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Cytotaxonomic revision of *Onosma* series *Aleppica* (Boraginaceae) in Iran

Massoud RANJBAR [*1], Maryam ALMASI [1]

[1] Department of Biology, Faculty of Science, Bu-Ali Sina University, P. O. Box 65175/4161, Hamedan, Iran

Abstract

In this paper a key, descriptions, morphological characters, chromosome number and meiotic behavior are presented for *Onosma* series *Aleppica* in Iran. The series characterized by biennial plants, erect-patent indumentum, glabrous tubercles, leaves without venation, density of inflorescence and the exerted anthers from corolla. *Onosma estahbanensis* and *O. soltanabadensis* are described and illustrated as new species based on morphological data and meiotic behavior. All populations of different species are diploid and possess chromosome number 2n = 2x = 18. Moreover, populations limits within *O*. series *Aleppica* are evaluated and determined by employing multivariate statistics and found a striking association between phenetic and meiotic behavior.

Key words: cytogenetics, new species*, onosma*, phenetic, pollen viability

1. Introduction

The family Boraginaceae consists of about 2500 species in 130 genera distributed throughout the tropical, subtropical and temperate regions. The centers of its highest diversity is in the North Temperate Zone are the Irano-Turanian and Mediterranean regions and in the tropics are Central America and northern and central South America. The tribe Lithospermeae is the second largest tribe in Boraginaceae, primarily distributed in the temperate region of the northern Hemisphere. It includes about 415 species in 24 genera, of which *Onosma* L. with more than 150 species represents the large genus (Al-Shehbaz, 1991). Based on the current knowledge, there are 51 species of the genus in Iran (Riedl, 1967; khatamsaz, 1992; Ghahreman and Attar, 1996; Attar and Joharchi, 2006; Attar and Hamzehee, 2007). The taxonomy of the genus continues to be subject of much confusion, mainly because of different approaches to species delimitation, resulting in varying numbers of recognized species. The genus has been divided into 3 sections according to indumentum type: *Haplotricha* Boiss., *Heterotricha* Boiss. and *Asterotricha* Boiss. (Boissier, 1879). Other morphological characters like leaf size and shape, bract shape, corolla shape, nutlet length, venation and corolla teeth size and anther length are the most variable morphological characters among *Onosma* species (Peruzzi and Passalacqua, 2008; Mehrabian et al., 2011).

Most cytological studies on *Onosma* have concerted on the chromosome number, with little work focused on detailed karyological analysis for taxonomic purposes. From these reports, it is evident that the chromosome number is known for just over the third of species. This paper represents a cytotaxonomic revision of the *O.* series *Aleppica* Riedl and includes full descriptions for all species of the series and their meiotic behaviors. In addition, two new species along with their meiotic behaviors are described from Iran. This article follows previous studies conducted on flora of Iran (Ranjbar & Negaresh 2013; Ranjbar et al., 2010a, b; 2011a, 2012b)..

2. Materials and methods

2.1. *Plant material*

The morphological study was mainly based on herbarium material. Several sheets have been examined for each species, from the following herbaria: B, BASU, E, FUMH, G, P, W and WU. Moreover, during several excursions in Iran, many species were studied in the field by the authors. The studied taxa and their localities are listed in Table 1. A total of 60 quantitative/qualitative morphological characters related to vegetative and reproductive organs were

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: 0098 811 8271541; Fax.: 0098 811 8381172; E-mail: massoud.ranjbar80@gmail.com
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studied in 40 plants from 8 populations of *Onosma bulbotricha*, 4 populations of *O. straussii*, 2 populations of *O. estahbanensis* and 1 population of *O. soltanabadensis* and then they were coded as binary/multi-state characters (Table 2).

Table 1. Studied taxa and their acronyms

Species	Locality	Altitude (m)	Voucher	Abbreviation
O. bulbotricha	Tehran: Ziaran	2050	BASU 27334	bul
O. bulbotricha	Kordestan: Dehgolan	1845	BASU 29164	bul
O. bulbotricha	Markazi: Vafs	2205	BASU 27662	bul
O. bulbotricha	Fars: Larizangan village	1835	BASU 24522	bul
O. bulbotricha	West Azerbaijan: Azar Shahr	1368	BASU 26472	bul
O. bulbotricha	Hamedan: Malayer	1660	BASU 12513	bul
O. bulbotricha	Hamedan: Avaj	2049	BASU 27334	bul
O. bulbotricha	West Azerbaijan: Bookan	1280	BASU 25057	bul
O. estahbanensis	Fars: Estahban	1720	BASU 23995	est
O. estahbanensis	Kohgiluyeh va boyer ahmad: Yasuj	1135	BASU 32736	est
O. soltanabadensis	Arak: Anjadan	1800	BASU 28676	sol
O. straussii	Lorestan: Dorud	1620	BASU 26985	str
O. straussii	Esfahan: Mobarakeh	1830	BASU 28677	str
O. straussii	Lorestan: Sefid Dasht	1300	BASU 28674	str
O. straussii	Arak: Anjadan	1800	BASU 28675	str

Table 2. Morphological features of the *Onosma* series *Aleppica* in this study

Stem length (cm)
***Characters related to setae**
Setae angle (inclination of setae: 1 = (80 – 100°) more or less patent; 2 = (5 – 80°) appressed to patent; 3 = (0 – 5°) appressed)
Tubercle size (width of tubercle, mm)
Setae length (length of bristles, mm)
Pilose length (length of short hair, mm)
Bristle density (glabrous = 0, scatter = 1, sparse = 2, loose = 3, dense = 4)
Pilose density (glabrous = 0, scatter = 1, sparse = 2, loose = 3, dense = 4)
Indumentum color
Characters related to leaves
Leaf length (length of basal and stem leaves, cm)
Leaf width (width of basal and stem leaves, mm)
Leaf shape (leaf length / leaf width)
With or without petiole
Leaf revolute (more or less flat = 1, revolute = 2)
Characters related to flowers/inflorescences
Bract length (mm)
Bract width (mm)
Inflorescence number
Pedicel length (mm)
Corolla length (mm)
Corolla maximum width (mm)
Corolla teeth length (mm)
Corolla teeth width (mm)
Corolla teeth shape
Corolla color
Corolla hair density (glabrous = 0, scatter = 1, sparse = 2, loose = 3, dense = 4)
Style length (mm)
Calyx length (mm)
Characters related to nutlet
Nutlet length (mm)
Nutlet width (mm)
Nutlet curve (absent = 0, present = 1)
Nutlet beak length (mm)
Nutlet shape
Nutlet color
Nutlet surface (smooth =1, wrinkle = 2, verrucose = 3)

* Characters related to setae were measured in different plant portions (stem, upper surface of basal and stem leaves, lower surface of basal and stem leaves, bract and calyx).

2.2. *Phenetics*

All populations studied here were used as operational taxonomic units (OTUs). A numerical taxonomic analysis of the different individuals from these populations was carried out based on 72 quantitative/qualitative characters. Data was entered into a Microsoft Excel version 10 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 was standardized to eliminate 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 (Ranjbar et al., 2010b, 2011c, 2012). This added the advantage of being able to handle missing data well.

