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Taxonomic importance of seed morphology of *Veronica* L. subsect. *Agrestes* Benth. (Plantaginaceae)

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Abstract. The study was conducted on five species of *Veronica* L. subsection *Agrestes* occurring in Central Europe: *Veronica agrestis* L., *V. polita* Fr., *V. opaca* Fr., *V. persica* Poir. and *V. filiformis* Sm. These species are very similar morphologically and are often misidentified. Last thorough studies of morphology and micromorphology of their seeds were conducted in 1960s. Based on 48 herbarium specimens, we examined SEM images of 422 seeds. We found that 22 of the 30 studied morphological traits differentiated five species at statistical level and *V. agrestis* and *V. persica* differed with the greatest number of features from other species. Our measurements of seed size were not fully congruent with previous studies, suggesting dependence of these features on external conditions. Seeds were usually cochlidiospermous and ovoid with a reticulate-verrucate seed coat, but these features were, at the same time, quite variable. The results of discriminant function confirmed that some characters of the seed size, shape and coat sculpture could be taxonomically useful in distinguishing species of *Veronica* subsect. *Agrestes*. In addition, we believe that similarity of the examined seeds may confirm unity of this group and the need of identifying a subsection.

Key words: *Veronica* taxonomy, SEM, micromorphology, seed coat, seed size, seed shape, seed sculpture, testa surface

1. Introduction

Veronica L. is the largest genus in the family Plantaginaceae sensu Angiosperm Phylogeny Group (2009) and comprises 450-500 species, including about 62 occurring in Europe (Walters & Webb 1972). In spite of a great deal of taxonomic research that has been done on the genus worldwide, several groups still require detailed investigation of additional characters to distinguish species and clarify their taxonomy. One such group is *Veronica* subsection *Agrestes* Benth.

The subsect. *Agrestes* of subgenus *Pocilla* (Dumort.) M. M. Mart. Ort., Albach & M. A. Fisch. consists of three cosmopolitan species: *V. polita* Fries, *V. persica* Poiret and *V. filiformis* Sm. and two representing the European type of range: *V. agrestis* L., and *V. opaca* Fries. Other species belonging to the subsect. *Agrestes* are restricted to the hyrcanian-caucasian region (Kulpa 1968; Albach *et al.* 2005).

Fruit and seed traits play a significant role in the taxonomy of many species (Stace 1989; Szkudlarz 2009; Davitashvili & Karrer 2010; Szkudlarz & Celka 2016). The morphology and micromorphology of seeds are often utilised for taxonomic purposes also in Plantaginaceae and related families, like Scrophulariaceae or Orobanchaceae (e.g.: Elisens & Tomb 1983; Dong *et al.* 2015; Richardson Ahedor & Elisens 2015).

Studies conducted on some of the species belonging to the subsect. *Agrestes* showed that seeds of this subsection differed from the others of subg. *Pocilla* by their smaller size – the seed length is less than 2 mm; by the specific cochlidiospermous (concave ventrally) type of seed, less frequently flattened (*V. ceratocarpa*); by occurrence of wrinkles on the dorsal surface of the seed; by the shape and size of the raphal ridge and chalazal plate and by reticulate-verrucate coat sculpturing with a polygonal cell and variable sizes, their anticlinal cell walls irregularly thickened forming, a more or less, convex reticulum and by periclinal cell walls with a central or eccentric papilla (Kulpa 1968; Juan *et al.* 1994; Muñoz-Centeno *et al.* 2006; Yilmaz 2013; Hassan & Khalik 2014).

The European species belonging to the subsect. *Agrestes* are considered as morphologically quite similar and, thus, they may be misidentified. Previous analyses of the species within the subsect. *Agrestes* showed several morphological characteristics which were useful in distinguishing some of them: the type (procumbent or decumbent) and thickness of the stem, the ratio of the length to the width of the capsule, the type of coverage by hairs and the ratio of the incision in a capsule to the length of its style, the number of seeds in a capsule, the shape and overlapping of calyx-segments (Tacik & Trzcińska-Tacik 1963; Walters & Webb 1972; Fischer 1981). Fruit morphology and anatomy (Juan *et al.* 1997; Saeidi Mehrvarz *et al.* 2001), as well as chemical characteristics (e.g.: Albach *et al.* 2005; Jensen *et al.* 2005; Saeidi Mehrvarz *et al.* 2008), pollen size (Sánchez Agudo *et al.* 2009), chromosome number (Saeidi Mehrvarz & Kharabian 2005), distribution of glycosides (Tomas-Barberan *et al.* 1988) and molecular data (e.g.: Albach & Greilhuber 2004; Albach *et al.* 2004) also provided important characters within the subsection.

However, none of these researchers examined and compared all the European species of the subsect. *Agrestes*, using SEM. Last thorough morphological and micromorphological examinations of seeds of all European species of the subsect. *Agrestes* were conducted in 1960s by Kulpa (1968). This is why we decided to study this group using methods that were not accessible at that time. We conducted our study on examples of Polish flora assuming they would make good representatives of European plants, due to central European location of Poland.

Our first aim was to characterize the seed morphology and seed coat sculpture of the species of subsect. *Agrestes*. Basing on the results of this examination, we wanted to determine if seed traits can help in identifying the subsection as the unity, or if they differ the species enough to have a taxonomic value.

2. Material and methods

The research was based on the study of herbarium specimens. Seeds of the following *Veronica* species were examined: *V. persica* (130 seeds), *V. agrestis* (92), *V. polita* (90), and *V. filiformis* (14) (Appendix 1). The latter species, as an invasive kenophyte, has been spreading through Europe since 1930s (Müller & Sukopp 1993; Pielech *et al.* 2012; Scalone & Albach 2012; Tokarska-Guzik *et al.* 2012). It is rare in Poland and, at the moment, can be found only in the southern part of the country (Żukowski 1959; Zając, Zając 2001). *V. filiformis* is regarded as propagating mainly asexually and rarely producing seeds, thus, the presence of capsules in some specimens in KRA herbarium should be treated as quite exceptional. This is why we decided to use the small number of seeds of this species that were available in our investigation.

Observations and measurements of seeds were carried out on images from an S-3000N Hitachi Co SEM at the Institute of Plant Protection in Poznań. Dry seeds were attached to table mounts using double sided adhesive carbon discs. The samples were then sputter coated with gold and photographed using SEM. Seeds of *Veronica* have a ventro-dorsal construction, with one axis of symmetry. Therefore, observations were made on three sides: the ventral (2-6 seeds per individual), dorsal (1-4 seeds per individual) and lateral (1-3 seeds per individual). In total, 184 seeds were photographed on ventral, 125 on dorsal and 113 on lateral surfaces. We used magnification from 60x to 150x, depending on the seed size. The coat sculpture was investigated using two magnifications: 500x and 1000x.

In order to statistically characterize seeds we selected 29 quantitative traits, some following already published research (e.g.: Tacik & Trzcińska-Tacik 1963; Kulpa 1968; Walters & Webb 1972). Thirteen characters were measured on the photographs using digiShape software (Moraczewski 2005): the perimeter (*P*), the length (*L*), the width measured at ½, ¼ and ¾ of the length (*W½*, *W¼*, *W¾*), the thickness at ½, ¼ and ¾ (*T½*, *T¼, T¾*), angles of micropylar and chalazal top (*AMi*, *ACh*), the length of chalaza (*LCh*) and the length and the width of chalaza plate (*LPl*, *WPl*). Two features were counted directly from the images: the number of wrinkles on the dorsal side (*WrN*) and the average number of angles in the centrally located 5 cells of the seed coat (*ACN*). In addition, fourteen traits were obtained from the calculations: *P/L*, *P/W½*, *L/W½*, *L/T½ L/W¼*, *L/W¾*, *L/T¼*, *L/T¾*, *W¼/W¾*, *T¼/T¾*, *LCh/L*, *LCh/LPl*, *LPl/WPl* and *L/WrN*.

