ORIGINAL ARTICLE

Seed morphology and endosperm structure of selected species of Primulaceae, Myrsinaceae, and Theophrastaceae and their systematic importance

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Abstract Seed size and shape, seed coat surface pattern, seed coat thickness, and endosperm structure were investigated in *Androsace septentrionalis, Cortusa matthioli, Hottonia palustris, Primula elatior, Soldanella carpatica* (Primulaceae), *Anagallis arvensis, A. minima, Cyclamen purpurascens, Glaux maritima, Lysimachia nemorum, L. vulgaris, Trientalis europaea* (Myrsinaceae), and *Samolus valerandi* (Theophrastaceae). Three seed size categories were distinguished on the basis of biometric measurements. Almost all seeds examined were found to be small with an angular shape classified as sectoroid or polyhedral. A new type of seed shape, suboval, was identified for *H. palustris*. *Cyclamen purpurascens* seeds differed from seeds of all other species examined because of their large size, subglobose shape, and concave hilar area. Despite the different shape types, the length/width ratio of the seeds examined was constant, while their hilum length/width ratio was a highly variable feature. Three types of seed surface patterns were observed: (1) reticulate, (2) tuberculate with secondary striations, and (3) poroid-alveolate with the presence of a spongy outer layer. For seeds of *Anagallis arvensis, A. minima, Cortusa matthioli, Lysimachia nemorum*, and *Soldanella carpatica*, secondary seed sculpture was described. The seed coats of all species examined were two-layered, and great differences in testa thickness were found (9.9–128.6 μm). In general, seeds of the Myrsinaceae species were more often characterized by thick testa. Different proportions in thickness of the inner and outer testa layers were observed. In seeds with reticulate seed patterns, the inner testa layer was twice to several times thicker than the outer layer, while the opposite proportions were observed in seeds with the tuberculate or poroid-alveolate seed sculpture pattern. In seeds of all species examined, oxalate crystals were present on the surface of the inner testa layer. Thus, the presence or absence of oxalate crystals in testa is not a feature distinguishing species with angular or subglobose seeds. Four types of endosperm structure were identified according to the thickness of the endosperm cell walls and the relief of their inner surface: (1) with evenly thickened and smooth cell walls, (2) with evenly thickened cell walls and circular or helical thickenings on their inside surfaces, (3) with very thick, but not evenly thickened cell walls with constrictions ("pitted"), and (4) with very thin papery and undulate cell walls. There is no rule concerning the seed shape, type of the seed sculpture, testa thickness, or endosperm structure that corresponds to the family affiliation of the species examined.

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Introduction

The phylogenetic relationships within the group of three families—Primulacaeae, Myrsinaceae, and Theophrastaceae—based either on DNA sequences from plastid (*atp*B, *ndh*F, *rbc*L, and *trn*L-F), nuclear (ITS), and mitochondrial (*atp*1 and *mat*R) genes or morphological data have been analyzed in many papers (Anderberg and Ståhl 1995; Anderberg et al. 1998, 2000, 2002; Källersjö et al. 2000; Caris et al. 2000; Hao and Hu 2001; Mast et al. 2001; Källersjö and Ståhl 2003; Martins et al. 2003; Hao et al. 2004; Ståhl and Anderberg 2004; Manns and Anderberg 2005, 2007; Oh et al. 2008). As a result of some of these studies, strong support for a sister group relationship between Myrsinaceae s.str. and the tribe Lysimachieae of Primulaceae was found. Källersjö et al. (2000) recognized four families among the "primuloid" families of the Ericales s.l., namely Primulaceae, Myrsinaceae, Theophrastaceae, and Maesaceae. In their opinion the recognition of four families was the best way of preserving taxonomic stability but required some generic realignments. These realignments concerned *Samolus*, which was transferred to Theophrastaceae; *Maesa*, which was raised to family level; and *Lysimachia, Anagallis, Trientalis, Glaux, Asterolinon, Pelletiera, Coris, Ardisiandra,* and *Cyclamen*, which were included in Myrsinaceae. In other works on molecular phylogeny of the Primulales s.l., many close or distant relationships of certain taxa were described. Martins et al. (2003) found that *Anagallis minima* is rather distantly related to *A. arvensis*, which, along with *Asterolinon* and *Pelletiera*, is closely related to *Lysimachia* sect. *Lerouxia*. These results also supported the monophyly of a group consisting of the four genera *Hottonia, Soldanella, Bryocarpum,* and *Omphalogramma*; provided evidence against monophyly of the large genera *Primula, Androsace,* and *Lysimachia*; and showed that *Cyclamen* is not a member of the Myrsinaceae-Lysimachieae clade such that its position remains unclear. Hao et al. (2004) found only *Glaux* to be placed within *Lysimachia*, and Anderberg et al. (2007) suggested that in addition to *Glaux*, such genera as *Anagallis, Asterolinon,* and *Pelletiera* should also be nested within *Lysimachia*. Recent work by Oh et al. (2008) concerning seed morphology of some species from the family Myrsinaceae, especially the genus *Lysimachia* and a few related taxa, showed that mapping of seed characters onto a previously obtained phylogenetic tree may provide potentially synapomorphic character states for those subclades of *Lysimachia* that were examined. The general conclusion of these authors was that seed morphology may provide some important evidence for understanding the phylogeny of the taxa being examined.

Because the phylogenetic interrelationships within the primuloid families are still not completely resolved, we have investigated seed structure of some species of Primulaceae, Myrsinaceae, and Theophrastaceae. The aims of our study were (1) to examine and describe seed morphology and endosperm structure of some genera from Primulaceae, Myrsinaceae, and Theophrastaceae, (2) to find seed characters of systematic importance, and (3) to evaluate their usefulness in confirming or constraining a new systematic classification of the genera studied.

Materials and methods

Biometric measurements

Morphological and anatomical seed characters of *Androsace septentrionalis*, *Cortusa matthioli*, *Hottonia palustris*, *Primula elatior*, *Soldanella carpatica* (Primulaceae), *Anagallis arvensis*, *A. minima*, *Cyclamen purpurascens*, *Glaux maritima*, *Lysimachia nemorum*, *L. vulgaris*, *Trientalis europaea* (Myrsinaceae), and *Samolus valerandi* (Theophrastaceae) were studied using seeds collected during the years 2005–2007 from living plants in natural localities and from herbarium collections (Table 1). In natural localities, plants grew in habitats optimal for them. In each locality, seeds were collected from 15 individuals, and the representative seed sample was used for biometric measurements. Herbarium specimens were used to obtain seeds of two species; for *Anagallis arvensis*, a representative seed sample was composed of seeds from 20 individuals, and for *Cyclamen purpurascens*, seeds were obtained from the few individuals available. Measurements of seed and hilum size were carried out with light microscopy (LM) on 30 seeds of each species. Seed coat thickness was measured on five seeds of each species with the help of scanning electron microscopy (SEM) pictures. Seed length was measured along the longest axis in the plane of the hilum and microphyle, seed width along the longest axis at a 90° angle to the plane of the hilum and microphyle, and seed thickness along the dorsiventral axis. Length and width of the hilum were also measured, and length/width ratio for both seed and hilum was calculated. For testa thickness, the inner (IL) and outer (OL) layers were measured on the longitudinal seed sections.

Seed preparations for SEM

Before cutting, 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 (two 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.

