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A contribution to *Onobrychis* sect. *Hymenobrychis* (Fabaceae) in East of Iran

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Abstract

Chromosome number, meiotic behavior and morphological characters related to habit and pollen grains were studied in *Onobrychis bojnurdensis* Ranjbar & Hajmoradi as a new species belonging to *Onobrychis* sect. *Hymenobrychis* from East of Iran. The new species is related to *O. sintenisii* but differs from it in a few morphological characters such as plant indumentums, size of stipule, bract and different part of the flower. We evaluated and determined the species limits within Eastern species of *O*. sect. *Hymenobrychis*, employing multivariate statistics. Results obtained from morphological and micromorphological studies confirm novelty of *O. bojnurdensis*. Cytogenetic study indicates that the *O. bojnurdensis* is a diploid plant and possesses 2n = 2x = 14 chromosome number. The general meiotic behavior was regular, with bivalent pairing and normal chromosome segregation at meiosis. This report is the first cytogenetic analysis of the new taxon.

Key words: Fabaceae, Iran, meiosis, new species, *Onobrychis* sect. *Hymenobrychis*

1. Introduction

Onobrychis Miller belongs to the family Fabaceae, subfamily Faboideae, tribe Hedysareae, and consists of about 342 species well-distributed in southern Europe and temperate western Asia. *Onobrychis* includes annual or perennial, mostly caulescent herbs (rarely spiny shrubs), which have an indumentum with simple hairs or rarely are glabrous. A few taxa of the genus are cultivated as fodder or for ornamental value (Lock and Simpson, 1991; Yakovlev et al., 1996; Mabberley, 1997). Recently some new taxa have been described in the genera *Onobrychis* from Iran (Ranjbar et al., 2004, 2007; Ranjbar, 2009; Ranjbar et al., 2009b, 2010e, 2010f, 2011).

Pollen grains of *Onobrychis* have been ascribed to a unique type (the *Onobrychis* type) by Faegri (1956), Faegri and Iversen (1989), as well as Moore et al. (1991) characterized by 3 apertures and the suprareticulate ornamentation of the exine. Several studies (Erdtman, 1960; Melhem, 1971; Ohashi, 1971; Pire, 1974; Pavlova and Manova, 2000; Karamian et al., 2009) of the Pollen morphology of *Onobrychis* shows validity of such data in taxonomic delimitation.

In recent years cytologycal analyses have an important role in solving taxonomic problems. Most of cytological studies in the genus have concentrated on the chromosome count (Baltisberger, 1991; Karshibaev, 1992), with little work focused on detailed karyological criteria for taxonomic purposes (e.g. Khatoon and Ali, 1991; Mesicek and Sojak ,1992). Studies on the impact of karyotypic and meiotic behavior data on the interspecific and phylogenetic relationships in the genus are still limited (Ranjbar et al., 2009a; Hesamzadeh Hejazi and Ziaei Nasab, 2010; Ranjbar et al., 2010a, 2010b, 2010c, 2010d, 2011, 2012). Two basic chromosome numbers (x = 7 and x = 8) and three ploidy levels (2n = 2x = 14, 2n = 4x = 28, 2n = 8x = 56 and 2n = 2x = 16, 2n = 4x = 32) are present in the genus *Onobrychis*.

During taxonomical studies on the genus *Onobrychis*, a new species belong to *O.* sect. *Hymenobrychis* was identified from Bojnurd, Khorasan province, E Iran. This study is mainly based on the herbarium specimens of the Bu-Ali Sina University Herbarium (BASU) as well as living materials. Flora Iranica (Rechinger 1984), Flora Orientalis (Boissier, 1872) and also deposed type specimens in W, G and PR were used as the main literatures and references for determination of specimens.

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2. Materials and methods

2.1. *Morphology*

This study is mainly based on the herbarium material and field observations during excursions in N, W and NW Iran (Figure 1, Table 1). Plants were collected from Bojnurd, Khorasan Province, E Iran and the vouchers preserved in BASU. A numerical taxonomic analysis of different species of *O*. sect. *Hymenobrychis* from E Iran, carried out based on 48 quantitative/qualitative characters related to vegetative and reproductive organs. Data were analyzed by using MVSP software version 3.2.

2.2. *Pollen morphology*

For light microscopy, pollen samples were obtained from herbarium specimens of the Bu-Ali Sina University herbarium (BASU, Table 1) and prepared using the standard method described by Erdtman (1960). Then, they were mounted in unstained glycerin jelly and observations were made with an Olympus BX-41 photomicroscope. Polar axis (P) and equatorial diameter (E), colpus length (L), colpus width (S), mesocolpium (M) and shape index (P/E) were measured. Data were analyzed by MVSP and SPSS software and the relationships between different populations were discussed by PCO. The terminology used here are according to Faegri (1956).

2.3. *Cytogenetics*

For cytogenetic study, 15 flower buds from at least 5 plants were fixed in modified Carnoy's solution in ethyl alcohol (96%), chloroform and propionic acid (6:3:2) for 24 h at room temperature and then stored in 70% ethyl alcohol at 4°C until used. Anthers were squashed and stained with 2% (w/v) acetocarmine. All slides were made permanent by the Venetian turpentine. Photographs of chromosomes were taken on an Olympus BX-41 photomicroscope at an initial magnification of × 1000. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs.

