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A new species of *Linum* (Linaceae) from Iran with a focus on description of *Linum turcomanicum* in view of morphological and molecular analyses

MARYAM BEHROOZIAN[1*], JAMIL VAEZI[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*
[*]*Corresponding author: maryam.behroozian@stu.um.ac.ir*

Abstract

The genus *Linum* consists of 15 species in Iran. A new species as well as a new record from Iran is described and illustrated here as *L. khorassanicum* and *L. turcomanicum,* respectively. The original description of the latter species is incomplete and ambiguous, probably due to incomplete taxon sampling. In this work, after a comprehensive taxon sampling from the entire distribution range of the species in northeast of Iran, the taxonomic description of *L. turcomanicum* is completed. The present study considers morphological and molecular (the nrITS region) analyses of the both species. In phylogenetic analysis based on the molecular data, we included these species and some related *Linum* species to examine the phylogenetic relationship of the new species and *L. turcomanicum* with other members of the genus. *Linum turcomanicum* is morphologically almost similar to *L. austriacum* and *L. perenne*, but it can be distinguished from them on the basis of several traits such as fruiting-pedicel form and length of petal. Likewise, results obtained from the molecular phylogenetic tree are consistent with those obtained from the morphological data. *Linum khorassanicum* is well characterized morphologically by having erect fruiting pedicels and inflorescence with few flowers. Our results suggest that the morphological data are in agreement with the molecular phylogenetic tree in which the taxonomic status of *L. khorassanicum* is confirmed as a new species. Based on IUCN Red List categories and criteria, *L. khorassanicum* and *L. turcomanicum* are evaluated as Endangered and Near Threatened species, respectively.

Keywords: biodiversity, conservation, Khorassan-Kopet Dagh, molecular phylogeny, taxonomy

Introduction

Linum Linnaeus (1753: 277) (subfamily Linoideae) with about 180 species worldwide is the most important genus in the family Linaceae. *Linum* species are distributed throughout the temperate and subtropical regions of the northern hemisphere, most abundantly in Europe, Asia and America. A few species also occupy habitats within the tropics as well (McDill *et al.* 2009, Kubitzki 2014). The species have played an important role in industry and medicine as fibers, oil, food and treatment of cancer and cardiovascular diseases (Gill 1987, Rickard-Bon & Thompson 2003, Mabberley 2008).

Linum consists of five sections in the Flora Iranica area, including sect. *Syllinum* Grisebach (1843: 115), sect. *Linastrum* (Planchon 1847: 597) Winkler (1931: 114), sect. *Dasylinum* Planchon (1847: 598), sect. *Linum* and sect. *Cathartolinum* Planchon (1847: 598) (Rechinger 1974, Sharifnia & Assadi 2001). Several taxonomic studies were performed on section *Linum* such as taxonomy of the *Linum perenne* Linnaeus (1753: 277) group in Europe (Ockendon 1971), revision of American and South African *Linum* (Rogers 1963, 1981; Mildner & Rogers 1978), seed surface patterns of Turkish *Linum* (Özcan & Zorlu 2009) and taxonomic review of the genus *Linum* in Iran (Talebi *et al.* 2012). The section *Linum* comprises six species in Iran including *L. nervosum* Waldstein & Kitaibel (1805: 109), *L. bienne* Miller (1768: No. 8), *L. usitatissimum* Linnaeus (1753: 277), *L. peyronii* Post (1892: 6), *L. glaucum* Boissier & Noë in Boissier (1856: 66) and *L. austriacum* Linnaeus (1753: 278) (Sharifnia & Assadi 2001).

There are various ways used by researchers, such as morphological characteristics and molecular markers, in order to taxonomically evaluate the interspecific boundaries (Stegnii *et al.* 2000, Diederichsen 2001, McBreen *et al.* 2003, Diederichsen & Raney 2006, Diederichsen *et al.* 2006, Fu & Allaby 2010, Farsi *et al.* 2013, Vaezi *et al.* 2014). More

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than 100 species of *Linum* present a wide range of variation in morphological characters, which makes the taxonomic work phenotypically difficult (Gill 1987). However, identification and description of new species using morphological traits alone may not be sufficient to address this goal. Recently, molecular markers are successfully applied to resolve the problem and represent considerable and powerful tools for species delimitation (Duminil *et al.* 2012, Farsi *et al.* 2013, Vaezi *et al.* 2014, Xu *et al.* 2015). The molecular markers such as nrITS and chloroplastic DNA particularly use for studying closely related species and also consider as a source of plant DNA barcoding sequences (Kress *et al.* 2005). Likewise, model-based clustering methods can discriminate species boundaries to establish evolutionary relationship (Duminil *et al.* 2012).

Most molecular studies within the Linoideae and *Linum,* in particular, have been accomplished as genetic diversity (Fu *et al.* 2002, Diederichsen & Fu 2008, Abou El-Nasr & Mahfouze 2013), while phylogenetic investigations are limited. A study of molecular phylogenetic analysis using the non-coding regions of chloroplast DNA sequences was done to establish the relationship among 16 *Linum* species (Fu & Allaby 2010). Furthermore, McDill *et al.* (2009) presented a phylogenetic analysis within subfamily Linoideae and *Linum* using data from the chloroplast (*ndh*F, *trn*L-F, *trn*K3′intron) and the nuclear ITS, with *Hugonia* Linnaeus (1753: 675) (Linaceae: Hugonioideae) as the outgroup. Some studies were carried out on the taxonomy of the genus *Linum* in Iran (Sharifnia & Albouyeh 2002, Hassanzadeh *et al.* 2007, Talebi *et al.* 2012, Sheidai *et al.* 2014). Sharifnia & Assadi (2001) recorded only one perennial *Linum* species, *L. austriacum*, from Khorassan, E and NE Iran. During a taxonomic revision of some voucher specimens determined as *L. austriacum* in NE Iran (mainly deposited in Ferdowsi University of Mashhad Herbarium (FUMH)), we found several specimens which were differed in some characters compared with the description of the species (Shishkin 1949, Davis 1967, Rechinger 1974, Sharifnia & Assadi 2001). *Linum austriacum* can be characterized by drooping fruiting pedicels from other species in section *Linum*, while all observed specimens were different by having the erect fruiting pedicels. For a more detailed review, field surveys were carried out in different regions of northeastern Iran. Along with more morphological differences between the characteristics of the specimens and the description of *L. austriacum* (see below), some specimens were identified as a new species named here as *L. khorassanicum* .

Moreover, *L. turcomanicum* Juzepczuk in Shishkin (1949: 720) was also recognized as a new record for Iran by using the Flora of U.S.S.R (Shishkin 1949). The most important morphological character which distinguishes *L. turcomanicum* from *L. austriacum* was fruiting-pedicel form. While *L. turcomanicum* has been described incompletely in Flora of U.S.S.R (Shishkin 1949), we completed here the description of the species based on a comprehensive taxon sampling from the type location in Misinov (Massinev) Mount and accurate observation of the mature specimens.

Here, we investigated the taxonomic status of the two above-mentioned species in NE Iran. Following the initial surveys, we used morphological characters in a frame of a morphometric analysis as well as sequence data obtained from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA.

Materials and Methods

Taxon sampling and conservation assessment

Herbarium specimens of three *Linum* species, *L. austriacum, L. turcomanicum* and *L. khorassanicum* were collected from different regions of Iran (Fig. 1, Table 1). The specimens were preserved in the Ferdowsi University of Mashhad Herbarium (FUMH), the Herbarium of Research Institute of Forest and Rangelands (TARI), and herbarium of Halophytes and C4 Plants Research Laboratory (Hb. Akhani, in University of Tehran).

Distribution data points were used to prepare distribution map of the species in DIVA-GIS 7.3 software (Hijmans *et al.* 2001). The geographical ranges of the taxa in the form of the extent of occurrence (EOO) and area of occupancy (AOO) in criterion B were applied to categorize the threat status (IUCN 2011). We used the occurrence data of the species in GeoCAT in order to calculate EOO and AOO for Red Listing (Bachman *et al.* 2011).

Morphometric study

Morphological analyses were carried out with 55 herbarium and field-collected *Linum* specimens (31, 16, and 8 individuals of *L. turcomanicum*, *L. austriacum*, and *L. khorassanicum*, respectively). For each sample, we measured fifty-two morphological traits including 26 quantitative and 26 qualitative ones (Table 2). Quantitative traits were measured using a ruler with the precision of 0.1 mm and qualitative traits were numerically codified using multi-status criteria (from 0 to 4). Univariate analyses were performed to discriminate the species effectively by the characters. Distributions of the quantitative characters were tested for normality using Kolmogorov-Smirnov test. This analysis indicated that some quantitative characters were not normally distributed. Therefore, non-normal variables were normalized using transformation methods. The Mann-Whitney U test was also used to detect statistical significance of morphological differences between all species pairs (*L. turcomanicum* and *L. khorassanicum*, *L. austriacum* and *L. khorassanicum*, *L. turcomanicum* and *L. austriacum*). Due to the fact that data matrix consists of both qualitative and quantitative characters, a multidimensional scaling analysis (MDS) was implemented. Indeed, MDS was used to obtain the general view on the morphological variation pattern among the individuals of the three species using the module ALSCAL. All univariate and multivariate analyses were performed using SPSS release 18.0.0 (SPSS Inc., Chicago, USA).

