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Microsporangia and pollen morphology of *Ginkgo biloba* cultivars

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Abstract: Morphological features of microsporangia and pollen grains from cultivated plants of *Ginkgo biloba* were examined using light and scanning microscopy. The sporophylls bear mainly two pendulous microsporangia; three or four were rarely found. The sporangia dehisce along a longitudinal slit and are characterized by reticulate primary sculpture. The basic shape of pollen grains is prolate and perprolate (rarely spheroidal) and they possess a single aperture, which extends from one extremity of the pollen grain to the other. The surface is rugulate, folded, psilate, psilate-slightly striate, regularly striate and fossulate. Perforations are present. Different types of sculpture were found on the same specimens. Our results suggest that although some cultivated plants are morphologically well characterized by habit and shape of leaves, they cannot be separated based on microsporangia and pollen grain morphology. In our investigations the microsporangia and pollen grain micromorphology of eight cultivars of *Ginkgo* were studied for the first time, providing some important new data.

Additional key words: microsporangia, pollen grains, morphology, SEM.

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Introduction

Living Ginkgoales are represented by a single species, *Ginkgo biloba* L. *G. biloba* is now a rare species in the wild but has been widely and long cultivated as an ornamental. *G. biloba* is a dioecious plant. Pollen cones are borne in the axils of the scale, and foliar leaves on the short shoots on male plants. The number of pollen cones (strobili) in one short shoot is almost six and this number is similar in different male trees. The cone is catkin-like and consists of a main axis with spirally arranged sporophylls and oval, elongate-elliptical, or boat-shaped sporangia (Sporne 1971; Crane 1985). The sporophylls bear two pendulous microsporangia but sometimes three and rarely four (Liu et al. 2006). The pollen grains of *G. biloba* possess one simple aperture that extends from one extremity of the pollen grain to the other. The terms used to describe the aperture of these pollen grains vary from monocolpate (Erdtman 1954; Nakamura 1978, Liu et al. 2006) to 1-sulcate (Yamazaki and Takeoka 1962) or anacolpate (Erdtman 1965). The desiccated grains are boat-shaped with a single longitudinal furrow while the fully swollen grains are almost spherical with a rounded or oval germinal aperture. Sahashi and Ueno (1986) investigated pollen with scanning electron microscopy (SEM) and concluded that the pollen surface (ulcerate) and the pollen grains are bordered by a rim from the proximal hemisphere and differ in sculpture. Audran and Masure (1978), Sahashi and Ueno (1986), Tekleva et al. (2007) and Zavialova et al. (2011) illustrated only the overall shape and type of sculpturing of spheroidal pollen grains of cultivated plants from France and Japan, from the Sochi arboretum and from the arboretum of the health resort 'Belye nochi', Sochi (Russia). To date there is limited research on other varieties.

The present study was conducted to illustrate the microstructure of mature microsporangia and dehydrated pollen grains of various cultivated *G. biloba*, with the aim of providing new diagnostic features for their characterization. The aims of our study were to examine and describe microsporangia and pollen morphology and to determine their characters of systematic importance.

Material and methods

Microsporangia of *G. biloba* were collected on 5–14 May, 2010–2012 from eight ornamental cultivated plants and one male clone (Table 1) by the authors from the collection of the Department of Dendrology and Nursery, University of Life Sciences in Poznań.

Each specimen was represented by 10 pollen cones. The pollen grains were studied with light microscopy (LM) and SEM to obtain comprehensive information about the general morphology and exine sculpture. The investigations with SEM were made on pollen grains which were dried in air, and for LM pollen was treated with 10% KOH (Frederiksen 1978). The lengths of the polar axis (P) and equatorial axis (E) were measured in 100 pollen grains per specimens and the P/E ratio was calculated.

The arithmetic mean, standard deviation and coefficient of variation for each mentioned trait were calculated (Table 1). The biometric data were analysed statistically. For each pollen grain feature, one-factor analysis of variance (ANOVA) was used to examine differences in means among cultivars studied. If significant differences were noted, multiple comparisons were carried out based on Tukey's test for equal sample sizes. Statistical analyses were performed using JMP 8.0 (SAS Institute, Inc. Cary, NC, USA: http://www.sas.com/).

The SEM micrographs were taken with a Zeiss EVO 40 microscope at the Electron Microscopy Laboratory, Faculty of Biology, Adam Mickiewicz University, Poznań. Prior to observation, the prepared material was sputtered with gold using an SCB 050 ion sputter. The study was documented with photographs taken during observation, primarily at magnification from × 250 to × 400 and surface × 2000 for microsporangia, for pollen grains × 7500 and × 20000 for exine. Micromorphological features of microsporangia were observed on the dorsal surface and exine sculpturing in the proximal and distal view. The terminology used to describe microsporangia/anther dehiscence followed Radford et al. (1974). With regard to the microsporangia surface, mainly the terminology of Barthlott (1981) was applied. The pollen terminology was adopted from Faegri and Iversen (1989), while the shape classification followed that of Erdtman (1952) based on the P/E ratio (Table 1).

