

## Pollen grain morphology in Iranian Hedysareae (Fabaceae)

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### ABSTRACT

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Pollen grain morphology of 15 taxa of the Hedysareae tribe distributed throughout Iran was studied using light and electron microscopy to identify major taxonomical characteristics of pollen grains. The pollen grains were tricolpate and tricolporate, prolate and perprolate. The ectocolpi were elongated, shallow or deep, narrowing at the poles. The colpus membrane was covered by large granules. Ornamentation was reticulate, and lumina differed in shape and size. In equatorial view, the pollen grains were elongated, elliptic to rectangular-obtuse, while in polar view they were circular, triangular-obtuse or triangular and trilobed. Two pollen types with two tentative subtypes were identified based on ornamentation and polar view.

**Key words:** pollen grains, Hedysareae, ornamentation, taxonomical characteristics

### INTRODUCTION

From De Candolle (1825) onward, the taxonomic delimitation of the Hedysareae tribe has undergone several modifications by various authors (Bentham, 1865; Hutchinson, 1964; Polhill, 1981; Choi and Ohashi, 2003; Lock, 2005). Recently, Lock (2005) expanded the tribe to 12 genera including *Alhagi* Adans., *Calophaca* Fisch ex DC., *Caragana* Fabr. and *Halimodendron* Fisch ex DC., formerly treated in the Galegeae (Polhill, 1981), in addition to *Corethroedendron* Basin., *Ebenus* L., *Eversmannia* Bunge, *Hedysarum* L., *Onobrychis* Mill., *Sartoria* Boiss. & Heldr., *Sulla* Medik. and *Taverniera* DC. The Hedysareae tribe as a whole occurs in dry, open localities with continental temperate or Mediterranean climate, and is restricted to Eurasia, North America and the Horn of Africa, including Socotra. After *Hedysarum*, with ca. 160 species, *Onobrychis*, with ca. 130 species, is the second largest genus within the tribe (Mabberley, 1990; Lock, 2005). These genera are restricted to Eurasia and well represented in the continental temperate and warm-temperate zones of the Irano-Turanian region. In Iran, the Hedysareae tribe comprises six genera, including *Ebenus* (1 species), *Alhagi* (1), *Taverniera* (4), *Eversmannia* (1), *Onobrychis* and *Hedysarum*. Iran is one of the main centers of diversity for *Onobrychis* and *Hedysarum*. *Onobrychis* comprises over 69 species in two subgenera divided into eight sections, viz. *Dendrobrychis* (3), *Lophobrychis* (5), *Onobrychis*

(15), *Laxiflorae* (1), *Anthyllium* (3), *Afghanicae* (3), *Heliobrychis* (28) and *Hymenobrychis* (11). The genus *Hedysarum* is divided into three sections, namely *Multicaulia* (6), *Subacaulia* (4) and *Crinifera* (6) (Rechinger 1984). They are distributed all over the country.

The pollen morphology of the Hedysareae has not been thoroughly investigated. Several general surveys of the family have been conducted (Erdtman, 1966; Melhem, 1971; Ohashi, 1971; Pire, 1974; Pavlova and Manova, 2000, Ghanavati *et al.*, 2007; Amirabadizadeh *et al.*, 2009). Pollen of *Onobrychis* and *Hedysarum* has been ascribed to one pollen type (the *Onobrychis* type) (Faegri, 1956; Faegri and Iversen, 1989; Moore *et al.*, 1991) based on the three apertures and the reticulate (suprareticulate) ornamentation of the exine.

In this study, pollen characteristics of the Hedysareae tribe, almost all of which is found exclusively in Iran, were analyzed to identify relationships among species that can be used in systematic studies and in sainfoin breeding programs.

### MATERIALS AND METHODS

Pollen morphology of 15 taxa of the tribe Hedysareae was studied. Pollen material was obtained from collections in the Herbarium Research Center of Khorasan-e-Razavi Agricultural and Natural Resources Center (MRCH), Mashhad, Iran, and the Herbarium of National Plant GeneBank of

Iran (NPGBI) (Table 1).

Pollen samples were obtained from the five herbarium specimens. For light microscopy (LM), 50 measurements of each trait were made at X1000 magnification in different pollen samples. Five characters were measured: P (polar diameter), E (equatorial diameter), L (colpus length), S (colpus width), and P:E ratio. Microphotographs were taken with a Leo-1455 vp scanning electron microscope (SEM) using the standard methods described by Erdtman (1966). Ten measurements of each trait were made at X 4000 magnification. Exine sculpturing was studied at X 15000 magnification. Pollen terminology in general follows Faegri and Iversen (1989) and Punt *et al.* (1994). Pollen grains studied with scanning electron microscopy were coated with gold using a Polaron-SC7620 sputter coater.

