Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (8):3885-3893
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

Palynological diversity in the genus *Onosma* L. (*Boraginaceae*) of Iran

Ahmad-Reza Mehrabian[1*], Masoud Sheidai[1], Zahra Noormohammadi[2], Valeyollah Mozafarian[3] and Younes Asrei[3]

[1]Shahid Beheshti University, GC., Faculty of Biological Sciences, Tehran, Iran
[2]Biology Department, School of Basic Sciences, Science and Research Branch, Islamic Azad University (SRBIAU), Poonak, Tehran, Iran.
[3]Research Institute of Forestd and Rangelands, Tehran, Iran

---

ABSTRACT

Pollen characteristics were studied in 24 species of Onosma L. (Boraginaceae) in Iran by scanning electron microscopy (SEM), showing the occurrence of the Tricolporate, Stenopalynous, (syncolporate), Tricolporate, prolate subprolate, ellipsoidal, isopolar or heteropolar pollen grains in these species. The pollen surface was granular except in O. rostellatum having micro granulating pollen surface. In mentioned study 7 characters including : Pollen size in Polar and equtourial view, relative size of Polar/equtourial size, Pollen shape in polar and equtourial view, Wall thickness and granulating size were assessed. Our result show pollen characters are useful for species differentiation of Onosma and relatively differentiation in Sections.

Key Words: *Onosma*, Pollen, Taxonomy, SEM, Iran

INTRODUCTION

Onosma is a genus with about 150 species occurring in dry, cliffy and sunny habitats, distributed mainly in Eurasia and Mediterranean regions, having its center of distribution and maximum concentration of species in Iran [2,25].

The genus *Onosma* contained biennale or perennial, hispid herbs, with flowers in terminal cymes, calyx accrescent, stamens inserted at the middle of the corolla and generally 4 nutlets flat at the base [4,15]. It contains about 60 species in Flora Iranica [22] However, Khatamsaz (2002)[13] described 37 species for Flora of Iran.

The data on pollen morphology of the genus *Onosma* is relatively poor [15] and only few works are available from Pakistan and Turkey [4,5,6].

According to many workers, for example, Clarke (1977)[7], Diez (1986)[8] and Diez and Valdes (1991)[9], the *Boraginaceae* is a eurypalynous family and a large number of its species can be recognized by their pollen characters.

Studying the species relationship by palynological data has been performed in Boraginaceae [19,4,5].

Moreover Johnston (1954)[12] studied light microscopy of the pollens in different genera of Lithospermae, including 45 species of *Onosma*, Qureshi and Qaiser (1987)[20] studied pollen characteristics of 9 *Onosma* species while, Perveen et al. (1995)[19] studied pollen morphology of 49 species from 20 genera in Boraginaceae, including 5 *Onosma* species. Binzet (2011)[4] also reported pollen characteristics of some *Onosma* species in Turkey. Similar studies are totally lacking from Iran. Therefore, the present study considers numerical analysis of pollen characteristics in 24 *Onosma* species growing in Iran and tries to evaluate the usefulness of the palynological data in the taxonomy of the genus and also use such data to illustrate the species affinity.

MATERIALS AND METHODS

The 24 samples studied were obtained from specimens in the herbaria HSBU, IRAN and W (acronymus follows [11]).

Pollen slides for light microscopy (LM) were prepared after acetolysis in the conventional way [10].Thirty measurements of pollen grains were made for each specimen. Pollen samples were removed from mature anthers and used for scanning electron microscopy (SEM), then mounted on aluminum stubs and coated with gold in an Emitech EMK 550 sputter. Observations and measurements were made with a Zeiss Axiophot light microscope and a Philips XL 20 SEM at 20kV.

LM observations were made on pollen grains prepared according to the Erdtman (1952)[9] acetolysed methods and photographed with Olympus CH2. The Descriptive terminology of Wodehouse (1956)[26] is followed in this study. Image tool ver.3 software was used for pollen measurements and data obtained were coded. For multivariate analyses the mean of quantitative characters were used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean=0, variance=1) were used for multivariate statistical analyses [20]. The average taxonomic distance and squared Euclidean distance were used as dissimilarity coefficient in cluster analysis of data [20].

Analysis of variance (ANOVA) was performed for quantitative characters used to get species groupings [20]. NTSYS ver. 2.1 [23]. UPGMA clustering methods were performed for grouping of the species by using NTSYS ver. 2.1 [23].

