ex Benth.) Spach (Chen & al., 2022b). In general, the incongruities between plastid and nuclear DNA markers within Lamiaceae have been explained by incomplete lineage sorting (ILS) and/or hybridization (Albaladejo & al., 2005; Drew & Sytsma, 2013; Drew & al., 2014; Deng & al., 2015; Walker & al., 2015; Hu & al., 2018; F. Zhao & al., 2021b; Chen & al., 2022b).

Only a few instances of hybridization in *Phlomooides* have been reported in previous studies (Popov, 1940), but several hybridization events have been reported in the sister genus *Phlomis* (e.g., Aparicio, 1997; Aparicio & al., 2000; Albaladejo & al., 2004; Mathiesen & al., 2011), and hybridization may be responsible for the phylogenetic incongruence found here. For both *Phlomooides congesta* (C.Y.Wu) Kamelin & Makhm. and *P. franchetiana* (Diels) Kamelin & Makhm. we sampled three accessions, and each species was monophyletic in the nrITS tree (Fig. 4). However, the accessions of each species did not group together in the plastid tree (Fig. 3). The sympatric distribution of the above species suggests that chloroplast capture may have occurred between these species in the past, and that intrageneric hybridization may be common within *Phlomooides*, particularly among geographically proximal taxa. Rapid speciation events often lead to ILS, and may have a confounding influence on accurately estimating phylogenetic relationships (Enard & Paabo, 2004; Pollard & al., 2006). *Phlomooides* seems to have undergone a rapid diversification since many branches in the phylogeny are very short (Figs. 3, 4). It is worth noting that the habitats of the three diversity centers of the genus are very different (humid forest margins or alpine zones in the Himalaya-Hengduan Mountains of East Asia, arid or semiarid zones in Central Asia, and steppe/arid mountains in the Iranian highlands). These heterogeneous habitats may have contributed to the rapid diversification of *Phlomooides*, as reported by Ye & al. (2019), and ILS following rapid diversification events is likely another reason for the topological discordance seen here.

## ■ TAXONOMIC TREATMENT

Based on our molecular phylogenetic analyses, *Phlomooides* as currently circumscribed is not monophyletic, as *Metastachydium* is shown to be embedded within the genus. The most parsimonious way to render a monophyletic classification is to reduce *Metastachydium* to a synonym of *Phlomooides* and transfer *M. sagittatum* to *Phlomooides*, as done below.



**Fig. 5.** Tangram comparing the combined plastid dataset (ML tree inferred from the CP8 dataset) and the nrITS dataset (ML tree inferred from the nrDNA dataset) trees. All clades with BS < 50% have been collapsed. Support values displayed on the branches follow the order ML-BS/BI-PP (“-” indicates PP < 0.9 and “\*”) indicates BS = 100% or PP = 1.00).

*Phlomis* Moench, Methodus: 403. 1794 – Type: *Phlomis tuberosa* (L.) Moench.  
= *Metastachydium* Airy Shaw ex C.Y.Wu & H.W.Li in Acta Phytotax. Sin. 13(1): 73. 1975, **syn. nov.**

*Phlomis sagittata* (Regel) C.L.Xiang & Y.Zhao, **comb. nov.** ≡ *Phlomis sagittata* Regel in Trudy Imp. S.-Peterburgsk. Bot. Sada 6(2): 373. 1880 ≡ *Metastachydium sagittatum* (Regel) C.Y.Wu & H.W.Li in Acta Phytotax. Sin. 13(1): 73. 1975 – Holotype: CHINA. Kuldscha, 13 May 1877, *A. Regel s.n.* (K barcode K000509295!; isotype: P barcode P00738149!).

