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Short Communication: Description and molecular diagnosis of a new species of *Delphinium* (Ranunculaceae) from Northeast Iran

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Abstract. *Hasanbarani M, Sharifnia F, Nejadsattari T, Assadi M. 2017. Short Communication: Description and molecular diagnosis of a new species of* Delphinium *(Ranunculaceae) from Northeast Iran. Biodiversitas 18: 639-644. Delphinium khorasanicum* Sharifnia & Hasanbarani sp. nov., a species distributed in NE Iran, is described and provided with a molecular diagnosis. It is morphologically related to *D. turkmenum* Lipsky. The most important morphological differences between the new species and *D. turkmenum* are mainly in the shape of spur, plant length and epidermal patterns. This species is compared with close taxa (they are close and relative in point of view morphologically, geographically and molecular characters). Illustration and distribution map are also presented.

Keywords: *Delphinium,* Iran, molecular study, new species

INTRODUCTION

Delphinium L. (Larkspur) belongs to the tribe Delphinieae and Ranunculaceae family, has ca. 370 species, it is distributed in the North temperate regions of the world (Agnihotri et al. 2014). Munz (1967) documented 244 species from Asia. *Delphinium* species are perennial, biennial or annual herbs. Leaves alternate, palmately divided. Perianth biserial, outer petaloid(sepals) and inner petaloid(petals). Flowers zygomorphic, borne in racemes. Sepals 5, the posterior sepal spurred. Petals 4, in 2 dissimilar pairs, the upper pair produced into nectariferous spur contained within the sepal spur. Stamins numerous. Follicles 3(-5). (Davis, 1965).

In the Flora Iranica, 53 species of *Delphinium* in three subgenera (*Delphiniasrtum* (DC.) Peterm, *Oligophyllon* Dimitrova and *Delphinium*) are mentioned (Iranshahr 1992). Differences between these subgenera area in seed form and habit. 29 species of *Delphinium* and 2 subgenera have been found in Iran. A difficult genus, many of taxa being connected by intermediates (Davis 1965). Several studies have been done on the *Delphinium* genus in the world such as palynological, morphological, molecular phylogeny and biosystematic (Ilarslan et al. 1997; Bursali and Dogan 2005; Jabbour and Renner 2012; Sharifnia et al. 2013).

In this survey, while revising the taxonomy of the genus *Delphinium* in Iran using the Flora of USSR (Neveskii 1937) and Flora Iranica (Iranshahr 1992), and based on the observation of herbarium specimens collected from west of Khorassan Province, we describe the new species *Delphinium khorasanicum*. Khorasan province is located in NE Iran an area of 13.335 km^2. It is the largest province of the country, includes on the fifth of country area. The new specimens were compared with its closest relative (*D. turkmenum*, *D. aquilegifolium* and *D. griseum*) based on morphological, tepal epidermal pattern; the patterns of epidermis tepal in angiosperm have important characteristics in identifying species were close together (Christensen and Hansen, 1998) and molecular characters. The sequence of *Delphinium khorasanicum* after the Blast in GenBank was to closest to *Delphinium griseum* but it is distributed in Afghanistan, we used sequence of this species for comparison bases. *Delphinium turkmenum* and *Delphinium aquilegifolium* morphologically and geographically close to *Delphinium khorasanicum.*

MATERIALS AND METHODS

The study has been done using herbarium specimens of TARI and newly collected specimens (Table 1). *Delphinium griseum* Gilli description was taken from Flora Iranica; the sequence of new species was close to *Delphinium griseum* using BLAST in GenBank (https://www.ncbi.nlm.nih.gov/Blast.cgi). Images of herbarium specimens were searched in the herbarium sites of the world such as Kew (Royal Botanical Garden Kew, http://www.kew.org/), Edinburgh (Royal Botanical Garden Edinburgh, http://www.rbge.org.uk), Missouri (Missouri Botanical Garden, http://www.tropicos.org), Geneva (Conservatoire et Jardin botaniques de la Ville de Genève, http://www.ville-ge.ch/musinfo/bd/cjb/chg/index.php?lang=en) and Wien (University of Vienna, http://herbarium.univie.ac.at/database/search.php). For

micromorphological study, outer tepals (lateral sepals) were stabled on the aluminum Stub and were covered by golden coat after freezing. Samples were observed via SEM (EM 3200). Terminology for tepal epidermal pattern is according Christensen and Hansan (1998). Total DNA was extracted from leaves using the MBST kit (Shayan et al. 2007). Amplification and cycle sequencing of *trnL-F* used primers c and f (Taberlet et al. 1991). After sequencing, sequences were checked and edited with Sequencher 4.1.4 software (Gene Codes Corporation, Ann Arbor, Michigan. U.S.A.). The *trn*L-F sequence of *D. griseum* was downloaded from GenBank (Table.1).