Results

The main morphological features of the investigated pollen grains are summarized in Table 1, while selected SEM micrographs of microsporangia and pollen grains are shown in Figures 1–28.

Morphological descriptions and interpretations

Pollen cone, shape and ornamentation

General morphology – The cone is catkin-like and consists of a main axis with spirally arranged sporophylls. The sporophylls bear two pendulous microsporangia in most cases (Figs 1A–9A) but sometimes, very rarely, three (Fig. 10) or four. Three sporangia were found in 'Saratoga', 'Fastigiata', 'Jan III Sobieski' and 'Władysław Łokietek' and four in 'Jan III Sobieski'. The sporangia of studied specimens of *G. biloba* based on their mode of dehiscence are longitudinal (Figs 5A, 7A).

The shape of microsporangia shows low variability. Generally, most sporangia are oval (Figs 1A–4A), elongate-elliptical (Figs 5A, 6A, 10B), rarely, almost round in 'Fastigiata', 'Pendula' and in the male clone (Figs 7A–9A) and boat-shaped when mature and open with almost acute (Figs 1A, 10A) or rounded apices (Figs 1A, 4A, 6A–9A). The average size of sporangia was 1.3–3.25 × 0.37–1.5 mm. The colour is yellow and the entire surface is uniformly coloured and glabrous. The dorsal and ventral surface is convex.

SEM investigation at higher magnification revealed one type of microsporangium sculpture – reticulate in all studied specimens of ornamental cultivars (Fig. 2B) and the male clone. In the dried state their surface is rugose (Figs 1B, 3B–9B). The cell shapes are polygonal, irregular, elongate in one direction, with straight or slightly undulating walls (Figs 1B–9B).

Table 1. Ranges (minimum-maximum), mean values (± SE), and coefficient of variation (CV) of morphological features of *Ginkgo biloba* pollen grains

