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PALYNOLOGICAL FEATURES OF ELEVEN *AETHIONEMA* TAXA FROM TURKEY AND THEIR SYSTEMATIC IMPLICATIONS

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Abstract

Pollen morphology of 11 taxa, including 2 endemic of the genus *Aethionema* W.T. Aiton from Turkey was examined under light and scanning electron microscopes. The pollens of *Aethionema* are mostly isopolar and bilaterally symmetric; spheroidal, prolate, perprolate and subprolate with the polar axes 14.07-26.41 µm and the equatorial axes 7.85-22.02 µm; mostly tricolpate, rarely 2-colpate; surface ornamentation is micro or macro reticulate. The exine thickness varies between 0.66 and 1.91 µm, and in tine thickness ranges from 0.27 to 0.85 µm. It is found that dimension of pollen grains, surface ornamentation, apocolpidium and amb diameter are taxonomically significant.

Introduction

The family Brassicaceae (Cruciferae), one of the largest angiosperm families, consists about 340 genera and 3350 species distributed mostly in temperate Northern Hemisphere (Al-Shehbaz, 1986; Karaismailoglu, 2016). The genus *Aethionema* W.T. Aiton represents with about 45 taxa in Turkey, including 20 endemic taxa (Guner *et al*., 2012). Turkey is one of the biodiversity rich centers of the genus, and its number in outside Anatolia declines gradually (Davis, 1965; Pinar *et al.*, 2007). *Aethionema* having relatively few morphological characters and dimorphism in fruits among individuals of some species poses some taxonomic problems in classification of taxa within the genus (Al-Shehbaz *et al.*, 2007). Besides, some of taxa within genus are of the common convergence in fruits and seeds in the family (Mummenhoff *et al.*, 1997). Using morphological characters in infrageneric delimitation becomes problematic in the genus. Therefore, additional features could make useful contribution to the solution of taxonomical problems in the genus.

The significance of palynological information has been stressed by several workers in the family Cruciferae, *viz.* Inceoglu and Karamustafa (1977), Brochmann (1992), Pinar *et al*. (2009), and Mutlu and Erik (2012). There has been no comprehensive palynological study in the genus *Aethionema*. However, recently Atceken *et al*., (2016) investigated pollen morphology of four species of the genus. Therefore, the present investigation aims to enhance current palynological knowledge of the genus and to evaluate their taxonomic significance as taxonomic characters.

Material and Methods

Plant samples used for investigation were collected from different natural habitats of Turkey. A list of studied specimens is given in Table 1 with collection localities and collection numbers, and specimens were stored in the ISTF (Istanbul University Science Faculty Herbarium).

Specimens for scanning electron microscopy were prepared mounting with silver adhesive on the stub, covered by gold, and examined with a JEOL Neoscope-5000 scanning electron microscope (Karaismailoglu, 2015).

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Pollen slides for light microscope were prepared following the technique of Wodehouse (1935). Pollen grains were dyed by safranin, and mounted with a cover slip, examined with Olympus CX21FS1 light microscope, and photographed by Kameram Imaging Software.

Table 1. The examined taxa of *Aethionema* and their locations

Taxa	Locality	Collection no.
Aethionema froedinii Rech. (A1)	Gumushane, Kelkit	Karaismailoglu 213
A. arabicum (L.) Andrz. ex DC. (A2)	Mugla, Koycegiz	Karaismailoglu 194
A. eunomioides (Boiss.) Bornm. (A3)*	Artvin, Yusufeli	Karaismailoglu 169
A. fimbriatum Boiss. (A4)	Nigde, Camardi	Karaismailoglu 275
*A. speciosum*Boiss. et Huet ssp. *speciosum*(A5)	Artvin, Savsat	Karaismailoglu 67
A. speciosum Boiss. et Huetssp. *compactum* Hartvig et Strid (A6)*	Mugla, Koycegiz	Karaismailoglu 260
A. saxatile (L.) R. Br. (A7)	Trabzon, Of	Karaismailoglu 94
A. oppositifolium (Pers.) Hedge (A8)	Agri, Downtown area	Karaismailoglu 164
A. iberideum Boiss. (A9)	Erzurum, Ispir	Karaismailoglu 170
A. armenum Boiss. (A10)	Kahramanmaras, Goksun	Karaismailoglu 206
A. grandiflorum Boiss. et Hohen. (A11)	Mugla, Marmaris	Karaismailoglu 191

