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Journal of Science and Technology

E-ISSN 2146-7706

Pollen morphology of some *Alyssum* L. (Brassicaceae) from Turkey

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ARTICLE INFO

Article history:
Received 07 March 2018
Received in revised form 07 Sep. 2018
Accepted 11 Sep. 2018

Keywords:
Alyssum
Brassicaceae
LM
Pollen morphology
SEM

ABSTRACT

The present study evaluated the taxa *A. linifolium* Stephan ex. Willd. var. *teheranicum* Bornm., *A. simplex* Rudolph, *A. trichocarpum* T.R. Dudley & Hub.-Mor., *A. armenum* Boiss., *A. praecox* Boiss. & Bal., *A. lepidoto-stellatum* (Hausskn. &Bornm.) T. R. Dudley, *A. sulphureum* T. R. Dudley & Hub. - Mor., *A. murale* Waldst. & Kit. subsp. *murale, A. pateri* Nyar. subsp. *pateri* of the genus *Alyssum* L. from the family Brassicaceae in terms of palynological characteristics. Samples of these taxa were collected in field studies. 9 taxa from this genus were examined using light microscope (LM) and scanning electron microscope (SEM). Results of the study revealed some common characteristics such as tectate pollens, subprolate, prolate shapes of pollens, heteropolar symmetry. Aperture type was tricolpate. SEM microphoto also indicated that ornamentation structures were reticulate.

1. Introduction

Turkey is the country having the richest flora in temperate climate zone with 11.707 natural flowering plants and fern species. Almost one third of the flora (33.4%) consists of 3649 endemic species (Davis et al., 1988; Güner et al., 2012). This family includes 49 tribes, approximately 321 genera, and 3660 species across the world (Al-Shehbaz, 2012). This family has 88 genera and 539 species (210 endemic species) in Turkey (Erik and Tarıkahya, 2004). The genus *Alyssum* L. is among the large genera of Turkey's Flora and represented by 90 species. 54 of these species are endemic (Davis 1985; Davis et al., 1988; Güner et al., 2012). İnceoğlu and Karamustafa (1977) studied on pollen morphology of the family Brassicaceae (Cruciferae) found in Ankara. Bahrami et al., (2012) analyzed pollen morphology (sec. Gamosepalum) of 8 species of *Alyssum* by using light microscope. Khan (2003) investigated pollen morphology of 7 *Alyssum* species from the family Brassicaceae under light microscope. Khalik (2005) focused on the importance of morphology, structure, and classification of trichomes of 82 species from 9 tribes of Egyptian Brassicaceae by using light microscopy (LM) and scanning electron microscopy (SEM). Bolurian (2009) studied 8 species of *Alyssum* L. and identified 3 pollen types (subprolate, prolate-spheroidal and prolate) and reticulate exine ornaments. İnce and Vural (1994) studied on pollen morphology of *Alyssum pateri* Nyar. and *A. praecox* Boiss. & Bal. İnceoğlu and Karamustafa (1977) studied pollen morphology of the species *Alyssum blepharocarpum* Dudley&Hub-Mor., *A. murale* Waldst. & Kit.,*p*, *A. sibiricum* Willd., and *A. umbellatum* Desv. Orcan and Binzet (2003) determined anatomic and palynological characteristics of *Alyssum obtusifolium* Steven ex D.C. Orcan and Binzet (2004) studied anatomy and pollen morphology of *Alyssum floribundum* Boiss. & Balansa. Kurşat et al., (2008) investigated morphological, anatomic, and palynological characteristics and chromosome number of *Alyssum harputicum* Dudley (Brassicaceae).

The present study aims to give a detailed account of the palynological features morphology of *Alyssum*.

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2. Materials and Methods

List of the species and this locality are provided below.