To describe the seeds, we also used other features. The degree of folding of the edge (*FE*) at the ventral side of seeds was assessed with a three-level scale: 1 – not folded, 2 – medium folded, 3 – strongly folded. Following the nomenclature of other authors (e.g.: Yamazaki 1957; Kulpa 1968), we found all seeds in subsection *Agrestes* to be cochlidiospermous. We considered seeds to be deep-cochlidiospermous, when $L/T½ < 2.5$, whereas $L/T½ \geq 2.5$ were shallow-cochlidiospermous. We also estimated the seed shape in outline from ventral and dorsal surfaces (e.g.: Martinez-Ortega & Rico 2001; Stearn 2004). We defined three main types of shapes: obovoid, elliptical, suborbicular and two transitional types: broadly obovoid and broadly elliptical. We defined elliptical seed shape when $0.9 < W_{1/4}/W_{3/4}$

< 1.1 and obovoid – when $0.9 > W_{1/4}/W_{3/4} > 1.1$ value. Seed shapes were considered as broadly obovoid and broadly elliptical when the ratios of $W_{1/2}/L$ and $W_{3/4}/L \geq 0.67$. Finally, we described seed shapes as suborbicular when $W_{1/4}/L$, $W_{1/2}/L$ and $W_{3/4}/L > 0.80$.

On the basis of images with the largest magnification (1000x), we described the sculpture of the coat surface of seeds and micromorphology of testa ornamentation for anticlinal and periclinal cell walls (e.g.: Muñoz-Centeno *et al.* 2006, 2007; Hassan & Khalik 2014).

Because of violations of ANOVA assumptions by some of the features, we decided to use the Kruskal–Wallis one-way analysis of variance by ranks (Kruskal & Wallis 1952). This nonparametric analysis was performed to determine which of the 29 studied traits were significantly different between pairs of species. The post-hoc Dunn's test was carried out to establish differences between pairs of species for each characteristic (Dunn 1964).

Interactions between the 29 features were checked with the Pearson's linear correlation coefficient to assess potentially redundant characteristics (Pearson 1900). The statistical significance of *FE* (in a three-level scale) was evaluated using the chi-square test (Pearson 1900).

Relationships among 48 herbarium specimens and among five species were tested on the standardized data using the discriminant function (McLachlan 2004) and the cluster analysis (Cattell 1943; Sokal & Rohlf 2003). Both analyses were made on average values of traits, which had fulfilled all the assumptions and had a statistically significant influence on the discrimination between groups. The dispersion of specimens between the first two discriminant variables was shown on a scatterplot. The taxonomic distance between species was illustrated by the UPGMA dendrogram constructed on the Euclidean distances.

All calculations were made using STATISTICA 10.0 for Windows (StatSoft, Inc. 2013).

3. Results

The comparison between species based on seed morphological characteristics revealed that *V. agrestis* and *V. persica* had the largest seeds with the average length (*L*) of 1.76 and 1.65 mm, respectively, while seeds of *V. filiformis* were the smallest and only 1.24 mm long. *V. persica* and *V. agrestis* also had the longest chalaza (*LCh*) equalling 1.12 and 1.11 mm on average and the longest chalazal plate (*LPl*) with the mean of 0.51 mm for both species. The smallest values were measured for *V. polita* with the mean *LCh* of 0.76 mm and *LPl* of 0.39 mm. The greatest number of wrinkles (*WrN*) were found for *V. polita* and *V. agrestis* with median equalling 12.0 and 11.5, respectively, and *V. persica* had the smallest *WrN* ranging from 6 to 14 and with the median of 10.0. We found *V. agrestis* as the most different from other species with regard to the measured/counted characteristics (Table 1, Appendix 2).

The mean ratio of the perimeter to the seed length (*P/L*) ranged from 3.28 to 3.63 for all species, and was the greatest for *V. agrestis* and the smallest for *V. persica* and *V. filiformis*. The ratio of the perimeter to the seed width measured at the ½ of the length (*P/W½*) also was the biggest for *V. agrestis* (5.1 in average) and the smallest for *V. filiformis* (4.49). The latter species also had the smallest value of the ratio of the length to the width at ½ (*L/W½*), which was 1.37, while the greatest value was 1.51 and was calculated for *V. persica*. All

Table 1. Morphological characteristics and coat sculpturing of seeds of five *Veronica* species, subsect. *Agrestes*

species	size ranges [mm]			dominating type	dominanting shape	dominanting *FE*	*WrN* ranges [number]	*ACN* ranges [number]	testa surface		
	L	*W½*	*T ½*						general sculpture	anticlinal cell walls	papilla
V. agrestis	1.19-2.10	0.83-1.61	0.46-1.12	deep-cochlidiospermous	obovoid	1	8-15	4.6-6.6	reticulate-verrucate, reticulate	convex	convex collapsed absent
V. filiformis	1.06-1.45	0.72-1.00	0.45-0.60	shallow-cochlidiospermous	broadly obovoid	1	10-13	5.6-6.6	reticulate-verrucate	convex	convex collapsed
V. opaca	0.88-2.22	0.47-1.60	0.37-1.11	deep-cochlidiospermous	elliptical, broadly obovoid	1	9-14	4.4-6.4	reticulate-verrucate, reticulate	convex flat	convex collapsed absent
V. persica	1.29-2.09	0.73-1.53	0.39-1.00	deep-cochlidiospermous	obovoid	1	6-14	5.0-6.4	reticulate-verrucate, reticulate	convex collapsed	convex collapsed absent
V. polita	0.86-1.71	0.58-1.20	0.39-1.20	deep-cochlidiospermous	obovoid	3	8-15	5.0-6.4	reticulate-verrucate, reticulate	convex	convex collapsed absent

Explanations: *L* – the length, *W½* – the width at ½, *T½* – the thickness at ½, *WrN* – the number of wrinkles on the dorsal side, *ACN* – the average number of angles in the centrally located 5 cells of the seed coat, *FE* – the degree of folding of the edge (1 – not folded, 3 – strongly folded) the ratios of the seed length to the width measured at different points were more or less related as were the ratios of the length to the thickness. The most differing among species was *L/T¼*, with averages ranging from 2.49 for *V. opaca* and *V. polita*, to 3.58 for *V. filiformis*. The ratio of the chalaza length to the seed length (*LCh/L*) was another characteristic important for differentiating between species, and the greatest mean value of this trait was again calculated for *V. filiformis* (0.71), while the smallest for *V. polita* (0.57). Similarly, the biggest value of the ratio of the length to the width of the chalazal plate (*LPl/WPl*) was determined for *V. persica* (1.60) and *V. filiformis* (1.55), and the smallest – for *V. polita* (1.33) (Appendix 2).

The results of chi-square tests made for *FE* showed that there were differences in the degree of folding of the seed edge between taxa. The value of the test statistic was $\chi2 = 56.57699$ and the value of probability was $p < 0.000001$. *V. polita* seeds had the most strongly folded edge, the majority of *V. filiformis* and *V. persica* were flat, and the seeds of *V. agrestis* and *V. opaca* were intermediate.

