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Morphological characters of the seed coat in selected species of the genus *Hypericum* L. and their taxonomic value

Piotr Szkudlarz* & Zbigniew Celka

Department of Plant Taxonomy, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61-614 Poznań, Poland
* corresponding author (e-mail: szkudl@amu.edu.pl)

Abstract. Eight *Hypericum* species are native to Poland: *H. elegans* Stephan ex Willd., *H. hirsutum* L., *H. humifusum* L., *H. maculatum* Crantz, *H. montanum* L., *H. perforatum* L., *H. pulchrum* L., and *H. tetrapterum* Fr. Only seeds of *H. elegans* were investigated in detail in Poland before, so here we present results of qualitative and quantitative analyses of seed morphology of the other 7 species, based on characters like seed length, width, and shape, seed coat sculpture, shape of epidermal cells of the testa, and number of epidermal cells along the seed axis. The results show that seeds of the studied species are small, 0.56-1.15 mm long and 0.26-0.49 mm wide. In SEM images, seed coat sculpture is reticulate in 5 species, papillate in *H. hirsutum*, and cup-shaped in *H. pulchrum*. The differences are caused by the varied final development of the testa epidermis, which constitutes the outer layer of the seed coat. The mean number of epidermal cells along the seed axis ranges from 22 to 33. Results of cluster analysis, based on the agglomeration method and including also published data on seeds of *H. elegans*, show that the variation in the investigated characters of seeds is reflected in the taxonomic division of the genus into sections.

Key words: Hypericaceae, seed sculpture, seed coat, taxonomic analysis, scanning electron microscope

1. Introduction

Hypericum L. is a large genus of flowering plants, including from 420 (Stevens 2007) to nearly 500 species (Nürk & Blattner 2010; Crockett & Robson 2011). Most of them are herbaceous plants, both perennials and annuals, but some are shrubs, rarely small trees (Robson 1981; Stevens 2007).

The genus *Hypericum* is distributed worldwide, primarily in the temperate zone and at higher altitudes in the mountains of the tropical and subtropical zones (Robson 1981; Stevens 2007). *Hypericum* spp. are found all over the Northern Hemisphere, in South America, in tropical areas, and in the south of Africa, Madagascar, and in southern Asia. Several species are also reported from Australia (Robson 1981; Stevens 2007). The centre of diversity of the genus *Hypericum* is located in the Palaearctic region, where 45% of the described species exist. The second centre of diversity is in the Neotropical region, where the genus is represented by about 30% of species. A lower diversity is observed in the Indo-Malayan region (10%), Nearctic region (8.5%), and Afrotropical region (6.4%) (Nürk 2011).

The taxonomic classification of the genus has aroused much controversy and has been discussed for many years. The genus has been included in the widely defined family Guttiferae, as one of 9 genera of the subfamily Hypericoideae (Robson 1977, 1981), in the family Clusiaceae, or in the narrowly defined family Hypericaceae (Stevens 2007; Crockett & Robson 2011).

Seeds of this genus provide useful information for identification of species or even subspecies (Hageman 1987; Alonso *et al.* 2013). Moreover, seed characters are extremely useful for identification of fossil specimens (Meseguer & Sanmartín 2012). The general morphological features of *Hypericum* seeds, on the basis of selected examples, have been presented by Robson (1981) in relation to the sections distinguished within the genus. In Poland, the only detailed report on seeds of this genus concerned *H. elegans* Stephan ex Willd. (Szkudlarz 2014).

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Table 1. Origin of the examined seed samples of *Hypericum* species

Species	Locality, habitat	Collector
H. hirsutum	20 km SE of Olkusz, Małopolska region, Poland, mixed forest	P. Szkudlarz
H. humifusum	4 km SW of Torzym, Lubuskie region, Poland; edge of a road across a forest plantation	S. Lisowski, P. Urbański
H. maculatum	Tarnawa Niżna, Podkarpackie region, Poland; mixed forest	P. Szkudlarz
H. montanum	Mełpin, Wielkopolska region, Poland; broad-leaved forest	A. Czarna
H. perforatum	2 km NE of Milicz, Wielkopolska region, Poland; roadside	P. Szkudlarz
H. pulchrum	near Torm Jutland (Denmark); oak forest	H. Piotrowska
H. tetrapterum	Komorów, 5 km S of Mikstat, Wielkopolska region, Poland; mid-forest meadow, drainage ditch	P. Szkudlarz

This study was primarily aimed to make a qualitative and quantitative analysis of morphological seed variation of *Hypericum* species native to Poland. The study also assumed additional aims: (*i*) to use any observed differences for taxonomic analysis and particularly (*ii*) to verify if the variation in the seed coat corresponds to the subdivision of the genus; and (*iii*) to construct a key to species identification on the basis of seed characters.

