



Photograph by Jim Mann Taylor

Phylogeny and biogeography of the lamioid mint genus *Phlomis* L.

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Forord

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Abstract

Classification of the lamioid mint genus *Phlomis* L., which comprises more than 100 species distributed in Asia, southern Europe and northern Africa, has so far been the subject of few, mainly morphological studies. The present research represents the first molecular phylogenetic study of the genus in which taxa representative of the entire genus are included. In contrast to considerable morphological and ecological variation among taxa, sequence variation in the *trnL* intron and the *trnL-trnF* intergenic spacer was remarkably low, which when analysed, resulted in sparse phylogenetic resolution, especially among the south-west Asian and the Mediterranean taxa. However, these phylogenetic results were still informative regarding *Phlomis* classification and biogeography. The results support a split of the genus into two separate groups; the taxonomic ranks of these groups as either genera or sections can be disputed. I choose here to recognise these clades as separate genera in order to decrease the taxonomic complexity of *Phlomis*. The close relationship between *Phlomis* and *Eremostachys* Bunge, pointed out by several authors, is confirmed in this study, and in a noteworthy new finding, the small Asian genus *Notochaete* Benth. is more closely related to *Phlomis* than previously assumed. Both *Eremostachys* and *Notochaete* are in fact nested within one of the clades of *Phlomis* s.l., here recognised as the genus *Phlomoides* Moench. Character optimisation analyses based on geographic distributions of all taxa show that the groups obtained by molecular phylogenetic analysis correlate well with biogeography. The results suggest that *Phlomis* s.l. has a south-west Asian origin in the area around Western China.

Introduction

Molecular phylogenetic studies of flowering plants have in many cases suggested relationships different from those proposed by the classical system builders. Many groups believed to be morphologically distinct have not turned out monophyletic in molecular phylogenies, and some groups that share only a few unique morphological characters have been robustly supported as monophyletic. One major example of the former is the evolution of the monocot lineage within the dicots, the dicots being a group that was long believed to be monophyletic based on one key characteristic, namely their two juvenile leaves (APG 1998; APGII 2003; Soltis and Soltis 2004). A counter example is the eudicots, a large lineage of angiosperms that is most often monophyletic in molecular studies (APG 1998; Källersjö et al. 1998; APGII 2003), but is united morphologically by only a single, common derived character: triaperturate pollen.

Lamiaceae (the mint family) is a large group of angiosperms in the Euasterid I order Lamiales that is easily identified by morphological characters. However, molecular studies have altered the circumscription of Lamiaceae, and some members of another Lamiales family, Verbanaceae, had to be transferred to Lamiaceae in order to make the two families monophyletic (APG 1998; Wagstaff et al. 1998). The same circumscription issue applies to the Lamiaceae subfamily Lamioideae, which must include the subfamily Pogostemoideae in order to be monophyletic (Bendiksby, Scheen, Mathiesen, Lindqvist and Albert, unpublished data; cf. Harley et al. 2004).

Stachys L. is a large lamioid genus that shows extensive infrageneric variation in morphological and cytological features and has no particular gestalt that immediately tells you it is a *Stachys*. Recent molecular studies suggest that the genus *Stachys* (as well as the tribe *Prasieae* Benth., to which it was earlier thought to belong to; Bentham 1832-1836) is polyphyletic because of both the independent origin of *S. officinalis* Franch. (and related species) and the inclusion of several other lamioid mint genera within it, e.g., *Sideritis* L., *Prasium* L. and *Phlomidioschema* Benth. (Lindqvist and Albert 2002; Bendiksby et al. unpublished data). *Phlomis* L. is another large group of lamioid mints. Unlike *Stachys*, it is easily recognised. *Phlomis sensu lato* (s.l.) is morphologically unique

as a lamioid group, but this morphology is not variable enough within the genus to make an infrageneric classification simple (Bentham 1832-1836; Kamelin and Makhmedov 1990a, b).

As early as 1794 Moench recognised morphological differences within *Phlomis* that he believed to be characteristic enough to split the taxon into two separate genera, *Phlomis* and *Phlomoides* (Moench 1794). These features have also been recognised by many other authors (Link 1829; Bentham 1832-1836; Boissier 1879; Briquet 1897; Kamelin et al. 1990a, b), and the discussion since has been to which level in the taxonomic hierarchy the two *Phlomis* groups should be assigned (see Appendix 1 for a complete overview of the classification history).

The division of *Phlomis* species into two groups has been based on several traits, including habit (the *Phlomis* group are shrubs or sub-shrubs, while members of the group *Phlomoides* are herbaceous), certain leaf characters, the shape and colour of the corolla and strong cytological data.

A brief description of *Phlomis* s.l. will highlight these differences. The leaves of *Phlomis* species are entire, corrugated and opposite. In the *Phlomis* group the leaves are lanceolate, oblong-ovate or elliptical. They are densely hairy and often leathery, while in the *Phlomoides* group the leaves are large, triangular, ovate-lanceolate, deeply cordate and membranous with sparse hairs (Azizian and Moore 1982a). The flowers are usually sessile and arranged in axillary verticillasters in both groups. The calyx is tubular or tubular-campanulate with 5 or 10 veins and 5 usually equal teeth. The 2-lipped corolla is yellow, pink or white and the upper lip is laterally compressed in the *Phlomis* group, while in the *Phlomoides* group the corolla is pink or purple, and it is not compressed but has an uneven margin and is densely bearded (see Appendix 2). In both groups the lower lip is 3-lobed with the middle lobe wider than the lateral ones, but in the *Phlomis* group the smaller size of the lateral lobes is distinct. In the *Phlomis* group the nutlets are mostly glabrous or pubescent, with simple hairs, while in the *Phlomoides* group they usually have dense, short stellate, apical hairs (Azizian et al. 1982a). The two groups within *Phlomis* differ also in chromosome number. The *Phlomis* group generally is $2n=20$ while

the *Phlomooides* group is $2n=22$. The chromosomes in section *Phlomis* are also larger than those of section *Phlomooides* (Azizian and Cutler 1982b)

The genus *Phlomis* s.l. has a wide distribution from China through Eurasia to the Mediterranean. Two centres of diversity can be recognised, south and east Anatolia and NW Iran, where all species belong to the *Phlomis* group and from the Central Asian parts of the old Soviet to E China, where all species of the group *Phlomooides* occur (Azizian et al. 1982a).

Previous studies on morphology and cytology have shown that another lamioid mint genus, *Eremostachys* Bunge, shares many characters with *Phlomis* s.l., especially with the *Phlomooides* group (Azizian et al. 1982b; Azizian et al. 1982a), and some taxonomists have suggested a complete re-circumscription of these genera to be necessary (Adylov, Kamelin, and Makhmedov 1986; Kamelin et al. 1990a, b; Makhmedov 1990).

Our study is a molecular phylogenetic one based on DNA sequences of the *trnL* intron and the *trnL-trnF* intergenic spacer. The purpose of this study is to evaluate phylogenetic relationships of *Phlomis* s.l., i.e., 1) Is *Phlomis* a monophyletic group? 2) Are the taxonomic groups described by Bentham or Kamelin and Makhmedov (1990a, b) supported? 3) What morphological features may correlate with classical vs. newly discovered internal groups? 4) Does biogeography correlate with the groups obtained in this study, and is it possible to determine the geographic origin of a common ancestor? and 5) Can morphological variation in the context of phylogeny be ascribed to speciation or ecological specialisation?

Materials and Methods

Plant materials

Most DNAs were isolated from silica gel dried samples of fresh leaf material collected at the NCCPG National Collection of *Phlomis*, UK, held by Jim Mann Taylor. Some DNAs were isolated from herbarium specimens held at Smithsonian Institution (US), Naturhistoriska Riksmuseet (S), Botanical Museum in Oslo (O), University of Uppsala (UPSV) as well as Jim Mann Taylor's private specimens (JMT). Vouchers of the JMT specimens are kept at the Botanical Museum, National History Museum, University of Oslo or at the NCCPG National Collection of *Phlomis*, UK. One sequence (*Eremostachys labiosa*) was obtained from GenBank (from Lindqvist and Albert 2002). See also Table 1 for details.

DNA extraction

Total DNA was isolated from the dried leaf tissue using the DNeasy[®] Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with two modifications made:

- 1) 1-2 cm² of the dried plant material was placed in 2 ml tubes together with two tungsten carbide beads. The plant material was then ground into a fine powder (about 2 min) at 20Hz on a mixer mill (MM301, Retsch GmbH & Co, Haan, Germany).
- 2) Step 2 was performed adding 400µl of Buffer AP1 to each sample before they were vortexed and placed in a -80°C freezer for approximately 10 min. After thawing, 4µl of RNase A was added.

PCR amplification and cycle sequencing

The *trnL* intron and the *trnL-trnF* intergenic spacer were amplified using the universal primers of Taberlet et al. (1991). For some of the herbarium samples the *trnL* intron and *trnL-trnF* intergenic spacer were amplified separately using primers "c" and "d" and primers "e" and "f", respectively. This was done due to reduced DNA quality of the samples. However, the DNA quality of the silica dried material proved to be high enough to amplify the regions together (hereafter called the *trnLF*-region) using primers "c" and "f".

PCR reactions were set up in volumes of 25 μ l using the AmpliTaq DNA polymerase buffer II kit (Applied Biosystems, Foster City, California, USA) containing 0.2 mM of each dNTP, 0.04% bovine serum albumen (BSA), 0.01 mM tetramethylammonium chloride (TMACl), 0.8 μ M of each primer and 2 μ l DNA.