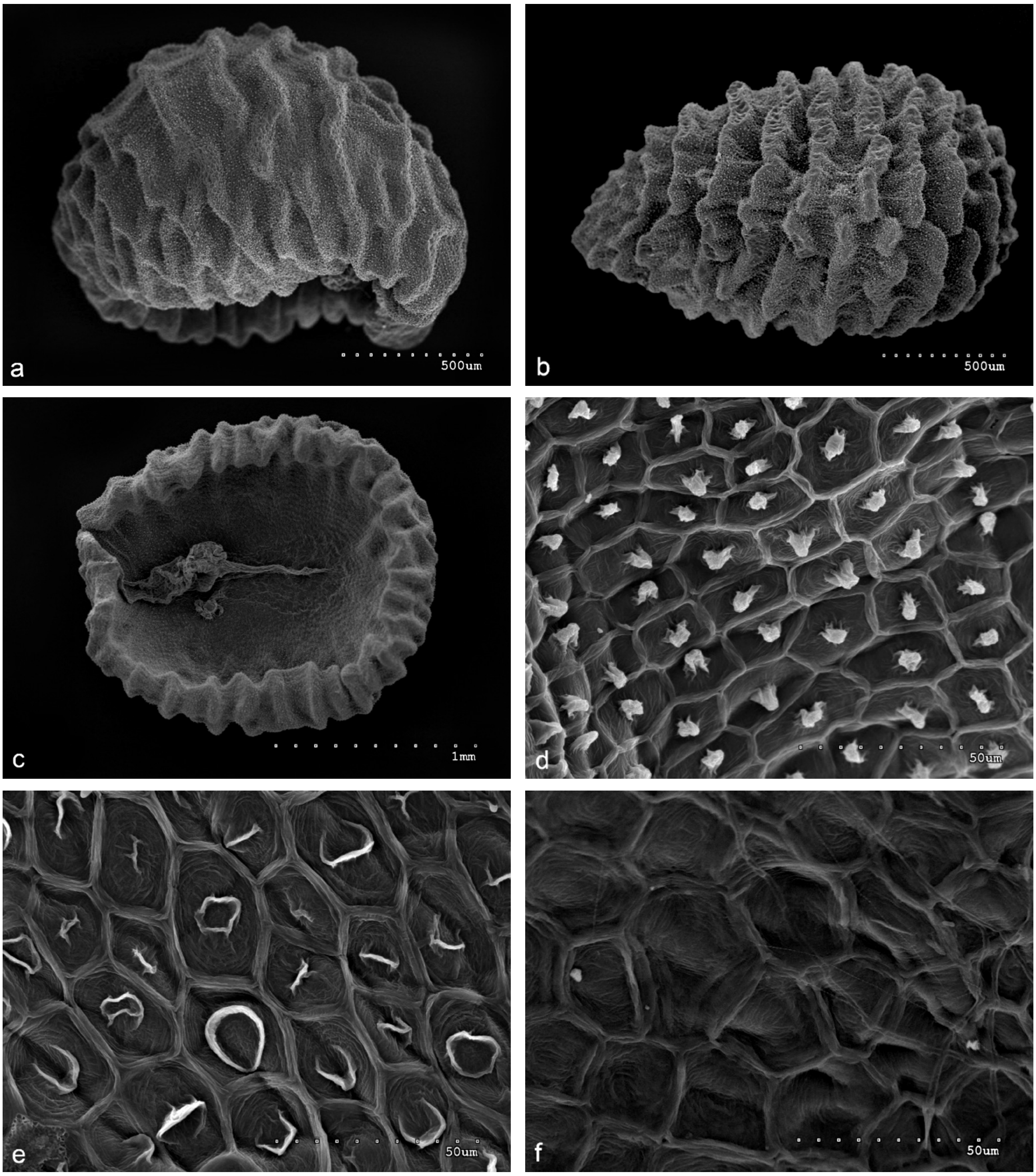

Fig. 1. The morphology of *Veronica agrestis* seeds
Explanations: a – lateral surface of individual no. 24, deep-cochlidiospermous type, b – dorsal surface of individual no. 16, obovoid shape, c – ventral surface of individual no. 23, suborbicular shape, d-f – testa surface, d – reticulate-verrucate of individual no. 21, e – reticulate-verrucate, with papillae partly collapsed of individual no. 22, f – reticulate, with no papilla of individual no. 20

All the compared species of subsect. *Agrestes* had seeds of cochlidiospermous type. Our studies showed that seeds of *V. agrestis*, *V. persica*, *V. opaca* and *V. polita* had the ratio of length to thickness (*L/T½*) varying from 1.98 to 2.32 (on average) and were deep-cochlidiospermous, while seeds of *V. filiformis* had value of *L/T½* equaling 2.55 (on average) and were shallow-cochlidiospermous (Table 1, Figs. 1a, 2a, 3a, 4a, 5a). The differences between species were not statistically significant in distinguishing taxa with regard to these characteristics; however, we found a statistically significant effect of *L/T¼* on the ability to differentiate. *V. filiformis* seeds were distinguishable from the rest with regard to this feature with the value of 3.58, while in other species, this value was smaller, from 2.49 to 2.92 (on average) (Appendix 2).

Shapes of most seeds in subsect. *Agrestes* were obovoid (34.53%) or elliptical (24.43%). Broadly obovoid or broadly elliptical seeds together accounted for 26.38%. The suborbicular seeds were in minority