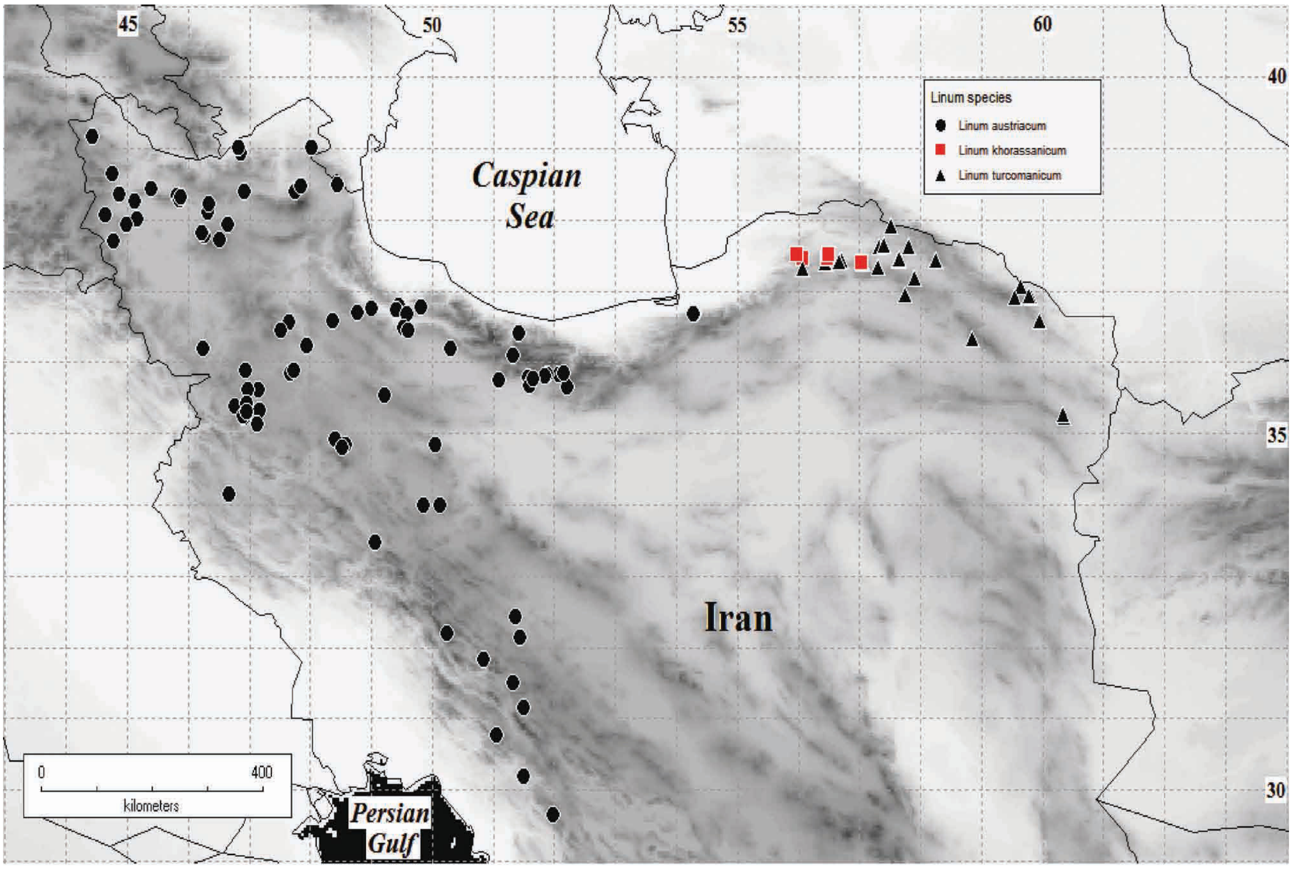

FIGURE 1. Geographical distribution of *Linum khorassanicum* (squares), *L. turcomanicum* (triangles) and *L. austriacum* (circles) in the region under study. The localities are based on herbarium records and also distribution data in Flora Iranica (Rechinger 1974).

TABLE 1. Voucher specimens included in the morphological (Mor.) and molecular (Mol.) analyses, plus GenBank accessions of haplotypes sequenced in the present study. The letters above the species name represent the voucher specimens included in the phylogenetic analyses (Fig. 3).

Species	Locality	Lat./ Long.	Mor.	Mol.	GenBank Acc.
L. turcomanicum	SE Kalat-e Naderi, between Jalil-Abad & Qaleh-Now, 1200 m, Joharchi 43013 (FUMH)	N 36° 55' 21.25" E 59° 48' 40.48"	√	-	-
L. turcomanicum	NW Kalat-e Naderi, 10 km from Kalat-e Naderi towards Archangan, 1100 m, Faghihnia & Zangooei 28939 (FUMH)	N 37° 4' 40.8" E 59° 38' 52.8"	√	-	-
L. turcomanicum	N Mashhad, 50 km on the road towards Kalat-e Naderi, 1500 m, Faghihnia & Zangooei 22090 (FUMH)	N 36° 35' 45.6" E 59° 56' 34.8"	√	-	-
L. turcomanicum[A]	W Torbat-e Jam, between Kalateh-Sefid and Dakal, SE mountains of Revenj, 1650 m, Joharchi 34158 (FUMH)	N 35° 14' 2.4" E 60° 20' 45.6"	√	√	KY661901
L. turcomanicum	W Bojnurd, 80 km west of Bojnurd between Chaman-Bid and Jowzak, 1300 m, Joharchi & Zangooei 16626 (FUMH)	N 37° 26' 2.4" E 56° 41' 45.6"	√	-	-

......*continued on the next page*

TABLE 1. (Continued)

Species	Locality	Lat./ Long.	Mor.	Mol.	GenBank Acc.
L. turcomanicum	W Bojnurd, southern slopes between Jowzak and Chaman-Bid, 1200 m, Joharchi & Zangooei 32670 (FUMH)	N 37° 24’ 57.6” E 56° 39’ 50.4”	√	-	-
L. turcomanicum	NE mountains of Neyshabour, 1600–1900 m, Assadi & Mozaffarian 36080 (TARI)	N 36° 20’ 14.23” E 58° 51’ 12.54”	√	-	-
L. turcomanicum	E Bojnurd, Sisab, Agricutural Research Station, 1450 m, Joharchi 16682 (FUMH)	N 37° 26’ 16.8” E 57° 38’ 16.8”	√	-	-
L. turcomanicum[B]	W Bojnurd, Ghorkhod Protected Area, west of Chaman-Bid, 1700 m, Memariani & Arjmandi 45034 (FUMH)	N 37° 23’ 49.1” E 56° 26’ 48.8”	√	√	KY661902
L. turcomanicum[C]	Kalat-e Naderi, Hammam-Qala bifurcation road, 1070 m, Joharchi & Behroozian 45044 (FUMH)	N 36° 56’ 2.4” E 59° 46’ 34.6”	√	√	KY661903
L. turcomanicum	W Bojnurd, Ghorkhod Protected Area, on the road towards Ternuli, 1700 m, Memariani & Arjmandi 44355 (FUMH)	N 37° 23’ 28.1” E 56° 26’ 27.4”	√	-	-
L. turcomanicum	W Bojnurd, Ghorkhod Protected Area, in *Juniperus* woodlands of Ternuli valley, 1859 m, Memariani & Arjmandi 43915 (FUMH)	N 37˚ 24’ 28.2” E 56˚ 25’ 33.2”	√	-	-
L. turcomanicum	N Bojnurd, 2 km south of Qezelqan, 1170–1190 m, Memariani & Zangooei 42569 (FUMH)	N 37º 39’ 01.9” E 57º 24’ 27.8”	√	-	-
L. turcomanicum	NW Quchan, Faruj towards Oqaz-e Kohneh, Qaleh-Safa mountains, 1750 m, Faghihnia & Zangooei 29334 (FUMH)	N 37° 26’ 6” E 58° 14’ 56.4”	√	-	-
L. turcomanicum[D]	N Mashhad, Hezar-Masjed mountains, 1km north of Kharkat, 2015–2020 m, Joharchi & Behroozian 45042 (FUMH)	N 36° 54’ 30.1” E 59° 32’ 37”	√	√	KY661904
L. turcomanicum	SW Torat-e Jam, southern mountains of Kalate-Sefid, 1805 m, Joharchi & Behroozian 45055 (FUMH)	N 35° 15’ 55.8” E 60° 19’ 49”	√	-	-
L. turcomanicum	SW Bojnurd, between Hessar-e Hosseini and Shoghan, 2005 m, Joharchi & Memariani 45028 (FUMH)	N 37° 19’ 6.5” E 56° 3’ 32.6”	√	-	-
L. turcomanicum	SW Bojnurd, between Rein and Arkan, Qaranqazo, 2050 m, Joharchi & Memariani 45026 (FUMH)	N 37° 23’ 47.7” E 57° 3’ 9.6”	√	-	-
L. turcomanicum	SW Bojnurd, Rein towards Arkan, eastern & northeastern slopes of Qaranqazo, 2050 m, Memariani & Zangooei 37548 (FUMH)	N 37° 23’ 55.9” E 57° 03’ 06.1”	√	-	-
L. turcomanicum	E Bojnurd, Sisab, 1450 m, Rashed 18492 (FUMH)	N 37° 27’ 28.8” E 57° 39’ 14.4”	√	-	-
L. turcomanicum	S Shirvan, Gelian towards Estarkhi, 1600 m, Joharchi & Zangooei 10426 (FUMH)	N 37° 11’ 34.8” E 57° 53’ 42”	√	-	-
L. turcomanicum	N Shirvan, Sevalli (Sevaldi) towards Loujalli, 1400 m, Faghihnia & Zangooei 25886 (FUMH)	N 37° 37’ 26.4” E 57° 47’ 56.4”	√	-	-
L. turcomanicum	S Bojnurd, Mehnan, 1500 m, Joharchi & Mahvan 10276 (FUMH)	N 37° 21’ 00” E 57° 18’ 7.2”	√	-	-
L. turcomanicum	SE Esfarayen, Sarigol, Tangeh-e Baba-Qodrat elevations, 1700m, Rafei & Zangooei 31659 (FUMH)	N 36° 57’ 14.4” E 57° 45’ 10.8”	√	-	-
L. turcomanicum	Bojnurd, NE Gifan, Misinov Mount, 2282 m, Joharchi 45130 (FUMH)	N 37˚ 55’ 13.1” E 57˚ 30’ 35”	√	-	-
L. turcomanicum	Bojnurd, NE Gifan, Misinov Mount, 2282 m, Joharchi 45131 (FUMH)	N 37˚ 55’ 13.1” E 57˚ 30’ 35”	√	-	-
L. turcomanicum[E]	Bojnurd, NE Gifan, Misinov Mount, 2282 m, Joharchi 45132 (FUMH)	N 37˚ 55’ 13.1” E 57˚ 30’ 35”	√	√	KY661905
L. turcomanicum	Bojnurd, NE Gifan, Misinov Mount, 2282 m, Joharchi 45133 (FUMH)	N 37˚ 55’ 13.1” E 57˚ 30’ 35”	√	-	-
L. turcomanicum	Bojnurd, NE Gifan, Misinov Mount, 2282 m, Joharchi 45134 (FUMH)	N 37˚ 55’ 13.1” E 57˚ 30’ 35”	√	-	-