2. 3. *Cytogenetic*s

Meiotic chromosome number and behavior were analyzed in 2 populations of *Onosma bulbotricha*, 2 populations of *O. straussii*, 1 population of *O. estehbanensis* and 1 population of *O. soltanabadensis*. For each population, 15 flower buds from at least 5 plants at an appropriate stage of development were fixed in Piennr's fluid containing ethanol (96%), chloroform and propionic acid, 6:3:2 (v/v/v), for 24 h at room temperature and then stored in 70% alcohol at 48° C until used. Anthers were squashed and stained with 2% acetocarmine. All permanent slides were made using Venetian turpentine (Wilson, 1945). Photographs of chromosomes were taken by Olympus 3030 digital Camera mounted on a BX-51 Olympus microscope. Chromosome counts were made from well-spread metaphases in intact cells, by direct observation and from photomicrographs. For grouping, the taxa showing similar meiotic behavior, principal coordinate's analysis (PCO) using MVSP ver. 3.2 were performed on standardized data (Kovach, 1985-2002).

2.4. *Pollen viability*

Pollen stain ability was considered as an indication of pollen viability. For this purpose pollen grains were first obtained from flowers of herbarium specimen and then stained with acetocarmin/glycerin (1:1). Slides were stored at room temperature for 24-48 hours. The stain ability was determined using samples of 1000 pollen grains per flower. Slides were examined and documented with an Olympus 3030 digital Camera mounted on a BX-51 Olympus microscope.

3. Results

3.1. *Taxonomical note on Onosma series Aleppica Riedl*

Onosma series Aleppica belongs to section *Haplotricha* and includes four species: *O. bulbotricha* DC., *O. estahbanensis* Ranjbar & Almasi, *O soltanabadensis* Ranjbar & Almasi and *O. straussii* (Riedl) Khat. The series is distinguished by biennial plants, glabrous tubercles, erect-patent indumentum, long leaves without venations, rich inflorescences of mostly 5-7 cymes, hairy nectary and exerted anthers from corolla (Riedl, 1967; 1979).

3.2. *Key to the species of Onosma series Aleppica*

1a- Corolla white to yellow; style 1 – 3 mm longer than corolla……..……..2
1b- Corolla pinkish to red; style ca. 5 mm longer than corolla……..……...…….3
2a- Rosette leaf absent; stem more than 30 cm long; bristle up to 3 mm long; exserted anther from corolla ca. 1 mm long…….……....……1. *O. bulbotricha*
2b- Rosette leaf present; stem up to 20 cm long; bristle up to 8 mm long; exserted anther from corolla ca. 5 mm long……..…..…….....…..2. *O. estahbanensis*
3a- Spatulate rosette leaf ca. 11 × 3 cm; bristle ca. 10 mm long; absent short hair; corolla ca. 25 mm long, stem reddish colored in dry state.....3. *O. soltanabadensis*
3b- Rosette leaf absent; basal leaf ca. 10 × 1 cm; seta up to 4 mm long; present short hair; corolla ca. 20 mm long, stem brown or straw colored in dry state…….....4. *O. straussii*

3.3 *Description*

Onosma bulbotricha DC. Prodr. 10: 64. 1846. (Figure 1)
Type: Iran. Near to Teheran, Aucher 3103 (holotype: G!).
= *O. echinata* Aucher ex DC. Prodr. [A. P. de Candolle] 10: 58. 1846.

Biennial plants, 30 – 47 cm tall. Stem single to several, erect to ascending, branched above, straw-colored when dry, covered by a mixture of erect-patent bristle 1.5 – 3.5 mm long, white and glabrous tubercles, 0.25 – 0.5 mm wide, and very short hairs between the tubercles. Leaves without venations; basal and lower stem leaves sessile, linear, 4 – 8 × 0.4 – 1 cm, subacute at the apex, margins strongly revolute, loosely covered adaxially with ascending white bristles 1.5 – 3.5 mm long, arising from tubercle; tubercles white, nearly flat, composed of 1 or 3 circles of roundish to radially cell elongated 0.25 – 0.75 mm wide and sparse short hairs, abaxially covered with similar bristles mainly along the midrib and only sparsely on other parts with dense short hairs, 0.25 – 0.5 mm long as those on stem. Middle and upper stem leaves similar but smaller than basal ones, 3 – 5 cm long, widest near base. Inflorescence consists of 1 – 3 terminal or lateral cymes, scorpioid, then elongated, up to 15 cm high in fruit. Lower bracts similar to upper stem leaves, up to 15 cm long, wide at base, decreasing in size upward. Pedicel 1 – 3 mm long in flower, up to 10 mm long in fruit, loosely covered by patent white bristles ca. 3 mm long. Calyx 15 – 18 mm long in flower, with patent white bristles to 3 mm long, lobes linear to oblong, acuminate, 1 – 2 mm wide. Corolla white to pale yellow, sometimes turning brown when dry, cylindrical to campanulate, ca. 22 × 21 mm, outside glabrous, lobes ca. 2 × 3 mm, acute at apex. Nectaries hairy. Stamens borne ca. 2/3 from corolla base; anthers linear, 8 – 9 mm long, coherent only at base, with sterile tips exserted ca. 1 mm long; filament 4 – 5 mm long. Style ca. 1 mm longer than corolla; stigma very small, distinctly bilobed. Nutlet ca. 6 × 4.5 mm, with gibbous shoulders and with sharp ventral and indistinct dorsal keel, beak strongly incurved, obtuse, pale grey to brown.

3.4. *Specimens seen*

Iran: Tehran, Ziaran to Taleghan, 5 km after Ziaran, 2050 m, 22.6.2011, *Ranjbar & Almasi 27334* (BASU!); Kordestan, Dehgolan to Qorveh, 20 km to Qorveh, 1845 m, 19.6.2011, *Ranjbar & Almasi 29164* (BASU!); Markazi, Vafs, 2205 m, 23.6.2011, *Ranjbar & Almasi 27662* (BASU!); Fars, Larizangan village, 1835 m, 30.4.2011, *Ranjbar & Almasi 24522* (BASU!); West Azerbaijan, Azar Shahr toward Ajab Shir, 15 km to Ajab Shir, 1368, 28.5.2011, *Ranjbar & Almasi 26472* (BASU!); West Azerbaijan, Bookan toward Miandoab, 1280 m, 17.5.2011, *Ranjbar & Almasi 25057* (BASU!); Kermanshah, Sonqor to Qorveh, 10 km after Sonqor, 1745 m, 20.6.2011, *Ranjbar & Almasi 27637* (BASU!); Hamedan, Malayer, 1660 m, 20.5.2009, *Ranjbar & Almasi 12513* (BASU!); Hamedan, Hamedan to Avaj, 17 km to Avaj, 2049 m, 22.6.2011, *Ranjbar & Almasi 27637* (BASU!); Kermanshah, Qasr Shirin, *Sharif 5230* (IRAN!); Karaj, mountains Halkedar near to Mardabad, 1300 m, *Rechinger 1042* (B!); Gilan, 45 km to Bostanabad, 1650 m, *Furse 2420* (W!); Turkey. Erzincan, Sipikör, Bendola, 1890 m, 30.6.1890, *Sintenis 3066 (P!)*.

Onosma estahbanensis Ranjbar & Almasi **sp. nov.** (Figure 2)
Type: Iran. Fars, Estahban, 1720 m, 29.4.2011, *Ranjbar & Almasi 23995* (holotype: BASU!).