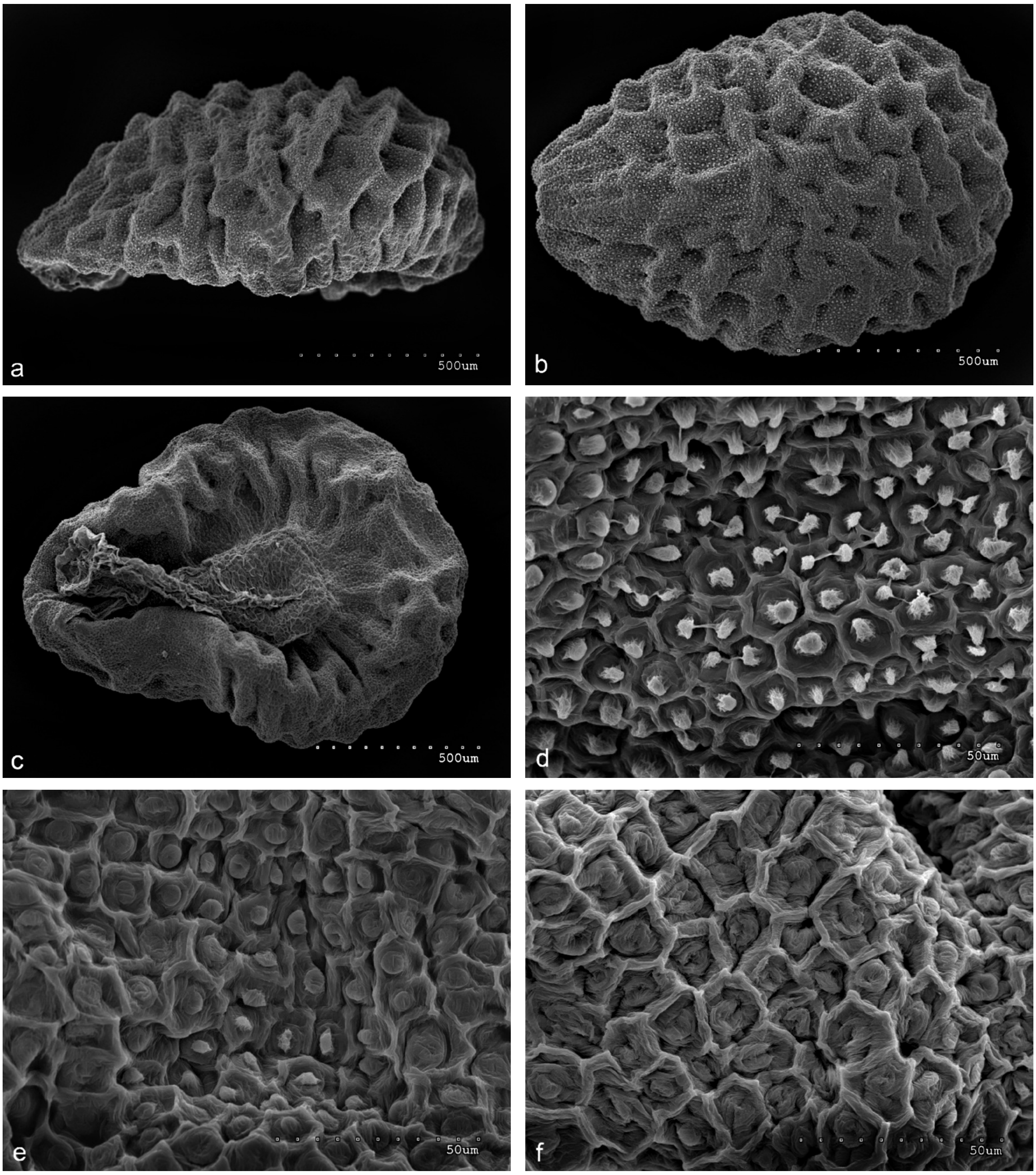

Fig. 2. The morphology of *Veronica filiformis* seeds
Explanations: a – lateral surface of individual no. 48, shallow-cochlidiospermous type, b – dorsal surface of individual no. 48, broadly obovoid shape, c – ventral surface of individual no. 47, obovoid shape, d-f – testa surface, d – reticulate-verrucate, with bigger papillae relative to the cells area of individual no. 48, e – reticulate-verrucate, with papillae in some part of area partly collapsed of individual no. 47, f – reticulate-verrucate, with papillae partly collapsed of individual no. 47

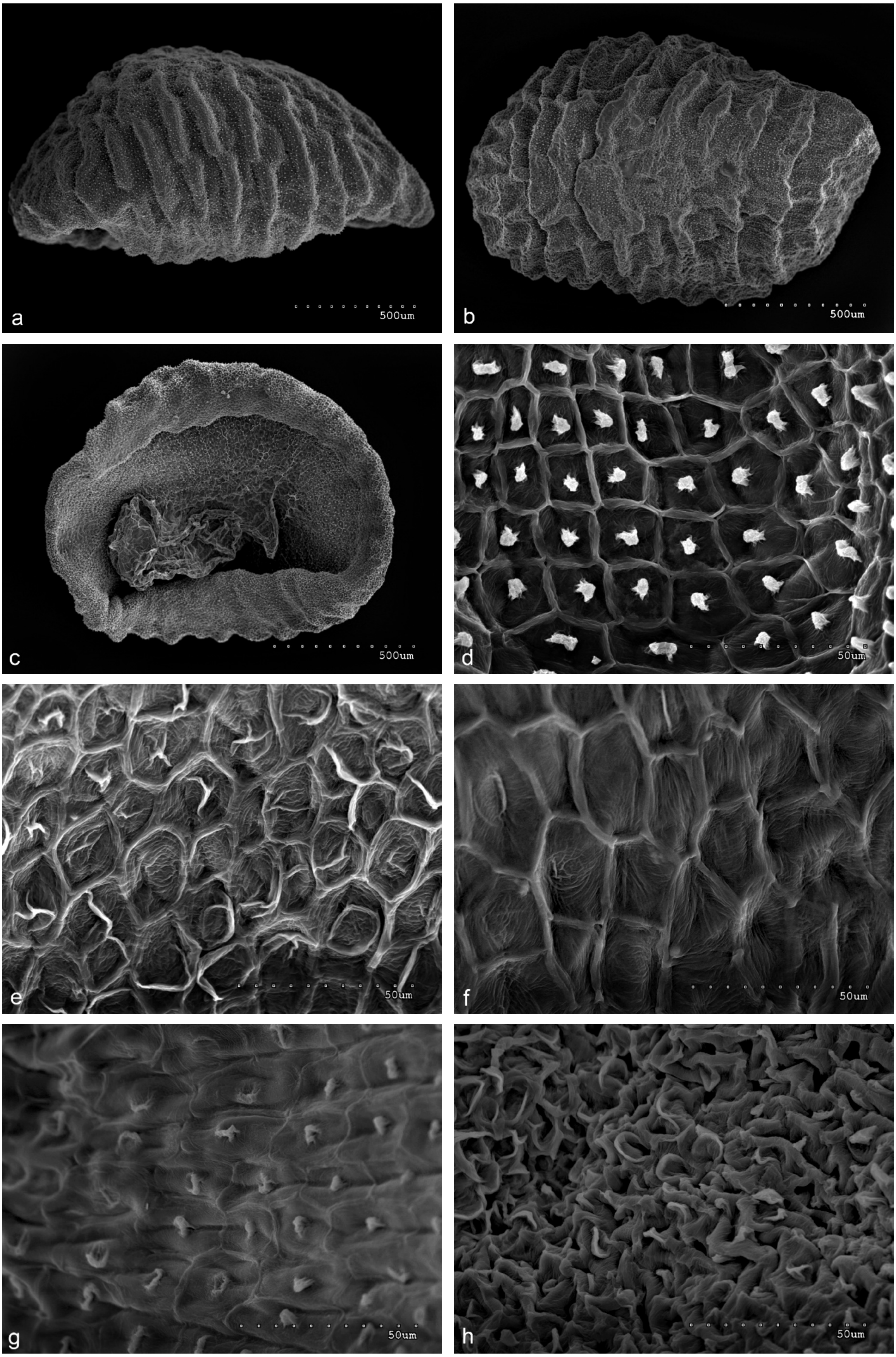

Fig. 3. The morphology of *Veronica opaca* seeds
Explanations: a – lateral surface of individual no. 45, deep-cochlidiospermous type, b – dorsal surface of individual no. 37, elliptical shape, c – ventral surface of individual no. 44, broadly elliptical shape, d-h – testa surface, d – reticulate-verrucate of individual no. 46, e – reticulate with papillae partly collapsed of individual no. 46, f – reticulate, with papillae collapsed or absent of individual no. 36, g – reticulate-verrucate, with cell walls partly flat of individual no. 43, h – reticulate-verrucate, irregular of individual no. 45