## RESULTS

Measurements of five traits of the studied species are shown in Table 2. Pollen grains of the Hedysareae are usually symmetrical and isopolar. Pollen grains of most Hedysareae species are tricolpate and of the *Onobrychis* type (Moore *et al.*, 1991), and only the *Lophobrychis* section has pollen grains that are tricolporate. We were thus able to recognize two types of pollen grains.

### Tricolpate type

These pollen grains were tricolpate, prolate and perprolate, generally triangular-obtuse, triangular, trilobed, and circular in polar view. In equatorial view, the grains were elongated, elliptic to rectangular-obtuse. The ectocolpi were elongated, shallow or deep and narrowing at the poles. The colpus membrane was covered by both large and small granules. Ornamentation was reticulate or perforate, and the lumina differed in shape and size. Based on the shape in polar and equatorial view, and on ornamentation, two tentative subtypes could be identified.

### Subtype I

Pollen grains were circular in polar view, with shallow colpi; the lumina of the reticulum sharply decreased in size at the extreme margin of the colpi.

Pollen grains were prolate (*O. arnacantha*, *O. shahpurensis* and *O. hohenackeriana*) and perprolate (*O. laxiflora*, *O. tavernieraefolia*, *O. aucheri* and *Ebenus stellata*) (P: E=1.86-2.30, dimensions  $P \times E = 23.27-37.39 \times 10.79-11.69 \mu\text{m}$ ) (Figs. 1 and 2). Pollen grains were elliptical and elongated in equatorial view. Ectocolpi were long and shallow, and the colpus membrane was covered by large and small sculptural elements ( $L \times S = 20.25-36.90 \times 0.50- 2.81 \mu\text{m}$ ). Ornamentation was

reticulate, with the largest lumina in the intercolpia. The lumina around the apertures were less than  $1 \mu\text{m}$  in diameter and resembled perforations.

Pollen grains of *E. stellata*, *O. arnacantha*, *O. shahpurensis*, *O. laxiflora*, *O. tavernieraefolia*, *O. aucheri* and *O. hohenackeriana* belong to this subtype, but they show some differences. We observed that *Onobrychis shahpurensis* had the smallest pollen grains, while *O. arnacantha* had the largest.

### Subtype II

Pollen grains were triangular in polar view, with deep colpi, and the lumina of the reticulum were the same size at the extreme margin of the colpi (Figs. 1 and 2).

Pollen grains were perprolate (P:E=2.13-2.70), very large in size and dimensions ( $P \times E = 18.24-47.73 \times 7.17-16.28 \mu\text{m}$ ). Pollen grains were triangular-obtuse, triangular or trilobed in polar view, and elliptical elongated to rectangular-obtuse in equatorial view. Ectocolpi were long and deep, and the colpus membrane was covered by large and small sculptural elements ( $L \times S = 14.90-40.32 \times 0.04-1.00 \mu\text{m}$ ).

Pollen grains of *Alhagi camelorum*, *Taverniera cuneifolia*, *Eversmannia subspinoso*, *Hedysarum kopetdaghi*, *H. damghanicum*, *Onobrychis susiana* and *O. ptolemaica* belong to this subtype (Figs. 2 and 3). We observed that *A. camelorum* had the smallest pollen grains, while *O. susiana* had the largest.

### Tricolporate type

Pollen grains had three colpi and three pores at the end of the colpi, rectangular in polar view, and elliptical elongated in equatorial view. The lumina of the reticulum were of the same size at the extreme margin of the colpi. Pollen grains were perprolate (P:E=2.35) and large in size (dimensions:  $P \times E = 38.96-39.13 \times 15.57-17.57 \mu\text{m}$ ). Ectocolpi were long and deep ( $L \times S = 35.58-36.50 \times 0.41- 0.47 \mu\text{m}$ ). Groups of columella and large sculptural elements were visible in the lumina of the reticulum. Pollen grains of *O. crista-galli* belong to this type, which is the largest pollen grain of the Hedysareae (Fig. 3).

Results of our study showed that pollen grain morphology of the Iranian representatives of the Hedysareae was comparatively homogenous and confirmed the general description presented by Ohashi (1971), Ferguson and Skvarda (1981), Faegri and Iversen (1989), Moore *et al.* (1991), Choi and Ohashi (1996), and Pavlova and Manova (2000). However, our observations showed that in *Onobrychis crista-galli*, pollen grains were different and tricolporate.

