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Pollen Micro-morphology of the *Minuartia* Species (Caryophyllaceae) in Iran

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Abstract The present study compared pollen micro-morphological characters among 20 Iranian *Minuartia* species. For this purpose, mature pollen grains taken from unopened flowers, were prepared, fixed and exhaustively investigated using Scanning Electron Microscopy (SEM). In order to perform the pollen micro-morphology of *Minuartia*, and to find its significance in taxonomy of the group, qualitative and quantitative variables related to the shape, size, ornamentations and pores were studied. Cluster and PCA analyses of qualitative and quantitative data were performed to demonstrate the pollen grain similarities among the species. According to our results, *Minuartia* species exhibit either sub-spherical or polyhedral pollen shapes. Pollen size also varies among different species. The longest polar axis length (P) belongs to *Minuartia meyeri* Bornm. (34.3±0.26µm) and the smallest one to *M. montana* L. (15.8±0.26µm). Pore ornamentations differ from prominent granular to slightly or distinctly sunken granular. The number of pores also varies considerably depending on species. It ranges from 10 (in *M. meyeri* and *M. acuminata* Turrill) to 24 (in *M. subtilis* Hand.-Mazz.) on two pollen hemispheres. The most reliable characters in this study were pore diameter (annulus included) (D), equatorial diameter (E), polar axis length (P), the distance between two pores (d), pollen outline, Pore diameter (annulus excluded) (R), annulus diameter (a), P/E ratio, Puncta diameter and Echini diameter respectively. Echini vs puncta (Ec:Pu) diameter ratio appeared to be crucial for the distinction of some closely related species such as *M. sublineata* Rech.f. and *M. lineata* (Boiss.) Bornm., as well as *M. montana* and *M. sclerantha* (Fisch & C. A. Mey.) Thell. Moreover, three out of the 20 species have D: d ratio (pore diameter: the distance between two pores ratio) only ≥ 1µm. According to our results, some differences in quantitative and qualitative palynological characters of similar species were observed that could be useful. Despite the diagnostic value of palynological data at the species rank, it was not useful to circumscribe any taxonomic group at the higher ranks.

Keywords Caryophyllaceae, Iran, micromorphology, Minuartia, Morphological similarity, Palynology, SEM micrographs

1. Introduction

Caryophyllaceae Juss., is a cosmopolitan family with 86 genera and approximately 2200 species. Most of these genera occur in temperate regions of the northern hemisphere and comprise both annual and perennial herbaceous plants, sub shrubs, more rarely shrubs or small trees that are usually monocious and rarely diocious (Bittrich 1993).

The genus *Minuartia* L. [(Caryophyllaceae, subfam. Minuartioideae (Alsinoideae), Tribe Alsineae according to Takhtajan 1997)], is one of the smallest genera within the family; however, it includes many taxonomically difficult species with unclear taxa delimitations. *Minuartia* spp., have an extensive distribution and are endemic to Asia, Europe and North America (Schischkin 1936; Halliday 1964; Mc Neill 1967; Meikle 1977; Kamari 1997). The genus is recognized via its ex-stipulate leaves, veined sepals, three valves in capsule, three styles and also nearly rounded petal apex (Mc Neill 1967; Rechinger 1988). According to Rechinger (1988) only six out of the 21 species are endemic to the Iranian plateau, among which two species i.e., *M. aucheriana* Bornm. and *M. acuminata*, are endemic to the Iran. *Minuartia* was introduced by Linnaeus (1753) with three species *M. dichotoma, M. campestris* and *M. montana* among which only the last one exists in Iran. Fourteen out of 35 species listed in *Flora Orientalis* currently exists in Iran (Boissier 1867). According to Rechinger (1988), it comprises 21 species in Iran in two different subgenera, i.e., *Spergella* (Fenzl) Mc Neill, (with one annual species i.e., *M. picta* Bornm. and *Minuartia* (with 20 species in six sections) in Iran. Considering morphological similarities among the Iranian species, their taxonomic positions had been controversial for a long time. For this purpose, complementary studies such as seed micro-morphology

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(Mostafavi et al. 2013), pollen micro-morphology, karyology, molecular phylogeny are necessary to support or confirm current taxonomical data. Fior et al. (2006) worked on six *Minuartia* species belonging to three different subgenera, i.e. *Minuartia, Rhodalsine* (J.Gay) McNeill, and *Spergella.* Among the species studied by them, only *Minuartia picta*, was in common with the Iranian species. This species showed closely relationships to some *Arenaria* species in the parsimony strict-consensus tree based on simultaneous analysis of ITS and matK sequences. Also, Harbaugh et al. (2010) confirmed that Minuartioideae is not monophyletic and they called some genera, such as *Minuartia* and *Arenaria,* polyphyletic based on chloroplast genome sequences. The results of Greenberg and Donoghue (2011), based on nuclear ribosomal ITS region and five chloroplast genes and intergenic spacers, with a few exceptions, support the tribal classification presented by Harbaugh et al. (2010). Greenberg and Donoghue (2011) worked on only 20 species of the genus (none of them except *M. picta* existed in Iran) and found that *Minuartia* species appear in tribes Sclerantheae (A.L. Juss.) DC., Sagineae Tanf., Sperguleae Dumort., and Eremogoneae Rabeler & W.L.Wagner. Therefore, they are not strictly monophyletic. Caryophyllaceae pollen morphology has been studied (Erdtman 1952; Chanda 1962; Mc Neill and Bassett 1974; Moore and Web 1978; Ghazanfar 1984; Bittrich 1993; Taia 1994; Yildiz 2001; Perveen and Qaiser 2006; Ataslar, Potogluerkara, and Tokur 2009; Külköylüoglu, Yildiz, and Minareci 2009; Eröz and Ataslar 2010; Yildiz, Çirpici, and Dadandi 2010; Yildiz et al. 2011). These studies revealed various pollen shapes for plants belonging to this family. Depending on species, pollen grain could be tricolpate, sub-oblate to sub-prolate, porate, pantocolpate, spherical or rounded polyhedral (Mc Neill and Bassett 1974). Tectum is mostly punctitegillate, sometimes anulopunctate, rarely reticulate (*Silene* L. spp. and *Cerastium indicum* Wight & Arnott), spinulate or microechinate. Pollen shape in genus *Minuartia* (Subgen. *Rhodalsine* (J. Gay) Mc Neill is trizonocolpate with rather thin exine layer and is completely different from other species belonging to Alsinoideae. All the species placed in this subfamily obviously have pantoporate pollen grains with thick exine layer (other *Minuartia* species is included). Pore number is variable in Alsinoideae (12-40) and Caryophylloideae (15-38) (Erdtman 1968; Bittrich 1993; Mc Neill and Bassett 1974). Therefore this character could be more useful for determining taxa. In the Caryophyllaceae family most of the species like *Arenaria* L.*, Minuartia, Dianthus* L., *Stellaria* L., *Gypsophila* L. and so forth exhibit pantoporate pollen grains and spinulose-scabrate to punctate tectum (Bittrich 1993; Perween and Qaiser 2006). According to the recent Turkish study (Ataslar, Potogluerkara, and Tokur 2009), *Gypsophila* species have spheroidal and pantoporate pollen grains. The exine is tectate and displays granulate-microechinate ornamentations in most species. It has been shown that the most important characters in genera with pantoporate pollen grains like *Minuartia*, are pore diameter, presence or absence of annulus, pore diameter (annulus included), the distance between two pores, pollen size and finally surface ornamentations (Punt and Hoen 1995). Based on apertural type and exine surface ornamentations, some *Minuartia* species were placed in *Moehringia trinervia* (L.) Clairv. type and some of them fell into *Minuartia rubra* Mc Neill type (Punt and Hoen 1995). Among *Minuartia* species belonging to the *Moehringia trinervia* type only *M. hybrida* (Vill.) Schischk. exists in Iran. In this type, pollen shape is usually ellipsoidal and less frequently spheroidal. Pores are circular to slightly elliptic, medium in size, slightly or distinctly sunken, annulus is narrow, operculum a shield beset with small microechinate granules with different size. The distance between two pores is larger than or sometimes equal to the pore diameter, annulus included. *Minuartia hybrida* group (*M. hybrida* and *M. verna* (L.) Hiern) remains nevertheless a rare exception since the distance between two pores is always shorter or equal to the pore diameter (annulus included). The exine is thick to very thick and usually become distinctly thinner towards the pores. Its ornamentations are microechinate with distinct puncta. Echini are distinct and irregularly arranged all over the surface. Its size and density is variable but it is usually small and usually not decreasing in number towards the pores. Puncta is usually larger or sometimes smaller than echini. Columella varies in size and is usually crowded. The *Moehringia trinervia* type is characterized by its narrow annulus, a distinctly larger distance between two pores than the pore diameter, its ellipsoidal shape and finally the presence of an operculum consisting of a shield beset with granules. The pore diameter is often relatively small (2-4 μm but in some species up to 6.5 μm). The outer margin of the annulus is frequently irregular because of the attached columellae. In this type, the number of pore ranges from 12-30. The *Minuartia rubra* type is *M. setacea* (Thuill.) Hayek and *M. rubra*, both absent from Iran. In these species, pollen grains are often small (with about 22 to 38 μm in diameter), spheroidal, pantoporate with a variable pore number ranging from 11 to 13 (always less than 15) (Punt and Hoen 1995). The pores are either circular or elliptic in outline, medium in size, sunken, with narrow or medium size annulus. Its operculum is consisted of a number of granules. In this type columella is usually small, uniform in size, more or less circular and often densely crowded (rarely widely spaced). The thick exine is microechinate with small puncta (much smaller than echini) and shows a decreasing thickness toward the pore. Finally, the *Minuartia rubra* type is characterized by its few small pores (usually around 12), distinct polygonal outline, large distance between two pores and relatively thin exine (2.5-3.5 μm). The pollen type has some resemblances with the *Moehringia trinervia* type but differs in the outline and number of pores. Based on the latest morphological studies on the taxonomy of the Iranian species, seven sections are detected for Subgen. *Minuartia.*, among which three sections i.e., sect. *Sabulina*, sect. *Acutiflorae* and sect. *Minuartia,* have very closely related species (Based on an unpublished data). *Minuartia*

