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POLLEN MORPHOLOGY OF TWENTY THREE SPECIES OF *ALLIUM* L. (AMARYLLIDACEAE) FROM TURKEY

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Keywords: *Allium*; Amaryllidaceae; Pollen morphology; LM; SEM; TEM; Flora of Turkey.

Abstract

The pollen morphology of 23 *Allium* L. species, grown in Turkey, belonging to the sections; *Rhizirideum*, *Schoenoprasum*, *Cepa*, *Molium*, *Brevispatha*, *Scorodon*, *Acanthoprason* and *Melanocrommyum*, were investigated by LM (light microscopy), TEM (transmission electron microscope), and SEM (scanning electron microscopy). This paper presents detailed pollen morphological features are given for these taxa. Common characteristics of pollen of *Allium* species investigated are ellipsoidal and heteropolar, bilateral symmetry; pollen grains are shed as monads. They are monosulcate (extended sulcate types) and monosulcate-operculate. The sculpturing of the exine, pollen membrane ornamentation, lumina shape, and sulcus membrane with a fragmented operculum are found to be characteristic features for separating species. Based on these properties three main pollen types were determined with three different exine sculpturing. The characteristic structures of pollen ornamentation, observed in SEM micrographs, are striate-perforate, striate-regulate-perforate and regulate-perforate. Sulcus membrane ornamentations are psilate, psilate-perforate, rugulate and regulate-perforate. The exine is semitectate and the tectum perforate. Columellae were found to be simplicolumellate.

Introduction

The genus *Allium* L. is one of the most diverse and taxonomically difficult groups of the monocots. The most recent classifications are based on morphological characters and molecular data, recognized more than 920 species in 15 subgenera and 80 sections (Friesen *et al.*, 2006). *Allium* is a taxonomically quite complex and has unresolved nomenclatural problems. In the last decades, many *Allium* taxa were newly described for Turkey and the certain number of *Allium* species and subspecies raised the number to about 220, classified into 15 sections, ca. one-third of which are endemic to this territory, demonstrating that Turkey is a prominent part of the southeastern Asian center of *Allium* diversity (Kollmann, 1984**;** Davis, 1984, 1988; Güner *et al*., 2000; Friesen *et al*., 2006; Koyuncu and Eker 2011; Celep *et al.*, 2012; Güner *et al*. 2012; Özhatay and Genç, 2013 and Govaerts *et al*., 2013; Ekşi *et al.*, 2015, 2016; Duman *et al*., 2017; Fırat *et al*., 2018; Govaerts *et al*., 2019; Özdöl *et al*., 2022). Cytological and data on pollen morphology of representatives of *Allium* genus has been subjected to earlier investigations (Nair and Sharma, 1965; Kuprianova, 1967; Radulescu, 1973; Kuprianova and Aliev, 1979; Schulze, 1980, 1980a; Pastor, 1981; Diez *et al*., 1987; El-Sadek *et al*., 1994; Kosenko and Kudryashova, 1995; Tolgor, 1995; Hosseinzadeh *et al*., 2009; Namin *et al*., 2009; Neshati *et al*., 2009, Maassoumi *et al*., 2014; Wrońska-Pilarek *et al*., 2016; Perveen and Qaiser, 2015; Hosseini, 2018). Palynological studies on *Allium* in Turkey; detailed studies of the pollen morphology of 14 species of *Allium* were made by Güler and Pehlivan (2006), 23 species of *Allium* were examined by Özhatay and Koçyiğit

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(2009) and 16 species of *Allium* were examined by Özler and Pehlivan (2010), 12 taxa belonging to Sect. Codonoprasum were examined by Koçyiğit (2014) and later, 10 species *Allium* L. taxa, 6 of which are endemic to Turkey, were examined by Başer *et al*. (2019), respectively. In literature on the *Allium* genus, the following pollen grain features are considered as having the highest diagnostic value. Turkey *Allium* an extremely polymorphous and taxonomically difficult genus species show a great morphological diversity and has unresolved nomenclatural problems therefore many taxonomical problems remain unsolved (Kollmann, 1984; Gurushidze *et al*., 2007). To solve the systematic problems of these polymorphic groups further cytological, anatomical and palynological studies are urgently needed.

In order to obtain more morphological data to solve taxonomical problems, detailed study of pollen morphology in Alliaceae family always has been suggested and pollen structure of some species of the genus *Allium* has been subjected to earlier investigations. In literature on the *Allium* genus, the following pollen grain features are considered as having the highest diagnostic value. Pollen information of some species from Turkey Güler and Pehlivan (2006) and Özler and Pehlivan (2010) suggested the usefulness of pollen characteristics for the systematics of the genus, and observed the possible use of these characters for solving certain taxonomical problems as well. Güler and Pehlivan (2006) recognized the sulcus type, sulcus structure, exine ornamentation, ekzin structure, presence or absence of operculum and operculum type, presence or absence of perforations on the pollen surface, density of perforations, size of perforations, and size of the pollen grains characteristics, which are also important for separating taxa at different taxonomic ranks.

The present study adds pollen morphological data for a further 23 species (including 14 sections and to compare the details of sculpturing), again using light, scanning electron microscopy and transmission electron microscope. An overview of pollen characteristics for all the Turkish representatives of the genus *Allium*, thus concluding a series of studies aimed at describing the characteristics of all the Turkish *Allium* genera, and enabling an overall review of the pollen data both from this study and from our previously published studies (Güler and Pehlivan 2006).

Material and Methods

The pollen grains were obtained from the Faculty of Pharmacy of Ankara University (AEF) herbarium. The complete list of the investigated taxa with sample provenance is reported in Table 1. Pollen morphological terminology of Walker (1974a, 1974b), Faegri and Iversen (1989), Punt *et al*. (1994, 2007); El-Sadek *et al*. (1994) and Hesse *et al*. (2009) was followed.

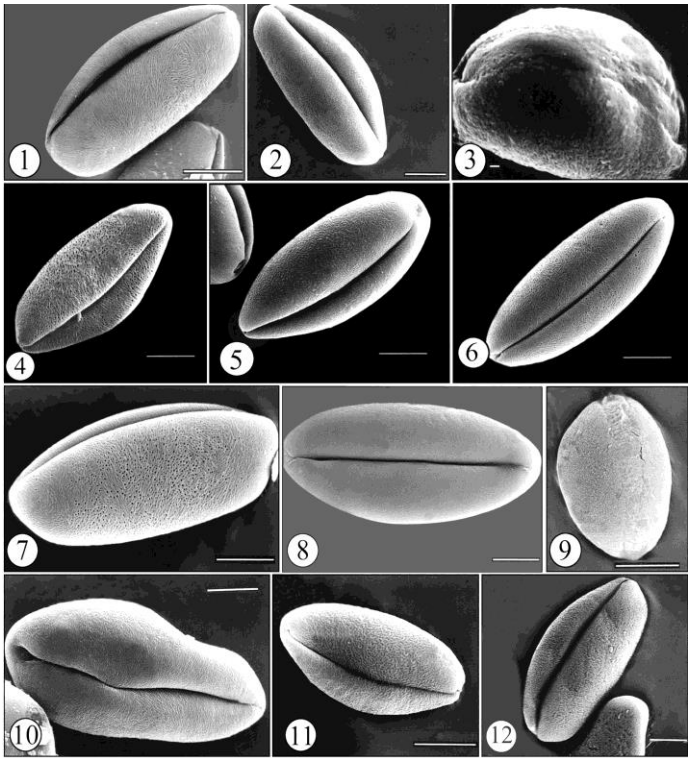
LM investigation

Samples were taken from herbarium specimens. For LM observations, the pollen was first treated with 70% ethyl alcohol to remove oily substances, and then embedded in glycerine-jelly, stained with safranin. For morphological analysis, pollen grains of specimens for LM investigations were prepared according to the methods of Wodehouse (1935) and Erdtman (1960). Pollen dimensions of all species were measured in such amounts that the resulting data followed Gaussian curves these measurements which were made in acetolysed, nonacetolysed pollen. In each sample, 50 pollen grains were measured in order to obtain the maximum and average value of the size. LM studies were made using a Nikon Alphaphot-2 YS2 microscope, under (E40, 0.65) and oil immersion (E100, 1.25), using 15 eye piece (ocular) and the following paremeters, as which pollen size i.e. long axis (LA) and short axis (SA), length of the sulcus (slg), width of the sulcus (slt) sulcus width for all types and operculum type, exine and intine thickness for all types.

