

Palynological study of *Nonea* (Boraginaceae-Boragineae) in Iran

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Nonea Medik. is a genus of the Boraginaceae tribe Boragineae, with approximately 35 species worldwide. Examination of their palynological characters supported the separation of taxa, which is usually based on morphological and micromorphological reproductive features. Pollen grains of 10 taxa representing three groups of taxa in Iran, the *N. caspica* group, the *N. persica* group and the *N. lutea* group were studied, but *N. rosea* and *N. turcomanica* were not included. The results differed from previous studies in Iran. The *N. caspica* group, including *N. caspica*, *N. melanocarpa*, and *N. diffusa* has 5-6-zonocolporate pollen grains with a psilate-punctate tectum. The *N. persica* group, including, *N. p.* var. *persica*, var. *suchtelenioides*, and *N. pulla* have 4-zonocolporate pollen grains with a psilate-punctate tectum. Finally the *N. lutea* group, including *N. lutea* var. *lutea* and *N. l.* var. *flavescens*, have 5-zonocolporate pollen grains with a reticulate-micro reticulate tectum. *N. rosea* and *N. turcomanica* have 5-zonocolporate pollen grains with a scrobiculate tectum © 2011 Progress in Biological Sciences. Vol. 1, No. 2, 36-43.

KEY WORDS: micromorphology, equatorial reticulum, pollen grains, *Nonea*, Boraginaceae, Iran

Introduction

Nonea Medik. is a genus of the Boraginaceae tribe Boragineae, with approximately 35 species (Selvi et al., 2006) distributed from central-western Asia to the Atlantic area of southern Europe and northern Africa. Its main center of diversity extends through the Pontic-Caucasian mountain systems and the Irano-Turanian-Anatolian highlands, while fewer species occur in Europe and around the Mediterranean, especially in the semiarid parts of the Maghreb region from Libya to Morocco (Selvi et al., 2006). The *Flora Iranica* (Riedl, 1967) lists 12 species. *Nonea caspica* and *N. pulla* each including 4 subspecies.

Nonea is taxonomically problematic mainly due to difficulty in observation and identification of morphological characters with diagnostic value. De Candolle (1846) proposed three sections for *Nonea*; Sect. *Orthocaryum* A. DC, including the species with erect nutlets; Sect. *Cytrocaryum* A. DC, characterized by curved, reniform nutlets;

and Sect. *Nonea* (= *Cryptanthera* A. DC) comprising all species with anthers hidden in the corolla tube. The latter includes the great majority of species of *Nonea* (De Candolle, 1846). Nevertheless, this infrageneric classification does not reflect the remarkable morphological and chromosomal diversity that is emerging from recent studies. For example, a palynological survey of Boragineae has shown that *Nonea* contains the greatest diversity in the tribe with five pollen types none of which fits into De Candolle's sections (Bigazzi and Selvi, 1998). A survey of stigma forms in the tribe has confirmed *Nonea* as the most variable genus, with six types largely corresponding to the pollen types (Bigazzi and Selvi, 2000). *Flora Iranica* (Riedl, 1967), *Flora of Iran* (Khatamsaz, 2002), and other floras describe vegetative characters such as form and size of plants, leaf and bract, whereas our study showed that these characters are highly variable and unreliable for species determination (Pakravan et al., 2009).

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Table 1. List of *Nonea* species and their localities.

