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POLLEN MORPHOLOGY OF *PINUS MUGO* TURRA × *PINUS SYLVESTRIS* L. HYBRIDS AND PARENTAL SPECIES IN AN EXPERIMENTAL CULTURE

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Pollen grains of *Pinus mugo*, *P. sylvestris* and *P.* ×*rhaetica* (= *P. mugo* × *P. sylvestris*) were examined by light and scanning electron microscopy. The pollen grains were bisaccate and monosulcate. The corpus-saccus attachment was distinct. The pollen corpus exine sculpture was verrucate-rugulate and deeply sculptured. The surface of the tectum was covered with small grana and it was perforate. The saccus sexine ornamentation was reticulate and irregularly perforate. The tectum surface characters in the proximal and distal view of the corpus and saccus were less variable and they did not provide good criteria to identify the species under study. However, some differences were observed in the size of elevation on the corpus between pollen grains of the same species and between the parental species. This study of the pollen grain morphology of the corpus and saccus provided some important new data.

Key words: Hybrid, pollen morphology, LM, SEM, *Pinus mugo*, *Pinus sylvestris*

INTRODUCTION

Natural hybridisation between *Pinus mugo* Turra and *P. sylvestris* L. in the regions of their sympatric occurrence has been known for a long time. The respective hybrid, named *Pinus* ×*rhaetica*, has been analysed and described by many researchers (Pravdin, 1964; Christensen, 1987a, b, c; Kanak, 1994; Bobowicz et al., 2000). At present the best known habitats of this hybrid are in the mountains of Central and Eastern Europe, i.e. in the Nowy Targ Valley (Staszkiewicz and Tyszkiewicz, 1969a, b) and in the Tatra Mountains in Poland (Staszkiewicz, 1996), the Czech Republic, Moravia (Staszkiewicz and Tyszkiewicz, 1972), Slovakia (Musil, 1977; Viewegh, 1981; Staszkiewicz, 1993a, b, c, 1994; Businský, 1999; Kormuťák et al., 2007), in the Rila Planina and Rhodope Mountains in Bulgaria (Dobrinov, 1965; Dobrinov and Jahzidis, 1971), in the Pyrenees (Christensen, 1987c), and in the Swiss Alps (Christensen, 1987c; Neet-Sarqueda et al., 1988; Kormuťák et al., 2005), where it was described for the first time.

Pinus ×*rhaetica* causes serious taxonomic problems. The hybrid swarm populations have already been subjected to morphologic studies of cones and morphologic-anatomical studies of needles (Staszkiewicz and Tyszkiewicz, 1969a, b, 1972; Szweykowski, 1969; Christensen, 1987a; Bobowicz, 1990; Staszkiewicz, 1996; Christensen and Dar, 1997), studies on the enzymatic variability or the variability of phenolic compounds (Szweykowski and Urbaniak, 1982; Krzaczek and Urbaniak, 1985; Bobowicz et al., 2000). The results of these studies, which were based on different traits, confirmed the hybrid character of the populations under investigation or they proved greater similarity of hybrids to one of the parental species. The above mentioned authors indicated the traits which differentiated *Pinus mugo* and *P. sylvestris* significantly. However, they did not find any trait whose presence would fully prove the hybrid origin of a particular specimen. The hybrid character could only be proved by indirect development of the traits under analysis, as compared with the typical traits of *Pinus mugo* and *P. sylvestris*.

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In gymnosperms, pollen grains represent developing male gametophytes, which are surrounded by a complex pollen wall, composed of an outer layer, i.e. exine (which is subdivided into ectexine and endexine) and an inner layer, i.e. intine (Fægri and Iversen, 1989). The exine is thicker at the proximal end and it is thinner but continuous in the distal region (Fernando et al., 2010).

There are bisaccate and monosulcate pollen grains in the *Pinus* genus. Historically, the pollen grain shape was used to distinguish between the pollen of the *Strobus* and *Pinus* subgenera. Pollen grains from the *Strobus* subgenus tend to have an outline in which the sacci are more or less continuous with the corpus, whereas the pollen grains of the *Pinus* subgenus have sacci that are distinct from the corpus in outline view. These have been referred to as haploxylon-type and diploxylon-type respectively, reflecting the subgeneric epithets from Shaw's classification system (1914). This division includes the morphometric characteristics related to the attachment and size of sacci (Zanni and Ravazzi, 2007). Haploxylon-type pollen grains are further characterised by a sculptured (verrucate) apertural membrane and a thickened ridge at the saccus aperture junction, whereas diploxylon-type pollen grains lack both apertural ornamentation and a prominent ridge (Bagnell, 1975).

The palynological literature provides little data concerning the size of pollen and exine sculpture on the corpus and saccus of pollen grains in the taxa under study. Klaus (1978) described the tectum of the corpus and saccus in pollen grains of *P. sylvestris* collected from pure stands in Lower Austria near Paudorf and *P. mugo* collected about 16 km south of the Austrian border on the slopes of Mount Mojstrovka on limestone rocks at an elevation of about 1600 m. Nakagawa et al. (2000) presented the size (the corpus breadth and basal width of the saccus) and the surface structure of the corpus pollen grains of three species of the French Alpine *Pinus*, including *P. sylvestris*. Kormuťák et al. (2008) provided data on the pollen size in *P. mugo*, *P. sylvestris* and their hybrid populations at Habovka and Suchá Hora in the western part of the High Tatras.

The main aim of this study was to: (1) describe variation in the pollen grain morphology of the *Pinus mugo* × *P. sylvestris* hybrid and parental forms; (2) describe and document the pollen grain micromorphology; (3) supply new data which can be used for identification of the species under study.

MATERIAL AND METHODS

Pollen grains were collected from 20 pine specimens growing in an experimental plot in the Dendrological Garden, Poznań University of Life Sciences and the Forest Arboretum in Zielonka, Poland. All the pines came from seeds collected from natural habitats. Of 79 shrubs growing at the Forest Arboretum in Zielonka, pollen was collected from all the specimens which bloomed in 2013, and the samples were numbered 1 to 16 (Tab. 1). Each sample was represented by 30 pollen grains, and they were analysed for eight quantitative traits (Tab. 2, Fig. 1) in polar view, according to Erdtman (1957), and for the following qualitative traits: the shape of the corpus and saccus and the type of the exine sculpture on the corpus and saccus.

