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EXAMINATION OF POLLEN MORPHOLOGY OF SOME AETHIONEMA (BRASSICACEAE), FROM TURKEY

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Abstract. Pollen morphology of 23 species of taxonomically difficult genus Aethionema W.T.Aiton was examined by light microscopy (LM) and scanning electron microscopy (SEM). Pollen grains have showed significant variation in aperture number and type. Generally pollen is tricolpate, but tricolpate-syncolpate, tricolpate-syncolpate-tetracolpate, syncolpate-tetracolpate, or merely syncolpate apertures also occur and make a heteromorphic assemblage. Pollen shape is prolate, prolate-spheroidal, subprolate, spheroidal or suboblate, and pollen ranges from 9.4 to 29 ˇm (P), 6.2 to 23.9 ˇm (E). Five main pollen types were identified based on the sculpturing of the exine. The pollen shape, size, surface ornamentation, muri and lumina shape and size are found as important and useful features for distiguishing of the taxa.

1. Introduction

The genus *Aethionema* W.T.Aiton (Brassicaceae) has 75 species in the worldwide, of which 31 species are endemic to Turkey [1,2]. Turkey is gene-center of the genus and maintains 51 taxa [3,4,5,6]. *Aethionema* is a taxonomically difficult genus and few macromorphological characters are available for species delimitation. Life span (annual or perennial) and fruit morphology are very importance at the species level [3]. Brassicaceae contains nectary plant taxa, and these are very important for honey bees [7].

Many researchers have emphasized the importance of pollen morphology for the Brassicaceae family [8,9,10,11,12]. İnceoğlu and Karamustafa (1977) was studied the pollen morphology of *Aethionema arabicum* (L.) Andrz. Ex DC and *A. armenum* Boiss. with light microscopy [13]. Atçeken (2016) studied pollen morphology of four taxa (*A. karamanicum* Ertugrul & Beyazoglu*, A. cordatum* Boiss.*, A. arabicum* and

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A. armenum) of genus [14]. Karaismailoğlu (2017) examined pollen morphology of eleven *Aethionema* taxa (*A. froedinii* Rech. Fil.*, A. arabicum, A. eunomioides* (Boiss.) Bornm.*, A. fimbriatum* Boiss.*, A. speciosum* Boiss *ssp. speciosum, A. speciosum ssp. compactum, A. saxatile, A. oppositifolium* (Pers.) Hedge*, A. iberideum* (Boiss.) Boiss.*, A. armenum* and *A. grandiflorum* Boiss. & Hohen.) distributed in Turkey [15].

Our objective was to examine the pollen morphology of 23 *Aethionema* species and determine the contributions of pollen morphology to taxonomy of the genus.

2. Material & Method

All pollen samples were taken from herbarium specimens in the GAZI collection. The voucher specimens are listed in the Appendix.

The pollen grains were studied by light microscopy (LM) and also by scanning electron microscopy (SEM) in order to investigate the exine sculpturing in detail. Pollen for LM was prepared by the methods of Wodehouse (1935) [16]. The LM studies were performed using a Nikon E-600 microscope. The following parameters were measured: polar (P) and equatorial (E) axis, colpus length (Clg) and width (Clt) and exine and intine thickness. For most specimens, the measurements of P (polar axis) and E (equatorial axis) represent the mean of 50 or more pollen grains, and of some 10 measurements for exine and intine thickness. The LM photographs were taken with a Nikon FDX–35 camera connected to a Nikon E-600 photomicroscope. For SEM, the air-dried pollen were covered with gold during exaporation prior to examination on the screen of a jeol electron microscope. Terminology follows that of Faegri-Iversen [17] and Brochmann [8]. The Simpson and Roe graphical test was used for graphical calculations [18]. The order of the species in the list adopted to Davis [19]. All samples have been deposited by Adıgüzel, N. in the Gazi University Herbarium. Specimens examined were given in Table 1.