Taxon	Locality	Collector & Herbarium ID.
<i>N. caspica</i> (Willd.) Don.	Esfahan, 30km after Ardestan to Kashan, 113 m, N 33° 33' E 52° 10'	Pakravan 2900-AIUH.
<i>N. diffusa</i> Boiss. and Buhse	Tehran, Karadj to Ghazvin, 109 km after Tehran, 1220 m	Falatoury 2921-AIUH.
	Markazi, Tehran to Saveh, 22 km to Saveh, 1290 m	Falatoury 2932-AIUH.
	Tehran, 15 km after Ziaran to Taleghan, 2100 m	Falatoury 2904-AIUH.
<i>N. lutea</i> (Desr.) DC. var. <i>flavescens</i> Lipsky	Gilan, Bandar Anzali, -28 m	Mohamadjany 2905-AIUH.
	Mazandaran, Ramsar, -25 m	Gholizadeh 2906- AIUH.
<i>N. lutea</i> (Desr.) DC. var. <i>lutea</i>	Gilan, Rasht to Asalem, Laksar, -28 m	Falatoury 2907- AIUH.
	Mazandaran, Minodasht, 5 km after Jangal- deh	Nattaj 2908- AIUH.
<i>N. melanocarpa</i> Boiss.	Tehran, Saveh highway, to Firouzbahram, 1090 m	Falatoury 2909- AIUH.
	Tehran, Kan, Park-é Kohsar, 2116 m, N 35° 56' 09" E 50° 57' 03"	Keshavarzi 2926- AIUH.
<i>N. persica</i> Boiss. var. <i>persica</i>	Tehran, Lar (preserve), 2440 m	Falatoury 2911- AIUH.
	Esfahan, Kashan, Ghamsar	Pakravan 2912- AIUH.
<i>N. persica</i> Boiss. var. <i>suchtelenioides</i> (Riedl) Falatoury and Pakravan	Tehran, Lavasanat, Naran, 1700 m	Falatoury 2914- AIUH.
	Tehran, Ziaran to Taleghan, 15 km after Ziaran, 2100 m	Falatoury 2923- AIUH.
<i>N. pulla</i> (L.) DC.	Azarbaiejan, Meshkinshahr to Ahar, 30 km to Ahar, 1200 m	Pakravan 2915- AIUH.
	Hamedan, Ganjname	Rastipisheh 2916- AIUH.
<i>N. rosea</i> (Bieb.) Link	Azarbaiejan, Arasbaran, Kaleibar to Ma- keidi, 1200 m	Pakravan 2917- AIUH.
	Tehran, Mahdasht to Karadj, 1195 m	Falatoury 2919- AIUH.
<i>N. turcomanica</i> M. Pop.	Tehran, Khalij-e Fars highway	Falatoury 2924- AIUH.

AIUH, Herbarium of Alzahra University

Pollen morphology is useful at all levels of the taxonomic hierarchy due to the strong selection forces at work in dispersal, water-stress, pollination, germination, and stigmatic interactions (Bigazzi and Selvi, 1998). Previous palynological studies of the Boraginaceae were valuable in delimiting genera and in the study of evolutionary trends within the family (Clarke et al., 1979).

A comparative survey of pollen morphology in the Boragineae by light scanning, and transmission electron microscopy was made by Bigazzi and Selvi (1998) and multivariate analyses lead to the recognition of 15 pollen morphotypes. *Nonea* showed a wide variation in tectum ornamentation, shape of grains and number of apertures.

A micromorphological study of *Anchusa* L. and *Nonea* Medik. by Jamalou et al. (2006) reported pollen grains of all *Nonea* species as 4-colporate. In the present study the relationship between morphological, micromorphological and palynological characters was examined to distinguish the taxa of *Nonea* more precisely.

Material and methods

Pollen sampling

Pollen samples were taken from approximately 19 specimens belonging to 11 specific or sub-specific taxa. The complete list of these taxa with sampling locations is reported in Table 1. Up to 4 populations of each species and 30 pollen grains of each population were examined. For description of pollen characters we referred