The following PCR program was used both when the region was amplified as one (primers “c” and “f”) or as two (primers “c” and “d”, and “e” and “f”) fragments: 30 cycles of 50 sec 95°C (denaturation), 50 sec 60°C (annealing) and 1 min 50 sec 72°C (extension), ending with a final 4 min 72°C (extension). Successful reactions were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) following the manufacturers instructions.

Cycle sequencing was performed with BigDye Terminator (BDT) Kit v1.1 (Applied Biosystems, Foster City, California, USA) using 4 μ l BDT, 2 μ l H₂O, 1 μ l primer and 3 μ l purified PCR product and the program suggested by the manufacturer. The amount of BDT was later reduced to 2 μ l, with no noted reduced quality of the final sequences. 2 μ l of 10xBuffer was then added to compensate for the loss of reaction volume. The products were then purified either by 1) using CENTRI SEP™ Columns (Princeton Separations, Adelphia, New Jersey, USA) and Sephadex G-50 DNA Grade F (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA) or 2) precipitation in ethanol and sodium acetate using the Applied Biosystems’ protocol for 96-well MicroAmp trays (Applied Biosystems, Foster City, California, USA), only modified slightly to account for the use of PCR strips and different centrifuges. In both cases the dried samples were dissolved in 10 μ l HiDi formamide, and the fragments were separated using an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and analysed with ABI Prism® DNA Sequencing Analysis Software version 3.6.

Forward and reverse sequences were edited and aligned using the software program Sequencher™ version 4.1.4. (Gene Codes Corporation, Ann Arbor, Michigan, USA) and the consensus sequences were accessioned in GenBank.

Alignment and indel coding

The *trnLF*-region sequences were aligned automatically using the program Sequencher™ version 4.1.4 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and then manually adjusted. Alignment was unambiguous, with only relatively few nucleotide differences. The resulting matrix included 108 taxa, of which 100 *Phlomis* taxa, 3 *Eremostachys* taxa, 1 *Notochaete* taxon, 2 *Paraphlomis* taxa and 2 *Lagochilus* taxa. The latter 4 taxa were used as outgroups and *Paraphlomis javanica* was used to root the trees. For three of the taxa, *Phlomis fruticosa* (EJx1), *Eremostachys labiosa* (AF502027) and *Paraphlomis javanica* (SAG1262), only the *trnL* intron was included and the remaining sequence was coded as missing data. Insertion/deletion events (indels) were coded according to Simmons and Ochoterena's (2000) 'simple' indel coding using the program SeqState (Müller 2005) and added to the matrix as binary characters.

Phylogenetic analyses

The two *trnLF*-region matrices (with and without simple indel coding) were subjected to parsimony analysis using the program TNT (Goloboff, Nixon, and Farris 2003a). The Memory Maximum was set to 10,000 trees and all analyses were run using the Traditional Search option with default settings (Wagner Tree with 100 random replications and saving 10 trees per replication). Trees obtained were further swapped using tree bisection reconnection (TBR). Default settings were used to estimate strict (=Nelsen) consensus.

To estimate support for internal branches, parsimony jackknifing (Farris et al. 1996) was performed using the New Tech Search option in the program TNT. It was performed with parameters set to 1000 replicates, P=36, absolute values, 5 add seqs, find minimum length 1 time, and cut off point=50.

The indel coded matrix was subjected to implied weighting (with a weak weighting function where $k=50$; P. Goloboff, personal comment) and a parsimony analysis was performed on the resulting matrix. Again, trees obtained were further swapped using TBR. A strict consensus tree was estimated using default settings.

Symmetric resampling must replace jackknifing when character weights are used (Goloboff et al. 2003b). To estimate support for internal branches for the implied weighting consensus tree, symmetric resampling was performed using the New Tech Search option with parameters set to 1000 replicates, P=33, absolute values, 5 add seqs, find minimum length 1 time, and cut off point=50.

Biogeographic analyses

In search of an ancestral geographic origin of the genus, a character optimisation analysis was run in Winclada (Nixon 1999). The taxa were assigned to five groups (character states 0-4); *Group 0, Mediterranean*: the countries bordering the Mediterranean Sea. *Group 1, SW Asia*: Cyprus, Turkey, Sinai, Iran, Iraq, Israel, Jordan, Syria and Lebanon. *Group 2, Middle Asia*: Caucasus, Turkmenistan, Kazakhstan, Uzbekistan, Kyrgyzstan, Tajikistan, Afghanistan, Pakistan, Mongolia, Russia, and the Chinese provinces of Xinjiang, Qinghai, Gansu, Ningxia, Inner Mongolia, Shaanxi and Shanxi. *Group 3, South Asia*, India, Pakistan, Bhutan, Nepal, Myanmar and the Chinese provinces of Xizang, Sichuan, Yunnan, Guizhou and Guangxi. *Group 4, East Asia*: Thailand, Laos, Indonesia, Philippines, Vietnam and the Chinese provinces of Heilongjiang, Jilin, Liaoning, Hebei, Shandong, Henan, Jiangsu, Anhui, Hubei, Hunan, Jiangxi, Zhejiang, Fujian, Taiwan, Guangdong and Hainan. See also Appendix 6 for a map of China and recognised geographic regions.

In the character dialog box the default settings were used (nonadditive, activated and unselected). Only state changes with unambiguous optimisations were considered.

Table 1 List of all taxa included in this study. Herbarium codes: JMT=Jim Mann Taylor's garden or private pressed specimens, O=Botanical Museum, Oslo, S=Svenska Riksmuseet, UPSV=Uppsala University, US=Smithsonian Institution, A=Arnold Arboretum. Distributions and morphological descriptions are taken from Flora of the USSR (Knorring 1954a, b), Flora of China (Hsi-wen and Hedge 1994a, b, c, d), Flora Iranica (Rechinger 1982a, b), Flora of British India (Hooker 1885) and *Phlomis* the Neglected Genus (Taylor 1998). For a more detailed Chinese distribution see Appendix 5. For group descriptions in the optimisation analysis see Fig. 2 and the material and methods chapter.

Name	Section	Collector ID	Type of material	Geographic Distribution	Placement in character optimisation analysis	GenBank Accession #
<i>Eremostachys labiosa</i> Bunge		AF502027 (VGx2)	Sequence from NCBI	Iran & Afghanistan, Turkmenistan (+ more in C-Asia?) - Collected in Kazakhstan - <i>trnL</i> -intron only	SW Asia, Middle Asia	AF502027
<i>Eremostachys laciniata</i> Bunge		MKO200	Herb. mat. from US	Turkey, Caucasus, Iran, Jordan & Syria - Collected in Jordan	SW Asia, Middle Asia	
<i>Eremostachys laevigata</i> Bunge		JF202	Herb. mat. from UPSV	Iran and Iraq - Collected in Kurdistan	SW Asia	
<i>Lagochilus cabulicus</i> Benth	Outgroup	EE2456	Herb. mat. from S	Caucasus, Turkmenistan, Iran & Afghanistan (+more) Collected in Pakistan	Middle Asia	
<i>Lagochilus hirtus</i> Fisch. & Mey	Outgroup	IOBx1	Herb. mat. from UPSV	China & Kazakhstan Collected in Kazakhstan	Middle Asia	
<i>Notochaete hamosa</i> Benth.		SSW3649	Herb. mat. from UPSV	China, Bhutan, India, Myanmar & Nepal - Collected in Nepal	S Asia	
<i>P. × almijarensis</i> Pau	Phlomis	CM04	Silica dried from JMT	Iberian Peninsula - Collected in Spain	Mediterranean	
<i>P. × margaritae</i> A. Aparicio & Silvestre	Phlomis	CM20	Silica dried from JMT	Sierra Margarita (Spain)	Mediterranean	
<i>P. × termessi</i> P.H. Davis	Phlomis	CM40	Silica dried from JMT	Endemic to Turkey - Collected in Turkey	SW Asia	
<i>P. × trullenquei</i> Pau	Phlomis	CM73	Silica dried from JMT	Iberian Peninsula	Mediterranean	
<i>P. aff. armeniaca</i> Willd.	Phlomis	CM13	Silica dried from JMT	Endemic to Turkey	SW Asia	
<i>P. alpina</i> Pall.	Phlomoïdes	CM89	Silica dried from JMT	China, Kazakhstan & Russia	Middle Asia	
<i>P. angustissima</i> Boiss. & Balansa ex Boiss.	Phlomis	CM14	Silica dried from JMT	Endemic to Turkey	SW Asia	
<i>P. anisodonta</i> Boiss. (1)	Phlomis	CM35	Silica dried from JMT	Endemic to Iran & Iraq	SW Asia	
<i>P. anisodonta</i> Boiss. (2)	Phlomis	CM77	Silica dried from JMT	Endemic to Iran & Iraq	SW Asia	
<i>P. armeniaca</i> Willd. (1)	Phlomis	CM15	Silica dried from JMT	Endemic to Turkey	SW Asia	
<i>P. armeniaca</i> Willd. (2)	Phlomis	CM93	Silica dried from JMT	Endemic to Turkey	SW Asia	
<i>P. atropurpurea</i> Dunn (1)	Phlomoïdes	CM25	Silica dried from JMT	China	S Asia	
<i>P. atropurpurea</i> Dunn (2)	Phlomoïdes	CM26	Silica dried from JMT	China	S Asia	
<i>P. aurea</i> Decne.	Phlomis	CM91	Silica dried from JMT	Sinai, possibly in Jordan - Collected in Sinai	SW Asia	
<i>P. betonicoides</i> Diels	Phlomoïdes	CM31	Silica dried from JMT	China	S Asia	
<i>P. bourgaei</i> Boiss.	Phlomis	CM51	Silica dried from JMT	Endemic to Turkey	SW Asia	