Table 1. The information of specimens and localities

A. lepidioides (Hub-Mor.)	B6 Sivas
A. carneum (Banks & Sol.) B.Fedtsch.	C7 Şanlıurfa
A. syriacum (Boiss.) Bornm.	C7 Şanlıurfa
A. heterocarpum J.Gay	C5 Adana
A. virgatum (Boiss) Hedge	Iran Zanjan
A. lycium I.A.Andersson et al.	C3 Antalya
A. stylosum DC.	C4 İçel
A. trinervium (DC.) Boiss.	B9 Van, Iran Tehran
A. spicatum Post	B5 Niğde
A. caespitosum (Boiss.) Boiss.	C5 Kayseri
A. subulatum (Boiss. & Heldr.) Boiss.	C3 Antalya
A. glaucinum Greuter et al.	B5 Niğde
A. membranaceum DC.	B9 Van, C3 Antalya
A. schistosum Boiss. & Kotschy	B4 Konya, C4 İçel
A. capitatum Boiss. & Balansa	B7 Kahramanmaraş
A. diastrophis Bunge	B8 Erzurum
A. coridifolium DC.	B6 Kahramanmaraş
A. munzurense Davis & Yıld.	B7 Tunceli
A. marashicum P.H.Davis	C6 Kahramanmaraş
A. huber-morathii P.H.Davis & Hedge	C5 Adana
A. turcicum H.Duman & Aytaç	B4 Ankara
A. dumanii Vural & Adıgüzel	B4 Ankara
A. alanyae H.Duman	C3 Antalya

3. Results

The main palynological features of the *Aethionema* species examined are summarized in Table 2 and Figure 1.

FIGURE 1. Simpson and Roe test for P, E and P/E.

3.1 Size, Symmetry and Shape

Pollen are isopolar, radialy symmetric. Pollen shape is prolate, prolate-spheroidal, subprolate, spheroidal or suboblate; P: 9.4–29 μm; E: 6.2–23.9 μm; P/E: 0.82–1.67. Outline is elliptic or circular in equatorial view and usually semiangular, rarely more or less circular in polar view (Figures 2,3 and 4).

3.2 Apertures

The pollen grains are tricolpate, tetracolpate or syncolpate. The colpus is long, usually wide. Apertural membrane is generally psilate or rarely granular (Figures 2,3 and 4). Pollen grains of some taxa are surrounded by colpus (syncolpate pollen grains) (Figures 2,3 and 4).

Exine

Exine is semitectate with a reticulate sculpturing. Lumina are generally 0.1 μm or less than 0.1 μm wide (Table 2). Lumina shape is irregular, elliptic or polygonal (Figure 4). Muri is less than 0.01 μm thick. Exine thickness is 0.8–2.3 μm. Ectexine is slightly thicker than endexine. Intine is 0.8–2.3 μm (Table 2). On the basis of reticulate exine sculpturing, 5 pollen types are recognised in the genus *Aethionema*:

Type I: The pollen grains of this type have a characteristic, vermiculate reticulation with relatively wide muri (mean 0.04 μm) and irregularly shaped lumina. This type was observed in *A. lepidioides* and *A. marashicum* (Figures 2, 3 and 4). The species included in type I are characterised by tricolpate, spheroidal or prolate-spheroidal pollen grains (Table 2).

Type II: This type has characteristic pollen grains with wide muri (mean 0.06 μm) and regularly shaped lumina. This type was characterized by tricolpate-syncolpate aperture types and observed in *A. trinervium*, *A. diastrophis* and *A. spicatum* (Figure 2, 3 and 4). All these species show heteromorphy.

Type III: Pollen grains have narrow muri (mean 0.025 μm) and wide lumina (mean 0.05 μm). This type was observed in *A. heterocarpum*, *A. lycium, A. caespitosum, A. subulatum, A. membranaceum, A. schistosum, A. dumanii* and *A. alanyae* (Figure 2, 3 and 4). Pollen grains are often tricolpate.

Type IV: This type has characteristic pollen grains narrow muri (mean 0.025 μm) and polygonal lumina. This type was observed in *A. virgatum, A. stylosum, A. glaucinum, A. capitatum*, *A. coridifolium, A. munzurense, A. huber-morathii and A. turcicum* (Figures 2, 3 and 4).

