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POLLEN MORPHOLOGICAL STUDY ON SOME RARE *ALLIUM* L. (AMARYLLIDACEAE) TAXA IN TURKEY

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Abstract

The pollen morphology of 10 *Allium* L. taxa, 6 of which are endemic to Turkey, were investigated in detail by light and scanning electron microscopy. According to LM and SEM, the pollen grains of genera were monad, monosulcate percolate, heteropolar with bilateral symmetry, 25.30 to 53,85 µm long axis (LA) and 17.55 to 36.86 µm short axis (SA), the form was prolate (mean of LA/SA ratio 1.30 to 1.70 and in polar view boat-shaped. Three types of ornamentation were determined. Striate-rugulate-perforate type in *Allium longisepalum, A. oreophilum, A. anacoleum, A. microspathum, A. shirnakiense, A. purpureoviride* and *A. armenum*, Rugulate–perforate type in *A. pervariense* and *A. gabardagense* and Rugulate–reticulate-perforate type in *A. arlgirdense*. Sulcus membrane ornamentations were rugulate or psilate. The sulcus extends from the distal to proximal ends in *A. anacoleum, A. arlgirdense* and *A. pervariense*. The present study on some Turkish species of *Allium* showed that several morphological pollen characters may possess taxonomical value.

Introduction

The *Allium* L. genus was formerly included in the Liliaceae family, but the Angiosperm Phylogeny Group (APG) reassessed the taxonomic position of this genus and finally *Allium* was placed in the Amaryllidaceae family (APG III 2009). The genus *Allium* comprises more than 850 species, making it one of the largest petaloid monocotyledonous genera (Fritsch *et al.,* 2010; Keusgen *et al.*, 2011; Herden *et al.,* 2016). It is a variable group that is widely spread across the Holoarctic region from the dry subtropics to the boreal zone (Li *et al.*, 2010). Turkey has approximately 200 *Allium* taxa in 14 sections, c. one-third of which are endemic to this territory, demonstrating that Turkey is a prominent part of the southeastern Asian center of *Allium* diversity (Koyuncu 2012; Eksi *et al.*, 2015; 2016; Fırat 2015; 2017; Fırat *et al*., 2018). Turkey is very rich in terms of biodiversity. The main reasons are: i) It is the meeting point of three phytogeographical regions. ii) Asian part of Turkey is a passageway and a migration route between Southern Europe and the flora of South-West Asia allowing the penetration of Asiatic elements into South Europe. iii) Many taxa have their center of origin and/or center of diversity in Anatolia. iv) The high endemism ratio, presumably connected with the climatic and topographical diversity of the country (Davis, 1965, 1971).

The family Amaryllidaceae is more or less stenopalynous taxon and pollen grains are generally monocolpate, bilateral symmetrical, boat shaped with subpsilate or rugulate-foveolate rarely reticulate tectum (Erdtman, 1952). Data on pollen morphology of representatives of *Allium* genus as acquired under LM microscope were given by Nair and Sharma (1965), Radulescu (1973), Diez (1987) and El-Sadek *et al.*, (1994). In recent years, several researchers have

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investigated this area, their studies have focused on some selected, constantly very rare species representing Guler and Pehlivan (2006), Namin *et al.* (2009), Neshati *et al.* (2009), Ozhatay and Kocyigit (2009), Ozler and Pehlivan (2010) and Maassoumi *et al.* (2014).

Pollen morphoplogy of the *Allium* species, which is the most difficult Monocotyledon family from systematic and taxonomic point of view (Guler and Pehlivan, 2006). In order to solve these problems, detailed pollen morphological studies of 10 species of the genus *Allium* have been investigated. Further attempts should additionally be undertaken to rate whether earlier not recognized pollen characters may be useful taxonomic markers at infrageneric or even species level in *Allium*.

Materials and Methods

Pollen sampling

Pollen samples were taken from specimens deposited in Herbarium of Van, Yüzüncü Yıl University (VANF). The complete list of the investigated taxa with sample provenance is reported in Table 1.