2.4. *Phenetics*

Ten populations of different species of *O*. sect. *Hymenobrychis* from east E of Iran were used as operational taxonomic units (OTUs) (Table 1). A numerical taxonomic analysis of the different individuals from these populations was carried out based on 48 quantitative/qualitative characters related to vegetative and reproductive organs. Data was entered into a Microsoft Excel version 7 spreadsheet. This spreadsheet was later converted into a file format suitable for phenetic analysis by MVSP software version 3.2 (Kovach 1985–2002). Principal coordinate analysis (PCO) was carried out using MVSP, with a matrix of standardized data. The data were standardized to eliminate the distorting effects in the output results caused by different measurement scales. Standardization was performed by subtracting the character mean and dividing by the standard deviation. For PCO, an average-distance-matrix of standardized data was obtained. The average distance was used because the data set contained both metric and binary (mixed) data. The distance matrix was double centered and the eigenvectors were calculated and plotted. The PCO gives the distances between OTUs rather than the correlation between the characters. This method is therefore suitable for mixed character data, as it will not be distorted by binary characters. For determining the value of characters in separating of the taxa, data were analyzed with SPSS ver. 10.0 soft ware (SPSS, 1999). This added the advantage of being able to handle missing data well.

Table 1. Specimens examined and their localities

Taxon	Locality	Voucher specimen	Alt. (m)	Abbreviation	Collector
O. bojnurdensis	Khorasan Province, 40 km to Bojnurd	23459 (BASU)	1715	bojn59	Ranjbar & Hajmoradi
O. meshhedensis	Sabzevar to Esfarayen, Afchang village	13641 (BASU)	1700	mesh41	Ranjbar & Hajmoradi
O. meshhedensis	Ghuchan to Sabzevar, 97 km before Sabzevar	13642 (BASU)	1492	mesh42	Ranjbar & Hajmoradi
O. kuchanensis	Ghuchan to Sabzevar, 97 km before Sabzevar	1492 (BASU)	1492	kuch92	Ranjbar & Hajmoradi
O. chorassanica	Ghuchan to Sabzevar	13636 (BASU)	1586	chor36	Ranjbar & Hajmoradi
O. chorassanica	Mashhad to Chenaran, Ferazy village, after Abgheh	13639 (BASU)	1540	chor39	Ranjbar & Hajmoradi
O. sintenisii	Ashkhaneh to Bojnurd, 26 km before Bojnurd	13634 (BASU)	925	sint34	Ranjbar & Hajmoradi
O. sintenisii	Bojnurd to Mamaljeh	13633 (BASU)	1200	sint33	Ranjbar & Hajmoradi
O. sintenisii	Bojnurd to Esfarayen, Mahnan village	13635 (BASU)	1420	sint35	Ranjbar & Hajmoradi
O. sintenisii	Shahrud to Bojnurd, 85 km to Ashkhaneh	13638 (BASU)	925	sint38	Ranjbar & Hajmoradi

3. Results

According to Hedge (1970), *O.* sect. *Hymenobrychis* is separated by some characters such as wings equal to or shorter than calyx, ovary 2- rarely 1-ovulate, fruit stipitate with a curved suture and a broad flattened crest, often toothed from other sections. According to our results different species of *O.* sect. *Hymenobrychis* can be distinguished from each other mainly in the color and size of flowers and characters of pods. Founded diversity in color of the flower in addition to being valuable in taxonomic studies for determination of specimens, with the concern of this fact that nearly all species of this genus are self pollinating, so probably this difference in color of flower play significant role in attracting pollinator insects to have a successful pollination.

3.1. *Key to species of the Onobrychis sect. Hymenobrychis in eastern Iran*

1a- Standard yellowish green without darker venation or purple maculae, calyx 2.5-3 mm long ..... ……1. *O. kuchanensis*
1b- Standard yellow with darker purple venation or with red maculae, calyx longer than 3 mm ……..2
2a- Plant sparsely to completely glabrous, standard yellow with red maculae..…...……..…3
2b- Plant hairy, standard yellow with darker purple venation ..……..……...…4
3a- Stipule 4-13 mm, leaflets 3-4 pairs, corolla relatively small, lower than 8 mm……..….2. *O. meshhedensis*
3b- Stipule 17-24 mm, leaflets 6-7 pairs, corolla longer than 16 mm ……..…….…..3. *O. bojnurdensis*
4a- Plant densely hairy, leaflets ovate to orbiculate, corolla 14-17 mm, calyx 8-12 mm, pod with prickles at the margin and disc .....4. *O. chorassanica*
5a- Plant spreading hairy, leaflets lanceolate, corolla 10-13 mm, calyx 4-5 mm, pod without prickles at the margin and disc …… ...……..…….……5. *O. sintenisii*

3.2. *New species*

Onobrychis bojnurdensis Ranjbar &F. Hajmoradi, sp. nov. (Figures 2, 3)

Type: Iran. Khorasan, 40 km to Bojnurd, 1715 m, 15.5.2010, Ranjbar & F. Hajmoradi 23459 (BASU! !; isotype: TARI!; W!; photo: W).

Perennial, plant 30-60 cm tall. Stems woody at base, branching, more or less glabrous. Stipules free, 17-24 mm long, triangular-lanceolate or lanceolate, more or less densely-hairy. Leaves with 6-7 pairs of leaflets; rachides remote, slender to thickened, straight or curved and ascending. Petiole 5-14 cm long; leaflets oblong-lanceolate, round to cuneate at base, acute to acuminate at apex, 28-35 mm long, 9-12 mm wide, glabrous above, more or less sparsely hairy beneath. Peduncles two to three times as long as leaves. Racemes more or less loose, composed of 34-36 flowers. Bracts papery, brown, 5-7 mm long, oblong to lanceolate or triangular, acute to acuminate, sparsely pubescent. Bracteoles narrowly linear, 1-1.4 mm long. Pedicels short, up to 2 mm long. Calyx 7-8 mm long, more or less densely covered with appressed hairs, 0.1-0.3 mm long; teeth ovate-triangular, as long as tube, densely covered with appressed hairs. Standard cream-yellowish, with darker spots, 16 × 11 mm, oval, emarginate, as long as keel. Wings 3-4 mm long, ciliate, shorter than standard and keel; limb 2.5 × 2.5 mm, briefly oblong, rounded at tip; claw filiform, 1-1.2 mm. Keel 13-15 mm long; limb 10 × 6 mm; claw 4-4.2 mm long. Stamens 15-16 mm long, the free portion 4-4.5 mm long.