TABLE 1. Location of samples used in the investigation

Locality	Taxa	Origin
Dendrological Garden, Poznań University of Life Sciences (DG)	*Pinus mugo* (no. 0351)	Tatra Mountains
	Pinus ×rhaetica (no. 0387)	Bór na Czerwonem
Forest Arboretum, Zielonka (FA)	*Pinus sylvestris*	Puszcza Zielonka
	Pinus mugo	Karkonosze Mountains
	Pinus ×rhaetica no. 1–16	Bór na Czerwonem

The pollen grains were macerated in 10% KOH (Frederiksen, 1978) and investigated with a light microscope Olympus BX SC30 (LM). Scanning electron microscopy (SEM) was used to obtain comprehensive information about the general morphology and exine sculpture. The SEM observa-

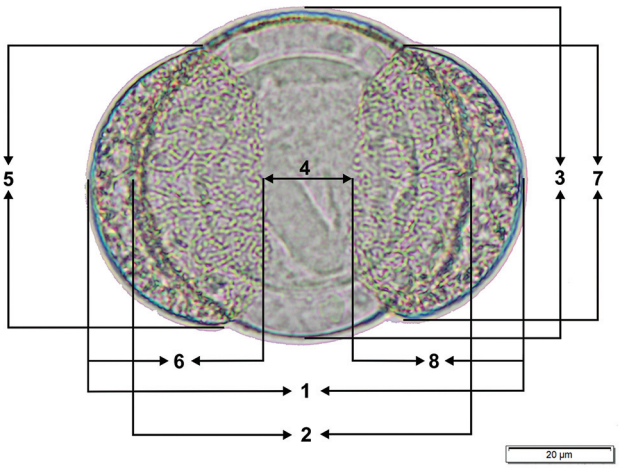


Fig. 1. Pollen grain dimension measurement method: 1 – pollen grain width; 2 – corpus width; 3 – corpus depth; 4 – distance between sacci; 5 – left saccus width; 6 – left saccus depth; 7 – right saccus width; 8 – right saccus depth (after Erdtman 1957, modified)

tions were conducted on air-dried pollen grains. The SEM micrographs were taken with a Zeiss EVO 40 microscope at the Electron Microscopy Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland. Prior to the observation, the prepared material was sputtered with gold by means of an SCB 050 ion sputter. The study was documented with photographs taken during the observation, mostly magnified ×5000 for the shape and ×20 000 for the exine sculpture. The micromorphological traits of the pollen grains were observed in proximal (polar) view of the corpus and saccus.

The pollen terminology was adopted from Erdtman (1957), Kremp (1965), Bagnell (1975), and Fægri and Iversen (1975).

The biometric data were analysed statistically. For each pollen trait, univariate analysis of variance (ANOVA) was used to examine mean differences between the species under study. When there were significant differences observed, the ANOVAs were followed by Tukey's HSD test at $\alpha = 0.05$. The statistical analyses were performed with STATISTICA 10 (StatSoft, Inc. 2011).

RESULTS AND DISCUSSION

PINUS MUGO

We analysed pollen from two shrubs which considerably differed from each other in habit and height. The total width of the pollen grains (the corpus and two sacci) in polar view of the *P. mugo* growing in the Dendrological Garden (DG) ranged from 60.06 to 74.36 μm. The *P. mugo* pollen grains from the Forest Arboretum (FA) were bigger and their width ranged from 62.92 to 77.22 μm. The corpus was similar in shape, i.e. prolate-spheroidal, and in size, i.e. 48.83 × 43.00 μm in *P. mugo* (DG) and 49.88 × 45.28 μm in *P. mugo* (FA). The size of the left and right sacci in the pollen grains of both specimens under study was almost identical (the *P. mugo* (DG) sacci were slightly wider). There were significant differences in the trait concerning the distance between the sacci (Tab. 2).

The proximal exine surface of the cappae was verrucate-rugulate, perforate, with small granules (Figs. 2b, 3b) and there were very sparse micrograna irregularly distributed over it. The elevations on the cappae were irregular in size and height, and the pattern was evident on the majority of pollen grains. The caps separated from each other and a few chambers fused (Fig. 3b). The comparison of the exine on the proximal surface of the cappae between the pollen grains from the Dendrological Garden (the Tatra Mountains) (Fig. 2b) and from the Forest Arboretum (the Karkonosze Mountains) (Fig. 3b) revealed noticeable differences in the size, shape and height of the elevation. The sexine sculpture of the sacci was reticulate and perforate (Figs. 10, 14, 15).

The shape of the corpus and sacci varied depending on the view of the pollen grains, i.e. equatorial, polar or lateral view. In none of the studies published so far have the authors specified the position of the measured pollen grains (Tab. 3). It was only Christensen (1987c) who specified the width of the pollen grains in *P. mugo* subsp. *P. mugo* – it ranged from 68.0 to 86.7 μm. These dimensions were much greater than the ones we measured in our study (Tab. 3). Kormuťák et al. (2008) reported that the mean width of the *P. mugo* corpus in equatorial view was 45.47 μm, the corpus depth was 35.21 μm and the width/depth ratio was 1.30 – the same as in *P. sylvestris*. In our study, the width and depth of the *P. mugo* corpus (measured in polar view) were greater, and the average width/depth ratio was mean 1.10 or 1.14 (in *P. sylvestris* 1.15). Chira (1971) reported that in non-acetolysed *P. mugo* pollen grains the corpus width ranged between 62.7 μm and 75.9 μm. In our study the pollen corpus widths in both specimens under study were about 25–50% smaller (Tab. 3). Contrary to our study, according to Klaus (1978), there were hardly any perforations on the cappae.

PINUS SYLVESTRIS

The pollen grains were bisaccate, with the average width of 65.49 μm, including the sacci. The corpus width ranged from 39.23 to 53.91 μm and the depth ranged from 37.18 to 45.76 μm. The pollen corpus was subprolate in shape (Fig. 4a). Both sacci were the same size and they were prolate in shape. The distance between the left and right saccus ranged from 14.30 to 22.88 μm (Tab. 2).

The corpus exine on the cappae was irregularly verrucate-rugulate (Fig. 4b). The tectum was perforate, with small granules. The elevations on the cappae were irregular in size and height and the pattern was evident on the majority of grains. The caps separated from each other and a few chambers fused (Fig 4b). The sacci ornamentation was reticulate, while the sexine was perforate with more numerous perforations than on the cappae (Fig. 11, 16). Sparse grana were found. The saccus sexine surface was partly flat in the middle part and slightly undulate in the region of the margin attachment (Fig. 16).

The comparison of our data with those published by other authors revealed some differences (Tab. 4). Christensen (1987c) reported that the width of pollen grains (from saccus to saccus) ranged from 71.4 to 88.4 μm (mean 80.4 μm). In our study the *P. sylvestris* pollen was over 20% smaller. The pollen grains in our study were also smaller than in both *P. mugo* specimens, which was in

TABLE 2. Mean values (±SE) and ranges (minimum-maximum) of the morphological traits of *Pinus* pollen grain and saccus.

