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POLLEN MORPHOLOGY OF *PINUS ULIGINOSA* NEUM. AND *PINUS UNCINATA* RAMOND EX DC IN AN EXPERIMENTAL CULTURE

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Abstract. Pollen grains of *Pinus uliginosa* and *P. uncinata* 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 with or without 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. Among the *P. uncinata* from the Forest Arboretum there were differences observed in the size, shape and height of elevation and sculpture on the corpus between pollen grains of the same specimen. This study of the pollen grain morphology of the corpus and saccus provided some important new data.

Key words: *Pinus uliginosa*, *Pinus uncinata*, pollen morphology, LM, SEM

INTRODUCTION

Pinus uliginosa and *Pinus uncinata* are two of more than a dozen pine species that can naturally be found in Europe (Staszkiewicz 1966, Seneta 1987). Their taxonomic position is complicated and it has not been fully determined. Over the years they were treated as separate species or they were included into the *Pinus mugo* complex due to suggestions pointing to their hybrid origin. Staszkiewicz & Tyszkiewicz (1972) found *Pinus uncinata* and *Pinus mugo* to be strongly related species, whereas Szweykowski (1969) called the population of *Pinus uliginosa* from the Great Peat Bog of Batorów a relic of a hybrid swarm of *Pinus mugo* and *Pinus sylvestris*. On the other hand, Krzakowa et al. (1984) hypothesised that *P. uliginosa* might be an isolated, marginal population of *P. uncinata*.

The literature provides numerous elaborations which investigate and/or determine the range of variability of *P. uliginosa* and *P. uncinata*. Studies on the morphology and anatomy of *P. uliginosa* and *P. sylvestris* needles were conducted by Boratyńska & Lewandowska (2009) and Boratyńska et al. (2011), whereas Sobierajska & Boratyńska (2008) studied *P. mugo* needles. Studies on the *P. uliginosa* cone morphology were conducted by Marcysiak et al. (2003), whereas Marcysiak (2004) studied *P. uncinata* cones. According to Boratyńska et al. (2011), the combination of needle and cone morphological traits is a good tool for distinguishing between *P. sylvestris*, *P. uncinata*, *P. mugo* and *P. uliginosa* with very high probability.

The palynological literature provides little data concerning the size 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 of *P. uncinata* collected in the Pyrenees on the eastern French side near Col de Llose (1740 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 *P. uncinata* from the southern French Alps. No information on the micromorphology of *P. uliginosa* pollen has been found.

The main aims of this study were to: 1. describe variation in the pollen grain morphology of *P. uliginosa* and *P. uncinata*; 2. describe and document the pollen grain micromorphology; 3. supply new data which can be used for identification of the species under study.

Table 1. Mean values (±SE) and ranges (minimum-maximum) of the morphological traits of *Pinus uliginosa* and *P. uncinata* pollen grain and saccus

Character	*P. uliginosa* (DG)	*P. uncinata* (DG)	*P. uncinata* (FA)	ANOVA
Pollen grain				
Width of the pollen grain (μm)	69.02±2.5 b 62.92–80.08	65.11±2.8 a 60.06–68.64	73.50±3.4 c 65.78–80.08	F = 63.09 P < 0.01
Width of the corpus (μm)	47.78±4.8 a 33.84–55.29	50.79±4.9 b 36.28–58.95	58.81±3.7 c 50.57–65.63	F = 48.03 P < 0.01
Depth of the corpus (μm)	46.05±2.3 a 40.04–48.62	45.86±2.3 a 42.90–51.48	49.19±3.1 b 40.04–54.34	F = 15.46 P < 0.01
Shape of the corpus (width/depth ratio)	1.05±0.13 a 0.74–1.28	1.10±0.14 b 0.85–1.37	1.19±0.09 c 0.99–1.35	F = 15.18 P < 0.01
Distance between the sacci (μm)	17.64±2.0 b 14.30–20.02	15.73±2.1 a 11.44–20.02	15.63±1.8 a 14.30–20.02	F = 9.89 P < 0.01
Left saccus				
Width (μm)	36.04±2.8 b 31.46–42.90	34.42±2.3 a 31.46–37.18	39.66±2.5 c 34.32–45.76	F = 34.00 P < 0.01
Depth (μm)	25.55±2.4 a 22.88–31.46	24.79±1.9 a 22.88–28.60	28.70±1.6 b 25.74–31.46	F = 33.01 P < 0.01
Shape (width/depth ratio)	1.42±0.12 a 1.10–1.63	1.39±0.10 a 1.20–1.50	1.38±0.08 a 1.27–1.60	F = 0.84 P = 0.44
Right saccus				
Width (μm)	36.80±2.3 b 31.46–42.90	34.99±2.1 a 31.46–40.04	39.85±2.9 c 37.18–45.76	F = 29.78 P < 0.01
Depth (μm)	25.83±1.4 b 22.88–28.60	24.60±1.4 a 22.88–25.74	29.17±2.0 c 25.74–34.32	F = 61.70 P < 0.01
Shape (width/depth ratio)	1.43±0.10 a 1.22–1.63	1.42±0.10 a 1.33–1.75	1.37±0.09 a 1.18–1.56	F = 3.48 P = 0.04