*=endemic

Pollen terminology based on Erdtman (1952 and 1969), Brochmann (1992) and Punt *et al.* (1994). Ten palynological characters have been determined to discriminate the 11 taxa of *Aethionema* genus (Table 2), and applied Duncan's multiple range tests for each of them (SPSS, 2006), and dissimilarity matrix was consisted (Table 3). Afterwards, the variations of the determined characters are presented in Figure 3 with the whisker graph. Cluster analysis of taxa was performed in accordance with UPGMA (Fig. 4) (Mohammadi and Prasanna, 2003). Besides, the ordination of taxa is performed with Principal Component Analysis (PCA) (Fig. 5). Computations except Duncan's multiple range tests were made with the MVSP software (Kovach, 2007).

Results and Discussion

The morphological characters of pollen grains of the examined *Aethionema* taxa are presented in Table 2, and photographs are presented in Figures 1 and 2. The pollens are isopolar and vary between prolate (prolate (72.73%), perprolate (9.09 %), and subprolate (9.09%) and spheroidal (9.09%). The polar axes range from 14.07-26.41 µm and the equatorial axes 7.85-22.02 µm. Their dimensions are smaller in *A. arabicum* and *A. speciosum* ssp. *compactum*, but larger in *A. iberideum* and *A. armenum* (Table 2, Figs 1&2).

The most common shape in the examined taxa is prolate (eight taxa), subprolate (one taxon), perprolate (one taxon) and rarely spheroidal (one taxon), respectively (Figures 1-2). The result is compatible with the result of Mutlu and Erik (2012), found in the genus *Arabis* L. of the family Cruciferae.

The aperture and exine characters of taxa can reflect the major criterion for the determination of the phylogenetic relationships (Cronquist, 1968; Ocak *et al*., 2013). The number of aperture ranges from 2 to 4; and aperture type is mostly tricolpate (basic type), however; dicolpate type is

Table 2. Pollen characteristics of the studied *Aethionema* taxa

Taxa	pollen shape	Polar axes (P)(µm)			Equatorial axes (E)(µm)			(P/E)	Colpus number	Aperture type	Ornamentation
		Min	Max	Mean (1)*	Min	Max	Mean (2)*				
A1	subprolate	14.07	17.11	16.57±0.12g	10.45	13.17	12.49±0.17c	1.32	3	tricolpate	micro reticulate
A2	prolate	15.16	17.29	16.44±0.18g	7.85	9.51	8.76±0.24g	1.87	3	tricolpate	reticulate
A3	prolate	16.58	19.22	17.85±0.24f	10.86	13.25	11.91±0.08d	1.49	3	tricolpate	reticulate
A4	prolate	18.61	20.54	19.13±0.33e	9.77	11.65	10.33±0.27e	1.85	3	tricolpate	coarsely reticulate
A5	prolate	17.53	20.42	18.91±0.24ef	8.96	10.09	9.70±0.11ef	1.94	3	tricolpate	reticulate
A6	prolate	13.59	16.15	14.99±0.18h	8.15	9.91	8.76±0.18g	1.71	2-3	%10 dicolpate, %90 tricolpate	reticulate
A7	prolate	18.21	20.94	19.82±0.12e	9.13	10.86	10.08±0.15e	1.96	2-3	%10 dicolpate, %90 tricolpate	reticulate
A8	prolate	20.16	24.72	22.34±0.16c	13.17	15.90	14.26±0.12b	1.56	3	tricolpate	reticulate
A9	prolate	23.44	25.51	24.05±0.12b	12.87	14.56	13.94±0.08b	1.72	2-3	%5 dicolpate, %95 tricolpate	coarsely reticulate
A10	spheroidal	20.11	22.18	21.45±0.21d	19.56	22.02	21.22±0.16a	1.01	2-3	%5 dicolpate, %95 tricolpate	coarsely reticulate
A11	perprolate	23.19	26.41	25.04±0.15a	8.76	9.46	9.05±0.12f	2.76	3	tricolpate	micro reticulate

