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# MORPHOLOGICAL, GENETICAL AND ECOGEOGRAPHICAL CHARACTERIZATION OF LONG-WINGED SPECIES OF ONOBRYCHIS SECT. ONOBRYCHIS (FABACEAE) IN IRAN

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In this study, 30 specimens (6 populations) from long-winged species of *Onobrychis* sect. *Onobrychis* including *O. verae* Širj., *O. gontscharovii* Vassilcz., *O. ptychophylla* Širj. & Rech. f., *O. sosnovskyi* Grossh. and *O. araxina* Schischkin were collected from their natural habitats in Iran. The specimens were biometrically assessed using 45 quantitative and 15 qualitative morphological characters. In order to study the genetic variations of different species, nucleotide sequence data from the internal transcribed spacer of the nuclear rRNA genes (ITS) were obtained. Data regarding longitude, latitude, altitude, slope inclination, slope direction, substrate, min/max annual temperatures, number of rainy days, annual precipitation as well as soil traits including texture, electrical conductivity, organic carbon, total nitrogen, available phosphorus and potassium, total neutralizing value, pH, and saturation percentage were recorded in each sample site. Cluster analysis of morphological characters showed two major groups separating northwest species from northeast ones. Furthermore, the ITS data analysis results showing two main groups, which are exactly in concordance with morphological groups. Also canonical correspondence analysis (CCA) of ecological data showed the precipitation, rainy days, sand% and silt% as the most effective factors for this separation. Furthermore it showed the effect of ecological factors on each species. *Onobrychis araxina* Schischkin is recorded for the first time from Azerbaijan Gharbi province, Iran. An identification key to the long-winged species of section *Onobrychis* in Iran is presented.

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**ویژگی‌های مورفولوژیکی، ژنتیکی و اکوجغرافیایی گونه‌های بال بلند بخش *Onobrychis* از جنس *Onobrychis* Mill. در ایران**

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در این بررسی، ۳۰ نمونه (۶ جمعیت) از گونه‌های بال بلند بخشه *Onobrychis* از جنس *Onobrychis* Mill. شامل گونه‌های *O. verae* Širj.، *O. gontscharovii* Vassilcz.، *O. ptychophylla* Širj. & Rech. f.، *O. sosnovskyi* Grossh. و *O. araxina* Schischkin، از زیستگاه‌های طبیعی خود در ایران جمع‌آوری شدند. این نمونه‌ها با استفاده از ۴۵ ویژگی کمی و ۱۵ ویژگی کیفی مورفولوژیکی مورد ارزیابی قرار گرفتند. به منظور بررسی تنوع ژنتیکی گونه‌های مورد نظر، از داده‌های مربوط به توالی نوکلئوتیدی ناحیه ITS ژن nrRNA استفاده شد. در هر محل جمع آوری، اطلاعاتی شامل طول و عرض جغرافیایی، ارتفاع، جهت و میزان شیب، بستر، حداقل و حداکثر درجه حرارت سالانه، تعداد روز های بارانی، بارش سالانه و همچنین ویژگی‌های مربوط به خاک از جمله بافت، رسانایی الکتریکی، کربن آلی، نیتروژن کل، فسفر و پتاسیم در دسترس، ارزش مواد خنثی شونده، pH و درصد اشباع ثبت شد. بررسی و مقایسه نتایج حاصل از تجزیه و تحلیل خوشه ای داده‌های مورفولوژیکی، منجر به تشخیص دو گروه اصلی گردید. این گروه‌بندی مبین جدایی گونه‌های شمال غربی از شمال شرقی کشور

ایران است. علاوه بر این، نتایج حاصل از تجزیه و تحلیل داده‌های مربوط به توالی نوکلئوتیدی ITS نیز نتایج به دست آمده از آنالیز داده‌های مورفولوژیکی را تایید می‌کند. همچنین نتایج حاصل از آنالیز داده‌های اکولوژیکی به روش CCA، تعداد روزهای بارانی، بارش سالانه، درصد شن و درصد سیلت خاک را به عنوان مهمترین عوامل مؤثر در جدایی این دو گروه معرفی کرد. همچنین چگونگی اثر عوامل زیست محیطی را برای هر یک از گونه‌ها نشان داد. در این بررسی *O. araxina* Schischkin برای اولین بار از ایران گزارش می‌شود. کلید شناسایی گونه‌های بال بلند بخش *Onobrychis* در ایران نیز ارائه شده است.

