POLLEN CHARACTERISTICS IN SOME PERSIMMON SPECIES (*DIOSPYROS* spp.)

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Aim of the work was to study the general characteristics and essential morphological traits of pollen grains, as the size, shape of pollen grains and number, form and position of apertures in *Diospyros kaki* L. f., *Diospyros virginiana* L., *Diospyros lotus* L. species and interspecies hybrid of *Diospyros virginiana* L. × *Diospyros kaki* L. f. The studies were performed on pollen obtained from genotypes cultivated in Slovakia (*D. kaki* L. f. a *D. lotus* L.) and Ukraine (*D. virginiana* L., *D. virginiana* L. × *D. kaki* L. f.). Pollen grains morphological traits were evaluated using the scanning and transmission electron microscopy. Mean length of polar axis varied between 48.0 µm (*D. lotus* L.) and 71.19 µm (*D. virginiana* L. × *D. kaki* L. f.) and equatorial axis (pollen width) from 23.56 µm (*D. virginiana* L.) to 33.14 µm (*D. virginiana* L. × *D. kaki* L. f.). The highest values of both axes were observed in the pollen grains of interspecies hybrid *Diospyros virginiana* L. × *Diospyros kaki* L. *Diospyros* spp. genotypes tested pollen grains polar axis variation range were classified as medium-sized to large. Regarding the shape index values, which ranged from 1.75 (*D. kaki* L. f.) to 2.18 (*D. virginiana* L.), are the pollen grains predominantly prolate or prolate spheroidal. Significant differences were detected among the tested species and the interspecific hybrid as well as between individual genotypes of *D. lotus* L., especially in the equatorial axis. The significant differences in the length of polar axis and equatorial diameter axis indicate the interspecific and intraspecific pollen variability of *Diospyros* species. Generally, the tested species pollen grains are solitary, isopolar, radially symmetric. According to shape and number of apertures the pollen grains are tricolporate. This study of pollen grains in species of genus *Diospyros* enlarged the currently sparse knowledge on taxonomical, palinological and practical use in bee-keeping oriented on identification of pollen grains occurring in the corbicular bee-pollen.

Key words: *Diospyros* spp.; *D. kak*i L. f; *D. virginiana* L.; *D. lotus* L.; *D. virginiana* L. × *D. kaki* L. f.; pollen grains; morphological traits of pollen grains

INTRODUCTION

Species of genus *Diospyros* belong to an extensive class of family *Ebenaceae* consisting of 7 genera. Yonemori et al. (2000) listed over 400 species forming the *Diospyros* genus and several of these species are of economic importance. The mild climatic region is suitable for growing and use in horticulture of three persimmon species – *Diospyros kaki* L. f. (japanese persimmon), *Diospyros virginiana* L. (persimmon virgin-

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iana) and *Diospyros lotus* L. (date persimmon) (Bellini & Giordani 2005). Japanese persimmon is prevalently occurring in the northern China, growing up to 1830–2500 m of altitude above the sea level (Morton & Kincaid 1995). From these original places it expanded to Japan, USA, Ukraine, Russia, Balkan region (Bellini & Giordani 2005) and Italy (Giordani 2002). Freely growing populations of date persimmon are widespread over many territories in western Asia (Turkey, Armenia, Azerbaijan), central Asia (Tajikistan, Turkmenistan, Uzbekistan) and in China, Nepal and Pakistan (Giordani 2002). Northern America is a region of natural occurrence of persimmon virginiana (Capon 1990). The first introduction of this species to Europe was realized in the 18th century – first to England and subsequently to France, Italy and to southern Ukraine (Rubcov 1974).

In Slovakia, persimmon species are cultivated in botanical gardens as well as by many small growers. The present knowledge on growing of these originally exotic plants clearly show the possibility of successful cultivation in local climatic conditions, preferably in the southern part of Slovakia. Research work and most studies of these species were concentrated for several years in the Slovak University of Agriculture in Nitra in close cooperation with the National Botanic Garden of the Ukraine National Academy of Sciences in Kiev (Grygorieva et al. 2009).

