COMPARATIVE MORPHOLOGY AND ANATOMY OF SEEDS OF SOME AETHIONEMA W.T. AITON (BRASSICACEAE) TAXA FROM TURKEY

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Abstract

Seed morphology and anatomy are taxonomically significant in Brassicaceae. The seed structures of *Aethionema* from Turkey, which include 12 taxa (*Aethionema syriacum*, *A. froedinii*, *A. arabicum*, *A. eunomioides*, *A. fimbriatum*, *A. speciosum* subsp. *speciosum*, *A. speciosum* subsp. *compactum*, *A. saxatile*, *A. oppositifolium*, *A. iberideum*, *A. armenum*, *A. grandiflorum*) were studied for knowing seed morphological and anatomical features with one-way analysis of variance, cluster analysis and principal component analysis. Seed size, shape and color were examined with stereomicroscopy. The surface patterns of seed were observed using Scanning Electron Microscopy (SEM). In addition, structure, and thicknesses of testa and endosperm were investigated anatomically. Thickness of testa and endosperm were of major significance to illustrate interspecific relations among the examined taxa.

Introduction

The Brassicaceae is one of the largest angiosperm families, including ca. 340 genera and 3350 species distributed throughout the world mainly in temperate regions of the Northern Hemisphere (Al-Shehbaz, 1986; Khalik and Maesen, 2002; Karaismailoğlu, 2017).

Taxonomically the genus *Aethionema* W.T. Aiton is problematic. Turkey is one of the centers of biodiversity and its number in outside Anatolia diminishes progressively (Davis, 1965; Pinar *et al.*, 2007). In Turkey, the genus represented with 45 *Aethionema* species, 20 species of which are endemic to Turkey (Davis, 1965; Guner *et al.*, 2012; Karaismailoğlu, 2018). The genus is extremely variable in habit, fruit and floral morphology, and chromosome number (Appel and Al-Shehbaz, 2003; Al-Shehbaz *et al.*, 2006). In addition, genus has a widespread convergence in traditional characters used in taxonomy, especially in fruit (Mummenhoff *et al.*, 1997). This factor cause some problems in classification of the genus.

Micromorphological features are taxonomically significant in the species delimitation (Brochmann, 1992; Pinar *et al.*, 2007), in determining evolutionary relationships and solving taxonomic problems (Khalik and Maesen, 2002). Especially, the seed coat variation is very important in infrageneric classification. Structures of the epidermal cells are also good diagnostic characteristics for the lower taxonomical categories (Barthlott, 1981; Khalik and Maesen, 2002). Likewise, seed and fruit anatomical characters are also used in systematic in determination of the natural limits of the genera (Karaismailoğlu, 2015a). However, there are only few taxonomical works on seeds structure of *Aethionema* and they are limited to few characteristics (Pinar *et al.*, 2007; Atceken *et al.*, 2016). Hence, the aim of this work is to test potency morphological and anatomical characters of seeds of some *Aethionema* taxa and their use in the classification within the genus using multivariate analyses and principal component analysis.

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Material and Methods

The seeds of 12 taxa of *Aethionema* were utilized for the morphological and anatomical studies. The specimens were collected from natural populations and stored in SUFAF (Siirt University Fauna and Flora Center). 50 ripe seeds of each taxon were taken. All examined taxa are listed in Table 1 with their locations.

Table 1.	The	examined	taxa	and	their	locations.