## INTRODUCTION

The genus *Onobrychis* Miller belongs to *Fabaceae-Hedysareae* (Polhill 1981; Lock 2005). Many confusions and contradictions are found in the taxonomy of *Onobrychis*. It comprises about 130 species and is mainly distributed in the north temperate regions, but its biodiversity centers are in the eastern Mediterranean area and western Asia especially Iran and Anatolia (Lock 2005, Yildiz & al. 1999). The genus was subdivided into two subgenera and nine sections (Rechinger 1984). The section *Onobrychis* (subgenus *Onobrychis*), is the second largest section of genus *Onobrychis* in Iran with 15 species. (Rechinger 1984). The species of this section are important perennial species used for high-protein fodder for ruminants and equines, for increasing the nutritive value of drought-resistant pastures due to nitrogen fixation, and for soil conservation (Abou-El-Enain 2002; Elena 2006). This section seems to be the most problematic and the boundary of species is not completely clear due to the polymorphism in morphological characters.

In conjunction with other taxonomic studies on *Onobrychis* spp. in Iran (e.g., Ranjbar & al. 2004, 2007, 2009, 2010; Ahangarian & al. 2007; Toluei & al. 2010; Amirabadi-zadeh, 2011), the present investigations were carried out to study the interspecific relationships between different long-winged species of section *Onobrychis* including *O. verae* Širj, *O. gontscharovii* Vassilcz., *O. ptychophylla* Širj. & Rech. f., *O. sosnovskyi* Grossh. and *O. araxina* Schischkin based on evidence from morphological characters and nucleotide sequence data from the internal transcribed spacer of the nuclear rRNA genes (ITS) also for showing the ecological characterization of each species. In this study, the genetic variations were investigated by means of nucleotide sequence data from the ITS region. Among the nuclear markers, ITS regions have been especially useful in recent phylogenetic studies at lower taxonomic levels in many angiosperm groups, including Fabaceae (Wojciechowski & al. 1993; Käss & Wink 1997a, b; Ainouche & Bayer 1999; Davis & al. 2002; Ahangarian & al. 2007). Ecogeographic data regarding the habitats of these species in Iran are reported here for the first time. In addition, *O. araxina* Schischkin is recorded for the first time from Iran.

Specifically, four main aims are addressed in this paper: (i) to detect the distribution of long-winged species of section *Onobrychis* in Iran; (ii) to study the morphological variation and diagnostic characters of long-winged species (iii); to assess the genetic variation of different long-winged species and (iv) to establish the relationships between species distribution and environmental gradients defined by several ecogeographic variables.

## MATERIALS AND METHODS

### Sample collection

We extensively studied the distribution of long-winged species of section *Onobrychis* in Iran: 30 specimens (6 populations) from *O. verae*, *O. gontsharovii*, *O. ptychophylla*, *O. sosnovskyi* and *O. araxina* were collected from their natural habitats (6 localities) during 2008 and 2009 (April–July). Voucher specimens are deposited in the herbarium of Bu-Ali-Sina University (BASU). The according collection sites are listed in Table 1. Identification of the specimens was performed using the regional Flora (Rechinger 1984; Hedge 1970; Boissier 1872; Grossheim 1972) and a relevant monograph (Širjaev 1926).

### Morphological data

The studied specimens (five individuals: a, b, c, d and e, in each population) were assessed by biometric study. Forty-five quantitative and fifteen qualitative morphological characters were chosen and evaluated (Table 2). For statistical analysis, the qualitative characters were initially encoded according to the multi-state method, and the related means were considered for quantitative characters and these were standardized. Phenetic analysis was carried out using MVSP Vers. 3.2 (Kovach 1985–2002) with unweighted pair-group method using arithmetic averages (UPGMA) (Sneath & Sokal 1973) and phenogram of these populations were prepared by analyzing the morphological character variations in all individuals of each population.

### Molecular data

Sequence data from nuclear ribosomal DNA internal transcribed spacers (nrDNA ITS) were obtained in order to analyze the genetic variation and species

Table 1. Localities and voucher numbers of *Onobrychis* materials studied.

| Species | Locality | Voucher number | Abbreviation | Gen Bank accession numbers of ITS |
|---|---|---|---|---|
| *O. sosnovskyi* | Azerbaijan sharghi, Sufian to Marand | Toluei&Ranjbar 23156 (BASU) | sos | JQ780469 |
| *O. araxina* | Azerbaijan Gharbi, before Chaldoran, Alimardan village | Toluei&Ranjbar 23157 (BASU) | ara | JQ780470 |
| *O. gontsharovii* | Khorasan Razavi, Kalat to Mashhad | Toluei&Ranjbar 23119 (BASU) | gon | JQ780471 |
| *O. ptychophylla* | Khorasan Razavi, Quchan, 15 km to Bajgiran | Toluei&Ranjbar 23152 (BASU) | pty | JQ780472 |
| *O. verae* | North Khorasan, Shirvan, Kouseh bifurcate | Toluei&Ranjbar 23164 (BASU) | ver | JQ780474 |
| *O. verae* | Khorasan Razavi, 60 km from Quchan to Dargaz, Tandooreh National Park | Toluei&Ranjbar 23165 (BASU) | ver | JQ780473 |

