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STIGMA MORPHOLOGY IN *ONOSMA* SPP. (BORAGINACEAE) WITH EMPHASIS ON ITS SYSTEMATICS IMPLICATION IN IRAN

AHMAD-REZA MEHRABIAN*, MAHNAZ ARABAMERI AND SAMIRA SADEGHI

Department of Plant Science and Biotechnology, Life Sciences and Biotechnology, Shahid Beheshti University GC, P. O. Box 1983963113, Tehran, Iran

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Abstract

The stigma morphology of some Iranian *Onosma* taxa were studied using SEM techniques, and were subsequently defined, illustrated and used for cluster analyses. Three types of stigmata structures were used in separating the taxa. Regardless that there are some taxa that are not distinguishable only by mentioned evidence, however, stigmatic features as subsidiary characters seem to be useful for delimitation of several taxa as well as solving some taxonomic challenges.

Introduction

Onosma L. is the largest genus in Boraginaceae, consisting of about 150 species (Kolarčik *et al*. 2010) mainly distributed in Asia and the Mediterranean region. These taxa are accosted to several taxonomic challenges (Teppner 1996). These taxa showing high similarities led to many errors in their identification (Akcin and Binzet 2011). According to the mentioned complexities, there was a necessity to analyze important morphological evidences especially reproductive features for solving related taxonomic problems. Heslop-Harrrison (1981) assessed the stigma characteristics in angiosperms and emphasized on their roles as valuable evidence to evaluate phylogenetic relationships. Moreover, typification of this diversity had been suggested by various authors for flowering plants especially in Boraginaceae (For example Heslop-Harrrison and Shivanna 1977).

For the first time taxonomic investigation on stigma was carried out by Raspail (1824) on Gramaine. In addition, an extensive study on stigma morphology over 250 angiosperm families was reported by Heslop-Harrison and Shivanna (1977). Further, stigma was applied by many authors in taxonomy of Boraginaceae (Bigazi and Selvi 2000) and showed high diversification in morphology and provided valuable evidence for several taxonomic complexities as well as clarifying the phylogenetic relationships within the tribe. Moreover, some authors (Riedl 1967, Davis 1978, Khatamsaz 2002) accentuated on the stigma features in *Onosma* for taxonomy among them.

Nevertheless, little attention has been paid to stigma micro-morphology of *Onosma* in scale of Iran. Therefore, the recent study was carried out using Scanning Electron Microscopy (SEM) to analyze the valuable evidences. Besides, these features are discussed with regard to their use as taxonomic evidence for simplifying some taxonomic complexities.

Materials and Methods

The materials were taken from wild populations as well as Herbarium samples in HSBU, W and TARI. Voucher data can be found in Table 1. For SEM studies, stigmas were mounted on stabs using double-sided adhesive tape and were coated with 12.5 - 15 nm of gold. The coated

*Author for correspondence: <a_mehrabian@sbu.ac.ir>.

samples were examined and photographed with Cam Scan- MV 2300 Electron Microscope. All papilla measurements were taken from at least 10-15 papillae from 3-5 stigmas. Qualitative data were assessed based on Harris and Harris (2001). Quantitative characters were coded as multistate characters used for further morphological analyses. The mentioned data were used for cluster analyses, including the UPGMA (Unweight Paired Group using Average method) SPSS version 16 software was used for all statistical analyses.

