

SEM AND STEREOSCOPE MICROSCOPE OBSERVATIONS ON THE SEEDS OF THE POLISH SPECIES OF THE GENUS *SORBUS* L. (ROSACEAE)

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ABSTRACT

This paper presents the results of the study on seed morphology of five Polish species of the genus *Sorbus* L. (*S. aria* (L.) Crantz, *S. aucuparia* L. Emend. Hedl., *S. chamaemespilus* (L.) Crantz, *S. intermedia* (Ehrh.) Pers. and *S. torminalis* (L.) Crantz). Observations and measurements were carried out with stereoscope and scanning microscopes. Four types of seed microsculpture were distinguished on the ground of SEM observations. The key to determine Polish species, based on morphological characters of seeds is given too.

KEY WORDS: *Sorbus*, seed morphology, SEM, stereoscope microscope.

INTRODUCTION

The genus *Sorbus* L. (Rosaceae, Maloideae) includes about 250 species, occurring mainly in temperate regions of the Northern Hemisphere (Phipps et al. 1990). The number of species growing wild in Europe has not been determined yet. According to Canadian botanists, who revised the subfamily Maloideae (Phipps et al. l.c.), even 91 species occur within this continent. Warburg and Kárpáti (1968) take into consideration 19 species in Europe. Basing on a multivariate morphometric study of the genus *Sorbus* (Aldasoro et al. 1998) only 12 species may be easily recognized in the area.

In Poland, seven species have been recognized: *S. aria* (L.) Crantz, *S. aucuparia* L. Emend. Hedl., *S. carpatica* Borbás, *S. chamaemespilus* (L.) Crantz, *S. graeca* (Spach) Kotschy, *S. intermedia* (Ehrh.) Pers. and *S. torminalis* (L.) Crantz (Mirek et al. 2002).

S. aucuparia is the most common species, widespread throughout Poland. In lowlands it is represented by the type subspecies *aucuparia*, while in the mountains by subspecies *glabrata* (Wimm. & Grab.) Cajander. *S. torminalis* is a rare, scattered tree having in Poland north-eastern limit of its range. *S. intermedia* as a northern species occurring naturally only on a few localities on the Baltic coast, is commonly planted as roadside tree and often naturalizes.

The latter four species grow in the wild only locally in southern Poland. *S. aria* occurs in the Pieniny and the Tatra Mountains. *S. chamaemespilus* grows in the Tatra Mountains where it is extremely rare – about 50 individuals on several localities have only been observed (Kaźmierczakowa and Zarzycki 2001). *S. graeca*, mistaken for *S. aria*, has been recorded from the Pieniny Mountains by Gabrielian (1977). Similarly *S. carpatica*, also included into the *S. aria* complex, has been noted only in the Pieniny and the Tatra Mountains (Pawłowska and Pawłowski 1970).

The genus *Sorbus* is characterized by pome fruit. The inner part of the fruit, termed by Rohrer, Robertson and Phipps (1991) as a core, arises from the inner ovary wall. It is membranous to cartilaginous and forms 2-5 loculi, with two ovules per each locule. The ovules of *Sorbus* species are anatropous, crossinucellate, with two integuments (Corner 1976). Not more than 25-50% ovules per fruit develop into matured seeds (Rohrer et al. l.c.).

The seed coat arises from outer integument. It usually consists of (4-)8-14(-16) cell layers. The cells of outer layer (epiderma) gradually become mucilaginous and disappear. Then there are about 4-6 layers of subepidermal sclerotic cells with thickened walls and a few layers of thin-walled cells with large intercellular spaces. The innermost layer of testa is crushed, similarly the cells of tegmen (originated from outer integument) are more or less wasted. The

seeds have an achlorophyllous embryo, surrounded by three cells thick endosperm (Corner 1976; Gabrielian 1978; Jankun 1993; Watson and Dallwith 2003).

The structure of the seed coat is considered to be of important character in *Sorbus* taxonomy (Gabrielian 1978; Jankun 1993). On the other hand Aldasoro and the others (1998) noted that seed characters (as well as fruit ones) are under the strong influence of selection for species dispersal strategy and this process may reduce their phylogenetic significance.

The main aim of the work was to describe the seeds of Polish species of *Sorbus* and to evaluate the utility of seed morphology in the taxonomy and in identification of examined taxa.

MATERIALS AND METHODS

The seed morphology of five Polish *Sorbus* species (*S. aria*, *S. aucuparia*, *S. chamaemespilus*, *S. intermedia* and *S. torminalis*) has been analysed. In the case of *S. aucuparia*, seeds of subspecies *aucuparia* and *glabrata* have been studied separately. Because of the lack of materials *S. graeca* and *S. carpatica* have not been investigated.

TABLE 2. Seed characters of the investigated species of *Sorbus*.

