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POLLEN MORPHOLOGY OF SOME ALLIUM SPECIES (AMARYLLIDACEAE) FROM IRAN

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Pollen morphology of 10 *Allium* L. taxa growing in Iran, representing three subgenera and six sections, were investigated via scanning electron microscopy. Pollen morphological characteristics indicated that pollen grains are monosulcate, heteropolar, and ellipsoidal. Polar axis revealed variation between 13.84 and 20.01 µm while Equatorial axis ranged from 20.4 to 42.37 µm. The forms of pollen grains were oblate, peroblate, and suboblate (mean of P/E ratio was 0.43 to 0.80). The aperture type and exine ornamentation were not homogeneous among the studied species. It was also observed that the sulcus extended from distal to proximal in two species of sect. *Allium* and one species of sect. *Melanocrommyum*. The exine was semitectate and the tectum was perforate. The variation in the main pollen characteristics in the investigated taxa allowed for recognizing two types of pollen grains in subg. *Allium*, *Melanocrommyum* and *Amerallium*: The first type had perforate rugulate trimming and high density of pollen perforations while the second type had perforate-striate-rugulate ornamentation and a low number of exine surface perforations.

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Key words: *Allium*; Amaryllidaceae; Iran; Pollen grains; Pollen morphology

ریخت‌شناسی دانه گرده در تعدادی از گونه‌های سرده پیاز (تیره نرگسیان) در ایران

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ریخت‌شناسی دانه گرده در ده تاکسون بومی در ایران که نماینده سه زیرجنس و شش بخش هستند، با میکروسکوپ الکترونی نگاره مورد مطالعه قرار گرفت. خصوصیات ریختی دانه گرده نشان داد که گرده‌ها تک شیاری، ناجور قطب و بیضوی هستند. محور قطبی بین ۱۳/۸۴ و ۲۰/۰۱ میکرومتر در حالیکه محور استوایی بین ۲۰/۴ و ۴۲/۳۷ میکرومتر بود. شکل دانه‌های گرده oblate، peroblate و suboblat بود (میانگین نسبت محور قطبی به محور استوایی، ۰/۴۳ تا ۰/۸۰ بود). نوع دریچه و تزیینات سطح اگزین در میان گونه‌های مورد مطالعه یکنواخت نبود. همچنین دریچه گسترش یافته از سطح دیستال به پروکسیمال در دو گونه از بخش Allium و یک گونه از بخش Melanocrommyum مشاهده شد. اگزین دارای یک تکتوم منفذدار و ناپیوسته بود. براساس تنوع موجود در صفات اصلی دانه گرده گونه‌های مطالعه شده، دو نوع دانه گرده در زیر جنس‌های Allium، Melanocrommyum و Amerallium مشخص شد. نوع اول دارای تزیینات شبکه‌ای و چین‌های ظریف و کوچک همراه با منافذ بسیار زیاد است در حالیکه نوع دوم دارای تزیینات مخطط و ظریف همراه با منافذ کمتر در سطح اگزین است.

INTRODUCTION

The genus *Allium* L. comprises more than 900 species (Fritsch & Abbasi 2013) which are naturally distributed in the northern hemisphere. It is a variable group widely spread across the holarctic region ranging from the dry subtropics to the boreal zone. These species prefer relatively dry sites in arid and moderately humid climates and regions that are seasonally dry

(Fritsch & Friesen 2002). New records for Iran and a certain number of *Allium* species and subspecies (Fritsch & Abbasi 2013; Fritsch & Amini Rad 2013; Akhavan & al. 2014) raised the number to about 148 species and subspecies, belonging to eight subgenera and 32 sections (Dolatyari & al. 2018). *Allium* species show a great morphological diversity, and therefore many taxonomical problems remain unsolved (Gurushidze & al. 2007).