Nevertheless, based on the shape of polar and

**Table 1. Hedysareae species and their locality.**

Section-Species	Locality	Voucher number
<i>Ebenus stellata</i> Boiss.	Kerman: 27Km from Jiroft towards Mahan, near Mohammadabad village, 1680m.	MRCH 7442
<i>Alhagi camelorum</i> Fisch.	Ghazvin: 10 Km from Ghazvin towards Rasht, 1360m.	MRCH 5809
<i>Taverniera cuneifolia</i> (Roth) Arn.	Boshehr: Kangan towards Cham, 10m.	MRCH 6792
<i>Eversmannia subspinosa</i> (Fisch.) B. Fedtsch.	Semnan: 28 Km from Shahrood towards Azadshahr, 1500m.	MRCH 8983
Sect. <i>Multicaulia Hedysarum kopetdaghi</i> Boriss.	Khorasan: 80 Km from Bojnord towards Gorgan, 1360m.	MRCH 6799
Sect. <i>Crinifera: Hedysarum damghanicum</i> Rech. F.	Semnan: 36Km from Semnan towards Damghan, Ahovan jugo, 1960m.	MRCH 8490
Sect. <i>Dendrobrychis: Onobrychis arnacantha</i> Bunge	Khorasan: Neishabour, north of Dizbade Olya, 2500m	MRCH 7489
Sect. <i>Lophobrychis: Onobrychis crista-galli</i> (L.) Lam	Kermanshah: Sare Pole zahab, Gardano village, 550m.	NPGBI 188
Sect. <i>Onobrychis: Onobrychis shahpurensis</i> Rech.f.	West Azerbaijan: NW of Orumiyeh, 26 Km from Orumiyeh towards Sero, 1700m.	MRCH 6046
Sect. <i>Laxiflora: Onobrychis laxiflora</i> Baker subsp. <i>taftanica</i> Rech. f.	Khorasan: North of Birjand, Between Birjand and Sedeh, Piranj village, 1910m.	MRCH 9344
Sect. <i>Anthyllium: Onobrychis susiana</i> Nab.	Khozestan: 35 Km from Masjedsoleiman towards Lali, 270m.	MRCH 6502
Sect. <i>Afghanica: Onobrychis tavernieraefolia</i> Stocks ex Boiss.	Sistan and Baluchestan: Khash, 1400m.	TARI 42756
Sect. <i>Heliobrychis Onobrychis aucheri</i> Boiss subsp. <i>teheranica</i> (Bornm.) Rech. F.	Esfehen: 22 Km from Kashan towards Natanz, 1200m.	TARI55952
Sect. <i>Hymenobrychis: Onobrychis ptolemaica</i> (Del.) DC	Khozestan: 40 Km from Ramhormoz towards Baghmalek, 950m.	MRCH 7758
Sect. <i>Hymenobrychis: Onobrychis hohenackeriana</i> Grossh.	West Azerbaijan: 10 Km from Hasratan towards Khodaafarin, 460m.	MRCH 6090

**Table 2. Hedysareae species examined for pollen grain morphology: polar (P) and equatorial (E) axes, length (L) and width (S) and the shape index (P:E).**