2. Material and methods

The analysis of seed morphology involved 7 species of the genus *Hypericum*, which are native to Poland: *H. hirsutum* L., *H. humifusum* L., *H. maculatum* Crantz, *H. montanum* L., *H. perforatum* L., *H. pulchrum* L., and *H. tetrapterum* Fr. Seeds used in this study were collected for this purpose by the authors or were extracted from dried specimens deposited in the Herbarium of the Department of Plant Taxonomy of Adam Mickiewicz University in Poznań (POZ) (Table 1). In the case of *H. pulchrum*, the seed sample originated from Denmark, as in Poland the species is extinct (Kaźmierczakowa *et al.* 2016).

The following characters were taken into account: seed length, width, and shape (length to width ratio), seed coat sculpture, shape of epidermal cells of the testa, and number of epidermal cells along the seed axis. Qualitative characters were investigated on the basis of optical analysis of dry seeds with the use of a stereo microscope and scanning electron microscope (SEM) after sputtering with gold. For SEM observations, 5 seeds from each sample were used. SEM images enabled us to describe and analyse qualitative characters of the seed coat. Quantitative analysis was based on measurements of 50 seeds of each species, by using a stereo microscope and software for digital analysis of

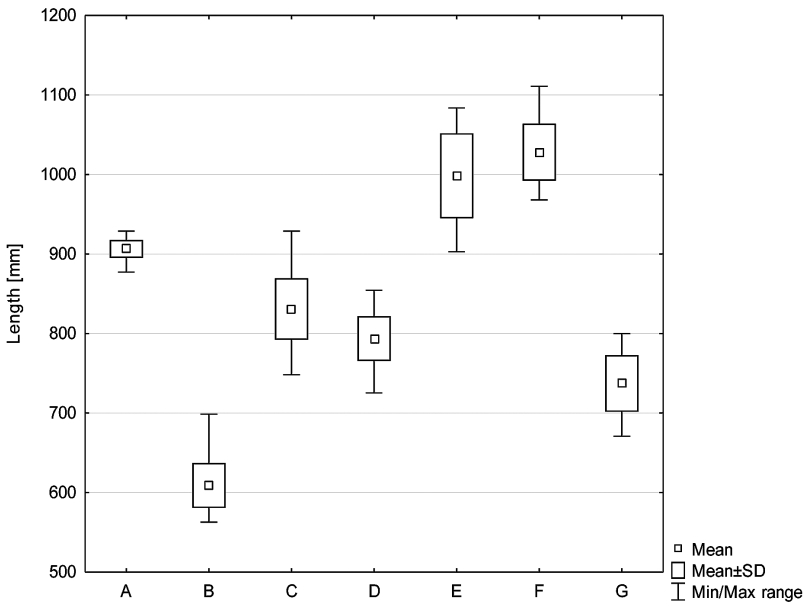

Fig. 1. Variation in seed length in the studied *Hypericum* species
Explanations: A – *H. hirsutum*, B – *H. humifusum*, C – *H. maculatum*, D – *H. montanum*, E – *H. perforatum*, F – *H. pulchrum*, G – *H. tetrapterum*

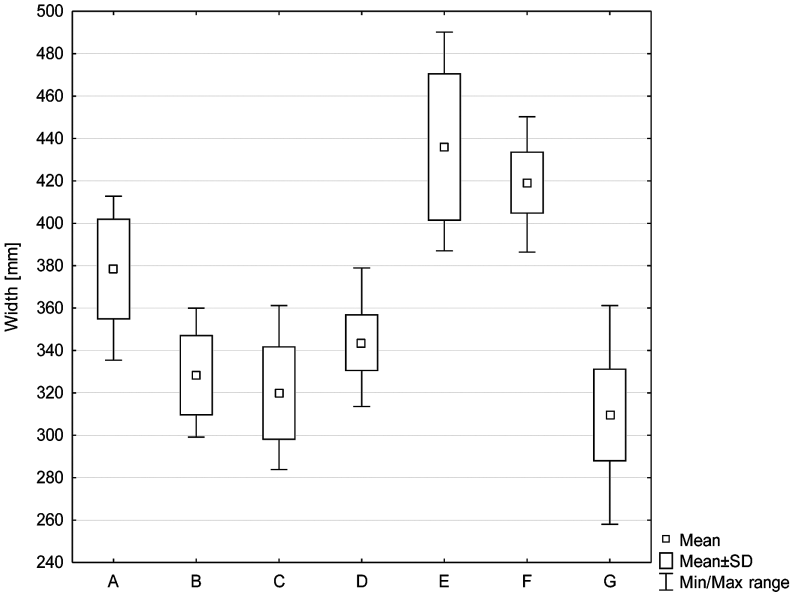

Fig. 2. Variation in seed width in the studied *Hypericum* species
Explanations: see Fig. 1

microscopic images (NIS-Elements Basic Research).

