Taxonomical investigation on some species of genus *Allium* based on the pollen grain micromorphology

ABSTRACT

Aims: The main aim of this research was to investigate the micromorphological characteristics of the pollen grains in seven species from genus *Allium* belong to three subgenera including *Melanocrommyum*, *Polyprason* and *Cepa* (seven sections).

Methodology: The pollen grains were examined by using Light Microscopy (LM), Scanning Electron Microscopy (SEM) and the pollen grains of one species under TEM (Transmission Electron Microscopy).

Results: The pollen grains were oblate and medium in shape and size. The pollen ornamentation of exine surface, exine ornamentation on sulcus edge, number of exine surface lumina and the state of pollen grain apex in the examined species were different. Semitectate and columellate ectexine with discontinuous endexine were seen in the pollen wall structure (sporoderm).

Conclusion: The dendrogram obtained from the pollen characters in SEM observations by using the numerical taxonomy system (NTSYS) software confirmed phylogram of the studied species obtained from recent phylogenetic research. Our palynological dendrogram can be used for segregation the sections and subgenera taxonomical levels in the studied species of genus *Allium*.

Keywords: Allium, Amaryllidaceae, Monocotyledons, Palynology, Phylogeny, Taxonomy

1. INTRODUCTION

The genus *Allium* is one of the largest Monocotyledons with a wide dispersion in central and south-east Asia, where the species of this genus constitute a great part of herbaceous societies [1, 2]. Approximately, fifty species of this genus are planted extensively or in local level that economically have great importance. Some of the wild species of this genus are

used as edible, medicinal and even decorative plants. Moreover, the wild species of this genus have valuable potential for supplying of human consuming [3].

The taxonomical position of *Allium* as the polymorph genus is sophisticated [4, 5]. Lately, the phylogentical and biogeographical examination on this genus has been done been done on the endemic species on china [1] and confirmed monophyly in *Allium* by using the phylogenetic analysis of molecular data (ITS nuclear marker and *rps16* chloroplast marker); but, the obtained phylogram from the phylogenetic analysis wasn't confirmed for some subgenera. In their research, three main monophyletic groups were specified: the first clade includes subgenera *Nectaroscordum*, *Amerallium* and *Microscordum*; the second clade includes subgenera *Caloscordum*, *Anguinum*, *Vvedenskya*, *Porphyroprason* and *Melanocrommyum* and the third clade includes subgenera *Butomissa*, *Cyathophora*, *Rhizirideum*, *Allium*, *Reticulatobulbosa* and *Polyprason* [1].

The palynological study of genus *Allium* is sophisticated but driven characteristics from some researches are able to classify taxonomical level on this genus. Majority of these studies were investigated the following characters: exine ornamentation to specify position of the tribe *Allieae* [6, 7], being single sulcus and having extensive sulcus as a predominant morphological pollen character in the genus *Allium* is determined [8]. [9] examined the species belong to sections *Codonoprasum* and *Allium* and specified the homogeneity of sulcus and pollen grain ornamentation in the genus *Allium*. Moreover, they indicated that some morphological characters of the pollen grain such as sulcus and presence or lack of operculum that have taxonomic importance in the section level. [2] examined the pollen grain morphology in some species of *Allium* (six sections including *Molium*, *Scorodon*, *Brevispatha*, *Codonoprasum*, *Allium* and *Melanocrommyum*) and characterized the shape of pollen grains was prolate and subprolate. Also, in all species extensive sulcus and the smallest and largest pollen grains are belonged to *A. guttatum* (section *Allium*) and *A.*

roseum (section *Molium*), respectively. Pollen grains from 30 *Allium* taxa belonging to 15 sections were recognized in Iran [10]. In this research, the pollens were heteropolar, peroblate to suboblate shape, rugulate to microrugulate, perforate to striate in subgenus *Melanocrommyum* and striate exine ornamentation [10]. According to result of [11], the morphological character of sulcus in investigated species in Iran was observed in genus *Allium* and section *Allium* that this character wasn't observed about the other sections. [12] observed an extensive sulcus from the beginning to the end of the pollen grains in all taxa in sections of *Rhizirideum*, *Codonoprasum* and *Allium*. In ultrastructure wall of the pollen grains, the exine semitectate and simplicolumellate were observed in all investigated sections. In addition, the exine ornamentation in these sections and related species were heterogeneous and weren't synchronize in classification of these sections and three exine ornamentation including striate-perforate, striate-rugulate-perforate and rugulate-perforate were observed in these species. They also stated that availability of operculum as an apomorphic character and narrow endexine layer as a taxonomic character in the genus *Allium* determined in the sections of *Rhizirideum*, *Codonoprasum* and *Allium* [12]. [15] separated *A. ursinum* in subspecies level based on the exine ornamentation and stated that this character is an appropriate taxonomic character (granulate-rugulate, rugulate-striate ornamentation in the subspecies *Ucrainicum* and perforate-rugulate ornamentation in the subspecies *Ursinum*). [14] examined three types of pollen grains in seven subgenera and thirteen sections of *Allium* in Pakistan that weren't in agreement with the mentioned classifications and determined the types of *A. fedtschenkoanum* (reticulate ornamentation), *A. grifthianum* (rugulate-foveolate ornamentation) and *A. roylei* (subpsilate ornamentation). [15] demonstrated characteristics of the pollen grains including qualitative and quantitative characters of some European species of genus *Allium* including three subgenera (*Allium*,

Amerallium and *Rhizirideum*) and five sections that explicitly being synchronize with the sections classification.