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 analysed taxa according to Tukey's a posteriori test (P < 0.05).

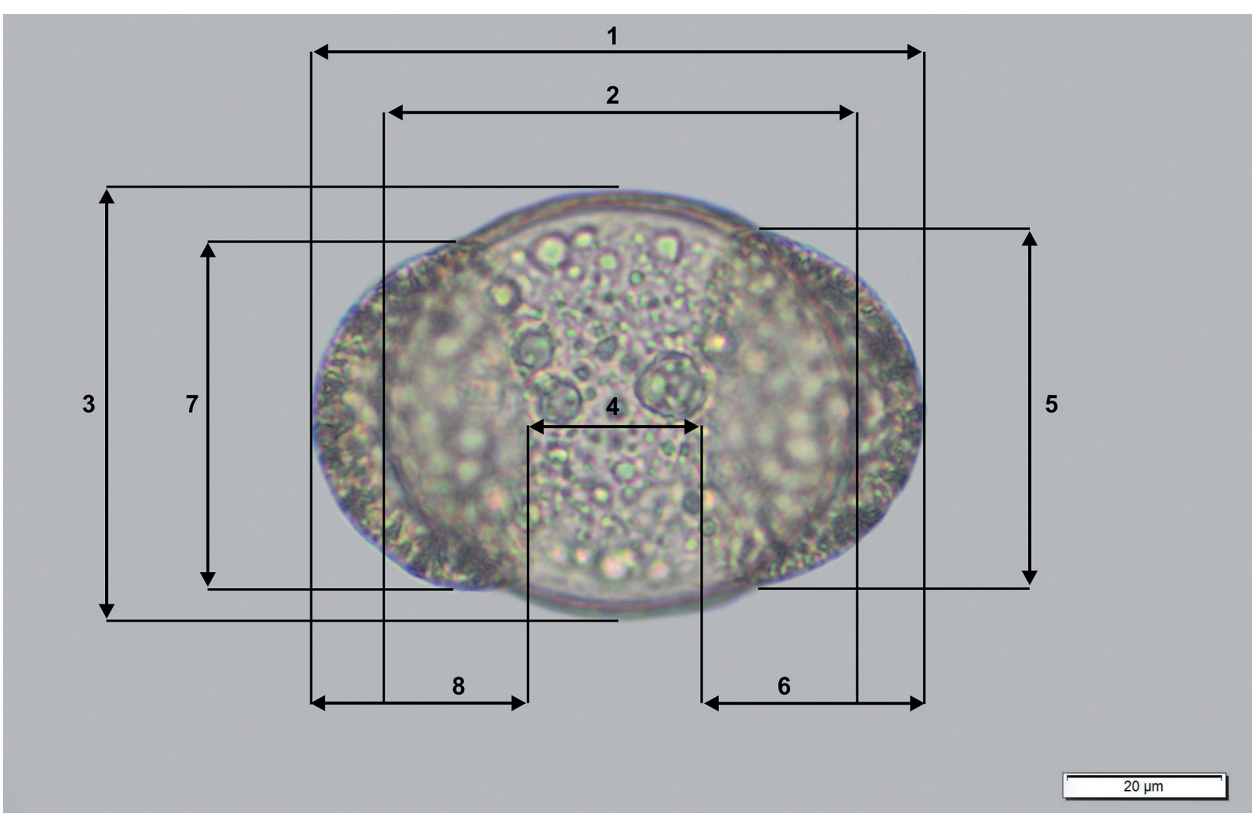

Fig. 1. Pollen grain dimension measurement method: 1 – pollen grain width, 2 – corpus width, 3 – corpus depth, 4 – distance between sacci, 5 – right saccus width, 6 – right saccus depth, 7 – left saccus width, 8 – left saccus depth (after Erdtman 1957, modified)

