

POLLEN MORPHOLOGY IN SELECTED CUPRESSACEAE GRAY. AND  
SCIADOPITYACEAE LUERSS. SPECIES IN AN EXPERIMENTAL CULTURE

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ABSTRACT. Pollen grains of *Cunninghamia lanceolata*, *Cryptomeria japonica* and *Sciadopitys verticillata* were examined by light and scanning electron microscopy. The pollen grains were non-saccate, generally oblate-spheroidal, spheroidal, prolate-spheroidal or subprolate. The pollen grains had papillae of different sizes and shapes on the distal face. The papillae were more conspicuous in *C. japonica* than in *C. lanceolata*. The pollen exine sculpture in the proximal view was verrucate-granulate and verrucate with microechinate suprasculpture in SEM. The surface of the exine was covered with echinate orbicules in Cupressaceae species. There were some differences in the size of the verrucae, perforation on the proximal surface and distal face between *S. verticillata* pollen grains and other species. This study of the pollen grain morphology provided some important new data which have not been reported in literature so far.

KEY WORDS: *Cunninghamia lanceolata*, *Cryptomeria japonica*, *Sciadopitys verticillata*, pollen morphology, LM, SEM

## INTRODUCTION

*Cunninghamia lanceolata* Hook., *Cryptomeria japonica* D. Don and *Sciadopitys verticillata* Sieb. et Zucc. are original gymnospermous trees of the Taxodiaceae family. Over the past decades scientists postulated the dissimilarity of *Sciadopitys verticillata*. As a result, from time to time the species was classified as a different family, i.e. Sciadopityaceae (HAYATA 1931, XI 1986, SCHULZ & STÜTZEL 2007, CHRISTENHUSZ et al. 2011, UEHARA & SAIKI 2011). However, species of the Taxodiaceae family are sometimes classified as the Cupressaceae family due to the characteristic features of their cones and due to the findings of some molecular and morphological phylogenetic studies (SENETA & DOLATOWSKI 2000, SCHULZ & STÜTZEL 2007, CHRISTENHUSZ et al. 2011).

*Cunninghamia lanceolata*, *Cryptomeria japonica* and *Sciadopitys verticillata* are native Asian species. There are natural habitats of *C. lanceolata* in central and southern China. *S. verticillata* has its natural habitat only in the Japanese mountains on the islands of Honshu, Shikoku and Kyushu (SENETA 1981, GRÍMSSON & ZETTER 2011). *Cryptomeria japonica* can naturally be

found in Japan, but its botanic variety (var. *sinensis*) can also be found in China (SENETA 1981, UEHARA & SAHASHI 2000). Conspicuous, old *C. japonica* trees grow near Japanese temples and palaces in impressive alleys (SENETA 1981). Thanks to its decorative values the species has often been planted as an ornamental tree. Due to its rapid growth and valuable wood it is still one of the most important forest trees in Japan (SENETA 1981, PAGE 1990, UEHARA & SAHASHI 2000). Forests with *C. japonica* occupy nearly 18% of the total land area of Japan (YONEKURA et al. 2012). Due to the large number of trees with light pollen, which can be carried by wind even as far as several dozen kilometres, nowadays the *C. japonica* pollen is an onerous allergen in spring (TANAKA et al. 2012, WANG et al. 2012, 2013).

*Cunninghamia lanceolata*, *C. japonica* and *S. verticillata* were introduced to Poland in the 19<sup>th</sup> century. However, due to their limited resistance to frost and poor commercial availability the species can only be found as collectors' specimens in Poland. Nevertheless, in Austria (ZETTER 1998, GRÍMSSON & ZETTER 2011), Denmark (LARSSON et al. 2010), Germany (FERGUSON et al. 1998) and Poland (MAČKO 1957, STUCHLIK et al. 2002, TROĆ & SADOWSKA 2006) *S. verticillata* pollen

grains were noted in Miocene fossil specimens. Similarly, specimens of *C. lanceolata* pollen grains were found in Austria (GRÍMSSON & ZETTER 2011).