Table 2. Morphological comparison between *Onobrychis bojnurdensis* and *O. sintenisii*

Character	*O. bojnurdensis*	*O. sintenisii*
Habit indumentum	glabrous	hairy
Stipule length (mm)	up to 24	up to 20
Corolla color	yellow with red maculae	yellow with darker purple
Standard length (mm)	16-17	9-13
Keel length (mm)	13-15	7-11
Calyx length (mm)	7-8	4-5
Wing indumentum	ciliate	glabrous

3.3. *Distribution and ecology*

O. bojnurdensis appears to be extremely restricted in its geographic distribution. It is currently known only from around its type locality (Figure 3). One small population with nearly 30 individuals was observed in the hilly and rocky region around the city Bojnurd.

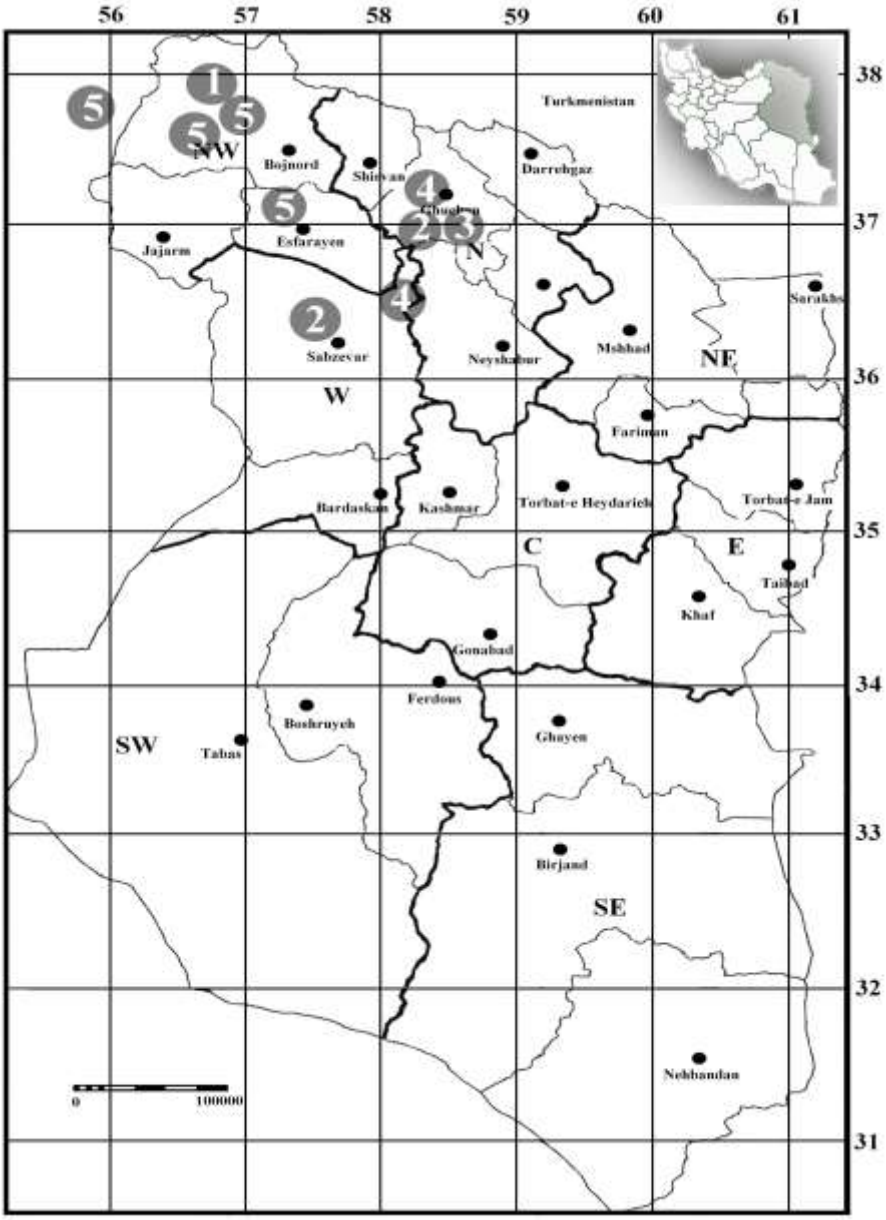

Figure 1. Distribution of different population of eastern species of *O*. sect. *Hymenobrychis* in E Iran. 1 - *O. bojnurdensis*, 2 - *O. meshhedensis*, 3 - *O. kuchanensis*, 4 - *O. chorassanica*, 5 - *O. sintenisii*

3.4. *Etymology*

The specific epithet refers to Mt Bojnurd, where the new species is found.

3.5. *Conservation status*

We observed a low frequency of the new species in its locality, which consisted of no more than 30 plants scattered near the Bojnurd. It is therefore evaluated as Vulnerable (VU) according to IUCN Red List criteria (IUCN, 2001) to identify taxa with small populations that could be at risk.

3.6. *Notes*

O. bojnurdensis represents the main morphological characteristics of *O*. sect. *Hymenobrychis* and is especially close to *O. sintenisii*. However, it differs mainly by its glabrous habit, the size of stipule, standard and calyx (Figure 3). The new species were compared with the isotype material of *O. sintenisii* in W and also with its detailed original description. A diagnostic morphological comparison between the taxa is presented in Table 2.

3.7. *Specimens examined*

Known only from the type material.