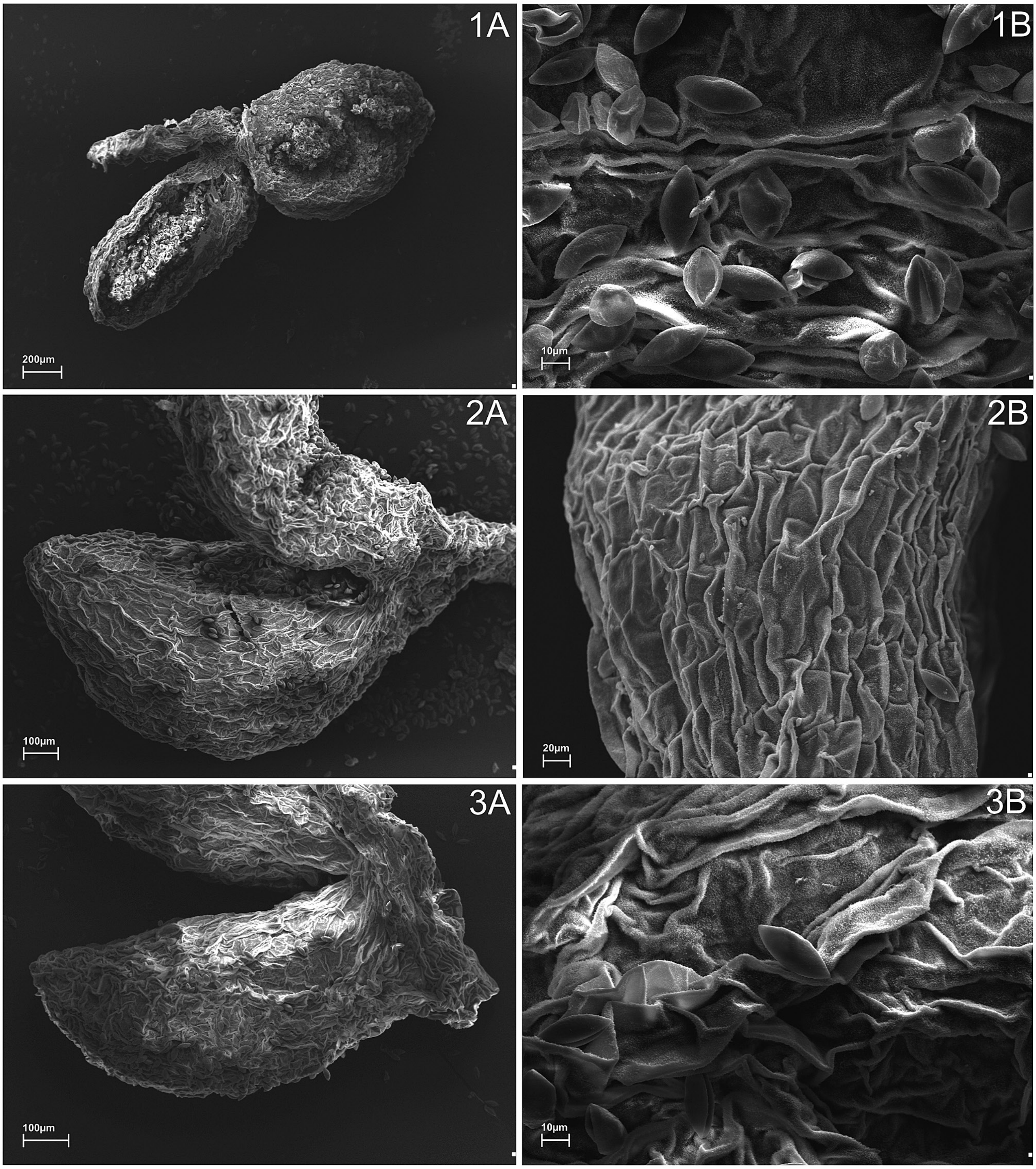
Cultivars of Ginkgo biloba	Pollen feature											
	Polar length (P.)				Equatorial width (E)				P / E ratio			
	Min	Max	Mean (±SE)	CV%	Min	Max	Mean (±SE)	CV%	Min	Max	Mean (±SE)	CV%
'Fastigiata'	35.1	40.5	37.71 (0.39) abc	5.6	13.5	18.9	15.70 (0.24) a	8.3	2.1	2.8	2.41 (0.03) ab	7.3
'Jan III Sobieski'	32.4	40.5	36.72 (0.43) c	6.4	13.5	18.9	15.79 (0.25) a	8.7	2.0	2.8	2.34 (0.04) ab	10.1
'Kazimierz Wielki'	32.4	40.5	36.72 (0.49) c	7.3	13.5	18.9	16.02 (0.23) a	7.9	2.0	2.8	2.30 (0.03) b	7.5
Clon of Male	32.4	48.6	39.33 (0.64) a	8.9	13.5	18.9	15.84 (0.24) a	8.4	2.1	2.7	2.49 (0.03) a	6.0
'Pendula'	35.1	45.9	38.25 (0.49) abc	7.0	13.5	18.9	16.02 (0.21) a	7.3	2.0	2.8	2.40 (0.03) ab	7.7
'Przemysław II'	32.4	43.2	36.77 (0.58) bc	8.7	13.5	18.9	15.03 (0.26) a	9.6	2.0	3.0	2.47 (0.06) ab	13.0
'Saratoga'	32.4	43.2	38.39 (0.42) abc	6.0	13.5	17.55	15.57 (0.20) a	7.1	2.2	3.0	2.48 (0.04) a	9.0
'Szamotuły'	29.7	43.2	38.92 (0.56) ab	7.8	13.5	17.55	15.88 (0.17) a	5.8	2.0	2.8	2.46 (0.04) ab	8.4
'Władysław Łokietek'	32.4	43.2	37.13 (0.43) bc	6.3	13.5	17.55	15.35 (0.22) a	7.8	2.2	2.8	2.43 (0.03) ab	7.6
ANOVA			F 4.0362	P 0.0002			F 2.0833	P 0.0378			F 2.7538	P 0.0062

ANOVAs were performer separately for each pollen features to determine the differences among cultivars studied. Same letters indicate a lack of statistically significant differences between analyzed cultivars according to Tukey's a posteriori test ($P<0.05$).

Anticlinal cell wall boundaries are generally well developed. Based on the relief of cell boundaries, one type was distinguished – raised (Figs 1B–9B). The curvature of outer periclinal cell walls is slightly concave (Figs 1B–9B). The surface of the outer cell wall (secondary sculpture) shows low variation among examined specimens; it varies from smooth to folded (Fig. 2B). The cuticle is smooth and covered with wax platelets (Figs 1B–9B). Our study indicated that microsporangium sculpture was remarkably similar in cultivars of *G. biloba* and can be taxonomically significant.