Alyssum simplex; Malatya, Darende, Darende-Gürün road 5. Km, 1100 m, steppe. 22.04.2010, BŞ 3912. ***A. armenum***; Sivas, Gürün, Karakuyu village, steppe, 1900 m, 27.05.2010, BŞ 4254. ***A. sulphureum***; Malatya, Darende, Darende-Gürün road, 5. km, steppe, 1100 m, 29.05.2010, BŞ 4369. ***A. lepidoto-stellatum***; Malatya, Darende, Darende-Hekimhan road, 2. km, steppe, 1200 m, 05.05.2011, BŞ 4880. ***A. linifolium* subsp. *teheranicum***; Malatya, Darende, Darende-Gürün road, 5. km, steppe, 1100 m, 05.05.2011, BŞ 4883. ***A. pateri* subsp. *pateri***; Malatya, Darende, Darende-Hekimhan road, 2. km, steppe, 1200 m, 01.06.2011, BŞ 5034. ***A. praecox***; Sivas, Gürün, Hezanlı Mountain, Çandil hill, steppe, 2000 m., 02.06.2011, BŞ 5058. ***A. murale* subsp. *murale***; Sivas, Gürün, Karakuyu village, Sinekkonmaz hill, steppe, 2000 m, 17.06.2011, BŞ 5262. ***A. trichocarpum***; Malatya, Darende, Başdirek village Külali hill, steppe, 1700-1900 m, 21.06.2011, BŞ 5311.

The pollen grains were prepared for light (LM) by the standard methods described by Wodehouse (1935). For light microscopy, the pollen grains were mounted in unstained glycerine jelly and observations were made with a Olympus BX 31. For scanning electron microscopy investigations, the pollen grains were put on stubs, stutter-coated with gold plate. The SEM examination was carried out on a ZEISS supra 55 scanning electron microscope in the SEM laboratory of the Mersin University Central Research Laboratory (MEİTAM). The measurements were based on 30 readings from each specimen. Polar axis (P), equatorial diameter (E), P/E ratio, exine (ex), intine (i), colpi long axis (clg), colpi short axis (clt), mezocolpia (L) and Apocolpia (t), were also measured.

The terminology used is in accordance with Faegri & İversen (1989), Moore et al. (1991), Punt et al. (2007) and Erdtman (1952)

3. Results

Alyssum L.

9 samples from 90 taxa of this genus found in Turkey were examined palynologically. Pollens are 3-colpate, colporate, inter-subangular, subangular, and isopolar, Pollen shapes are prolate-spheroidal, and prolate, Polar axis is 19-40 µm and equatorial axis is 19-26 µm. Apertures vary from long-very long colpus to very narrow on the edges. Membrane of colpus is reticulate and terminates with a sharp and spherical end (table 1, figure 1-2-3).

A. linifolium Stephan ex. Willd. var. ***teheranicum*** Bornm.

The pollen shape was prolate (P/E: 1.34) with a polar axis of 26.01 µm and an equatorial diameter of 19.41 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 19.99 µm and width of 1.24 µm. Exine thickness was 0.86 µm. intine thickness was 0.46 µm. Ornamentation was reticulate and reticulum was shallow. 6-9 perforations per µm^2, lumina 0.17– 0.77 µm thick, muri 0.23–0.29 µm thick in average (table 1, figure 1-a, 2-a, 3-a, b).

A. simplex Rudolph

The pollen shape was prolate (P/E: 1.74) with a polar axis of 36.55 µm and an equatorial diameter of 31.04 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 31.04 µm and width of 1.63 µm. Exine thickness was 1.30 µm. intine thickness was 0.42 µm. Ornamentation was reticulate and reticulum was shallow. 1-3 perforations per µm^2, lumina 0.30-1.05 µm thick, muri 0.25-0.30 µm thick in average (table 1, figure 1-b, 2-b, 3-c, d).

A. trichocarpum T.R. Dudley & Hub. - Mor.

The pollen shape was prolate (P/E: 1.56) with a polar axis of 40.73 µm and an equatorial diameter of 26.14 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 33.66 µm and width of 1.61 µm. Exine thickness was 1.30 µm. intine thickness was 0.48 µm. Ornamentation was reticulate and reticulum was shallow. 2-5 perforations per µm^2, lumina 0.27-0.67 µm thick, muri 0.16-0.24 µm thick in average (table 1, figure 1-c, 2-c, 3-e, f).

A. armenum Boiss.

The pollen shape was prolate (P/E: 1.40) with a polar axis of 28.59 µm and an equatorial diameter of 20.47 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 23.29 µm and width of 1.27 µm. Exine thickness was 1.07 µm. intine thickness was 0.46 µm. Ornamentation was reticulate and reticulum was shallow. 3-4 perforations per µm^2, lumina 0.34-0.91 µm thick, muri 0.20-0.29 µm thick in average (table 1, figure 1-d, 2-d, 3-g, h).

A. praecox Boiss. & Bal.

The pollen shape was prolate (P/E: 1.49) with a polar axis of 30.74 µm and an equatorial diameter of 20.64 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 25.19 µm and width of 1.22 µm. Exine thickness was 0.95 µm. intine thickness was 0.41 µm. Ornamentation was reticulate and reticulum was shallow. 2-4 perforations per µm^2, lumina 0.35-0.80 µm thick, muri 0.20-0.28 µm thick in average (table 1, figure 1-e, 2-e, 3-k, m).