The main aim of our research was to evaluate the pollen grain micromorphological characters and its comparison with the taxonomy and phylogeny of genus *Allium* including seven species belong to seven different sections. The pollen grain characters were reported in some species for the first time. In addition, compared with the previous researches, more pollen grain micromorphological characters were evaluated.

2. MATERIAL AND METHODS

The pollen grains were provided from the collected samples of Main Botanical Garden of Russian Academy of Sciences (MHA), Moscow, Russia. The speciements were collected since May and June 2003 and identified by E. Kalikov(Table 1). Also, taxonomic relationships among the studied species in this research showed in Table 1 [1]. For LM observations, the pollen grains were acetolyzed based on Erdtman method [16]. Then, mounted on glycerine jelly glass slides. Thirty pollen grains were measured by Leitz Light Microscopy (HM-LUX3) from each studied species and images were taken by Dino camera (AM-423). For SEM observations, non-acetolyzed pollen grains were put on metal legs and transferred to the EMITECH sputter coater for coating by gold-paladium (K450X). Finally, the obtained micrographs were taken by VEGA-TESCAN Scanning Electron Microscopy. Terminology for LM and SEM observations were explored accrording to the following references [17, 18, 19]. For construction the dendrogram, the obtained characters by SEM observations from the pollen grains were coded (Table 4). Finally, the NTSYS Software [20] was used along based on Single Linkage method and UN1 similarity coefficient (binary coefficient) (Table 5). For TEM preparation, the pollen grains were fixed by 2% osmium tetroxide and stained by uranylacetate solution [21]. Then, dehydrated in ethanol series and

were put in acetone. Finally, embedded in Epon mixture (Epon 812, Epon Harter DDSA, Epon Harter MNA) according to the standard method of [22]. Ultrathin sections of the pollen grains were obtained by an ultramicrotome (LKB 8800), then stained with lead citrate (LKB 8800, Ultratome III) [21]. The micrographs were made by using a JEOL-JEM-100B Transmission Electron Microscopy.

3. RESULTS AND DISCUSSION

Based on the LM observations, the pollen grains in the studied species were observed oblate in shape and medium in size (Table 2 and Fig. 1). In SEM micrographs, the pollen grain characters were different in these species (Table 3, Figs. 2 and 3). The exine ornamentation on surface and the exine ornamentation on sulcus edge were different and these characters for each species are expressed as follows: striate exine ornamentation on surface and sulcus edge for *A. altissimum*, perforate-striate exine ornamentation on surface and perforate on sulcus edge for *A. fetisowii*, striate-perforate exine ornamentation on surface and striate on sulcus edge for *A. backhousianum*, exine ornamentation on surface and sulcus edge of macrostriate for *A. karataviense*, striate-psilate exine ornamentation on surface and psilate-striate exine ornamentation on sulcus edge for *A. obliquum*, perforate-striate exine ornamentation on surface and psilate exine ornamentation on sulcus edge for *A. rosenbachianum*, perforate-microstriate exine ornamentation on surface and sulcus edge for *A. schoenoprasum* (Table 3). The pollen grains of *A. fetisowii* and *A. schoenoprasum* species were acute in the end and in the other species were obtuse (Table 3). The size of lumina and muri in the studied species also was different. The scope of changes in lumina's size in surface of the pollen grain almost was observed similar in *A. altissimum, A. backhousianum* and *A. karataviense* (0.06- 0.13 µm) (Table 3). The number of exine surface lumina, was the least (4 lumina at 2 µm ^{2}) for *A. altissimum* and the most

(30 and 31 lumina at 2 μm ^{2}) for *A. fetisowii* and *A. backhousianum* species, respectively (Table 3).

The results of *A. schoenoprasum* TEM micrographs belongs to section *Schoenoprasum* and subgenus *Cepa* revealed that the ectexine is semitectate, infratectum is simplicolumellate, foot-layer is discontinuous and endexine layer is very thin and discontinuous (Fig. 4).

This research indicated that there were less differences about the LM observations (shape and size); but, the dendrogram of pollen micromorphological characters from SEM observations provided useful valuable taxonomical characters on the subgenera and sections classifications (Table 4 & 5, Fig. 5).

Various shapes of pollen grains were observed in this genus on previous investigations and the dominant shape of the pollen grains in this genus is oblate shape. Medium pollen type was observed in the most species of this genus during this research and previous research [2, 9, 10, 11, 13, 14, 15].

