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PAN POLSKA AKADEMIA NAUK ODDZIAŁ W KRAKOWIE

POLLEN MORPHOLOGY OF SELECTED SPECIES OF THE *CEPHALOTAXUS* SIEB. ET ZUCC., *TAXUS* L. AND *TORREYA* ARN. GENERA

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This study is the first comparison of the morphology of pollen grains in ten cultivars of three species of the *Taxus*, *Torreya nucifera* and *Cephalotaxus harringtonia* var. *drupacea* genera. The material came from the Botanical Garden of Adam Mickiewicz University in Poznań, Poland. Each measurement sample consisted of 50 pollen grains. In total, 750 pollen grains were analyzed. Light and electron scanning microscopy was used for the morphometric observation and analysis of pollen grains. The pollen grains were inaperturate and classified as small and medium-sized. They were prolate-spheroidal, subprolate to prolate in shape. The surface of the exine was microverrucate-orbiculate, perforate in *Cephalotaxus harringtonia* var. *drupacea*, granulate-orbiculate, perforate in all *Taxus* taxa and granulate-microverrucate-orbiculate, perforate in *Torreya*. The orbicules were rounded to oval in surface view, and the size was considerably diversified. The pollen features were insufficient to distinguish between individual *Taxus* members – only groups were identified. The values of the coefficient of variability of three features (LA, SA and LA/SA) were significantly lower than the orbicule diameter. The pollen surface of all *Taxus* specimens was similar, so it was not a good identification criterion. The pollen grains of the *Taxus* taxa were smaller and had more orbicules than *Cephalotaxus* and *Torreya*. Palynological studies provided taxonomic support for recognition of two different genera of the Cephalotaxaceae and Taxaceae families, which are closely related.

Keywords: micromorphology, pollen variability, taxonomy, SEM

INTRODUCTION

The Cephalotaxaceae and Taxaceae families represent two closely related groups of conifers of considerable economic and pharmacological importance.

The Cephalotaxaceae family is represented by a single genus, *Cephalotaxus* Sieb. et Zucc., with 7 species. Its distribution is restricted to southern and eastern Asia, including Japan, South Korea, central and eastern China, Hainan, Taiwan, India, Myanmar, Laos and parts of Vietnam (Hils, 1993; Lang et al., 2013). The taxonomic position of the Cephalotaxaceae family has been controversial for a long time. Some authors consider it either a group of the Taxaceae family or a separate family with its own phylogenetic origin (Florin, 1948). According to a recent classification of extant gymnosperms based on molecular and morphological phylogeny, the *Taxus*, *Torreya* and *Cephalotaxus* genera have been placed in the Taxaceae family (Christenhusz et al., 2011). However, the Cephalotaxaceae family is elevated to the rank of its own order, Cephalotaxales Takht. ex Reveal (Reveal, 1993).

Plants of the Taxaceae family grow mainly in the northern hemisphere. The *Taxus* genus alone is reported to include 21–24 species and 55 varieties of trees or shrubs (Fu et al., 1999; Thomas and Polward, 2003; Spjut, 2007), while *Torreya* comprises 5–6 species and is mostly distributed in North America, China and Japan (Seneta, 1981).

In Poland mainly the native *Taxus baccata*, with about 30 varieties, and hybrid *T.* ×*media*, with more than 20 varieties are cultivated. The species *T. cuspidata* and its variety 'Nana', along with *Torreya* and *Cephalotaxus* are very rare and they can be found almost exclusively in collections.

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The recognition of yew varieties is rather difficult due to very high variability of *T. baccata* and mutual similarity of its varieties. The problem was compounded by breeding of shrubs from seeds and formation of intermediate forms (Seneta, 1981).

The pollen morphology of *Cephalotaxus* species from Japan was studied by means of a light microscope (LM) and a scanning electron microscope (SEM) by Ueno (1959), Yamazaki and Takeoka (1962) and Zhen (1993). The species from India was studied by Yadav et al. (2013), the species *T. cuspidata* and *Torreya* spp. from Japan by Ueno (1959) and Yamazaki and Takeoka (1962), and the species *T. baccata* and *Torreya* spp. from Romania by Predan and Toniuc (2009).

The pollen of Cephalotaxaceae and Taxaceae is generally spherical, without air bladders, microscabrate-microverrucate with orbicules all over the surface (Yamazaki and Takeoka, 1962), and with either granular-orbicular exine (Kuprianova and Gumbatov, 1988) or verrucous exine (Ikuse, 1956; Ueno, 1959; Predan and Toniuc, 2009). The light microscopy observations made by Ikuse (1956) and Ueno (1959) revealed a single aperture in *Taxus* pollen, whereas Kurmann and Zavada (1994) stated that the pollen was inaperturate. Ueno (1959) and Yamazaki and Takeoka (1962) reported that the pollen of *Cephalotaxus* and *Torreya* spp. was aperturate (primitive germ furrow). There were also ultrastructural studies of pollen development in *T. baccata* (Rohr, 1977; Pennell and Bell, 1985, 1986).

The main aims of this study were: 1) to describe variation in the morphology of pollen grains in ten cultivars of three species within the *Taxus* genus and one species of the *Torreya* and *Cephalotaxus* genera; 2) to describe and document the pollen grain micromorphology; 3) to supply new data, which can be used for identification of inaperturate pollen grains of the gymnospermae taxa.

MATERIAL AND METHODS

The study was conducted on pollen grains of *Cephalotaxus harringtonia* var. *drupacea* Koidz., *Taxus baccata* L. and its six varieties, *T. cuspidata* Siebold et Zucc. and its one variety, *T.* ×*media* Rehder – and its three varieties and *Torreya nucifera* Siebold et Zucc. The materials came from the Botanical Garden of Adam Mickiewicz University in Poznań, Poland (BG AMU). Table 1 shows a list of the analyzed taxa.

TABLE 1. A list of the analyzed taxa. Abbreviations: BG AMU – Botanical Garden of Adam Mickiewicz University, Poznań; BG Powsin – Polish Academy of Sciences Botanical Garden, Center for Biological Diversity Conservation in Powsin; KA – Kórnik Arboretum, Institute of Dendrology, Polish Academy of Sciences; n.d. – no data.