All the above mentioned persimmon species are deciduous plants. They are economically utilized as fruit trees. Persimmon fruit is edible, suitable for consuming when reaching softness and are fully ripe (Heaton 1997). Matured fruit is of „meaty" consistence with a very delicious taste. It is consumed in raw and/or dried state, further in the form of jams, marmalades, syrups, beverages, fruit salads, distilled drinks eventually as additives to ice-creams, yogurts and other food products (Heaton 1997). Fruits, flowers and other plant parts contain many biologically active substances having often importance even from the medicinal viewpoint e.g. triterpenoides (Thuong et al. 2008), diospyrine (Hazra et al. 2005), phenolic compounds, flavone glycosides, flavonoids, carotenoids (Hosotani et al. 2004) and tannins (Park et al. 2004).

For practical reasons, concerning the use and distribution of mentioned plant species in Slovakia, it is quite important to learn the biological peculiarities connected with flowering as well as with interspecific variability and reproduction capacity in local climatic conditions. It is known, the persimmon flowers are prevailingly unisexual, only exceptionally being bisexual. It means, the plant bears both, the male and female flowers. Sometimes the dioecism occurs. Many individuals have only female flowers and the fruits can arise without fertilization – by parthenocarpy and they are seedless. Flowering biology complexity is for these species symptomatic, and it brings questions concerning several topics like the pollen grains characteristics, designated in palinology as sporoforms. Information on *Diospyros* genus pollen grain traits occurs scarcely in a few papers (Erdtman 1966; Kodela 2006; Geeraerts et al. 2009).

The morphological characteristics of pollen grains are utilized for taxonomic classification purposes, further for phylogenetic evaluation of plant species and detection of hybrid and polyploid forms (Erdtman 1966; 1986; Erdtman & Roger 2007).

Then aim of this work was determination of pollen grains general characteristics and basic morphological traits in selected species of *Diospyros* genus including the variability of tested species.

MATERIAL AND METHODS

The pollen grains morphology was studied in *Diospyros kaki* L. f., *Diospyros virginiana* L., *Diospyros lotus* L. species and interspecies hybrid *D. virginiana* L. × *D. kaki* L. f. We have evaluated the basic morphological characteristics of tested persimmon species pollen grains – the size and shape, position, number and form of apertures (aperture – the thinned region of the sporoderma – wall pollen grain, usually function as sites of the germination).

The pollen grain size has been determined by measuring the length of polar axis (P – the straight line between the distal and proximal poles of a pollen grain) and length equatorial axis (E – pollen width, the distance between the poles in equatorial part of pollen). The shape index (SI – the ratio of the length of the polar axis and equatorial axis) allowed to determine the pollen grains shape. The pollen grains are classified according to shape index into 9 shape classes (Dostál et al. 1966). The pollen size (length of polar axis and equtorial axis – width of pollen) and shape (SI – P/E) were measured in micrometers.

Analyzed morphological traits were measured and evaluated in 40–60 pollen grains (in polar position) of each species. Electron microscopes ZEISS EVO LS 15 and PEMMA-102 (SELMI) enabled to distinguish and identify of pollen surface – structure, special ornamentation upmost thickness exine – sexine and aperture membrane structure.

Morphological traits were evaluated on pollen samples collected in year 2010 from female flowers during full flowering from the persimmon trees of species *Diospyros virginiana* L., cultivated in Kiev (Ukraine), *D. lotus* L. in Arborétum Mlyňany (Slovakia) and *D. kaki* L. f. in Nitra (Slovakia). Pollen samples released from dry male flowers were further dried under laboratory conditions. For microscopic study of morpholo§gical characteristics the dry pollen was used.