Table 1. Species studied and their respective sections and subsections divisions

Section	Species	Locality	Collector	Herbarium
Sect. *Protonosma*	*Onosma orientale* L.	Masjed Soleyman, Andika,	Mozaffarian	TARI-63017
Sect. *Podonosma*	*Onosma rostellatum* Lehm.	Kermanshah, Bayangan	Mehrabian	HSBU-2010244
Sect. *Onosam*	*Onosma albo-roseum* Fisch.	Kerned-Gharb, 45km West of Kerend, Rijab	Lashkar& Hatami	TARI-167
Sect.*Onosam*	*Onaosma armenum* DC.	Azarbaidjan,Maku to Khoy	Assadi& Mozaffarian	TARI-30353
Sect. *Onosam*	*Onosma bisotunensis* Attar. & Hamzee.	Kermabshah, Biston MT.	Mehrabian	HSBU-2010231
Sect. *Onosam*	*Onosma caurelescens* Boiss.	Azarbaidjan	-	Wien-08929
Sect. *Onosam*	*Onosma dasytrichum* Boiss.	Kermanshah, Paveh to Javanroud	Mehrabian	HSBU-2010248
Sect. *Onosam*	*Onosma rasychaenum* Boiss.	Mahneshan, Angoran, Belgheis Mountain	Mehrabian	HSBU-281
Sect. *Onosam*	*Onosma elwendicum* Wettst.	Tehran, Lashkarak	Mehrabian	HSBU-2010247
Sect. *Onosam*	*Onosma macrophyllum* Bornm.	Kermanshah, Gahvareh, Baba Shah Ahmad Mt.	Mehrabian	HSBU-2010235
Sect. *Onosam*	*Onosma olivieri* Boiss.	Kurdistan, Nosud to Nodesheh	Mehrabian	HSBU-2010246
Sect. *Onosam*	*Onosma straussii* H.Riedl.	Markazi, Arak, Gavar	Mehrabian	HSBU-2010232
Sect. *Onosam*	*Onosma bulbotrichum* DC.	Zanjan To Mahneshan	Mehrabian	HSBU-2010238
Sect. *Onosam*	*Onosma chrysochaetum* Bornm.	Isfahan	Bornm	IRAN-1040
Sect. *Onosam*	*Onosma cornutum* H.Riedl.	Tehran, Lashkarak	Mehrabian	HSBU-2010236
Sect. *Onosam*	*Onosma dichroanthum* Boiss.	Golestan, Gorga, between Tash and Gharabagh	Assadh& Hamdi	TARI-85460
Sect. *Onosam*	*Onosma kilouyense* Boiss.	Kermanshah, Gahvareh	Mehrabian& Mohamadi	HSBU-2011104
Sect. *Onosam*	*Onosma Kotschy* Boiss.	South of Estahbanat	Moaffarian	TARI-46999
Sect. *Onosam*	*Onosma nervosum* H.Riedl.	Esfahan, Ferydonshahr, Venizan Mountain	Mozaffarian	TARI-77244
Sect. *Onosam*	*Onosma microcarpum* DC.	Markazi, Arak, Gavar	Mehrabian	HSBU-2010240
Sect. *Onosam*	*Onosma Pachypodum* Boiss.	Tehran, Sohanak	Mehrabian	HSBU-2010241
Sect. *Onosam*	*Onosma sabalanicum* Ponert	Aerdabil, Meshkin Shahr, Sabalan Mt	Mehrabian	HSBU-2010249
Sect. *Onosam*	*Onosma sericeum* var. *sericeum*. Willd.	Teharan, Lashkarak	Mehrabian	HSBU-2010250
Sect. *Onosam*	*Onosma stenosiphon* Boiss.	Kerman	Kanani	HSBU-2010237

Table 2: Pollen characteristics in *Onosma* species studied. P: Polar axis, E: Equatorial axis, GS: Gland Size, WT: Wall thickness, Polar View: PV, Equatourial view:EV