Figure. 2. *Onobrychis bojnurdensis* sp. nov. A - habit, B - calyx, C - standard, D - keel, E - wings, F - androecium, G - ovary (bar: A = 2 cm, B-G = 1 cm). Drawn after the type collection

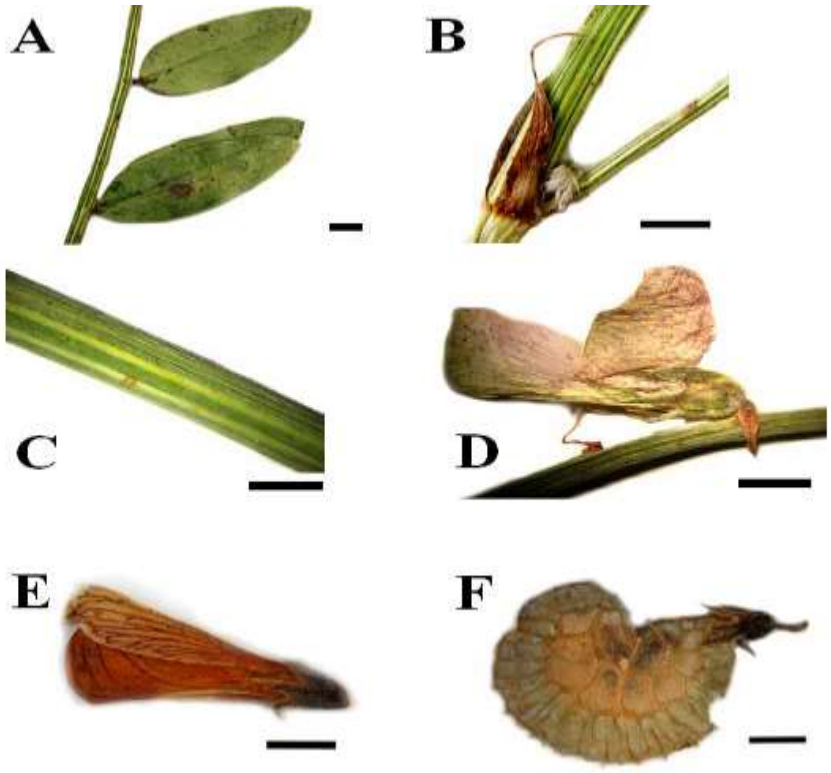

Figure 3. Different vegetative and reproductive parts of *Onobrychis bojnurdensis* sp. nov. (A-D) and *O. sintenisii* Bornm. *725a* (E-F), A – leaflet, B – stipule, C – stem, D – flower, E – Flower, F - Pod (bar: A-D = 4 mm, E-F = 2.5 mm)

3.8. *Morphological analysis*

Analyzing morphological characters of eastern species of *O*. sect *Hymenobrychis* show intrspecific variation. Results from PCO analysis of the matrix of correlations are presented in Figure 4. It is possible to distinguish 3 main groups when plotted on the first 2 eigenvectors. Group I with *O. chorassanica*, group II with *O. meshhedensis*, *O. kuchanensis* and *O. sintenisii* and group III with *O. bojnurdensis* distinguished. As it is clear, *O. bojnurdensis* because of its distinctive characters separated from others species of *O*. sect *Hymenobrychis* and make a distinct group alone.

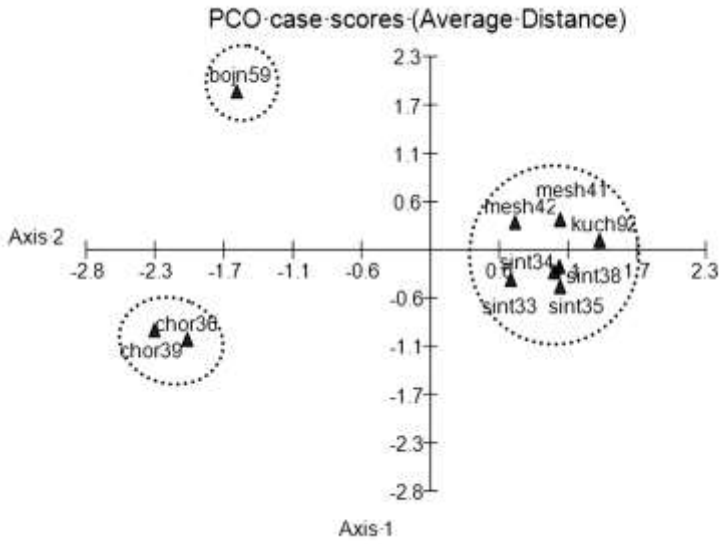

Figure 4. Relationships between different populations of species of *O*. sect. *Hymenobrychis* illustrated by the first and second egenvectors of PCO analysis based on morphological characters (abbreviations are as listed in Table 1)

3.9. *Pollen morphoogy*

Pollen grains of Eastern species of *O*. sect. *Hymenobrychis* are small, rarely medium, sized ranging from: P = 28 to 34.6 µm, E = 13.4 to 19.7 µm. The smallest pollen grains belong to *O. kuchanensis*, while the largest ones belong to *O. chorassanica* (Table 3). They are 3-colpate, prolate and perprolate the ectocalpi are elongated shallow or deep, narrowing at the poles. The cops membrane is covered with large granules. In equatorial view the pollen grains are elongated, elliptic to rectangular-obtuse; and in polar view they are circular, triangular-obtuse or triangular (Figure 5). PCO analysis of pollen morphological characters which were performed using average distance revealed the phenetic relationships between the taxa. Analysis using average distance showed phenetic relationships between different studied species of *O. Hymenobrychis* (Figure 6). Three main groups resulted from the analysis: two population of *O. chorassanica* in group 1; different population of *O. meshhedensis*, *O. kuchanensis* and *O. sintenisii* in group 2 and *O. bojnurdensis* in group 3. These results are consisting with morphological grouping and based on both data new species stand in a separate plot (Figure 6).