Taxon	No.	Year of planting in BG AMU in Poznań	Origin
Cephalotaxus harringtonia var. *drupacea*	1873	n.d.	n.d.
Taxus baccata	64	1924	BG AMU
Taxus baccata 'Adpressa'	1426	1968	KA
Taxus baccata 'Adpressa Aurea'	2167	1976	KA
Taxus baccata 'Aurea'	1427	1968	KA
Taxus baccata 'Aurea Nova'	4022	1990	n.d.
Taxus baccata 'Aurea Nova'	2736	1994	KA
Taxus baccata 'Erecta'	6883	n.d.	n.d.
Taxus baccata 'Fastigiata'	4018	1990	KA
Taxus cuspidata	506	1951	KA
Taxus cuspidata 'Nana'	6535	n.d.	n.d.
Taxus ×*media* 'Hatfieldii'	4000	1990	KA
Taxus ×*media* 'Profesor Kobendza'	7371	2004	BG Powsin
Taxus ×*media* 'Wojtek'	6367	2001	BG AMU
Torreya nucifera	63	1938	KA

The pollen grains were examined using light microscopy (LM) and scanning electron microscopy (SEM). Fresh pollen grains macerated in 10% KOH (Dyakowska, 1959; Frederiksen, 1978) or in glycerine were used for LM investigations. The length of the long axis (LA) and the length of the short axis (SA) were measured under an Olympus BX 43 light microscope with a camera. The orbicule diameter was measured under an SEM. A total of 50 pollen grains per specimen were used for the measurements. The LA/SA ratio was calculated. In total, 750 pollen grains were examined. Air-dried pollen without any special preparation, coated with gold by means of an SCB 050 ion sputter was used for SEM investigations. Micrographs were taken with an EVO 40 scanning electron microscope (Carl Zeiss, Jena, Germany) at an accelerating voltage of 10–15 kV, at the Confocal and Electron Microscopy Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland. The study was documented with photographs taken during the observations, mostly magnified ×40 and ×100 in LM, ×7000 for the shape and ×35000 for the exine sculpture of the pollen grains in SEM. The authors used the pollen terminology according to Faegri and Iversen (1989), Punt et al. (2007) and Hesse et al. (2009), and the shape classification according to Erdtman (1952).

The minimum and maximum values of the characteristics, arithmetic means and the coefficient of variability were calculated. Before one-way analysis of variance (ANOVA) the distribution of each feature was studied. A one-way ANOVA and post-hoc HSD Tukey's test were used to examine differences in the mean values of the traits under study. Statistica 13.1 for Windows software was used for statistical analyses.

RESULTS

CEPHALOTAXUS HARRINGTONIA VAR. *DRUPACEA*

The pollen grains of the species were mainly monads. The length of the long axis (LA) of *C. harringtonia* var. *drupacea* pollen grains ranged from 27.8 to 44.2 μm, while the length of the short axis (SA) ranged from 25.6 to 42.9 μm (Table 2). The outline was circular in polar proximal and distal views and slightly elliptical in equatorial view (Fig. 1a). Pollen grains sized 37.1–50.5 x 34.4–46.6 μm with a pollen tube were found in glycerine (Figs. 1a, b). Polyhedral pollen was found in SEM (Fig. 1c). The shape of the pollen grains was spheroidal and prolate-spheroidal (LA/SA = 1.0–1.1) (Table 2, Figs. 1a, b). The pollen grains were inaperturate and medium-sized, according to Erdtman's classification (1952). The values of the coefficient of variability of three features (LA, SA and LA/SA) were significantly lower (from 3.8 to 14.4%) than the orbicule diameter (39.2%) (Table 2).

All the pollen grains of *C. harringtonia* var. *drupacea* had uniform ornamentation. The exine ornamentation in LM was psilate to granulate (Figs. 1a, b) and irregularly granulate-verrucate, covered with orbicules in SEM (Figs. 1c, d), circular or oval in shape (Fig. 1d). The mean diameter of the orbicules was 0.74 μm (range: 0.3–1.2 μm). The surface of the orbicules was microverrucate with a slightly irregular margin (Fig. 1d). The exine surface perforation varied in size and shape.

TAXUS BACCATA

The general morphology of pollen grains in the species type and cultivars was similar. The pollen grains of the examined taxa were mainly monads. The mean length of the long axis (LA) of *T. baccata* pollen grains ranged from 21.95 μm in *T. baccata* 'Erecta' to 25.00 μm in *T. baccata* 'Aurea Nova', while the mean length of the short axis (SA) ranged from 19.72 μm in *T. baccata* 'Erecta' to 22.69 μm in *T. baccata* 'Aurea' (Table 2). The outline was circular in polar proximal and distal views and elliptical in equatorial view (Fig. 2a). Pollen grains sized 27.6–37.7 x 26.6–34.3 μm with a pollen tube were found in glycerine (Figs. 2a–c, e, f). Polyhedral pollen was found in SEM (Figs. 3m, o). The shape of the pollen grains ranged from prolate-spheroidal (mean LA/SA = 1.06–1.14) to subprolate (mean LA/SA = 1.15–1.21) (Table 2, Fig. 3). The pollen grains in the samples were deformed (Figs. 3a, e, g, i, k). The pollen grains were inaperturate and small or medium-sized, according to Erdtman's classification (1952). The values of the coefficient of variability of three features (LA, SA and LA/SA) were significantly lower (from 3.8 to 12.7%) than the orbicule diameter (from 20.5 to 37.8%) (Table 2).

All the pollen grains of *T. baccata* and its cultivars had uniform ornamentation. The exine ornamentation in LM was psilate or microscabrate (Fig. 2) and irregularly granulate, covered with orbicules in SEM (Fig. 3). The orbicules were circular or oval in shape. The mean diameter of the orbicules was 0.46 μm (range: 0.2–0.8 μm). The surface of the orbicules was microverrucate, with a slightly irregular margin (Figs. 3b, d, f ,h, j, l, n, p). The exine surface perforation varied in size and shape.

TAXUS CUSPIDATA

The pollen grains of the examined taxa were mainly monads. The mean length of the long axis (LA) ranged from 24.86 μm in *T. cuspidata* to 26.50 μm in 'Nana' variety, while the mean length of the short

TABLE 2. Characteristic features of *Cephalotaxus*, *Taxus* and *Torreya* pollen grains with the results of one-way ANOVA.

Taxon	Length of the						LA/SA ratio			Orbicule diameter		
	long axis (LA)			short axis (SA)								
	mean (µm)	min. max. (µm)	cv (%)	mean (µm)	min. max. (µm)	cv (%)	mean (µm)	min. max. (µm)	cv (%)	mean (µm)	min. max. (µm)	cv (%)
Cephalotaxus harringtonia var. *drupacea*	34.45	27.8 44.2	13.4	32.86	25.6 42.9	14.4	1.05	1.0 1.1	3.8	0.74	0.3 1.2	39.2
Taxus baccata	23.30	19.2 29.6	9.4	20.78	17.8 24.6	7.4	1.12	1.0 1.4	8.9	0.46	0.3 0.8	28.3
Taxus baccata 'Adpressa'	24.13	21.2 29.3	7.5	21.51	18.8 26.3	6.8	1.12	1.0 1.3	8.0	0.45	0.2 0.8	37.8
Taxus baccata 'Adpressa Aurea'	23.86	19.7 28.4	10.1	21.02	16.0 24.3	8.9	1.14	1.0 1.3	9.6	0.44	0.3 0.6	20.5
Taxus baccata 'Aurea'	24.11	20.2 32.7	12.7	22.69	18.6 28.7	12.1	1.06	1.0 1.1	3.8	0.50	0.3 0.8	24.0
Taxus baccata 'Aurea Nova'	25.00	18.7 30.1	10.0	20.77	17.0 27.8	11.9	1.21	1.0 1.5	12.4	0.45	0.3 0.8	24.4
Taxus baccata 'Aurea Nova'	24.50	20.1 29.2	9.3	21.24	16.9 25.7	8.8	1.16	1.0 1.4	7.8	0.47	0.2 0.7	23.4
Taxus baccata 'Erecta'	21.95	19.2 30.3	9.8	19.72	16.7 30.5	12.5	1.12	1.0 1.3	8.0	0.45	0.2 0.6	22.2
Taxus baccata 'Fastigiata'	24.16	20.5 29.5	9.9	21.04	17.9 24.8	7.7	1.15	1.0 1.5	9.6	0.47	0.3 0.6	21.3
Taxus cuspidata	24.86	18.6 33.0	13.0	22.78	17.3 28.5	12.8	1.09	1.0 1.3	7.3	0.70	0.3 1.7	34.3
Taxus cuspidata 'Nana'	26.50	21.6 33.7	10.6	23.67	19.5 32.7	12.0	1.13	1.0 1.4	9.7	0.55	0.3 0.9	21.8
Taxus ×*media* 'Hatfieldii'	23.90	20.1 29.8	9.2	20.77	17.3 28.4	12.0	1.16	1.0 1.3	7.8	0.50	0.2 0.7	26.0
Taxus ×*media* 'Profesor Kobendza'	22.87	17.9 28.3	11.5	20.47	14.9 28.0	13.2	1.12	1.0 1.3	7.1	0.44	0.2 0.7	27.3
Taxus ×*media* 'Wojtek'	24.31	19.8 30.0	9.9	22.37	18.7 27.8	10.5	1.09	1.0 1.3	7.3	0.56	0.3 0.9	23.2
Torreya nucifera	31.15	24.6 37.3	9.0	29.25	23.5 33.7	8.5	1.07	1.0 1.2	3.7	0.64	0.2 1.3	45.3
ANOVA	F = 43.96 p < 0.01			F = 59.32 p < 0.01			F = 6.75 p < 0.01			F = 10.883 p < 0.01		