MATERIAL AND METHODS

Pollen grains were collected from three pine specimens growing in an experimental plot in the Dendrological Garden, Poznań University of Life Sciences (DG) and the Forest Arboretum in Zielonka (FA), Poland. Two pines came from seeds collected from natural habitats. *Pinus uliginosa* (no. 0526) growing in the Dendrological Garden comes from the Nature Reserve Peat-Bog near Węgliniec, and *P. uncinata* growing in the Forest Arboretum was obtained from seeds collected in the Pyrenees. The origin of the third tree under analysis, i.e. *P. uncinata*, no. 0790, is unknown.

Each sample was represented by 30 pollen grains. Table 1 lists the traits measured (in the polar view), and Figure 1 shows the measurement method. The pollen terminology was adopted from Erdtman (1957), Kremp (1965), Bagnell (1975) and Fægri & Iversen (1975).

The pollen grains were examined with a light microscope Olympus BX SC30 (LM) and with a scanning electron microscope (SEM) to obtain comprehensive information about the general morphology, cappae exine sculpture and saccus sexine sculpture. The SEM investigation was conducted on the pollen grains which were dried in the air, whereas the LM pollen was treated with 10% KOH (Frederiksen 1978). 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 the proximal view of the corpus and saccus.

The biometric data were analysed statistically with STATISTICA 10 (StatSoft, Inc. 2011). 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 $\alpha = 0.05$.

RESULTS

PINUS ULIGINOSA (TABLE 1, FIG. 2)

The width of the pollen grains measured with both sacci ranged from 62.92 to 80.08 μm. On average the corpus was about 48 μm wide and about 46 μm deep. Its shape was prolate-spheroidal (Fig. 2A) (the average width/depth of corpus ratio was 1.05). Both prolate-shaped sacci were similar in size (Fig. 2E). The distance between them was very large, on average 17.64 μm.

The proximal exine surface of the cappae was verrucate-rugulate, perforate (Fig. 2B, D). There were very sparse micrograna irregularly distributed over the surface of the cappae. 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. 2B). The surface of the sacci was reticulate (Fig. 2E, F), slightly undulate in the region of the saccus attachment (Fig. 2A, C). The sexine sculpture of the sacci was perforate, small puncta and holes were found (Fig. 2F).

PINUS UNCINATA (TABLE 1, FIGS 3, 4)

We analysed the size of the *P. uncinata* pollen grains from two locations. There were surprising differences in the measurement results. The pollen grains collected in the Forest Arboretum in Zielonka were the biggest of all the pines under study – in terms of the total width (73.5 μm on average), the size of the corpus (58.81 × 49.19 μm on average) and the size of the sacci (about 40 × 29 μm on average). The pollen grains collected in the Dendrological Garden were significantly different. They were over 10% smaller: their average total width was 65.11 μm and the corpus was 50.79 × 45.86 μm in size. Apart from that, the corpus of the pollen grains of both *P. uncinata* specimens differed in shape. It was subprolate in the pine from the Forest Arboretum (the mean width/depth of corpus ratio is 1.19), whereas in the pine from the Dendrological Garden the shape was prolate-spheroidal (with the width/depth of corpus ratio of 1.10). The only trait where the measured values were similar was the distance between the sacci. On average it was 15.73 μm in *P. uncinata* DG and 15.63 μm in *P. uncinata* FA. However, it was much smaller than in *P. uliginosa*.

