

MORPHOMETRIC DATA ABOUT SEED COAT ORNAMENTATION WITH TAXONOMIC CONSIDERATIONS IN *JOVIBARBA HEUFFELII* (CRASSULACEAE) POPULATIONS FROM SOUTHERN CARPATHIANS

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The seed coat ornamentation pattern was generally used in the systematics of other Crassulacean genera. Previous work showed a considerable variation of this character within the genus *Jovibarba* Opiz. In this paper it was investigated by optical and electron microscopy SEM the seed coat ornamentation constancy among 9 Romanian populations of *Jovibarba heuffelii* (Schott) A&D Löve to evaluate the infraspecific reliability of this character. Morphometric data and SEM micrographs of a representative seed coat and ornamentation details are presented about the Romanian material, showing homogeneity of seed size, shape and pattern. The seed coat shows a more or less irregular network of crisscrossing longitudinal and transversal broken ridges, without constant cell pattern or any wart-like protuberances but fitting the general characters of Knapp's "Leitermodel". Although quite homogeneous within the populations examined by us, the Romanian material departs from the "Jovibarba Type" a.3. pattern reported by Knapp, being rather similar to the a.3. "Net structure" he described in *Orostachys japonicus*, but with sinuous longitudinal ridges exceeding 20 per seed. The dissimilarity with Knapp's "Jovibarba Type" existing in the Romanian populations suggests that the seed coat morphology has a considerable intraspecific variation between distant populations, a fact that could not be neglected when assessing its taxonomic value even at infraspecific level.

Key words: *Jovibarba heuffelii* (Schott) A&D Löve, seed coat morphometry, SEM, systematics.

INTRODUCTION

Jovibarba heuffelii (Schott) A&D Löve belongs to the polymorphic family of Crassulaceae. Its infrafamilial classification is disputed and it was assigned to several supraspecific taxa, based on various morphological characters. It is generally accepted that it is closely related to *Sempervivum* s.s., being treated either as an independent (sister) genus, or as infrageneric units of various levels subordinated to *Sempervivum* s.l.

The seed coat (lat. samen testa) ornamentation often has features usable as taxonomic criterion and its pattern was successfully used by other authors as taxonomic criterion in the systematics of various other plant groups.

Studying the seeds of some *Jovibarba heuffelii* (Schott) A&D Löve specimens from the Banat and Domogled Mountain it was unexpectedly realized

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that the ornamentation pattern differs towards that reported by Knapp in his 1994 comprehensive monograph on Crassulacean seed coat ornamentation (11). This observation has raised the question whether the pattern of seed coat ornamentation could indeed be used as a reliable taxonomic criterion within the genus *Jovibarba* and for *Jovibarba heuffelii* (Schott) A&D Löve populations. Moreover, within the Sempervivoideae there are still questions about the validity of samens testa ornamentation as taxonomic criterion, as in Knapp's classification one *Sempervivum* s.s. species groups together with *Jovibarba* species.

Barthlott, discussing in his work published in 1981 (2) some aspects referring to the evolution and the systematic applicability of the seed surface characters in plants, argued in favour of the use of seed coat ornamentation characters as taxonomic criteria in plant systematics.

The ornamentation of the seed testa in the family Crassulaceae shows a wide variation suggesting that it might be used as a taxonomic criterion in this family. Despite this promising attribute, seed coat characters have not received the appropriate attention until quite recently because the seeds of most species are only about 1 mm long or even smaller and are therefore difficult to observe and measure.

Occasional references to Crassulacean seeds could be found in literature, mostly as illustrations than as descriptions.

Froderstrom (7) for the first time during the 30's described the seeds of a large number of *Sedum* species and used them for systematic purposes, recognizing papillose, mamillate, glabrous, and smooth seeds.

In 1956, Ravarut (14) gave some brief descriptions of features of the seed without using them as diagnostic characters in Romanian Crassulaceae, with no illustration.

Clausen in his 1959 and 1976 works (5, 6) described in detail the seed coat ornamentation of several North American *Sedum* species distinguishing glabrous, smooth, alveolate, reticulate, verrucose, papillate, longitudinally ribbed or ridged seeds and seeds with a scalariform ornamentation.

Brouwer and Stahlin, in their 1975 handbook of seed-science, described glabrous, ribbed or sulcate seeds in *Sedum*.

In 1977, Maire (12) described in some North African species of *Sedum* glabrous, verrucose, papillose seeds or seeds decorated with longitudinal striations. In the same year, Meikle (13) distinguished in some Cyprian *Sedum* species rugulose-papillose seeds and seeds having longitudinal ribs.

Hart (1978) studied by scanning electron microscopy (SEM) the seed micromorphology of the acre-group species and of the series *Rupestris* Berger. He distinguished seeds with a reticulate, reticulopapillose, papillose with the papillae in parallel longitudinal paired rows, and costate testa. He hypothesized that the papillose seeds with the papillae arranged in longitudinal paired rows could be considered as a primitive form of the seeds with a costate testa.

A later study from 1980 published by Hart't and Berendsen (9) shed new light on the ornamentation of the seed coat in *Sedum* s.l. applying electron microscopy techniques (SEM) to show that the seed coat ornamentation is indeed a valid criterion in *Sedum* systematics.