Table 1 List of species analyzed in the study

Family	Species	Specimen collection data	Figures
Myrsinaceae	*Anagallis minima* L.	N 53°35′ E 18°35′ leg. Aneta Czarna	4f–m
Myrsinaceae	*Cyclamen purpurascens* Mill.	TRN Herbarium, PL leg. Andrzej Zielski	3r–v
Myrsinaceae	*Glaux maritima* L.	N 52°10′ E 17°41′ leg. Aneta Czarna	3m–q
Myrsinaceae	*Lysimachia nemorum* L.	N 49°08′ E 22°34′ leg. Aneta Czarna	4n–r
Myrsinaceae	*Lysimachia vulgaris* L.	N 52°21′ E 15°52′ leg. Maria Morozowska	3a–d
Myrsinaceae	*Trientalis europaea* L.	N 53°29′ E 22°42′ leg. Aneta Czarna	3e–l
Myrsinaceae	*Anagallis arvensis* L.	POZNB Herbarium, PL leg. Aneta Czarna	4a–e
Primulaceae	*Androsace septentrionalis* L.	N 53°46′ E 20°28′ leg. Aneta Czarna	5a–d
Primulaceae	*Cortusa matthioli* L.	N 49°17′ E 19°56′ leg. Aneta Czarna	6f–i
Primulaceae	*Hottonia palustris* L.	N 52°06′ E 17°04′ leg. Aneta Czarna	6j–n
Primulaceae	*Primula elatior* (L.) Hill	N 49°08′ E 22°34′ leg. Aneta Czarna	5e–l
Primulaceae	*Soldanella carpatica* Vierh.	N 49°17′ E 19°56′ leg. Aneta Czarna	6a–e
Theophrastaceae	*Samolus valerandi* L.	N 52°10′ E 17°41′ leg. Aneta Czarna	6o–r

For SEM, seeds and seed cuttings were covered with gold and examined with a Zeiss EVO 40 electron microscope at 8–15 kV depending on the species.

Statistical analysis

The biometric data were analyzed statistically. For each seed trait, one-factor analysis of variance (ANOVA) was used to examine differences in means among species studied. If critical differences were noted, multiple comparisons were carried out based on Tukey's test for equal sample sizes. To show similarities and differences among taxa studied, Ward's hierarchical clustering method was used to compute cluster groups of species based on the seed characters analyzed. Statistical analyses were performed using JMP 8.0 (SAS Institute, Cary, NC, USA; http://www.sas.com/).

The specimens and the seed material are deposited in the herbarium of the Botany Department (POZNB), Poznan University of Life Sciences, Poland.

Results

Seed shape and size

To describe seed shape for the species examined, we have adopted two of the three major shape types proposed by Oh et al. (2008). These were the sectoroid seed shape, which was observed for seeds of *Androsace septentrionalis*, *Cortusa matthioli*, *Soldanella carpatica* (Primulaceae), *Anagallis arvensis*, *A. minima*, *G. maritima*, *Lysimachia nemorum*, *L. vulgaris*, and *T. europaea* (Myrsinaceae), and the polyhedral seed shape, which was observed for seeds of *P. elatior* (Primulaceae) and *Samolus valerandi* (Theophrastaceae) (Table 2). We have described two other seed shape types: suboval and subglobose with a concave hilar area (Fig. 1). The suboval shape corresponds to the lateral view of the seed and is typical for *H. palustris*. Seeds are laterally flattened, and the lateral faces are slanted towards the ventral margin. The hilum is located along the ridge on the top side (Fig. 6j, k). Subglobose seeds are typical for *Cyclamen purpurascens*. Their shape is similar to rugose seeds described by Oh et al. (2008) for *Asterolinon linum-stellatum*, but their surface is not rugose. In dorsal view they are elliptic, and on the ventral side, they have a concave hilar area (Fig. 3r, s). Three seed size categories were distinguished: small, medium, and large. Most of the species examined have small seeds with the following length, width, and thickness ratios: 0.5–1.6 × 0.3–1.2 × 0.4–0.8 mm. *T. europaea* has medium size seeds (1.6 × 1.4 × 0.6 mm), and seeds of *Cyclamen purpurascens* are substantially larger than all other species (2.8 × 2.4 × 1.3 mm) (Table 2).

Statistical analyses showed significant differences ($P < 0.0001$) among species studied for all features analyzed (Tables 3, 4). Ward's agglomerative grouping method distinguished three main groups of taxa (Fig. 2). Two of them comprise species representing both Primulaceae and Myrsinaceae, and *Cyclamen purpurascens* is isolated in the third one. In the first group, the closest similarities were found for species representing the same family, while in the second group, the closest similarities were observed for species representing different families and different shape types. Multiple comparisons based on Tukey's test showed the presence of two uniform groups of species based on mean values of the length/width ratio. The first group included all species of Myrsinaceae examined and only one species of Primulceae, i.e., *P. elatior*, while the second group included all other species of Primulaceae (except *Soldanella carpatica*) and *Samolus valerandi* of Theophrastaceae (Table 3). Despite the different sizes and shape types of seeds, length/width proportions remained fairly similar within particular families (CV = 23.4%). In contrast, the hilum length/width ratio was found to be a highly variable feature (CV = 53.7%) (Table 4).

The species examined were characterized by either narrow, narrowly elliptic, or elliptic hilum shapes (ratios from longest axis to shortest axis ranged from 8:1 to 5:1, 4.5:1 to 2.5:1, and 2:1 to 1.5:1, respectively).

Most of the examined species of *Primulaceae* and *Myrsinaceae* have narrowly elliptic or elliptic hila more or less flush with the surface. *Soldanella carpatica, Cortusa matthioli*, and *Samolus valerandi* have narrow hilum (Fig. 6a, b, f, o), and *Cyclamen purpurascens* has extremely different, elliptic immersed concave hila (Fig. 3s; Table 2). Seeds of *Cyclamen purpurascens* were also significantly different from seeds of Myrsinaceae, Primulaceae, and Theophrastaceae in all other biometric features (Tables 3, 4). In turn, seeds of *Samolus valerandi* were found to be very similar to seeds of *Anagallis minima* according to testa thickness, and no significant differences were noted between these species for seed length, width, or thickness.

Seed surface and testa thickness

Three types of the seed sculpture were observed among all species examined. The most common was reticulate ornamentation of the seed surface, observed for *G. maritima* (Fig. 3n), *Cyclamen purpurascens* (Fig. 3t), *Androsace septentrionalis* (Fig. 5b), *Soldanella carpatica* (Fig. 6c), *Cortusa matthioli* (Fig. 6h), *H. palustris* (Fig. 6l), and *Samolus valerandi* (Fig. 6q). The reticulate and partly vesiculose seed pattern of *Cortusa matthioli* and the reticulate seed sculpture of *Soldanella carpatica* were also characterized by secondary sculpture; in *Cortusa matthioli* the surface of the outer cell walls exhibited a striate micro-ornamentation (Fig. 6h) and in *Soldanella carpatica* small secondary protrusions were observed (Fig. 6d). A tuberculate seed surface pattern characterized *P. elatior* seeds, and tuberculate sculpture with secondary striations was observed for *Anagallis arvensis*, *A. minima*, and *Lysimachia nemorum* (Fig. 4d, i, p). Two species, *Lysimachia vulgaris* and *T. europaea*, were characterized by the presence of a sponge-like outer layer with a poroid-alveolate surface pattern. On *L. vulgaris* seeds, this outer layer has a columnar look (Fig. 3b) that was previously described by Oh et al. (2008). The observed thickness of the outer layer was about 80–110 μm (Table 2). The sponge-like outer layer on *T. europaea* seeds consists of rectangular cells (RC) with a roof-like upper wall and longitudinal thickenings on the inner cell wall surface (Fig. 3j). The thickness of the outer layer for this species was about 30–62 μm (Table 2). The surface of the sponge-like outer layer on *T. europaea* sometimes showed a reticulate pattern with secondary striations (Fig. 3g). Testa structure of all species examined was characterized by two layers, an inner layer (IL) and outer layer (OL) of different thicknesses, which