Exine ornamentation was varied in genus *Allium* [2, 9, 10, 11, 13, 14, 15]. Our results show that varied exine ornamentation with the other characters on SEM observation as useful taxonomic characters in section level.

Our research on the genus *Allium* confirms previous palynological research on pollen wall structure on *Codonoprasum*, *Allium* and *Rhizirideum* sections [9, 12].

The perforate-striate exine surface ornamentation for two species including *A. fetisowii* and *A. rosenbachianum* belong to the subgenus *Melanocrommyum* was alike; but, these two species were different in the exine ornamentation on sulcus edge, the number of exine surface lumina, the state of pollen grain apex and the size of lumina and muri. The

variations of exine ornamentation on the surface and sulcus edge for the other species were in agreement with the performed sections classification. Moreover, the number of exine surface lumina for each species was also different and the least and the most number of lumina was counted in the subgenus *Melanocrommyum*. Our results also indicated that *A. obliquum* belong to section *Oreiprason* and subgenus *Polyprason* had no lumina in the exine surface. In the studied species in Iran, the exine surface ornamentation without lumina was observed in the subgenus *Reticulatobulbosa* in section *Campanulata* and also in the subgenus *Polyprason* in section *Falcatifolia* [11]. Therefore, the exine surface ornamentation without lumina can be defined as a useful micromorphological character in the subgenus *Polyprason* and its related sections. In flora of Pakistan *A. rosenbachianum* on section *Megaloprason*, *A. schoenoprasum* belongs to section *Schoenoprasum* and *A. roylei* in section *Oreiprason* were placed on *A. roylei* types based on the pollen grain morphology [14].

The dendrogram of the palynological characters analysis was in agreement with the taxonomical ranking and previous phylogram by [1] on these taxa in subgenus and section levels. The phylogram of the studied species were distinguished based on [1] research. [1] had done the extensive phylogenetic analysis by molecular data (ITS nuclear marker and *rps*16 chloroplast marker) on *Allium* genus. The final dendrogram of our palynological investigation was in agreement with the obtained phylogram by [1] research. According to the pollen grain dendrogram obtained from analysis by SEM observations in subgenus *Melanocrommyum*, *A. fetisowii* introduced as a sister group with the other species in this subgenus. In clade *Melanocrommyum*, *A. fetisowii* in section *Longibidentata* determined as the sister group of the remaining species of the subgenus *Melanocrommyum* based on the phylogenetic information of molecular markers [1]. *A. schoenoprasum* belongs to subgenus *Cepa* and section *Schoenoprasum* was different from the other species in SEM

observation, although in the state of pollen grain apex was similar to *A. fetisowii.* The results also indicated that *A. altissimum, A. backhousianum* and *A. karataviense* species with similar size in lumina were placed in the subgenus *Melanocrommyum*. The palynological dendrogram in this study put the species *A. altissimum* and *A. backhousianum* from subgenus *Melanocrommyum* and in sections *Procerallium* and *Acmepetala* together. Moreover, according to the molecular studies, these two sections were put together [1].

4. CONCLUSION

The dendrogram of palynological analysis data in the related species was in parallel with the divisions of sections and subgenera taxonomical rank and the phylogram of phylogenetic studies. Also, our research on the genus *Allium* confirms some previous palynological research.

COMPETING INTERESTS

The authors of this manuscript declare that they have no competing interests.

REFERENCES

1. Li QQ, Zhou SD, He XJ, Yu Y, Zhang YC, Wei XQ. Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. Ann Bot. 1990; 106:709-33.
2. Özhatay N, Kocyigit M. Pollen morphology of *Allium* species (Lilliaceae) in European Turkey and around Istanbul. Phytol. 2009;15(2):199-208.
3. Fritsch RM, Abbasi M. Taxonomic Review of *Allium* subg. *Melanocrommyum* in Iran, Germany, Leibniz-Institut fur Pflanzengenetik und Kulturpflanzenforschung Gatersleben, 2013.