Species-Character	P(µm)	E(µm)	P/E(µm)	GS(µm)	WT(µm)	PV	EV
O.albo-roseum	20.01 18.68-21.91	16.07 13.32-17.79	1.25 1.23-1.40	0.18 0.12-0.25	0.138 0.110-0.150	subprolate	heteropolar
O.armenum	15.60 13.61-16.66	12.55 11.11-14.40	1.25 1.22-1.56	0.11 0.05-0.14	0.135 0.120-0.160	prolate	heteropolar
O.bistonensis	13.75 12.02-14.53	12.45 11.68-14:00	1.1 1.02-1.03	0.17 0.14-0.26	0.133 0.11-0.150	subprolate	heteropolar
O.bulbotrichum	16.67 15.30-18.59	13.37 12.69-14.05	1.25 1.20-1.32	0.12 0.08-0.15	0.127 0.09-0.150	prolate	heteropolar
O.caurelescens	14.83 12.09-16.43	11.74 10.11-13.39	1.11 1.20-1.22	0.13 0.07-0.15	0.110 0.090-0.140	subprolate	heteropolar
O.chrysochaetum	14.71 12.44-15.66	11.97 10.42-13.25	1.30 0.79-1.81	0.10 0.07-0.19	0.130 0.100-0.190	subprolate	heteropolar
O.cornutum	16.14 15.31-16.89	11.75 11.31-12.05	1.38 1.35-1.40	0.15 0.09-0.20	0.127 0.100-0.150	prolate	heteropolar
O.dasytrichum	12.96 11.60-13.92	10.31 9.58-10.22	1.25 0.83-1.36	0.11 0.06-0.07	0.145 0.130-0.180	subprolate	heteropolar
O.dichoroanthum	15.35 13.62-16.50	12.69 11.53-13.55	1.20 1.81-1.21	0.11 0.08-0.20	0.137 0.120-0.170	prolate	heteropolar
O.elwedicum	15.39 13.09-17.55	12.46 10.39-13.59	1.24 1.23-1.29	0.15 0.08-0.23	0.140 0.110-.160	prolate	heteropolar
O.kilouyense	15.66 13.69-17.10	12.64 11.52-13.22	1.24 1.18-1.29	0.15 0.10-0. 20	0.140 0.120-0.160	subprolate	heteropolar
O.kotschy	16.32 14.49-15.77	12.13 10.75-13.76	1.35 1.34-1.40	0.16 0.08-0.24	0.130 0.110-0.150	subprolate	heteropolar
O.macrophyllum	16.76 13.95-18.08	11.90 10.83-12.53	1.40 1.28-1.44	0.13 0.08-0.24	0.122 0.110-0.170	prolate	heteropolar
O.nervosum	14.25 12.37-14.96	10.58 9.25-11.77	1.34 1.33-1.27	0.09 0.07-0.15	0.131 0.110-0.170	prolate	heteropolar
O.microcarpum	17.49 16.06-19.78	15.42 10.83-12.53	1.13 1.12-1.58	0.15 0.08-0.24	0.133 0.110-0.170	prolate	heteropolar
O.olivieri	15.55 14.20-16.63	12.60 11.36-13.85	1.23 1.22-1.24	0.10 0.07-0.12	0.136 0.110-0.180	prolate	heteropolar
O.orientale	13:00 12.08-14.31	11.55 9.91-12.46	1.12 1.09-1.14	0.09 0.07-0.10	0.125 0.100-0.160	subprolate	heteropolar
O.pachypodum	16.91 16.39-17.57	11.94 11.15-12.58	1.41 1.39-1.46	0.13 0.08-0.20	0.127 0.100-0.170	subprolate	heteropolar
O.rasychaenum	13.09 12.81-13.85	11.28 11.12-11.48	1.16 1.14-1.51	0.13 0.08-0.24	0.080 0.070-0.100	subprolate	heteropolar
O.rostellatum	20:00 16.92-22.36	14.21 13.23-17.24	1.50 1.27-1.56	0.06 0.04-0.11	0.141 0.110-0.160	ellipsoidal	isopolar
O.sabalanicum	13.81 12.47-14.62	10.60 9.86-11.68	1.37 1.26-1.39	0.09 0.05-0.13	0.122 0.110-0.170	prolate	heteropolar
O.sericeum	18.37 14.90-20.56	14.32 11.60-16.10	1.28 1.27-1.29	0.16 0.10-0.22	0.107 0.080-0.230	prolate	heteropolar
O.stenosiphon	17.77 16.54-19.47	13.16 12.61-13.72	1.35 1.31-1.41	0.18 0.09-0.26	0.960 0.900-1.100	subprolate	heteropolar
O.straussii	14.44 13.25-15.94	10.73 9.61-12.25	1.34 1.30-1.37	0.11 0.06-0.17	0.136 0.110-0.160	prolate	heteropolar