Species	P				E				L				S				P:E ratio
	μm				μm				μm				μm				
1 <i>Ebenus stellata</i>	27.47	(27.70)	27.90	†	12.40	(12.74)	13.24		26.30	(26.70)	27.00		0.50	(0.98)	1.70		2.17
2 <i>Alhagi camelorum</i>	18.24	(18.37)	18.50		7.17	(7.28)	7.40		14.90	(15.25)	15.60		0.14	(0.23)	0.31		2.52
3 <i>Taverniera cuneifolia</i>	21.38	(22.00)	22.65		8.00	(8.27)	8.55		21.50	(12.19)	21.88		0.04	(0.04)	0.05		2.60
4 <i>Eversmannia subspinosa</i>	21.65	(22.27)	22.90		9.15	(9.47)	9.80		20.60	(21.05)	21.50		0.21	(0.26)	0.30		2.35
5 <i>Hedysarum kopetdaghi</i>	21.21	(22.73)	25.53		8.00	(8.60)	9.67		19.45	(20.94)	23.44		6.00	(0.65)	0.70		2.64
6 <i>Hedysarum damghanicum</i>	23.32	(23.42)	23.55		10.53	(10.97)	11.20		20.25	(20.87)	21.06		0.56	(0.72)	1.00		2.13
7 <i>Onobrychis arnacantha</i>	28.23	(30.80)	32.77		14.40	(15.57)	16.22		20.83	(24.02)	26.90		1.40	(2.10)	2.81		1.97
8 <i>Onobrychis crista-galli</i>	38.96	(39.04)	39.13		15.57	(16.57)	17.57		35.58	(36.00)	36.50		0.41	(0.44)	0.47		2.35
9 <i>Onobrychis shahpurensis</i>	23.27	(24.30)	23.81		12.71	(13.01)	13.31		21.50	(22.71)	22.90		0.85	(0.98)	1.10		1.86
10 <i>Onobrychis laxiflora</i>	24.50	(30.94)	37.39		14.00	(14.31)	14.60		36.50	(36.86)	36.90		1.27	(1.30)	1.40		2.16
11 <i>Onobrychis susiana</i>	30.17	(40.49)	47.73		13.49	(14.51)	16.28		27.96	(34.84)	40.32		0.26	(0.35)	0.37		2.70
12 <i>Onobrychis tavernieraefolia</i>	27.79	(28.11)	28.43		11.97	(12.55)	13.14		24.91	(25.31)	25.71		1.13	(1.27)	1.50		2.3
13 <i>Onobrychis aucheri</i>	24.07	(25.70)	27.09		10.79	(11.19)	11.69		21.76	(23.10)	25.40		0.82	(0.90)	0.99		2.29
14 <i>Onobrychis ptolemaica</i>	24.51	(26.33)	27.15		11.46	(11.80)	12.38		21.64	(22.79)	23.49		0.24	(0.30)	0.36		2.39
15 <i>Onobrychis hohenackeriana</i>	22.77	(23.85)	24.94		11.70	(12.07)	12.44		19.52	(20.89)	22.27		0.87	(1.13)	1.28		1.98

Note: Numbers in each column give the “lowest (average) highest” values for each characteristic.

equatorial views, ornamentation, and presence of pores and colpi, two types as well as two subtypes could be distinguished. The observed differences in the pollen grain morphology of these groups did not suggest any change in the adopted taxonomical scheme of the genera.

It has been reported that the small southwest Asian genus *Alhagi* has 3-5 species (Rechinger, 1984; Lock and Simpson, 1991; Lock, 2005). Ahangarian *et al.* (2007) suggested that *Alhagi* may be represented by three species and form its own strongly supported clade positioned at the base of the Hedysareae. *Alhagi* has an obtuse keel, one-foliolate leaves and a non-Hedysaroid fruit venation pattern, and seems to be different from others. But pollen grains show that the genus is rectangular in equatorial view, having tricolpate apertures, triangular in polar view, with deep colpi; lumina of the reticulum of the same size at the extreme margin of the colpi suggested that *Alhagi* may be related to the Hedysareae tribe.

*Ebenus*, a genus having ca. 20 species growing in Eurasia, with Turkey as its main center of diversity (Huber-Morath, 1971; Lock, 2005), was also analyzed in our study. Morphological traits including one segmented pod with 1 to 2 seeds (Huber-Morath, 1970; Polhill, 1981), and nrDNA ITS phylogeny shows that the genus is closely related to other genera of the tribe (Ahangarian *et al.*, 2007). Our pollen grain analysis showed that *Ebenus* had tricolpate apertures, circular in polar view, with shallow colpi, and lumina of the reticulum sharply decreased in size at the extreme margin of the colpi. This suggests that it is related to the Hedysareae.

One species of *Taverniera*, a medium-sized genus consisting of 16 species distributed over northeast Africa and southeast Asia (Mozaffarian, 1988), was included in our study. *Taverniera* is morphologically characterized by shrubby habit, 1, 3-foliolate leaves and short stipitate pods enclosed in the corolla (Polhill, 1981; Thulin, 1985; Choi and Ohashi, 2003). Gross morphological (Polhill, 1981; Thulin, 1985; Choi and Ohashi, 2003), pollen morphological (Thulin, 1985; Choi and Ohashi, 1996, 2003) and nodal anatomical features (Choi *et al.*, 1998) together with an nrDNA ITS tree (Ahangarian *et al.*, 2007) suggested that *Taverniera* is closely related to *Hedysarum*. Our study also showed that *Taverniera* had pollen grains triangular in polar view, with deep colpi, lumina of the reticulum of the same size at the extreme margin of the colpi, and confirmed the finding of other researchers.

The genus *Hedysarum* comprises about 100

species distributed in temperate to boreal regions of the northern hemisphere. Plants of this genus occur in different habitats and show large variation in pod features, notably being covered with ribs, spines or prickles, which have been regarded as one of the most important diagnostic characters separating infrageneric taxa in *Hedysarum* (Basiner, 1845; Fedtschenko, 1902; Choi and Ohashi, 2003). According to the classification made by these authors, *Hedysarum* is circumscribed and composed of four sections, two of which were included in our study. The pollen grains showed that this genus had tricolpate apertures, triangular in polar view, with deep colpi, and lumina of the reticulum of the same size at the extreme margin of the colpi, which suggested that *Hedysarum* is associated with genera of the Hedysareae tribe.