Table 1. The examined species of *Onosma.*

Taxa	Locality	Collector	Voucher number
O. rostellata Lehm.	Kurdistan, Bayangan, 1450 m	Mehrabian	HSBU-2010244
O. orientale Lehm.	Khuzistan, Masjed Soleyman, 600 m,	Mozaffarian	TARI-63017
O. asperrima Bornm.	Fars, Nurabad: Doshman-Ziari Region, AbZalu Village, Kuhe Tasak,2200 m	-	TARI-45772
O. bodeana Bornm.	Tehran, Sohanak, 2200m	Mehrabian	HSBU-2010256
O. bulbotrica De Candoll.	Zanjan To Mahneshan, 2200 m	"	HSBU-2010238
O. cornuta H. Riedl.	Kurdistan, Bijar to Takab, 1600 m	"	HSBU-2013876
O. dichroanta Boiss.	Golestan National Park1500 m	Heidari, Ghorbani & Habibi	HSBU- 2007300
O. gauba Bornm.	Tehran, Damavand, 2500 m	Mozaffarian	TARI-37319
O. kilouyense Boiss.	Khuzestan, Dehdez, KuhSefid, 2700 m	"	TARI-74528
O. kotschyi Boiss.	Fars, South of Estahbanat, 2200 m	"	TARI-46999
O. longiloba Bge.	Semnan, 20km Mohamadabad	Pahlevani	HSBU-2012-100
O. microcarpa De Candoll.	Markazi, Arak, Gavar, 2000 m	Mehrabian	HSBU-2010244
O. pachypoda Boiss.	Azarbaijan, Yam,MishoDagh Mt.	"	HSBU-2010602
O. sabalanica Ponert	Aerdabil, Sabalan Mt. 2900 m	"	HSBU-2010249
O. sericea Willd.	Kurdistan, Sanandaj, Abidar, 1730 m	"	HSBU-2010273
O. stenosiphon Boiss.	Kerman, Chopar Mt.	Kanani	HSBU-2010237
O. azarbaidjanensis Mehrabian & Noormohamadi	Azarbayjan-khalkhal-hashtchin		HSBU-20121
O. wheeler-hainesi H. Riedl	Kermanshah-gahvare-gozaran		HSBU-2012546
O. sheidai Mehrabian	Kermanshah-15 km paveh		HSBU-20123
O. chlorotricha Boiss**.**			
O. elwendica wettst.	Tehran,Lashkarak, 1900 m	Mehrabian	HSBU-2010247
O . lanceolata Boiss. & Hausskn.			
O. macrophyla Bornm.	Kermanshah, Malavi to Eslam Abad	Mozaffarian	TARI-64384
O. olivieri Boiss.	Kermanshah, 1500m		IRAN-2901
O. straussii H.Riedl.	Markazi, Arak, Gavar, 2178m	Mehrabian	HSBU-2010-232
O. armena De Candoll.	Azarbaijan, Maku to Khoy, 2400m	Assadi & Mozaffarian	TARI-30353
O. bisotunensis Attar & Hamzeh'ee.			HSBU-2012343
O. caeulescens Boiss.			
O. iranshahri Ghahreman & Atar.	Kurdistan, Marivan to Paveh, Gardan-e Tat,	Mozaffaraian	TARI-75701
O. rascheyana Boiss.	Zanjan, Mahneshan, Angoran Protected Area, Belgheismt. 2700 m	Mehrabian	HSBU-2010281
O. mozaffariani Mehrabian.	Kermanshah-gahvare-babashahahmad		

Results and Discussion

Based on the evidences, three types of stigma were identified in the *Onosma* taxa.

Type I: Globose stigma, sculpturing verrucate/tuberculate ornate at the top and rugose-striate at the base. *O. longiloba, O. stenosiphon,* and *O. orientale* appeared type (Fig. 1).

Type II: Parted lobes, immersed, shoal gapped to absence. Sculpturing showing verrucate/tuberculate at the top and rugose-striate at the base. *O. dichrantha, O. cornuta, O. rascheyana*are symbols of this type (Fig. 1).

Type III: Connected lobes, deeply gapped. Sculpturing showing verrucate/tuberculate at the top and rugose-striate at the base. This type covers a dominant portion of the studied species including *O. pachypoda, O. asperrima, O. gaubae* and *O. sericea* (Fig. 1).

