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معرفی گونه جدید *Phelipanche pouyanii* (تیره گل جالیزیان) از ایران

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چکیده. *Phelipanche pouyanii* به عنوان یک گونه جدید از استان خراسان جنوبی واقع در شرق ایران معرفی می شود. صفات ریخت شناسی تشخیصی شامل دندانه‌های کاسه خیلی بلندتر از لوله کاسه و بی‌کرک بودن میله های پرچم، به خوبی گونه جدید را از گونه‌های خویشاوند نظیر *P. mutelii*، *P. angustelaciniata* و *P. nana* جدا می‌کنند. نتایج حاصل از مطالعات گرده‌شناسی و ریزریخت‌شناسی دانه نشان می‌دهد که این صفات ارزش آرایه‌شناختی در مرزبندی بین گونه جدید و گونه های خویشاوند ندارند. با این حال، توالی های حاصل از نشانگر ریبوزومی ITS، مرزبندی آشکاری را بین گونه جدید و گونه های خویشاوند نشان می‌دهند.

واژه‌های کلیدی. آرایه‌شناسی تلفیقی، بومی، تبارزایی، حفاظت، خراسان جنوبی

Phelipanche pouyanii (Orobanchaceae), a new species from Iran

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Abstract. *Phelipanche pouyanii* is described here as a new species from South Khorassan Province, East of Iran. Its diagnostic morphological features are the calyx teeth being far longer than the calyx tube and staminal filaments being glabrous. These characters clearly differentiated the new species from its closely related taxa, i.e., *P. mutelii*, *P. angustelaciniata* and *P. nana*. Results obtained from the pollen and seed micromorphological characters showed no significant taxonomic value in the delimitation of the new species from its closely related species. The internal transcribed spacer (ITS) sequences, however, showed sufficient differences to delineate the new species from its closely related species.

Keywords. conservation, endemic, integrated taxonomy, phylogeny, South Khorassan

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INTRODUCTION

The family Orobanchaceae comprises approximately 90 genera and 2060 species worldwide, excluding a few non-parasitic autotrophs (McNeal et al., 2013). The number of species of the holoparasitic genus *Orobanche* L. *sensu lato* varies from 36 (Schiman-Czeika, 1964), 33 (Gilli, 1979) and 39 (Saeidi Mehrvarz et al., 2010), up to 41 (Iranshahr, 2008, referred to Novopokrovsky, 1958) in Iran. These species belong to two traditionally accepted sections *Trionychon* Wallr. (= *Phelipanche* Pomel) and *Orobanche* (= sect. *Osproleon* Wallr.). However, according to the morphological, karyological and molecular studies, these sections have been treated as separate genera, *Phelipanche* and *Orobanche*, respectively (Holub, 1990; Schneeweiss et al., 2004a, b; Carlón et al., 2005; Weiss-Schneeweiss et al., 2006; Park et al., 2008; noted also by Joel, 2009; Li et al., 2019).

The representatives of the genus *Orobanche* s.l. occupy the arid, semi-arid to humid tropical habitats (Molau, 1995). The distribution of the members of the genus depends on very narrow to broad ranges of host plants parasitized by them (Fernández-Aparicio et al., 2009). Saeidi Mehrvarz et al. (2010) listed the common host plants which are parasitized by the *Orobanche* s.l. species in Iran; Some of *Orobanche* s.l. species have been adapted to parasitize a wide range of host plants.

During field investigations and taxonomic revision of the genus *Orobanche* s.l. in the Provinces of North Khorassan, Razavi Khorassan and South Khorassan, east and northeast of Iran, we discovered an unknown specimen belonging to the genus *Phelipanche*. At the first glance, it appeared to be phenotypically similar to *P. nana* (Reut.) Soják and *P. angustelaciniata* (Gilli) Holub. For instance, the characters “branched stem”, “densely hairy style in the lower half” and “densely villous stigma” are common with those of *P. nana*, whereas “branched stem”, “moderately dense inflorescence”, “bract length”, “linear-lanceolate shape of scales”, “the lower corolla lip longer than the upper one”, and “indumentum type of corolla” revealed its close affinity to *P. angustelaciniata*. Moreover, its branched stem and corolla length and color seem to be similar with those of *P. mutelii* (F.W.Schultz) Pomel. Consequently, comparative morphological studies revealed that the unknown specimen indeed belong to an undescribed species.

