TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2017) 41: 392-403

doi:10.3906/bot-1608-38

Phlomis iranica (Lamiaceae: Lamioideae), a new species from the Khorassan-Kopet Dagh floristic province, NE Iran

Jamil VAEZI[1,*], Maryam BEHROOZIAN[1], Mohammad Reza JOHARCHI[2], Farshid MEMARIANI[2]
[1]Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
[2]Department of Botany, Research Centre for Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

Received: 24.08.2016 • **Accepted/Published Online:** 11.02.2017 • **Final Version:** 18.07.2017

Abstract: The genus *Phlomis* L. consists of three species, *Ph. cancellata* Bunge, *Ph. chorassanica* Bunge, and *Ph. herba-venti* L., in the Khorassan-Kopet Dagh floristic province, NE Iran. A new species is described and illustrated here as *Ph. iranica* Joharchi & Vaezi sp. nov. from this area. We included voucher specimens of the new and allied species in a morphological and molecular (nrDNA ITS) framework to examine the taxonomic status of the new taxon. Results showed that *Ph. iranica* can be morphologically distinguished from the species distributed in NE Iran by seven diagnostic traits including length and width of calyx tube, flower numbers in the lowermost verticillaster, length of branches in lowest and highest inflorescences, width of lowest leaf in branches, and width of lower lip corolla. Results obtained from the molecular phylogenetic tree are consistent with those obtained from the diagnostic morphological characters in which the taxonomic status of *Ph. iranica* is confirmed as a new species. The conservation status of the new species was evaluated as Critically Endangered.

Key words: Lamiaceae, *Phlomis*, new species, morphology, molecular phylogeny, Iran

1. Introduction

According to previous taxonomic studies, the number of species of the genus *Phlomis* L. (Lamiaceae: Lamioideae) has been estimated to be from over 100 (Azizian and Cutler, 1982) to about 90 (Salmaki et al., 2012, after excluding some synonymous species reported by Govaerts et al., 2010). The genus has a continuous distribution from the Mediterranean basin to East China through the westernmost and northeastern parts of Iran (Azizian and Moore, 1982). Southern and eastern Anatolia and northwestern Iran are presented as the centers of *Phlomis* species diversity (Azizian and Moore, 1982). Representatives of the genus grow as shrubs or subshrub plants with the sessile flowers organized in axillary verticillasters. The calyx form is tubular and distinctly five-toothed. The upper lip of the corolla is laterally compressed while the lower one is three-lobed with the middle lobe wider than the lateral ones. Moreover, the inside of the corolla margin is not bearded (Azizian and Moore, 1982).

The genus is represented by 28 species including four hybrids in the *Flora Iranica* area (Rechinger, 1982), of which 20 species (including three hybrids) occur in Iran. Jamzad (2012) in *Flora of Iran* reported 22 species including three hybrids for Iran. However, according to *Flora Iranica* (Rechinger, 1982), three *Phlomis* species including *Ph. cancellata* Bunge, *Ph. herba-venti* L., and *Ph. chorassanica* Bunge are distributed in NE Iran. Moreover, Jamzad (2012) represented a new hybrid (*Ph.* × *wendelboi* Jamzad) for this region. Of these species, *Ph. chorassanica* was described by Bunge (1873) based on a specimen collected in July 1858 from the Tus region of NE Iran and preserved in the G-BIOSS herbarium (Rechinger, 1982). To date, no further collections were reported for this species. Nonetheless, after several fieldtrips at the type locality, we could not find the species. It seems that its type locality was possibly destroyed before the species could extend its local distribution range.

The Khorassan-Kopet Dagh floristic province in the Irano-Turanian region is located in the mountainous areas of northeastern Iran and somewhat in southern Turkmenistan. The area is a transition zone and a corridor connecting different provinces of the Irano-Turanian region and also the Hyrcanian mountain forests of the Euro-Siberian region. The unique combination of Irano-Turanian species and also the presence of a local center of endemism are evidence of a separate biogeographic entity (Memariani et al., 2007, 2016a, 2016b).

* Correspondence: vaezi@um.ac.ir

In a recent field examination of some unexplored mountainous areas of the easternmost limits of the Khorassan-Kopet Dagh floristic province, we found an individual of *Phlomis* restricted to Zaloo Mountain (Figure 1). After an exhaustive investigation in the area, we found a few scattered plants indicating that these individuals have possibly been geographically isolated for a long time. At first glance, it appeared that the new species (hereafter referred to as *Ph. iranica*) was morphologically somewhat similar to *Ph. herba-venti* due to their almost identical growth form and corolla color, while when we used the *Phlomis* identification key presented in *Flora Iranica* (Rechinger, 1982), *Ph. iranica* seemed to be more related to *Ph. persica* Boiss. The latter species is completely distributed in western Iran with nonoverlapping geographical distribution with *Ph. iranica*. Likewise, when we utilized the *Phlomis* identification key presented in the *Flora of Turkey* (Huber-Morath, 1982), the new species appeared to be related to *Ph. integrifolia* Hub.-Mor., a species endemic to Malatya, Turkey, and related to *Ph. persica* with regard to the growth form, cauline and floral leaf form/size, and corolla color. We also compared the new species with the type photograph of *Ph. chorassanica* obtained from the herbarium G-BIOSS. We found no similarity between them in terms of several traits such as the growth form, leaf size, number and density of verticillasters, and calyx size. Moreover, when we used the *Phlomis* identification key presented in *Flora of the USSR* (Shishkin, 1954), *Ph. iranica* tended to nest into the series *Salicifoliae* Knorr. based largely upon the characteristics of leaves, bracteoles, and verticillasters. However, in order to ascertain the exact taxonomic status of the new species as an independent taxon, we included individuals of *Ph. persica*, *Ph. herba-venti*, and *Ph. cancellata* in the current study.