Various aperture types have been observed in this type: tricolpate (*A. virgatum, A. stylosum, A. glaucinum, A. munzurense* and *A. turcicum), tricolpate-syncolpate (A. coridifolium), syncolpate* (*A. capitatum)* and tetracolpate (*A. huber-morathii*) (Table 2). The pollen shape is often prolate-spheroidal or subprolate (Figure 3).

Type V: Pollen grains have narrow muri and irregularly shaped lumina. This type was observed in *A. carneum* (Figure 2, 3 and 4) . The pollen type is prolate-spheroidal (Figure 3) and tricolpate-syncolpate (Table 2).

4. Result & Discussion

The pollen of the *Aethionema* species examined in this study showed considerable variations with respect to features such as the number and type of apertures and thickness of muri and width of lumina. In *Aethionema,* 16 species were found with merely tricolpate (*A. lepidioides, A. marashicum, A. heterocarpum, A. coespitosum, A. subulatum, A. membranaceum, A. schistosum, A. dumanii, A. virgatum, A. stylosum, A. glaucinum, A. munzurense, A. turcicum* and *A. syriacum*), one with merely syncolpate (*A. capitatum*) and one with merely tetracolpate pollen (*A. huber-morathii*). The other species were tricolpate-syncolpate (*A. trinervium, A. diastrophis, A. lycium, A. alanyae, A. caridifolium* and *A. carneum*) (Table 2). Rollins and Banerjee have observed that the most common pollen type in the family Brassicaceae is tricolpate with grooves varying in length in different genera [20]. In addition, tetracolpate form in the pollen of *Dithyrea californica* and pentacolpate forms characteristic of many species of the genus *Querella*, *Physaria* and *Dimorphocarpa* of Brassicaceae have been seen. Doğan and İnceoğlu also found tricolpate, syncolpate, tetracolpate and pentacolpate grains in the genus *Isatis* L. of the family Brassicaceae [21]. Tricolpate pollen grains of the species *A. stylosum, A. glaucinum, A. schistosum, A. turcica* and *A. dumanii* show considerable variations in pollen size (Table 2 and Figure 1). In each specimen of these species both 50% larger and 50% smaller pollen grains have been observed. Variation in pollen size and aperture type was attributed to heteromorphy in pollen grains by some researchers [22,23,24,25]. Heteromorphy is caused by either anomalies in meiosis [22, 26], colchicine [27,28], or hybridization [29,30].

Table 2. Pollen morphological parameters of Aethionema (values in μm)