Feature/species	<i>S. aria</i>	<i>S. aucuparia</i> subsp. <i>aucuparia</i>	<i>S. aucuparia</i> subsp. <i>glabrata</i>	<i>S. chamaemespilus</i>	<i>S. intermedia</i>	<i>S. torminalis</i>
Length (mm)	4.82 (3.80-5.80)	3.88 (3.00-5.00)	3.91 (2.90-4.70)	6.07 (4.40-6.80)	5.69 (4.10-7.10)	6.22 (3.20-8.10)
Width (mm)	2.31 (1.60-3.10)	1.80 (1.20-2.40)	1.76 (1.50-2.30)	3.25 (2.40-3.90)	2.52 (1.80-3.40)	3.24 (1.70-5.70)
Thickness (mm)	1.75 (1.00-2.50)	1.07 (0.70-2.10)	1.05 (0.80-1.40)	2.28 (1.30-3.30)	2.09 (1.20-3.20)	2.31 (1.10-4.20)
Length to width ratio	2.14 (1.57-2.72)	2.18 (1.43-3.46)	2.18 (1.43-3.46)	1.88 (1.37-2.43)	2.28 (1.70-3.35)	1.96 (1.02-3.20)
Outline	Oblanceolate ($\pm 38\%$ analysed seeds) or obovate ($\pm 26\%$), less frequently oblong ($\pm 20\%$) or elliptical ($\pm 16\%$)	Elliptical ($\pm 85\%$ analysed seeds), sometimes oblong ($\pm 10\%$) or obovate ($\pm 5\%$), only sporadically roundish or oblanceolate	Elliptical ($\pm 88\%$ analysed seeds), oblong ($\pm 6\%$), obovate ($\pm 4\%$) or oblanceolate ($\pm 2\%$)	Most often obovate ($\pm 92\%$ observed seeds), sporadically wide obovate ($\pm 8\%$)	Mainly elliptical ($\pm 60\%$ analysed seeds), less frequently obovate ($\pm 19\%$), oblong ($\pm 12\%$) or oblanceolate ($\pm 9\%$)	Obovate ($\pm 77\%$ analysed seeds), sometimes elliptical ($\pm 12\%$) or oblanceolate ($\pm 6\%$), rarely wide obovate ($\pm 4\%$) and only sporadically oblong or roundish
Apex	Roundish, often with the central, shallow cavity	Obtuse, rarely slightly subacute; often with poorly marked shallow, elliptical cavity, sometimes situated \pm asymmetrically	The same, as in <i>S. aucuparia</i> subsp. <i>aucuparia</i>	Obtuse, sometimes roundish, with poorly marked, \pm elliptical, shallow cavity in the middle	Subacute to obtuse; with elliptical cavity, situated \pm asymmetrically, rarely in the middle	Roundish to obtuse, with poorly marked, central cavity
Base	Most often beak-like, sometimes roundish	Slightly subacute or beak-like, only sporadically roundish; asymmetric due to laterally placed hilum	Slightly subacute or beak-like, sporadically roundish; asymmetric as in <i>S. aucuparia</i> subsp. <i>aucuparia</i>	Obtuse to roundish	Mainly beak-like, with laterally placed hilum	Acute or subacute, rarely roundish
Hilum	Elliptical in outline, laterally placed	\pm well visible, having a form of elliptical cavity	Well visible, having a form of elliptical cavity	Circular in outline, often laterally placed	\pm well visible, elliptical, most often laterally placed	Elliptical, most often laterally placed, only sometimes well marked

TABLE 1.

Outline	Length to width ratio	Position of the widest part of seed
Roundish	<1.5	In the middle of its length
Wide obovate	<1.5	In the upper half of its length
Elliptical	1.5-2.5	In the middle of its length
Obovate	1.5-2.5	In the upper half of its length
Ovate	1.5-2.5	In the lower half of its length
Oblong	>2.5	In the middle of its length
Oblanceolate	>2.5	In the upper half of its length

The samples of fruits with mature seeds were mainly collected from natural localities and sporadically from cultivated trees. The number of investigated samples depended on the material accessibility and comprised one sample for *S. chamaemespilus*, four for *S. aria*, four for *S. intermedia*, 12 for *S. torminalis* and 27 for *S. aucuparia* (22 for type subspecies and five for subsp. *glabrata*).

The total of 1980 seeds from five species were characterised in respect to four quantitative features: length of seed (mm), its max. width (mm), thickness (mm), ratio of seed length to seed width and one qualitative character – position of the widest part of seed in relation to seed axis.