It appears that using the assemblage of two or more independent sources of data could ideally strengthen and support our understanding of the taxonomic status of a new or problematic species. From this point of view, several investigators demonstrated that using both morphological and molecular evidence provides valuable features for species delimitation (Duminil et al., 2012; Farsi et al., 2013; Vaezi et al., 2014; Dutta et al., 2015; Korkmaz et al., 2015; Xu et al., 2015). The purpose of this study was to describe

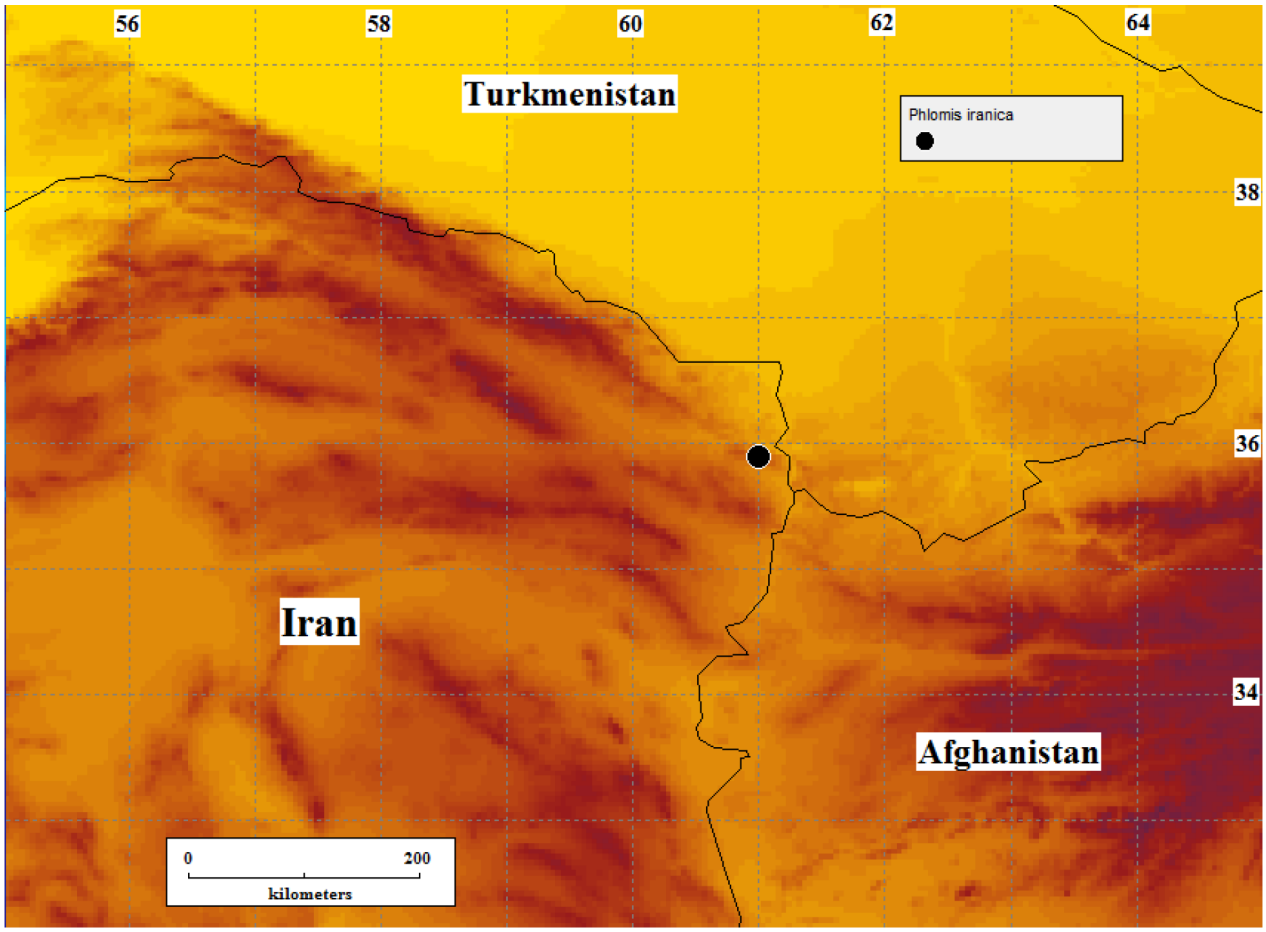

Figure 1. Geographical distribution of *Phlomis iranica* in Khorassan-Kopet Dagh, NE Iran.

and illustrate *Phlomis iranica* as a new species using a combination of morphological and molecular datasets.