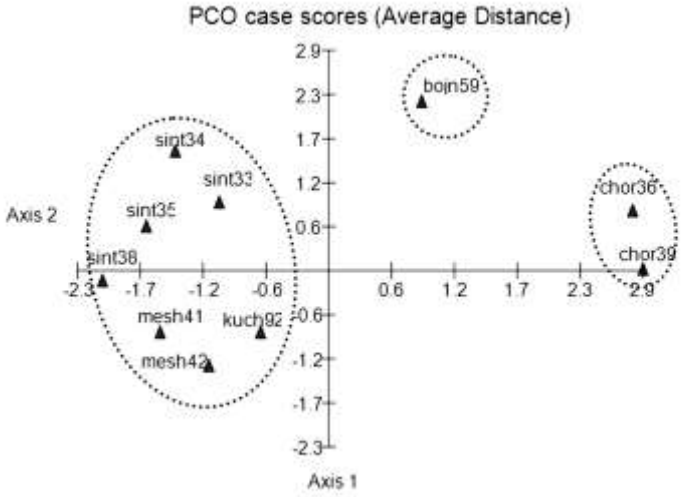

Figure 6. Relationships between different populations of eastern species of *O*. sect. *Hymenobrychis* illustrated by the first and second eigenvectors of PCO analysis based on pollen characters (abbreviations are as listed in Table 1)

The mean values and ranges of five quantitative characters are given in Table 3. Analysis of pollen morphological data with SPSS software (Figure 7) indicates that except for a slight overlap, four characters of polar axis length (P), equatorial diameter (E), mesocolpium (M) and colpus length (L) were occasionally useful in separating *O. bojnurdensis* as a new taxon.

Table 3. The mean values and ranges of the pollen characters in different population of eastern species of *O.* sect. *Hymenobrychis*

Taxa	P	E	M	W	l	P/E
O. bojnurdensis(23459)	31(32.6)35	17(18.3)21	7(10.5)11	1(1.5)1	23(25.35)29	1.7
O. meshhedensis (13641)	28(29.3)30	13(14.5)15	3(3.1)5	1(1)1.5	25(26.3)28	2
O. meshhedensis (13642)	24(28.1)32	13(15.6)18	2(4.7)9	1(1.2)1.5	22(23.6)29	1
O. kuchanensis (1492)	28(28)31	12(13.4)15	3(4.2)4	1(1.2)1.5	22(25.8)27	2
O. sintenisii (13634)	26(28.8)30	12(14)16	3(4.3)7	1(1.1)1.5	22(25.5)28	2
O. sintenisii (13633)	28(29.9)32	13(13.7)15	3(4.3)6	1(1.1)1.5	26(27.3)30	2.1
O. sintenisii (13635)	27(28.6)32	12(13.5)15	3(3.8)6	1(1.1)1.5	26(27.3)29	2.1
O. sintenisii (13638)	28(29.2)30	13(14.1)15	2(3.5)5	1(1.4)1.5	25(26.3)28	2
O. chorassanica (13636)	31(34.6)36	16(18.6)20	6(7.6)9	1(1.1)1.5	29(31.7)33	1.8
O. chorassanica (13639)	33(34.4)36	17(19.7)22	7(7.9)9	1(1)1	30(31.6)33	1.7

Abbreviations: P = Polar axis; E = Equatorial diameter; M = Mesocolpium; W = Colpus width; L = Colpus length; P/E = Shape index.

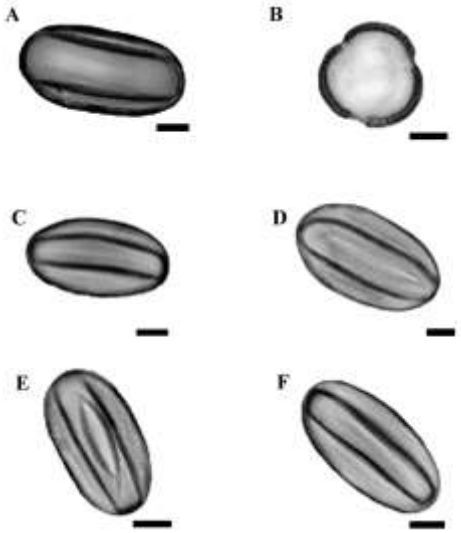

Figure 5. Light microscopy micrographs of pollen grains. A & B - *O. bojnurdensis* (23459), C - *O. sintenisii* (13633), D - *O. chorassanica* (13636), E - *O. meshhedensis* (13641), F - *O. kuchanensis* (1492) (bar = 6 µm)

3.10. *Cytogenetics*

Chromosome number and meiotic behavior of the new species were studied here. A wide range of meiotic stages was observed in anthers within the same flower. A total of 547 diakinesis/metaphase I (D/MI), 290 anaphase I/telophase I (AI/TI), 202 methaphase II (MII) and 407 anaphase II/telophase II (AII/TII) cells were analyzed. The D/MI cells were usually regular with predominant bivalent (II) pairing. However, varied degrees of meiotic irregularities included chromosome stickiness, precocious division of centromeres, cytomixis, B chromosome and desynapsis in D/MI; chromosome bridges resulting from stickiness in AI/TI, asynchronous nucleus in MII, tripolar cells in AII/TII were observed (Figure 8).

Sticky chromosomes along with laggards were found in 6.18% of diakinesis cells of *O. bojnurdensis* (Figure 8B). Chromosome stickiness may be caused by genetic and environmental factors, and several agents have been reported to cause chromosome stickiness (Pagliarini, 2000).