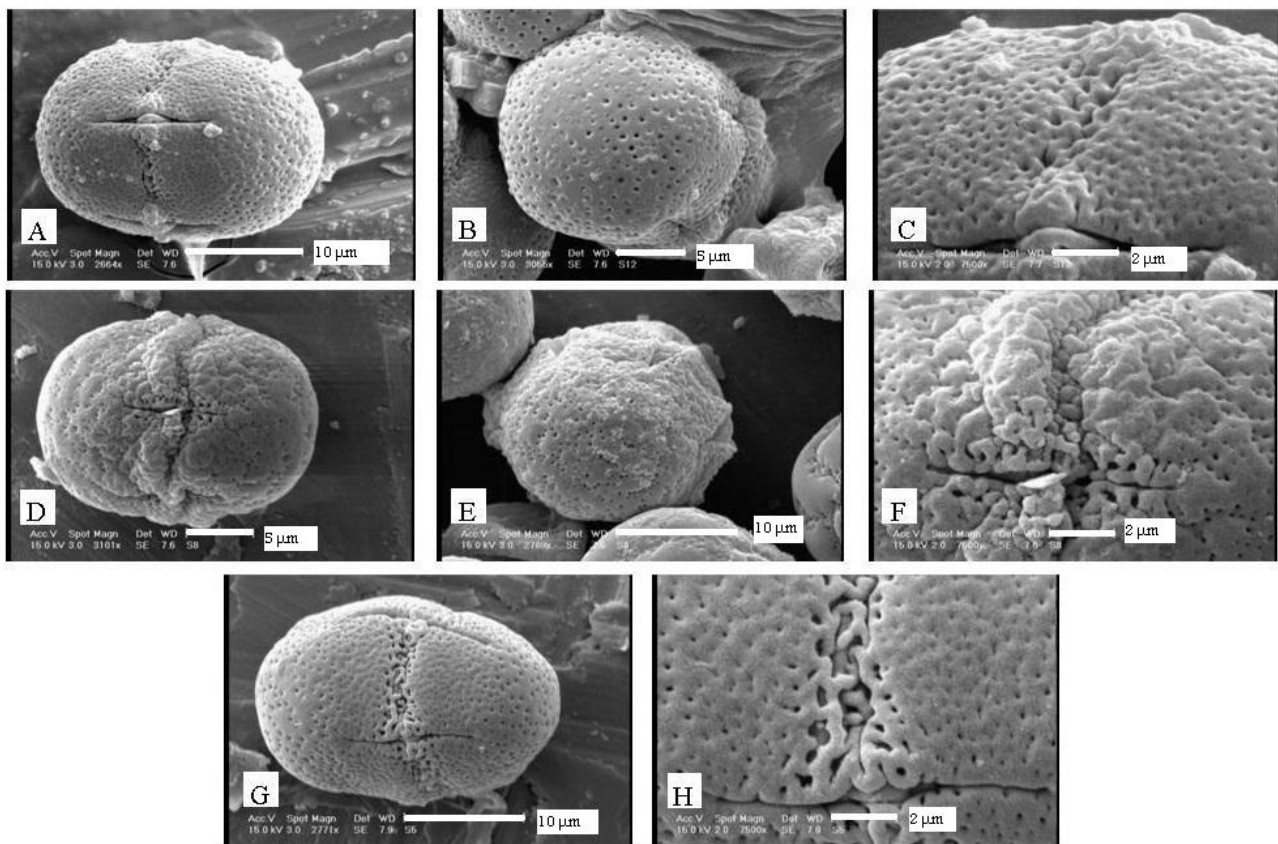


Fig. 1. SEMs of *Nonea caspica* group. A, *N. caspica*, equatorial view; B, *N. caspica* polar view; C, *N. caspica* aperture feature showing the thickened margins. D, *N. diffusa* equatorial view; E, *N. diffusa*, polar view; F, *N. diffusa*, aperture showing the thickened margins and equatorial reticulum. G, *N. melanocarpa* equatorial view; H, *N. melanocarpa* aperture feature.

to the glossary of pollen and spore terminology by punt et al. (2007).

Sample preparation

Samples were taken from herbarium specimens. Pollen grains for scanning electron microscopy were mounted on standard aluminum stubs, sputter-coated with gold and observed with a Philips XL30. We used this technique to study the tectum and 3D shape of pollen grains.

