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Ornamentation of the Testa of Some Eastern Asian Sedoideae (Crassulaceae)

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Abstract Ornamentation of the testa of 25 taxa in the genera *Hylotelephium*, *Aizopsis*, *Rhodiola*, *Orostachys* and *Meterostachys* (Crassulaceae) found in Eastern Asia is studied by SEM. The seed coats of these species can be grouped into four types according to their combined characteristics of cell shape, relief of cell boundaries, the morphology of anticlinal cell wall, and the curvature of outer periclinal wall of exotesta. Following types are recognised: longitudinally costate, reticulate, colliculate and multipapillate.

Key words: Crassulaceae, *Hylotelephium*, *Aizopsis*, *Rhodiola*, *Orostachys*, *Meterostachys*, testa ornamentation, typology

The subfamily Sedeoideae is well known for the morphologic variability of its representatives and associated difficulty of species demarcation. Traditional morphologic characteristics do not always permit precise identification of taxa. Evaluating various micro-morphologic features of seeds might help to overcome this problem with species differentiation.

The arrangement of a seed's epidermal cells is usually of minor taxonomic interest. However in some taxa assessing this feature may facilitate the discrimination of taxa between at species and genus level (Barthlott, 1981). This characteristic has been used successfully for taxonomic purpose in such taxa as the genera *Begonia* (Bouman & Delange, 1982), *Ornithogalum* (Moret *et al.*, 1990), and *Gardenia* (Persson, 1995), the family Saxifragaceae (Kulbaeva, 1992), and others.

Differences in size, epidermal pattern, and shape of these cells and the sculpturing of testa provide benchmarks that can be used to delimit species and genera of the subfamily Sedeoideae. Studies on these characteristics have shown that almost every studied genus of the subfamily has a specific and consistent set of features (Hart & Berendsen, 1980; Knapp, 1994).

The first detailed description of the seed morphology of *Sedum* species was made by H. Fröderström (1930–1936), who used features of seed surface architecture to segregate Central European species into sections and series. Later, data on the ornamentation of the seeds of *Sedum* (s. l.) species appeared in the papers of Clausen (1984) and others.

Applying scanning electron microscopy to the study of the seed coat architec-

ture of *Sedum* (s. l.) has shown the diversity of this characteristic within the genus and its invariability among all species of a genus (Hart & Berendsen, 1980). In light of the results from 40 European and five Asian species these authors recognised three types of exotestal ornamentation–unipapillate, bipapillate, and multipapillate–reflecting the size of the testa cells and the number of papillae they bear.

Another typology of exotesta for the family Crassulaceae was proposed by U. Knapp (1994), who performed an extensive study on the seed micro-morphology of 225 species of 31 genera of the family. He recognised four types of exotesta by using the morphologic features of the anticlinal cell walls and named the categories according to the genera in which the pattern is most common. The four classification are Kalanchoe-type, Echeveria type, Umbilicus-type and Crassula-type (Knapp, 1994).

Information about seed morphology of Eastern-Asian species of Sedoideae is rather limited, only for the following taxa: *Aizopsis aizoon* (L.) Grulich, *A. maximowiczii* (Regel) S. Gontch., *A. middendorfiana* (Maxim.) Grulich ssp. *middendorfiana*, *A. middendorfiana* (Maxim.) ssp. *sichotensis* (Vorosch.) S. Gontch., *A. selskiana* (Regel et Maack) Grulich, *Hylotelephium pallescens* (Freyn) H. Ohba, *H. ussuriense* (Kom.) H. Ohba, *Orostachys japonica* (Maxim.) Berger, *O. spinosa* (L.) C. A. May., *Rhodiola quadrifida* (Pall.) Fisch. & C. A. May. and *Rh. rosea* L. (Hart & Berendsen, 1980; Knapp, 1994; Abankina & Gontcharova, 1997). In the present paper, we describe the micro-morphologic features of 25 taxa of Eastern Asian Sedeoideae and group them according to their characteristic properties.