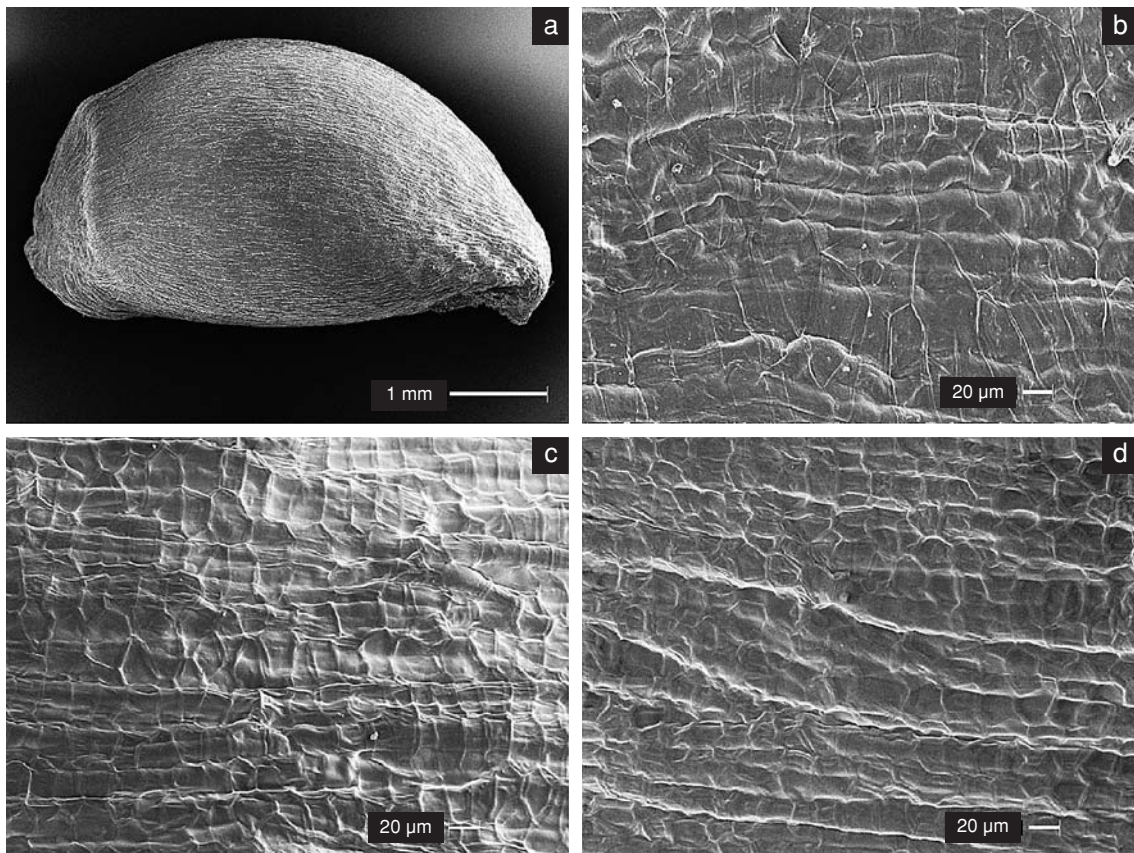


Fig. 1. (SEM) – *Sorbus aria* (L.) Crantz: a – the seed of elliptical outline, the apex roundish, the base beak-like ($\times 35$); b-d – the close up of seed surface; sclerenchymatic fibres covered by gentle net of isodiametric cells of outer layer of testa, intermediate type of microsculpture ($\times 550$).

Morphological descriptions of seeds were based on the measurements and observations with the stereoscope microscope type SZ-PT Olympus and scanning microscope type LEO 435 VP.

The outline of seed was determined according to the length to width ratio and the position of the widest part of the seed in relation to hilum, as given in Table 1.

RESULTS

Both, size and outline of seeds of *Sorbus* species appeared to be very variable, even in individual samples of the same species. Though the seeds of investigated species differed in average dimensions but their ranges of variability overlapped (Table 2).

The largest seeds were observed in *S. torminalis*. Somewhat smaller seeds were in *S. chamaespilus* and *S. intermedia*, than in *S. aria*. The smallest ones were noted in *S. aucuparia* and at the same time the seeds of the subspecies *glabrata* were on average a little longer than the seeds of type subspecies *aucuparia*, which were somewhat wider and thicker. In general there was no significant difference in seed size between investigated subspecies of *S. aucuparia*. The most slender seeds (basing on length/width ratio) occurred in *S. intermedia* samples and the thickest ones in *S. chamaespilus* (Table 2).

The seeds of elliptical or obovate outline prevail in investigated species. Elliptical seeds were most often observed in *S. aucuparia* (in both subspecies) and in *S. intermedia*

and obovate in *S. chamaespilus* and *S. torminalis*. *S. aria* had the most differentiate seeds in respect of outline. They were as well oblanceolate as obovate, oblong and elliptical and percentage of seeds of particular outline ranged from 16 to 38. Additionally seeds of *S. aria*, *S. aucuparia* and *S. intermedia* were asymmetric as a result of unequally formed, lateral margins, with one side convex and the other one \pm straight. Asymmetric forms of seeds were rarely observed in *S. chamaespilus* and *S. torminalis* (Table 2).

Usually the seed apex of all investigated species were roundish, obtuse, often with shallow, elliptical cavity. Sometimes *S. intermedia* and *S. aucuparia* had seeds with slightly subacute apex. The base of *Sorbus* seeds was also slightly subacute or roundish and obtuse; in *S. aucuparia*, *S. aria* and *S. intermedia* often beak-like, asymmetric due to laterally placed trace of funicular attachment. Hilum was usually well visible, only poorly marked in *S. torminalis*; having a form of shallow, elliptical or circular cavity. Instead, a micropyle was not observed in any investigated species.

The colour of seed-coat of *Sorbus* species ranged from light brown tinge (usually in *S. aucuparia* and *S. aria*) to dark-brown (the other species). In all cases the surface of seed-coat, seen with the naked eye, seemed to be smooth and mat.

The most characteristic feature of seeds of investigated species of *Sorbus* appeared to be a microsculpture of seed-coat. Basing on SEM observations four types of seed microsculpture were distinguished:

1) With thin-walled reticulum. The cells of testa are isodiametric, pentagonal or hexagonal, about 20-30 μm in dia-

