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POLLEN MORPHOLOGY STUDIES ON SOME *GAGEA* SALISB. (LILIACEAE) SPECIES FROM TURKEY

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Abstract

In this study, palynological features of *Gagea granatellii, G. villosa* var. *villosa, G. juliae, G. bohemica, G. peduncularis, G. taurica, G. foliosa, G. villosa* var. *hermonis, G. bithynica* and *G. dubia* were investigated. Similarities and dissimilarities of the pollen grains of these taxa were compared. Scanning electron microscope and light microscope microphotograps of these 10 taxa were investigated for the first time. Pollen grains of all studied taxa were identified as prolate/subprolate and sulcate (monocolpate). Some differences in the exine ornamentation of these taxa were identified. *G. granatellii, G. villosa* var. *villosa* and *G. juliae* had reticulate type (Type I); in *G. bohemica* and *G. peduncularis* microreticulate-perforate (Type II); and in *G. taurica, G. foliosa, G. villosa* var. *hermonis, G. bithynica* and *G. dubia* microreticulte exine ornamentation were identified.

Key words: *Gagea* Salisb. (Liliaceae), Pollen morphology, Biodiversity, Flora, Turkey.

Introduction

Liliaceae is one of the largest monocotyledon families having 15 genera and ca. 600 species. Members of the family are widely distributed in Northern Hemisphere, temperate zones of Eastern Asia and North America. In recent studies, the family of Liliaceae is treated in Monocots/Liliales according to the angiosperm phylogeny group (APG, 1998, 2003, 2009, 2016).

The genus *Gagea* comprises of ca. 300 species and it is the biggest genus of Liliaceae. It includes 15 sections and 9 of them have natural distribution in Turkey. First revision of Turkish *Gagea* was done by Rix (1984) in the Flora of Turkey. He reported 26 taxa from Turkey. This number grew to 31 with the recent studies performed by other researchers. 5 of these 31 taxa are endemic to Turkey and endemism ratio of *Gagea* is 16.13% (Rix, 1984; Hamzaoğlu *et al.*, 2008; Kayıkçı *et al.*, 2014; Tekşen & Kahraman Erkul, 2015a, 2015b; Tekşen *et al.*, 2015; Peruzzi, 2016; Tekşen & Eker, 2017).

Taxonomy of *Gagea* has great confusions due to the great variation in vegetative and generative characters which are used for delimitation of *Gagea* taxa. Also ratio of the interspecific hybridisation is too high in *Gagea* when compared with other Liliaceae genera. Because of these reasons, discrimination between *Gagea* taxa could be unclear for researchers and many published *Gagea* taxa are overloaded with synonyms (Levichev, 1990a, 1990b, 1999).

Palynological studies are important for plant taxonomy to classify problematic groups such as in *Gagea*. Until today, some palynological studies were performed by native or foreign researchers on *Gagea* taxa which are distributed in Turkey (Ekici, 2014; Sezer *et al.*, 2018). However, number of these studies are still too limited and many *Gagea* taxa are not palynologically investigated.

In this study, detailed palynological features of 10 *Gagea* taxa (*Gagea taurica, G. foliosa, G. granatellii, G. villosa* var. *villosa, G. villosa* var *hermonis, G. bithynica, G. bohemica, G. Peduncularis, G. juliae* and *G. dubia*) are investigated for the first time. We believe that, this study will make contributions to classification of *Gagea* taxa.

Materials and Methods

Gagea samples were collected from Muğla and Eskişehir provinces between 2016-2017 years (Table 1). Polleniferous material was acquired from the herbarium of Eskisehir Osmangazi University (OUFE) pollen grains of 10-15 distinct flowers from each plant specimen were also collected from different localities were used. Light microscopy and SEM were used for investigate the pollen morphology of taxa. The terminology of Faegri and Iversen was used (Faegri & Iversen, 1975). For the investigations by light microscopy, pollen grains which were obtained from *Gagea* specimens were prepared according to the Wodehouse (1935) and Erdtman (1969) techniques. 10x and 40x plan objectives were used for the aim of identifications and counts. Detailed investigations and identifications of pollen grains was performed by 100x plan oil-immersion objective. Each space in the scale bar of ocular micrometer is equal to 0.98 µm. Exine and intine thickness of the taxa were measured in compliance with Wodehouse (1935) and Erdtman (1969). Terminologies for pollen morphology were used (Wodehouse, 1935; Pokrovskaia, 1958; Kuprianova, 1967; Erdtman, 1969; Faegri & Iversen, 1975).