Name	Section	Collector ID	Type of material	Geographic Distribution	Placement in character optimisation analysis	GenBank Accession #
<i>P. bovei</i> de Noé	Phlomis	CM66	Silica dried from JMT	Algeria & Tunisia	Mediterranean	
<i>P. bovei</i> de Noé ssp. <i>maroccana</i> (1)	Phlomis	CM41	Silica dried from JMT	Morocco & Algeria	Mediterranean	
<i>P. bovei</i> de Noé ssp. <i>maroccana</i> (2)	Phlomis	CM78	Silica dried from JMT	Morocco & Algeria	Mediterranean	
<i>P. brevibracteata</i> Turrill	Phlomis	CM46	Silica dried from JMT	Endemic to Cyprus	SW Asia	
<i>P. breviflora</i> Benth.	Phlomoïdes	CM58	Silica dried from JMT	Eastern Himalayas	S Asia	
<i>P. bruguieri</i> Desf.	Phlomis	FR5769	Herb. mat. from US	Turkey, Lebanon, Iran & Iraq - Collected in Iran	SW Asia	
<i>P. cancellata</i> Bunge	Phlomis	CM21	Silica dried from JMT	Iran, Turkmenistan & Caucasus - Collected in Iran	SW Asia, Middle Asia	
<i>P. capitata</i> Boiss.	Phlomis	CM22	Silica dried from JMT	Endemic to Turkey	SW Asia	
<i>P. cashmeriana</i> Royle ex Benth. (1)	Phlomis	CM67	Silica dried from JMT	Afghanistan, Kashmir & Pakistan	Middle Asia	
<i>P. cashmeriana</i> Royle ex Benth. (2)	Phlomis	CM79	Silica dried from JMT	Afghanistan, Kashmir & Pakistan	Middle Asia	
<i>P. chrysophylla</i> Boiss. (1)	Phlomis	CM72	Silica dried from JMT	Lebanon, Syria & Jordan	SW Asia	
<i>P. chrysophylla</i> Boiss. (2)	Phlomis	CM80	Silica dried from JMT	Lebanon, Syria & Jordan	SW Asia	
<i>P. cretica</i> Presl (1)	Phlomis	CM23	Silica dried from JMT	Crete, Greece & Aegean Islands - Collected in Crete	Mediterranean	
<i>P. cretica</i> Presl (2)	Phlomis	CM49	Silica dried from JMT	Crete, Greece & Aegean Islands	Mediterranean	
<i>P. crinita</i> Cav.	Phlomis	MT4781	Herb. mat. from UPSV	Spain, Morocco, Algeria & Tunisia - Collected in Andalucia, Spain	Mediterranean	
<i>P. cypria</i> Post var. <i>cypria</i>	Phlomis	CM28	Silica dried from JMT	Endemic to Cyprus - Collected in Cyprus	SW Asia	
<i>P. cypria</i> Post var. <i>occidentalis</i> Meikle	Phlomis	CM63	Silica dried from JMT	Endemic to Cyprus - Collected in Cyprus	SW Asia	
<i>P.</i> 'Edward Bowles'	Phlomis	CM54	Silica dried from JMT	(- Alleged hybrid between <i>P. russeliana</i> and <i>P. fruticosa</i>)	-	
<i>P. ferruginea</i> Tenore	Phlomis	CM08	Silica dried from JMT	Endemic to S Italy	Mediterranean	
<i>P. floccosa</i> D. Don	Phlomis	CM11	Silica dried from JMT	Tunisia, Egypt, Libya, Karpathos & Kos - Collected in Karpathos	Mediterranean	
<i>P. fruticosa</i> L. (1)	Phlomis	CM52	Silica dried from JMT	S Europe, Turkey, Crimea & Caucasus - Collected in Portugal	Mediterranean, SW Asia, Middle Asia	
<i>P. fruticosa</i> L. (2)	Phlomis	CM53	Silica dried from JMT	S Europe, Turkey, Crimea & Caucasus (Received as <i>P. grandiflora</i>)	Mediterranean, SW Asia, Middle Asia	
<i>P. fruticosa</i> L. (3)	Phlomis	CM81	Silica dried from JMT	S Europe, Turkey, Crimea & Caucasus (Received as <i>P. angustifolia</i>)	Mediterranean, SW Asia, Middle Asia	
<i>P. fruticosa</i> L. (4)	Phlomis	EJx1	Herb. mat. from UPSV	S Europe, Turkey, Crimea & Caucasus - Collected in Crete	Mediterranean, SW Asia, Middle Asia	

Name	Section	Collector ID	Type of material	Geographic Distribution	Placement in character optimisation analysis	GenBank Accession #
<i>P. grandiflora</i> H.S. Thompson (1)	Phlomis	CM32	Silica dried from JMT	Greece & Turkey - Collected on Samos Isl.	Mediterranean, SW Asia	
<i>P. grandiflora</i> H.S. Thompson (2)	Phlomis	CM55	Silica dried from JMT	Greece & Turkey - Collected in Turkey	Mediterranean, SW Asia	
<i>P. grandiflora</i> H.S. Thompson (3)	Phlomis	CM90	Silica dried from JMT	Greece & Turkey	Mediterranean, SW Asia	
<i>P. herba-venti</i> L.	Phlomis	CM70	Silica dried from JMT	France, Spain, Portugal, Sicily & Italy - Collected in France	Mediterranean	
<i>P. italica</i> L. (1)	Phlomis	CM07	Silica dried from JMT	Endemic to Balearic Islands	Mediterranean	
<i>P. italica</i> L. (2)	Phlomis	CM87	Silica dried from JMT	Endemic to Balearic Islands	Mediterranean	
<i>P. jeholensis</i> Nakai & Kitag.	Phlomoïdes	CM92	Silica dried from JMT	China - Collected in China	E Asia	
<i>P. cf. kurdica</i> Rech.f.	Phlomis	CM48	Silica dried from JMT	Turkey, Israel, Lebanon, Syria, Iran & Iraq - Collected in Turkey	SW Asia	
<i>P. lanata</i> Willd.	Phlomis	CM34	Silica dried from JMT	Crete & Karpathos	Mediterranean	
<i>P. lanceolata</i> Boiss.	Phlomis	CM24	Silica dried from JMT	Iran, Iraq & Turkey - Collected in Turkey	SW Asia	
<i>P. leucophracta</i> P.H. Davis & Hub.-Mor.	Phlomis	CM38	Silica dried from JMT	Endemic to Turkey - Collected in Turkey	SW Asia	
<i>P. linearis</i> Boiss. & Balansa var. <i>plumosa</i> Boiss.	Phlomis	CM29	Silica dried from JMT	Endemic to Turkey - Collected in Turkey	SW Asia	
<i>P. longifolia</i> Boiss. & Blanche (1)	Phlomis	CM74	Silica dried from JMT	Lebanon, Turkey & Cyprus? - Collected in Turkey	SW Asia	
<i>P. longifolia</i> Boiss. & Blanche (2)	Phlomis	CM75	Silica dried from JMT	Lebanon, Turkey & Cyprus? - Collected in Turkey	SW Asia	
<i>P. longifolia</i> Boiss. & Blanche (3)	Phlomis	CM85	Silica dried from JMT	Lebanon, Turkey & Cyprus?	SW Asia	
<i>P. lunarifolia</i> Sibth. & Sm. (1)	Phlomis	CM39	Silica dried from JMT	Cyprus & Turkey - Collected in Turkey	SW Asia	
<i>P. lunarifolia</i> Sibth. & Sm. (2)	Phlomis	CM82	Silica dried from JMT	Cyprus & Turkey - Collected in Turkey	SW Asia	
<i>P. lychnitis</i> L. (1)	Phlomis	CM10	Silica dried from JMT	France, Spain & Portugal - Collected in Spain	Mediterranean	
<i>P. lychnitis</i> L. (2)	Phlomis	CM56	Silica dried from JMT	France, Spain & Portugal (-Possible cross with <i>P. crinita</i>)	Mediterranean	
<i>P. lychnitis</i> L. (3)	Phlomis	CM143	Herb. mat. from JMT	France, Spain & Portugal - Collected in Valencia, Spain	Mediterranean	
<i>P. lychnitis</i> L. x <i>crinita</i>	Phlomis	CM06	Silica dried from JMT	- Garden hybrid	Mediterranean	
<i>P. lycia</i> D. Don	Phlomis	CM83	Silica dried from JMT	Endemic to Turkey	SW Asia	
<i>P. macrophylla</i> Wall.	Phlomoïdes	CM64	Silica dried from JMT	Temperate Himalayas	S Asia	
<i>P. megalantha</i> Diels (1)	Phlomoïdes	CM16	Silica dried from JMT	China - Collected in China	Middle Asia, S Asia, E Asia	
<i>P. megalantha</i> Diels (2)	Phlomoïdes	CM43	Silica dried from JMT	China - Collected in China	Middle Asia, S Asia, E Asia	