Biennial plants, 13 – 20 cm tall. Stem single to several, erect to ascending, covered by a mixture of antrorse bristle up to 6 mm long; tubercles white and glabrous, 0.25 – 0.5 mm wide, and patent short hairs. Sterile shoots short to moderately elongated, ca. 2 cm long, with rosette leaves 7 – 10 × 0.5 – 1 cm, without venation, narrow toward the base, 2 – 3 cm, spatulate to oblanceolate, subacute at the apex; densely covered adaxially with ascending bristles, ca. 8 mm long, arising from white and glabrous tubercles, ca. 0.5 mm wide, and densely short hairs between the tubercles; abaxially covered with similar bristles mainly along the midrib and only sparsely on other parts with dense short hairs, 0.25 – 0.5 mm long as those on stem. Stem leaves similar but smaller than basal ones. Inflorescence consists of 5 – 7 terminal or lateral cymes, 4 – 10 flowers on each scorpioid, then elongated. Bracts lanceolate-linear, ca. 20 mm long, wide at base, decreasing in size upward, to 15 mm long, bristle 1 – 2 mm long. Pedicel ca. 5 mm long in flower, with densely patent yellow bristles. Calyx ca. 13 mm long at anthesis, densely covered with patent yellow bristles, lobes linear to oblong, acute, ca. 1 mm wide. Corolla cream-colored, cylindrical to campanulate, ca. 18 × 12 mm, sparsely hairy in outside; lobes ca. 2 × 3 mm wide, acute at apex. Nectaries hairy. Stamen borne ca. 4/5 from corolla base; anthers linear, ca. 8.5 mm long, coherent only at base, with fertile tips exserted ca. 5 mm long; filament ca. 5 mm long. Styles ca. 5 mm longer than corolla; stigma very small, distinctly bilobed. Nutlets not seen.

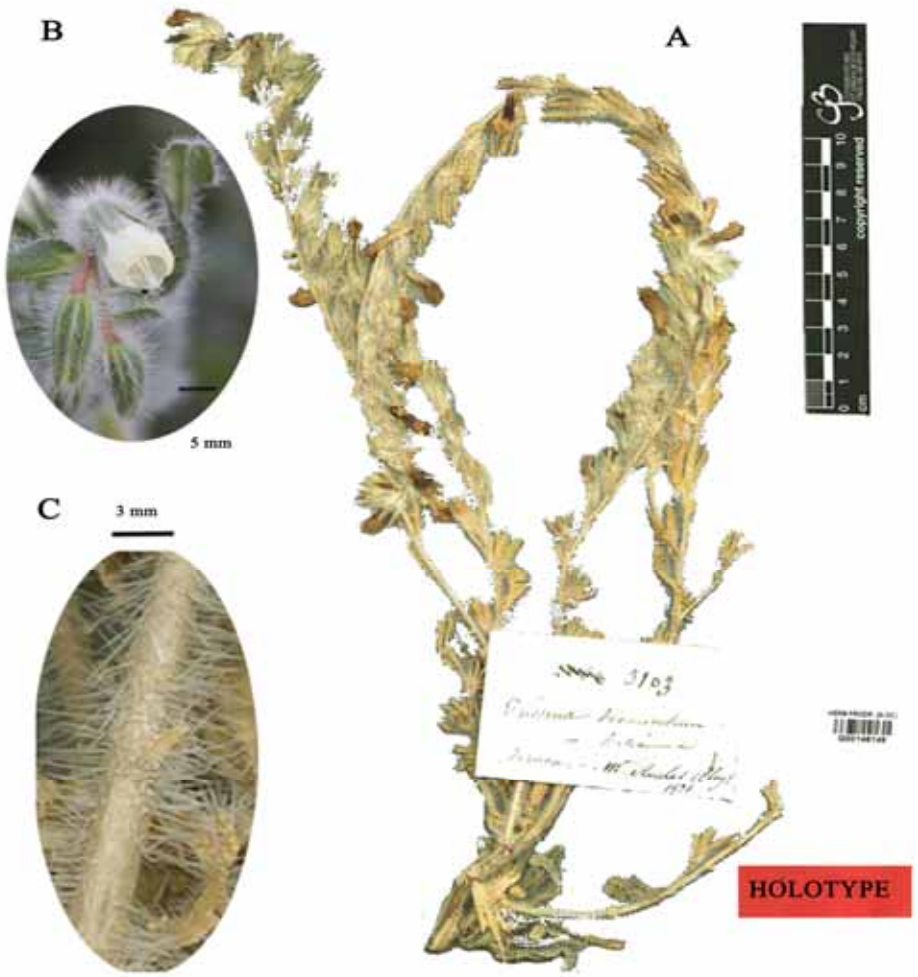

Figure 1. *Onosma bulbotricha* DC. (*Auch. 3103*). (A) habit, (B) corolla with exserted anther, (C) bristle on the stem. Holotype of photograph is provided by G

3.5. *Taxonomic and distribution remarks*

Onosma estahbanensis distributed in south of Iran and appear to be the closest relative to *O. bulbotricha* (Figures 1, 2) because of indumentum, color and size of corolla, however, they differ from each other mainly in short stem, present of rosette leaves, density of short hairs, and pedicel length. Important differences between the new species and *O. bulbotricha* are presented in Table 3. The new species is a local endemic to Iran, known from dry-steppe zone near to Estahban, Fars province while, *O. bulbotricha* has a wide distribution in Afghanistan, Iran, Iraq and Turkey.

3.6. *Etymology*

The species is named after the Estahban, in Fars Province, Iran.

3.7. *Specimens seen*

Iran: Kohgiluyeh va Boyer Ahmad, 10 km from Yasuj toward Baba Meidan, 1135 m, 28.4.2011, *Ranjbar & Almasi 32736* (BASU!).

Onosma soltanabadensis Ranjbar & Almasi sp. nov. (Figure 3)
Type: Iran. Markazi, Anjadan, 1800 m, 14.6.2012, *Ranjbar & Almasi 28676* (holotype: BASU!).

Biennial 14 – 16 cm tall. Stems several, erect to ascending, pink to reddish in fresh plant, reddish colored in dry state, covered by a mixture of antrorse bristles 3 – 4 mm long, arising from white and glabrous tubercles, 0.25 – 0.5 mm wide, without short antrorse hairs between the tubercles. Sterile shoots short to moderately elongated, 2 – 3 cm

long, with rosette leaves ca. 11 × 3 cm, spatulate, without venations, tapering into a petiole like base, revolute at margin, round at apex, loosely covered adaxially with antrorse white bristles ca. 10 mm long, arising from tubercle, tubercle white, nearly flat or convex, composed of 3 or 5 circles of roundly cell elongated, 0.25 – 0.75 mm wide the tubercles; abaxially covered with similar bristles mainly along the midrib and only sparsely on other parts. Middle and upper stem leaves lanceolate 4.5 × 1 cm, sessile, revolute at margin, acute at apex. Inflorescence consists of 4 – 5 terminal or lateral cymes, scorpioid, and then elongated. Lower bracts similar to upper stem leaves, to 4 cm long, decreasing in size upward. Pedicel 5 – 7 mm in flower, densely covered by patent white bristles up to 4 mm long. Calyx ca. 17 mm long in flower, white bristles up to 4 mm long in outside, lobes linear to oblong, acuminate, 1 – 2 mm wide. Corolla pinkish to red sometimes pale yellow in basal part, cylindrical to campanulate, ca. 25 × 17 mm, sparsely short hairs in outside, lobes ca. 2.5 × 3 mm, acute at apex. Nectaries hairy. Stamens borne ca. 4/5 from corolla base; anthers linear, ca. 9 mm long, coherent only at base, exserted from corolla ca. 5 mm long; filament 10 mm long. Style ca. 3 mm longer than the corolla; stigma very small, distinctly bilobed. Nutlets not seen.