Pollen grains are surrounded by complex pollen walls with the outer granular layer (ectexine) and the inner lamellar layer (endexine) (UEHARA & SAHASHI 2000). In the Taxodiaceae family pollen grains are generally spherical and granular (UEHARA & SAHASHI 2000), non-saccate (KURMANN 1990, UEHARA & SAHASHI 2000), frequently with a papilla in the middle of the leptoma (GRÍMSSON & ZETTER 2011). Several authors have reported on the morphology and ultrastructure of *C. lanceolata* pollen grains (XI 1986, SUROVA & KVAVADZE 1988, XI & WANG 1989, KURMANN 1990 and JIA et al. 1998); *C. japonica* – by UENO (1951, 1960), YAMADA et al. (1980), XI & WANG (1989), KURMANN (1992), UEHARA & SAHASHI (2000) and *S. verticillata* – by KVAVADZE (1986) and HANSEN et al. (2001).

The main aims of this study were to: 1. describe variation in the pollen grain morphology of *C. lanceolata*, *C. japonica* and *S. verticillata*; 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

In March and April 2015 *Cunninghamia lanceolata*, *Cryptomeria japonica* and *Sciadopitys verticillata* pollen grains were collected from trees growing in the collection of the Department of Dendrology and Nursery, Poznań University of Life Sciences, Poland. Each sample was represented by 100 pollen grains, which were analysed for two quantitative traits (Table 1) in the polar view, according to ERDTMAN (1965), and for the following qualitative traits: the shape of the

pollen grains, and the type of the exine sculpture on the proximal and distal face.

The pollen grains were macerated in 10% KOH (FREDERIKSEN 1978) or in glycerine and were investigated with a light microscope Olympus BX SC30 (LM) to obtain comprehensive information about the general morphology. SEM observations were conducted on the pollen grains which were dried in the air. SEM micrographs were taken with a Zeiss EVO 40 microscope at the Electron and Confocal 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  $\times 5000$  for the shape and  $\times 25\,000$  for the exine sculpture in SEM and  $\times 40$  in LM. The micromorphological traits of the pollen grains were observed in the proximal and distal view.

The pollen terminology was adopted from ERDTMAN (1965), BAGNELL (1975), FÆGRI & IVERSEN (1975), PUNT et al. (2007) and HESSE et al. (2009).

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

The main morphological features of the pollen grains under investigation are summarised in Table 1. A description of the pollen grains morphology is given

Table 1. Mean values ( $\pm$ SE) and ranges (minimum-maximum) of the morphological traits of pollen grains

Species	Method of pollen grain preparation	Length ( $\mu$ m)	Width ( $\mu$ m)	Length/Width ratio (shape)
<i>Cunninghamia lanceolata</i> Hook.	KOH	40.71 $\pm$ 4.12 c	40.76 $\pm$ 3.59 c	1.02 $\pm$ 0.06 b
		31.92–35.12	32.40–36.00	0.92–1.16
		38.32–48.26	36.16–46.00	
	glycerine	35.73 $\pm$ 2.52 a	36.50 $\pm$ 2.90 ab	1.00 $\pm$ 0.04 ab
		27.57–39.74	27.10–39.11	0.88–1.07
		42.00–50.56	40.46–46.40 (53.99)	
<i>Cryptomeria japonica</i> D. Don	KOH	38.33 $\pm$ 3.23 b	38.36 $\pm$ 3.44 b	0.98 $\pm$ 0.07 a
		(22.29) 25.03–29.33	(21.76) 25.57–29.75	0.83–1.14
		35.44–39.27	32.35–39.42	
<i>Sciadopitys verticillata</i> Sieb. et Zucc.	KOH	42.28–49.22	41.28–48.85	
		35.45 $\pm$ 3.28 a	34.85 $\pm$ 3.73 a	1.00 $\pm$ 0.03 ab
		29.05–35.36	27.21–36.00	0.90–1.04
		36.32–42.96	36.48–42.64	
ANOVA		F = 21.88 P < 0.01	F = 21.92 P < 0.01	F = 3.95 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 ( $P < 0.05$ ).