Table 2 Seed morphological and anatomical characters of the species examined from Primulaceae, Myrsinaceae, and Theophrastaceae

| Species	Seed shape	Spongy outer layer	Surface	Hilum shape	Edges	Testa thickness (μm)			Endosperm cell walls	
						Testa	Outer layer	Inner layer	Type of thickness	Inner surface
Anagallis arvensis	Sectoroid	Absent	Tuberculate with secondary striations	Narrow	Not winged or keeled; weakly or strongly winged	71.6	49.9	21.7	Evenly thickened	Smooth or with little protrusions
Anagallis minima	Sectoroid	Absent	Tuberculate with secondary striations	Narrowly elliptic	Not winged, rarely weakly keeled	18.6	12.5	6.1	Very thin, paper-like	Strongly undulate, partly with circular thickenings
Androsace septentrionalis	Sectoroid	Absent	Reticulate	Narrow	Not winged or keeled	19.0	4.0	15.0	Evenly thickened or "pitted"	If pitted, then with "hollows"
Cortusa matthioli	Sectoroid (± angled)	Absent	Reticulate/vesiculose with secondary striations	Narrow	Not winged or keeled	31.5	6.4	25.1	Evenly thickened	Smooth
Cyclamen purpurascens	Subglobose	Absent	Strongly reticulate	Narrowly elliptic immersed concave hilum	–	45.8	32.3	13.5	Very thick, not evenly thickened, "pitted"	With "hollows"
Glaux maritima	Sectoroid	Absent	Reticulate	Narrowly elliptic	Weakly keeled	93.1	34.2	58.9	Evenly thickened or very thin, paper-like	Smooth, or if thin, then with circular thickenings
Hottonia palustris	Suboval (± angled)	Absent	Weakly reticulate	Elliptic	–	10.5	3.0	7.5	Thin, paper-like	Smooth, slightly undulate
Lysimachia nemorum	Sectoroid, polyhedral	Absent	Tuberculate with secondary striations	Elliptic	Not winged or keeled	121.9	63.2	57.7	Evenly thickened	With helical thickenings
Lysimachia vulgaris	Sectoroid	Present	Poroid-alveolate	Narrowly elliptic	Not winged or keeled	101.1–128.6	82.5–110.0	18.6	Thin, paper-like	Smooth or slightly undulate
Primula elatior	Polyhedral	Absent	Tuberculate	Elliptic	Not winged or keeled	87.0	69.0	18.0	Evenly thickened and rarely "pitted" or very thin	Smooth, slightly undulate, if pitted, then with "hollows"
Samolus valerandi	Polyhedral	Absent	Reticulate to weakly reticulate	Narrow	Not or very weakly keeled or winged	10.7	3.6	7.1	Evenly thickened	Smooth
Soldanella carpatica	Sectoroid (± angled)	Absent	Weakly reticulate with secondary protrusions	Narrow	Not winged or keeled	9.9	4.6	5.3	Thin, paper-like	Smooth, strongly undulate
Trientalis europaea	Sectoroid/ polyhedral	Present	Poroid-alveolate (± reticulate, with secondary striations)	Narrowly elliptic to elliptic	Not winged or keeled	66.6–96.1	32.5–62.0	34.1	Evenly thickened or very thin, paper-like	Strongly undulate with circular thickenings
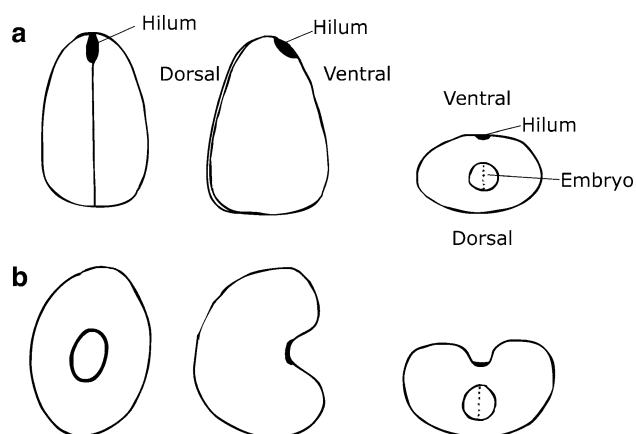
Fig. 1 Schematic illustration of seed shapes of **a** *Hottonia palustris* (suboval laterally flattened, side faces, cavernous, and slanted towards the ventral margin) and **b** *Cyclamen purpurascens* (subglobose with concave hilar area). Seed shape in ventral view (*left column*), lateral view (*middle column*), and transverse section (*right column*)

closely adhere to each other. Primulaceae seeds had a smaller range of testa thickness (9.9–87.0 μm) than seeds of Myrsinaceae (18.6–128.6 μm). Seeds with especially thin testa were found for *Soldanella carpatica, H. palustris*, and *Samolus valerandi* for which testa thickness ranged from 9.9–10.7 μm (Table 2). For all species examined with reticulate seed coat patterns, the inner testa layer was thicker than the outer testa layer (ratios of inner layer to outer layer ranged from 1.7:1 to 3.9:1). The opposite pattern was found for seeds with tuberculate seed sculpture and seeds with presence of the sponge-like outer layer. For example, in seeds with rather thick testa (71.6–96.1 μm), such as *P. elatior* (Fig. 5h, i, j) (Primulaceae), *Anagallis arvensis* (Fig. 4e), and *T. europaea* (Fig. 3j) (Myrsinaceae), the ratio of the thickness of the inner/outer testa layers was 1:3.8, 1:2.3, and 1:1.8, respectively. In seeds of *Lysimachia nemorum* (Fig. 4q) and *L. vulgaris* (Fig. 3a, b), in which the testa was very thick (121.9–128.6 μm), the same ratios were 1.1:1 and 5.9:1, respectively (Table 2). The feature common for all species examined was the presence of oxalate crystals on the surface of the inner testa layer (Figs. 3j, o, u; 4e, m; 5h, l; 6m). For *Androsace septentrionalis* (Fig. 5b), *Soldanella carpatica*, and *Cortusa matthioli* (Fig. 6c, h), outlines of crystals were visible on the ventral view of the seed surface, under the cuticle cell layer.

Endosperm structure

Endosperm structure differed strongly among species examined. We have defined two endosperm characters: cell wall thickness and the relief of the inside cell wall surfaces. Four main types of endosperm were distinguished: (1) with evenly thickened cell walls and smooth inside cell wall surfaces, (2) with evenly thickened cell walls and circular or helical thickenings on their inside surfaces, (3) with very thick, unevenly thickened cell walls with constrictions ("pitted") and with dark deep hollows on the inside cell wall surfaces, (4) with very thin, paper-like cell walls slightly or strongly undulate. The first type of endosperm was found in seeds of *Anagallis arvensis*, for which little protrusions were also observed on the inner cell wall surfaces (Fig. 4e). The same type of endosperm, but without protrusions, was typical for *G. maritima, Cortusa matthioli*, and *Samolus valerandi* seeds (Figs. 3p; 6i, r). The endosperm structure of *T. europaea* and *G. maritima* seeds was very variable, as two different types of endosperm structure were present in them (Fig. 3k, l, p, q). The second endosperm type was found in *Lysimachia nemorum* seeds (Fig. 4r). The third type was found in seeds of *Cyclamen purpurascens, Androsace septentrionalis*, and *P. elatior* (Figs. 3v; 5d, i, j, k). It is worth mentioning that in seeds of *P. elatior*, the endosperm structure was not uniform, as its cells located on the end of the longest axis were characterized by papery thin cell walls (Fig. 5j). The fourth endosperm type was observed in seeds of *G. maritima* (Fig. 3q), *Anagallis minima* (Fig. 4k, l), and *Soldanella carpatica* (Fig. 6e), consisting of cells with very strongly undulate walls. The same type of endosperm was found in seeds of *Lysimachia vulgaris, T. europaea* (Fig. 3d, k), and *H. palustris* (Fig. 6m, n), in which the cell walls were only slightly undulate.