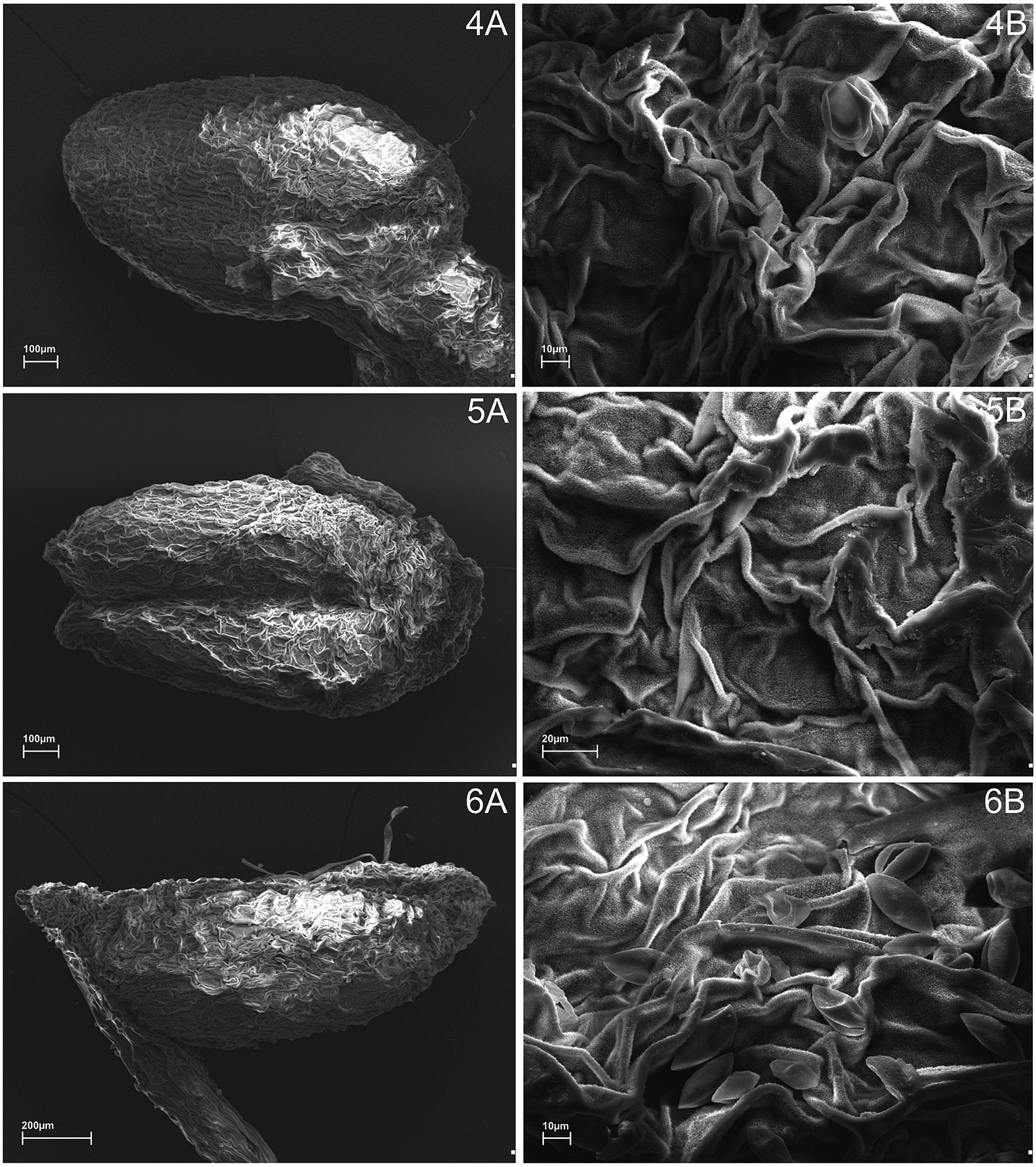
Pollen

General morphology – Dehydrated pollen grains vary in shape; most were boat-shaped, bilaterally