Taxon	Pollen grain				
	Width of the pollen grain (1)	Width of the corpus (2)	Depth of the corpus (3)	Shape of the corpus (2/3 ratio)	Distance between the sacci (4)
P. mugo DG	66.83 ± 3.0 c-f 60.06-74.36	48.83 ± 2.9 b-e 41.89-56.02	43.00 ± 2.1 c-g 40.04-45.76	1.14 ± 0.09 a-d 1.02-1.40	15.73 ± 2.2 bcd 11.44-20.02
P. mugo FA	69.78 ± 3.5 g 62.92-77.22	49.88 ± 2.8 c-f 44.05-57.52	45.28 ± 2.4 g-j 40.04-48.62	1.10 ± 0.09 abc 0.97-1.36	18.02 ± 2.1 e 11.44-20.02
P. sylvestris FA	65.49 ± 3.9 b-e 57.20-71.50	48.58 ± 3.3 a-e 39.23-53.91	42.80 ± 2.3 c-f 37.18-45.76	1.15 ± 0.10 bcd 0.86-1.33	17.73 ± 2.5 e 14.30-22.88
P. ×rhaetica DG	66.92 ± 2.1 c-f 62.92-74.6	46.47 ± 3.5 ab 39.66-54.24	43.38 ± 2.7 d-h 40.04-48.62	1.09 ± 0.11 ab 0.94-1.35	14.78 ± 1.7 ab 11.44-17.16
P. ×rhaetica no. 1 FA	69.21 ± 3.6 fg 62.92-71.50	50.40 ± 2.2 d-g 45.20-55.05	45.95 ± 3.6 j 37.18-54.34	1.12 ± 0.09 abc 0.95-1.27	14.40 ± 1.8 ab 11.44-17.16
P. ×rhaetica no. 2 FA	64.35 ± 3.0 abc 62-92-80.08	47.05 ± 2.9 abc 39.61-52.89	43.57 ± 2.5 e-i 40.04-48.62	1.06 ± 0.06 a 0.97-1.18	14.20 ± 1.6 ab 11.44-17.16
P. ×rhaetica no. 3 FA	66.45 ± 2.9 c-f 57.20-68.64	49.28 ± 3.0 b-f 43.22-55.50	44.81 ± 2.7 f-j 40.04-48.62	1.11 ± 0.11 abc 0.93-1.35	15.35 ± 2.1 abc 11.44-20.02
P. ×rhaetica no. 4 FA	63.59 ± 3.0 ab 60.06-71.50	47.85 ± 3.0 a-d 42.01-58.10	41.09 ± 2.5 a-d 37.18-45.76	1.17 ± 0.13 cde 0.96-1.56	17.06 ± 1.9 cde 14.30-20.02
P. ×rhaetica no. 5 FA	66.83 ± 2.2 c-f 60.06-71.50	47.17 ± 3.2 abc 40.23-53.71	45.00 ± 2.0 f-j 40.04-48.62	1.06 ± 0.09 a 0.93-1.25	17.35 ± 1.7 de 14.30-22.88
P. ×rhaetica no. 6 FA	69.21 ± 3.1 fg 62.92-77.22	52.09 ± 1.7 fgh 48.66-56.40	45.66 ± 2.2 hij 40.04-48.62	1.16 ± 0.05 b-e 1.06-1.24	13.63 ± 2.5 a 8.58-17.16
P. ×rhaetica no. 7 FA	68.16 ± 3.0 efg 62.92-74.36	51.48 ± 2.2 efg 47.04-56.65	45.86 ± 2.7 ij 40.04-51.48	1.14 ± 0.09 a-d 1.01-1.27	14.01 ± 1.9 ab 11.44-17.16
P. ×rhaetica no. 8 FA	64.83 ± 2.8 a-d 60.06-71.50	52.36 ± 2.8 fgh 45.55-57.08	45.00 ± 2.9 f-j 40.04-51.48	1.17 ± 0.10 cde 1.00-1.34	15.35 ± 1.6 abc 11.44-17.16
P. ×rhaetica no. 9 FA	67.69 ± 2.5 efg 62.92-74.3	53.28 ± 4.4 ghi 40.86-61.29	45.86 ± 2.3 ij 40.04-48.62	1.15 ± 0.11 bcd 0.89-1.36	14.97 ± 1.6 ab 11.44-17.16
P. ×rhaetica no. 10 FA	64.64 ± 3.9 a-d 60.06-71.50	45.63 ± 1.4 a 42.89-47.48	40.14 ± 2.7 ab 37.18-45.76	1.15 ± 0.08 bcd 0.95-1.28	16.78 ± 2.2 cde 11.44-20.02
P. ×rhaetica no. 11 FA	62.92 ± 2.9 ab 57.20-68.64	51.28 ± 4.2 efg 41.32-57.88	39.85 ± 2.6 a 34.32-45.76	1.25 ± 0.14 f 1.03-1.56	13.82 ± 1.1 a 11.44-14.30
P. ×rhaetica no. 12 FA	62.25 ± 3.1 a 57.20-68.64	49.79 ± 3.7 c-f 43.96-59.79	40.90 ± 2.0 abc 37.18-45.76	1.20 ± 0.09 def 1.02-1.34	14.49 ± 1.8 ab 11.44-17.16
P. ×rhaetica no. 13 FA	67.31 ± 2.5 d-g 62.92-71.50	56.14 ± 4.8 i 42.41-63.61	45.66 ± 2.1 hij 42.90-51.48	1.23 ± 0.11 ef 0.93-1.48	14.59 ± 1.9 ab 11.44-17.16
P. ×rhaetica no. 14 FA	63.21 ± 2.5 ab 57.20-68.64	50.32 ± 5.4 d-g 40.53-59.77	42.42 ± 3.0 b-e 37.18-48.62	1.17 ± 0.12 cde 0.94-1.32	17.64 ± 1.9 e 14.30-20.02
P. ×rhaetica no. 15 FA	67.40 ± 3.0 d-g 60.06-71.50	48.57 ± 4.7 a-e 39.90-56.50	45.76 ± 3.0 ij 40.04-51.48	1.09 ± 0.11 ab 0.92-1.26	14.40 ± 2.7 ab 11.44-20.02
P. ×rhaetica no. 16 FA	70.07 ± 3.7 g 62.92-77.22	55.11 ± 3.7 hi 48.23-62.01	46.62 ± 2.5 j 42.90-51.48	1.18 ± 0.10 c-f 0.99-1.42	13.73 ± 1.6 a 11.44-17.16
ANOVA	F = 18.09 P < 0.01	F = 19.65 P < 0.01	F = 20.68 P < 0.01	F = 9.95 P < 0.01	F = 17.36 P < 0.01

agreement with the observations made by Kormuťák et al. (2008). Apart from that, the corpus width values we measured in polar view were much smaller than the values reported by Chira (1971), but they were greater than the values reported by Nakagava et al. (2000) and by Kormuťák et al. (2008). According to these authors, the corpus width values ranged from 62.7 to 85.8 μm (non-acetolysed pollen grains), where the mean width of acetolysed pollen grains was 44 μm and 43.58 μm, respectively. In comparison with the values reported by Kormuťák et al. (2008), the *P. sylvestris* pollen corpora in our study were bigger and the width/depth ratio was much lower. The authors did