Pollen grains were treated with Farmer's fixative for 24 h and preserved in 70% ethanol. For light Microscopy, grains were observed with an Olympus DP12 video camera. We used this technique to measure distances and dimensions.

Results

General morphology

Grains were single, zonocolporate, and isopolar, with a polar length of 20-44 μm and equatorial length of 15-33 μm . The shape was mostly prolate or subprolate, occasionally prolate-spheroidal. The number of apertures ranged from 4 to 6. In equatorial outline, grains were elliptic, whereas in polar view the outline depended on the number of apertures, being square (4-colporate), to pentagonal or hexagonal (5-colporate or 6-colporate). Grains were always isocolporate, with equatorial apertures. Ectoa-pertures were 7.5-11.5 μm long and fusiform (slightly broader at the equator). The apertures were smooth or thickened by the presence of granules. The sculpture type of grain changed

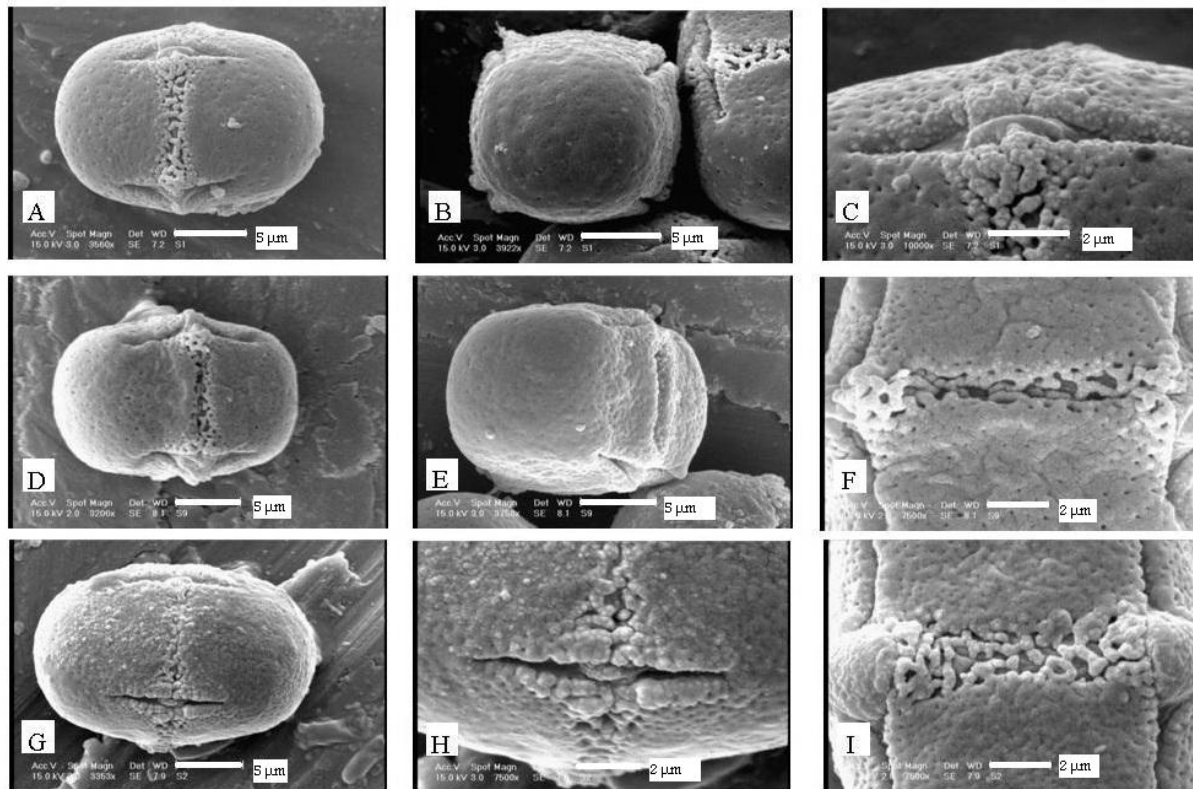


Fig. 2. SEMs of *Nonea persica* group. A, *N. persica* var. *persica* equatorial view; B, *N. persica* var. *persica* polar view; C, *N. persica* var. *persica* aperture feature showing the granular surface of equatorial reticulum and margins of aperture. D, *N. persica* var. *suchtelenioides* equatorial view; E, *N. persica* var. *suchtelenioides* polar view; F, *N. persica* var. *suchtelenioides* equatorial reticulum; G, *N. pulla* equatorial view; H, *N. pulla* aperture feature showing the thickened margins of aperture; I, *N. pulla* equatorial reticulum.