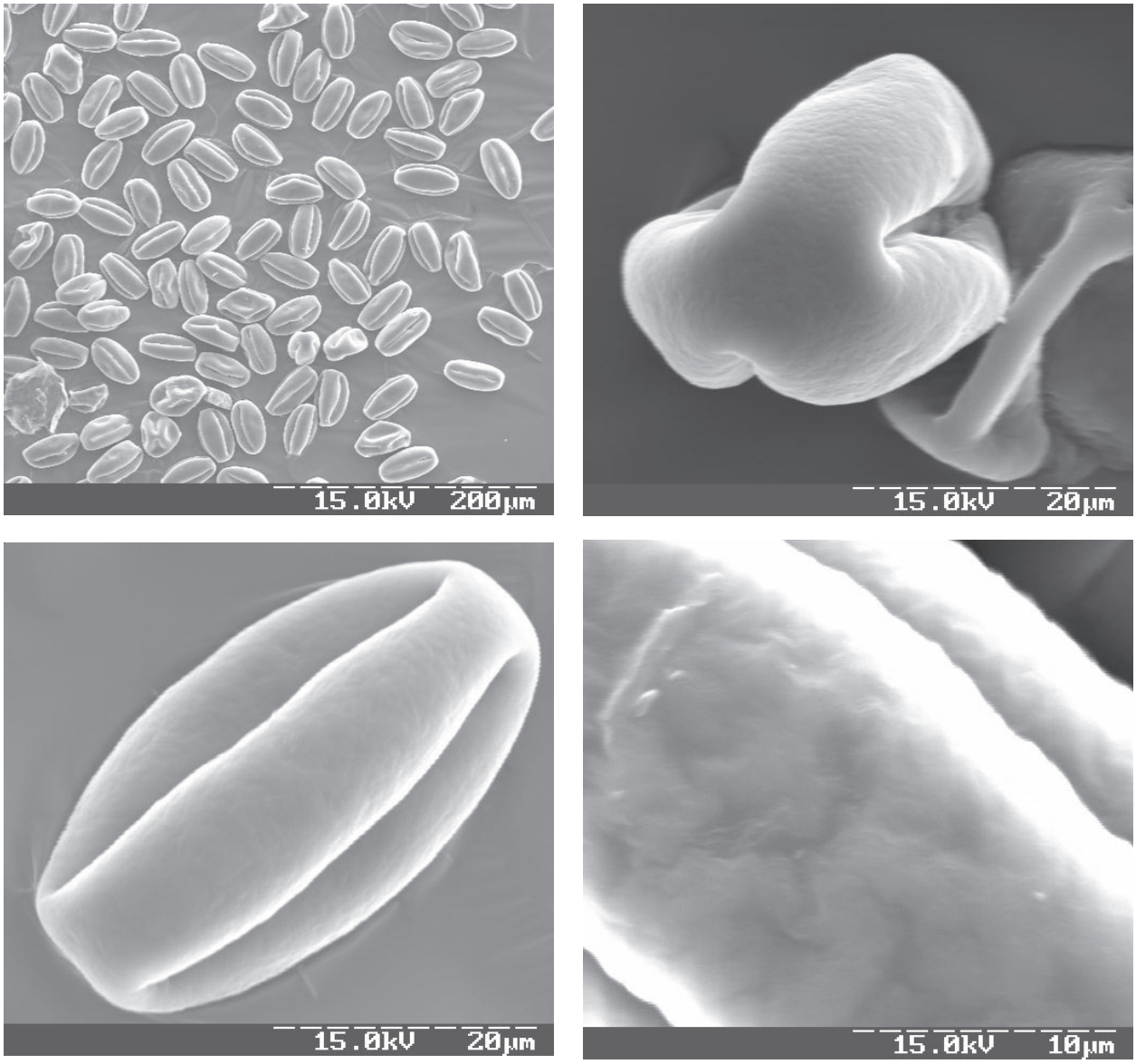

Fig. 1. Pollen grains of *Diospyros lotus* L. species in different position
(Photo: Gurnenko 2010)

RESULTS AND DISCUSSION

Pollen grains of the genus *Diospyros* occur individually – solitery, defined as monades. This study on pollen morphology of tested plant species showed that pollen grains are radially symmetrical, isopolar and according to the localization of apertures are zono-tricolporate. Three compound apertures are according to distribution equidistant. The size, shape of pollen grains and number of apertures are documented on Figures 1–4. Pollen grains possess three prolonged apertures – colpi which are narrowed at poles. Additional pole is in the colpus middle at the equatorial centre (circular equatorial aperture). This classification correlates with literature data (Sowunmi 1995; Kodela 2006; Geeraerts et al. 2009). In some pollen grains were observed annuluses – an area of the exine surrounding a pore with thickened periphery or operculum – part of exine over the pore (Fig. 1–4). The exine membrane in an area of the colpus is granulated. From the cytological view the pollen grains of *Diospyrus* species are bicellular with exception of *Diospyrus kaki* L. f. and *Diospyrus lotus* L., which are unicellular (Kamelina et al. 1983). It means that generative cell division on sperm cells is accomplished in the pollen pocket.