2. Materials and methods

2.1. Sampling and conservation survey

For the morphological study, we included voucher specimens of *Phlomis cancellata*, *Ph. herba-venti*, and *Ph. persica* as well as the new species (*Ph. iranica*) collected from different localities in NE Iran or obtained from herbarium specimens of FUMH and TUH (Table 1). All field-collected voucher specimens were preserved at FUMH. For the molecular study, fresh-collected materials were preserved in silica gel. Moreover, we integrated new sequences obtained from the current work with those obtained from GenBank belonging mostly to some closely related *Phlomis* species distributed in Iran (Table 2).

Table 1. List of taxa included in the morphological (Mor.) and molecular (Mol.) study, voucher information, and GenBank accessions of ribotypes sequenced in the current work.

Species	Locality	Lat./ long.	Voucher no.	Mor.	Mol.	GenBank acc.
Ph. cancellata	North Khorassan, Faruj towards Oghaz-e Kohne (FUMH)	36°22′30.60″N 60°25′35.82″E	29382	√	-	-
Ph. cancellata	Khorassan Razavi, Sarakhs, around the Bazangan cave (FUMH)	37°27′47.58″N 58°13′41.06″E	27149	√	-	-
Ph. cancellata	Khorassan Razavi, NE Mashhad, Azhdarkuh, Joghri (FUMH)	36°29′N 59°52′E	23242	√	-	-
Ph. cancellata	North Khorassan, N Faruj, Najafabad (FUMH)	37°17′N 58°13′E	42751	√	√	KY088279
Ph. herba-venti	East Azarbayjan, N slopes of Mishoudagh, south of the main road near Yam (S of Marand) (TUH)	45°48′29″N 38°20′E	55260	√	-	-
Ph. herba-venti	West Azarbayjan, Sardasht, Zamziran pass (TUH)	36°20′N 45°34′E	7558	√	-	-
Ph. herba-venti	East Azarbayjan, Arasbaran Protected Area, 1 km from Kaleybar towards Hejrandoust (TUH)	38°53′N 47°00′E	39808	√	-	-
Ph. herba-venti	Mazandaran, 10 km from Marzanabad towards Kelardasht (TUH)	36°26′38.44″N 51°15′19.60″E	8429	√	-	-
Ph. herba-venti	North Khorassan, W Bojnurd, Almeh (FUMH)	37°31′17.42″N 56°23′14.34″E	2176	√	-	-
Ph. herba-venti	West Azarbayjan, Rezaeiyh-Ghasemlu (TUH)	37°20′N 45°09′E	7557	√	-	-
Ph. herba-venti	Mazandaran, Marzanabad, on the road towards Kojur (TUH)	36°27′11.98″N 51°19′41.38″E	20464	√	-	-
Ph. herba-venti	East Azarbayjan, Arasbaran Protected Area (TUH)	38°50′48.2″N 46°54′34.4″E	39830	√	-	-
Ph. herba-venti	West Azarbayjan, Sardasht, Zamziran pass (TUH)	36°20′N 45°34′E	7563	√	√	KY088280
Ph. persica	Kermanshah, between Kuzaran and Gahvareh (TUH)	34°26′17.93″N 46°28′01.12″E	18351	√	-	-
Ph. persica	Chaharmahal and Bakhtiyari, Shahrekord, Hafshejan (TUH)	32°13′N 50°47′E	14399	√	√	KY088282
Ph. persica	Markazi, between Shazand and Arak (TUH)	34°01′01.23″N 49°33′56.13″E	7575	√	-	-
Ph. iranica	Khorassan Razavi, Torbat-e Jam, NW Salehabad, Zaloo Mount (FUMH)	35°53′30.8″N 60°59′50.2″E	45824	√	-	-
Ph. iranica	Khorassan Razavi, Torbat-e Jam, NW Salehabad, Zaloo Mount (FUMH)	35°53′31.6″N 60°59′50.3″E	45061	√	√	KY088281

Table 2. List of *Phlomis* and *Phlomoides* species and their GenBank accession numbers used in this study.

Species	GenBank acc.
Phlomis purpurea	AY792819
Phlomis elliptica	JN680365
Phlomis anisodonta	JN680363
Phlomis bruguieri	JN680366
Phlomis lychnitis	AY792793
Phlomis composita	AY839236
Phlomis crinita	AY792808
Phlomis brevibracteata	KP828804
Phlomis floccosa	EU827091
Phlomis cretica	KP828808
Phlomis fruticosa	KF529539
Phlomis lanata	KP828815
Phlomoides strigosa	EU827110
Phlomoides umbrosa	EU827108
Phlomoides ornata	EU827104

A distribution map of *Ph. iranica* was prepared using DIVA-GIS version 7.3 software (Hijmans et al., 2001) based on distribution data points. Moreover, the threat status (IUCN, 2014) was evaluated using the geographical ranges of the new species in the form of the extent of occurrence (EOO) and the area of occupancy (AOO) for criterion B. For this purpose, we used GeoCAT to calculate EOO and AOO for the Red Listing (Bachman et al., 2011).