......*continued on the next page*

TABLE 1. (Continued)

Species	Locality	Lat./ Long.	Mor.	Mol.	GenBank Acc.
L. turcomanicum	Bojnurd, NE Gifan, Misinov Mount, 2282 m, Joharchi 45135 (FUMH)	N 37˚ 55’ 13.1” E 57˚ 30’ 35”	√	-	-
L. turcomanicum	Bojnurd, NE Gifan, Misinov Mount, 2282 m, Joharchi 45136 (FUMH)	N 37˚ 55’ 13.1” E 57˚ 30’ 35”	√	-	-
L. khorassanicum [A]	SW Bojnurd, Rein, western slope of Tupal-Rayeh, 2015 m, Memariani, Zangooei & Arjmandi 37679 (FUMH)	N 37° 24’ 06.9” E 57° 02’ 26.2”	√	√	KY661899
L. khorassanicum	W Bojnurd, Ghorkhod Protected Area, 9–10 km from Zard towards Kastan, 1560 m, Memariani & Arjmandi 43877 (FUMH)	N 37˚ 30’ 50.7” E 56˚ 29’ 00.4”	√	-	-
L. khorassanicum	W Bojnurd, Ghorkhod Protected Area, 9–10 km from Zard towards Kastan, 1560 m, Memariani & Arjmandi 43878 (FUMH)	N 37˚ 30’ 50.7” E 56˚ 29’ 00.4”	√	-	-
L. khorassanicum	W Bojnurd, Ghorkhod Protected Area, 9–10 km from Zard towards Kastan, 1560 m, Memariani & Arjmandi 43879 (FUMH)	N 37˚ 30’ 50.7” E 56˚ 29’ 00.4”	√	-	-
L. khorassanicum	W Bojnurd, Ghorkhod Protected Area, northern slopes of Ghorkhod Mount, Memariani & Arjmandi 44460 (FUMH)	N 37˚ 27’ 42.2” E 56˚ 28’ 11.9”	√	-	-
L. khorassanicum	W Bojnurd, Ghorkhod Protected Area, 7 km west of Zard towards Kastan, 1510 m, Joharchi, Memariani & Behroozian 45035 (FUMH)	N 37º 30́ 56.5″ E 56º 29́ 02.0″	√	-	-
L. khorassanicum	W Bojnurd, Ghorkhod Protected Area, 7 km west of Zard towards Kastan, 1510 m, Joharchi, Memariani & Behroozian 45036 (FUMH)	N 37º 30́ 56.5″ E 56º 29́ 02.0″	√	-	-
L. khorassanicum [B]	W Bojnurd, Ghorkhod Protected Area, 7 km west of Zard towards Kastan, 1510 m, Joharchi, Memariani & Behroozian 45037 (FUMH)	N 37º 30́ 56.5″ E 56º 29́ 02.0″	√	√	KY661900
L. austriacum	NE Tehran, Lar valley, 2450–2550 m, Wendelbo & Assadi 13239 (TARI)	N 35˚50’ 08.66” E 51˚ 52’ 23.69”	√	-	-
L. austriacum	NE Tehran, Taloo, 1800 m, Dini 8937 (TARI)	N 35˚46’ 26.08” E 51˚ 38’ 40.51”	√	-	-
L. austriacum	NE Tehran, Lar valley, 2500 m, Dini & Arazm 14462 (TARI)	N 35˚49’ 16.72” E 51˚ 50’ 33.93”	√	-	-
L. austriacum	NE Tehran, Lar valley, 1800 m, Dini & Arazm 14464 (TARI)	N 35˚46’ 26.08” E 51˚ 38’ 40.51”	√	-	-
L. austriacum[A]	Chaharmahal and Bakhtiari, Sabzkooh, Chahartagh, 2350 m, Mozaffarian 59960 (TARI)	N 31˚50’ 15” E 50˚ 50’ 06”	√	√	KY661906
L. austriacum	Chaharmahal and Bakhtiari, Lordegan, road to Dorahoon, Abvanak bridge, 1800 m, Mozaffarian 54998 (TARI)	N 31˚30’ 25.93” E 51˚ 18’ 21.24”	√	-	-
L. austriacum[B]	East Azarbaijan, Arasbaran, NW Vinagh, 1300–1400 m, Assadi & Vosughi 24653 (TARI)	N 39˚01’ 44.05” E 46˚ 48’ 34.55”	√	√	KY661907
L. austriacum	East Azarbaijan, Arasbaran, Vaighan towards Vinagh, 1000 m, Assadi & Maassoumi 20473 (TARI)	N 38˚56’ 40.58” E 46˚ 51’ 03.97”	√	-	-
L. austriacum	West Azarbaijan, Bazargan, Siah- Cheshmeh road, Beduli, 2000 m, Assadi 85280 (TARI)	N 39˚10’ 53.27” E 44˚ 25’ 28.24”	√	-	-
L. austriacum	East Azarbaijan, Tabriz road to Tehran, near GhoriGol, Shebli pass, 2300 m, Assadi 85347 (TARI)	N 37˚56’ 33.47” E 46˚ 38’ 39.88”	√	-	-
L. austriacum	Kordestan, west of Sanandaj, towards Marivan, 1750 m, Assadi 84927 (TARI)	N 35˚19’ 17.99” E 46˚ 57’ 20.16”	√	-	-
L. austriacum	Kermanshah, towards Eslamabad Gharb, Hovaroo Mount, 1600–1900 m, Mirabdali & Heidari 2993 (TARI)	N 34˚09’ 12.54” E 46˚ 39’ 06.16”	√	-	-

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TABLE 1. (Continued)

Species	Locality	Lat./ Long.	Mor.	Mol.	GenBank Acc.
L. austriacum	Markazi provence, Arak towards Mohallat, Lattedar Mount, 2100–2500 m, Mozaffarian & Maassoumi 47911(TARI)	N 33˚59' 49" E 50˚ 06' 51"	√	-	-
L. austriacum	Gilan, between Barresar and Deilaman, 1500 m, Assadi 86404 (TARI)	N 36˚47' 12.91" E 49˚ 48' 39.28"	√	-	-
L. austriacum	Golestan, Jahan-Nama Protected Area, Gholgholi and Khersdarreh, 1772 m, Jafari 1915 (Hb. Akhani)	N 36˚40' 69" E 54˚ 16' 60"	√	-	-
L. austriacum	Golestan, Jahan-Nama protected area, Atashan rocks towards Imamverdi hills, 1753 m, Jafari 2306 (Hb. Akhani)	N 36˚40' 59" E 54˚ 16' 29"	√	-	-