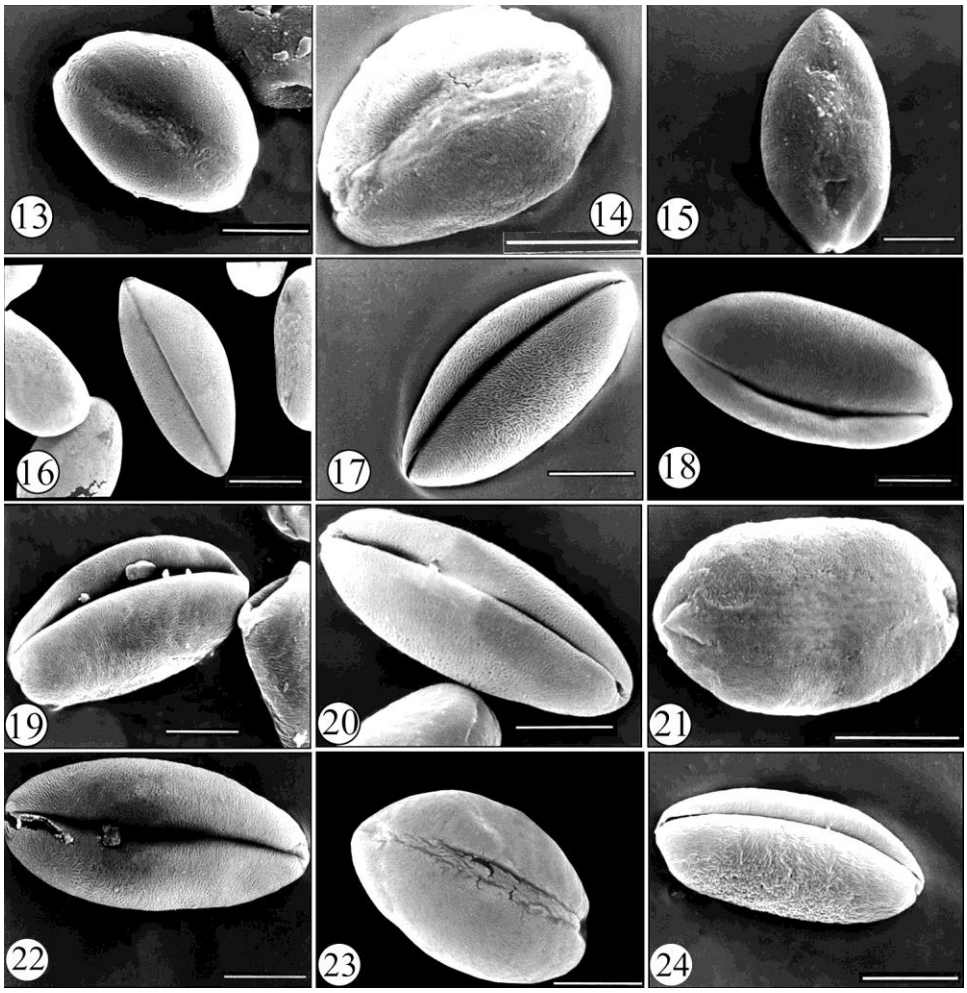
The used eyepiece and lens scales require a conversion of the measurement results to micrometers (μm). These measurements which were made in acetolysed, nonacetolysed pollen and on LM micrographs are given in (Figs. 67-98). The LM photomicrographs were taken with an Orthomat-w camera connected to a Carl Zeiss-photo binocular light microscope.

SEM investigation

For SEM investigations, the pollen was first treated with 70% ethyl alcohol then air-dried before being mounting on SEM specimen stubs subsequently coated with gold plate, and examined under a Jeol JSM-840A (Turkish Petroleum Corporation, TPAO, Turkey) scanning electron microscope. The clearest SEM photographs representing each pollen type and the main pollen features were selected for this paper (Figs 1-24, Figs 25-48, Figs 49-56). SEM micrographs were used mainly for studying the overall shape, type of sculpturing, and more detailed information on the sculptures.



Figs 1-12: SEM photographs of the pollen grains of *Allium* species, showing pollen grain in distal view. 1. *A. scabriscapum*: Pollen grain in distal view, SEM x 2500; 2. *A. szovitsii*: Pollen grain in distal view, SEM x 1500; 3. *A. cepa*: Pollen grain in distal view with psilate-perforate of the sulcus membrane, SEM x 4000; 4. *A. schoenoprasum*: Pollen grain in distal view with operculum, SEM x 2500; 5. *A. cassium*: Pollen grain in distal view, SEM x 2000; 6. *A. zebdananse*: Pollen grain in distal view, SEM x 2000; 7. *A. subhirsutum*: Pollen grain in distal view, perforate-striate ornamentation, SEM x 2200; 8. *A. subhirsutum*: Pollen grain in distal view, SEM x 1800; 9. *A. cupani* subsp. *hirtovaginatum*: Pollen grain in distal view with rugulate-perforate of the sulcus membrane, SEM x 2500; 10. *A. longisepalum*: Pollen grain in distal view, SEM x 2000; 11. *A. callidictyon*: Pollen grain in distal view, SEM x 2500; 12. *A. callimischon* subsp. *haemostictum* Pollen grain in distal view, SEM x 1500; Scale bar = 10 μm (1,2,4,5,6,7,8,9,10,11,12); 1 μm (3).

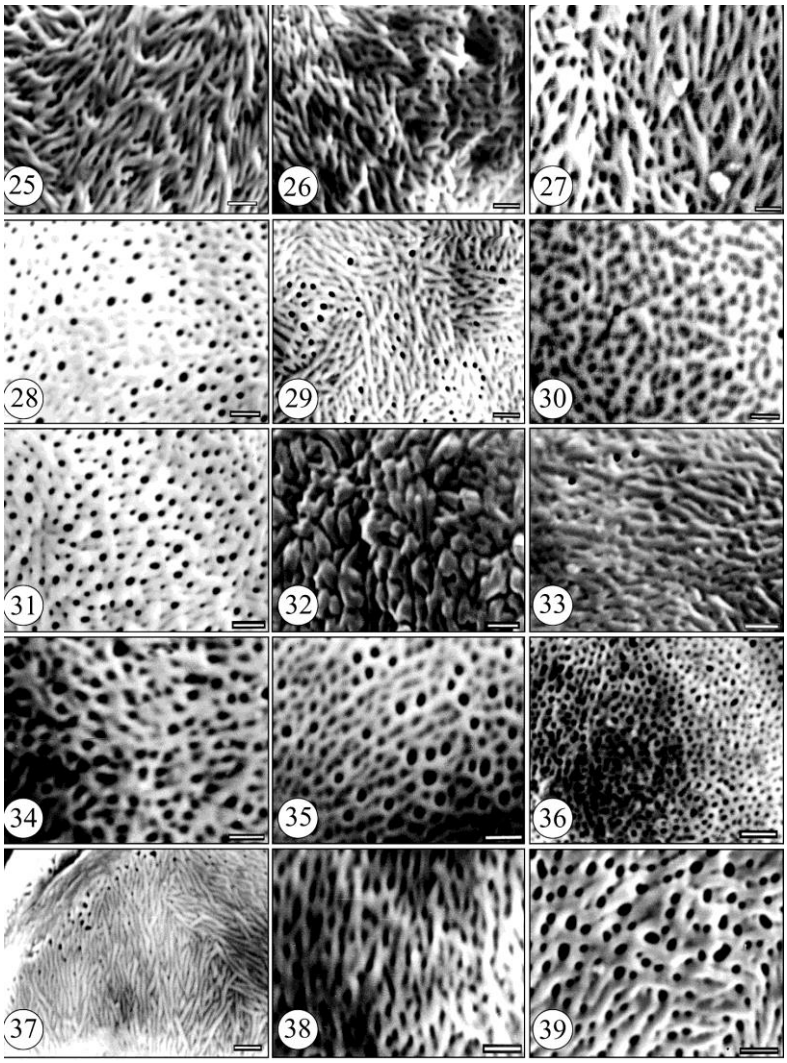


Figs 13-24: 13. *A. frigidum*: Pollen grain in distal view with psilate-perforate of the sulcus membrane, SEM x 2500; 14. *A. kossoricum*: Pollen grain in distal view with psilate ornamentation of the sulcus membrane, SEM x 2700; 15. *A. akaka*: Pollen grain in proximal view with sulcus extending to the proximal face and with broad and rounded ends view with rugulate-perforate ornamentation, SEM x 2000; (16) *A. akaka*: Pollen grain in distal view SEM x 1900; 17. *A. akaka*: Pollen grain in distal view with striate-rugulate-perforate ornamentation, SEM x 2300; 18. *A. chrysanterum*: Pollen grain in distal view, SEM x 2500; 19. *A. cardiostemon*: Pollen grain in distal view with operculum, SEM x 2500; 20. *A. colchicifolium*: Pollen grain in distal view, SEM x 2500; 21. *A. decipiens*: Pollen grain in distal view with psilate ornamentation of the sulcus membrane SEM x 3500; 22. *A. kharputense*: Pollen grain in distal view, with operculum, SEM x 2500; 23. *A. noëanum*: Pollen grain in distal view with rugulate ornamentation of the sulcus membrane SEM x 2500; 24. *A. lycaonicum*: Pollen grain in lateral view, SEM x 2500 with rugulate ornamentation. Scale bar = 10 µm.