**Description.** – Perennial herbs, rhizomatous. Stems 20–70 cm tall, erect, subquadrangular, robust, few-branched, brown to brown-purple, densely stellate pilose, denser below nodes. Basal leaves sagittate, base strongly cordate, margin coarsely crenate, petioles 1–12 cm long, densely stellate pilose, basal leaf blade 6–8 × 3–5 cm, adaxially sparsely hispid, with simple and stellate trichomes, abaxially gray; stem leaves opposite, lower stem leaves similar to basal leaves, upper stem leaves triangular, petioles 0.5–12 cm long, clasping, stem leaf blade 3–5 × 2–3 cm, trichomes similar to basal leaves, bases cordate, margins crenate. Verticillasters axillary, 4–14-flowered; lower verticillasters widely spaced; floral leaves 0.5–5 × 0.4–2.5 cm, with petioles 4–6 mm long, blade triangular to lanceolate, gradually reduced upward; bracts subulate, 6–9 mm long, indumentum dense with stellate and pilose simple hairs. Calyx tubular, 10–12 × 3 mm, densely stellate and simple pilose and pubescent outside, 10-veined; teeth 5, triangular, subequal, margin pilose, 1.5–2 mm long, apical spine 2 mm long. Corolla purple to violet, ca. 2 cm long, 2-lipped; posterior lip erect, ca. 1 cm long, margin 2-lobed, stellate tomentose outside, with sparse short simple trichomes inside, denser and longer near margin; anterior lip 3-lobed, ca. 10–11 × 5–6 mm, middle lobe largest, broadly reniform, margin unequally denticulate or undulate; lateral lobes ovate-oblong, much shorter than middle lobe; tube ca. 1 cm long, sparsely stellate tomentose outside, annulate pilose inside near throat. Stamens 4, 1–2 mm long, included, filaments extremely short, densely tufted hairy, triangular-dilated at base. Style included, 8–10 mm, apex unequally 2-lobed. Mericarps brown, elliptic, glabrous.

**Phenology.** – Flowering from May to June and fruiting from July to August.

**Distribution and habitat.** – The species is distributed in Central Asia (Xinjiang of northwest China; Terskey Alatau, Kyrgyzstan). It usually grows in river valleys, on meadows or grassy slopes at an altitude between 1000 m and 1400 m (suppl. Fig. S8).

**Additional specimens examined.** – CHINA. Xinjiang: Xinyuan County, the valley of Qiapuhe, 43°20'21.03"N, 83°14'34.68"E, elevation 1077 m, 4 Jun 2016, *J.D. Ya, Q.R. Zhang & X.J. Hu 16CS12989* (KUN!); Tekes County, from Tekes to Yining, near the 82 km milestone of G220, 43°22'38.12"N, 81°51'48.91"E, elevation 1490 m, 13 Jun 2016, *J.D. Ya, Q.R. Zhang & X.J. Hu 16CS12196* (KUN!); Nileke County, Keling Town, 43°47'02.57"N, 82°24'39.46"E, elevation 1166 m, *Y. Zhao &*

*J.F. Xiao ZY24* (KUN!). KYRGYZSTAN. Terskey Alatau: near lake Tuz-Kul, 28 Jun 1950, *L.I. Medvedeva 951* (US!).

**Conservation status.** – *Phlomis sagittata* is distributed in Ili Kazak Autonomous Prefecture, Xinjiang Province, China, and Terskey Alatau, Kyrgyzstan. With only three collections from China since its initial publication and one collection from Kyrgyzstan, data on the population of the species is insufficient. Thus *P. sagittata* should be classified as Data Deficient (DD) based on the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Subcommittee, 2019). Nonetheless, we have found overgrazing is universal in Ili, and most individuals we found in Nilka County (China) had been grazed by livestock. It is possible that after collecting more data on potential threats and more accurately describing population parameters, this species will be attributed to the category Near Threatened (NT).

## CONCLUSION

This is the first study to infer the systematic position of *Metastachydium sagittatum* based on chloroplast and nuclear DNA data, as well as morphological characters. *Metastachydium sagittatum* should be transferred to *Phlomis* (tribe Phlomisae), and a new combination, *Phlomis sagittata* is proposed. Monophyly of the redefined *Phlomis* s.l. is confirmed, but the monophyly for the two largest sections (i.e., *P. sect. Phlomis* and *P. sect. Filipendula*) was not recovered, indicating a new infrageneric classification should be considered. Future studies should include a wider breadth of taxon sampling in a low-copy molecular phylogenetic framework, as well as additional morphological data, for stable sectional delimitations.