The proximal exine surface of the cappae was verrucate-rugulate, perforate, with (Fig. 3B, D) or without small granules (Fig. 4B, D). The pollen grains from the specimen in the Dendrological Garden had an exine elevation with most grana clearly visible but irregularly distributed over the surface of the cappae (Fig. 3B, D). In the pollen grains from the specimen from Zielonka (FA) there were differences observed in the size, shape and height of the elevation on the corpus and the number of puncta (Fig. 4B, D). The exine elevation (Fig. 4B) was similar to the exine from the specimen in the Dendrological Garden (Fig. 3B, D) but in some pollen grains the elevation was bigger and smooth (Fig. 4D). The height of the neighbouring caps was almost identical. The ornamentation of the sacci was similar, reticulate, irregularly perforate, and there were small puncta and small holes observed. There were more perforations than on the cappae (Figs 3F, 4F).

DISCUSSION

The available literature lacks detailed information on the morphology and micromorphology of *Pinus uliginosa* and *P. uncinata* pollen grains (Table 2 and 3).

As far as *P. uliginosa* is concerned, only Christensen (1987) reported that the width of pollen grains of this species (with both sacci) ranged from 68.0 to 86.7 μm, the mean value being 74.1 μm. Our findings were different, but it may have been so because the measurements were taken in a different view of the pollen grains (Table 2).

The comparison of the size of *P. uliginosa* pollen grains obtained in our research with the results obtained by Bykowska & Klimko (2015) for *P.* × *rhaetica*, which *P. uliginosa* is often identified with, and with its potential parental species, i.e. *P. mugo* and *P. sylvestris,* revealed that the average total width of the pollen