umbellulifera (Boiss. & Balansa) Mc Neill, *M. juniperina* Maire & Petitm., *M. glandulosa* (Boiss. & Huet.) Bornm., *M. aucheriana, M. sublineata, M. lineata* and finally *M. sabalanica* Assadi & Mostafavi are closely related taxa that are placed in sect. *Acutiflorae*. Also, due to many morphological similarities, species *M. micrantha* Schischk., *M. hamata* Mattf., *M. meyeri, M. decipiens, M. sclerantha* and *M. montana* are all placed in the separate section i.e., sect. *Minuartia*. Section *sabulina* is another section of the subgen. *Minuartia* with four species i.e., *M. hybrida*, *M. mesogitana* Hand.-Mazz., *M. subtilis* and *M. urumiensis* Mattf. These species are also very closely related ones taxonomically. In this study, pollen micro-morphology of 20 *Minuartia* species belonging to two subgenera is investigated for the purpose of identifying some closely related taxa morphologically. For this reason, some characters that were mentioned as important in the previous studies on pollen grains of the family were used.

2. Materials and Methods

Materials used in this study are listed and their vouchers are mainly preserved at TARI (Table 1).

Micro-morphological data were obtained using SEM (LEO 440i). Each sample was coated with 550Å-thick layer of gold in a Polaron SC7610 vacuum coating apparatus for 180s. Three pollens of each species were measured at the average using Carnoy, a digital measurement tool (Schols et al. 2002) and Standard deviation (SD) was added for each character using SPSS (version 22.0.0) software. We used some qualitative and quantitative characters i.e., polar axis length, equatorial diameter, P:E ratio, pore number, pore diameter (annulus including), annulus diameter, pollen shape, pollen ornamentations pore ornamentations as depicted in Tables 2 and 3. The terminology used follows that recommended by Punt et al. (1994).

Cluster analysis (Ward`s Hierarchical Clustering method) and Principal Component Analysis (PCA) were performed using SPSS software. Cluster analysis is a convenient method for identifying homogenous groups of cases called clusters. Prior to the cluster analysis, our data were standardized (Range of 0 to 1). Squared Euclidean distance was used to analyze interval scaled data (The Euclidean distance could only be applied when all of the variables are continuous). Measure was rescaled in 0 to 1 ranges. PCA is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. It is a variable- reduction technique that shares many similarities to exploratory factor analysis. Its aim is to reduce a larger set of variables into principal components. In the present study, we used Varimax Rotation Method in PCA. Two factors were extracted. Factors were divided into two axes X and Y. Simple scatter plot was designed for species based on characters used in this study to show the relationships among taxa.

3. Results

Quantitative and qualitative characters are presented in Tables 2 and 3 (terminology according to Punt et al. 1994).

3.1. Quantitative Characters

3.1.1. Pollen Size

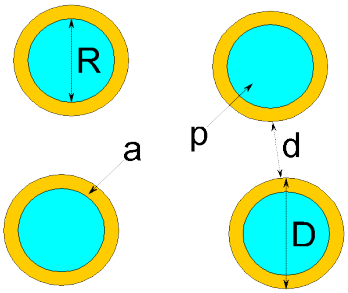
Polar axis length (P), equatorial diameter (E) and P:E ratio were measured precisely. As shown in the table 2, the longest and smallest P were both devoted to annual species i.e., *M. meyeri* (34.3±0.26 µm) and *M. montana* (15.8±0.26 µm) respectively (Table 2). Pollen grains of the perennial *Minuartia glandulosa* (27.2±0.3 µm) and the annual *M. montana* (13.5±0.1 µm) had the longest and smallest E, respectively. P:E ratio was adjustable in all species from ~1 µm (*M. picta* and *M. aizoides* Bornm.) to 1.38±0.01 µm (*M. meyeri*).

3.1.2. Pore Size and Number

Minuartia aucheriana (6.4±0.754 µm) and *M. mesogitana*, (2.4±0.1 µm) had longest and smallest pore diameter (annulus included) among the other taxa respectively. The largest annulus diameter belonged to *M. meyeri* and *M. aucheriana* and two species i.e., *M. sublineata* Rech. f. and *M. decipiens* had no annulus in around. The smallest distance between pores was observed in *M. lineata* (3.4±0.26 µm) while *M. meyeri* had the largest one (7±0.2 µm). The distance between two pores in *M. aucheriana, M. lineata* and *M. sublineata* was approximately equal or slightly shorter than pore diameter. In the other species this distance was longer than the pore diameter (Table 2).

The number of pores was variable from five to 12 in one hemisphere therefore 10-24 pores are present in two hemispheres (counting based on Monoszon 1952). *Minuartia subtilis* had maximum pore number and *M. meyeri* as well as *M. acuminata* showed minimum pore number among the studied species. Overall, *M. subtilis, M. mesogitana, M. hybrida, M. montana, M. lineata* and finally *M. picta* pore numbers were 16 or more than 16. Others had 10 to 14 pores on two pollen hemispheres.

The relation between pore diameter and pore distance obtained through our SEM analysis is shown (Figure 1).