## AUTHOR CONTRIBUTIONS

CLX, YPC, and CZL conceived this research. YZ, FZ, YS, AP, JC, BT, CM, PE, OTT, YPC, and CLX collected materials. YZ and FZ performed the experiments. YZ, FZ, YPC, and CLX analyzed the data. All co-authors contributed to the manuscript and revised it critically. All authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work. — YZ, <https://orcid.org/0000-0001-6850-5070>; FZ, <https://orcid.org/0000-0001-7328-2262>; YS, <https://orcid.org/0000-0002-0901-0761>; AP, <https://orcid.org/0000-0002-6052-6675>; JC, <https://orcid.org/0000-0003-1627-3700>; BT, <https://orcid.org/0000-0001-7248-2799>; CM, <https://orcid.org/0000-0002-1008-9569>; PE, <https://orcid.org/0000-0003-2926-255X>; OTT, <https://orcid.org/0000-0001-7120-1895>; CZL, <https://orcid.org/0000-0003-1250-7098>; YPC, <https://orcid.org/0000-0002-7502-1848>; CLX, <https://orcid.org/0000-0001-8775-6967>

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#### Appendix 1. The GenBank accession numbers of the taxa from CP8 and nrITS in this study and their voucher information.

Information is presented in the following order and format: **Taxon**; *Voucher information* (Herbarium), nrITS, *atpB-rbcL*, *psbA-trnH*, *rpl16*, *rpl32-trnL*, *rps16*, *trnK*, *trnL-trnF*, *trnT-trnL* (\* indicates sequences obtained in this study; – indicates missing sequences).

*Metastachyidium sagittatum* (Regel) C.Y.Wu & H.W.Li 1; Xinyuan County, Xinjiang, China, *Ya J.D. & al. 16CS12989* (KUN), ON890911\*, ON820578\*, ON820620\*, ON835603\*, ON835650\*, ON835697\*, ON835744\*, ON843207\*, ON843254\*. *Metastachyidium sagittatum* (Regel) C.Y.Wu & H.W.Li 2; Tekes County, Xinjiang, China, *Ya J.D. & al. 16CS12196* (KUN), ON890910\*, ON820577\*, ON820619\*, ON835602\*, ON835649\*, ON835696\*, ON835743\*, ON843206\*, ON843253\*. *Phlomis composita* Pau; The Royal Botanic Gardens, Kew, British (cultivated), *Xiang C.L. & Chen Y.P. s.n.* (KUN), ON890890\*, ON820555\*, ON820616\*, ON835580\*, ON835627\*, ON835674\*, ON835721\*, ON843184\*, ON843231\*. *Phlomis fruticosa* L.; The Royal Botanic Gardens, Kew, British (cultivated), *Xiang C.L. & Chen Y.P. s.n.* (KUN), ON890891\*, ON820556\*, ON820615\*, ON835581\*, ON835628\*, ON835675\*, ON835722\*, ON843185\*, ON843232\*. *Phlomis herba-venti* subsp. *pungens* (Willd.) Maire ex DeFilippis; Russia, *Wuzman E.I. s.n.* (MW), ON890892\*, ON820557\*, ON820614\*, ON835582\*, ON835629\*, ON835676\*, ON835723\*, ON843186\*, ON843233\*. *Phlomoidea adylovii* Lazkov;