4. Klaas M. Applications and impact of molecular markers on evolutionary and diversity studies in the genus *Allium*. Plant Breed. 1998;117:297-308.
5. Fritsch RM, Friesen N. Evolution, domestication and taxonomy. In: Rabinowitch, H. D. & Currah L. (eds.): *Allium* crop science: recent advances,Wallingford, New York, 5–30, 2002.
6. Kosenjo VN, Kudryashova GL. Palynomorphology of the family Alliaceae, Bot. Zhurnal (st. Petersburg), 1995; 80(6):5-17.
7. Radulescu D. Recherches morpho-palynologiques sur la famille Liliaceae, Acta Bot. Horti. Bucur. 1973; 133-248**.**
8. Harley MM, Zavada MS. Pollen of the monocotyledons: Selecting characters for cladistic analysis. *In*: Monocots: Systematics and Evolution, K.L, Melbourne, Wilson and D.A Morrison (Eds). CSIRO. 2000; 194-213.
9. Güler U, Pehlivan S. Pollen morphology of some species belonging to *Codonoprasum* and *Allium* sections of *Allium* (Liliaceae-Alliaceae) genus, Biologia. 2006; 61(4):449-55.
10. Neshati F, Fritsch RM, Zarre S. Pollen morphology of some *Allium* L. species (Alliaceae) from Iran. Plant Diversity and Evolution. 2009; 127 (4): 433–451.
11. Namin HH, Mehrvarz SS, Zarre S, Fritsch RM. Pollen morphology of selected species of *Allium* (Alliaceae) distributed in Iran, Nordic J. Bot.2009;27(1):54-60.
12. Özler H, Pehlivan S. Pollen morphology of *Allium* L. (Lilliaceae) taxa in Turkey, Bangladesh J. Bot. 2010; 39(1):37-46.
13. Bareemizadeh F, Maassoumi SM, Ghasempour H, Karimi N. Pollen morphology of the genus *Allium* in comparison with genus *Calochortus* of order Liliales, Int. J. Biosci. 2014; 4(12):237-43.
14. Perveen A, Qaiser M. Pollen flora of Pakistan–lxviii. Alliaceae, Pak. J. Bot. 2015; 47(1): 263-68.

15. Wronska-Pilarek D, Halbrither H, Krazyminska A, Rzyminska L, Bednorz J. Bocianowskij J. Pollen morphology of selected European species of the genus *Allium* L. (Alliaceae), Acta Sci. Pol. Hortorum Cultus. 2016; 15(4):65-84.

16. Eerdtman G. The acetolysis technique: a revised description, Svensk Botanisk Tidskrift, 1960; 54:561-64.

17. Tokarev P. Morphology and ultrastructure of pollen grain. Moscow, KMK Sci, Press Ltd., 2003.

18. Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A. Glossary of pollen and spore terminology, Rev. Palaeobot. Palynol. 2007; 143:1-81.

19. Hesse M. Pollen Terminology (An illustrated handbook). Vienna, Springer, 2009.

20. Rohlf FJ. NTSYS-PC: Numerical Taxonomy and Multivariate Analysis System. Exeter Software, Setauket, NY, USA, 1993.

21. Reynalds ES. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of cell biology. 1963; 17: 208–212.

22. Weakley BS. A Beginner's Handbook in biological Electron Microscopy. Churchill Livingstone, Edinburg, 252 pp, 1972.

Table 1. Taxonomic relationships and collecting data from the studied species [1].

Taxon	Section	Subgenus	Collector and date of collecting
Allium altissimum Regel.	*Proceallium*	*Melanocrommyum*	E. Kalikov, 30.5.2003, gathering from MHA
Allium fetisowii Regel.	*Longibidentata*	*Melanocrommyum*	E. Kalikov, 30.5.2003, gathering from MHA
Allium backhousianum Regel.	*Acmepetala*	*Melanocrommyum*	E. Kalikov, 10.6.2003, gathering from MHA
Allium karataviense Regel.	*Miniprason*	*Melanocrommyum*	E. Kalikov, 30.5.2003, gathering from MHA
Allium obliquum L.	*Oreiprason*	*Polyprason*	E. Kalikov, 30.5.2003, gathering from MHA
Allium rosenbachianum Regel.	*Megaloprason*	*Melanocrommyum*	E. Kalikov, 30.5.2003, gathering from MHA
Allium schoenoprasum L.	*Schoenoprasum*	*Cepa*	E. Kalikov, 30.5.2003, gathering from MHA

Table 2. The obtained pollen data from the Light Microscopy studies of the studied species.

Taxon	Pollen size (µm)	Polar axis (µm) Min–Mean±SD–Max	Equatorial axis (µm) Min–Mean±SD–Max	P.E	Pollen shape	Pollen type
A. altissimum	35.18±6.52	17.00-18.28±1.24-21.00	27.50-35.18±6.52-46.00	0.5	Oblate	Medium
A. fetisowii	30.74±5.13	15.00-16.16±1.51-20.00	22.50-30.74±5.13-37.50	0.5	Oblate	Medium
A. backhousianum	29.97±2.34	12.00-16.22±1.79-17.50	27.50-29.97±2.34-35.00	0.5	Oblate	Medium
A. karataviense	31.39±4.92	12.00-16.84±2.03-20.00	22.50-31.39±4.92-41.00	0.5	Oblate	Medium
A. obliquum	31.56±6.09	12.50-16.16±1.60-20.00	25.00-31.56±6.09-41.00	0.5	Oblate	Medium
A. rosenbachianum	33.70±1.92	14.00-19.60±3.63-25.00	30.00-33.70±1.92-37.50	0.6	Oblate	Medium
A. schoenoprasum var. *sibiricum*	30.82±5.08	15.00-16.46±2.24-20.00	25.00-30.82±5.08-39.00	0.5	Oblate	Medium

Table 3. The obtained pollen data from the Scanning Electron Microscopy from the studied species.