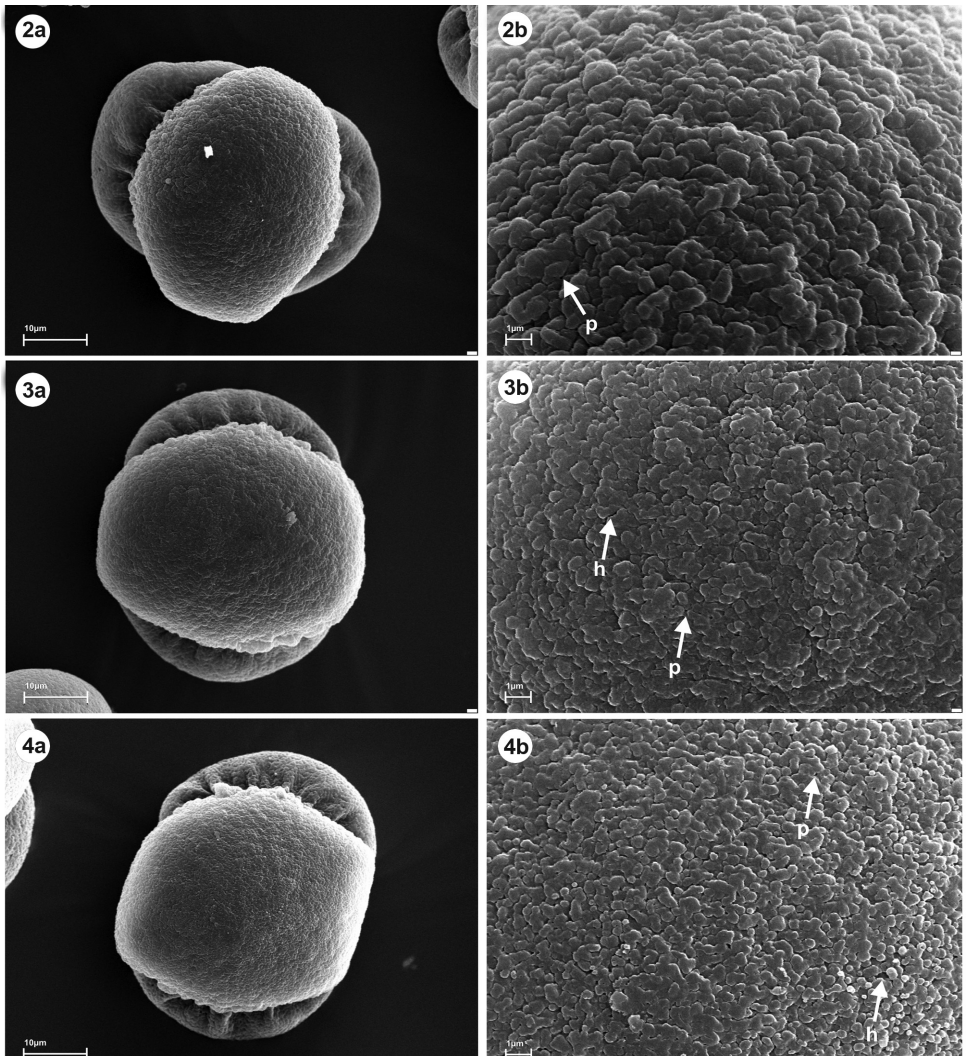
TABLE 2. Continued

Taxon	Left saccus			Right saccus		
	Width (5)	Depth (6)	Shape (5/6 ratio)	Width (7)	Depth (8)	Shape (7/8 ratio)
P. mugo DG	35.56 ± 2.2 b-h 31.46-40.04	25.74 ± 1.8 cde 22.88-28.60	1.39 ± 0.12 a-e 1.20-1.75	35.65 ± 2.7 b-f 31.46-40.04	25.36 ± 2.1 c-f 20.02-28.60	1.41 ± 0.12 a-e 1.20-1.71
P. mugo FA	33.94 ± 2.5 abc 28.60-37.18	26.12 ± 2.3 de 22.88-28.60	1.31 ± 0.12 a 1.11-1.50	34.32 ± 2.6 a-d 28.60-40.04	25.74 ± 2.3 d-g 22.88-28.60	1.34 ± 0.14 a 1.10-1.63
P. sylvestris FA	33.94 ± 2.3 abc 28.60-37.18	23.55 ± 2.2 ab 20.02-28.60	1.45 ± 0.12 c-f 1.25-1.71	34.13 ± 2.4 abc 28.60-37.18	24.12 ± 2.2 a-d 20.02-28.60	1.42 ± 0.13 a-f 1.20-1.71
P. ×rhaetica DG	36.42 ± 2.6 d-i 31.46-42.90	25.93 ± 1.7 de 22.88-28.60	1.41 ± 0.11 a-f 1.20-1.41	36.42 ± 1.7 d-g 34.32-40.04	26.22 ± 1.7 efg 22.88-28.60	1.39 ± 0.09 a-d 1.20-1.39
P. ×rhaetica no. 1 FA	39.75 ± 1.9 j 37.18-42.90	27.27 ± 2.1 efg 22.88-31.46	1.46 ± 0.10 def 1.27-1.63	40.99 ± 2.3 h 37.18-45.76	27.55 ± 2.4 gh 22.88-34.32	1.49 ± 0.11 def 1.30-1.75
P. ×rhaetica no. 2 FA	34.42 ± 2.3 a-e 28.60-37.18	24.88 ± 1.9 bcd 22.88-28.60	1.39 ± 0.08 a-e 1.22-1.50	34.13 ± 2.1 abc 28.60-37.18	25.26 ± 1.7 c-f 22.88-28.60	1.35 ± 0.10 ab 1.11-1.50
P. ×rhaetica no. 3 FA	37.66 ± 2.9 hij 34.32-45.76	25.36 ± 1.6 cd 20.02-28.60	1.49 ± 0.11 ef 1.33-1.78	37.94 ± 2.2 g 31.46-42.90	25.74 ± 2.3 d-g 20.02-28.60	1.48 ± 0.12 c-f 1.30-1.86
P. ×rhaetica no. 4 FA	34.61 ± 2.2 a-f 31.46-37.18	23.26 ± 2.1 ab 17.16-28.60	1.50 ± 0.15 f 1.22-1.86	35.08 ± 2.4 b-f 31.46-40.04	23.26 ± 2.1 ab 20.02-28.60	1.52 ± 0.18 f 1.33-2.00
P. ×rhaetica no. 5 FA	36.80 ± 2.1 f-i 34.32-40.04	24.98 ± 1.7 bcd 22.88-28.60	1.48 ± 0.12 ef 1.30-1.75	36.89 ± 1.7 efg 34.32-40.04	24.50 ± 1.4 a-e 22.88-25.74	1.51 ± 0.09 ef 1.33-1.75
P. ×rhaetica no. 6 FA	37.56 ± 2.7 g-j 34.32-42.90	28.03 ± 2.2 fg 25.74-31.46	1.34 ± 0.09 ab 1.09-1.56	37.08 ± 2.2 fg 31.46-40.04	27.55 ± 2.2 gh 22.88-31.46	1.35 ± 0.12 a 1.18-1.63
P. ×rhaetica no.7 FA	36.61 ± 2.4 e-i 31.46-42.90	27.17 ± 1.6 efg 22.88-28.60	1.35 ± 0.09 abc 1.20-1.56	36.32 ± 3.1 c-g 31.46-48.62	26.98 ± 1.9 fgh 22.88-31.46	1.35 ± 0.10 a 1.18-1.70
P. ×rhaetica no. 8 FA	35.37 ± 2.7 b-g 31.46-40.04	24.88 ± 1.5 bcd 22.88-28.60	1.42 ± 0.10 b-f 1.22-1.75	35.18 ± 2.6 b-f 31.46-42.90	24.60 ± 1.6 b-e 22.88-28.60	1.44 ± 0.14 a-f 1.22-1.88
P. ×rhaetica no. 9 FA	36.70 ± 2.1 f-i 31.46-40.04	25.93 ± 1.7 de 22.88-28.60	1.42 ± 0.10 b-f 1.20-1.63	36.23 ± 2.6 c-g 28.60-40.04	26.79 ± 1.9 fgh 22.88-31.46	1.35 ± 0.10 ab 1.11-1.56