Molecular methods

DNA extraction, PCR amplification and sequencing

Two, two and five individuals of geographically distant populations were included in the molecular study for the species *L. khorassanicum*, *L. austriacum* and *L. turcomanicum*, respectively (Table 1). Silica-dried leaves of the plant materials were used for DNA extraction following a modification of the Doyle & Doyle (1987) CTAB protocol (Joly *et al.* 2006). Amplification of the nrITS region (ITS1-5.8S-ITS2) was done in 25 µl reactions containing 2.5 µl 10X PCR buffer (Fermentas, Lithuania), 2.5 µl $MgCl_2$ (25 mM, Fermentas, Lithuania), 0.2 mM of each dNTP, 2U of Taq polymerase, 100 µmol/L of the universal primers ITS4 and ITS5 (White *et al.* 1990), and ca. 200 ng genomic DNA. An initial denaturation step at 95 ºC for 5 min was followed by 35 cycles of denaturation (30 s at 95 ºC), annealing at 52 ºC for 30 s, elongation at 72 ºC for 1 min and a final extension at 72 ºC 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, Michigan). Furthermore, BLAST was used to perform similarity searches comparing the sequences generated in the current work with those in GenBank. Consequently, we combined new sequences collected in the present study with *Linum* sequences from the two sections, *Linum* and *Dasylinum*, available in GenBank (*L. stelleroides* Planchon (1848: 178), FJ169516; *L. viscosum* Linnaeus (1762: 398), FJ169517; *L. pubescens* Banks & Solander (1794: 268), FJ169518; *L. hypericifolium* Salisbury ex Steudel (1821: 484), FJ169519; *L. hirsutum* Linnaeus (1753: 277), FJ169520; *L. lewisii* Pursh (1814: 210), FJ169523; *L. perenne*, FJ169524; *L. grandiflorum* Desfontaines (1798: 278), FJ169525; *L. usitatissimum*, FJ169526; *L. bienne*, FJ169527; *L. marginale* Cunningham (1825: 357), FJ169528) and one outgroup (*Reinwardtia indica* Dumortier (1822:19), FJ169514) to make a single alignment.

Phylogenetic analyses

ITS sequences were aligned with Clustal W (Thompson *et al.* 1994) as implemented in BioEdit Sequence Alignment Editor (Hall 1999), followed by manual adjustments. The gaps were coded as simple indels (Simmons & Ochoterena 2000) using SeqState version 1.25 (Müller 2005) and they appended to the sequence matrix as binary characters.

ITS sequences were used to produce phylogenies using Maximum Parsimony (MP) and Bayesian Inference (BI). MP was implemented in PAUP* version 4.10b (Swofford 2002). All characters were treated as unordered and gaps as missing data. A heuristic search was performed with 1000 random replicates and TBR branch swapping. One thousand bootstrap replicates were analysed as implemented in PAUP* using the heuristic search option with 10 random taxon additions. Bayesian optimality criterion was conducted using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). To determine the evolutionary model that best fitted for the three partitions (ITS1, 5.8S, and ITS2), the hierarchical likelihood ratio test was computed using MrModeltest 2.2 (Nylander 2004) with executable MrModelblock file in PAUP*. Among the 24 available models, the SYM+G**,** K80+I, and GTR+G substitution models were chosen as the best fitting models considering the Akaike Information Criterion (AIC) (Vaezi & Brouillet 2009; Vaezi *et al.* 2014) for the ITS1, 5.8S, and ITS2 partitions, respectively. However, the Bayesian MCMC inference was performed for ten million generations, sampling every 100 generations. The convergence and burn-in phases were confirmed by comparing the posterior probabilities of different splits among runs and by plotting the log likelihood values from each run as implemented in Tracer version 1.4 (Rambaut & Drummond 2007). Trees were visualized using TreeView version 1.6.6 (Page 2001).

Results

Morphological analyses

According to univariate results, 8 of 52 morphological characters (2 quantitative and 6 qualitative) did not significantly discriminate paired species *L. turcomanicum* vs *L. khorassanicum* , *L. austriacum* vs *L. khorassanicum* , and *L. turcomanicum* vs *L. austriacum* (indicated by the symbol † in Table 2). Therefore, these traits were excluded from the subsequent analyses. Results of the statistical Mann-Whitney test indicated that 35 morphological characters clearly differentiate between the species pairs, *L. turcomanicum* vs *L. khorassanicum* and *L. austriacum* vs *L. khorassanicum* (indicated by the symbols * and §, respectively in Table 2). Furthermore, 14 traits significantly discriminate the individuals of *L. turcomanicum* from those of *L. austriacum* (indicated by the symbol ¥ in Table 2).

Multidimensional scaling analysis resulted in an ordination that plotted individuals in a coordinate system with two dimensions (Fig. 2). This analysis had a low stress value (0.07). This value represents a measure of fit where the goodness of fit ranges from 0 to 0.4, the closer to 0 this value is, the better the fit to the data (Rohlf 2000, Hout *et al.* 2013). The MDS analysis showed a clear segregation between individuals of the three taxa. The first dimension completely discriminated the individuals of *L. khorassanicum* from those of the species pair, *L. turcomanicum* and *L. austriacum*. The second one moderately differentiated the individuals of *L. turcomanicum* from those of *L. austriacum*. These results are largely congruent with those obtained from the univariate analyses (Table 2).

TABLE 2. Description and results of the quantitative and qualitative morphological traits used in the present study. Differentiating characters between the species, *Linum turcomanicum* (LT), *L. khorassanicum* (LKH), and *L. austriacum* (LA) are marked with symbols above the abbreviated characters. P-Value less than 0.05 is used to reject the null hypothesis of non-significantly differentiating characters. Symbols: * the characters differentiated *L. turcomanicum* from *L. khorassanicum*; § the characters differentiated *L. austriacum* from *L. khorassanicum*; ¥ the characters differentiated *L. turcomanicum* from *L. austriacum;* † the characters that did not significantly differentiate the taxa under study.

Character	Abbrev.	Mean			Min			Max			Std. dev.			Mann Whitney Test (P-Value)		
		LT	LA	LKH	LT	LA	LKH	LT	LA	LKH	LT	LA	LKH	LT/LKH	LA/LKH	LT/LA
Plant height (mm)	PLHT*§¥	371	497	276	190	290	200	670	890	350	124	153	58	0.04	<0.0001	<0.0001
Length of lower leaf (mm)	LOLL*§	12.7	12.7	5.9	6.0	7.0	4.5	27.0	26.0	7.0	4.7	5.1	1.0	<0.0001	<0.0001	0.81
Width of lower leaf (mm)	WOLL*	2.0	1.6	1.1	0.8	0.8	1.0	5.0	2.5	1.3	0.8	0.5	0.1	<0.0001	0.08	0.07
Length of middle leaf (mm)	LOML*§	15.4	16.9	8.7	1.4	7.5	6.5	22.5	26.0	11.0	4.9	6.1	1.9	<0.0001	<0.0001	0.51
Width of middle leaf (mm)	WOML*§	2.3	1.9	1.1	0.7	0.9	0.9	10.5	3.8	1.8	1.6	0.8	0.3	<0.0001	0.01	0.27
Length of upper leaf (mm)	LOUL*	8.8	8.5	6.8	4.0	5.5	5.0	20.5	15.0	8.0	3.1	2.5	1.1	0.04	0.08	0.83
Width of upper leaf (mm)	WOUL†	1.4	1.3	1.4	0.6	0.7	1.0	2.3	2.5	1.8	0.5	0.5	0.2	1	0.26	0.2
Lower internode length (mm)	LINL*§	6.5	6.5	3.6	1.3	4.0	2.5	19.0	14.0	8.0	3.7	2.6	1.8	<0.0001	<0.0001	0.39
Middle internode length (mm)	MINL¥	7.8	9.9	9.7	4.0	6.0	4.0	13.0	19.5	25.0	2.5	3.3	6.7	0.77	0.36	0.02
Upper internode length (mm)	UINL¥	10.3	13.1	11.8	4.0	8.0	5.0	16.5	19.5	21.5	3.0	3.8	4.9	0.43	0.47	0.02
Stem diameter (mm)	STDI§¥	1.5	1.9	1.0	0.6	1.2	0.8	8.5	2.5	1.1	1.4	0.4	0.1	0.1	<0.0001	<0.0001
Pedicel length (mm)	PELE§¥	13.1	18.1	14.5	8.0	13.5	10.0	17.5	22.5	21.0	2.2	2.7	3.6	0.37	0.02	<0.0001
Length of outermost sepal (mm)	LOLS*§	5.1	5.0	6.7	4.0	3.5	6.0	6.0	7.0	8.0	0.6	1.0	0.7	<0.0001	<0.0001	0.38
Width of outermost sepal (mm)	WOLS*§¥	2.1	2.4	3.9	1.5	2.0	3.5	2.8	3.5	5.0	0.3	0.4	0.5	<0.0001	<0.0001	0.03
Length of petal (mm)	LOPT*§¥	16.1	13.0	18.8	11.5	1.5	15.0	24.0	18.0	21.0	3.0	3.4	1.9	<0.0001	<0.0001	<0.0001
Width of petal (mm)	WOPT*§	9.1	9.5	12.9	5.0	8.0	12.0	13.0	12.0	15.0	2.1	0.9	1.4	<0.0001	<0.0001	0.63
Length of filament (mm)	LOFI*§	5.9	5.1	7.6	3.0	3.6	2.0	8.0	8.0	10.0	1.7	1.5	2.4	0.01	0.01	0.17
Length of style (mm)	LOST*§	5.0	5.8	10.9	2.5	3.0	9.5	7.5	8.0	12.0	1.3	1.6	0.9	<0.0001	<0.0001	0.15
Number of nerves on outermost sepal	NNOS†	3.2	3.0	3.0	3.0	3.0	3.0	5.0	3.0	3.0	0.7	0.0	0.0	0.31	1	0.14
Length of capsule (mm)	LOCA*§¥	6.8	6.2	9.1	5.5	5.0	5.0	9.0	7.0	11.0	0.6	0.6	1.8	<0.0001	<0.0001	<0.0001
Width of capsule (mm)	WOCA*§	5.9	5.6	8.2	5.0	4.0	5.0	7.0	7.0	9.0	0.5	1.0	1.4	<0.0001	<0.0001	0.09
Length of innermost sepal (mm)	LOIS*§	5.4	5.4	7.3	4.0	4.5	7.0	6.5	7.0	8.0	0.6	0.8	0.5	<0.0001	<0.0001	0.64
Width of innermost sepal (mm)	WOIS*§	3.5	3.6	5.7	3.0	3.0	5.0	4.0	4.0	6.0	0.4	0.4	0.5	<0.0001	<0.0001	0.41
Margin thickness of innermost sepal (mm)	MTOS*§	0.6	0.6	1.0	0.3	0.3	0.8	1.0	0.9	1.3	0.1	0.1	0.2	<0.0001	<0.0001	0.97
Length of anther (mm)	LOAN*§	1.6	1.5	3.0	1.4	1.0	2.6	2.0	2.0	3.2	0.2	0.2	0.2	<0.0001	<0.0001	0.36
Width of anther (mm)	WOAN*§	0.6	0.6	1.2	0.5	0.5	0.9	0.7	1.0	1.5	0.1	0.1	0.2	<0.0001	<0.0001	0.26
Branch of stem (1: low branches above 2: ramosus from the upper half)	BOSM*§	—	—	—	2	2	1	2	2	2	—	—	—	<0.0001	<0.0001	1