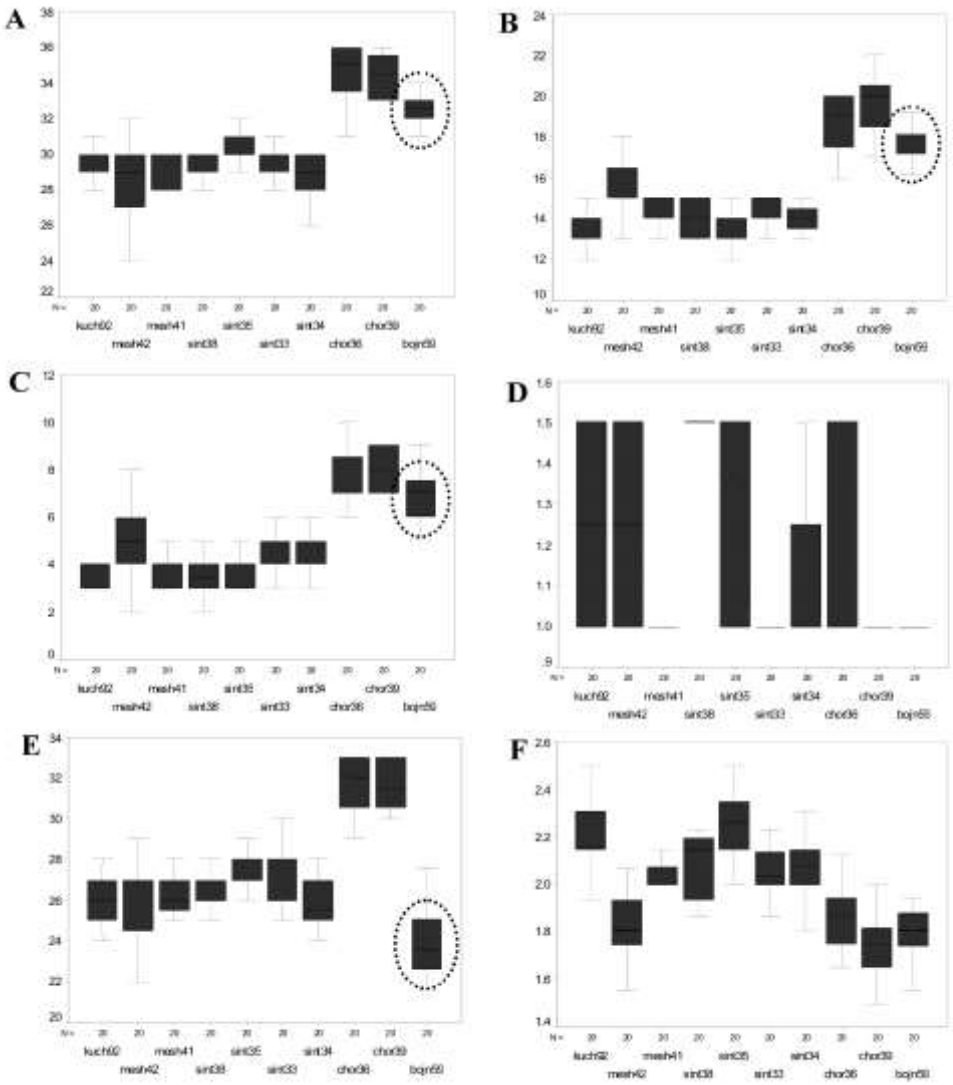

Figure 7. Box and whisker plots depicting the pollen characters in different populations of eastern species of *O.* sect. *Hymenobrychis*. A - polar axis, B - equatorial diameter, C – mesocolpium, D - colpus width, E - colpus length, F - shape index (abbreviations are as listed in Table 1)

Precocious division of centromeres is another abnormality that was found in 0.18% of metaphase I cells (Figure 8F). Desynapsis is considered as the precocious separation of bivalents in metaphase of meiosis I leading to the formation of varied degrees of univalents. Desynapsis was observed in 0.18% of D/MI cells. The phenomenon of cytomixis consists in the migration of chromosome between meiocytes through cytoplasmic connection. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered a mechanism of evolutionary significance (Ghaffari, 2006). This phenomenon occurred in 0.18% of D/MI cells. B-chromosomes or accessory chromosomes that occur in addition to the standard or A-chromosomes in some of the plants, are smaller than other chromosomes and do not form any association with them. B-chromosomes when present in high numbers affect negatively the growth and vigor of the plants, while in low numbers may benefit the plant possessing them (Jones and Houben, 2003). The B-chromosomes were observed in 0.36% of D/MI cells (Figure 8C). Chromosome bridges resulting from stickiness were observed in 3.1% of anaphase I cells (Figure 8E). The thickness of bridges and the number of chromosomes involved in their formation varied among different meiocytes. Genetic as well as environmental factors has been considered as the reason for chromosome stickiness in different plant species. The spindle apparatus is normally bipolar and acts as a single unit, playing a crucial role in chromosome alignment during metaphase. Any distortion or breakage in the spindle may result in random sub-grouping of the chromosome (Nimala and Rao, 1996). Tripolar cells which were observed in 2.21% of TII cells (Figure 8L) may lead to the formation of abnormal tetrads and infertile pollen grains.

The basic chromosome number in *Onobrychis* sect. *Hymenobrychis* is either x = 7 or x = 8. In this study, 2n = 2x = 14 is recorded for *O. bojnurdensis*. The members of *O.* sect. *Hymenobrychis* are diploid with 2n = 2x = 14 and 2n

= 2x = 16 chromosome numbers (Ranjbar et al., 2012), whereas those of *O.* sect. *Onobrychis* are diploid or tetraploid with 2n = 2x = 14, 2n = 2x = 16, 2n = 4x = 28 and 2n = 4x = 32 chromosome numbers (Ranjbar et al., 2009a, 2010a, 2010c, 2010d, 2012) and of *O*. sect. *Heliobrychis* are diploid with 2n = 2x = 16 chromosome number (Ranjbar et al., 2010b). The results from the present study increase our knowledge about the basic chromosome numbers in the genus *Onobrychis*, especially in *O*. sect. *Hymenobrychis* that can be helpful in taxonomic delimitation and represents a new taxon by establishing relationships between cytogenetic and morphological criteria.