In this work, we describe and illustrate *P. pouyanii* as a new species using morphological, palynological, seed surface micromorphological and molecular data. In addition, the relationships of the new species with its morphological relatives (*P. nana*, *P. mutelii* and *P. angustelaciniata*) are discussed.

MATERIALS AND METHODS

Plant specimens were first collected by M.R. Joharchi during a fieldwork in Birjand, South Khorassan Province (Iran) in 2012. Herbarium vouchers from field-collected specimens were deposited in the Ferdowsi University of Mashhad Herbarium (FUMH, acronym according to Thiers, 2016). The specimens were checked against the relevant literature (Schiman-Czeika, 1964; Gilli, 1979; Iranshahr, 2008; Saeidi Mehrvarz & Shahi Shavon, 2017). Moreover, we included one individual of *P. nana* collected from South Khorassan Province, Tabas, Chirook village (Zokaei 729-FUMH), one specimen of *P. mutelii* sampled from South Khorassan Province, SE Birjand (Joharchi & Zangooei 10784-FUMH) (incorporated in the morphological comparison) and one individual of *P. angustelaciniata* collected from South Khorassan Province, Tabas, Ozabgoo, Alimorad mine (Ayatollahi & Zangooei 13874-FUMH) in the present study. Furthermore, in order to perform the molecular study, we included herbarium or silica-dried leaves of the new species, *P. angustelaciniata* and *P. orientalis* (Beck) Soják (0004711-W, collected from Golestan National Park, E. Golestan province, Iran). We incorporated the new molecular data obtained from the present study with those belonging to some closely related *Phelipanche* species including *P. nana* and *P. mutelii* obtained from GenBank.

For the morphological descriptions, the fully developed structures of plant materials were examined. Due to darkening of the colors during desiccation, coloration characters were directly noted and photographed in the field. Flowers, bracts, and bracteoles were rehydrated before morphological examinations and measurements were made.

Pollen morphology of *P. pouyanii*, *P. nana* and *P. angustelaciniata* was examined by means of light microscopy (LM) and scanning electron microscopy (SEM). Pollen grains were obtained from dried specimens used in the morphological study. The pollen grains were acetolysed following Erdtman (1960). They were observed with an Olympus microscope model BX-50 (Olympus Co., Tokyo, Japan) equipped with an oil-immersion objective. The characters “polar axis”, “equatorial axis”, “exine thickness”, and “the ratio polar axis/equatorial axis” were measured for at least 30 pollen grains of each taxon. Pollen size and shape classes were used following Erdtman (1952). For SEM, the acetolyzed pollen grains were first dehydrated in a graded ethanol series. Then, they were mounted on aluminum stubs and coated with the gold-palladium alloy in a sputter coater (SC

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7620, Quorum Technologies Ltd, UK). Subsequently, they were observed and photographed with a VP LEO 1450 SEM at 20 KV (Zeiss Co., Oberkochen, Germany). The SEM micrographs were used for descriptions of the pollen grains following the terminology of Erdtman (1952).

The length, width, shape, epidermal cell shape, the anticlinal and the periclinal walls, and testa pattern of seeds were measured and described for 20 seeds per taxon using SEM (model VP LEO 1450 at 20 KV). Seed preparation for SEM was similar to that of pollen as described above.

Total DNA was extracted using the standard CTAB method (Doyle & Doyle, 1987). Each included taxon, i.e., *P. pouyanii*, *P. angustelaciniata*, and *P. orientalis*, was represented by one voucher specimen. The forward and reverse universal primers ITS5 and ITS4 (White et al., 1990), respectively were used to amplify the ITSnrDNA region. The amplification was performed in 25 µL volumes containing 12.5 µL of Taq DNA polymerase Master Mix Red 2X (Amplicon A/S, Denmark), 100 µmol/L of each primer, and ca. 200 ng genomic DNA. PCA amplification was carried out under the following conditions: a preliminary denaturation step at 95 ºC for 5 min followed by 35 cycles of denaturation at 95 ºC for 30 s, annealing at 55 ºC for 45 s, elongation at 72 ºC for 1 min and a final extension at 72 ºC for 7 min. PCR-product purification and direct sequencing were performed using Macrogen's sequencing service (Macrogen Inc., Korea). Sequencher ver. 5.2.4 (Gene Codes Inc., Ann Arbor, Michigan) was used to edit sequences.