Taxa	Location	Voucher
Aethionema syriacum	Hatay, Dörtyol, Yahyalı plateau, meadows, 750 m, 19.03.2017	Karaismailoğlu 346
A. froedinii	Gümüşhane, Kelkit, Akdağ, inclined slopes, 2100 m, 09.07.2015	Karaismailoğlu 213
A. arabicum	Muğla, Köyceğiz, Ağla-Eren villages, roadsides, 1753 m, 05.06.2015	Karaismailoğlu 194
A. eunomioides	Artvin, Yusufeli, Kılıçkaya, roadside, rocky slopes, 704 m, 22.05.2015	Karaismailoğlu 169
A. fimbriatum	Niğde, Çamardı, Yelatan village tops, 2083 m, 12.06.2016	Karaismailoğlu 275
A. speciosum subsp. speciosum	Artvin, Şavşat, Ciritdüzü village, stones slopes, 1182 m, 10.07.2014	Karaismailoğlu 67
A. speciosum subsp. compactum	Muğla, Köyceğiz, Sandras mount, subalpin regions, slopes, 1819 m, 04.06.2016	Karaismailoğlu 260
A. saxatile	Trabzon, Of, roadside, meadows, 10 m, 17.07.2014	Karaismailoğlu 94
A. oppositifolium	Ağrı, airport environments, open fields, meadows, 1600 m, 16.05.2015	Karaismailoğlu 164
A. iberideum	Erzurum, İspir-İkizdere, rocky slopes, 1154 m, 22.05.2015	Karaismailoğlu 170
A. armenum	Kahramanmaraş, Göksun, Berit mountain, humid areas, 1750 m, 19.06.2015	Karaismailoğlu 206
A. grandiflorum	Muğla, Marmaris, Kırzeytin mountain, serpentine stones, 494 m, 05.06.2015	Karaismailoğlu 191

Macromorphological features such as shape, size, and color of the seeds were studied using Olympus ZS51 stereomicroscope and Kameram Imaging Software. For micromorphological examinations of seed surface ornamentation, specimens were prepared for Scanning Electron Microscopy by mounting with silver adhesive on the stub, and gold coated, and examined with JEOL Neoscope-5000 Scanning Electron Microscope (Karaismailoğlu, 2015b).

The cross sections were taken from the middle part of the seed with an automatic microtome (Thermo Shonda Met Finesse). The specimens were placed in FAA for a minimum of 24 hrs, then dehydrated through ethanol and xylene series, and stained with hematoxylin (harris-RRSP67-E) in a dyeing apparatus (ASC 720 Medite), and were mounted with Entellan to observe anatomical features (Karaismailoğlu, 2015a, b). The anatomical characters (including testa and endosperm thicknesses) were observed and photographed with using Olympus CX21FS1 microscope and Kameram Imaging Software.

Data analyses presented in Tables 2 and 3 were made with Duncan's multiple-range test in SPSS computer program to determine the statistical significance of differentiations among the data to evaluate interspecific relationships (SPSS, 2006). Grouping of taxa was performed using the

Taxa						Features			
	Colour	Shape	Seed	Seed sizes	Ratio	Ornamentation	Cell types on the	Anticlinal	Periclinal
			L (mm)	W (mm)	L/W	on the surface	surface	cell wall	cell wall
Aethionema syriacum	Dark Brown	Elliptic	1.19±0.05cd	0.87±0.04cd	1.36	Verrucate	Alveolate	Sunken	Convex or flat
A. froedinii	Light Brown	Broadly Ovate	1.21±0.11cd	1.05±0.12bc	1.15	Reticulate	Rectangular and pentagonal	Raised	Concave
A. arabicum	Brown- Gray	Elliptic	1.21±0.13cd	0.51±0.18de	2.37	Verrucate	Alveolate or polygonal	Sunken	Concave or flat
A. eunomioides	Brown	Ovate	1.37±0.11c	0.68±0.09d	2.01	Reticulate- foveate	Alveolate or polygonal	Raised	Convex
A. fimbriatum	Brown- Gray	Elliptic	1.71±0.16b	1.08±0.12bc	1.58	Ocellate	Alveolate	Sunken	Convex or flat
A. speciosum subsp. speciosum	Brown- Black	Elliptic	2.02±0.08a	1.49±0.14a	1.35	Verrucate	Alveolate	Sunken	Concave or flat
A. speciosum subsp. compactum	Light Brown	Ovate	1.41±0.11c	0.95±0.08c	1.48	Ocellate	Alveolate	Sunken	Concave or flat
A. saxatile	Brown	Elliptic	1.19±0.10cd	0.91±0.08c	1.30	Verrucate	Alveolate	Sunken	Concave or flat
A. oppositifolium	Dark Brown	Elliptic	2.04±0.16a	1.49±0.13a	1.36	Ruminate	Irregular wrinkles	Sunken	Convex
A. iberideum	Black	Ovate	1.41±0.12c	0.85±0.06cd	1.65	Reticulate	Polygonal	Raised	Concave
A. armenum	Brown	Ovate	1.26±0.08cd	0.89±0.06cd	1.41	Reticulate- ocellate	Polygonal and alveolate	Raised or sunken	Concave or flat
A. grandiflorum	Dark Brown	Ovate	1.65±0.15b	1.21±0.08b	1.36	Reticulate- foveate	Polygonal and alveolate	Sunken	Concave
*Different letters are significant at $p = 0.05$ level (Duncan's multiple-range test), length=L, width=W	significant a	tt p = 0.05 le	yvel (Duncan's m	ultiple-range tes	st), length	i=L, width=W.			