*Eversmannia*, a small genus consisting of four species, one of which, *E. subspinosa*, was analyzed in this study, is closely allied with the *Onobrychis* type. Fruit pericarp anatomy suggested that *E. subspinosa* is closely related to *Hedysarum* (Mironov and Sokoloff, 2000), while pollen morphology identified it as a sister of *Alhagi* (Choi and Ohashi, 1996). In contrast to other members of the Hedysareae tribe, *Eversmannia* is characterized by non-lomentaceous fruits that are rather dehiscent, that is, they do not break into segments as is typical of the tribe and have transversal venation pattern of valves (Mironov and Sokoloff, 2000). Our study illustrated that *Eversmannia* had trilobbed pollen grains in polar view, with deep colpi, and the lumina of the reticulum were the same size and very small at the extreme margin of the colpi, which suggested it is related to the Hedysareae.

The genus *Onobrychis* comprises ca. 130 species (Mabberley, 1990) classified into 2 subgenera, and 9 sections (Rechinger, 1984). The subgenus *Sisyrosema* differs from the subgenus *Onobrychis* by its large, crescent/kidney-shaped ovaries and pods, hairy vexillum, large persistent flowers and epidermis of calyx without crystals (Rechinger, 1984; Yildiz 2002). The nrDNA ITS data (Ahangarian *et al.*, 2007) demonstrated that the subgenus *Sisyrosema*, represented here by four of its five constitutive sections, appears to be a well supported monophyletic group, whereas the subgenus *Onobrychis* is not monophyletic due to the sister group relationship of its two representative species to the subgenus *Sisyrosema* and the inclusion of the two species of *Hedysarum* within it. Yildiz (2002) suggested, however, that the monophyly of these two subgenera was not supported by a phylogenetic analysis of fruit characters. In our study, pollen grains of nine