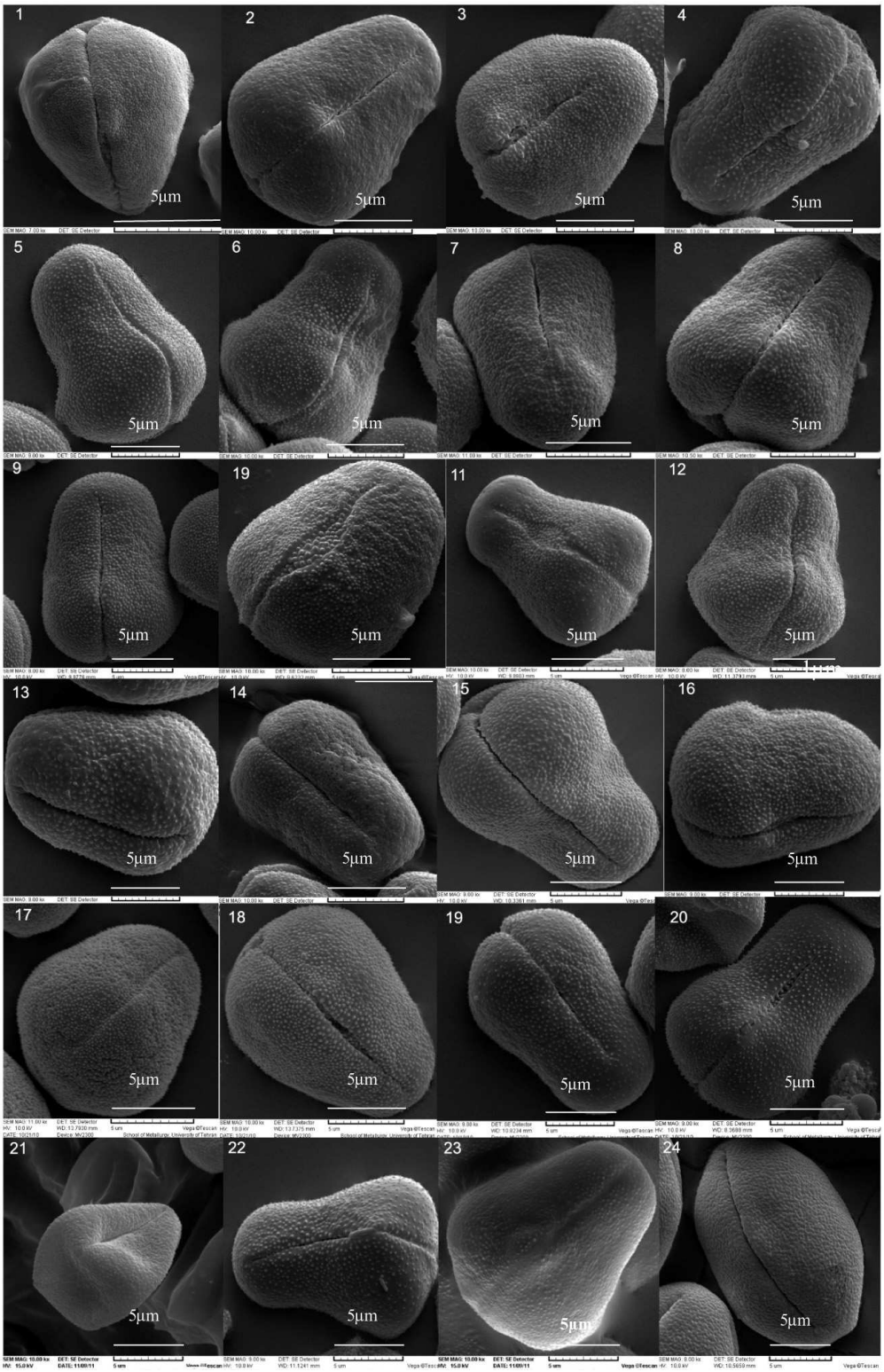

Fig 1. SEM of pollen in *Onosma* species studied (Equtourial View).

1- *O.albo-roseum.*, 2- *O.armenum* DC., 3- *O.bistonensis* 4-*O.bulbotrichum* DC., 5-*O.cornutum*,6-*O.dichroanthum.*,7-*O.dasytrichum.*,8-*kilouyense.*,9-*O.kotschyi.*,10-*O.rascheyanum.*,11-*O.sablanicum.*,12-*O.sericeum.*,13-*O.stenosiphon.*,14-*O.straussii.*,15-*O.microcarpum.*,16-*O.olivieri.*,17-*O.orientale.*,18-*O.pachypodum.*,19-*O.macrophyllum.*,20-*O.nervosum.*,21-*O.caerulescens.*,22-*O.elwendicum.*,23-*O.chrysochaetum.*, 24-*O.rostellatum*

Fig. 2
1- *O.bulbotrichum*, 2- *O.dichroanthum*, 3- *O.macrophyllum*, 4-*O. sbalanicum*,, 5- *O. chrsochaetum*, 6- *O.rostellatum*