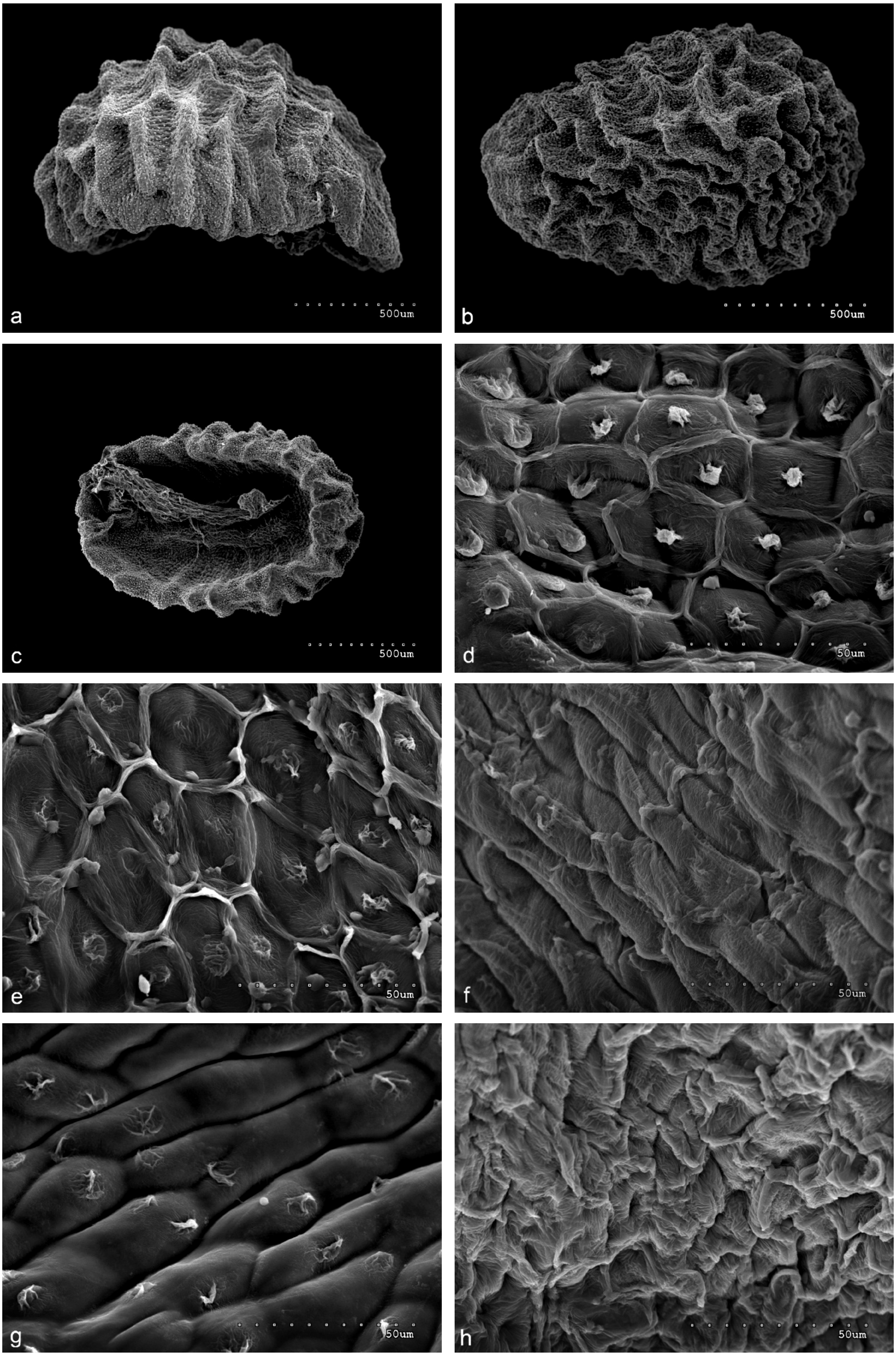

Fig. 4. The morphology of *Veronica persica* seeds

Explanations: a – lateral surface of individual no. 11, deep-cochlidiospermous type, b – dorsal surface of individual no. 14, obovoid shape, c – ventral surface of individual no. 1, elliptical shape, d-h – testa surface, d – reticulate-verrucate of individual no. 4, e – reticulate-verrucate, with papillae partly collapsed of individual no. 7, f – reticulate, with no papilla and cell walls partly collapsed of individual no. 11, g – reticulate-verrucate, with papillae partly collapsed and cell walls collapsed of individual no. 4, h – reticulate-verrucate, irregular of individual no. 12

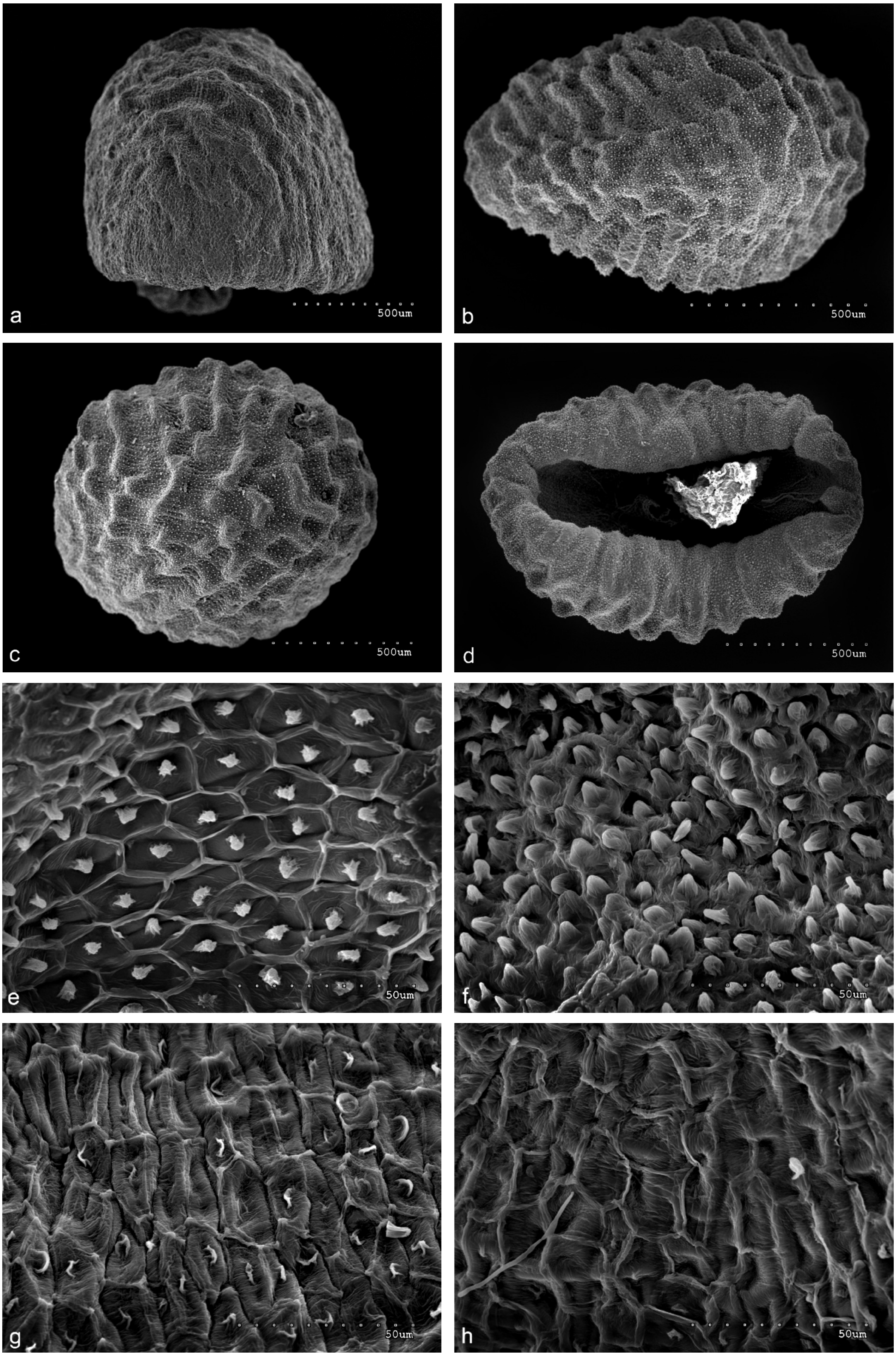

Fig. 5. The morphology of *Veronica polita* seeds

Explanations: a – lateral surface of individual no. 26, deep-cochlidiospermous type, b – dorsal surface of individual no. 34, obovoid shape, c – dorsal surface of individual no. 32, suborbicular shape, d – ventral surface of individual no. 29, elliptical shape, e-h – testa surface, e – reticulate-verrucate of individual no. 33, f – reticulate-verrucate, with bigger papillae relative to the cell area of individual no. 32, g – reticulate-verrucate, with papillae and cell walls partly collapsed of individual no. 34, h – reticulate, with collapsed or no papilla of individual no. 33