from the apocolpial to the mesocolpial regions. The tectum was continuous, psilate-punctate or scrobiculate with puncta of varying size; reticulate or microreticulate. The taxa showed a reticular sculpturing covered by granular processes along an equatorial furrow forming a more or less pronounced constriction. Four groups were recognized based on the morphology of pollen grains.

N. caspica group. Grains 5-(6) - zonocolporate, from small to small-medium sized, polar diameter (P)= 20-45 μm , equatorial diameter (E)= 15-35 μm . Shape from subprolate to prolate-spheroidal, P/E= 1-1.03. Outlines were elliptic or elliptic-circular in equatorial view and pentagonal or hexagonal with rounded margins in polar view. Ecto-apertures were fusiform, 10 μm

long and 0.5-1 μm wide; endo-apertures were lalongate. Tectum was psilate-punctate, with the exception of the equatorial reticulum, which was thickened on the margins of apertures. This group included *N. caspica*, *N. diffusa* and *N. melanocarpa* (Fig. 1, Table 2).

N. persica group. Grains 4 - zonocolporate, from small to medium sized, P = 20-30 μm , E = 16-25 μm , shape subprolate, P/E = 1.20. Outlines were elliptic or elliptic-rectangular in equatorial view and square-circular with rounded margins in polar view. Ecto-apertures were fusiform, 10 μm long and 1-2.5 μm wide, endo-apertures were lalongate. Tectum was diffusely psilate-punctate, with the exception of the equatorial reticulum which was covered by more or less pronounced granular processes and thickened in the margins of apertures. This group includes