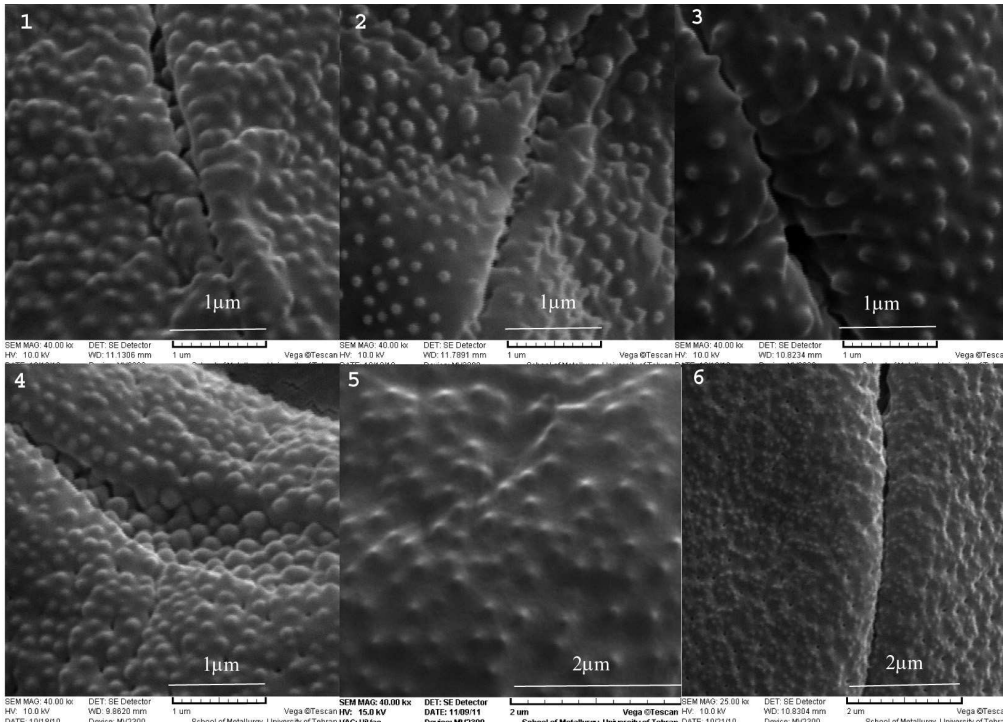

Fig 3. Pictures (polar and equatorial view) of acetolysed pollens in *Onosma* species studied (Scale bars: 11µm).1,2-*O.albo-roseum* Fisch., 3,4- *O.bisotunensis* Attar.& Hamzehee., 5,6-*O.bulbotrichum* DC., 7,8-*O.caerulescens* Boiss., 9,10 *O.cornutum* H.Riedl., 11,12-*O.dasytrichum* Boiss., 13,14-*O.dichroanthum* Boiss., 15,16-*O. elwendicum* Wettst., 17,18-*O.kilouyense* Boiss., 19,20-*O.kotschy* Boiss., 21,22- *O.macrophyllum* Bornm., 23,24- *O.nervosum* H.Riedl., 25,26-*O. microcarpum* DC., 27,28-*O.olivieri* Boiss., 29,30-*O.orientale* L., 31,32 *O.pachypodum* Boiss., 33,34-*O.rascheyanum*., 35,36- *O.rostellatum* Lehm.,37,38-*O.sericeum*., 39-40- *O.stenosiphon* Boiss., 41-42-*O.straussii* (H.Riedl.) Khatamsaz.

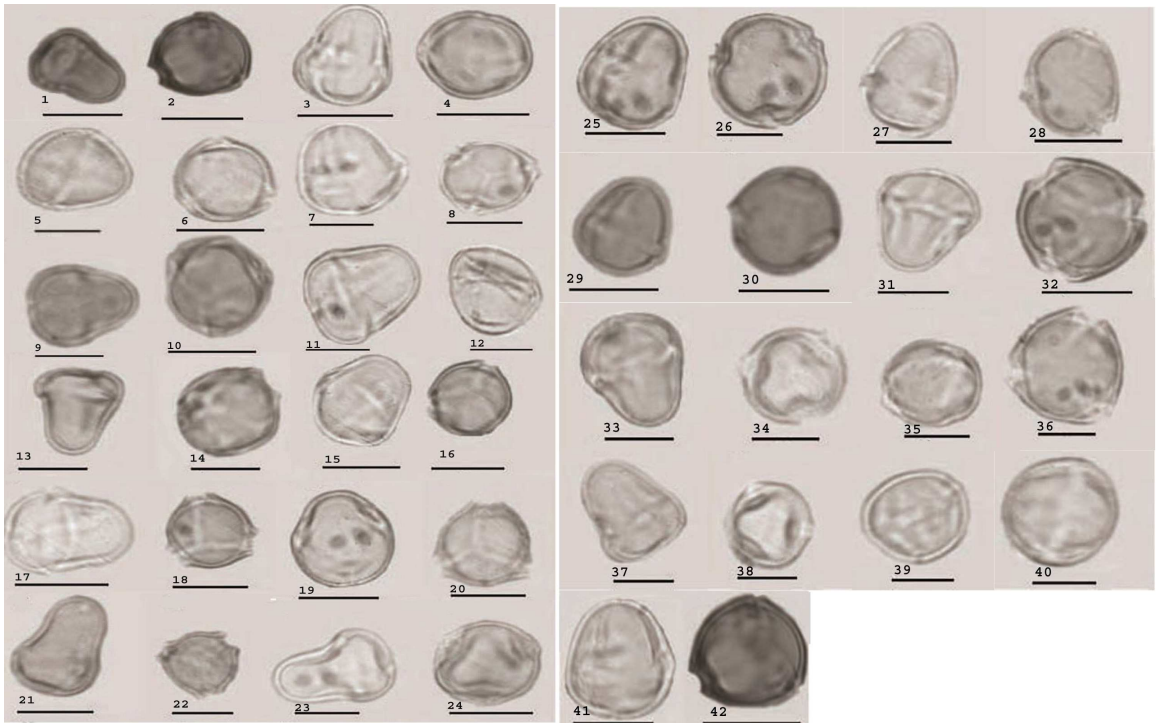

Fig. 4 . UPGMA of pollen data.