Table 1. Localities of *Delphinium* specimen (specimens included in the morphological, tepal epidermal patterns and molecular study indicated by an asterisk* and other specimens for more study) + GenBank accession number.

Species	Locality
Delphinium khorasanicum Sharifnia and Hassanbarani (tree flowers were observed for describe the new species)	Khorassan: North west of Neyshabur, Bar fall, 2004 m, Sharifnia and Hassanbarani 16155 IAUNT.* collection date: 30/5/2016 Khorassan Province, Neyshabur,Garmab mountain, 1900m, Sharifnia and Hasanbarani 17001 IAUNT, Same area, 1800 m, Sharifnia and Hasanbarani 17002 IAUNT. collection date: 3/6/2016 **GenBank accession: LC194958.1**
Delphinium turkmenum Lipsky	Semnan: Turan protected area, 22 km from Ghazaran to Miandasht, 1240 m, Feritag and Jadidi 28987 TARI. collection date: 11/5/1987 Khorassan: Northwest of Neyshabur, Bar fall, 2004 m, Sharifnia and Hassanbarani, 16159- IAUNT.* collection date: 30/5/2016 **GenBank accession: KY425588**
Delphinium aquilegifolium (Boiss.) Bornm.	Hamadan: Kabudar- Ahang, Ghohrod, Sarayjogh, khuh-e Siah, 2000-2240m, Mozaffarian 64628-TARI.* Collection date: 8/6/1988 **GenBank accession: KY425589** Mazandaran: Lar vally, 2450-2550m, Wendelbo and Assadi 13264-TARI. collection date: 2/7/1974 Tehran:10 km from Karaj, On Chalus road, 1750m Babakhanlu and Amin 20004-TARI. collection date: 23/5/1973
Delphinium griseum Gilli	**Afghanistan:** Kabul. Voucher of the specimen: Volk 1873, kept at Munich Herbarium (M) **GenBank accession: JN573556.1**

Note: IAUNT= herbarium code of Islamic Azad University, Tehran North Branch

RESULTS AND DISCUSSION

Taxonomic treatment

Delphinium khorasanicum Sharifnia & Hasanbarani sp.nova (Figure 1).

Holotypus: Iran. Khorassan, NW of Neyshabur, Bar Fall, 2004 m, Sharifnia and Hasanbarani, 16155 TARI, Isotypous IAUNT.