Discussion

Recent molecular and morphological phylogenetic studies on the relationships among taxa of "primuloid" families of the Ericales s.l. (Primulaceae, Myrsinaceae, and Theophrastaceae) have shown that, despite molecular evidence for close relationships of certain taxa, the addition of morphological information may lead to a slight increase, or decrease, in support for phylogenetic relationships of some groups (Anderberg et al. 2007; Oh et al. 2008). Based on our observations, the testa thickness, presence or absence of oxalate crystals in the outer testa layer, and the endosperm structure are very important features that may be potentially informative characters of systematic importance. According to the general rule (Anderberg and Ståhl 1995), the subglobose seeds of the former Myrsinaceae-Myrsinoideae have "pitted" endosperm cell walls and their testa are devoid of crystals, while the angular seeds of the former Primulaceae have evenly thickened, smooth endosperm cell walls and rather thick, usually distinctly two-layered testa, generally with rhomboid crystals. However, among species we examined for this study, we found angular seeds of the former Primulaceae with either thin or

Table 3 Ranges (minimum–maximum), mean values (±SE), and coefficients of variation (CV) of seed morphological features of the species studied (e.g., seed length, seed width, length/width ratio, and seed thickness)

Species	Seeds (n)	Seed length (mm)				Seed width (mm)				Length/width ratio				Seed thickness (mm)			
		Min.	Max.	Mean (±SE)	CV (%)	Min.	Max.	Mean (±SE)	CV (%)	Min.	Max.	Mean (±SE)	CV (%)	Min.	Max.	Mean (±SE)	CV (%)
Anagallis arvensis	30	0.91	1.56	1.235 (0.024)g	10.7	0.68	1.10	0.944 (0.021)ef	12.5	1.028	1.794	1.318 (0.023)d	9.6	0.52	0.94	0.668 (0.020)cd	16.4
Anagallis minima	30	0.41	0.62	0.551 (0.009)j	9.1	0.36	0.73	0.424 (0.012)hi	16.0	0.562	1.590	1.325 (0.034)d	14.1	0.31	0.56	0.356 (0.009)hi	13.9
Androsace septentrionalis	30	0.91	1.25	1.046 (0.018)h	9.3	0.47	0.78	0.621 (0.016)g	14.1	1.269	2.383	1.715 (0.050)b	15.8	0.41	0.73	0.560 (0.017)efg	17.0
Cortusa matthioli	30	1.07	2.08	1.636 (0.038)bc	12.6	0.57	1.43	1.001 (0.039)de	21.5	1.068	2.754	1.698 (0.070)b	22.6	0.44	0.78	0.635 (0.017)cde	14.7
Cyclamen purpurascens	30	1.82	3.42	2.803 (0.069)a	13.6	1.79	2.85	2.373 (0.049)a	11.3	1.015	1.465	1.184 (0.022)d	10.0	0.95	1.75	1.320 (0.034)a	14.1
Galax maritima	30	1.09	1.38	1.237 (0.014)fg	6.2	0.73	1.07	0.903 (0.019)ef	11.3	1.101	1.781	1.384 (0.027)cd	10.6	0.39	0.73	0.505 (0.014)g	15.0
Hottonia palustris	30	0.57	0.99	0.759 (0.014)i	10.3	0.34	0.62	0.486 (0.010)h	11.4	1.036	2.294	1.586 (0.047)bc	16.2	0.31	0.47	0.378 (0.007)h	10.0
Lysimachia nemorum	30	1.17	1.53	1.398 (0.018)de	7.1	0.86	1.38	1.095 (0.018)cd	9.0	1.026	1.571	1.284 (0.022)d	9.2	0.39	0.70	0.552 (0.016)fg	15.6
Lysimachia vulgaris	30	1.22	1.82	1.523 (0.029)cd	10.5	0.91	1.38	1.200 (0.025)c	11.6	1.047	1.824	1.285 (0.037)d	15.7	0.62	0.83	0.697 (0.010)c	7.9
Primula elatior	30	1.10	1.72	1.372 (0.030)ef	11.9	0.48	1.43	1.095 (0.037)cd	18.3	0.846	2.542	1.317 (0.073)d	30.3	0.57	1.00	0.817 (0.017)b	11.4
Samolus valerandi	30	0.39	0.57	0.481 (0.008)j	9.2	0.23	0.42	0.314 (0.010)i	16.9	1.000	2.130	1.576 (0.054)bc	18.8	0.23	0.34	0.282 (0.005)i	10.4
Soldanella carpatica	30	1.38	1.87	1.684 (0.021)b	7.0	0.70	0.91	0.834 (0.012)f	7.8	1.516	2.414	2.035 (0.043)a	11.7	0.39	0.88	0.619 (0.023)cdef	20.2
Trientalis europaea	30	1.30	1.82	1.583 (0.025)bc	8.5	0.91	1.51	1.358 (0.025)b	10.1	1.013	1.857	1.177 (0.031)d	14.3	0.39	0.73	0.611 (0.016)def	14.5
ANOVA	df	F		P		F		P		F		P		F		P	
Species	12	428.6		<0.0001		427.6		<0.0001		32.7		<0.0001		222.4		<0.0001	
df error	378																

One way ANOVAs were performed separately for each of the seed features to determine the differences among species studied. *Same letters* indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test ($P < 0.05$)

Table 4 Ranges (minimum–maximum), mean values (±SE), and coefficients of variation of seed morphological features of the species studied (e.g., hilum length, hilum width, and length/width ratio)

Species	Seeds (n)	Hilum length (mm)				Hilum width (mm)				Length/width ratio			
		Min.	Max.	Mean (±SE)	CV (%)	Min.	Max.	Mean (±SE)	CV (%)	Min.	Max.	Mean (±SE)	CV (%)
Anagallis arvensis	30	0.39	0.78	0.511 (0.017)cd	18.2	0.08	0.18	0.114 (0.005)c	25.1	3.056	6.125	4.616 (0.138)de	16.3
Anagallis minima	30	0.06	0.14	0.094 (0.004)h	21.1	0.05	0.09	0.066 (0.002)e	19.2	0.875	2.000	1.454 (0.063)i	23.6
Androsace septentrionalis	30	0.30	0.43	0.378 (0.006)ef	8.5	0.04	0.12	0.074 (0.004)de	27.3	2.917	9.000	5.442 (0.271)bcd	27.3
Cortusa matthioli	30	0.39	0.91	0.626 (0.027)b	23.6	0.05	0.08	0.078 (0.001)de	9.8	4.875	11.400	8.102 (0.362)a	24.5
Cyclamen purpurascens	30	0.38	1.60	1.045 (0.056)a	29.6	0.19	0.61	0.371 (0.017)a	25.8	1.000	7.000	3.028 (0.228)g	41.3
Galax maritima	30	0.23	0.78	0.411 (0.020)ef	27.1	0.08	0.13	0.100 (0.004)cd	19.3	2.300	6.000	4.176 (0.177)ef	23.2
Hottonia palustris	30	0.10	0.18	0.121 (0.004)gh	20.0	0.02	0.13	0.075 (0.005)de	38.6	0.769	6.500	1.987 (0.225)hi	62.1
Lysimachia nemorum	30	0.34	0.57	0.429 (0.011)de	13.5	0.16	0.26	0.195 (0.005)b	12.7	1.619	3.167	2.226 (0.063)ghi	15.6
Lysimachia vulgaris	30	0.31	0.52	0.420 (0.010)def	13.0	0.05	0.16	0.093 (0.005)cde	27.8	2.750	8.800	4.885 (0.311)cde	34.9
Primula elatior	30	0.26	0.44	0.323 (0.010)f	16.6	0.05	0.16	0.110 (0.005)c	25.7	1.625	7.800	3.173 (0.221)fg	38.2
Samolus valerandi	30	0.13	0.26	0.194 (0.005)g	15.0	0.03	0.03	0.030 (0.000)f	0.0	4.333	8.667	6.478 (0.178)b	15.0
Soldanella carpatica	30	0.31	0.80	0.576 (0.023)bc	22.0	0.08	0.13	0.100 (0.002)cd	10.2	3.100	8.500	5.848 (0.281)bc	26.3
Trientalis europaea	30	0.26	0.60	0.458 (0.015)de	18.0	0.13	0.23	0.175 (0.005)b	14.4	1.714	3.563	2.648 (0.088)gh	18.2
ANOVA	df			F	P			F	P			F	P
Species	12			135.8	<0.0001			199.7	<0.0001			78.0	<0.0001
df error	378												