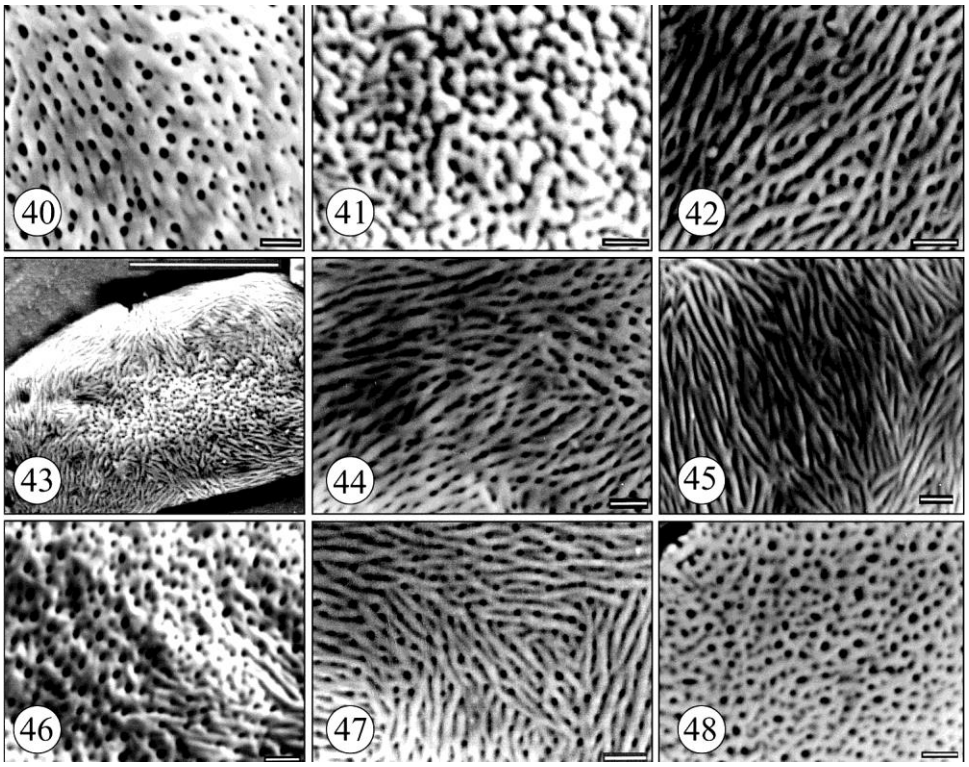
TEM investigation

Acetolyzed pollen grains were stained with 2% OsO_4 and uranyl acetate, dehydrated and embedded in epon araldite according to the method described by Skvarla and Turner (1966). Ulthrathin sections of the pollen grains were obtained with a glass knife in a Reichert Supernova microtome (Gazi University, Faculty of Medicine, Ankara Turkey). Post-staining was done with

lead citrate for 5 minutes (Reynolds, 1963), and the sections were examined under a Zeiss EM9 (Figs 57-66). TEM micrographs were used mainly for studying the overall shape, pollen walls type of sculpturing, and more detailed information on the sculptures.



Figs 25-39: Ornamentation of *Allium* pollen grains. 25. *A. scabriscapum*: Striate-perforate ornamentation, SEM x 10000; 26. *A. szovitsii*: Striate-perforate ornamentation, SEM x 10000; 27. *A. shoenoprasum*: Striate-perforate ornamentation, SEM x 10000; 28. *A. subhirsutum*: Striate-perforate ornamentation, SEM x 10000; 29. *A. cassium*: Striate-rugulate-perforate ornamentation SEM x 10000; 30. *A. cupani* subsp. *hirtovaginatum*: Striate-rugulate-perforate ornamentation SEM x 10000; 31. *A. zebdananse*: Striate-rugulate-perforate ornamentation SEM x 10000; 32. *A. longisepalum*: Distal face showing perforate-rugulate ornamentation, SEM x 10000; 33. *A. longisepalum*: Proximal face showing perforate-rugulate ornamentation, SEM x 10000; 34. *A. callidictyon*: Perforate-striate-rugulate ornamentation, SEM x 10000; 35. *A. callimischon* subsp. *haemostictum*: Perforate-striate-rugulate ornamentation, SEM x 10000; 36. *A. frigidum*: Rugulate-perforate ornamentation SEM x 10000; Perforate-striate-rugulate ornamentation, SEM x 10000; 37. *A. kossoricum*: Striate-perforate ornamentation, SEM x 10000; 38. *A. akaka*: Striate-rugulate-perforate ornamentation SEM x 10000; 39. *A. cardiostemon*: Perforate-striate ornamentation, SEM x 10000; Scale bar = 10 µm.



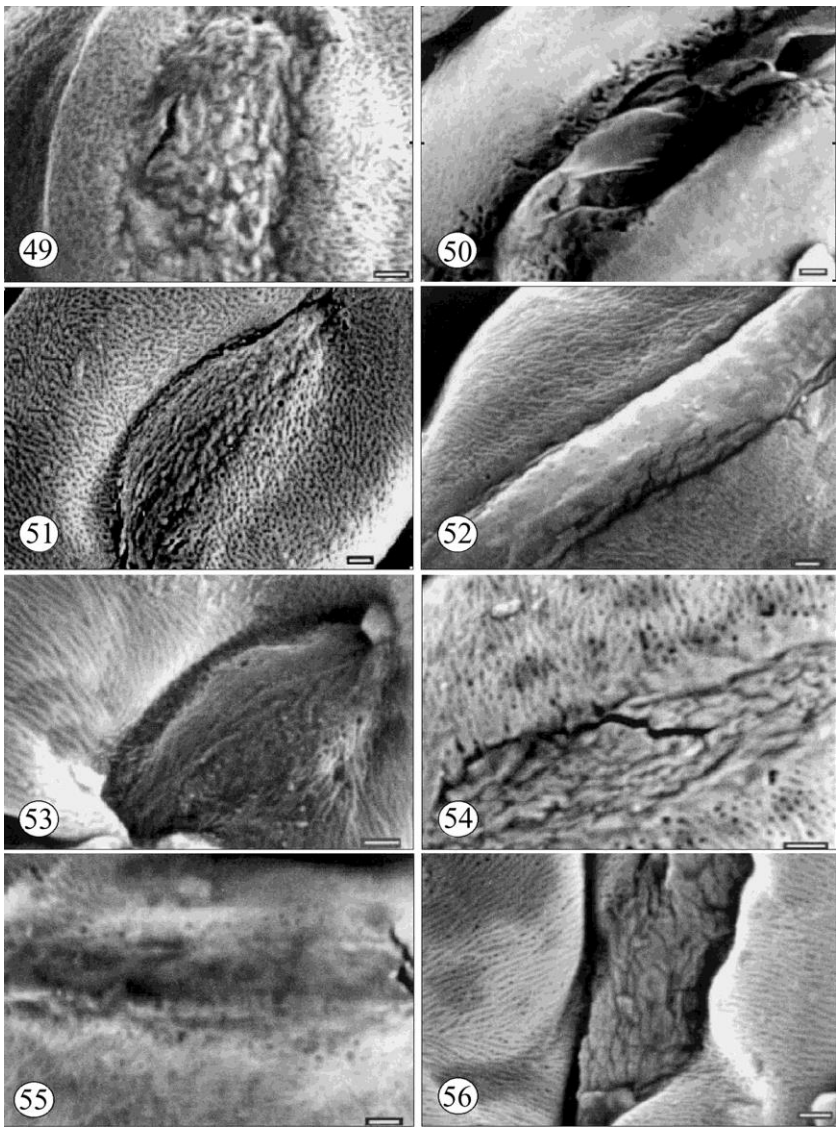
Figs 40-48: Ornamentation of *Allium* pollen grains. 40. *A. colchicifolium*: Striate-perforate ornamentation, SEM x 10000; 41. *A. decipiens*: Rugulate-perforate ornamentation SEM x 10000; 42. *A. orientale*: Distal face showing Striate-rugulate-perforate ornamentation SEM x 3500; 43. *A. orientale*: Proximal face showing Striate-rugulate-perforate ornamentation SEM x 3500; 44. *A. kharputense*: Striate-perforate ornamentation, SEM x 10000; 45. *A. noëanum*: Striate-perforate ornamentation, SEM x 10000; 46. *A. lycaonicum*: Proximal face showing perforate-rugulate ornamentation, SEM x 10000; 47. *A. lycaonicum*: Distal face showing sitriate-perforate ornamentation, SEM x 10000; 48. *A. hirtifolium*: Distal face showing sitriate-perforate ornamentation, SEM x 10000; Scale bar = 10 µm.