Materials and Methods

Most of the seeds used in this study were collected from their natural habitats in the Russian Far East (Kamtchatka, Sakhalin, Khabarovsky, and Primorsky Territories), Hokkaido, and Northern Honshu. Some seeds were obtained from the collection of the Botanical Garden of the Far-Eastern Branch of the Russian Academy of Sciences (FEB RAS, Vladivostok), received throughout seed exchange programme or from the Herbariums of the Komarov's Botanical Institute RAS (LE; St.-Petersburg) and the Institute of Biology and Soil Science of the FEB RAS (VLA; Vladivostok). Seeds of the following taxa were examined: *Aizopsis aizoon*, *A. kamtchatica* (Fisch.) Grulich, *A. maximowiczii* (Regel) S. Gontch. *A. middendorfiana* ssp. *middendorfiana*, *A. middendorfiana* ssp. *sichotense*, *A. selskiana*, *Hylotelephium erythrostictum* (Miq.) H. Ohba, *H. pallescens* (Freyn) H. Ohba, *H. sordidum* (Maxim.) H. Ohba, *H. triphyllum* (Haw.) Holub, *H. verticillatum* (L.) H. Ohba, *H. viviparum* (Maxim.) H. Ohba, *H. ussuriense* (Kom.) H. Ohba, *Meterostachys sikokiana* (Makino) Nakai, *Orostachys iwarenge* (Makino) H. Hara, *O. japonica*, *O. malacophylla* (Pall.) Fisch., *O. spinosa* (L.) C.A. May., *Rhodiola alsia* (Fröd.) Fu, *Rh. angusta* Nakai, *Rh. chrysanthemifolia* (Léveillé) Fu, *Rh. integrifolia* Raf., *Rh. ishidae* (Miyabe et Kudo) H. Hara, *Rh. rosea* L. var. *rosea*, *Rh. rosea* var. *sachalinense* (Boriss.) S. Gontch. The names of the

species are in accordance with the nomenclature of Borissova (1939), Ohba (1978, 1995), Grulich (1984), and Gontcharova (1999).

The terminology used to describe seed morphology and architecture is that of Barthlott (1981) and Stern (1992).

For scanning electron microscopy seeds were mounted on holders by using double-sided adhesive tape and sputtered with gold. The samples were examined and photographed by using a JEOL S 5800 microscope.

Results

The exotesta of all studied species of Eastern Asian Sedoideae consists of regular longitudinal rows of isodiametric or transversely oblong, quadrate to hexagonal cells. The cells, except for those near the raphe, are uniform in appearance throughout the entire surface and are isodiametric as a rule. The cells forming raphe are longitudinally oblong (Abankina & Gontcharova, 1997). Seeds of studied taxa are characterised as follows.