Name	Section	Collector ID	Type of material	Geographic Distribution	Placement in character optimisation analysis	GenBank Accession #
<i>P. milingensis</i> C.Y. Wu & H.W. Li (1)	Phlomoïdes	CM17	Silica dried from JMT	China (Tibet) - Collected in China	S Asia	
<i>P. milingensis</i> C.Y. Wu & H.W. Li (2)	Phlomoïdes	CM42	Silica dried from JMT	China (Tibet) - Collected in China	S Asia	
<i>P. mongolica</i> Turcz.	Phlomoïdes	RO159	Herb. mat. from US	China & Mongolia - Collected in Mongolia	Middle Asia, E Asia	
<i>P. monocephala</i> P.H. Davis	Phlomis	CM37	Silica dried from JMT	Endemic to Turkey - Collected in Turkey	SW Asia	
<i>P. nissolii</i> L.	Phlomis	CM30	Silica dried from JMT	Turkey, Lebanon & Syria - Collected in Turkey	SW Asia	
<i>P. oreophila</i> Kar. & Kir.	Phlomoïdes	CM47	Silica dried from JMT	China, Kazakhstan, Kyrgyzstan, Mongolia, Russia & Tajikistan	Middle Asia	
<i>P. platystegia</i> Post	Phlomis	CM71	Silica dried from JMT	Israel - Collected in Israel	SW Asia	
<i>P. pratensis</i> Kar. & Kir. (1)	Phlomoïdes	CM18	Silica dried from JMT	China, Kazakhstan & Kyrgyzstan - Collected in China	Middle Asia	
<i>P. pratensis</i> Kar. & Kir. (2)	Phlomoïdes	CM44	Silica dried from JMT	China, Kazakhstan & Kyrgyzstan - Collected in China	Middle Asia	
<i>P. purpurea</i> L. (1)	Phlomis	CM03	Silica dried from JMT	Portugal, Spain & Morocco - Collected in S Spain	Mediterranean	
<i>P. purpurea</i> L. (2)	Phlomis	CM62	Silica dried from JMT	Portugal, Spain & Morocco	Mediterranean	
<i>P. purpurea</i> L. (3)	Phlomis	CM86	Silica dried from JMT	Portugal, Spain & Morocco	Mediterranean	
<i>P. purpurea</i> L. (4)	Phlomis	MT4782	Herb. mat. from UPSV	Portugal, Spain & Morocco	Mediterranean	
<i>P. purpurea</i> L. ssp <i>almeriensis</i> (Pau) Losa & Rivas Goday ex Rivas Mart.	Phlomis	CM02	Silica dried from JMT	Spain - Collected in Spain	Mediterranean	
<i>P. purpurea</i> × <i>lychnitis</i>	Phlomis	CM01	Silica dried from JMT	- Garden hybrid	Mediterranean	
<i>P. rigida</i> Labill.	Phlomis	CM33	Silica dried from JMT	Lebanon, Turkey, Syria, Iran & Iraq - Collected in Turkey	SW Asia	
<i>P. rotata</i> Benth. ex Hook.f. (<i>Lamiophlomis rotata</i> Kudo 1929)	Phlomoïdes	FL15600	Herb. mat. from US	China & Alpine Sikkim Himalaya - Collected in Tibet	Middle Asia, S Asia	
<i>P. russeliana</i> Lag. ex Benth.	Phlomis	CM69	Silica dried from JMT	Endemic to Turkey	SW Asia	
<i>P. samia</i> L. (1)	Phlomis	CM50	Silica dried from JMT	Former Yugoslavia, Greece & Turkey - Collected in Greece	Mediterranean	
<i>P. samia</i> L. (2)	Phlomis	CM76	Silica dried from JMT	Former Yugoslavia, Greece & Turkey - Collected in Turkey	Mediterranean	
<i>P. samia</i> L. (3)	Phlomis	CM84	Silica dried from JMT	Former Yugoslavia, Greece & Turkey	Mediterranean	
<i>P. sp.</i>	?	CM12	Silica dried from JMT	- Collected in Turkey	-/-	
<i>P. tatsienensis</i> Bureau & Franck var. <i>hirticalyx</i> (Hand.-Mazz.) C.Y. Wu (2)	Phlomoïdes	CM45	Silica dried from JMT	China - Collected in China	S Asia	
<i>P. tatsienensis</i> Bureau & Franck var. <i>hirticalyx</i> (Hand.-Mazz.) C.Y. Wu (1)	Phlomoïdes	CM19	Silica dried from JMT	China - Collected in China	S Asia	

Name	Section	Collector ID	Type of material	Geographic Distribution	Placement in character optimisation analysis	GenBank Accession #
<i>P. taurica</i> Hartwiss ex Bunge	Phlomis	CM36	Silica dried from JMT	Crimea, Caucasus	Middle Asia	
<i>P. tuberosa</i> L. (1)	Phlomoides	CM09	Silica dried from JMT	Hungary, former Yugoslavia, Bulgaria, Greece, Turkey, Iran, China, Kazakhstan, Kyrgyzstan, Mongolia & Russia	Mediterranean, SW Asia, Middle Asia, E Asia	
<i>P. tuberosa</i> L. (2)	Phlomoides	CM88	Silica dried from JMT	Hungary, former Yugoslavia, Bulgaria, Greece, Turkey, Iran, China, Kazakhstan, Kyrgyzstan, Mongolia & Russia - Collected in Portugal	Mediterranean, SW Asia, Middle Asia, E Asia	
<i>P. tuberosa</i> L. (3)	Phlomoides	TE6937	Herb. mat. from US	Hungary, former Yugoslavia, Bulgaria, Greece, Turkey, Iran, China, Kazakhstan, Kyrgyzstan, Mongolia & Russia - Collected in Siberia	Mediterranean, SW Asia, Middle Asia, E Asia	
<i>P. umbrosa</i> Turcz var. <i>australis</i> Hemsley (1)	Phlomoides	CM68	Silica dried from JMT	China	Middle Asia, S Asia, E Asia	
<i>P. umbrosa</i> Turcz var <i>australis</i> Hemsley (2)	Phlomoides	BB1360	Herb. mat. from US	China - Collected in China	Middle Asia, S Asia, E Asia	
<i>P. umbrosa</i> Turcz. var. <i>umbrosa</i>	Phlomoides	CM65	Silica dried from JMT	China	Middle Asia, S Asia, E Asia	
<i>P. urodonta</i> Popov	Phlomoides	CM27	Silica dried from JMT	Kyrgyzstan	Middle Asia	
<i>P. viscosa</i> Poir (2)	Phlomis	CM60	Silica dried from JMT	Lebanon, Syria, Palestine & Turkey	SW Asia	
<i>P. viscosa</i> Poir (3)	Phlomis	CM61	Silica dried from JMT	Lebanon, Syria, Palestine & Turkey	SW Asia	
<i>P. viscosa</i> Poir. (1)	Phlomis	CM59	Silica dried from JMT	Lebanon, Syria, Palestine & Turkey	SW Asia	
<i>Paraphlomis javanica</i> Prain	Outgroup	SAG1262	Herb. mat. from A	China, India, Indonesia, Laos, Malaysia, Myanmar, Pakistan, Philippines, Thailand & Vietnam -Collected in China (Guizhou) - <i>trnL</i> -intron only	Middle Asia, E Asia, S Asia	
<i>Paraphlomis rugosa</i> Prain	Outgroup	JFR1097	Herb. mat. from US	-Collected in Thailand	S Asia	

Results

Sequence divergence and alignments

The length of the *trn*LF-region sequences varied from 862 bp (*Phlomis purpurea*) to 886 bp (*Paraphlomis rugosa*). The complete matrix, including *Paraphlomis*, *Lagohilus*, *Notochaete*, *Eremostachys* and *Phlomis* taxa, had an aligned length of 932 bp. The program SeqState (Müller 2005) recognized 48 indels, which were coded as present/absent. The resulting matrix contained 980 characters.

Phylogeny reconstruction

The maximum parsimony analysis of the *trn*LF-region without indel coding produced 32 most parsimonious trees (MPTs) of 88 steps (results not shown). The maximum parsimony analysis of the *trn*LF-region with indel coding produced 1360 MPTs with 159 steps (Fig. 1). The maximum parsimony analysis of the indel coded matrix with implied weighting (k=50) produced 870 MPTs with a best score of 0.62138. This analysis gave a slightly more resolved tree (see Fig. 1 for strict consensus).

The results of this cpDNA study showed that the species of *Phlomis*, *Eremostachys* and *Notochaete* together formed a monophyletic group (marked as ‘Ingroup’ in Fig. 1) with jackknife/symmetric resampling support of 86/91%, respectively. *Phlomis* s.l. is paraphyletic with regard to *Notochaete* and *Eremostachys*, while the *Eremostachys* taxa included formed a monophyletic group, albeit with no jackknife/symmetric resampling support.

This expanded ingroup was split in two monophyletic sister groups (Fig. 1). Group I, corresponded to the “*Phlomoides* group” plus the three *Eremostachys* taxa and the single *Notochaete* taxon, and it received jackknife/symmetric resampling support of 61/70%. Group II corresponded to the “*Phlomis* group” and received jackknife/symmetric resampling support of 99/99%.

Within groups I and II twelve monophyletic subgroups were recognised (I_A-II_H, Fig. 1). These groups are mainly based on the geographical distributions of the taxa included.

Within group I, four subgroups were recognised (groups I_A-I_D, Fig. 1). Members of group I_A are distributed in the northern and western parts of China, Kazakhstan, Kyrgyzstan, Mongolia, Russia and Tajikistan. The taxa in group I_B are distributed in Kyrgyzstan, Turkmenistan,

Caucasus, Iran and Iraq. Group I_C includes taxa distributed in northern China, Kazakhstan, Mongolia, Kyrgyzstan and Russia, as well as *Phlomis tuberosa*, a taxon that is distributed throughout almost the entire *Phlomis* s.l. distribution range, from China to South-eastern Europe. Members of group I_D are distributed throughout China (except in the north-western province of Xinjiang), Northern India, Bhutan Myanmar, Nepal and the Himalayas.