Table 2. Statistically significant seed traits in the non-parametric Kruskal-Wallis test and significant results of post-hoc Dunn's tests among the pairs of five *Veronica* species, subsect. *Agrestes*

Character abbreviation	*p*	*V.a.* *V.f*	*V.a.* *V.o*	*V.a.* *V.pe*	*V.f.* *V.o*	*V.a.* *V.po*	*V.f.* *V.pe*	*V.pe.* *V.po*	*V.o.* *V.po*	*V.o.* *V.pe*	*V.f.* *V.po*
P	< 0.0001	**	**	**	**	**	**	**	**		
L	< 0.0001	**	**	**	**	**	**	**	**	**	
W½	< 0.0001	**	**	**	*	**	*	**	**		
T½	0.0001	**	*			**					
W¼	< 0.0001	**	*	**	**	**	**	**	**		
W¾	< 0.0001	**	**	**	*	**	*	**	**		
T¼	0.0009	**			*	*					
T¾	< 0.0001	**	**	**		**					
LCh	< 0.0001		*			**	*	**		**	
LPl	0.0006					**		**			
WrN	< 0.0001			**				**		**	
P/L	< 0.0001	*	**	**		*					
P/W½	0.0009	*	**								
L/W½	0.0008			**						**	
L/W¼	0.0030			*						**	
L/W¾	0.0003			**						**	
L/T¼	0.0034				*					*	
L/T¾	0.0152			*				*			
T¼/T¾	0.0090				*						
LCh/L	< 0.0001				**	*		**		**	**
LPl/WPl	0.0126							*			
L/WrN	< 0.0001					**	*	**		**	

Explanations: *p* – significance of non-parametric Kruskal-Wallis test; statistical significant level of Dunn's test, * – p<0.05, ** – p<0.01; *V.a.* – *Veronica agrestis*, *V.f.* – *V. filiformis*, *V.o.* – *V. opaca*, *V.pe.* – *V. persica*, *V.po.* – *V. polita*; traits of seeds, *P* – perimeter, *L* – length, *W½*, *W¼*, *W¾* – widths, *T½*, *T¼*, *T¾* – thicknesses, *LCh* – length of chalaza, *LPl* – length of chalaza plate, *WPl* – width of chalaza plate, *WrN* – number of wrinkles

(14.66%, in total). Most of *V. agrestis*, *V. persica* and *V. polita* seeds were obovoid. *V. persica* and *V. polita* also showed the second largest share of elliptical seeds, while *V. agrestis* had the second largest share of broadly obovoid seeds. Most of *V. opaca* seeds had the same proportion of elliptical and broadly obovoid shapes, whereas most of *V. filiformis* seeds were broadly obovoid. The largest share of broadly elliptical seeds occurred in *V. opaca*, and the largest share of suborbicular – in *V. agrestis* (Table 1, Figs. 1b, 1c, 2b, 2c, 3b, 3c, 4b, 4c, Figs. 5b-d).

General sculpture of testa of all five species from subsect. *Agrestes* was mostly reticulate-verrucate and regular, sometimes irregular – in *V. opaca* and *V. persica* and sometimes reticulate – in *V. agrestis*, *V. opaca*, *V. persica* and *V. polita*. Anticlinal cell walls of seeds in most cases were convex, but sometimes, they were flat or collapsed. Periclinal cell walls were flat with more or less centrally located papillae, which were more or less convex, sometimes collapsed or absent (Table 1, Figs. 1d-f, Figs. 2d-f, Figs. 3d-h, Figs. 4d-h, Figs. 5e-h).

The number of angles of seed coat cells (*ACN*) was not statistically significant in distinguishing species. However, calculations showed that seeds of *V. filiformis* had relatively more angles than other species, while cells of *V. opaca* had the smallest number of angles (Table 1).

The Kruskal-Wallis test showed that 22 out of the 29 studied traits differentiated species of the *Agrestes* subsection on a statistically significant level (Table 2). Dunn's tests made for pairs of species showed that *V. agrestis* and *V. persica* differed with the greatest number of features from other species, whereas only one characteristics – the ratio of the chalaza length and the seed length (*LCh/L*) – allowed *V. polita* to be distinguished from *V. filiformis*. In general, taxa differed most with regard to traits describing seed size, like the perimeter, length, width at ½, ¼ and ¾ (*P*, *L*, *W½* , *W¼*, *W¾*; Table 2).

Differences in seed morphological structure of *Veronica* subsect. *Agrestes* were reflected in the results of discriminant function. From among eight analyzed characters, two had the greatest influence on species discrimination: the length of seed (*L*) on the first discriminant variable (U_1) and the ratio of perimeter and length (*P/L*) on the second discriminant variable (U_2; Table 3). These four discriminant variables covered

Table 3. Discriminant power testing made on the average values of eight traits that met all the assumptions, for seeds of five *Veronica* species, subsect. *Agrestes*

Trait abbreviation	partial λ	*p*	U_1 72.6%	U_2 12.4%
L	0.27	0.000003	11.53	0.62
P/L	0.29	0.000007	2.31	5.85
T¼/ T¾	0.49	0.001793	0.17	2.39
P/W½	0.66	0.039311	0.00	0.26
AMi	0.39	0.000150	0.48	0.19
T¼	0.56	0.007209	2.80	0.10
W¼/W¾	0.64	0.030232	0.91	0.06
LPl/WPl	0.67	0.045696	0.27	0.71

Explanations: λ – partials Wilks' lambda, *p* – levels of significance, U_1, U_2 – the first two discriminant variables, *L* – the length, *P/L* – the ratio of the perimeter and *L*; *T¼/T¾* – the ratio of thickness at ¼ and ¾; *P/W½* – the ratio of the perimeter and width at ½; *AMi* – the angle of micropylar top, *T¼* – thickness at ¼, *W¼/W¾* – the ratio of width at ¼ and ¾, *LPl/WPl* – the ratio of the length and width of chalaza plate

100% of the total variation. The classification of individuals to species revealed that the ordination was 94.3%, on average. 100% of individuals were classified properly to *V. agrestis*, *V. filiformis* and *V. polita*, 92.3% – to *V. persica* (the rest to *V. opaca*) and 83.3% – to *V. opaca* (the rest to *V. persica*). In the space between U_1 and U_2 (Fig. 6), individuals of *V. filiformis*, *V. agrestis* and *V. polita* species formed three separate agglomerations and the fourth group consisted of mixed specimens of *V. opaca* and *V. persica*. The UPGMA method of agglomeration made on the shortest Euclidean distances among 5 species confirmed the closest similarity of *V. persica* and *V. opaca* and the largest separateness of *V. filiformis* seeds (Fig. 7).