Table 2. The macro- and micro morphological features of the examined taxa.

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Таха			Fe	Features		
	Outer epidermis	Inner epidermis	Testa	Compressed tissue	Parenchyma	Ja
	cell type	cell type	thickness	structure	type	thickness
A. syriacum	Rectangular cells	Cubic cells	51.77±5.47f	Single layer coarsely flat cells	Single layer flat cells	10.94±2.11e
A. froedinii	Cylindrical shaped cells	Elongated rectangular shaped cells	104.12±4.11a	Single layer coarsely flat cells	Single layer cubic cells	35.40±2.54a
A. arabicum	Irregularly flat cells	Rectangular cells	61.28±7.26e	Single layer flat cells	Single layer flat cells	8.26±0.77ef
A. eunomioides	Flat cells	Elongated rectangular shaped cells	43.08±3.10g	3-4 layers crushed cells	Single layer coarsely flat cells	30.79±1.25b
A. fimbriatum	Irregularly flat cells	Elongated rectangular shaped cells	70.25±2.45d	Single layer coarsely rectangular cells	Single layer flat cells	10.05±1.19e
A. speciosum subsp. Ovoid speciosum	Ovoid cells	Flat cells	88.19±2.18c	Single layer crushed cells	Single layer flat cells	18.49±1.35d
A. speciosum subsp. Ovoid compactum	Ovoid cells	Elongated rectangular shaped cells	96.23±5.01b	1- or 2-layers rectangular cells	Single layer flat cells	11.08±1.13e
A. saxatile	Coarsely flat cells	Rectangular cells	105.49±4.09a	Single layer flat cells	Single layer rectangular cells	9.27±0.84e
A. oppositifolium	Flat cells	Crushed cells	50.69±1.98f	Single layer flat cells	Single layer flat cells	34.85±1.45a
A. iberideum	Flat cells	Elongated rectangular shaped cells	31.43±0.49h	Single layer flat cells	Single layer flat cells	23.84±0.71c
A. armenum	Tuberculate or flat cells	flat cells	60.43±6.43e	Single layer flat cells	Single layer flat cells	9.25±0.22e
A. grandiflorum	Ovoid or flat cells	Rectangular cells	51.42±0.99f	Single layer flat cells	Single layer flat cells	17.26±0.85d

Table 3. The seed anatomical features of the examined taxa.

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clustering analysis method (Unweighted Pair Group Method with Arithmetic mean, UPGMA) (Fig. 3). In addition, coordination and similarity matrix based upon Principal Component Analysis (PCA) were done (Fig. 4 and Table 3). All computations except Duncan's multiple range tests were performed by the MVSP software (Kovach, 2007).