Table 3. Diagnostic morphological characters of *Onosma bulbotricha* and *O. estahbanensis*

Species	*O. bulbotricha*	*O. estahbanensis*
Rosette leaf	*without rosette leaf*	*with rosette leaf*
Stem	*more than 30 cm*	*up to 20 cm*
Leaf shape	*linear*	*spatulate-oblanceolate*
Leaf base	*sessile*	*2 – 3 cm*
Indumentum color	*white*	*yellowish to white*
Bristle hair density	*loose*	*dense*
Short hair density	*sparse*	*dense*
Bristle hair length (mm)	*less than 4*	*ca. 8*
Bract length (mm)	*ca. 15*	*ca. 20*
Inflorescence number	*1 – 3*	*5 – 7*
Pedicel length in flower (mm)	*1 – 3*	*ca. 5*
Exserted style length (mm)	*2 – 3*	*ca. 5*
Exserted anther from corolla(mm)	*ca. 1*	*ca. 5*
Borne stamen from corolla base	*2/3*	*4/5*

Figure 2. *Onosma estahbanensis* sp. nov. (A) habit, (B) corolla with exserted anther, (C) style, (D) calyx with yellow bristle

3.8. *Taxonomic and distribution remarks*

Onosma soltanabadensis distributed in west of Iran and appear to be the closest relative to *O. straussii* (Figures 3, 4) because of indumentum, color of corolla, however, they differ from each other mainly in present of rosette leaves, length of hairs, and exerted anther from corolla. Important differences between the new species and *O. straussii* are presented in Table 4. The new species is a local endemic to Iran, known from dry-steppe zone and stony slopes near of the mountainous regions around Anjadan village near Arak in Markazi Province while, *O. straussii* grows in Markazi, Lorestan, Khuzestan, Esfahan, Kohgiluyeh and Boyer-Ahmad Provinces.

3.9. *Etymology*

The species is named after the Soltanabad, old name of Arak city, in Markazi Province, Iran.

Figure 3. Holotype of *Onosma soltanabadensis* Ranjbar & Almasi. (A) close up of flowers, (B) close up of rosette leaf, (C) habit form. Scale bar: 1cm.

Table 4. Diagnostic morphological characters of *Onosma straussii* and *O. soltanabadensis*

Species	*O. straussii*	*O. soltanabadensis*
Rosette leaf	without rosette leaf	with rosette leaf
Basal leaf shape	linear-oblanceolate	spatulate
Basal leaf width (mm)	ca. 10	ca. 30
Leaf apex	acute	Round
Bristle hair length (mm)	to 4 mm	to 10 mm
Short hair density	sparse	glabrous
Tubercle circles	2 – 4	3 – 5
Pedicel length in flower (mm)	3 – 4	5 – 7
Calyx length (mm)	ca. 15	ca. 17
Corolla length (mm)	ca. 20	ca. 25
Corolla width (mm)	ca. 15	ca. 17
Exerted anther from corolla(mm)	ca. 3	ca. 5
Borne stamen from corolla base	2⁄3	4⁄5
Filament length (mm)	ca. 6	ca. 10
Chromosome number	9	9 + 1B

Onosma straussii (Riedl) Khat. Iran. J. Bot. 5 (2): 76. 1992. (Figure 4)
basionym: *O. cyrenaica* E.A.Durand & Barratte subsp. *straussii* Riedl.
= *O. bulbotricha* DC. var. *rubriflora* Bornm., in Beih. Bot. Centrb1. 20/B: 186. 1906.
Type: Iran. Markazi, Arak, Sultanabad in montibus near to Khomein, 15.6.1896, *Strauss* (holotype: JE!, isotype: B!).

Biennial 20 – 42 cm tall. Stem single to several, erect to ascending, branched above, green color in fresh plant, brown or straw-colored when dry, covered by a mixture of antrorse bristle, 2.5 – 4 mm long, arising from white and glabrous tubercles, 0.25 – 0.75 mm wide, and short antrorse hairs between the tubercles. Leaves without venation. Basal and lower stem leaves sessile, linear-oblanceolate, 7 – 14 × 0.7 – 1 cm, acute at the apex, margins strongly revolute, loosely covered adaxially with antrorse white bristles 2 – 4 mm long, tubercle white, nearly flat, composed of 2 or 4 circles of roundly cell elongated, 0.25 – 0.75 mm wide and sparsely short hairs between the tubercles, abaxially covered

with similar bristles mainly along the midrib and only sparsely on other parts, with sparse short hairs as those on stem. Middle and upper stem leaves similar, to basal leaves only a little narrowed toward base, 3 – 5 cm long. Inflorescence consists of 5 – 7 terminal or lateral cymes, then elongated. Lower bracts similar to upper stem leaves, to 3 cm long, decreasing in size upward. Pedicel 3 – 4 mm long in flower, densely covered by patent white and yellow bristles to 3 mm long. Calyx ca. 15 mm long in flower, with dense patent white and yellow bristles to 3 mm in outside, lobes linear to oblong, acuminate, 1 – 2 mm wide. Corolla pinkish to red and sometimes pale yellow in basal part, cylindrical to campanulate, ca. 20 × 15 mm, with sparsely short hairs, lobes ca. 2 × 3 mm wide, acute at apex. Nectaries hairy. Stamens borne ca. 2/3 from corolla base; anthers linear, 7 – 9 mm long, coherent only at the base, sterile tips exserted ca. 3 mm long; filament 5 – 7 mm long. Style ca. 5 mm longer than corolla; stigma very small, distinctly bilobed. Nutlets 6 × 4.5 mm, with gibbous shoulders and with sharp ventral and indistinct dorsal keel, beak strongly incurved, obtuse, pale grey to brown.

3. 10. *Specimens seen*

Iran: Lorestan, Dorud, 10 km after Natural Resources Office, 1602 m, 18.6.2011, *Ranjbar & Almasi 26985* (BASU!); Esfahan, Mobarakeh, Dehnu mountains 1830 m, 18.6.2011; Lorestan, Khoram Abad to Sefid Dasht, Darreh Ashkaft 1300 m, 28.5.2012, *Ranjbar & Almasi 28674* (BASU!); Markazi, Anjadan, 1800 m, 14.6.2012, *Ranjbar & Almasi 28675* (BASU!); Markazi, Arak, Sultanabad in mountains near to Khomein, 1700 m, 15.7.1896, *Strauss 5488* (JE!); Markazi, Arak, Raswend, 1800 m, 1.8.1899, *Strauss 5488* (B!).