P. ×rhaetica no. 10 FA	34.32 ± 2.7 a-d 31.46-40.04	24.02 ± 2.6 abc 20.02-28.60	1.43 ± 0.10 b-f 1.20-1.63	34.70 ± 2.3 a-e 31.46-40.04	23.83 ± 1.9 abc 20.02-25.74	1.46 ± 0.11 b-f 1.33-1.71
P. ×rhaetica no. 11 FA	33.37 ± 2.4 ab 28.60-40.04	24.79 ± 1.7 bcd 22.88-28.60	1.35 ± 0.07 abc 1.22-1.50	33.56 ± 2.0 ab 31.46-40.04	24.31 ± 1.6 a-d 22.88-28.60	1.38 ± 0.09 abc 1.22-1.50
P. ×rhaetica no. 12 FA	32.70 ± 2.2 a 28.60-37.18	23.55 ± 1.8 ab 20.02-28.60	1.39 ± 0.12 a-e 1.20-1.71	32.60 ± 1.9 a 28.60-37.18	24.21 ± 2.3 a-d 20.02-28.60	1.35 ± 0.12 ab 1.20-1.57
P. ×rhaetica no. 13 FA	36.04 ± 2.9 c-i 22.88-28.60	26.41 ± 1.8 d-g 31.46-42.90	1.37 ± 0.13 a-d 1.20-1.88	36.23 ± 2.8 c-g 22.88-31.46	26.31 ± 1.7 efg 11.44-17.16	1.38 ± 0.13 abc 1.09-1.67
P. ×rhaetica no. 14 FA	33.56 ± 2.2 ab 20.02-28.60	22.88 ± 1.8 a 28.60-40.04	1.47 ± 0.12 ef 1.25-1.71	33.46 ± 2.7 ab 20.02-25.74	22.69 ± 2.0 a 14.30-20.02	1.48 ± 0.11 c-f 1.33-1.71
P. ×rhaetica no. 15 FA	37.08 ± 2.1 ghi 22.88-28.60	26.31 ± 2.3 def 34.32-42.90	1.42 ± 0.15 b-f 1.20-1.75	37.28 ± 2.3 fg 22.88-28.60	26.69 ± 1.7 fgh 11.44-20.02	1.40 ± 0.11 a-d 1.20-1.63
P. ×rhaetica no. 16 FA	38.13 ± 2.3 ij 22.88-31.46	28.12 ± 2.1 g 31.46-42.90	1.36 ± 0.10 a-d 1.18-1.56	37.94 ± 2.7 g 22.88-31.46	28.22 ± 2.3 h 11.44-17.16	1.35 ± 0.08 a 1.18-1.50
ANOVA	F = 17.92 P < 0.01	F = 18.58 P < 0.01	F = 6.98 P < 0.01	F = 19.94 P < 0.01	F = 17.84 P < 0.01	F = 8.07 P < 0.01

One way ANOVAs were performed separately for each features to determine the differences among taxa studied. Same letters indicate a lack of statistically significant differences between analyzed taxa according to Tukey's a posteriori test (P<0.05).
Abbreviations: DG - Dendrological Garden, Poznań University of Life Sciences; FA - Forest Arboretum in Zielonka

not notice any differences from the *P. mugo* in the latter trait.

The cappa exine sculpture described by Nakagava et al. (2000) was the same as in our study, i.e. verrucate to rugulate. The authors illustrated the corpus ornamentation, and the tectum granules were clearly visible. Our study showed that the tectum surface was smooth, without granules or with



Figs. 2–4. Scanning electron micrographs (SEM) of pollen grains in polar view. **Fig. 2**. *Pinus mugo* DG. **Fig. 3**. *P. mugo* FA. **Fig. 4**. *P. sylvestris* FA. DG – Dendrological Garden; FA – Forest Arboretum; h – holes; p – puncta.