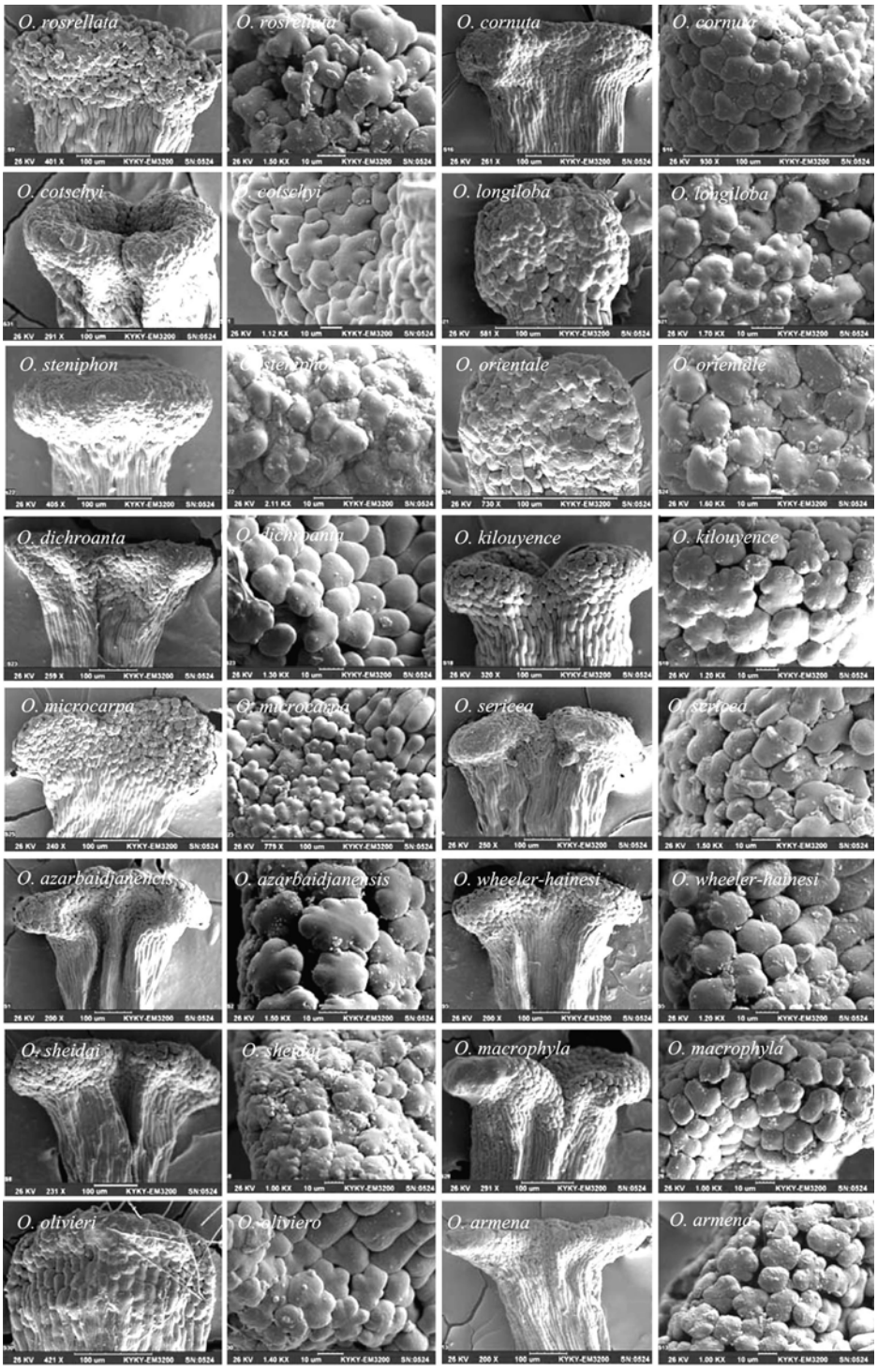
The average values of the mentioned characters represent significant variation between the studied taxa. The data are shown in Table 2. Stigma shape and length, cape lobe numbers, width of style ornamentation, and cape lobe shape as the most variable characters respectively. Based on stigmatic evidence, taxa divided into two major clusters using the UPGMA clustering method (Fig. 2). The first sub cluster consists of some taxa of sect. *Onosma, O. orientale* (sect. *Podonosma*) and *O. rostellata* (Sect. *Protonosma*), and the second sub cluster covers other species of sect. *Onosma* only.