Collection date: 30/5/2016

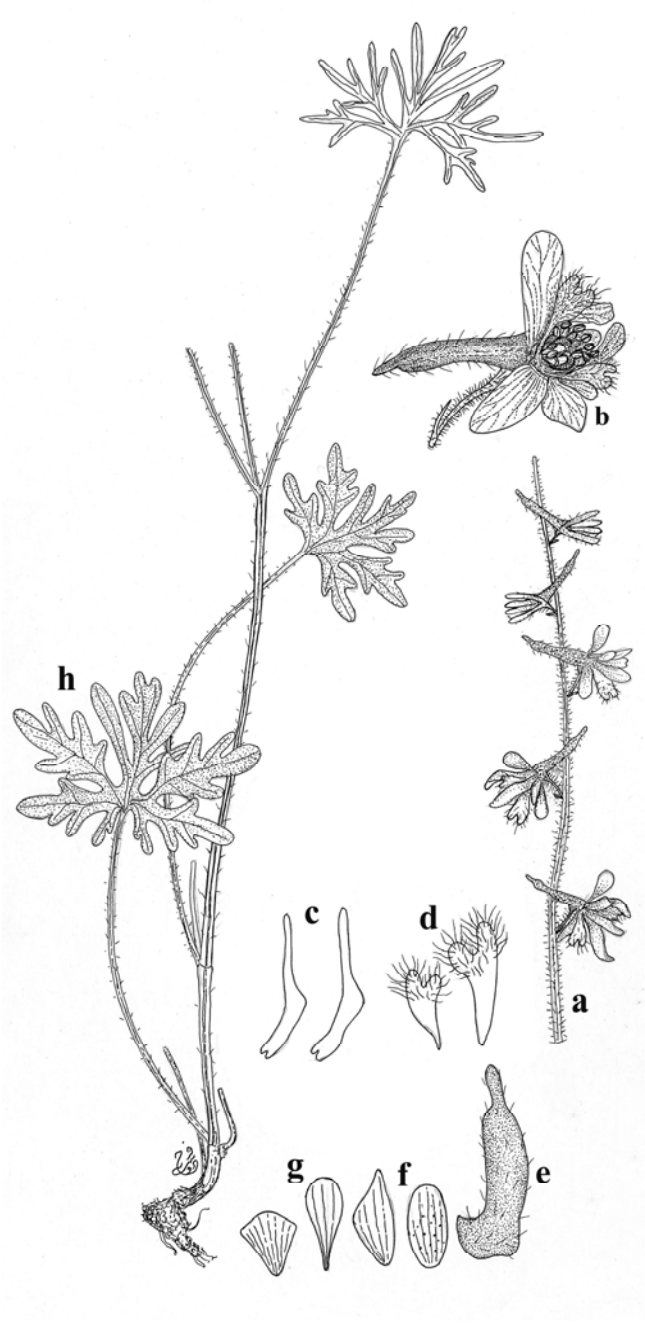

Figure 1. *Delphinium khorasanicum*. a: raceme, b: flower (×1), c: upper petal (×2), d: lower petal (×2), e: upper sepal (× 1.2), f: lateral sepals (×1), g: lower sepals (×1), h: radical leaves (×2), i: stem leaves (×2)

Figure 2. Spur shape in *Delphinium*. A. *D. khorasanicum* and B. *D. turkmenum*

Description

Perennial, up to 100 cm high. Stem sulcate, villous puberulent below, spreading pillose pubescent above. Leaves long petiolated (petiole up to c. 15 cm long) palmatisect, segment lobe into cuneate division, segments to c. 20mm in diameter; cauline leaves palmatisect; segments lanceolate, up to 10mm long. Raceme lax, paniculate. Bracts linear lanceolate, 2-4 mm long. Pedicel up to 6mm long. Bracteoles born near the flower, 1-2 mm long, lanceolate. Flowers dark blue, 30-40 mm long, spur 21 mm, horizontal and papillate at apex; Sepals elliptic, lower petals 10 ×5 mm long, deeply bifid, densely barbate, white-blue; upper petal labia, c. 18-20mm long, yellow. Anther dark violet, numerous. Gynoecium three carpels.

Additional specimens

Khorassan Province, Neyshabur, Garmab mountain, 1900m, Sharifnia and Hasanbarani 17001 IAUNT,

Same area, 1800m, Sharifnia and Hasanbarani 17002 IAUNT.

Collection date: 3/6/2016

Morphological and outer tepal epidermal patterns study

Delphinium khorasanicum is related to *D. turkmenum*, both of them are distributed in NE of Iran. The two species are similar in terms of morphological. One of the few characters that distinguish *D. turkmenum* from *D. khorasanicum* is the shape of spur which suddenly narrowed at the base in the new species (Figure 2). Some diagnostic differences between *D. khorasanicum, D. turkmenum*, *D. griseum* (distributed in east of Afghanistan) and *D. aquilegifolium* that their sequences using BLAST were close to *D. khorasanicum* listed in Table 2. Outer Tepal (lateral sepal) epidermal patterns in *D. khorasanicum* is rugose, in *D. turkmenum* striate and in *D. aquilegifolium* is papillose that is clear in SEM image (Figure 3). Barthlott (1981, 1990) in a general review of epidermal characters of plants, confirmed the systematic significance of epidermal characters. The distribution of these species is presented on Figure 4.

Molecular study

Molecular comparison results using BLAST in NCBI showed that *D. griseum* and *D. aquilegifolium* are the closest to *D. khorasanicum*. *Delphinium griseum is* distributed in the east of Afghanistan (Iranshahr 1992), Holotypus of this species was observed from Wien Herbarium and its description was compared with *D. khorasanicum* and other relatives which showed morphological differences (Table 2). Comparison of different bases in chloroplast DNA sequences of these species are presented (Table 3). *trnL-F* sequence of the new species differs from other species by eight bases. These sequences that we use to compare with *D. khorasanicum* were selected of a phylogenetic study including many species of Persian *Delphinium* (data unpublished).

Table 2. Morphological and micromorphological characters used to distinguish *D. khorasanicum* from its relatives.