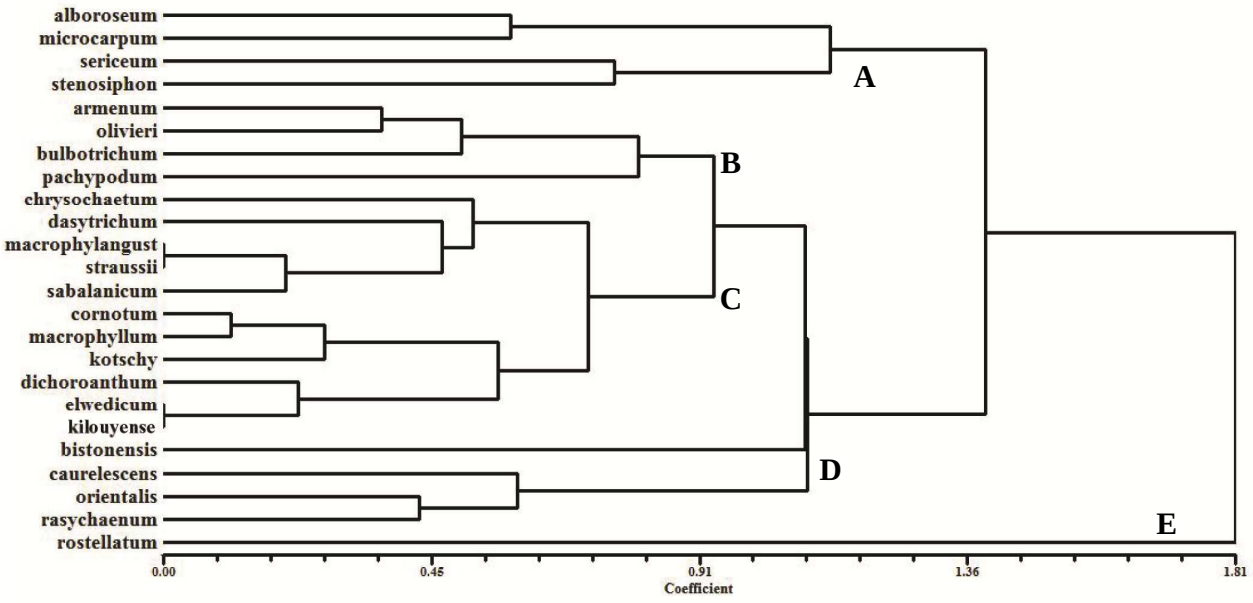

Table3: ANOVA analysis Between, Within and Total in Groups.

		Sum of Squares	df	Mean Square	F	Sig.
P	Between Groups	483.977	23	21.042	14.025	0.001
	Within Groups	144.031	96	1.500		
	Total	628.008	119			
E	Between Groups	276.544	23	12.024	14.413	0.001
	Within Groups	80.088	96	.834		
	Total	356.632	119			
RATIO	Between Groups	.944	23	4.106E-02	3.085	0.001
	Within Groups	1.278	96	1.331E-02		
	Total	2.222	119			
GLAND	Between Groups	.106	23	4.623E-03	2.586	0.001
	Within Groups	.172	96	1.788E-03		
	Total	.278	119			
WALL	Between Groups	1366.283	23	59.404	63.946	0.001
	Within Groups	89.180	96	.929		
	Total	1455.463	119			

RESULTS AND DISCUSSION

The results of pollen analyses in *Onosma* species studied are presented in Table 2 and Figs. 1-4. The pollen characteristics were defined according to Wodehouse (1956)[26]. The SEM study showed the occurrence of the Stenopalynous, colporate, prolate, subprolate, Ellipsoidal, isopolar or heteropolar pollen grains in the *Onosma* species studied (Fig. 1). The size of polar axis varied in the species studied; the largest polar axis was observed in *O. albo-roseum*, *O. rostellatum* (20.01 µm and 20:00 µm respectively), while the smallest polar axis occurred in *O. orientale* and *O. dasytrichum* (13:00 µm and 12.96. µm respectively). Similarly the largest equatorial axis was observed in *O. albo-roseum* (16.07µm) while the smallest one occurred in *O. dsytrichum* and *O.nervosum* (10.31 µm and 10.58 µm respectively).

In Polar view 4 types of pollen shape were recognized in *Onosma* species studied according to description of Wodehouse (1956)[26] : 1- Prolate, 2- subprolate, 3- ellipsoidal. The species of *O.rostellatum* was the only species with ellipsoidal shape pollen grains, while the other species showed prolate and subprolate pollen shape (Table 2).

In Equtourial view 1 types of pollen shape were recognized that including kind of heteropolar.