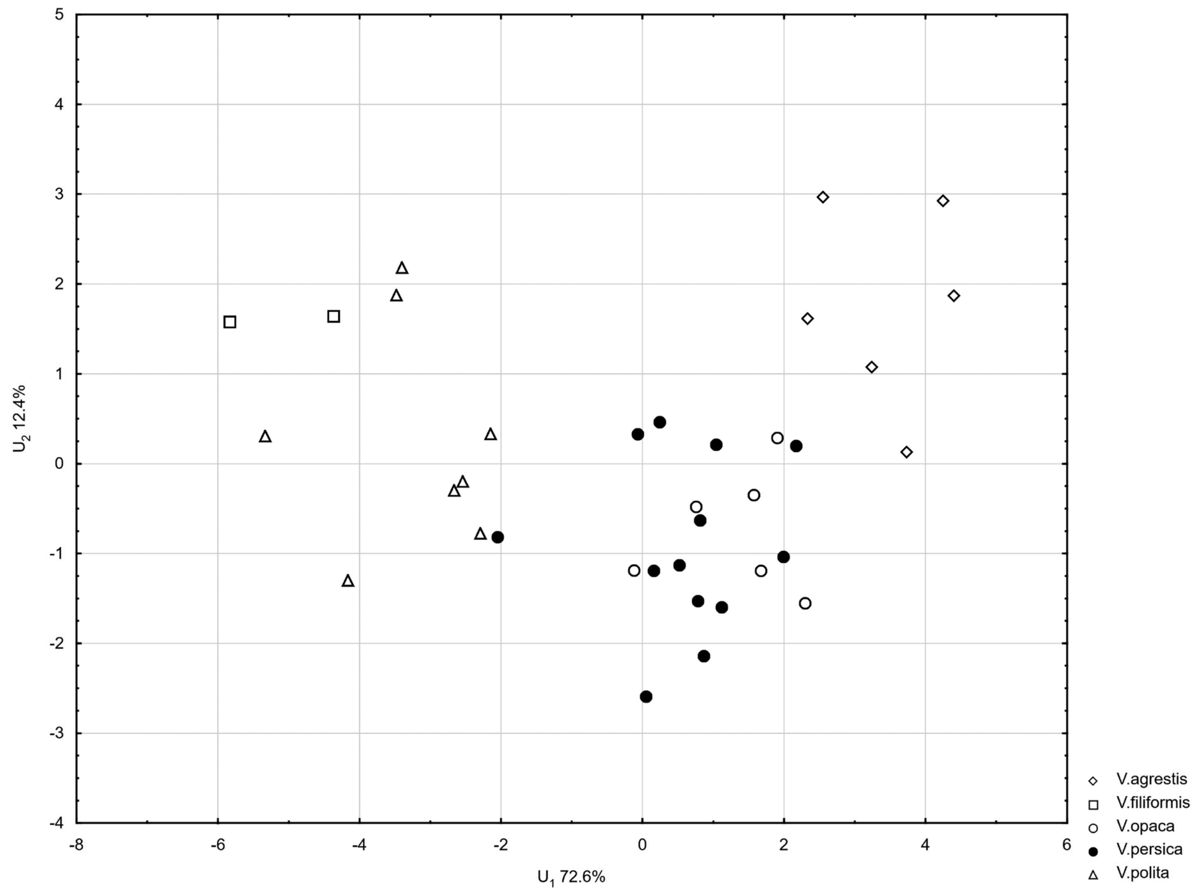

Fig. 6. Result of the discriminant analysis based on eight seed characteristics of five species of the genus *Veronica*, subsect. *Agrestes* made between the first two discriminant variables (U_1, U_2), which accounted for 85% of the total variation

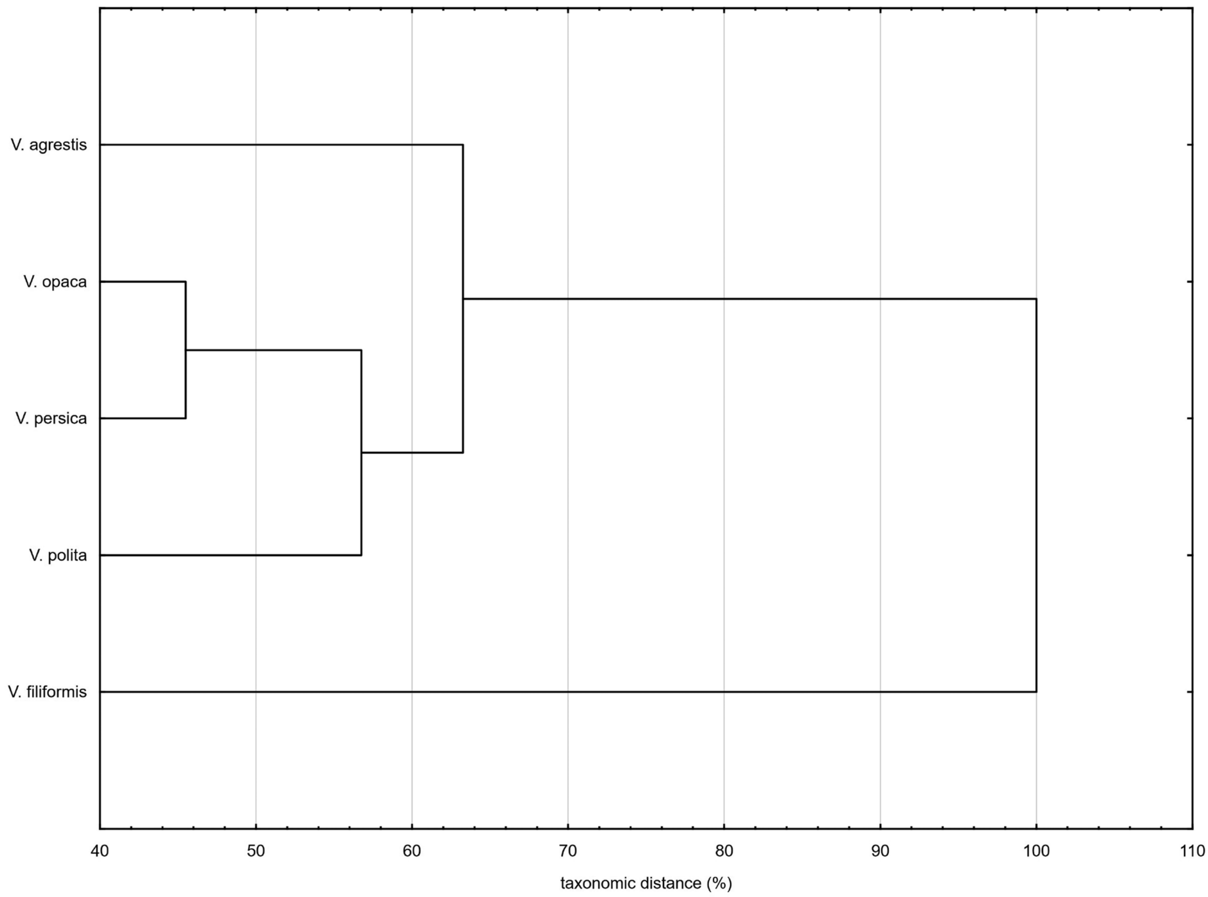

Fig. 7. The dendrogram constructed on the shortest Euclidean distances according to the UPGMA method using eight seed characteristics of five species of the genus *Veronica*, subsect. *Agrestes*

4. Discussion

The study carried out on 30 seed morphological characteristics of five *Veronica* species from the *Agrestes* subsection occurring in the central part of the European range, revealed that some morphological traits, such as size, shape and testa surface characters showed differences between species and could be taxonomically useful. We found that 22 of the 30 features differentiated species on a statistically significant level. We did not find any characters that would allow to distinguish each of the five compared taxa but thirteen characters, including mainly features describing the seed size and the size of chalazal parts, allowed to distinguish *V. agrestis* from *V. polita* and twelve – to distinguish *V. persica* from *V. agrestis* and *V. polita* (Table 2).