Results and Discussions

Pollen grains of all studied taxa are observed as monad, mono-sulcate, heteropolar, oblong shaped and oblate or peroblate. All of these are also bilateral and anisobisymetric. Width of non-acetolysed pollen grains changes from 44.40 µm to 56.20 µm and length 34.83-43.10 µm. P/E ratio is measured between 0.68-0.78. Dimensions of the acetolysed pollen grains are measured as 31.66-38.93 µm height and 44.96-53.40 µm width. P/E ratio ranges from 0.66 to 0.77. Aperture is simple, elongated and apex of it is acute or obtuse (Table 2).

Table 1. Investigated specimens of *Gagea* taxa.

Species	Locality
Gagea taurica Steven	B3: Eskişehir, Hekimdağ Village, Steppe and stony slopes, 1360 m, N: 39° 53'39" - E: 30°34' 33", 10.04.2016 (O. Koyuncu) (OUFE: 21173)
Gagea foliosa (C. Presl) Schult. & Schult.f.	B3: Eskişehir, Mihallıççık, *Pinus nigra* forrest, open areas, 1550 m, N: 39° 51'45" - E: 31°36' 02", 12.04.2017 (O. Koyuncu) (OUFE:21181)
Gagea granatellii (Parl.) Parl.	B3: Eskişehir, Alpu, Bozan plantation area, N: 39° 49' 41" - E: 31° 09' 08", 950 m, 10.03.2016 (O. Koyuncu) (OUFE: 21172)
Gagea villosa var. *villosa* (M. Bieb) Sweet	A5: Çorum, Boğazköy, Alacahöyük National Park, Boğazkale, Big Temple, 1085m, N: 40°14'04"- E: 34°41'51, 06.05.2017 (O. Koyuncu) (OUFE: 21180)
Gagea villosa var. *hermonis* Dafni & Heyn	B3: Eskişehir Alpagut, 2 km to Demirciler Village, N: 40° 00' 45" - E: 30° 31' 16", 259 m, 25.02.2017 (O. Koyuncu) (OUFE: 21176)
Gagea bithynica Pascher	B3: Eskişehir, Türkmen Mountain, Kalabak Village, Çimenharmanı area, 900 m, N: 39° 43' 05" - E: 30° 38' 88", 06.05.2016 (O. Koyuncu) (OUFE: 21175)
Gagea bohemica (Zauschn.) Schult. & Schult.f.	B3: Eskişehir, Tandır Village, 1286 m, N: 39° 55' 16" - E: 34° 41' 19", 16.04.2016 (O. Koyuncu) (OUFE: 21174)
Gagea peduncularis (C. Presl) Pascher	B3: Eskişehir, Kalabak camping area, 1007 m, N: 39° 30' 48" - E: 30° 68' 98", 01.04.2017 (O. Koyuncu) (OUFE: 21179)
Gagea juliae Pascher	C2: Muğla, Yaraş Billage, 741 m, N: 37° 08' 35' - E: 28° 29' 47", 26.03.2017 (A.C. Yıldız) (OUFE: 21178)
Gagea dubia A. Terracc.	C2: Muğla, Fethiye, Babadağ road, 644 m, N: 36° 56' 49" - E: 29° 16' 18", 22.03.2017 (A.C. Yıldız) (OUFE: 21177)

Reticulate, Microreticulate and Microreticulate-Perforate exine ornamentation types were identified from studied taxa. Muria is single or composed; simpli, dupli or pluricolumellate. Exine structure is tectate. In the light of the obtained data from study, polen grains of the studied taxa can be classified in 3 groups according to their exine ornamentation types (Figs. 1-2).

Pollen types: Three pollen types are recognized on the basis of exine ornamentation.

Type I: Reticulate

In this type lumenae are similar, they wider like muri or sometimes wider than it. Lumenae has wider structure than microreticulate type pollen grains. Muri are simplicolumellate or whole in some species. Following species belong to this type: *G. granatellii, G. villosa* var. *villosa* and *G. juliae* (Fig. 2). *Gagea villosa* var. *villosa* has the highest width between type I pollen grains with 56.20 µm. The lowest of these is *G. juliae* with 46.76 µm. Height of *G. villosa* var. *villosa* which is measured the highest as 43.10 µm. *G. granatellii* has the lowest value with 36.23 µm (Table 2).