Within group II, two monophyletic sister groups were formed (Fig.1), Group II_A and a large, mainly unresolved group, the “SW Asian clade”. Group II_A included two accessions of *P. cashmeriana* and received a jackknife/symmetric resampling support of 87/92%. *Phlomis cashmeriana* is distributed in Afghanistan, Pakistan and Kashmir. The large “SW Asian clade” received a jackknife/symmetric resampling support of 60/69% and it included seven subgroups, II_B-II_H (Fig.1). Group II_B is a non-geographically coherent group that received jackknife/symmetric resampling support of 64/68%. It included one hybrid accession and two *P. fruticosa* accessions. The taxa of group II_C are distributed in Iran, Iraq, and Afghanistan, Turkmenistan and Caucasus and the group received jackknife/symmetric resampling support of 60/67%. Group II_D is also a non-geographic group and included two *P. italica* taxa and one alleged *P. purpurea*. The group received jackknife/symmetric resampling support of 65/76%. Group II_E included three *P. longifolia* accessions and received jackknife/symmetric resampling support of 62/66%. *Phlomis longifolia* is distributed in Lebanon, Asian Turkey and Cyprus. Members of group II_F are distributed in the Middle East, Iran, Iraq and Asian Turkey, and the group received jackknife/symmetric resampling support of 86/93%. Group II_G included taxa that are all part of a putative Spanish hybrid complex (see Aparicio and Albaladejo 2003; Albaladejo, Aparicio, and Silvestre 2004); this group received a jackknife/symmetric resampling support of 51/62%. Group II_H included taxa distributed in Asian Turkey, Cyprus and Greece and received a jackknife/symmetric resampling support of 79/83%.

The results of the character optimisation analysis (Fig. 2) gave a detailed picture of the geographical groups described above. The analysis assigned a Middle Asian ancestral geographic origin of *Phlomis* s.l. Within group I, the “*Phlomooides* group”, a South Asian origin is assigned to group I_D. Within group II, the “*Phlomis* group”, a South West Asian origin was assigned to the “SW Asian clade”.

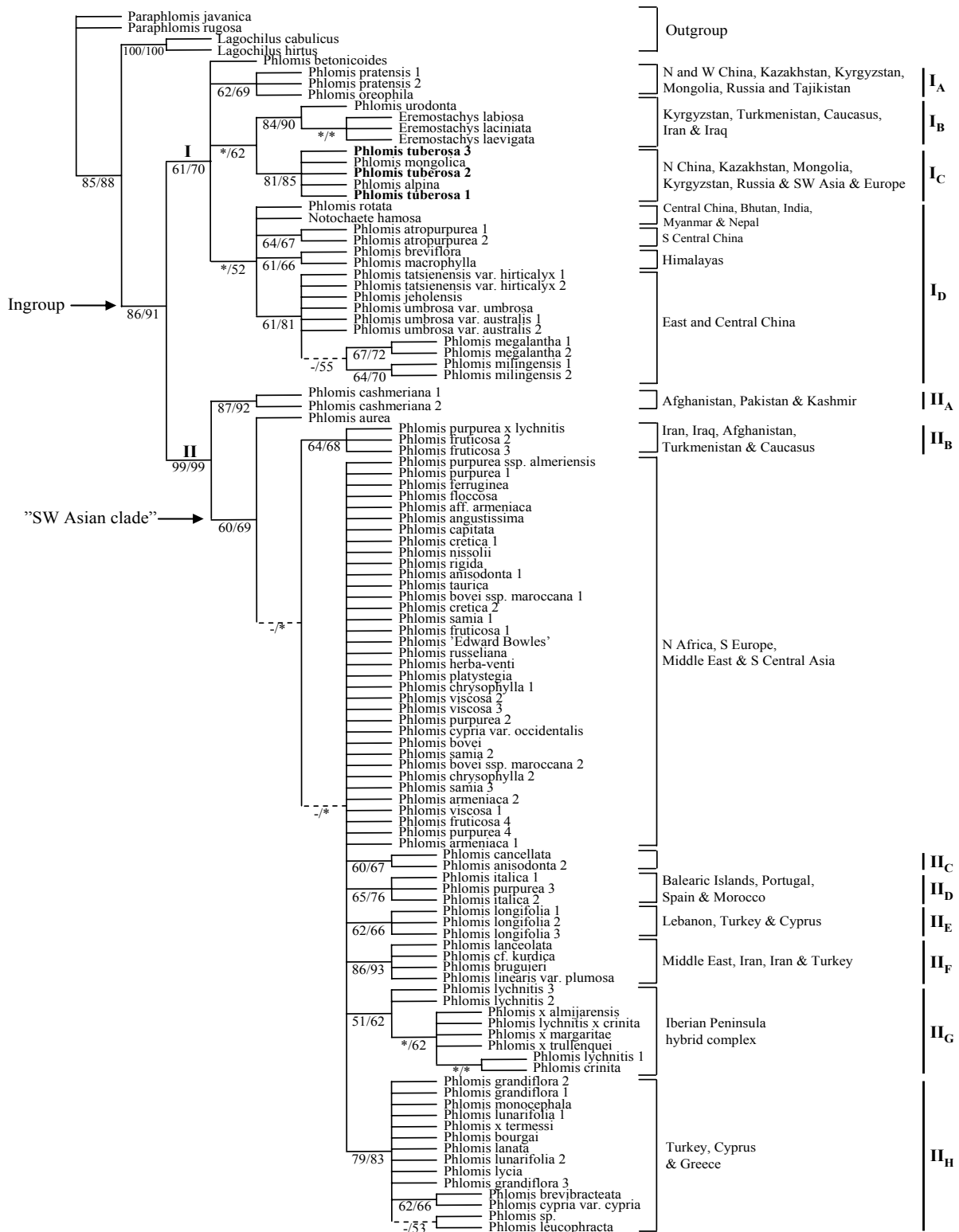


Figure 1. Phylogeny of *Phlomis* and related taxa based on *trnLF*-region sequence data. The strict consensus of 870 trees derived from implied weighting ($k=50$) is shown. Stippled lines denote branches that collapse in the strict consensus without implied weighting. Jackknife/symmetric resampling values above 50% are shown (in that order) below branches. Branches that collapse in the jackknife/symmetric resampling analyses are denoted with an asterisk (*). A dash (-) denotes that the group was not identified in the analysis without implied weighting and hence received no jackknife support. Geographical distributions are shown to the right of the tree. *Phlomis tuberosa* (**bold**) has a distribution range from SE Europe spreading through the former USSR, China and the Middle East. Numbers following taxon names refer to individual accessions.

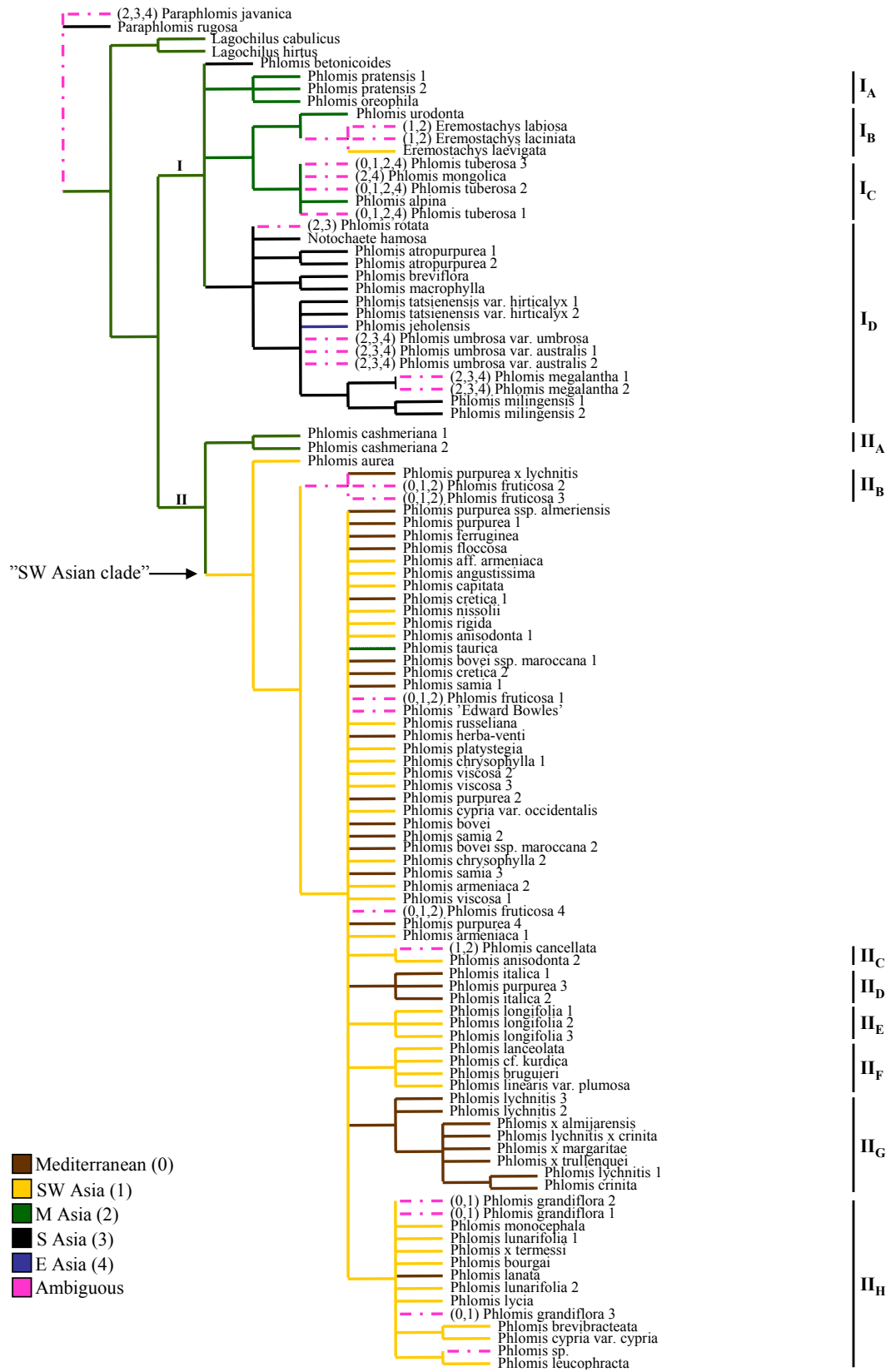


Figure 2. Strict consensus tree with character optimisations (unambiguous changes only) based on general geographic distributions (see Materials and Methods section). Numbers in brackets refer to character states in Winclada (Nixon 1999)