Palynological data were successfully applied to the point at a large systematic distance between *Allium* and members of true Liliaceae (Oliveira Maia 1941; Schulze 1980), where they are only available for relatively few species. Nevertheless, it can be tested whether only these characteristics could provide strategies for solving taxonomic problems (Neshati & al. 2009). Former palynological studies on *Allium* (Oliveira Maia 1941; Nair & Sharma 1965; Radulescu 1973; Kuprianova & Aliev 1979; Schulze 1980; Pastor 1981; El-Sadek & al. 1994; Kosenko & Kudryashova 1995; Tolgor 1995; Güler & Pehlivan 2006) mainly explored species from other geographic areas. Meanwhile, pollen information of some species from Iran (Namin & al. 2009; Neshati & al. 2009) 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.

Palynological studies on *Allium* in Iran investigated by Neshati & al. (2009) suggested several morphological pollen characteristics, which may possess taxonomical value. Namin & al. (2009) recognized the sulcus 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.

This study examines the pollen morphological studies of 10 taxa of the genus *Allium*, which is the most difficult monocotyledon family from systematic and taxonomic point of view. The main aim of this study is to evaluate the significance of pollen characteristics for the systematics of the genus.

MATERIALS AND METHODS

Pollen grains from 10 *Allium* taxa belonging to six sections were taken from herbarium specimens deposited in the Herbarium of University of Kurdistan (UOK) and Herbarium of Kurdistan Research Center of Agriculture and Natural Resources (HOK), Sanandaj (IRAN). Taxonomic affiliation, origin of the plants, and voucher data are provided in table 1.

Table 1. Voucher specimen information of examined *Allium* species. The subgenera and sections follow Friesen & al. (2006), Fritsch and Abbasi (2013).

Taxonomic groups and species	Collection data
Subgen. *Allium*	
Sect. *Allium*	
A. macrochaetum Boiss. and Hausskn	Kurdistan, Baneh to Sardasht, Kanibard village, 1250 m, 7747-HOK
A. fuscoviolaceum Fomin	Kurdistan, Marivan, Zaryvar Lake, 1500 m, 3248-HOK
Subgen. *Melanocrommyum*	
Sect. *Melanocrommyum*	
A. fedtschenkoi Nábělek	Kurdistan, 38 km from southern east of Sanandaj, Naran village, 1500-2400 m, 811-HOK
A. mozaffarianii Maroofi. and R.M Fritsch	30 km from Marivan to Paveh (Tangeh Dezli), 1400 m, 923-HOK
Sect. *Acanthoprason*	
A. haemanthoides Boiss. and Reut	Kurdistan, Marivan, Almaneh village, 1400 m, 8464-HOK
Sect. *Pseudoprason*	
A. hooshidaryae Mashayekhi, Zarre and R.M. Fritsch (E1)	Kurdistan, Divandarreh, Doozakhdarreh village, 1500 m, 1234-UOK
A. hooshidaryae Mashayekhi, Zarre and R.M. Fritsch (E2)	East Azerbaijan, Bookan,2200 m, 1235-UOK
A. koelzii (Wendelbo) Perss. And Wendelbo	Kurdistan, Marivan, Almaneh village, 1400 m, 8013-HOK
Sect. *Procerallium*	
A. remediorum (R.M Fritsch) R.M Fritsch	Kurdistan, Sanandaj, Shian Village, 2000 m, 1236-UOK
Subgen. *Amerallium*	
Sect. *Molium*	
A. longisepalum Bertol.	Kurdistan, Kamyaran, Divaznav Village, slopes of shahoo mountain, 1000 m, 13415-HOK

Flowers were prepared for scanning electron microscopy (SEM) using the methods described in Harley (2004). The pollen grains were acetolysed according to the method of Erdtman (1960) with some minor modifications. All pollen samples were cooked for three to five minutes at 100° C during acetolysis. For SEM, after acetolysis pollen grains were soaked in absolute ethanol, and were then pipetted directly onto 12.5 mm diameter stubs, they were air-dried at room temperature and then, coated in a sputter coater with approximately 25 nm of Gold–Palladium. The specimens were examined and photographed with a TESCAN MIRA 3 scanning electron microscope. Pollen assessment was performed on 20-30 pollen grains, which included the overall shape, size of polar and equatorial axis, type of sculpturing, and more detailed information on the sculptures. Principally, the terminology of Erdtman (1960) and Punt & al. (2007) was applied.