Type II: Microreticulate-Perforate

In type II pollen grains, perforate ornamentation was observed as both of the proximal and distal surfaces. Muri is complete or compound and so it is duplicolumellate or pluricolumellate structured.

None of the pollen grains of studied species have only perforate ornamentation. Microreticulate-perforate ornamentation has been observed only in *Gagea bohemica* and *G. peduncularis* (Fig. 2).

Gagea peduncularis has the highest pollen width with 56.13 µm and so *G. bohemica* has the lowest width value with 45.36 µm between microreticulate-perforate species. The smallest height value was measured as 33.06 µm from *G. bohemica*. The highest value was determined as 41.83 µm from *G. peduncularis* (Table 2).

Type III: Microreticulate

Lumen is wide like muri or smaller than it. Muri is complete or compound, simplicolumellate or pluricolumellate. Width of muri decreases from proximal to distal surface. Another important difference is the decrease at the number of lumen from proximal to distal surface.

Gagea taurica, G. foliosa, G. villosa var. *hermonis, G. bithynica* and *G. dubia* have microreticulate ornamentation (Fig. 2). *G. taurica* has the highest width value between type III ornamented pollen grains with 52.96 µm. *G. dubia* has the lowest value with 44.40 µm. Like in width, height comparison of type III pollen grains gave same results. The smallest value was measured in *G. dubia* with 31.66 µm and highest value was measured in *G. taurica* with 40.33 µm (Table 2).

Table 2. Pollen data of investigated *Gagea* taxa.

Taxa	*P*	*Eq*	*P/E*	*clg*	*clt*	*Exine*	*Intine*	*Ornamentation*
G. granatellii (W)	38.96 ± 5.99 µm	55.06 ± 4.25 µm	0.70	46.50 ± 6.79 µm	12.21 ± 3.66 µm	1.12 ± 0.21 µm	0.75 ± 0.30 µm	Reticulate
G. granatellii (E)	36.23 ± 3.94 µm	52.66 ± 4.42 µm	0.68	44.06 ± 5.75 µm	9.50 ± 5.25 µm	1.18 ± 0.24 µm	-	Reticulate
G. villosa var. *villosa* (W)	43.10 ± 2.95 µm	56.20 ± 3.24 µm	0.76	43.83 ± 4.28 µm	9.17 ± 3.99 µm	1.10 ± 0.25 µm	0.73 ± 0.28 µm	Reticulate
G. villosa var. *villosa* (E)	38.93 ± 3.61 µm	53.40 ± 4.03 µm	0.72	43.50 ± 3.71 µm	6.93 ± 1.73 µm	1.12 ± 0.21 µm	-	Reticulate
