Journal of Applied Biological Sciences 10 (3): 13-19, 2016
ISSN: 1307-1130, E-ISSN: 2146-0108

Monitoring of Pollen Micromorphology of *Acanthophyllum* C. A. Mey. (Caryophyllaceae) Genus from Central Anatolian in Turkey

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Received: October 04, 2016
Accepted: December 18, 2016

Abstract

Pollen morphology of *Acanthophyllum* C. A. Mey(Caryophyllaceae) species in Turkey were investigated by light and scanning electron microscopy in this study. There are 5 species in Turkey belonging this genus: *Acanthophyllum mucronatum* C. A. Meyer, *Acanthophyllum acerosum* Sons., *Acanthophyllum microcephalum* Boiss., *Acanthophyllum oppositiflorum* Aytaç, *Acanthophyllum verticillatum* (Willd.) Hand.-Mazz. The pollen grains of *Acanthophyllum* species are radially symmetrical and isopolar, oblate-spheroidal and prolate-spheroidal, operculate, and pantoporate, their pores have conical spinules on operculum. Pore numbers and diameter, exine thickness, pollen ornamentation and operculum ornamentationvary between *Acanthophyllum* species.

Keywords: *Acanthophyllum* C.A.Mey., Light Microscope, Morphology, Pollen, Scanning Electron Microscope

INTRODUCTION

Caryophyllaceae is one of large angiosperm families. It contains86 genera and almost 2200 species. These species are distributed mainly Mediterrenean region[1-3]. There are 32 genera which includes over 470 species in Turkey [4].Conventionally, there are 3 subfamilies among Caryophyllaceae: Alsinoideae Burnett, Caryophylloideae Arn., and Paronychioideae A.St.[1]. *Acanthophyllum* C. A. Mey. is a member of Caryophyllaceae family, subfamily Caryophylloideae[5].Genus *Acanthophyllum* contains approximately 60 species and is distributed in the Irano-Turanian region[1]. In Turkey, there are 5 species representing the genus:*Acanthophyllum acerosum* Sons.,*Acanthophyllum microcephalum* Boiss., *Acanthophyllum mucronatum* C.A.Mey., *Acanthophyllum verticillatum* (Willd.) Hand.-Mazz., *Acanthophyllum oppositiflorum* Aytaç (endemic) [6, 7].

The Caryophyllaceae family has medicinal and ornamental properties which brings an importance[8]. This genus includes small pulvinate shrubs with spiny leaves that grow in open habitats[9].

In this study, pollen morphology of 5 taxa, one of which is endemic belonging to Turkish *Acanthophyllum* C. A. Mey.were investigated by using light microscope (LM) and scanning electron microscope (SEM). The different and similar characteristics of the species were determined.

MATERIALS and METHODS

Specimens were collected in 2014 and 2016 from the localities given in "Table 1".The palynological investigations are based on the herbarium specimens obtained from Gazi University Faculty of Science Herbarium. Pollen slides were prepared according to Wodehouse (1935) from mature flowers. At least 50 pollen grains for each species were examined in light microscope and micrographs were taken by Leica DM 750 digital imaging system. Different morphological characters of pollen parts such as pollen diameter, pore diameter, distance between two pores and exine thickness were measuredby means of light microscopy.

Table 1. List of investigated taxa and localities.