An important morphological characteristic is the size of pollen grains. Our data showed that the pollen grains of the species *Diospyros kaki* L. f., *Diospyros virginiana* L., *Diospyros lotus* L. species and interspecies hybrid *D. virginiana* L. × *D. kaki* L. f. are according to Dostál et al. (1966) classification the medium-sized to large. The length of polar axis (P) measured in the mentioned three species (Table 1) varied from 42.25 to 55.41 µm and the width of equatorial axis (E) was in range of 21.40–32.47 µm. The highest values of mean length of polar axis (71.19 µm) and equatorial axis (33.14 µm) was recorded in pollen of the interspecies hybrid *D. virginiana* L. × *D. kaki* L. f. Differences in values of the both indicators of pollen grain size (P and E) between the hybrid *D. virginiana* L. × *D. kaki* L. f. and other tested species were statistically significant. The significance of differences was observed also in the mean length of polar and equatorial axes in all analyzed species with exception of mean length of polar axis in *Diospyros kaki* L. f. and *Diospyros lotus* L. species. The values of variation coefficient were in the range of 3.63–6.50% for polar and in the range of 6.15–7.56% for equatorial axes. This suggests a low variability in pollen grains size of studied species, dispite statistically significant intraspecific variability, illustrated in Table 1 (P = 48.00–51.39 µm and E = 23.56–27.78 µm).

Results of morphological analysis of pollen in selected genotypes of *Diospyros lotus* L. species from

T a b l e 1

The measured pollen morphological traits of some *Diospyros* species

Species	n	min	max	$\bar{x}$	$s_{\bar{x}}$	V%	SI	Homogeneous groups
P – polar axis [µm]								
Diospyros kaki L. f.	56	43.97	54.06	48.73	0.91	6.50	–	c
Diospyros lotus L.	60	42.50	50.50	48.00	0.50	3.63	–	c
Diospyros virginiana L.	48	49.28	55.41	51.39	0.59	4.00	–	bc
D. virginiana L. *x D. kaki* L. f.	59	63.17	80.54	71.19	0.48	6.30	–	a
E – equatorial axis [µm]								
Diospyros kaki L. f.	56	24.58	32.47	27.78	0.57	7.20	1.75	b
Diospyros lotus L.	60	23.97	31.17	26.06	0.56	7.56	1.84	bc
Diospyros virginiana L.	48	21.40	25.94	23.56	0.41	6.15	2.18	d
D. virginiana L. *x D. kaki* L. f.	59	30.20	36.51	33.14	0.50	5.00	2.14	a

min. – minimum value, max. – maximum value, $\bar{x}$ – arithmetic mean, SI – shape index (P/E),
$s_{\bar{x}}$ – standard error, V – variation coefficient (%), n – number of the individuals

Arborétum Mlyňany (Table 2) confirmed a higher intraspecific variability of pollen grains size as evidenced by the values of variation coefficients for polar (9.76 –21.64 %) and equatorial (7.09–9.29%) axes. It was found, that pollen grains size varies considerably in one individual plant even (Ostrolucká & Križo 1989).

Shape index (SI) of pollen grain depends on parameters of polar (P) and equatorial (E) axis. Shape index (the P/E ratio) of tested species varied from 1.75 (*Diospyros kaki* L. f.) to 2.18 (*Diospyros virginiana*

T a b l e 2

The measured pollen morphological traits of selected genotypes of *Diospyros lotus* L. species

Genotypes	n	min	max	$\bar{x}$	V%	SI	Homogeneous groups
P – polar axis [µm]							
DL – AM 1	40	10.07	63.44	44.37	21.64	–	a
DL – AM 2	40	26.92	54.09	46.93	9.76	–	ab
DL – AM 5	40	22.93	54.22	44.03	11.20	–	a
E – equatorial axis [µm]							
DL – AM 1	40	6.06	34.63	25.01	19.29	1.77	a
DL – AM 2	40	16.46	34.00	25.57	9.34	1.83	ab
DL – AM 5	40	12.17	30.70	24.44	7.09	1.80	b

DL – *Diospyrus lotus* L., AM – locality (Arborétum Mlyňany), 1, 2, 5 – genotypes, n – number of the individuals