Table 2 continued

Taxa	Colpus sizes*		Clt/Clw	Apo*	Amb*	Lumina*	Muri*	Intine*	Exine*
	Clt(µm) (3)	Clw(µm) (4)		(µm) (5)	(µm) (6)	(µm) (7)	(µm) (8)	(µm) (9)	(µm) (10)
A1	9.81±0.16f	1.08±0.04g	9.08	2.13±0.04d	9.01±0.12f	0.41±0.04d	0.65±0.06a	0.45±0.02c	1.25±0.04cd
A2	14.26±0.27b	2.25±0.08e	6.33	2.25±0.06d	13.05±0.18d	0.66±0.08bc	0.38±0.11cd	0.32±0.06d	0.89±0.08e
A3	9.29±0.44fg	2.86±0.12d	3.24	2.38±0.04d	17.24±0.27ab	0.89±0.06a	0.61±0.8ab	0.68±0.06a	1.47±0.04b
A4	14.15±0.16b	2.14±0.08ef	6.61	4.11±0.12b	10.83±0.16e	0.51±0.08cd	0.71±0.12a	0.44±0.04c	1.08±0.10d
A5	10.86±0.33e	2.51±0.14e	4.32	3.57±0.15c	14.62±0.33c	0.62±0.06c	0.48±0.10abc	0.76±0.12a	1.44±0.08b
A6	13.74±0.27bc	3.53±0.12c	3.89	3.86±0.21bc	14.22±0.15c	0.69±0.04c	0.49±0.06c	0.43±0.04c	1.21±0.06cd
A7	12.55±0.24d	2.62±0.12de	4.79	4.02±0.24bc	13.54±0.18d	0.85±0.08a	0.69±0.05a	0.69±0.06a	1.38±0.08bc
A8	14.77±0.12b	4.96±0.24a	2.97	4.45±0.15b	14.46±0.21c	0.51±0.11cd	0.42±0.08c	0.85±0.06a	1.79±0.04a
A9	9.86±0.18f	4.21±0.16b	2.34	3.41±0.12c	9.41±0.18f	0.28±0.02e	0.45±0.06c	0.27±0.04e	0.66±0.02f
A10	14.83±0.22b	2.05±0.14f	7.23	6.08±0.18a	17.65±0.27a	0.85±0.06a	0.28±0.04d	0.71±0.08a	1.85±0.08a
A11	15.21±0.25a	4.37±0.16b	3.48	4.17±0.24b	15.61±0.21b	0.73±0.04b	0.43±0.06c	0.68±0.10ab	1.91±0.12a

*Different letters are significant at p = 0.05 level (Duncan's multiple-range test), colpus length=clt, colpus width=clw, Apocolpidium=Apo, Outline of a pollen grain as viewed from pole=Amb, apocolpium=Apo, Standard Deviation=±, for the taxa abbreviations see Table 1.

also encountered in some taxa e.g. *A. speciosum* ssp. *compactum* (A6), *A. saxatile* (A7), *A. iberideum* (A9) and *A. armenum* (A10). Besides, some taxa showed heteromorphic characters such as 90 % tricolpate and 10 % dicolpate in taxa *A. speciosum* ssp. *compactum* (A6) and *A. saxatile* (A7), 95% tricolpate and 5% dicolpate in taxa *A. iberideum* (A9) and *A. armenum* (A10). These variations in pollen aperture type are declared as heteromorphy in pollens by Inceoglu and Karamustafa (1977) and Ceter *et al*. (2013). Also, the colpus sizes range from 9.29 (*A. eunomioides*) to15.21 (*A. grandiflorum*) µm in length, from 1.08 (*A. froedinii*) to 4.96 (*A. oppositifolium*) µm in width. The colpus membranes are more or less granulate (Table 2, Figures 1&2).