R = pore diameter; D = pore diameter, annulus included; d = the distance between two pores; a = annulus; P=pore (according to Punt & Hoen, 1995)

Figure 1. Relation between pore diameter and pore distance

Table 1. The list of the species used for pollen micro-morphological study and their localities

Taxa	Localities
M. picta Bornm.	Yazd: Nodushan to Hafthar, ca. 1400 m, *Mozaffarian* 77388 (TARI) Fars: Shiraz, Pachenar, 1480m, *Foroughi* 3829 (TARI) Tehran: Kavir protected area, Siahkuh, ca. 1250m, *Runemark, Foroughi & Assadi* 19477 (TARI)
*M. aizoides*Bornm.	Azarbayejan: Shabil, Sabalan Mts., 3450m, *Forough & Assadi* 13927 (TARI) Azarbayejan: Ardabil, Ghotursuii, Sabalan Mts., 2400m, *Termeh & Musavi* 16359 (E)
M. acuminata Turrill	Azarbayejan: Urumieh, Silvana, Mavana, 2600 m, *Mozaffarian* 69893 (TARI) Hamadan: North slopes of Alvand mountain, 2500-3300m, *Mozaffarian* 64946 (TARI) Azarbayejan: Arasbaran protected area, Kalan mountain, *Assadi & Sardabi* 24297 (TARI)
*M. recurva*Schinz. & Thell.	Azarbayejan: Arasbaran protcted area, Kalan mountain on the west side of gaurd station, 2470 m, *Jamzad, Zehzad & Izadpanah* 70234 (TARI) Mazandaran: S Ramsar, 3100m, Runemark & Masoumi 21797 (TARI) Tehran: Shemshak, Dizin, 3000-3500m, Mozaffarian & Mohammadi 49074 (TARI)
*M. meyeri*Bornm.	Isfahan: Semirom, 35 km Semiron to Chaharrah, 2200 m, *Nowrouzi* 3760 (TARI) Azarbayejan: Urumieh, Razhan, 1800m, Mostafavi 1250 (IAUH) Hormozgan: Bandar-Abbas, above Geno mountain, 2300m, Wendelbo & Foroughi 15385 (TARI) Fars: Bamu protected area, 1800-2000m, Wendelbo & Assadi 17760 (TARI) Tehran: Telu, 1930m, Mehregan, Mostafavi & Pursakhi 1243 (IAUH)
M. decipiens (Fenzl) Bornm.	Hormozgan: Bandar-Abbas, ca. 50 km north of Senderk Araghein, 1100 m, *Mozaffarian* 44598 (TARI)
M. micrantha Schischk.	Azarbayejan: Maku, 1230 m, *Foroughi* 3871 (TARI) Azarbayejan: W Bazargan, 1500-1700m, Assadi & Mozaffarian 30199 (TARI) Azarbayejan: ca. 15km Maku, in Marand way, 1200-1400m, Assadi & Mozaffarian 30158 (TARI)
M. montana L.	Azarbayejan: Arasbaran protected area, mountains south of Vaighan, 1200 m, *Assadi & Masoumi* 20364 (TARI) Lorestan: Sefid-kuh, 1800-1900m, Wendelbo & Assadi 16664 (TARI)
M. sclerantha (Fisch &C. A. Mey.) Thell.	Azarbayejan:Arasbaran protected area, Veinagh to Ghaghalu, ca. 1000 m, *Assadi & Masoumi* 20496 (TARI)
*M. hamata*Mattf.	Tehran: Damavand rocky mountains, north of Veliran village, 1850 m, *Mozaffarian* 32271 (TARI) Fars: Bamu protected area, 1800-2000m, *Wendelbo & Foroughi* 17732 (TARI) Azarbayejan: Urumieh, Silvana, 1600m, *Mostafavi* 1255 (IAUH) Tehran: Telu, 1930m, *Mehregan, Mostafavi & Pursakhi* 1242 (IAUH) Mazandaran: Kelardasht, 1200m, *Foroughi* 1373 (TARI) Kermanshah: Bisotun to Songhor, 1550-1600m, Hamzeh & Asri 87798 (TARI)
M. hybrida (Vill.) Schischk.	Lorestan: Sefid-kuh mountain, Farah-kash, 1800-1900 m, *Wendelbo & Assadi* 16685 (TARI) Khorassan: ca. 45km of Sirvan, Golul-sarani protected area, 1600-2300m, *Assadi & Masoumi* 50495 (TARI) Azarbayejan: 18 km from Sardasht to Piranshahr, 1000m, *Runemark & Foroughi* 19883 (TARI)
M. mesogitana Hand.-Mazz.	Azarbayejan: 62 km from Ahar, on the road to Tabriz, 1400 m, *Assadi & Masoumi* 20582 (TARI)
M. subtilise Hand.-Mazz.	Tehran: Haraz road, Lar valley, 2400m, *Sanii & Assadi* 14167 (TARI) Isfahan: Natanz, Karkas mountain, 3000-3300m, *Foroughi & Assadi* 18074 (TARI) Azarbayejan: SW of Khalkhal, 1800m, *Wendelbo & Assadi* 27853 (TARI)
M. umbellulifera (Boiss.& Balansa) Mc Neill	Azarbayejan: Urumieh, Rajhan, 2500-3000m, *Zehzad* 2377 (TARI)
M. sabalanica Assadi & Mostafavi	Azarbayejan: Sabalan mountain, 2900 m, *Foroughi* 6120 (TARI)
*M. aucheriana*Bornm.	Isfahan: Fereidoon shahr, near Sibak village, 2900 m, *Assadi & Khatamsaz* 76484 (TARI) Lorestan: Dorud, between Saravand & Gohar lake, 2300-3500m, *Mozaffarian & Sardabi* 42331 (TARI) Bakhtiari: Shahre-kurd, Kelar mountain, 2750-2956m, *Mozaffarian* 57740 (TARI)
M. glandulosa (Boiss. & Huet) Bornm.	Azarbayejan: Urumieh, Pesan, Bani top mountain, 2495 m, *Mozaffarian* 87504 (TARI) Mazandaran: S Ramsar, 2950m. *Runemark & Masoumi* 21640 (TARI) Azarbayejan: between Targevar & Oshnavieh, 1850-2100m, *Mousavi & Zargari* 36829 (E)
M. lineata(Boiss.)Bornm.	Azarbayejan: South slope of Sahand-kuh mountain, 2600-3500 m, *Assadi & Mozaffarian* 30761 (TARI) Azarbayejan: Ardabil, Sabalan Mts., Shabil, 3000m, *Foroughi & Assadi* 13936 (TARI) Tehran: Karaj-Chalus way, above Kandovan, 3200m, Assadi & Heidarnia 95697 (TARI) Khorassan: W Bojnourd, 2100m, *Joharchi & Aydani* 35608 (FUMH) Hamadan: Mishan Sq., 2700-3000m, *Rostami* 1276 (TARI) Semnan: Nizva mountain, 3500-3900m, *Assadi & Ranjbar* 82115 (TARI)
*M. sublineata*Rech.f.	Fars: North of Dena mountain, 2800-3600 m, *Assadi & Mozaffarian* 31483 (TARI) Azarbayejan: Sahand, 2500-2850m, *Mozaffarian & Mohammadi* 37508 (TARI) Azarbayejan: 34km Giwi to Ardabil, 2250-2600m, *Mozaffarian & Nowrouzi* 31483 (TARI)
M. khorassanica Assadi & Mostafavi	Khorassan: west of Ghaen, Tajan mountains, 1900 m, *Raafei* 30924 (FUMH)

Table 2. Comparison of quantitative pollen micro-morphological data in *Minuartia* species (measuring M±SD for each parameter): Abbreviation: P: polar axis length; E: equatorial diameter; N: pore number in equatorial surface; D: pore diameter (annulus included); R: pore diameter (annulus excluded); a: annulus diameter; d: the distance between two pores