......*continued on the next page*

TABLE 2. (Continued)

Character	Abbrev.	Mean			Min			Max			Std. dev.			Mann Whitney Test (P-Value)		
		LT	LA	LKH	LT	LA	LKH	LT	LA	LKH	LT	LA	LKH	LT/LKH	LA/LKH	LT/LA
Stem status (1: erect and ascending 2: curved at base 3: both)	STST†	—	—	—	1	1	1	1	1	1	—	—	—	1	1	1
Sterile stem (1: exist 2: not exist)	STSM*	—	—	—	1	1	1	2	2	1	—	—	—	0.05	0.22	0.18
Leaf status (1: erect and imbricate 2: spreading)	LEST*§	—	—	—	1	1	2	1	1	2	—	—	—	<0.0001	<0.0001	1
Tip shape of lower leaf (1: obtuse; 2:acute; 3:acuminate)	TSLL§¥	—	—	—	2	2	2	3	3	2	—	—	—	0.31	0.01	<0.0001
Tip shape of middle leaf (1: obtuse; 2:acute; 3:acuminate)	TSML†	—	—	—	2	3	3	3	3	3	—	—	—	0.25	1	0.09
Tip shape of upper leaf (1: obtuse; 2:acute; 3:acuminate)	TSUL*	—	—	—	3	3	2	3	3	3	—	—	—	0.04	0.15	1
Margin thickness of lower leaf (1: scabrid; 2: smooth)	MTLL*¥	—	—	—	1	2	1	1	2	2	—	—	—	<0.0001	0.15	<0.0001
Color of leaf (1: blue-green; 2: gray-green; 3:green 4; 1,2)	COLF¥	—	—	—	2	2	2	3	3	3	—	—	—	0.7	0.1	0.01
Margin thickness of sepal (1: herbaceous; 2:membranous)	MTIS†	—	—	—	2	2	2	2	2	2	—	—	—	1	1	1
Margin color of sepal (1: white; 2: green-herbacous)	MCOS†	—	—	—	1	1	1	1	1	1	—	—	—	1	1	1
Tip shape of outermost sepal (1: acuminate; 2: obtuse: 3:obtuse-rouded; 4:1,2)	TSOS*§	—	—	—	1	3	2	3	3	2	—	—	—	<0.0001	<0.0001	0.06
Pedicel status of fruit (1:erect; 2: drooping)	PSOF§¥	—	—	—	1	2	1	1	2	1	—	—	—	1	<0.0001	<0.0001
Tip shape of petal (1: obtuse; 2: obtuse-rounded)	TSOP†	—	—	—	2	2	2	2	2	2	—	—	—	1	1	1
Color of capsule (1: yellow-brown; 2:dark brown; 3:yellow-green)	COCE*	—	—	—	1	1	1	3	3	1	—	—	—	0.05	0.14	0.36
Shape of outermost sepal (1: oblong-elliptic; 2: wide-round ovate; 3: ovate)	SOOS*§	—	—	—	1	1	3	1	1	3	—	—	—	<0.0001	<0.0001	1
Shape of innermost sepal (1: oblong-elliptic; 2: wide- round ovate; 3: ovate)	SOIS§¥	—	—	—	1	2	2	3	3	2	—	—	—	0.39	0.02	0.01
Shape of capsule (1: ovate; 2: wide ovate; 3: orbiculate)	SOCE*§	—	—	—	1	1	2	1	1	2	—	—	—	<0.0001	<0.0001	1
Tip shape of innermost sepal (1: acuminate; 2: obtuse: 3:obtuse-rouded; 4:1,2)	TSIS†	—	—	—	4	3	4	4	4	4	—	—	—	1	0.49	0.16
Shape of style (1: clavate; 2: capitate)	SOSE*§	—	—	—	2	2	1	2	2	2	—	—	—	<0.0001	0.01	1
Color of style (1: yellow; 2: blue; 3: both)	COSE*§	—	—	—	1	1	2	1	1	2	—	—	—	<0.0001	<0.0001	1
Color of anther (1: yellow-brown; 2: yellow-blue; 3: blue)	COAR*§	—	—	—	1	1	2	1	1	2	—	—	—	<0.0001	<0.0001	1
Nerve thickness of sepal (1: specified; 2: non-specified and thin; 3: both)	NTOS*§	—	—	—	1	1	1	1	1	2	—	—	—	<0.0001	<0.0001	1
Number of nerve on leaf (1: 1–3(–5); 2: 3; 3: 0–1)	PFOI§¥	—	—	—	1	2	1	1	2	1	—	—	—	1	<0.0001	<0.0001
Position of fruit on inflorescence (1: two sides of inflorescence; 2: one sides of inflorescence; 3: both)	MN0L*§	—	—	—	1	1	3	4	4	3	—	—	—	0.01	<0.0001	0.2
Position of stamens and styles (1: stamens long; 2: styles long; 3: stamens and styles equal)	SOSS*§	—	—	—	1	1	2	1	1	2	—	—	—	<0.0001	<0.0001	1

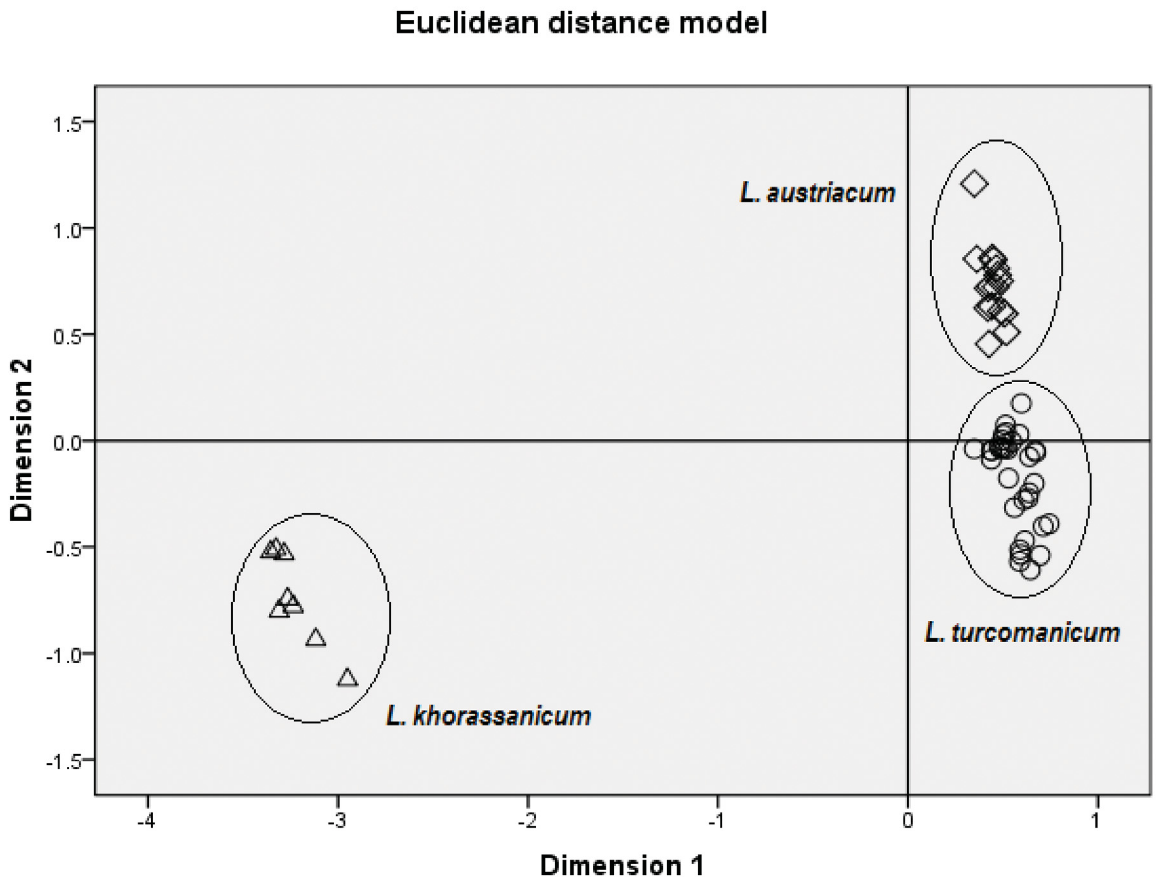

FIGURE 2. Ordination diagram of the multidimensional scaling analysis of 44 differentiating morphological characters comprising 31, 8, and 16 specimens of *Linum turcomanicum, L. khorassanicum* , and *L. austriacum,* respectively.