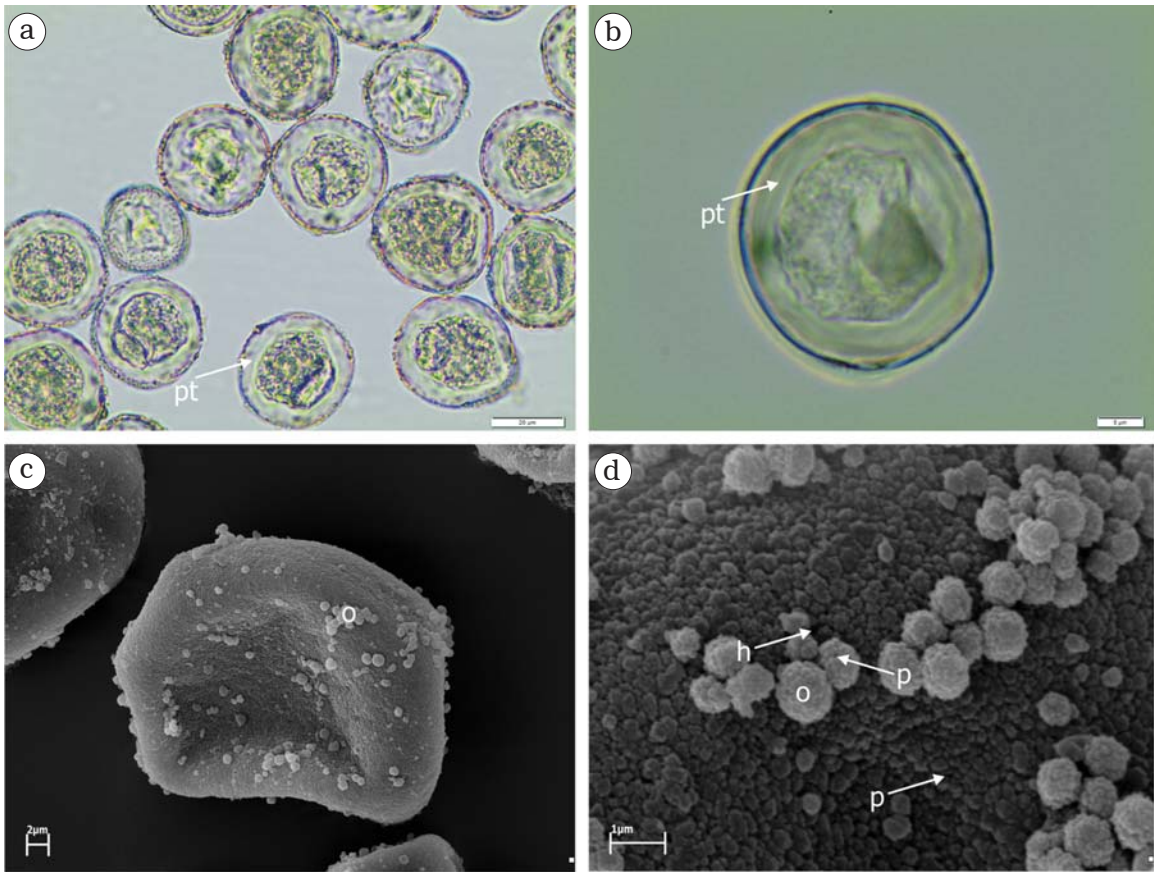

Fig. 1. LM and SEM microphotographs of *Cephalotaxus harringtonia* var. *drupacea* pollen grains. (**a**) group of pollen grains, prolate spheroidal in distal view, (**b**) in distal view with pollen tube, (**c**) polyhedral pollen, (**d**) pollen grain with orbicules on surface; o – orbicule, pt – pollen tube, p – puncta.

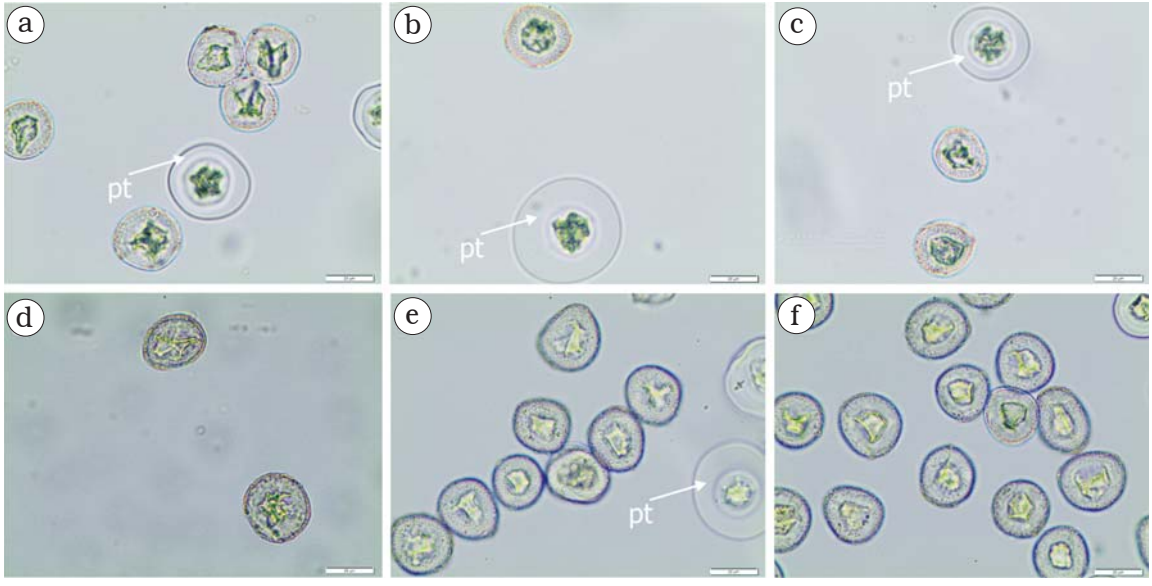

Fig. 2. LM microphotographs of *Taxus baccata* pollen grains. (**a**) *T. baccata*, (**b**) 'Adpressa', (**c**) 'Aurea', (**d**) 'Aurea Nova', (**e**) 'Erecta', (**f**) 'Fastigiata'; pt – pollen tube.