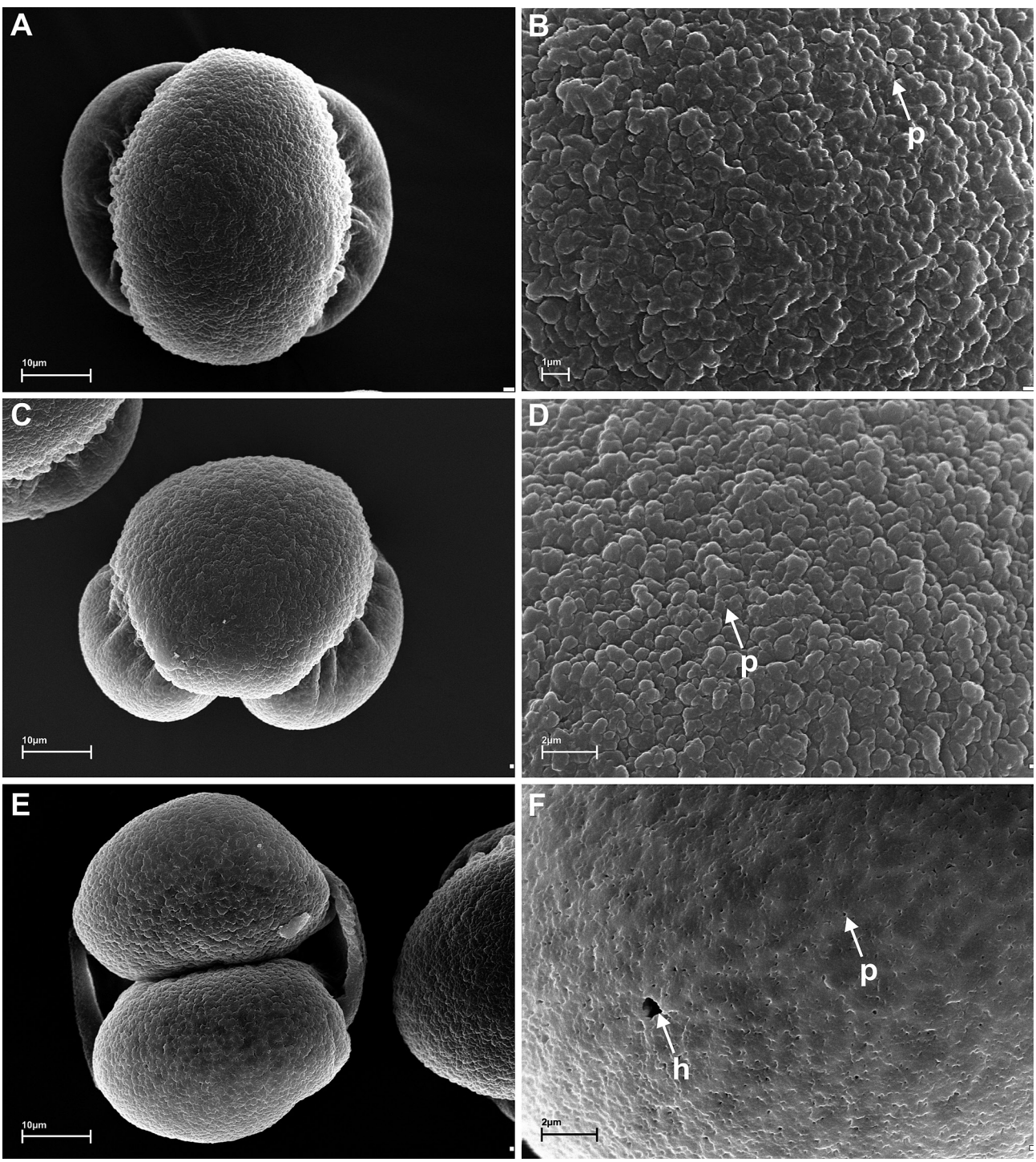

Fig. 2. Scanning electron micrographs (SEM) of pollen grains of *Pinus uliginosa* (DG): A – polar view, B – proximal polar area of corpus with exine ornamentation, C – equatorial view, D – proximal equatorial area of corpus with exine ornamentation, E – distal view, F – ornamentation of sacci (h – holes, p – puncta)

grains in *P. uliginosa* was similar to the average value in the *P. mugo* from the Forest Arboretum and *P.* × *rhaetica* no. 1 FA and 6 FA. The width of the corpus in *P. uliginosa* was similar to that in *P. mugo* DG, *P. sylvestris* and *P.* × *rhaetica* no. 2, 4, 5 and 15 from the Forest Arboretum, whereas the shape of its corpus was similar to that of *P.* × *rhaetica* no. 2 FA and 5 FA. The same distance between the sacci as in *P. uliginosa* (17.64 μm on average) was noted in *P.* × *rhaetica* No. 14 FA. Similar sizes were observed in *P. sylvestris* (17.73 μm), *P. mugo* FA (18.02 μm), *P.* × *rhaetica* no. 4 (17.06 μm) and no. 5 FA (17.35 μm).

Christensen (1987) and Businský (1999) observed that *P. uliginosa* was morphologically intermediate between *P. uncinata* and *P. mugo*. This study and the study by Bykowska & Klimko (2015) showed that