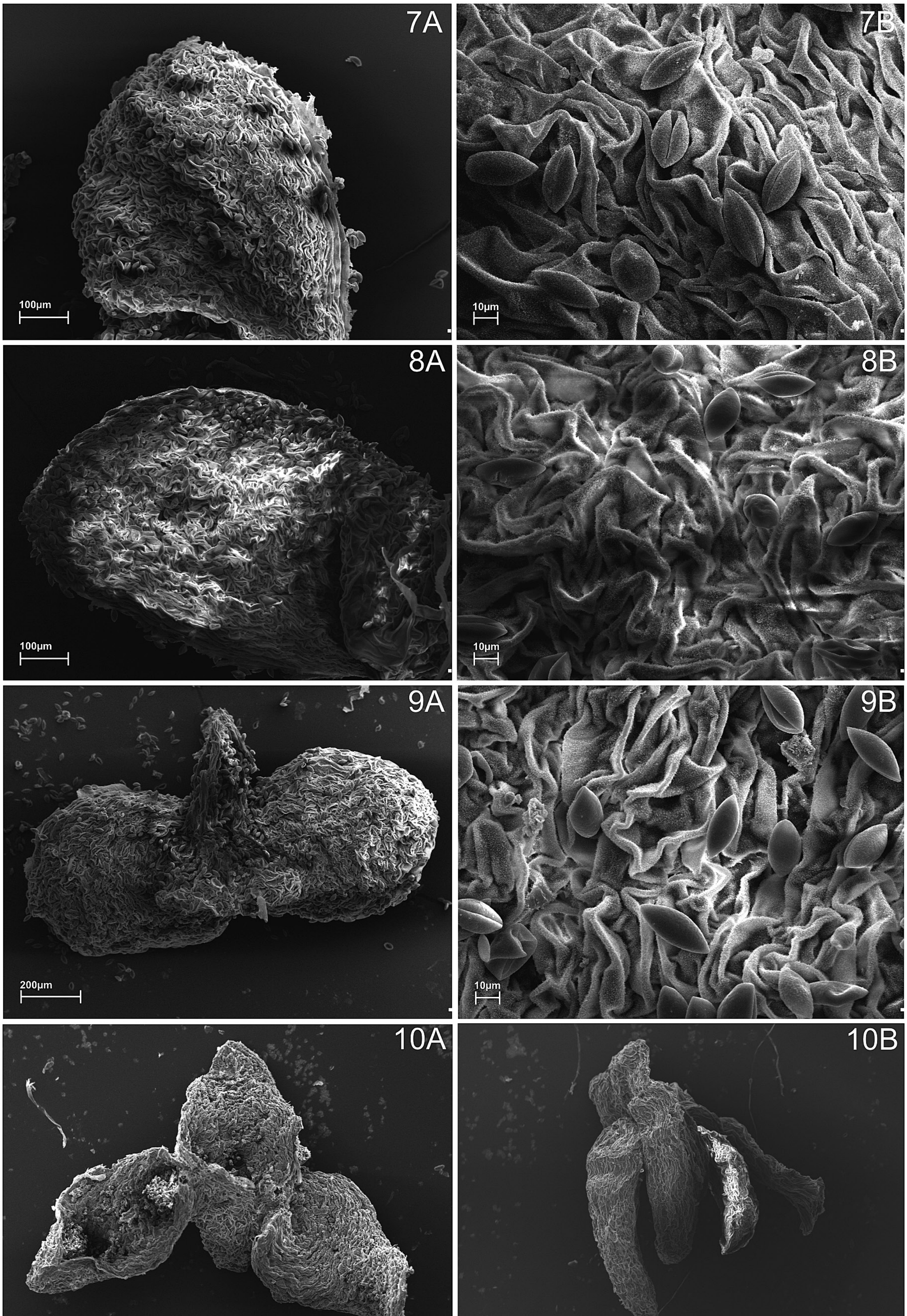


Figs 1–3. SEM micrographs of the microsporangia of *Ginkgo biloba*: 'Saratoga' (1), 'Jan III Sobieski' (2), 'Władysław Łokietek' (3)

