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MICROSTRUCTURE OF FRUITS AND SEEDS OF SELECTED SPECIES OF Hydrangeaceae (Cornales) AND ITS SYSTEMATIC IMPORTANCE

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Abstract. The paper presents the results of the study on fruit and seed morphology and anatomy of some chosen species of Hydrangeaceae. Fruit micromorphology, seed shape and size, seed coat pattern, testa thickness, and endosperm cells were investigated in *Hydrangea heteromalla, H. paniculata, Philadelphus californicus, P. delavayi, P. incanus, P. inodorus, P. pubescens* var. *verrucosus, P. tenuifolius, Deutzia compacta, D. rubens,* and *D. scabra.* Differences of potential taxonomic significance were found in the ornamentation of the fruit and the pistil surface in the *Hydrangea* species. The examined seeds were characterized by reticulate primary sculpture with different size and shape of the testa cells. The protrusive secondary sculpture was observed on the *Hydrangea* seeds. The seeds of the *Philadelphus* species were characterized by the rugate secondary sculpture. The seeds of the *Deutzia* species had sunken secondary sculpture of different patterns and these differentiations are of systematic importance. The endosperm cell walls were very thin in the *Hydrangea* and *Philadelphus* seeds, while they were evenly thick in the *Deutzia* seeds. That difference may be of systematic importance. The average thickness of the two-layered testa was 3.51–11.50 µm. Its inner and outer layers thickness varied from 2.27–9.25 µm and 1.24–4.05 µm, respectively. Most of the differences were found to be significant.

Key words: fruit micromorphology, seed sculpture, testa, endosperm cells

INTRODUCTION

Taxonomic descriptions of the family Hydrangeaceae and the phylogenetic relationships within it have been the subject of many studies. On the basis of several phylogenetic analyses using sequences of the plastid genes *rbc*L [Chase et al. 1993, Olmstead et al. 1993, Xiang 1999], *atp*B [Savolainen et al. 2000], and *ndh*F [Olmstead et al. 2000],

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as well as a combination of plastid and nuclear DNA [Soltis et al. 2000, Albach et al. 2001], in agreement with Angiosperm Phylogeny Group III System (APG III) [2009], the family Hydrangeaceae is classified in the order Cornales, clade Asterids. However, it is still a family with many not fully resolved systematic and evolutionary problems. Detailed analysis of the phylogenetic relationships in the Cornales [Soltis et al. 1995] based on DNA sequences of plastid gene *rbc*L showed that Hydrangeaceae comprises *Broussaisia, Cardiandra, Carpenteria, Decumaria, Deinanthe, Deutzia, Dichroa, Fendlera, Fendlerella, Hydrangea, Jamesia, Kirengeshoma, Philadelphus, Pileostegia, Platycrater, Schizophragma* and *Whipplea.* Hufford [1995], analyzing seed morphology of Hydrangeaceae for its phylogenetic implications, found that the morphological data supported the hypotheses of the monophyly of *Deutzia* and *Philadelphus*, although relevant character states appeared to display parallelisms. According to his results, seed morphology, like other examined characteristics, failed to support the monophyly of *Hydrangea*, as it has been traditionally circumscribed. He found that a hypothesis of monophyly for a group that includes the climbing *Decumaria, Pileostegia* and *Schizophragma*, as well as *Hydrangea heteromalla* and *H. paniculata*, is supported by long spindle-form seeds with a micropylar flange. Hufford [1997] and Hufford et al. [2001], in further studies based on morphological data and molecular and morphological data, respectively, proposed to treat *Fendlera* and *Jamesia* as subfamily (Jamesioideae) and showed that the remaining genera of hydrangeoids should be treated as the subfamily Hydrangeoideae, which is divided into two large tribes/subclades: Philadelpheae and Hydrangeeae. Philadelpheae includs three clades: *Philadelphus* + *Carpenteria*; *Deutzia* + *Kirengeshoma* and *Fendlerella* + *Whipplea*. Hydrangeeae clade includes species of *Hydrangea* and other genera like *Broussaisia, Cardiandra, Decumaria, Deinanthe, Dichroa, Pileostegia, Platycrater, Schizophragma* and that clade was recognized, in such description, as monophyletic group [Hufford et al. 2001]. From previous studies of Hydrangaceae, as well as many other studies of different angiosperm groups [e.g. Molvray and Kores 1995, Watanabe et al. 1999, Johnson et al. 2004, Oh et al. 2008, Morozowska et al. 2011] seed morphology has been shown to have systematic significance at different phylogenetic levels [Barthlott 1981, 1984].

With the aim of finding some additional characteristics and new morphological data, which according to Hufford et al. [1997] might be helpful in resolving more fully the phylogeny of Hydrangeaceae, we have investigated fruit and seed structures of some chosen species representing genera *Hydrangea*, *Philadelphus* and *Deutzia.*

The main goal of this study was 1) to describe variation in the fruit micromorphology of two *Hydrangea* species from a *Heteromalle* subsection; 2) to describe and document the seed morphology and anatomy of chosen *Hydrangea*, *Philadelphus* and *Deutzia* species; 3) to supply new morphological and anatomical data with the aim of improving the understanding of the morphological evolution of the examined species.

MATERIAL AND METHODS

Biometric measurements. The morphological and anatomical fruit and seed characteristics of *Hydrangea heteromalla* D. Don*., H. paniculata* Sieb*., P. californicus* Benth.,

P. delavayi Henry, *Philadelphus incanus* Koehne*, P. inodorus* L.*., P. pubescens* Loisel. var. *verrucosus* (Schrad.) Hu, *P. tenuifolius* Rup. & Maxim.*., Deutzia compacta* Carib.*, D. rubens* Rehder and *D. scabra* Thunb. were studied using seeds collected during the years 2009–2010 from living plants and from herbarium collections (tab. 1). In each locality, the seeds used for biometric measurements were collected from 5–10 individuals, dependent on the availability of the material and the representative seed sample of 30 seeds of each species was used. When herbarium specimens were used, the representative seed sample was also composed of 30 seeds. To describe the seed shapes of the

Table 1. List of species from the family Hydrangeaceae analyzed in the study
Tabela 1. Wykaz gatunków z rodziny Hydrangeaceae objętych badaniami

Species Gatunek	Specimen collection data[1] Informacje o pochodzeniu nasion[1]
Deutzia compacta Carib.	Ireland, Dublin, NBBG[2], 2010
Deutzia rubens Rehder	Ireland, Dublin, NBBG[2], 2010
Deutzia scabra Thunb.	Portugal, Coimbra, JBUC[3], 2010
Hydrangea heteromalla D. Don.	Poland, Poznań, AMUBG[4], 2008
Hydrangea paniculata Sieb.	Poland, Poznań, AMUBG[4], 2008
Philadelphus californicus Benth.	Poland, Poznań, AMUBG[4], 2008
Philadelphus delavayi Henry	Poland, Poznań, AMUBG[4], 2008
Philadelphus incanus Koehne	Portugal, Coimbra, JBUC[3], 2010
Philadelphus inodorus L.	Poland, Poznań, AMUBG[4], 2009
Philadelphus tenuifolius Rupr. & Maxim.	Russia, Blagoveschensk, ABGI[5], 2010
Philadelphus pubescens var. *verrucosus* (Schrad.) Hu	Poland, Poznań, AMUBG[4], 2009

[1] country, city, name of plant collection, year of collecting – państwo, miasto, nazwa kolekcji roślinnej, rok zbioru
[2] National Botanic Gardens, Glasnevin, Dublin 9, Ireland
[3] Jardim Botânico da Universidade de Coimbra
[4] Adam Mickiewicz University Botanical Garden in Poznan – Ogród Botaniczny Uniwersytetu im. Adama Mickiewicza w Poznaniu
[5] The Russian Academy of Sciences Far East Branch. The Amur branch of Botanical Garden – Institute.

examined species we adopted seven basic shape forms given for Hydrangeaceae by Hufford [1995]. Measurements of the seed size were carried out with light microscopy (LM). Seed length was measured along the longest axis in the plane of the chalazal and microphylar ends and seed width along the longest axis at a 90° angle to the plane of the chalaza and microphyle. Seed coat thickness was measured on eight seeds of each species with the help of scanning-electron-microscope (SEM) photographs. For testa (T) thickness, the inner (IL) and outer (OL) layers were measured on the longitudinal seed sections. The specimens and the seed material are deposited in the herbarium of the Botany Department (POZNB), Poznan University of Life Sciences, Poland.