One-way ANOVAs were performed separately for each seed feature to determine the differences among species studied. *Same letters* indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test ($P < 0.05$)
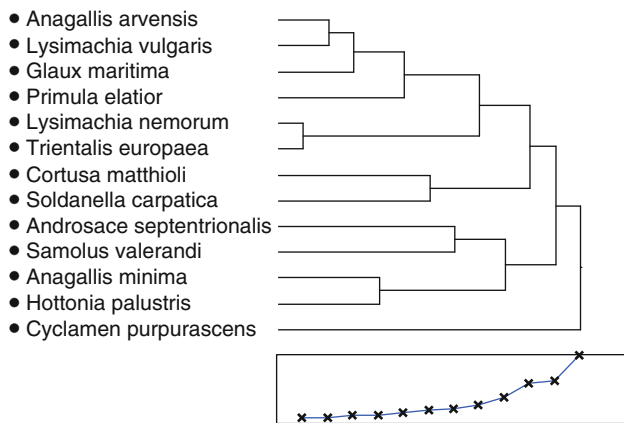
- Anagallis arvensis
- Lysimachia vulgaris
- Glaux maritima
- Primula elatior
- Lysimachia nemorum
- Trientalis europaea
- Cortusa matthioli
- Soldanella carpatica
- Androsace septentrionalis
- Samolus valerandi
- Anagallis minima
- Hottonia palustris
- Cyclamen purpurascens

Fig. 2 Dendrogram of cluster groupings of the species studied on the basis of seed morphological features

thick testa. After the reclassification of that family and transferring such species as *Lysimachia, Anagallis, Trientalis, Glaux*, and *Cyclamen* to Myrsinaceae (Källersjö et al. 2000), only *P. elatior* (among those species examined) was characterized by thick testa. This is in agreement with the results of our previous study in which the presence of rather thick testa was also described for *P. veris* seeds (Klimko et al. 2001). In this study we observed endosperm with irregularly thickened cell walls with constrictions in seeds of not only *Cyclamen purpurascens* (which fits well with the description of Myrsinaceae), but also in small angular seeds of *P. elatior* and *Androsace septentrionalis*. Anderberg and Kelso (1996) in their earlier investigations on phylogenetic implications of endosperm cell wall morphology in *Douglasia, Androsace*, and *Vitaliana* (Primulaceae), noted that the endosperm of *Primula* and *Androsace* is characterized by evenly thickened cell walls without constrictions. According to the same authors, the endosperm cell walls with irregular thickening and narrow constrictions characterizes all *Douglasia* and *Vitaliana* species and supports the monophyly of these genera. However, a few years later Ståhl and Anderberg (2004) found that in seeds of some *Androsace*, the endosperm consists of cells with unevenly thickened cell walls with narrow constrictions. We have found *Androsace septentrionalis* to be another example of an *Androsace* species with "pitted" endosperm cell walls. According to our observations, the feature of the irregularly thickened endosperm cell walls is due to the presence of the deep dark hollows on the inner surface of the cell walls, and such hollows were observed by us in seeds of *P. elatior* and *Androsace septentrionalis*.

The presence of endosperm with very thin, paper-like cell walls and slight or strong undulations, which was observed in seeds of *Anagallis minima*, *G. maritima*, and *T. europaea,* the three species transferred recently to Myrsinaceae (Källersjö et al. 2000), as well as in seeds of

Lysimachia vulgaris, may suggest that this type of endosperm structure is common among Myrsinaceae species. However, in seeds of *Lysimachia nemorum*, although the endosperm cell walls were rather thin and undulate, the relief of their inside wall surfaces was rather different, characterized by the presence of very distinct helical thickenings.

Results of this study show that rhomboid crystals can be present in testae of subglobose and angular seeds, so that this feature is not exclusive to small angular seeds, and lack of crystal presence in subglobose seeds of *Cyclamen purpurascens* before now does not support the transfer of that species to Myrsinaceae. Our results concerning seed structure of other species of the present Myrsinaceae, such as *Anagallis arvensis* and *A. minima*, for which there are many different reports concerning their molecular and morphological similarity (Martins et al. 2003; Manns and Anderberg 2005), showed on one hand very close similarity of their tuberculate seed sculpturing with secondary striations, while on the other hand, the results on other features examined did not confirm that similarity. For example, seeds of these two species differed in the presence or absence of winged and keeled seed edges, respectively. The testa was more than three times thicker in seeds of *Anagallis arvensis* than in seeds of *A. minima*, and striking differences in endosperm structure of these two species were observed. In *Anagallis arvensis*, seed endosperm consisted of cells with evenly thickened smooth cell walls, while in seeds of *A. minima*, the endosperm cell walls were papery, thin, and strongly undulate. Differences in the seed structure observed in these two species are in agreement with the results of molecular studies showing their distant phylogenetic relationships (Martins et al. 2003).

Our results concerning description and comparison of the seed structure of *Anagallis arvensis* and *Lysimachia nemorum,* for which there is strong molecular evidence of a very close relationship (Källersjö et al. 2000; Manns and Anderberg 2007; Oh et al. 2008), add some more morphological support for the reclassification of *Anagallis* from Primulaceae to Myrsinaceae. Similarities in the seed shape, seed sculpture, presence of oxalate crystals as well as thickness of endosperm cell walls within these two species were observed. However, we found one obvious difference between them: the relief of the inside surface of endosperm cell walls, which in *L. nemorum* is characterized by helical thickenings. One other difference concerns the testa thickness, which was more than 1.5 times thicker in seeds of *L. nemorum* than in seeds of *A. arvensis*. To resolve the differentiation between these two species, more taxa from both genera should be examined.