Molecular analyses

Overall, 22 sequences were incorporated in the analyses, including 726 aligned characters, of which 349 were constant and 255 parsimony informative. The remaining 122 characters were parsimony uninformative. Maximum parsimony analysis of inferred sequences yielded three equally most parsimonious trees (L = 687; CI = 0.77; RI = 0.86; RC = 0.66). The strict and 50% majority rule consensus ITS trees obtained from parsimony and Bayesian analyses, respectively, produced congruent tree without any major difference (Fig. 3). Four and three intraspecific variations were found between the individuals of *L. austriacum* and *L. turcomanicum*, respectively. No such polymorphisms, however, were observed between the two ribotypes of the new species. Thus, these identical ribotypes were collapsed to a branch sister to clade I. The latter clade consists of polytomous subclade II comprising five individuals of *L. turcomanicum* (named A–E in Fig. 3) and subclade III, where the ribotypes of the three species *L. austriacum, L. lewisii*, and *L. perenne* are nested within it.

Discussion

The morphological results appear to be consistent with those of the molecular (Figs. 2 and 3) in terms of first, a clear segregation between *Linum turcomanicum* and *L. austriacum* and second, the taxonomic status of *L. khorassanicum* as a new species. In general, the Man-Whitney test results have shown that 8 of 21 (38%) vegetative traits significantly discriminate *L. turcomanicum* from *L. austriacum*, in contrast to 6 of 31 (19%) floral characters (Table 2). *Linum turcomanicum* is consistently grouped with *L. austriacum* and *L. perenne* (Fig. 3). All the three species are morphologically almost similar but they can be distinguished from each other by several morphological features such as the fruiting-pedicel form and apex of the 3-outermost sepals (Table 3). These species have inflorescences composed of many

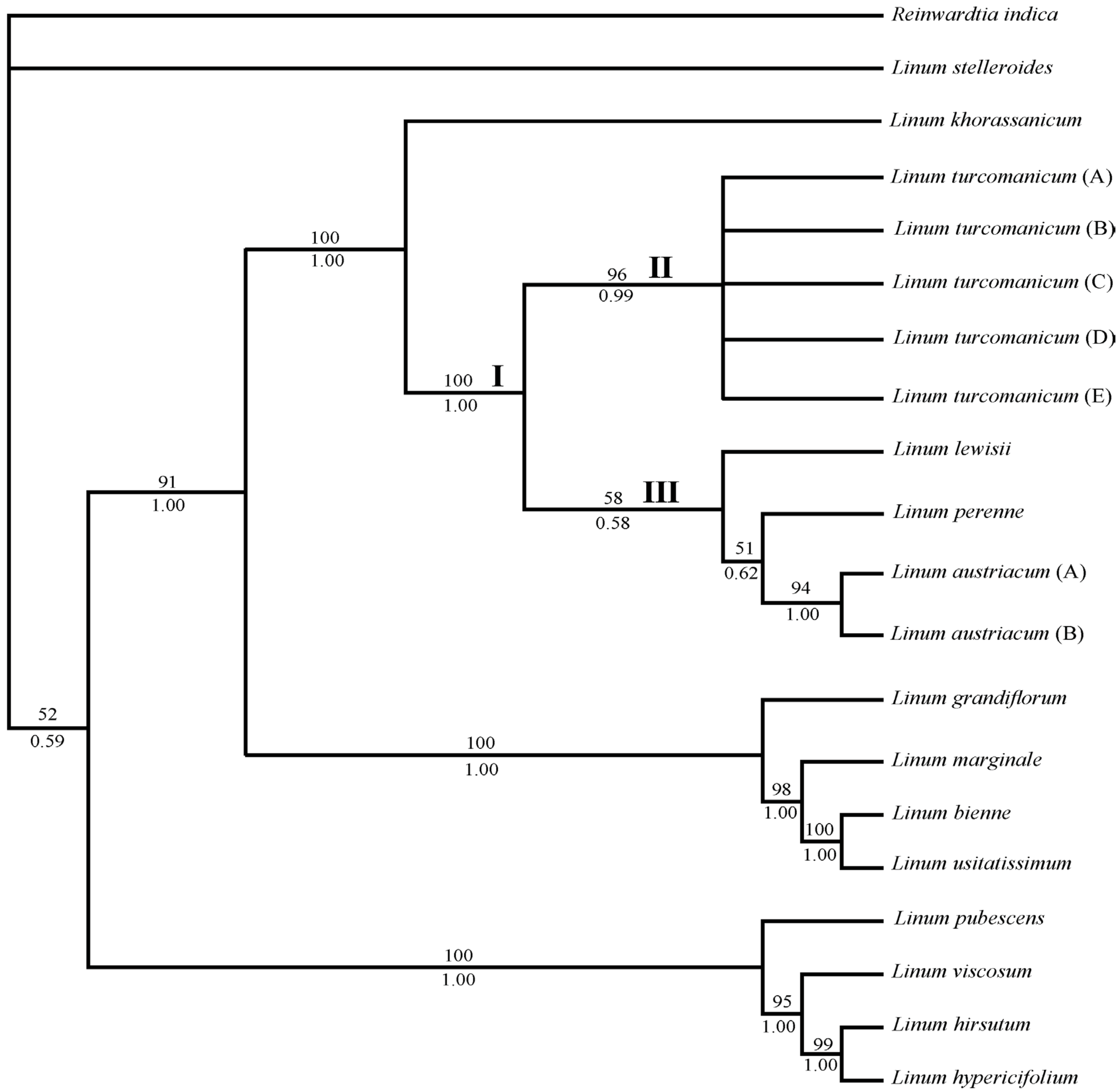

FIGURE 3. Phylogenetic relationships among the *Linum* species under study and related species resulting from the ITS data set based on Maximum Parsimony and Bayesian analyses. The number above and below the branches represent bootstrap supports and Bayesian posterior probabilities, respectively. The roman numbers (I–III) on the branches are explained in the text. The letters in parentheses correspond to the accessions represented in the Table 1.

flowers (2–12 flowers) and similar size in flowers, anthers and capsules. However, *L. turcomanicum* morphologically differs from *L. austriacum* by 14 vegetative and floral traits. Moreover, the molecular evidence obtained from this study provided a valuable set of analytical characters for delimitation of these species (Fig. 3). Geographically, *L. austriacum* is distributed widely in the western and partly central mountainous areas of Iran, whereas *L. turcomanicum* is dispersed in the northeast of Iran without any overlapping localities with *L. austriacum* (Fig. 1). Detection of intraspecific polymorphisms in *L. austriacum* and *L. turcomanicum* seems to be related more to the large geographic range occupied by the species, particularly by the first (see Wendel & Albert 1992). These intraspecific molecular variations, however, had no effect on decreasing species delimitation in the consensus tree (*i.e.*, alleles coalesce within the species).

Based on the univariate morphological results, several non-overlapping variables discriminated *L. khorassanicum* from the species *L. turcomanicum* and *L. austriacum* (Table 2). Among them, branch of stem, tip shape of outermost sepals, length and width of sepals, petals, anther and capsule, shape of capsule and color of anther and style are the best discriminating morphological features. Furthermore, the multivariate analysis of morphological variations supports segregation of *L. khorassanicum* from the species, *L. turcomanicum* and *L. austriacum* (Fig. 2). *Linum khorassanicum* is well characterized morphologically by having erect fruiting pedicels and inflorescence with few flowers. Although within the most species of *Linum* both the long and short-style forms are found (McDill *et al.* 2009), *L. khorassanicum* is assigned only with the long-style form. Moreover, in *L. khorassanicum* an increase in the size of flowers, anthers and capsules can be observed.

The ITS sequences used here provided enough power of distinction at the specific level (see also, Albaladejo *et al.* 2005, Kress *et al.* 2005). The ITS phylogenetic tree obtained from this study (Fig. 3) showed that *L. khorassanicum* occupies a unique position basal to the clade I comprising the species *L. turcomanicum*, *L. lewisii, L. perenne* and *L. austriacum* with strong bootstrap (100%) and Bayesian posterior probability (1.00) supports. In other words, the phylogenetic tree supports delimiting the new species from the other *Linum* species included in the current study. This agrees well with the results obtained from the morphometric analyses (Fig. 2, Table 2). As a result, our morphological and molecular data suggested that *L. khorassanicum* can be recognized as a distinct species.