2.2. Morphological methods

For the morphological description of the new species and evaluating its relationships with morphologically related species (*Ph. cancellata*, *Ph. herba-venti*, and *Ph. persica*), we assessed 25 fully developed quantitative and qualitative morphological traits (Table 3). In this regard, a total of 18 well-developed specimens belonging to the above-mentioned species were evaluated (Table 1). Quantitative morphological traits were measured using a ruler with precision of 1 mm. Unfortunately, due to the inaccessibility of samples of *Ph. integrifolia*, in this study we compared morphological features of *Ph. iranica* with those available for *Ph. integrifolia* as described in *Flora of Turkey* (Huber-Morath, 1982) (Table 3).

2.3. Molecular methods

2.3.1. DNA extraction and sequencing

DNA extraction of silica-dried leaves was carried out using a modified CTAB procedure (Doyle and Doyle, 1987) described by Joly et al. (2006). For the three species *Ph. cancellata*, *Ph. persica*, and *Ph. iranica*, at least one representative voucher was included in the study. The voucher specimens of the two first species were identified according to *Flora Iranica* (Rechinger, 1982). Species names and accession numbers of the samples used in this study are listed in Table 1.

The internal transcribed spacer (ITS) region of the nuclear ribosomal DNA was PCR-amplified using universal primers ITS4 and ITS5 as described by White et al. (1990). Amplification of DNA sequences was performed in volumes of 25 µL containing 10X PCR buffer (Fermentas, Lithuania), 2.5 µL of $MgCl_2$ (25 mM, Fermentas), 0.2 mM of each dNTP, 2 U of Taq polymerase, 100 µmol/L of the universal primers ITS4 and ITS5, and about 200 ng of genomic DNA. PCR amplifications were performed under the following conditions: one initial denaturation step at 95 °C for 5 min followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 45 s, and extension at 72 °C for 1 min with a final extension at 72 °C for 7 min. PCR products were purified according to PEG purification (Joly et al., 2006). Direct sequencing was conducted using Macrogen's sequencing service (Macrogen Inc., Korea). Sequences were edited using Sequencher version 5.2.4 (Gene Codes Inc., Ann Arbor, MI, USA). ITS sequences from 12 *Phlomis* and three *Phlomoides* species were obtained from GenBank and they were added to the molecular matrix (Table 2).

2.3.2. Phylogenetic analyses

ITS sequence alignments were performed using Clustal W (Thompson et al., 1994) as implemented in BioEdit Sequence Alignment Editor (Hall, 1999) followed by further manual adjustments. We coded the indels following the simple indel coding method (Simmons and Ochoterena, 2000) using SeqState version 1.25 (Müller, 2005). An evolutionary model that was best fitted for all nucleotide datasets was determined using MrModeltest 2.2 (Nylander, 2004). The model GTR+I was selected for the total nucleotide data considering the Akaike information criterion (Vaezi and Brouillet, 2009; Vaezi et al., 2014).

Maximum parsimony (MP) analysis was performed using a heuristic search with 1000 random addition replicates and tree-bisection reconnection branch swapping. Characters were equally weighted and character states were specified as unordered. Most parsimonious trees were summarized using the consensus tree methods available in PAUP* (Swofford, 2002). Maximum likelihood (ML) analysis was also performed using a heuristic search with five random sequence addition replicates and the model selected. Bootstrap branch support in MP and ML analyses was performed using 1000 bootstrap replicates. Both the MP and ML analyses were implemented in PAUP*.

Table 3. Diagnostic morphological characters of the taxa included in the present study. Measurement units are specified in parentheses.

Character/species	*Ph. iranica*	*Ph. persica*	*Ph. cancellata*	*Ph. herba-venti*	*Ph. integrifolia*
Number of inflorescence branches	2	3	0	2–7	NA
Flower numbers in lowermost verticillaster	6	10	14–20	10	NA
Length of branches in lowest verticillaster (mm)	40–42	138–209	0	225–355	NA
Length of branches in highest verticillaster (mm)	25–27	46–95	0	100–227	NA
Floral leaf length in uppermost verticillaster (mm)	68–71	25–37	45–70	27–50	NA
Floral leaf length in lowermost verticillaster (mm)	60–62	28–68	0	27–37	NA
Gland on floral leaves	Absent	Absent	Present	Present/absent	NA
Width of lowest leaf in branches (mm)	8–10	15–21	0	16–45	NA
Length of calyx (mm)	14–16	7–8	8–10	7–9	10–12
Width of calyx tube (mm)	5–5.5	3–3.5	3.5–4	2.5–3	NA
Length of three-branched bracteole (mm)	12–17	7–11.5	12–15	10–21	5–7
Length of single-branched bracteole (mm)	0	0	14–18	0	NA
Corolla length (mm)	25–27	14–17	14–19	15–25	20–25
Length of corolla tube (mm)	13–14	7–9	6–9	7–13	NA
Length of upper lip of corolla (mm)	10–11	5–6	6–8	8–12.5	NA
Length of lower lip of corolla (mm)	10–11	4.5–5	7–8	5–11	NA
Width of lower lip of corolla (mm)	11–12	4–5	5–5.5	4–8	NA
Trichome type on stem	Short stellate	Short stellate	Short stellate	Branched stellate with densely monoradial hairs-short stellate with articulate hairs	Stellate-tomentose
Length of monoradial trichome on floral leaf (mm)	0	0	1–2	1–2.25	NA
Trichome type on floral leaves	Short stellate	Short stellate	Short stellate with articulate hairs	Branched stellate with densely monoradial hairs-short stellate with articulate hairs	Tomentellous-canescent
Length of monoradial trichome on calyx (mm)	0	0	1–2	1–2.75	NA
Trichome type on calyx	Short stellate	Short stellate	Branched stellate with loosely/densely monoradial hairs	Branched stellate with loosely/densely monoradial hairs-short stellate with articulate hairs	Stellate-tomentose
Monoradial trichome length on calyx teeth (mm)	0	0	0.8–1.5	0.5–2.25	NA
Trichome type on calyx teeth	Short stellate	Short stellate	Branched stellate with loosely monoradial hairs	Branched stellate with loosely/densely monoradial hairs-short stellate with articulate hairs	NA
Cilium on upper lip of corolla	Short and few	Short and few	Long and many	Short and few	NA