RESULTS AND DISCUSSION

Pollen morphology of 10 taxa of *Allium* was investigated under SEM (fig. 1). Palynogical structures of the examined *Allium* specimens are summarized in tables 2-4. A general description can be given as follows:

According to SEM investigations, the pollen grains are monad, monosulcate, and heteropolar with bilateral symmetry. The pollen shapes (based on P/E ratio; Polar axis/Equatorial axis 0.43-0.80) were oblate, suboblate, and peroblate (fig. 1, table 1). The pollen sizes given for this genus were 55.2 × 19.3 µm (Radulescu 1973), 25.75 µm × 17.48 µm - 51.19 µm × 32.30 µm (Özhatay 1977), 25.50 µm × 18.44 µm - 44.52 × 26.80 µm (Güler & Pehlivan, 2006) and 27.5 µm × 17 µm (Neshati & al. 2009). In this study, the pollen was medium in size with a long axis (20.4–42.37 µm) and short axis (13.84–20.01 µm). The largest pollen size was found in *A. longisepalum* (42.37 × 20.01 µm), while the smallest size belonged to *A. mozaffarianii* (20.4 × 14.12 µm) (fig. 2). The P/E ratio varied between 0.43 (*A. hooshidaryae* (E2)) and 0.80 (*A. remediorum*). Also, the sulcus extended from distal to proximal in species of sect. *Allium* (*A. macrochaetum* and *A. fuscoviolaceum*) and sect. *Melanocrommyum* (*A. fedtschenkoi*). However, the former studies (conducted by El-Sadek & al. 1994; Pastor 1981; Guler & Pehlivan 2006) confirmed existence of this character only in examined taxa of subgen. *Allium*.

Sulcus ends were blunt and rounded in all species. In general, the sulcus became narrow in the equator and widened in the poles, especially in species with non-extended sulcus. The longest sulcus extension was measured in *A. longisepalum* (33.82 µm), while the shortest dimension was observed in *A. mozaffarianii* (18 µm), and the widest sulcus dimension was seen in *A. remediorum* (8.74 µm) (table 3). Sulcus membrane as a normal characteristic of pollen grains might be useful in taxonomy of *Allium*. However, it may be usually destroyed through acetolysis. Güler & Pehlivan (2006) reported sulci with an operculum covering the sulcus membrane in some species of sect. *Codonoprasum*. However, we did not find this structure among the species investigated.

The acetolyzed pollen walls, the exine, is composed of a membrane which contains perforations. The lumina are almost circular in *Allium* species. In this study the shape of lumina was mostly circular or irregular. There are also differences in the number of perforation, diameter of perforation, and thickness of lirae. In this study, the number of perforation in *Melanocrommyum* subgenus was very low compared with *Allium* and *Amerallium* subgenera. The diameter of exine perforation was 0.03 µm in *A. koelzii* as the smallest, while 0.1 µm in *A. longisepalum* as the widest. The thickness of continuous series of transition between exine perforations (lirae) was measured as well, which varied between 0.12 µm in *A. koelzii* (sect. *Pseudoprason*) and 0.2 µm in *A. fedtschenkoi* (sect. *Melanocrommyum*) (table 4).

Exine sculpturing and pollen types

Two different ornamentation types of exine sculpturing (perforate-rugulate and perforate-striate-rugulate, table 2) and as a consequence, two pollen types can be distinguished in the investigated *Allium* species:

Type I: Perforate rugulate ornamentation was seen in *A. fuscoviolaceum* and *A. macrochaetum* from subgen. *Allium* and sect. *Allium*. This type of sculpturing has been previously reported for sect. *Allium* (Nair and Sharma 1965; Pastor 1981; El-Sadek & al. 1994; Gu¨ler & Pehlivan 2006; Namin & al. 2009; Neshati & al. 2009). Based on investigated species in former studies (Nair and Sharma 1965; Kuprianova & Aliev, 1979; Pastor 1981; Namin & al. 2009; Neshati & al. 2009) extended sulcus is very characteristic for the whole section of *Allium*. In the present study, this characteristic along with sculpturing type and high number of perforation on the exine surface can be used to distinguish species of sect. *Allium*. The pollen characters of the two species studied here do not differ significantly from one another, but with few difference from the data presented by Kuprianova & Aliev (1979), Neshati & al. (2009) and Namin & al. (2009) (Sulcus length and perforation diameter were between 30-35 µm and 0.12- 0.47 µm respectively). However, it was 20 to 24 µm and 0.08 to 0.12 µm here. *Allium fuscoviolaceum* and *A. macrochaetum* were studied here for the first time.

Table 2. Characteristic features of the investigated pollen in species of *Allium* (values in μm). SD: Standard Deviation

Taxa	equatorial axis (EA)			Polar axis (PA)			PA/EA ratio	Shape	Exin ornamentation
	min	max	mean ± SD	min	max	mean± SD			
A. mozaffarianii	19.2	21.6	20.4± 1.16	12.98	15.26	14.12± 1.40	0.69	Oblate	perforate-striate-rugulate
A. remediorum	23.5	26.13	24.81± 1.108	19.36	20.59	19.97± 0.49	0.80	Suboblate	perforate-striate-rugulate
A. fuscoviolaceum	24.31	25.12	24.71± 1.04	12.40	16.80	14.6± 1.62	0.59	Oblate	perforate-rugulate
A. fedtschenkoi	25.9	30.53	28.21± 2.004	15.70	20.52	18.11± 1.97	0.64	Oblate	perforate-striate-rugulate
A. haemanthoides	28.20	30.45	29.32± 1.37	14.61	18.72	16.66± 1.56	0.56	Oblate	perforate-striate-rugulate
A. longisepalum	38.44	46.30	42.37± 3.18	18.97	21.06	20.01± 1.12	0.47	Peroblate	perforate-rugulate
A. hooshidaryae (E1)	28.41	31.94	30.17± 1.56	13.69	13.99	13.84± 0.40	0.45	Peroblate	perforate-striate-rugulate
A. hooshidaryae (E2)	30.88	32.99	31.93± 1.24	12.23	15.85	14.04± 1.45	0.43	Peroblate	perforate-striate-rugulate
A. macrochaetum	26.12	28.53	27.32± 0.96	14.3	15.76	15.03± 0.69	0.55	Oblate	perforate-rugulate
A. koelzii	25.01	28.95	26.98± 1.51	13.54	14.90	14.22± 0.76	0.52	Oblate	perforate-striate-rugulate

Table 3. Aperture morphological characters of *Allium* taxa on SEM (values in μm). SD: Standard Deviation

Taxa	Sulcus length			Sulcus width		
	Min	Max	mean± SD	min	max	mean± SD
A. mozaffarianii	17.45	18	17.72± 0.40	2.72	3.69	3.2± 0.40
A. remediorum	21.88	26.13	24.09± 1.82	5.59	11.90	8.74± 2.32
A. fuscoviolaceum	20.19	21.29	20.74± 0.54	2.32	6.22	4.27± 1.66
A. fedtschenkoi	22.13	25.09	23.61± 1.36	3.34	5.09	4.21± 0.76
A. haemanthoides	24.51	28.51	26.51± 1.66	13.69	16.69	15.19± 1.22
A. longisepalum	33.21	34.44	33.82± 0.47	3.67	5.82	4.74± 0.97
A. hooshidaryae (E1)	19.21	20.27	19.74± 0.51	5.22	5.86	5.54± 0.36
A. hooshidaryae (E2)	26.37	22.9	24.63± 1.69	5.20	8.55	6.87± 1.36
A. macrochaetum	23.57	25.19	24.38± 0.71	3.76	4.71	4.23± 0.37
A. koelzii	23.12	28.95	26.03± 2.13	5.17	8.52	6.84± 1.31

Table 4. Microsculpturing features of perforate pollen ornamentation (values in μm). SD: Standard Deviation