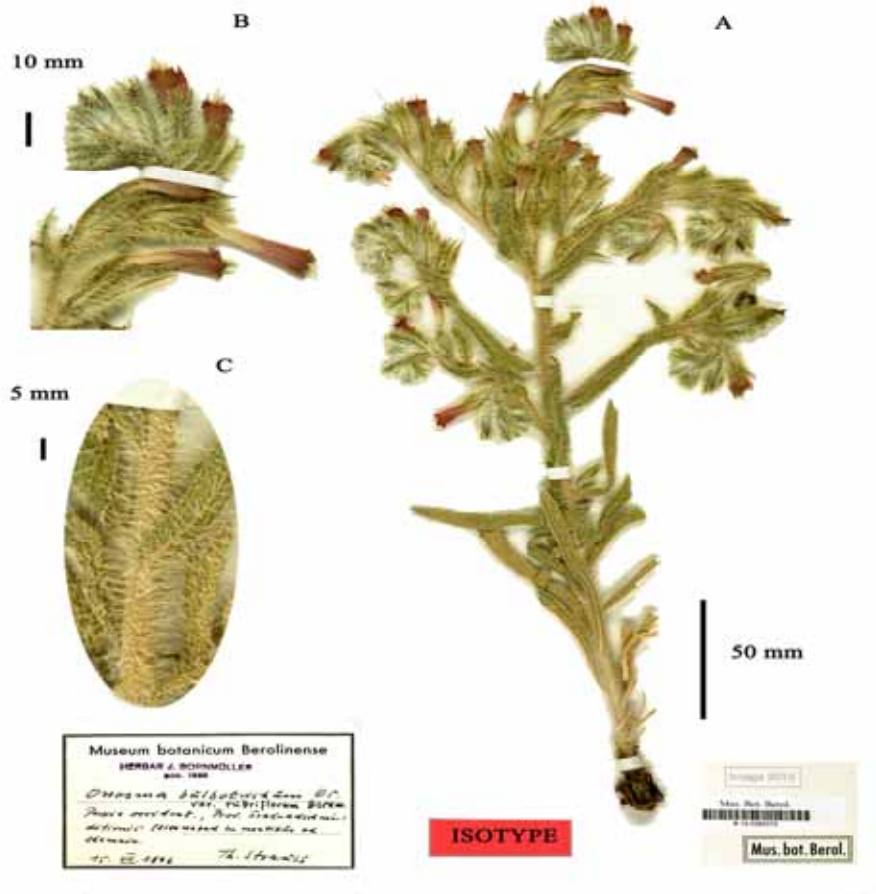

Figure 4. *Onosma straussii* (Riedl) Khat. (Strauss, B). (A) habit, (B) inflorescence, (C) bristle of stem. Isotype photograph is provided by B

3.11. *Morphology*

Results from PCO analysis on the matrix of correlations are presented in Figure 5. It is possible to distinguish 4 main groups when plotted on the first 2 eigenvectors. Group 1 with bul13, bul57, bul164, bul22, bul364, bul34, bul72 and bul62; group 2 with str75, str85, str74 and str77; group 3 with sol76 and group 4 with est95 and est35 is placed separately because of the size and color of corolla, exserted anthers, size and shape of leaves and hairs length (Tables 3, 4). A distribution map showing the locations of different populations of the species is presented in Figure 6.

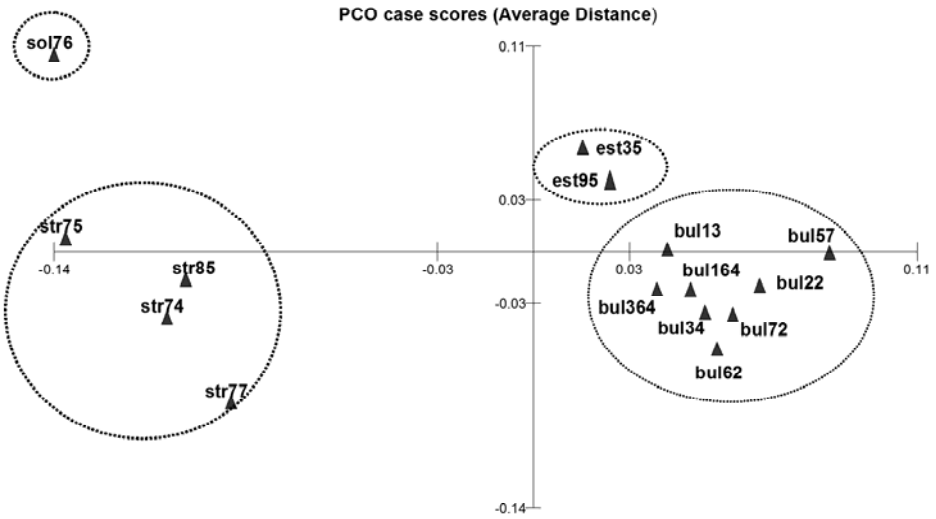

Figure 5. The phenetic grouping obtained from PCO analysis of the morphological characters in *Onosma* series. *Aleppica* based on the average distance coefficient (MVSP 3.2).

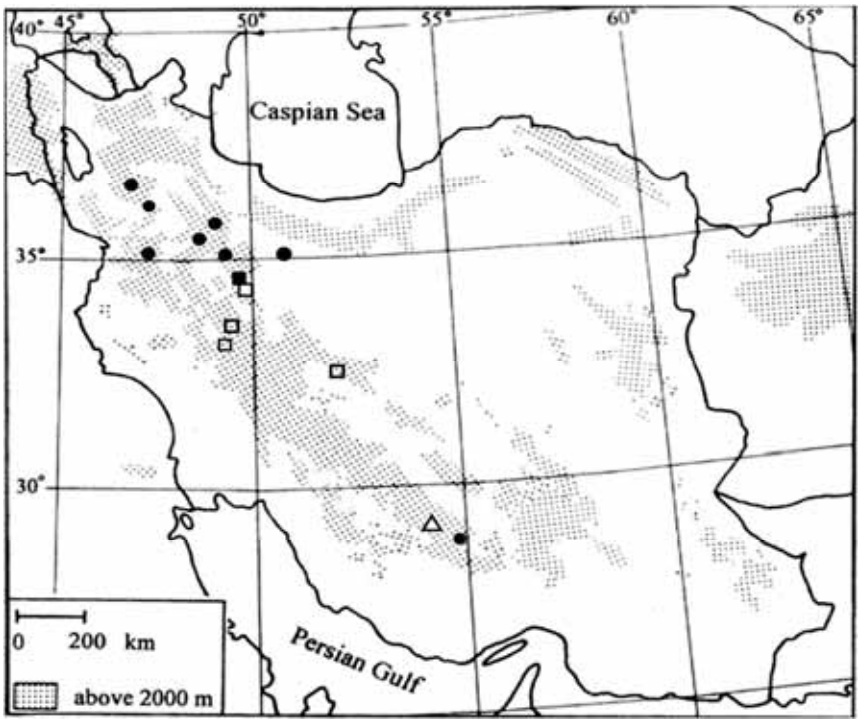

Figure 6. Distribution of *Onosma bulbotricha* (black circle), *O. estahbanensis* (triangular), *O. straussii* (with square) and *O. soltanabadensis* (black square)

3.12. *Cytogenetics*

Results from the cytogenetic study on 6 species showed that they are diploid with 2n = 2x = 18 chromosomes, consistent with the proposed base number x = 9. Almost all the studied material displayed regular bivalent pairing and chromosome segregation at meiosis. However, meiotic irregularities were observed in some taxa, including the ring and rod bivalents in diakinesis, sticky chromosomes and cytomixis in metaphase I, laggard chromosomes and bridges in anaphase I, II and telophase I, II. Results from PCO analysis on the matrix of correlations are presented in Figure 7. It is possible to distinguish 4 main groups when plotted on the first 2 eigenvectors. Group 1 with est95; group 2 with str75 and str74; group 3 with sol76 and group 4 with bul57 and bul72, are placed separately because of almost 30% cytomixy, 50% ring and rod, 7% B-chromosome, and variation in abnormalities with less frequency, respectively (Figure 7).