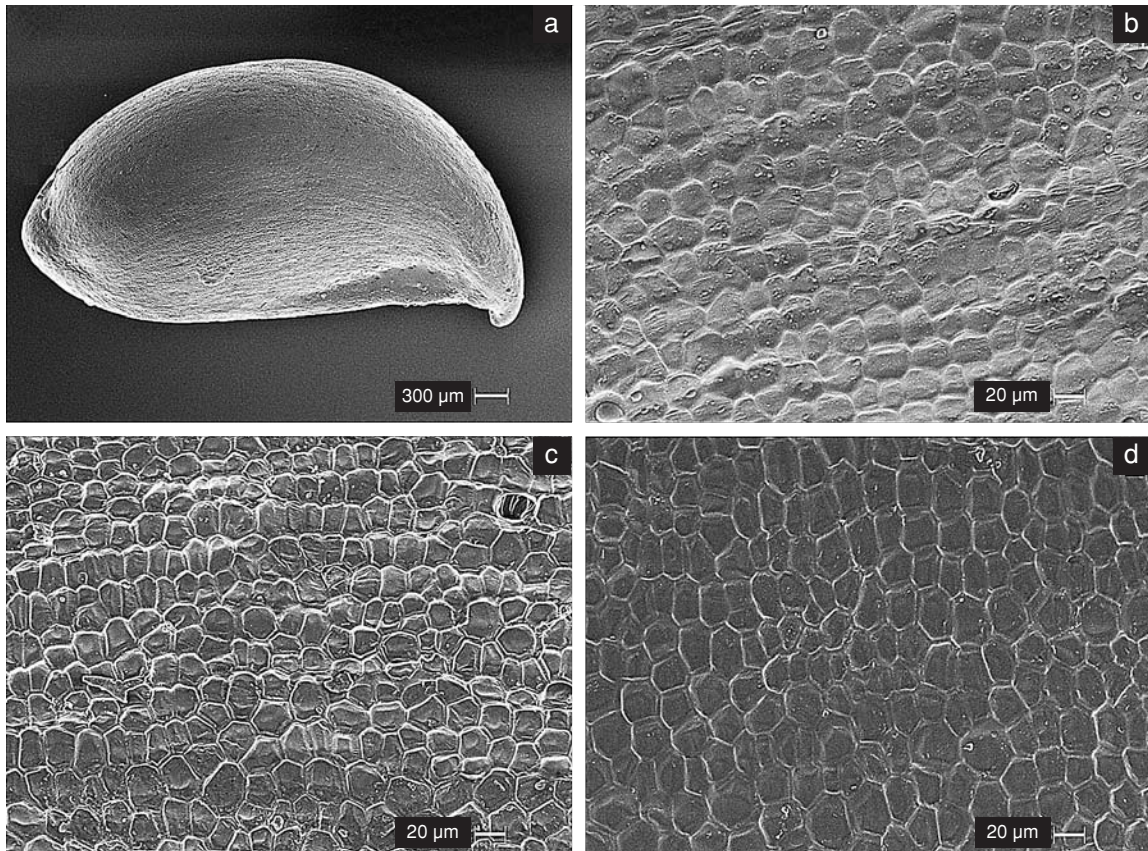


Fig. 2. (SEM) – *Sorbus aucuparia* subsp. *aucuparia*: a – the seed of elliptical outline, the apex slightly subacute, the base beak-like ($\times 40$); b-d – the close up of seed surface; thin-walled reticulate microsculpture visible ($\times 550$).

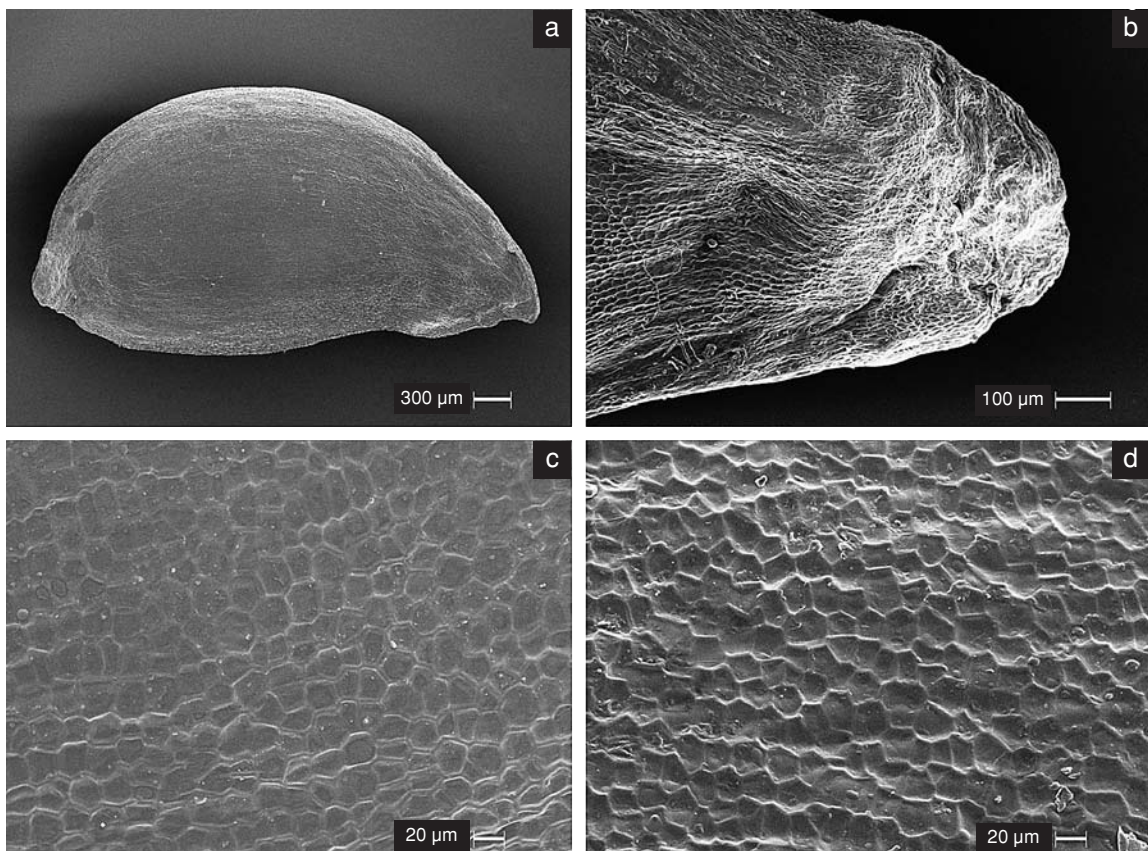


Fig. 3. (SEM) – *Sorbus aucuparia* subsp. *glabrata* (Wimm. & Grab.) Cajander: a – the seed of obovate outline, the apex roundish, the base beak-like ($\times 45$); b – the close up of the base zone of the seed with hilum ($\times 197$); c-d – the close up of seed surface with thin-walled reticulate microsculpture ($\times 550$).