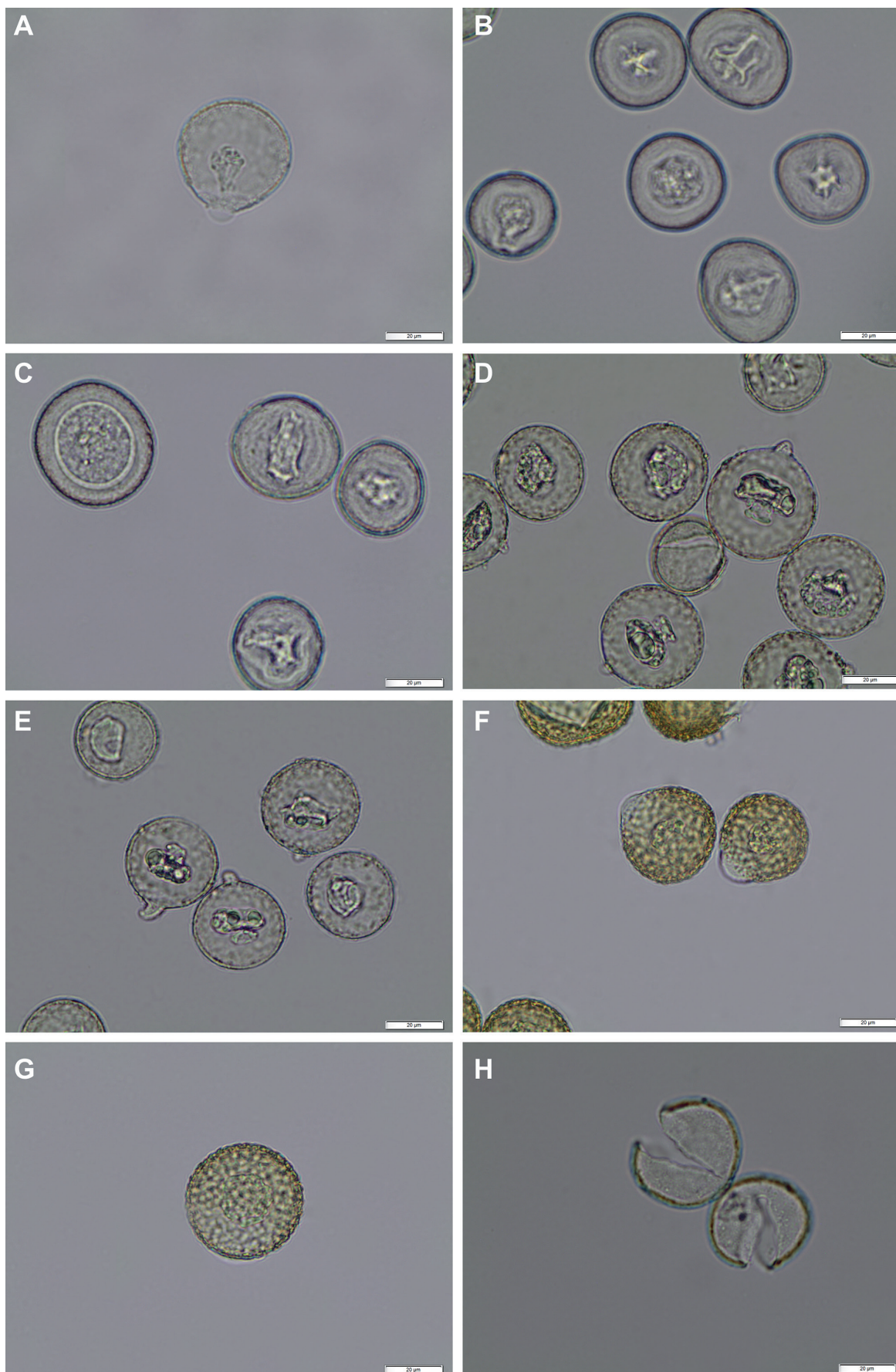


Fig. 1. Light microscope micrographs (LM) of pollen grains in the equatorial and polar view: A, B – *Cunninghamia lanceolata*; C, D, E – *Cryptomeria japonica*; F, G – *Sciadopitys verticillata*; H – *Cunninghamia lanceolata*, split pollen; *pa* – papilla



below and illustrated with selected SEM and LM photographs (Figs 1–4).