Table 1. List of voucher specimens belonging to the genus *Allium*.

Section	Taxa	Localities	Satus
Molium G. Don ex W.D.J. Koch	*Allium longisepalum* Bertol.	Turkey. Şırnak, Gabar Mountain, open oak forest, 700 m, 2 May 2014, M. Fırat 30635	Rare
Porphyroprason Ekberg.	*A. oreophilum* C.A. Meyer, Verz.	Turkey. B9 Van, Başkale discrict, İspiriz mountain, Rocky places, scree, 3300 m, 7 June 2014, M. Fırat 31010	Rare
Scorodon C. Koch	*A. anacoleum* Hand.-Mazz.	Turkey. Hakkari, Sat Mountain, rocky region, 2900 m, 8 August 2014, M. Fırat 30987	Rare
	A. microspathum Ekberg	Turkey. Hakkari, Sat Mountain, rocky region, 2900 m, 8 August 2014, M. Fırat 30985	Endemic
	A. arlgirdense Blakelock	Turkey. Hakkari, Sat Mountain, rocky region, 2900 m, 8 August 2014, M. Fırat 30986	Rare
Codonoprasum Reichb.	*A. armenum* Boiss. & Kotschy	Turkey. B9 Van, Bahçesaray discrict, Agirov mountain, Rocky places, 2400 m, 1 July 2015, M. Fırat 31010	Endemic
Melanocrommyum Webb & Berth.	*A. shirnakiense* L. Behçet & Rüstemoğlu	Turkey. C9, Şırnak, Beytüşşebap district, Cevam region, rocky area, 1450 m, 30 May 2014, M. Fırat 30843	Endemic
	A. purpureoviride M. Koyuncu & İ. Genç	Turkey. B7 Elazığ, from Elazığ to Pertek 22 km, near field, 669 m, 13 May 2015 M. Fırat 32691	Endemic
Allium Boiss.	*A. pervariense* Fırat & Koyuncu	Turkey. C9 Siirt: Pervari, Botan River, opposite to Bedar (Beğendik) Village, fields converted from the Quercus forests, 1500-1700 m, 4 July 2012. M. Fırat 29712 (AEF 26722).	Endemic
	A. gabardagense Fırat	Turkey. C9 Şırnak: Cizre, Gabar mountain slopes, 410 m, limestone rocks, 01 May 2014, M. Fırat. 30515)	Endemic

For LM studies

Samples were taken from herbarium specimens. For pollen morphological analysis, pollen grains were prepared according to the methods of Wodehouse (1935). The following parameters, as which pollen size i.e. long axis (LA) and short axis (SA), sulcus width, exine thickness and intine thickness were measured. In each sample, 30 pollen grains were measured in order to obtain the maximum and average value of the size. Photomicrographs were made with a Olympus BX31 binocular light microscope.

For SEM studies

For SEM study, pollen grains obtained from each specimen were transferred onto stubs and coated with platinum. The SEM micrographs were taken with a ZEISS supra 55. The terminologies for pollen morphology were used in accordance with Kosenko (1991a,b) and Hesse *et al.,* (2009).

Results and Discussion

Pollen morphology of 10 taxa of *Allium* was investigated by LM (Fig. 1) and SEM (Figs 2-3). The following characters were emphasized as important for separating taxa at different taxonomic value: the sulcus, presence or absence of perforations on the pollen surface, size of perforations, size of the pollen grains and the sulcus extends from distal to proximal end. Present pollen data is based on 10 species of the genus *Allium* representing 6 sections: Sect. *Molium*; *A. longisepalum.* Sect. *Porphyroprason*; *A. oreophilum.* Sect. *Scorodon*; *A. anacoleum, A. microspathum, A. arlgirdense*. Sect. *Codonoprasum; A. armenum.* Sect. *Melanocrommyum*; *A. shirnakiense, A. purpureoviride*. Sect. *Allium*; *A. pervariense, A. gabardagense.* The monosulcate pollen grains, which are regarded as primitive among seed plants, occur widely among the monocotyledons (Ozler and Pehlivan, 2007; Ozhatay and Kocyigit, 2009; Fırat, 2015; 2017; Fırat *et al*. 2018). The common characteristics of pollen grains were monads, monosulcate, ellipsoidal, and heteropolar in *Allium* genus. In this study the exine ornamentational characteristics observed in their SEM micrographs were perforate-striate, perforate-rugulate and perforate-striate-rugulate. These results show that there were several pollen characters of taxonomic significance in *Allium*.