We followed the nomenclature widely accepted in works on seed morphology (Robson 1981; Bojňanský & Fargašová 2007; Meseguer & Sanmartín 2012).

The collected data were subjected to statistical analysis in STATISTICA 10.0 for Windows (StatSoft, Inc. 2013). It involved cluster analysis based on the agglomeration method. Considering the close relations between the compared taxa and the small expected differences, we used Ward's (1963) method. In the analysis of agglomeration for the analysed characters, numerical values were assigned to specific states of the characters: seed shape, i.e. length/width ratio (1 = up to 2, 2 = higher than 2), seed coat sculpture (1 = reticulate, 2 = papillate, 3 = cup-shaped), shape of epidermal cells of the testa (1 = isodiametric, 2 = elongated, 3 = wider transversely than longitudinally), and number of epidermal cells along the seed axis (1 = below 23, 2 = 23-28, 3 = higher than 28). In the cluster analysis, we used also results of research on seeds of *H. elegans*, which were reported earlier (Szkudlarz 2014).

The studied characters were used for taxonomic analysis and allowed us to construct a key to species identification on the basis of seeds.

3. Results

3.1. Seed shape and its variation in the studied *Hypericum* species

Seeds of the studied members of the genus *Hypericum* are elongated, cylindrical, rounded at both ends, sometimes with protruding mucros, straight or slightly curved. The raphe is nearly as long as the seed. Seed length in the analysed species ranges from 560 µm to 1150 µm (Fig. 1), and width from 260 µm to 490 µm (Fig. 2). Among the studied species, the most distinct were the seeds of *H. humifusum*, which were the smallest, and those of *H. perforatum* and *H. pulchrum*, with the largest seeds (Fig. 1). In respect of seed width, the most different from the others were also *H. perforatum* and *H. pulchrum* (Fig. 2), while seeds of the other species did not differ in width.

3.2. Seed structure and variation among species

In SEM micrographs, seed sculpture is reticulate, an only in single species it is papillate or cup-shaped. This is due to differences in final development of the testa epidermis, which constitutes the outer layer of the seed coat.

Hypericum hirsutum L.

Seeds straight, cylindrical, ends rounded, without mucros (Fig. 3A). Seed length 870-930 µm, width 340-415 µm. Under a stereo microscope, seed surface dull, in some places shiny, covered with irregular, sharply pointed papillae. Seed colour rusty-orange (Fig. 3A). In SEM images slightly different, seed surface wrinkled (Fig. 4A). Observation under a greater magnification (1000×) shows that the wrinkles are sunken papillae. Each epidermal cell is initially convex, but during seed coat drying the outer wall collapses. Since the seed surface is densely covered with papillae, it is difficult to determine the shape of epidermal cells of the testa, but in some parts of the seed coat it is noticeable that