TABLE 3. Morphological comparison among *Linum khorassanicum, L. perenne*, *L. austriacum*, and *L. turcomanicum.*

	L. khorassanicum	*L. perenne*	*L. austriacum*	*L. turcomanicum*
Leaves	pale green to canescens	glaucescent to usually pure-green	glaucous or gray- green	green or glaucescent in dry
Inflorescence	few-flowered (1–3-flowered)	many-flowered (2–12-flowered)	many-flowered (2–12-flowered)	many-flowered (2–12-flowered)
Fruiting pedicels	always erect	erect, sometimes curved	recurved or strongly deflexed	erect or hardly recurved
Flowers	36–48 mm in diameter	20–40 mm in diameter	20–30 mm in diameter	26–45 mm in diameter
Calyx	6.5–8 × 3–5 mm	3.5–5 × 2.5–3.5 mm	4–6 × 2.25–3.5 mm	4–6 × 1.5–2.5 mm
Apex of outer sepals	acute to rounded, median nerve extended to apex	obtuse or acuminate, median nerve disappeared to apex	acute, median nerve disappeared to apex	obtuse or acuminate, median nerve disappeared to apex
Corolla	18–24 × 12–15 mm	10–20 × 8–15 mm	10 × 15 mm	13–22 × 6–13 mm
Styles forms	only long-styled	long and short-styled	long and short-styled	long and short-styled
Anthers	3–3.2 mm long	1.5 mm long	1.5–2 mm long	1.2–2mm long
Capsules	10–11 × 9 mm, broadly ovoid	5–7 × 4–6 mm, ovoid	5–7 × 5 mm, ovoid	5.5–7.5 × 5.5–7 mm, broadly ovoid
Fruit	few	partly many	many	partly many

Taxonomy

Linum khorassanicum **Joharchi & Behroozian, *sp. nov*. (**Fig. 4)

Type:—IRAN. North Khorassan: W Bojnurd, Ghorkhod Protected Area, 7 km west of Zard towards Kastan, 1510 m, N 37º 30́ 56.5″, E 56º 29́ 02.0″, 23 May 2013, Joharchi, Memariani & Behroozian 45035 (Holotype: FUMH, Isotype: TARI); Paratypes: *ibid.* 45036, 45037 (FUMH).

Perennial. Roots thick and robust, becoming woody. Flower-bearing stems few, 22–35 cm high, erect, otherwise nearly prostrate or decumbent at base, thick, rigid, cylindrical, pale green; sterile stems with short, many and nearby imbricate leaves, unbranched or branched at apex. Leaves 3–12 × 0.7–1.5(2) mm, erect, linear, nerveless or 1-nerved, usually involute at margins, acute, green. Inflorescence 1–3-flowered; pedicels erect, straight, thick, short in flowering, 9–20 mm long in fruiting. Flowers 36–48 mm in diameter. Sepals rather large, three outer sepals 6.5–8 × 3–5 mm , ovate, acute to round at apex, narrowly white- membranous at margin, two inner sepals broadly ovate, rounded at apex, broadly white-membranous at margin, 0.9–1.5 mm wide, 3–5-nerved, hardly protruding below, median nerve extended to apex, pale green. Petals 18–24 × 12–15 mm, broadly obovate, rounded at apex, blue, gradually tapering below to yellowish claw. Stamens only in long-styled forms, 7.5–8 mm long; anthers 3–3.2 mm long. Styles filiform. Stigma capitate. Capsules 10–11 × 9 mm, broadly ovoid, tapering at apex and acuminate, becoming cream-brown; septa ciliate. Seeds 5–5.5 × 2.5–2.7 mm, flattened, obliquely ovate- elliptic, shiny brown. May–July.

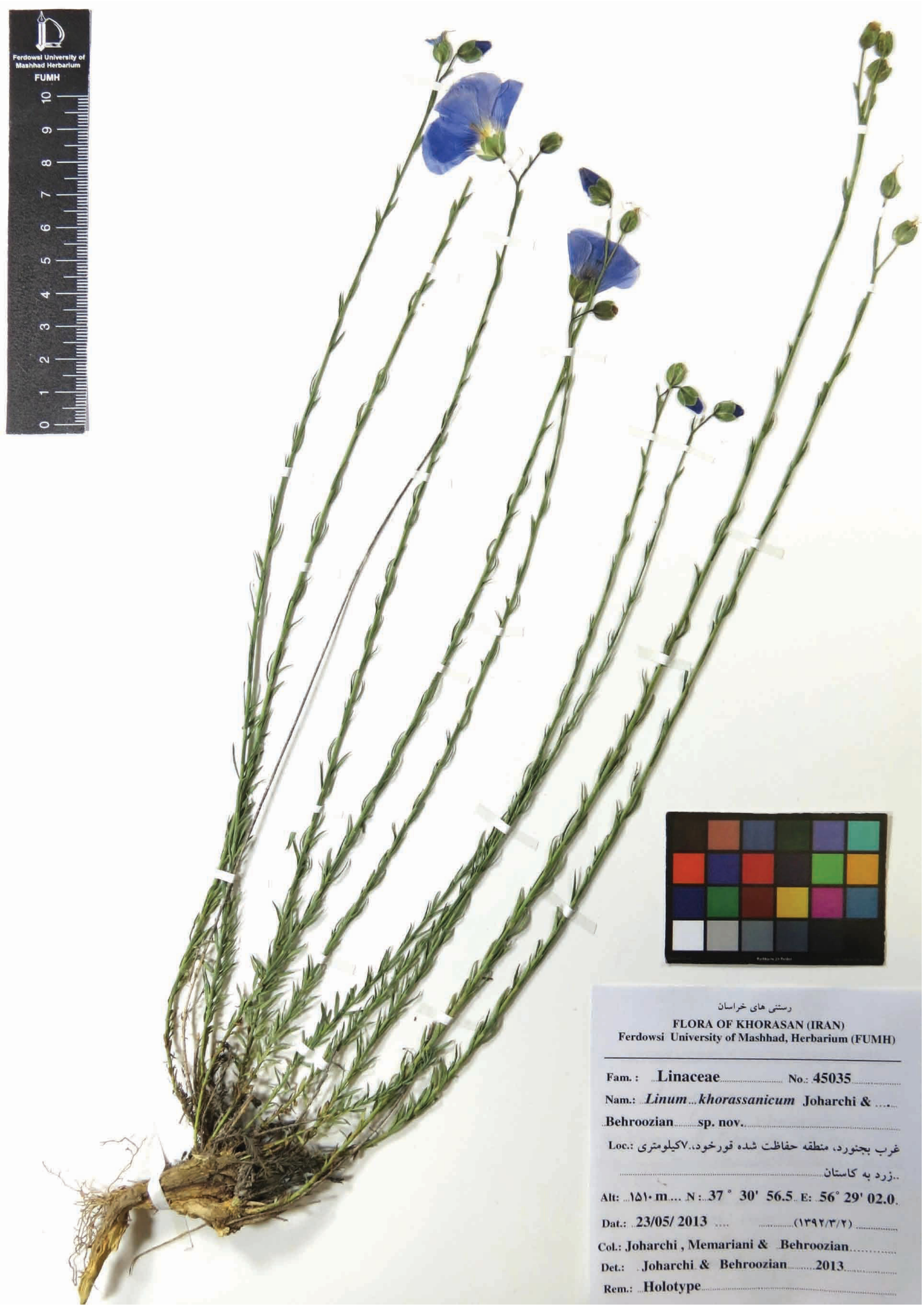

FIGURE 4. Holotype of *Linum khorassanicum* sp. nov. (Joharchi, Memariani & Behroozian 45035 (FUMH)).

Additional specimens examined:—IRAN. W Bojnurd, Ghorkhod Protected Area, 2 km on the bifurcation road towards Ternuli valley, on the hills of silviculture plan, 1540-1600 m, N 37º 23́ 28.1″, E 56º 26́ 27.4″, 18 May 2010, *Memariani & Arjmandi 43677b* (FUMH); W Bojnurd, Ghorkhod Protected Area, northern slopes of Ghorkhod Mt., 2000-2700 m, 18 July 2003, *Assadi & Hamdi 85589* (TARI); W Bojnurd, Ghorkhod Protected Area, Ghorkhod Mt., 1600 m, 17 July 2003, *Assadi & Hamdi 85489* (TARI); Northeastern part of Golestan National Park, *ca.* 7 km west of Soolgerd, in open *Juniperus* wooldland and *Artemisia* steppe, 1300- 1600 m, N 37º 27', E 56º 4', 28 April 1995, *Akhani 10541* (Hb. Akhani); Northern part of Golestan National Park, near Koilar, on gypsum marl, 1131 m, N 37˚ 31' 14", E 55˚ 58' 47", 23 June 2003, *Akhani 16864* (Hb. Akhani). For the other specimens refer to table 1.

Etymology:—The specific epithet refers to the distribution range of the new species in North Khorassan province in the northeast of Iran.