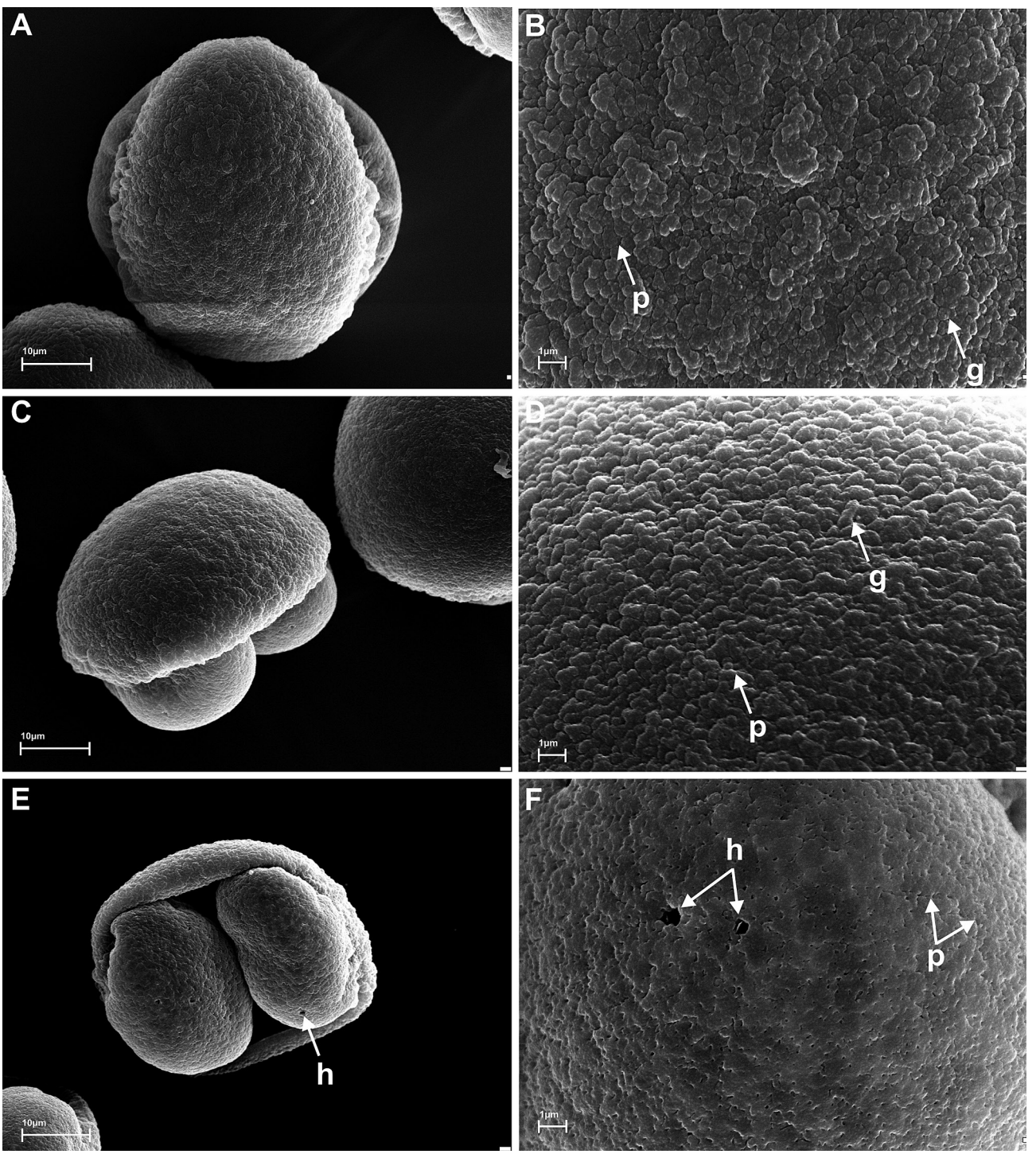

Fig. 3. Scanning electron micrographs (SEM) of pollen grains of *Pinus uncinata* (DG): A – polar view, B – proximal polar area of corpus with exine ornamentation, C – equatorial view, D – proximal equatorial area of corpus with exine ornamentation, E – distal view F – ornamentation of sacci (g – grana, h – holes, p – puncta)