T a b l e 3

Literature data on pollen morphological traits in some *Diospyros* species

Plant species	axis	min	max	$\bar{x}$	SI	Authors
Diospyros kaki L. f. – G1	P	51.6	53.7	52.6	1.79	Evrenosoglu & Misirli (2009)
	E	29.0	29.5	29.4		
Diospyros kaki L. f. – G2	P	50.8	54.2	52.6	1.82	Evrenosoglu & Misirli (2009)
	E	28.4	29.6	28.9		
Diospyros abyssinica (Hiern) F. White	P	–	–	22.9	1.18	Geeraerts et al. (2009)
	E	–	–	11.4		
Diospyros kirkii Hiern	P	–	–	31.6	1.05	Geeraerts et al. (2009)
	E	–	–	30.2		
Diospyros lotus L.	P	–	–	50.6	0.98	Geeraerts et al. (2009)
	E	–	–	51.5		
Diospyros squarrosa Klotzsch	P	–	–	37.4	1.48	Geeraerts et al. (2009)
	E	–	–	25.3		
Diospyros virginiana L.	P	–	–	41.4	1.33	Geeraerts et al. (2009)
	E	–	–	31.2		
Diospyros virginiana L.	P	–	–	59.0	1.28	Erdtman 1966
	E	–	–	46.0		

P – length of polar axis, E – length of equatorial axis, SI – shape index

L.) (Table 2). According to the values of shape index the pollen grains can be included into the shape class classified as prolate or prolate-spheroidal. The shape indexes ranging from 1.77 to 1.83 in the genotypes of *Diospyros lotus* L. species confirmed a certain degree of identity substantiating thus the classification to shape class of prolonged pollen grains, i. e. with the vertical axis longer than equatorial one. In comparison with the literature data by Geeraerts et al. (2009) our results concerning the shape indexes of tested species are generally higher. On the other hand, the data shown in Table 1 and the values of shape indexes (1.28–1.33) presented in Table 3 verify, that the pollen grains of *Diospyros* genus with exception of species *Diospyros lotus* L. (Table 3) are prolonged sporoformes (Erdtman 1966; Geeraerts et al. 2009). Interesting values of shape index (1.79–1.82) for *Diospyros kaki* L. f. are reported by Evrenosoglu & Misirli (2009), what is consistent with our result for this species (1.75) given in Table 1. Morphological traits variability could be in-