## Appendix 1. Continued.

Kyrgyzstan, *Lorgeav G.A. 7265* (LE), ON890907\*, ON820572\*, ON820627\*, ON835597\*, ON835644\*, ON835691\*, ON835738\*, ON843201\*, ON843248\*, *Phlomoidea alpina* (Pall.) Adylov, Kamelin & Makhm.; Hejing County, Xinjiang, China, *Zhang T. & al. 08CS491* (KUN), ON890938\*, MN814865, MN814865, MN814865, MN814865, MN814865, MN814865. *Phlomoidea azerbaijiana* (Rech.f.) Kamelin & Makhm.; Iran, *Mahmoudi R.A. & Ranjbar P.R. 25996* (BASU), ON890921\*, ON820588\*, ON820636\*, ON835613\*, ON835660\*, ON835707\*, ON835754\*, ON843217\*, ON843264\*. *Phlomoidea bamianica* (Rech.f.) Kamelin & Makhm.; Afghanistan, *Hewer T.F. 1262* (LE), ON890928\*, ON820595\*, ON820634\*, ON835620\*, ON835667\*, ON835714\*, ON835761\*, ON843224\*, ON843271\*. *Phlomoidea betonicoides* (Diels) Kamelin & Makhm.; Lijiang City, Yunnan, China, *Xiang C.L. 1289* (KUN), ON890935\*, MN617020, MN617020, MN617020, MN617020, MN617020, MN617020, MN617020, MN617020. *Phlomoidea burmanica* (Mukerjee) Kamelin & Makhm.; Lushui County, Yunnan, China, *Chen Y.P. & al. EM1560* (KUN), ON890898\*, ON820563\*, ON820630\*, ON835588\*, ON835635\*, ON835682\*, ON835729\*, ON843192\*, ON843239\*. *Phlomoidea canescens* (Regel) Adylov, Kamelin & Makhm.; Kyrgyzstan, *Lazkov G.A. 7266* (MW), ON890914\*, ON820581\*, ON820613\*, ON835606\*, ON835653\*, ON835700\*, ON835747\*, ON843210\*, ON843257\*. *Phlomoidea chinghoensis* (C.Y.Wu) Kamelin & Makhm.; Qinghe County, Xinjiang, China, *Chen Y.P. & al. EM857* (KUN), ON890913\*, ON820580\*, ON820611\*, ON835605\*, ON835652\*, ON835699\*, ON835746\*, ON843209\*, ON843256\*. *Phlomoidea congesta* (C.Y.Wu) Kamelin & Makhm. 1; Meigu County, Sichuan, China, *Fang W. & al. DE40303* (KUN), ON890901\*, ON820566\*, ON820609\*, ON835591\*, ON835638\*, ON835685\*, ON835732\*, ON843195\*, ON843242\*. *Phlomoidea congesta* (C.Y.Wu) Kamelin & Makhm. 2; Weixi County, Yunnan, China, *Chen Y.P. & al. EM1626* (KUN), ON890904\*, ON820569\*, ON820623\*, ON835594\*, ON835641\*, ON835688\*, ON835735\*, ON843198\*, ON843245\*. *Phlomoidea congesta* (C.Y.Wu) Kamelin & Makhm. 3; Kunming City, Yunnan, China, *Xiang C.L. & al. 1822* (KUN), ON890902\*, ON820567\*, ON820608\*, ON835592\*, ON835639\*, ON835686\*, ON835733\*, ON843196\*, ON843243\*. *Phlomoidea cordifolia* (Regel) Adylov, Kamelin & Makhm.; Kyrgyzstan, *Lorgeav G.A. 7265* (LE), ON890934\*, ON820601\*, ON820633\*, ON835626\*, ON835673\*, ON835720\*, ON835767\*, ON843230\*, ON843277\*. *Phlomoidea eriocalyx* (Regel) Adylov, Kamelin & Makhm.; near mountain Nurata, Uzbekistan, *Beshko N.Yu. s.n.* (TASH), ON890916\*, ON820583\*, ON820648\*, ON835608\*, ON835655\*, ON835702\*, ON835749\*, ON843212\*, ON843259\*. *Phlomoidea fetisovii* (Regel) Adylov, Kamelin & Makhm.; Kyrgyzstan, *Lazkov G.A. s.n.* (TASH), ON890929\*, ON820596\*, ON820641\*, ON835621\*, ON835668\*, ON835715\*, ON835762\*, ON843225\*, ON843272\*. *Phlomoidea franchetiana* (Diels) Kamelin & Makhm. 1; Kunming City, Yunnan, China, *Chen Y.P. & al. EM592* (KUN), ON890895\*, ON820560\*, ON820606\*, ON835585\*, ON835632\*, ON835679\*, ON835726\*, ON843189\*, ON843236\*. *Phlomoidea franchetiana* (Diels) Kamelin & Makhm. 