Taxa	The number of perforations in 1 μm^2	Diameter of perforations			Lirae thickness		
		min	max	mean± SD	min	max	mean± SD
A. mozaffarianii	7	0.03	0.05	0.04± 0.01	0.13	0.21	0.17± 0.03
A. remediorum	14	0.04	0.05	0.045± 0.01	0.16	0.18	0.17± 0.01
A. fuscoviolaceum	27	0.04	0.13	0.08± 0.03	0.11	0.19	0.15± 0.04
A. fedtschenkoi	7	0.03	0.09	0.06± 0.02	0.14	0.27	0.20± 0.05
A. haemanthoides	14	0.04	0.06	0.05± 0.01	0.09	0.19	0.14± 0.04
A. longisepalum	16	0.07	0.14	0.1± 0.03	0.15	0.20	0.17± 0.02
A. hooshidaryae (E1)	6	0.04	0.08	0.06± 0.01	0.13	0.20	0.16± 0.02
A. hooshidaryae (E2)	6	0.04	0.08	0.06± 0.01	0.12	0.22	0.17± 0.03
A. macrochaetum	40	0.05	0.12	0.08± 0.02	0.18	0.23	0.20± 0.02
A. koelzii	4	0.01	0.06	0.03± 0.02	0.10	0.14	0.12± 0.01

The perforate regulate ornamentation was observed in *A. longisepalum* as well, the only representative species of subgen. *Amerallium* in sect. *Molium*. This is a first report for sect. *Molium*. This species had the largest pollen grains among the species studied. In addition, *A. longisepalum* possessed the longest sulcus and the biggest lumina among perforate exine type. Based on pollen and sulcus dimensions, it can be another pollen type, but, since only one representative species was studied for this subgenus, further investigation with more species of subgen. *Amerallium* is necessary. It is worth mentioning that according to Namin & al. (2009), the only species of sect. *Briseis* of this subgenus, *A. paradoxum*, did not show any diagnostic palynomorphological feature.

Type II: Perforate-striate-rugulate pollen sculpture type was assigned to subgen. *Melanocrommyum*. A set of variable morphological characteristics was identified in subgen. *Melanocrommyum*, with most of them enjoying a great potential to solve taxonomic issues. Nevertheless, the anthers of subgen. *Melanocrommyum* are far less variable in their shape, dimensions, and color, but they may show usable taxonomic characteristics in some cases according to electron microscopy (Fritsch & Abbasi 2013). This may allow to detect many variable features. However, the knowledge of these microscopic characteristics, especially the extent of intraspecific variation is still incomplete. The present study involved six species of subgen. *Melanocrommyum*. *Allium mozaffarianii*, *A. fedtschenkoi*, *A. hooshidaryae*, *A. remediorum*, *A. koelzii* and *A. haemanthoides*. This is a first report on pollen morphological characteristics of those species. The most important characteristics of the pollen grains in all species of subgen. *Melanocrommyum* include the low number of exine surface perforations and a tendency towards striate sculpturing and small dimension of lumina. This was well in line with Neshati & al. (2009) results. Conversely, based on the results of Namin & al. (2009), the most important characteristic of the pollen grains in *A. cristophii*, a member of the sect. *Kaloprason* and *Melanocrommyum* subgenus, is the high density of perforations on the exine surface at both the distal and proximal faces. Both Species of sect. *Pseudoprason* (*A. hooshidaryae* and *A. koelzii*) had the least number of exine perforation while *A. haemanthoides*, a member of *Acanthoprason* sect. had the most number of perforation. The only species of sect. *Procerallium* (*A. remediorum*) had the longest axis, so the pollen shape was suboblate. There was no other report for this section to compare. Therefore, the pollen characteristics of this section needs to be studied further. The pollen characters of the two species of section *Melanocrommyum* (*A. mozaffarianii* and *A. fedtschenkoi*) had almost same features. Pollen morphological characters (pollen shape, exine sculpture and diameter of perforation) of *A. elburzense* and *A. kuhsorkhense* from sect. *Acanthoprason* which has been previously reported (Neshati & al. 2009) was totally same with *A. haemanthoides*. This information will provides evidence for the possible close relationship between those species and perhaps confirmed their current classification as proposed by Fritsch & Abbasi (2013). Based on our data, extended sulcus type from distal to proximal end was observed in sect. *Allium*. However, this characteristic was also found in *A. fedtschenkoi* in sect. *Melanocrommyum* as a new report. Therefore, it cannot be considered as a synapomorphic characteristic only for sect. *Allium*, as suggested by Neshati & al. (2009). As a conclusion, the present study on some Iranian species of *Allium* indicated that the most informative characteristics were found within exine pattern of pollen grain and pollen sulcus type. These characteristics may possess taxonomical value. However, more materials should be investigated to consider the real taxonomic value of these characteristic combinations.