ITS sequences obtained from the present study (*P. pouyanii*, MW524124; *P. angustelaciniata*, MW524122; *P. orientalis*, MW524123) were combined with some closely related *Phelipanche* sequences available in GenBank (*P. aegyptiaca* Pomel, KC811164; *P. gratiosa* (Webb & Berthelot) Carlón, G.Gómez, M.Laínz, Moreno Mor., Ó.Sánchez & Schneew, EU581793; *P. mutelii* (F.W.Schultz) Pomel, AY209340; *P. lavandulacea* Pomel, EU581718; *P. inexspectata* Carlón, G.Gómez, M.Laínz, Moreno Mor., Ó.Sánchez & Schneew, AY960739; *P.* cf. *iberica* (Beck) Soják, LT715380; *P. rosmarina* (Beck) Banfi, Galasso, Soldano, MK026668; *P. ramosa* (L.) Pomel, KY513946; *P. nana*, AY209318; *P. oxyloba* (Reut.) Soják, AY209319; *P. tunetana* (Beck) Soják, AY209324 and *Orobanche alba* Stephan ex Willd., AY209249 was included as an outgroup. The integrated sequences were aligned using Clustal W (Thompson et al., 1994) as implemented in BioEdit Sequence Alignment Editor (Hall, 1999) followed by manual corrections. SeqState ver. 1.25 (Müller, 2005) was used to code the indels using the "simple indel coding" method (Simmons & Ochoterena, 2000). Evolutionary model selection was carried out to determine the best-fitted nucleotide substitution model for the dataset using MrModeltest 2.2 (Nylander, 2004). Subsequently, the Bayesian inference was run for one million generations for the ITS matrix as implemented in MrBayes 3.2.1 (Ronquist et al., 2012). The first 25% of generations were discarded as determined by Tracer ver. 1.4 (Rambaut & Drummond, 2007). Trees were visualized using TreeView ver. 1.6.6 (Page, 2001). In general, the resulting ITS phylogenetic tree showed low resolution, particularly among the species *P. ramosa*, *P. rosmarina*, *P.* cf. *iberica*, *P. inexspectata*, *P. pouyanii*, *P. angustelaciniata*, *P. oxyloba*, *P. nana* and, *P. tunetana*. Therefore, to demonstrate more sequence resolution, the network analysis was used for the species mentioned above using the statistical parsimony on the dataset with gaps treated as missing data and a 92% connection limit (Tempelton et al., 1992). This analysis was performed in the TCS program ver. 1.21 (Clement et al., 2000).

RESULTS

By the presence of bracteole and connected calyx parts, the new species is clearly nested within the genus *Phelipanche*. The close relationships and affinity among *P. pouyanii*, *P. mutelii*, *P. angustelaciniata*, and *P. nana* are well supported by their similar morphological traits such as the branched stem, bracteole length, stamen and style length, scale length/width, the length of the longest calyx dent in comparison with the length of the calyx tube, the number of calyx dents, sparse indumentum of petal margin, and mucronate and glabrous anthers. However, *Phelipanche pouyanii* can be distinguished from *P. mutelii*, *P. angustelaciniata* and *P. nana* by multiple morphological characters, summarized in Table 1.

Among the diagnostic vegetative and reproductive morphology ical traits in *P. pouyanii* are subulate floral bract, bract being as long as or shorter than the calyx, the calyx teeth far longer than the calyx tube, bracteoles lanceolate to linear, and glabrous filaments. These morphological characters are used frequently in the diagnosis of *Orobanche* s.l. species (e.g., Domina & Soldano, 2015; Piwowarczyk, 2015).