Characters	Species			
	D. aquilegifolium (three samples)	***D. griseum***	***D. khorasanicum*** (three sample)	***D. turkmenum*** (two samples)
Plant length (cm)	30-60	25-70	90-100	25-70
Spur tips	Cylindrical	Cylindrical	With long papilla	Cylindrical
Spur width (mm)	2-3	2-3	4-5	2-3
Leaf apex	Obtuse	Acute	Obtuse	Acute
Epidermal pattern	Papillose	-	Rugose	Striate

Table 3. Comparison of different bases in chloroplast DNA sequences *(trnL-F*) between *D. khorasanicum*, *D. turkmenum, D. griseum* and *D. aquilegifolium.* Each column represents position of bases

	0 2 6	0 2 7	0 7 3	0 7 5	0 7 7	0 8 5	1 4 6	2 5 7	5 7 0	8 3 6	8 3 8	8 3 9	9 6 1	9 8 2
D. khorasanicum	C	A	T	G	A	A	T	A	T	A	G	G	G	A
D. turkmenum	A	C	G	A	T	A	G	A	T	A	G	G	A	G
D. aquilegifolium	C	A	T	G	A	T	G	C	A	G	C	T	G	G
D. griseum	A	C	G	A	T	A	G	A	A	A	G	G	G	G

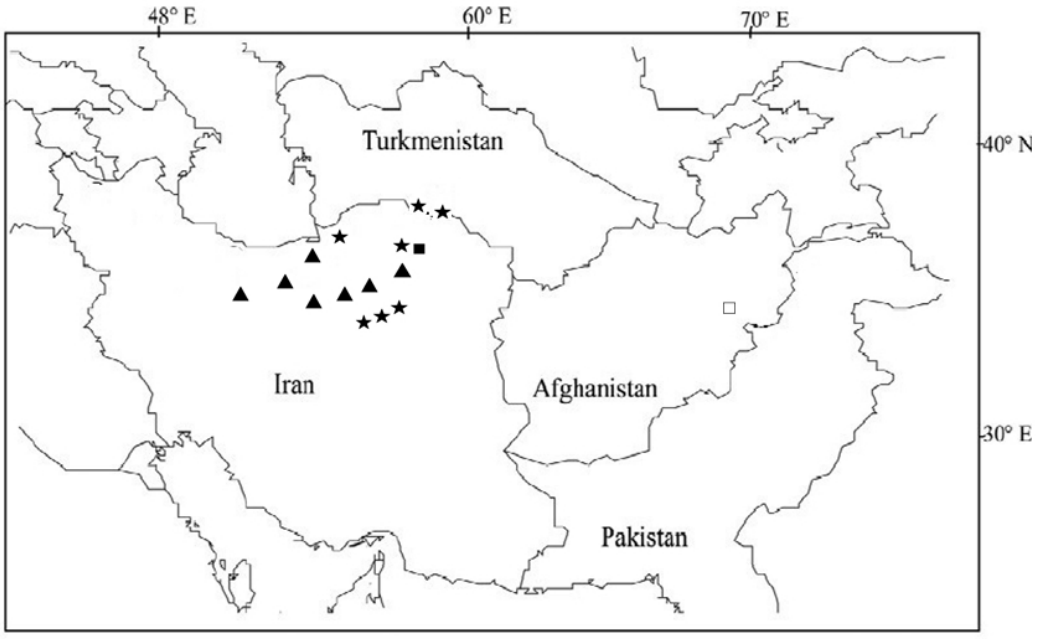

Figure 4. Distribution map of some *Delphinium* species from Northeast Iran, i.e., *D. turkmenum* ★, *D. khorasanicum* ■, *D. griseum* □, and *D. aquilegifolium* ▲