Species	Polar axis (P)			Equatorial axis (E)			P/E Ratio	Colpus length (Clg)			Colpus width (Clgt)			Shape	Exine Thickness	Intine Thickness	Aperture type	Muri width	Lumen Width	Pollen Type
	min	mean	max	min	mean	max	mean	min	mean	max	min	mean	max					mean	mean	
A. lepidioides(A1)	16,2	16.6	16.8	16.3	16.6	16.7	1	9.2	11.4	12.4	3.7	4.2	4.8	Spheroidal	1	0.4	tricolpate	0.04	0.07	I
A. marashicum(A2)	15.6	17.3	18.7	12.5	14.6	15.6	1.18	12.3	15.6	16.9	2	2.6	3.1	Prolate-Spheroidal	1	0.5	Tricolpate, 6 % tetracolpate	0.04	0.04	I
A. trinervium(A3)	17.7	18.2	19.8	17.7	18.2	19.8	1	14.4	16.1	17.6	3.7	4.2	4.9	Spheroidal	1.1	0.3	Tricolpate, 40% syncolpate	0.06	0.03	II
A. spicatum(A4)	16.6	17.9	18.7	17.7	17.9	18.7	1	10.4	12.2	14.1	3.9	4.9	5.2	Spheroidal	0.8	0.3	Tricolpate, 3% tetracolpate	0.06	0.05	II
A. diastrophis(A5)	13.5	15.2	17.7	8.3	9.6	11.4	1.58	9.4	11.2	12.1	0.9	1.1	1.7	Subprolate	1	0.3	Tricolpate, 10% syncolpate	0.06	0.05	II
A. heterocarpum (A6)	22.9	27.3	29	17.9	17.8	18.7	1.53	15.7	22.4	23.4	1.9	2.8	3.0	Subprolate	1	0.3	tricolpate	0.02	0.05	III
A. lycium(A7)	13.5	15.6	16.6	9.4	11.4	12.5	1.37	9.7	12.5	13.7	1.9	2.1	2.8	Subprolate	1.6	0.3	Tricolpate, 2% syncolpate	0.03	0.04	III
A. coespitosum(A8)	13.5	16.4	17.7	14.6	15.1	16.6	1.09	9.1	12	13.4	4.8	5.2	6.1	Prolate-Spheroidal	1.4	0.3	tricolpate	0.02	0.06	III
A. subulatum(A9)	14.6	15	15.6	13.5	14.6	15.6	1.03	9.2	10.4	11.1	3.1	3.5	3.9	Prolate-Spheroidal	0.9	0.3	tricolpate	0.01	0.06	III
A. membranaceum (A10)	14.6	17.1	19.8	8.3	10.4	13.5	1.64	9.7	13.3	14.7	1.9	2.1	3.2	Subprolate	0.9	0.3	tricolpate	0.02	0.06	III
A. schistosum(A11)	11.4	15.2	26	6.2	11.1	18.7	1.38	8.1	12.5	21.4	4.2	5.3	6.1	Subprolate	0.85-1.2	0.3	tricolpate	0.007-0.01	0.05-0.07	III
A. dumanii(A12)	17.7	20	25	12.5	14.6	15.6	1.45	12.4	16.6	20.2	1.8	2.6	3.0	Subprolate	1	0.3	tricolpate	0.04	0.07	III
A. alanyae(A13)	13.5	16.1	16.6	12.5	15.4	15.6	1.05	8.6	15.1	16.2	2.0	2.3	2.7	Prolate-Spheroidal	0.9	0.3	Tricolpate,	13.5	16.1	IV
A. virgatum(A14)	16.6	17	17.7	12.5	15.9	17.7	1.07	10.9	12	13.1	4.8	5.2	5.7	Prolate-Spheroidal	1	0.6	tricolpate	0.03	0.2	IV
A. stylosum(A15)	15.6	21.1	23.9	17.7	20.6	23.9	1.02	10.2	18	20.4	5.1	9	9.8	Prolate-Spheroidal	1.3	0.3	tricolpate	0.02	0.06	IV
A. glaucinum(A16)	14.6	20.7	23.9	15.6	20.2	20.9	1.02	11.1	17.3	20.2	3.9	4.6	5.7	Prolate-Spheroidal	1	0.5	tricolpate	0.03	0.2	IV
A. capitatum(A17)	14.6	15.9	17.7	8.3	11	12.5	1.45	10.3	14.1	15.2	1.2	2.1	2.8	Subprolate	1.6	0.3	syncolpate	0.02	0.07	IV
A. caridifolium (A18)	16.6	17.6	18.7	14.6	15.3	15.6	1.15	13.1	15.6	16.2	2.4	3.1	3.8	Prolate-Spheroidal	2.1	0.3	Tricolpate, 10% syncolpate	0.02	0.09	IV
A. munzurense(A19)	19.8	20.5	21.8	17.7	18.5	19.8	1.11	14.1	14.8	16.1	3.4	4.2	5.1	Prolate-Spheroidal	2	0.3	tricolpate	0.02	0.1	IV
A. huber-morathii (A20)	14.6	15	15.6	15.6	15.8	16.6	0.9	8.3	10.4	11.2	4.3	5.2	5.9	Oblate-Spheroidal	0.8	0.3	tetracolpate	0.02	0.05	IV
A. turcicum(A21)	17.7	21.7	27.1	15.6	20	22.9	1.09	14.7	18.7	24.2	2.0	2.4	3.2	Prolate-Spheroidal	1.8	0.5	tricolpate	0.03	0.1	IV
A. carneum(A22)	17.7	18.7	20.8	15.6	18.4	19.8	1.02	14.2	15.6	16.7	5.8	6.2	7.1	Prolate-Spheroidal	1	0.3	Tricolpate, 2% Syncolpate	0.015	0.03	V
A. syriacum (A23)	25	26	27	14	15.6	17	1.67	19.7	20.8	21.2	1.1	1.6	2.0	Subprolate	1	0.3	tricolpate	0.01	0.03	II