Collector Number	Species	GPS	Altitude	Collection Date	Habitat	Phytogeographic Region	Localities
MA 5693	*Acanthophyllum verticillatum* C.A.Mey.	39°09'29.6"N 39°29'01.7"E	970	24.7.2014	Step, Hillside	Irano-Turanian	B7Tunceli: Tunceli, Between Tunceli - Ovacık (Valley Munzur) 10. km
MA 5693	*Acanthophyllum oppositiflorum* Aytaç	38°53'41.2"N 39°14'52.9"E	875	13.8.2014	Step	Irano-Turanian Endemic	B7Tunceli: Pertek, Between Pertek - Çemişgezek 9. km, 2.5 km before Singeç Bridge
MA 6758	*Acanthophyllum mucranatum* C.A.Mey.	37° 47' 47.5"N 44° 05' 04.2"E	1825	31.7.2016	Naked Hillsides	Irano-Turanian	C10Van: Başkale, Hakkari Provincial Border, 33 km to Hakkari from Başkale, Right-hand Hills
MA 6758	*Acanthophyllum microcephalum* Boiss.	37° 47' 47.5"N 44° 05' 04.2"E	1825	31.7.2016	Naked Hillsides	Irano-Turanian	C10Van: Başkale, Hakkari Provincial Border, 33 km to Hakkari from Başkale, Right-hand Hills
MA 6758	*Acanthophyllum acerosum* Sosn.	37° 47' 47.5"N 44° 05' 04.2"E	1825	31.7.2016	Naked Hillsides	Irano-Turanian	C10Van: Başkale, Hakkari Provincial Border, 33 km to Hakkari from Başkale, Right-hand Hills

For SEM, pollen grains were directly mounted on stubs and covered withgold and surface ornamentations of pollen grains were examined in detail with Jeol Tescan MAIA3 XMU model electron microscope in Bartın University Central Research Laboratory.

The pollen morphology terminology adopted by Fagri and Iversen (1989), Punt et al.(2007), Moore et al. (1991) was used[10-12]. Shape classification follows Erdtman (1969), based on P/E ratio in "Table 2-3". [13].

Table 2. Pollen morphology of species *Acanthophyllum* M: median, V: variation, S: standart deviation.

	Spheroidal	***A. acerosum***	***A. microcephalum***	***A. mucronatum***	***A. oppositiflorum***	***A. verticillatum***
Polar axes (µm)	M V	35.03 (28.54-45.82)	30.11 (28.87-34.16)	30.32 (26.24-33.87)	28.95 (25.81-30.65)	33.9 (26.78-42.17)
	S	± 2.95	± 2.11	±1.65	± 1.48	± 2.16
Equatorial axes (µm)	M V	35.08 (27.74-46.82)	29.28 (26.39-31.44)	30.38 (27.97- 32.68)	27.59 (25.48-30.01)	34.17 (25.66-42.89)
	S	± 2.23	± 1.68	± 1.38	± 1.12	± 2.57
Exine thickness (µm)	M V	1.96 (1.43-2.29)	2.14 (1.92-2.37)	1.79 (1.40 -2.11)	1.88 (1.47-2.39)	1.86 (1.47-2.38)
	S	± 0.20	± 0.19	± 0.17	± 0.23	± 0.2
Intine thickness (µm)	M V	0.51 (0.38-0.66)	0.57 (0.51-0.64)	0.51 (0.41-0.64)	0.52 (0.44-0.64)	0.52 (0.41-0.69)
	S	± 0.07	± 0.05	± 0.07	± 0.05	± 0.06
Pore length (µm)	M V	4.43 (2.43-7.48)	3.57 (2.42-4.16)	5.43 (3.49-7.49)	4.13 (2.84-5.12)	4.24 (2.11-6.65)
	S	± 1.5	± 0.78	± 0.88	± 0.61	± 0.16
Pore width (µm)	M V	4.46 (2.74-8.75)	3.34 (3.14-3.69)	5.55 (3.97-7.76)	4.12 (3.09-5.32)	4.52 (2.76-7.53)
	S	± 1.64	± 0.24	± 0.83	± 0.54	± 1.33
Pore distance (µm)	M V	7.19 (4.38-11.10)	4.13 (3.07-4.2)	8.12 (5.97-10.39)	7.65 (4.96-10.29)	6.94 (3.71-9.17)
		± 1.91	± 0.17	± 1.09	± 1.06	± 1.32