Discussion

Circumscription of *Phlomis*

A large parsimony analysis of the entire subfamily Lamioideae (Bendiksby, Scheen, Mathiesen, Lindqvist and Albert, unpublished data) shows *Phlomis* to be the sister group to a diverse group of mints, including *Lamium*, *Lagochilus/Leonurus*, *Ballota/Marrubium* and a broadly defined clade containing the genus *Leucas*. The analysis shows two possible scenarios: 1) *Phlomis* comes out unresolved with *Paraphlomis* and the diverse group (without implied weighting, results not shown) or 2) *Paraphlomis* is resolved as the sister group of *Phlomis* plus the diverse group of mints (with implied weighting; Fig. 3).

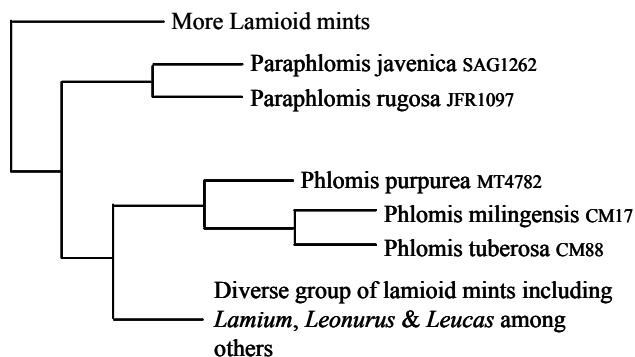


Figure 3. Schematic phylogeny of the Lamioideae, showing the position of *Phlomis* with regard to its closest relatives in a strict consensus tree with implied weighting ($k=50$) (Bendiksby et al. unpublished data).

In the present cpDNA study *Phlomis* (marked as 'Ingroup' in Fig.1.) is not monophyletic, since members of the genera *Eremostachys* and *Notochaete* are nested within the genus.

Within the extended *Phlomis* group we find two sister groups, group I and II (Fig.1), corresponding to the two sections *Phlomoides* and *Phlomis* (Bentham 1832-1836), respectively. The three *Eremostachys* taxa group with *Phlomis urodonta* with high resampling support (I_B, Fig 1). This close relationship between *Eremostachys* and *Phlomoides* is in congruence with the opinions of earlier workers based on morphological, anatomical, cytological, phytochemical and palynological data (Azizian et al. 1982b; Azizian et al. 1982a; Adylov, Kamelin, and Makhmedov 1987; Kamelin et al. 1990a, b). In fact, in the work of

Azizian and Cutler (1982b), it is stated that “*Phlomis* and *Eremostachys* are so similar in their anatomy that one description will cover both genera.”

In the works by Adylov, Kamelin and Makhmedov (Adylov et al. 1986, 1987; Kamelin et al. 1990a, b; Makhmedov 1990), the two sections *Phlomis* (86 spp.) and *Phlomoides* (136 spp.) were raised to the generic level and the old genus *Eremostachys* Bunge was split into three different genera: the restored *Phlomoides* Moench, (73 spp.), the new *Paraeremostachys* T.A. Adylov, Kamelin. & A.M. Makhmedov (15 spp.), and *Eremostachys* Bunge (9 spp.).

The close relationship between *Notochaete* Benth. (a genus with only two species) and *Phlomis* (I_D, Fig.1) has not been proposed previously. Since the one species of *Notochaete* included here, *Notochaete hamosa*, is part of a polytomy, it is not possible at present to determine the precise relationship of *Notochaete* to any particular species within the “*Phlomoides* group”. However, the taxon has an affinity with the taxa of group I_D, which correlates well with regard to distribution range. *Notochaete hamosa* is found in China, Bhutan, India, Myanmar & Nepal, a geographic range which overlaps with that of group I_D. The fact that the type species of the genus *Notochaete*, *N. hamosa*, groups with *Phlomis* in all the analyses of the large lamioid mint data set of Bendiksby et al. (unpublished data) makes the placement of the genus within *Phlomis* relatively certain, and a recombination of *Notochaete hamosa* is necessary (see Appendix 7 for the new combination).

Infrageneric relationships

The descriptions of the infrageneric taxonomic groups of Bentham (1832-1836) and Kamelin and Makhmedov (1990a; 1990b) were based on morphological characters: corolla colour, number of flowers in the verticillasters and the shape and indumentum of leaves and bracts, and on habitat. None of the infrageneric taxonomic groupings of these authors are supported in this study. Within group II, the “*Phlomis* group”, it is difficult to state anything definite because it is highly unresolved, but the clades that receive some jackknife/symmetric resampling support do not correspond to any established infrageneric groups.

Bentham (1832-1836) did not recognise any taxonomic levels below his section *Phlomidopsis*, which corresponds to our group I, the “*Phlomoides* group”. In the classification system of Kamelin and Makhmedov (1990b) the genus *Phlomoides* is split into two sections, section *Phlomoides* and section *Filipendula*. Section *Phlomoides* is comprised of all the previous

Phlomis section *Phlomooides* species and one *Eremostachys* species, *E. vulnerantes*, while section *Filipendula* is comprised of previous *Eremostachys* species and one newly described species, *P. lanatifolia* (Makhmedov 1986). The sections were split into nine and twelve subsections, respectively. *Eremostachys vulnerantes* was placed in a monotypic subsection of section *Phlomooides*, while the three *Eremostachys* species included in this analysis were placed in two different subsections of section *Filipendula*. Within the “*Phlomooides* group” the infrageneric classification proposed by Kamelin and Makhmedov (1990b) is not congruent with our results.

Biogeography

The genus *Phlomis* s.l., as well as both groups I and II, were assigned a Middle Asian geographic ancestral origin in the character optimisation analysis (Fig. 2). The first shift to a different ancestral origin comes within group II wherein the “SW Asian clade” is assigned a South West Asian origin. The two different geographic origins seen here correlate well with the two main centres of diversification noted by Azizian and Moore (1982a). In a character optimisation analysis where all the provinces of China were assigned to one group (results not shown) the origin of Group I turned out to be Chinese, which may suggest the origin of this group to be in currently Chinese regions of Middle Asia.

The topology of the cpDNA phylogeny is correlated with the geographical distribution of the different taxa (groups I_{A-H} in Figs. 1 and 2 mainly denote groups of geographic distribution among the two groups within *Phlomis* s.l.). The four subgroups of group I, I_A-I_D (Figs. 1 and 2), are monophyletic groups that are more or less supported in the jackknife/symmetric resampling analyses. Group I_A contains taxa distributed in the Central Asian countries of Kazakhstan, Kyrgyzstan, and Tajikistan, Russia, and the northern and western parts of China, and were assigned a Middle Asian origin in the character optimisation analysis (Fig. 2). Group I_B includes taxa from Kyrgyzstan, Turkmenistan, Caucasus, Iran and Iraq and was assigned a Middle Asian origin as well. Group I_C includes taxa from a wide distribution range, mostly due to *Phlomis tuberosa*, the only *Phlomis* species to be found throughout the entire distribution range, from the north-westernmost province in China (Heilongjiang) through northern China, the Central Asian countries and the Middle East to Southern Europe (Knorring 1954a; Huber-Morath 1982; Hsi-wen et al. 1994c; Taylor 1998). This group is assigned a middle Asian origin

in the optimisation analysis (Fig. 2). The species in groups I_A-I_C grow mainly in alpine meadows and grassy slopes at elevations from 800-3000 m, except *P. tuberosa* which grows in wet grasslands and valleys. In group I_D we find *Phlomis* taxa distributed throughout China, except the Xinjiang province, and in Bhutan, India, Myanmar and Nepal (see also Appendices 5 and 6 for details on Chinese distribution and map of China). This group has been assigned a South Asian origin (Fig. 2). Many of the species of group I_D grow in moist soil or in forests, a trait that distinguishes them from the members of group I_{A-C}, and at elevations from 300-4400 m.