*CUNNINGHAMIA LANCEOLATA* (TABLE 1, FIGS 1A, B, H, 2)

The pollen grains were single. The size of the pollen grains depended on the method of specimen preparation. In the polar view they were on average 35.73 or 40.71  $\mu\text{m}$  long and 36.50 or 40.76  $\mu\text{m}$  wide. The pollen grains macerated in KOH were about 5  $\mu\text{m}$  bigger than those fixed in glycerine (Table 1). The pollen size varied considerably. There were small grains, which diameters were less than 30  $\mu\text{m}$ , and large grains, which diameters ranged up to more than 50  $\mu\text{m}$  (Table 1). The papilla in the equatorial view was 4–5  $\mu\text{m}$  long and the basal width was 10  $\mu\text{m}$ . The shape of the pollen grains fixed in KOH ranged from oblate-spheroidal to subprolate, whereas the shape of the pollen grains fixed in glycerine ranged from oblate-spheroidal to prolate-spheroidal (Table 1, Fig. 1A, B).

In the proximal view the pollen grains were convex (Fig. 2A, B). The exine in the proximal view was verrucate-granulate with microechinate orbicules (Fig. 2A–C). The shape of the verrucae was rounded, with polygonal cover with microgranules. The tectum was sparsely perforate with small holes and puncta (Fig. 2C, F), similar as in *Pinus mugo*, *P. sylvestris*, *P. × rhaetica* (BYKOWSKA & KLIMKO 2015), *P. uliginosa* and *P. uncinata* (KLIMKO & BYKOWSKA 2015). In the proximal view the verrucae were irregular in size and height and the pattern was noticeable on the majority of grains. In SEM the distal face on the germinal apparatus was concave, rounded or ovate, bordered by a rim from the proximal hemisphere and it differed in sculpture

(Fig. 2D, E). In great magnification the area of the leptoma was microgranulate and there were echinate orbicules found (Fig. 2F). The echinate orbicules were similar as in the proximal view. We observed the leptoma with a short papilla on the distal face of the pollen grains in SEM (Fig. 2D–F), in LM in the equatorial view (Fig. 1A) and in the polar view (Fig. 1B).

The pollen size observed in our study was partly similar to other authors' observations. SUROVA & KVAVADZE (1988) researched pollen grains from the Botanical Garden in Georgia. They observed that the size of *C. lanceolata* pollen grains was 28.0–39.0  $\mu\text{m}$  (we observed larger grains, too). The leptoma was large and occupied nearly the entire distal surface. It was pore-shaped and 1.5–2.0  $\mu\text{m}$  in size. Part of the distal surface was smooth, the granules were very small and sparsely distributed. In the study by KURMANN (1990) pollen grains were larger and their diameters ranged from 31.0 to 53.0  $\mu\text{m}$ . In our study the diameter of the largest grains slightly exceeded 48  $\mu\text{m}$ . We also observed that in the specimens macerated in KOH 52.06% of the pollen grains were deformed (broken) (Fig. 1H).

The presence of a papilla on *C. lanceolata* pollen grains is disputable. According to ERDTMAN (1965), the pollen had a pore-like aperture. UENO (1951) reported that the papilla was absent in this species. Similarly to our study, WODEHOUSE (1935), HO & SZIKLAI (1973) and GRIMSSON & ZETTER (2011) observed the presence of a papilla.

*CRYPTOMERIA JAPONICA* (TABLE 1, FIGS 1C–E, 3)

The pollen grains were single. In the polar view they were on average 38.33  $\mu\text{m}$  long and 38.36  $\mu\text{m}$  wide.