Taxon	Exorn	Sd	S	L	M	P
A. altissimum	Striate	Striate	Obtuse	0.06-0.13	0.13-3.46	4
A. fetisowii	Perforate-striate	Perforate	Acute	0.06-0.20	0.13-0.86	30
A. backhousianum	Striate-perforate	Striate	Obtuse	0.06-0.13	0.13-0.40	31
A. karataviense	Macrostriate	Macrostriate	Obtuse	0.06-0.13	0.06-0.40	12
A. obliquum	Striate-psilate	Psilate-striate	Obtuse	-	-	-
A. rosenbachianum	Perforate-striate	Psilate	Obtuse	0.06-0.26	0.06-1.20	27
A. schoenoprasum var. *sibiricum*	Perforate-microstriate	Perforate-microstriate	Acute	0.06-0.40	0.06-1.60	22

Exorn: Exine ornamentation, Sd: Sulcus edge ornamentation, S: The state of pollen grain apex, L: Lumina size, M: Muri size, P: The number of exine surface lumina.

Table 4. The pollen traits from Scanning Electron Microscopy and coding of these characters by the analysis with NTSYS software.

Taxon	Exorn (code)	Sd (code)	S (code)	L (code)	M (code)	P (code)
A. altissimum	Striate (1)	Striate (1)	Obtuse (1)	0.06 (1)-0.13 (1)	0.13 (1)-3.46 (5)	4 (1)
A. fetisowii	Perforate-striate (2)	Perforate (2)	Acute (2)	0.06 (1)-0.20 (2)	0.13 (1)-0.86 (2)	30 (5)
A. backhousianum	Striate-perforate (3)	Striate (1)	Obtuse (1)	0.06 (1)-0.13 (1)	0.13 (1)-0.40 (1)	31 (6)
A. karataviense	Macrostriate (4)	Macrostriate (3)	Obtuse (1)	0.06 (1)-0.13 (1)	0.06 (2)-0.40 (1)	12 (2)
A. obliquum	Striate-psilate (5)	Psilate-striate (4)	Obtuse (1)	(0)	0	0
A. rosenbachianum	Perforate-striate (2)	Psilate (5)	Obtuse (1)	0.06 (1)-0.26 (3)	0.06 (2)-1.20 (3)	27 (4)
A. schoenoprasum var. *sibiricum*	Perforate-microstriate (6)	Perforate-microstriate (6)	Acute (2)	0.06 (1)-0.40 (4)	0.06 (2)-1.60 (4)	22 (3)

Table 5. The similarity coefficients of the studied species compared with the others by using the NTSYS software.

Taxon	a	b	c	d	e	f	g
a	1.00						
b	0.40	1.000					
c	0.76	0.40	1.00				
d	0.54	0.222	0.66	1.00			
e	0.22	0.00	0.22	0.22	1.00		
f	0.40	0.400	0.40	0.54	0.22	1.00	
g	0.22	0.40	0.22	0.40	0.00	0.40	1.00

a.*A. altissimum*, b. *A. fetisowii,* c. *A. backhousianum*, d. *A. karataviense*, e. *A. obliquum*, f. *A. rosenbachianum*, g. *A. schoenoprasum* var*. sibiricum*.