In this study, microperforations visible only on SEM microgaphs. The number of perforation in 1 μm^2 is 2-12, the diameter of perforation in average is 0.10–0.42 µm and the thicknes of lira in average is 0.20–0.60 µm (Fig. 3). The diameter of perforation was observed to be the biggest in *A. microspathum* (Fig. 3; 4). The number of perforations in 1 μm^2 was more in *A. armenum* (Fig. 3; 6). Intine 1.05–0.40 µm thick. The *A. longisepalum* had thicknest intine while *A. microspathum* had thinnest one (Table 2).

Some researchers have showed that the sulcus features and the presence of operculum may be a taxonomic value in some families (Chanda *et al.,* 1979; Kosenko, 1991a,b; Güler and Pehlivan, 2006; Ozler and Pehlivan, 2010). In SEM photomicrographs, sulcus membranes are psilate in *A. pervariense, A. oreophillum, A. arlgirdense, A. armenum* and *A. shirnakense*. Rugulate sulcus membrane ornamentation was found in *A. microsepalum, A. anacoleum, A. longisepalum, A. gabardagense* and *A. purpureoviride* (Fig. 3). The operculum was found to be fragmented on the sulcus membrane (Fig 1; 10). Similarly, Guler and Pehlivan (2006), Ozler and Pehlivan (2010) reported that sulcus membrane ornamentations were psilate, psilate-perforate and rugulate-perforate in *Allium* taxa and the operculum was found to be fragmented on the sulcus membrane or sometimes completely covering it.

The common characteristics of the pollen grains of *Allium* have been investigated. Species showed that their pollen apertures are monosulcate and monosulcate-operculate. The advantage of

a monosulcate aperture (extended sulcate) in monocotyledons with the inclusion of *Allium*, is underlined by Harley and Zavada (2000) and Ozler and Pehlivan (2010). In the present study, biggest pollen size was found in *A. longisepalum*, whereas the smallest was found in *A. arlgirdense* (Table 2). It was recognized that the sulcus extends from distal to proximal end in *A. anacoleum*, *A. arlgirdense* and *A. pervariense* investigated. The width of the sulcus ranged from 2.75 – 5.77 µm (Table 2). The extended sulcus type has been observed on *Allium* and the family

Fig. 1. LM microphotography of examined *Allium* pollen.1-2: *A. longisepalum*, 3-4: *A. oreophilum*, 5-6: *A. anacoleum*, 7-8: *A. microspathum*, 9-10: *A. arlgirdense*, 11-12: *A. armenum* 13-14: *A. shirnakiense*, 15-16: *A. purpureoviride*, 17-18: *A. pervariense*, 19-20: *A. gabardagense* (scale 20 µm).

Liliaceae (Guler and Pehlivan, 2006; Ozler and Pehlivan, 2007; 2010). The sulcus ends were sharp in *A. arlgirdense*, *A. anacoleum*, *A. armenum* and *A. gabardagense* (Fig. 2; 6, 10, 12, 20). The sulcus ends were rounded in the other investigated taxa (Fig. 2; 4, 8, 14, 16, 18). The longest