Table 3. Dissimilarity matrix of the examined taxa (for the taxa abbreviations see Table 1).

Taxa	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11
A1	0	-	-	-	-	-	-	-	-	-	-
A2	2.27	0	-	-	-	-	-	-	-	-	-
A3	2.71	2.34	0	-	-	-	-	-	-	-	-
A4	1.96	1.35	2.69	0	-	-	-	-	-	-	-
A5	2.24	1.52	1.30	1.62	0	-	-	-	-	-	-
A6	2.61	0.90	2.23	1.82	1.61	0	-	-	-	-	-
A7	2.25	1.41	1.85	1.04	0.72	1.67	0	-	-	-	-
A8	3.34	2.83	2.69	2.19	2.34	2.97	1.88	0	-	-	-
A9	2.64	3.50	3.29	2.50	2.76	3.85	2.47	2.36	0	-	-
A10	4.66	4.65	3.80	4.19	4.13	4.65	3.92	2.65	4.06	0	-
A11	4.14	3.01	3.21	2.57	2.48	3.26	2.07	1.91	3.07	4.17	0

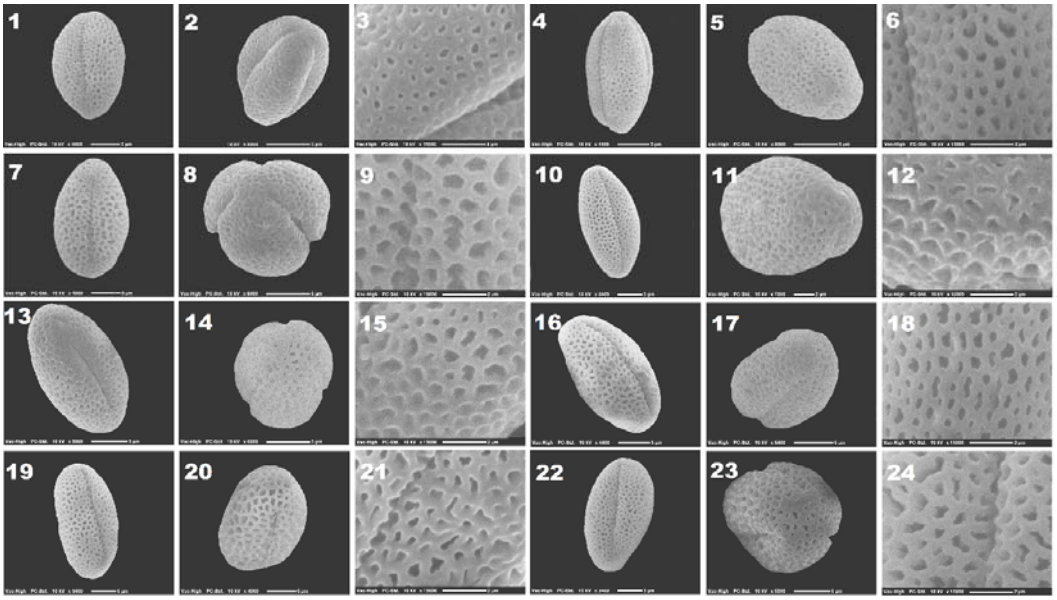

Fig. 1. SEM microphotograph of the studied *Aethionema*: A1: 1-3, A2: 4-6, A3: 7-9, A4: 10-12, A5: 13-15, A6: 16-18, A7: 19-21, A8: 22-24 (For abbreviation taxa see Table 1).