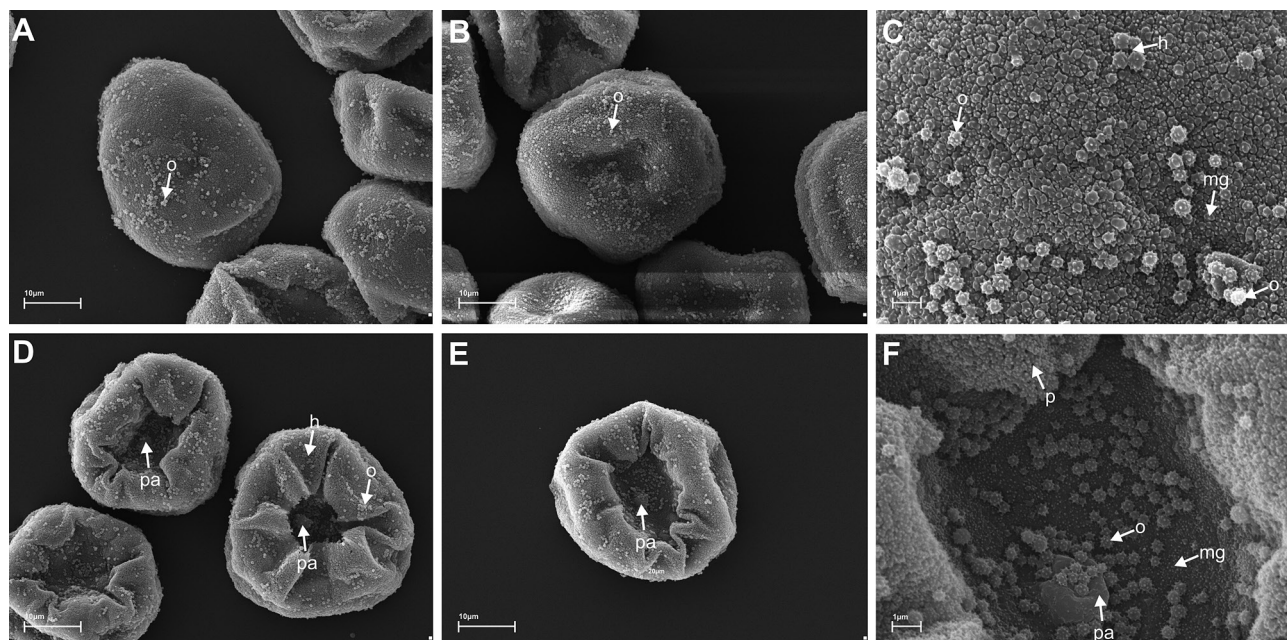


Fig. 2. Scanning electron micrographs (SEM) of *Cunninghamia lanceolata* pollen grains; *h* – holes, *mg* – micrograna, *o* – orbicule, *p* – puncta, *pa* – papilla

The pollen size varied considerably. The length of individual *C. japonica* pollen grains ranged from 22.29 to 49.22  $\mu\text{m}$ , and the width ranged from 21.76 to 48.85  $\mu\text{m}$ . As far as the size of the pollen grains, we can distinguish three groups (Table 1). It was very easy to notice the papilla in *C. japonica* (Figs 1D, E, 3C–E). Its length in the equatorial view ranged from 2.34 to 5.55(–6.27)  $\mu\text{m}$ , and the basal width ranged from 4.62 to 8.64  $\mu\text{m}$ . The size of the pollen grains and papilla were not positively correlated. The shape of the pollen grains in the polar view ranged from oblate-spheroidal to subprolate (Table 1, Fig. 1D, E).

The pollen grains were convex in the proximal view (Fig. 3A). The exine was verrucate-granulate, covered with echinate orbicules (Fig. 3A, B). The tectum was sparsely perforate, with small holes (Fig. 3B). In the proximal view the verrucae were irregular in size and height and the pattern was noticeable on the majority of grains. In SEM the distal face was concave and it had a straight papilla (Figs. 3C–F). In LM straight or curved papillae were observed. The surface of the papilla was microgranulate with echinate orbicules (Fig. 3D–F). In the distal view in great magnification the exine was microgranulate and there were echinate orbicules found (Fig. 3F). The echinate orbicules were similar as in the proximal view. In the specimens macerated in KOH and glycerine only individual grains were broken.

In our study the *C. japonica* pollen grains were larger than in the reference publications. According to WANG et al. (2012), the diameter of *C. japonica* pollen grains was about 30  $\mu\text{m}$ . UEHARA & SAHASHI (2000) reported that *C. japonica* pollen was generally spherical and 25–35  $\mu\text{m}$  in diameter. The surface of the body zone was granular. There were small spherical orbicules. The pa-