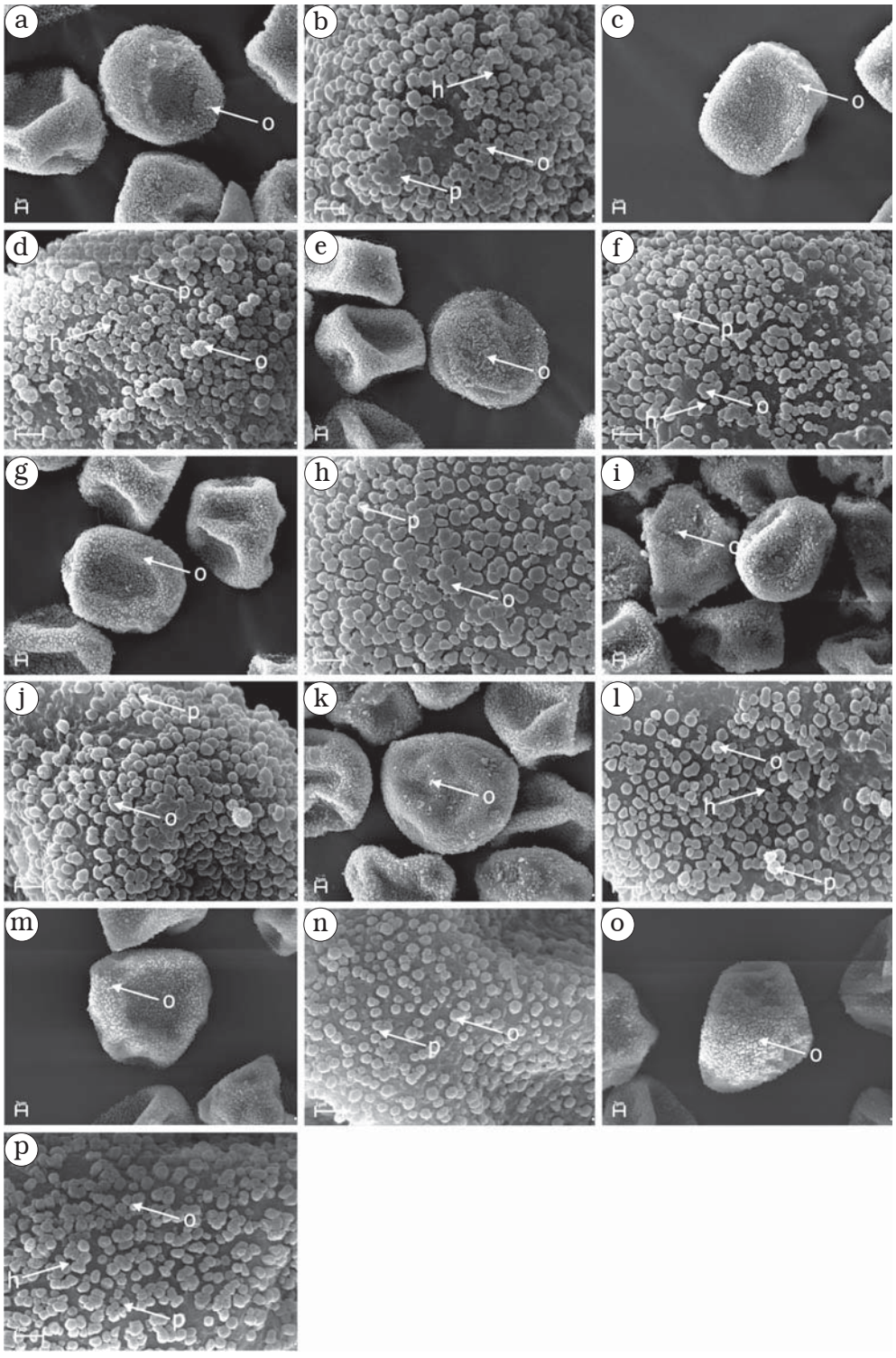

Fig. 3. SEM microphotographs of *Taxus baccata* pollen grains. (**a**,**b**) *T. baccata*, (**a**) group of pollen grains, one deformed, (**b**) detail of exine sculpturing; (**c**,**d**) 'Adpressa', (**c**) pollen grain, tetrahedral, (**d**) detail of exine sculpturing; (**e**,**f**) 'Adpressa Aurea', (**e**) group of pollen grains, three deformed, (**f**) detail of exine sculpturing; (**g**,**h**) 'Aurea', (**g**) group of pollen grains, three deformed, (**h**) detail of exine sculpturing; (**i**,**j**) 'Aurea Nova' (no. 4022), (**i**) group of pollen grains, some deformed, (**j**) detail of exine sculpturing; (**k**,**l**) 'Aurea Nova' (no 2736), (**k**) group of pollen grains, some deformed, (**l**) detail of exine sculpturing; (**m**,**n**) 'Erecta', (**m**) polyhedral pollen grain, (**n**) detail of exine sculpturing; (**o**,**p**) 'Fastigiata', (**o**) polyhedral pollen grain, (**p**) detail of exine sculpturing; h – holes, o – orbicule, p – puncta.

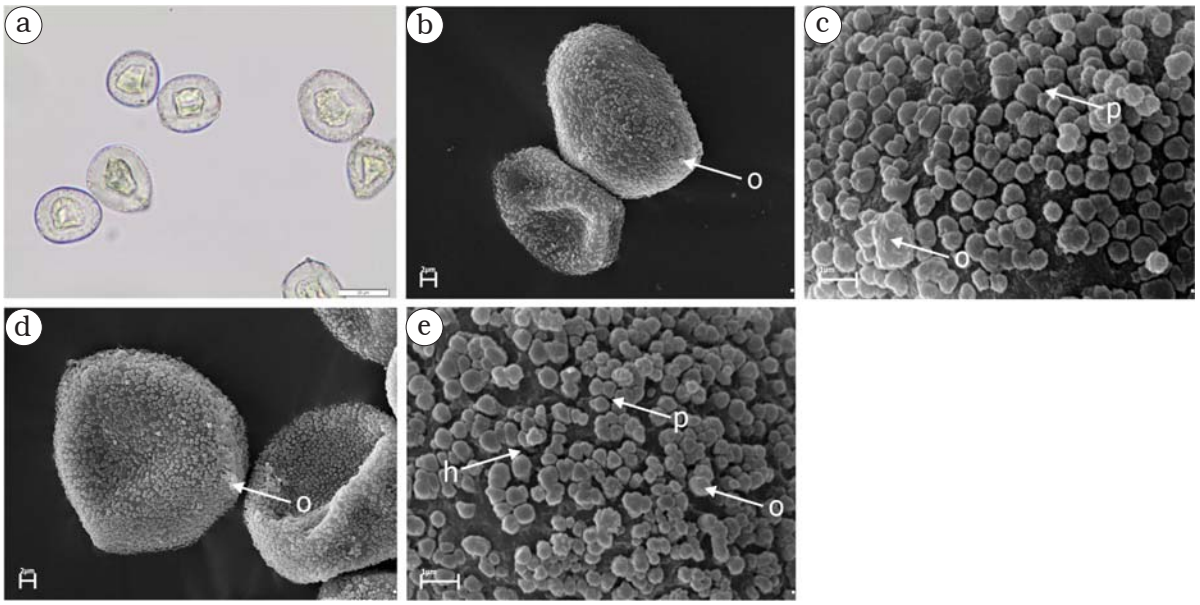

Fig. 4. LM and SEM microphotographs of *Taxus cuspidata* pollen grains. (**a**) group of pollen grains in distal view, (**b**) two pollen grains, (**c**) detail of exine sculpturing; (**d**,**e**) 'Nana', (**d**) two pollen grains, (**e**) detail of exine sculpturing; o – orbicule.