A. lepidoto-stellatum (Hausskn. &Bornm.) T. R. Dudley

The pollen shape was subprolate (P/E: 1.29) with a polar axis of 30.06 µm and an equatorial diameter of 23.39 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 23.77 µm and width of 1.00 µm. Exine thickness was 0.97 µm. intine thickness was 0.54 µm. Ornamentation was reticulate and reticulum was shallow. 3-6 perforations per µm^2, lumina 0.30-0.75 µm thick, muri 0.15-0.23 µm thick in average (table 1, figure 1-f, 2-f, 3-n, o).

A. sulphureum T. R. Dudley & Hub. - Mor.

The pollen shape was subprolate (P/E: 1.28) with a polar axis of 30.90 µm and an equatorial diameter of 24.21 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 24.82 µm and width of 1.54 µm. Exine thickness was 1.30 µm. intine thickness was 0.49 µm. Ornamentation was reticulate and reticulum was shallow. 4-7 perforations per µm^2, lumina 0.27-0.76 µm thick, muri 0.18-0.25 µm thick in average (table 1, figure 1-g, 2-g, 3-p, r).

A. pateri Waldst. & Kit. subsp. ***pateri***

The pollen shape was prolate (P/E: 1.39) with a polar axis of 21.90 µm and an equatorial diameter of 15.78 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 17.14 µm and width of 0.92 µm. Exine thickness was 0.81 µm. intine thickness was 0.40 µm. Ornamentation was reticulate and reticulum was shallow. 5-9 perforations per µm^2, lumina 0.21-0.60 µm thick, muri 0.12-0.21 µm thick in average (table 1, figure 1-h, 2-h, 3-s, t).

A. murale Nyar. subsp. ***murale***

The pollen shape was prolate (P/E: 1.45) with a polar axis of 24.79 µm and an equatorial diameter of 17.10 µm. The aperture type of pollen was trizonocolpate. The colpus was in long-acute ended with a colpus length of 18.73 µm and width of 0.99 µm. Exine thickness was 0.93 µm. intine thickness was 0.47 µm. Ornamentation was reticulate and reticulum was shallow. 3-7 perforations per µm^2, lumina 0.28-0.73 µm thick, muri 0.15-0.25 µm thick in average (table 1, figure 1-k, 2-k, 3-u, z).

4. Conclusions

Detailed pollen morphology of 9 taxa included in the sections Meniocus, Alyssum, Gamosepalum and Odontarrhena from the genus *Alyssum* of the family Brassicaceae was examined by light microscope and SEM. Brassicaceae is considered as a stenopalinous family (Erdtman, 1966). Ertdman (1952) described palynological characteristics of 55 genera and 50 species from this family. Appel and Al-Shehbaz (2003) identified that ornamentation of pollens was reticulate and tricolpate in the family Brassicaceae, on the other hand it was rare spinulosa, punctate tectum and 10-colpate in the genus *Heliophilia*.

In the study by İnceoğlu and Karamustafa (1977) carried out with light microscope, 23 species of Brassicaceae family are tricolpate however it was determined that the species *Matthiola oxyceras* DC. had no aperture and the species *Cardamine pratensis* L. was sincolpate. Moore and Webb (1987) classified pollens of the family Brassicaceae as reticulate and tricolpate. Khalik (2002) divided this family into three types of pollen based on lumina size. It was indicated that pollens of this family were more closely related to Tamaricaceae family morphologically and pollens of both families were reticulate tectum and tricolpate (Qaiser and Perveen, 2004). Ertdman (1952) reported that the family Brassicaceae was closer to the family Capparaceae. On the other hand, in their study, Qaiser and Perveen (2001) determined that the family Capparaceae was eurypalynous and its pollen shape was tricolpate.