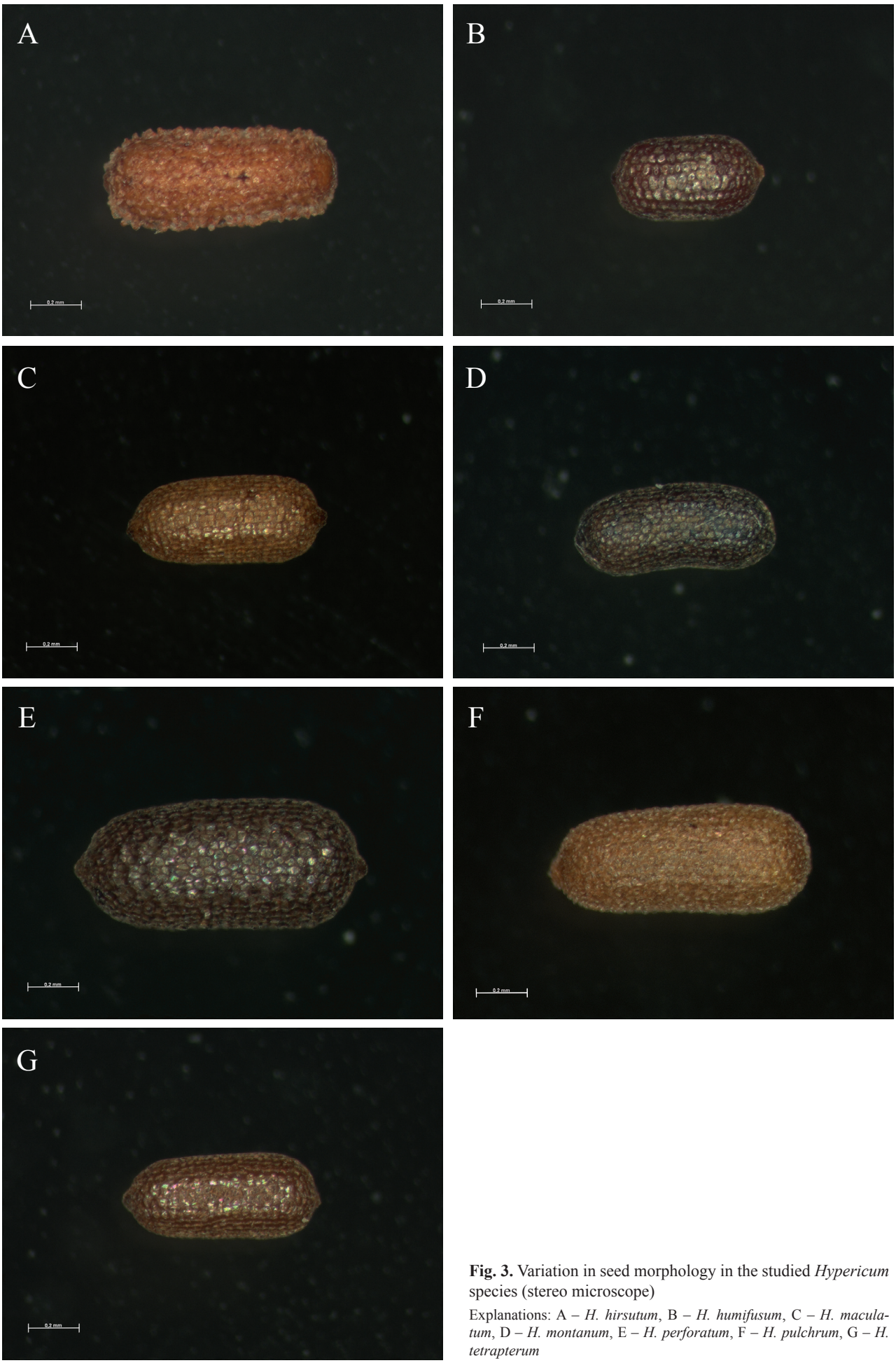

Fig. 3. Variation in seed morphology in the studied *Hypericum* species (stereo microscope)

Explanations: A – *H. hirsutum*, B – *H. humifusum*, C – *H. maculatum*, D – *H. montanum*, E – *H. perforatum*, F – *H. pulchrum*, G – *H. tetrapterum*

they are isodiametric (Fig. 5A). The mean number of epidermal cells along the seed axis is about 30.

Hypericum humifusum L.

Seeds straight, cylindrical, rounded at both ends, with conspicuous mucros (Fig. 3B). Seed length 575-698 µm, width 296-370 µm. Under a stereo microscope, surface shiny, dark brown (Fig. 3B). In SEM images, reticulate sculpture is visible (Fig. 4B). The testa epidermis is so delicate that the seed coat sculpture shows both epidermal cells and the outlines of subepidermal cells, with thickened radial walls. Epidermal cells small, polygonal (usually pentagonal or hexagonal), isodiametric, with straight radial walls (Fig. 5B). The mean number of epidermal cells along the seed axis is about 25.

Hypericum maculatum Cr.

Seeds slightly curved, cylindrical, with rounded ends, sometimes with minute, protruding mucros (Fig. 3C). Seed length 735-902 µm, width 328-351 µm. Under a stereo microscope, surface smooth, shiny. Seed colour from dark green or green-brown to brown (Fig. 3C). In SEM images, reticulate sculpture is visible. The pattern is formed by closely adhering epidermal cells (Fig. 4C), which are isodiametric, minute, rounded to elliptic, slightly elongated longitudinally (Fig. 5C). The mean number of epidermal cells along the seed axis is about 23.

Hypericum montanum L.