axis (SA) ranged from 22.78 μm in *T. cuspidata* to 23.67 μm in the 'Nana' variety (Table 2). The outline was circular in polar proximal and distal views and elliptical in equatorial view (Figs. 4a, b). Pollen grains sized 32.3–44.6 x 31.3–43.8 μm with a pollen tube were found in glycerine. The shape of the pollen grains was prolate-spheroidal (mean LA/SA = 1.09–1.13) (Table 2, Fig. 4). The pollen grains were inaperturate and small or medium-sized, according to Erdtman's classification (1952). The values of the coefficient of variability of three features (LA, SA and LA/SA) were significantly lower (from 7.3 to 13.0%) than the orbicule diameter (from 21.8 to 34.3%) (Table 2).

All the pollen grains had uniform ornamentation. The exine ornamentation in LM was psilate to granulate (Fig. 4a) and irregularly granulate, covered with orbicules in SEM (Figs. 4b–e). The orbicules were circular or oval in shape (Figs. 4c, e). The mean diameter of the orbicules was 0.62 μm (range: 0.3–1.7 μm). The surface of orbicules was microverrucate (Figs. 4b–e), and the margin was slightly irregular. The exine surface perforation varied in size and shape.

TAXUS ×MEDIA

The pollen grains of the examined taxa were mainly monads. The mean length of the long axis (LA) of *T. ×media* pollen grains ranged from 22.87 μm in the 'Profesor Kobendza' variety to 24.31 μm in 'Wojtek', while the mean length of the short axis (SA) ranged from 20.47 μm in the 'Profesor Kobendza' variety to 22.37 μm in 'Wojtek' (Table 2). The outline was circular in polar proximal and distal views, elliptical in equatorial view (Fig. 5a) and rounded triangular (Figs. 5b, c). Pollen grains sized 28.5–41.6 x 27.6–40.8 μm with a pollen tube were found in glycerine. The shape of the pollen grains was prolate-spheroidal (mean LA/SA = 1.09–1.12) and subprolate (mean 1.16) (Table 2, Fig. 5). The pollen grains were inaperturate and small or medium-sized, according to Erdtman's classification (1952). The values of the coefficient of variability of three features (LA, SA and LA/SA) were significantly lower (from 7.1 to 13.2%) than the orbicule diameter (from 23.2 to 27.3%) (Table 2).

The *T. ×media* pollen grains had uniform ornamentation. The exine ornamentation in LM was psilate or microscabrate (Figs. 5a, b) and irregularly granulate, covered with orbicules (Figs. 5c–i) in SEM. The shape of the orbicules was circular or oval (Figs. 5c–i). The mean diameter of the orbicules was 0.50 μm (range: 0.2–0.9 μm). The surface of the orbicules was microverrucate, with an irregular margin. The exine surface perforation varied in size and shape.

TORREYA NUCIFERA

The pollen grains of the examined species were mainly monads. The length of the long axis (LA) of *Torreya* pollen grains ranged from 24.6 to 37.3 μm,

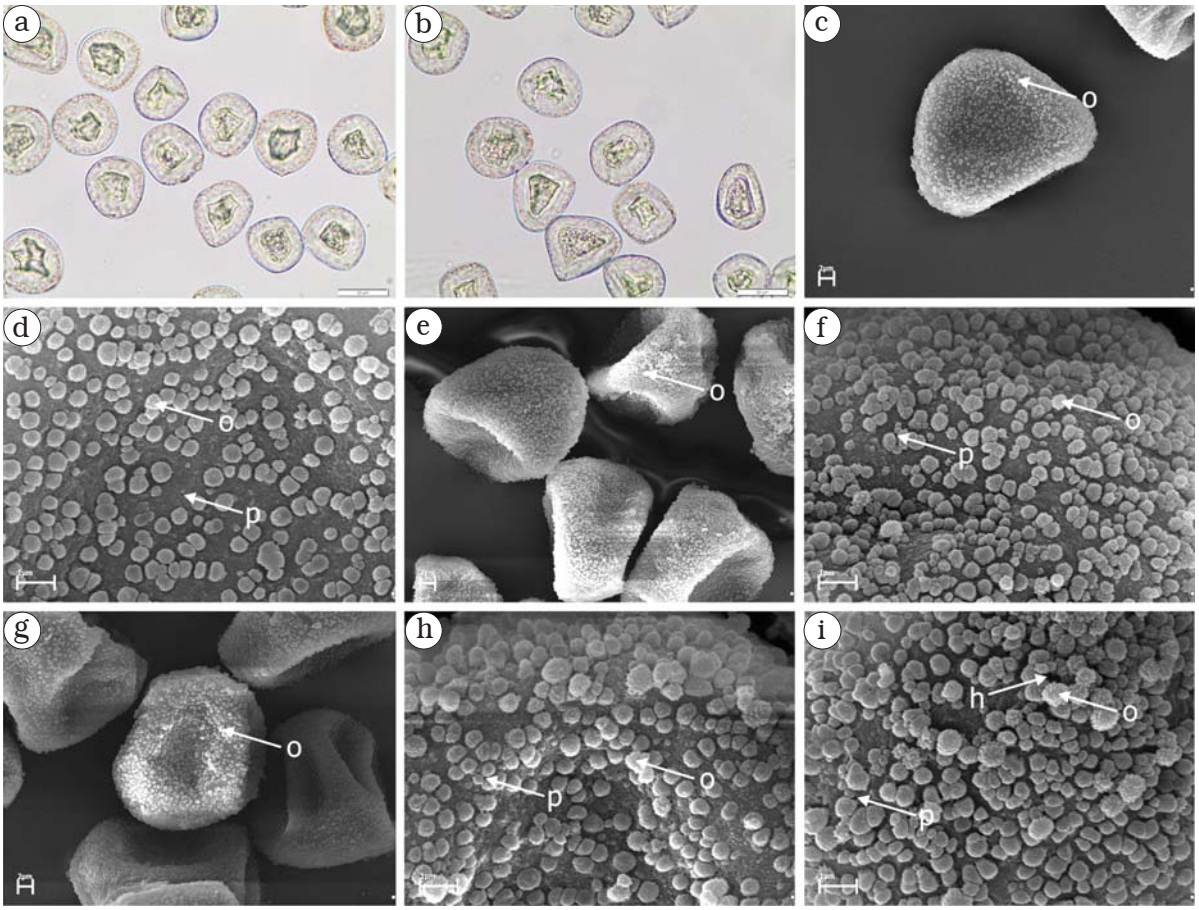

Fig. 5. LM and SEM microphotographs of *Taxus* ×*media* pollen grains. (**a**) 'Hatfieldii' – group of pollen grains in distal view, (**b**) 'Wojtek' – group of pollen grains in distal view; (**c**,**d**) 'Hatfieldii', (**c**) rounded triangular pollen grain, (**d**) detail of exine sculpturing; (**e**,**f**) 'Profesor Kobendza', (**e**) group of pollen grains, some deformed, (**f**) detail of exine sculpturing; (**g**–**i**) 'Wojtek', (**g**) group of pollen grains, some deformed, (**h**,**i**) detail of sculpturing; h – holes, o – orbicule, p – puncta.