Pollen morphology of this genus was studied by some scientists (Moore et al., 1991; Reille, 1992; and Beug, 2004). Pollens of *Alyssum* species were described as Hornungia type by Faegri and Iversen (Faegri and Iversen 1989). Based on this result, it was seen to have 3-zonocolpate pollen and reticulate ornamentation. It was identified in the study on taxa of Alyssum that pollen morphology was homogenous and pollen shape varied from subprolate to prolate (İnceoglu and Karamustafa, 1977; Faegri and Iversen, 1989, Moore et al., 1991; Anchev and Deneva, 1997; Orcan and Binzet, 2003; Perveen et al., 2004). Pavlova et al., (2016) determined that colpi of all species was long, shallow, and got narrower towards poles. Additionally, exine is 1-1.5 µm-thick and was determined to vary in terms of ornamentation and size lumen. In their study, Anchev and Deneva (1997) did not observe perforation for taxa of *Alyssum*. In the present study, ornamentations of taxa were reticulate and there was no perforation. The change in pollen size between populations studied is a result of a polyploidy mentioned before for species of Brassicaceae (Anchev and Deneva, 1997).

Pollens of *Alyssum linifolium* var*. teheranicum* from Meniocus section are prolate and 3-colpate with radial symmetry. Polar axis is 26.01 µm and equatorial axis is 19.41 µm. Polar axis is longer than equatorial axis. Equatorial appearance of pollens are subrectangular. Colpus is thin and long and ends narrowing through the ends. Thickness of colpus is 1.24 µm. Exine is 0.86 µm and tectate. Detailed SEM analysis revealed that ornamentation was reticulate. Intine is thin, 0.46 µm (table 1, fig. 1a-2a). İnceoglu and Karamustafa (1977) reported that regarding palynological characteristics of *A. blepharocarpum* T. R. Dudley & Hub. Mor. species from Meniocus section, the pollen shape was subprolate, polar axis was 19.1 µm, equatorial axis was 16.3 µm, exine was 1.2 µm, intine was 0.80 µm, and exine ornamentations were reticulate. (Khan 2003) In this study, *A. linifolium* species was prolate spheroidal, polar axis was 22 µm, equatorial axis was 21 µm, colpus length was 16.5 µm. Exine was found to be 3.3 µm which was considerably thick. In the present study, polar axis was 26.01 µm, equatorial axis was 19.41 µm, and colpus length was 19.99 µm.

Pollens of A. simplex, *A. trichocarpum, A. armenum* and *A. praecox* from Alyssum section are 3-colporate with radial symmetry. Polar axis is prolate and much longer than equatorial axis. Equatorial appearances of pollens are circular and oval. Exine is tectate, ornamentation is reticulate according to detailed SEM analysis. Exine varies from 0.95 µm to 1.30 µm. Intine is thin and varies between 0.41 and 0.48 µm. (table 1, fig. 1-2(b,c,d,e) fig. 3 c-d, e-f, g-h, k-m). İnceoglu and Karamustafa (1977) studied on *A. umbellatum* Desv. species of Alyssum section. Characteristics of pollens were prolate, polar axis was 37.1 µm, equatorial axis was 26.2 µm, exine was 1.7 µm, intine was 0.70 µm and ornamentation is reticulate. It is compatible with the present study. In the study by Pakvaran et al., (2011) in Iran it was found that pollens of *A. simplex* varieties were tricolpate and there were differences in sizes and morphology. *A. simplex* var*. mazandaranicum, A. simplex* var. *micranthum* were prolate and differences were found in size. For *A. simplex var. micranthum*, polar axis was 47.73 µm and equatorial axis

was 33.89 µm. In their study, Karabacak et al., (2016) considered that *Alyssum amasianum* which is a new species of Alyssum section was related to the species *A. hirsutum* subsp. *caespitosum,* and *A. hirsutum* subsp*. hirsutum* and compared them. In conclusion, pollens were tricolpate reticulate tectate and perprolate. Structure, size, and shape of exine were not important for distinguishing taxa. In their study, İnce and Vural (1994) found that pollens of *A. preacox* were tricolpate and prolate, polar axis was 24.8 µm, equatorial axis was 18.33 µm, exine was 1.02 µm and intine was 0.94 µm. These results are compatible with the present study.

Pollens of the taxa *A. lepidoto-stellatum* and *A. sulpureum* from Gamosepalum Section have radial symmetry and are 3-colpate. Polar axis is much longer than equatorial axis, while *A. sulpureum* is prolate, *A. lepidoto-stellatum* is subprolate. Equatorial appearance of pollens is circular. Exine's structure is tectate, ornamentation is reticulate according to detailed examination made under light microscope and by SEM. Exine is 0.97 µm -1.30 µm. Intine is 0.49 µm -0.54 µm (table 1, fig. 1-2(f-g) fig. 3 n-o, p-r). In their study, İnceoglu and Karamustafa (1977) studied the species *A. sibiricum* Willd. from Gamosepalum section. Pollens are subprolate, polar axis is 20 µm, equatorial axis is 15.3 µm, exine is 1.3 µm, intine is 0.60 µm and ornamentation is reticulate. It is compatible with the present study.