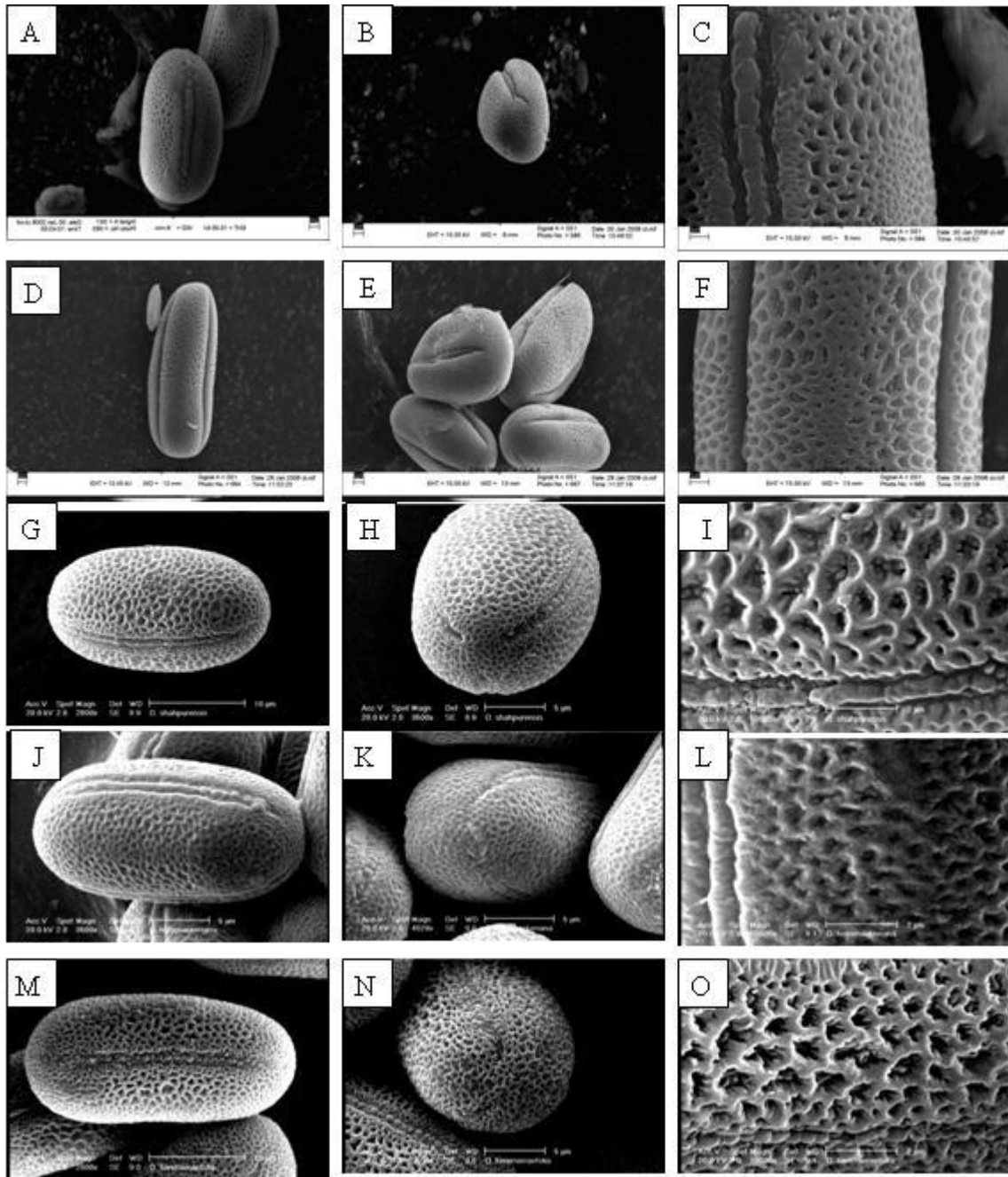


Fig. 1. A-C: pollen grains of *O. arnacantha*. A: equatorial view; B: polar view; C: colpus and ornamentation. D-F: pollen grains of *O. laxiflorae*. D: equatorial view; E: polar view; F: colpus and ornamentation. G-I: pollen grains of *O. shahpurensis*. G: equatorial view; H: polar view; I: colpus and ornamentation. J-L: pollen grains of *O. hohenackeriana*. J: equatorial view; K: polar view; L: colpus and ornamentation. M-O: pollen grains of *O. tavernieraefolia*. M: equatorial view; N: polar view; O: colpus and ornamentation.