while the length of the short axis (SA) ranged from 23.5 to 33.7 μm (Table 2). The outline was circular in polar, proximal and distal views and elliptical in equatorial view (Fig. 6). Pollen grains with a pollen tube were found in LM. The shape of the pollen grains ranged from spheroidal to prolate-spheroidal (LA/SA = 1.0–1.2) (Table 2, Fig. 6a). The pollen grains observed in SEM were prolate-spheroidal, subprolate and prolate (Fig. 6b). The pollen grains were inaperturate and medium-sized, according to Erdtman's classification (1952). The values of the coefficient of variability of three features (LA, SA and LA/SA) were significantly lower (from 3.7 to 9.0%) than the orbicule diameter (45.3%) (Table 2).

All *T. nucifera* pollen grains had uniform ornamentation. The exine ornamentation in LM was psilate or microscabrate (Fig. 6a) and microverrucate, covered with orbicules in SEM (Figs. 6b–d). The orbicules were circular or oval in shape (Figs. 6c, d). The mean diameter of the orbicules was 0.64 μm (range: 0.2–1.3 μm). The surface of the orbicules was microverrucate, with a slightly irregular margin. The exine surface perforation varied in size and shape. We distinguished two size categories: puncta and small holes (Figs. 6c, d).

Comparison of the morphological features of the pollen of *Cephalotaxus*, *Taxus baccata*, *T. cuspidata*, *T.* ×*media* and *Torreya* taxa according to the Tukey's post-hoc tests ($p < 0.05$) showed that the studied taxa formed six homogenous groups for the SA, five homogeneous groups for the LA, four groups for the LA/SA and three for the orbicule diameter (Table 2, Fig. 7). The *Taxus* and *Cephalotaxus* genera differed significantly from *Torreya* in the LA and SA (ANOVA, $p < 0.01$).

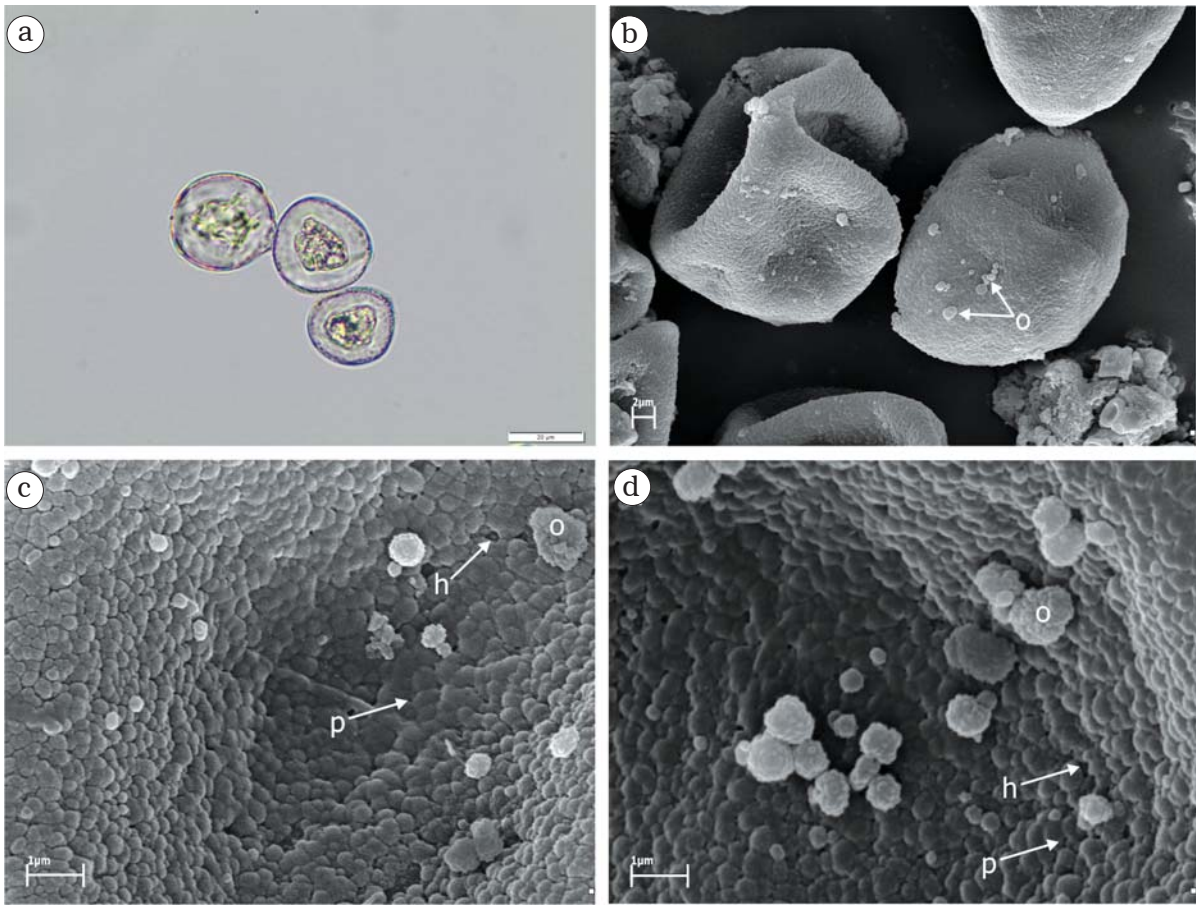

Fig. 6. LM and SEM microphotographs of *Torreya nucifera* pollen grains. (**a**) three pollen grains in distal view, (**b**) three pollen grains, one deformed, (**c**,**d**) detail of exine sculpturing; h – holes, o – orbicule, p – puncta.