sulcus extension dimension was measured in *A. pervariense*, whereas the shortest dimension was observed in *A. arlgirdense* (Table 2). The widest sulcus dimension was seen in *A. pervariense*. The longest length dimension of sulcus was seen in *A. longisepalum* and the shortest dimension was seen in *A. arlgirdense* (Table 2). The thickest exine dimension was found in *A. longisepalum* and the thinnest exine dimension was found in *A. shirnakiense* (Table 2). According to SEM, the exine sculpturing was striate-perforate, striate-rugulate-perforate and rugulate-perforate (Table 2, Fig 2, 1-19). Perforate-striate, perforate-rugulate and perforate-striate-rugulate exine structure have been reported in previous investigations (Guler and Pehlivan, 2006; Ozler and Pehlivan, 2007; 2010).

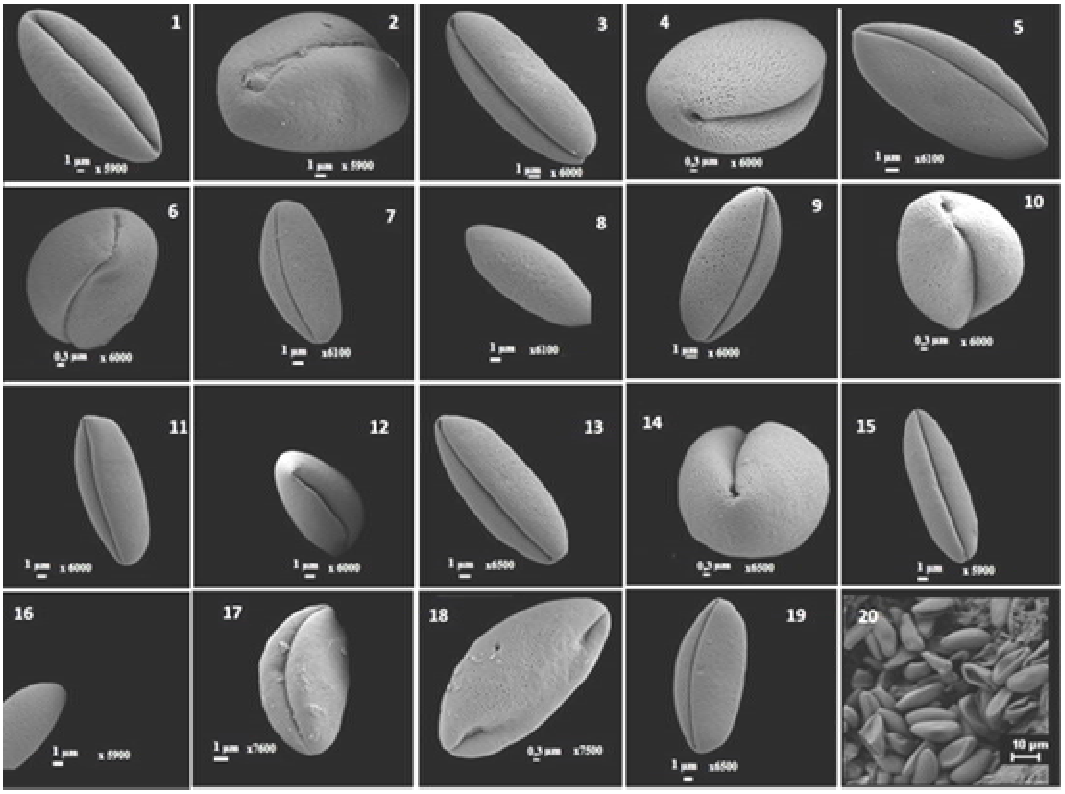

Fig. 2. SEM microphotography of the examines *Allium* pollen.1-2: *A. longisepalum,*3-4: *A. oreophilum,* 5-6: *A. anacoleum,* 7-8: *A. microspathum,* 9-10: *A. arlgirdense,* 11-12: *A. armenum,* 13-14: *A. shirnakiense,* 15-16: *A. purpureoviride,* 17-18: *A. pervariense,* 19-20: *A. gabardagense.*