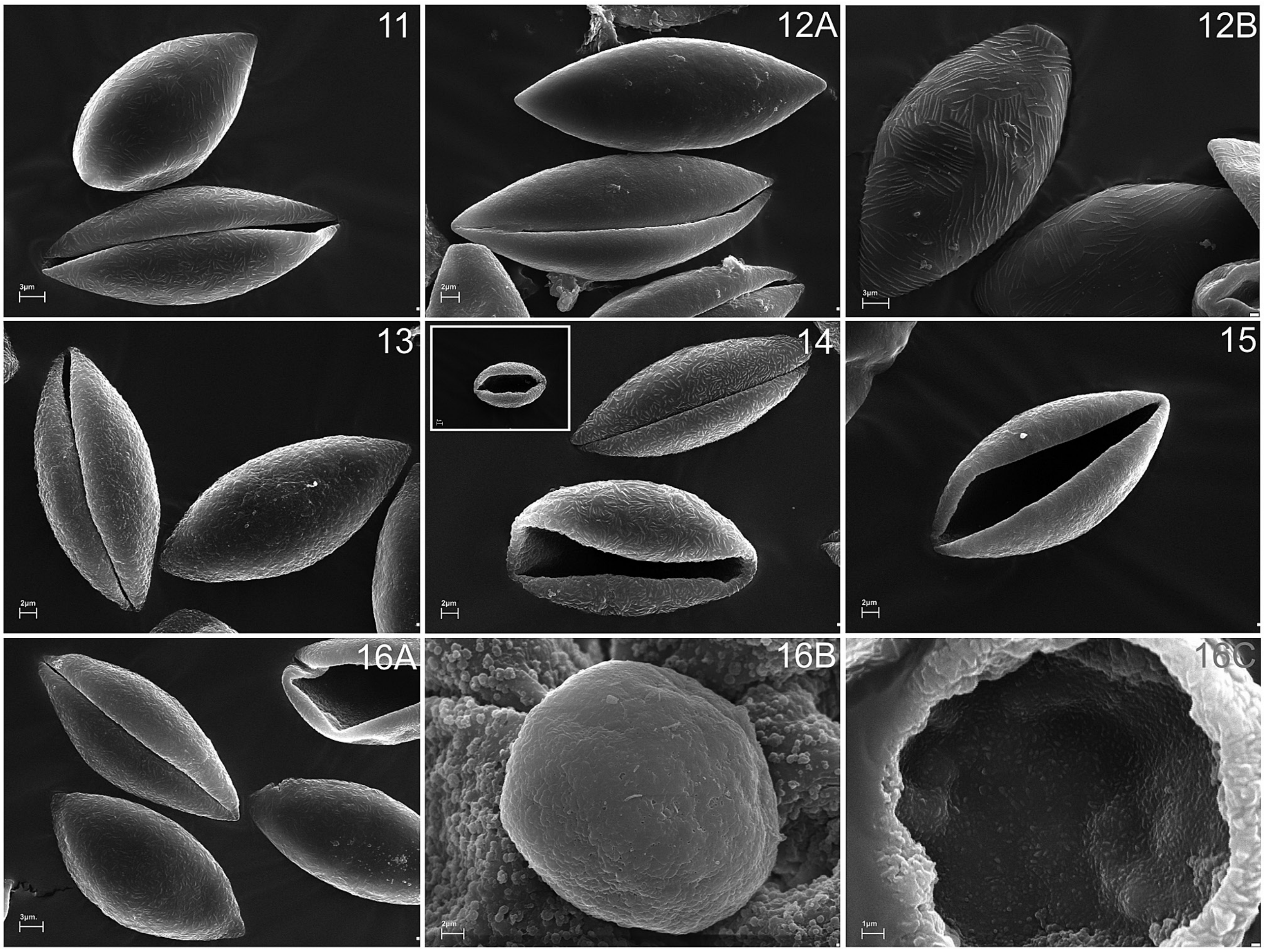
symmetrical as observed in SEM and rarely circular (Fig. 16B). The mean length was from 36.72 to 38.93 μm and width from 15.03 to 16.02 μm (Table 1). Perprolate pollen generally dominated, with a P/E ratio from 2.30 in 'Kazimierz Wielki' to 2.48 in 'Saratoga' and 2.49 in the male clone. Prolate pollen occurred in four cultivars: 'Jan III Sobieski' (16.6%), 'Kazimierz Wielki' (10%), 'Szamotuły' (6.6%) and 'Pendula' (3.3%). Values of P, E, and P/E ratio showed low variability (P – 5.6–8.9%; E – 5.8–9.6%; P/E – 6.0–13.0%) (Table 1). The shape of hydrated pollen grains observed in LM was suboblate, oblate spheroidal to spheroidal (most pollen grain) and P/E varied from 0.75 to 1.0 (more rarely prolate and perprolate). The proximal side of the subcircular pollen was more convex (Fig. 16B) and the distal face was concave



Figs 4–6. SEM micrographs of the microsporangia of *Ginkgo biloba*: 'Kazimierz Wielki' (4), 'Przemysław II' (5), 'Szamotuły' (6)



Figs 7–10. SEM micrographs of the microsporangia of *Ginkgo biloba*: 'Fastigiata' (7), 'Pendula' (8), male clone (9), 'Saratoga' (10A), 'Fastigiata' (10B)



Figs 11–16. SEM micrographs of pollen grains of *Ginkgo biloba*: 'Fastigiata' (11), 'Przemysław II' (12), 'Szamotuły' (13), 'Jan III Sobieski' (14), male clone (15), 'Saratoga' (16)