Seed preparations for SEM. Before cutting, the seeds were fixed in a mixture of ethyl alcohol and acetic acid in a 3:1 proportion for 12 h. After rinsing in distilled water (2 times for 10 min), they were sectioned at -15°C in a cryostat LEICA CM 1850, washed several times to remove the freezing medium, and dried using an acetone sequence in the following concentrations: 30, 50, 70, 90, and 100%, three times for 6 min in each. For SEM, fruit and seed cuttings were covered with gold and examined with a Zeiss EVO 40 electron microscope at 8–15 kV, depending on the species.

The collected data were used to carry out a single factor variance analyses. The significant differences were calculated using the Duncan test at a significance level $\alpha = 0.05$.

RESULTS

Fruits of both examined *Hydrangea* species are capsular, many-seeded (30–100/fruit) and are characterized by interstylar fruit dehiscence [Reed 2004]. The microornamentation of the capsule surface of the examined *Hydrangea* species varied. The shapes of the cuticle cells on *H. heteromalla* fruits were difficult to describe, as their anticlinal walls are not very distinct, and their microornamentation is very irregular and strongly reticulate. A microsculpturing surface pattern of the cuticle cells on *H. paniculata* fruits is distinctly striate. The cells are significantly elongated and parallel-orientated. Five valvate sepals are persistent on the fruits of both examined taxa at the fruiting time. The shape of the sepals also differs in each of the examined *Hydrangea* species. On *H. heteromalla* fruits they are triangular, longer than they are broad, and toothed. On *H. paniculata* fruits, the sepals are shorter than they are broad, and blunt (fig. 1 A–D). The microornamentation of the sepals' surface is similar in both examined species of *Hydrangea*, and it is strongly papillate, with distinct striations on the surface (fig. 1 E, F). However, the microornamenttion of the style surface is different for both examined taxa. The *H. heteromalla* style surface is faintly papillate with striations, while on the surface of the *H. paniculata* style strong striations covering long flat cuticle cells are visible. Anomocytic stomata were present on style surfaces of both examined *Hydrangea* species (fig. 2 A–D). The stigma of both species curve a little over the style apex; this is generally restricted to the distal region of the style. Its surface is of a dry type with multi-celled long papillae (fig. 2 E, F).

The seeds of *Hydrangea heteromalla* are ellipsoidal, slightly compressed, with a peg-like form at the micropylar pole and a wing at the chalazal end. The seeds of *H. paniculata* have a spindle form; they are also slightly compressed and narrowly tailed at the chalazal pole. A flangelike elaboration in the micropylar region of the *H. paniculata* seeds was present. The primary sculpture of both examined *Hydrangea* species had reticulate patterns with not very elongated or broad individual cell shapes on the *H. heteromalla* seeds and with significantly elongated and narrow individual cell shapes, with pointed ends on the *H. paniculata* seeds. The anticlinal walls of the seed coat cells of both species were straight and strongly protruding above the surface of the periclinal walls. The latitudinal walls in the central body of the seed were infrequent on the *H. heteromalla* seeds, while on the *H. paniculata* seeds they were frequently present

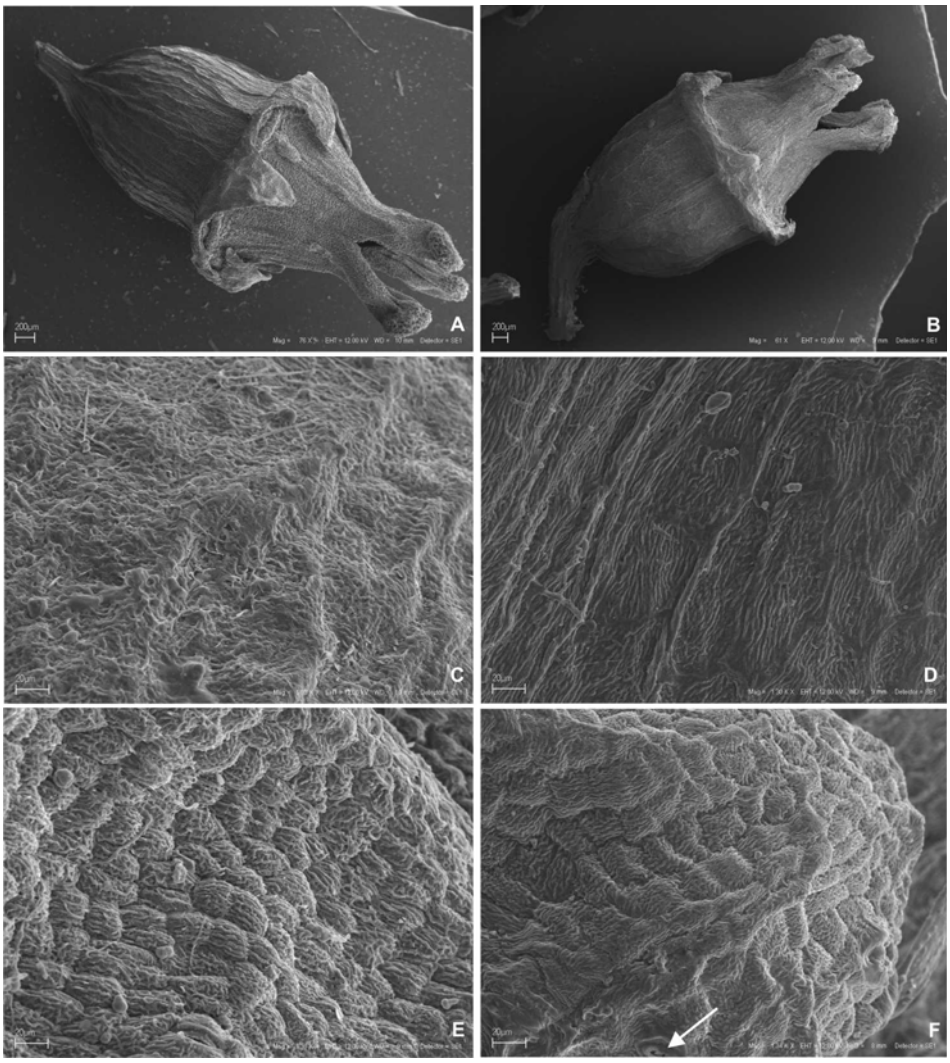


Fig. 1. Fruits of *Hydrangea heteromalla* (A, C, E) and *H. paniculata* (B, D, F); microstructure of capsule surface (C, D); microstructure of calyx sepals surface with stomata (arrow) visible (E, F)

Ryc. 1. Owoce *Hydrangea heteromalla* (A, C, E) *H. paniculata* (B, D, F); mikrostruktura powierzchni torebki (C, D); mikrostruktura powierzchni działek kielicha z widocznym (strzałka) aparatem szparkowym (E, F)

(tab. 2; fig. 3 A, B, E). A protrusive secondary sculpture, visible at higher magnification, was observed on both *Hydrangea* seeds, with a minute rugate form. On the *H. heteromalla* seeds, on the ridges of their longitudinal anticlinal walls, sunken rhomboidal features situated in regular rows were observed (fig. 3 B, D, F). The average

Table 2. Seed characters and character states of the examined taxa
Tabela 2. Wykaz i opis cech badanych nasion

Species Gatunek	Seed shape Kształt nasion			Primary sculpture Skulptura pierwotna					Secondary sculpture Skulptura wtórna	
	Overall seed shape Ogólna forma nasion	Form of micropylar pole Kształt bieguna mikropylarnego	Form of chalazal pole Kształt bieguna chalazalnego	Shapes of cells Kształt komórek	Enclosure of outer periclinal wall Zarys zewnętrznej ściany peryklinalnej	Boundaries of cells Granice komórek	Form of anticlinal walls Ukształtowanie ścian antyklinalnych	Frequency of antyclinal walls Częstość wyst. ścian antyklinalnych	Protrusive secondary sculpture Wypukła skulptura wt.	Sunken secondary sculpture Wklęsła skulptura wt.
Deutzia compacta Carib.	A	F	Aw	A	B	A	A	A	D	A
Deutzia rubens Rehder	A	F	Aw	A	A	A	B	A	D	A
Deutzia scabra Thunb.	A	F	Aw	A	A	A	A	A	D	A
Hydrangea heteromalla D. Don.	A	C	Aw	A	A	A	A	B	A	C
Hydrangea paniculata Sieb.	B	E	At	A	A	A	A	A	A	C
Philadelphus californicus Benth.	D	E	Aw	A	B	A	A	A	A	C
Philadelphus delavayi Henry	D	E	Aw	A	A	A	A	A	A	C
Philadelphus incanus Koehne	D	E	Aw	A	A	A	A	A	A	C
Philadelphus inodorus L.	D	E	At	A	A	A	A	A	A	C
Philadelphus pubescens Loisel. var. *verrucosus* (Schrad.) Hu	D	E	At	A	B	A	A	A	A	C
Philadelphus tenuifolius Rupr. & Maxim.	D	E	Aw	A	A	A	A	A	A	C