Seeds straight, cylindrical, rounded at both ends, with hardly visible mucros (Fig. 3D). Seed length 725-822 µm, width 322-379 µm. Under a stereo microscope, surface shiny, dark brown (Fig. 3D). In SEM images, reticulate sculpture is visible, formed by delicate epidermal cells (Fig. 4D), which are minute, polygonal (pentagonal or hexagonal), isodiametric, with straight radial walls. Cell walls of the testa epidermis do not seem to adhere at their angles (Fig. 5D). The mean number of epidermal cells along the seed axis is about 22.

Hypericum perforatum L.

Seed shape highly variable, straight, sometimes slightly curved, usually cylindrical. Some seeds very long and narrow, some shorter and wider. Both ends rounded, with protruding mucros (Fig. 3E). Seed length 914-1043 µm, width 319-479 µm. Under a stereo microscope, shiny surface with minute pits, which are darker than whole seeds (Fig. 3E). Seed colour highly variable, ranging from light brown or dark green to dark brown or black. In SEM images, reticulate sculpture is visible (Fig. 4E). Cells isodiametric, polygonal, irregular, wider transversely than longitudinally. Outer cell walls of the testa epidermis form a delicate fold around each cell. As a result, the cells seem to be rounded at their angles, not adhering to one another (Fig. 5E). The mean number of epidermal cells along the seed axis is about 30.

Hypericum pulchrum L.

Seeds straight or sometimes slightly curved, cylindrical, rounded at both ends, with inconspicuous mucros (Fig. 3F). Seed length 968-1111 µm, width 386-442 µm. Under a stereo microscope, their surface shiny, with minute pits (Fig. 3F). Seed colour brown. In SEM images, seed coat sculpture unique, so far not reported in this genus, described here as cup-shaped (Fig. 4F): seed surface densely covered with minute cups. The structure results from collapse of outer cell walls of the testa epidermis. The outline of cell shape formed by radial walls is not visible (Fig. 5F), only the isodiametric, cup-shaped outer cell walls can be seen. The mean number of epidermal cells along the seed axis is about 33.

Hypericum tetrapterum Fr.
(syn. *Hypericum acutum* Moench)

Seeds straight, cylindrical, rounded at both ends, with protruding mucros (Fig. 3G). Seed length 670-800 µm, width 260-340 µm. Under a stereo microscope, surface shiny, dark brown (Fig. 3G). In SEM images, reticulate sculpture is visible, formed by delicate epidermal cells of the testa (Fig. 4G), which are minute, polygonal (usually pentagonal) isodiametric, with straight radial walls. The cell walls of the testa epidermis do not seem to adhere to one another at their angles (Fig. 5G). The mean number of epidermal cells along the seed axis is about 25.

3.3. Taxonomic analysis

The performed cluster analysis based on seed characters allowed us to group the studied species. Three groups can be clearly distinguished: the first one composed of *H. hirsutum* and *H. pulchrum*, the second one consisting of *H. humifusum*, and the third one including all the other species (Fig. 6). The groups partly correspond to the earlier described sections within the genus.

3.4. Key to species identification on the basis of SEM images of seeds

With the use of the analysed seed characters, we have constructed a key to identification of *Hypericum* species native to Poland.

1 – seed coat surface reticulate 3
1* – seed coat surface not reticulate 2
2 – seed coat surface papillate ..... *H. hirsutum*
2* – seed coat surface cup-shaped ..... *H. pulchrum*