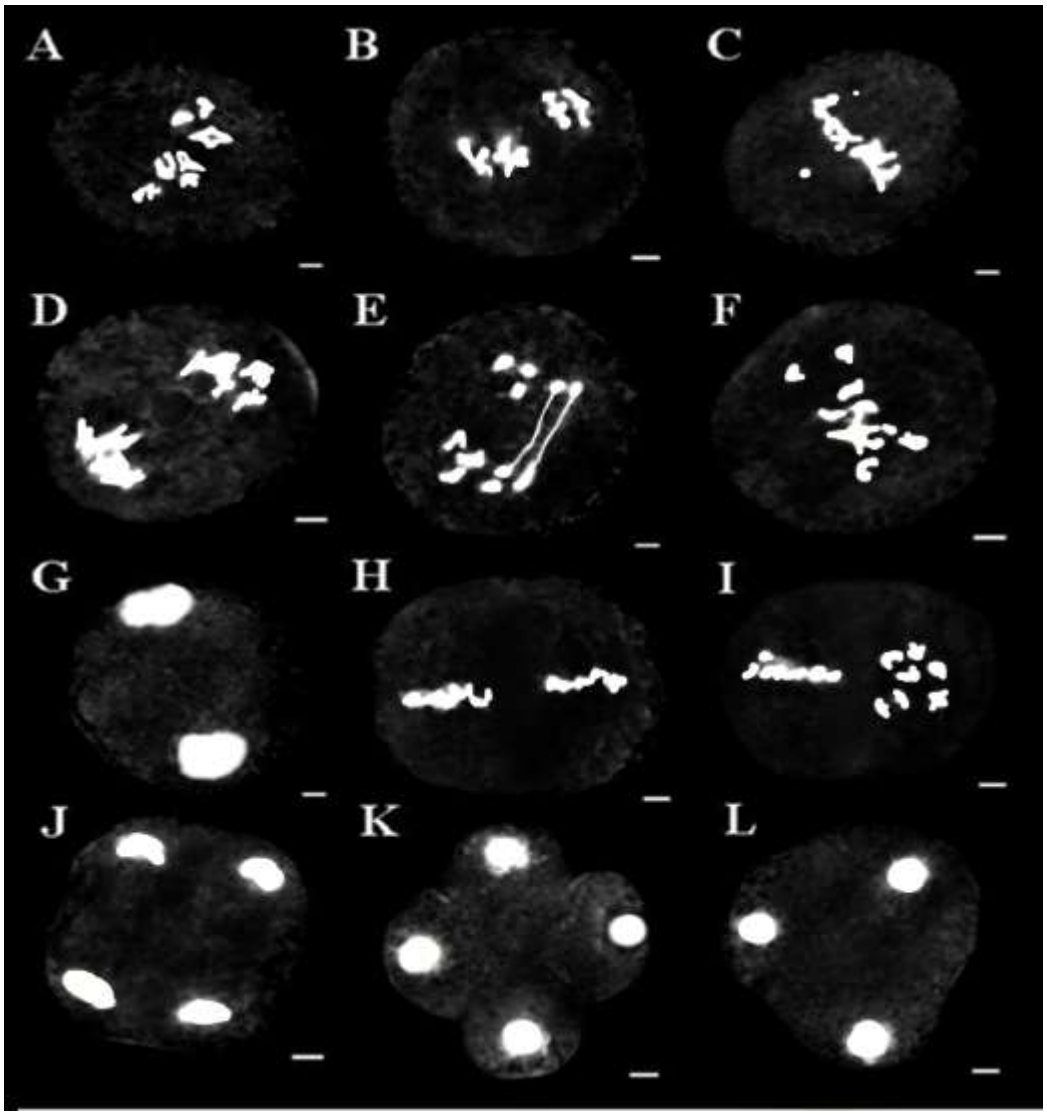

Figure 8. Representative meiotic cells in *Onobrychis bojnurdensis.* A - diakinesis, B - sticky and laggard, C - B chromosome, D - anaphase I, E - bridge F precocious ascension in metaphase I, G - telophase I, H - metaphase II, I - Asynchronous nucleus, J - anaphase II, K - telophase II, L - tripolar cell (bar = 6 µm)

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References

Baltisberger, M. 1991. IOPB chromosome data 3. IOPB Newsletter. 17: 5-7.

Boissier, E. 1872. *Onobrychis.* In (Ed) Boissier, E. Flora Orientalis. Geneva, Basilease and Lugundi. pp. 545-553.

Erdtman, G. 1960. The acetolysis method. Svensk Botanisk Tidskrift. 54: 561-564.

Faegri, K. 1956. Palynological studies in NW European Papilionaaceae. Botany Museum Bergen.

Faegri, K., Iversen, J. 1989. Textbook of pollen analysis. Blackwell Science Publishing, Chichester.

Ghaffari, S. M. 2006. Occurrence of diploid and polyploidy microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. African J. Biotech. 5: 1450-1453.