G. juliae (W)	36.56 ± 2.75 µm	46.76 ± 3.63 µm	0.78	32.70 ± 2.67 µm	11.29 ± 1.89 µm	1.28 ± 0.38 µm	0.80 ± 0.25 µm	Reticulate
G. juliae (E)	37.80 ± 3.82 µm	48.76 ± 4.80 µm	0.77	38.76 ± 2.04 µm	7.38 ± 2.02 µm	1.18 ± 0.24 µm	-	Reticulate
G. peduncularis (W)	41.83 ± 3.30 µm	56.13 ± 3.54 µm	0.74	44.21 ± 4.44 µm	10.71 ± 3.24 µm	1.66 ± 0.27 µm	0.92 ± 0.26 µm	Microreticulate-Perforate
G. peduncularis (E)	33.23 ± 7.68 µm	49.70 ± 5.59 µm	0.66	42.87 ± 5.01 µm	9.19 ± 4.13 µm	1.15 ± 0.23 µm	-	Microreticulate-Perforate
G. bohemica (W)	35.23 ± 4.29 µm	51.06 ± 4.81 µm	0.68	42.26 ± 4.44 µm	8.60 ± 0.98 µm	1.05 ± 0.15 µm	0.67 ± 0.23 µm	Microreticulate-Perforate
G. bohemica (E)	33.06 ± 3.40 µm	45.36 ± 7.60 µm	0.72	38.25 ± 2.26 µm	7.33 ± 1.43 µm	1.08 ± 0.19 µm	-	Microreticulate-Perforate
G. taurica (W)	40.33 ± 4.23 µm	52.96 ± 3.89 µm	0.76	43.84 ± 3.64 µm	11.38 ± 3.22 µm	1.22 ± 0.25 µm	0.73 ± 0.25 µm	Microreticulate
G. taurica (E)	37.70 ± 6.62 µm	52.53 ± 6.16 µm	0.71	42.41 ± 6.43 µm	9.75 ± 3.76 µm	1.18 ± 0.24 µm	-	Microreticulate
G. foliosa (W)	35.90 ± 2.59 µm	47.40 ± 2.74 µm	0.75	41.08 ± 1.72 µm	9.08 ± 2.15 µm	1.32 ± 0.30 µm	0.70 ± 0.25 µm	Microreticulate
G. foliosa (E)	38.33 ± 3.01 µm	49.90 ± 4.85 µm	0.76	39.41 ± 3.65 µm	9.33 ± 2.14 µm	1.32 ± 0.24 µm	-	Microreticulate
G. villosa var. *hermonis* (W)	39.93 ± 2.03 µm	51.23 ± 2.06 µm	0.77	42.60 ± 3.29 µm	8.73 ± 3.21 µm	1.20 ± 0.25 µm	0.93 ± 0.20 µm	Microreticulate
G. villosa var. *hermonis* (E)	35.93 ± 3.96 µm	49.80 ± 1.54 µm	0.72	41.53 ± 3.07 µm	8.46 ± 2.60 µm	1.18 ± 0.25 µm	-	Microreticulate
G. bithynica (W)	34.83 ± 2.79 µm	48.23 ± 4.95 µm	0.72	42.01 ± 5.18 µm	8.57 ± 2.82 µm	1.13 ± 0.27 µm	0.87 ± 0.22 µm	Microreticulate
G. bithynica (E)	35.66 ± 2.68 µm	47.50 ± 3.92 µm	0.75	40.57 ± 2.87 µm	7.64 ± 1.54 µm	1.17 ± 0.24 µm	-	Microreticulate
G. dubia (W)	34.86 ± 2.75 µm	44.40 ± 3.59 µm	0.78	37.07 ± 4.03 µm	8.85 ± 3.02 µm	1.25 ± 0.34 µm	0.82 ± 0.28 µm	Microreticulate
G. dubia (E)	31.66 ± 2.86 µm	44.96 ± 3.01 µm	0.70	38.09 ± 3.36 µm	6.08 ± 2.22 µm	1.20 ± 0.24 µm	-	Microreticulate