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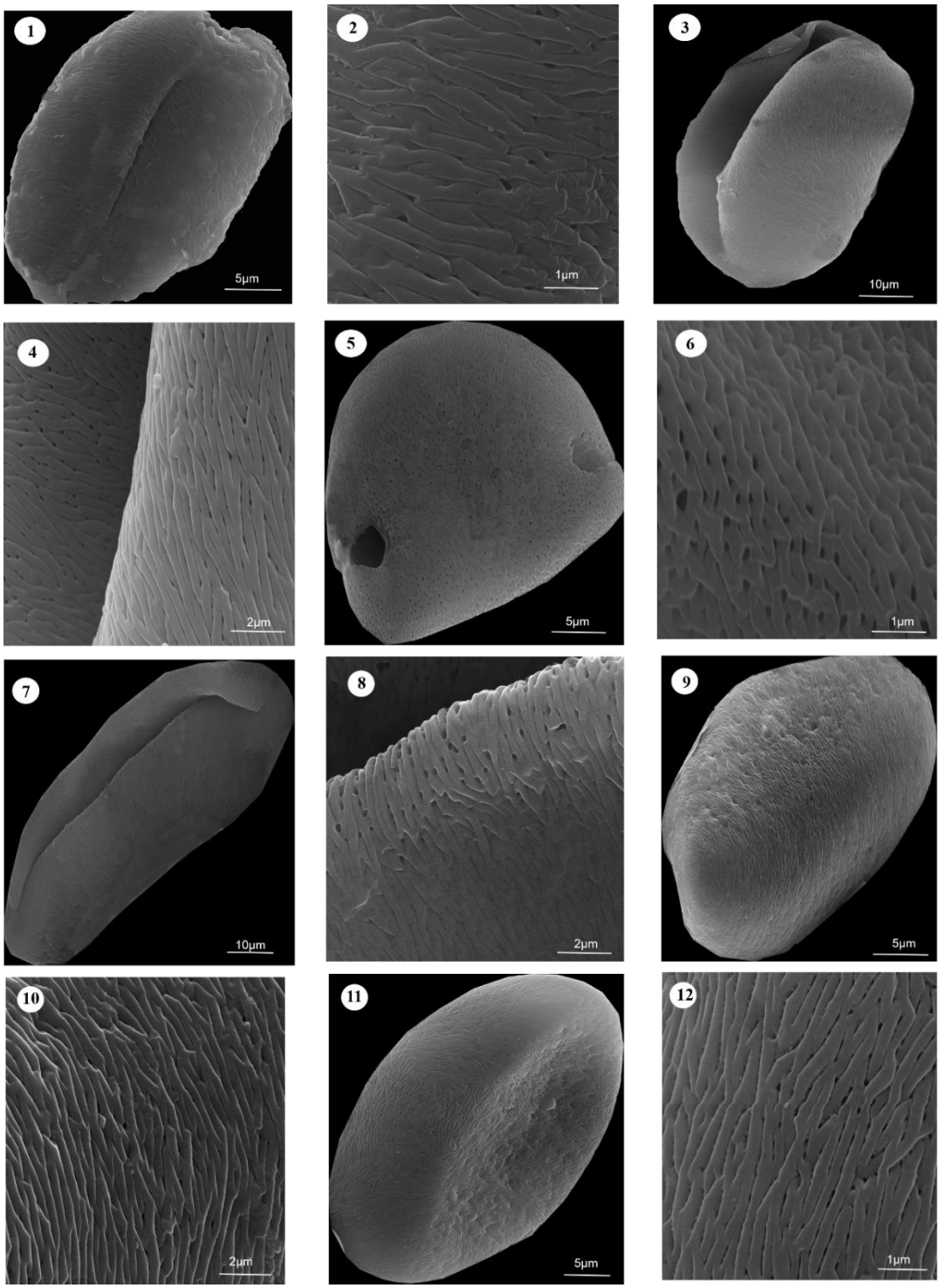