Pollens of the taxa *A. murale* subsp*. murale* and *A. pateri* subsp. *pateri* from Odontarrhena section have radial symmetry and are 3-colpate. Both species are prolate. Polar axis is much longer than equatorial axis, equatorial appearance of pollens is circular. Exine's structure is tectate, ornamentation is reticulate according to detailed examination made under light microscope and by SEM. Exine is 0.81 µm -0.93 µm, intine is 0.40 µm -0.47 µm (table 1, fig. 1-2(h-k) fig. 3 s-t, u-z). In their study, İnceoglu and Karamustafa (1977) studied the species *A. murale* Waldst et Kit. subsp. *murale* and *A. pateri* Nyar subsp. *pateri* from Odontarrhena section. Pollens are subprolate, polar axis is 19.1 µm, equatorial axis is 16.3 µm, exine is 1.2 µm, intine is 0.80 µm, and ornamentation is reticulate. These are compatible with the present study. In the study by Orcan and Binzet (2003), pollens of *A. obtusifolium* Steven ex D.C. in the same section were subprolate, polar axis was 15.4 µm, equatorial axis was 13.5 µm, exine was 1.09 µm. It is compatible with the present study.

The present study revealed morphological characteristics of pollens of 9 taxa belonging to the genus *Alyssum* from the family Brassicaceae. Pollens of some taxa (*A. sulphureum, A. trichocarpum, A. lepido-stellatum* and *A. armenum*) analyzed in this study have been studied for the first time. We think that it would be beneficial to several fields particularly plant taxonomy.

Acknowledgements

The authors thank to Doç. Dr. Rıza BİNZET who is helper to take of electron photographs of pollen surface,

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Table 1. Quantitative pollen morphological parameters of *Alyssum* taxa

Taxa	P (µm)	E (µm)	P/E ratio	Ex (µm)	In (µm)	clt (µm)	clg (µm)	t	L	Or.
A. linifolium var. *teheranicum*	26.01 ±1.85	19.41 ±1.53	Prolat	0.86±0.16	0.46±0.10	1.24±0.38	19.99±1.79	4.23±0.69	20.23±1.52	Reticulate
A. simplex	36.55 ±1.99	21.01±0.76	Prolat	1.30±0.27	0.42±0.12	1.63±0.30	31.04±1.85	3.46±0.44	22.78±1.32	Reticulate
A. trichocarpum	40.73 ±1.84	26.15±2.40	Prolat	1.42±0.31	0.48±0.19	1.61±0.38	33.66±2.33	5.15±0.69	27.85±1.57	Reticulate
A. armenum	28.59 ±2.13	20.47±1.85	Prolat	1.07±0.17	0.46±0.18	1.27±0.30	23.29±2.13	5.24±0.18	20.09±1.15	Reticulate
A. praecox	30.74 ±0.75	20.64±1.75	Prolat	0.95±0.11	0.41±0.12	1.22±0.19	25.19±1.97	4.71±0.13	21.52±1.57	Reticulate
A. lepidoto-stellatum	30.06 ±2.74	23.39±2.64	Subprolat	0.97±0.10	0.54±0.08	1.00±0.13	23.77±3.03	4.91±0.62	23.43±1.30	Reticulate
A. sulphureum	33.90 ±2.27	24.21±1.67	Subprolat	1.30±0.33	0.49±0.18	1.55±0.36	24.82±2.59	4.51±0.64	23.97±1.19	Reticulate
A. pateri subsp. *pateri*	21.90 ±0.99	15.78±1.22	Prolat	0.81±0.20	0.40±0.13	0.92±0.12	17.14±1.77	3.45±0.44	15.81±1.06	Reticulate
A. murale subsp. *murale*	24.79 ±1.32	17.10±1.06	Prolat	0.93±0.12	0.47±0.09	0.99±0.19	18.73±1.96	3.42±0.43	17.68±2.11	Reticulate

P: Polar axis, **E:** Equatorial diameter, **clg:** Colpus longitude(length), **clt:** Coplus latitude(width), **Ex**: Exine thikness, **In:** Intine thikness, **t:** mezocolpia, **L:** Apocolpia, **Or:** Ornamentation