W: Non-acetolised; E: Acetolised; P: Polar diameter; Eq: Equatorial diameter; plg: Porus length; plt: Porus width; clg: Colpus length; clt: Colpus width; t: One edge of polar triangle

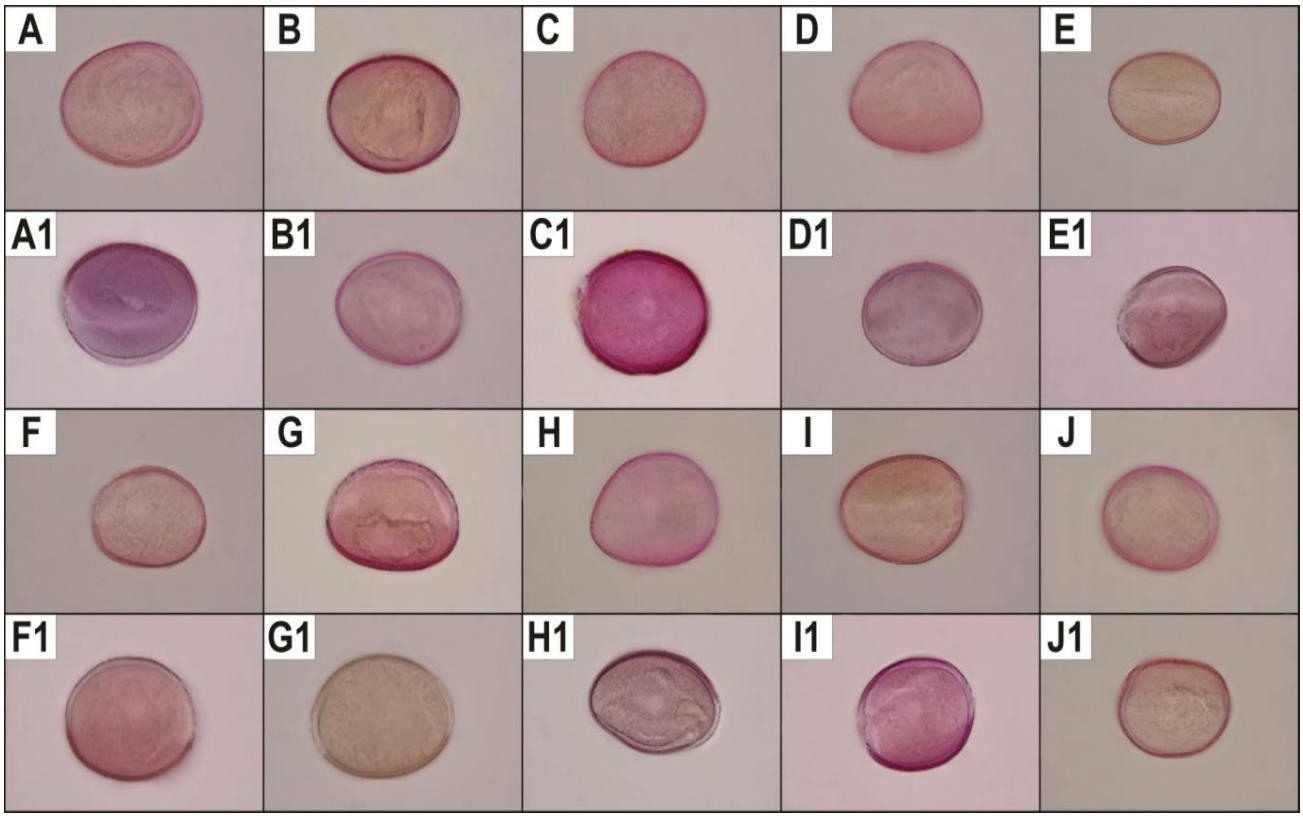

Fig. 1. Non acetolised (A, B, C, D, E, F, G, H, I, J) and acetolised (A1, B1, C1, D1, E1, F1, G1, H1, I1, J1) pollen grains of *Gagea* taxa (*G. granatellii*: A, A1; *G. villosa* var. *villosa*: B, B1; *G. juliae*: C, C1; *G. pedunculares*: D, D1; *G. bohemica*: E, E1: *G. taurica*: F, F1; *G. foliosa*: G, G1: *G. villosa* var. *hermonis*: H, H1: *G. bithynica*: I, I1: *G. dubia*: J, J1).