	Characters												
Taxa	**subgenera-sections**	**P (µm)**	**E (µm)**	**P/E (µm)**	**N**	**D (µm)**	**R (µm)**	**a (µm)**	**d (µm)**	**D/d (µm)**	**Echini Diameter (µm)**	**Puncta diameter (µm)**	**Echini/ Puncta diameter (µm)**
M. aucheriana	*Minuartia-Acutiflorae*	31.4±0.2	25.5±0.5	1.22±0.208	14	6.4±0.754	4.8±0.4	1.5±0.173	6.2±0.2	1.03±0.151	0.16±0.04	0.15±0.602	1.06±0.06
M. umbellulifera	*Minuartia-Acutiflorae*	26.8±0.72	23.7±1.17	1.12±0.03	12	5±0.5	4.2±0.75	0.8±0.3	5.9±0.45	0.84±0.08	0.22±0.02	0.21±0.01	1.01±0.09
M. sabalanica	*Minuartia-Acutiflorae*	23.9±0.95	20.3±1.3	1.17±0.06	14	4.2±0.52	3.2±0.4	1±0.3	5±0.6	0.85±0.21	0.27±0.02	0.1±0.01	2.56±0.46
M. lineata	*Minuartia-Acutiflorae*	25.3±0.98	20±1.05	1.2±0.11	18	3.25±0.25	3±0.5	0.6±0.1	3.4±0.26	0.95±0.14	0.16±0.005	0.15±0.05	1.2±0.44
M. sublineata	*Minuartia-Acutiflorae*	21.9±0.1	19.2±0.34	1.13±0.02	14	5.3±0.26	5.3±0.26	0	4±0.5	1.33±0.1	0.18±0.02	0.1±0.02	1.75±0.52
M. glandulosa	*Minuartia-Acutiflorae*	33.7±0.52	27.2±0.3	1.2±0.11	12	5.7±0.17	5.2±0.26	0.5±0.1	6.9±0.36	0.8±0.1	0.2	0.12±0.25	1.62±0.34
M. acuminata	*Minuartia-Lanceolatae*	28.8±0.26	22.6±0.26	1.273±0.25	10	4.483±0.43	4.4±0.36	0.3±0.1	6.7±0.26	0.666±0.09	0.246±0.05	0.16±0.03	1.53±0.50
M. recurva	*Minuartia-Plurinerviae*	25.7±0.36	19.9±0.17	1.28±0.01	12	2.5±0.1	2±0.1	0.5±0.1	5.5±0.1	0.45±0.01	0.17±0.01	0	0
M. aizoides	*Minuartia-spectabilis*	28±0.5	26.8±0.26	1.04±0.01	12	4.8±0.2	3.8±0.17	1±0.1	5.9±0.1	0.8±0.02	0.28±0.17	0.2±0.01	1.35±0.005
M. picta	subgen. *Spergella*	19.7±0.1	19.4±0.26	1.01±0.01	18	3.8±0.17	3.6±0.26	0.29±0.04	3.7±0.13	1.02±0.03	0.15±0.01	0.15±0.01	1.04±0.14
M. mesogitana	*Minuartia-sabulina*	18.6±0.45	16.8±0.35	1.1±0.01	18	2.4±0.1	2±0.2	0.4±0.08	3.6±0.1	0.66±0.02	0.26±0.02	0.11±0.01	2.45±0.48
M. subtilis	*Minuartia-sabulina*	26.4±0.17	20.1±0.1	1.31	24	3±0.1	2.2±0.1	0.8±0.1	4.18±0.16	0.71±0.04	0.21±0.01	0.11±0.01	1.91±0.16
M. hybrida	*Minuartia-sabulina*	24.5±0.26	20.3±0.1	1.19±0.01	18	3±0.26	2.4±0.1	0.7±0.04	4.6±0.2	0.64±0.06	0.15±0.005	0.13±0.01	1.15±0.13
M. micrantha	*Minuartia-Minuartia*	28.5±0.26	23.4±0.2	1.2±0.02	12	4.28±0.33	3.3±0.17	1±0.2	6.8±0.17	0.6±0.06	0.15±0.01	0.18±0.01	0.86±0.15
M. hamata	*Minuartia-Minuartia*	18.5±0.4	14.5±0.3	1.27±0.03	12	4±0.1	3.7±0.43	0.29±0.04	5±0.2	0.79±0.01	0.22±0.01	0.2±0.01	1.06±0.77
M. montana	*Minuartia-Minuartia*	15.8±0.26	13.5±0.1	1.16±0.01	16	2.5±0.17	1.9±0.1	0.59±0.12	3.9±0.1	0.63±0.05	0.24±0.005	0.14±0.02	1.78±0.22
M. decipiens	*Minuartia-Minuartia*	23.6±0.1	19.1±0.1	1.23	12	3.7±0.17	3.7±0.3	0	5.56±0.3	0.66±0.45	0.19±0.47	0.11±0.01	1.7±0.5
M. meyeri	*Minuartia-Minuartia*	34.3±0.26	24.6±0.1	1.38±0.01	10	6±0.2	4.6±0.1	1.49±0.16	7±0.2	0.85±0.03	0.22±0.01	0.16±0.0	1.37±0.1
M. sclerantha	*Minuartia-Minuartia*	23.6±0.1	18.4±0.1	1.28±0.01	12	3.4±0.17	2.8±0.17	0.59±0.12	5.3±0.23	0.63±0.005	0.22±0.01	0.08±0.02	2.8±1.03
M. khorassanica	*Minuartia-Khorassanica* (based on an unpublished data)	25.7±0.2	21.6±0.17	1.18±0.005	12	4.6±0.08	3.5±0.1	1±0.1	6.6±0.1	0.69±0.005	0.24±0.01	0.22±0.0	1.08±0.08

Table 3. Comparison of qualitative pollen micro-morphological data in *Minuartia* sp

	characters							
Taxa	**subgenus-section**	**Pore ornaments**	**Pollen outline**	**Pollen ornaments**	**Echinidensity**	**Echini arrangement**	**Puncta status**	**Echini status**
M. aucheriana	*Acutiflorae*	sunken granular	sub spherical	microechinate-punctate	medium	irregular	indistinct	indistinct
M. umbellulifera	*Acutiflorae*	sunken granular	polyhedral	microechinate-punctate	dense	regular	almost indistinct	distinct
M. sabalanica	*Acutiflorae*	sunken granular	polyhedral	microechinate-punctate	dense	rather regular	almost indistinct	distinct
M. lineata	*Acutiflorae*	prominent granular	sub spherical	microechinate-punctate	dense	rather regular	indistinct	distinct
M. sublineata	*Acutiflorae*	prominent granular	sub spherical	microechinate-punctate	very dense	irregular	indistinct	distinct
M. glandulosa	*Acutiflorae*	prominent granular	sub spherical	microechinate-punctate	medium	irregular	indistinct	indistinct
M. acuminata	*Lanceolatae*	prominent granular	polyhedral	microechinate-punctate	medium	irregular	distinct	distinct
M. recurva	*Plurinerviae*	sunken granular	polyhedral	microechinate	dense	regular	no puncta	distinct
M. aizoides	*spectabilis*	sunken granular	polyhedral	microechinate-punctate	medium	irregular	distinct	distinct
M. picta	subgen. *Spergella*	prominent granular	sub spherical	microechinate-punctate	medium	irregular	distinct	distinct
M. mesogitana	*sabulina*	sunken granular	polyhedral	microechinate-punctate	dense	regular	Almost indistinct	distinct
M. subtilis	*sabulina*	sunken granular	polyhedral	microechinate-punctate	dense	irregular	distinct	distinct
M. hybrida	*sabulina*	sunken granular	polyhedral	microechinate-punctate	dense	irregular	distinct	distinct
M. micrantha	*Minuartia*	sunken granular	polyhedral	microechinate-punctate	medium	rather regular	indistinct	indistinct
M. hamata	*Minuartia*	prominent granular	polyhedral	microechinate-punctate	3low	completely irregular	distinct	distinct
M. montana	*Minuartia*	sunken granular	polyhedral	microechinate-punctate	medium	rather regular	indistinct	distinct
M. decipiens	*Minuartia*	prominent granular	sub spherical	microechinate-punctate	medium	irregular	almost indistinct	very distinct
M. meyeri	*Minuartia*	sunken granular	polyhedral	microechinate-punctate	medium	irregular	distinct	distinct
M. sclerantha	*Minuartia*	sunken granular	polyhedral	microechinate-punctate	medium	rather irregular	indistinct	indistinct
M. khorassanica	*Khorassanica* (unpublished data)	sunken granular	polyhedral	microechinate-punctate	medium	rather irregular	indistinct	indistinct

3.1.3. The Size of Echinus and Punctum

Echini to puncta diameter ratio (Ec:Pu) varied among taxa and therefore appeared to be a suitable character to differentiate similar species (Table 2). The lack of puncta was only observed in *M. recurva* Schinz & Thell. (sect. *Plurinerviae*) (Figure 2(f)). *Minuartia sclerantha* (sect. *Minuartia*) and *M. micrantha* (sect. *Minuartia*) had the largest and the smallest E:P ratio, respectively (Figure 3(f) Figure 3(e)). All other taxa as mentioned above, had microechinate-punctate ornamentations.

3.2. Qualitative Characters

3.2.1. Shape

Two different shapes of pollen i.e., sub spherical and polyhedral could be found in the genus *Minuartia*. Sub-spherical pollen grains were observed in *M. aucheriana, M. sublineata* (Figure 5(a)), *M. lineata* (Figure 4(i)), *M. glandulosa* (Figure 4(h)) (sect. *Acutiflorae*), *M. picta* (subgen. *Spergella*) (Figure 2(a)) and *M. decipiens* (sect. *Minuartia*) (Figure 2(i)), while all other species had polyhedral pollen grains (Figures 2-5).