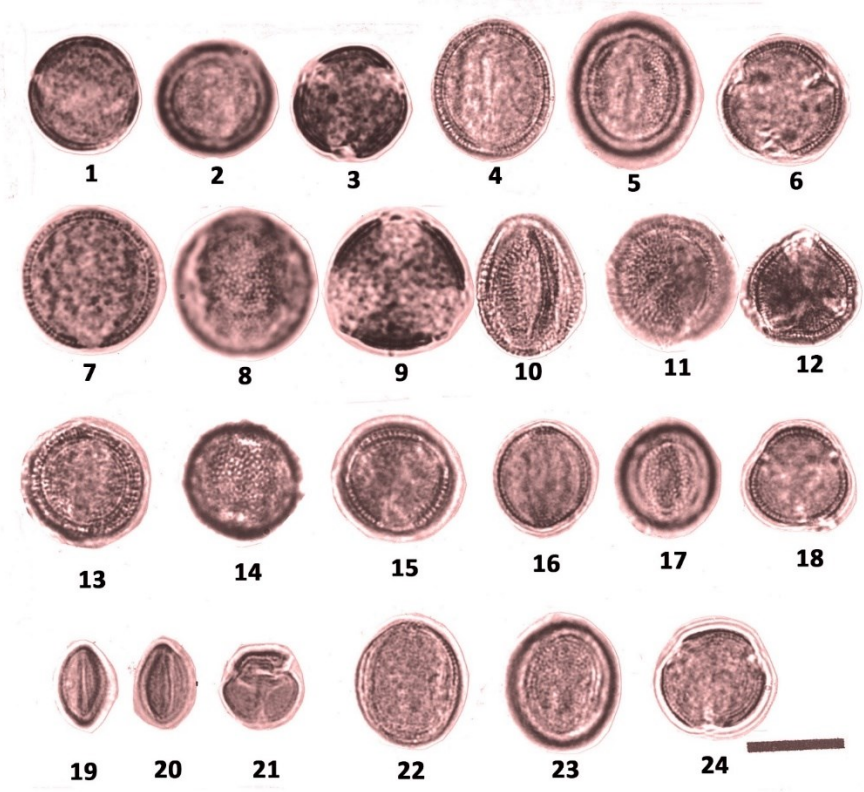

FIGURE 2. LM pollen microphotograph of *Aethionema* taxa. **1–3:** *A. carneum*, 1; Meridional, optical section, outline, 2; Aperture and ornamentation, 3; Polar view. 4–6: *A. heterocarpum*. 4: Meridional, optical section, outline. 5: Aperture and ornamentation. 6: Polar view. 7–9: *A. stylosum*. 7: Meridional, optical section, outline. 8: Ornamentation, 9: Polar view. 10–12: *A. trinervium*. 10: Meridional, optical section, outline. 11: Aperture and ornamentation. 12: Polar view. 13-15: *A. caespitosum*, 13; Meridional, optical section, outline, 14; Ornamentation. 15; Polar view. 16-18: *A. membraneceum,* 16; Meridional, optical section, outline, 17; Ornamentation, 18; Polar view. 19-24: *A. schistosum*. 19; Meridional, optical section, outline, 20; Aperture. 21; Polar view, 22; Meridional, optical section, outline. 23; Ornamentation. 24; Polar view (Scale bar: 10 µm).