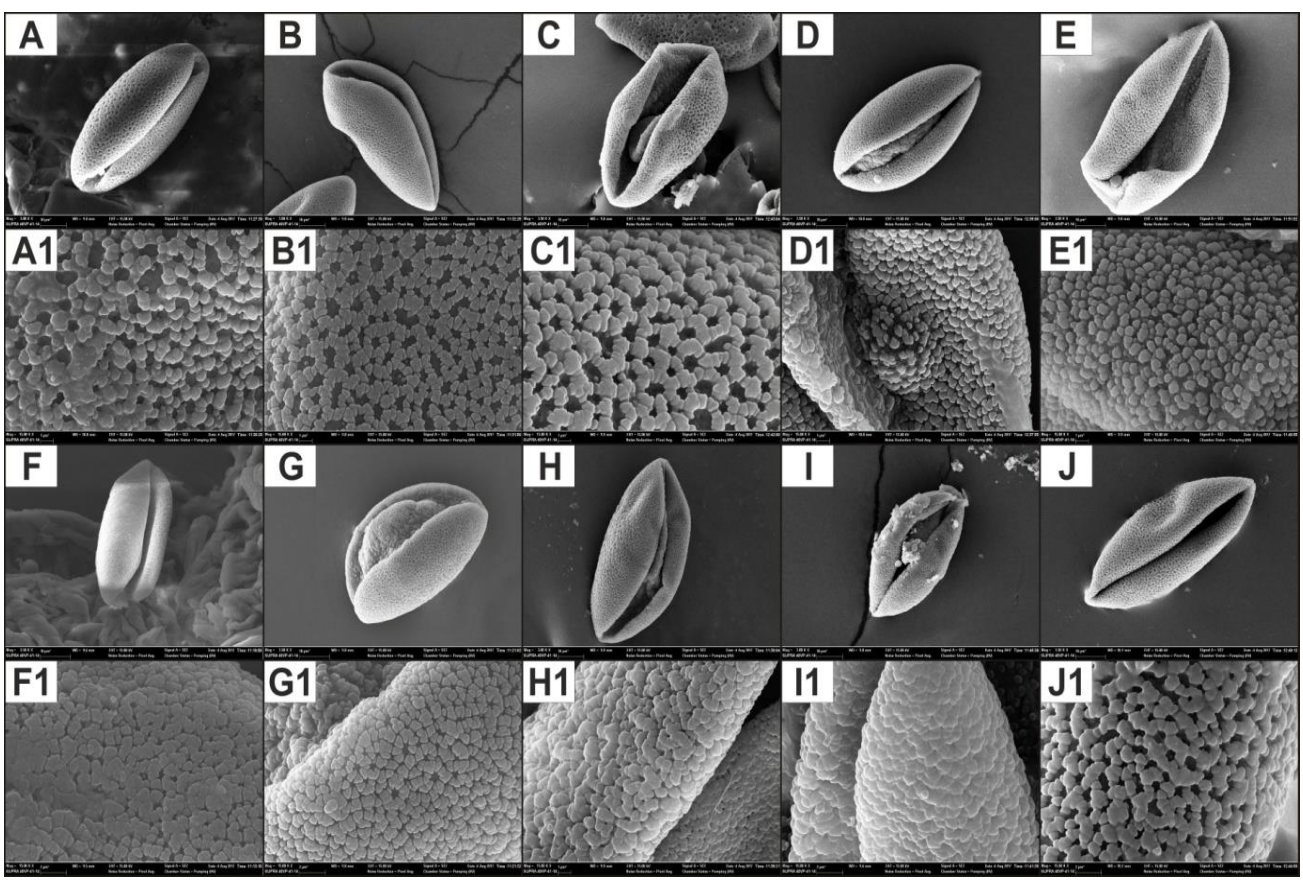

Fig. 2. General view and ornamentation of *Gagea* taxa in SEM (*G. granatellii*: A, A1; *G. villosa* var. *villosa*: B, B1; *G. juliae*: C, C1; *G. pedunculares*: D, D1; *G. bohemica*: E, E1: *G. taurica*: F, F1; *G. foliosa*: G, G1: *G. villosa* var. *hermonis*: H, H1: *G. bithynica*: I, I1: *G. dubia*: J, J1).

Conclusions

Turkey has a higher floristic diversity than many other countries of the world. Flora of Turkey has also a great importance because of its high endemism ratio. Until today ca. 13000 vascular plant taxa have been identified in Turkey and more than 3000 of these are endemic to Turkey. Main reasons of this high endemism ratio of Turkey are geological, geomorphological and edaphic features and so geographic location of Turkey. Due to these reasons, Turkey is diversification center for many genera and many of these genera have more endemism ratio than many other countries like in *Gagea* (Erik & Tarıkahya, 2004). Until today many taxonomists tried to classify *Gagea* taxa. Number of studies including different disciplines such as general morphology, cytology, hybridization and palynology were conducted on *Gagea* taxa (Rechinger, 1986; Levichev, 1999; Kosenko, 1999; Caparelli *et al*., 2006).

Taxonomy of the *Gagea* genus is harder than other Liliaceae genera owing to some problems. Vegetative and generative character are mainly used for the classification of *Gagea* taxa. Unfortunately, these characters showed great variations in different stages of ontogeny and so under the different ecological conditions (Zarrei & Zarre 2005).

Subgeneric grouping in the genus are not clear and it creates conflicts between researchers. According to the Kosenko (1999) and Kosenko & Levichev (1988), palynomorphological characters are important tools for the classification of Liliaceae taxa and specially in *Gagea*. Findigs of recent studies support also this idea of Kosenko and Levichev (Kosenko & Levichev, 1988; Kosenko, 1999).

Pollen grains of *Gagea* taxa showed heterogeneous distribution pattern under groups and subgroups according to the palynomorphological data which was obtained from this study. This palynomorphological grouping shows partly conformation with macromophological characters based on groupings under *Gagea* genus.

As a result, only palynomorphological characters are not enough to classify *Gagea* taxa. On the other hand, palynomorphological characters are important for plant taxonomy specially in problematic groups such as *Gagea*.

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