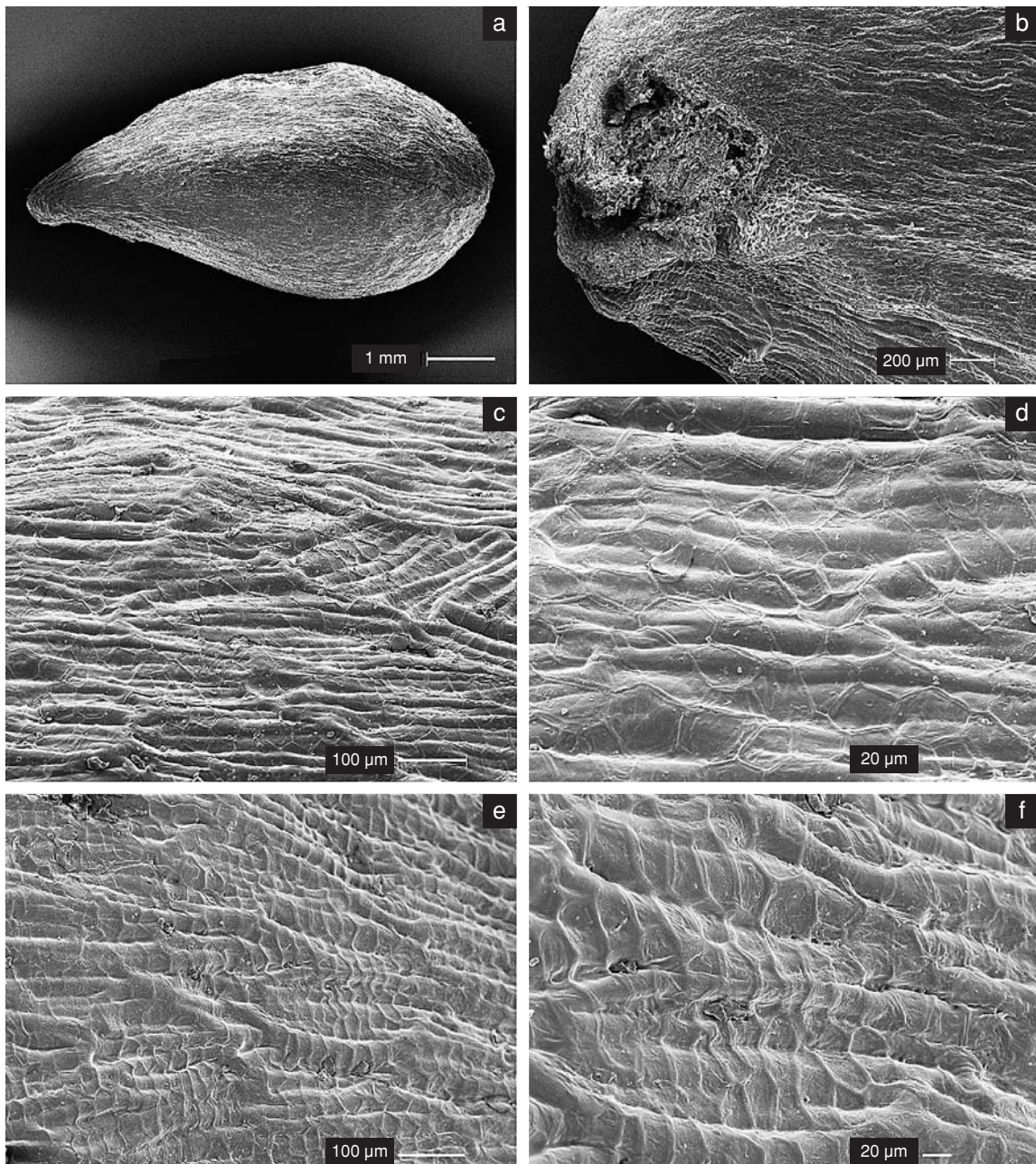


Fig. 4. (SEM) – *Sorbus chamaemespilus* (L.) Crantz: a – the seed of obovate outline, the apex and base roundish ($\times 27$); b – the close up of the apex zone of the seed with micropyle ($\times 86$); c-f – the close up of seed surface with very distinct, ribbed-reticulate microsculpture; sclerenchymatic fibers covered by gentle net of izodiametric cells of outer layer of testa (c – $\times 305$, d – $\times 695$, e – $\times 300$, f – $\times 651$).

meter. They are arranged in regular rows, orientated longitudinally to seed axis, looking like a honeycomb. Their outer walls are somewhat concave or almost flat, rarely slightly convex. The lateral walls are weakly thickened, though well visible, forming more or less protruding margins over the surface of testa.

This type of seeds was characteristic for *S. aucuparia* – both for type subspecies and subsp. *glabrata* (Figs 2, 3).