Some of our results showed similarities in the seed structure of *H. palustris* and *Soldanella carpatica*. These

Fig. 3 Seeds of **a–d** *Lysimachia vulgaris*, **e–l** *Trientalis europaea*, **m–q** *Glaux maritima*, and **r–v** *Cyclamen purpurascens*. **m, s** Ventral view showing narrowly elliptic (**m**) hilum (*H*) and concave hilar area (**s**). **e, r** Dorsal view showing seed outline and the reticulate surface pattern. **c, g, h, i** Surface of the spongy outer layer of *Lysimachia vulgaris* (**c**) and *Trientalis europaea* seeds (**g, h, i**). **n, t** Reticulate (**n**) and strongly reticulate (**t**) pattern of the outer layer (*OL*). **f** Partly removed spongy outer layer showing presence of oxalate crystals (*OxC; arrow*). **a, b, j, o, u** Longitudinal sections (L.S.) showing testa (*T*) thickness and rhomboid crystals (*arrows*) on the surface of the inner layer (*IL*). **b, j** L.S. showing columnar cells (*CC*) and rectangular cells (*RC*) of spongy outer layers of *Lysimachia vulgaris* (**b**) and *Trientalis europaea* (**j**) seeds. **d, k** L.S. showing very thin, papery walls of endosperm (*End*). **l** L.S. showing endosperm cells with evenly thickened, undulate walls with circular thickenings on the inner cell walls surface. **p, q** L.S. showing *Glaux maritima* endosperm cells with smooth evenly thickened (**p**) and papery thin, with circular thickenings on the inner cell wall surface (**q**). **v** L.S. showing "pitted" endosperm structure with unevenly thickened endosperm cell walls with dark hollows on the inside cell wall surfaces (*arrows*)
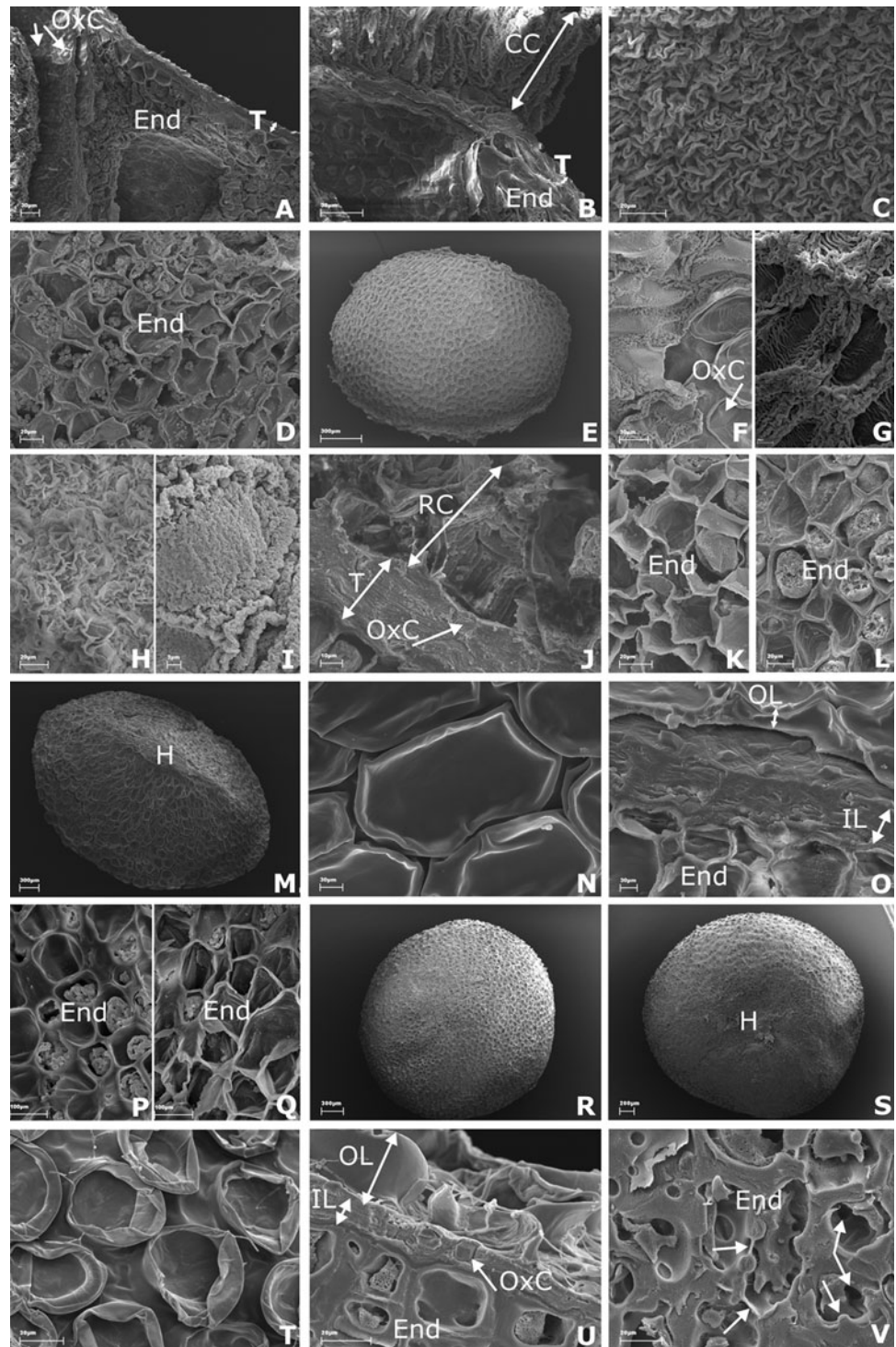

included the reticulate seed pattern, very thin testae, presence of rhomboid crystals, and very thin, papery, and smooth endosperm cell walls. According to some earlier molecular studies (Trift et al. 2002; Martins et al. 2003), there is some support for the assumption of common ancestry of *Hottonia* and *Soldanella*. Similarities in the

seed structure of these two species we describe here adds support for that hypothesis.

The inclusion of *Samolus valerandi* in the family Theophrastaceae is the subject of much discussion, as there is very weak morphological support for its transfer to that family (Källersjö and Ståhl 2003; Martins et al. 2003; Caris