Aizopsis Grulich

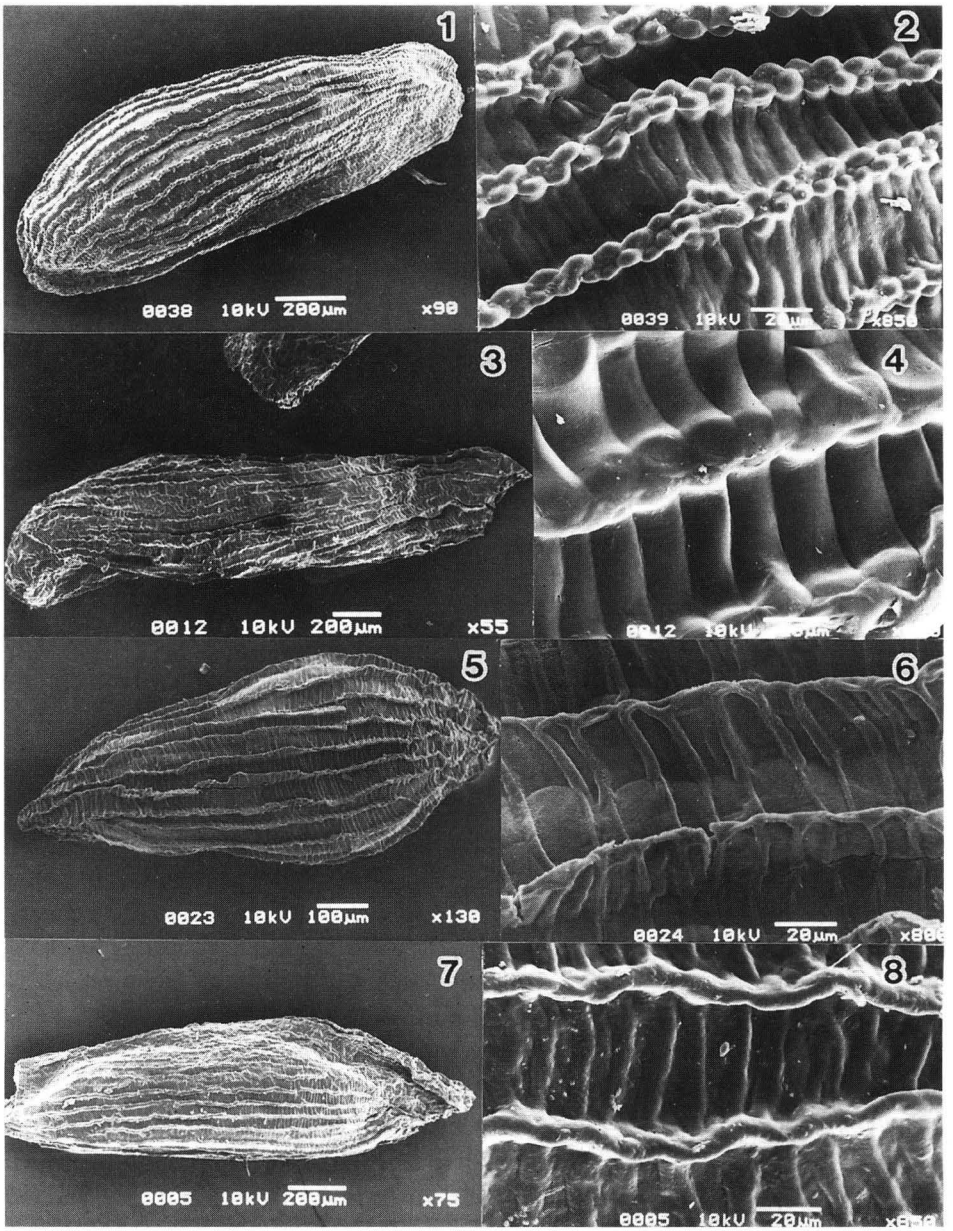
The seeds are small, dark-brown, and ovoid to slender-ellipsoidal in shape slightly flattered with obviously narrowed micropylar and rounded chalazal ends. Their surface is covered with longitudinal undulated broad ribs formed by the entirely free or partially fused and raised distal ends of the exotesta cells.

Two types of exotesta were revealed in the genus:

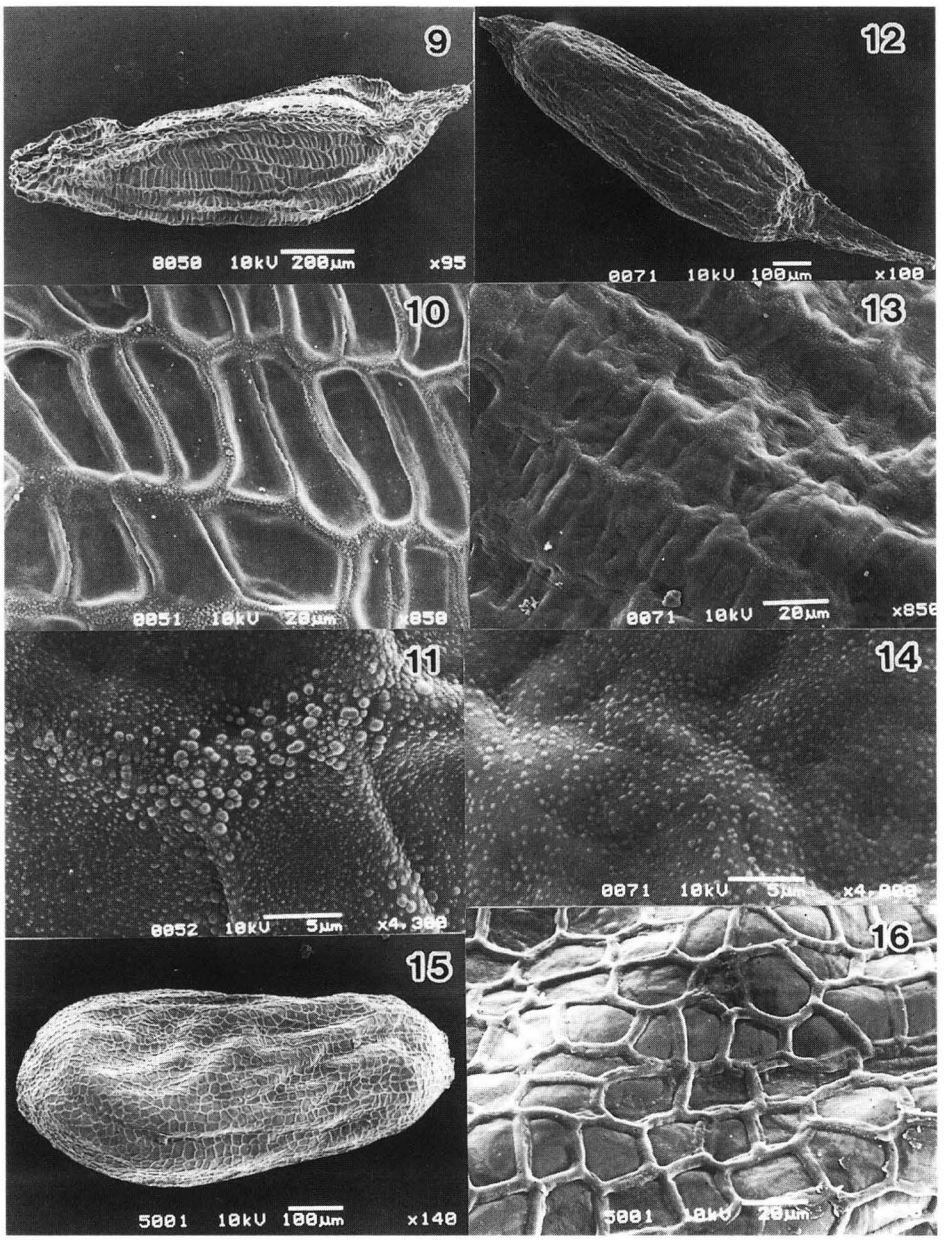
1. Cells that are at least three or more times longer than wide. The seed surface looks longitudinally laticostate. The distal ends of the cells are always raised and thickened, and their apexes are rounded. These ends are either entirely free or partially fused which determines the more or less undulated appearance of the ribs. The proximal anticlinal boundaries are channelled. The extent of fusion of the distal anticlinal walls and the degree of their thickening are species-specific. The outer periclinal cell wall is slightly convex. The cells near the micropyle may have one or two papillae, which are small nipple-like protuberances on the periclinal cell wall. Species: *A. aizoon*, *A. kamtchatica*, *A. maximowiczii*, *A. middendorfiana* ssp. *middendorfiana*, *A. middendorfiana* ssp. *sichotense*.

2. Cells are less than three times longer than wide. The distal ends are not raised. Anticlinal boundaries are pronounced, and channelled. The periclinal walls have two papillae near the distal ends. Sometimes an additional papilla occurs in the middle of the cell. Because the presence of these papillae, the seed surface looks faintly papillate. This type of exotesta was found only in one species, *Aizopsis selskiana*.