differentiation. Total genomic DNAs were isolated from dried leaf material using the standard CTAB (hexadecyl trimethyl ammonium bromide) method (Doyle & Doyle 1987). The complete nrDNA ITS region was amplified using the ITS4 and ITS5 primers (White et al. 1990). PCR amplifications were performed in 50 µl reaction volumes containing: 0.5–1 µg DNA, 10 pmol of each primer, 1.5 µl dNTPs (10 mM), 5 µl Taq polymerase buffer, 10 mg/ml bovine serum albumin (BSA), 0.8 units Taq DNA polymerase (Pharmacia Biotech, Freiburg, Germany). PCR amplifications were carried out on a thermal cycler (Biometra, Göttingen, Germany) using the following parameters: initial denaturation at 94 °C for 5 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 48 °C for 1 min and extension at 72 °C for 90 s and a final extension at 72 °C for 5 min. PCR products were precipitated with 4 mol/l NH4Ac and ethanol (1:1:6) followed by centrifugation for 15 min. Sequencing was performed using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Freiburg, Germany). For each sample the nrDNA ITS region was sequenced using the ITS4 primer. Sequences were analyzed using capillary electrophoresis with a Mega-BaceTM 1000 sequencer (Molecular Dynamics Inc., Amersham Pharmacia). The nrDNA ITS sequences were aligned with Bioedit Vers. 7.0.5 (Hall 1999) and adjusted manually. The aligned data matrix was reconstructed by parsimony optimality criterion using PAUP* ver. 4.0b10 (Swofford 2002). The heuristic search option was selected using 100 replications of simple addition sequence and TBR branch-swapping with MulTrees on and steepest descent off. Support for clades were evaluated by bootstrapping using 1000 replications with the heuristic search option, simple addition sequence and TBR branch swapping. The ITS sequence of *Hedysarum wrightianum* retrieved from GenBank (GQ246059) was included in the analyses as an outgroup. A phylogenetic tree was drawn using Tree View (Page 1996).

**Ecogeographic data**

Data obtained from each site included: altitude, longitude, latitude, slope inclination, slope orientation, substrate, min. and max. annual temperature, rainy days per year, mean annual precipitation and soil traits including texture (sand%, silt% and clay%), electrical conductivity (EC), organic carbon (OC%), total nitrogen (Total N%), available phosphorus (av. P%), available potassium (av. K%), total neutralizing value (TNV%), pH and saturation percentage (SP). Soil samples were collected from 0–30 cm depth and measured in the soil laboratory with routine methods of soil analysis. Climatic information for each collecting site was obtained from the Islamic Republic of Iran Meteorological Organization (IRIMO), see table 3. These data were converted to numerical values for canonical correspondence analysis (CCA), a technique for direct gradient analysis. Variables constraining the scores of populations are represented by arrows, the correlations of these variables to CCA axes being proportional to length, direction, and angle of arrows to axis.

## RESULTS

**New record**

**Onobrychis araxina** Schischkin, Ber. Tomsker Staats-Univ. 80: 489 (1928).

*Specimen seen*. Azerbaijan Gharbi: Chaldoran, Alimardan village, 2002 m, Toluei & Ranjbar 23157 (BASU).

*O. araxina* belongs to the sect. *Onobrychis*. *O. araxina* is characterized by the longer wings than calyx, purplish corolla, short height and fruit denticulate on crest. It was formerly known as an endemic eastern Anatolian element which is rather close to the Iranian gathering (Hedge 1973).

Table 2. List of characters and related numerical codes used in morphological studies.

| No. | Characters | Numerical code |
|---|---|---|
| 1 | Plant height | cm |
| 2 | Stem length | cm |
| 3 | Length of stem hairs | mm |
| 4 | Leaf length | cm |
| 5 | Petiole length | cm |
| 6 | Number of leaflets | In no. |
| 7 | Leaflet length | mm |
| 8 | Leaflet width | mm |
| 9 | Hair length of adaxial surface of leaflet | mm |
| 10 | Hair length of abaxial surface of leaflet | mm |
| 11 | Stipule length | mm |
| 12 | Stipule width | mm |
| 13 | Length of stipule hairs | mm |
| 14 | Inflorescence length | cm |
| 15 | Peduncle length | cm |
| 16 | Length of peduncle hairs | mm |
| 17 | Number of flowers per raceme | In no. |
| 18 | Bract length | mm |
| 19 | Bract width | mm |
| 20 | Length of bract hairs | mm |
| 21 | Pedicel length | mm |
| 22 | Length of pedicel hairs | mm |
| 23 | Calyx length | mm |
| 24 | Calyx width | mm |
| 25 | Length of calyx tube | mm |
| 26 | Length of calyx teeth | mm |
| 27 | Length of calyx hairs | mm |
| 28 | Standard length | mm |
| 29 | Standard width | mm |
| 30 | Keel length | mm |
| 31 | Keel width | mm |
| 32 | Keel claw length | mm |
| 33 | Wing length | mm |
| 34 | Wing width | mm |
| 35 | Wing claw length | mm |
| 36 | Wing auricle length | mm |
| 37 | Pod length | mm |
| 38 | Pod width | mm |
| 39 | Number of crest spines | mm |
| 40 | Spine length | mm |
| 41 | Spine width | mm |
| 42 | Number of areole per pod | In no. |
| 43 | Length of pod hairs | mm |
| 44 | Seed length | mm |
| 45 | Seed width | mm |

