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POLLEN MORPHOLOGY OF SELECTED EUROPEAN SPECIES OF THE GENUS *Allium* L. (ALLIACEAE)

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Abstract. The pollen morphology of eight species from three subgenera and five sections of the genus *Allium* L. was studied by LM and SEM (i.e.: *A. angulosum*, *A. carinatum*, *A. senescens* subsp. *montanum*, *A. oleraceum*, *A. scorodoprasum*, *A. ursinum*, *A. victorialis* and *A. vineale*). The material came from natural sites of these species located in Poland, Czech Republic, Austria and Italy. For measurements a sample consisted of 30 pollen grains. In total, 240 pollen grains were analysed. They were analysed in respect to six quantitative features (i.e.: length of long axis – LA, length of short axis – SA, thickness of exine along long axis – Ex, SA/LA and Ex/LA ratios and length of sulcus) and the following qualitative ones: pollen outline and shape, exine ornamentation. Taxonomic value of these pollen features is considerable, especially on the sections level. On the basis of these features, it is impossible to distinguish individual *Allium* species but only their groups. The examined features were characterized by moderate (LA, SA and SA/LA) or high variability (Ex, Ex/LA). Among studied species the lowest variability was found in *A. victorialis* and the highest in *A. vineale* and *A. oleraceum*.

Key words: pollen variability, palynology, micromorphology, taxonomy, SEM

INTRODUCTION

Allium L. is a very diverse and species-rich (about 700–800 species) genus distributed all over the whole northern hemisphere, with the main centre of diversity in the mountains of Southwest and Central Asia [Fritsch and Friesen 2002, Erhardt et al. 2008, Friesen 2008, Block 2010].

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The species from genus *Allium* are morphologically very diversified, therefore, many taxonomical problems remain unsolved [Gurushidze et al. 2007]. In the 19th century, the *Allium* genus was divided into 6 groups. In the second half of the 20th century, first 3 subgenera and 12 sections were separated, next – 5 subgenera and 16 sections and, later on – 6 subgenera and 54 sections and subsections [Kamenetsky and Rabinowitch 2004]. Systematics was based on the origin of plants as well as morphological, anatomical, cytological and serological traits. Among others, numerical taxonomy was applied [El-Gadi and Elkington 1977, Fritsch 1996]. With the course of time, thanks to the performed genetical analyses, verification of systematic affiliation became easier. Fritsch and Friesen [2002] divided genus *Allium* into 14 subgenera. In the most recent publications of Friesen et al. [2006] and Neshati et al. [2009], the genus is divided into 15 subgenera and 72 sections.

Our knowledge concerning pollen morphology of species from the *Allium* genus is far from being complete. Although, in recent years, numerous researchers have investigated this area, their studies have focused on some selected, frequently very rare species representing the flora of a given country or even region [Namin et al. 2009, Neshati et al. 2009, Özhatay and Koçyiğit 2009, Özler and Pehlivan 2010].

For our studies, species of extensive distribution, common all over Europe and some also in Asia representing three different subgenera and five sections were selected [Davies 1992, Rabinowitch and Currah 2002].

Majority of palynologists consider the sulcus (ectoaperture) features as diagnostic characters of pollen grains of the *Allium* genus. Other important features include: exine ornamentation, length of pollen grains, pollen shape, number and diameter of perforations, operculum presence or absence and operculum type [Nair and Sharma 1965, Radulescu 1973, Kupriyanova and Aliev 1979, Pastor 1981, Diez 1987, El-Sadek et al. 1994, Kosenko and Kudryashova 1995, Tolgor 1995, Koç 2001, Bogdanović et al. 2008, Namin et al. 2009, Neshati et al. 2009, Özhatay and Koçyiğit 2009, Özler and Pehlivan 2010, Maassoumi et al. 2014].

The aim of the present study was to describe the morphology of pollen grains of eight selected European *Allium* species and to define the rank of the diagnostic features of grains of the species under study. In addition, attempts were also made to establish to what extent pollen morphology of the examined species corroborates the division of the *Allium* genus into subgenera and sections adopted in classical taxonomy [Friesen et al. 2006]. Another goal was to analyse the variability of pollen grains of the selected *Allium* species because, so far, it has not been the object of separate palynological studies.

MATERIAL AND METHODS

Research material. The study was conducted on eight *Allium* species which represent three subgenera and five sections of the genus *Allium*. A list of the analysed species, with their affiliation to particular subgenera and sections, is shown in table 1. Due to the lack of a consistent taxonomic system of the *Allium* genus, in this paper, the taxonomic classification of the studied species was adopted from Ohri et al. [1998] and Friesen et al. [2006].

The palynological terminology used in the study follows Punt et al. [2007] and Hesse et al. [2009].

Pollen samples of eight species of *Allium* came from natural sites located in Poland, the Czech Republic, Austria and Italy. Plant material was collected in the herbariums of the W. Szafer Institute of Botany in Kraków (KRAM), Institute of Botany of Jagiellonian University in Kraków (KRA), Adam Mickiewicz University in Poznań (POZ) (tab. 1).

Table 1. List of localities of *Allium* species studied

Subgenus	Section	Species	Localities and position	Collector, herbarium
Allium	*Allium*	*A. scorodoprasum* [Ohri et al. 1998]	Poland, Karpaty Mts, Pawlikowice 49°55'0"N, 20°5'0"E	Bartoszek W., KRA
	Allium	*A. vineale* [Ohri et al. 1998]	Poland, Leśno 53°56'58"N, 17°42'0"E	Borek R., KRA
	Codonoprasum	*A. oleraceum* [Friesen 2008]	Poland, Roczyny 49°51'0"N, 19°19'0"E	Zając A.M., KRA
	Codonoprasum	*A. carinatum* [Ohri et al. 1998]	Italy, Dolomites Mts 46°26'0"N, 11°51'0"E	Zając A.M., KRA
Amerallium	*Arctoprasum*	*A. ursinum* [Pich et al. 1998]	Poland, Klaczowa 49°52'9"N, 21°48'6"E	Towparz K., KRA
Rhizirideum	*Rhizirideum*	*A. angulosum* [Ricroch et al. 2005]	Czech Republic, Kojetice 49°8'57"N, 15°49'32"E	Reitmayer J.L., KRAM
	Anguinum	*A. victorialis* [Ohri et al. 1998]	Poland, Glinianka 50°28'28"N, 22°18'25"E	Michalewska A., Nobis M., KRA
	Rhizirideum	*A. senescens* subsp. *montanum* [Ricroch et al. 2005]	Czech Republic, Štramberk 49°35'30"N, 18°7'2"E	Otruba J., POZ

KRA – Institute of Botany of Jagiellonian University in Kraków, KRAM – W. Szafer Institute of Botany in Kraków, POZ – Adam Mickiewicz University in Poznań

For the measurements samples were acetolysed according to Erdtman's method [1960]. The acetolysing mixture was made up of 9 parts of acetic acid anhydride and one part of concentrated sulphuric acid and the process of acetolysis lasted 2.5 minutes.

A sample consisted of 30 mature, correctly formed pollen grains. For each species after 30 grains was measured. In total, 240 pollen grains were analysed.

Pollen grains were analysed for six quantitative features, i.e. length of long axis (LA), length of short axis (SA), thickness of exine along long axis (Ex), and SA/LA and Ex/LA ratios and length of sulcus, as well as the following qualitative traits: pollen outline and shape, exine ornamentation.

Light and scanning electron microscopy. The observations were carried out with light microscope (LM) (Biolar 2308, Nikon HFX-DX). Pollen grains were prepared in glycerine gelly and measured using the eyepiece (ocular) with scale. Then the direct results of measurements were recalculated into micrometers by multiplying them by 2. The used eyepiece and lens scales require a conversion of the measurement results to micrometers (µm).