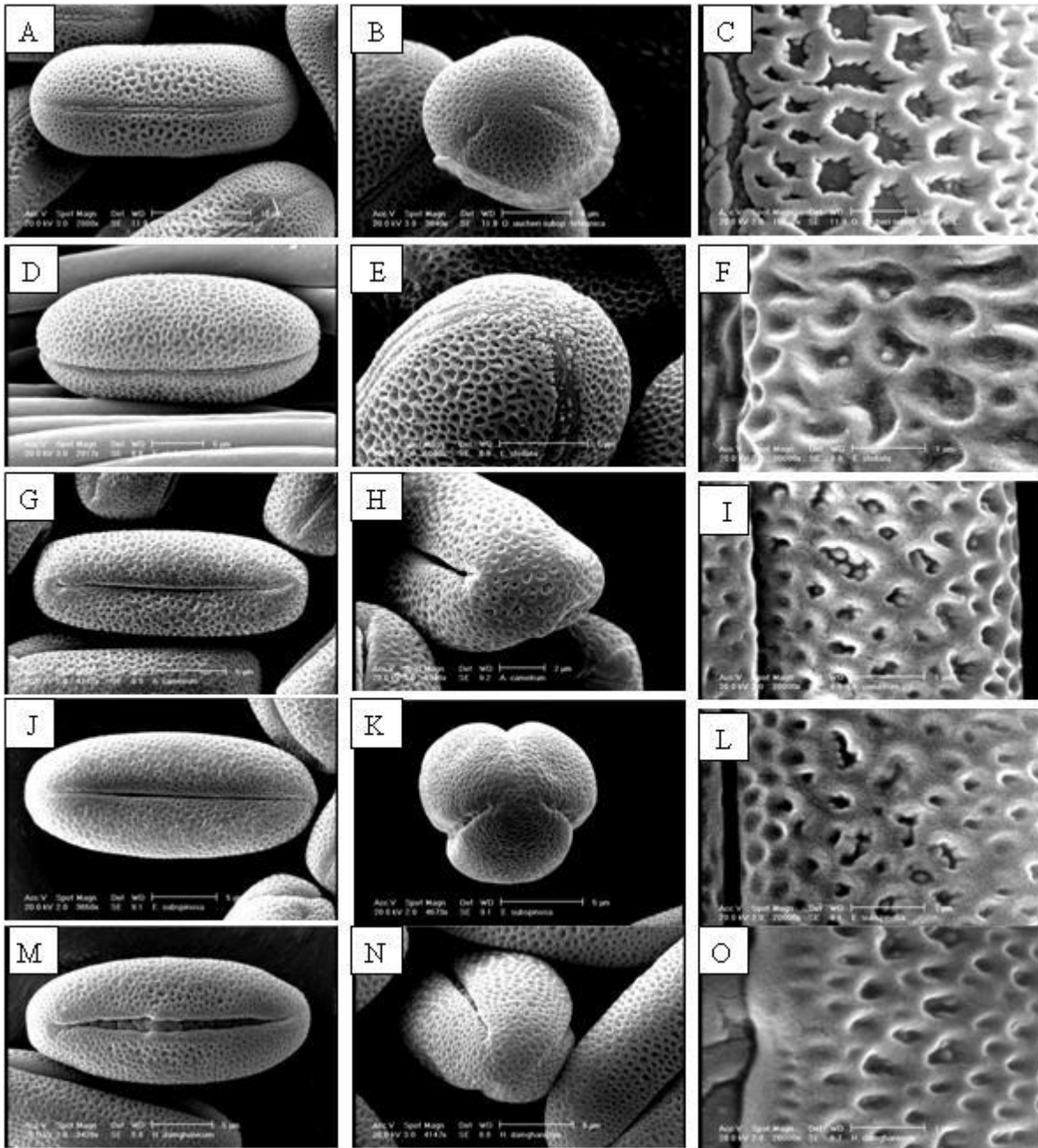


Fig. 2. A-C: pollen grains of *O.aucheri*. A: equatorial view; B: polar view; C: colpus and ornamentation. D-F: pollen grains of *Ebenus stellata*. D: equatorial view; E: polar view; F: colpus and ornamentation. G-I: pollen grains of *Alhagi camelorum*. G: equatorial view; H: polar view; I: colpus and ornamentation. J-L: pollen grains of *Eversmannia subspinosa*; J: equatorial view; K: polar view; L: colpus and ornamentation. M-O: pollen grains of *Hedysarum damghanicum*. M: equatorial view; N: polar view; O: colpus and ornamentation.