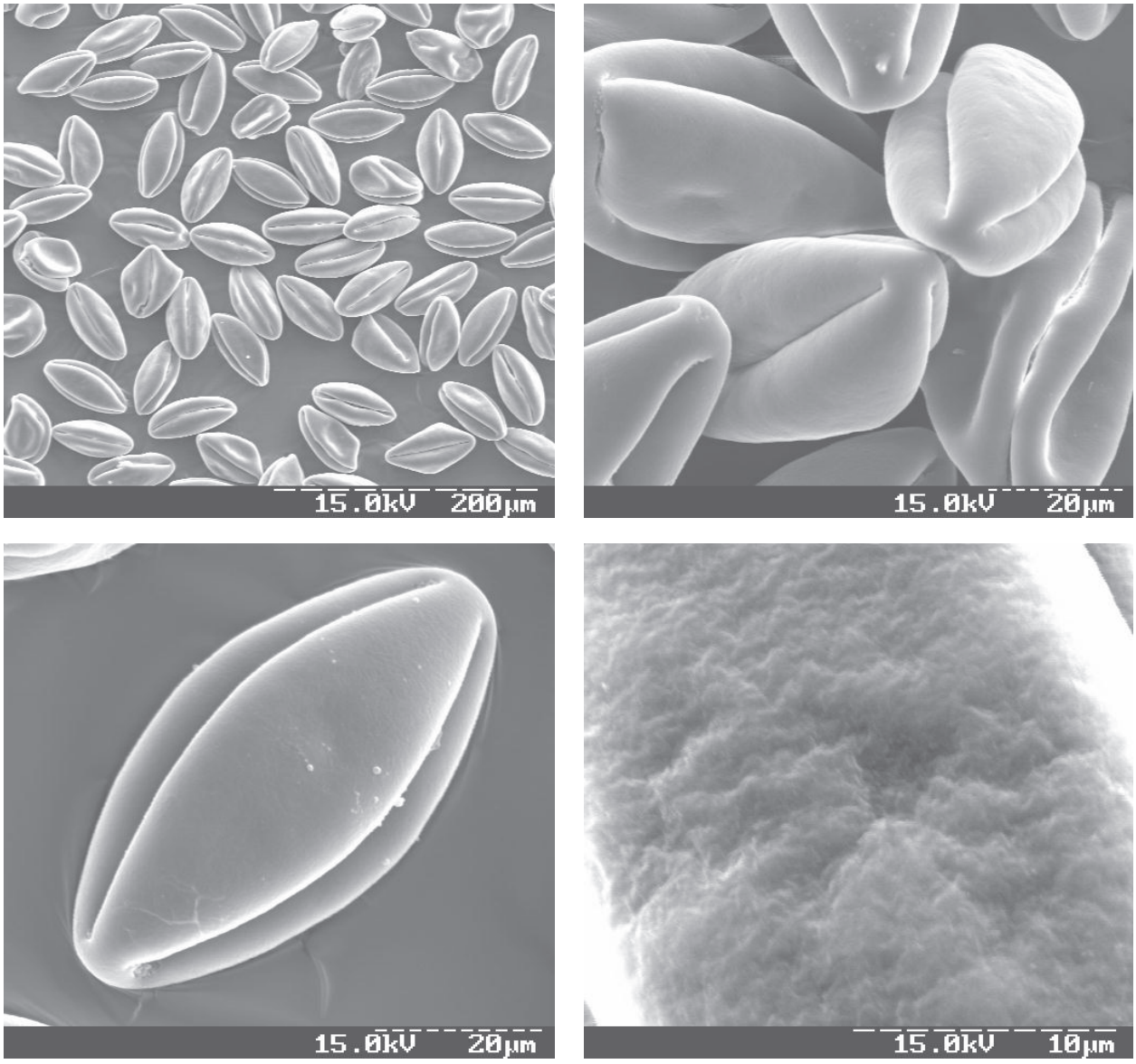

Fig. 2. Pollen grains of *Diospyros virginiana* L. species in different position (Photo: Gurnenko 2010)

fluenced by the environmental factors, such as temperature, humidity and mineral nutrition. In case of pollen grain size, except for the above mentioned factors, the inner factors are also of importance – especially the chromosomes number. Polyploid species are characterized by larger pollen grains with increased aperture numbers. Pollen size could be influenced even by different methodical procedures and approaches to pollen samples processing e.g. the pollen elaboration by acetolysis, using various agents like acetocarmine, KOH or processed without any chemicals, applying the dry pollen method (Ostrolucká & Križo 1989). These factors can significantly influence the pollen size even of course species. For example Sowunmi (1995) in *Diospyros mespiliformis* Hochst. Ex A.D.C. reported the mean value for polar axis length (P) 42.4 µm (in range 39.0–46.0 µm) and the equatorial axis (E) 31.2 µm (27.0–33.0 µm) whereas Geeraerts et al. (2009) for the same species found the mean values for the length P – 32.3 µm and E – 24.8 µm. There were found also