Distribution, habitats and conservation:—So far, *L. khorassanicum* has been found in the middle mountain steppes of Aladagh range, Ghorkhod Mount and adjacent north-eastern parts of Golestan National Park in North Khorassan Province (Fig. 1). It usually grows on north, northwest and west facing slopes, between 1100–2500 m *a.s.l.* Based on a phytosociological relevé in the type locality in Mt. Ghorkhod and observations of the habitats in northern slopes of Aladagh range, the new species grows in grassy mountain steppes dominated by *Festuca valesiaca* Schleicher ex Gaudin (1811: 242) community with a dense vegetation coverage of nearly up to 100%. The habitats are usually very rich in annuals and herbal perennials with scattered woody species (Table 4). The specimens 10541 (Hb. Akhani) and 43877 (FUMH) recorded as *Linum austriacum* in previous publications (Akhani 1998 and Memariani 2016c, respectively) belong to the new species *L. khorassanicum*. Akhani (1998) referred to the necessity of further studies on the specimen from Golestan National Park named as *L. austriacum* because of some morphological differences in the easternmost populations of the recorded species.

TABLE 4. Species associated with *Linum khorassanicum*, based on a 225 m^2 (15 m ×15 m) relevé in the type locality: date: 23V2013, elevation: 1510 m, gradient: 10–15°, aspect: N-NE, total cover: 90%. Cover-abundance scales are based on Braun-Blanquet (1964).

Species	Cover-abundance	Species	Cover-abundance
Festuca valesiaca Schleich. ex Gaudin	4	*Allium rubellum* M.Bieb.	+
Cousinia decipiens Boiss. &Buhse	2	*Asperula arvensis* L.	+
***Linum khorassanicum* sp. nova**	1	*Bongardia chrysogonum* (L.) Spach	+
Asperula glomerata (M.Bieb.) Griseb.	1	*Bromus danthoniae* Trin.	+
Astragalus jolderensis Fedtsch.	1	*Erysimum ischnostylum* Freyn & Sint.	+
Astragalus khoshjailensis Sirj. & Rech.f.	1	*Fumana procumbens* (Dun.) Gren. & Gordon	+
Berberis integerrima Bunge	1	*Iris fosteriana* Aitch. & Baker	+
Bromus kopetdagensis Drobov	1	*Lappula microcarpa* (Ledeb.) Guerke	+
Cerasus pseudoprostrata Pojark.	1	*Leopoldia caucasica* (Griseb.) Losinsk.	+
Convolvulus calvertii Boiss.	1	*Marrubium parvifolorum* Fisch. & C.A.Mey.	+
Crucianella sintenisii Bornm.	1	*Muscari neglectum* Guss.	+
Dactylis glomerata L.	1	*Plantago lanceolata* L.	+
Echinops ritrodes Bunge	1	*Prangos latiloba* Korov.	+
Galium verum L.	1	*Ranunculus oxyspermus* Willd.	+
Hypericum scabrum L.	1	*Scorzonera leptophylla* (DC.) Krasch. & Lipsch.	+
Onobrychis cornuta (L.) Desv.	1	*Scorzonera mucida* Rech.f.	+
Onosma dichroanthum Boiss.	1	*Turgenia latifolia* (L.) Huffm.	+
Onosma longilobum Bunge	1	*Valerianella oxyrrhynca* Fisch. & C.A.Mey.	+
Poa bulbosa L.	1	*Ziziphora tenuior* L.	+
Rhamnus pallasii Fisch. & C.A.Mey.	1	*Helianthemum ledifolium* (L.) Miller	r
Klasea latifolia (Boiss.) L.Martins	1	*Helichrysum oocephalum* Boiss.	r
Stachys turcomanica Trautv.	1	*Rumex tuberosus* L.	r
Stipa holosericea Trin.	1	*Tulipa undulatifolia* Boiss. var. *micheliana* (Hoog) Wilford	r
Verbascum cheiranthifolium Boiss.	1	*Tulipa montana* Lindl.	r
Ziziphora clinopodioides Lam.	1		

Biogeographically, the new species is a narrow endemic element of western Khorassan-Kopet Dagh (KK). The Khorassan-Kopet Dagh floristic province is located in mountainous areas of northeastern Iran and partly in southern Turkmenistan. The area is a transition zone and a corridor connecting different phytogeographical units of the Irano-Turanian region with a high rate of 14% endemism in its total flora (Memariani *et al.* 2016a, 2016b). According to IUCN Red List Categories and Criteria (IUCN, 2011), *L. khorassanicum* is here assessed as Endangered (EN, B1+2ac (i,iii)). Its extent of occurrence (EOO) is about 400 km^2 with few and severely fragmented locations. The habitats in Aladagh range are not officially protected and the main localities in Ghorkhod Protected Area, including the type locality, are situated in poorly protected parts of the northern borders of the area recently damaged by extending the agricultural fields and road construction (Memariani *et al.* 2016c). The new species is therefore considered to be facing a very high risk of extinction in the wild and needs very urgent *in situ* and *ex situ* conservation efforts.

Linum turcomanicum **Juzepczuk in Shishkin (1949: 720)** (Fig. 5).

Type:—TURKMENISTAN. Massinev Mount, Androssov *s.n.*

Perennial. Stems 20–67 cm high, ± numerous, ascendens, in the upper half to the fourth branching, sterile stems many, rather densely leafy, with erect or spreading linear leaves. Leaves of flower-bearing shoots 5–25 mm long, 0.6–3 mm wide, spreading to erect, linear-lanceolate to lanceolate, margins slightly thin-denticulate and scabrous, rarely flat, involute, green or glaucescent in dry, lower leaves acute, rarely acuminate, upper leaves long-acuminate, 1–3-nerved. Inflorescence composed of rather few or many-flowered cymes; pedicels erect, short in flowering, long in fruiting, 10–25 mm, thin, straight or hardly recurved in fruit. Flowers 26–45 mm in diameter. Sepals glaucescent or pale green, darker in bud, the outer ones 4–6 mm long and 1.5–2.5 mm wide, ovate-elliptic, obtuse or acuminate, with narrow white-membranous margin, inner sepals 4.5–6.5 mm long and 3–4 mm wide, broadly ovate, rounded at apex, broadly white-membranous at margin, shortly mucronulate, dorsally with 3–5 prominent nerves below. Petals 13–22 mm long, 6–13 mm wide, obovate or broadly obovate, almost three times as long as sepals, cuneately tapering at base, obtuse or orbicular above, blue to pale blue, sometimes whitish blue, with yellowish claw, overlapping at margins. Stamens in long-styled forms (as styles in short-styled forms) 3–4.5 mm long, stamens in short-styled forms (as style in long-styled forms) 6.5–8 mm long, styles 4–5 or 6–7 mm long, respectively; anthers 1.2–2 mm long and 0.5–0.7 mm wide. Stigma capitate. Capsules 5.5–7.5 mm long and 5.5–7 mm wide, usually broadly ovoid, shortly mucronate at apex; yellow-straw, septa ciliate. Seeds 5–6 mm long and 2–2.5 mm wide, obliquely oblong-ovate, flattened, dark brown, shiny.

Specimens examined:—IRAN. NE Bojnurd, 21 km on road towards Gifan, 1000 m, N 35° 54' 35", E 57° 22' 37.07", *Assadi & Maassuumi 50211* (TARI). For the other specimens refer to table 1.

Distribution, habitats and conservation:—*Linum turcomanicum* was hitherto known from the type locality in Misinov (Massinev) Mount in Turkmenistan near the Iranian borders. So, this species is recorded here 69 years after its description from the type location in Turkmenistan. The type specimen is in early flowering stage; therefore, the original description of the species is partly incomplete and ambiguous especially in morphological characters of the fruits. The revision of the *Linum* specimens in FUMH revealed that this species is an endemic plant widely distributed throughout Khorassan-Kopet Dagh floristic province in northeastern Iran and southern Turkmenistan (Fig. 1). In this work, after a comprehensive taxon sampling from the entire species range in northeast of Iran, the taxonomic description of *L. turcomanicum* was completed. Specimens collected near the type locality (45730-37 FUMH) grow in understory of *Acer monspessulanum* Linnaeus (1753: 105) shrubs in higher mountain slopes of Misinov Mt. However, the other populations, usually with few individuals, occur in a wide range of habitats and vegetation types from the moist mountain steppes in western parts of its distribution range (in North Khorassan province) to the dry gypsum and marl hills in the east (in Razavi Khorassan province).

L. turcomanicum was previously known as a Central Khorassan- Kopet Dagh (KK) endemic and evaluated as DD (Data Deficient) threat category (Memariani *et al.* 2016b). However, according to the discovery of relatively wide distribution range throughout KK (omni-KK), the species is re-evaluated here as a non-threatened plant. The maximum distance between any pair of distribution point is 420 km and the calculated EOO is 38106 km^2. Based on IUCN criteria and categories, it is evaluated as NT (Near Threatened) and it is likely to be qualified for a threatened category in the near future. A reduction analysis by GeoCAT showed that the loss of the south-easternmost populations in Torbat-e Jam (34158 FUMH; Fig. 1) can reduce the EOO down to 19474 km^2 (49% reduction) and re-evaluate the threat status up to VU (Vulnerable). Therefore, conservation of the satellite populations of the species is of great importance to ensure protection of the genetic diversity across its distribution range with relative diverse habitats.

FIGURE 5. A specimen of *Linum turcomanicum* collected from Misinov Mt. (near the type locality) in northeast of Iran (Joharchi & Behroozian 45130 (FUMH)).

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