2; Dali City, Yunnan, China, *Yin Z.J. & al. YDX1420* (KUN), ON890896\*, ON820561\*, ON820621\*, ON835586\*, ON835633\*, ON835680\*, ON835727\*, ON843190\*, ON843237\*. *Phlomoidea franchetiana* (Diels) Kamelin & Makhm. 3; Kunming City, Yunnan, China, *Li Y.Y. & Jiang L.Q. LJ17* (KUN), ON890903\*, ON820568\*, ON820610\*, ON835593\*, ON835640\*, ON835687\*, ON835734\*, ON843197\*, ON843244\*. *Phlomoidea hamosa* (Benth.) Mathiesen; Jingdong County, Yunnan, China, *Peng H. & al. PH8093* (KUN), ON890893\*, ON820558\*, ON820604\*, ON835583\*, ON835630\*, ON835677\*, ON835724\*, ON843187\*, ON843234\*. *Phlomoidea hybrida* (Zelen.) Kamelin & Makhm.; Kherson, Ukraine, *Tsvetlev N.N. & al. 1498* (LE), –, ON820573\*, ON820626\*, ON835598\*, ON835645\*, ON835693\*, ON835739\*, ON843202\*, ON843249\*. *Phlomoidea hyoscyamoides* (Boiss. & Buhse) Kamelin & Makhm.; Khorasan, Iran, *Mahmoudi R.A. 59252* (BASU), ON890926\*, ON820593\*, ON820639\*, ON835618\*, ON835665\*, ON835712\*, ON835759\*, ON843222\*, ON843269\*. *Phlomoidea isochila* (Pazij & Vved.) Salmaki; Kazakhstan, *Pimenov M.G. & al. 134* (MW), ON890931\*, ON820598\*, ON820629\*, ON835623\*, ON835670\*, ON835717\*, ON835764\*, ON843227\*, ON843274\*. *Phlomoidea kurpsaica* Lazkov; Kyrgyzstan, *Lorgeav G.A. 350* (LE), ON890915\*, ON820582\*, ON820644\*, ON835607\*, ON835654\*, ON835701\*, ON835748\*, ON843211\*, ON843258\*. *Phlomoidea labiosiformis* (Popov) Adylov, Kamelin & Makhm.; Iran, *Mahmoudi R.A. & Ranjbar P.R. 16711* (BASU), ON890927\*, ON820594\*, ON820638\*, ON835619\*, ON835666\*, ON835713\*, ON835760\*, ON843223\*, ON843270\*. *Phlomoidea laciniata* (L.) Kamelin & Makhm.; Tavush, Armenia, *Zernov A. & al. 7082* (MW), ON890922\*, ON820589\*, ON820635\*, ON835614\*, ON835661\*, ON835708\*, ON835755\*, ON843218\*, ON843265\*. *Phlomoidea lehmanniana* (Bunge) Adylov, Kamelin & Makhm.; Tajikistan, *Kisemwa K. (MW)*, ON890930\*, ON835692\*, ON835701\*, ON820645\*, ON835622\*, ON835669\*, ON835716\*, ON835763\*, ON843226\*, ON843273\*. *Phlomoidea longiaristata* (C.Y.Wu & H.W.Li) Salmaki; Fugong County, Yunnan, China, *Peng H. & al. NJ072* (KUN), ON890894\*, ON820559\*, ON820603\*, ON835584\*, ON835631\*, ON835678\*, ON835725\*, ON843188\*, ON843235\*. *Phlomoidea maximoviczii* (Regel) Kamelin & Makhm.; Tonghua County, Jilin, China, *Chen Y.P. & al. EM1457* (KUN), ON890900\*, ON820565\*, ON820622\*, ON835590\*, ON835637\*, ON835684\*, ON835731\*, ON843194\*, ON843241\*. *Phlomoidea mongolica* (Turcz.) Kamelin & A.L. Budantzev; Kangbao County, Hebei, China, *Chen Y.P. & al. EM1475* (KUN), ON890909\*, ON820576\*, ON820617\*, ON835601\*, ON835648\*, ON835695\*, ON835742\*, ON843205\*, ON843252\*. *Phlomoidea ornata* (C.Y.Wu) Kamelin & Makhm.; Kunming City, Yunnan, China, *Xiang C.L. & al. 1815* (KUN), ON890905\*, ON820570\*, ON820618\*, ON835595\*, ON835642\*, ON835689\*, ON835736\*, ON843199\*, ON843246\*. *Phlomoidea pratensis* (Kar. & Kir.) Adylov, Kamelin & Makhm.; Fukang County, Xinjiang, China, *Chen Y.P. & al. EM770* (KUN), ON890912\*, ON820579\*, ON820612\*, ON835604\*, ON835651\*, ON835698\*, ON835745\*, ON843208\*, ON843255\*. *Phlomoidea puberula* (Krylov & Serg.) Adylov, Kamelin & Makhm.; Kazakhstan, *Tsvetlev N.N. & al. 