Results and Discussion

Macro and micro morphological data on the seeds of the examined *Aethionema* taxa indicate a wide variation (Table 2). Color, shape, and size features of the examined taxa were studied macromorphologically. Seeds are elliptic (*A. syriacum, A. arabicum, A. fimbriatum, A. speciosum* subsp. *speciosum, A. saxatile* and *A. oppositifolium*) and ovate (*A. eunomioides, A. speciosum* subsp. *compactum, A. iberideum, A. armenum* and *A. grandiflorum*) or broadly ovate (*A. froedinii*) in shape and light Brown (*A. froedinii* and *A. speciosum* subsp *compactum*) or dark Brown (*A. syriacum, A. oppositifolium* and *A. grandiflorum*), Brown-Gray (*A. arabicum* and *A. fimbriatum*), Brown-Black (*A. speciosum* subsp. *speciosum*) and Black (*A. iberideum*) in color. Surface of the seeds is slightly rough or straight (Table 2). Seed sizes are variable, which range between 1.19 and 2.04 mm in length, and between 0.51 and 1.49 mm in width. Particularly, *A. arabicum, A. speciosum* and *A. oppositifolium* are of the greater variability among *Aethionema* taxa.

Seed surfaces of the taxa were examined as micromorphologically, and prominent characters such as surface ornamentations and cell types, periclinal and anticlinal cell walls of the seeds were recorded (Table 2 and Fig. 1). Six types of seed surface ornamentations were determined: verrucate in *A. syriacum, A. arabicum, A. speciosum* subsp. *speciosum* and *A. saxatile*, reticulate in *A. froedinii* and *A. iberideum*, reticulate-foveate in *A. eunomioides* and *A. grandiflorum*, ocellate in *A. fimbriatum* and *A. speciosum* subsp. *compactum*, ruminate in *A. oppositifolium*, and reticulate-ocellate in *A. armenum*. The most common type is verrucate, and, the least common types are ruminate and reticulate-ocellate (Table 2). This character is found to be taxonomically important in the delimitation of the taxa within the genus. Besides, cell shapes on the seed surfaces are very diverse, and consisted of alveolate cells, polygonal, rectangular, or pentagonal and irregular wrinkles. The predominant cell type is alveolate; the rare types are rectangular or pentagonal. At the same time, anticlinal cell walls in examined taxa are sunken or raised and flat or undulated, periclinal cell walls are convex, concave, and flat as well.

The outcomes of anatomical studies are given in Table 3 and Fig. 2. The testa epidermis in the examined taxa is scleranchymatic type and consists of two layers including outer and inner epidermis. The outer epidermis indicated markedly variations among the examined taxa. They are rectangular in A. syriacum, cylindrical in A. froedinii, flat in A. arabicum, A. eunomioides, A. fimbriatum, A. oppositifolium, A. iberideum, A. armenum and A. grandiflorum, ovoid in A. speciosum subsp. speciosum, A. speciosum subsp. compactum and A. grandiflorum, and tuberculate in A. armenum, waved or straight and thick or thin-walled, regular or irregular cells in the cross-sections (Fig. 2). However, the inner epidermis is in the form such as cubic in A. syriacum, rectangular in A. froedinii, A. arabicum, A. eunomioides, A. fimbriatum, A. speciosum subsp. compactum, A. saxatile, A. iberideum and A. grandiflorum, flat in A. speciosum subsp. speciosum and A. armenum, and crushed in A. oppositifolium. Besides, the mean values of the testa thickness range between 105.49 µm and 31.43 µm. Accordingly, testa covers the most location in testa based on other examined anatomical characters. As well as, parenchyma (endosperm) cells in the examined taxa are single-layered and are composed of the flattest, rarely cubic, and rectangular cells. Endosperm thickness in examined taxa range between 35.40 µm and 8.26 µm; the widest endosperm is noted in A. froedinii; however, it is the narrowest in A. arabicum (Table 3).