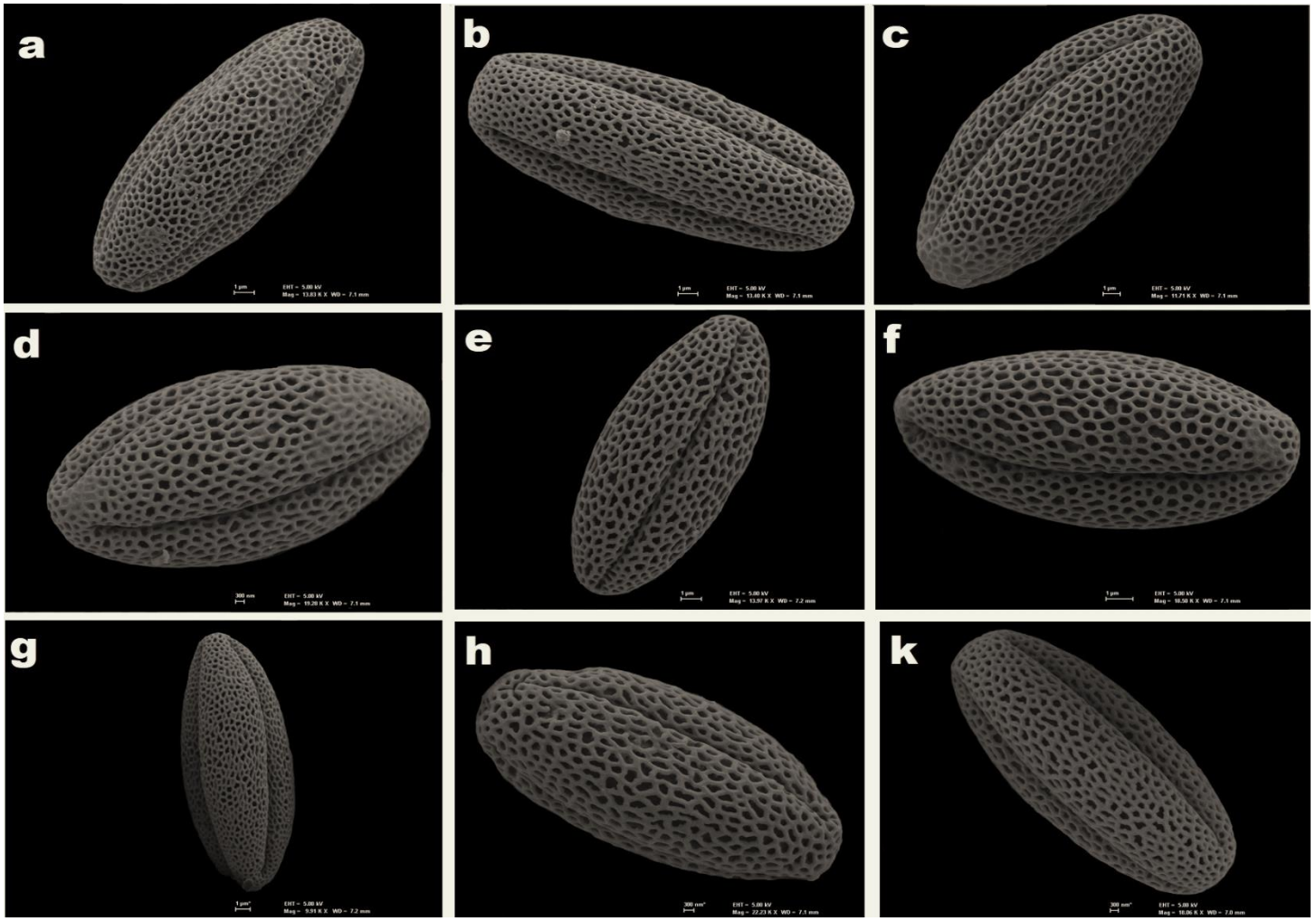

Figure 1. SEM micrograph of ***Alyssum***; Equatorial view and apertures a: ***A. linifolium* var. *teheranicum*** b: ***A. simplex***, c: ***A. trichocarpum***, d: ***A. armenum***, e: ***A. praecox***, f: ***A. lepidoto-stellatum***, g: ***A. sulphureum***, h: ***A. pateri* subsp. *pateri***, k: ***A. murale* subsp. *murale.***