Results and Discussion

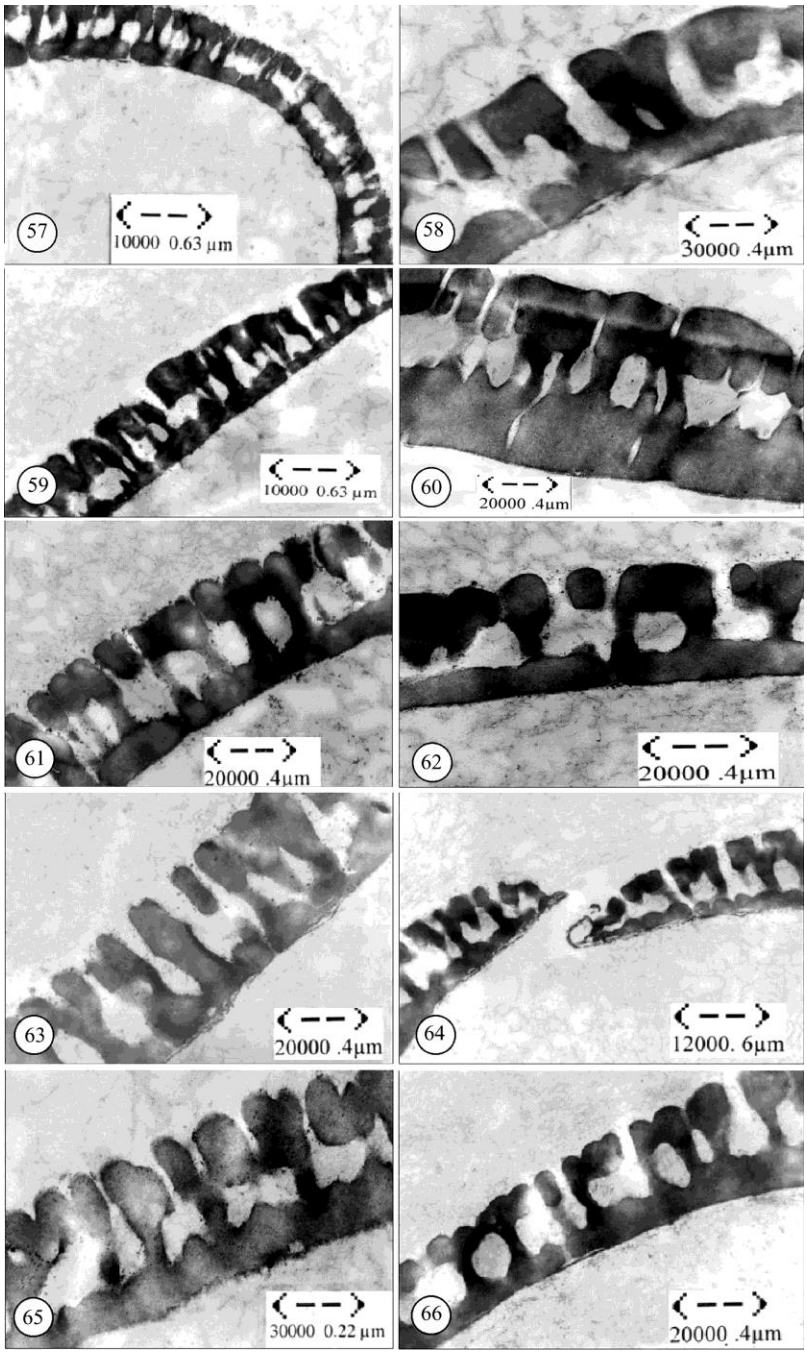
All studied pollen grains are medium-sized (25-50 µm) with more or less bilateral symmetry. The sulcus is either as long as the half of the circumference of the pollen grain (longest axis), or much longer and extends to the proximal face.

The main palynogical features of Turkish *Allium* taxa (and specimens) examined are summarized in Table 2. According to LM and SEM investigations, the pollen grains are monad, monosulcate, monosulcate-operculate, ellipsoidal, heteropolar with more or less bilateral symmetry; 25.98 to 50.32 µm long axis (LA) and 16.26 to 32.10 µm short axis (SA), the form was prolate (mean of LA/SA ratio 1.12 to 1.69). The pollen shapes (based on long axis (LA)/ short axis (SA) ratio) prolate or subprolate, outline more or less circular in polar view and boat-shaped. Pollen morphological parameters are given in Table 2. Medium to large in size with LA 25-50 µm and SA 17-36 µm. The sulcus extends from distal to proximal in all species. Sulcus ends are sharp, blunt, broad and rounded. Sulcus membrane ornamentations were rugulate or psilate. In most of the species with operculum, there are fragmented operculum within the sulcus of *A. szovitsii*, *A. shoenoprasum* (Fig. 69), *A. cupani* subsp. *hirtovaginatum* (Fig. 80), *A. callidictyon* (Fig. 82), *A.*