Table 2. continued.

| No. | Characters | Numerical code |
|---|---|---|
| 46 | Leaflet shape | 1- oblong-elliptic, linear-oblong, oblanceolate 2- oblong-elliptic, linear-oblong 3- oblong-elliptic, oblanceolate 4- oblong-elliptic, oblanceolate, orbicular 5- oblong-elliptic, linear 6- linear-oblong |
| 47 | Hair state of adaxial surface of leaflet | 1- glabrous 2- glabrous and sparse 3- glabrous, sparse and loose 4- sparse and loose 5- loose |
| 48 | Hair state of abaxial surface of leaflet | 1- loose 2- loose and dense |
| 49 | Standard shape | 1- obovate 2- obovate and elliptic 3- orbicular |
| 50 | Standard apex | 1- retuse 2- emarginated 3- truncate 4- obtuse |
| 51 | Comparison of standard and keel size | 1- equal 2- equal or standard longer than keel 3- standard longer than keel 4- keel longer than standard |
| 52 | State of stem hair | 1- sparse 2- sparse-loose 3- sparse, loose and dense 4- loose 5- dense |
| 53 | Flower color | 1-rose-pink 2- purplish |
| 54 | State of peduncle hair | 1- sparse 2- sparse and loose 3- loose and dense 4- loose 5- dense |
| 55 | State of stipule hair | 1- loose and dense 2- sparse, loose and dense 3- sparse and ciliate 4- sparse, ciliate and loose 5- sparse and loose 6- glabrous, sparse, ciliate and loose 7- sparse 8- dense |
| 56 | State of bract hair | 1- ciliate, sparse and loose 2- sparse and ciliate 3- loose 4- sparse and loose 5- sparse, loose and dense 6- ciliate 7- sparse 8- dense |
| 57 | Bracteole shape | 1- lanceolate 2- subulate |
| 58 | State of calyx hair | 1- tube is sparse and dentate is sparse and ciliate 2- tube is sparse and dentate is sparse and loose 3- tube is sparse and dentate is loose 4- both tube and dentate are loose 5- tube is sparse and dentate is dense 6- tube is sparse and loose, dentate is loose |
| 59 | Existence of black hairs | 1- black hairs on calyx and pedicel 2- black hairs on pod, calyx and pedicel |
| 60 | Pod shape | 1- Crest with small teeth, disk spineless 2- Crest with small spines, disk with small spinules 3- Crest with small spines, disk with small spinules or spineless 4- crest with spines and disk with hammate teeth |

**Morphologic data**

*Onobrychis verae* occurs in Middle Asia, Turkmenistan and Iran. It is a perennial plant usually erect or ascending, somewhat branching at base, with 2–7 pairs of leaflets, leaflets elliptic-oblong, linear-oblong or lanceolate, standard 8–11 mm, keel 7–11 mm, wings 6–9.5 mm, pods 5–7 mm and crest 4–6 toothed, without or with very short tuberculed prickles on disc. *O. gontscharovii* occurs in Central Asia. It is a perennial plant; stem woody at base, with 2–9 pairs of leaflets, leaflets elliptic or elliptic-oblong, corolla 9–10 mm long, wings 6–7 mm, pods 7–9 mm with few hammate teeth on disc and crest 4–8 toothed. *O. ptychophylla* is a perennial plant from near Ghoochan in Khorasan Province. It has stem intensely woody, with 2–11 pairs of leaflets, leaflets linear, lanceolate or elliptic-oblong, standard 8–9 mm, keel 7–9 mm, wings 6–7 mm, pods 6–7 mm long, with thin and irregular teeth on crest. *O. sosnovskyi* occurs in Caucasus, Turkey and Iran. It is a perennial plant usually erect, branching at base, with linear or narrowly linear leaflets, corolla 9–11 mm, wings 8–9 mm and pods 5–7 mm long, denticulate at margin. *O. araxina* occurrs in Turkey and Iran. It is a perennial plant with erect-ascending stems, leaves with 7–9 pairs of linear-oblong or oblong-elliptic leaflets. Corolla purplish, standard 9–11 mm, keel 9–11 mm, wing 8–9 mm and pods 5–6 mm long, denticulate on crest and with or without short hooks on the disc.