The Pollen surface was granulating in all *Onosma* species studied except in *O. rostellatum* which was micro granulating. The size of pollen granules varied in the species studied, for example *O. albo-roseum* (0.18 µm), *O. stenosiphon* (0.18 µm) and *O. bisotunensis* (0.17 µm) had large size granules while *O. orientale* (0.09 µm) *O. nervosum* (0.09µm), showed the presence of medium granule size and *O. rostellaum* (0.06 µm) was the only species that showing small granule size (Table 2).

The species studied also varied in thickness of exine. *O. dasytrichum* (0.145 µm), *O. rostellatum* (0.141 µm) have the most thickness and *O. rasychaenum* (0.080) has the least thikness (Table 2).

Based on Fig 4, 4major clusters are formed:
The first major cluster (C) contains *O. chrysochaetum, O. dasytrichum, O. nervosum, O. straussii, O. sabalanicum, O. cornutum, O. macrophyllum, O. kotschy, O. dichroanthum, o.elwendicum* and *O.kilouyense*.
O. albo-roseum, O. stenosiphon, O. sericeum, O. microcarpum form the second major cluster (A).
O. armenum, O. olivieri, O. bulbotrichum and *O. pachypodum* formed the second major cluster (B).
The Forthth major cluster (D) contains *O. bisotunensis, O. caurelesecens, O. orientale* and *O.rasychaenum.*
O. rostellatum formed fifth group (E) that is a different group in mentioned analysis.

Mentioned groups mostly are classified based on Sizes and are not according Classification of Sections and Subsections in Flora Iranica [21].

ANOVA test showed significant difference ($p<0.001$) or quantitative pollen characters among the species studied (Table 3.)

In this study, twenty-four *Onosma* (Boraginaceae) species from 3 sections of *Podonosma* (Boiss.) Gurke, *Onosma* L. and *Protonosma* M.Popov. were investigated from pollen characteristics point of view, showing variations in details of pollen characteristics. These differences may be used in the species delimitation as revealed by factor analysis showing that pollen characters like gland size, equatorial axis and pollen shape are the most variable characters among the *Onosma* species studied.

The UPGMA tree obtained in the present study show relationship among the species which are not considered as the members of a single section/subsection in the Flora Iranica [22]. For example, in the first major cluster, although *O. bulbotrichum, O. pachypodum* from the sect. *Onosma*, subsect. *Haplotricha* and *Onosma armenum* is belonging subsect. *Asterotricha* are grouped together. In other groups *O. bisotunenis, O. caurelescens* and *O. rasychaenum* from the sect. *Onosma*, subsect. *Asterotricha* shows affinity to *O. orientale* of the sect. *Protonosma*. Exceptionally *O. rostellatum* that really is different species than other studied species and show a different group in dendrograms, the pollen characteristics although differentiate the *Onosma* species from each other, they do not show the species relationship.

In a our recent study about mentioned species based on morphological and molecular evidences [15], presence of common bands in two or more species, for example ISSR bands obtained in *O. bistonensis* and *O. bulbotrichum*, as well as in, *O. rostellatum* and *O. sericeum* indicate the presence of synapomorphic characters to be used in sister group identification. Moreover, UPGMA trees of morphological characters show affinity of species and sections. For example *O. pachypodum and o. sericeum* are as sister group and *O. microcarpum, O. sabalanicum and O. longilobum* as a other sister groups are belonging to *Haplotricha* section. While, *O. rostellatum* stands far from the other species.

The NJ and UPGMA trees obtained from morphological and molecular data partly agrees with each other. In both *O. dasytrichum* and *O. microcarpum* are placed close to each other *O. pachypodum* shows affinity to *O. bistonensis* and *O. bulbotrichum* are placed close to each other while, *O. rostellatum* stands far from the other species. The tree of combined morphological and ISSR data clearly separates the members of three sections of *Onosma, Podonosma* and *Protonosma* from each other. But UPGMA tree obtained from palynological data in our study are not in agreement with the Section ans subsection classification of Flora Iranica [22].

It was previously reported that the three colpi of syncolporatae pollen in some members of the genus *Onosma* converge in one pole or even two poles [18]. Binzet et al (2011) [5] reporeted that all *Onosma* taxa except *O. orientale* were observed to have three colpies of syncolporatae pollen at large pole and colpies are joined at the only

large poles (heteropolar). In addition to the colpies of *O. orientale* dont converge in one or two poles .Moreover we observerd similar results in the present study.

Wodehouse [26] and Lee [14] have reported that a direct correlation between flower and pollen size. However, Pandey (1971)[18] demonstrated in the genus *Nicotiana* that there was no correlation between flower and pollen size and the species with the largest flowers had small pollen grains. We found that the pollen of *O. sabalanicum* and *O. rasychaenum*, are smaller (13.81 µm (N) and 13.09 µm (N), respectively) but with medium flowers whereas, in *O. dasytrichum* and *O. nervosum* are relatively smaller (14.44 µm (N)) with larger flowers in this study. Our results are in agreement with the results Pandey (1971)[18].