Table 3. Pollen morphology of species *Acanthophyllum*

	A. acerosum	***A. microcephalum***	***A. mucronatum***	***A. oppositiflorum***	***A. verticillatum***
Pore number	7-13	7-11	6-9	6-13	6-12
Spinules on operculum	8-10	8-10	10-15	8-14	6-9
Spinules (100 µm^2)	55-60	45-50	55-58	90-95	50-55
Plg/plt	0.92	1.06	0.97	1	0.94
Operculum shape	Oblate-spheroidal	Prolate- spheroidal	Oblate-spheroidal	Spheroidal	Oblate- spheroidal
Pollen shape	Oblate- spheroidal	Prolate- spheroidal	Oblate-spheroidal	Prolate- spheroidal	Oblate- spheroidal
Pollen dimension(P/E)	0.99	1.02	0.99	1.01	0.99
Pollen class	Pantoporate	Pantoporate	Pantoporate	Pantoporate	Pantoporate
Pollen aperture type	Tectate	Tectate	Tectate	Tectate	Tectate
Exine structure	Spinulose-punctate	Spinulose-punctate	Spinulose-punctate	Spinulose-punctate	Spinulose-punctate
Pollen ornamentation	Scabrat-punctate	Scabrat-punctate	Scabrat-punctate	Scabrat-punctate	Scabrat-punctate

RESULTS

***Acanthophyllum acerosum* Sons.**

Pollen grains were radially symmetrical and isopolar. Polar axes were 35.03 µm (28.54-45.82µm), equatorial axes were 35.08 µm (27.74-46.82µm). Pollen shape was P/E:0.99, oblate-spheroidal.The pollen grains were operculate and pantoporate, generally with 7-13 porate. Pores had polar length of 4.43(2.43-7.48) µm and equatorial length 4.46(2.74-8.75) µm. plg/plt: 0.92, oblate-spheroidal.There were 8-10 conical spinules on operculum. Distance between two pori was7.19 (4.38-11.10)µm.The exine was tectate and 1.96 (1.43-2.29) µm in thickness. Intine thickness was 0.51(0.38-0.66) µm. Exine structure was spinulose-punctate. Polen ornamentation was scabrate-punctate. There were 55-60 spinules in 100 µm² area.

***Acanthophyllum microcephalum* Boiss**

Pollen grains were radially symmetrical and isopolar. Polar axes was30.11 (28.87-34.16) µm, equatorial axes was29.28 (26.39-31.44) µm. Pollen shape was P/E:1.02, prolate-spheroidal.The pollen grains were operculate and pantoporate, generally 7-11 porate. Pores polar length of 3.57 (2.42-4.16)µm, equatorial length 29.28 (26.39-31.44) µm (plg/plt):1.06, prolate-spheroidal. There were 8-10 conical spinules on operculum. Distance between two pori was 4.13 (3.07-4.2)µm.The exine was tectate and 2.14(1.92-2.37) µm in thickness. Intine thickness was 0.57 (0.51-0.64) µm. Exine structure was spinulose-punctate. Polen ornamentation was scabrate-punctate.There were 45-50 spinules in 100 µm2 area.

***Acanthophyllum* mucronatum C. A. Mey.**

Pollen grains were radially symmetrical and isopolar. Polar axes was30.32 (26.24-33.87) µm, equatorial axes was30.38 (27.97- 32.68)µm. Pollen shape was P/E:0.99, oblate-spheroidal. The pollen grains were operculate and pantoporate, generally 6-9 porate. Pores had polar length of 5.43 (3.49-7.49)µm and equatorial length 5.55 (3.97-7.76) µm (plg/plt):0.97, oblate-spheroidal. There were 10-15 conical spinules on operculum. Distance between two poriwas 8.12 (5.97-10.39)µm. The exine was tectate and 1.79 (1.40 -2.11)µm in thickness. Intine thickness was0.51 (0.41-0.64) µm. Exine structure was spinulose-punctate. Polen ornamentation was scabrate-punctate.There were 53-58 spinules in 100 µm2 area.