283* (LE), –, ON820574\*, ON820625\*, ON835599\*, ON835646\*, ON835693\*, ON835740\*, ON843203\*, ON843250\*. *Phlomoidea rotata* (Benth. ex Hook.f.) Mathiesen 1; Yadong, Tibet, China, *Chen Y.P. & al. EM1072* (KUN), ON890899\*, ON820564\*, ON820602\*, ON835589\*, ON835636\*, ON835683\*, ON835730\*, ON843193\*, ON843240\*. *Phlomoidea rotata* (Benth. ex Hook.f.) Mathiesen 2; Tibet, China, *2020MacC24* (XZ), ON890937\*, MZ150795, MZ150795, MZ150795, MZ150795, MZ150795, MZ150795, MZ150795. *Phlomoidea schugnanica* (Popov) Adylov, Kamelin & Makhm.; Tajikistan, *Kacar A.E. & al. 335* (LE), ON890918\*, ON820585\*, ON820640\*, ON835610\*, ON835657\*, ON835704\*, ON835751\*, ON843214\*, ON843261\*. *Phlomoidea seravschanica* (Regel) Adylov, Kamelin & Makhm. 1; Tajikistan, *Klyuykov E.V. 465* (LE), ON890920\*, ON820587\*, ON820646\*, ON835612\*, ON835659\*, ON835706\*, ON835753\*, ON843216\*, ON843263\*. *Phlomoidea seravschanica* (Regel) Adylov, Kamelin & Makhm. 2; Tajikistan, *Anonymus s.n.* (LE), ON890919\*, ON820586\*, ON820647\*, ON835611\*, ON835658\*, ON835705\*, ON835752\*, ON843215\*, ON843262\*. *Phlomoidea sogdiana* (Pazij & Vved.) Salmaki; Uzbekistan, *Pimenov M.G. & al. 61* (MW), ON890932\*, ON820599\*, ON820628\*, ON835624\*, ON835671\*, ON835718\*, ON835765\*, ON843228\*, ON843275\*. *Phlomoidea speciosa* (Rupr.) Adylov, Kamelin & Makhm. 1; Kyrgyzstan, *Mkryakii Z. & al. s.n.* (LE), ON890924\*, ON820591\*, ON820632\*, ON835616\*, ON835663\*, ON835710\*, ON835757\*, ON843220\*, ON843267\*. *Phlomoidea speciosa* (Rupr.) Adylov, Kamelin & Makhm. 2; Uzbekistan, *Turginov O.T. s.n.* (TASH), ON890925\*, ON820592\*, ON820631\*, ON835664\*, ON835671\*, ON835711\*, ON835758\*, ON843221\*, ON843268\*. *Phlomoidea subspicata* (Popov) Adylov, Kamelin & Makhm.; Turkmenistan, *Polevova S. 7265* (MW), ON890923\*, ON820590\*, ON820637\*, ON835615\*, ON835662\*, ON835709\*, ON835756\*, ON843219\*, ON843266\*. *Phlomoidea tianschanica* (Popov) Adylov, Kamelin & Makhm.; Kyrgyzstan, *Pertti U. 48127* (MW), ON890933\*, ON820600\*, ON820643\*, ON835625\*, ON835672\*, ON835719\*, ON835766\*, ON843229\*, ON843276\*. *Phlomoidea tibetica* (C.Marquand & Airy Shaw) Kamelin & Makhm.; Cona County, Tibet, China, *Hu G.X. & al. 1209009* (KUN), ON890897\*, ON820562\*, ON820607\*, ON835587\*, ON835634\*, ON835681\*, ON835728\*, ON843191\*, ON843238\*. *Phlomoidea tuberosa* (L.) Moench; Burqin County, Xinjiang, China, *Chen Y.P. & al. EM852* (KUN), ON890908\*, ON820575\*, ON820624\*, ON835600\*, ON835647\*, ON835694\*, ON835741\*, ON843204\*, ON843251\*. *Phlomoidea umbrosa* (Turcz.) Kamelin & Makhm.; Beijing, China, *Zhang B.Y. & Zhao Y. LBJ2018001* (KUN), ON890906\*, ON820571\*, ON820605\*, ON835596\*, ON835643\*, ON835737\*, ON843200\*, ON843247\*. *Phlomoidea uniflora* (Regel) Adylov, Kamelin & Makhm.; Uzbekistan, *Mkryakii Z. & al. s.n.* (LE), ON890917\*, ON820584\*, ON820642\*, ON835609\*, ON835656\*, ON835703\*, ON835750\*, ON843213\*, ON843260\*. *Phlomoidea younghusbandii* (Mukerjee) Kamelin & Makhm.; Nagarzê County, Tibet, China, *Chen Y.P. & al. EM1033* (KUN), ON890936\*, MW405448, MW405448, MW405448, MW405448, MW405448, MW405448, MW405448, MW405448, MW405448, MW405448.