pilla was in the centre of the germinal zone and the tip of papilla was slightly curved. In our study the tip of the papilla was straight, blunt or slightly curved. GRIMSSON & ZETTER (2011) reported that *C. japonica* fossil pollen grains were 25–33  $\mu\text{m}$  wide in LM and 24–27  $\mu\text{m}$  wide in SEM, whereas the polar axis was 20–26  $\mu\text{m}$  long in LM (the diameter of our pollen grains ranged up to about 45  $\mu\text{m}$ ). The sculpturing was scabrate in LM, microverrucate in SEM. The surface of the leptoma was granulate, with large irregularly distributed microechinate orbicules (SEM). HO & SZIKLAI (1973) described dry pollen of *Cryptomeria* as cherry-like. The distal face of the pollen was concave, with a slightly bent papilla. The sculpture pattern was microverrucate and the granules of the sculptural element were ornamented, spinulose. The papilla was microscabrate rather than microverrucate.

#### SCIADOPITYS VERTICILLATA (TABLE 1, FIGS 1F, G, 4)

The pollen grains were single. On average they were 35.45  $\mu\text{m}$  long and 34.85  $\mu\text{m}$  wide (measured in the polar view). The pollen size varied considerably. Two pollen groups were distinguished – small grains sized about 29–35  $\times$  27–36  $\mu\text{m}$  and big grains, which were about 36–43  $\mu\text{m}$  long and 36–43  $\mu\text{m}$  wide. The papilla in the equatorial view was 4–5  $\mu\text{m}$  long, and the basal width was 17  $\mu\text{m}$ . The shape of the pollen grains in the polar view ranged from oblate-spheroidal to prolate-spheroidal (Table 1, Fig. 1F, G).

The pollen grains were convex in the proximal view (Fig. 4A, B). The exine was verrucate (Fig. 4A–C). All verrucae were with microgranules, microechinate, irregularly dispersed. No echinate orbicules

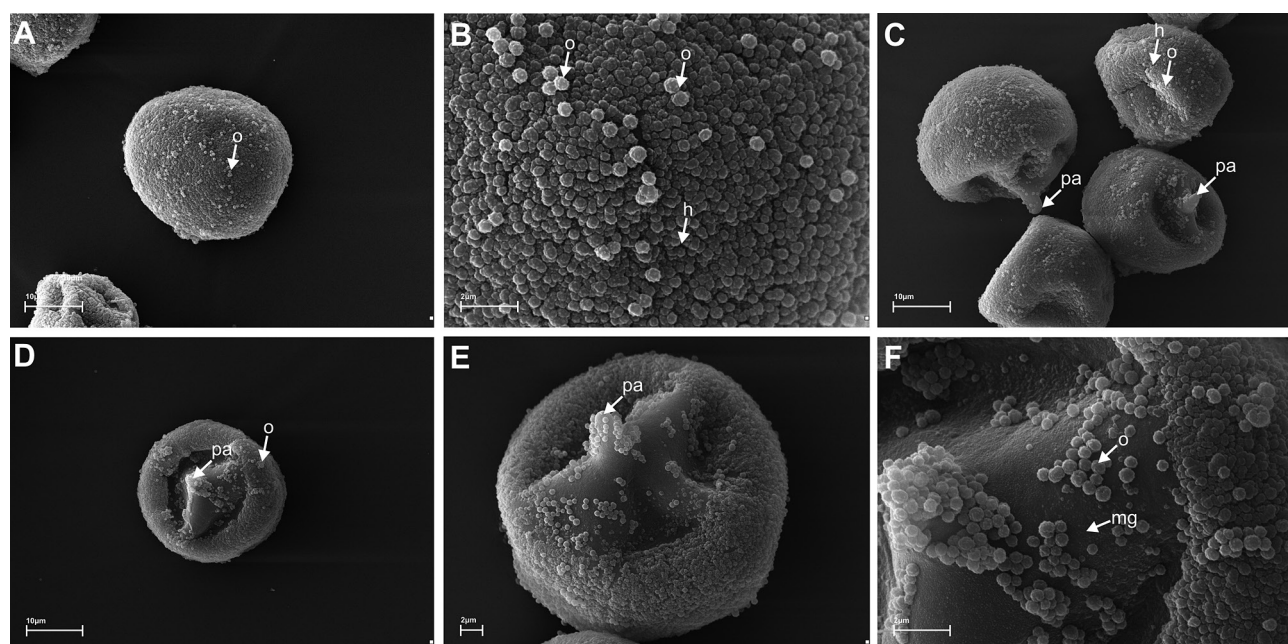


Fig. 3. Scanning electron micrographs (SEM) of *Cryptomeria japonica* pollen grains; *h* – holes, *mg* – micrograna, *o* – orbicule, *p* – puncta, *pa* – papilla