For SEM (scanning electron microscope) investigations pollen samples from flowers at the beginning of anthesis were taken from field collections or from herbarium specimens. Pollen was put in small paper pouches and rehydrated for a few seconds to obtain the turgescent pollen state. The material was dehydrated in 2,2-dimethoxypropane and critical-point dried according to Halbritter [1998]. Air-dried pollen was used without any special preparation. All samples were mounted on stubs with double-faced adhesive tape, sputter coated with gold, and examined using a JEOL JSM-6390 scanning microscope. The SEM photos of studied *Allium* species were taken by Heidemarie Halbritter and are collected in the basis PalDat – a palynological database (http://www.paldat.org).

Statistical analysis. The normality of LA, SA, SA/LA, Ex, Ex/LA data distributions were tested using Shapiro-Wilk's normality test [Shapiro and Wilk 1965]. A one-way analysis of variance (ANOVA) was carried out to reveal significant differences in LA, SA, SA/LA, Ex, Ex/LA across species. The minimal and maximal values of characteristics as well as arithmetical means were calculated. When critical differences were noted, multiple comparisons were carried out using Tukey's honestly significant difference (HSD) test for each trait; based on this, homogeneous groups (not significantly different from each other) were determined for the analyzed features. The relationships between LA, SA, SA/LA, Ex, Ex/LA were estimated using Pearson correlation coefficients [Sokal and Rohlf 1995]. The Mahalanobis distance [Mahalanobis 1936] between the species, determined using LA, SA, SA/LA, Ex, Ex/LA, can be treated as the phenotypic distance between species. The differences among analysed individuals were verified by the cluster analysis using the nearest neighbour method and Euclidean distances [Camussi et al. 1985]. All data analyses were performed using the statistical package GenStat v. 10.1 [GenStat 2007].

RESULTS

General pollen morphological description. A description of the pollen grain morphology of the *Allium* species studied here is given below and illustrated with scanning electron micrographs (figs 1–4). Pollen of *Allium* is dispersed as monads. The pollen grains are sulcate, heteropolar and oblate (figs 1–4 a, b, e, f). 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 sulcus has parallel borders and the narrow ends of it are often characteristically rounded (figs 1, 3 a–c, e, f, 2 a–c, e, f, g, 4 a, b, e–g). Exine ornamentation can be described best as rugulate or finely striate and perforate or even microreticulate (figs 1, 3 d, g, 2 d, h, 4 c, h). In some species ornamentation differs between proximal face and distal pollen regions. In dry pollen and also after acetolysis the sulcus is infolded due to the relatively thin pollen wall and the pollen is boat-shaped.

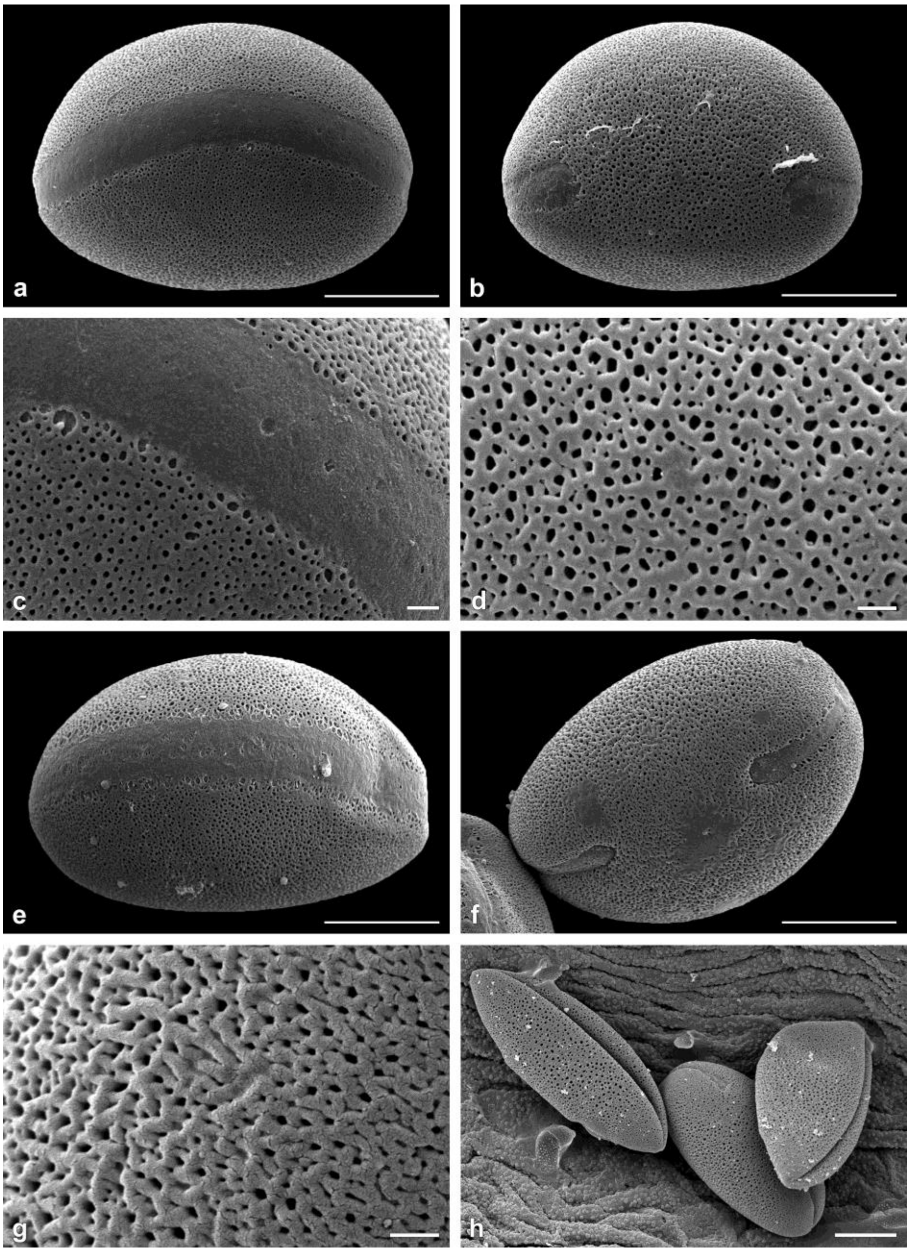

Fig. 1 a–h. *A. scorodoprasum* (a–d) – a – polar distal view, b – polar proximal view with extended sulcus, c – sulcus detail with smooth sulcus membrane, d – proximal polar area with microreticulate exine ornamentation. *A. vineale* (e–h) – e – polar distal view, f – polar proximal view with extended sulcus, g – rugulate perforate exine ornamentation in polar proximal area, h – dry, boat-shaped pollen grains; Bar = 10 μm in a, b, e, f, h, Bar = 1 μm in c, d, g