Analyses revealed that stigma characters could not be used in determining and delimiting natural groups in *Onosma*, but showing their efficiency for delimitation within a wide range of taxa. Moreover, *O. rostellata* (sect. *Protonosma*) and *O. orientale* (sect. *Podonosma*) as a different taxa than sect. *Onosma* are grouped together in a joint sub-cluster. This arrangement basis on stigmatic features is not accordant to provided classifications in Flora Iranica (Riedl 1967), Flora Turkey (Davis 1978) and Flora of USSR (Shishkin 1953). Besides, the results of cluster analyses on morphology and ISSR evidence (Mehrabian *et al*. 2011) palynology (Mehrabian *et al*. 2012), petal and corolla micro-morphology (Arab Ameri *et al*. 2015), trichome micro-morphology (Mehrabian *et al*. 2013) did not confirm the new grouping based on stigma structures, whereas new studies on stigmatic morphology of Boraginaceae (Bigazi and Selvi 2000) confirms our logic. Moreover, these structures provide morpho-types that are peculiar to some genera and have proven efficient for differentiation in generic levels and phylogenetic relationships in Boraginaceae (Bigazi and Selvi 2000). Bigazi and Selvi (2000) noted that stigma diversity is comparable with pollen diversity, and provided a new classification in Boragniaceae based on ultra-structural features of stigma. Our study proposed three morpho-types in the studied taxa that type I covers sect. *Podonosma* and sect. *Protonosma* and some species of Sect. *Onosma* (sub sect. *Haplotricha* and *Asterotricha*). Type II and Type III include three sub sections (*Haplotricha*, *Heterotricha* and *Asterotricha)* belonging to Sect. *Onosma.* Therefore, the recent character cannot be used for delimitation of sections and sub sections in *Onosma*.

Cuticles ornamentation showed overall homogeneity (smooth surface) in the studied taxa with the exception of *O. chlorotricha* having granulate ornamentations; therefore, we suggest that it may not be valuable as a differential character in *Onosma.* Bigazzi and Selvi (2000) suggested that this character is valuable to distinguish the genera with taxonomic affinity.

Papilla aggregation include pressed to separate, so that our results indicate that the recent character is not effective in the infrageneric level. Bigazzi and Selvi (2000) pointed out that recent evidence clearly use in phylogenetic correlations in generic level in Boraginaceae.

The shape of cap lobes is amorphous in sect. *Podonosma* and Sect. *Protonosma*, but is globular in sect. *Onosma* with the exception of *O. gaubae* showing an oval structure. The aforementioned micro-morphological structures accompanied with other differential evidence confirms presence of the natural groups (sections) in *Onosma* introduced by Shishkin (1953),


O. rosrellata
O. rosrellata
O. cornuta
O. cornuta
O. cotschyi
O. cotschyi
O. longiloba
O. longiloba
O. steniphon
O. steniphon
O. orientale
O. orientale
O. dichroanta
O. dichroanta
O. kilouyence
O. kilouyence
O. microcarpa
O. microcarpa
O. sericea
O. sericea
O. azarbaidjanensis
O. azarbaidjanensis
O. wheeler-hainesi
O. wheeler-hainesi
O. sheidai
O. sheidai
O. macrophyla
O. macrophyla
O. olivieri
O. olivieri
O. armena
O. armena

(Contd.)