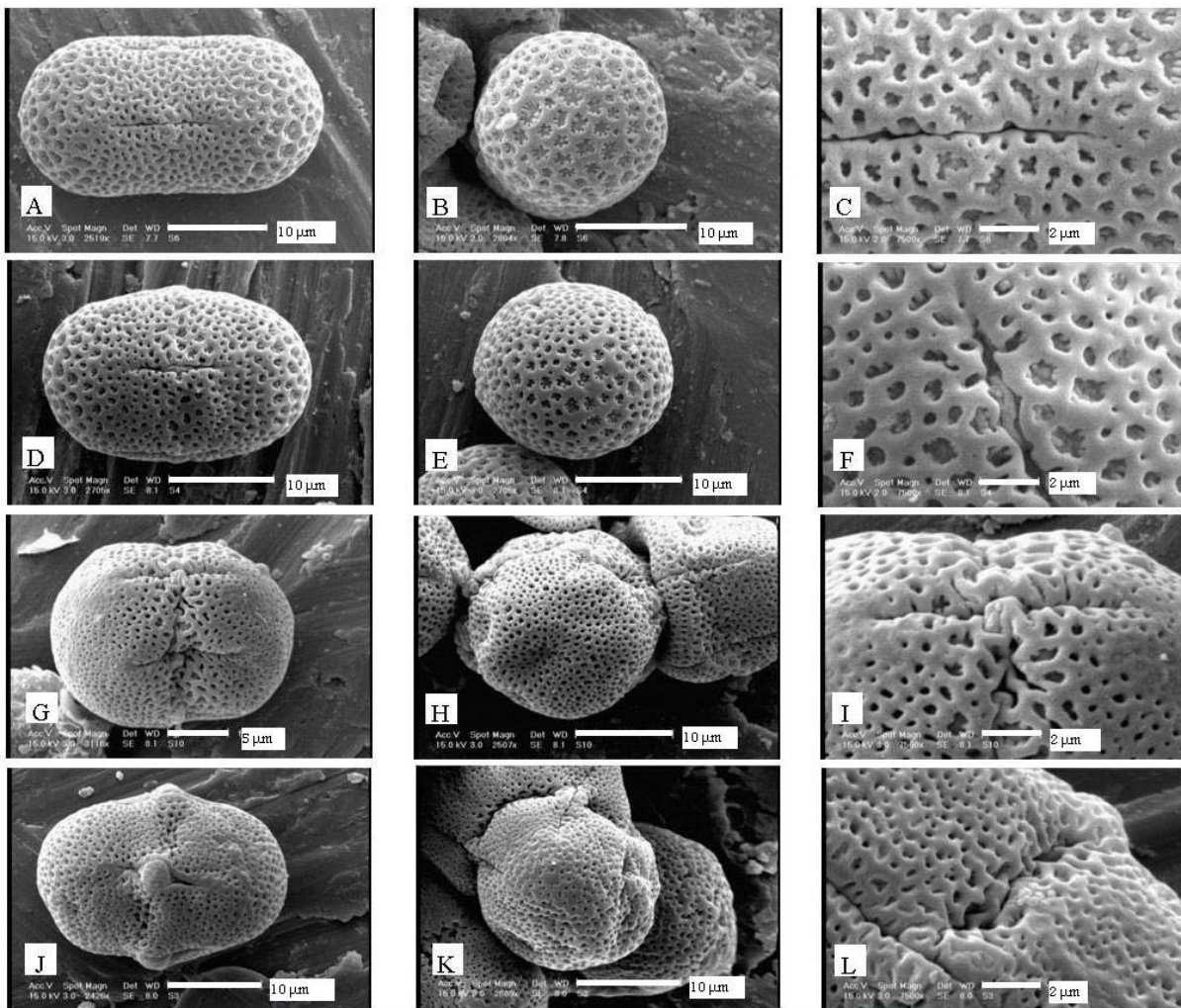


Fig. 3. SEMs of *Nonea lutea* group, *N. rosea* and *N. turcomanica*. A, *N. lutea* var. *lutea* equatorial view; B, *N. lutea* var. *lutea* polar view; C, *N. lutea* var. *lutea* aperture feature; D, *N. lutea* var. *flavescens* equatorial view; E, *N. lutea* var. *flavescens* polar view; F, *N. lutea* var. *flavescens* aperture feature; G, *N. turcomanica* equatorial view; H, *N. turcomanica* polar view; I, *N. turcomanica* equatorial reticulum and aperture feature; J, *N. rosea* equatorial view; K, *N. rosea* polar view; L, *N. rosea* equatorial reticulum and aperture feature.

N. persica var. *persica* and -var. *suchtelenioides* and *N. pulla*. This group was highly similar to *Pulmonaria obscura*-type (Bigazzi and Selvi, 1998; Selvi and Bigazzi, 2001) (Fig. 2, Table 2). *N. lutea* group. Grains 5-zonocolporate, medium sized, P = 35-45 μm, E = 25-32 μm, shape prolate, P/E = 1.40. Outlines were elliptical in equatorial view and pentagonal with rounded margins in polar view. Ecto-apertures were slightly fusiform, 10-11 μm long; endo-apertures were alongate. Tectum ranged from microreticulate to reticulate at the polar regions and the

equatorial reticulum. This group consists of *N. lutea* var. *lutea* and var. *flavescens*. Contrary to what has been previously reported (Bigazzi and Selvi, 1998) *N. rosea* shows distinct differences from this group (Fig. 3. A-F and M; Table 2). *N. rosea*. Grains 5-zonocolporate, medium sized, P = 25-35 μm, E = 18-28 μm, shape subprolate, P/E = 1.30. Outlines were elliptic-circular in equatorial view and pentagonal with rounded margins in polar view. Ecto-apertures were fusiform, 11 μm long and 1-2.5 μm; endo-apertures were alongate. Tectum was scrobicu