Overall seed shape A. ellipsoidal; B. spindle-form; C. obovate; D. funnel-form; E. bottle-form; F. urceolate; G. horn-shaped – Ogólny kształt nasion A. elipsoidalny; B. wrzecionowaty; C. jajowaty; D. lejkowaty; E. butelkowaty; F. dzbankowaty; G. w kształcie rogu

Form of micropylar pole A. round; B. flat; C. peglike; D. pointed; E. flange; F. cowl – Kształt bieguna mikropylarnego A. okrągły; B. płaski; C. hakowaty; D. zaostrzony; E. kołnierzowaty; F. kapturowaty

Form of chalazal pole A. differentiated from body as flattened wing (Aw) or slender tail (At); B. undifferentiated from body – Kształt bieguna chalazalnego A. w formie płaskiego skrzydełka (Aw) lub smukłego ogona (At); B. niewyróżniający się

Shapes of cells A. largely rectangular with length greater than width; B. polygons with length nearly equal to width – Kształt komórek A. głównie prostokątne o długości większej niż szerokość; B. wielokątne o niemal równej długości i szerokości

Enclosure of outer periclinal wall A. anticlinal walls perpendicular to tangential surface of seed coat cells and do not tend to cover the tangential surface; B. anticlinal walls largely overarch and cover the tangential surface of the seed coat cells – Zarys zewnętrznych ścian peryklinalnych A. ściany antyklinalne prostopadłe do stycznej powierzchni komórek łupiny nasiennej, raczej nie otaczają powierzchni stycznej; B. ściany antyklinalne głównie wygięte, szczelnie otaczają styczną powierzchnię komórek łupiny nasiennej

Boundaries of cells A. anticlinal walls that form the boundary between seed coat cells protrude above the outer tangential surfaces of the cells; B. anticlinal walls that form the boundary between seed coat cells depressed below the outer tangential surfaces of the cells – Granice komórek A. ściany antyklinalne, które tworzą granicę pomiędzy komórkami łupiny nasiennej wystają ponad zewnętrzną powierzchnię styczną komórek; B. ściany antyklinalne, które tworzą granicę pomiędzy komórkami łupiny nasiennej są zapadnięte poniżej zewnętrznej powierzchni stycznej komórek

Form of anticlinal walls A. anticlinal walls largely straight; B. anticlinal walls sinuate – Ukształtowanie ścian antyklinalnych A. ściany antyklinalne głównie proste; B. ściany antyklinalne faliste

Frequency of anticlinal walls A. latitudinal cross walls common in the body of the seed; B. latitudinal cross was infrequent or absent in the body of the seed – Częstość występowania ścian antyklinalnych A. ściany poprzeczne częste na powierzchni nasion; B. ściany poprzeczne rzadkie lub nieobecne na powierzchni nasion

Protrusive secondary sculpture A. rugae; B. elongate parallel striations; C. papillate knobs; D. absent (without raised sculptural elements) – Wypukła skulptura wtórna A. pofałdowana; B. z wydłużonymi, równoległymi bruzdami; C. brodawkowata; D. brak (bez wyniesionych elementów skulptury)

Sunken secondary sculpture A. shallow depressions; B. cylindrical pits; C. absent (without sunken sculptural features) – Wklęsła skulptura wtórna A. płytkie zagłębienia; B. cylindryczne zagłębienia; C. brak (brak zapadniętych elementów skulptury)

(after Hufford 1995, modified) – (za Huffordem 1995, zmienione)

Table 3. Biometrical measurements of selected seed features
Tabela 3. Pomiary biometryczne wybranych cech nasion

Species – Gatunek	Seed length Długość nasion mm ± SD	Seed width Szerokość nasion mm ± SD	Testa – Łupina nasienna			Figures Zdjęcia
			Testa thickness Grubość łupiny nasiennej µm ± SD	Inner layer thickness Grubość zewn. warstwy łupiny nasiennej µm ± SD	Outer layer thickness Grubość wew. warstwy łupiny nasiennej µm ± SD	
Deutzia compacta Carib.	2.28 ± 0.41 de*	0.52 ± 0.10 d	7.88 ± 0.07 g	3.90 ± 0.05 f	3.90 ± 0.04 g	7 B-D; 8 C, D
Deutzia rubens Rehder	0.90 ± 0.1 b	0.44 ± 0.05 c	-	-	-	7 E, F
Deutzia scabra Thunb.	2.04 ± 0.28 c	0.64 ± 0.10 f	5.83 ± 0.18 c	2.90 ± 0.08 b	2.90 ± 0.11 c	7 A; 8 A, B
Hydrangea heteromalla D. Don.	0.77 ± 0.14 a	0.30 ± 0.09 a	4.83 ± 0.11 b	3.52 ± 0.10 e	1.29 ± 0.08 a	1 A, C, E; 2 A, C, E; 3 A-D
Hydrangea paniculata Sieb.	2.28 ± 0.19 d	0.39 ± 0.06 b	7.17 ± 0.15 e	4.15 ± 0.06 g	3.06 ± 0.14 d	1 B, D, F; 2 B, D, F; 3 E-H;
Philadelphus californicus Benth.	3.42 ± 0.48 h	0.52 ± 0.08 d	7.22 ± 0.14 e	3.24 ± 0.08 c	3.99 ± 0.09 g	5 B, D, F
Philadelphus delavayi Henry	2.20 ± 0.25 cd	0.58 ± 0.09 ef	3.51 ± 0.16 a	2.27 ± 0.13 a	1.24 ± 0.09 a	6 A, C, E
Philadelphus incanus Koehne	2.79 ± 0.41 f	0.62 ± 0.08 f	6.52 ± 0.16 d	3.22 ± 0.07 c	3.30 ± 0.11 e	5 A, C, E
Philadelphus inodorus L.	3.23 ± 0.25 g	0.53 ± 0.10 de	7.46 ± 0.12 f	3.39 ± 0.17 d	4.05 ± 0.13 g	4 A-C
Philadelphus pubescens Loisel. var. *verrucosus* (Schrad.) Hu	3.56 ± 0.35 h	0.41 ± 0.08 bc	9.22 ± 0.09 h	5.64 ± 0.11 h	3.58 ± 0.13 f	4 D-H
Philadelphus tenuifolius Rupr. & Maxim.	2.54 ± 0.21 e	0.53 ± 0.07 d	11.50 ± 0.11 i	9.25 ± 0.15 i	2.31 ± 0.08 b	6 B, D, F

SD – standard deviation – SD – odchylenie standardowe
*Values marked with the same letter do not differ significantly at α = 0.05 – *Wartości oznaczone tę samą literą nie różnią się istotnie dla α = 0,05