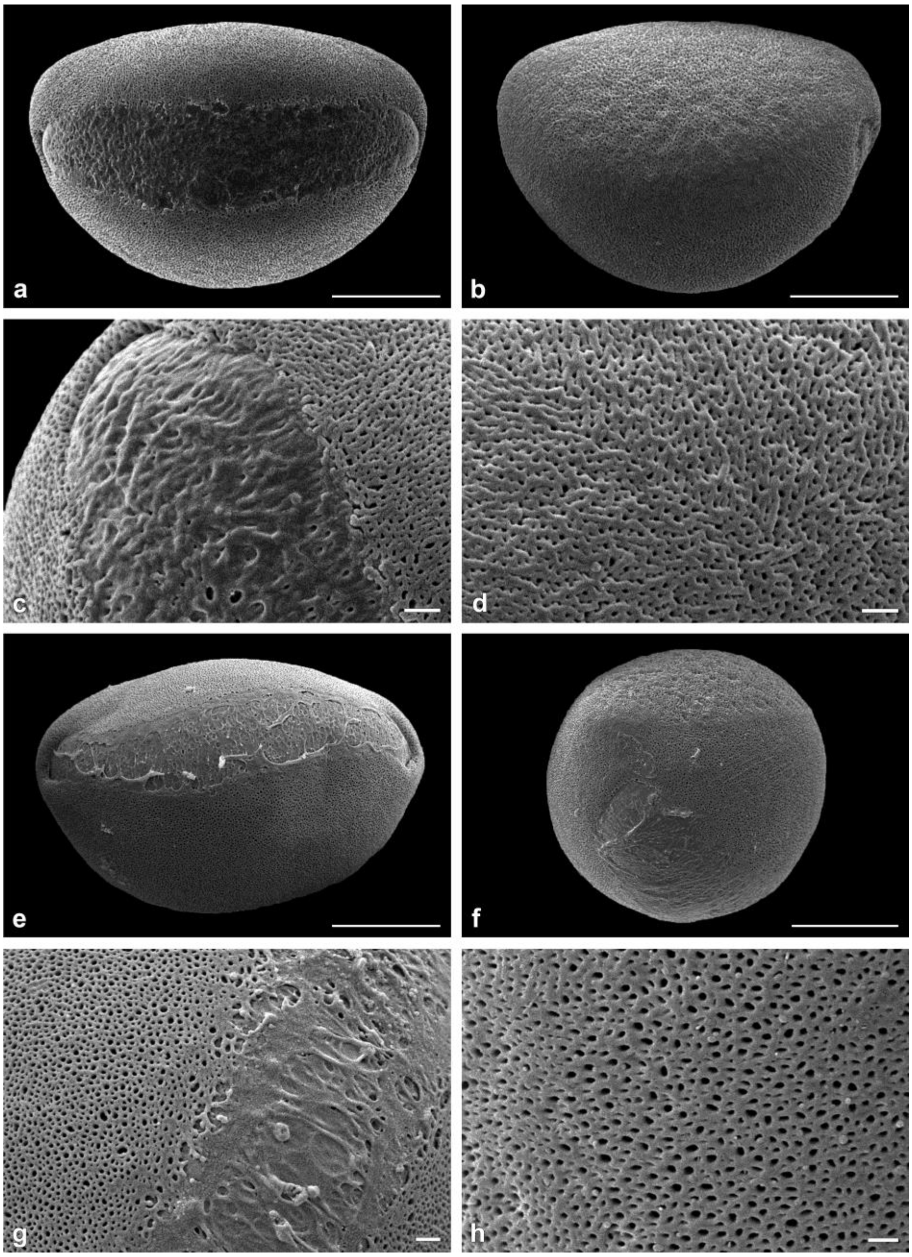

Fig. 2 a–h. *A. oleraceum* (a–d) – a – polar distal view, b – oblique polar proximal view, c – sulcus detail with characteristically rounded narrow end, d – rugulate perforate exine ornamentation in polar proximal area. *A. carinatum* (e–h) – e – polar distal view, f – equatorial view, g – sulcus detail, expanded intine with tubular intine channels, h – exine in equatorial region with microreticulate ornamentation; Bar = 10 μm in a, b, e, f, Bar = 1 μm in c, d, g, h

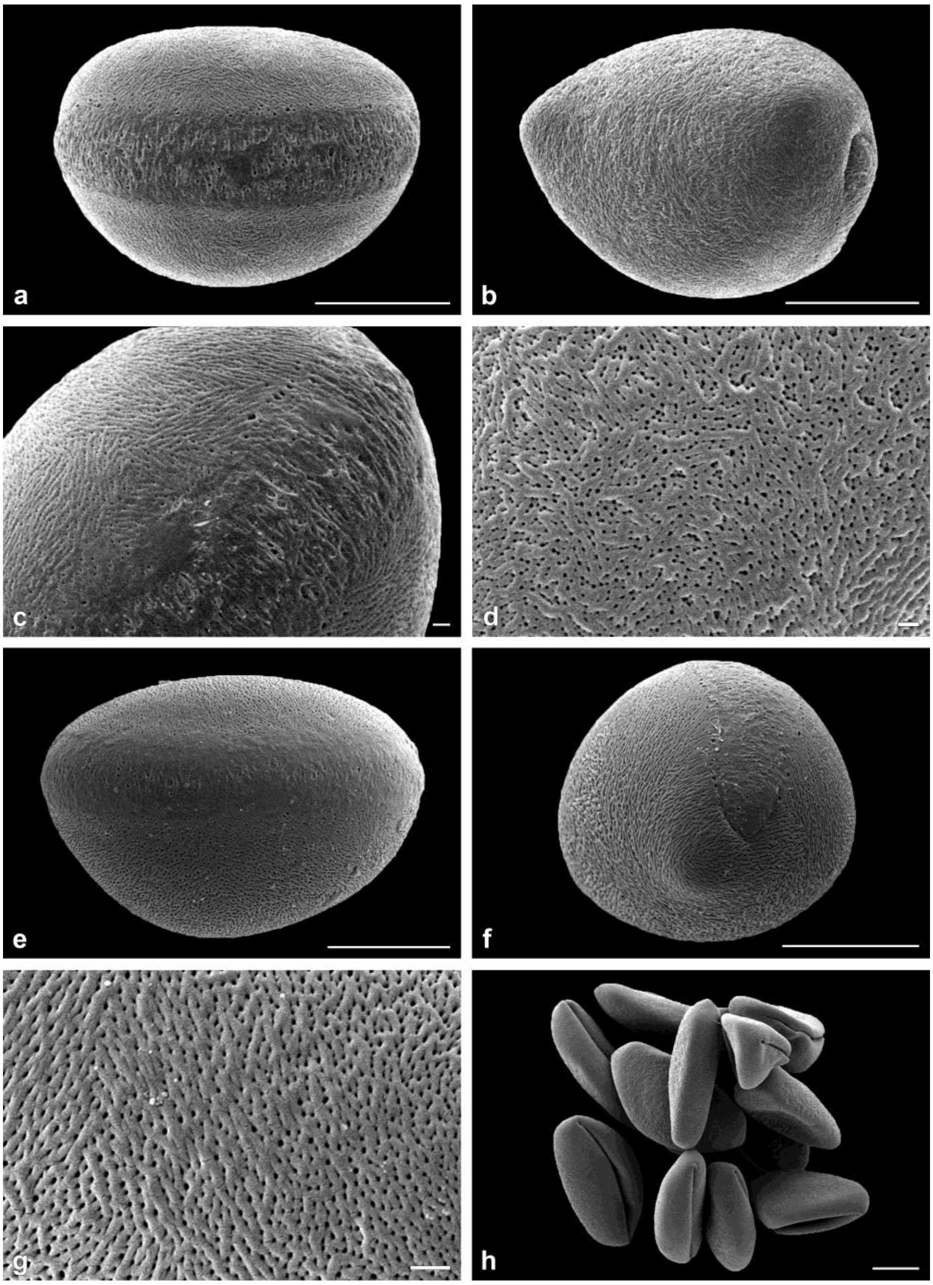

Fig. 3 a–h. *A. ursinum* (a–d) – a – polar distal view, b – oblique polar proximal view, c – sulcus detail, d – polar proximal area with rugulate perforate exine ornamentation. *A. angulosum* (e–h) – e – polar distal view, f – equatorial view, g – polar proximal area with striate perforate exine ornamentation, h – dry, boat-shaped pollen grains; Bar = 10 µm in a, b, e, f, h, Bar = 1 µm in c, d, g