***Acanthophyllum verticillatum* (Willd.)Hand.-Mazz.**

Pollen grains were radially symmetrical and isopolar. Polar axes was 33.9 (26.78-42.17)µm, equatorial axes was34.17 (25.66-42.89)µm. Pollen shape was P/E:0.99, oblate-spheroidal. The pollen grains were operculate and pantoporate, generally 6-12 porate. Pores had polar length of 4.24 (2.11-6.65)µm and equatorial length 4.52 (2.76-7.53)µm. (plg/plt):0.94, oblate-spheroidal. There were 8-14 conical spinules on operculum. Distance between two poriwas 6.94 (3.71-9.17)µm. The exine was tectate and 1.86 (1.47-2.38) µm in thickness. Intine thickness was 0.52 (0.41-0.69) µm. Exine structure was spinulose-punctate. Polen ornamentation was scabrate-punctate.There were 50-55 spinules in 100 µm2 area.

***Acanthophyllum oppositiflorum* Aytaç**

Pollen grains were radially symmetrical and isopolar. Polar axes was 28.95 (25.81-30.65) µm, equatorial axes was27.59 (25.48-30.01) µm.Pollen shape was P/E:1.01, prolate-spheroidal. The pollen grains were operculate and pantoporate, generally 6-13 porate. Pores had polar length of 4.13 (2.84-5.12) µm and equatorial length 4.12 (3.09-5.32) µm. (plg/plt):1, spheroidal. There were 6-9 conical spinules on operculum. Distance between two poriwas 7.65 (4.96-10.29)µm. The exine was tectate and 1.88 (1.47-2.39) µm in thickness. Intine thickness was0.52 (0.44-0.64) µm. Exine structure was spinulose-punctate. Polen ornamentation was scabrate-punctate. There were 90-95 spinules in 100µm2 area .

The pollen grains of *Acanthophyllum* were radially symetrical and isopolar.Shape was oblate-spheroidal and prolate-spheroidal. Polar axes was 25.81-45.82 µm and the equatorial axes was 28.48-46.82 µm (Table 2, Figures 1-2). *A. acerosum*, *A. mucronatum* and *A. verticillatum* pollen shape was oblate-spheroidal while *A. microcephalum* and *A. oppositiflorum* pollen shape was prolate-spheroidal. *Acanthophyllum acerosum* had the biggest pollen grains with35.03 µm polar axis and 35.08 µmequatorial axis lengths, while *Acanthophyllum oppositiflorum* had the smallest-sized pollen grains with28.95 µm polar axes and 27.59 µm equatorial axis lengths.

The pollen grains were operculate and pantoporate, generally 6-13 porate. Pores length was 2.11-7.49 µm pore width of 2.74-8.75 µm. *A. mucronatum* had the largest pollen grains with 5.43 µm pore length and 5.55 µm pore widths while *A. microcephalum* had the smallest pollen pore 3.57 µm pore length and 3.34 µm pore width. *A. mucronatum* had the biggest pore distance of two pori 8.12 µm while *A. microcephalum* had the smallest pore distance of two pori 4.13 µm. Polar length/equatorial length (Plg/Plt) ratio was between 0.92 µm and 1.06 µm. *A. acerosum*, *A. mucronatum* and *A. verticillatum* had oblate –spheroidal operculum shape while *A. microcephalum* prolate-spheroidal and *A. oppositiflorum* was spheroidal.

The exine structure was scabrat-punctate and 1.79-2.14 µm in thickness. Intine thickness ranged between 0.51-0.57 µm.There were spinules in the form of conical that vary from 6-15 in number over the operculum, according to the species.