NA: Not available.

All datasets were further investigated using the Bayesian optimality criterion as implemented in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). The Bayesian MCMC inference was run for five million generations, sampling each 100 generations. The number of generations to be discarded was determined using Tracer version 1.4 (Rambaut and Drummond, 2007). Trees were visualized using TreeView version 1.6.6 (Page, 2001).

3. Results

3.1. Morphological study

Twenty-five morphological characters vary among the species included in the study. Of these, 19 and 6 are quantitative and qualitative traits, respectively. Overall, 21 of 25 (84%), 15 of 25 (60%), and 15 of 25 (60%) characters differentiated the species pairs *Phlomis cancellata* vs. *Ph. iranica*, *Ph. herba-venti* vs. *Ph. iranica*, and *Ph. persica* vs. *Ph. iranica*, respectively (Table 3).

3.2. Molecular study

A total of 19 aligned ITS sequences including three accessions of the outgroups were added to the matrix used in the molecular analyses. The length of the entire ITS region was 686 and 662 bp with and without coded gaps, respectively. A total of 433 bp was obtained from *Ph. cancellata* due to missing some nucleotides from both the 5′ and 3′ ends of its sequence. Of the 686 aligned characters, only 63 were parsimony-informative, while the remaining 623 were constant and parsimony-uninformative characters. The alignment of 41 polymorphic sites for the four species included in the morphological study is shown in Table 4.

Analysis of the ITS sequences using Bayesian inference with the GTR+I substitution model resulted in a 50% majority rule consensus tree that was topologically quite congruent with those inferred from the MP and ML trees (Figure 2). The MP analysis of inferred sequences yielded 114 equally most parsimonious trees (L = 156; CI = 0.801; RI = 0.806; RC = 0.646). In the phylogenetic tree constructed (Figure 2), two main clusters (excluding the three outgroup species) were observed. The first cluster (I) included *Ph. purpurea*, *Ph. cancellata*, and *Ph. persica*. *Phlomis iranica* (the new species) and the remaining species included in the molecular study formed the second main cluster (II). *Phlomis iranica* appeared as a sister to the remaining species of the second cluster.

4. Discussion

Previous studies indicated that the phytogeographical region of the Khorassan-Kopet Dagh floristic province may have had a potential impact on speciation, probably due to its transitional zone and high diversity of habitats (Memariani et al., 2007, 2016a, 2016b). *Allium xiphopetalum* Aitch. & Baker is distributed from the central Zagros and Alborz mountains to Afghanistan and Pamir-Alai through the transitional Khorassan-Kopet Dagh zone (Memariani et al., 2007). Furthermore, *Dianthus polylepis* Bien. ex Boiss. and *D. pseudocrinitus* Behrooz. & Joharchi have been considered to be endemic to this region (Farsi et al., 2013; Vaezi et al., 2014).

Based on the morphological results, *Phlomis iranica* is characterized by seven species-specific diagnostic traits consisting of length of calyx, flower numbers in lowermost verticillaster, width of calyx tube, length of branches in lowest inflorescence, length of branches in highest inflorescence, width of lowest leaf in branches, and width of lower lip corolla (Table 3). Accordingly, 7 out of the 25 (28%) differentiating morphological characters (Table 3) effectively discriminated the new species from those included in the morphological study. Interestingly, 9 out of the 41 (22%) variable nucleotide sites (54, 65, 73, 74, 118, 404, 422, 609, and 615; Table 4) exclusively discriminate *Ph. iranica* from the three closely related species, *Ph. persica*, *Ph. cancellata*, and *Ph. herba-venti*. Compared with the description available for *Ph. integrifolia* presented in *Flora of Turkey* (Huber-Morath, 1982), the bracteoles, calyx, and corolla are longer in *Ph. iranica* (Table 3). In addition, the shape of the leaf base and leaf apex is attenuate and acute, respectively, in *Ph. iranica*, while they are cuneate and suboblate in *Ph. integrifolia*. Therefore, at the taxonomic level, the results obtained from both the phylogenetic and morphological studies seem largely congruent in showing the new species as generally distinct. Similarly, previous studies have shown that the molecular analyses of the ITS sequences strongly support the results of morphological studies (Farsi et al., 2013; Mytnik-Ejsmont et al., 2014; Vaezi et al., 2014). According to the results obtained from the morphological study (Table 3), the most closely related species to *Phlomis iranica* are *Ph. persica* and *Ph. herba-venti* with 15 (60%) differentiating traits, followed by *Ph. cancellata* with 21 (84%) discriminating characters.