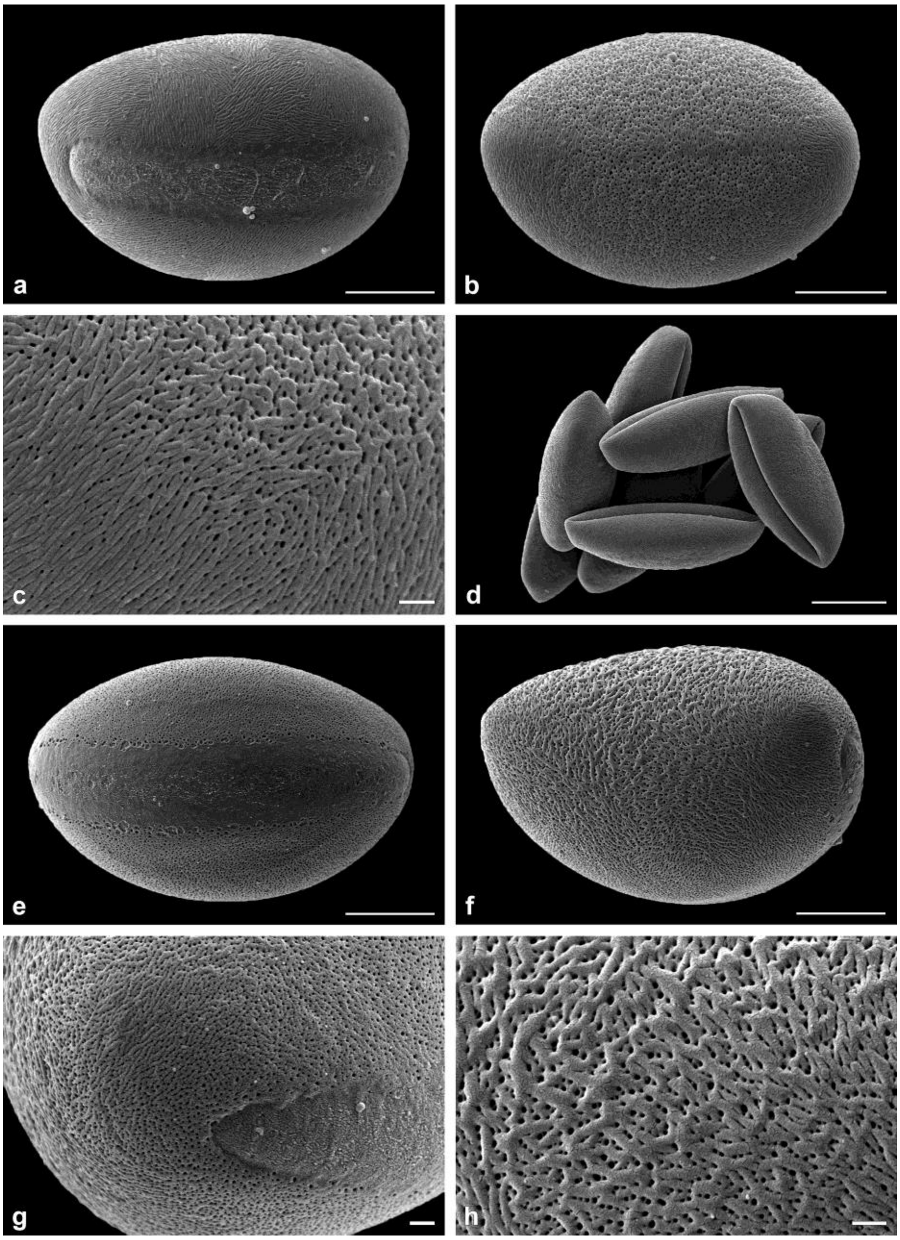

Fig. 4 a–h. *A. victorialis* (a–d) – a – polar distal view, b – polar proximal view, c – exine ornamentation, transition zone between polar proximal area (microreticulate) and equatorial area (striate perforate), d – dry, boat-shaped pollen grains. *A. senescens* subsp. *montanum* (e–f) – e – polar distal view, f – oblique polar proximal view, g – detail of sulcus, narrow end, h – polar proximal region with rugulate perforate exine ornamentation; Bar = 10 μm in a, b, d, e, f, Bar = 1 μm in c, g, h

Table 2. Range (min-max), mean values and coefficient of variation (cv) of studied traits

Species	LA (μm)			SA (μm)			SA/LA			Ex (μm)			Ex/LA		
	min–max	mean	cv	min–max	mean	cv	min–max	mean	cv	min–max	mean	cv	min–max	mean	cv
A. angulosum	26–34	29.40e	6.95	16–24	19.53c	8.37	0.529–0.786	0.667a	9.43	0.6–1.6	1.00ab	35.23	0.018–0.062	0.034a	37.30
A. carinatum	34–44	39.40a	5.68	20–28	23.13a	8.99	0.500–0.706	0.588cd	9.01	0.4–1.6	0.80bc	49.56	0.009–0.047	0.020b	51.03
A. senescens subsp. *montanum*	32–38	34.47cd	4.22	18–24	20.80bc	6.96	0.500–0.706	0.605bcd	8.36	0.4–1.6	0.71bc	54.48	0.011–0.050	0.021b	56.46
A. oleraceum	32–42	38.27ab	6.25	20–28	22.33ab	10.27	0.476–0.824	0.587cd	13.46	0.4–1.6	0.80bc	49.56	0.010–0.044	0.021b	50.36
A. scorodoprasum	26–34	30.87e	5.81	16–24	20.87bc	9.65	0.533–0.769	0.677a	8.60	0.4–1.0	0.57c	44.52	0.012–0.038	0.019b	48.47
A. ursinum	34–38	35.53c	4.10	20–24	22.33ab	7.09	0.526–0.706	0.629abc	7.90	0.6–2.0	1.15a	39.65	0.016–0.056	0.032a	38.65
A. victorialis	34–40	37.27b	4.98	18–24	21.47b	8.09	0.450–0.667	0.577d	8.18	0.4–1.0	0.78bc	29.62	0.010–0.029	0.021b	29.51
A. vineale	28–38	33.07d	6.69	18–26	21.13bc	9.20	0.500–0.800	0.642ab	11.24	0.2–1.6	0.63c	56.29	0.006–0.057	0.020b	62.40
$HSD_{0.001}$	–	1.69	–	–	1.60	–	–	0.05	–	–	0.31	–	–	0.009	–
ANOVA P > F	–	< 0.001	–	–	< 0.001	–	–	< 0.001	–	–	< 0.001	–	–	< 0.001	–

One-way ANOVA's were performed separately for each of traits. Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's *post hoc* test ($p < 0.001$)

Nomenclature: LA – length of long axis; SA – length of short axis; Ex – exine thickness