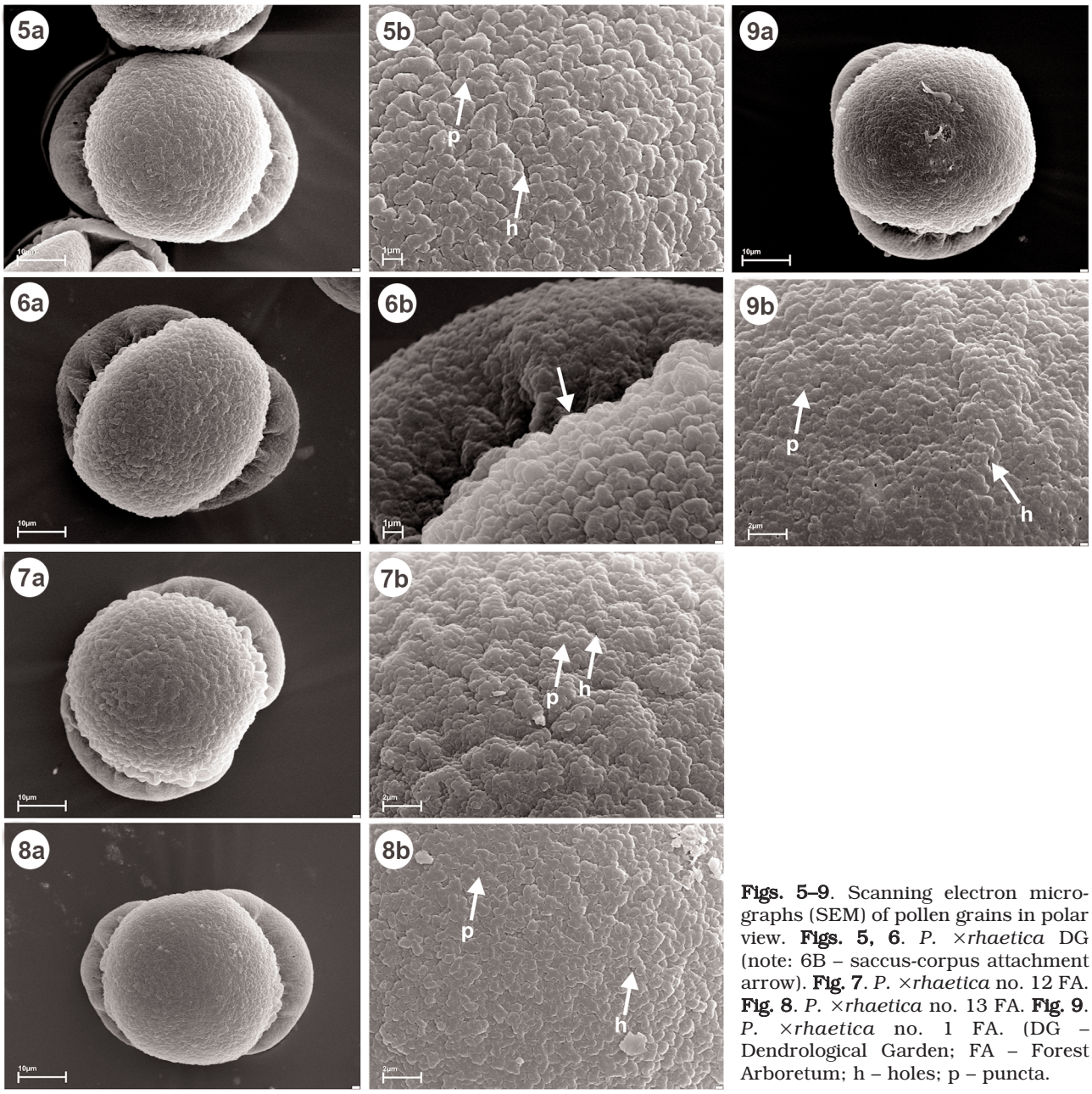
simple, poorly visible granules. Klaus (1978) reported that the cappa shape was irregular, polygonal and there were numerous microgranula. Some perforations did occur, but very seldom. The chamber cappae on the saccus were occasionally flattened, with some microgranules present. There may be absolutely no perforations or there may be moderate numbers of them.

HYBRID *PINUS* ×*RHAETICA*

Seventeen specimens of *P.* ×*rhaetica* were studied. The pines were grown in two stands, but all of them came from the 'Bór na Czerwonem' reserve. There were significant differences in the size of pollen grains between individual specimens. Apart from that, we observed that some hybrids were similar to the other pine species under study.

The total width of the *P.* ×*rhaetica* pollen grains with two sacci ranged from 62.25 μm to 70.07 μm on average. The pollen grain corpus ranged from 45.63 to 56.14 μm in width and from 39.85 to 46.62 μm in depth (mean values). The shape of the pollen grain corpus was identified as subprolate (Figs. 6A) in 10 specimens, whereas in the other 7 specimens it was identified as prolate-spheroidal (Figs. 5, 7, 9). On average, the air sacci were 32.60–40.99 μm wide and 22.69–28.22 μm deep. Their shape was identified as prolate in all the 17 specimens, although the values of the width/depth of the corpus ratio were variable. The average distance between the left and right saccus ranged from 13.63 to 17.64 μm.

Of the 17 pine specimens, no. 1 FA and no. 6 FA were the most similar to each other in terms of the pollen grain size (Tab. 2). *P.* ×*rhaetica*



Figs. 5–9. Scanning electron micrographs (SEM) of pollen grains in polar view. **Figs. 5, 6**. *P.* ×*rhaetica* DG (note: 6B – saccus-corpus attachment arrow). **Fig. 7**. *P.* ×*rhaetica* no. 12 FA. **Fig. 8**. *P.* ×*rhaetica* no. 13 FA. **Fig. 9**. *P.* ×*rhaetica* no. 1 FA. (DG – Dendrological Garden; FA – Forest Arboretum; h – holes; p – puncta.

no. 16 FA was distinguished by the large size of its pollen grains and sacci. The only exception was the distance between the sacci. The average value of this trait (13.73 μm) was one of the lowest values, and it was significantly different from the distances between the sacci noted in *P. mugo* and *P. sylvestris*. A similar short distance between the sacci could be observed only in *P.* ×*rhaetica* no. 6 FA and 11 FA (Tab. 2). *P.* ×*rhaetica* no. 1 FA was distinguished by big sacci. On the other hand, the sizes of the pollen grains and sacci in *P.* ×*rhaetica* DG and shrubs no. 3 FA and 5 FA were very similar to those in *P. mugo* DG and *P. sylvestris*.