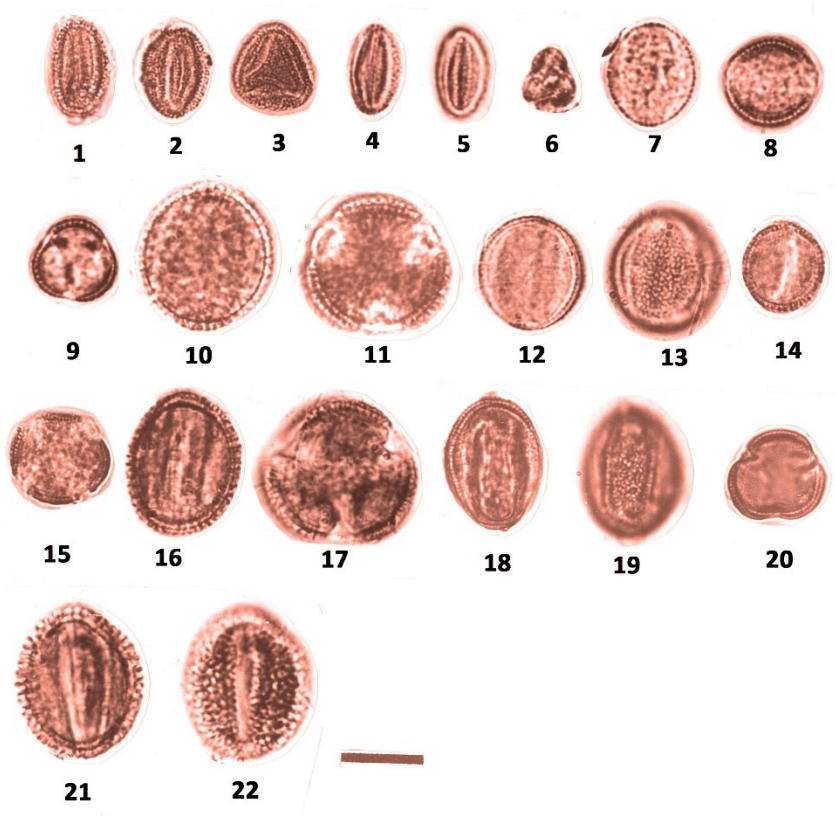

FIGURE 3. LM pollen microphotograph of *Aethionema* taxa. 1-3: *A. capitatum*, 1; Meridional, optical section, outline. 2; Aperture and ornamentation, 3; Polar view. 4-6: *A. diastrophis*, 4; Meridional, optical section, outline. 5; Aperture and ornamentation, 6; Polar view. 7-9: *A. coridifolium*, 7; Meridional, optical section, outline. 9; Syncolpate aperture, 9; Polar view. 10-11: *A. munzurense,* 10; Meridional, optical section, outline. 11; Polar view. 12-13: *A. marashicum*, 12; Meridional, optical section, outline. 13; Ornamentation. 14-15: *A. huber-morathii*, 14; Meridional, optical section, outline. 15; Polar view. 16-17: *A turcica*, 16; Meridional, optical section, outline. 17; Polar view. 18-20: *A. dumanii*, 18: Meridional, optical section, outline. 19; Ornamentation, 20; Polar view. 21-22: *A. alanyae*, 21; Meridional, optical section, outline. 22; Aperture and ornamentation (Scale bar: 10 µm)