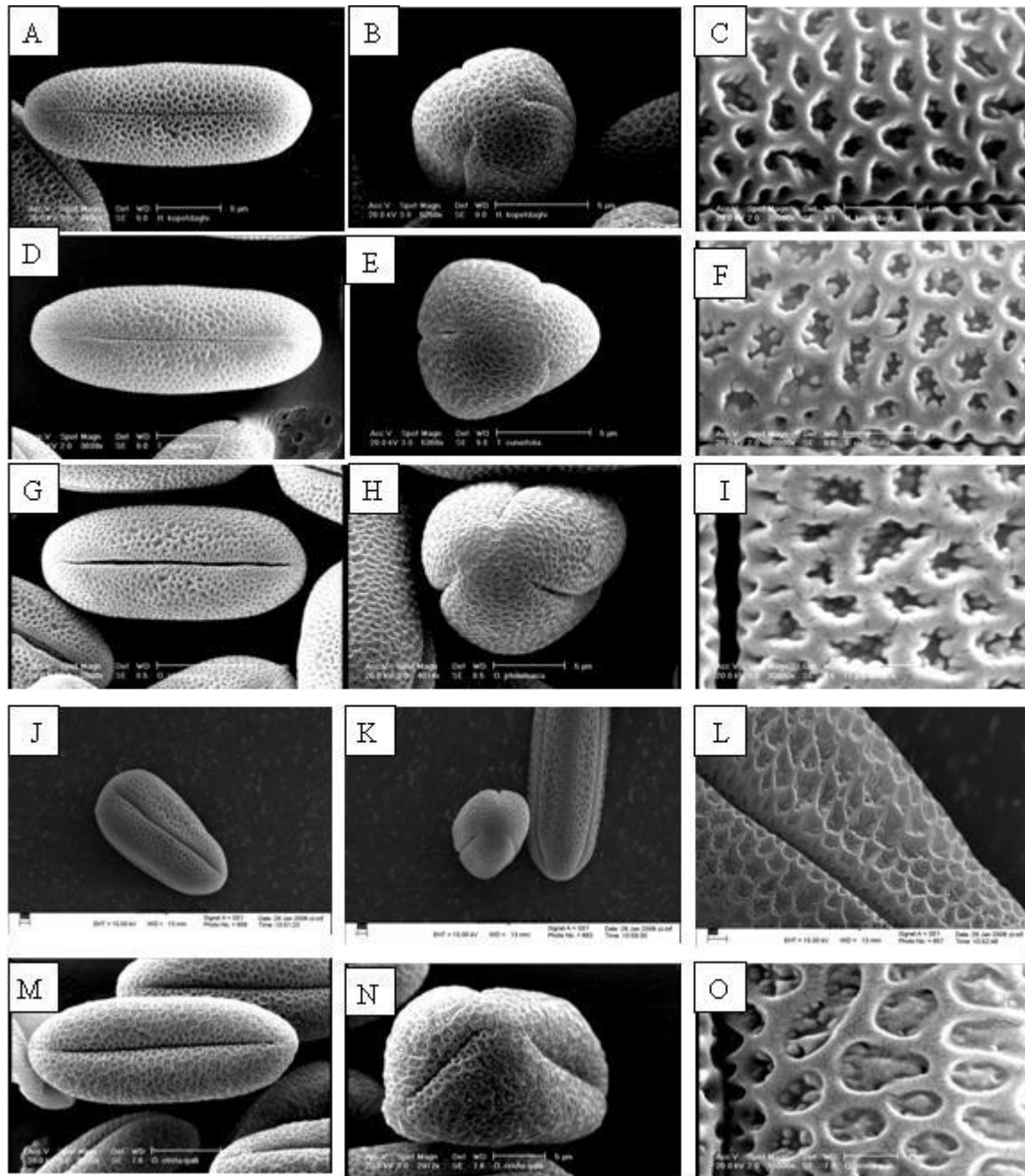


Fig. 3. A–C: pollen grains of *Hedysarum kopetdaghii*. A: equatorial view; B: polar view; C: colpus and ornamentation. D–F: pollen grains of *Taverniera cuneifolia*. D: equatorial view; E: polar view; F: colpus and ornamentation. G–I: pollen grains of *O. ptolemaica*. G: equatorial view; H: polar view; I: colpus and ornamentation. J–L: pollen grains of *O. susiana*. J: equatorial view; K: polar view; L: colpus and ornamentation. M–O: pollen grains of *O. crista-galli*. M: equatorial view; N: polar view; O: colpus and ornamentation.

species were analyzed and results showed that section *Lophobrychis* (*O. crista-galli*) differs from the others, for it has three colpi and three pores at the end of the colpi, which were rectangular in polar view. In section *Hymenobrychis* (for example; *O. ptolemaica* and *O. hohenackeriana*), all of the species had wax on the their exines, which is different from others; these species were distinguished by this character, which seems effective in their pollination. In this section the species belong to subtype of tricolpate, however, these species are belonged to subtype of tricolpate type. *Onobrychis ptolemaica* and *O. susiana* are closely related to other genera of the Hedysareae, such as *Hedysarum*, *Alhagi*, *Eversmannia* and *Taverniera*, while *O. hohenocheriana* and other species of this section are related to another subtype.

The types of pollen apertures in the Hedysareae corresponded to those of its habit, that is, all perennials are tricolpate and annual herbs have tricolporate apertures, which is in agreement with Choi and Ohashi (1996). Although in this study we found some annual herbs that had tricolporate apertures, this type can only be found in section *Lophobrychis* of *Onobrychis*.

Increasing pollen grain size is a general trend among members of the Hedysareae tribe that has been described in a number of genera and families by many authors (Wodehouse, 1928; Van Campo, 1966; Nair, 1970; Walker, 1975; Saad and Taia, 1988). *Onobrychis* and *Ebenus* had bigger pollen grains than the other genera. Although the annual species of *Onobrychis* generally have larger pollen grains than the perennials, in our study pollen grain size showed no correlation with the annual and perennial growing habits.

Collected data showed that there might be a positive correlation between pollen grain morphology and habitat, which needs more investigation. The presence of a thick tectum with narrow lumina makes some species of the Hedysareae, such as *Ebenus stellata*, *Taverniera cuneifolia*, *Onobrychis crista-galli*, *Onobrychis susiana*, *Onobrychis tavernieraefolia* and *Onobrychis ptolemaica*, suitable for xerophytic habitats, and the ecological distribution of these species in southern Iran confirms this hypothesis. In addition, species such as *Onobrychis shahpurensis*, *Eversmannia subspinosa* and *Onobrychis laxiflora*, which are mesophytic, have a thinner tectum.

Results of this study showed that pollen grain is one of the most important tools for identifying and appropriately separating Hedysareae species from one another. Due to its diverse climatic conditions, Iran is one of the greatest centers of origin and genetic diversity for species of the Hedysareae tribe.

Species identification may increase the probability of selecting appropriate genes that contribute to environmental adaptation. They could be transferred to crop species for developing stress tolerant cultivars in plant breeding programs using conventional and molecular techniques.

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