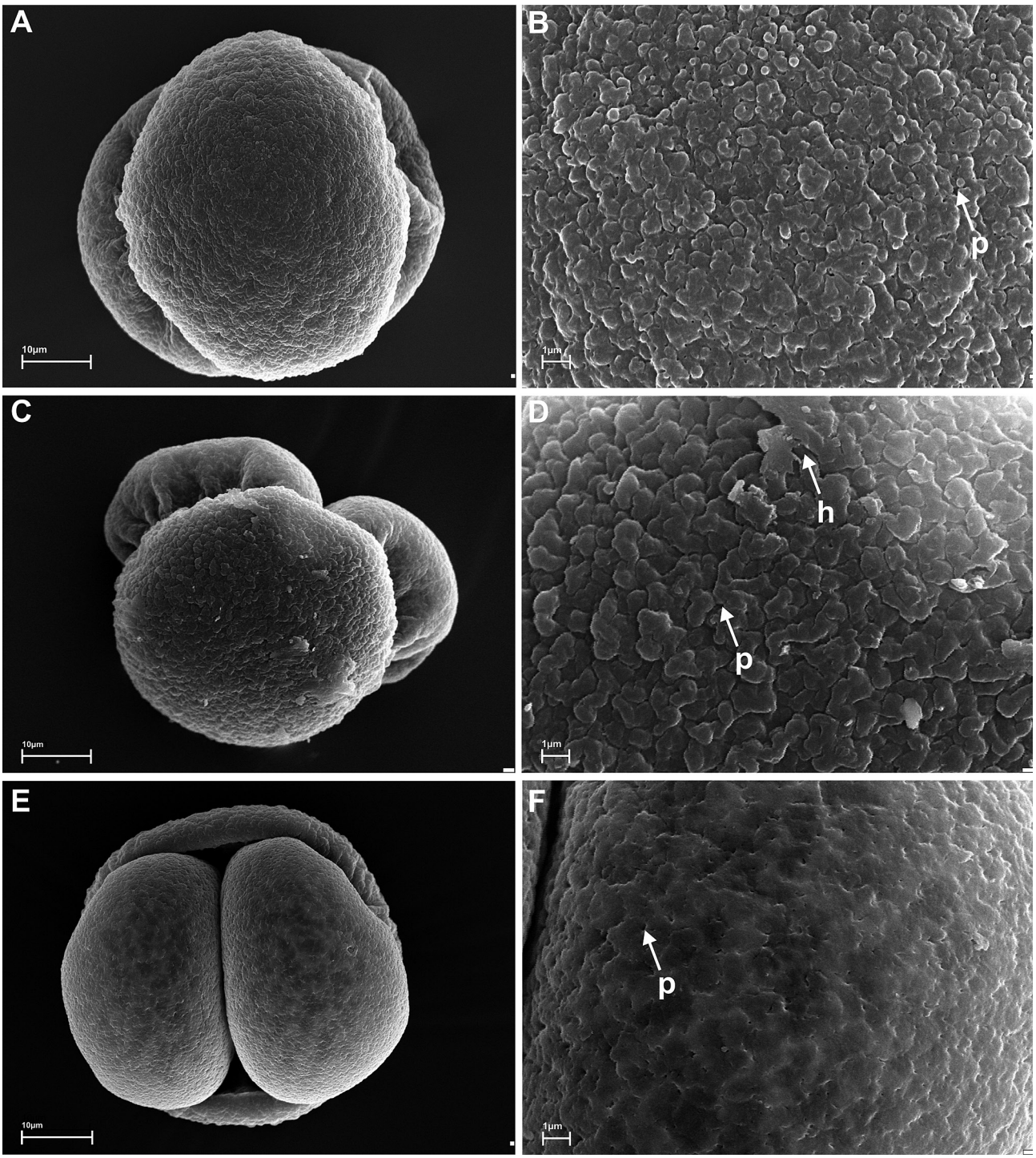

Fig. 4. Scanning electron micrographs (SEM) of pollen grains of *Pinus uncinata* (FA): A – polar view, B – proximal polar area of corpus with exine ornamentation, C – equatorial view, D – proximal equatorial area of corpus with exine ornamentation, E – distal view, F – ornamentation of sacci (h – holes, p – puncta)

only the size of the corpus (47.78 × 46.05 μm) of *P. uliginosa* was in the intermediate position. In *P. uncinata* the average size of the corpus was 54.80 × 47.52 μm, whereas in *P. mugo* it was 49.35 × 44.14 μm. There were relatively big differences in the distance between the sacci. The distance in *P. uliginosa* was 17.64 μm, whereas the average distance in *P. uncinata* was 15.68 μm, and in *P. mugo* it was 16.87 μm.

In contrast to *P. uliginosa,* the available literature provided more information on the *P. uncinata* pollen morphology. Christensen (1987) reported that the width of the *P. uncinata* pollen grains (with both sacci) ranged from 71.4 to 85.0 μm (mean 78.9 μm). In our study the pollen grains of both specimens were smaller. According to Nakagawa et al. (2000), in the equatorial view the corpus breadth of the acetolysed

Table 2. A comparison of individual trait values of *Pinus uliginosa* reported by the authors cited and the values found in our study