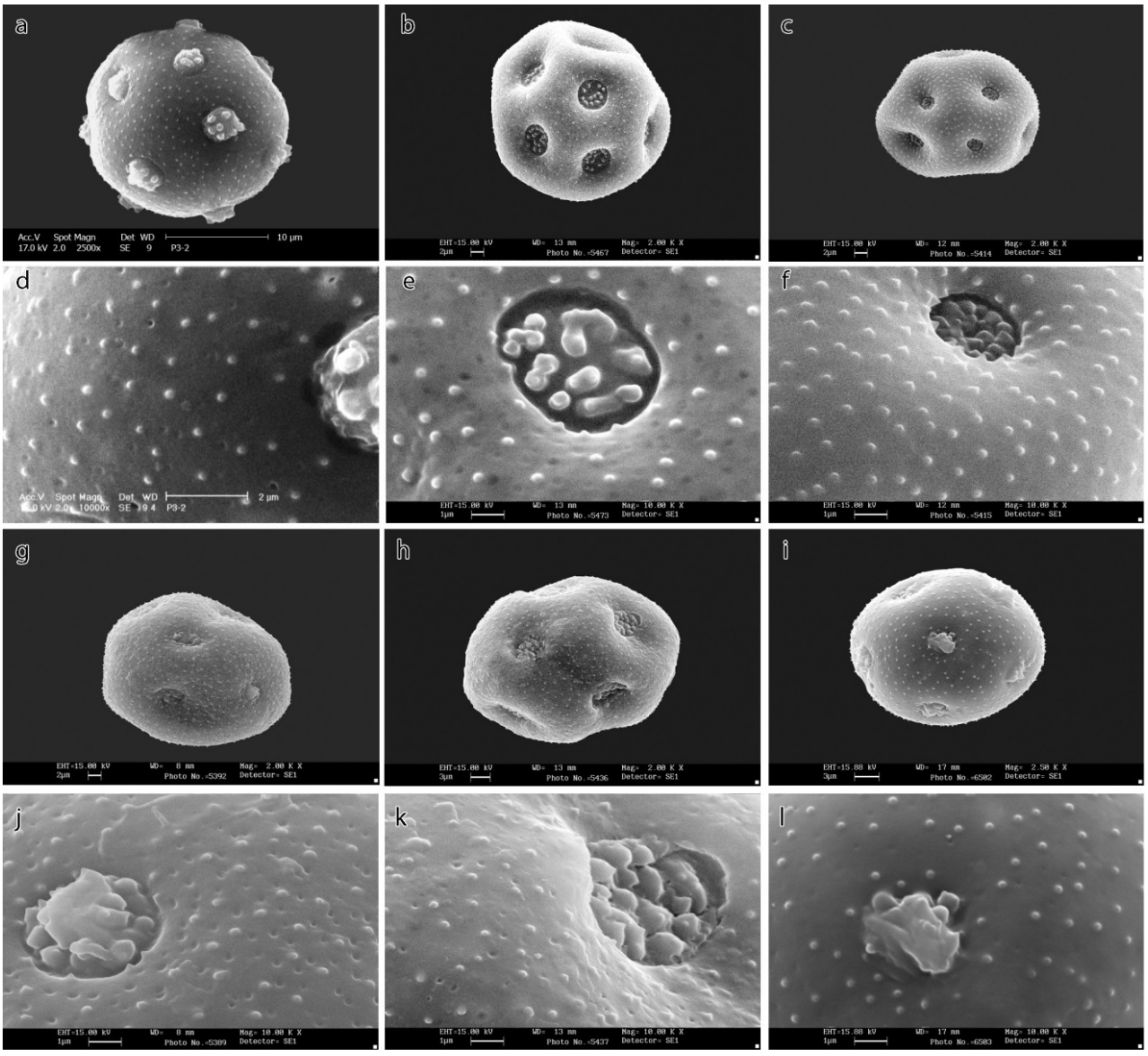


Figure 2. SEM micrographs of pollen morphology in *Minuartia* species: a, b, c, g, h, i- general appearance; d, e, f, j, k, l- ornamentations. a and d- *Minuartia picta*, b and e- *M. aizoides*, c and f- *M. recurva*, g and j- *M. acuminata*, h and k- *M. meyeri*, i and l- *M. decipiens*. Scale bars: a, g=2; b, c, h, i=3; d, e, f, j, k, l=1 μm

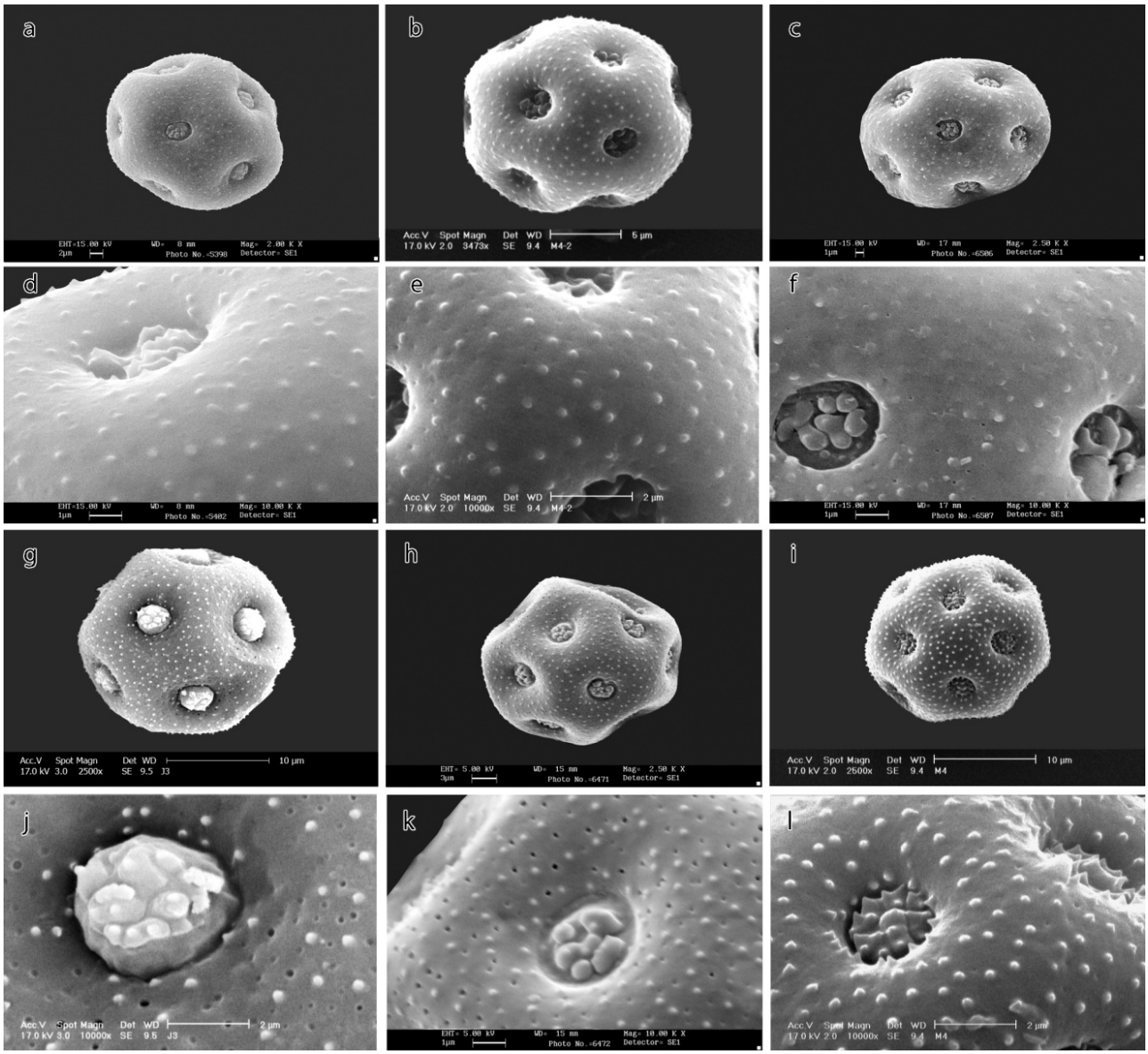


Figure 3. SEM micrographs of pollen morphology in *Minuartia* species: a, b, c, g, h, i- general appearance; d, e, f, j, k, l- ornamentations. a and d- *Minuartia micrantha*, b and e- *M. montana*, c and f- *M. sclerantha*, g and j- *M. hamata*, h and k- *M. hybrida*, i and l- *M. mesogitana*. Scale bars: a, e, j, l=2; h=3; b=5; c, d, f, k= 1; g, i=10 µm