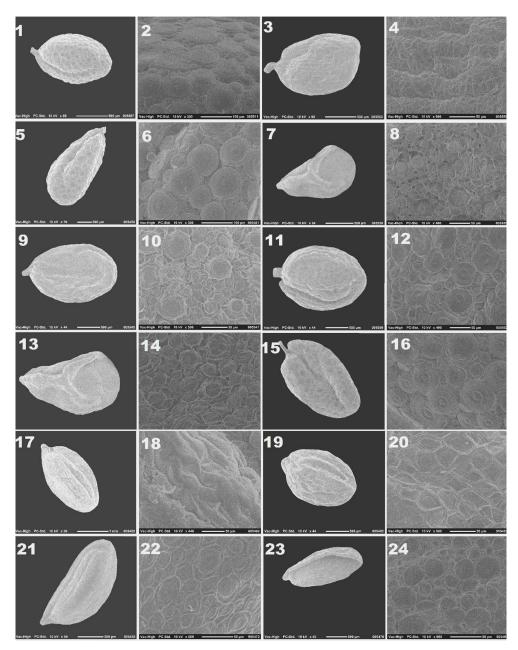


Fig. 1. SEM micrographs of the examined taxa: 1-2. A. syriacum, 3-4. A. froedinii, 5-6. A. arabicum, 7-8. A. eunomioides, 9-10. A. fimbriatum, 11-12. A. speciosum subsp. speciosum, 13-14. A. speciosum subsp. compactum, 15-16. A. saxatile, 17-18. A. oppositifolium, 19-20. A. iberideum, 21-22. A. armenum, 23-24. A. grandiflorum.

The UPGMA dissimilarity clustering dendrogram for the studied taxa is shown in Fig. 3. A. speciosum subsp. speciosum, A. speciosum subsp compactum, A. saxatile and A. froedinii form cluster A; other eight taxa form cluster B. In cluster A three taxa, A. speciosum subsp. speciosum,

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A. speciosum subsp. compactum and A. saxatile formed a subclade A, where A. froedinii is distantly related to them. Cluster B further formed two distinct subclades. B1 consists of A. *iberideum*, A. oppositifolium and A. eunomioides; B2 grouped A. fimbriatum, A. armenum, A. arabicum, A. grandiflorum and A. syriacum.

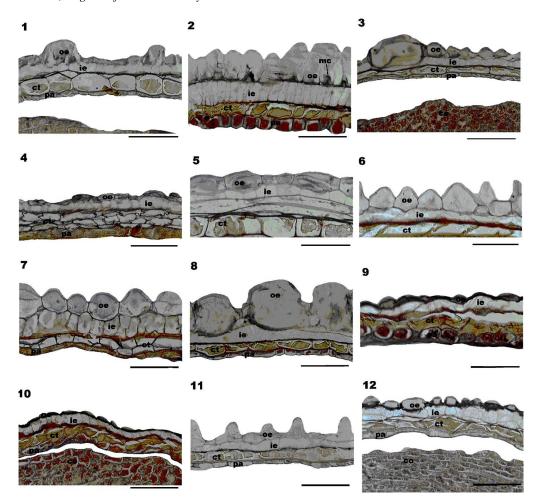


Fig. 2. The seed anatomical structures of the examined taxa; 1. A. syriacum, 2. A. froedinii, 3. A. arabicum, 4. A. eunomioides, 5. A. fimbriatum, 6. A. speciosum subsp. speciosum, 7. A. speciosum subsp. compactum, 8. A. saxatile, 9. A. oppositifolium, 10. A. iberideum, 11. A. armenum, 12. A. grandiflorum (oe: outer epidermis, ie: inner epidermis, ct: compressed tissue, pa: parenchyma, co: cotyledon, scale bars: 100 μm).

As shown in dendrogram, A. froedinii, A. iberideum and A. fimbriatum were prominently different from other taxa based on the examined characteristics (Table 2). The clades contained closely related taxa such as A. arabicum-A. armenum and A. syriacum-A. grandiflorum in compatible with the traditional taxonomic rank of Aethionema taxa in Turkey. It means that the macro and micro morphological and anatomical characteristics of the seeds are suitable with the used characters in the infra-generic separation of the Aethionema species in Flora of Turkey (Davis, 1965).

PCA ordination and dissimilarity matrix in accordance with morphological and anatomical characters of seeds are given in Table 3 and in Fig. 4. The closest and the most distant taxa are determined. *A. arabicum* and *A. armenum* are the most closely related taxa (dissimilarity percentage: 1.15), as *A. saxatile* and *A. iberideum* are the most distant taxa (dissimilarity percentage: 19.56) (Table 4 and Fig. 4). In addition, the cumulative variance value of principal components achieved 59.41% (Axis 1: 39.23%, Axis 2: 20.18%).