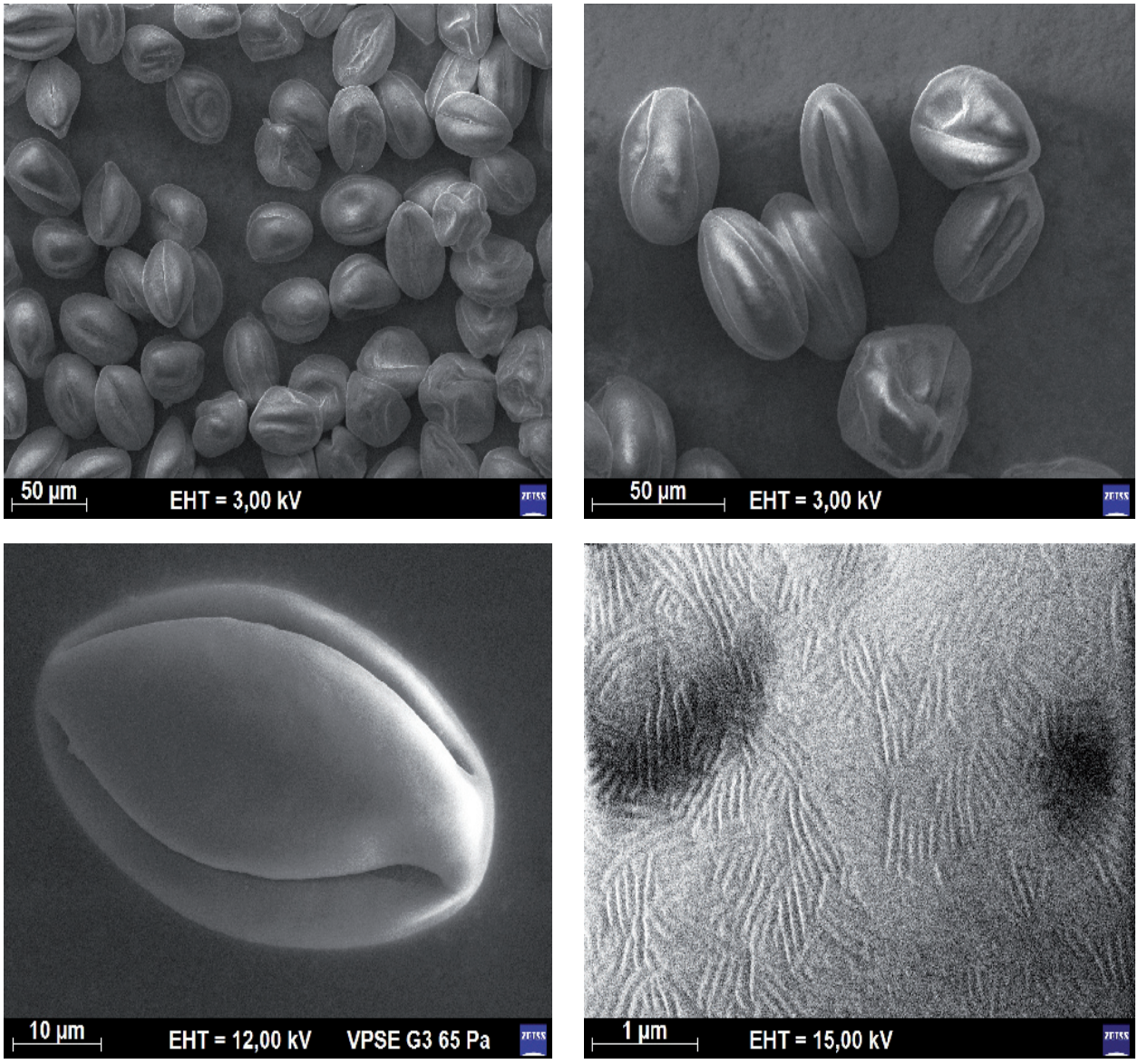

Fig. 3. Pollen grains of *Diospyros kaki* L. species in different position (Photo: Ostrovský 2010)

significant different values of the mentioned traits in species *Diospyros lotus* L. and *Diospyros virginiana* L. presented in our study as well as in the study published by Geeraerts et al. (2009). The lengths of polar and equatorial axis were larger in pollen samples collected on localities of Slovakia. In the paper of Geeraerts et al. (2009) an extensive knowledge on pollen morphological diversity was accumulated for different persimmon species.

Kodela (2006) reports of evaluation of the species *Diospyros australis* (R. Br.) Hiern., where the mean length of polar axis reached 36.9 μm and the equatorial one 29.7 μm. Our results suggest a possible polymorphism of pollen obtained from individual genotypes of a given species grown in different ecological and geographical conditions, respectively. Colour of pollen grains is taken as a relevant trait from the taxonomic point of view. The tested samples of persimmon pollen were of different yellow shades.