**Appendix 2.** The GenBank accession numbers of the newly sequenced taxa from CP80 in this study and their voucher information. Information is presented in the following order and format: *Taxon*; *Voucher information* (Herbarium), GenBank accession number.

*Betonica officinalis* L. 2; Nanjing City, Jiangsu, China, *Chen Y.P. & al. EM467* (KUN), ON815615. *Metastachyidium sagittatum* (Regel) C.Y.Wu & H.W.Li 1; Xinyuan County, Xinjiang, China, *Ya J.D. & al. 16CS12989* (KUN), ON815617. *Metastachyidium sagittatum* (Regel) C.Y.Wu & H.W.Li 2; Tekes County, Xinjiang, China, *Ya J.D. & al. 16CS12196* (KUN), ON815616. *Paraphlomis foliata* (Dunn.) C.Y.Wu & H.W.Li; Jiangle County, Fujian, China, *Xiang C.L. & al. 441* (KUN), ON815618. *Paraphlomis hispida* C.Y.Wu; Malipo County, Yunnan, China, *Zhu X.X. s.n.* (KUN), ON815619. *Phlomis herba-venti* subsp. *pungens* (Willd.) Maire ex DeFilipps; Russia, *Wuzman E.I. s.n.* (MW), ON815620. *Phlomooides adylovii* Lazkov; Kyrgyzstan, *Lorgeav G.A. 7265* (LE), ON815621. *Phlomooides chinghoensis* (C.Y.Wu) Kamelin & Makhm.; Qinghe County, Xinjiang, China, *Chen Y.P. & al. EM857* (KUN), ON815622. *Phlomooides congesta* (C.Y.Wu) Kamelin & Makhm.; Meigu County, Sichuan, China, *Fang W. & al. DFA0303* (KUN), ON815623. *Phlomooides lehmanniana* (Bunge) Adylov, Kamelin & Makhm.; Tajikistan, *Kisemwa K.* (MW), ON815624. *Phlomooides longiaristata* (C.Y.Wu & H.W.Li) Salmaki; Fugong County, Yunnan, China, *Peng H. & al. NJ072* (KUN), ON815625. *Phlomooides pratensis* (Kar. & Kir.) Adylov, Kamelin & Makhm.; Fukang County, Xinjiang, China, *Chen Y.P. & al. EM770* (KUN), ON815626. *Phlomooides rotata* (Benth. ex Hook.f.) Mathiesen; Yadong, Tibet, China, *Chen Y.P. & al. EM1072* (KUN), ON815627. *Phlomooides sogdiana* (Pazij & Vved.) Salmaki; Uzbekistan, *Pimenov M.G. & al. 61* (MW), ON815628. *Phlomooides speciosa* (Rupr.) Adylov, Kamelin & Makhm.; Uzbekistan, *Turginov O.T. s.n.* (TASH), ON815629. *Phlomooides subspicata* (Popov) Adylov, Kamelin & Makhm.; Turkmenistan, *Polevova S. 7265* (MW), ON815630. *Phlomooides tuberosa* (L.) Moench; Burqin County, Xinjiang, China, *Chen Y.P. & al. EM852* (KUN), ON815631. *Phlomooides uniflora* (Regel) Adylov, Kamelin & Makhm.; Uzbekistan, *Mkryakii Z. & al. s.n.* (LE), ON815632.