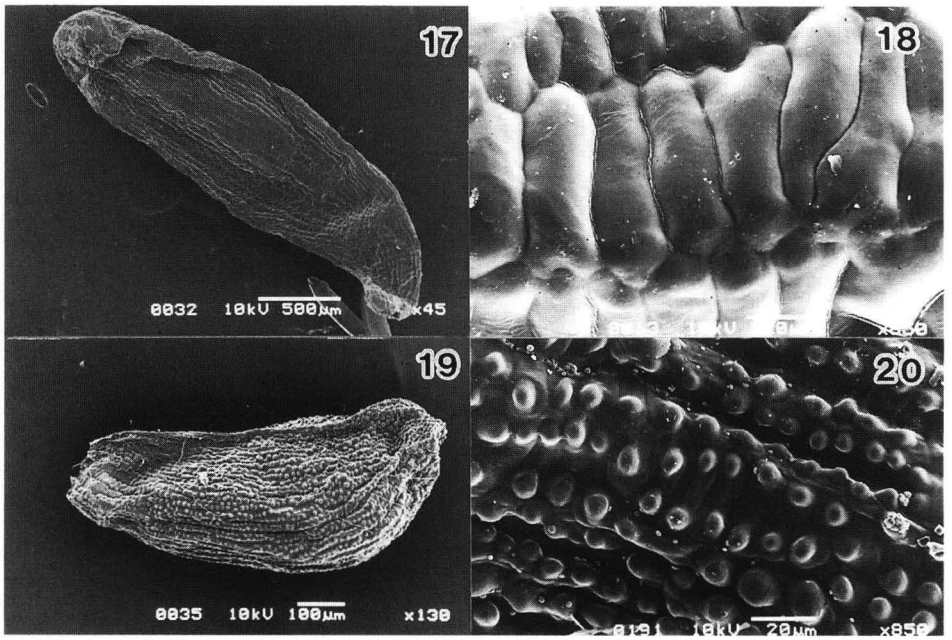
Studied species do not possess secondary sculpture of the cell wall.



Figs. 1–4. Laticostate type of testa ornamentation. 1 & 2. *Aizoopsis kamtschatica*; 1. Whole seed; 2. Detail of seed coat ornamentation. 3 & 4. *Rhodiola rosea*; 3. Whole seed; 4. Detail of seed coat ornamentation. Figs. 5–8. Tenuicostate type of testa ornamentation. 5 & 6. *Hylotelephium ussuriense*; 5. Whole seed; 6. Detail of seed coat ornamentation. 7 & 8. *Rhodiola integrifolia*; 7. Whole seed; 8. Detail of seed coat ornamentation.



Figs. 9–16. Reticulate type of testa ornamentation. 9–11. *Hylotelephium verticillatum*; 9. Whole seed; 10. Detail of seed coat ornamentation; 11. Secondary structure of the cell wall. 12–14. *Meterostachys sikokiana*; 12. Whole seed; 13. Detail of seed coat ornamentation; 14. Secondary structure. 15 & 16. *Orostachys malacophylla*; 15. Whole seed; 16. Detail of seed coat ornamentation.



Figs. 17 & 18. Colliculate type of testa ornamentation in *Rhodiola ishidae*; 17. Whole seed; 18. Detail of seed coat ornamentation. Figs. 19 & 20. Multipapillate type of testa ornamentation in *Aizopsis selskiana*; 19. Whole seed; 20. Detail of seed coat ornamentation.

Hylotelephium H. Ohba

The seeds are small, ellipsoidal (*H. ussuriense*) or slender-ellipsoidal (*H. pallescens*), and light brown or yellowish brown (Abankina & Gontcharova, 1997). Exotesta cells are at least three times longer than wide. Cell boundaries can be pronounced or not pronounced. The anticlinal cell walls are equally thickened and completely fused. The proximal anticlinal walls are straight or slightly curved. The periclinal walls are slightly concave.

Two types of exotesta were revealed in the genus:

1. Those in which the distal walls are much higher than the proximal ones. The seed surface is tenuicostate. Species: *H. ussuriense*, *H. erythrostictum*, and *H. verticillatum*.

The secondary sculpture of the cell walls is micro-papillate.

2. Those in which the distal and proximal anticlinal walls are raised approximately equally. The seed surface is reticulate. Species: *H. pallescens*, *H. sordidum*, *H. triphyllum*, and *H. viviparum*.

The secondary sculpture of the cell walls is not pronounced (*H. triphyllum*) or it is micro-papillate. Species: *H. verticillatum* and others (Fig. 11).

Orostachys Fisch. ex Berger

The seeds are small, ellipsoidal or ovoidal, and yellowish-brown with narrowed micropylar and rounded chalazal ends.