Within the “*Phlomis* group” eight monophyletic subgroups are found, II_{A-H}. Of these, only four groups, II_A, II_C, II_F and II_H, show a geographic pattern. Group II_A includes *P. cashmeriana* alone and is distributed in Afghanistan, Pakistan and Kashmir. This ancestral geographic origin of this group follows that of the base of the tree and is hence Middle Asian. Group II_C is distributed in Afghanistan, Turkmenistan, Caucasus and Iran (*P. cancellata*) and Iran and Iraq (*P. anisodonta*) and the group is assigned a south-west Asian origin (Fig. 2). Both group II_F and II_H are assigned a South West Asian origin. In Group II_H all but three taxa, *P. lanata*, *P. brevibracteata* and *P. cypria* var. *cypria* are found in Turkey. The latter two are endemic to Cyprus and come out as a group with jackknife/symmetric resampling support of 62/66%.

The Iberian Peninsula hybrid complex (group II_G)

In Andalucia and Levante (southern and eastern Spain, respectively) the two species *P. lychnitis* and *P. crinita* hybridise, and morphologically intermediate plants have been recognised since the early 20th century (Pau 1918a). The hybrid complex has been treated by many authors (Pau 1918a, b, 1922; Mateu 1986; Rivas-Martínez et al. 1991) and all with different taxonomic scenarios. The study by Albaladejo, Aparicio and Silvestre (2004), however, presented a scenario with three parental taxa, *P. lychnitis*, *P. crinita* ssp. *crinita* (from Levante) and *P. crinita* ssp. *malacitana* (from Andalucia), and only one hybrid taxon, *P. x composita*, including all morphological intermediates among the parental taxa. This is also in accordance with the results of Mateu (1986) who named all hybrids between the three taxa mentioned above *P. composita* Pau. Hence all three hybrid taxa included in group II_G belong to the same taxon sensu Mateu, but their biological relationships may differ from their taxonomic recognition.

The hybrid taxa mentioned above and four parental individuals are included in group II_G (Fig. 1), although with very low jackknife/symmetric resampling support values (51/62%).

Phlomis x margaritae is a hybrid between *P. purpurea* and *P. composita* (Aparicio 1997; Aparicio et al. 2000). From the placement of the taxa in our results one can deduce that, if the aforementioned parents are correct, *P. composita* is the maternal parent of the hybrid (given maternal plastid inheritance in Lamiaceae; Corriveau and Coleman 1988). A study of the Iberian parental taxa by Albeladejo et al. (2005) showed that the pattern of chloroplast haplotype variation is geographic rather than taxonomic. This result can also be discerned to some extent in this study; however, analyses of the *trnL*F-region have also yielded taxonomic groups.

Non-geographical groups that deserve special attention

Group II_B is a monophyletic group of three taxa that received jackknife/symmetric resampling support of 64/68%. It includes *P. purpurea x lychnitis*, *P. fruticosa* 2 and *P. fruticosa* 3 (Fig.1). According to Jim Mann Taylor, *P. fruticosa* 2 was received as *P. grandiflora* and *P. fruticosa* 3 as *P. angustifolia* (J. M. Taylor, personal notes), but morphologically they are not similar to *P. grandiflora* nor *P. angustifolia*. Taylor believed they could be *P. fruticosa*, but he did not exclude the possibility that the two accessions were hybrids (J. M. Taylor, personal comment). The placement of these two *P. fruticosa* accessions makes it likely that they are more related to the hybrid taxon *P. purpurea x lychnitis* than the remaining two *P. fruticosa* accessions included.

Although it is not possible to state anything definite about these two taxa based on the present results, the obvious difficulty in determining their identity based on morphology and their phylogenetic placement suggests they may be hybrids of unknown parental origin. The parental (maternal) status of *P. purpurea x P. lychnitis* cannot be confirmed from our results, since the accession does not group with any of the hypothesised parents. This may imply that neither *P. purpurea* nor *P. lychnitis* is the maternal parent, however, this part of the phylogenetic tree is highly unresolved and other possibilities cannot be excluded.

Group II_D includes two *P. italica* accessions and one *P. purpurea* accession. However, the latter accession was a gift to Jim Mann Taylor from a garden in Greece, and only alleged to be *P. purpurea* (J. M. Taylor, personal notes). Taylor himself has based on morphological

comparisons, strong doubts that the plant is *P. purpurea* (Taylor, personal comment). In our analyses this accession does not group with any of the other *P. purpurea* accessions included (which are all part of the large polytomy of the “SW Asian clade”, Fig.1), an indication that it might be distinct from *P. purpurea* and more closely related to *P. italica*.

Ecological specialisation: at the clade or species level?

Within the genus *Phlomis* s.l. there is little correlation between the morphological differences noted by, e.g., Bentham (1832-1836) and Kamelin and Makhmedov (1990a; 1990b) and the genetic variation seen in the *trnLF*-region studied here (see also Appendix 1 and 2). Habit (herbaceous versus woody) is a notable exception. In the work by Azizian and Moore (1982a), where several morphological and palynological characters were studied, the infrageneric groups obtained supported those of previous workers (Bentham 1832-1836; Boissier 1879; Briquet 1897; Post 1932; Knorring 1954a). However, morphological differences with regard to various leaf characters (e.g., indumentum and shape) and flower characters (e.g., calyx shape, calyx-teeth shape and corolla colour) appear much more likely to correlate with ecological specialisation rather than genetic relationships. For example in Bentham (1832-1836) *P. lychnitis* is placed in a separate subsection based on the shape of the bracteoles, while in our results the taxon is nested deep within the “SW Asian clade”. Bentham also placed *P. cashmeriana* together with *P. lunarifolia* among others in a subsection based on the numbers of flowers in the verticillasters and the shape of the bracts and apices.

Phlomis rotata Benth. ex Hook (Group I_D, Fig. 1) was transferred to a monotypic genus, *Lamiophlomis* (as *L. rotata*) by Kudo (1929) since he believed its morphological features to be unique: it is monocarpic and has a basal rosette pressed flat to the ground as well as characteristic differences in leaf shape of young plants versus flowering plants (Hsi-wen et al. 1994d; Taylor 1998). In Flora of China (Hsi-wen et al. 1994d), although described as a *Lamiophlomis*, it is stated that it is “perhaps best regarded as a specialized species of *Phlomis*, especially as the differences noted between the genera seem to be quantitative only”. The close affinity to *Phlomis* is supported in this molecular study, where *Phlomis rotata* is nested within group I, the “*Phlomoides* group”, a placement that also fits perfectly with its distribution range. The unique features of this taxon may be due to its habitat; *Phlomis rotata* grows in weathered alpine alluvial fans, stony alpine meadows and floodplains at elevations of 2700-4900 m (Hsi-

wen et al. 1994d). The taxon is not included in Kamelin and Makhmedov's description of their genus *Phlomooides* (1990b) and a new combination of the species is therefore necessary (Appendix 7).

Concluding remarks

The genus *Phlomis* proved to show relatively low interspecific genetic variation in the cpDNA *trnL*F-region, very similar to the *Stachys* results of Lindqvist and Albert (2002). The results of the present study nevertheless support splitting of *Phlomis* s.l. into two separate genera, *Phlomooides* Moench and *Phlomis* sensu stricto. *Phlomis* s.l. is already a large genus and its taxonomic complexity would increase even further with the inclusion of the approximately 73 *Eremostachys* species as suggested by Kamelin and Makhmedov (1990b). Recognition of *Phlomis* sensu stricto and *Phlomooides*, which are substantially supported morphologically (Knorring 1954a; Azizian et al. 1982b; Azizian et al. 1982a; Hsi-wen et al. 1994c) and now also molecularly, would result in a major decrease in the taxonomic complexity of *Phlomis* as it is circumscribed today. Further research with more molecular markers, especially nuclear loci, will be needed to specify the generic composition of *Phlomooides* with regard to *Eremostachys* and *Notochaete*. The groups obtained here correlate well with geographic distribution, however, the infrageneric relationships, especially within *Phlomis* s.str., remain more or less unresolved in this study due to poor variation in the molecular marker used.

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Appendices

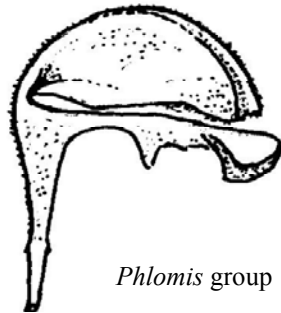
Appendix 1. Classification history of the genus *Phlomis* L.

	Genus/Genera	Section (s)	Subsection (s)	References
Common classification	<i>Phlomis</i>	<i>Phlomis</i> <i>Phlomoides</i>		
Linné	<i>Phlomis</i>			(Linné 1735, 1753)
Moench	<i>Phlomis</i> ----- <i>Phlomoides</i>			(Moench 1794)
Link	<i>Phlomis</i> ----- <i>Phlomidopsis</i>			(Link 1829)
Bentham	<i>Phlomis</i>	<i>Euphlomis</i> ----- <i>Phlomidopsis</i>	<i>Lychnitis</i> <i>Gymnophlomis</i> <i>Dendrophlomis</i> <i>Oxyphlomis</i>	(Bentham 1832-1836)
Boissier	<i>Phlomis</i>	<i>Euphlomis</i> ----- <i>Phlomidopsis</i>	<i>Gymnophlomis</i> <i>Dendrophlomis</i> <i>Oxyphlomis</i>	(Boissier 1879)
Briquet	<i>Phlomis</i>	<i>Euphlomis</i> ----- <i>Phlomoides</i>	<i>Lychnitis</i> <i>Gymnophlomis</i> <i>Dendrophlomis</i> <i>Oxyphlomis</i>	(Briquet 1897)