4.1. Taxonomic treatment

Phlomis iranica Joharchi & Vaezi, **sp. nov.** (Figures 3 and 4)

Typus: Iran. Khorassan Razavi province, Torbat-e Jam, NW Salehabad, Zaloo Mount, 1263 m, 35°53′31.6″N, 60°59′50.3″E, 02.06.2013, Joharchi & Behroozian 45061 (Holotype FUMH, Isotype TUH).

Perennial, 15–25 cm high; roots woody, multicipital; stems erect, densely stellate-hairy with equal and unequal hairs, mixed monoradial long articulate hairs; radical leaves 8.5–17.5 cm long and 6–12 mm broad, linear-lanceolate, entire, at base attenuate, acute in apex; cauline leaves absent, rarely one pair; floral leaves 3–13 cm long and 4–12 mm broad, resembling the radical leaves; upper surface of leaves pale green with scattered stellate hairs; lower surface of leaves with very densely appressed stellate and monoradial hairs. Verticillasters 1–3, 2–6-flowered; bracteoles 12–17 mm long, three-branched, subulate, spinescent, covered by equal and unequal stellate hairs. Calyx ±15 mm long, tubular-campanulate, covered by dense stellate hairs; tube ±12 mm long, posterior teeth ±3 mm long (spines 2 mm), interior teeth ±4 mm long (spines 3 mm), emarginated. Corolla lilac-pink, 25–27 mm long; upper lip ±11 mm, ciliate, lower lip as long as upper lip; middle lobe of the lower lip 12 mm wide; the lateral lobes triangular, teeth 3 mm long; filaments glabrous; nutlets

Table 4. Sequence alignment (polymorphic sites only) of ITS sequences of four *Phlomis* accessions. Dots indicate matched nucleotide states to the first ribotype, hyphens indicate alignment gaps, and question marks indicate missing data.

Species	Aligned polymorphic positions																																										
	46	48	54	61	65	73	74	82	94	118	164	167	176	191	196	202	354	390	402	404	410	414	421	422	423	451	454	455	456	458	503	505	543	564	570	571	575	576	577	609	615
Ph. herba-venti	C	A	C	A	C	C	T	A	T	G	A	T	G	T	G	G	T	C	C	T	–	T	G	G	C	A	–	–	–	A	G	G	T	T	T	A	C	T	A	C	C
Ph. cancellata	?	?	?	?	?	?	?	?	C	.	G	C	A	T	A	T	.	T	–	.	C	.	.	.	.	.	–	–	–	.	C	A	?	?	?	?	?	?	?	?	?
Ph. persica	–	G	.	C	.	.	.	G	C	.	G	C	T	C	.	T	C	.	.	.	C	C	T	A	T	–	A	T	C	C	.	.	C	C	C	G	T	C	G	.	.
Ph. iranica	–	G	G	.	A	A	C	G	C	T	G	C	.	C	.	.	.	.	.	C	–	C	.	C	.	.	–	–	–	.	.	.	C	C	C	G	T	.	G	T	A