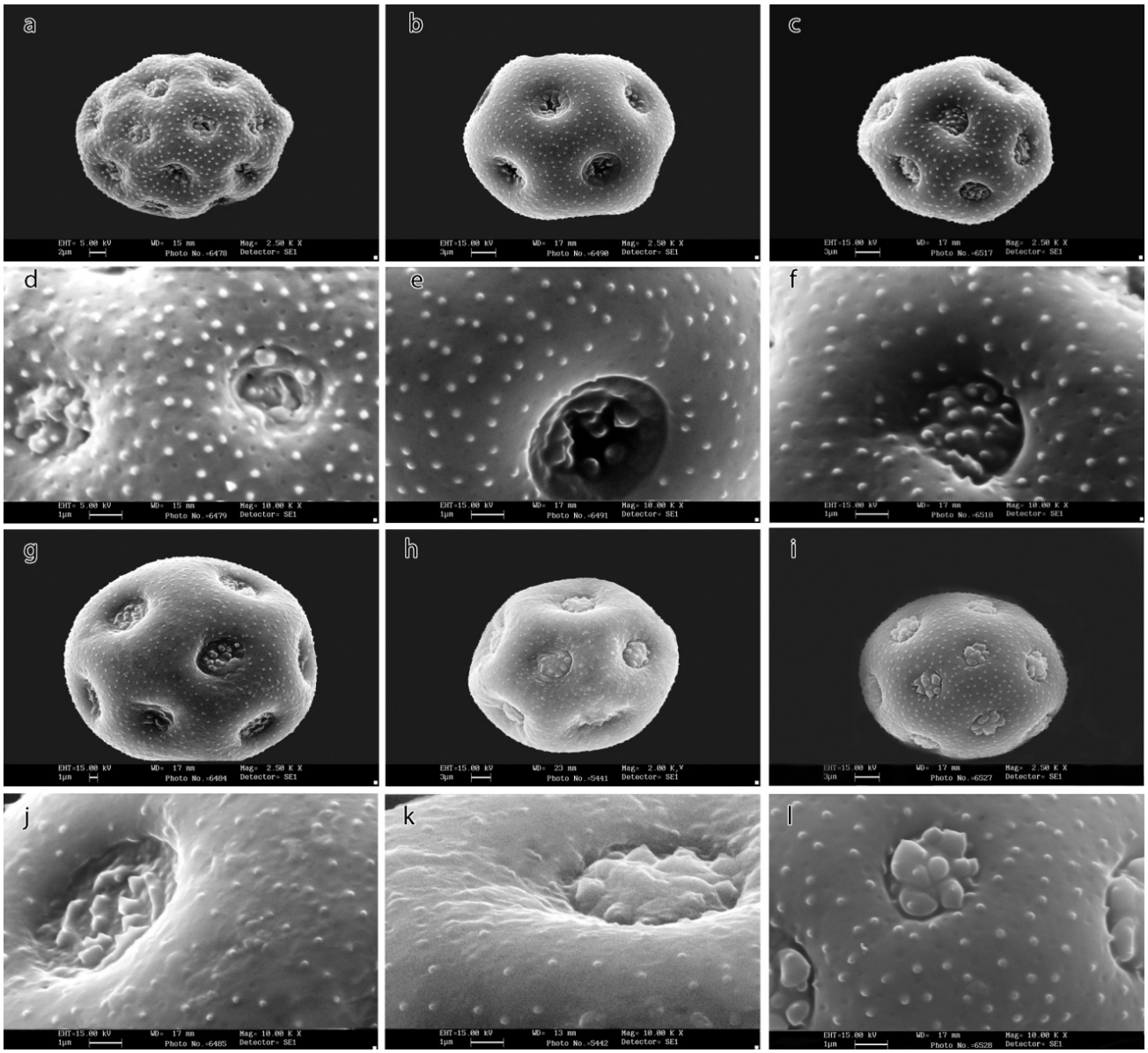


Figure 4. SEM micrographs of pollen morphology in *Minuartia* species: a, b, c, g, h, i- general appearance; d, e, f, j, k, l- ornamentations. a and d- *Minuartia subtilis*, b and e- *M. umbellulifera*, c and f- *M. sabalanica*, g and j- *M. aucheriana*, h and k- *M. glandulosa*, i and l- *M. lineata*. Scale bars: a=2; d, e, f, g, j, k, l=1; b, c, h, i=3 μm

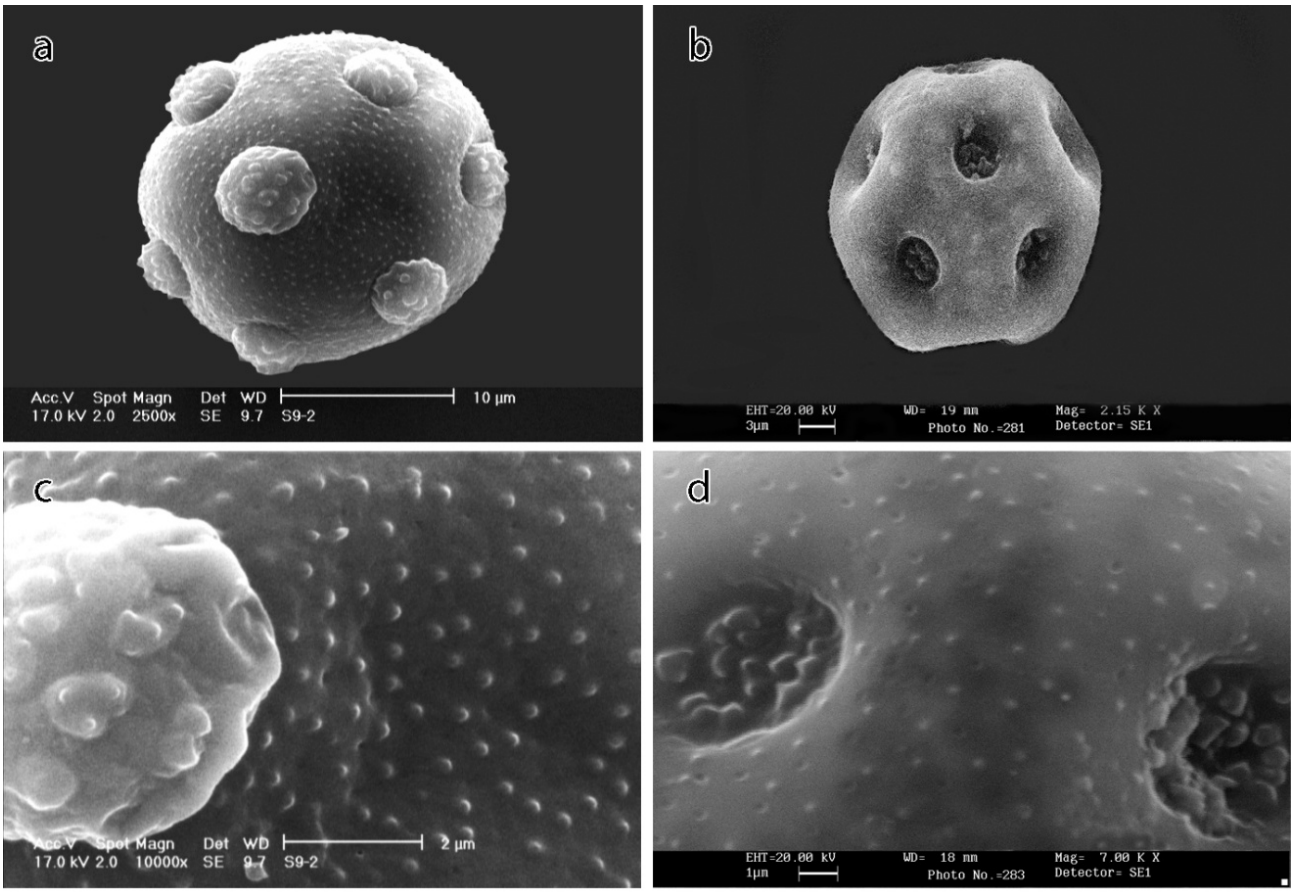


Figure 5. SEM micrographs of pollen morphology in *Minuartia* species: a, b- general appearance; c, d- ornamentations. a and c- *Minuartia sublineata*, b and d- *M. khorassanica*. Scale bars: a=10; b=3; c=2; d=1µm

3.2.2. Ornamentations

In almost all investigated species, pollen grain ornamentations were microechinate-punctate. As previously mentioned, only *Minuartia recurva* had no puncta on its pollen surface that could be a suitable character to distinguish it. In this species the operculum was ornamented with granules. Pores were slightly (approximately flattened) to distinctly sunken or completely prominent.