Figure 1 represents an UPGMA phenogram with average distance coefficients resulting from analyzing the morphologic characters of five individuals of each population; apparently five groups are separated and each of the species separated from the other taxa in a

Table 3. Ecogeographical variables for different studied populations of the long-winged species of *Onobrychis* sect. *Onobrychis* in Iran.

| Ecogeographical factors | species | | | | | |
|---|---|---|---|---|---|---|
| | sos 23156 | ara 23157 | gon 23119 | pty 23152 | ver 23164 | ver 23165 |
| Altitude (m) | 1626 | 2002 | 1734 | 1792 | 1697 | 2155 |
| Latitude | N 38° 20. 540' | N 39° 02. 544' | N 36° 35. 879' | N 37° 39. 621' | N 37° 39. 417' | N 37° 32. 816' |
| Longitude | E 45° 52. 033' | E 44° 40. 613' | E 59° 56. 836' | E 57° 49. 191' | E 57° 49. 132' | E 58° 25. 909' |
| Slope inclination | 50% | 0 | 35% | 10% | 5% | 80% |
| Slope orientation | N | 0 | SE | S | NW | SE |
| Maximum temperature (°C) | 16.5 | 15.5 | 20.2 | 17.5 | 17.5 | 17.5 |
| Minimum temperature (°C) | 7.6 | 5.3 | 6.6 | 6.8 | 6.8 | 6.8 |
| Rainy days (In no) | 96.7 | 118.3 | 77.2 | 90.2 | 90.2 | 66 |
| Annual precipitation (mm) | 338.3 | 294.5 | 212.6 | 272.4 | 272.4 | 252.7 |
| Substrate | Soil | Soil and gravel | Soil | Soil | Soil | Soil and gravel |
| Texture | L | SCL | L | SIL | SIL | L |
| Clay% | 24 | 26 | 22 | 15 | 23 | 14 |
| Sand% | 40 | 49 | 29 | 31 | 21 | 39 |
| Silt% | 36 | 25 | 49 | 54 | 56 | 47 |
| EC ($\times 10^3$ mmhos/cm) | 0.448 | 0.439 | 0.31 | 0.323 | 0.342 | 0.457 |
| OC% | 2.55 | 1.81 | 0.29 | 2.16 | 1.05 | 0.23 |
| pH | 7.44 | 7.47 | 7.53 | 7.34 | 7.52 | 7.23 |
| av. K (ppm) | 728.6 | 659.4 | 184.5 | 560.4 | 461.5 | 827.6 |
| av. P (ppm) | 11.4 | 7.2 | 5.2 | 11 | 10.6 | 31.6 |
| Total N% | 0.25 | 0.18 | 0.02 | 0.21 | 0.1 | 0.02 |
| SP | 35 | 36.2 | 33.1 | 27.1 | 34.3 | 30 |
| TNV% | 25.5 | 22.5 | 28 | 8.5 | 23 | 1 |

single group. The northeast species including *O. ptychophylla, O. verae* and *O. gontsharovii* are positioned adjacently and completely separated from the long-winged species in northwest of Iran, including *O. sosnovskyi* and *O. araxina*.

**Molecular data**

The aligned complete ITS region data matrix (including outgroup) consisted of 489 characters of which 445 characters are constant. Twenty-eight variable characters are parsimony-uninformative and number of parsimony-informative characters is 16. A bootstrap consensus tree was generated by analyzing the aligned sequences of nrDNA ITS region using PAUP (Fig. 2). Our study showed that there is not intrapopulation variation for nrDNA ITS sequence.

*Onobrychis sosnovskyi* and *O. araxina* are closest species with high bootstrap value of 100 and weakly allied with other species. Also the long-winged species in northeast form a well-supported clade with bootstrap support value of 90. The ITS polymorphism in different species is indicated in Table 4. In addition to nucleotide substitutions, several diagnostic indels were observed in the aligned ITS sequence data. A one bp indel (insertion and deletion) at the position 76 and a 2- bp indel at positions 94-95 occurred in the northeast species clade and deleted in *O. sosnovskyi* and *O. araxina*. Also a one base-pair (bp) indel situated at position 416 of the ITS alignment was inserted in *O. sosnovskyi* and *O. araxina* but deleted in northeast species.