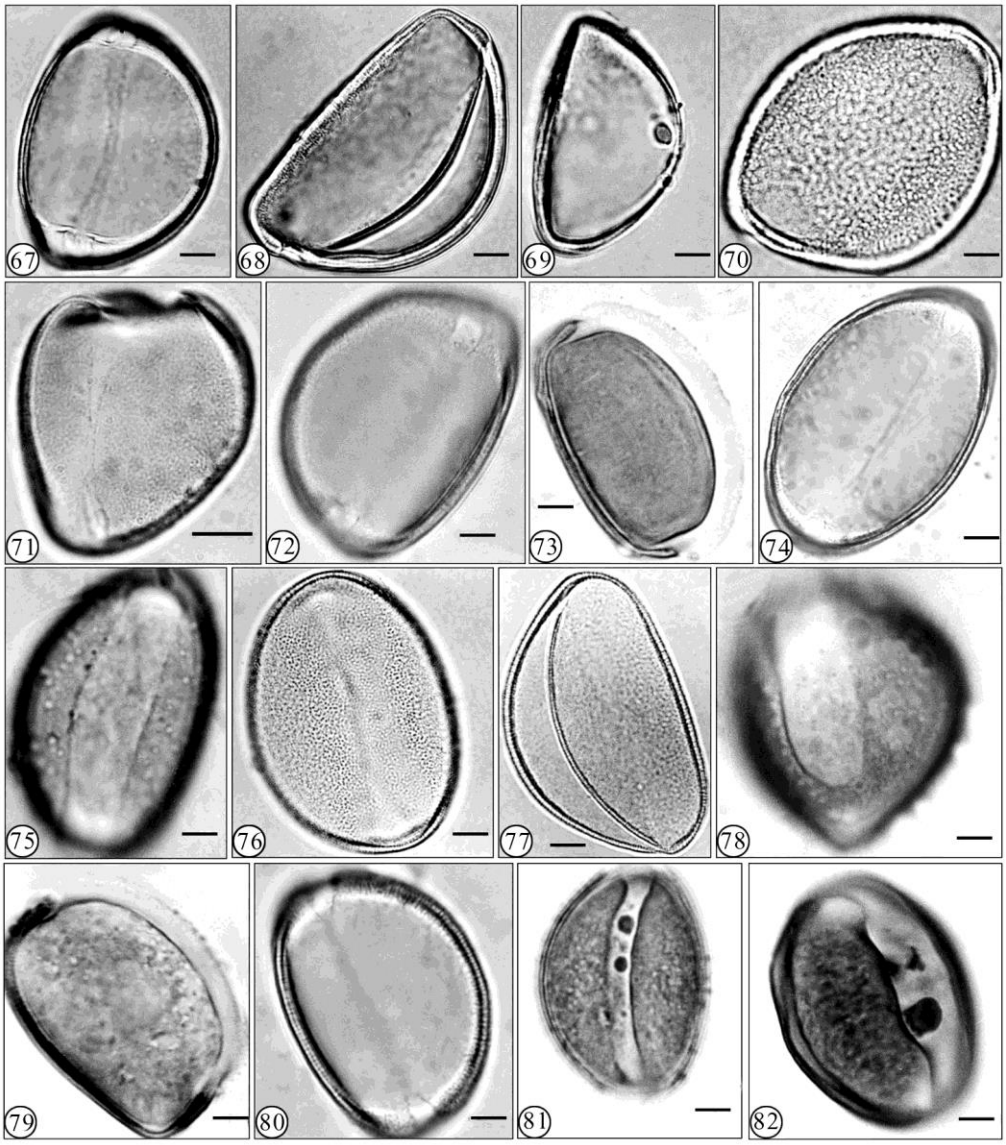
callimischon (Fig. 86), *A. akaka* (Fig. 89), *A. chrysantherum* (Fig. 91), *A. cardiostemon* (Fig. 19), *A. decipiens* (Fig. 94), *A. orientale* (Figs 95-96), *A. kharputense* (Fig. 22), *A. lycaonicum* (Fig. 97), and *A. hirtifolium* (Fig. 98). Sulcus membrane ornamentations are psilate, psilate-perforate, rugulate and and rugulate-perforate (Figs 49-56).



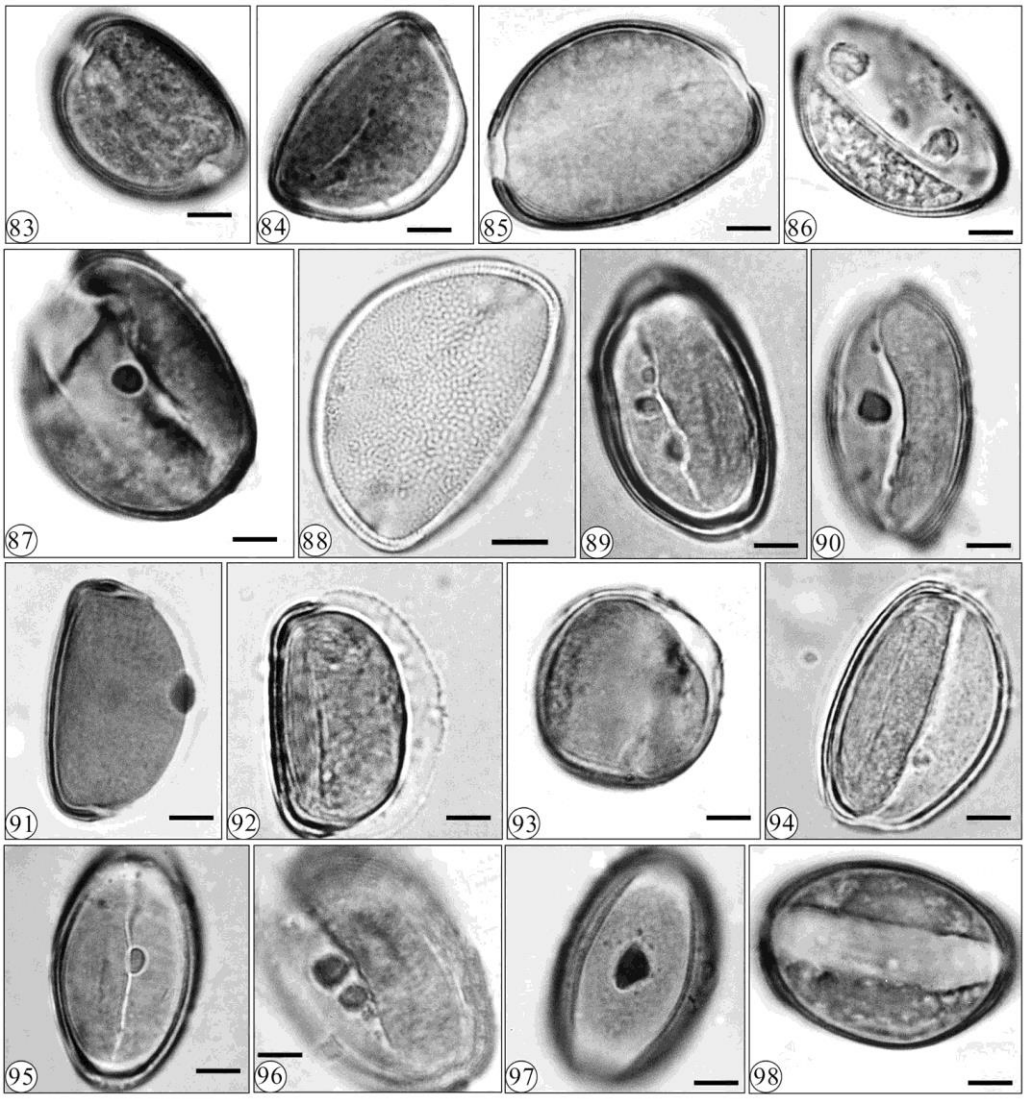
Figs 49-56: Ornamentation of the sulcus membrane of *Allium* pollen grains. SEM photographs of the pollen grains of *Allium* species, showing pollen grain in distal view. 49. *A. szovitsii*: rugulate-perforateof the sulcus membrane SEM x 7000; 50. *A. subhirsutum*: Psilate-perforate of the sulcus membrane, SEM x 5000; 51. *A. zebdananse*: Rugulate-perforate ornamentation of the sulcus membrane, SEM x 5000; 52. *A. longisepalum*: Psilate-perforate of the sulcus membrane, SEM x 5000; 53. *A. cardiostemon*: Rugulate-perforate ornamentation of the sulcus membrane, SEM x 8000; 54. *A. colchicifolium*: Rugulate-perforate of the sulcus membrane, SEM x 8000; 55. *A. kharputense*: Psilate of the sulcus membrane, SEM x 6500; 56. *A. lycaonicum*: Rugulate-perforate ornamentation of the sulcus membrane, SEM x 6000 Scale bar = 1 µm.



Figs 57-66: TEM photomicrographs of exine structure (Pollen wall stratification and morphology of *Allium* pollen grains). 57. *A. scabriscapum*: TEM x 10000; 58. *A. schoenoprasum*: TEM x 30000; 59. *A. cepa*: TEM x 10000; 60. *A. subhirsutum*: TEM x 20000; 61. *A. cupani* subsp. *hirtovaginatum*: TEM x 20000; 62. *A. callidictyon*: TEM x 20000; 63. *A.*: TEM x 20000; 64. *A. frigidum*: TEM x 12000; 65. *A. cardiostemon*: TEM x 30000; 66. *A. decipiens*: TEM x 20000.