DISCUSSION

According to Shamsabadi et al. (2013), palynological studies for 11 species of the *Acanthophyllum* sect.Oligosperma in northeastern Iran including *A. borsczowii* Litw., *A. speciosum* Rech. f. & Schiman-Czeika, *A. korshinskyi* Schischk., *A.pachystegium* Rech. f., *A. adenophorum* Freyn., *A. lilacinum* Schischk., *A. brevibracteatum* Lipsky., *A. diezianum* Hand.-Mzt., *A. laxiusculum* Schiman-Czeika, *A. squarrosum* Boiss., and *A. heratense* Schiman-Czeika[14]. Pollen grains were radially symmetrical and spheroidal and their type was pantoporate. The grains had median size (23-31 µm), which was different from 24.5µm polar axes and 23.8 µm equatorial axis in *A. borsczowii* (smallest) to 30.7 µm polar axes and 30.3µm equatorial axis in *A. adenophorum* (largest). While *Acanthophyllum* grains have median size (27-35 µm), this character is different from 28.95 µm polar axes and 27.59 µm equatorial axis in *Acanthophyllum oppositiflorum* (smallest) to 35.03 µm polar axes and 35.08 µmequatorial axis in *Acanthophyllum acerosum* (largest). Pollen ornamentation was scabrate-punctateand exine structure was spinulose-punctate. Our results are similar with M. Mahmoudi Shamsabad, H. Ejtehadi, J. Vaezi & F. Memariani results but our results include more knowledge. Also this is the first study to determine polar length/ equatorial length (Plg/Plt) ratio is between 0.92 µm and 1.06 µm, number of spinules on operculum is between 6-15, *Acanthophyllum* species.

Except these studies that investigated pollen morphology of *A. borsczowii* Litw.,*A. speciosum* Rech. f. & Schiman-Czeika, *A. korshinskyi* Schischk.,*A.pachystegium* Rech. f., *A. adenophorum* Freyn., *A. lilacinum* Schischk., *A. brevibracteatum* Lipsky., *A. diezianum* Hand.-Mzt., *A. laxiusculum* Schiman-Czeika, *A. squarrosum* Boiss., and *A. heratense* Schiman-Czeika, there are no reported data about pollen morphology of other *Acanthophyllum* species.

Pollen morphology of some *Velezia* L. species has been studied by Poyraz, Ataşlar (2010) and they reported the pollen grains of *V. tunicoides*, *V. hispida*., *V. quadridentata*, *V. pseudorigida* and *V. rigida* has 11-14 porate while *Acanthophyllum* has6-13 pores. *Velezia* L. has oblate-spheroidal shape with the polar axes of 27.92-39.64 µm and the equatorial axes of 28.21-39.80 µm. *Acanthophyllum* has oblate-spheroidal and prolate-spheroidal shape. Polar axes of 25.81-45.82 µm and the equatorial axes of 28.48-46.82 µm. *Acanthophyllum*has bigger pollen grains than *Velezia*. Pollen grains of *Velezia* species are oblate-spheroidal, operculate, polypantoporate with scabrate-microperforate or scabrate-foveolate ornamentation [15]. *Acanthophyllum* mainly differs from these taxa withexine structure is scabrat-punctate.

El Naggar (2004) reported the pollen grains of *Gypsophila pilosawere* monade, apolar, hexagonal-polyhedral or spheroidal, 18-19 x 18-19 µm. Aperture was pantoporate. Pores were circular, 3-4 µm and tectum