The results obtained from palynological and seed micromorphological investigation of the current study are presented in Figures 3-4 and Table 2. Clearly, the basic type of pollen grain of *Phelipanche* (radially symmetrical trizonocolpate, Abu Sbaih et al., 1994) is also found to be present in the specimens studied. According to Erdtman (1952), the pollen shape of *P. pouyanii* in monads is

prolate-spheroidal (P/E: 1.01-1.14), whereas that of *P. angustelaciniata* and *P. nana* is subprolate (P/E: 1.15-1.33) and prolate (P/E: 1.34-2.00), respectively. Abu Sbaih et al. (1994) reported the oblate-spheroidal pollen shape for *P. nana*. This discrepancy may reflect intraspecific variation in the pollen shape. Based on the results, the surface sculpture of pollen in *P. pouyanii*, *P. angustelaciniata* and *P. nana* is found to be scabrate-perforate (Fig. 1).

The seed micromorphological characters of *P. pouyanii*, *P. angustelaciniata* and *P. nana* as shown by SEM (Fig. 2) are reviewed in Table 2. The results demonstrate that the seed surface sculpture of the three species is alveolate as shown for *Orobanche owerinii* Beck and *O. reticulata* Wallr. by Zare & Dönmez (2013). The seed dimension almost differentiates *P. pouyanii* from *P. angustelaciniata* in which the seed length and width in *P. pouyanii* is nearly two times greater than those in *P. angustelaciniata*, while these characters cannot distinguish *P. pouyanii* from *P. nana*. In all the species studied, the testa pattern and seed shape are isodiametric to irregular and ovate-elliptic, respectively. The anticlinal walls are raised with a smooth surface. Nevertheless, top of the anticlinal walls is slightly waved in *P. pouyanii* and *P. nana*. Moreover, the periclinal wall in the three species is reticulate-perforate with a sparsely papillate surface pattern.

In overall, our results indicate that the micromorphological characteristics resulted from pollen and seed surface studies are of no conclusive taxonomic value in the differentiation of *Phelipanche* species studied, while they can be helpful if combined with other morphological features to have a correct circumscription of each species. This finding is in accordance with the works of Abu Sbaih et al. (1994), Plaza et al. (2004), Halamski (2011), and Zare & Dönmez (2013). In contrast, in a micro-morphological framework, Parsapanah & Saeidi-Mehrvarz (2015) showed that the palynological characters are useful in delimitation of some *Pedicularis* species incorporated in the study.

Table 1. Diagnostic morphological characters among three closely related *Phelipanche* species included in the present study.

Character/Species	*P. pouyanii*	*P. mutelii*	*P. angustelaciniata*	*P. nana*
Inflorescence	moderately dense	dense	moderately dense	very dense
Scale	8-10 mm long, linear-lanceolate	up to 17 mm long, lanceolate to subulate	10-13 mm long, linear-lanceolate	5-13 mm long, ovate
Bract	9-14 mm long, narrow ovate-lanceolate	5-12 mm long, subulate or lanceolate	13-15 mm long, ovate	7-14 mm long, lanceolate or subulate
Bracteoles	lanceolate to linear	linear or subulate	narrow lanceolate to long subulate	linear or subulate
Calyx	10-14 mm long, dents very longer than tube	7-12 mm long, dents equal, a little shorter or longer than tube	10-17 mm long, dents longer than tube	9-13 mm long, dents equal or a little longer than tube
Corolla	17.5-23 mm long, cylindrical-infundibuliform	18-22 mm long, infundibular	10-17 mm long, cylindrical-infundibuliform	14-17 mm long, campanulate or infundibular
Filament	Glabrous	sparsely villous	sparsely villous	sparsely villous
Host	*Prunus armeniaca* (Juvenile)	*Nicotiana tabacum, Parrotia persica, Prunus dulcis, Prunus armeniaca, Cousinia multiflora, Carpinus* spp., *Salvia* spp., *Stachys* spp.	*Prunus* spp.	*Artemisia* spp., *Centaurea* spp., *Astragalus* spp., *Eryngium* spp., *Prunus scoparia*