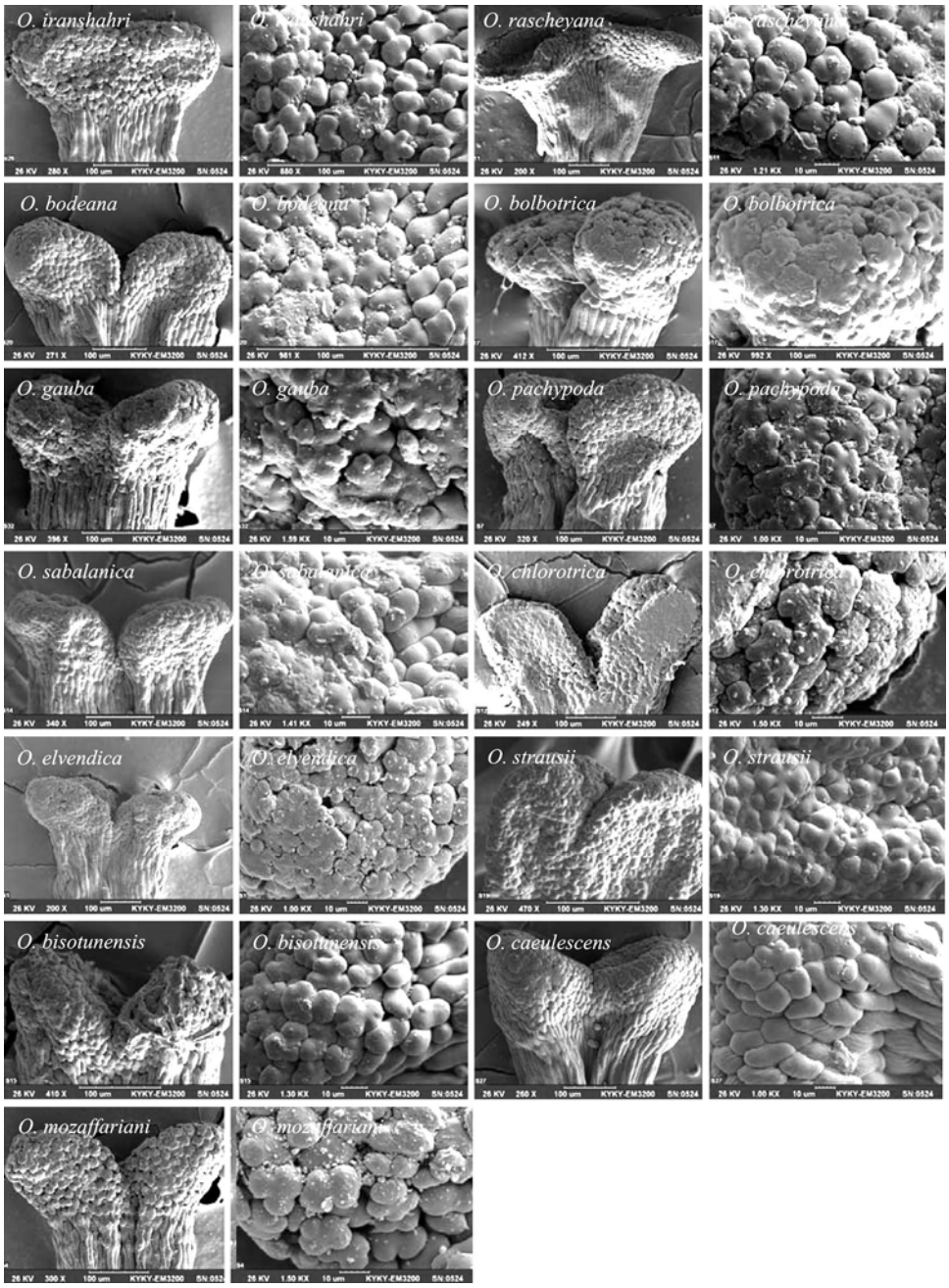


Fig 1. The SEM pictute of stigma. Taken by Arabameri & Sadeghi.

Riedl (1967), Dvais (1978), Teppner (1972), Kolarčik *et al*. (2010), Mehrabian (2011, 2012, 2013) and Arab-Ameri *et al*. (2013).

Table 2. Stigma characters of studied taxa.