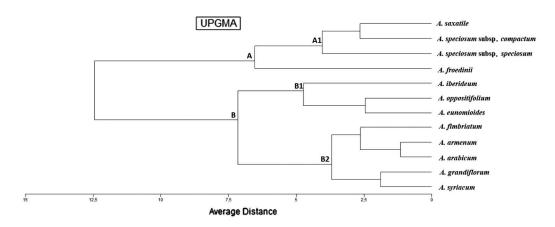


Fig. 3. UPGMA of the examined taxa.

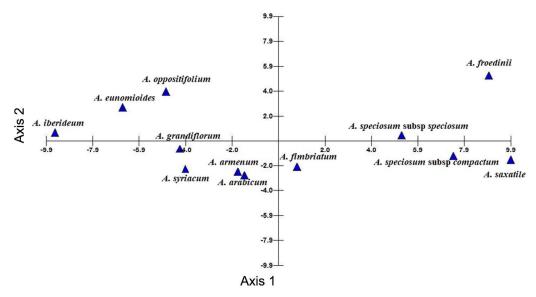


Fig. 4. Principal component analysis of the examined taxa.

Таха	1	2	3	4	5	9	7	8	6	10	11	12
Aethionema syriacum (1)	0	т	ī	x	I		,		1			
A. froedinii (2)	14.94	0	ı	,	Ţ	,	,	,	ı	,	,	ī
A. arabicum (3)	2.61	13.12	0	,	ŗ	,	,	,		Ţ		ī
A. eunomioides (4)	5.65	15.83	7.52	0	t	ı	ı	ı	ι	ſ	ī	r
A. fimbriatum (5)	4.86	10.97	2.53	8.84	0	ī	ī	ī	a	·	,	а
A. speciosum subsp. speciosum (6)	9.64	6.97	7.46	12.10	5.20	0	ı	ı	ī	ŗ	ï	
A. speciosum subsp. compactum (7)	11.53	6.88	9.11	14.60	6.73	2.97	0	ı	ı	ı	ï	,
A. saxatile (8)	13.89	6.80	11.40	17.10	9.16	5.12	2.65	0			,	,
A. oppositifolium (9)	6.45	13.85	7.52	2.46	8.20	10.60	13.30	15.68	0		ï	,
A. iberideum (10)	6.25	19.05	8.84	3.69	10.70	14.80	17.10	19.56	5.79	0	ï	
A. armenum (11)	2.67	13.18	1.15	7.20	2.75	7.64	9.31	11.69	7.12	8.42	0	,
A. grandiflorum (12)	1.87	14.42	3.57	4.24	5.35	9.55	11.70	14.15	4.71	5.59	3.18	0

Table 4. Dissimilarity matrix of the examined taxa.

The classification of the *Aethionema* taxa is based on fruit morphology (Davis, 1965), however; the main differences in fruit morphology and the widespread convergence in this character within family drift into complexity systematics of genus in Turkey (Mummenhoff *et al.*, 1997). To illuminate this problem, the morphological (macro and micro) and anatomical characters of the seeds, which are rarely referred to systematics of genus and no comprehensive researches have been performed so far, are used as an additional character in this study.

The morphological features of the seeds present valuable data in the evolutionary classification of flowering plants (Corner, 1976; Kaya *et al.*, 2011). The seed morphology of the examined *Aethionema* taxa includes important information in the identification. The seed morphological variations have been found at the species level, especially in seed color and sizes. Seed color ranges from light brown, dark brown, brown-black to brown-grey (Table 2). Seed color is diagnostic at the generic and specific level for some extent. The information of seed color is consistent with some previous studies such as Barthlott (1984), Pinar *et al.* (2007, 2009), Kasem *et al.* (2011), Kaya *et al.* (2011) and Karaismailoğlu (2016). Also, the observed morphological variations in the seed are compatible with the diagnostic characters in Flora of Turkey (Davis, 1965) for *Aethionema* taxa.