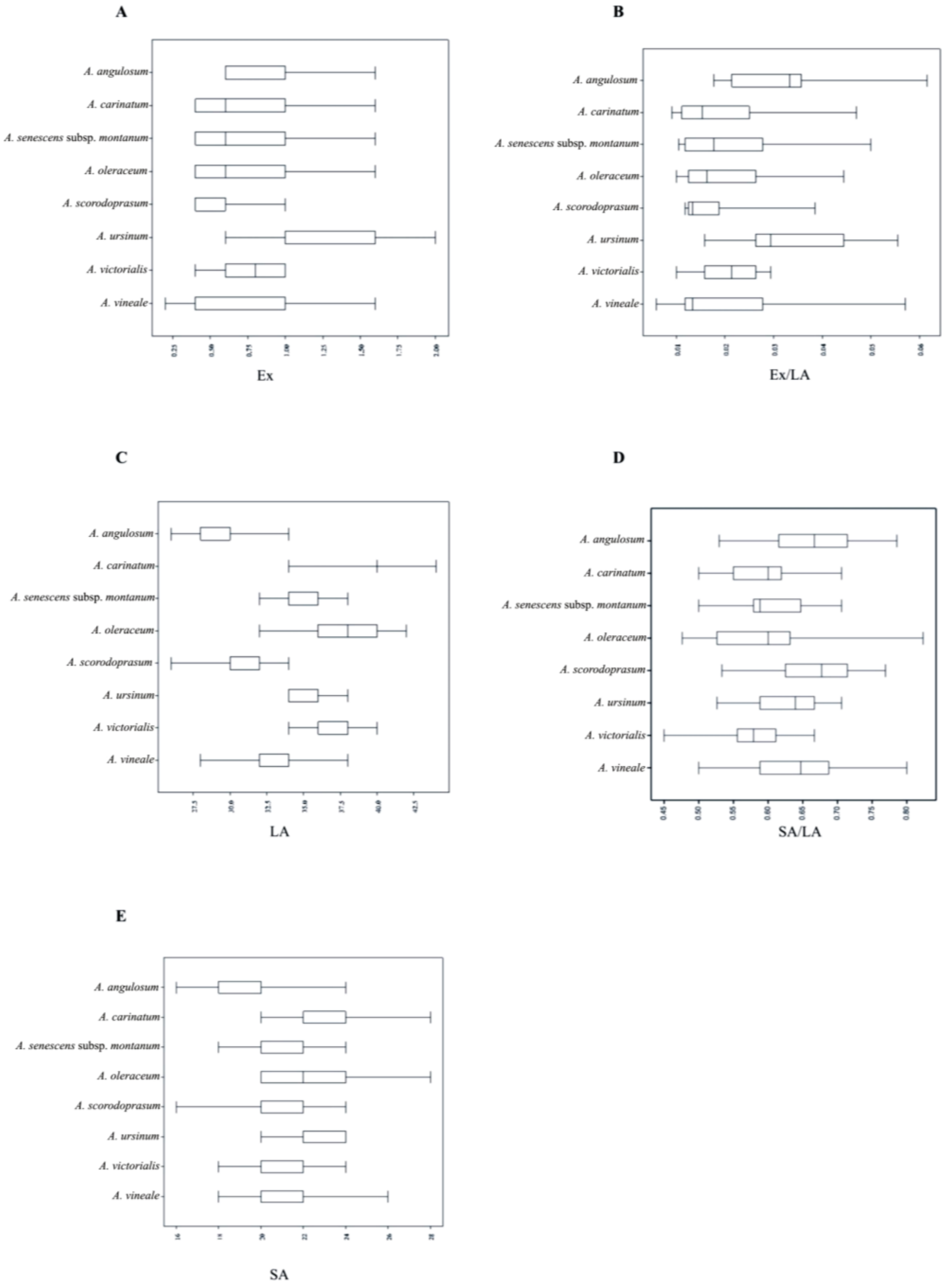

Fig. 5 A–E. Scatterplots of the relationship between observed traits for studied species

All studied pollen grains, according to Erdtman's [1952] pollen size classification, are medium-sized (25.1–50 µm). The average length of the long axis (LA) was 34.78 µm (range: 26–44 µm). The smallest mean length of the long axis was found for the pollen of *A. angulosum* (29.40 µm) and *A. scorodoprasum* (30.87 µm), and the largest – for *A. carinatum* (39.40 µm) and *A. oleraceum* (38.27 µm) (tab. 2).

Majority of the smallest pollen grains occurred in the *A. angulosum* sample (all measured pollen grains were relatively small, at a narrow range of the long axis; 26–34 µm; tab. 2). The longest pollen grains were found in *A. carinatum* (77% pollen grains have LA ≥40 µm). Such large pollen grains were also found in *A. oleraceum* and *A. victorialis*. The smallest range of the described feature was determined in *A. ursinum* (34–38 µm) and *A. victorialis* (34–40 µm), while the greatest was recorded in *A. scorodoprasum* (26–34 µm) (tab. 2, fig. 5 C).

The mean length of the short axis (SA) was 21.45 µm (range: 16–28 µm). The shortest mean short axis (SA) occurred in the pollen of *A. angulosum* (19.53 µm), while the longest – in *A. carinatum* (23.13 µm) (tab. 2). As in the case of the LA feature, a distinctly smallest range of the SA feature was found in *A. ursinum* (20–24 µm), while the greatest range of 8 µm was determined in several of the examined species (tab. 2, fig. 5 E).

The outline in polar proximal and distal views was mostly elliptic, and in equatorial view, it was elliptic or circular (figs 1–4 a, b, e, f).

The mean SA/LA ratio was 0.62, and ranged from 0.45 in *A. victorialis* to 0.82 in *A. oleraceum* (tab. 2, fig. 5 D). All studied pollen grains were elongated; pollen shapes (based on mean SA/LA ratio) were most frequently oblate (93.3–100%), rarely suboblate (3.3–6.7%) or peroblate (3.3–10%) (tab. 3, fig. 5 D). In *A. carinatum, A. senescens* subsp. *montanum* and *A. ursinum*, all pollen grains were oblate. A single pollen grain from suboblate class occurred in *A. angulosum*, *A. oleraceum*, *A. scorodoprasum*, and *A. vineale*, and peroblate pollen grains were found to occur in *A. oleraceum* and *A. victorialis*.

Mean exine thickness was 0.8 (0.2–2.0) µm. Exine was the thinnest in *A. vineale* (0.2 µm) and the thickest in *A. ursinum* (2.0 µm) (tab. 2, fig. 5 A). In the case of these two species, the authors also found the greatest value range of this feature, respectively: 0.2–1.6 µm and 0.6–2.0 µm. The smallest range of the described feature (0.4–1.0 µm) was determined in *A. scorodoprasum* and *A. victorialis* (fig. 5 A). The relative thickness of the exine (Ex/LA ratio) averaged 0.02 (0.01–0.06) (tab. 2, fig. 5 B).

Exine ornamentation is variable and there are smooth transitions between all possible ornamentation features. Proximal and equatorial pollen regions are often significantly different. Microreticulate ornamentation is present in *A. scorodoprasum* and *A. vineale* (section *Allium*) as well as in *A. carinatum* (section *Codonoprasum*) (figs 1 d, g, 2 h). The lumina width is decreasing slightly from proximal to distal face and towards the sulcus borders. In *A. victorialis* the proximal polar area is rather microreticulate whereas the equatorial and distal areas are finely striate and perforate (fig. 4 a–c). In *A. oleraceum*, *A. ursinum* and *A. senescens* ssp. *montanum* the proximal area is rugulate changing to striate and perforate more distally (figs 2 b, d, 4 f, h). The ornamentation of *A. angulosum* is striate and perforate throughout (fig. 3 g).

All studied pollen grains are sulcate. The sulcus is about as long as the half of the circumference of the pollen grains long axis in *A. oleraceum, A. carinatum, A. ursinum,* *A. angulosum*, *A. victorialis* and *A. senescens* ssp. *montanum* (figs 2 a, e, f; 3 a, b, e, f; 4 a, e). Only in *A. scorodoprasum* and *A. vineale* (section *Allium*) pollen has an extended sulcus reaching to the proximal face (fig. 1 a–c, e, f). The sulcus membranes are psilate without any ornamentation. In expanded pollen grains a kind of perforations can be detected sometimes, but these are the ends of radial channels of the intine, which is thickened beneath the aperture.

Interspecific variability of pollen grains. Statistically significant differences were determined among the species with regard to all the analysed features ($p < 0.001$) (tab. 2).

The examined features were characterized by moderate or high variability (tab. 2). Three features: LA, SA and SA/LA exhibited significantly lower values of the coefficient of variability (from 4.1 to 12.35%) than exine thickness features (Ex, Ex/LA; from 29.51 to 56.46%). On the other hand, analyses of coefficients of variation of pollen features for individual *Allium* species showed that pollen grains of *A. victorialis* were characterised by the lowest variability, whereas the highest variability was found in *A. vineale* and *A. oleraceum*.

Table 3. Percentage participation of pollen grains in shape classes distinguished for the *Allium* species analysed in the study

Species	Pollen shape classes (%)		
	peroblate	oblate	suboblate
A. angulosum	–	96.7	3.3
A. carinatum	–	100.0	–
A. senescens subsp. *montanum*	–	100.0	–
A. oleraceum	10.0	86.7	3.3
A. scorodoprasum	–	96.7	3.3
A. ursinum	–	100.0	–
A. victorialis	3.3	96.7	–
A. vineale	–	93.3	6.7

Table 4. The correlation matrix for the observed features

Feature	LA	SA	SA/LA	Ex	Ex/LA
LA	1				
SA	0.441***	1			
SA/LA	0.616***	-0.428***	1		
Ex	-0.015	0.019	0.040	1	
Ex/LA	-0.251***	-0.090	0.187**	0.964***	1

, * – significant at 0.01, 0.001. Nomenclature as in the Table 2

Positive statistically significant correlations between LA and SA (r = 0.441), LA and SA/LA (r = 0.616), SA/LA and Ex/LA (r = 0.187), Ex and Ex/LA (r = 0.964), as well as negative correlations between LA and Ex/La (r = -0.251), SA and SA/LA (r = -0.428), were observed (tab. 4).