Taxa	AP	CLS	SS	GDS	COS	CLN	ALP	AWP	WSO	ST	SW	SL
O. rostellata Lehm.	Pr	Am	Tw	No	Sm	4.5	22.8	15.6	9.6	I	279	130
O. orientale Lehm.	Pr	Am	Gl	Sh	Sm	4	17.8	13.1	7.7	II	134	81
O. asperrima Bornm.	Pr	Gl	Tw	Sh	Sm	6.5	28.2	17.3	8.3	II	446	242
O. bodeana Bornm.	Se	Gl	Tw	De	Sm	5.5	23.3	18.2	11.3	III	425	122
O. bulbotrica De Candoll.	Pr	Gl	Tw	De	Sm	8	23.5	19.3	10.6	III	260	100
O. cornuta H. Riedl.	Pr	Gl	Gl	No	Sm	5	19.8	16.3	10.2	I	412	101
O. dichroanta Boiss.	Se	Gl	Tw	Sh	Sm	4	19.4	16.2	8.2	II	437	128
O. gauba Bornm.	Pr	Ov	Tw	De	Sm	6	24.1	13.9	10.4	III	364	99
O. kilouyense Boiss.	Se	Gl	Tw	Sh	Sm	6.5	22.7	17.6	11.2	II	359	65
O. kotschyi Boiss.	Pr	Gl	Gl	No	Sm	6	21.15	14	12.8	I	258	50
O. longiloba Bge.	Pr	Gl	Gl	No	Sm	6.5	18.5	15.5	7.5	I	143	130
O. microcarpa De Candoll.	Se	Gl	Tw	Sh	Sm	6	24.7	20	11.2	II	448	152
O. pachypoda Boiss.	Pr	Gl	Tw	De	Sm	5	21.7	17.4	12.9	III	343	126
O. sabalanica Ponert	Pr	Gl	Tw	De	Sm	6	17.2	14.2	9.05	III	336	102
O. sericea willd.	Pr	Gl	Tw	Sh	Sm	6	17.4	13.5	10.8	II	418	75
O. stenosiphon Boiss.	Pr	Gl	Gl	No	Sm	6	15.9	13.4	6.4	I	271	89
O. azarbaidjanensis Mehrabian & Noormohamadi	Se	Gl	Tw	Sh	Sm	5	22.8	16.8	9.2	II	522	113
O. wheeler-hainesi H. Riedl.	Se	Gl	Tw	Sh	Sm	4.5	20.7	14	9	II	500	92
O. Sheidai Mehrabian.	Se	Gl	Tw	Sh	Sm	5.5	24.3	19.6	12.7	II	480	85
O. chlorotricha Boiss.	Se	Gl	Tw	De	Gr	4	17.1	13.3	13.3	III	457	226
O. elwendica wettst.	Pr	Gl	Tw	De	Sm	6	23	18.2	11.3	III	430	110
O. lanceolata Boiss. & Hausskn.	Pr	Gl	Tw	De	Sm	6.5	20.9	16.3	8.3	III	270	104
O. macrophyla Bornm.	Se	Gl	Tw	Sh	Sm	4	18	14.9	8.2	II	397	90
O. olivieri Boiss.	Pr	Gl	Tw	Sh	Sm	6	26.2	19.3	13.7	II	460	142
O. straussii H. Riedl.	Pr	Gl	Tw	De	Sm	7	17.8	15.3	-	III	232	150
O. armena De Candoll.	Se	Gl	Tw	Sh	Sm	4.5	20.2	15	9.2	II	550	112
O. bisotunensis Attar & Hamzeh'ee.	Pr	Gl	Tw	De	Sm	6	17.21	13.5	7.4	III	274	152
O. caeulescens Boiss.	Pr	Gl	Tw	De	Sm	7	21.9	17.3	13	III	409	175
O. iranshahri Attar & Ghahreman.	Se	Gl	Tw	Sh	Sm	6	20.7	17.8	10.1	II	380	150
O. rascheyana Boiss.	Se	Gl	Tw	Sh	Sm	4	18.4	14.9	11.5	II	573	80
O. mozaffariani Mehrabian.	Se	Gl	Tw	De	Sm	3	19	14.4	8.5	III	373	160

AP: Aggregation in papilla, CLS: The cap lobe shape, SS: The stigma shape, GDS: Gap depth in tip of stigma, COS: Cuticle ornamentation in stigma surface, CLN: The cap lobe numbers, ALP: The average length of papilla cap, AWP: The average width of papilla cap µm**,** WSO**:** Width of style ornamentation µm, ST: Stigma type, SW: Stigma width µm, SL: Stigma length µm, Pr: Pressed, Se: Separate, Am: Amorphous, Gl: Globular Ov: Oval, Tw: Two lobar, No: No gap, Sh: Shallow, De: Deep , Sm: Smooth, Gr: Granula.