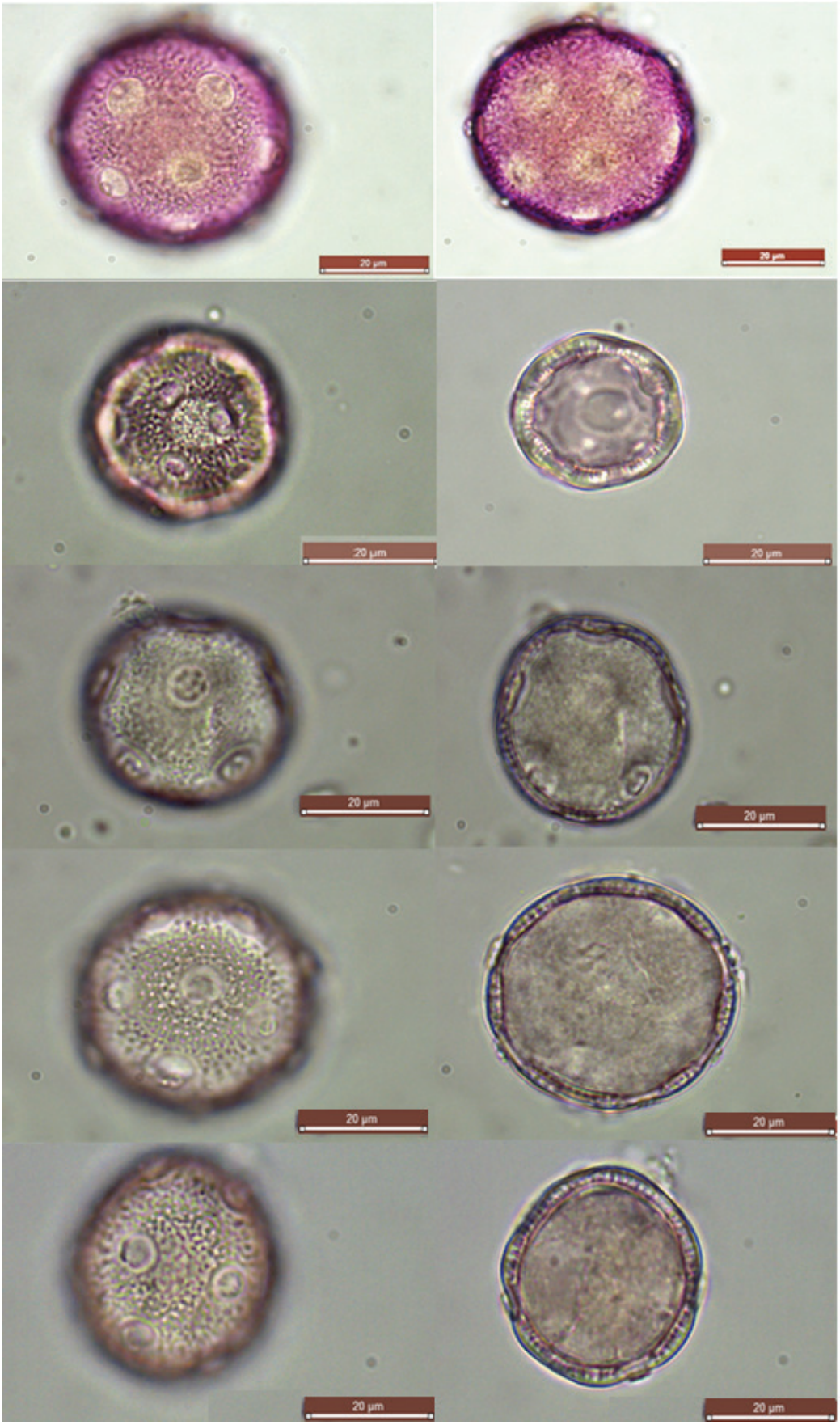

Figure 1. LM photos of pollen grains of *Acanthophyllum* species: A.acerosum A-B, *A. microcephalum* C-D, *A mucronatum*. E-F, *A. verticillatum* G-H and *A. oppositiflorum* I-J.