Minuartia lineata, M. sublineata, M. glandulosa, M. acuminata, M. aizoides, *M. hamata* and *M. decipiens* exhibited a prominent granular type. All other species had an impressed granular form (Figures 2-5).

3.3. Cluster Analysis and PCA

The results of cluster analysis and Principal Component Analysis for *Minuartia* species are shown in Tables 4 and 5. The cluster dendrogram demonstrates the species similarities. The phenoline drown at the top of each dendrogram, shows the taxonomical distances. High correlation coefficients indicate a high degree of similarity among taxa and therefore show low distance between them.

In cluster analysis, the species firstly were divided into two major groups (Table 4). *Minuartia aizoides, M. acuminata, M. khorassanica* Assadi & Mostafavi, *M. micrantha, M. umbellulifera, M. meyeri, M. glandulosa* and *M. aucheriana* were classified as one major group (i.e., group I) and other 12 species were included in the group II. Between 0-5 taxonomical distances, the group I, was divided into two secondary groups. The species *Minuartia meyeri, M. aucheriana* and *M. glandulosa* were placed in the group A and other mentioned above species, were categorized in the group B of the secondary group. Also, in 5-10 taxonomical distances, group II was divided into two secondary groups. The group A was consisted of *M. montana, M. hamata, M. mesogitana* and *M. picta*. The group B was also divided into two secondary groups in taxonomical distance of 5. The species *M. subtilis, M. hubrida and M. lineata* were placed in the separate group and *M. sublineata, M. recurva, M. sabalanica, M. sclerantha* and finally *M. decipiens* were placed in juxtaposition in the other secondary group (Table 4).

In Principal Component Analysis (PCA), characters were grouped in two components. Based on these two components, the species were scattered. The results of PCA were in line with the cluster analysis. The spatial arrangement of taxa is shown in Table 5.

The characters with positive variance from 0.3 to near to 1 in each component were respected as the most effective ones. Some characters such as pore diameter (annulus included) (D) (0.919), equatorial diameter (E) (0.863), polar axis length (P) (0.853), the distance between two pores (d) (0.830), pollen

outline (0.815), Pore diameter (annulus excluded) (R) (0.809), annulus diameter (a) (0.553), P/E ratio (0.443), Puncta diameter (0.415), Echini diameter (0.374) had significant role in diagnosing adjacent species in two components respectively. All the above characters (except pollen outline) respected as the most important, were quantitative (Table 6).

Table 4. Cluster dendrogram showing species similarities based on pollen micro-morphological data

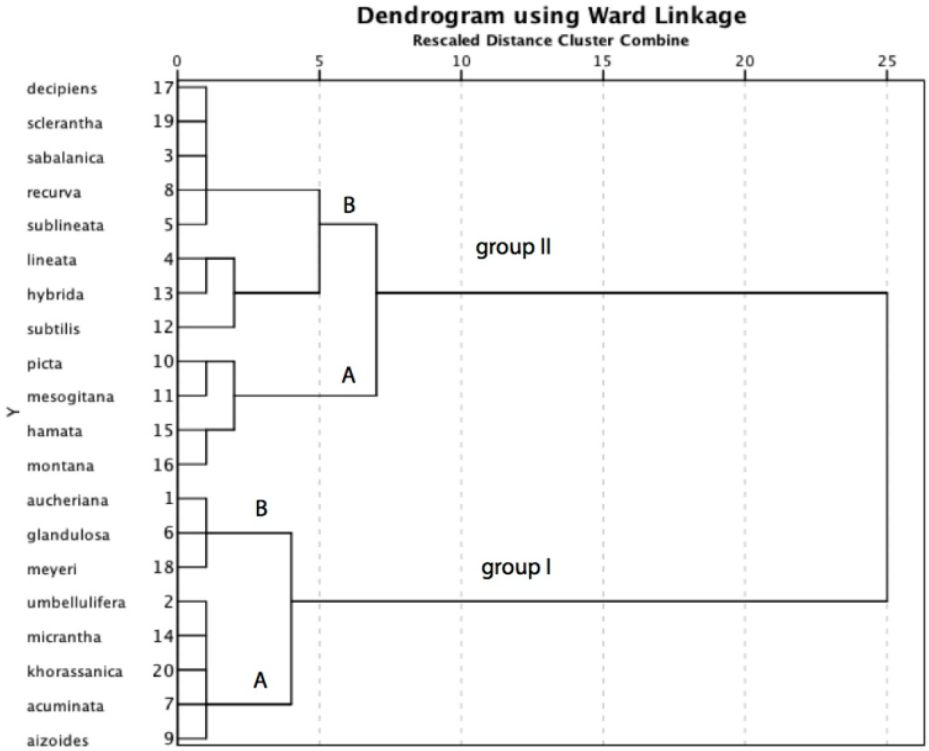


Table 5. PCA, showing similarities among taxa in two dimensions based on pollen micro-morphological data

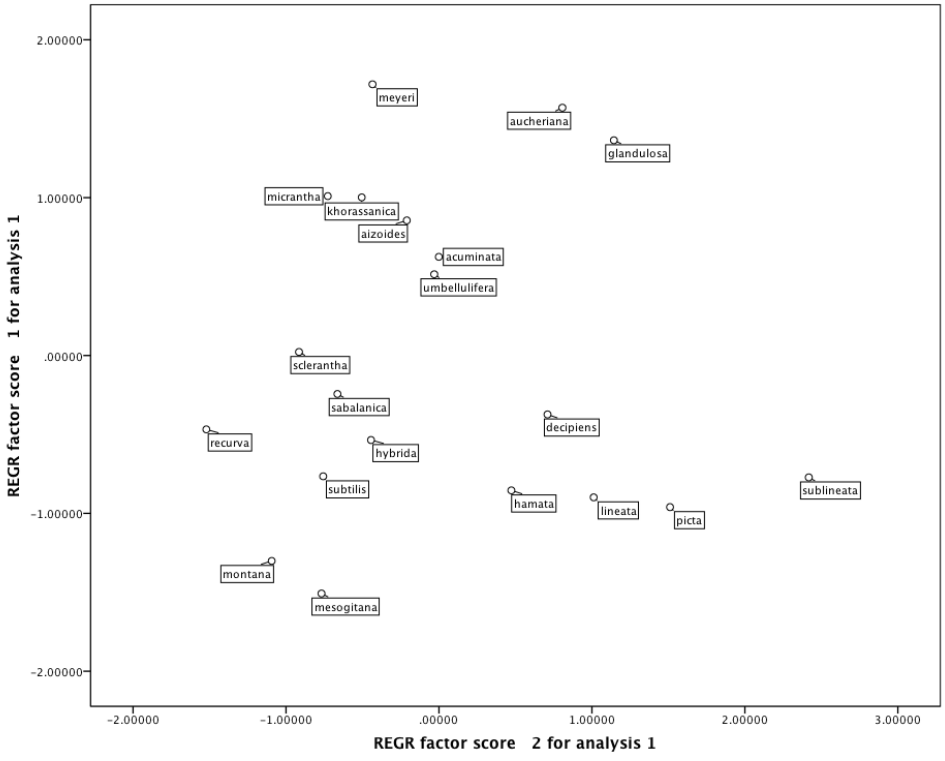


Table 6. The comparison of the variance between 18 quantitative and qualitative characters by use of Principal Component Analysis extraction method

Component Matrix[a]		
	Component	
	1	2
P	.853	.226
E	.863	.108
P/E	.077	.443
N	-.589	-.188
D	.919	-.241
R	.809	-.500
a	.512	.553
d	.830	.421
D/d	.285	-.777
Echini diameter	-.135	.374
Puncta diameter	.415	-.084
Echini/Puncta diameter	-.249	.002
Pore ornaments	.038	-.806
Pollen outline	-.243	.815
Echini density	-.433	-.340
Echini arrangement	-.418	.298
Puncta status	-.145	.220
Echini status	-.555	-.196

4. Discussion

According to Punt and Hoen (1995) two types of pollen grains were identified for *Minuartia* species considered in that article.