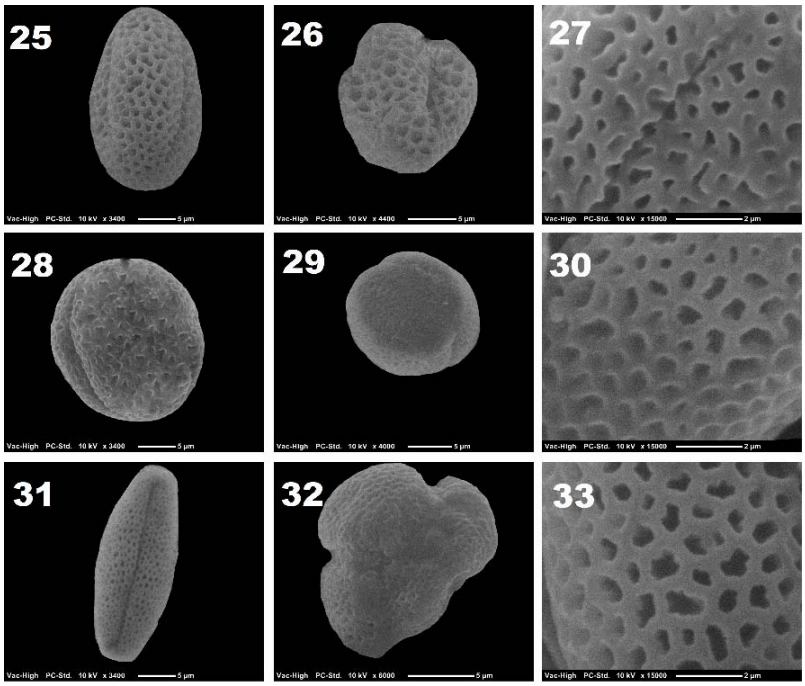

Fig. 1. SEM microphotograph of the studied *Aethionema*: A9: 25-27, A10: 28-30 and A11: 31-33 (For abbreviation taxa see Table 1).

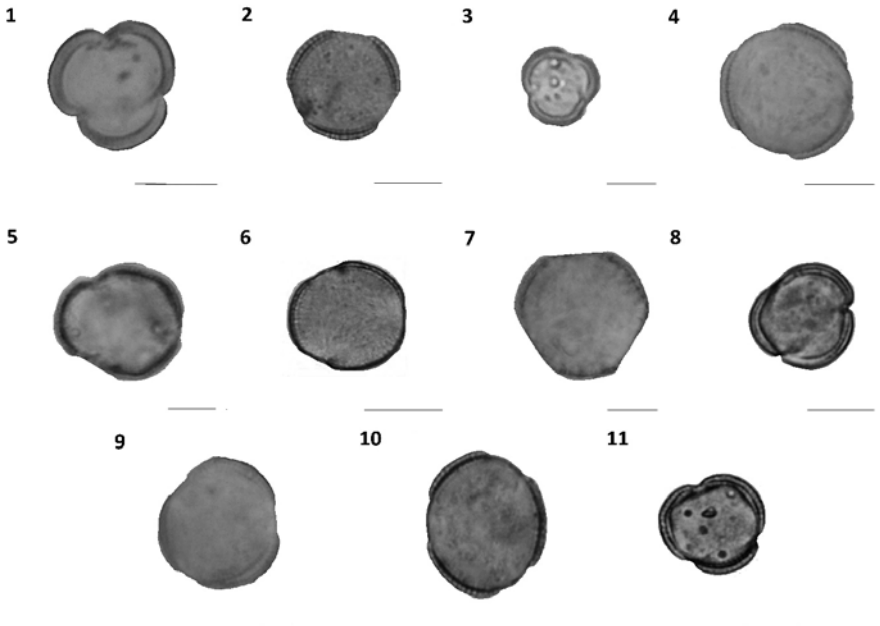

Fig. 2. Light microscope photographs of the studied *Aethionema*: A1:1, A2:2, A3:3, A4:4, A5:5, A6:6, A7:7, A8:8, A9:9, A10:10 and A11:11(Scale bars=5 µm) (For abbreviation taxa see Table 1).