were observed in the proximal view. The exine was clearly perforate with puncta and small holes (Fig. 4C). The size of the verrucae in the proximal view was irregular in size and the pattern was noticeable on the majority of grains. The distal face of the pollen grains was concave in SEM (Fig. 4D, E, G). In great magnification the exine on the distal face was microgranulate (Fig. 4F, H) or almost smooth and there were echinate orbicules found (Fig. 4I). The orbicules on the germinal zone of some pollen grains were diversified in size and shape (Fig. 4F, I) or almost invisible (Fig. 4H). There were no orbicules in the proximal view in SEM. We observed the papilla in the pollen grains in the equatorial view in SEM (Fig. 4B) and in LM (Fig. 1F). We observed that in the specimens macerated in KOH 53.85% of the pollen grains were deformed (broken).

According to GRÍMSSON & ZETTER (2011), *S. verticillata* pollen was comparable in size or slightly larger than the largest pollen of the Cupressaceae, Cephalotaxaceae and Taxaceae families. Our research findings partly confirm these observations, as well as the observations made by UENO (1951), who noted that the diameter of *S. verticillata* pollen grains was 33–38  $\mu\text{m}$  (we observed larger grains, too). KVAVADZE

(1986) and SUROVA & KVAVADZE (1988) reported that the diameter of pollen grains (subjected to acetolysis) was 36–71  $\mu\text{m}$  (in our study the diameter of the largest grains did not exceed 45  $\mu\text{m}$ ). The researchers distinguished the following three forms of grains, depending on their shape and size of orbicules on the distal side: small orbicules of identical size, large orbicules of different sizes and adjacent or merging orbicules. Our research also showed considerable diversification in the size of orbicules in the germinal zone (Fig. 4F, H, I).

XI (1986) described the pollen surface ornamentation as rugulate and this tectum can be distinguished from the pollen grains of other genera. Ho & SZIKLAI (1973) observed gemma on the proximal surface. We did not make this observation in our study. MAĆKO (1957) described *S. verticillata* fossil pollen grains from lower Miocene in Poland. The average diameter of the pollen grains was 33–55  $\mu\text{m}$  or 46–55  $\mu\text{m}$ , the pollen exine was densely wrinkled superficially. The author saw no differences between the features of fossil and recent forms. GRÍMSSON & ZETTER (2011) reported that the fossil pollen grains from Austria were 38–45  $\mu\text{m}$  wide in LM, 35–40  $\mu\text{m}$  wide in SEM, and the polar axis was 31–34  $\mu\text{m}$  long in LM.