Table 5. Phenotypic distance between the species calculated on the basis LA, SA, SA/LA, Ex, Ex/LA by Mahalanobis distance

Species	*A. angulosum*	*A. carinatum*	*A. senescens* subsp. *montanum*	*A. oleraceum*	*A. scorodoprasum*	*A. ursinum*	*A. victorialis*	*A. vineale*
A. angulosum	0							
A. carinatum	5.28	0						
A. senescens subsp. *montanum*	2.83	2.81	0					
A. oleraceum	4.70	1.04	2.15	0				
A. scordoprasum	2.08	4.67	2.02	4.06	0			
A. ursinum	3.64	2.34	1.63	1.85	3.07	0		
A. victorialis	4.12	1.42	1.5	0.81	3.48	1.52	0	
A. vineale	2.37	3.47	0.82	2.81	1.29	2.10	2.25	0

Nomenclature as in the Table 2

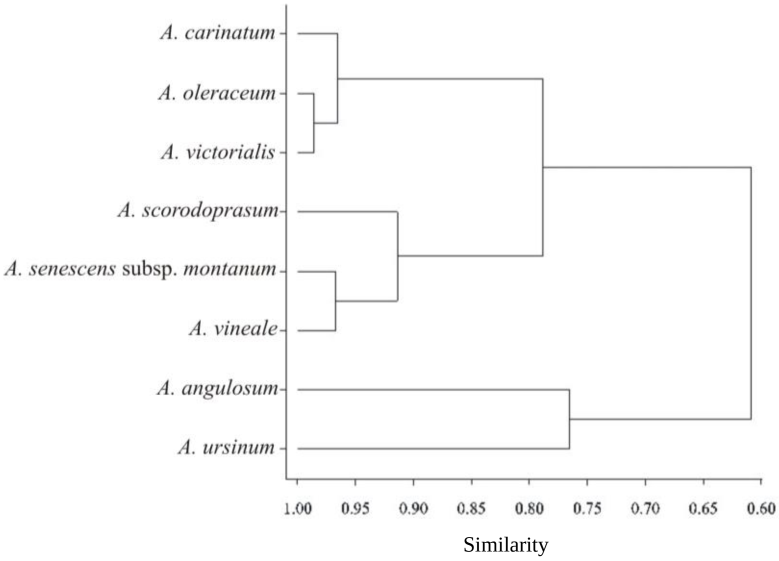

Fig. 6. Dendrogram of cluster grouping of eight species on the basis of five traits (LA, SA, SA/LA, Ex, Ex/L)

The highest phenotypic similarity (Mahalanobis distance equal 0.81) was revealed between *A. oleraceum* (section *Codonoprasum*) and *A. victorialis* (section *Anguinum*) and the lowest (Mahalanobis distance equal 5,28) was found for *A. angulosum* (section *Rhizirideum*) and *A. carinatum* (section *Codonoprasum*) (tab. 5).

The above data were confirmed by the dendrogram obtained as a result of agglomeration grouping using Ward method (fig. 6). All examined *Allium* species were divided into two groups. The first group comprised *A. angulosum* and *A. ursinum*, while the second one included six *Allium* species and was divided into two subgroups: *A. carinatum*, *A. oleraceum*, *A. victorialis* and *A. scorodoprasum*, *A. senescens* subsp. *montanum* and *A. vineale*.

DISSCUSION

In literature on the *Allium* genus, the following pollen grain features are considered as having the highest diagnostic value: sulcus structure, exine ornamentation, presence or absence of perforations and operculum, pollen size and shape [Kupriyanova and Aliev 1979, Pastor 1981, Diez 1987, Cabezudo et al. 1992, El-Sadek et al. 1994, Koç 2001, Güler and Pehlivan 2006, Özler and Pehlivan 2007, Namin et al. 2009, Neshati et al. 2009, Özhatay and Koçyiğit 2009, Özler and Pehlivan 2010, Maassoumi et al. 2014]. Our results corroborated the diagnostic value of the majority of these features.

The diagnostic value of the analysed quantitative and qualitative features of pollen grains was considerably higher on the section level than on the species level. The same opinion was expressed by other palynologists investigating the *Allium* genus [Koç 2001, Özler 2001, Güler and Pehlivan 2006, Bogdanović et al. 2008, Neshati et al. 2009].

All researchers agree that sulcus features are the most important characters in the diagnosis of *Allium* genus pollen grains. From our findings the sulcus in *Allium* is a simple one. According to Halbritter and Hesse [1993] classification, simple sulcus occurs in all *Allium* species studied in this investigation. Two sulcus types were identified in them. The first one – when the sulcus is about as long as the half of the circumference of the pollen grains long axis and the second type – when the sulcus is longer as the half of the circumference and extends to the proximal face ending near the proximal pole. The first sulcus type occurred in six of eight of the studied *Allium* species from four sections (*Anguinum, Arctoprasum, Codonoprasum* and *Rhizirideum*). Other researchers found it in species from the same *Allium* sections but also in representatives of other sections. Güler and Pehlivan [2006] reported it in species from *Codonoprasum* and *Allium* sections, Namin et al. [2009] – in *Allium* species belonging to 12 different sections, Özhatay and Koçyiğit [2009] – in *Codonoprasum*, *Molium*, *Brevispatha*, *Scorodon* and *Melanocrommyum* sections and Özler and Pehlivan [2010] – in selected species from *Rhizirideum*, *Codonoprasum* and *Allium* sections. The second extended sulcus type was recorded only in two species from the *Allium* section. Also other papers confirmed the occurrence of this sulcus type only in species from the *Allium* section [Kupriyanova and Aliev 1979, Güler and Pehlivan 2006, Özhatay and Koçyiğit 2009, Namin et al. 2009, Neshati et al. 2009].

The sulcus ends in the *Allium* species studied by us which derive from five sections were not strongly varied. They exhibited rounded sulcus ends, usually equal to the width of the sulcus (sections *Allium*, *Anguinum*, *Arctoprasum* and *Rhizirideum*); they were narrowing slightly only in two species from the *Codonoprasum* section. According to the other authors, the sulcus ends can be broad and rounded or acute [Güler and Pehlivan 2006, Namin et al. 2009], mostly rounded, rarely sharp or very rarely truncate [Özler and Pehlivan 2010]. On the other hand, Özhatay and Koçyiğit [2009] maintain that the sulcus ends are narrowed and rounded only in section *Molium*, broad and rounded in section *Brevispatha* and *Scorodon* and rounded in section *Melanocrommyum*.

Güler and Pehlivan [2006] and Özler and Pehlivan [2010] reported that in *Allium* species, the sulcus membranes ornamentation could be psilate, psilate-perforate, striate-reticulate, granulate, rugulate or rugulate-perforate. Similarly to Namin et al. [2009], sulcus membranes were not observed in *Allium* pollen grains. According to Maassoumi et al. [2014] sulcus membrane ornamentation were regulate-perforate or perforate-regulate.

The sulcus membrane is not ornamented and more or less psilate. This can be observed in well preserved and well prepared material after critical – point drying with SEM only. Due to the relatively thin pollen wall the sulcus is infolded in dry pollen condition (herbarium material) and in acetolysed pollen. Acetolysis causes additional sulcus membrane damage. Sulcus ornamentations as described by some authors must be seen under this aspect. Perforations seen on the sulcus are not to compare with usual exine perforations. In the sulcus region the intine is thickened and tubular channels pretend exine perforations especially when the sulcus is well expanded. All other reported onamentations of the sulcus are at best shrinking artefacts.

Özler and Pehlivan [2010] reported an operculum in three species from sections *Allium*, *Codonoprasum* and *Rhizirideum*. Güler and Pehlivan [2006] as well as Özhatay and Koçyiğit [2009] described a fragmented operculum only in the *Codonoprasum* section. As already indicated by Namin et al. [2009], no operculum could be seen in any species from the *Allium* genus. The presence of an operculum or even of different operculum types is very questionable. Authors describe these features in acetolysed pollen grains taken from herbarium specimens. The figures in this papers are not convincing.