DISCUSSION

The comparison of our data with those published by other authors revealed some differences (Table 3). In contrast to smaller grains with microspinulate orbicules described so far (Ueno, 1959; Yamazaki and Takeoka, 1962; Zhen, 1993; Yadav et al., 2013), we found the pollen grains of *Cephalotaxus* to be larger, with microverrucate exine ornamentation on orbicules. Zhen (1993) described pollen in seven *Cephalotaxus* species and found that the pollen grain diameters in polar view ranged from 22.6 to 34.8 µm and they were spherical or subspheroidal. The surface of pollen grains (LM) was nearly psilate or weakly granulate. Under SEM the exine was covered with fine, dense granules, and sparse Ubisch bodies were found on the granular layer. The Ubisch bodies had minute gemmate processes on the surface. Ueno (1959) reported that the pollen diameter in *C. harringtonia* was 30–33 µm, with a primitive bilateral germ furrow, and the sexina was granulate. Yamazaki and Takeoka (1962) reported that pollen grains of this species were aperturate, and their size was 25.5–28.5 x 27–30 µm. The surface was covered with small orbicules, which easily separated from the surface. The diameter of the orbicules was ca. 0.25–0.9 µm. Yadav et al. (2013) reported that in other *Cephalotaxus* species the exine was psillate to granulate (LM), microverrucate (SEM) and the orbicules were microspinulate. In our study the pollen grains were inaperturate, the mean diameter of the orbicules was 0.74 µm and the orbicules were microverrucate. The pollen grains were similar to those of the Taxaceae as they were spheroidal in shape and characterized by tenuity on their distal face. The *Cephalotaxus* pollen grains differed significantly in the LA and SA from the *Taxus* and *Torreya* pollen grains. Hesse et al. (2009) published photos of *Cephalotaxus* pollen grain with a pollen tube which was very similar to the one observed in our study (Figs. 1a, b).

There are some data concerning the size of *T. baccata* species, but there are no available

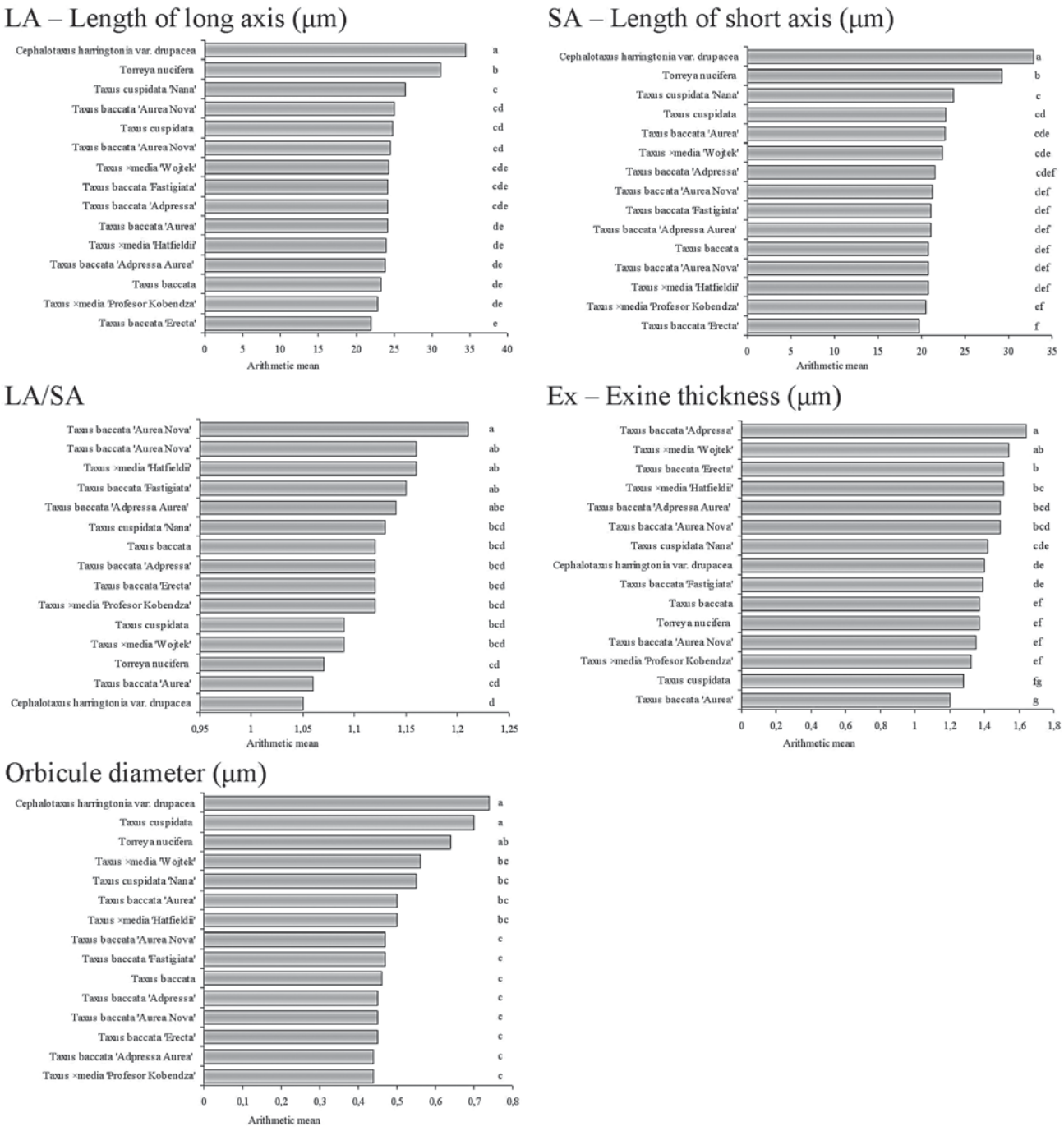

Fig. 7. Arithmetic means and results of Tukey's test for quantitative characteristics of *Cephalotaxus*, *Taxus baccata*, *T. cuspidata*, *T. ×media* and *Torreya* pollen grains. Taxa marked with the same letter(s) do not differ significantly at $p < 0.01$.

studies on cultivated ornamental plants. Erdtman (1965), Predan and Toniuc (2009), and Halbritter (2016) reported that the pollen grains in *Taxus* were small. West (1962) and Beug (1963) observed medium-sized pollen grains. The values in our study (19.2–29.6 µm) did not differ considerably from the data reported in the species description in these palynological studies. SEM photos of *T. baccata* collected in PalDat, a palynological database (http://www.paldat.org), were similar to ours. With regard to the exine sculpturing of *T. baccata* and ornamental plants, one basic type

TABLE 3. A comparison of individual characteristics of *Cephalotaxus*, *Taxus* and *Torreya* reported by other authors with the values found in our study. Abbreviations: LA – length of long axis, SA – length of short axis, Ex – exine thickness; * diameter.