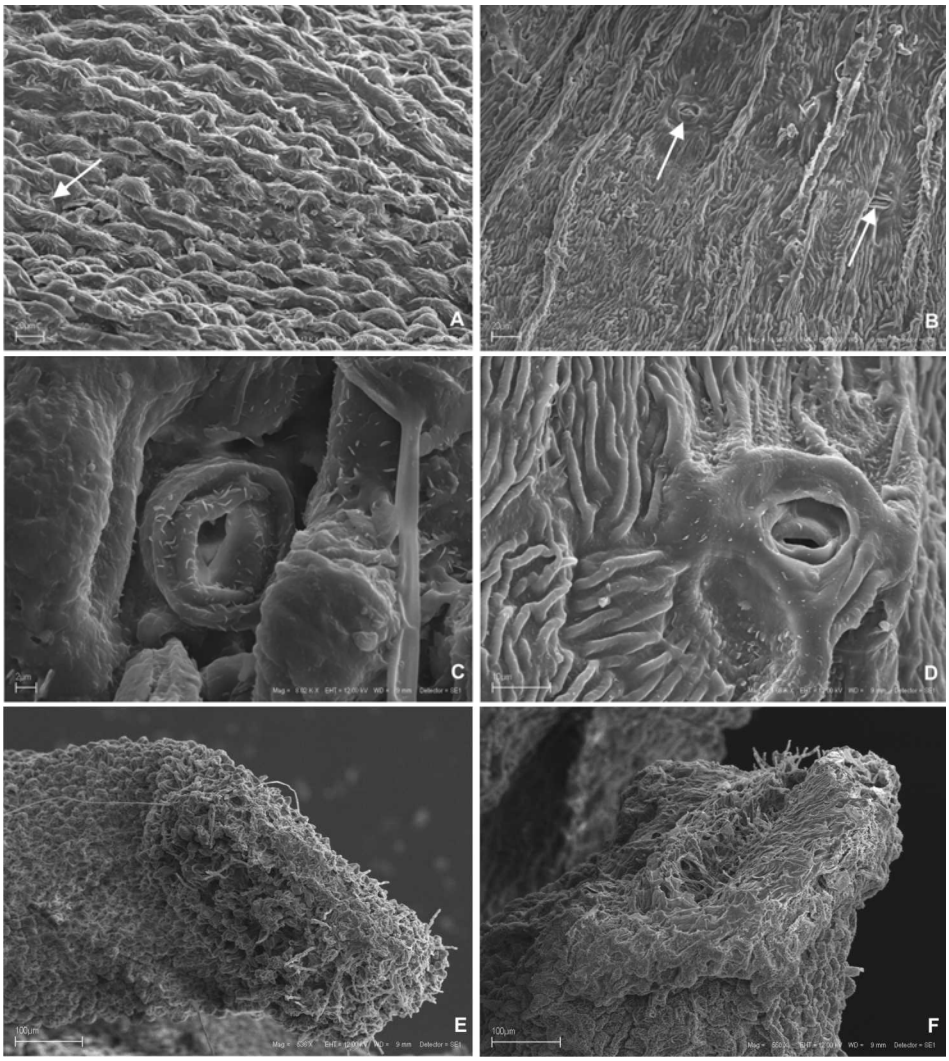
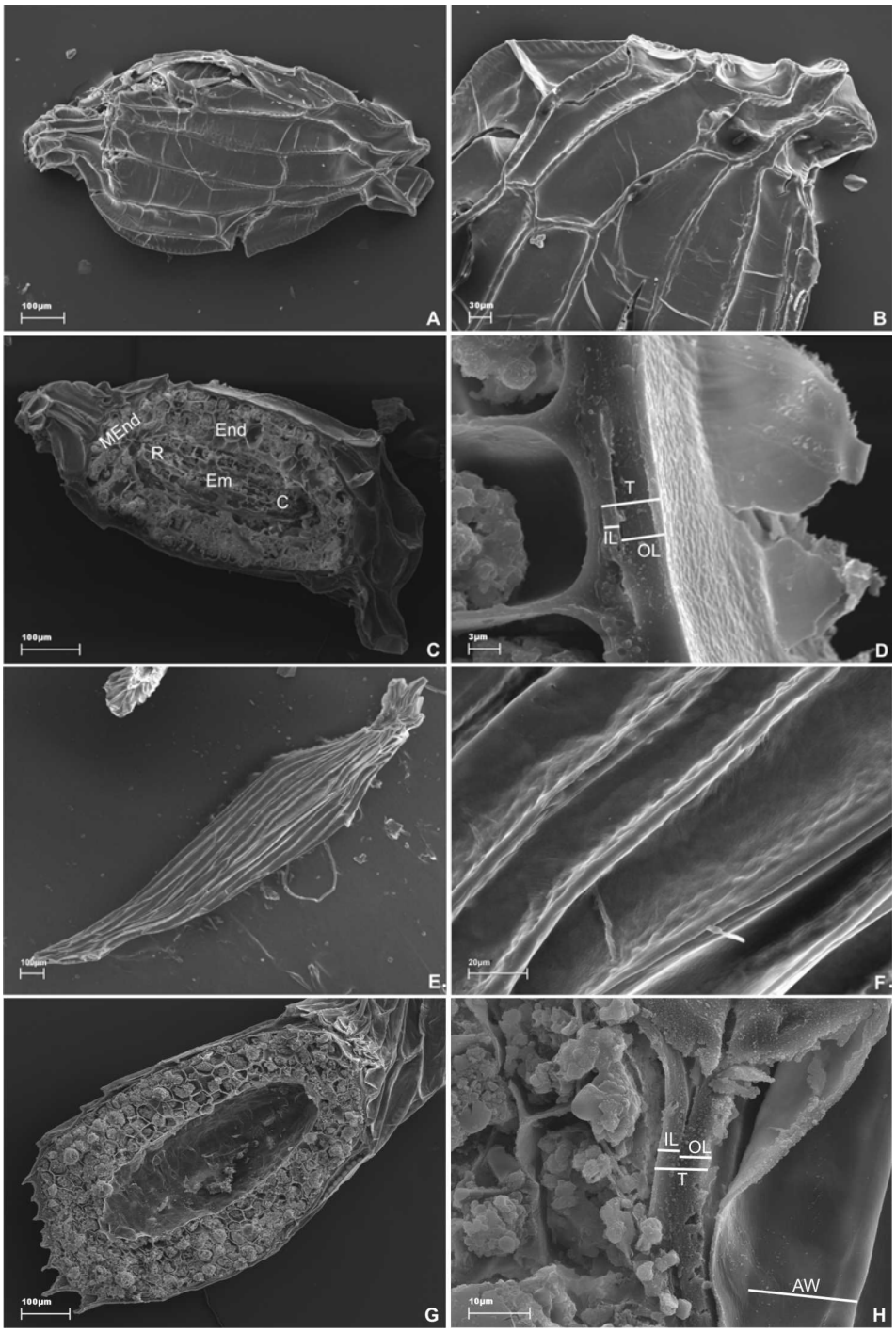


Fig. 2. Fruits of *Hydrangea heteromalla* (A, C, E) and *H. paniculata* (B, D F); microstructure of style surface with visible (arrows) stomata (A, B); stomata on the style surface (C, D); stigma surface with papillae (E, F)

Ryc. 2. Owoce *Hydrangea heteromalla* (A, C, E) *H. paniculata* (B, D F); mikrostruktura powierzchni szyjki słupka z widocznymi (strzałki) aparatami szparkowymi (A, B); aparaty szparkowe na powierzchni szyjki słupka (C, D); znamię słupka z papillami (E, F)

length and width of examined *Hydrangea* seeds ranged from 0.77 to 2.28 mm and 0.30 mm to 0.39 mm, respectively. The average thickness of the two-layered seed testa (T) of the two examined *Hydrangea* species ranged from 4.83 µm to 7.17 µm, with the average thickness of the inner testa layer (IL) varying from 3.52 µm to 4.15 µm and of


100µm
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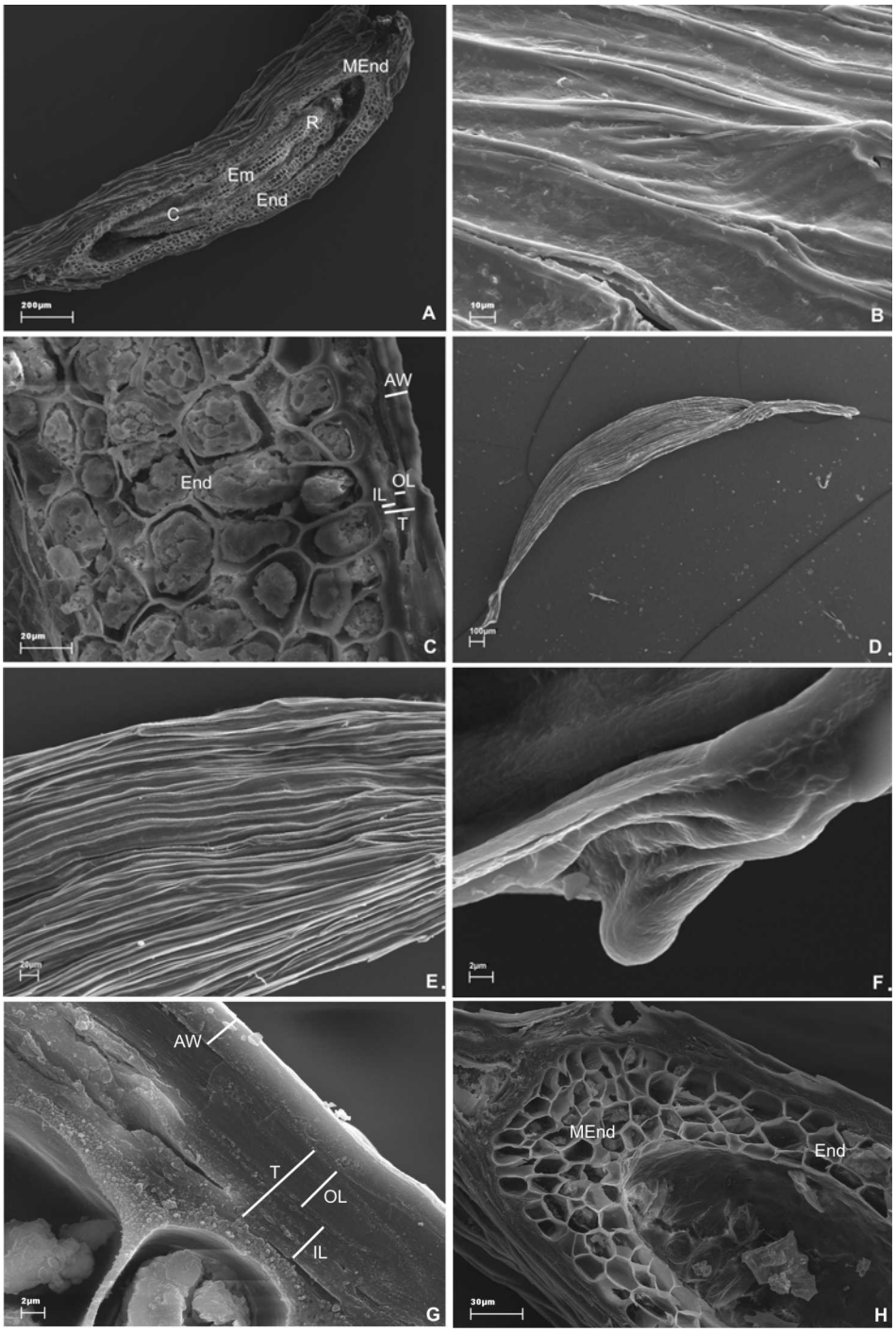
the outer testa layer (OL) from 1.29 µm to 3.06 µm, respectively. All found differences were significant (tab. 3; fig. 3 D, H). On the longitudinal seed cuttings, the endosperm cells were polygonal in shape with very thin, paper-like cell walls. No differentiation in the shape and size of the endosperm cells or thickness of their walls was observed, in dependence of the seed region (fig. 3 C, G).