2) With thick-walled reticulum. The cells are izodiametric, pentagonal or hexagonal, about 20-30 μm in diameter, their outer walls are concave and lateral ones are distinctly thick.

This type of seeds was characteristic for *S. torminalis* (Fig. 6).

3) With ribbed-reticulum. Microsculpture is formed by two-cell layers of testa. The outer layer consists of izodia-

metric cells, pentagonal or hexagonal in outline, about 30-50 μm in diameter and with unequally thickened walls. All walls of these cells are relatively thin, but the lateral ones are more clearly marked. This layer of seed-coat looks like a very gentle net, covering the underneath layer of testa, composed of sclerenchymatic fibres, having acute, overlapping ends. They make distinct ribs, \pm longitudinally elongated to seed axis. There are some narrow depressions among the fibres.

This type of seeds was characteristic for *S. chamaemespilus* (Fig. 4).

4) With intermediate microsculpture. Microsculpture is formed by two-cell layers of testa. The outer layer of seed-coat forms a very gentle net, consisting of izodiametric cells, pentagonal or hexagonal in outline, about 20-30

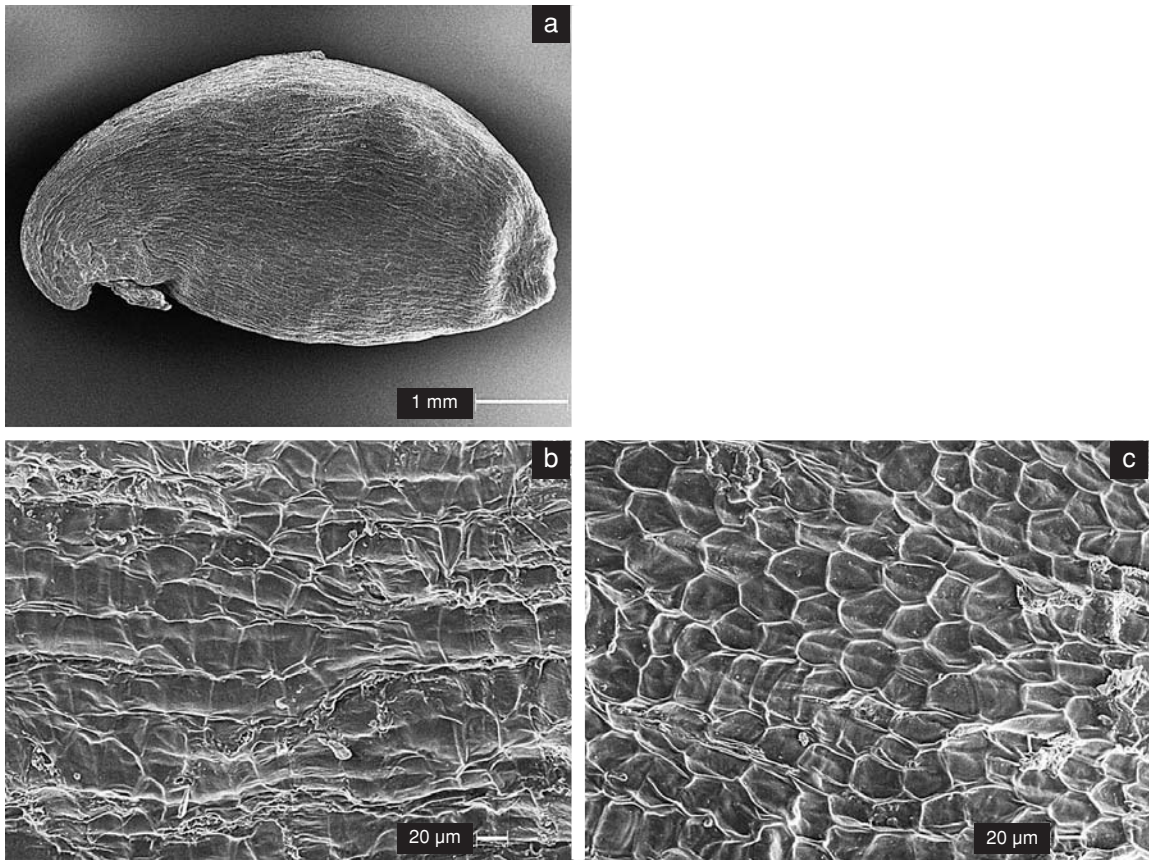


Fig. 5. (SEM) – *Sorbus intermedia* (Ehrh.) Pers.: a – the seed of elliptical outline, the apex obtuse, the base beak-like ($\times 33$); b – (SEM) – the close up of seed surface; intermediate type of microsculpture ($\times 550$); c – the close up of net of izodiametric cells of outer layer of testa ($\times 550$).