		Christensen (1987)	Our study
Pollen grain view		–	polar
Pollen grain acetolysis		–	non-acetolysed
Type of microscope		–	LM* SEM
Characters	Pollen grain width (μm)	68.00–86.70; mean 74.10	62.92–80.08; mean 69.02*
	Corpus width (μm)	–	33.84–55.29; mean 47.78*
	Corpus depth (μm)	–	40.04–48.62; mean 46.05*
	Corpus shape (width/depth ratio)	–	0.74–1.28; mean 1.05*
	Cappa exine sculpture	–	verrucate-rugulate; rarely granulate; slightly perforate on the proximal surface
	Saccus sexine sculpture	–	reticulate; slightly undulate; tectum perforate, punctate, with small holes

pollen grains was 37-(41)-44 μm. In our study the value of this trait in the polar view was greater in both of the specimens under analysis. Nakagawa et al. (2000) reported that the exine sculpture of the corpus was the same as in our study. In the study by Klaus (1978) conducted on *P. uncinata* from the Pyrenees there were differences between the pollen grains in the saccus sculpture. Our research showed that the ornamentation of the cappa in the *P. uncinata* pollen grains from the Dendrological Garden was the same as in the study by Klaus (1978), but the sculpture of the corpus of the pollen grains from the Pyrenees grown in the Forest Arboretum in Zielonka was different.

Some authors include *P. uncinata* into the *P. mugo* complex and/or regard it as a hybrid species. In order to check it we compared the results of our research with the results of analyses by Bykowska & Klimko (2015). As far as the morphological traits are concerned, we noted that the *P. uncinata* (DG) pollen grains were similar to the *P. mugo* (DG) grains analysed by Bykowska & Klimko (2015). The total width of the *P. uncinata* (DG) pollen grains was slightly smaller, but the corpus was bigger. In both specimens the distance between the sacci was identical, and the shape and size of the sacci was similar.

There was a considerable similarity between *Pinus* × *rhaetica* no. 16 FA analysed by Bykowska & Klimko

Table 3. A comparison of individual trait values of *Pinus uncinata* reported by the authors cited and the values found in our study

		Klaus (1978)	Christensen (1987)	Nakagava et al. (2000)	Our study
Pollen grain view		–	–	equatorial	polar
Pollen grain acetolysis		acetolysed	–	acetolysed	non-acetolysed
Type of microscope		SEM	–	SEM	LM* SEM
Characters	Pollen grain width (μm)	–	71.40–85.00; mean 78.90	–	60.06–80.08; mean 69.30*
	Corpus width (μm)	–	–	37.00–44.00; mean 41.00	36.28–65.63; mean 54.80*
	Corpus depth (μm)	–	–	–	40.04–54.34; mean 47.52*
	Corpus shape (width/depth ratio)	–	–	–	0.85–1.37; mean 1.14*
	Cappa exine sculpture	micrograna single or almost invisible; micrograna in low numbers; perforations regularly	–	verrucate to rugulate, deeply sculptured	verrucate– rugulate; perforate, with puncta; grana – clearly, irregularly distributed or absent
	Saccus sexine sculpture	micrograna slightly visible or almost invisible; micrograna in the majority of grains clearly but not numerous; perforation present (puncta and holes), sometimes more numerous than on the cappa	–	–	reticulate; irregularly perforate with puncta and holes, more perforations than on the cappa

(2015) and *P. uncinata* (FA) (in terms of the large size of its pollen grains and sacci). The only exception was the distance between the sacci, i.e. 15.63 μm in *P. uncinata* (FA) vs 13.73 μm in *P.* × *rhaetica* no. 16 FA. Moreover, *P. uncinata* (FA) was distinguished by big sacci (ca. 40 × 29 μm). Their size was similar to the size of the sacci in *P.* × *rhaetica* no. 1 FA.

Apart from that, we observed a large width of pollen grain corpuses in both *P. uncinata* specimens under analysis (50.79 and 58.81 μm on average). In the study by Bykowska & Klimko (2015) the width of pollen grain corpuses also exceeded 50 μm in nine *P.* × *rhaetica* specimens.

CONCLUSIONS

To conclude, our study of the pollen grain morphology of *P. uliginosa* and *P. uncinata* 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 eight quantitative traits were helpful with identification and the differences in the size and shape of the pollen grains and saccus were statistically significant. The shape of the corpus in *P. uliginosa* was prolate-spheroidal and in *P. uncinata* it was subprolate and prolate-spheroidal.

The tectum surface traits in the proximal view of the corpus and saccus were less variable (except *P. uncinata* FA) and they were not a good criterion for identification of the species under study. Our study shows that several morphological traits of pollen can be of taxonomical value. Thus, this detailed analysis has greatly increased our knowledge of individual species.

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