Figs 67-82: LM photographs of selected *Allium* species. 67. *A. szovitsii*: Proximal view with ornamentation LM x 1000 (A); 68. *A. szovitsii*: oblique polar proximal view, showing sulcus border, LM x 1000 (A); 69. *A. schoenoprasum*: oblique polar proximal view, showing operculum, LM x 1000 (A); 70. *A. schoenoprasum*: Pollen grain in proximal view, Striate-perforate ornamentation, LM x 1000 (A); 71. *A. cepa*: Pollen grain Equatorial distal view LM x 1000 (A); 72. *A. cepa*: Proximal view LM x 1000 (A); 73. *A. cepa*: oblique polar proximal view, LM x 1000 (N); 74. *A. subhirsutum*: Proximal view with ornamentation LM x 1000 (A); 75. *A. cassium*: Pollen grain Equatorial distal view LM x 1000 (N); 76. *A. cassium*: Pollen grain in distal view, striate-rugulate-perforate ornamentation LM x 1000 (A); 77. *A. cassium*: oblique polar proximal view, LM x 1000 (A); 78. *A. cassium*: Equatorial distal view LM x 1000 (A); 79. *A. zebdananse*: oblique polar proximal view, LM x 1000 (N); 80. *A. cupani* subsp. *hirtovaginatum*: Pollen grain in proximal view with sulcus extending to the proximal face, LM x 1000 (A); 81. *A. cupani* subsp. *hirtovaginatum*: Pollen grain in distal view with fragmented operculum, LM x 1000 (N); 82. *A. callidictyon*: Pollen grain in distal view with fragmented operculum, LM x 1000 (N); Scale bar = 1µm (40-52). (A) = acetolysed, (N) = not acetolysed.



Figs 83-98: LM photographs of selected *Allium* species. 83. *A. callidictyon*: pollen grain in proximal view with sulcus extending to the proximal face, LM x 1000 (N); 84. *A. callidictyon*: oblique polar proximal view, LM x 1000 (N); 85. *A. callimischon* subsp. *haemostictum*: Pollen grain in distal view LM x 1000 (N); 86. *A. callimischon* subsp. *haemostictum*: Pollen grain in distal view with fragmented operculum, LM x 1000 (N), 87. *A. callimischon* subsp. *haemostictum*: Equatorial distal view with operculum, LM x 1000 (N); 88. *A. frigidum*: Proximal view with ornamentation LM x 1000 (A); 89. *A. akaka*: Pollen grain in distal view with fragmented operculum, LM x 1000 (N); 90. *A. akaka*: Pollen grain in distal view with fragmented operculum, LM x 1000 (N); 91. *A. chrysantherum*: oblique polar proximal view, view showing operculum, LMx1000 (N); 92. *A. cardiostemon*: oblique polar proximal view, LMx1000 (N); 93. *A. decipiens*: Pollen grain in equatorial distal view, LMx1000 (N); 94. *A. decipiens*: Pollen grain in distal view with fragmented operculum, LM x 1000 (N); 95. *A. orientale*: Pollen grain in distal view with operculum, LM x 1000 (N); 96. *A. orientale*: Pollen grain in equatorial distal view with fragmented operculum, LM x 1000 (N); 97. *A. lycaonicum*: Pollen grain in distal view with operculum, LM x 1000 (N); 98. *A. hirtifolium*: Pollen grain in distal view showing sulcus, LM x 1000 (N). Scale bar = 1 µm (83-98). (A) = acetolysed, (N) = not acetolysed.

The exine, is mainly composed of columellae covered by the tectum which contains perforations (eutectate and microperforate pollen grains). These microperforations were only visible on SEM micrographs. There are also differences in the number of perforation, diameter of perforation and thickness of lirae. Fine perforation is getting bigger through sulcus side (Figs 13, 14, 21), (The perforations small ca. 0.08-0.35 µm the width of the intervening tectum). The most typical (separate species) have structured muri, simplicolumellate muri and were formed by lirae. The lumina are almost circular in *Allium* species (Figs 25-48). Schulze (1980) and Pınar *et al.* (2009) have shown that muri and lumen shapes of the pollen are taxonomically important characters. Small convex sculpture elements (supratectal muri) on the surface are more or less irregularely arranged and short (rugulate to rugulate-perforate pattern, sometimes muri very shallow) with perforations of variable diameter in between, rarely long and parallel (transitions to striate condition) These were formed by striae of different length and orientation and detail of the exine showing irregular striate-perforate ectexine (perforation only on the outside of the tectum) are presented. The number of perforation in 1µm² is 4-14, the diameter of perforation on average is 0.08-0.35 µm and the thickness of lira on average is 0.16-0.33µm (Figs. 25-48). The diameter of perforation was observed to be the highest in *A. frigidum* (Fig. 36). Intine is 0.52-0.96µm thick. The *A. longisepalum* and *A. subhirsutum* had thickest intine while *A. schoenoprasum, A. kossoricum,* had thinnest one (Table 2).

According to SEM survey, exine ornamentation can be described best as the exine sculpture was striate-perforate, striate-rugulate-perforate and rugulate-perforate (Figs 25-56). However, in *A. longisepalum,* one of the lateral surfaces of the pollens is striate-rugulate-perforate and the other surface of the pollen grains was striate-perforate (Figs 10, 32-33); *A. akaka* (Figs 15-17, 38), *A. orientale* (Figs 42-43), *A. lycaonicum* (Figs 24, 46-47), one of the lateral surfaces of the pollens are striate-perforate and the other surface of the pollen grains was rugulate-perforate Therefore, these pollens grains are para-isopolar. Perforate-striate, perforate-rugulate-perforate and perforate-striate-rugulate exine structure have been reported in previous investigations (Pastor 1981; Diez *et al.*, 1987; El-Sadek *et al*., 1994; Güler and Pehlivan, 2006; Namin *et al*., 2009; Neshati *et al*., 2009; Özhatay and Koçyiğit, 2009; Özler and Pehlivan, 2010; Koçyiğit, 2014; Maassoumi *et al*., 2014; Wrońska-Pilarek *et al*., 2016; Hosseini, 2018 and Başer *et al*., 2019). A rugulate-perforate ornamentation was observed in *A. frigidum* (Fig. 36) and *A. decipiens* (Fig. 41), while striate-rugulate-perforate ornamentation was seen in *A. cepa* (Fig. 3), *A. cassium*, (Fig. 29) and *A. zebdananse* (Fig. 31), *A. longisepalum* (Fig. 32) *A. cupani* subsp. *hirtovaginatum* (Fig. 30), *A. callidictyon* (Fig. 34), *A. callimischon* subsp. *haemostictum* (Fig. 35), *A. akaka*, *A. orientale* (Fig. 42), *A. lycaonicum* (Fig. 47) Whereas in the other species striate-perforate ornamentation was observed.

The present study revealed that *Allium* taxa possess three types of ornamentational characteristics as follows:

(i) Striate-perforate: *A. scabriscapum, A. szovitsii, A. schoenoprasum, A. subhirsutum, A. kossoricum, A. chrysanterum, A. cardiostemon, A. colchicifolium, A. kharputense, A. noëanum, A. hirtifolium.*

(ii) Striate-regulateperforate: *A. cepa, A. cassium A. zebdananse, A. longisepalum, A. cupani* subsp. *hirtovaginatum, A. callidictyon, A. callimischon s*ubsp. *haemostictum, A. akaka, A. orientale, A. lycaonicum.*

(iii) Rugulate-perforate: *A. frigidum, A. decipiens.*

Table 1. List of *Allium* species investigated with vouchers..

Taxonomic groups	*Taxa*	Collection data
Subgenus *Rhizirideum* Section *Rhizirideum* G.Don ex W.D.J.Koch	*A. scabriscapum* Boiss. & Kotschy.	B9 Van, Van-Hakkari castle crossroad, after 3 km from Gürpınar district, Çavuştepe village (Urartu castle), limestone and dry solepes, ca. 1850 m, M. Koyuncu & M. Çoşkun AEF 12585.
	A. szovitsii Regel	A8 Rize, İkizdere district, Anzer plateau, stony slopes, ca. 2200-2300 m, 26.7.1993, M. Koyuncu, T. Ekim, A. Güner AEF 17869.
Subg. *Cepa* Sect. *Schoenoprasum* (Kunth) Dumort.	*A. schoenoprasum* L.	A8 Rize, İkizdere district, Ovit plateau, stony slopes, ca. 2800-3100 m, 21.9.1993, M. Koyuncu AEF 17968
Subg. *Cepa* Sect. *Cepa* (Mill.) Prokh.	*A. cepa* L.	B7 Elazığ, Elazığ-Malatya crossroad, from Elazığ to Malatya 22 km, in a cultivated field, 29.6.1974, M. Koyuncu & K. Karamanoğlu, AEF 02134
Subg. *Amerallium* Sect. *Molium* G.Don ex Koch	*A. subhirsutum* L	C1Muğla, around Marmaris-İçmeler district, ca. 20 m, 17.4.1992, M. Koyuncu AEF 17088.
	A. cassium Boiss.	C4 Konya, Kartalçık neighbourhood, stony slopes, ca. 1550 m, 10.6.1996, M. Koyuncu AEF 12124.
	A. zebdananse Boiss. & Noë	A8 Artvin, rocky region, in crevices of rocks, ca. 1000 m, M. Koyuncu AEF 19480.
	A. longisepalum Bertol.	C9 Siirt, Şirvan district, the highest pass on the road between Cevizli and Siirt district, cultivated in field, ca. 1000 m, M. Koyuncu & A. Güner AEF 12809
Sect. *Brevispatha* Valsecchi.	*A. cupani* Raf. subsp. *hirtovaginatum* (Kunth) Stearn	C4 Mersin, around Mut district dry slopes, shrubbery, ca. 100 m, M. Koyuncu & M. Çoşkun, AEF 2142.
	A. callidictyon C.A.Mey. ex Kunth	C5 İçel, Aslanköy Çoçak district, Yıldız Mountain, Gökbel plateau, ca. 2400 m, M. Koyuncu AEF 12818.
	A. callimischon subsp. *haemostictum* Stearn	C2 Muğla, between Bayır-Tırnaz village ridge, open Pinus burutia forest, limestone rocks, ca. 450 m, M. Koyuncu & A. Güner AEF 4587.