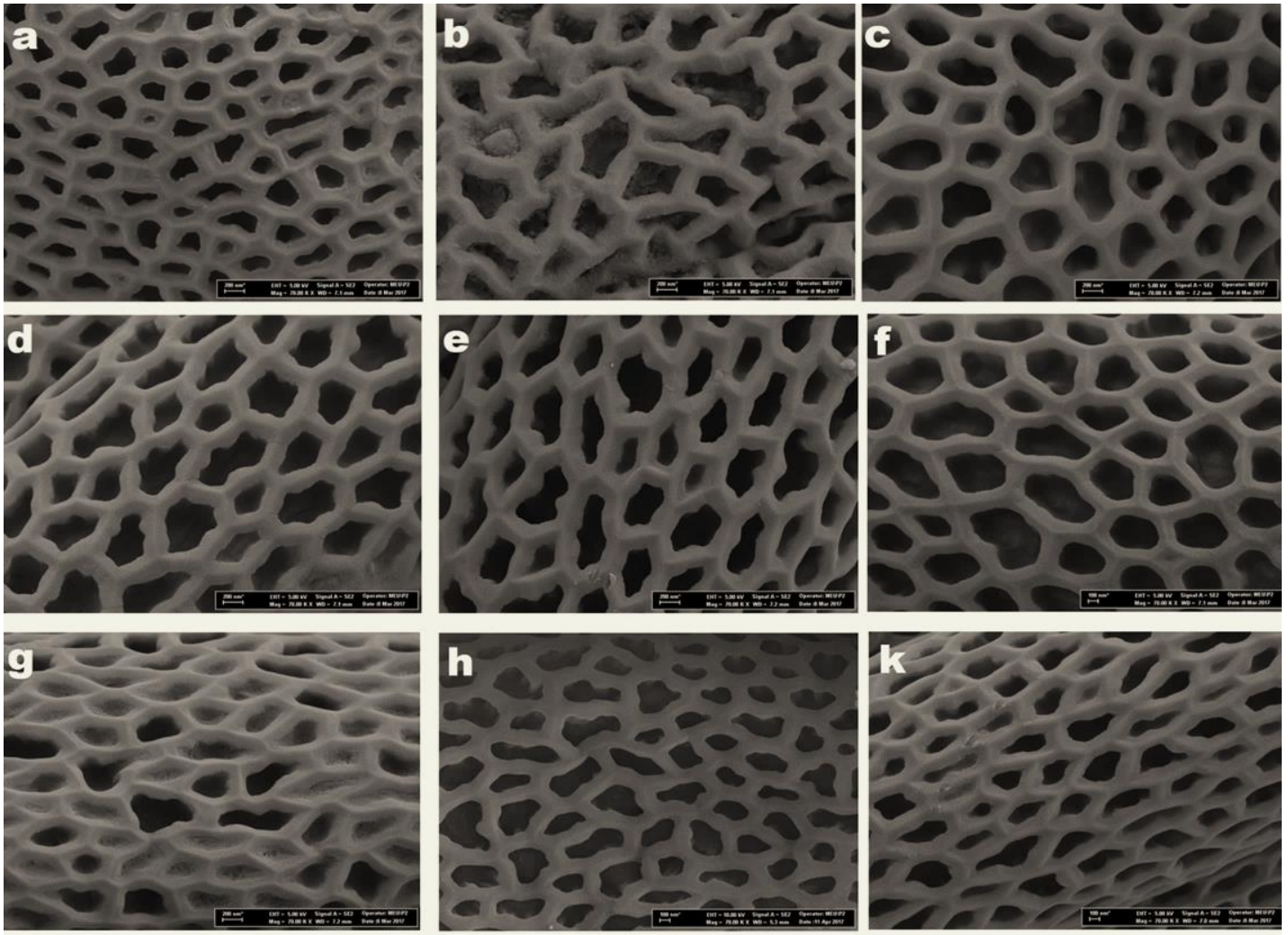

Figure 2. SEM micrograph of ***Alyssum***; Ornamentation. a: ***A. linifolium* var. *teheranicum*** b: ***A. simplex***, c: ***A. trichocarpum***, d: ***A. armenum***, e: ***A. praecox***, f: ***A. lepidoto-stellatum***, g: ***A. sulphureum***, h: ***A. pateri* subsp. *pateri***, k: ***A. murale* subsp. *murale.***