Allium taxa are of three types ornamentational characteristics as follows; Striate-rugulate-perforate: *A. longisepalum*, *A. oreophilum*, *A. anacoleum*, *A. microspathum*, *A. shirnakiense*, *A. purpureoviride* and *A. armenum*; Rugulate-perforate: *A. pervariense*, *A. gabardagense*; Rugulate–reticulate-perforate: *A. arlgirdense.*

The main palynological differences have been registered at the section level. These results are similar to the earlier studies (Guler and Pehlivan, 2006; Ozler and Pehlivan, 2007; 2010; Neshati *et al.,* 2009; Ozhatay and Kocyigit, 2009).

Table 2. Pollen morphological parameters of the investigated *Allium* species.

Species	LA (µm)		SA (µm)		LA/SA	Slg (µm)		Slt (µm)		Sp		Exine (µm)		Intine (µm)		Orn.
	M	SD	M	SD		M	SD	M	SD	M	SD	M	SD	M	SD	
Sect. *Molium*																
A. longisepalum	53.85 ± 2.93		36.86 ± 3.29		Prolate	47.50 ± 3.15		5.77 ± 0.78		-		1.76 ± 0.43		1.05 ± 0.23		St-rg-pf
Sect. *Porphyroprason*																
A. oreophilum	30.94 ± 1.40		18.18 ± 1.23		Prolate	27.34 ± 1.64		3.27± 0.88		-		1.34 ± 0.33		0.68± 0.15		St-rg-pf
Sect. *Scorodon*																
A. anacoleum	30.53 ± 1.42		19.58 ± 1.59		Prolate	34.37 ± 2.21		4.11± 0.70		4.08 ± 0.80		1.48 ± 0.38		0.72 ± 0.21		St-rg-pf
A. microspathum	28.24 ± 2.12		17.55 ± 1.79		Prolate	25.87 ± 2.22		3.82 ± 0.59		-		1.15± 0.22		0.40 ± 0.10		St-rg-pf
A. arlgirdense	25.30 ± 2.75		17.97 ± 1.48		Prolate	30.77 ± 2.74		2.75 ± 0.49		3.54 ± 0.37		1.18 ± 0.20		0.67± 0.17		Rg-rec-pf
Sect. *Codonoprasum*																
A. armenum	35.16 ± 1.73		24.07 ± 2.78		Prolate	32.19 ± 2.14		3.76± 0.59		-		1.10 ± 0.26		0.55 ± 0.09		St-rg-pf
Sect. *Melanocrommyum*																
A. shirnakiense	30.09 ± 2.73		18.90 ± 2.05		Prolate	26.01 ± 2.22		4.79 ± 0.64		-		0.82 ± 0.28		0.44 ± 0.19		St-rg-pf
A. *purpureoviride*	33.08 ± 2.01		19.65 ± 1.99		Prolate	29.89 ± 1.80		3.26 ± 0.50		-		1.33 ± 0.23		0.58 ± 0.24		St-rg-pf
Sect. *Allium*																
A. pervariense	29.68 ± 1.99		19.86 ± 1.91		Prolate	33.44 ± 2.43		3.30 ± 0.68		5.72 ± 0.93		1.04 ± 0.43		0.66 ± 0.10		Rg-pf
A. gabardagense	34.03 ± 2.18		21.73 ± 2.56		Prolate	29.14 ± 2.24		3.52 ± 0.52		-		1.25± 0.35		0.74 ± 0.18		Rg-pf

Nomenclature: (LM); SA – Short axis; LA – Long axis; M – Mean value; SD – Standard deviation; Slg – Length of the sulcus; Slt – Width of the sulcus; Sp – proximal extension of the sulcus, st- rg -pf – striate-rugulate- perforate; rg- pf– rugulate-perforate; Rg-rec-pf – rugulate-reticulate-perforate; Orn. - ornamentation