Appendix 1 continued

	Genus/Genera	Section (s)	Subsection (s)	References
Adylov, Kamelin & Makhmedov	<i>Phlomis</i>	<i>Phlomis</i>	<i>Phlomis</i> <i>Viscosae</i>	(Kamelin et al. 1990a)
		<i>Platyphlomis</i>		
<i>Oncophlomis</i>				
<i>Gymnophlomis</i>		<i>Gymnophlomis</i> <i>Angustissimae</i> <i>Macrodongta</i> <i>Oppositiflorae</i>		
<i>Lychnites</i>				
<i>Oxyphlomis</i>		<i>Oxyphlomis</i> <i>Paradendrophlomis</i> <i>Samiae</i> <i>Rigidae</i> <i>Orientalis</i> <i>Australes</i> <i>Brevispiniae</i> <i>Albiflorae</i> <i>Trineurae</i>		
	<i>Phlomoides</i>	<i>Phlomoides</i>	<i>Phlomoides</i> <i>Orientalis</i> <i>Melananthae</i> <i>Mulienses</i> <i>Tetragonae</i> <i>Vulnerantes</i> <i>Alpinae</i> <i>Tianschanicae</i> <i>Annuae</i>	(Kamelin et al. 1990b)
		<i>Filipendula</i>	<i>Cordatae</i> <i>Integrifoliae</i> <i>Rhodanthae</i> <i>Hissaricae</i> <i>Spectabilis</i> <i>Lyratae</i> <i>Fulgentes</i> <i>Speciosae</i> <i>Laciniatae</i> <i>Labiosae</i> <i>Gymnocalyces</i>	

Appendix 2. Drawings and photographs demonstrating the difference in corolla shape within the two *Phlomis* groups



Phlomis group



Phlomoides group

Drawings by Jim Mann Taylor



Photo from
www.dipbot.unict.it

Phlomis fruticosa (lectotype for the *Phlomis* group)



Photograph by Anne-Cathrine Scheen

Phlomis tuberosa (lectotype for the *Phlomoides* group)

Appendix 3. Key to *Phlomis*, *Phlomoides*, *Eremostachys* and *Paraeremostachys*
 (Adylov et al. 1986) Translated by Jan Wesenberg, University of Oslo

- 1. Upper lip of corolla laterally compressed, flattened, sickle-shaped.....*Phlomis* L.
- + Upper lip not laterally compressed, not flattened, arch-shaped.....2.
- 2. Main root swollen into turnip-shaped tubers or 2-3 differently sized tubers placed close together by the root neck. Side roots not swollen.....3.
- + Main root not swollen. Side roots in the form of small tubers, sometimes like a strand of pearls, more rarely roots almost non-swollen.....*Phlomoides* Moench.
- 3. Calyx funnel-shaped, already during flowering with very broad membranous wheel-shaped rim distinct from the calyx tube.....*Eremostachys* Bunge.
- + Calyx tube-shaped, tube-bell-shaped or bell-shaped, without rim, or teeth bend first during fruit stage and form a short, indistinct rim
*Paraeremostachys* Adyl. R. Kam. et Machmedov.

**Appendix 4. Key to *Notochaete*, *Eremostachys*, *Leucas*, *Phlomis* and
*Lamiophlomis***
(modified from Hsi-wen and Hedge 1994e)

- Upper lip of corolla convex or galeate, rarely nearly flat, always densely hairy.
1. Calyx teeth 5, spinescent, hooked at apex.....*Notochaete* Benth.
 - + Calyx teeth 5-10, not spinescent and hooked at apex.....2.
 2. Nutlets prominently hairy at apex; filaments with comblike-fimbriate appendages at base; calyx teeth truncate, apiculate at apex; desert-meadow plants.....
.....*Eremostachys* Bunge
 - + Nutlets slightly hairy or glabrous; other characters not as above.....3.
 3. Calyx 10-toothed.....*Leucas* R. Brown
 - + Calyx 5 toothed.....4.
 4. Upper corolla lip always hairy or fringed -incised; posterior stamens not appendiculate at base; mostly erect herbs or subshrubs; leaf venation not fan-shaped; verticillasters axillary, lax or dense.....*Phlomis* L.
 - + Upper corolla lip not fringed or incised; posterior stamens not appendiculate at base; stemless herbs; leaf venation fan-shaped; verticillasters in a dense scapose capitulum or short spike.....*Lamiophlomis* Kudo

Appendix 5. Detailed geographical distributions of taxa in the “Phlomoides group”

Species	Habitat	Geographical distribution	Reference
<i>Phlomis betonicoides</i>	Grassy slopes, forested grassland, forests	Sichuan, Xisang (=Tibet) & Yunnan	Flora of China (Hsi-wen et al. 1994c)
<i>P. pratensis</i>	Alpine meadows, 1500-3000m	Xinjiang Kazakhstan & Kyrgyzstan	Flora of China (Hsi-wen et al. 1994c) & Flora of Russia (Knorring 1954a)
<i>P. oreophila</i>	Grassy slopes, 2100-3000m	Xinjiang Kazakhstan, Kyrgyzstan, Mongolia, Russia & Tajikistan	Flora of China (Hsi-wen et al. 1994c) & Flora of Russia (Knorring 1954a)
<i>P. urodonta</i>	Woodland and scrub zone	Lake Sary-Chilek area (Kyrgyzstan)	Flora of Russia (Knorring 1954a)
<i>Eremostachys labiosa</i>	Foothills, on loess deposits	Turkmenistan	Flora of Russia (Knorring 1954b)
<i>E. laciniata</i>	Stony mountain slopes	Caucasus, Asia Minor & Iran	Flora of Russia (Knorring 1954b) & Flora Iranica (Rechinger 1982b)
<i>E. laevigata</i>		Iran & Iraq	Flora Iranica (Rechinger 1982b)
<i>Phlomis mongolica</i>	Grassy slopes, 800-2200m	Gansu, Hebei, Nei Mongol (=Inner Mongolia), Shaanxi & Shanxi	Flora of China (Hsi-wen et al. 1994c)
<i>P. alpina</i>	Alpine and forest zones, in meadows.	Xinjiang Kazakhstan & Russia	Flora of China (Hsi-wen et al. 1994c) & Flora of Russia (Knorring 1954a)
<i>P. tuberosa</i>	Wet grasslands, valleys, 1200-2100m	Heilongjiang, Nei Mongol, Xinjiang, Kazakhstan, Kyrgyzstan, Mongolia, Russia, SW Asia & Europe	Flora of China (Hsi-wen et al. 1994c) & Flora of Russia (Knorring 1954a)
<i>Notochaete hamosa</i>	Margins of subtropical evergreen forests, valleys, 1200-2500m	Yunnan, Central & Eastern temperate Himalaya	Flora of China (Hsi-wen et al. 1994a) & Flora of British India (Hooker 1885)
<i>Phlomis rotata</i> (<i>Lamiophlomis</i>)	Weathered alpine alluvial fans, stony alpine meadows, floodplains, 2700-4900m	Gansu, Qinghai, Sichuan, Xisang, Yunnan & Alpine Sikkim Himalaya	Flora of China (Hsi-wen et al. 1994d) & Flora of British India (Hooker 1885)
<i>P. atropurpurea</i>	Marshy meadows, 2800-3900m	Yunnan	Flora of China (Hsi-wen et al. 1994c)

Appendix 5 continued

Species	Habitat	Geographical distribution	Reference
<i>P. breviflora</i>	Does well in moist soil (JMT), 300-3600m	Eastern Himalaya	Flora of British India (Hooker 1885)
<i>P. macrophylla</i>	On open slopes, 3100-4100m	Temperate Himalaya	Flora of British India (Hooker 1885)
<i>P. tatsienensis</i> var. <i>hirticalyx</i>	Grassy slopes, forests, 2500-3400m	Yunnan	Flora of China (Hsi-wen et al. 1994c)
<i>P. jeholensis</i>	Hillsides, wet areas	Hebei	Flora of China (Hsi-wen et al. 1994c)
<i>P. umbrosa</i> var. <i>australis</i>	Hillsides, grasslands, stream sides, thickets; 1600-3200 m	Anhui, Gansu, Guizhou, Hubei, Hunan, Shaanxi, Sichuan & Yunnan	Flora of China (Hsi-wen et al. 1994c)
<i>P. umbrosa</i> var. <i>umbrosa</i>	Sparse forests, grassy slopes; 2000-3200 m	Gansu, Guangdong, Guizhou, Hebei, Hubei, Liaoning, Nei Mongol, Shaanxi, Shandong, Shanxi & Sichuan	Flora of China (Hsi-wen et al. 1994c)
<i>P. megalantha</i>	Abies forests, thickets, grassy slopes, 2400-4200m	Hubei, Shaanxi, Shanxi & Sichuan	Flora of China (Hsi-wen et al. 1994c)
<i>P. milingensis</i>	Picea forests, thickets, 3400-4400m	Xisang	Flora of China (Hsi-wen et al. 1994c)

Appendix 6. Map of the Provinces of China



Map of China. Red borders separate the three geographic areas used in the character optimisation analysis.

Appendix 7. New combinations

Phlomoides rotata (Benth. ex Hook.f.) C. Mathiesen, *comb. nov.*

Basionym: *Phlomis rotata* Benth. ex Hook.f. -- Fl. Brit. Ind. iv. 694.

Notes: *Phlomis rotata* is the type for *Lamiophlomis* Kudo

Phlomoides hamosa (Benth.) C. Mathiesen, *comb. nov.*

Basionym: *Notochaete hamosa* Benth. -- Pl. Asiat. Rar. i. 63.

Notes: *Notochaete longiaristata* C.Y.Wu & H.W.Li may eventually need transfer as well, but I have not seen this species, so no change is made at this time.