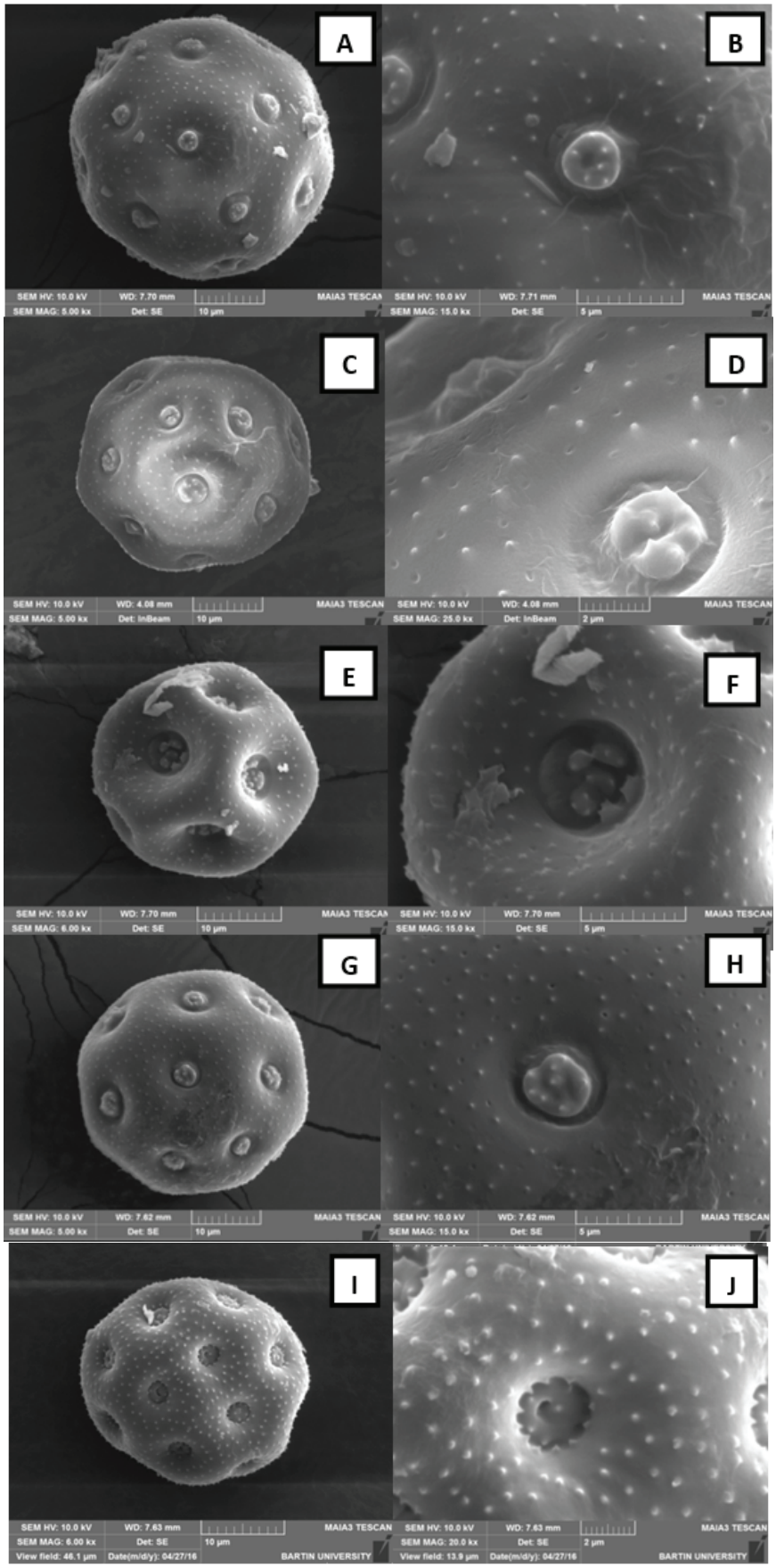

Figure 2. SEM photos of pollen grains of *Acanthophyllum* species: A.acerosum A-B, *A. microcephalum* C-D, *A mucronatum*. E-F, *A. verticillatum* G-H and *A. oppositiflorum* I-J.

was perforate [16]. *Acanthophyllum* mainly differs from these taxa with exine structure is scabrat-punctate and *Acanthophyllum* grains size is medium (27-35 µm). Also *Acanthophyllum* is spheroidal.

Yıldız, Minareci (2008) reported the pollen grains of *Silene urvillei* Schott. werespheroidal, numbers of pores were 15-24. The exine ornamentation was microechinate [17]. *Acanthophyllum* differs from these taxa with exine ornamentation is scabrat-punctate and numbers of pores are 6-13.