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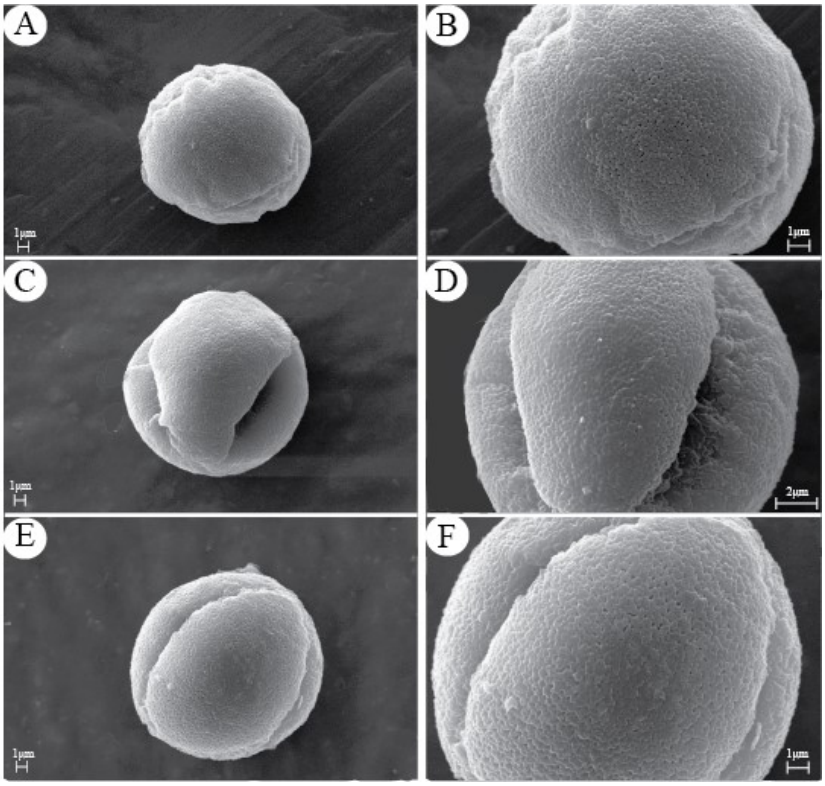

Figure 1. Scanning electron micrographs of the pollen grains of *Phelipanche* species. **A-B.** *P. pouyanii* (Joharchi 44746-FUMH). **C-D.** *P. angustelaciniata* (Ayatollahi & Zangooei 13874-FUMH). **E-F.** *P. nana* (Zokaei 729-FUMH)