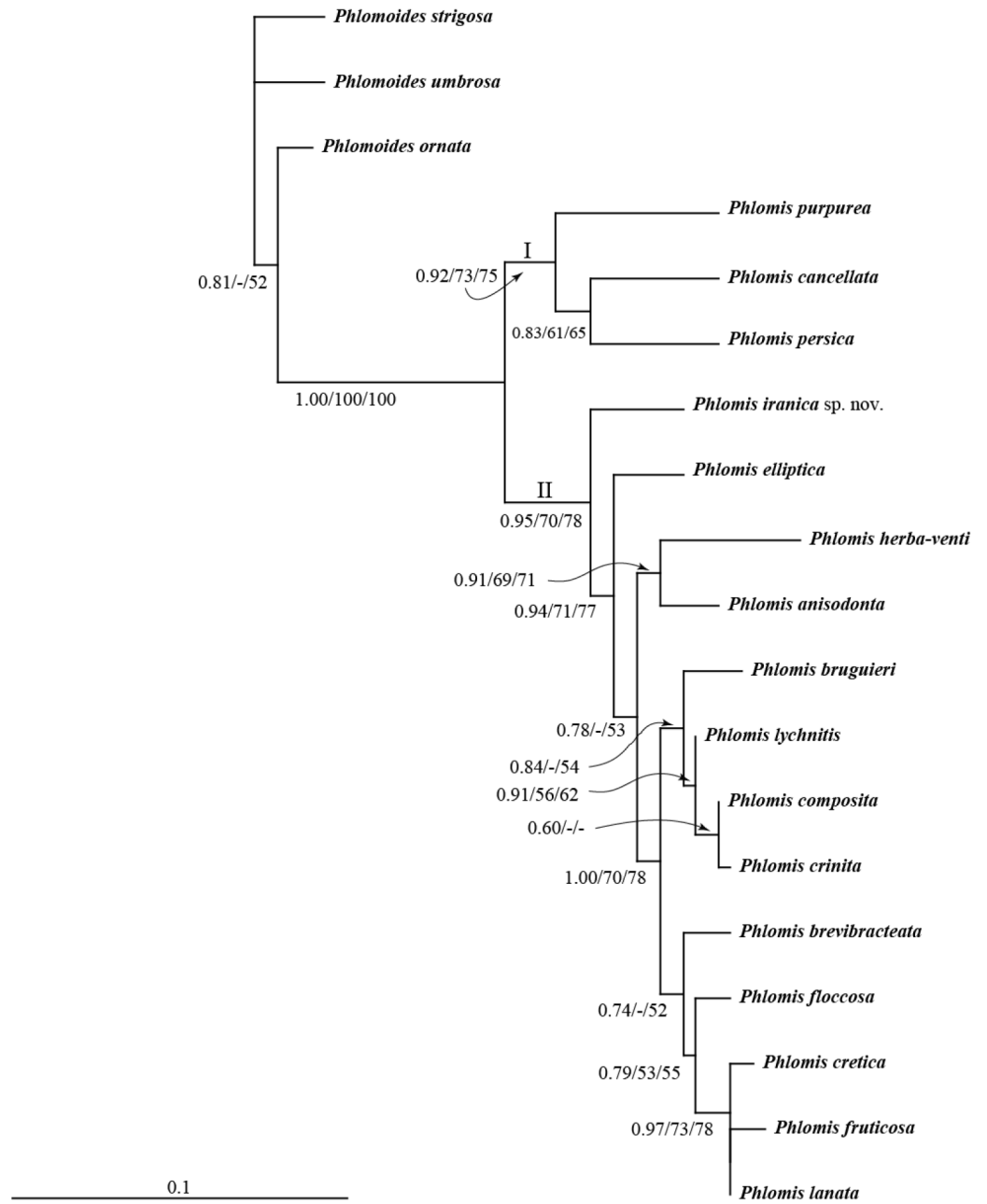

Figure 2. Phylogenetic relationships among the *Phlomis* species under study and allied species resulting from the ITS dataset based on Bayesian, ML, and MP analyses. The numbers above or below the branches represent Bayesian posterior probabilities/ML bootstrap supports/MP bootstrap supports, respectively. Hyphens indicate that the bootstrap support is below 50. The roman numbers (I and II) on the branches are explained in the text.

Figure 3. Holotype of *Phlomis iranica* sp. nov. deposited in the FUMH herbarium (Joharchi & Behroozian, 45061).