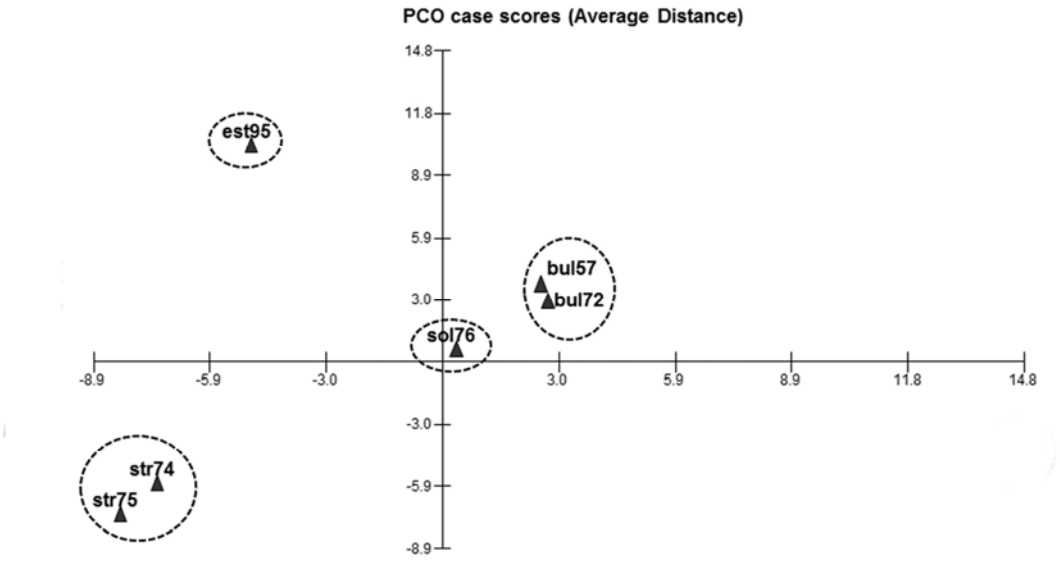

Figure 7. PCO analysis of *Onosma* series *Aleppica* populations based on cytogenetic data

3.13. *Ring and rod bivalents*

During meiotic prophase, replicated homologs identify one another and become progressively more intimately juxtaposed until they are connected along their entire lengths; they then separate, revealing a few remaining connections known as "chiasmata". Chiasmata are mechanically important to ensure bipolar alignment and regular segregation of homologs during the first (reduction) meiotic division. Each chiasma corresponds to the site of a reciprocal exchange, or "crossover", at the DNA level, as shown cytologically by differential staining of sister chromatid chromatin. Differential staining of the structural axes along which chromatin is organized reveals two further aspects of chiasmata: (1) reciprocal exchange also occurs between chromatin axes, which are morphologically continuous across chiasma sites; and (2) in accord with the fact that only one chromatid of each homolog participates in exchange sister chromatid axes are locally separated at each chiasma. The rod configuration describes only one chiasma while the ring configuration, two chiasmata (Blat et al., 2002; Senda et al., 2005). The highest frequencies of ring and rod bivalents were observed in *O. straussii* (Figures 8, 9, 10 Table 5).

3. 14. *B-chromosomes*

B-chromosomes or accessory chromosomes, that occur in addition to the standard or A-chromosomes in some plants, are smaller than other chromosomes and do not form any association with them. B-chromosomes when present in high numbers negatively affect the growth and vigor of the plants, while in low numbers they may be beneficial to the plant (Jones and Houben, 2003; Ranjbar et al., 2010a, b). B-chromosomes were observed only in *O. soltanabadensis* (Figure 9).

3. 15. *Cytomixis*

Cytoplasmic connection is a phenomenon widely described in angiosperms (Levan, 1941; Risueno et al., 1969). The first description was made by Gates (1908), who observed delicate threads of cytoplasm connecting adjacent pollen mother cells in *Oenothera*. Cytomixis consists in the migration of chromosomes between meiocytes through cytoplasmic connections. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered as a mechanism of evolutionary significance. Until now cytomixis in meiocytes has been investigated in numerous species (Ranjbar et al., 2009; 2011b). This phenomenon was observed with high frequency in *O. estahbanensis* (Figures 10, 11, 12, Table 5).

3. 16. *Chromosome stickiness*

Sticky chromosomes were observed in some studied populations with different frequencies (Figure 12, Table 5). They are characterized by their agglomeration in different stages of the cell cycle and can occur due to the presence of mutant genes or abiotic factors as X-rays (Steffensen, 1955; 1956), gamma irradiation (Rao & Rao, 1977; Al Achkar

et al., 1989), temperature, herbicides (Badr and Ibrahim, 1987) and some chemical elements present in soil (Steffensen, 1956; Zanella, 1991; Caetano-Pereira et al., 1995). Another hypothesize is that chromosome stickiness may be caused by a failure or a bad functioning in one or two types of non-histone chromosomal proteins (Peres Kiihl et al., 2011).

3.17. *Laggard chromosomes*

Laggard chromosomes were observed in anaphase/telophase I in some populations studied (Figure 13). Non-oriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers (Nicklas and Ward, 1994). It has been suggested that infertility of polyploids is not solely due to the production of aneuploid gametes formed by improper segregation of chromosomes during anaphase/telophase stages, genetic factors may also bring about pollen sterility as evidenced in different tetraploid species (Hazarika and Rees, 1967; Pagliarini 1990; 2000; Baptista-Giacomelli et al., 2000; Ranjbar et al., 2010a, b; 2011a).

3. 18. *Pollen viability*

The comparative results between meiotic behavior and pollen viability showed the highest (99.4) and lowest (75) percentages of the stained pollens in populations str74 and bul57, respectively (Figure 14, Table 5). This result indicates that irregularities observed at meiosis probably have a direct relation with species fertility.

Table 5. Meiotic behavior and pollen viability in some populations of *Onosma bulbotricha, O. straussii*, *O. soltanabadensis* and *O. estahbanensis*. D/MI = diakinesis/metaphase I, AI/TI = anaphase I/telophase I, MII = metaphase II, AII/TII = anaphase II/telophase II, n = chromosome number

Species	str74	srt75	sol76	bul57	est95	bul72
% D/MI	42	40.9	34	41.4	73.5	34.9
% B-chromosome	0	0	7.1	0	0	0
% Cytomixis	0	0	0	1.7	26.3	0.4
% Chromosome stickiness	1.8	0	1.4	10.21	0	3.6
% Ring and rod bivalent	57	50	16	0	7.6	0
% Desynapsis	0	0	1.7	3.5	0	0
% Precocious migration	2	1.5	1.7	5.9	0	2
% AI/TI	49	39.5	34.8	47.5	25	39.3
% Bridge	0.15	2.6	3	1.6	0	0.3
% Laggard chromosome	0	1.9	0	2.2	0	2.6
% Cytomixis	0.15	0	0	1.9	6.7	0
% Fragmented chromosome	0	0	0	0.32	0	0
% Forward	0	0	0	1.60	0	0.5
% MII	1	77	1.38	2.56	0.36	0.27
% Fragmented chromosomes	14	41	0	0.00	0	0
% AII/TII	8.2	18.9	029.4	7.2	0	24.7
% Pollen viability	99.4	99	95	75	94.3	97.7
N	9	9	9 + 1B	9	9	9