Fig. 1. SEM micrographs of pollen grains in *Allium* species. 1 – 2, *A. mozaffarianii* (distal view and exine surface); 3 – 4, *A. fedtschenkoi* (distal view and exine surface); 5 – 6, *A. Fuscoviolaceum* (proximal view and exine surface); 7 – 8, *A. hooshidaryae*. (distal view and exine surface); 9 -10, *A. hooshidaryae* (distal view and exine surface); 11- 12, *A. remediorum* (distal view and exine surface).

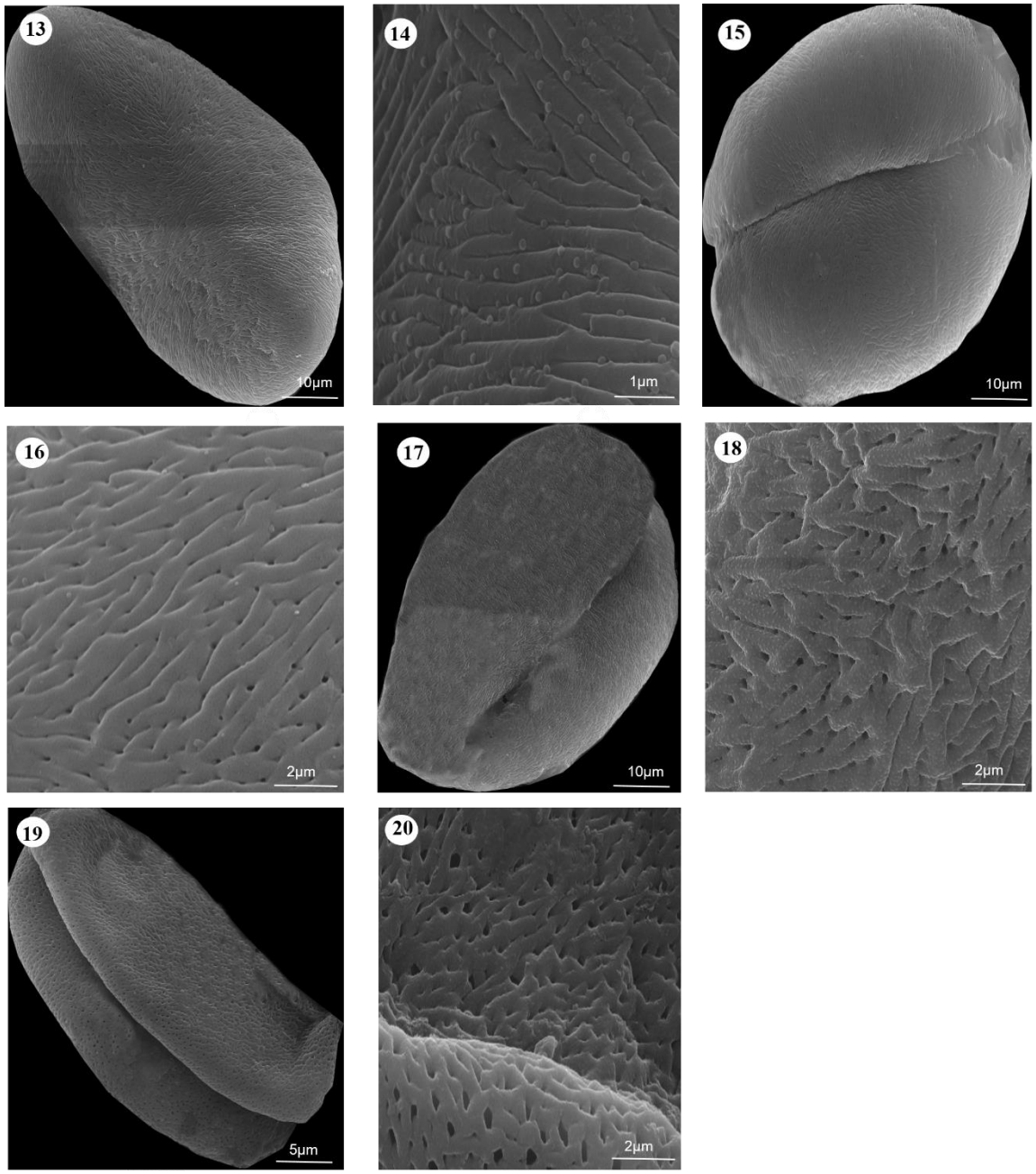

Fig. 1. Continued. 13 – 14, *A. koelzii* (distal view and exine surface); 15 – 16, *A. haemanthoides* (distal view and exine surface); 17 – 18, *A. longisepalum* (distal view and exine surface); 19- 20, *A. macrochaetum* (distal view and exine surface).

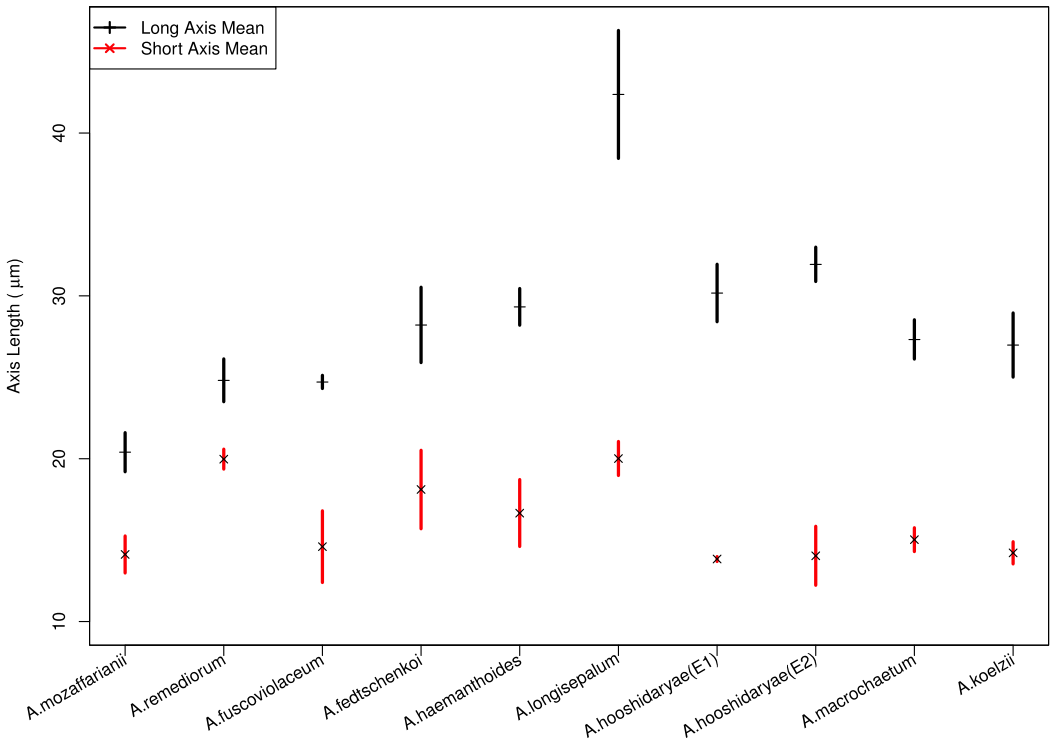

Fig. 2. Measurements of pollen grains in *Allium* species based on SEM.