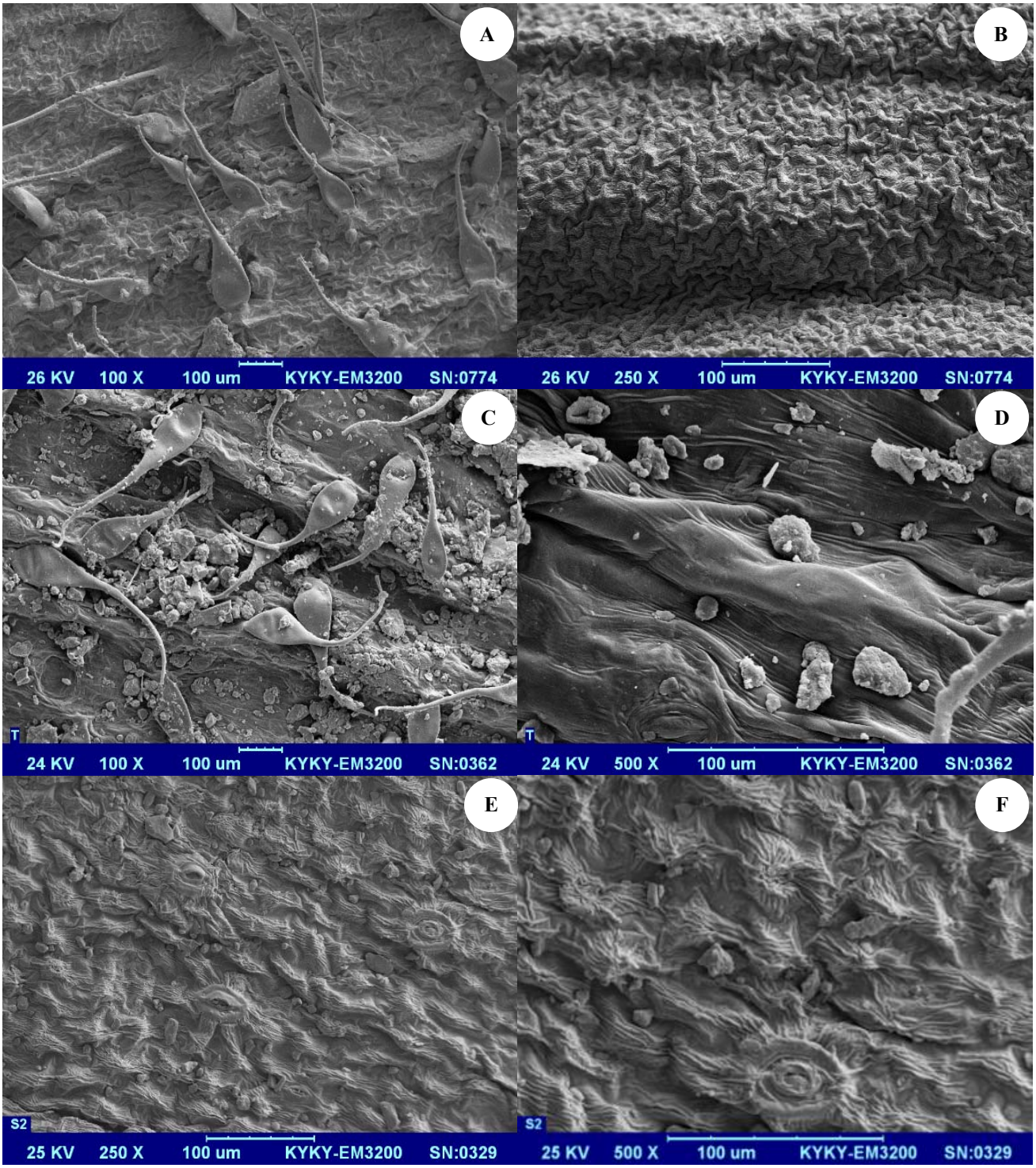

Figure 3. SEM micrographs of outer tepal epidermal patterns (lateral sepal). A-B. Outer tepal epidermal pattern in *D. khorasanicum*, C-D. Outer tepal epidermal patterns in *D. turkmenum*, E-F. Outer tepal epidermal patterns in *D. aquilegifolium*

In conclusion, according to morphological, epidermal patterns and molecular studies, *Delphinium khorasanicum* is a new species. In *Delphinium*, spur shape is one of the most important taxonomic character for identification (attenuate, cylindrical, obtuse, acute, etc.) that it could be a genetic characteristic, the spur of new taxa has clearly long papilla and differ from other *Delphinium* spp. in Iran plateau. Scanning Electron Microscope image of tepal in *D. turkmenum* and *D. khorasanicum* showed although the outer tepals (lateral sepal) of both species are hairy but epidermal patterns is striate and rugose respectively. Outer tepals (sepals) are in *D. khorasanicum* without glandular

pubescence but in *D. turkmenum* with glandular pubescence that is clear in SEM images (Figure 3). Differences between bases in *trnL-F* sequence with at least eight bases showed that *D. khorasanicum* is away from the close relative. *Delphinium khorasanicum* is a perennial plant with considering position of *D. griseum*, *D. turkmenum* and *D. aquilegifolium* in Flora Iranica, the sequence BLAST in GenBank and according to phylogeny study on Delphinieae species (Jabbour and Renner 2012) we concluded that *D. khorasanicum* is placed in *Oligophyllon* subgenus.

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