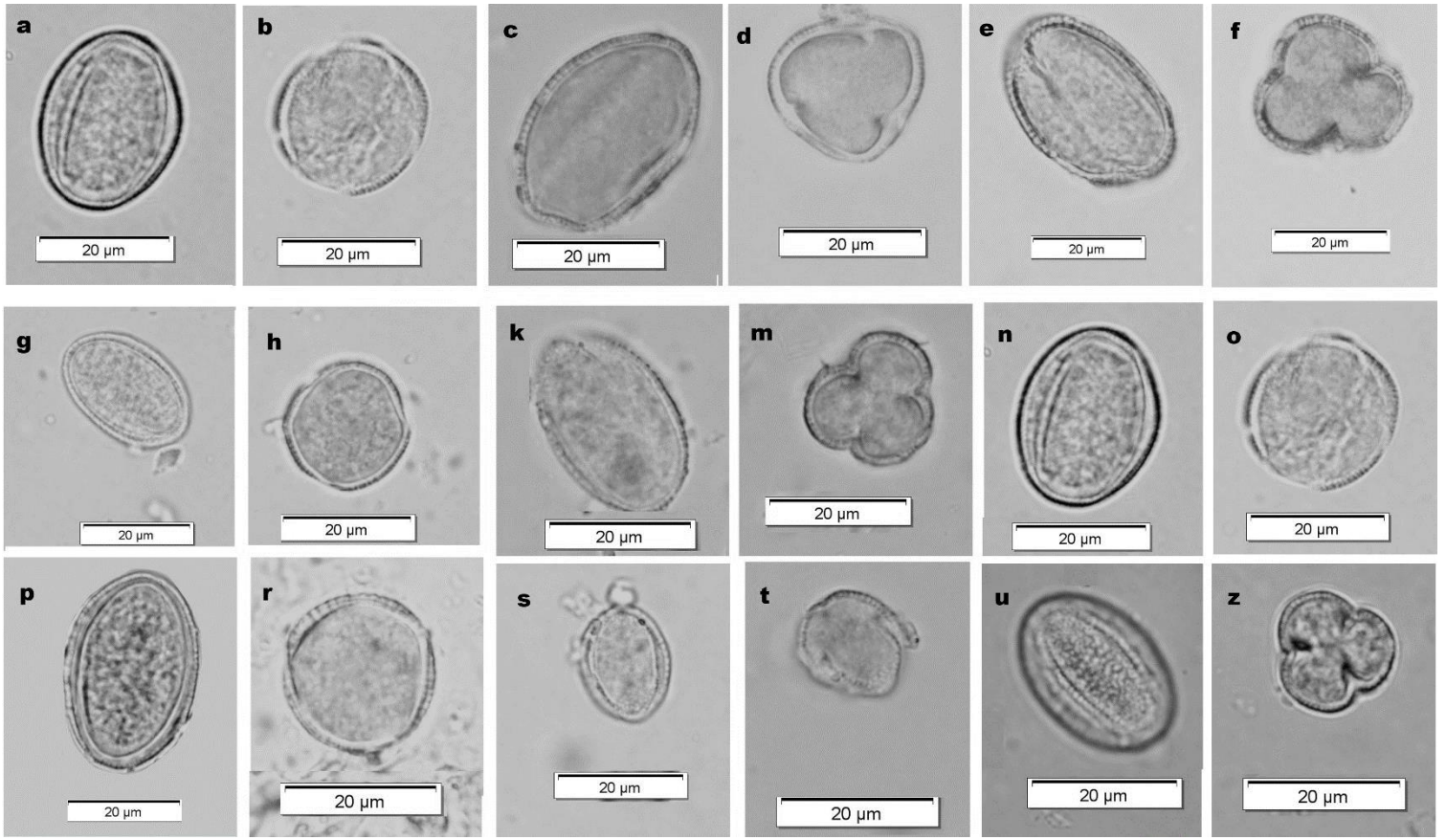

Figure 3. LM micrograph of the pollen of ***Alyssum***. a-b: ***A. linifolium*** var. *teheranicum*, a: Equatorial view, b: Polar view, c-d: ***A. simplex***, c: Equatorial view, d: Polar view, e-f: ***A. trichocarpum*** e; Equatorial view, f: Polar view, g-h: ***A. armenum***, g; Equatorial view, h: Polar view, k-m: ***A. praecox***, k: Equatorial view, m: Polar view, n-o: ***A. lepidoto-stellatum***, n: Equatorial view, o: Polar view, p-r: ***A. sulphureum***, p: Equatorial view, r: Polar view, s-t: **A. pateri subsp. *pateri***, s; Equatorial view, t: Polar view, u-z: ***A. murale* subsp. *murale***, u. Equatorial view, z: Polar view.