Fig. 4 Seeds of **a–e** *Anagallis arvensis*, **f–m** *A. minima*, and **n–r** *Lysimachia nemorum*. **a, f, o** Ventral view showing narrowly elliptic (**a, f**) and elliptic (**o**) hilum (*H*) shape. **b, g, n** Dorsal view showing lack of winged or keeled edges of dorsal faces. **c, h** Lateral view showing strongly winged edges of dorsal face (**c**) and lack of winged or keeled dorsal face (**h**). **d, i, p** Seed surface showing tuberculate pattern with secondary striations. **j** Seed surface with partly removed outer layer (*OL*) and crystal visible on the surface of the inner layer (*IL*). **e, m, q** Longitudinal section (L.S.) showing testa thickness and presence of oxalate crystals (*OxC; arrows*) on the surface of the inner layer (*IL*). **e, k, l, r** L.S. showing endosperm (*End*) structure with evenly thickened cell walls with little protrusions on the inner cell wall surfaces (**e**); very thin, papery cell walls (**k, l**); evenly thickened cell walls with helical thickening on the inner cell wall surfaces (**r**)
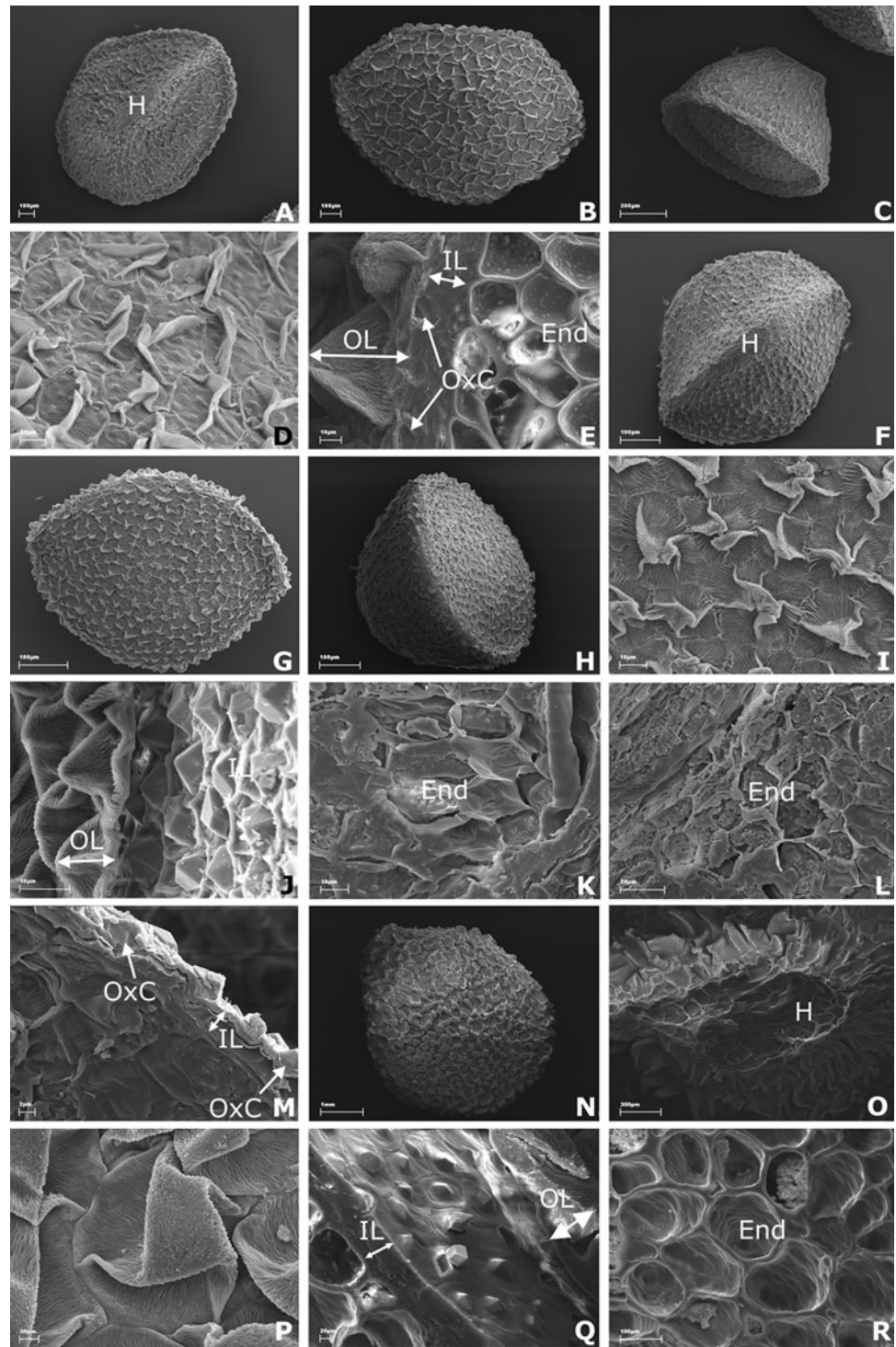
and Smets 2004). We found that seeds of *Samolus valerandi* were very similar to seeds of *H. palustris* and *Soldanella carpatica* (Primulaceae) in size proportions, faintly reticulate seed ornamentation, presence of oxalate crystals, and very thin testae. Some similarity (due to the presence of very thin testae) was observed between seeds of *S. valerandi* and seeds of *Androsace septentrionalis*

(Primulaceae) and *Anagallis minima* (Myrsinaceae). The structure of very thin testa in seeds of *S. valerandi* was described in our earlier study (Morozowska and Czarna 2006). On the other hand, thickness of the endosperm cell walls in seeds of *S. valerandi* differed from the endosperm structure in seeds of the species mentioned above; seeds of *A. septentrionalis* had endosperm with "pitted" cell walls

Fig. 5 Seeds of **a–d** *Androsace septentrionalis* and **e–l** *Primula elatior*. **a**, **f** Ventral view showing linear (**a**) and elliptic (**f**) hilum (*H*) shape. **e** Dorsal view showing lack of winged or keeled edges of dorsal face. **b** Outer layer (*OL*) surface with reticulate seed pattern and oxalate crystals (*OxC*) visible under cuticle cells (*arrows*). **g** Tuberculate seed pattern. **c**, **h**, **l** Longitudinal section (L.S.) showing testa (*T*) thickness and presence of oxalate crystals (**h**, **l**; *arrows*) on the surface of the inner layer (*IL*). **c**, **d**, **i**, **j**, **k** L.S. showing endosperm (*End*) structure with irregular thickenings of the cell walls and dark hollows on the inner cell wall surfaces (*arrows*)
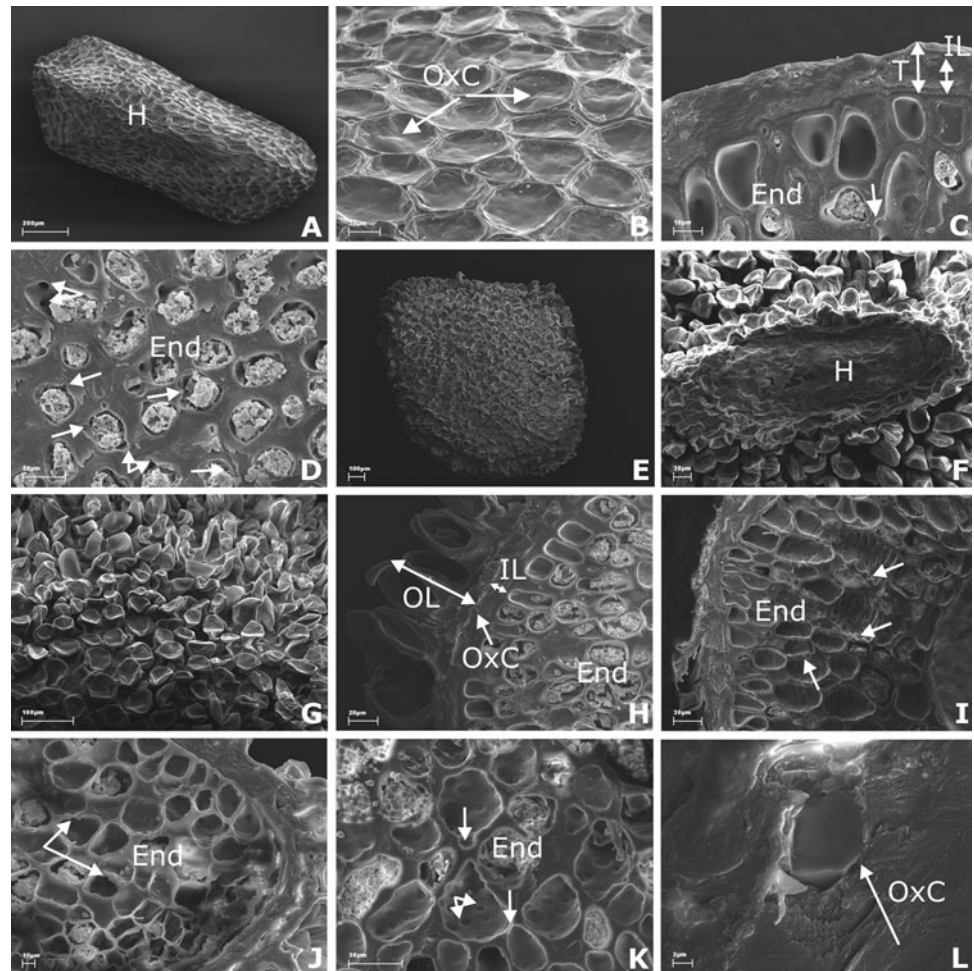

while the other species were characterized by papery thin endosperm cell walls. In general, the features of *S. valerandi* seeds we described compare well with the description of Theophrastaceae seeds given by Ståhl and Anderberg (2004), according to whom seeds of Theophrastaceae are characterized by faintly reticulate seed ornamentation and endosperm with smooth or irregularly thickened cell walls. In our opinion, the best way to find more, stronger morphological support for transferring *Samolus* to Theophrastaceae would be to examine the seed structure of the large number of species of that genus.