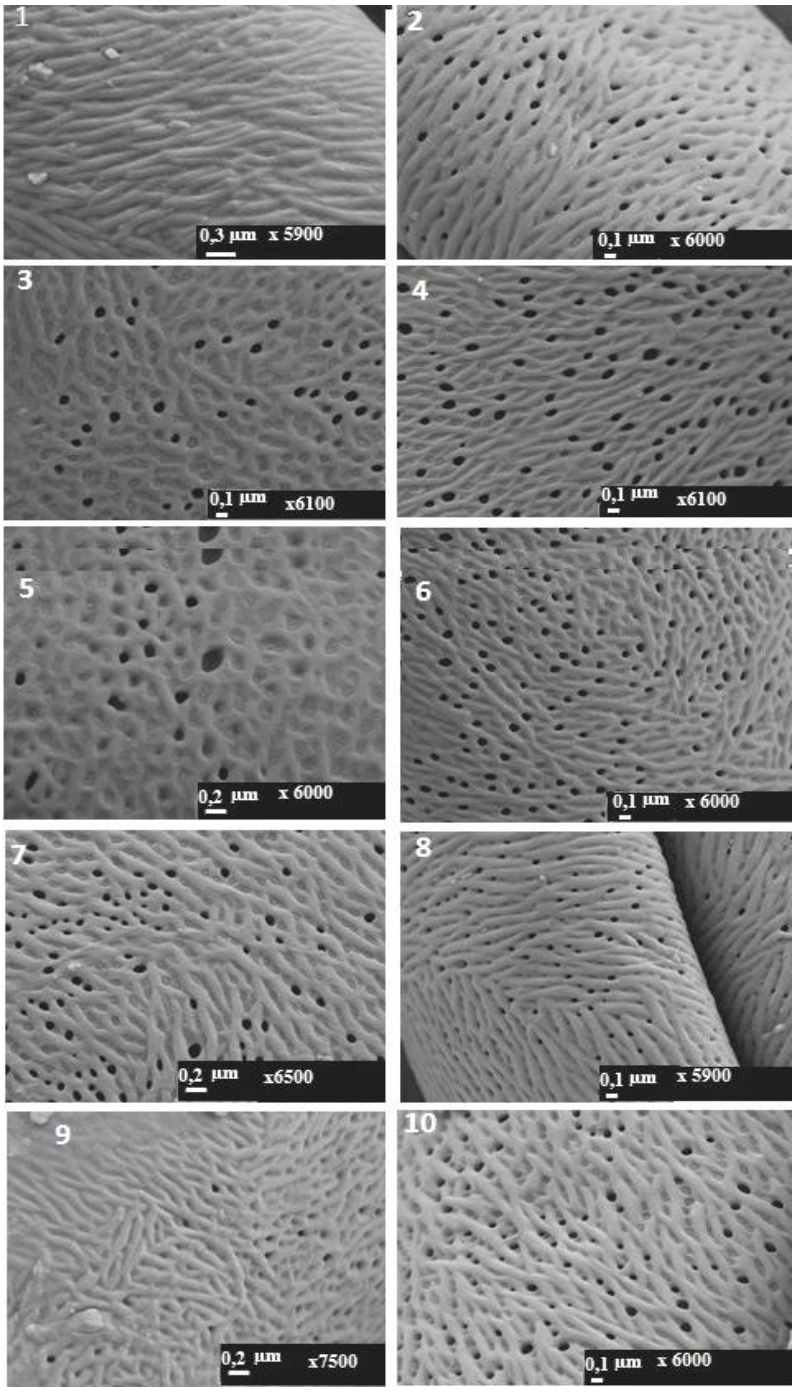

Fig. 3. SEM microphotography of the pollen ornamentation: 1. *A. longisepalum*; 2. *A. oreophilum*; 3. *A. anacoleum*; 4. *A. microspathum;* 5. *A. arlgirdense;* 6. *A. armenum;* 7. *A. shirnakiense;* 8. *A. purpureoviride;* 9. *A. pervariense;* 10. *A. gabardagense.*

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