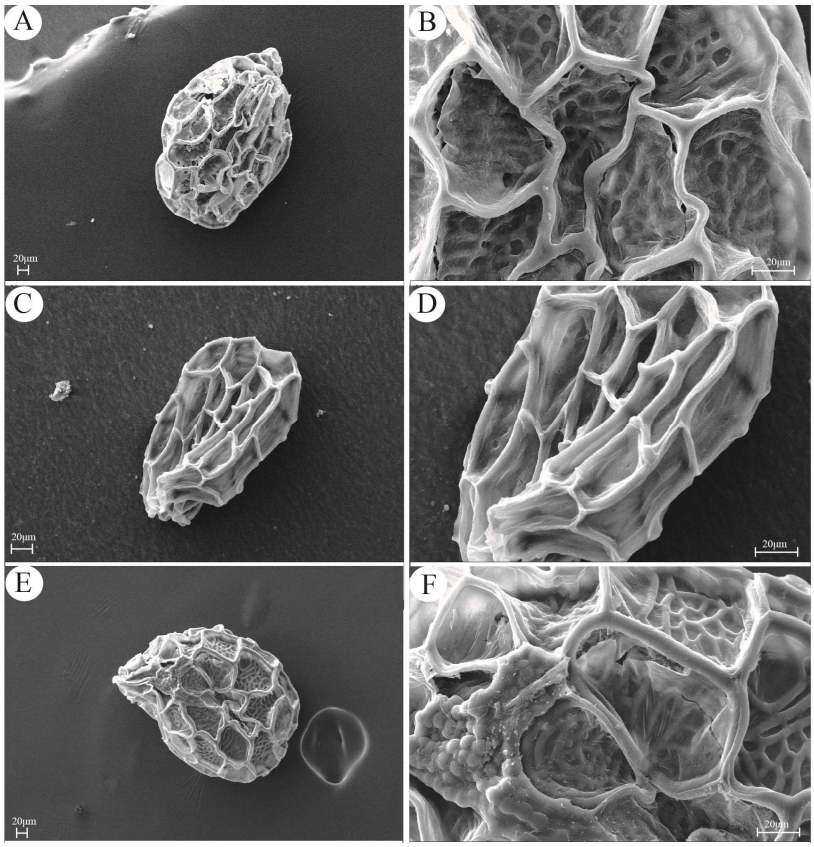

Figure 2. Scanning electron micrographs of seeds in *Phelipanche* species showing the shape (left) and ornamentation (right). **A-B.** *P. pouyanii* (Joharchi 44746-FUMH). **C-D.** *P. angustelaciniata* (Ayatollahi & Zangooei 13874-FUMH). **E-F.** *P. nana* (Zokaei 729-FUMH).

Table 2. Details of pollen and seed micromorphological characters of *Phelipanche* species included in the present study.

Character/Species	*P. pouyanii*	*P. angustelaciniata*	*P. nana*
Polar axis (P, µm)	26.6	22.5	27.1
Equatorial axis (E, µm)	24.9	17.9	17.3
P/E	1.07	1.26	1.57
Exine thickness (µm)	1.35	2.86	3.4
Pollen aperture	Tricolpate	tricolpate	tricolpate
Pollen sculpture	scabrate-perforate	scabrate-perforate	scabrate-perforate
Pollen shape	prolate-spheroidal	subprolate	prolate
Seed length (µm)	320	180	340
Seed width (µm)	220	110	200
Periclinal wall	reticulate-perforate	reticulate-perforate	reticulate-perforate-papilate
Testa pattern	isodiametric to irregular	isodiametric to irregular	isodiametric to irregular
Anticlinal wall	smooth to waved	Smooth	smooth to waved
Seed shape	ovate-elliptic	ovate-elliptic	ovate-elliptic

Totally, 637 nucleotide characters (including coded gaps) related to 15 ITS sequences were integrated into the Bayesian analysis, of which 487 were constant, 131 parsimony-uninformative and the 19 remaining nucleotide sites were parsimony-informative. Analysis of the ITS sequences using Bayesian inference with the SYM nucleotide substitution model (based on the Akaike information criterion) resulted in a 50% majority rule consensus tree (Fig. 3). The tree shows that all the *Phelipanche* species incorporated in the study form a monophyletic group with a strong posterior probability support (PP=1.00) with respect to the outgroup.

The *Phelipanche* species, however, make two subclades I and II (Fig. 3) where the new species, *P. pouyanii*, is nested within the subclade II. *Phelipanche mutelii* which is morphologically similar to *P. pouyanii*, is located within the subclade I. However, the meaningful signals provided by the ITS data do not support a close relationship between these two species.

In general, the Bayesian phylogenetic tree shows relatively low resolution among the *Phelipanche* species. Therefore, to provide more resolution, the members of the subclade II were reanalyzed using the network reconstruction algorithms. The results obtained from the simplified network (Fig. 4) show that no identical ribotypes were identified among the species included in the network analysis. The closest related species to *P. pouyanii* is *P.* cf. *iberica* connected with two mutational steps. Furthermore, two morphologically related species, *P. nana* and *P. angustelaciniata* are connected to *P. pouyanii* with three mutational steps. In spite of the relatively low sequence resolution resulted among the *Phelipanche* species, the network analysis has provided an adequate power to differentiate *P. pouyanii* as an independent species from its relatives. However, usage of chloroplastic markers might provide a phylogenetic relationship among the species under study with more resolution in comparison with that based on the nrDNA (ITS) data.

Description of the new species

Phelipanche pouyanii Joharchi & Vaezi, *sp. nov.* (Figs. 5, 6)

Type: IRAN. South Khorassan Province: SW Birjand, Bagheran Mountains, southwest of Omar Shah Dam. N: 32º 49' 39.6" E: 59º 10' 47.5", 1700 m, 6 May 2012, Joharchi 44746 (Holotype: FUMH; Isotype: FUMH).