These two types are characterized by some major characters such as the distance between two pores, annulus thickness, exine thickness, pore number, pollen outline, the number of granules in operculum region and also pore diameter.

According to our results in the present study, these two types are not sufficient to classify all *Minuartia* species. In some cases, the species display mixed characters and therefore, could not be placed in either *Minuartia rubra* or *Moehringia trinervia* type. Some of the most important characters (with the variance of > 0.8) in the present study were pore diameter (annulus included), equatorial diameter, polar axis length, the distance between two pores and pollen outline.

The results of cluster analysis and Principal Component Analysis showed that despite separation of some closely related taxa via pollen micro-morphological data, no distinct group is detected that could conform the correlation among taxonomic groups in the section and subgenus ranks. In other words, it could only separate similar taxa at the species level.

Here we compare some representatives of each section of *Minuartia* placed in subgen. *Minuartia* and the only species in sect. *Spergella* separately.

At first, the results obtained for six species belonging to sect. *Acutiflorae* i.e. *M. aucheriana, M. lineata, M. sublineata, M. umbellulifera* (Boiss.) Mc Neill, *M. sabalanica* and *M. glandulosa* are discussed:

Minuartia sabalanica, a new Iranian species (sect. *Acutiflorae*), (Mostafavi et al. 2011), showed a higher Ec: Pu diameter ratio compared to other species belonging to the section (2.56±0.46 µm) (Table 2). This ratio could separate this new species from its closely related species in section i.e., *M. umbellulifera* (1.01±0.09). It reflected the fact that echina is much larger than puncta in *M. sabalanica*. Despite the presence of quite smaller puncta in this newly identified species, D:d and P:E ratios did not show considerable differences compared to *M. umbellulifera*. Moreover, the difference in pore number on two pollen hemispheres between *M. umbellulifera* (12 pores) and *M. sabalanica* (14 pores) was also significant. Echinus density was the same in both species (Tables 2, 3). *Minuartia lineata* and *M. sublineata* belonging to sect. *Acutiflorae* are distinguishable using only a few characters such as plant color and pedicel length. Our results illustrated that these species could also be dissociated through pollen micro-morphological characters. The great majority of species belonging to the sect. *Acutiflorae* except for *M. aucheriana* and *M. sublineata*, have a D:d ratio less than 1µm. It means that the distance between two pores is larger than the pore diameter, annulus included. In *M. aucheriana* and *M. sublineata* this ratio was between 1-1.33 µm. The largest polar axis length in this section was observed in *M. glandulosa* and the smallest one in *M. sublineata*. *Minuartia aucheriana*, *M. sabalanica* and *M. umbellulifera* represented species displaying pores with slightly to distinctly sunken granular ornamentations while the other three species had prominent granular pores. Among species belonging to the sect. *Acutiflorae*, only *M. sabalanica* and *M. umbellulifera* had a polygonal outline. This character obviously proves their taxonomical relationships.

The next section including six Iranian species is sect. *Minuartia*: *Minuartia meyeri* showing important morphological similarities with *M. decipiens* subsp. *persica*. This fact explains their juxtaposition with each other. However, they have some morphological differences in their pedicel length and sepal base shape. The latter subspecies is endemic to the country and is reported from Southern Iran. My palynological investigations showed some obvious additional differences. According to the results, pollen grains of the two closely related species i.e., *M. decipiens* and *M. meyeri* have considerable differentiating characters and could be easily dissociated via pollen micro-morphology.

This, reflects the fact that we could not disregard the role of pollen morphology in distinguishing some species that have many morphological similarities.

Minuartia montana and *M. sclerantha* not only have many morphological resemblances but also have the same distribution (NW Iran). The results of this study revealed some differences in palynological characters such as E:P diameter ratio, echini arrangement, pollen size and also the number of pores. It should be mentioned that the Ec:Pu diameter ratio in *M. sclerantha* is 2.8±1.03µm, which is more than the other species in the genus.

Puncta size in *M. sclerantha* was very short (0.08±0.02

μm) but its puncta approximately was as large as *M. montana*. Pollen size in *M. sclerantha* was more than *M. montana* while the number of pores in *M. montana* was more than what observed in *M. sclerantha*. Other quantitative data like D:d ratio, P:E ratio and so on were not so variable. Echini in *M. sclerantha* was distinct and rather regularly arranged throughout the surface but in *M. montana* this status was inversed. The cluster dendrogram shows these differences via putting them in two separate secondary clusters A and B of the group II.

Another species, that falls into sect. *Minuartia,* is *Minuartia hamata* showing different morphological features in comparison to other species placed in this section. This species showed a few differences in pollen morphology such as its echini density. Echinus is distinct and is arranged irregularly with low density. It should be mentioned that the results of seed micro-morphology for the determination of its taxonomic position among the other species as well as pollen results was sine qua non not per se adequate (Mostafavi et al. 2011). More evidences such as molecular phylogeny could be effective to designate precisely its taxonomic position. The results of cluster analysis and PCA demonstrated that this species is near to the related species i.e., *M. montana* (cluster A of the group II) that are both placed in the sect. *Minuartia*. Other species belong to the mentioned above section, are scattered in different clusters.

Four species i.e., *M. mesogitana, M. hybrida, M. subtilis* and *M. urumiensis* is placed in sect. *Sabulina* according to Rechinger (1988). In this study, we investigated only three of them. *Minuartia hybrida*, *M. mesogitana* and *M. subtilis* had many morphological resemblances making their taxonomical dissociation difficult.

The results of palynological studies showed that *M. subtilis* could be separated from the two other species (24 pores in two hemispheres) because of its higher number of pores.

However, *Minuartia mesogitana* and *M. hybrida* could not be dissociated via this character as they have exactly the same pore number.

Minuartia subtilis was discernible from *M. hybrida* and *M. mesogitana* by its longest P:E ratio (1.31 μm). However, in this case, characters such as pollen shape and pore ornamentations were not such reliable (Table 2). Undeniably, one of the most important characters for detecting *M. mesogitana* was a higher E: P ratio compared to two other species. Moreover this pollen appeared to be smaller than those released from *M. hybrida* and *M. subtilis*. Separation of *Minuartia mesogitana* from two remained taxa is demonstrated in Table 4. It is placed in the cluster A of the major group II, while two remained species were in juxtaposition in a secondary group of the cluster B.

Minuartia khorassanica (Mostafavi et al. 2011b) was another new species showing polyhedral pollen grains and sunken granular pore ornamentations. Therefore this species considerably differs with the nearest one, *M. lineata* releasing sub spherical pollen grains with different pore ornamentations. Moreover pore numbers and annulus diameter was also different despite the same Ec: Pu ratio. Cluster dendrogram confirmed above statements about the separation of these two nearest taxa.

Minuartia recurva was the only species that could be easily differentiated from other *Minuartia* species by having no puncta on its pollen surface. The results of the cluster analysis could not separate it from all other taxa.

This is quite considerable that only in two distinguishable taxa, *M. aizoides* and *M. picta*, from two different subgenera (i.e., *Spergella* and *Minuartia* (sect. *Spectabilis*)), P:E ratio was equal to 1.

Although the results of cluster analysis and PCA could not show taxonomic similarities among the species placed in a certain group, it could be useful in determining some closely related taxa.

Considering these statements we reach a conclusion that pollen micro-morphological studies have had considerable role in distinguishing some related taxa at the species rank.

In the near future, karyological studies and molecular investigations will be also performed in order to confirm the taxonomic position of the *Minuartia* spp. growing in Iran.

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