Table 2. Pollen characteristics of *Nonea* species

Characters Taxon	(E) Equatorial diameter (µm)			(P) Polar diameter (µm)			P/E	Pollen shape	Mean Exoaperture length (µm)	Mean Exoaperture distances (µm)
	Min	Max	Mean	Min	Max	Mean				
<i>N. caspica</i>	19.82	32.72	26.70	28.38	43.39	34.55	1.29	subprolate	11.28	9.10
<i>N. diffusa</i>	15.20	30.00	21.56	19.87	32.00	22.66	1.06	Prolate-spheroidal	7.76	9.00
<i>N. lutea</i> var. <i>flavescens</i>	26.29	29.82	28.27	37.56	40.25	38.90	1.40	prolate	10.60	9.03
<i>N. lutea</i> var. <i>lutea</i>	26.66	30.41	29.20	35.59	42.99	39.44	1.40	prolate	10.52	8.04
<i>N. melanocarpa</i>	24.74	28.78	26.08	30.80	34.30	32.25	1.20	subprolate	11.32	10.20
<i>N. persica</i> var. <i>persica</i>	16.52	19.34	18.44	20.04	23.51	21.98	1.19	subprolate	8.69	9.82
<i>N. persica</i> var. <i>suchtelenioides</i>	18.48	21.44	20.52	23.51	25.79	23.84	1.21	subprolate	10.20	11.18
<i>N. pulla</i>	21.38	24.36	22.65	25.92	29.42	27.50	1.21	subprolate	8.63	9.86
<i>N. rosea</i>	19.52	27.48	23.02	26.29	33.61	29.77	1.30	subprolate	11.18	10.39
<i>N. turcomanica</i>	29.85	31.49	30.35	34.35	36.39	35.41	1.16	subprolate	8.93	11.14

late to reticulate at the equatorial reticulum (Fig. 3. J-L, Table2).

N. turcomanica. Grains 5-zonocolporate, medium sized, P = 33-37 µm, E = 29-32 µm, shape subprolate, P/E = 1.16. Outlines were elliptic-circular in equatorial view and pentagonal with rounded margins in polar view. Ecto-apertures were slightly fusiform, 9 µm long; endo-apertures were alongate. Tectum was scrobiculate, with the exception of the equatorial reticulum, which was thickened in the margins of apertures (Fig. 3. G-I and N; Table 2).

Key to pollen grains of *Nonea* species

1. Grains 4-zonocolporate, tectum diffusely psilate-punctate, some pores not hollow, equatorial reticulum covered by more or less pronounced granules.....*N. persica* group (*N. persica* var. *persica* and var. *suchtelenioides* and *N. pulla*)

1. Grains 5(6)-zonocolporate, various tectum, equatorial reticulum not covered by granules.....2

2. Grains 5-zonocolporate, prolate, tectum reticulate or microreticulate, equatorial reticulum obscure.....*N. lutea* group (*N. lutea* var. *lutea* and var. *flavescens*)