The seeds of all of the examined *Philadelphus* species had a funnel-form. They had characteristic flanges at the micropylar region and flattened wings (*P. incanus, P. californicus, P. delavayi, P. tenuifolius*) (fig. 5 and 6 A, B) or short tails (*P. inodorus*) (fig. 4 A) at the chalazal poles, except for *P. pubescens* var. *verrucosus* (fig. 4 D), which had the chalazal end of the seed prolonged as a narrow tail. The primary sculpture pattern of the seeds of all examined *Philadelphus* species was reticulate with markedly long, narrow cells and pointed ends (fig. 4 B, E; fig. 5 and 6 C, D). The nearly plicate form of the cells with lateral anticlinal walls tended to enclose the surface of the outer periclinal walls on the *P. pubescens* var. *verrucosus* and *P. californicus* seeds. Additionally, the endings of the anticlinal walls of the seed coat cells of these two species protruded much more over the seed coat surface in comparison with other examined species (fig. 4 E, F and fig. 5 D). The seed coats of all examined *Philadelphus* species were characterized by a rugate secondary sculpture with rather rough and widely scattered rugae on the cell surfaces (tab. 2, fig. 4 B, F). The average length and width of the *Philadelphus* seeds ranged from 2.20 to 3.56 mm and 0.41 mm to 0.62 mm, respectively. The statistical analysis of the average values of these features grouped examined *Philadelphus* species into several homogeneous groups. According to the seed lengths *P. californicus* and *P. pubescens* var. *verrucosus* seeds were similar. For all other species the differences were significant. According to the seed widths three homogeneous groups including *P. californicus* and *P. inodorus*, *P. delavayi* and *P. incanus* or *P. delavayi* and *P. inodorus* were found. *P. pubescens* var. *verrucosus* seeds were significantly narrow comparing with all other examined species representing that genus. The average thickness of the two-layered seed testa (T) of the examined *Philadelphus* species ranged from 3.51 µm to 11.50 µm, with the average thickness of the inner testa layer (IL) varying from 2.37 µm to 9.25 µm and of the outer testa layer (OL) from 1.24 µm to 4.05 µm. Average testa thickness was significantly different for all exam-

Fig. 3. Seeds of *Hydrangea heteromalla* (A–D) and *H. paniculata* (E–H); shape of seeds (A, E); primary (B, F) and secondary testa sculpture (D, F); seed longitudinal cutting with embryo (C) and endosperm visible (C, G: Em – embryo, R – radicula, C – cotyledons, End – endosperm, MEnd – micropylar endosperm); part of the seed longitudinal cutting with testa (D, H: T – testa, IL – inner testa layer, OL – outer testa layer, AW – anticlinal seed coat cell wall)

Ryc. 3. Nasiona *Hydrangea heteromalla* (A–D) i *H. paniculata* (E–H); kształt nasion (A, E); pierwotna (A, B, E) i wtórna skulputura łupiny nasiennej (D, F); przekrój podłużny z widocznym zarodkiem (C) i bielmem (C, G: Em – zarodek, R – radikula, C – liścienie, End – bielmo, MEnd – bielmo mikropylarne); fragment przekroju podłużnego z łupiną nasienną (D, H: T – łupina nasienna, IL – wewnętrzna warstwa łupiny nasiennej, OL – zewnętrzna warstwa łupiny nasiennej, AW – ściana antyklinalna komórki łupiny nasiennej)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ined *Philadelphus* species while its inner and outer layers thickness did not differ for *P. californicus* and *P.inodorus* as well as for *P. californicus* and *P. incanus* (tab. 3; fig. 4 C, G; fig. 5 and 6 E, F;). On the longitudinal seed cuttings, the endosperm cells were polygonal in shape with very thin, paper-like cell walls, especially in the micropylar seed region. In *P. inodorus*, the endosperm cell walls were delicately thicker in the lateral endosperm region, but the differences were not very distinct (fig. 4 C, H; fig. 5 F; fig. 6 E, F).

The seeds of the three examined *Deutzia* species had ellipsoidal form. They had a cowl at the micropylar pole and a flattened wing in the chalazal region. The examined seeds were characterized by a reticulate primary sculpture pattern, with the individual cell shapes very elongated on *D. compacta* seeds and much shorter and broader on *D. scabra* and *D. rubens* seeds. The anticlinal walls of the seed coat cells protruded above the outer periclinal wall in all three examined taxa and they were largely straight on *D. scabra* and *D. compacta* seeds, but slightly sinuate on *D. rubens* seeds. The outer periclinal walls were largely exposed on the seeds of *D. scabra* and *D. rubens*, while on *D. compacta* seeds the lateral anticlinal walls tended to enclose the surface of the outer periclinal walls. All of the three examined taxa had frequent latitudinal cross walls in the central body of the seed (fig. 7 A, C, E). On the seeds of the three examined *Deutzia* species a sunken secondary sculpture was present and it was characterized by shallow depressions. On the *D. scabra* seed coat they occurred less regularly and were present mostly on the ridges of the anticlinal walls (fig. 8 A, B). On the seeds of *D. compacta*, delicate shallow depressions were visible at higher magnifications on the whole surface of the outer periclinal walls, while the seed coat of *D. rubens* seeds was characterized by very distinct, regular depressions on both the anticlinal and peryclinal walls (tab. 2; fig. 7 D, F). The average length and width of examined *Deutzia* seeds ranged from 0.90 to 2.28 mm and 0.44 mm to 0.64 mm, respectively. The average thickness of the double-layered seed testa (T) of the examined *Deutzia* species ranged from 5.83 µm to 7.88 µm, with the average thickness of the inner testa layer (IL) varying from 2.90 µm to 3.90 µm and of the outer testa layer (OL) from 2.90 µm to 3.90 µm. All found differences were significant (tab. 3; fig. 8 A, D). The anticlinal walls and outer periclinal

Fig. 4. Seeds of *P. inodorus* (A–C)*, P. pubescens* var. *verrucosus* (D–H): seed longitudinal cutting with embryo and endosperm visible (A: Em – embryo, R – radicula, C – cotyledons, End – endosperm, MEnd – micropylar endosperm); shape of seeds (D); primary (E) and secondary testa sculpure (B, F); part of the seed longitudinal cutting with endosperm and testa (C, G, H: End – endosperm, MEnd – micropylar endosperm, T – testa, IL – inner testa layer, OL – outer testa layer, AW – anticlinal seed coat cell wall)

Ryc. 4. Nasiona *P. inodorus* (A–C)*, P. pubescens* var. *verrucosus* (D–H): przekrój podłużny z widocznym zarodkiem i bielmem (A: Em – zarodek, R – radikula, C – liścienie, End – bielmo, MEnd – bielmo mikropylarne); kształt nasion (D); pierwotna (B, E) i wtórna skulputura łupiny nasiennej (B, F); fragment przekroju podłużnego z bielmem i łupiną nasienną (C, G, H: End – bielmo, MEnd – bielmo mikropylarne, T – łupina nasienna, IL – wewnętrzna warstwa łupiny nasiennej, OL – zewnętrzna warstwa łupiny nasiennej, AW – ściana antyklinalna komórki łupiny nasiennej)