Table 1 contd.

Taxonomic groups	Taxa	Collection data
Subg. *Melanocrommyum* Sect. *Scorodon* W. D. J. Koch	*A. frigidum* Boiss. & Heldr.	C3 Isparta, Davras Mountain, cultivated in field and stony slopes, ca. 2000 m, M. Koyuncu AEF 5582.
	A. kossoricum Fomin	B9 Van, Başkale district, around Güzeldere crossing, steppe, ca. 2800 m, M. Koyuncu & M. Çoşkun AEF 12716.
Subg. *Melanocrommyum* Sect. *Acanthoprason* Wendelbo	*A. akaka* S. G. Gmelin ex Roem. & Schult. & Schult. f.	C9 Hakkari, Berçelen plateau, ca. 2400 m, 21.7.1994, M. Koyuncu AEF 02117.
Subg. *Melanocrommyum* Sect. *Melanocrommym*(Webb & Berthel.) Rouy	*A. chrysantherum* Boiss. & Reut.	B7 Malatya, between Quercus scrub and Juniperus, ca. 800-2150 m, M. Koyuncu, AEF 19243.
	A. cardiostemon Fisch. & C. A. Mey	C6 Adana, between Osmaniye-Fevzipaşa district ridge, Nur Mountain, forest openings Quercus shrub, ca. 1000 m, 5.6.1975, M. Koyuncu AEF 19244.
	A. colchicifolium Boiss.	B7 Elazığ, Yalnız Mountain, near river, steppe, ca. 1650 m, M. Koyuncu AEF 19244.
	A. decipiens Fisch. ex Schult. & Schult. f.	A8 Erzurum, Aşkale Pınar kapanı village, Kop Mountain, stony slopes, ca. 2100 m, 13.6.1998, M. Koyuncu AEF 10037
	A. orientale Boiss.	C1 Muğla, limestone hills and slopes, rocky places vineyards, ca. 600-1870 m, 10.6.1996, M. Koyuncu & T. Ekim AEF 19623.
	A. kharputense Freyn & Sint.	C7 Urfa, Siverek district, Otlu village, in a cultivated field, cultivated in field, ca. 1050 m, 6.5.1995, M. Koyuncu & T. Ekim AEF 19623.
	A. noëanum Reut. ex Regel	C7 Urfa, Siverek district, Karabahçe village, cultivated in field, ca. 1260 m, 3.5.1978, H. Mayer, AEF 19422.
	A. lycaonicum Siehe ex Hayek	C5 Adana, Osmaniye Zorkun plateau, in the meadows, ca. 1540 m, M. Koyuncu & M. Çoşkun AEF 02153
	A. hirtifolium Boiss.	C10 Hakkari, volcanic ash soil, dry slopes, ca. 1580-2600 m, 10.6.1996, M. Koyuncu AEF 12810.

The symbols AEF stand for herbarium sheets from the Faculty of Pharmacy of Ankara University.

Table 2. Characteristic features of the pollen of Turkish *Allium* species investigated.

Species	LA (µm) M SD	SA (µm) M SD	LA/SA	Slg (µm)	Slt (µm)	Aperture membrane	Exine thicknes (µm)	Exine Surface	İntine thickness (µm)	Operculum
Sect. *Rhizirideum*										
A. scabriscapum (N)	28.72±1.00	19.96+0.56	1.43	40.20±1.46	4.18±1.19	ps	1.71±0.11	St-pf	0.78±0.11	Absent
A. szovitsii (N)	41.31±2.17	27.13±2.18	1.12	54.60±2.57	3.17±1.63	rg-pf	1.44±0.13	St-pf	0.63±0.13	Present
Sect. *Schoenoprasum*										
A.schoenoprasum (N)	28.34±1.38	20.76±1.20	1.37	43.40±1.61	4.04±0.82	ps	1.30±0.11	St-pf	0.52±0.06	Present
Sect. *Cepa*										
A. cepa (N)	29.61±0.90	20.7±0.64	1.43	42.30±1.52	3.75±0.60	ps-pf	1.46±.0.11	St-rg-pf	0.75±0.14	Absent
Sect. *Molium*										
A. subhirsutum (N)	42.10±2.82	28.00±1.90	1.50	64.30±2.71	7.48±1.64	ps-pf	1.85±0.13	St-pf	0.86±0.12	Absent
A. cassium (N)	39.72±1.74	26.76±0.74	1.48	62.20±2.51	5.94±1.30	ps-pf	2.00±0.07	St-rg-pf	0.84±0.11	Absent
A. zebdananse (N)	37.67±1.00	25.65±0.60	1.47	54.20±1.61	6.24±0.79	rg-pf	1.49±0.1	St-rg-pf	0.72±0.08	Absent
A. longisepalum (N)	50.32±2.34	32.10±1.31	1.57	74.60±3.33	6.06±1.11	ps-pf	1.96±0.12	St-rg-pf	0.96±0.1	Absent
Sect. *Brevispatha*										
A. cupani subsp. *hirtovaginatum* (N)	29.02±1.12	20.62±1.05	1.40	40.30±1.46	3.10±1.92	rg-pf	1.61±0.12	St-rg-pf	0.68±0.12	Present
A. callidictyon (N)	28.94±1.16	21.86±1.41	1.32	40.20±1.70	3.32±1.48	ps-pf	1.36±0.23	St-rg-pf	0.79±0.11	Present
A. callimischon subsp. *haemostictum* (N)	34.10±0.98	23.92±1.15	1.43	46.70±1.41	3.96±0.80	rg-pf	1.71±0.15	St-rg-pf	0.80±0.14	Present
Sect. *Scorodon*										
A. frigidum (N)	32.46±1.76	22.66±2.27	1.43	47.10±2.33	2.99±1.02	ps-pf	1.20±0.13	rg-pf	0.64±0.12	Absent
A. kossoricum (N)	28.52±2.10	18.52±1.46	1.54	39.40±2.27	1.95±1.01	ps	1.07±0.13	St-pf	0.52±0.07	Present
Sect. *Acanthoprason*										
A. akaka (N)	34.60±1.88	20.48±1.28	1.69	50.60±2.87	3.86±1.48	rg-pf	1.42±0.21	St-rg-pf	0.77±0.12	Present
Sect.*Melanocrommym*										
A. chrysanterum (N)	27.28±1.04	16.26±1.05	1.68	45.20±1.25	3.68±1.17	rg	1.20±0.18	St-pf	0.72±0.12	Present
A. cardiostemon (N)	25.98±0.96	21.11±1.76	1.51	34.60±1.59	2.24±0.85	rg-pf	1.24±0.13	St-pf	0.73±0.09	Present
A. colchicifolium (N)	27.30±1.56	17.16±1.16	1.59	38.10±1.92	3.92±1.11	ps-pf	1.19±0.11	St-pf	0.68±0.12	Absent
A. decipiens (N)	30.70±1.19	19.96±1.47	1.54	42.80±1.62	2.82±1.45	ps	1.35±0.22	rg-pf	0.72±0.1	Present
A. orientale (N)	33.40±1.48	21.54±0.83	1.55	45.40±1.98	3.92±1.11	ps	1.75±0.12	St-rg-pf	0.74±0.09	Present
A. kharputense (N)	31.72±1.71	20.24±1.60	1.57	41.70±1.46	3.43±0.75	ps	1.36±0.18	St-pf	0.75±0.09	Present
A. noëanum (N)	30.36±1.39	18.66±1.50	1.63	40.40±1.25	1.29±1.64	rg	1.18±0.22	St-pf	0.70±0.1	Present
A. lycaonicum (N)	30.98±1.27	20.00±1.47	1.55	40.90±1.62	4.22±0.78	rg-pf	1.53±0.16	St-rg-pf	0.73±0.13	Present
A. hirtifolium (N)	29.26±1.18	19.86±0.82	1.48	40.60±1.50	3.92±1.00	rg-pf	1.52±0.11	St-pf	0.74±0.13	Present