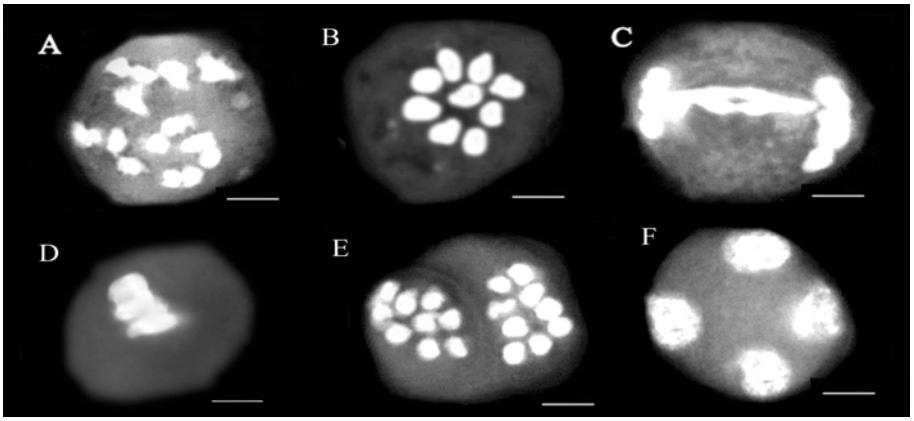

Figure 8. Representative meiotic cells in *Onosma strausii* 75 with n = 9. (A) ring and rod in diakinesis, (B) diakinesis, (C) bridge in telophase I, (D) metaphase I, (E) telophase I, (F) telephase II. Scale bar: 5 µm.

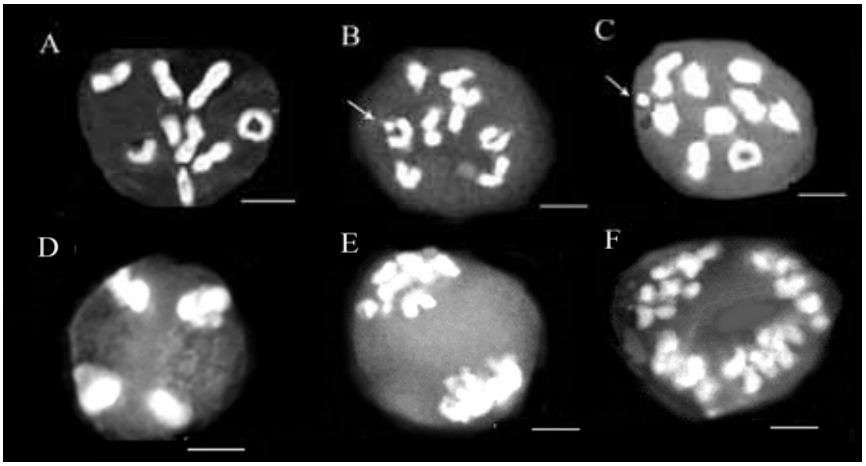

Figure 9. Representative meiotic cells in *Onosma soltanabadensis* with n = 9 + 1B. (A) Ring and rod in diakinesis, (B) B chromosome in diakinesis, (C) B chromosome in diakinesis, (G) telophase II, (H) telophase I, (F) telophase II. Scale bar: 5 μm.

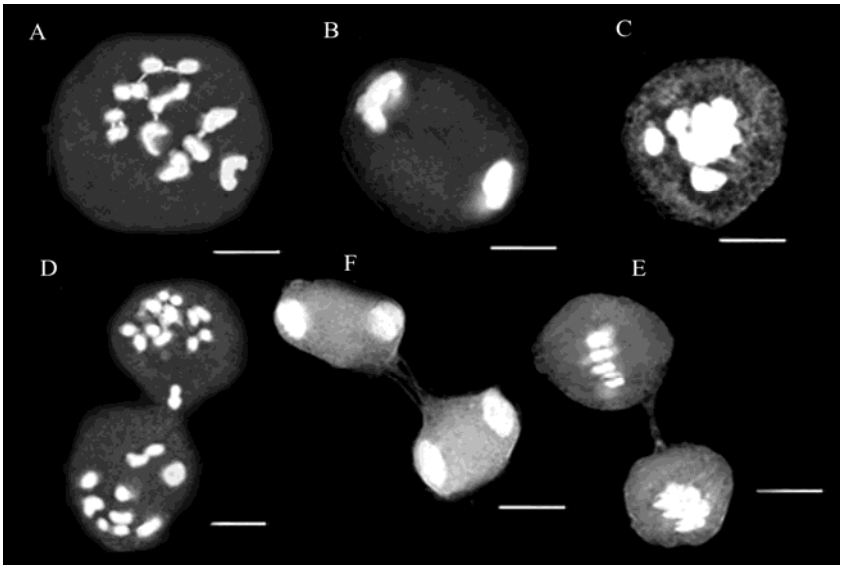

Figure 10. Representative meiotic cells in *Onosma estahbanensis* with n = 9. (A) diakinesis, (B) telophase I, (C) precocious migration in metaphase I, (D) cytomixis in diakinesis, (E) cytomixis in telophase I, (F), cytomixis in metaphase I. Scale bar: 5 μm.

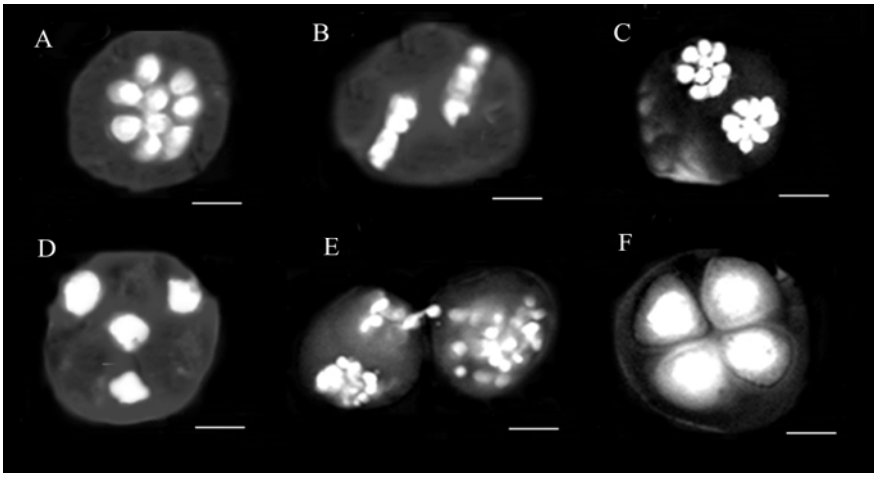

Figure 11. Representative meiotic cells in *Onosma strausii* 74 with n = 9. (A) diakinesis, (B) metaphase II, (C) telophase I, (D) telophase II, (E) cytomixis in telophase II, (F) tetrad. Scale bar: 5 μm.