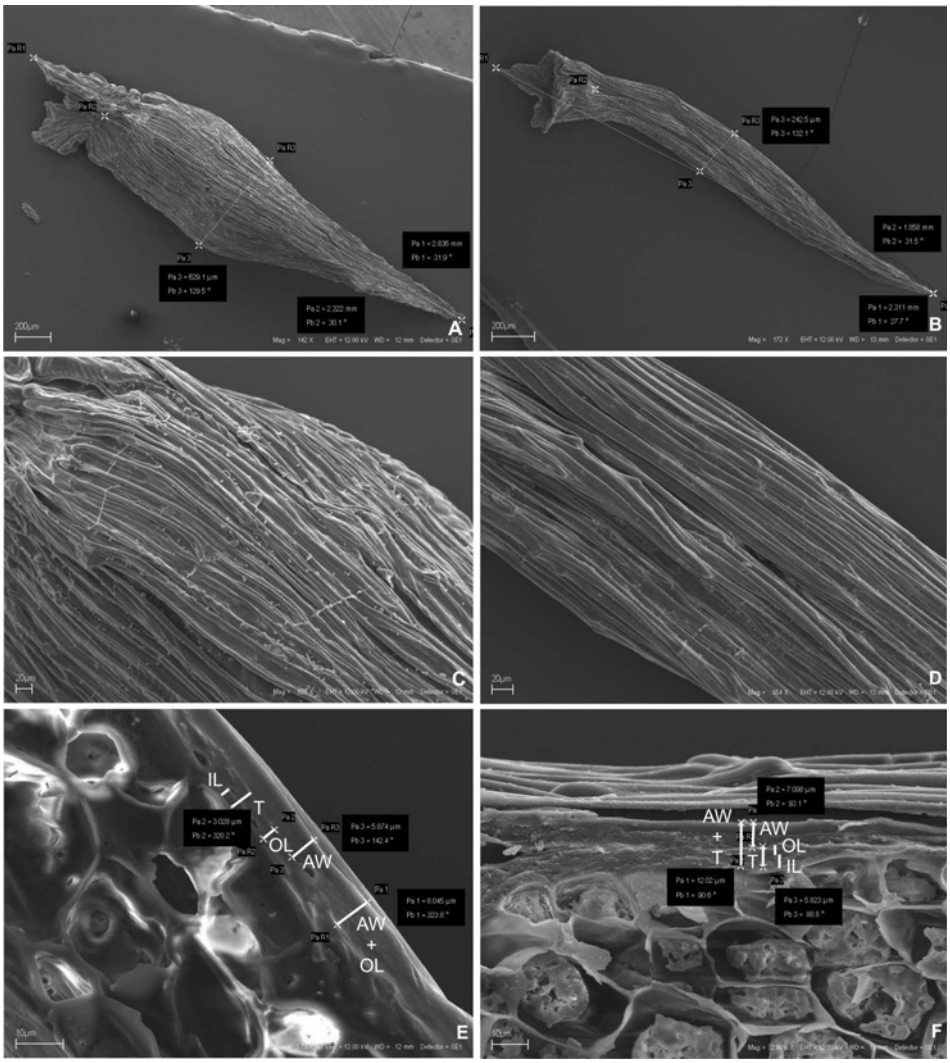

Fig. 5. Seeds of *Philadelphus incanus* (A, C, E)*, P. californicus* (B, D, F); shape of seeds (A, B); primary testa sculpure (C, D); part of the seed longitudinal cutting with endosperm and testa (E, F: End – endosperm, T – testa, IL – inner testa layer, OL – outer testa layer, AW – anticlinal seed coat cell wall)

Ryc. 5. Nasiona *Philadelphus incanus* (A, C, E)*, P. californicus* (B, D, F); kształt nasion (A, B); pierwotna skulputura łupiny nasiennej (C, D); fragment przekroju podłużnego z bielmem i łupiną nasienną (E, F: End – bielmo, T – łupina nasienna, IL – wewnętrzna warstwa łupiny nasiennej, OL – zewnętrzna warstwa łupiny nasiennej, AW – ściana antyklinalna komórki łupiny nasiennej)

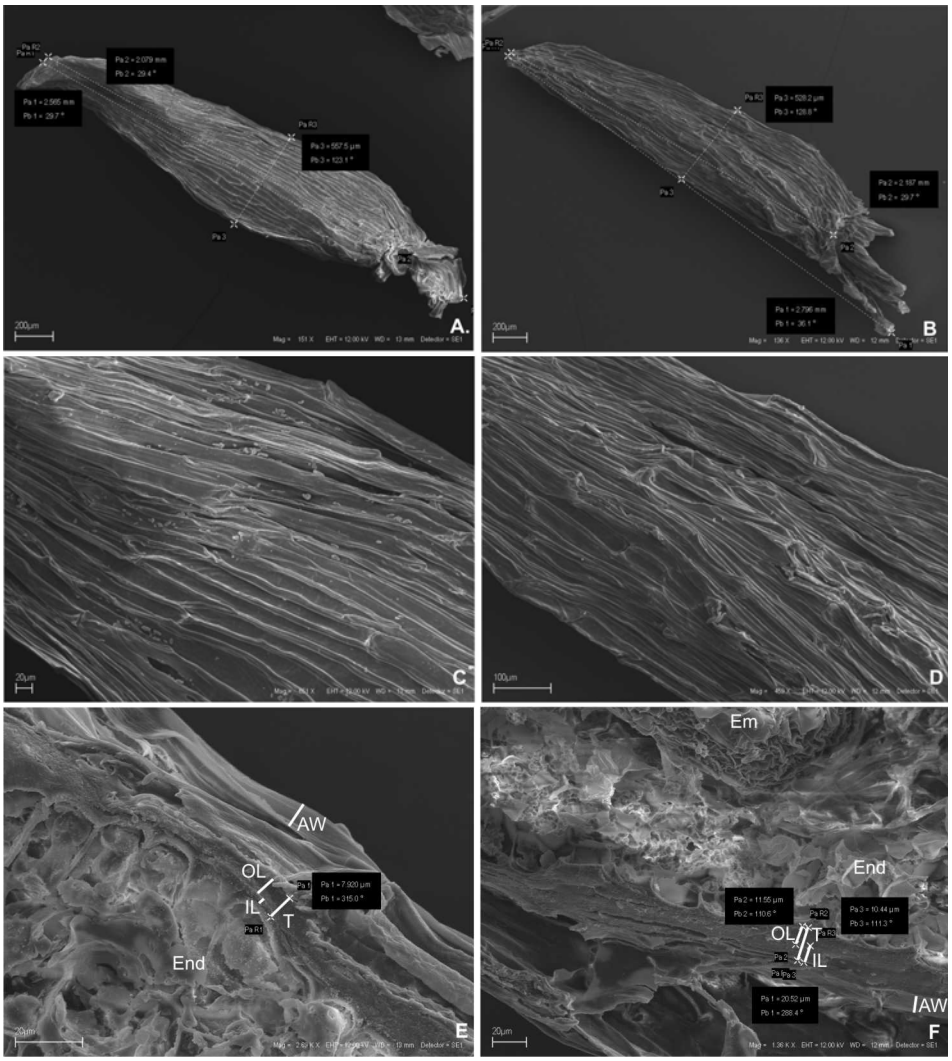

Fig. 6. Seeds of *P. delavayi* (A, C, E)*, P. tenuifolius* (B, D, F); shape of seeds (A, B); primary testa sculpure (C, D); part of the seed longitudinal cutting with endosperm and testa (E, F: End – endosperm, T – testa, IL – inner testa layer, OL – outer testa layer, AW – anticlinal seed coat cell wall)

Ryc. 6. Nasiona *P. delavayi* (A, C, E)*, P. tenuifolius* (B, D, F); kształt nasion (A, B); pierwotna skulputura łupiny nasiennej (C, D); fragment przekroju podłużnego z bielmem i łupiną nasienną (E, F: End – bielmo, T – łupina nasienna, IL – wewnętrzna warstwa łupiny nasiennej, OL – zewnętrzna warstwa łupiny nasiennej, AW – ściana antyklinalna komórki łupiny nasiennej)