With regard to *Cyclamen purpurascens*, whose placement within Myrsinaceae is still under discussion (Martins et al. 2003), we found that, although the genus *Cyclamen* is characterized by capsular fruits, its seed shape and hilum, rather thin testae and "pitted" endosperm cell walls bear a good resemblance to the description of seeds typical for drupaceous species from Myrsinaceae (Ståhl and Anderberg 2004). The only feature that contradicts that description was the presence of oxalate crystals observed on the surface of the inner testa layer.

In conclusion, our study of the seed structure of some species of Primulaceae, Myrsinaceae, and Theophrastaceae provided some important new data concerning seed shape and size, seed coat pattern, presence of oxalate crystals on the surface of the outer testa layer, thickness of testa, and endosperm structure. In general, seeds, as generative organs that are only slightly influenced by environmental conditions, are important diagnostic features (but see Janyszek et al. 2008; Janyszek and Jagodzinski 2009). Their high structural diversity provides the most valuable criteria for classification at species and family levels. Thus, a detailed analysis of the morphological and anatomical structure of seeds greatly increases our knowledge of individual species and may be helpful in better understanding the phylogeny of the taxa examined. According to our observations, the relief of the secondary wall thickenings forming the secondary seed sculpture on some of the seeds examined is of high systematic importance. In any case, for purposes of comparative systematics, it is necessary to define these secondary sculptures structurally. Their nature can often be revealed by TEM examinations of thin

Fig. 6 Seeds of **a–e** *Soldanella carpatica*, **f–i** *Cortusa matthioli*, **j–n** *Hottonia palustris*, and **o–r** *Samolus valerandi*. **a, f, j, o** Ventral view showing narrow (**a, f**) and narrowly elliptic (**j, o**) hilum (*H*) shape. **j, k** Ventral (**j**) and lateral (**k**) view showing suboval seed shape with cavernous lateral faces. **p** Dorsal view showing very weakly keeled edges between dorsal and lateral faces. **c, d** Outer surface layer with reticulate seed pattern with secondary protrusions. **g, h** Reticulate/vesiculose seed pattern with secondary striations and oxalate crystals (*OxC*) visible under cuticle cells (**h**) (*arrows*). **l, q** Weakly reticulate seed pattern with oxalate crystals visible under cuticle cells. **c, i, m, r** Longitudinal sections showing testa (*T*) thickness and presence of oxalate crystals (*arrows*) on the surface of the inner layer. **e, i, m, n, r** Longitudinal sections showing endosperm (*End*) structure with evenly thickened cell walls (**i, r**) and very thin, papery cell walls slightly (**m, n**) or very strongly (**e**) undulate
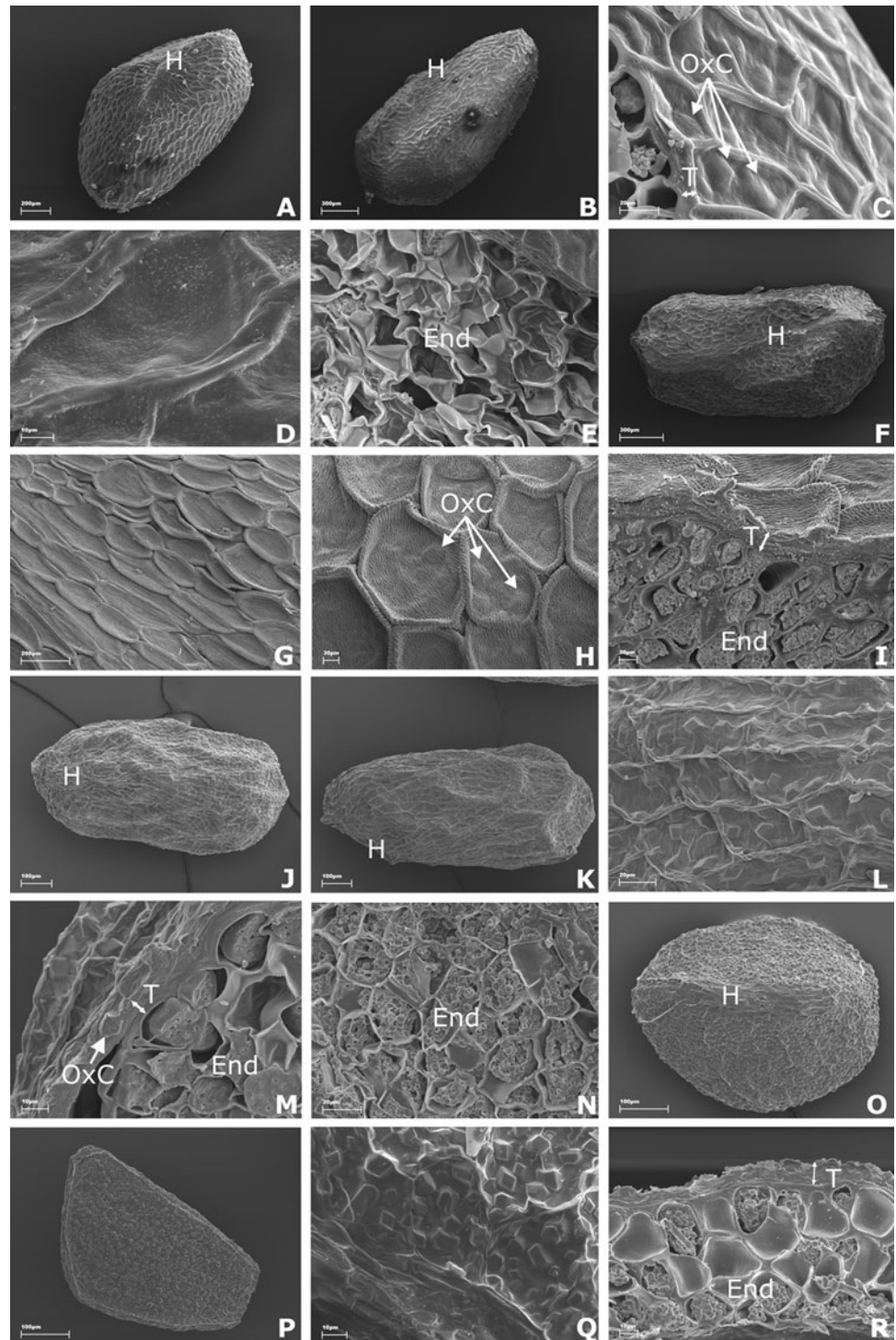
sections (Barthlott 1990). We also conclude that the presence of oxalate crystals in testae, which were observed in seeds of all species examined, should not be used as the feature distinguishing species with angular or subglobose seeds. The other character that in our opinion is of high systematic interest is the endosperm structure, especially the thickenings of the cell walls and their inner surface relief. In analyzing those features, we have shown some similarities in endosperm structure between species from different genera and families not reported earlier. However, as a single source of characters, endosperm structure cannot be expected to resolve all unclear relationships among

the families examined. Such studies should be continued, based on a broader sampling of taxa, and including both molecular and morphological evidence.

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