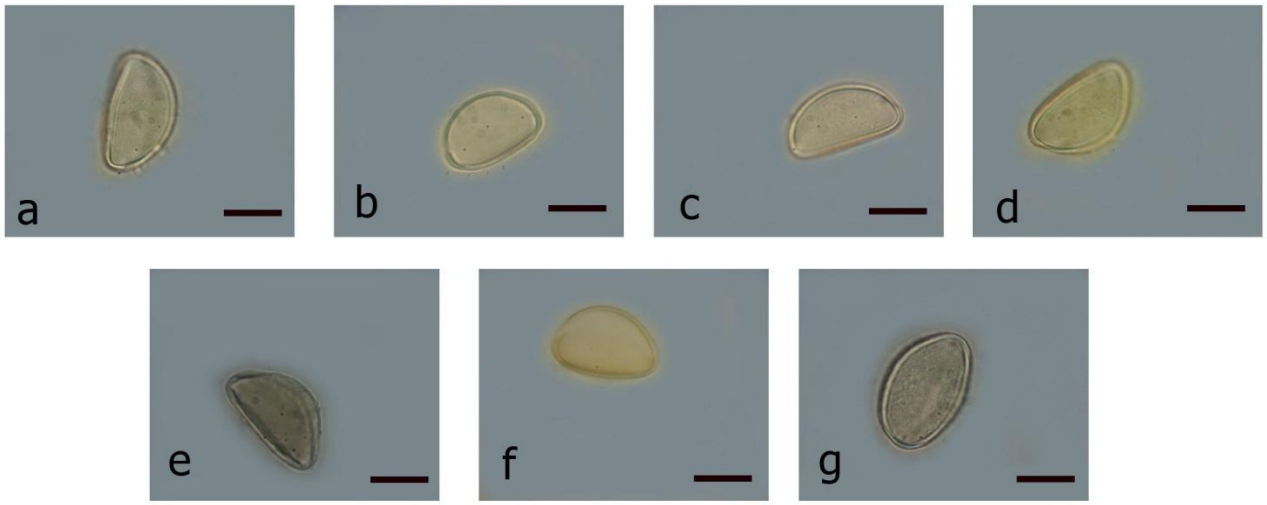

Fig. 1. Light microscopy photographs of the studied species. a. *A. altissimum*, b. *A. fetisowii,* c. *A. backhousianum*, d. *A. karataviense*, e. *A. obliquum*, f. *A. rosenbachianum*, g. *A. schoenoprasum* var. *sibiricum*.(Light Microscopy, 1000X, scale: 10 µm)

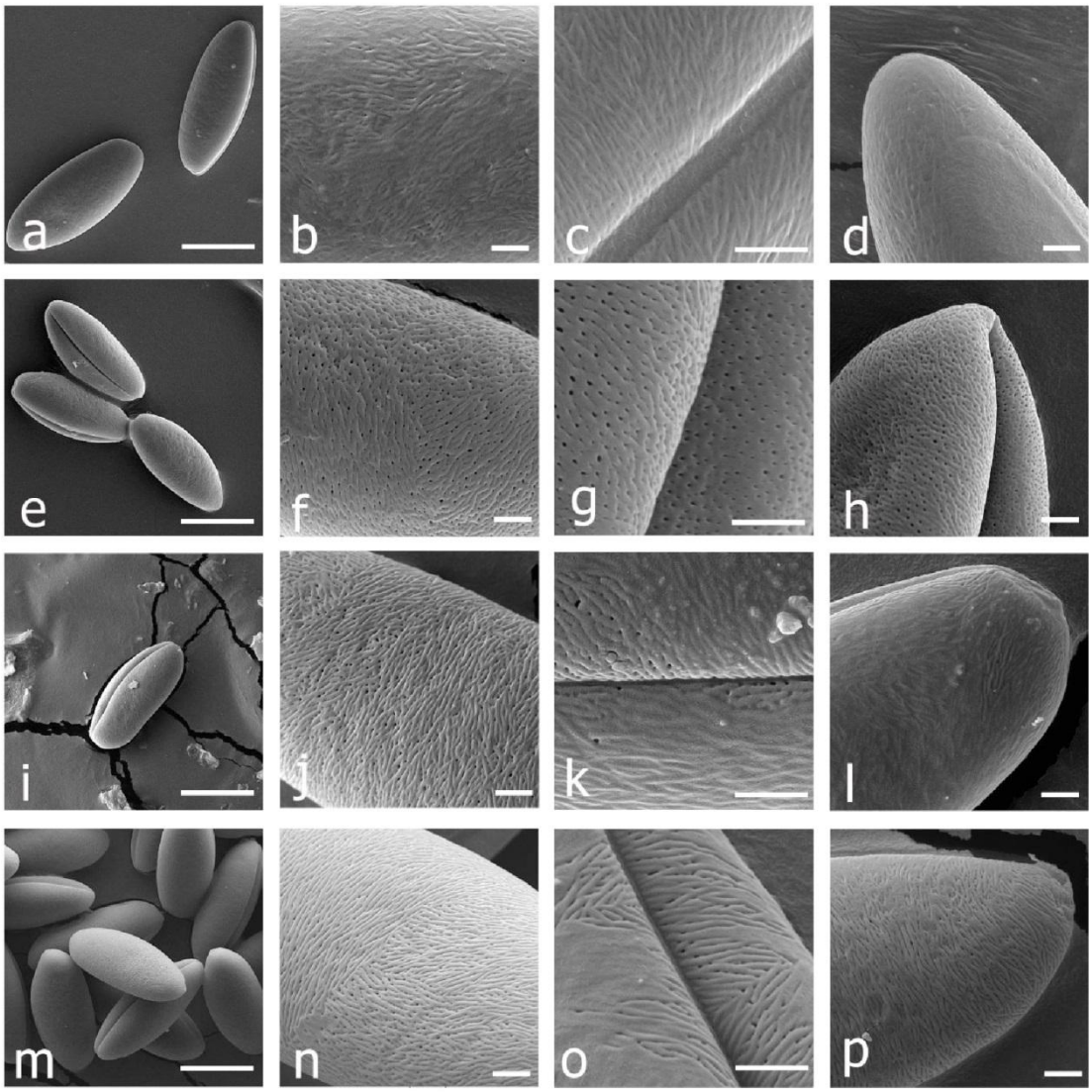

Fig. 2. The Scanning Electron Microscopy micrographs of the studied species: (for each species, the equatorial view of pollen grain, exine ornamentation, sulcus edge exine ornamentation and the state of pollen grain apex have been determined, respectively). *A. altissimum* (a–d), *A. fetisowii* (e–h), *A. backhousianum* (i–l) and *A. karataviense* (m–p). (Scale bar: 20 µm for a, e, i & m., scale bar: 2 µm for b, c, d, f, g, h, j, k, l, n, o & p.)