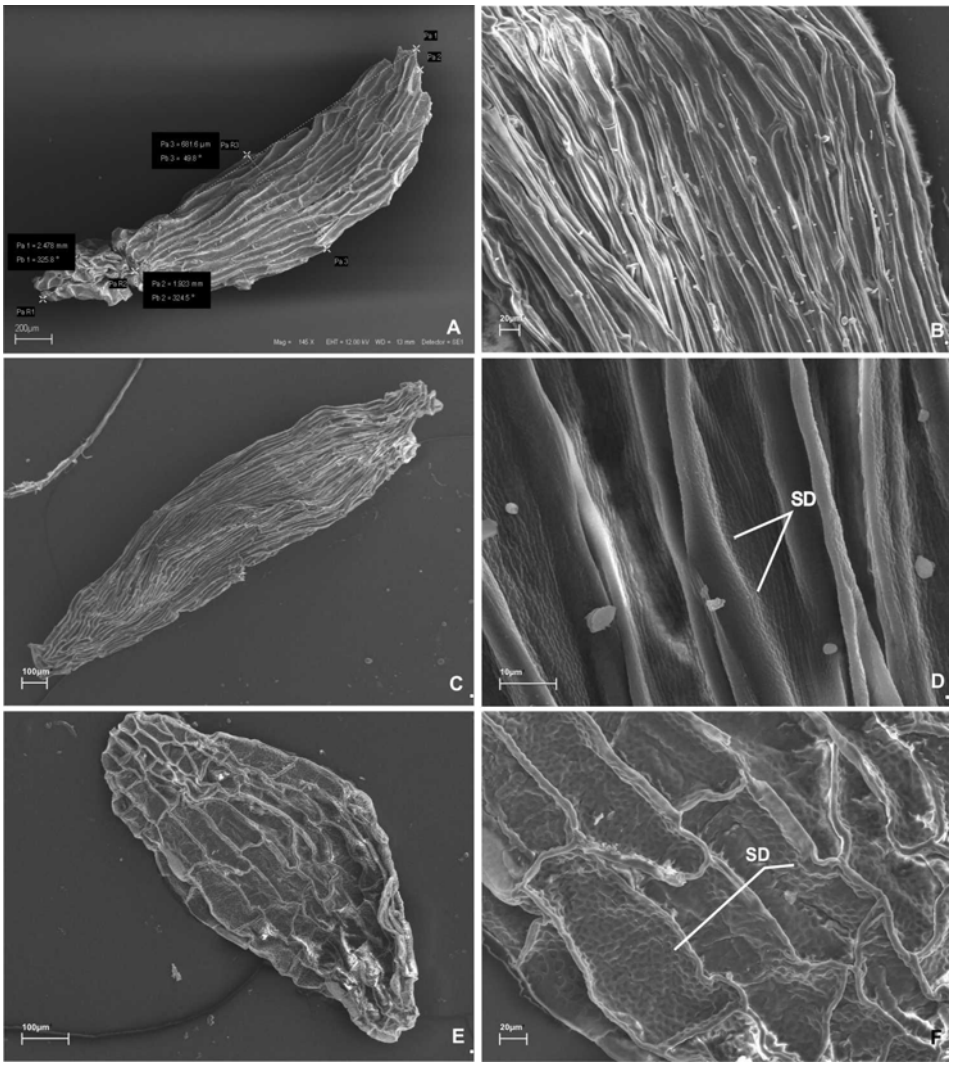

Fig. 7. Seeds of *Deutzia scabra* (A), *D. compacta* (B–D) and *D. rubens* (E, F); shape of seeds (A, C, E); primary (A, B, E) and secondary testa sculpture (D, F: SD – sunken depressions on the surface of the periclinal walls of the seed coat cell)

Ryc. 7. Nasiona *Deutzia scabra* (A), *D. compacta* (B–D) i *D. rubens* (E, F); kształt nasion (A, C, E); pierwotna (A, B, E) i wtórna skulputura łupiny nasiennej (D, F: SD – delikatne zagłębienia na powierzchni komórek łupiny nasiennej)

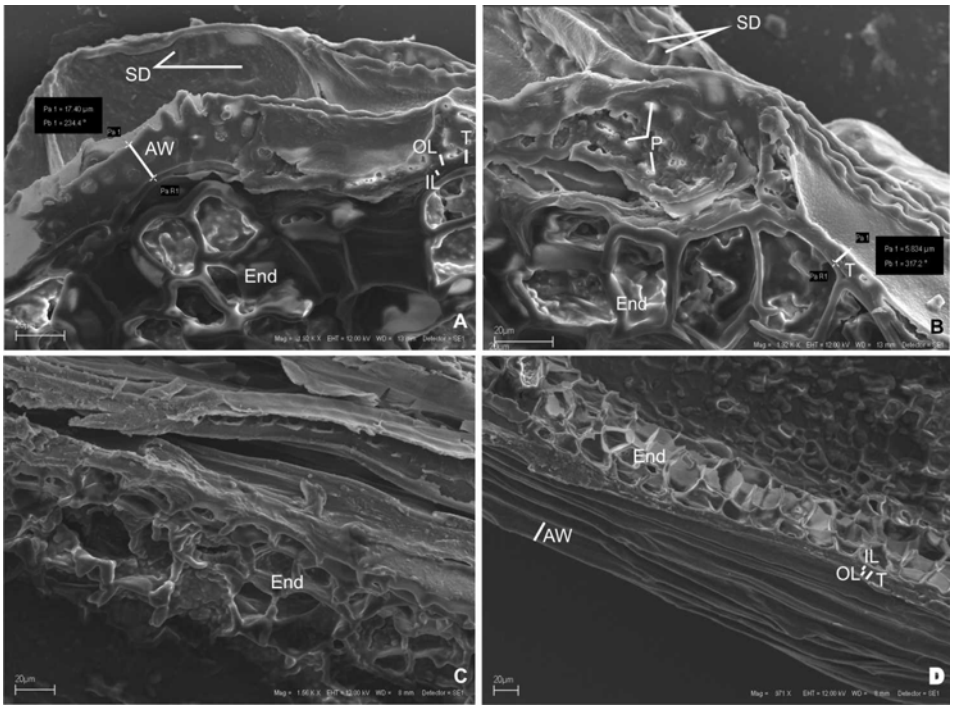

Fig. 8. Seeds of *Deutzia scabra* (A, B) and *D. compacta* (C, D); secondary testa sculpture (A, B: SD – sunken depressions on the surface of the periclinal walls of the seed coat cell); part of the seed longitudinal cutting with endosperm and testa (A–D: End – endosperm, T – testa, IL – inner testa layer, OL – outer testa layer, AW – anticlinal seed coat cell wall, P – pits)

Ryc. 8. Nasiona *Deutzia scabra* (A, B) i *D. compacta* (C, D); wtórna skulputura łupiny nasiennej (A, B: SD – delikatne zagłębienia na powierzchni komórek łupiny nasiennej); fragment przekroju podłużnego z bielmem i łupiną nasienną (A–D: End – bielmo, T – łupina nasienna, IL – wewnętrzna warstwa łupiny nasiennej, OL – zewnętrzna warstwa łupiny nasiennej, AW – ściana antyklinalna komórki łupiny nasiennej, P – jamki)

walls of the testa cells of *D. scabra* seeds were strongly thickened and a significant number of pits were visible on them. On the longitudinal seed cuttings, the endosperm cells were polygonal in shape with evenly thickened cell walls (fig. 8 A, B).

Among all other examined species no differentiation in the shape and size of endosperm cells, in dependence of the seed region, was observed except for the delicate diminutive of endosperm cells in its micropylar region.

DISCUSSION

The family Hydrangeaceae comprises 17 genera and about 220 species distributed in temperate and subtropical regions of the Americas, Pacific Islands, Asia and Europa. Most genera of Hydrangeaceae are small (1–4 species) and restricted to a narrow geographic region, with the exception of *Hydrangea* (32 species), *Philadelphus* (70), and *Deutzia* (70), which are relatively large and occur widely. These three large genera of the family are distributed in the northern hemisphere, with *Hydrangea* extending to South America [Hufford 2004].

The species of Hydrangeaceae that were examined in the present study represent different subgenera/sections/subsections recognized by different authors to be within *Hydrangea* (subsection *Heteromallae – H. heteromalla, H. paniculata*) [Hufford 1997], *Deutzia* (section *Deutzia – D. scabra*; section *Mesodeutzia – D. rubens*; *D. compacta*) and *Philadelphus* (subgenus *Philadelpus* – all examined taxa) [Hufford 2004, Seneta 1996, Huang et al. 2001]. Most of them are commercially available and very popular landscape plants [Rinehart and Reed 2008].

According to the literature, fruits of *Hydrangea heteromalla* and *H. paniculata* are similar in shape and size and their projected apical part is conical and of different length [Wei and Bartholomew 2001, Hufford 2004, Bojňanský and Fargašovă 2007]. According to our results, both *Hydrangea* species may be distinguished by the fruit and by style surface microornamentation pattern.