Plant 18-27 cm tall with branched stem at base, purple; scales 8-10 mm long, towards tip gradually

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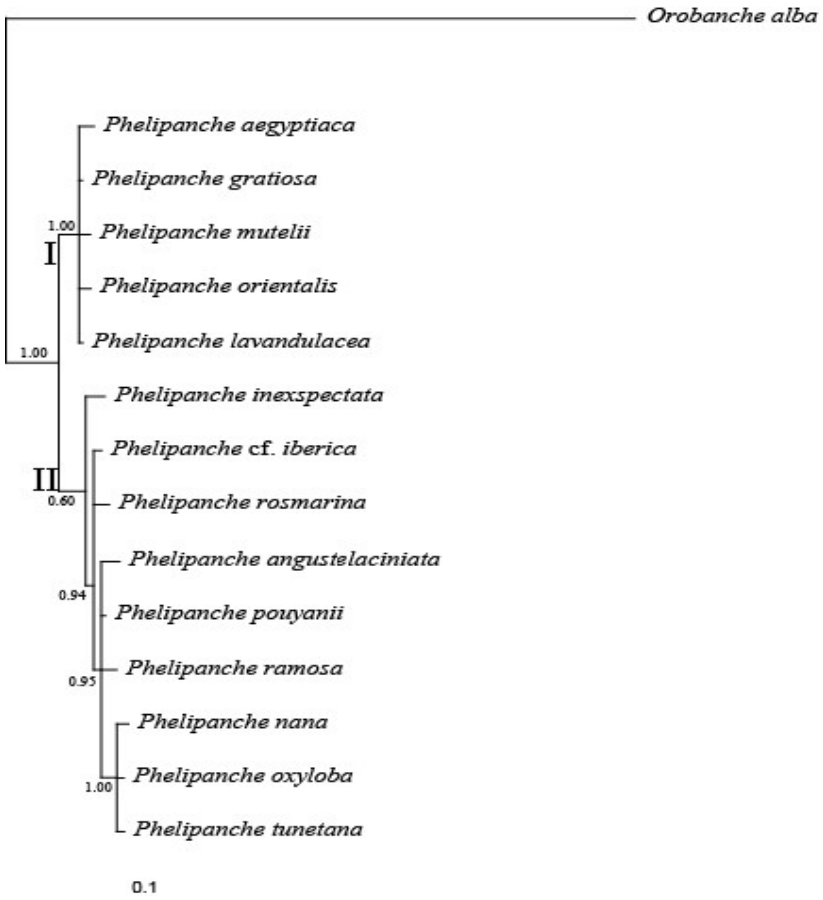

Figure 3. Phylogenetic tree of some *Phelipanche* species resulting from the Bayesian analysis of their ITS data. Subclade II comprises the unresolved sequences which are expanded in Figure 6. Numbers above or below branches are posterior probabilities.