Yıldız, Dadandı (2009) reported the pollen grains of *Silene cirpicii* were spheroidal, ornamentation was microechinate, microperforate, number of pores per pollen was 23-28 µm, number of granules on pore 9-15. *Acanthophyllum* differs from these taxa withexine ornamentation is scabrat-punctate and numbers of pores are 6-13[18].

Ataşlar, Erkara, Tokur (2009) were performed palynological studies for all 12 *Gypsophila* taxa which were polyporate and spheroidal. *Gypsophila* pollen grains ranged in size from 23.79 to 32.84 µm in non-acetolysed pollen and from 19.48 to 25.94 µm in acetolysed pollen. *Acanthophyllum* polar axes of 25.81-45.82 µm and the equatorial axes of 28.48-46.82 µm.The exine sculptures were clavate-microperforate ornamentation and granulate-microechinate - microperforate ornamentaion[19]. The major difference of *Acanthophyllum* from this species is the exine ornamentation which is only scabret-punctate.

Pollen morphology of some *Dianthus* species has been studied by Vural (2008). According to that study, pollen grains of *D. Aytachii* were periporate, long axis 34.9 mm (32–37 mm), short axis 35.1 mm (33–38 mm). Pollen shapewasoblate-spheroidal. Pore number was between 8–14, polar length/equatorial length (Plg/Plt):0.97, oblate-spheroidal, 5.5mm (4–6.5 mm) diameter, operculate with four to six echinae[20].The major difference of *Acanthophyllum* from this species is the pantoporatelong axes of 25.81-45.82 µm and the short axes of 28.48-46.82 µm.Number of pores are 6-13. Polar length/equatorial length (Plg/Plt) ratio is between 0.92 µm and 1.06 µm. *A. acerosum A. mucronatum* and *A. verticillatum* have oblate –spheroidal operculum shape while *A. microcephalum* Prolate-spheroidal and *A. oppositiflorum* is spheroidal.The exine structure is scabrat-punctate.

Bozchaloyi and Keshavarzi (2014) determined some pollen features of *Stellaria* speciesusing SEM. According to that study, pollen shapes in polar and equatorial views were spheroid. Average size of pollen grains in the *Stellaria* species and related gen era varied from 22.5 µm (*S. alsinoides*) to 42µm (*Myosoton aquaticum*). Pore diameters varied between 4.45 µm and 7.63 µm. The average exine thick ness was between 1.37 µm (*S. alsinoides*) and 2.61 µm (*S. holostea*)[21]. *Acanthophyllum* mainly differs from these taxa with long axes of 25.81-45.82 µm and the short axes of 28.48-46.82 µm. *Acanthophyllum* has1.79-2.14 µm exine thickness.

Ataşlar (2003) reported the pollen grains of *Saponaria kotschyi* Bois.hasspheroideae, 36 µm diameter, periporatae, 12-pored. Operculum has 3 µm diameters with spinulate. The exine sculpture is granulatae microechinatae-microperforatae[22]. *Acanthophyllum* has 6-13 pores and pollen grains of *Acanthophyllum* long axes of 25.81-45.82 µm and the short axes of 28.48-46.82 µm.

Çinbilgel, et al.(2007) described the pollen grains of *Saponaria pamphylica* Boiss. & Heldr as pollen grains are spheroideae, with isopolar symmetry, size middle, average 42.795 µm diameter, periporatae; numbers of pores are 11-12, average diameter 5.9 µm. Operculum is with 10-12 spinulate. The exine ornamentation is spinulatae (spinulosus) and microperforatae.[23] *Acanthophyllum* mainly differs from these taxa with pollen shape is oblate-spheroidal or prolate spheroidal.

In conclusion, according to the results of this study, pollen ornamentation, operculum ornamentation of pollen grains, pore numbers and diameter, exine and intine thickness are helpful characters in determining *Acanthophyllum* species.

Acknowledgments

I would like to thank to Z. Aytaç from the Herbarium of Gazi University (GAZI) and to I. Demir from Van (Turkey).

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