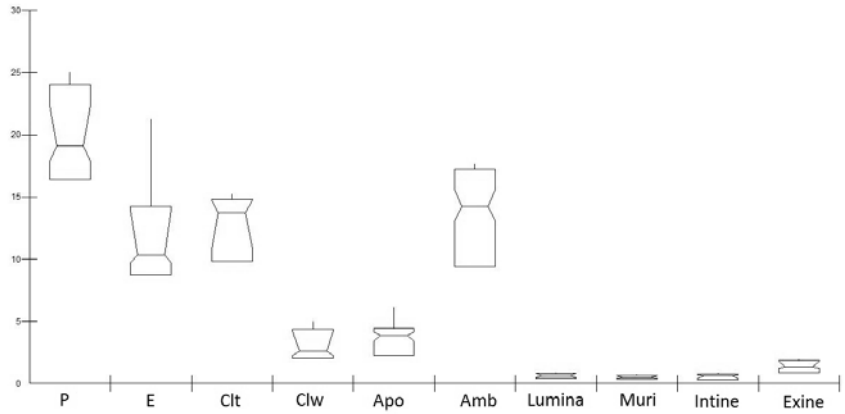

Fig. 3. Correlation among the palynological characters for the studied taxa (for abbreviations of the characters see Table 2).

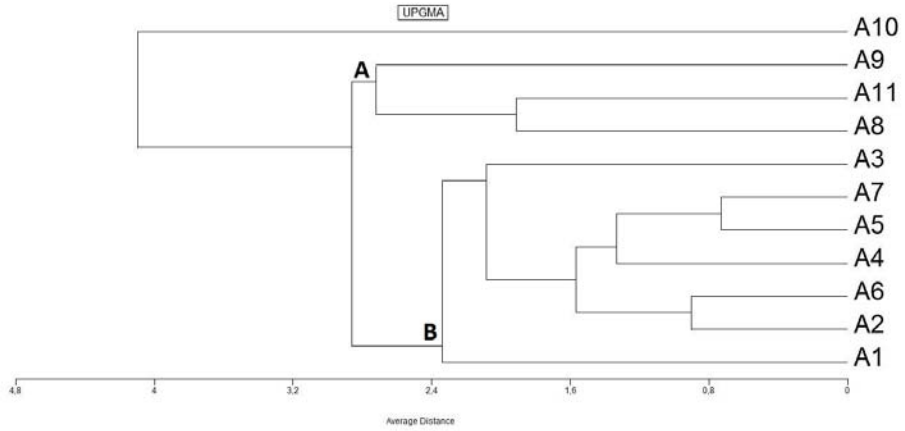

Fig. 4. UPGMA clustering of the examined taxa based on palynological characters (for taxa abbreviations see Table 1).

The exine thickness varies between from 0.66 (*A. iberideum*) and1.91 (*A. grandiflorum*) µm, and it frequently seems thicker in aperture. Besides, the in tine thickness ranges from 0.27 (*A. iberideum*) to 0.85 µm (*A. oppositifolium*). The surface ornamentation in the examined *Aethionema* taxa is reticulate and coarse or micro reticulate with straight or rough muri. Lumina includes 4-7 polygonal or irregular cells. Its diameter varies between 0.28 (*A. iberideum*) and 0.89 (*A. eunomioides*) µm.

The pollen exine ornamentations are of a significant role in delimitation of the some closely related taxa in Cruciferae (Khalik *et al.*, 2002). The present communication reveals that the pollen ornamentations of genus *Aethionema* are reticulate, coarsely reticulate, and micro-reticulate (Table 2). Anchev and Deneva (1997) reported ornamentation of the pollens of Cruciferae family is mostly reticulate and foveolate, which confirms the present result.