**Ecological data**

Ecological direct gradient analysis, as shown by CCA (Fig. 3) can establish the relative importance of each environmental variable in the distribution of the species. This figure shows the separation of the long-winged species in northeast and that ones in northwest of Iran, also precipitation, rainy days, sand% and silt% as the most effective factors for this separation. It shows that rainy days as well as the texture are the most effective factors for separation of *O. araxina*. Furthermore, it shows the precipitation being a prominent ecological factor for the separation of *O. sosnovskyi*. The slope orientation, SP and TNV are the most effective factors on *O. ptychophylla* and the most effective factor on *O. gontsharovii* is max. temperature. This figure introduces the number of rainy days, slope inclination and orientation as the most effective factor in species distribution and substrate, altitude and av. K are the lowest effective factors in species distribution. Table 5 includes interset correlations of canonical coefficients for twenty ecological variables. It should be noted that CCA axis 1 is positively correlated with OC and Total N also negatively correlated with slope inclination and orientation. CCA axis 3 is positively correlated with Total N and negatively correlated with slope orientation.

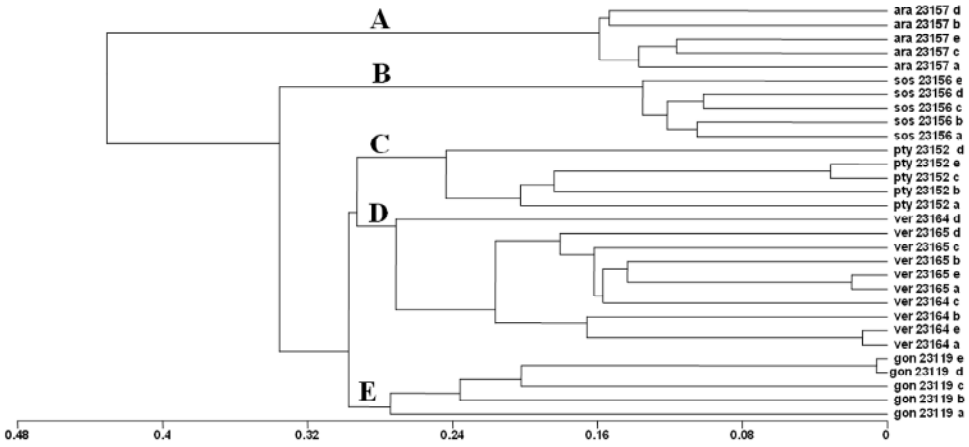


Fig. 1. The UPGMA phenogram based on the analyzing the morphological data in five individuals of each population of the long-winged species of *Onobrychis* sect. *Onobrychis* in Iran representing five groups A, B, C, D and E.

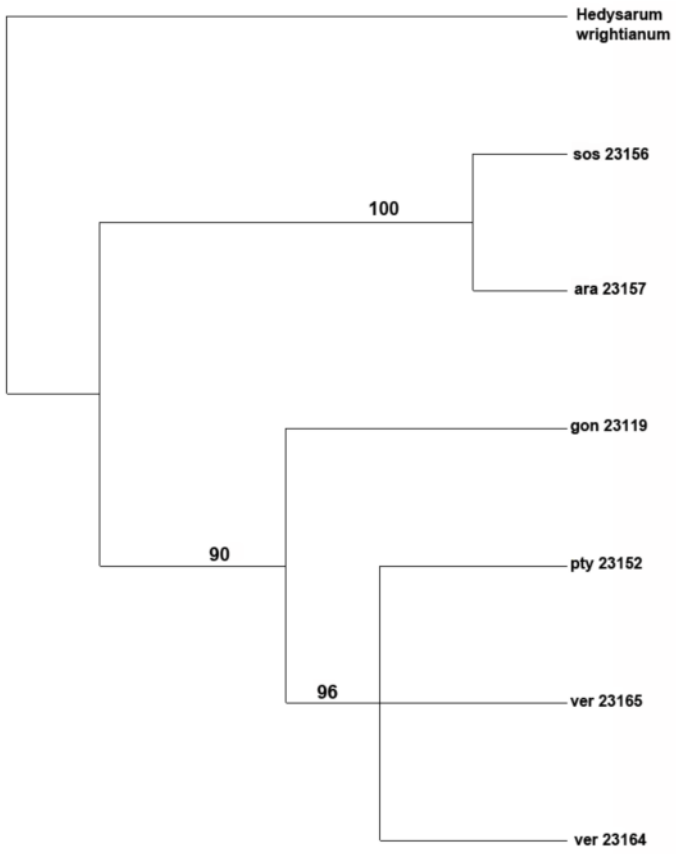


Fig. 2. A bootstrap consensus tree based on ITS sequences data of the long -winged species of *Onobrychis* sect. *Onobrychis* in Iran (abbreviations are as listed in Table 1). Numbers refer to bootstrap values. Bootstrap values greater than 50% were shown above the branches. (from 1000 replications).