Exotesta cells are 1.5 to 2 times longer than wide. The seed surface is reticulate. Anticlinal cell walls are completely fused, equally thickened and raised. The cell boundaries are not clearly detectable (slightly channelled). The periclinal walls are slightly concave (*O. japonica* and *O. spinosa*) or flat (*O. iwarenge* and *O. malacophylla* [Figs. 15 & 16]).

Micropapillate secondary sculpture of the cell walls was evident in all studied species.

Meterostachys Nakai

The seeds are small, slender-ellipsoidal, and yellowish brown with narrowed micropylar and chalazal ends.

The exotesta is composed of oblong quadrangular-hexagonal cells that are approximately three times longer than wide. The cell boundaries are not pronounced. Anticlinal cell walls are completely fused and equally thickened. The seed surface is faintly reticulate because of a thick cuticle.

The cell walls have micro-papillate secondary sculpture (Fig. 14). Species: *Meterostachys sikokiana*.

Rhodiola L.

These seeds are bigger than those in all other genera, slender-ellipsoidal, dark brown and often winged.

The exotesta consists of longitudinal rows of transversely oblong cells. The secondary sculpture was not pronounced in any studied species. Three types of exotesta were revealed in this genus.

1. Those with cells that are hexagonal, and at least three times longer than wide. The distal cell ends are raised and thickened have flattered apexes, and are entirely fused; however, their boundaries are pronounced and appear as grooves. The seeds surface is longitudinally laticostate. The periclinal walls are convex. Species: *Rh. angusta*, *Rh. chrysanthemifolia*, and *Rh. rosea* var. *rosea* (collected from Europe mainly).

2. Those in which cells are hexagonal, and at least three times longer than wide, but in which the distal cell ends are raised but not thickened. Distal anticlinal cell walls are entirely fused; their boundaries are not pronounced clearly. The proximal anticlinal cell walls are slightly raised, not thickened. Proximal cell boundaries appear as grooves or are not pronounced. The seeds surface is tenuicostate. The periclinal wall is slightly concave or straight. Species: *Rh. integrifolia*, *Rh. rosea* var. *rosea* (collected from Far East mainly), and *Rh. rosea* var. *sachalinense*.

3. Those in which the cells are 4-polygonal, 1.5 to 3 times longer than wide.

Cell boundaries are well pronounced, grooves-like, straight or irregularly curved. Periclinal wall is convex. Seed surface is colliculate. Species: *Rh. ishidae*, *Rh. alsia*.

Discussion

As noted previously, two classification systems of the seed coat architecture of Crassulaceae have been suggested (Hart & Berendsen, 1980; Knapp, 1994). Because these authors used different approaches and terminology to circumscribe exotesta types the assignments resulting from application of their classification schemes cannot be compared and some of the terms do not seem appropriate. For example, Hart and Berendsen (1980) based their classification on such characteristics as the number and placement of papillae on the exotesta cell surface. This schema seems quite logical for unipapillate and multipapillate seeds, but from my point of view the group of bipapillate seeds has nothing to deal with this character. I hardly can compare with papillae raised anticlinal cell walls, which form ribs on the seed surface (Fig. 2). Nevertheless, in my classification of seed coats types of the Eastern-Asian Sedoideae I basically accepted their scheme.

There are difficulties associated with applying the classification system presented by Knapp (1994). This system relies on a large number of morphologic characteristics, but the names of reflect the genus in which a particular type of exotesta is most common. Knapp placed *Rhodiola* and *Orostachys* together in one group. However, from my point of view Far Easterns representatives of these genera have markedly different exotestal architecutre (Figs. 4, 8 & 16).

My study on the seeds micromorphology of 25 Eastern Asian taxa of Sedoideae shows that in the vast majority of the studied taxa, the exotesta has an easily discernible cellular arrangement (*Rhodiola*, *Orostachys*, *Aizopsis* and *Hylotelephium*). However, in the genus *Meterostachys* the exotestal arrangement is masked by cuticle. The entire seed surface has a uniform architecture except cells situated near raphe and cells, which form it. Often these cells have different appearance.