In our opinion, exine ornamentation in *Allium* species is variable and diversified and can be determined because of its diminutiveness in well prepared material in SEM only. Ornamentation is not uniform all over the pollen grain but differs slightly between proximal face and distal pollen regions. The majority of researchers call exine ornamentation rugulate [Cabezudo et al. 1992, Güler and Pehlivan 2006, Namin et al. 2009, Neshati et al. 2009, Özhatay and Koçyiğit 2009, Özler and Pehlivan 2010], rarely rugulate-striate [Güler and Pehlivan 2006, Namin et al. 2009, Özler and Pehlivan 2010], striate [Güler and Pehlivan 2006, Neshati et al. 2009, Özler and Pehlivan 2010] or psilate [Özhatay and Koçyiğit 2009]; with or without perforations. Also other types of ornamentation can be found in the PalDat data base [Buchner and Weber 2000] such as: microreticulate, verrucate or striate-reticulate. The description of exine ornamentation as verrucate derives from the often bumpy impression of the proximal polar region. According to our experiments, exine ornamentation in *Allium* is more varied than majority of researchers claim and microreticulate or reticulate exine ornamentations occur here more often.

Statistical analyses indicate that, in the case of the examined *Allium* species, the feature exhibiting the highest diagnostic value was the length of pollen grains (LA), whereas the width of pollen grains (SA) as well as SA/LA ratio had a lower diagnostic rank. Even though these features fail to distinguish single species, nevertheless they divide them into groups, e.g. those with the largest pollen grains are *A. carinatum* and *A. oleraceum*, whereas those with the smallest – *A. angulosum* and *A. scorodoprasum*. The results of mean values of the pollen size, i.e. of LA and SA features, obtained in our investigation are similar to those reported by Żuraw [2007] for *A. victorialis* and *A. ursinum* and slightly higher in comparison with Özhatay and Koçyiğit [2009] who measured the mean pollen size of *A. scorodoprasum* and *A. vineale*. These features have also the smallest and similar variability and there are positive, statistically significant, correlations between them. Such result appears to be justified also methodologically because the length (LA) and width (SA) of pollen grains are easy to measure (under light microscope) and, therefore, measurement errors are rare here in contrast to exine thickness which precise measurement is more difficult and that is why it can be burdened with a higher error. Perhaps this explains why coefficient of variability for Ex and Ex/LA features is usually considerably higher. Similar variability results of pollen grains for individual features were obtained in studies on other taxa, e.g. *Rosa*, *Carex*, *Rubus* or *Crataegus* [Wrońska-Pilarek et al. 2010, 2012, 2013, Wrońska-Pilarek and Jagodziński 2011].

The results obtained on the dendrogram are not unequivocal because clusters were formed from both species derived from the same as well as from different sections. A separate position was occupied by those derived from different sections, *A. angulosum* from section *Rhizirideum* and *A. ursinum*, the only representative of the *Arctoprasum* section. The remaining species formed the second group with two subgroups. The first one was made up from *A. carinatum*, *A. oleraceum* from section *Codonoprasum* and *A. victorialis* from subgenus *Rhizirideum*, section *Anguinum* closely related with one another. The second subgroup was formed by *A. scorodoprasum*, *A. vineale* from the same section *Allium* and *A. senescens* subsp. *montanum* from section *Rhizirideum*.

CONCLUSIONS

1. The most important pollen grain features of the studied *Allium* species comprise pollen size (long pollen axis, LA), sulcus length and exine ornamentation.

2. The diagnostic value of the above-mentioned quantitative and qualitative pollen grain features is considerably higher on the sections level, than on the species level. This hypothesis was corroborated by the fact, that species from the *Allium* and *Codonoprasum* sections were isolated fairly clearly. Nevertheless, on the basis of these features, it was impossible to isolate individual *Allium* species but only their groups. In addition, species belonging to these groups did not always derive from the same sections. There-

fore, pollen grain morphology can only be used as an auxiliary feature for the diagnosis of these species.

3. The performed statistical analyses corroborated high phenotypic similarity between closely related *A. carinatum* and *A. oleraceum* from section *Codonoprasum* as well as *A. scorodoprasum* and *A. vineale* from section *Allium.*

4. The obtained results of statistical analyses fail to confirm fully the taxonomic division of the *Allium* genus adopted in this study and are not unequivocal. For example, the high phenotypic similarity can occur both between closely related *A. carinatum* and *A. oleraceum* from section *Codonoprasum* as well as between *A. oleraceum* (section *Codonoprasum*) and *A. victorialis* (section *Anguinum*) derived from other sections. Two groups of species can be distinguished on the dendrogram to which both related species from the same sections (e.g. *A. carinatum* and *A. oleraceum* from section *Codonoprasum*) as well as those taxonomically distant (e.g. *A. angulosum* from section section *Rhizirideum* and *A. ursinum* from section *Arctoprasum*) belong.

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REFERENCES

Block, E. (2010). Garlic and Other Alliums: The Lore and the Science. RSC Publishing, Cambridge.

Bogdanović, S., Brullo, S., Mitić, B., Salmeri, C. (2008). A new species of *Allium* (Alliaceae) from Dalmatia, Croatia. Bot. J. Linn. Soc., 158, 106–114.

Buchner, R., Weber, M., eds. (2000 onwards). *Alllium* L. PalDat – a palynological database (http://www.paldat.org).

Cabezudo, B., Pastor, J., Trigo M.M., Nieto Caldera, J.M. (1992). Observaciones sobre *Allium rouyi* Gautier. Acta Bot. Malac., 17, 123–126.

Camussi, A., Ottaviano, E., Caliński T., Kaczmarek, Z. (1985). Genetic distances based on quantitative traits. Genetics, 111, 945–962.

Davies, D. (1992). Alliums, the ornamental onions. BT Batsford Ltd., London.

Diez, M.J. (1987). Liliaceae. In: Atlas polinico de Andalucia, Valdes, B., Diez, M.J., Fernandez, I. (eds). Occidental Instituto de Desarrollo Regional Universidad de Sevilla y Excma Diputacion de Cadiz Sevilla, 379–395.

El-Gadi, A., Elkington, T.T. (1977). Numerical taxonomic studies on species in *Allium* subgenus *Rhizirideum*. New Phytol., 79, 183–201.

El-Sadek, L., El-Gazaly, G., Ayvad M. (1994). Cytology and palynology of common monocots in Mariut Egypt 1. Common species of the families Alliaceae and Liliaceae. Quatar Univ. Si. J., 14(2), 270–280.

Erdtman, G. (1952). Pollen morphology and plant taxonomy, Angiosperms. Almquist and Wiksell., Stockholm.

Erdtman, G. (1960). The acetolysis method. A revised description: Svensk. Bot. Tidskr., 54, 561–564.

Erhardt, W., Götz, E., Bödeker, N., Seybold, S. (2008). Zander Handwörterbuch der Pflanzennamen. Verlag Eugen Ulmer Stuttgart, Stuttgart.

Friesen, N. (2008). Die Gattung *Allium* – Taxonomisscher Überblick und wissenschaftliche Sammlung im Botanischen Garten der Universität Osnabrück. Osnabrücker Natruwiss. Mitteil., 33/34, 95–110.

Friesen, N., Fritsch, R.M., Blattner, F.R. (2006). Phylogeny and new infrageneric classification of *Allium* (Alliaceae) based on nuclear ribosomal DNA ITS sequences. Aliso, 22, 372–395.

Fritsch, R.M. (1996). Neue Ergebnisse zur Taxonomie und Evolution von *Allium* L. In: Evolution und Taxonomie von pflanzengenetischen Ressourcen: Festschrift für Peter Hanelt. (Schriften zu Genetischen Ressourcen 4), Fritsch, R., Hammer, K. (eds). IGR/ZADI, Bonn, 19–46.

Fritsch, R.M., Friesen, N. (2002). Evolution, domestication and taxonomy. In: Evolution und Taxonomie von Pflanzengenetischen Ressourcen. *Allium* crop science – recent advances, Rabinowitch, H.D., Currah, L. (eds). CABI Publishing, Oxon, 5–30.

GenStat. (2007). GenStat Release 10. Reference Manual Lawes Agricultural Trust, Rothamsted, UK.

Güler, Ü., Pehlivan, S. (2006). Pollen morphology of some species belonging to *Codonoprasum* and *Allium* sections of *Allium* (Liliaceae-Alliaceae) genus. Biologia, 61(4), 449–455.