The corpus exine on the cappa was irregularly verrucate-rugulate and it was deeply sculptured (Figs. 5–9). There might be some granulations present on the cappa (Figs. 5b, 6b, 8b, 9b), but they were few in number (Figs. 7b) or almost invisible (Fig. 6b). The caps separated from each other and a few chambers fused. The saccus ornamentation was reticulate. In the middle part the saccus was flat and slightly undulate on the margin. The surface of the saccus in the middle part was psilate-perforate

TABLE 3. A comparison of separate character values of *Pinus mugo* reported by the authors cited and the values found in our study.

				Characters					
	Pollen grain view	Pollen grain acetolysis	Type of microscope	Pollen grain width (µm)	Corpus width (µm)	Corpus depth (µm)	Corpus shape (width/ depth ratio)	Cappa exine sculpture	Saccus sexine sculpture
Chira (1971)	–	non-acetolysed	–	–	62.70-75.90	–	–	–	–
Klaus (1978)	–	acetolysed	SEM	–	–	–	–	no microgranula or very weakly developed; micrograna – clear, irregularly distributed; almost no perforations	–
Christensen (1987c)	–	–	–	68.00-86.70; mean 76.80	–	–	–	–	–
Nakagava et al. (2000)	equatorial	acetolysed	SEM	–	–	–	–	–	–
Kormuťák et al. (2008)	equatorial	acetolysed	LM	–	mean 45.47	mean 35.21	mean 1.30	–	–
our study	polar	non-acetolysed	LM* SEM	60.06-77.22; mean 68.30*	41.89-57.52; mean 49.35*	40.04-48.62; mean 44.14*	0.97-1.40; mean 1.12*	verrucate-rugulate; perforate; micrograna – very sparse, irregularly distributed	reticulate; perforate; puncta and small holes

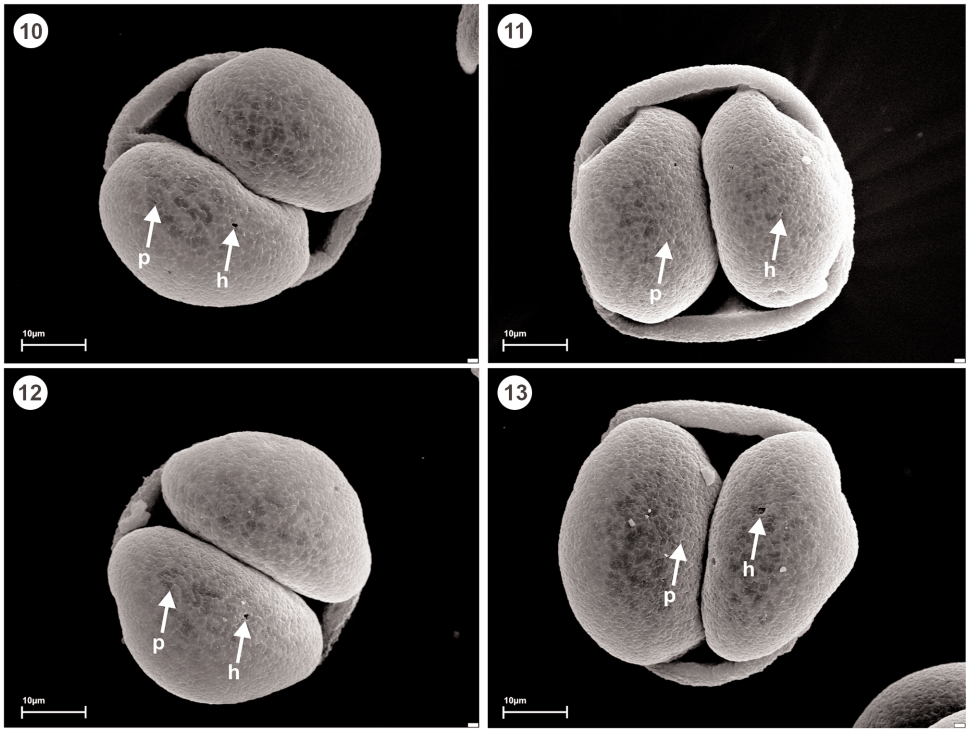
TABLE 4. A comparison of separate character values of *Pinus sylvestris* reported by the authors cited and the values found in our study.

				Characters					
	Pollen grain view	Pollen grain acetolysis	Type of microscope	Pollen grain width (µm)	Corpus width (µm)	Corpus depth (µm)	Corpus shape (width/ depth ratio)	Cappa exine sculpture	Saccus sexine sculpture
Chira (1971)	–	non-acetolysed	–	–	62.70-85.80	–	–	–	–
Klaus (1978)	–	acetolysed	SEM	–	–	–	–	numerous microgranula; almost no micrograna; very rare perforations	no micrograna; moderate or no perforations
Christensen (1987c)	–	–	–	71.40-88.40; mean 80.40	–	–	–	–	–
Nakagava et al. (2000)	equatorial	acetolysed	SEM	–	40.00-47.00; mean 44.00	–	–	verrucate to rugulate, deeply sculptured	–
Kormuťák et al. (2008)	equatorial	acetolysed	LM	–	mean 43.58	mean 33.75	mean 1.30	–	–
our study	polar	non-acetolysed	LM* SEM	57.20-71.50; mean 65.49*	39.23-53.91; mean 48.58*	37.18-45.76; mean 42.80*	0.86-1.33; mean 1.15*	irregularly verrucate-rugulate; perforate with small granules	reticulate; numerous perforations; punctate; sparse grana