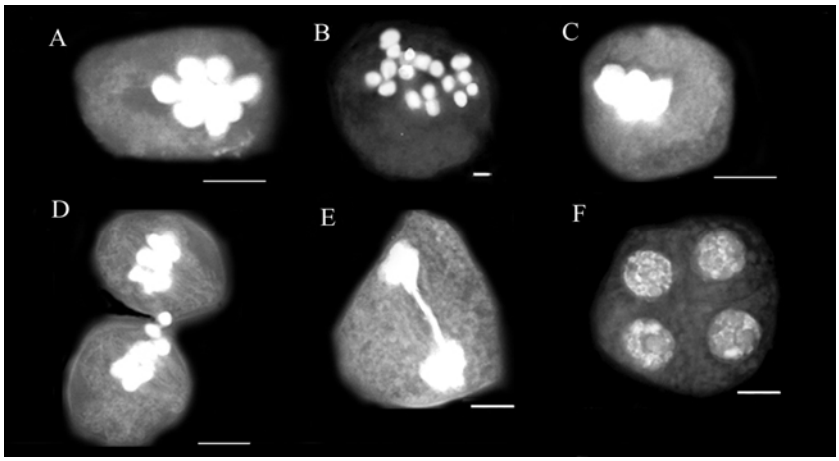

Figure 12. Representative meiotic cells in *Onosma bulbotricha* 57 with n = 9. (A) diakinesis, (B) early telophase I, (C) sticky chromosome in metaphase I, (D) cytomixis in metaphase I, (E) bridge in telophase I, (F) telophase II. Scale bar: 5 μm for D, E and F. Scale bar: 2 μm for B. Scale bar: 10 μm for A and C

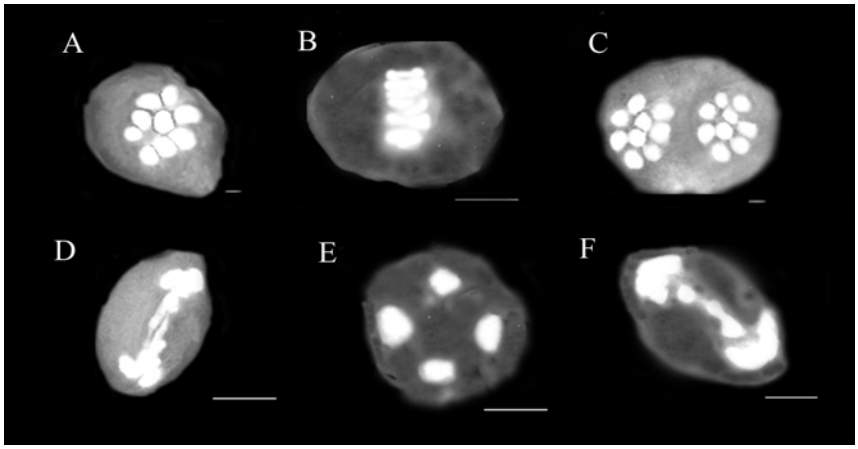

Figure 13. Representative meiotic cells in *Onosma bulbotricha* 72with n = 9. (A) diakinesis, (B) metaphase I, (C) telophase I, (D) bridge in telophase I, (E) telophase II, (F) laggard in telophase I. Scale bar: 5 μm for B, D, E and F. Scale bar: 2 μm for A and C

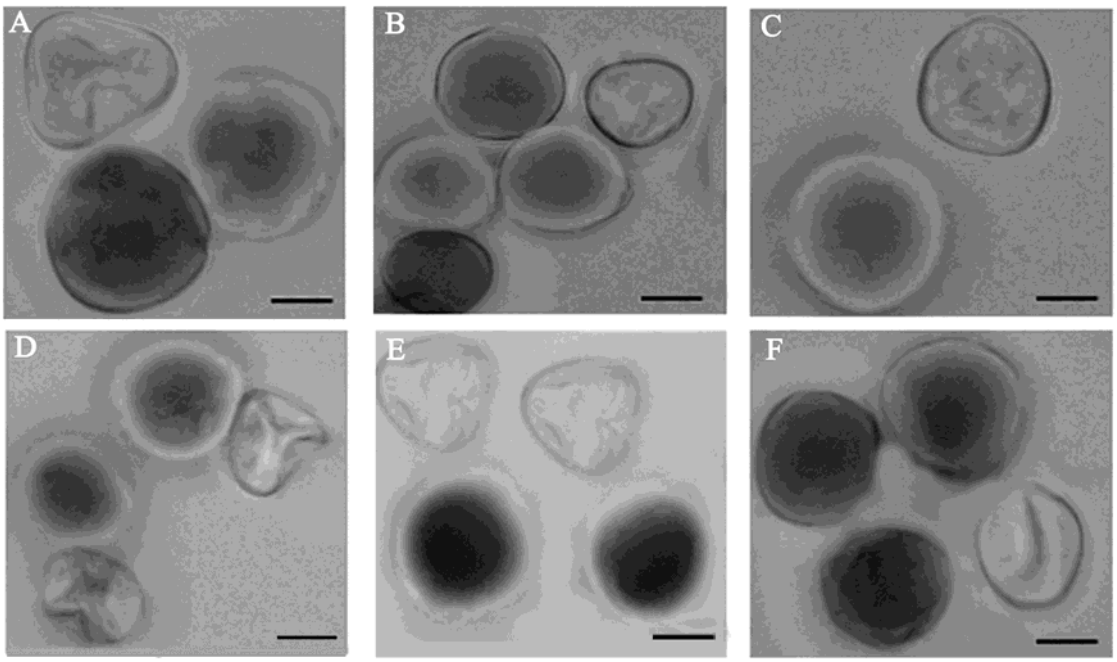

Figure 14. Pollen viability in different populations of *Onosma* series *Aleppica*, (A) *O. bulbotricha* 72, (B) *O. straussii* 74, (C) *O. estahbanensis*, (D) *O. bulbotricha* 57, (E) *O. straussii* 75, (F) *O. soltanabadensis*. Scale bar: 5 μm

4. Conclusions

Onosma estahbanensis, and *O. soltanabadensis* are new endemic species of *O.* sect. *Haplotricha*. Many examples of speciation are found in nature. In some cases this is caused by a physical barrier that forces a population to diverge into two different species (allopatric speciation) the same as *O. estahbanensis*. However, speciation without physical isolation can also occur, in this process new species arises from coexisting populations (sympatric speciation). Careful genetic modeling of sympatric speciation has suggested it is possible for populations to diverge even when they remain in physical contact. In these models populations can diverge even with low to moderate degrees of gene flow, if there is strong disruptive selection (Dieckmann and Doebeli, 1999; Luijtelaar et al., 2004; Rueffler, 2012). *O. soltanabadensis* is easily distinguished from sympatric *O. straussii* as both have corolla pinkish to red and their style ca. 5 mm longer than corolla (see Table 4). x = 6 and x = 8 are represented for *O*. sect. *Haplotricha* as dominant chromosome count in Europe and Asia respectively. The chromosome number x = 9 is less frequent and reported in *O. visianii* Clem. and *O. graeca* Boiss. (Teppner, 1971; 1974; 1991; Ghaffari, 1996; Mártonfi et al., 2008, Kolarčik et al., 2010). The observations of the present study as well as the available data on chromosome number in *O*. series *Aleppica* indicate that, x = 9 can be considered as basic choromosome number in the series.

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