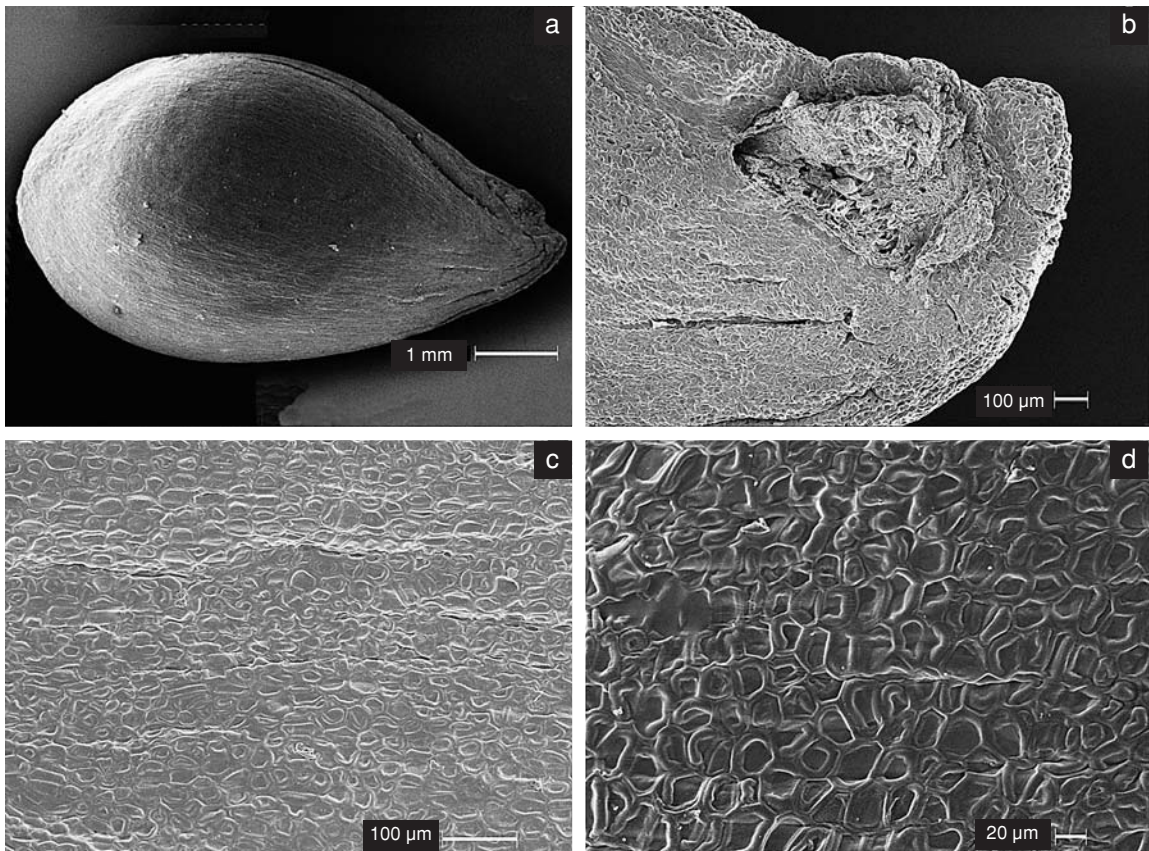


Fig. 6. (SEM) – *Sorbus torminalis* (L.) Crantz: a – the seed of obovate outline, the apex roundish, the base subacute ($\times 29$); b – the base zone of the seed with hilum ($\times 120$); c-d – the thick-walled reticulate type of microsculpture of cellulose pectin layer of izodiametric cells (c – $\times 300$) and its close up (d – $\times 550$).

µm in diameter, sometimes a little elongated perpendicularly to seed axis. Outer walls of these cells are very thin and lateral ones are gently thickened. This network covers the underneath layer of sclerenchymatic fibres, forming a system of cylindrical ribs, orientated \pm longitudinally to seed axis. Their arrangement is similar to the previous type of seed microsculpture, but the ribs are rather obscure as well as the depressions among them are weakly marked and not numerous.

This type of seeds was characteristic for *S. aria* and *S. intermedia* (Figs 1, 5).

The seeds of both species were very similar. However certain differences were observed. Seeds of *S. aria* were often oblanceolate, with ribs more or less developed and scattered depressions. On the other hand seeds of *S. intermedia* were often elliptical with obscure ribs and depressions were rather invisible.

Key to the Polish species of *Sorbus* based on morphological characters of seeds

1. Seeds small (3-)3.5-4.5(-5) mm long, (1.2-)1.5-2.0 (-2.4) mm wide, (0.7-)0.9-1.2(-2.1) thick, having thin-walled reticulum on the surface *S. aucuparia*
1*. Seeds larger, having thick-walled reticulum or ribbed-reticulum 2.
2. Seeds usually more than (3.2-)6(-8.1) mm long, (1.7-)3(-5.7) mm wide and (1.1-)2(-4.2) mm thick, microsculpture as thick-walled reticulum *S. torminalis*
2*. Seeds usually smaller, if not as above, then microsculpture as ribbed-reticulum 3.
3. Most of seeds obovate in outline, ribs and depressions among them distinctly marked *S. chamaemespilus*
3*. Seeds variable in outline, but rarely obovate, ribs as well as depressions among them rather poorly marked, sometimes depressions absent *S. aria*, *S. intermedia*

DISCUSSION

Systematic research on the many *Sorbus* species with the help of the morphological and anatomical analyses of flowers, pollen grains, fruits, buds and leaves as well as the anatomical structure of wood were carried out by different authors (Kovanda 1961; Gabrielian 1978; Rohrer et al. 1991; Aldasoro et al. 1998). Considering the importance of seed characters in *Sorbus* taxonomy, only the anatomic structure of the seed coat has been underlined till now (Gabrielian l.c.; Jankun 1993). This work shows that seed morphology, especially its microsculpture, may also be an important character in distinguishing species within genus *Sorbus*. The microsculpture characters together with the size and shape of seeds are good enough diagnostic features to separate the investigated species. Distinguishing seeds of *S. intermedia* and *S. aria*, may, however, cause some difficulties. Morphological similarity of seeds of those taxa are probably related to their close taxonomic position. According to McAllister (Jankun l.c.) *S. intermedia* as tetra-

ploid hybrid, has chromosome complex, consisting of two genomes of *S. aria* s.l., one of *S. aucuparia* and one of *S. torminalis*.