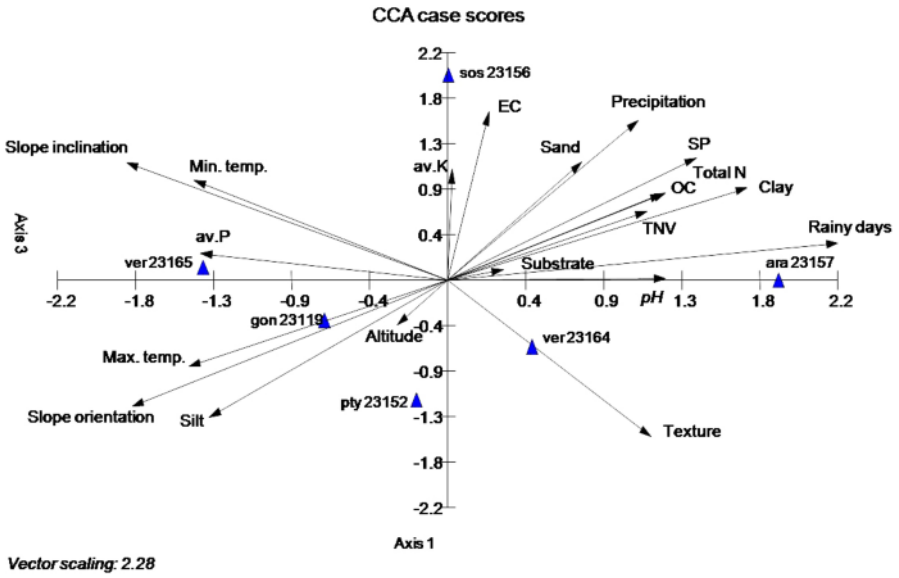


Fig. 3. Canonical correspondence analyses (CCA) of twenty ecogeographic data for collection sites of the long-winged species of *Onobrychis* sect. *Onobrychis* in Iran.

Table 4. ITS polymorphism in different populations of *Onobrychis verae*, *O. gontsharovii*, *O. ptychopylla*, *O. sosnovskyi* and *O. araxina* (abbreviations are as listed in Table 1). R= A or G, W=T or A, (−) = indel. Only sites that differed are shown.

| Population | Position in alignment | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 004 | 007 | 012 | 028 | 039 | 045 | 046 | 048 | 051 | 066 | 071 | 072 | 076 | 094 | 095 | 099 | 135 | 231 | 260 | 296 | 307 | 416 | 449 | 450 | 551 |
| sos 23156 | A | G | T | T | C | A | C | T | G | C | C | G | − | − | − | T | G | T | C | T | G | C | C | T | A |
| ara 23157 | A | G | T | T | C | T | C | T | G | C | C | G | − | − | − | W | G | T | C | T | G | C | C | T | A |
| gon 23119 | G | T | C | T | C | T | C | A | C | T | T | G | G | A | T | T | G | C | T | C | T | − | T | C | G |
| pty 23152 | G | T | C | C | T | T | G | A | C | T | C | A | G | A | T | T | G | C | C | C | T | − | T | C | G |
| ver 23165 | G | T | C | C | C | T | G | A | C | T | C | A | G | A | T | T | G | C | C | C | T | − | T | C | G |
| ver 23164 | G | T | C | C | C | T | G | A | C | T | C | A | G | A | T | T | R | C | C | C | T | − | T | C | G |

## DISCUSSION

In this study, cluster analysis of morphological characters in long-winged species of sect. *Onobrychis* showed two major groups separating northwest species (*O. sosnovskyi* and *O. araxina* ) from northeast ones (*O. ptychophylla, O. verae* and *O. gontsharovii)*. Based on morphological variations, each of the species separated from the other taxa (groups A, B, C, D and E in Fig. 1). The following key represents the distinguishing features of each species.

1. Plant with black hairs on pedicel, calyx and pod; calyx length 3–5 mm; wing 8–9 mm; adaxial surface of leaflets glabrous; pods with small teeth on crest and disk spineless 2

Plant with black hairs on pedicel and calyx; calyx length 4.5–8 mm; wing 6–9.5 mm; adaxial surface of leaflets with loose and sparse hairs and rarely glabrous; pods with spines on crest and disk with small spinules, hammate teeth or spineless 3

2. Plant height 14–30 cm; flower color purplish; standard as long as keel; leaf length 1.5–8 cm 1. *O. araxina*

Plant height 35–60 cm; flower color is rose-pink; standard is longer than keel; leaf length 4–15 cm 2. *O. sosnovskyi*

Table 5. Interset correlations for twenty environmental variables.