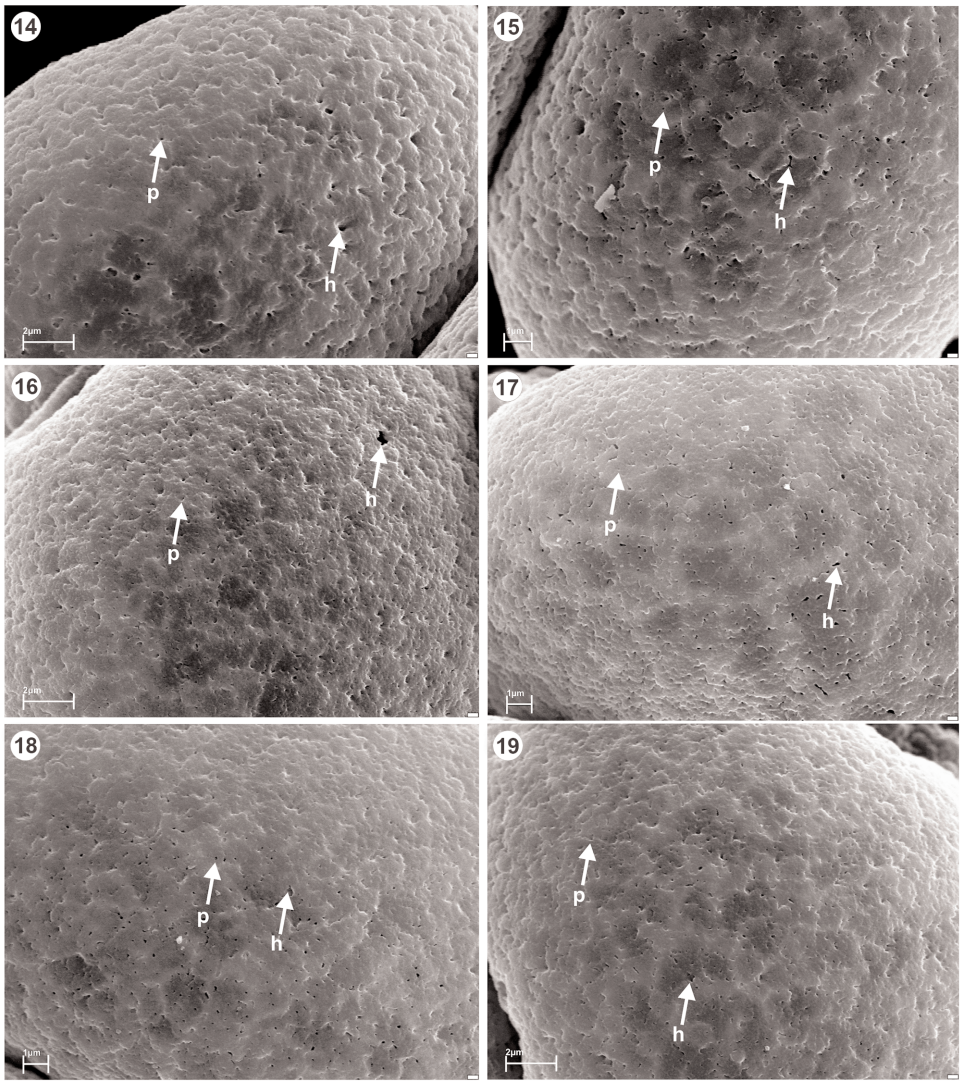
TABLE 5. A comparison of separate character values of *Pinus* ×*rhaetica* reported by the authors cited and the values found in our study.

				Characters					
	Pollen grain view	Pollen grain acetolysis	Type of microscope	Pollen grain width (µm)	Corpus width (µm)	Corpus depth (µm)	Corpus shape (width/ depth ratio)	Cappa exine sculpture	Saccus sexine sculpture
Chira (1971)	–	non-acetolysed	–	–	–	–	–	–	–
Klaus (1978)	–	acetolysed	SEM	–	–	–	–	–	–
Christensen (1987c)	–	–	–	68.00-88.40; mean 80.10	–	–	–	–	–
Nakagava et al. (2000)	equatorial	acetolysed	SEM	–	–	–	–	–	–
Kormuťák et al. (2008)	equatorial	acetolysed	LM	–	mean 42.42	mean 33.41	mean 1.28	–	–
our study	polar	non-acetolysed	LM* SEM	57.20-80.08; mean 66.18*	39.61-63.61; mean 50.25*	34.32-54.34; mean 43.97*	0.89-1.56; mean 1.15*	irregularly verrucate-rugulate; deeply sculptured; rare granulations or almost invisible	reticulate; variable perforations; puncta and small holes

Abbreviations (for tables 3–5): LM - Light Microscope; SEM - Scanning Electron Microscopy



Figs. 10–13. Scanning electron micrograph (SEM) of pollen grains on distal surface. **Fig. 10**. *Pinus mugo* DG. **Fig. 11**. *P. sylvestris* FA. **Fig. 12**. *P.* ×*rhaetica* DG. **Fig. 13**. *P.* ×*rhaetica* no. 12 FA. DG – Dendrological Garden; FA – Forest Arboretum; h – holes; p – puncta.



Figs. 14–19. Scanning electron micrographs (SEM) of saccus ornamentation. **Fig. 14**. *Pinus mugo* DG. **Fig. 15**. *P. mugo* FA. **Fig. 16**. *P. sylvestris* FA. **Fig. 17**. *P.* ×*rhaetica* DG. **Fig. 18**. *P.* ×*rhaetica* no. 11 FA. **Fig. 19**. *P.* ×*rhaetica* no. 12 FA. DG – Dendrological Garden; FA – Forest Arboretum; h – holes; p – puncta.

(Figs. 17–19). The perforation was variable in size and shape. We distinguished two size categories: puncta and small holes (Figs. 17–19).

Christensen (1987c) reported that the width of pollen grains (from saccus to saccus) ranged from 68.0 to 88.4 μm. In our study the average width of all grains ranged from 62 to 70 μm. Kormuťák et al. (2008) reported that the corpus width was 42.42 μm and the depth was 33.41 μm. The ratio of both traits was 1.28. The corpora of the pollen grains of the *P.* ×*rhaetica* specimens under study were larger (taking mean values into consideration). We noted the same value as the one reported by Kormuťák et al. (2008) for the corpus width to depth ratio (1.25 on average) only in one specimen (no. 11 FA). The values of this trait were smaller in the other hybrids (Tab. 5).

CONCLUSIONS

To conclude, our study of the pollen grain morphology of *P. mugo* × *P. sylvestris* hybrids and parental species provided some important new data concerning the corpus, saccus, their size and shape, distance between the saccus and the ornamentation on the proximal surface of the corpus and saccus. The biometric measurements of the eight quantitative traits were helpful for the identification and the differences in the size and shape of the pollen grains and saccus were statistically significant. In comparison with the parental species, the hybrids exhibit an extended range of variation in many features. The shape of the corpus in *P. mugo* × *P. sylvestris* was prolate-spheroidal and subprolate, whereas in

P. mugo it was prolate-spheroidal and subprolate and in *P. sylvestris* it was subprolate. The tectum surface characters in proximal view of the corpus and saccus were less variable and they were not a good criterion for identification of the species under study. However, these species differed in the height and shape of elevations on the corpuses. Our study shows that several morphological characters of pollen can be of taxonomical value. Thus, this detailed analysis has greatly increased our knowledge of individual species.

AUTHORS' CONTRIBUTIONS

Both authors contributed to the conception of the research as well as collection, analysis and data interpretation. JB material collection, morphological analysis and text editing; MK micromorphological studies, photographs and text editing. The authors declare that there are no conflicts of interest.

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