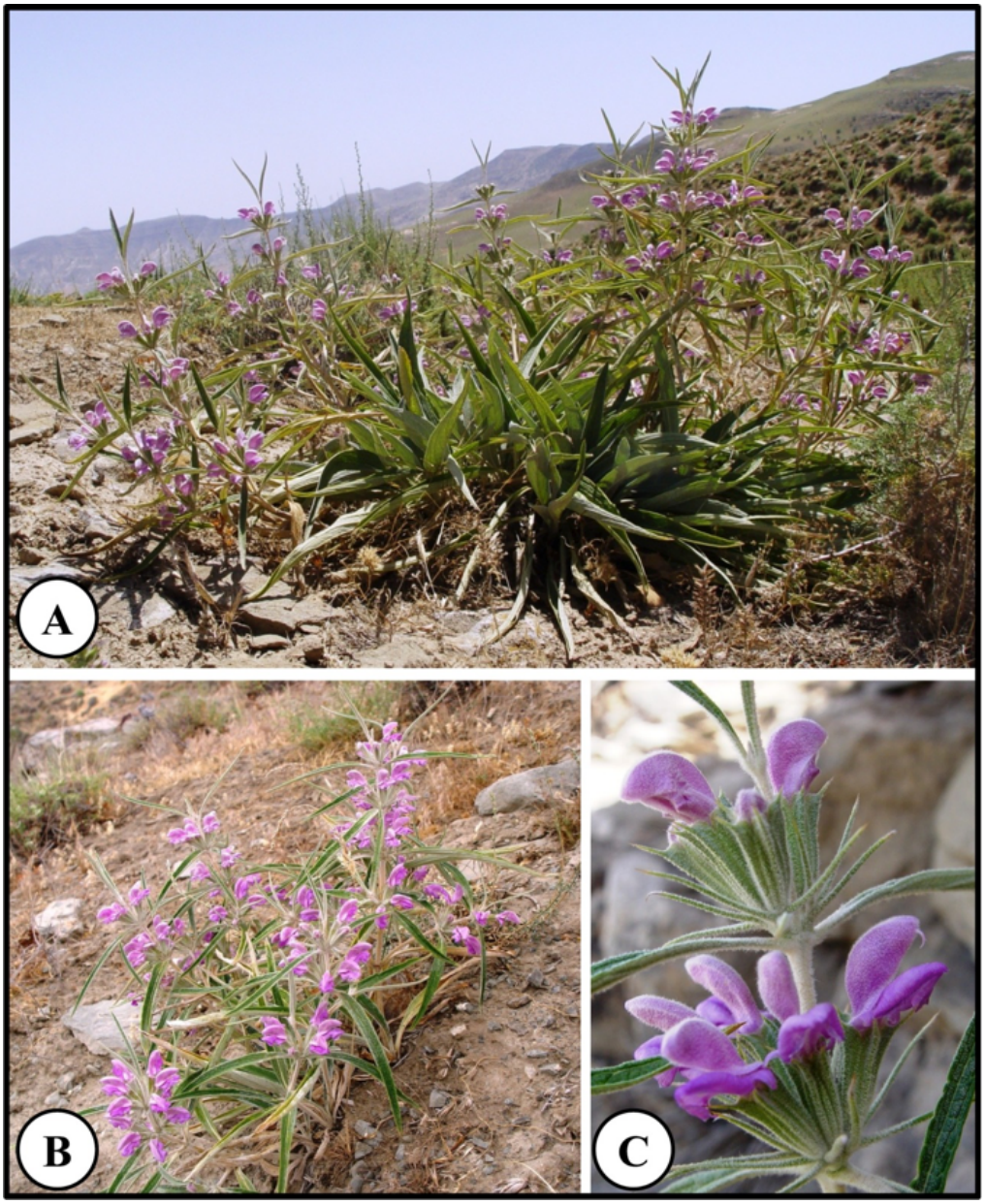

Figure 4. *Phlomis iranica*: A & B- in its natural habitat on the slopes of Zaloo Mountain, C- a close-up view of the inflorescence (photos by MR Joharchi).

glabrous, brown to dark brown, bearded in apex. June–July.

Specimens seen: Torbat-e Jam, NW Salehabad, Zaloo Mount, 1267 m, 35°53′30.8″N, 60°59′50.2″E, 05.06.2016, Joharchi 45824 (FUMH).

4.2. Biogeography and conservation status

Phlomis iranica is a local endemic to the eastern part of the Khorassan-Kopet Dagh floristic province in the Irano-Turanian region. The eastern Khorassan-Kopet Dagh is known as one of the main centers of plant endemism in the region (Memariani et al., 2016a, 2016b). This species is hitherto known only from the type locality near the borders of Turkmenistan and Afghanistan (NE Iran) at Zaloo Mountain, which is partly isolated in the easternmost extensions of the Khorassan-Kopet Dagh mountain system (Figure 1). We found two populations of the new species at the type location. The main one with about 200 mature individuals, some of them seeming very old with thick rootstocks, dominated an area of about 2 ha covered mainly by two creeping *Euphorbia* species: *E. granulata* Forssk. and *E. densa* Schrenk. The smaller population, with up to 15 mature individuals in a surface

area of about 100 m^2, is located near the main population. The area is mainly covered by *Artemisia-Astragalus* steppe with very scattered *Pistacia vera* L. shrubs.

Based on the IUCN categories and Red List criteria (IUCN, 2014), *Phlomis iranica* is evaluated as Critically Endangered [CR(B1+B2ab(iii,v))] indicating its very high risk of extinction in the near future because of the small population and very restricted AOO and EOO. Overgrazing and climatic aridization are two main threats to the conservation of the species. Searching for more populations in similar habitats, in situ conservation through fencing around the small populations, and ex situ conservation efforts such as seed banking, micropropagation, and cultivation in botanical gardens are highly recommended.

4.3. Identification key to the species *Phlomis iranica*

1. Corolla white 2
- Corolla purple or pink 3
2. Verticillasters many, contingence; anterior dents of calyx 7.5 mm long ..... *Phlomis chorassanica* Bunge
- Verticillasters few, remote; anterior dents of calyx 14–19 mm long ..... *Phlomis cancellata* Bunge
3. Margin of leaves dentate.....*Phlomis herba-venti* L.
- Margin of leaves entire 4
4. Verticillasters many; calyx 7–8 mm long; corolla 14–17 mm long ..... *Phlomis persica* Boiss.
- Verticillasters few; calyx 10–16 mm long; corolla 20–27 mm long 5
5. Bracteoles 5–7 mm long; calyx 10–12 mm long; corolla 20–25 mm long ..... *Phlomis integrifolia* Hub.-Mor.
- Bracteoles 12–17 mm long; calyx 14–16 mm long; corolla 25–27 mm long .....*Phlomis iranica* Joharchi & Vaezi

Acknowledgments

The authors wish to thank Dr F Attar for sending the TUH plant specimens. We gratefully acknowledge the G-BOISS herbarium staff and also Mr Salman Rahimi for providing pictures of the type specimen of *Ph. chorassanica*. The authors also thank the Vice President for Research and Technology of Ferdowsi University of Mashhad for financial support.

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