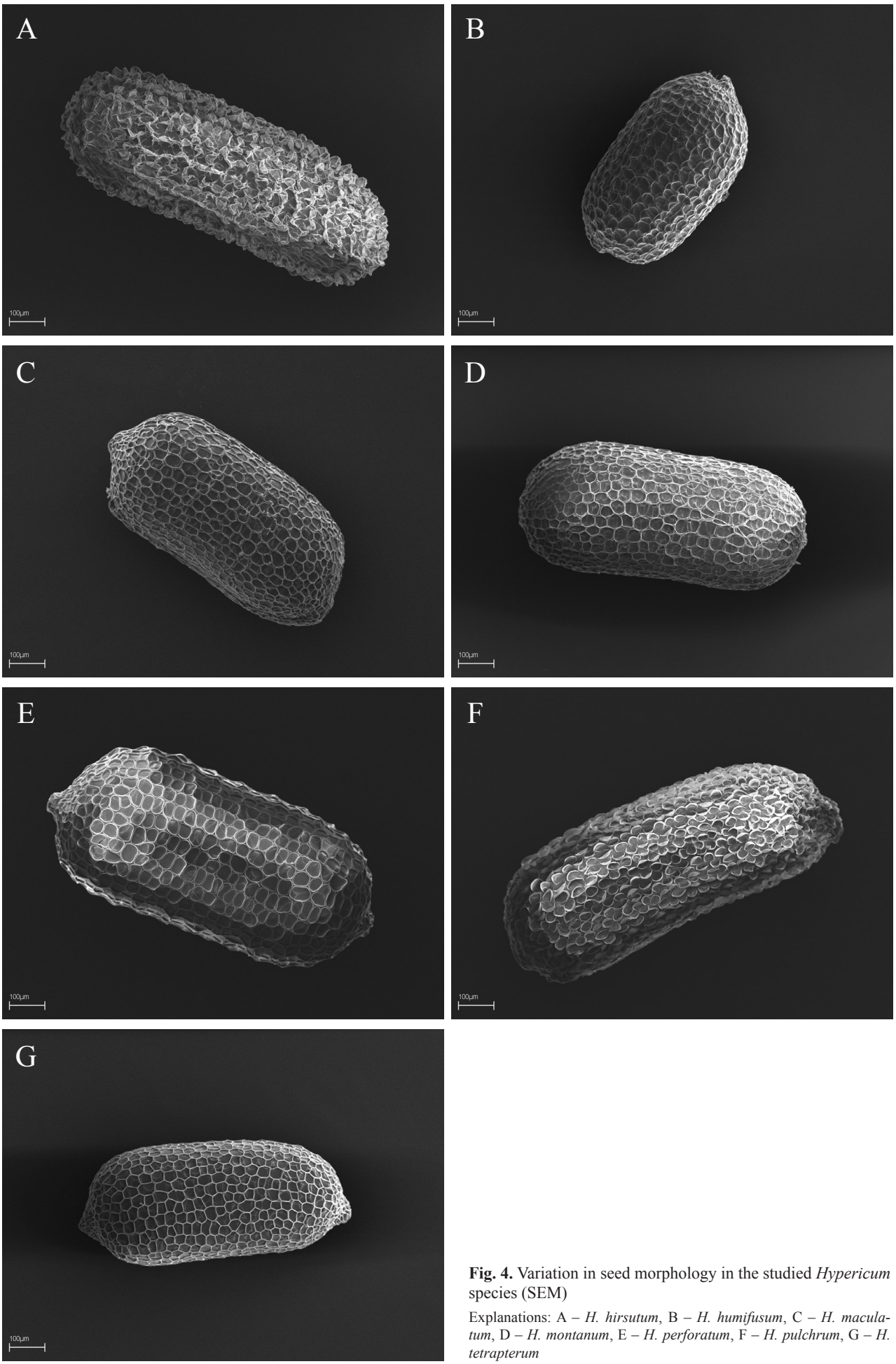

Fig. 4. Variation in seed morphology in the studied *Hypericum* species (SEM)

Explanations: A – *H. hirsutum*, B – *H. humifusum*, C – *H. maculatum*, D – *H. montanum*, E – *H. perforatum*, F – *H. pulchrum*, G – *H. tetrapterum*