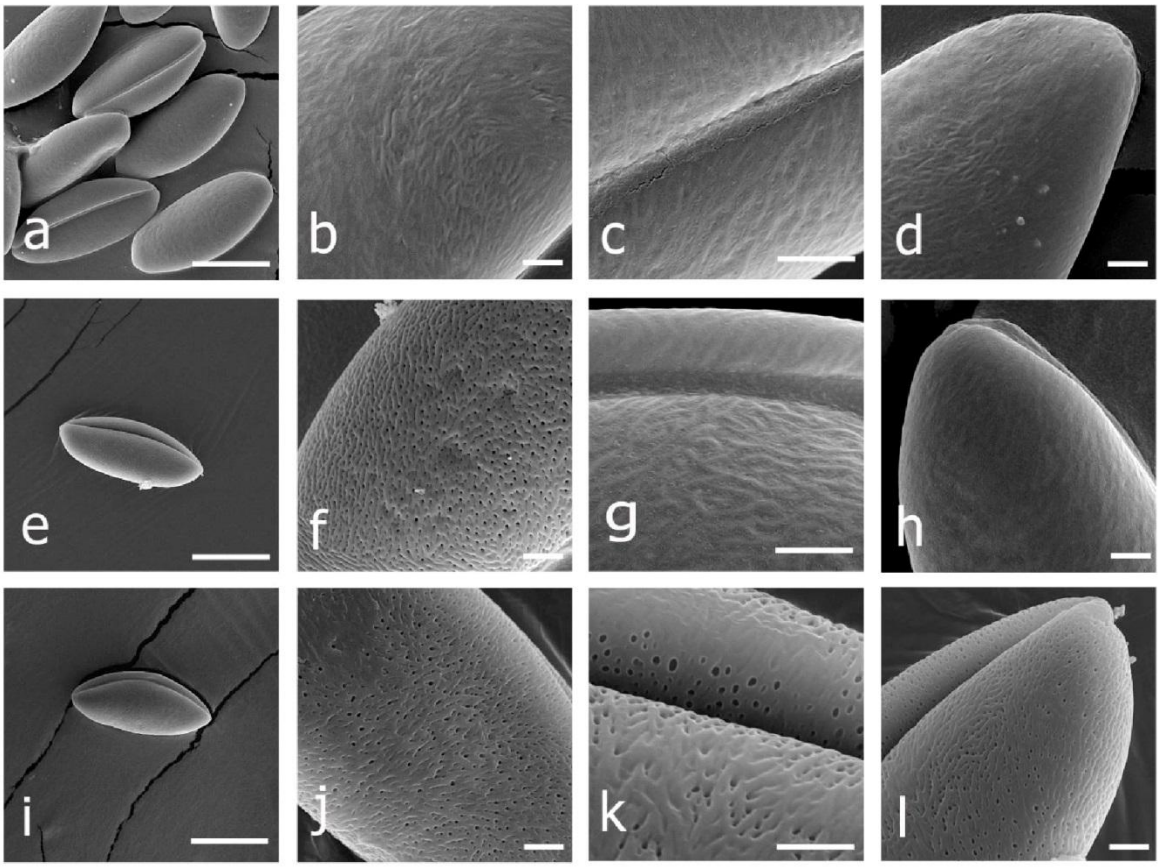

Fig. 3. The Scanning Electron Microscopy micrographs of the studied species: (for each species, the equatorial view of pollen grain, exine ornamentation, sulcus edge exine ornamentation and the state of pollen grain apex have been determined, respectively). *A. obliquum* (a–d), *A. rosenbachianum* (e–h) and *A. schoenoprasum* var. *sibiricum* (i–l). (Scale bar: 20 µm for a, e & i., scale bar: 2 µm for b, c, d, f, g, h, j, k & l)