Hedge, I. C. 1970. *Onobrychis.* In: (Ed) Davis, P. H., Flora Turkey and the Aegean Islands. Edinburgh: Edinburgh University Press. pp: 584-589.
Hesamzadeh Hejazi, S. M., Ziaei Nasab, M. 2010. Cytotaxonomy of some *Onobrychis* (Fabaceae) species and populations in Iran. Caryologia. 63: 18-31.
Karamian, R., Moradi Behjou, A., Ranjbar, M. 2009. Pollen morphology of the *Onobrychis* sect. *Heliobrychis* in Iran. T. B. J. 1: 63-72.
Karshibaev, H. K. 1992. Chromosome numbers of some Fabaceae in Uzbekistan. Tezisy 3 Soveshchanie Po Kariologii Rastenii. 27: 1-2.
Kovach, W. 1985-2002. Institute of Earth Studies, University College of Wales, Aberystwyth, (Shareware), MVSP Version 3.2, Kovach Computing Services http://www.kovcomp.com/MVPs/downl2.html.
Khatoon, S., Ali, S. 1991. Chromosome numbers in subfamily Papilionoideae (Leguminosae) from Pakistan. Willdenowia. 20: 159-165.
Jones, N., Houben, A. 2003. B-chromosomes in plant: escapees from the A-chromosome genome? T. P. S. 8: 417-423.
Lock, J. M., Simpson, K. 1991. Legumes of West Asia, a check-list. Royal Bot. Gardens, Kew.
Mabberley, D. J., 1997. The plant book. A portable dictionary of the vascular plants, 2nd ed. Cambridge University.
Melhem, T. 1971. Pollen grains of plants of the Cerrado- Leguminosae- Lotoideae Tribe Phaseoleae. Hoehnea 1: 119-151.
Mesicek, J., Sojak, J. 1992. Chromosome numbers of Mongolia angiosperms. Preslia. 64: 193-206.
Moore, P. D., Webb, J. E., Collinson, M. E. 1991. Pollen analysis (2 Ed.). Blackwell scientific publications, Oxford.
Nimala, A., Rao, P. N. 1996. Genetics of chromosome numerical mosaism in higher plants. The nucleus 39: 151-175.
Ohashi, H. 1971. A taxonomic study of the tribe Coronilleae (Leguminosae) with special reference to pollen morphology. Journal of the Faculty of Science, University of Tokyo 11: 25-92.
Pagliarini, M. S. 2000. Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. Genet. Mol. Biol. 23: 997-1002.
Pavlova, D. K., Manova, V. I. 2000. Pollen morphology of the genera *Onobrychis* and *Hedysarum* (Hedysareae, Fabaceae) in Bulgaria. Ann. Bot. Fen. 37: 207-217.
Pire, S. 1974. Studio palynologico de la tribu Hedysareae (Leguminosae). Bonplandia. 3(12): 143-168.
Ranjbar, M. 2009. *Onobrychis oshnaviyehensis* sp. nov. (sect. *Hymenobrychis*, Fabaceae) from Iran. Nordic J. Bot. 27: 1-5.
Ranjbar, M., Amirabadizadeh, H., Karamian, R., Ghahremani, M. A. 2004 Notes on *Onobrychis* sect. *Heliobrychis* (Fabaceae) in Iran. Willdenowia. 34: 187-190.
Ranjbar, M., Hajmoradi, F., Karamian, R. 2010a. Mitotic study of some species of *Onobrychis* sect. *Hymenobrychis* DC. in Iran. I. J. P. B. 1: 47-54.
Ranjbar, M., Hajmoradi, F., Karamian, R. 2011. Meiotic chromosome number and behaviour of *Onobrychis alborzensis* (Fabaceae): a new species from northern Iran. Feddes Repert. 122: 1-12.
Ranjbar, M., Hajmoradi, F., Karamian, R. 2012 An overview on cytogenetics of the genus *Onobrychis* (Fabaceae) with special reference to *O*. sect. *Hymenobrychis* from Iran. Caryologia. 65 (3): 187-198.
Ranjbar, M., Karamian, R., Afsari, S. 2010b. Meiotic chromosome number and behaviour of *Onobrychis avajensis* (Fabaceae): a new species from western Iran. Plant Ecol. Evol. 143: 170-175.
Ranjbar, M., Karamian, R., Hadadi, A. 2009a. Biosystematic study of *Onobrychis vicifolia* Scop. and *Onobrychis altissima* Grossh. (Fabaceae) in Iran. I. J. B. 15: 85-95.
Ranjbar, M., Karamian, R., Hadadi, A. 2010c. Cytosystematics of three *Onobrychis* species (Fabaceae) in Iran. Caryologia. 63: 237-249.
Ranjbar, M., Karamian, R., Hajmoradi, F. 2009b. Taxonomic notes on *Onobrychis* sect. *Hymenobrychis* (Fabaceae, Hedysareae) in Iran. Novon. 19: 215-218.
Ranjbar, M., Karamian, R., Hajmoradi, F. 2010d. Chromosome number and meiotic behavior of two populations of *Onobrychis chorassanica* Bunge sect. *Hymenobrychis* in Iran. J. Cell Mol. Res. 2: 49-55.
Ranjbar, M., Karamian, R., Tolui, Z., Amirabadizadeh, H. 2007. *Onobrychis assadii* (Fabaceae), a new species from Iran. Ann. Bot. Fen. 44: 481-484.
Ranjbar, M., Karamian, R., Vitek, E. 2010e. *Onobrychis bakuensis* (Fabaceae), a new species from Azerbaijan Ann. Bot. Fen. 47: 233-236.
Ranjbar, M., Karamian, R., Vitek, E. 2010f. Notes on *Onobrychis* sect. *Hymenobrychis* (Fabaceae) in Tajikistan, with the description of a new species. Nord. J. Bot. 28: 1-4.
Rechinger, K. H. 1984. *Onobrychis*. In (Ed) Rechinger, K. H. Flora Iranica. Akademische Druck- und Verlagsanstalt, Graz. 157. pp: 389-459.
Spss. 1999. Statistical software package of the social sciences vol. 10.0. spss Inc., Chicago, Illinois.
Yakovlev, G. P., Sytin, A. K., Roskov, Y. R. 1996. Legumes of Northern Eurasia, a check-list. Royal Bot. Gardens, Kew.

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