Characteristics (min.-max.)					
Author	**LA (μm)**	**SA (μm)**	**Orbicule**		**Ornamentation sculpture (SEM)**
			diameter	**surface**	
Cephalotaxus harringtonia					
Ueno (1959)	30–33	–	–	–	granular, sometimes rough corrugate
Yamazaki and Takeoka (1962)	27–30*	25.5–28.5	0.25–0.9	–	–
Lee (1979)	18–22	15–18	–	–	–
Zhen (1993)	22.6–34.8	–	–	minute gemmate	granulate
our study	28.0–44.2	25.6–42.9	0.3–1.2	microverrucate	irregulary granulate–verrucate
Taxus baccata					
Erdtman (1965)	22.5–25.8	–	–	–	granulate
Predan and Toniuc (2009)	20.2–22	–	small	–	verrucate
Halbritter (2016)	10–25	–	–	–	microgemmate
our study	19.2–29.6	17.8–24.6	0.2–0.8	microverrucate	granulate
Taxus cuspidata					
Ueno (1959)	18–20	–	–	–	granulate
Yamazaki and Takeoka (1962)	32–35	31–34	0.1–0.6	minute spinules (30–80 μm long)	–
Lee (1979)	18–26	18–24	–	–	–
our study	18.6–33.0	17.3–28.5	0.3–1.7	microverrucate	granulate
Torreya nucifera					
Ueno (1959)	30	–	–	–	granular, sometimes rough corrugate
Yamazaki and Takeoka (1962)	30–34	–	0.2–0.6	spinuliferous (70 μm length)	–
Predan and Toniuc (2009)	22.2–28.4	–	–	–	verruccate
our study	24.6–37.3	23.5–33–7	0.2–1.3	microverrucate	microverrucate

can be distinguished: granulate-orbiculate. Predan and Toniuc (2009) reported that the *T. baccata* pollen had verrucous exine, whereas Halbritter (2016) reported that the exine was microgemmate. However, different types of sculpture were found on some specimens of *Ginkgo biloba* cultivars, e.g., *G. biloba* 'Fastigiata', 'Jan III Sobieski', 'Pendula' (Korszun and Klimko, 2014) and in *G. biloba* 'Pragensis' (Klimko et al., 2016). In *Prunus* the striae thickness differs significantly between cultivars within species, while the groove width differs between some species and cultivars (Chwil, 2015).

The pollen grains of *T. cuspidata* species type from Kyoto (Japan) were described by Ueno (1959). Their diameter was 18–20 μm. The grains were more or less spheroidal, angular in outline. Yamazaki and Takeoka (1962) reported that the pollen grain diameter was 31–34 x 32–35 μm, nearly circular in polar view, and oblate-spheroidal in equatorial view. The exine was covered with small orbicules (diameter 0.1–0.6 μm) with minute spinules about 30-80 μm long. There were differences in the descriptions of the shape of *T. cuspidata* pollen (Table 2), because the authors applied the SA/LA ratio. In palynological studies the LA/SA and SA/LA ratios were applied (Bykowska and Klimko, 2016; Wrońska-Pilarek et al., 2016). The comparison of our data with those published by other authors revealed some differences. The surface of the orbicules was microverrucate. Yamazaki and Takeoka (1962) also investigated other *Taxus* species and obtained mostly the same results as in *T. cuspidata.*

Our study is the first comparison of the size, shape and sculptures of pollen grains in *T.* ×*media* cultivars. We found that the exine ornamentation of pollen grains in SEM was similar to *T. baccata* and *T. cuspidata.* The general morphology of pollen grains of the three taxa was similar. Kuprianova and Gumbatov (1988) reported that the diameter of orbicules of the *Taxus* genus was 0.7–0.9 (1.0) μm or more. In our study this diameter ranged from 0.3 to 0.9 (1.7) μm. The SEM observations showed that *Taxus* had more orbicules than *Cephalotaxus* and *Torreya.*

Ueno (1959) and Yamazaki and Takeoka (1962) described *Torreya nucifera* pollen grains. The pollen was more or less spheroidal (30–34 μm). In lateral view it was sometimes slightly ellipsoidal and aperturate. The surface of the pollen grain was covered with spinuliferous orbicules. The diameter of orbicules was about 0.2–0.6 μm and their spinules were 70 μm in length. Predan and Toniuc (2009) reported that the pollen grains from Romania were between 22.2 and 28.4 μm in diameter. In our study the pollen grains were between 24.6 and 37.3 μm in diameter (LA). The pollen was inaperturate and the exine surface was verrucous, like in the study by Predan and Toniuc (2009). The taxoid pollen exine ruptured during hydration at a specialized region (the tenuitas or papilla) in the centre of a circular leptoma and was consequentially shed (Hesse et al., 2009). Kuprianova and Gumbatov (1988) and Predan and Toniuc (2009) noted that many pollen grains of *T. baccata* and *Torreya* sp. from plants growing in the Caucasus Mountains and in Bucharest were deformed. We also observed deformed pollen grains in LM and SEM.

The orbicule diameters in our study were similar to those reported by Ueno (1959), Yamazaki and Takeoka (1962) and Zhen (1993). However, the results of our study showed a wider range of orbicule diameter values for *Cephalotaxus harringtonia*, *Taxus cuspidata* and *Torreya nucifera* than the data provided in the reference publications. Our study also supplemented information about the pollen grains of *Taxus baccata* and *T.* ×*media*, because their orbicule diameter was not measured in any of the cited reference publications (Table 3).

The presence of an aperture in *Cephalotaxus* and *Taxus* is disputable. According to Kodrul et al. (2006), the aperture is thought to be small and masked by the folds of the exine. The pollen grains in our study were inaperturate, but the data in reference publications are divergent. Some authors described the pollen grains of the taxa under study as aperturate (Ueno, 1959; Yamazaki and Takeoka, 1962), while others did not find any apertures (Kedves et al., 1991; Zhen, 1993; Predan and Toniuc, 2009; Yadav et al., 2013; Halbritter, 2016). Halbritter (2016) studied air-dried and critical point dried pollen grains of *Taxus baccata* in LM, SEM and TEM, and Yadav et al. (2013) studied critical point-dried pollen grains of *Taxus wallichiana*, *Cephalotaxus mannii* and *Cephalotaxus griffithii* in SEM, and found that the pollen was inaperturate. Zhen (1993) used LM, SEM and TEM in his study on pollen grains of 7 *Cephalotaxus* species, but did not mention the presence of an aperture in the research findings.

Our study revealed no correlation between the chromosome number and pollen grain size (*Cephalotaxus* n = 12, *Taxus* n = 12, and *Torreya* n = 11). The wall structure and pollen tube of Cephalotaxaceae and Taxaceae resembled those in Taxodiaceae and Cupressaceae (Ueno, 1959). The morphology and ultrastsructure of pollen grains in Cupressaceae and Taxaceae were similar (Surova and Gumbatov, 1986; Surova and Kvavadze, 1988; Kurmann, 1990). The pollen grains were spheroidal or flattened spheroidal, granular or verrucose ectexine, and lamellate endexine, with numerous orbicules (Kurmann,

1994). There were significant differences between Cupressaceae and Taxaceae on the distal face and in the ornamentation of orbicules (Bykowska and Klimko, 2016).

CONCLUSIONS

Our study showed that several morphological characteristics of pollen could be taxonomically valuable. Some features used for comparing species may be useful morphological characteristics of pollen grains described in this paper. The most important pollen grain features of the *Cephalotaxus*, *Taxus* and *Torreya* taxa were the size, exine ornamentation and the surface of orbicules. The pollen features were insufficient to distinguish between individual *Taxus baccata*, *T. cuspidata*, *T.* ×*media* and their cultivars – only groups were isolated. The values of the coefficient of variability of three features (LA, SA and LA/SA) were significantly lower than the orbicule diameter. The *Taxus* spp. pollen grains were smaller, with more orbicules than the *Cephalotaxus* and *Torreya* pollen grains. The *Taxus* and *Cephalotaxus* genera differed significantly from *Torreya* in the LA and SA. Palynological studies gave taxonomic support for recognition of two different genera of the Cephalotaxaceae and Taxaceae families which are closely related.

AUTHORS' CONTRIBUTIONS

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

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