Fruit or seed surface structure have been variously utilized for solving taxonomical problems, interpretation of evolutionary relationship and illumination of the adaptive characters of the fruit or seed surface (Sulaiman, 1995; Pinar *et al.*, 2007; Karaismailoğlu, 2015a). Seed coat surface features such as surface ornamentation, anticlinal and periclinal cell wall patterns, and epidermal cell structure, have been found as helpful in delimitation of taxa within some genera in Brassicaceae family (Murley, 1951; Vaughan and Whitehouse, 1971; Barthlott, 1981; Koul *et al.*, 2000; Moazzeni *et al.*, 2007; Karaismailoğlu, 2016).

In this study, seed surface ornamentations, which are verruculate, reticulate, reticulatefoveate, ocellate, ruminate and reticulate-ocellate, can be served as good diagnostic characters at the specific level. The seed surface patterns in *A. syriacum*, *A. froedinii*, *A. fimbriatum*, *A. speciosum* subsp. *speciosum*, *A. speciosum* subsp. *compactum*, *A. saxatile* and *A. oppositifolium* taxa are reported here for the first time. This character found useful in separation of the following taxa, *A. speciosum* subsp. *speciosum* and *A. grandiflorum* taxa. Reticulate type is the most common surface ornamentation found among the taxa studied. It has commonly used in generic level in the family Brassicaceae (Barthlott, 1981; Koul *et al.*, 2000; Zeng *et al.*, 2004; Moazzeni *et al.*, 2007; Karaismailoğlu, 2016).

The outcomes of this study are congruent with seed surface is reticulate or verrucate in *Aethionema iberideum, A. eunomioides, A. arabicum* and *A. armenum* (Pinar *et al.*, 2007; Atceken *et al.*, 2016). This paper is the first report to the ocellate seed surface ornamentations of *A. speciosum* subsp. *compactum*, and *A. fimbriatum*, ruminate ornamentation type for *A. oppositifolium* (Fig. 1). Seed surface ornamentation is reported as reticulate-verrucate (Pinar *et al.*, 2007) and verrucate (Atceken *et al.*, 2016) in *A. armenum*, while current investigation reports as reticulate-foveate. The unique morphological microcharacters have reflected a correlation between a molecular data and the morphological characters utilized in classification (Batur, 2014).

Characteristics of testa epiderma cells are taxonomically significant at the generic and subgeneric levels (Tegel, 2002; Karaismailoğlu, 2015a). These characteristics are found inconsistent in the present investigation. The outer epidermal cells are composed of rectangular, cylindrical, flat, ovoid, and tuberculate in forms with thick or thin wall. The inner epidermal cells are cubic, rectangular, flat, and crushed in forms as well. At the same time, the widest testa is noted in *A. saxatile*, whereas narrowest is in *A. iberideum*. Present investigation reveals that the testa epidermal features can be a helpful taxonomic character in separation of *Aethionema* taxa.

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Current results are compatible with previous studies such as Vaughan and Whitehouse (1971); Meyer (1973, 1979, 1991) and Moazzeni *et al.* (2007).

A dendrogram was created to evaluate the morphological and anatomical features of the seeds of the *Aethionema* taxa with UPGMA of cluster analysis. The morphological and anatomical characteristics of the seeds have supported the features utilized in the separation of *Aethionema* taxa of Turkey.

Principal component analysis may be helpful in providing information about the variability of the used characteristics. The obtained cumulative variance values of principal components indicate that the examined characteristics in *Aethionema* taxa may be utilized in explaining the differences among the taxa because of high variance value. Dissimilarity ratios among the taxa were determined. Accordingly, the closest relationship was seen between *A. arabicum* and *A. armenum*, however, the most distant relationship was found between *A. saxatile* and *A. iberideum*.

In the present study, significance of some characteristics in the infrageneric delimitation in *Aethionema* has been examined. The application of some seed characters, which are generally related with seed micromorphological and anatomical patterns, can be useful in the systematics of genus, and supportive ancestral characters in the infra generic classification. This is a preliminary investigation of systematic significance of some seed morphological and anatomical characteristics of the genus *Aethionema*; further extensive studies including all taxa of the genus would be helpful in better understanding systematic problems of the genus *Aethionema*.

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