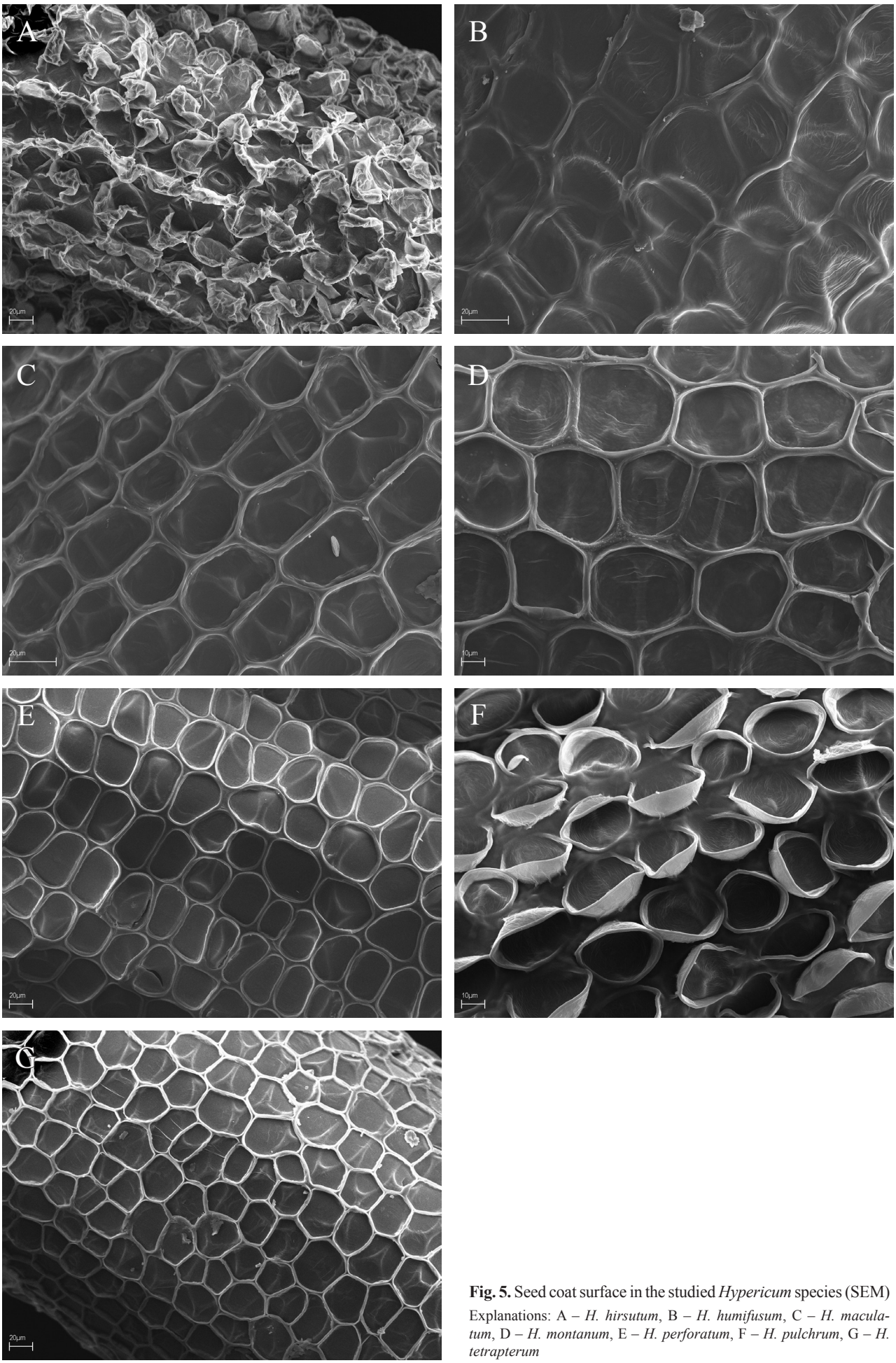

Fig. 5. Seed coat surface in the studied *Hypericum* species (SEM)

Explanations: A – *H. hirsutum*, B – *H. humifusum*, C – *H. maculatum*, D – *H. montanum*, E – *H. perforatum*, F – *H. pulchrum*, G – *H. tetrapterum*

Table 2. Comparison of seed size (length × width, in mm) in the studied *Hypericum* species, based on selected sources

Species	Bojňanský & Fargašová (2007)	Robson (1996, 2002, 2010a, 2010b)*	This study
H. elegans	1.0-1.2 × 0.40-0.50	0.8-1.0	1.20-1.30 × 0.42-0.46
H. hirsutum	0.9-1.1 × 0.35-0.45	ca. 1.0	0.87-0.93 × 0.34-0.42
H. humifusum	0.5-0.7 × 0.35-0.45	0.5-0.6	0.58-0.70 × 0.30-0.37
H. maculatum	0.9-1.1 × 0.40-0.50	0.8-1.2	0.76-0.90 × 0. 33-0.35
H. montanum	0.8-1.0 × 0.30-0.40	0.8	0.73-0.82 × 0.32-0.38
H. perforatum	1.0-1.2 × 0.50-0.60	ca. 1.0	0.91-1.04 × 0.32-0.48
H. pulchrum	1.0-1.2 × 0.40-0.50	0.8-1.0	0.97-1.11 × 0.39-0.44
H. tetrapterum	1.0-1.1 × 0.45-0.55	0.6-0.8 (1.1)	0.67-0.80 × 0.26-0.34