Nomenclature: N-Non acetolysed pollen grains (LM); LA-Long axis; SA-Short axis; SD-Standard deviation; M-Mean value; Slg-Length of the sulcus; Slt-Width of the sulcus; St-pf; Sitriate-perforate; St-rg; Striate-rugulate; St-rg-pf; Sitriate-regulate-perforate; rg; rugulate; rg-pf; rugulate-perforate; ps; Psilate; ps-pf; psilate-perforate.

In SEM photomicrographs, sulcus membranes are psilate in *A. scabriscapum*, *A. schoenoprasum*, *A. kossoricum* (Fig. 14), *A. decipiens* (Fig. 21), *A. orientale* and *A. kharputense* (Fig. 55); and psilate - perforate sulcus membrane ornamentation was seen in *A. cepa* (Fig. 3), *A. subhirsutum* (Fig. 50), *A. cassium*, *A. longisepalum* (Fig. 52), *A. callidictyon*, *A. frigidum* (Fig. 13) and *A. colchicifolium* (Figs. 20, 54); Rugulate sulcus membrane ornamentation was found in *A. chrysanterum* and *A. noëanum* (Fig. 23), and rugulate-perforate sulcus membrane ornamentations are seen in *A. szovitsii* (Fig. 49), *A. zebdananse* (Fig. 51), *A. cupani* subsp. *hirtovaginatum* (Fig. 9), *A. callimischon* subsp. *haemostictum*, *A. decipiens* (Fig. 21), *A. akaka* (Figs 15, 16), *A. cardiostemon* (Fig. 53), *A. lycaonicum* (Fig. 56) and *A. hirtifolium* similarly, Güler and Pehlivan (2006); Özler and Pehlivan (2010); Maassoumi *et al*. (2014) and Başer *et al*. (2019) reported that sulcus membrane ornamentations were psilate, striate-reticulate, rugulate and regulate-perforate in *Allium* taxa. Several researchers have emphasized that the sulcus membrane sculpturing may be a taxonomic value in some families (Kosenko, 1999; Güler and Pehlivan, 2006; Özler and Pehlivan, 2010).

The genus studied includes the sections; *Rhizirideum*, *Schoenoprasum*, *Cepa*, *Molium*, *Brevispatha*, *Scorodon*, *Acanthoprason* and *Melanocrommyum*. We aimed to elucidate the infra-familial positions of the *Allium*. On the basis of the pollen structure of *Allium* within Alliaceae it found to be homogenous genus from the pollen point of view and a heterogenous family from the morphological point of view. Pollen morphology of 23 taxa of *Allium* was investigated under LM (Figs 67-98), SEM (Figs 1-56) and TEM (Figs. 57-66). The common characteristics of pollen grains were monad, monosulcate (extended sulcate types), and monosulcate-operculate, ellipsoidal, heteropolar with bilateral symmetry. However, pollen of *A. longisepalum, A. akaka, A. orientale* and *A. lycaonicum* are paraisopolar.

This study shows that pollen characters have been significant value in classification of *Allium*. The main palynological differences have been found at the section level, especially in the sulcus membrane and the presence of an operculum. A distal fragmented operculum and extended sulcate type are typical for *Allium* of Alliaceae. Sulcus long, getting the ends of the pollen grain or spreading to the proximal side.

In the present study, the biggest pollen size was found in *A. longisepalum*, whereas the smallest was found in *A. cardiostemon*. There is a decrease in pollen size in the sequence of the subsections *Rhizirideum*, *Schoenoprasum*, *Cepa*, *Molium*, *Brevispatha*, *Scorodon*, *Acanthoprason*, *Melanocrommyum* .The sulcus extends from distal to proximal and the sulcus ends are braod and rounded only in *A. akaka* (Figs 15, 16) while they were sharp in other species such as *A. subhirsutum* (Figs. 8, 74), *A. zebdanense* (Fig. 6), *A. longisepalum* (Fig. 10), *A. cupani* subsp. *hirtovaginatum* (Fig. 80), *A. callidictyon* (Figs. 11, 83), *A. callimischon* (Fig. 87), *A. chrysantherum* (Fig. 18) and *A. orientale* and it's blund in other ones. The longest length dimension of sulcus was seen in in section *Molium* are *A. subhirsutum*, *A. cassium*, *A. zebdanense* and *A. longisepalum*. Figs 5, 6, 8, 10). The widest sulcus dimension was seen in *A. subhirsutum* (Figs 8, 74).

It was recognized that the sulcus extends from distal to proximal end in all the taxa investigated (Table 2). The operculum was found to be fragmented on the sulcus membrane (Figs 69, 81, 82, 86, 87, 89, 90, 91, 94, 95, 96, 97) or sometimes completely covering it. Three are in our previous unreported from this genus, the operculum was determined only in *A. pallens* subsp. *pallens*, *A. bassitense* and *A. hirtovaginum* under the Section *Codonoprasum* (Güler and Pehlivan 2006). In the other studies of this genus, the operculum was determined only in *A. albidum* subsp. *caucasicum* (Section *Rhizirideum*), *A. rupicola* (Section *Codonoprasum*), *A. asperiflorum* under the Section *Allium* (Özler and Pehlivan, 2010), 12 taxa belonging to Section *Codonoprasum*

(Koçyiğit, 2014) and *A. arlgirdense* under the Section *Scorodon* (Başer *et al*., 2019). According to Kosenko (1992), a non-operculate exine is a plesiomorphic peculiarity. Several researchers have emphasized that the sulcus features and the presence of operculum may be a taxonomic value in some families (Chanda *et al*., 1979; Halbritter and Hesse, 1993; Güler and Pehlivan 2006; Özler and Pehlivan, 2010). The advantage of a monosulcate aperture (extended sulcate) in monocotyledons with the inclusion of *Allium*, is underlined by Harley and Zavada (2000); Güler and Pehlivan (2006); Namin *et al*., 2009; Neshati *et al.* (2009); Özhatay and Kocyigit (2009); Özler and Pehlivan (2007, 2010); Maassoumi *et al*. (2014) and Başer *et al*. (2019).

Palynological data related to exine ornamentation indicate the heterogeneous characters of this genus. In this study we have determined that there are intraspecific variations among studied species as well which are based on exine sculpturing, and sulcus ornamentation. *Allium* species divided into 4 pollen types according to sulcus membrane sculpturing; among *Allium* species a psilate, psilate-perforate, rugulate and rugulate-perforate sulcus membrane is distinctive. In the present investigated taxa such as *A. scabriscapum* (Fig. 57), *A. schoenoprasum*, (Fig. 58) and *A. cepa* (Fig. 59). The exine is semitectate and the tectum perforate while the tectum is with intervals and is formed by simple columella. Exine thickness 1-2 µm. Ectexine is thicker than endexine and that endexine exhibits a very thin continuous structure. Tectum is thicker than foot layer with intervals (Figs 57-66).

The results show that there were several pollen characters of taxonomic significance in the genus *Allium*. There are also differences in the size of the pollen, exine sculpturing, ornamentation of sulcus membrane and lumen shape, size and murus size, exine thickness and number of perforation, diameter of perforation and thickness of lira. We recognized 3 main types, distinct by pollen sculpturing, lumina shape and sulcus membrane ornamentation. The main palynological differences have been registered at the section level. These results are similar to the earlier studies (Güler and Pehlivan, 2006; Özler and Pehlivan, 2010; Neshati *et al*., 2009; Özhatay and Kocyigit, 2009; Başer *et al*., 2019; Table 2; Figs 25-48).

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