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APPENDIX

Specimens studied:

S. aria: WIELKOPOLSKIE PROV. – Poznań, 24.10.2002, leg. L. Bednorz; MAŁOPOLSKIE PROV. – Pieniny Mts. (Kierowe Skałki), 15.09.2002, leg. G. Vončina; – Tatry Mts. (Mountain Botanical Garden – GOB Zakopane), 02.10.2002, leg. A. Delimat; – Tatry Mts. (Strażyska Valley), 17.09.2002, leg. L. Bednorz.

S. aucuparia subsp. *aucuparia*: ZACHODNIOPOMORSKIE PROV. – Podczele, 08.2003, I. Maciejewska-Rutkowska; – Resko forest inspectorate, 24.08.00, leg. Paweł Rutkowski; – Mieszkowice, 23.08.1998, leg. Ł. Nosal; POMORSKIE PROV. – Słowiński National Park (Mierzeja Łebska), 18.08.2002, leg. L. Bednorz; KUJAWSKO-POMORSKIE PROV. – Bory Tucholskie National Park (Lubiana), 14.09.1997, leg. I. Maciejewska; – Bory Tucholskie National Park (Charzykowy), 17.09.1997, leg. I. Maciejewska; – Włocławek forest inspectorate, 07.1997, leg. M. Jatczak; WIELKOPOLSKIE PROV. – Wielkopolski National Park, 30.10.1997, leg. I. Maciejewska, T. Maliński; – Borówiec, 23.09.1998, leg. I. Maciejewska, A. Zientarska; – Jarocin forest inspectorate 08.1997, leg. A. Andrzejewska, – Siemianice Experimental Forest (division Laski, 40a), 20.10.1998, leg. P. Rutkowski, – Wałcz, 09.1997, leg. P. Terlecki; LUBELSKIE PROV. – Stefankowice village, 01.10.1999, leg. S. Wejnar; – Kazimierz Dolny, 02.08.1999, leg. D. Wrońska-Pilarek; – DOLNOŚLĄSKIE PROV. – Bukowina Sycowska, 19.08.1998, leg. P. Rutkowski; Wołów forest inspectorate (Głębowice division), 10.1999. ŚLĄSKIE PROV. – Olsztyn near Częstochowa, 18.08.1999, leg. L. Ważyńska; – MAŁOPOLSKIE PROV. – Pieniny Mts. (Czertezik), 22.09.1997, leg. I. Maciejewska, A. Zientarska; – Małe Pieniny Mts., 22.09.1997, leg. I. Maciejewska, A. Zientarska; – Ojców Na-

tional Park (near Jerzmanowice), 25.09.1997, leg. I. Maciejewska, A. Zientarska; – Myczenice Małopolskie, 19.09.2002, leg. L. Bednorz; – Gorce Mts., 10.1997, leg. J. Lach.

S. aucuparia subsp. *glabrata*: DOLNOŚLĄSKIE PROV. – Karkonosze Mts. (Śnieżne Kotły), 10.10.2001, leg. L. Bednorz; – Karkonosze Mts. (Broniek Czech's glade), 09.1995, leg. W. Danielewicz; – Karkonosze Mts. (over Strzecha Akademicka), 09.1995, leg. W. Danielewicz; MAŁOPOLSKIE PROV. – Tatry Mts. (Sarnia Skała), 17.08.2002, leg. L. Bednorz; – Gorce Mts., 10.1997, leg. J. Lach.

S. chamaemespilus: MAŁOPOLSKIE PROV. – Tatry Mts. (GOB Zakopane), 22.09.2002, leg. A. Delimat.

S. intermedia: ZACHODNIOPOMORSKIE PROV. – Mielno, 10.1997, leg. A. Harat; POMORSKIE PROV. – 'Kępa Redłowska' reserve, 01.10.2002, leg. J. Szramka; – Kolbudy, 01.10.2002, leg. J. Szramka; WIELKOPOLSKIE PROV. – Poznań (Dendrological Garden), 22.10.2001, leg. L. Bednorz.

S. torminalis: ZACHODNIOPOMORSKIE PROV. – 'Bielinek nad Odrą' reserve, 04.09.2002, leg. L. Bednorz; POMORSKIE PROV. – Tuchola, 06.10.2000, leg. L. Bednorz; KUJAWSKO-POMORSKIE PROV. – Rogóźno, 05.10.2000, leg. L. Bednorz; WIELKOPOLSKIE PROV. – Goraj, 28.09.2000, leg. L. Bednorz; – 'Brzęki przy Starej Gajówce' reserve, 24.09.2000, leg. L. Bednorz; – Wielkopolski National Park, 25.09.1999, leg. L. Bednorz; – 'Kawęczyńskie Brzęki' reserve, 30.09.2000, leg. L. Bednorz; – Potarzyca, 27.09.2000, leg. L. Bednorz; – Piaski, 27.09.2000, leg. L. Bednorz; DOLNOŚLĄSKIE PROV. – Jawor, 02.10.2000, leg. L. Bednorz; OPOLSKIE PROV. – 'Kamień Śląski' reserve, 19.09.2002, leg. L. Bednorz; MAŁOPOLSKIE PROV. – 'Białowodzka Góra' reserve, Góra' reserve, 16.09.2002, leg. L. Bednorz.