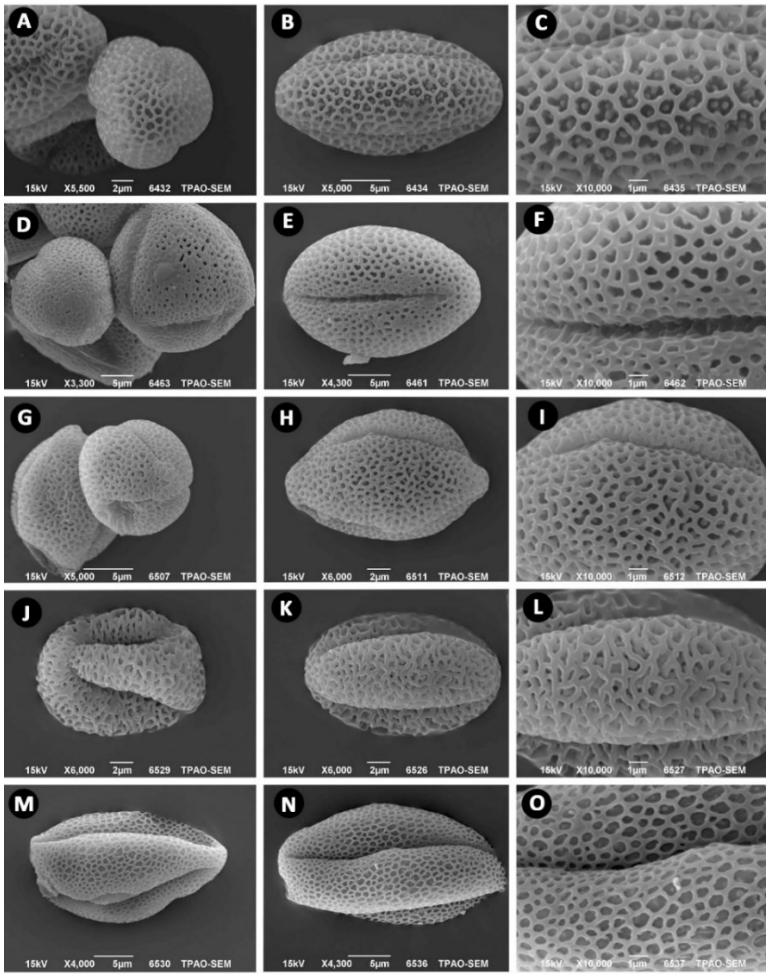

FIGURE 4. SEM pollen microphotograph of *Aethionema* taxa. A-C: *A. marashicum* (type I), A; Polar view, B; equatorial view, C; ornamentation. D-F: *A. trinervium* (type II), D; Syncolpate and tricolpate pollen in polar view, E; equatorial view, F; ornamentation. G-I: *A. schistosum* (type III), G; Syncolpate pollen in polar view, H; equatorial view, I; ornamentation. J-L: *A. capitatum* (type IV), J; Syncolpate pollen in polar view, K; equatorial view, L; ornamentation. M-O: *A. carneum* (type V), M-N; equatorial view, O; ornamentation.

There has been no chromosomal study of the *Aethionema* species so far. Thus, future cytological studies in the genus may provide further information about heteromorphy in pollen grains. İnceoğlu and Karamustafa investigated 32 Brassicaceae species pollen grains and these were divided into 3 reticulate types, according to the diameters of the lumina (faintly reticulate, reticulate and coarsely reticulate), using LM [13].

Brochmann divided the genus *Draba* L. of the family Brassicaceae into 5 pollen types with respect to exine sculpturing resembling *Aethionema* in pollen morphology. In this study 5 pollen types are recognised according to pollen exine sculpturing (types I, II, III, IV and V) These pollen type described as vermiculate reticulation with relatively wide muri and irregularly shaped lumina (Type I), reticulate pollen grain with wide muri and regularly shaped lumina (Type II), pollen grain have narrow muri and wide lumina (Type III), pollen grain of this type have narrow muri and polygonal lumina (Type IV), pollen grains have narrow muri and irregularly shaped lumina (Type V). Heteromorphic pollen grains are mostly observed in the species belonging to type IV [8]. İnceoğlu and Karamustafa described the general pollen morphological features of *A. arabicum* and *A. armenum* using LM. They described the sculpturing of these 2 species as faintly reticulate [13]. Karaismailoğlu, determined reticulate, microreticulate and coarsely reticulate exine sculpturing in eleven taxa of *Aethionema* [15]. Similarly Atçeken et al. determined reticulate and microreticulate ornamentation in four taxa of *Aethionema* [14]. In addition, in a study which is about pollen morphology of 39 species belonging to 23 genera of the tribes Arabideae, Euclidieae, Hesperideae, Lunarieae, Matthioleae and Sisymbrieae from Brassicaceae from Egypt were studied by using scanning electron microscope (SEM), the pollen grains were tricolpate and their shape varies from prolate spheroidal, subprolate to prolate. Three pollen types can be distinguished based on the size of the lumina. The ornamentation varies between genera within the tribes and between species within the same genus [31].

This study results show that pollen shape, size, surface ornamentation, muri and lumina shape and size are important and useful characters contributing to the differentiation of the taxa.

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