| Variables | Interset correlation | | |
|---|---|---|---|
| | Axis 1 | Axis 2 | Axis 3 |
| Altitude | 0.015 | 0.017 | -0.016 |
| Slope inclination | -0.503 | -0.064 | 0.189 |
| Slope orientation | -0.511 | -0.079 | -0.297 |
| Max. temp. (°C) | -0.001 | -0.008 | -0.022 |
| Min. temp. (°C) | -0.015 | -0.019 | 0.004 |
| Rainy days (In no) | 0.055 | -0.001 | -0.007 |
| Precipitation (mm) | 0.029 | 0.005 | 0.002 |
| Substrate | 0.243 | 1.379 | 0.064 |
| Texture | 0.136 | -0.022 | -0.149 |
| Clay% | 0.070 | -0.021 | 0.014 |
| Sand% | 0.034 | 0.049 | 0.024 |
| Silt% | -0.032 | -0.025 | -0.049 |
| EC | 0.033 | 0.099 | 0.084 |
| OC% | 0.348 | -0.147 | 0.140 |
| pH | 0.020 | 0.000 | -0.013 |
| av. K | 0.028 | 0.052 | 0.017 |
| av. P | -0.094 | 0.132 | 0.019 |
| Total N% | 0.393 | -0.165 | 0.218 |
| SP | 0.033 | 0.002 | 0.001 |
| TNV% | 0.234 | -0.248 | 0.040 |

3. Pods with spines (0.3–3 mm) on crest and disk with hammate teeth; wing 6–7.5 mm; standard as long as or shorter than keel 3. *O. gontsharovii*
Pods with spines (0.2–2 mm) on crest and disk with small spinules or spineless; wing 6–9.5 mm; standard as long as or longer than keel 4
4. Plant height 50–80 cm; wing 7–9.5 mm; pods with spines (0.2–2 mm) on crest 4. *O. verae*
Plant height 15–55 cm; wing 6–7 mm; pods with spines (0.2–1 mm) on crest 5. *O. ptychophylla*

Furthermore, the ITS data analysis results showing two main groups, which are exactly in concordance with morphological groups because it separating northwest species (*O. sosnovskyi* and *O. araxina*) with high bootstrap value of 100 from northeast ones (*O. ptychophylla, O. verae* and *O. gontsharovii)*. Also the long-winged species in northeast form a well-supported clade with bootstrap support value of 90. But these two well-supported clades (northwest and northeast ones) weakly allied with each other with bootstrap value less than 50%. Hence, it seems that the long-winged species in northeast and that ones in northwest of Iran cannot have a progenitor-derivative relationship. More studies are needed to confirm this result.

In this study, it was shown that nucleotide sequence data from the internal transcribed spacer of the nuclear rRNA genes (ITS) could be applied to investigate *Onobrychis* genetics, as indicated by other relevant studies (e.g., Käss & Wink 1997a; Ahangarian & al. 2007). It is shown to be of high potential to reveal genotypic diversity and in the longer term, to provide potential molecular markers that could be linked to phenotypic properties. The results obtained here suggest a substantial genetic diversity across the long-winged species of sect. *Onobrychis*. Also they were found to be variable in terms of morphological characteristics.

Morphological characters are the outer, visibly expressed features of an organism – in plants these are not only affected by genetic factors, but by environmental factors as well (Mal & Doust 2005). Studies on the morphological variations according tohabitat differences can help us to understand the manner, mechanism and determining factors of plant adaptation and evolution (Yang 1991). The present study shows that long-winged species of *Onobrychis* sect. *Onobrychis* exist under the following ecogeographic conditions: soil textures being loam (L), sandy clay loam (SCL) and silt loam (SIL), ranging from pH 7.23–7.53, EC 0.31–0.457, OC 0.23–2.55, clay 14–26%, sand 21–49%, silt 25–56%, av. K 184.5–827.6, av. P 5.2–31.6, Total N 0.02–0.25%, SP 27.1–36.2 and TNV 1–28%; substrate is soil or a mixture of soil and gravel; They exist on substrate without slope (0) or in N, NW, S and SE slope orientations with 5–80% inclination; lowest altitude 1626 m a.s.l., highest altitude 2155 m a.s.l.; max. annual temperature ranging from 15.5–20.2°C, min. annual temperature from 5.3–7.6 C; number of rainy days/year 66–118.3; annual precipitation 212.6–338.3 mm. CCA analysis introduced the number of rainy days, slope inclination and orientation as the main factors associated with the distribution of long-winged species of *Onobrychis* sect. *Onobrychis*. Furthermore it showed correlations

between two major morphologic-genetic groups and certain ecogeographical factors. It shows the separation of the long-winged species in northeast and that ones in northwest of Iran also precipitation, rainy days, sand% and silt% as the most effective factors for this separation. Thus there are ecogeographical patterns in the morphological and genetical variation of long-winged species of *Onobrychis* sect. *Onobrychis.*

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