The exine sculpture is also an important morpho-

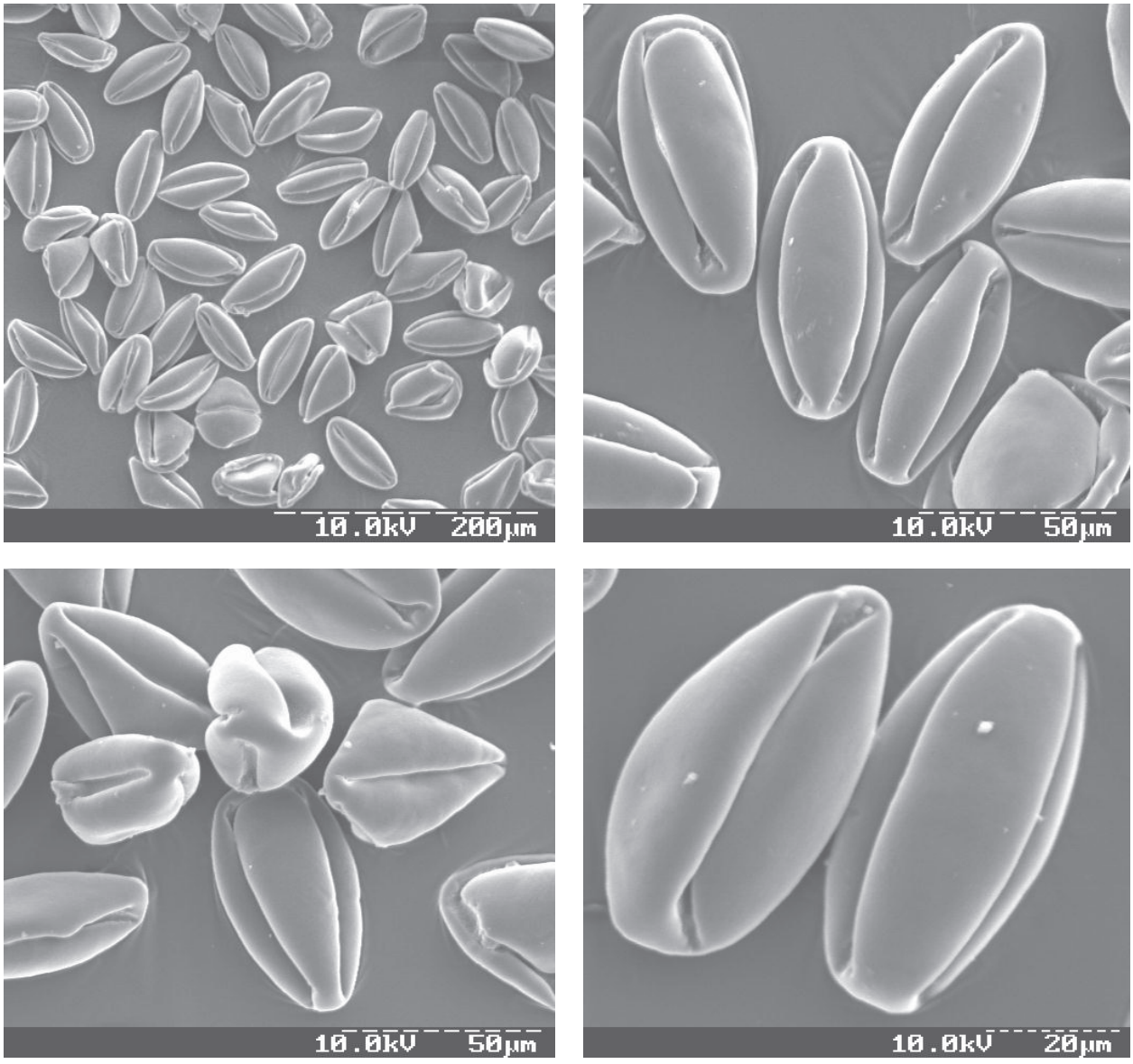

Fig. 4. Pollen grains of interspecies hybrid *Diospyros virginiana* L. × *D. kaki* L. f.
(Photo: Gurnenko 2010)

logical trait, but its structural elements could be observed in detail only by scanning electron microscopy (REM). Our studies using REM have shown that the sexine of tested species (Fig. 1–4) is not smooth but sculptured. There are dominating finely striation (striate), scarring (scabrate) or wrinkles, more markedly in *Diospyros virginiana* L. species, also with occurrence of perforations. Significantly was distinguished species *Diospyros kaki* L. f., its sexina is irregularly baculiform (Fig. 3). Similar characteristics of sculpture elements for some species of genus *Diospyros* are described by Geeraerts et al. (2009) and Kodela (2006).

CONCLUSION

Experimental study was oriented on morphological characterization of pollen grains (sporoforms) in *Diospyros kaki* L. f., *Diospyros virginiana* L., *Diospyros lotus* L. species and interspecies hybrid *D. virginiana* L. x *D. kaki* L. f., with special attention paid to the size and shape of pollen grains. Statistically significant differences in the length of polar and equatorial axes identifying their size and shape were detected between investigated taxons. According to values of shape index, the pollen grains can be included into the shape class classified as prolate or prolate-spheroidal. Pollen grains of tested species are radially symmetric and isopolar. Taking into account the appertures shape and their number they are classified as zono-tricolporate.

Our observations showed that not only pollen size, but also the type and number of apertures together with microstructural characteristic of exine sculpture are important morphological traits, characteristic for each species. Certain polymorphism of pollen morphological traits in *Diospyros* species was detected.

Analyses of tested species oriented to pollen grains morphology are of importance from various viewpoints – evolution and taxonomy of the genus provides new information and improves the knowledge about interesting plants, which despite of their exotic origin become a fully deserved concern of the Slovakia growers' community. This is closely connected with the fact, that persimmon has an extending economical importance leading to an increased exploitation in practice especially as fruit species suitable for the branch of ecological agriculture.

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