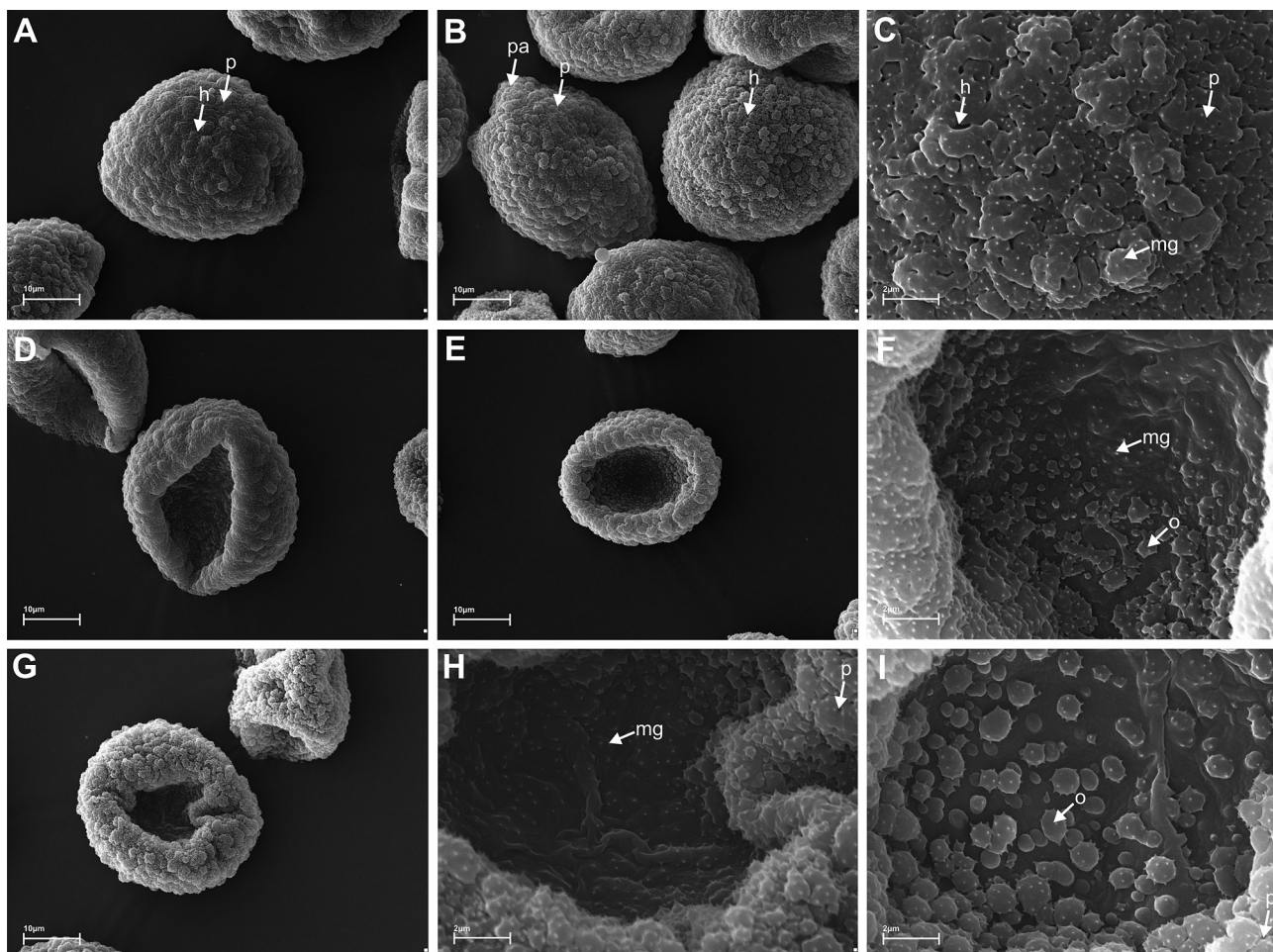


Fig. 4. Scanning electron micrograph (SEM) of *Sciadopitys verticillata* pollen grains; *h* – holes, *mg* – micrograna, *o* – orbicule, *p* – puncta, *pa* – papilla

The sculpturing was verrucate in LM, verrucate with microechinate suprasculpture in SEM, the verrucae were with perforation. Our research confirmed these observations.

KVAVADZE (1986) suggested that the polymorphism and high percentage of pollen deformation observed in *S. verticillata* growing in a culture were caused by unfavourable conditions for its normal development. We think that it was also caused by the preparation of specimens. When dried pollen grains were used in SEM, very few of them were deformed (in contrast to the grains macerated in KOH). There were similar results in LM (liquid preparation – glycerine).

## CONCLUSIONS

In conclusion, our study of the morphology of *Cunninghamia lanceolata*, *Cryptomeria japonica* and *Sciadopitys verticillata* pollen grains provided some important new data concerning the size, shape and ornamentation on the proximal surface and distal face. The biometric measurements of the two quantitative traits in the polar view were of little help for the identification. In general, the pollen grains were medium-sized, according to ERDTMAN'S classification (ERDTMAN 1965). The general shape of the pollen grains in the polar view (length/width ratio) was oblate-spheroidal, spheroidal and prolate-spheroidal. The traits of the exine surface in the proximal view were less variable and they were a good criterion of identification of the species under study. *C. lanceolata* and *C. japonica* differed in the shape and size of the papillae and in the shape of echinate orbicules on the proximal surface. Our study showed that several traits of the pollen morphology could be of taxonomical value. Thus, this detailed analysis greatly increased our knowledge of individual species.

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