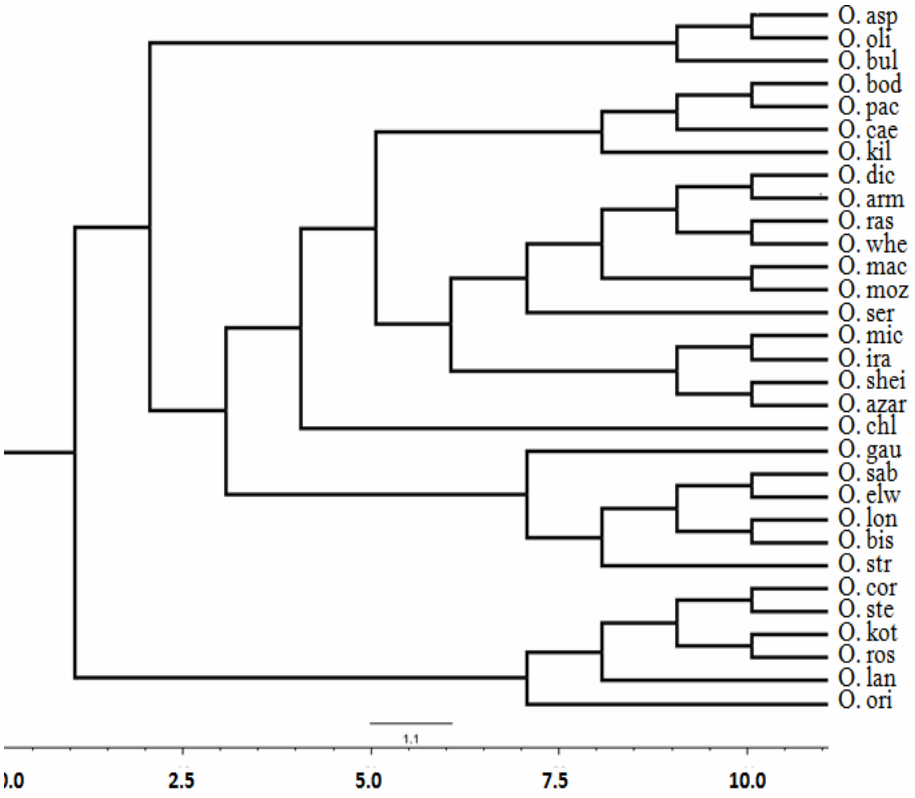


Fig 2. Neibour joning dendrogram of studied taxa based on stigma stigma evidences.

O. asp: O. asperrima, *O. oli: O. olivieri*, *O. bul: O. bulbotrica*, *O. bod: O. bodeana*, *O. pac: O. pachypoda*, *O. cae: O.caeulescens*, *O. kil: O. kilouyense*, *O. dic: O. dichroanta*, *O. arm: O. armena*, *O. ras: O.rascheyana*, *O. whe: O.wheeler-hainesi*, *O. mac: O. macrophyla*, *O. moz: O.mozaffariani*, *O. ser: O. sericea*, *O. mic: O. microcarpa*, *O. ira: O.iranshahri*, *O. shei: O. Sheidai*, *O. azar: O.azarbaidjanensis*, *O. chl: O. chlorotricha* , *O. gau: O. gauba*, *O. sab: O. sabalanica*, *O. elw: O. elwendica*, *O. lon: O. longiloba*, *O. bis: O. bisotunensis*, *O. str: O. straussii*, *O. cor: O. cornuta*, *O. ste: O. stenosiphon*, *O. kot: O. kotschyi*, *O. ros: O. rostellata*, *O. lan: O.lanceolata*, *O. ori : O. orientale* .

Based on our results, we propose length and width of papilla cap, since length and width of stigma is valuable in delimitation of some closest taxa only. Moreover, there is a strong correlation between pollen size and stigma-papilla size, so that certain types of cross-pollination are impossible. These specializations can thus play a part as isolating mechanisms (Heslop-Harrison 1981). Due to the mentioned fact, divergence of some species with diversity in papilla size is justifiable and would seem logical.