Cell shape, relief of cell boundaries, the morphology of anticlinal cell wall, and the curvature of outer periclinal wall of exotesta are the main features that characterise different taxa. Several types of anticlinal cell wall were revealed in the studied taxa: raised or channelled, and equally or unequally thickened. The periclinal cell wall was concave or convex, and either had or lacked papillae-like structures.

The particular ornamentation of the seed characterises almost every species and subspecies, and this attribute is what makes the micro-morphology of the seed so taxonomically valuable. Data obtained in the present study on the seed coat morphology of Eastern Asian Sedoidea confirm this statement. Of all studied species, only *Rhodiola rosea* varies in this feature. European specimens of this taxon was characterised in laticostate seed surface, while those originated from the Far East had tenuicostate seed surface. There are about 50 synonyms of *Rhodiola rosea* (Ohba 1981), and per-

haps some of them represent independent taxa at the subspecific level at least. However, additional characteristics are required to substantiate this hypothesis.

Almost every section and series in the genera *Aizopsis*, *Hylotelephium* and *Orostachys* sect. *Orostachys* is uniform in morphological characters of spermoderma while in the sect. *Rhodiola* of the genus *Rhodiola* these characters vary widely, high diversity of exotestal types within *Rhodiola* perhaps reflects an artificial nature to this taxon.

The seed coats of the studied species can be grouped into four types according to their combined characteristics:

1. Longitudinally costate–The distal cell ends or walls are raised and always higher than proximal ones.

1.1. Laticostate–The distal cell ends are raised and much thicker than the proximal one, their apexes are rounded or slightly flattered, in some species proximal cell boundaries are not pronounced or channelled. Distal anticlinal walls are fused only in the lower part (*Aizopsis* [Figs. 1 & 2]) or entirely fused (*Rhodiola* [Figs. 3 & 4]).

Species: *Aizopsis aizoon*, *A. kamtchatica*, *A. maximowiczii*, *A. middendorfiana* ssp. *middendorfiana*, *A. middendorfiana* ssp. *sichotense*, *Rhodiola angusta*, *Rh. chrysanthemifolia*, and *Rh. rosea* var. *rosea* (collected from Europe mainly).

1.2. Tenuicostate (Figs. 5–8)–The distal and proximal anticlinal cell walls are raised but not thickened. The distal walls are several times higher than are proximal ones and are entirely fused.

Species: *Hylotelephium ussuriense*, *H. erythrostictum*, *H. viviparum*, *Rhodiola integrifolia*, *Rh. rosea* var. *rosea* (collected from Far East mainly), *Rh. rosea* var. *sachalinense*.

2. Reticulate (Figs. 9–16)–The proximal and distal anticlinal cell walls have equal thickness and height. The periclinal cell wall is slightly concave; cell boundaries are either channelled or not discernible.

Species: *Hylotelephium pallescens*, *H. sordidum*, *H. triphyllum*, *H. verticillatum*, *Orostachys iwarenge*, *O. japonica*, *O. malacophylla*, *O. spinosa*, and *Meterostachys sikokiana*.

3. Colliculate (Figs. 17 & 18)–The periclinal cell wall is convex; cell boundaries are channelled.

Species: *Rhodiola ishidae* and *Rh. alsia*.

4. Multipapillate (Figs. 19 & 20)–The periclinal walls have two papillae placed near distal ends. Some times an additional papilla is placed in the middle of cell. The distal walls are not raised.

Species: *Aizopsis selskiana*.

As noted previously, two classification systems of the seed coat architecture of Crassulaceae have been suggested (Hart & Berendsen, 1980; Knapp, 1994). Because these authors used different approaches and terminology to circumscribe exotesta types the assignments resulting from application of their classification schemes can-

not be compared and some of the terms do not seem appropriate. For example, Hart and Berendsen (1980) based their classification on such characteristics as the number and placement of papillae on the exotesta cell surface. This schema seems quite logical for unipapillate and multipapillate seeds, but from my point of view the group of bipapillate seeds has nothing to deal with this character. I hardly can compare with papillae raised anticlinal cell walls, which form ribs on the seed surface (Fig. 2). Nevertheless, in our classification of seed coats types of the Eastern-Asian Sedoideae I basically accepted their scheme.

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