Biggazi et al (2006)[2] reported the importance of Pollen charecters in taxonomy and phylogeny of *Cynogolsseae*. Scheel et al (1996) [24] show pollen charecters as important evidences in order to delimitation of subfamilies in Boraginaceae. Morover Biggazi and Selvi (1998)[3] reported pollen eviedences as delimitation of plant Taxa in Tribes of Boraginaceae.

Binzet (2011)[4] reported the use of pollen characteristics in proper recognition of twenty-five *Onosma* taxa from two subsections *Haplotricha* and *Asterotricha* in Turkey. They show that the pollen morphology is a useful diagnostic tool in determination of *O. stenoloba* Hausskn.ex Riedl and *O. mersinana* Riedl, Binzet et Orcan which shows close affinity in morphology. Interestingly enough, these authors also state that the acetolysis methods resulted in a better explanation of the palynological relationship within the genus *Onosma* than the Wodehouse method (1956)[26].

CONCLUSION

Our study show some characters are more useful than others, for example pollen shape in polar view, Polar and Equatourial long are better characters than others in order to species differentiation. Our study show pollen characters are valuable in order to species differentiation but are not useful for delimitation of Sections and subsections, exceptionally some characters that including Shape (elliptic) and ornamentation structure (micro granulating) are useful for differentiation of Sect. *Protonosma* (*O. rostellatum*) from other sections.

Thus, morphological and molecular data shows better evidences for delimitation of Sections and subsections in *Onosma* species and Pollen characters are only useful for differentiate the *Onosma* species from each other.

Acknowledgement
We would like to thank Dr. Vitek from Botany Department of Natural History Museum of Wien for his helping. Moreover we are very grateful to Mr Amini Rad and Mr. Pahlevani from Iran Herbarium.

REFERENCES

[1]. P.W, Ball,. *Bot. J. linn. Soci*.,**1972**, 65: 353-354.
[2]. M, Bigazzi, , F. Selvi, *Pl.Sys.Evo.*,**1998**, 213-121-151.
[3]. M, Bigazzi, E. Nardi, F. Selvi, , *Willdenowia*., **2006**, 36 : 37-46.
[4]. R. Binzet, *Pak. J. Bot*., **2011**, 43(2): 731-741.
[5]. R. Binzet, N. Orcan., *Phytol. Balcan***., 2003a,** 9: 97-111.
[6]. R. Binzet, N. Orcan, *Herb. Jour. Syst.Bot.*, **2003b**,10: 57-76.
[7]. C.G.S, Clarke, *Rev. Palae. Palyno.*, **1977**, 24(2):NEPF. 59-101.
[8]. M. Diez, Valdés,M.G, Fernández. I. *Grana*., **1986**, 25: 171-176.
[9]. M.J, Diez, B. Valdés, *Bot.Jour. Lin.Soc*. , **1991**,107: 49-66.
[10]. G. Erdtman, Almqvist and Wiksell, Stockholm.,**1952**, 539 pp.
[11]. P.K. Holmgren, N.H. Holmgren, L.C. Barnett, L.C *Reg. Vegt.*, **1990**, 120:1-693.
[12]. I.M.Johnston ,J. Arnold Arbor., **1954,** 35: 1-81.
[13].M. Khatamsaz M, Flora of Iran (in Persian), Tehran, Research Institute of Forests and Rangelands, **2002**.
[14]. S.Lee, *Syst. Bo*., 2002, 3: 1-19.
[15]. F. Maggi, V. Kolarcik, P.Mártonfi, *Biologica*., **2008**, 63/2: 183-186 .
[16]. A.R.Mehrabian1, M. Sheidai, Z.NoormohammadiY. Asri, V.Mozafarian, *Afri. Jour. Biotech*., **2010**, Vol. 10(53):10831-10838.

[17]. J.C.Ning, Y.Z. Xi, Y.L. Zhang, *Act. Phyt. Sin.*, **1995**, 33: 52-57.
[18]. J. Heslop-Harrison (Ed.). *Development and Physiology* Butterworths, London,**19710**)5
[19]. A.Perveen, Qureshi. U.S, M.Qaiser, *Pakistan J. Bot*., **1995**, 27: 327-360.
[20]. J. Podani, Introduction to the Exploration of Multivariate Biological Data. pp. Backhys and Leiden Publishers, **2000**. 407pp.
[21]. U.S. Qureshi, M. Qaiser, *Pakistan J. Bot*., **1987**, 19: 99-105.
[22]. H. Riedl, Akademische Druck und Verlagsanstalt, Graz, Austria, **1967**.
[23]. F.J. Rohlf, NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 2.0.Exeter Software New York, New York, USA, **1998**.
[24]. R, Sceel, J.P. Ybert, O.M. Barth, Grana., **1996**, 35: 138-153.
[25]. J.C. Willis, JCUniversity Press, Cambridge, **1973.**
[26]. R.P. Wodehouse, Mc Graw Hill, New York, **1965.**