Explanation: * – Robson measured only seed length

3 – seeds elliptic in outline (length/width <2) .... *H. humifusum*
3* – seeds elongate in outline (length/width >2) 4
4 – epidermal cells usually wider transversely than longitudinally ..... *H. perforatum*
4* – epidermal cells usually isodiametric 5
4** – epidermal cells usually slightly elongate 6
5 – seeds with mucros, testa epidermis composed of polygonal cells ..... *H. tetrapterum*
5 – seeds with rounded ends, without mucros, testa epidermis composed of cells rounded at angles .... *H. montanum*
6 – seeds with mucros ..... *H. maculatum*
6 – seeds with rounded ends, without mucros .... *H. elegans*

4. Discussion

Detailed data on seed dimensions in individual species of the genus *Hypericum* are not commonly reported, so there are few sources of comparative data (see Bojňanský & Fargašová 2007). In the basic publication on *Hypericum* seeds (Robson 1981), they are described as small, 0.3-1.5 mm long. This is confirmed by our findings (Table 2), but our measurements have revealed some differences, as compared with data reported in the literature. In general, the data on seed length presented by Robson (1996, 2002, 2010a, 2010b) are to a large extent consistent with our results, but in the case of *H. maculatum* and *H. hirsutum*, our values are slightly smaller (see Table 2). In comparison to the work of

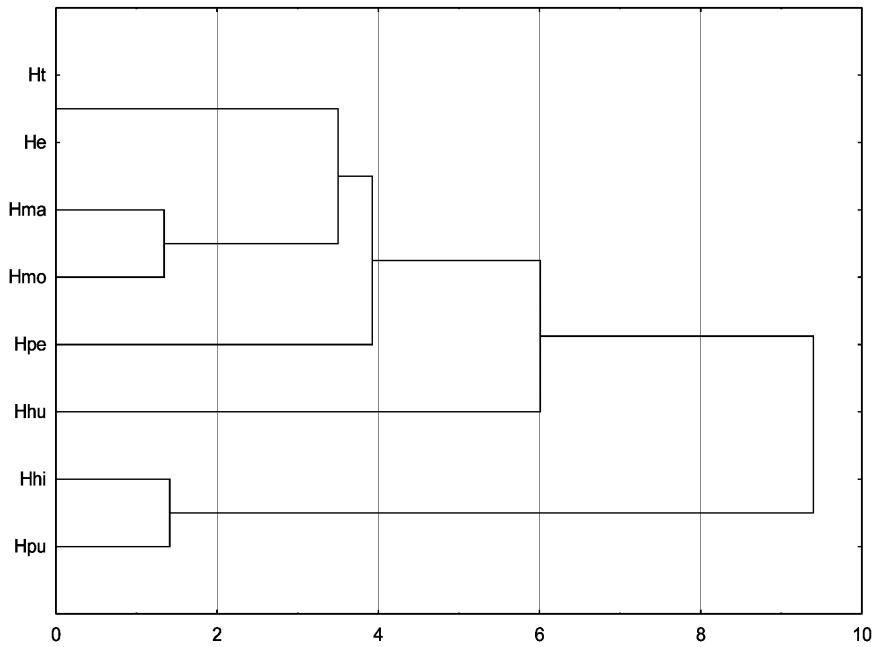

Fig. 6. Dendrogram of seed similarity of the studied *Hypericum* species, based on cluster analysis
Explanations: He – *H. elegans*, Hhi – *H. hirsutum*, Hhu – *H. humifusum*, Hma – *H. maculatum*, Hmo – *H. montanum*, Hpe – *H. perforatum*, Hpu – *H. pulchrum*, Ht – *H. tetrapterum*

Bojňanský & Fargašová (2007), presenting detailed data on seed length and width, differences are substantial in most cases (see Table 2). Also in the other species in our study, seed dimensions were slightly smaller. The differences may result from a high variation of seeds of the species within their geographic ranges, which has not been studied so far. Intraspecific variation has been reported for some of the studied species (Robson 2002; Mártonfi 2008). Our results markedly broaden the available knowledge about variation of the studied structures.

The performed cluster analysis based on seed characters allowed us to group the studied species (Fig. 6). The clearly distinguished group with *H. hirsutum* and *H. pulchrum* belongs to the section *Taeniocarpium*, while the isolated *H. humifusum* belongs to the section *Oligostema* (Robson 1981). The other species are included in the section *Hypericum*, except for *H. montanum* of the section *Adenosepalum* (Robson 1981). However, when analysing the correspondence between seed structure and taxonomic classification, it must be remembered that this study focused on only 8 species and the analysis was based on only few characters related to seed structure.

Our morphological description of the seeds can be used for comparison with other seeds of the genus *Hypericum* (see Robson 1981, 2002), and as a reference for identification of both recent and fossil materials (Meseguer & Sanmartín 2012).

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