It is noteworthy that the seed length (*L*) was the only feature distinguishing, on a highly statistically significant level ($p < 0.01$), all species with one exception: it failed to distinguish *V. polita* and *V. fifliformis* (Table 2). The results of the discriminant function confirmed its greatest impact on species variability. The width of seeds, measured at different points, could also be helpful in recognizing species, with the exception of two pairs of them: *V. opaca* – *V. persica* and *V. filiformis* – *V. polita*. Eight characters of seed size distinguished statistically significant, in the largest number of cases, pairs of species, while the number of wrinkles (*WrN*) allowed to distinguish *V. persica* from *V. agrestis*, *V. opaca* and *V. polita* (Table 2). The degree of edge folding (*FE*) distinguished *V. filiformis* seeds from seeds of other species. In addition, our research showed that the largest percentage of strongly folded edges occurred in *V. polita* (Table 1).

The similarity of *V. persica* and *V. opaca* demonstrated in our study was shown in earlier molecular studies (Albach *et al.* 2005). However, the size characters must be considered with some caution, as they are often described as dependent on environmental variables. The length and width of the seeds are the only size characters we can compare as they are commonly described in Floras. But as all the size traits were highly correlated, such comparison is sufficient to conclude on the size of seeds in general.

In our study, *V. agrestis* had the biggest seeds with the average length of 1.76 mm, which was a bit more than the mean data for Poland: 1.6 mm (Tacik & Trzcińska-Tacik 1963) and for Europe: 1.5 mm (Walters & Webb

1972). According to sources for Poland and Europe, the biggest seeds among the five analysed species, could be found in *V. persica*: 2.0 (Tacik & Trzcińska-Tacik 1963) and 1.75 (Walters & Webb 1972), while the average in our analysis equalled 1.65 (Appendix 2). The comparison of our data with other authors (e.g.: Fischer 1981; Hassan & Khalik 2014) brings more ambiguities and confirms that size characters cannot be treated as fully trustworthy.

The micromorphological characters of chalaza did not seem to be very useful in differentiating species, although the length of chalaza (*LCh*) can help to distinguish *V. polita* from *V. persica* and *V. agrestis*, *V. persica* from *V. opaca* and *V. filiformis* and also *V. opaca* from *V. agrestis* (Table 2).

The ratios describing shape of a plant organ are usually considered as more appropriate for taxonomical purposes, as they are less dependent on environmental variables. In our case, only few ratios could help to distinguish species, and they were usually correlated with the measured features.

All seeds of *Veronica* subsect. *Agrestes* were cochlidiospermous, but we found that only *V. filiformis* had shallow-cochlidiospermous type of seeds, while other taxa were characterized by deep-cochlidiospermous seeds (Table 1). This confirmed the results of previous studies, when seeds were described as follows: *V. persica* as cochlidiospermous (Yamazaki 1957); *V. persica* and *V.polita* as deep-cochlidiospermous, whereas *V. filiformis, V. agrestis* and *V. opaca* as shallow-cochlidiospermous (Kulpa 1968); *V. persica* and *V. polita* as deep cymbiformis, but *V. filiformis* as slighty cymbiformis (Fischer 1981); *V. agrestis*, *V. persica* and *V. polita* as cyathiform (Juan *et al.* 1994); *V. agrestis*, *V. persica*, *V. opaca* and *V. polita* as concave on one face, while *V. filiformis* as shallowly concave on one face (Walters & Webb 1972).

The shape of seeds in subsection *Agrestes* was variable and described differently in numerous sources. For example, Tacik & Trzcińska-Tacik (1963) characterized *V. agrestis*, *V. persica* and *V. polita* as obovoid but *V. opaca* as broadly obovoid. Kulpa (1968) presented almost all as broadly obovoid or suborbicular, with the exception of *V. filiformis*, whose seeds were described as obovoid or broadly obovoid. Walters & Webb (1972) identified seeds of four species as broadly elliptical, also with the exception of *V. filiformis* having elliptical shape of seeds. Fischer (1981) noted seeds of *V. polita* as elliptical, *V. persica* as broadly elliptical and, finally, *V. filiformis* as obovate. In recent years, Hassan & Khalik (2014) reported *V. persica* seeds as broadly elliptical, *V. agrestis* as elliptical to globose and *V. polita* as ovoid-oblong. Our research showed that ovoid seed shape was dominant in subsect. *Agrestes* (34.53%) and it was the most common in *V. agrestis*, *V. persica* and *V. polita*. In contrast, *V. opaca* seeds most often were broadly obovoid or elliptical, while seeds of *V. filiformis* were predominantly broadly obovoid (Table 1).

Many authors highlighted the systematic value of the structural form of the seed coat due to its low phenetic variation of the species (e.g.: Barthlott 1981; Elisens & Tomb 1983; Martinez-Ortega & Rico 2001; Muñoz-Centeno *et al.* 2006, 2007; Szkudlarz 2009; Szkudlarz & Celka 2016). The use of SEM made it possible to determine general types of seed coat sculpturing. Researchers observed cristate-papillatae (Juan *et al.* 1994) or reticulate-verrucate (Muñoz-Centeno *et al.* 2006) seed coat structures in subsect. *Agrestes*. Likewise, Kaplan *et al.* (2007) described the seed surfaces as rugose-reticulate in *V. persica*, Yilmaz (2013) as reticulate-verrugate in *V. polita* and Hassan & Khalik (2014) as strongly ridged with micro-papillae all over in *V. persica* and *V. polita*, but tuberculate in *V. agrestis*. In contrast, Kulpa (1968) described the structure of seeds in this subsection as acute-papillate, based on the fact that the length of warts was up to 2 times larger than the width. However, it must be remembered that Kulpa did not use SEM. Our observations confirmed that the reticulate-verrucate seed coat structure was the most common in all species of the studied subsection, but, sometimes, we also observed only reticulate forms (Table 1). The testa surface of the investigated seeds was relatively variable. In the same species, cell walls formed a reticulum, which was regular and, sometimes, irregular, the walls of cells were convex, flat or collapsed and the central papilla were convex or collapsed; sometimes we observed no papilla. Furthermore, the seed coat sculpture was not the same in one individual and around one seed.

We found *V. filiformis* seeds as the most different from all others. The greatest distinction of the species was shown both in molecular studies (Taskova *et al.* 2004) and earlier research on the morphology of seeds (e.g.: Kulpa 1968; Walters & Webb 1972; Fischer 1981).

The species is native in the Caucasus Mountains and Asia Minor (Walters & Webb 1972) and now, it is invading Europe reproducing mainly vegetatively. The recent study confirmed this feature and, additionally, experimentally proved seeds from the introduced area of the species to be smaller than seeds of native stands (Scalone & Albach 2012). What is more, the authors did not find any capsules in the course of their investigation. It is also very probable that the seeds in our study were immature, and this fact may be responsible for their distinctiveness.

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