Pollen and colpus dimensions and, diameter of the apo and amb figure are remarkably variable to separate taxa, and to be useful for the delimitation of taxa unlike muri, lumina and thickness of intine and exine (Fig. 3). The pollen morphology of *Aethionema* taxa shows a close

relationship with other genera of the family, for example; *Arabidopsis* Heynh.(Khan, 2004), *Hesperis* L. (Pinar *et al*.,2009), and *Arabis* (Mutlu and Erik, 2012).

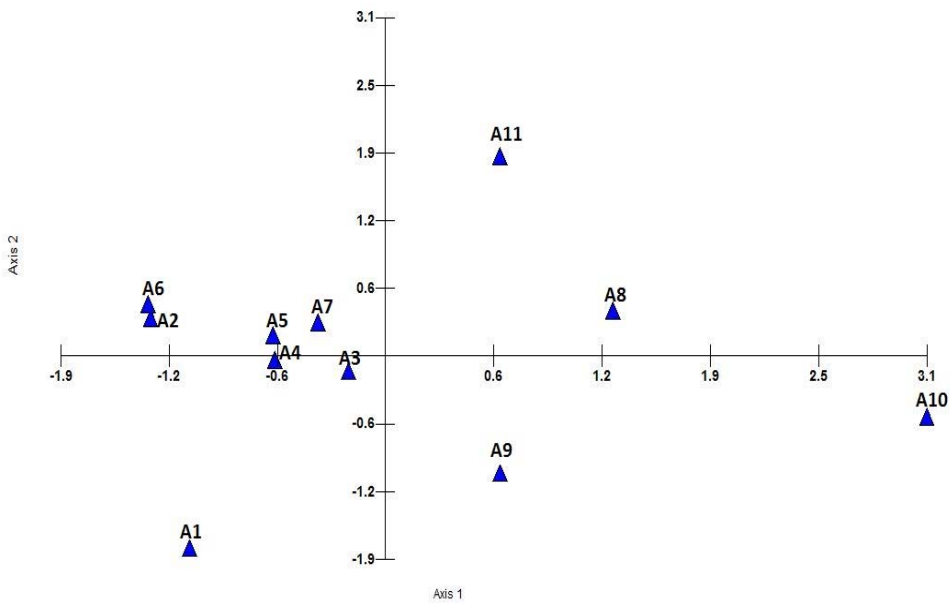

Fig. 5. PCA of the studied taxa based on palynological characters (for taxa abbreviations see Table 1)

A dendrogram of cluster analysis of 11 *Aethionema* taxa has been created based on 10 characters. To define the correlation of the dendrogram and the dissimilarity matrix (Table 3), the co-phenetic correlation coefficient is measured; the higher relationship reflects more preferable in terms of position in the hierarchy. In the cluster analysis of the studied taxa formed two distinctive clades, clade A and clade B. Clade A includes taxa *A. oppositifolium* (A8), *A. iberideum* (A9) and *A. grandiflorum* (A11). Clade B includes taxa *A. froedinii* (A1), *A. arabicum* (A2), *A. eunomioides* (A3), *A. fimbriatum* (A4), *A. speciosum* ssp. *speciosum* (A5), *A. speciosum* ssp. *compactum* (A6) and *A. saxatile* (A7). However, taxon *A. armenum* (A10) remains out the cluster (Fig. 4). It is found that the most closely related taxa are *A. speciosum* ssp. *speciosum* (A5) and *A. saxatile* (A7) (dissimilarity ratio: 0.72), while taxa *A. froedinii* (A1) and *A. armenum* (A10) are the most distantly related (dissimilarity ratio: 4.66) (Fig. 5, Table 3).Clade B consists of most of the taxa studied, which can be attributed as the taxa of this clade having primitive characteristics within *Aethionema*.

The current investigation reveals that palynological data has taxonomic significance, and it offers substantial contribution to the current classification of *Aethionema*.

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