The seed micromorphology of the *Hydrangea* species examined by us has been described earlier by Hufford [1995]. Regarding the *H. paniculata* seed shape and size, our results are consistent with that author's descriptions. However, regarding the *H. heteromalla* seeds, our results differ with those of Hufford [1995] in respect to seed shape, length, as well as to the primary and secondary sculpture and form of the micropylar seed pole. Additionally, a new characteristic of the *H. heteromalla* seeds has been described in the present work. It concerns the features of the secondary seed sculpture observed on the ridges of the seed coat's longitudinal anticlinal walls. The reason our description of the *H. heteromalla* seeds' morphology is different than Hufford's description [1995] may arise from the fact that *H. heteromalla* forms a complex species out of which a number of segregate species have been recognized [Wei and Bartholomew 2001], and a large assemblage of variable ecotypes occurs from the eastern Himalayas, through the interior, to eastern China [Hinkley 2003].

Three *Philadelphus* species that were examined in our work for the first time, such as *P. tenuifolius, P. delavayi* and *P. californicus* show typical seed shape and size for that genus, along with the form of the micropylar and chalazal regions. Additionally, as with *P. pubescens* var. *verrucosus*, *P. californicus* was characterized by significantly protruding anticlinal wall endings, which wasn't described previously by Hufford [1995]. Our measurements of the seed length of *Philadelphus inodorus* and *Philadelphus incanus,* two species examined earlier by Hufford [1995], were nearly the same or very similar to his results. That means that seed length is a stable seed character despite the different origins of the examined seeds and most probably the different environmental conditions of plant growth. Among all of the examined species of the genus

Philadelphus, the species *P. californicus* turned to be least differentiated according to all examined seed morphological features.

Three of the *Deutzia* species examined here showed seed shape, size, and type of seed sculpture typical for that genus, according to Hufford [1995], but some differences between the examined species were observed. The *D. compacta* and *D. rubens*, the two species examined for the first time, and being included in the same section *Mesodeutzia* showed different shape and length of the seed coat cells and the form of the anticlinal walls. The different appearance of the *Deutzia compacta* seeds' sculpture, which is very similar with that of the *Philadelphus* species, and the form of *D. rubens* seeds sculpture, similar to that of the *Hydrangea heteromalla*, may support the statement by Hufford [1997] and Hufford et al. [2001] that *Deutzia* wasn't derived from *Philadelphus*.

The seeds of all of the examined Hydrangeaceae species develop from anatropous, unitegmic, and tenuinucellate ovules with an endosperm formation of cellular type [Netolitzky 1926, Hufford 2004]. According to the results obtained from the statistical analysis of the seeds' length and width it was found the these two features did not differ significantly the examined species. Almost all of the examined species differed significantly according to the seed coat thickness. Our results showed that the seed coat of all the examined species is double-layered with easily distinguishable inner and outer layers. Krach [1977] stated that in the ripe seeds of Hydrangeaceae only 2 cell-layers remain traceable. According to Niemirovich-Danchenko and Lobova 1998], the anticlinal walls of the seed coat cells, which are the part of the outer testa layer (exotesta), are strongly lignified and, in some of the examined species, with a significant number of pits on their surface. In our observations, significantly thick seed coat cell walls with many pits were visible in the *Deutzia scabra* seeds; they were very similar to what has been observed by Niemirovich-Danchenko and Lobova [1998] in *Carpodetus serratus* (Carpodetaceae), a representative of the family that was included in Hydrangeales by Takhtajan [1997]. According to the obtained results the average seed coat thickness was thicker by 1.3–6.7 μm in the seeds of the *Philadelphus* species, in comparison with the *Hydrangea* and *Deutzia* species. However, the differences in testa thickness were found to be significant for almost all of the examined species, with the exception of the *Hydrangea paniculata* and *P. californicus* seeds. The reciprocal proportions of the thickness of the inner and outer testa layers were different within the examined species. In the *Hydrangea* species, the inner testa layer was thicker than the outer one. In three out of the six examined *Philadelphus* species, the inner testa layer was thicker than the outer one; and for another three of them, the reciprocal proportions in the thickness of the two testa layers were opposite. In the *Deuztia* seeds, both testa layers were of equal thickness. The average thickness of the inner and outer testa layers were significantly different for all of the examined species with the exception of the *Philadelphus californicus* and *P. incanus* as well as for *P. californicus* and *P. inodorus*. As it has been shown in other plant families and genera, differences in the thicknesses of the exo- and endotesta layers might be of systematic importance [Morozowska et al. 2011]. The analyses of the type of the endosperm cells shape and the wall thickness showed that the endosperm cell walls of the examined *Hydrangea* and *Philadelphus* species were very thin and paper-like, similar to that described by Morozowska et al. 2011] in some Primulaceae and Myrsinaceae species. Visibly thicker, evenly thickened endosperm cell

walls were observed in the seeds of the two examined *Deutzia* species. According to Krach [1977] this may be due to the storage of reserve-cellulose in the cell walls of the endosperm. However, no distinct differentiation in the shapes of cells or the endosperm cell wall thicknesses were observed in different endosperm regions in seeds of all the examined species. Such differences were found by Morozowska et al. [2011] and, according to these authors, the type of endosperm cell wall thickness and the relief of their inner surface might be of systematic importance. To find out the possible statistic significance and systematic importance of the analyzed seed anatomical features, more species of Hydrangeaceae representing different genera should be examined.

CONCLUSIONS

1. Microornamentation pattern of the fruits and style surface of examined *Hydrangea* species were different and of taxonomic significance.
2. The seeds of *Hydrangea heteromalla* and *H. paniculata* differed in size and in the form of the micropylar and chalazal seed ends.
3. The secondary sculpture of *Hydrangea heteromalla* seeds is characterized by the presence of shallow depressions on the ridges of the anticlinal walls.
4. The primary seed sculpture of all the examined *Philadelphus* species is of a reticulate type. Some differences concerning the degree with which the anticlinal walls enclosed the surface of the outer periclinal walls was observed.
5. Examined *Deutzia* seeds were characterized by a sunken secondary sculptures of different patterns and these differentiations are of systematic importance.
6. Differences in the endosperm cell walls thickness were observed between *Hydrangea* and *Philadelphus* seeds in comparison with *Deutzia* seeds, and this may be of systematic importance.
7. No differentiation in the shape and size of the endosperm cells in dependence of the seed region were observed.
8. The seed coats of all examined species were double-layered with different reciprocal proportions in the thickness of its inner and outer layers.

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MIKROSTRUKTURA OWOCÓW I NASION WYBRANYCH GATUNKÓW Z Hydrangeaceae (Cornales) I JEJ ZNACZENIE SYSTEMATYCZNE

Streszczenie. Mikromorfologia owoców, długość i kształt nasion, skulptura nasion, grubość łupiny nasiennej oraz struktura bielma zostały zbadane dla *Hydrangea heteromalla, H. paniculata, Philadelphus californicus, P. delavayi, P. incanus, P. inodorus, P. pubescens* var. *verrucosus, P. tenuifolius, Deutzia compacta, D. rubens* and *D. scabra.* Wzór urzeźbienia powierzchni owocu i szyjki słupka gatunków z rodzaju *Hydrangea* jest taksonomicznie istotnie zróżnicowany. Nasiona gatunków *Hydrangea* różnią się ponadto długością oraz formą wykształcenia bieguna mikropylarnego i chalazalnego. Wtórna skulptura nasion *H. heteromalla* charakteryzuje się obecnością płytkich zagłębień na brzegach ścian antyklinalnych. Nasiona wszystkich gatunków z rodzaju *Philadelphus* charakteryzują się siateczkowatym typem skulptury pierwotnej i skulpturą wtórną w postaci delikatnych zmarszczeń. Na nasionach gatunków z rodzaju *Deutzia* stwierdzono zapadnięty typ skulptury wtórnej o zróżnicowanym wzorze, co uznano za cechę o znaczeniu systematycznym. Ściany komórkowe komórek bielma były bardzo cienkie w nasionach gatunków z rodzajów *Hydrangea* i *Philadelphus*, natomiast w nasionach gatunków z rodzaju *Deutzia* były one równomiernie zgrubiałe. Różnice te uznano za cechę istotną systematycznie. Łupina nasienna wszystkich zbadanych gatunków jest dwuwarstwowa, a wzajemny stosunek grubości jej warstwy zewnętrznej i wewnętrznej jest zmienny.

Słowa kluczowe: mikromorfologia owoców, skulptura nasion, testa, komórki bielma

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