Gurushidze, M., Mashayekhi, S., Blattner, F.R., Friesen, N., Fritsch, R.M. (2007). Phylogenetic relationships of wild and cultivated species of *Allium* section Cepa inferred by nuclear rDNA ITS sequence analysis. Plant Syst. Evol., 269, 259–269.

Halbritter, H. (1998). Preparing living pollen material for scanning electron microscopy using 2,2-dimethoxypropane (DMP) and critical-point drying. Biotech. Histochem., 73, 137–143.

Halbritter, H., Hesse, M. (1993). Sulcus morphology in some monocot families. Grana, 32, 87–99.

Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner, R., Frosch-Radivo, A., Ulrich S. (2009). Pollen terminology. An illustrated handbook. Springer, Vienna.

Kamenetsky, R., Rabinowitch, H.D. (2004). The genus *Allium*: A development and horticultural analysis. Hortic. Rev., 32, 329–390.

Koç, F. (2001). Investigation of pollen morphology of some endemic *Allium* in Turkey. PhD thesis. Institute of Science and Technology. Gazi University.

Kosenko, V.N., Kudryashova, G.L. (1995). Palynomorphology of the family Alliaceae. Bot. Zhurnal (st. Petersburg)., 80(6), 5–17.

Kupriyanova, L.A., Aliev, T.A. (1979). Palynological data in the systematics of the genus *Allium* L. (Alliaceae JG Agardh). Bot. Zh. (St. Petersburg), 64(9), 1273–1284.

Maassoumi, S.M., Kiani, S., Bareemizadeh, F., Ghasempour, H., Karimi, N., Shohani, F., Amiri, S. (2014). Pollen morphology of the genus *Allium* in comparison with genus *Calochortus* of order Liliales. Int. J. Biosci., 4(12), 237–243.

Mahalanobis, P.C. (1936). On the generalized distance in statistics. Proc. Natl. Inst. Sci. India, 12, 49–55.

Nair, P.K.K., Sharma, M. (1965). Pollen morphology of Liliaceae. J. Palynology, 1, 38–61.

Namin, H.H., Mehrvarz, S.S., Zarre, S., Fritsch, R.M. (2009). Pollen morphology of selected species of *Allium* (Alliaceae) distributed in Iran. Nordic J. Bot., 27(1), 54–60.

Neshati, F., Fritsch, R.M., Zarre, S. (2009). Pollen morphology of some *Allium* L. species (Alliaceae) from Iran. Bot. Jahrb. Systemat., 127(4), 433–451.

Ohri, D., Fritsch, R.M., Hanelt, P. (1998). Evolution of genome size in *Allium* L. (Alliaceae). Plant Syst. Evol., 210, 57–86.

Özhatay, N., Koçyiğit, M. (2009). Pollen morphology of *Allium* species (Lilliaceae) in European Turkey and around Istanbul. Phytol. Balcan., 15(2), 199–208.

Özler, H. (2001). Study of pollen morphology structures of some species that belong to *Asparagus* L., *Allium* L., *Muscari* Miller and *Fritillaria* L. PhD thesis. Inst. Sci. & Technology, Gazi Univ. (in Turkish).

Özler, H., Pehlivan, S. (2007). Comparison of pollen morphological structuresof some taxa belonging to *Asparagus* L. and *Fritillaria* L. (Liliaceae) from Turkey. Bangladesh J. Bot., 36(2), 111–120.

Özler, H., Pehlivan, S. (2010). Pollen morphology of *Allium* L. (Lilliaceae) taxa in Turkey. Bangladesh J. Bot., 39(1), 37–46.

Pastor, J. (1981). Estudio palinologico del genero *Allium* en la Peninsula Iberica y Baleares. Bot. Macaronés., 8–9, 189–214.

Pich, U., Fritsch, R., Schubert, I. (1996). Closely related *Allium* species (Alliaceae) share a very similar satellite sequence. Plant Syst. Evol., 202, 255–264 Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A. (2007). Glossary of pollen and spore terminology. Rev. Palaeobot. Palynol., 1431(2), 1–81.

Rabinowitch, H.D., Currah, L. (2002). *Allium* crop science: recent advances CABI Publishing, Oxon.

Radulescu, D. (1973). Recherches morpho-palynologiques sur la famille Liliaceae. Acta Bot. Horti. Bucur., 1972–73, 133–248.

Ricroch, A., Yockteng, R., Brown, S.C., Nadot, S. (2005). Evolution of genome size across some cultivated *Allium* species. Genome, 48, 511–520.

Shapiro, S.S., Wilk, M.B. (1965). An analysis of variance test for normality (complete samples). Biometrika, 52, 591–611.

Sokal, R.R., Rohlf, F.J. (1995). Biometry: The Principles and practice of statistics in biological research. W.H. Freeman, New York.

Tolgor, M. (1995). Pollen morphology of *Allium* and its taxonomic significance. J. Jilin Agricult. Univ., 17(1), 36–40.

Wrońska-Pilarek, D., Jagodziński, A.M. (2011). Systematic importance of pollen morphological features of selected species from the genus *Rosa* (Rosaceae). Plant Syst. Evol., 295(1–4), 55–72.

Wrońska-Pilarek, D., Bocianowski, J., Jagodziński, A.M. (2013). Comparison of pollen grain morphological features of selected species of the genus *Crataegus* L. (Rosaceae) and their spontaneous, interspecific hybrids. Bot. J. Linn. Soc., 172, 555–571.

Wrońska-Pilarek, D., Jagodziński, A.M., Maliński, T. (2012). Morphological studies of pollen grains of the Polish endemic species of the genus *Rubus* L. (Rosaceae). Biologia, 67(1), 87–96.

Wrońska-Pilarek, D., Janyszek, M., Jagodziński, A.M. (2010). Pollen morphology of selected Central European species from *Vignea* and *Carex* subgenera (*Carex* L., Cyperaceae) and its relation to taxonomy. Bot. J. Linn. Soc., 164, 422–439.

Żuraw, B. (2007). Biological value and morphological traits of pollen of selected garlic species *Allium* L. Acta Agrobot., 60(1), 67–71.

MORFOLOGIA ZIARN PYŁKU WYBRANYCH EUROPEJSKICH GATUNKÓW Z RODZAJU *Allium* L. (ALLIACEAE)

Streszczenie. W pracy przedstawiono wyniki badań nad morfologią ziarn pyłku ośmiu gatunków z rodzaju *Allium* L. (*A. angulosum, A. carinatum, A. senescens* subsp. *montanum, A. oleraceum, A. scorodoprasum, A. ursinum, A. victorialis* i *A. vineale*) reprezentujących trzy podrodzaje i pięć sekcji, oparte na obserwacjach przy użyciu mikroskopu świetlnego (LM) i skaningowego (SEM). Materiał badawczy pochodził ze stanowisk naturalnych wymienionych gatunków zlokalizowanych w Polsce, Czechach, Austrii i we Włoszech. Pojedyncza próba pomiarowa składała się z 30 ziarn pyłku, a ogółem poddano analizie 240 ziarn. Ziarna pyłku przebadano pod względem sześciu cech ilościowych (długość osi długiej – LA, długość osi krótkiej – SA, grubość egzyny wzdłuż osi długiej – Ex, stosunek SA/LA i Ex/LA oraz długość „sulcus" – wydłużonego otworu ułożonego dystalnie) oraz cech jakościowych takich jak zarys i kształt ziarn pyłku oraz urzeźbienie powierzchni egzyny. Wykazano, że wartość taksonomiczna tych cech jest znacząca, szczególnie na poziomie sekcji, oraz że cechy te nie umożliwiają rozróżnienia pojedynczych gatunków *Allium*, a tylko ich grup. Analizowane cechy ziarn pyłku charakteryzował średni (LA, SA i SA/LA) lub wysoki (Ex, Ex/LA) poziom zmienności. Wśród badanych gatunków najniższy poziom zmienności reprezentowały ziarna pyłku *A. victorialis*, a najwyższy – *A. vineale* i *A. oleraceum*.

Słowa kluczowe: zmienność ziarn pyłku, palinologia, mikromorfologia, taksonomia, SEM

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