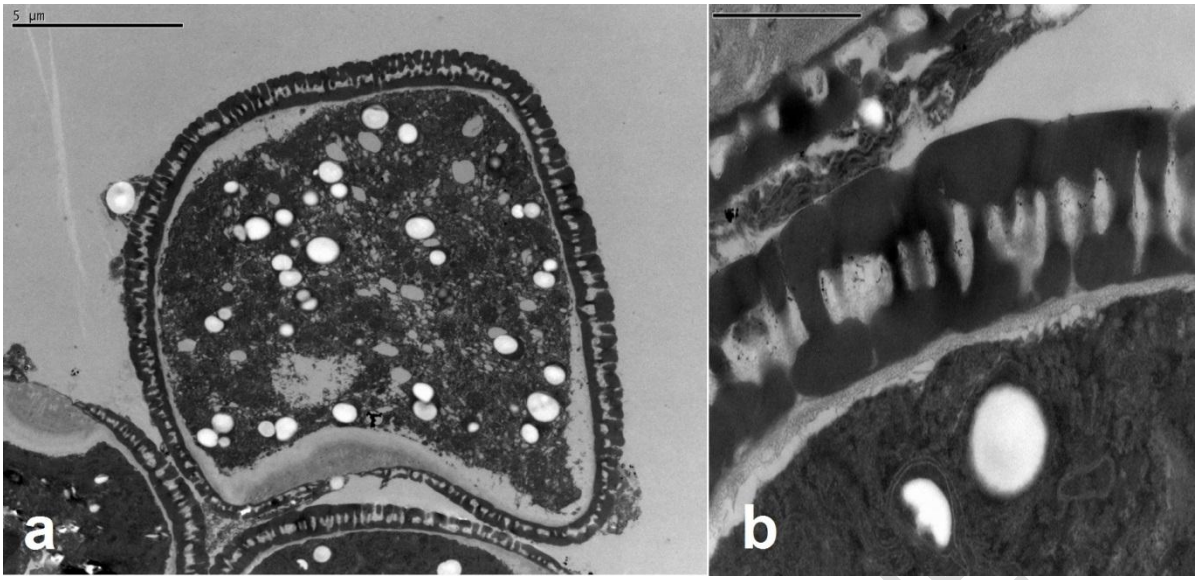

Fig. 4. The Transmission Electron Microscopy micrographs of *A. schoenoprasum* var. *sibiricum*: a. Cross section of pollen grain (Scale bar: 5 µm), b: Cross section of pollen wall (Scale bar: 1 µm).

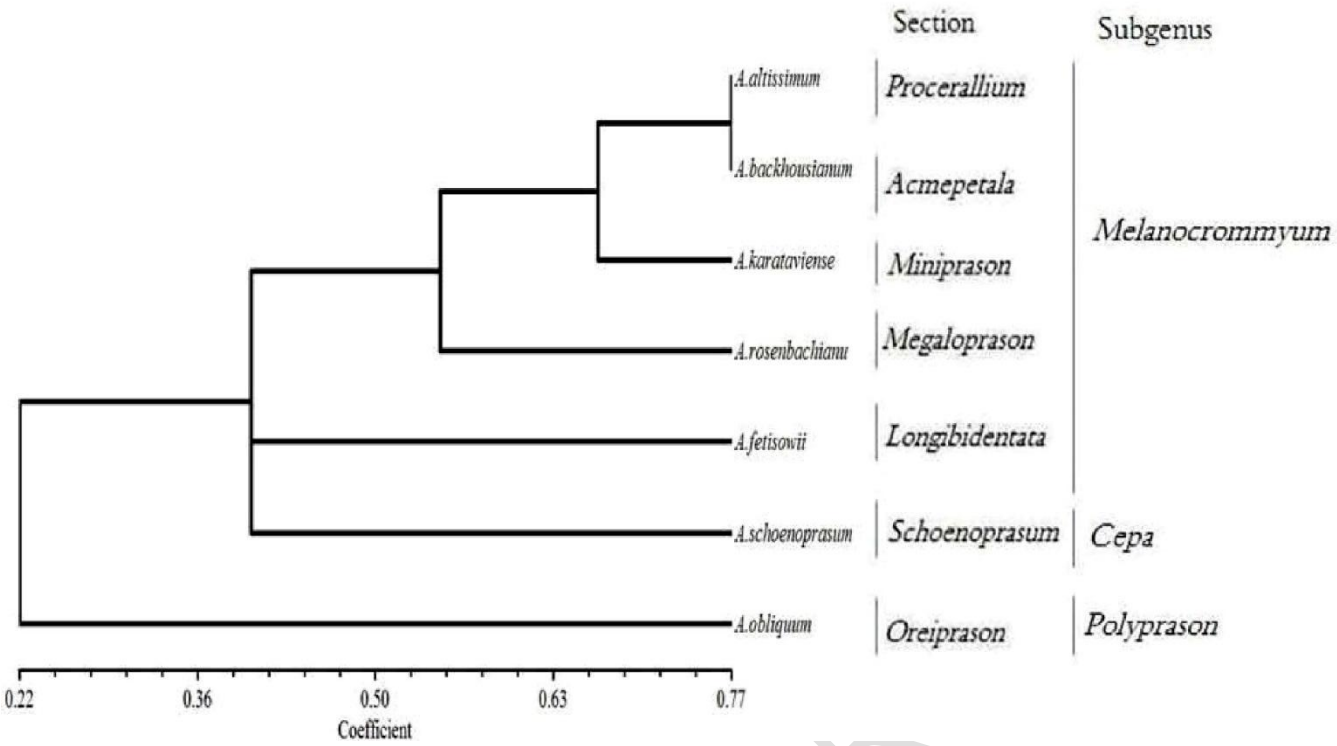

Fig. 5. Dendrogram obtained from the analysis of pollen data and taxonomic relationships of the studied species.