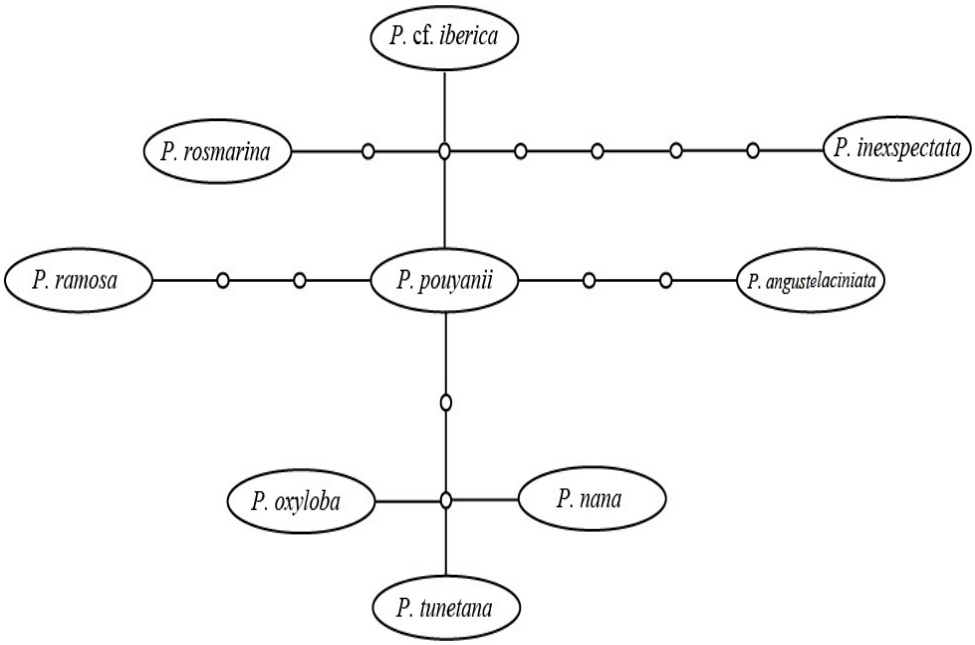

Figure 4. Simplified ribotype network of the ITS dataset using all ribotypes included in the subclade II of Figure 5. The small circles indicate unsampled ribotypes.

Figure 5. *Phelipanche pouyanii* in its natural habitat (Joharchi 44746-FUMH, photo by M.R. Joharchi).

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Figure 6. Holotype of *Phelipanche pouyanii* sp. nov. deposited in the FUMH herbarium (Joharchi 44746).

Figure 7. Distribution map of *Phelipanche pouyanii* in Bagheran Mountains, SW Birjand.

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narrow; inflorescence 10-12 cm long, fairly dense, triangular (base slightly broadened) forming a spike; flowers pedicellate, pedicel length in lower flowers up to 6 mm, in upper flowers 2-3 mm; bracts narrowly ovate-lanceolate, 9-14 mm long, as long as or shorter than calyx, glandular pubescent; bracteols linear-lanceolate, glandular pubescent; calyx cylindrical to campanulate 10-14 mm long, dents strongly developed, very longer than the tube, triangular in base, linear to filiform at the tip; corolla 17.5-23 mm long, pale yellow and inflated at the base, cylindrical-infundibuliform, deeply purple, lobes of the upper lip ovate-triangular with acute apex, lobes of the lower lip ovate-elliptic and longer than the upper lip, all lobes entire at margin, the lower lip between lobes densely pubescent; stamen 4-5 mm above the base of the corolla, filaments glabrous as the base, densely glandular-pubescent near the anthers; pollen aperturate, trisulcate-tricolpate, exine ornamentation scabrate-perforate, polar axis 26.6 µm, equatorial axis 24.9 µm, exine thickness 1.35 µm; style pubescent at the base, stigma densely pubescent; capsule 4-5 mm long; seed 0.358×0.247 mm, spheroidal, testa pattern irregularly triangle, anticlinal wall smooth to ribbed.

Etymology: The species is named in honor of Mr. Mohsen Pouyan, an eminent botanist in Birjand, South Khorassan province, where the species was collected.

Habitat and Host: *Phelipanche pouyanii* grows in a narrow valley in southern slopes of Bagheran Mountains in an area which is planted mainly by apricot and apple orchards. Based on current data, it is exclusively parasitic on *Prunus armeniaca* L.

Conservation status:—So far, the new species is known only from the type locality. Accordingly, it has a very restricted distribution range in the east of Iran (Fig. 7). Based on IUCN criteria and categories for Red Listing, the Data Deficient (DD) category is applied to taxa with insufficient information mainly due to poorly known distribution data, however, using the criterion D which deals with very small or restricted populations (IUCN, 2016), *Phelipanche pouyanii* is assessed here as Vulnerable (VU D1,2).

Key to the closely related species of the present study

1. Corolla length shorter than 17 mm …… 2
- Corolla length longer than 17 mm …….... 3
2. Upper and lower lips of corolla nearly equal in size, corolla limbs ovate, apiculate ..... *P. nana* (Reut.) Soják
- Upper lips of corolla very shorter than lower lips, corolla limbs linear …… ……..*P. angustelaciniata* (Gilli) Holub
3. Calyx dents equal, a little shorter or longer than tube, filaments sparsely villous …..……*P. mutelii* (F.W.Schultz) Pomel
- Calyx dents very longer than tube, filaments glabrous at base and towards apex …….. …….*P. pouyanii* Joharchi & Vaezi, sp. nov.

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