This research as a small part of a broad study on systematics of Iranian *Onosma* plays an important role in solving the complexities of *Onosma* and analyzes the most distinctive evidence. Regardless that some taxa are not distinguishable only by the stigma characteristics, they apparently play a complementary role in achieving detailed revision and perfect view on phylogenetic relationships of taxa. Besides, this study indicates that the stigma characteristics, as the basic structure in the reproduction system of Boraginaceae appears to be very effective in explaining the phylogenetic relationships in *Onosma*.

Refrences

Akcin E and Binzet R 2011. Micromorphological studies on nutlets of some *Onosma* L. (Boraginaceae) species from Turkey. Pak. J. Bot. **43**(2): 743-752.

Arab-Ameri M, Mehrabian AR and Sheidai M 2015. Nutlet and flower morphological studies on *Onosma* L. (Boraginaceae) in Iran. Iran. J. Bot. **20**(2): 211-227.

Bigazzi M and Selvi F 2000. Stigma form and surface in the tribe Boragineae (Boraginaceae): micro-morphological diversity, relationships with pollen and systematic relevance. Can. J. Bot.**78**: 388-408

Davis PH 1978. Flora of Turkey. Vol. 6. Edinburgh Univ. Press, Edinburgh.

Harris JG, Harris MW 1994 Plant identification terminology: an illustrated glossary. Spring Lake, Utah: Spring Lake Publishing.

Heslop-HarrisonY 1981. Stigma characteristics and angiosperm taxonomy. Nord. J. Bot. **1** (3): 401-420.

Heslop-Harrison Y and Shivanna KR 1977. The receptive surface of the Angiosperm stigma. Ann. Bot. **41**(6): 1233-1258.

Khatamsaz M 2002. *Boraginaceae* L. *In: Fl.Iran*, Assadi *et al.* (eds.), 39, pp. 114-168. Research Institute of Forests and Rangelands, Iran (In Persian).

Kolarčik V, Zozomova-Lihova J and Martonfi P 2010. Systematics and evolutionary history of the *Asterotricha* group of the genus *Onosma* (*Boraginaceae*) in central and southern Europe inferred from AFLP and nrDNA ITS data. Plant. Syst. Evol. **290** (1-4): 21-45.

Mehrabian AR Sheidai M and Mozaffarian V 2013. Micromorphology of leaf trichomes in *Onosma* (*Boraginaceae*) and their systematic relevance in Iran. Int. J. Balk. Flo. Veg. **20**(1): 33-48.

Mehrabian AR, Sheidai M, Noormohammadi Z, Mozaffarian V and Asri Y 2012. Interpopulations diversity in *Onosma microcarpa* (Boraginaceae): Morphological and molecular (ISSR) approach. Sci. Med. **3**: 187-198.

Mehrabian AR, Sheidai M, Noormohamadi Z, MozafarianV and Asri Y 2011. Palynological diversity in the genus *Onosma* L. (Boraginaceae) Palynological diversity in the genus *Onosma* L. (*Boraginaceae*) of Iran. Ann. Bio. Res. **3**(8): 3885-3893.

Raspail FV 1824. Essaid'une classification gtnerale des gramin CesfondtesurI'etudephysiologique des characttres de cettefamille. Ann. J. Sci. Nat. **4**: 423451; **5**: 1830.

Riedl H 1967. *Boraginaceae* L. *In: Flora Iranica*, Rechinger, KH (ed.), AkadDruck-u, pp. 169-212, Verlagsanstalt, Austria.

Shishkin B 1953. *Boraginaceae* L. *In: Flora Of USSR,* Komarov, VL (ed.), Keter Publishing House Jerusalem Ltd., Israel.

Teppner H 1972. Cytosystematische Studien an *Onosma* (Boraginaceae). Ber. Deut. Bot. Ges. **84**: 691-696.

Teppner H 1996. Blüten und Blütenbesucherbei *Onosma* (Boraginaceae– Lithospermeae).– *Fed Rep* **106**: 525–532.

Yousaf Z, Shinwari ZK, Qureshi RA and Perveen A 2008. Leaf epidermal anatomy of selected *Allium* species, family Alliaceae from Pakistan. Pak. J. Bot. **40**(1): 77-90.

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