2. Grains 5(6)-zonocolporate, tectum except in equatorial reticulum not reticulate, equatorial reticulum apparent.....3

3. Tectum scrobiculate.....4

3. Tectum psilate-punctate....*N. caspica* group (*N. caspica*, *N. diffusa* and *N. melanocarpa*)

4. Endoaperture unapparent, equatorial reticulum and aperture depressed..... *N. turcomanica*

4. Endoaperture apparent, equatorial reticulum slightly depressed.....*N. rosea*

Discussion

Morphological and micromorphological studies have altered the taxonomy of *Nonea* (Pakravan et al., 2009). In this study the relationship between morphological, micromorphological and palynological characters was examined. Results

showing differentiation of palynological characters supports the separation of taxa based on reproductive characters. The results of this study did not conform to a previous study that described all pollen grains of this genus as 4-colporate (Jamalou et al., 2006). Furthermore, *N. flavescens*, *N. anchusoides* and *N. turcomanica* have been illustrated as having curved nutlets (Jamalou et al., 2006), but in *Flora Iranica* (Riedl, 1967) and other studies they have been characterized with semi-curved or straight nutlets (Khatamsaz, 2002, Selvi, et al. 2002; Pakravan et al., 2009).

In the present study, the pollen grains of 10 taxa representing three groups of species of *Nonea*, *N. caspica*, *N. persica* and *N. lutea* were distinguished. Due to unique characteristics such as a scrobiculate tectum, *N. rosea* and *N. turcomanica* have not been assigned to any of these groups.

N. caspica group. *Nonea caspica* is highly variable, presently divided into four subspecies, based on vegetative characters such as shape and size of leaves (Riedl, 1967). Because of distinctive reproductive characters, separation of these taxa into three species as *N. caspica*, *N. melanocarpa*, and *N. diffusa* is more reasonable (Pakravan et al., 2009). This separation is supported by palynological characters. Although they show similarities that categorize them as a group, they are distinguishable from one another. In addition, an examination of specimens of *N. caspica* that differed in size and shape of leaves showed they possessed the same type of pollen grains. Contrary to what has been reported previously (Bigazzi and Selvi, 1998) 4-zonocolporate grains have never been found in this group, so was distinguishable from other members of *Pulmonaria obscura*-type which are 4-zonocolporate.

N. persica group. Due to their similarity, *N. suchtelenioides* Riedl and *N. persica* Boiss. have been considered two varieties of *N. persica* Boiss., and a new combination has been suggested, *N. persica* Boiss. var. *suchtelenioides* (Riedl) Falatoury and Pakravan (Pakravan et al., 2009; Selvi and Bigazzi, 2001). Palynological study confirms this. Taxa of this group are similar and cannot be separated by palynological

characters, but *N. pulla* shows small differences from the two other taxa in morphological and micromorphological characters such as shape of nutlets, faucal scale indumentums, and color of throat.

N. lutea. *Nonea lutea* and *N. flavescens*, formerly recognized as distinct species (Khatamsaz, 2002; Riedl, 1967), do not have sufficiently differing characteristics to be distinguishable as separate species, so have been treated as varieties of the same species (Pakravan et al., 2009). Remarkable similarity in their pollen grains supports the conclusion that they are not separate.

N. rosea. This taxon, contrary to what has been reported previously (Bigazzi and Selvi, 1998), is not similar to *N. lutea* in palynological characters. In our study, specific differences have been defined that discriminate it from the *N. lutea* group, for example, ornamentation of the tectum. *Nonea rosea* also has specific morphological and micromorphological characters that help to distinguish it, such as color of limb and shape of nutlets (Pakravan et al., 2009).

N. turcomanica. In addition to its unique morphological and micromorphological characters such as shape of nutlets, color and size of limb, and shape of leaves (Pakravan et al., 2009), *N. turcomanica* has particular palynological characters, so it can be easily distinguished from species of the *N. lutea* group by ornamentation of the tectum and the shape of pollen grains.

Consequently, palynological characters support the results of studies that are based on morphological and micromorphological reproductive characters (Pakravan et al., 2009), and, as expected, they do not correspond to vegetative characters. In other words, when two taxa differ in color of limb, color of throat, faucal scales and shape of nutlets, their pollen grains also have distinguishing characteristics, but variation in size of plant and shape and size of leaves may be seen in two taxa that have the same type of pollen grain.

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