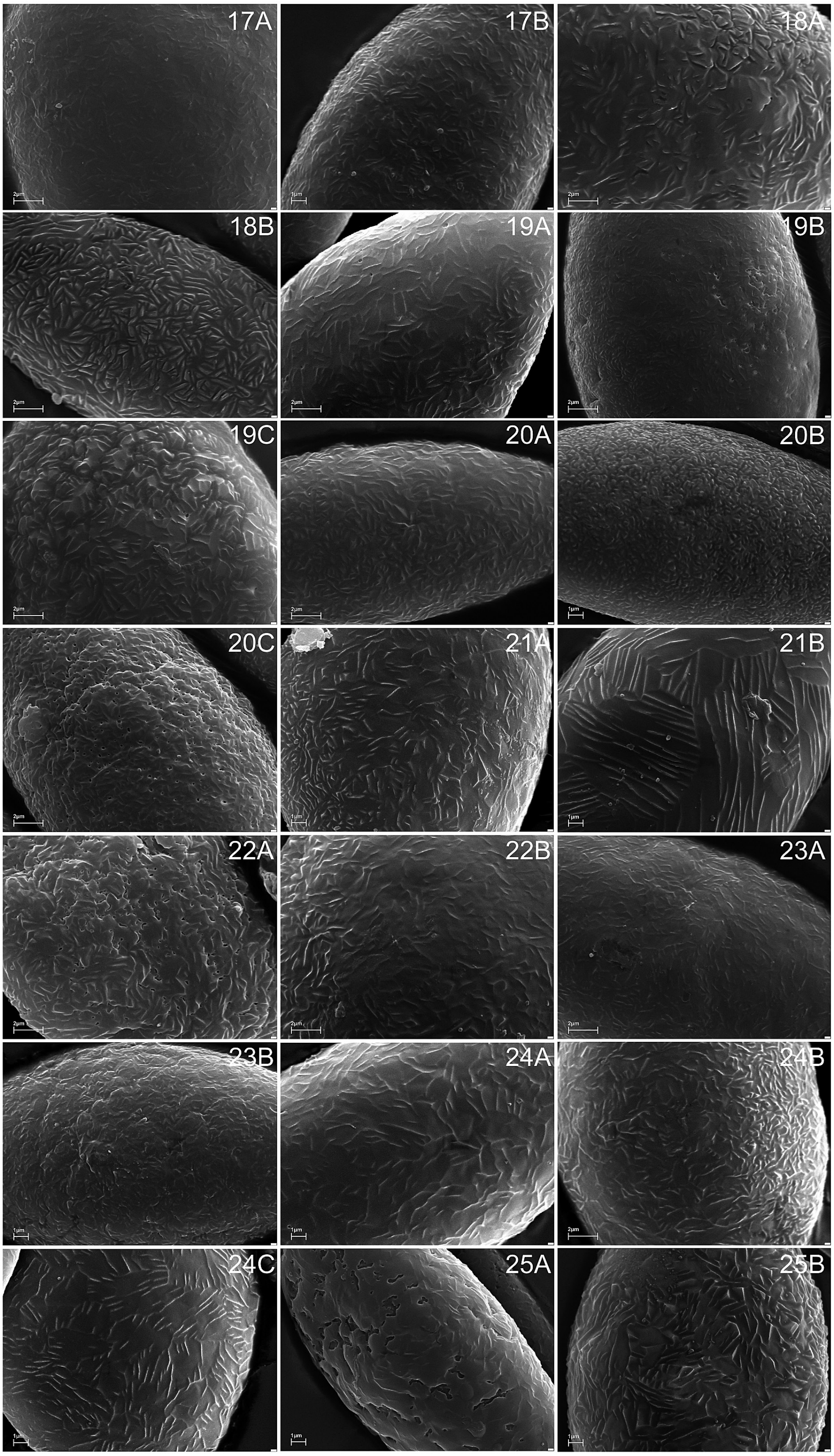
(Fig. 16C). Statistical analysis did not show a significant difference ($P > 0.05$) in the means between the cultivars (Table 1). Pollen grains are of medium size (29.7–48.6 × 13.5–18.9 μm) according to Erdtman's classification (1952).

The aperture – The pollen grains possess a monosulcate 'aperture', which is shrunken as a result of dryness. The ectoaperture is long, without a margo, straight or slightly folded. SEM show that it extends from one extremity of the pollen grain to the other. Pollen grains with a closed sulcus are narrow ellipsoidal (Figs 11, 12A, 13, 15, 16A) and those with an open sulcus are broadly ellipsoidal (Figs 12B, 14, 15) and their extremities are pointed (Figs 12A, 16A) or slightly rounded (Figs 12B, 14).

Exine sculpturing – Exine sculpturing was mainly observed in SEM in the proximal and distal view of boat-shaped pollen (Figs 17–25). The proximal surface has the same sculpturing as on the distal surface but various exine sculpturing between the non-apertural and apertural region in spheroidal pollen was found (Fig. 16B, C). The pollen grains do not have one strict morphological pattern even within the same tree. SEM observations showed that ornamentation can be: psilate (Figs 17A, 19B, 23A), psilate-slightly striate (Figs 19A, 20A, 24A, C), rugulate (Figs 17B, 18B, 20B–22B, 24B), folded (Figs 20C, 22A, 23B, 25A), fossulate (Figs 18A, 19C, 25B) or regularly striate (Fig. 21B). Perforations are rare, randomly scattered, occasionally arranged in groups and elongated and slit-like (Figs 19B, 25A). The extremities and margin of this pollen are irregularly striate. A striate sculpture is not affected by variants in processing and hydration of the pollen. The exine of the margin aperture is psilate (e.g. Figs 11–13) or slightly verrucate (Figs 14, 16C).

Discussion

Pollen cones in the study by Liu et al. (2006) were sampled from a mature tree in the Beijing Botanical Garden (China). In 4168 sporophylls considerable differentiation of sporangia numbers was found, e.g. two sporangia in 91.74%, three in 7.72% and four



Figs 17—25. SEM micrographs of ornamentation of pollen grains of *Ginkgo biloba:* 'Fastigiata' (17), 'Jan III Sobieski' (18), 'Kazimierz Wielki' (19), 'Pendula' (20), 'Przemysław II' (21), 'Saratoga' (22), 'Szamotuły' (23), 'Władysław Łokietek' (24), male clone (25)

sporangia in 0.55%. In our study the sporophylls bear two pendulous sporangia in most cases. Four and three simple sporangia were observed in 'Fastigiata', 'Saratoga', 'Jan III Sobieski' and 'Władysław Łokietek'. The pollen cones of *Ginkgo* have undergone a process of reduction of the number of sporangia from three or four to two, and therefore support the reduction hypothesis of reproductive organs (Liu et al. 2006). The shape of sporangia was oval, elongate-elliptical and boat-shaped and in our study some were also almost round (Liu et al. 2006). The microsporangia walls exhibit shrinkage of the epidermis, fibrous thickening of the endothecium and enzymatic dissolution of the tapetum during pollen dispersal, which contributes to sporangia opening (Keijzer 1987; Abid and Qaiser 2004; Lu et al. 2011). Based on the mode of dehiscence of microsporangia/anthers there are four types: longitudinal, poricidal, valvular and transverse (Radford et al. 1974). The sporangia dehisce along a longitudinal slit, confirming earlier observations, e.g. by Liu et al. (2006). The mode of dehiscence of sporangia/anther is a diagnostic feature and can be utilized for taxonomic determination at generic and family levels (Bano et al. 2008). There are only a few data concerning size of sporangia. The average length and width of sporangia were similar as reported earlier by Liu et al. (2006) (2–3 × 1–1.5 mm) but have a slightly wider range in our study (1.3–3.25 × 0.37–1.5 mm). The present study is the first to investigate the sporangium surface of *G. biloba* and detailed observations of sporangia sculpture on the dorsal surface provided new data.

There are also only scarce data concerning the size of cultivated pollen grains in *G. biloba*, but the ornamental cultivated plants have not been studied. Pollen size shows a wide range of variation. The pollen length was reported to vary from 25 to 47 μm (Audran and Masure 1978), from 27 to 29 μm (Tekleva et al. 2007) and from 14 to 34 μm (Zavialova et al. 2011). The values of particular characters obtained in our studies did not differ drastically from the data reported in the species description in these palynological works.

The spheroidal pollen grain has an almost rounded, convex or swollen aperture and slightly sunken margin (Tekleva et al. 2007; Zavialova et al. 2011). In our study we did not observe pollen grains like those described above. According to the literature the proximal surface of this pollen was rugulate with rare granules and rarely with small perforations and the surface of the aperture finely verrucate. In particular we noted the sculpture of boat-shaped pollen grains. Regarding sculpturing of exine, six basic types can be distinguished: psilate, psilate-slightly striate, rugulate, regularly striate, fossulate and folded. Zavialova et al. (2011) concluded that the proximal surface of boat-shaped pollen grains was rugulate. The pollen grains do not have one strict morphological pattern even within the same tree (Figs 17–25). The results of the present study demonstrated that the sculpture of pollen grains shows considerable variation and some cultivars may have more variable pollen than others. A single aperture at the distal pole characterizes the gymnosperm taxa of higher rank Cycadoideae, Bennettitopsida and Ginkgopsida (Zavialova et al. 2011), the primitive dicotyledons, e.g. *Magnolia* and *Degeneria*, and monocotyledons, e.g. *Dracaena*, *Sansevieria*, *Hypoxis* (Klimko et al. unpublished results). *Ginkgo* and *Cycas* have pollen grains that are extremely similar in external morphology but differ considerably in the ultrastructure (Tekleva et al. 2007). The sporoderm ultrastructure of modern *Ginkgo* was described by Tekleva et al. (2007) and Zavialova et al. (2011). According to the authors the proximal and distal pollen walls differ considerably in thickness and structure. The ectexine consists of solid and very thick tectum. The endexine is nearly uniform in thickness on both the proximal and distal sides. The endexine is thin, four to six times thinner than the ectexine. The intine is about half as thick as the exine and it varies in thickness on the proximal and distal sides. Pollen is characterised by infratectal elements of irregularly variable shape including columellate-like and granular elements (Zavialova et al. 2011).

Our results suggest that although some cultivated plants are morphologically well characterized by habit and shape of leaves (our observation), they cannot be separated based on microsporangia and pollen grain morphology. In conclusion, our study of the microsporangia and pollen grain micromorphology of some cultivars of *Ginkgo* provided some important new data, e.g. shape and ornamentation of microsporangia, psilate, psilate-striate and regularly striate, fossulate and folded sculpture of pollen grains. Thus, a detailed analysis greatly increases our knowledge of individual cultivars and our studies should be continued.

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