A 1994 comprehensive work of Knapp (11) studying by scanning electron microscopy (SEM) the seed coat ornamentation in a great number of species in several Crassulacean genera shows a considerable variation of this character within the family, but also within the genus *Jovibarba* Opiz. Using morphological features of the anticlinal cell walls of the seed coat he described four general categories in the family Crassulaceae and named them by the genus in which the pattern characterizing each category occurred most frequently thus leaving room for confusions and misinterpretations.

In this work, Knapp described an archetype called the "Leitermodel" within which *Jovibarba heuffelii* was assigned a specific model called "Jovibarba" type.

Abankina, M. N., Gontcharova, S. B. in 1997 (1) described some morphological and anatomical characters of seeds in some *Sedum* species from the Russian Far East.

Later, a 1999 SEM study by Gontcharova in Eastern-Asian Sedoideae (3), brought new data on the characters of samens testa in this large and polymorphic group within the family Crassulaceae.

MATERIAL AND METHODS

A number of at least 20 mature fully ripen seeds per plant from individual plants of *Jovibarba heuffelii* A&D Löve belonging to populations from locations in the Romanian Southern Carpathians were investigated. Most of the specimens were collected by the authors from populations in their natural habitat (Fig. 4). Some seeds were obtained from the collections of the herbarium of the Biology Institute of the Romanian Academy (BUCA), but they were not included in the morphometric and statistical study due to their insufficient number. Nevertheless, these specimens were used for visual comparison of exotestal morphology.

The similarity of morphological characters was first assessed by examination with conventional light microscopy using either stereomicroscopes or microscopes (Nikon E200 Nikon TE300 Nikon E600) and auxiliary light sources for direct illumination. The measurements were made by direct use of ocular and stage micrometric scales and also using a CCD camera attached to the Nikon SMZ1500 stereomicroscope and the provided image acquisition software.

From each plant 3 representative seeds were dehydrated in ethyl alcohol series, dried in air, critical point desiccated and coated with gold after being affixed with double-sided adhesive tape to aluminum holders. The selected seeds were photographed after being examined under SEM.

The parameters measured are described below as depicted in Figure 1:

1. the total maximal width of the seed,
2. the total length of the seed, measured between the two apices (ends) of the seed,
3. the width of the central (medial) longitudinal fold (costa),
4. the width of the space between the 2 paramedial longitudinal folds (costae), including the central (medial) longitudinal fold (costa) – comprising the 2 paramedial sulci and the medial costa, but excluding the 2 paramedial costae. Due to the sinuosity and irregularity of the longitudinal costae, this parameter was measured at the midpoint of the distance between the 2 apices (ends) of the seed,
5. the total number of costae on both sides of the seed.

RESULTS AND DISCUSSIONS

To answer the question of infraspecific of seed testa ornamentation variability, the samens testa of several populations of *Jovibarba heuffelii* (Schott) A&D Löve were comparatively investigated. The present work investigated the constancy of seed coat ornamentation among several local populations of *Jovibarba heuffelii* (Schott) A&D Löve in order to evaluate the reliability of this character at the infraspecific level, as it was suggested that at the infrageneric/supraspecific level it could be a reliable taxonomic character.

A number of 20–33 seeds from individual plants of *Jovibarba heuffelii* (Schott) A&D Löve from 9 locations in the Romanian Southern Carpathians were investigated. Most of the specimens were collected by the authors from populations in their natural habitat, a few seeds being obtained from the collections of the herbarium of the Institute of Biology Bucharest of the Romanian Academy, but due to their insufficient number they were used only for visual comparison of exotestal morphology and confirmed the conclusions of our study.

There were examined seeds from 22 individual plants collected at the following locations: Herculane – 3 ex., Domogled Mt. – 8 ex., Balta Cerbului/V. Cernei – 1 ex. Arjana Mt. – 2 ex., Buila Mt. (Santinela Cheii) – 2 ex., Barbatesti, VL (in cult) 1ex, Caineni/Valea Oltului 1 ex., Cozia Mt. (Turnu Monastery) 3 ex., Bucegi Mt. 1ex.

Preliminary examination with light microscopy of the Romanian material showed a high homogeneity of seed, shape and pattern, observations further confirmed by SEM examination and by measurements.

The seeds are small, brown varying in hue from light brown to dark brown, pyriform, ellipsoidal to ovoid, flattened, with a rounded chalazal extremity and with a more pointed micropylar end, not presenting any corona or a very vague pseudocorona at the micropylar end.

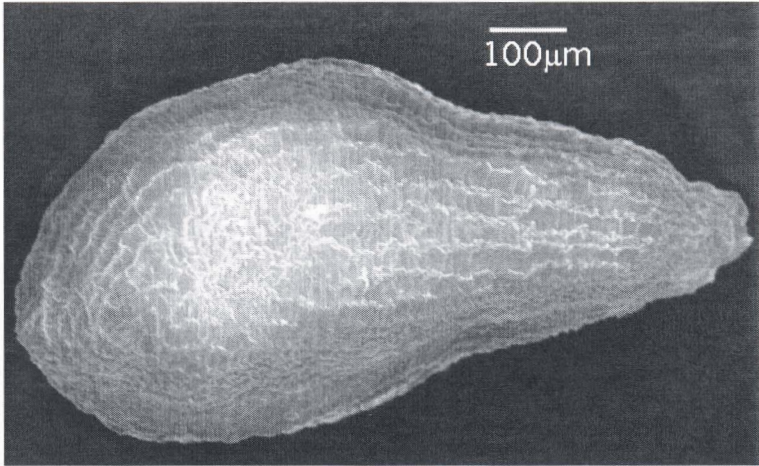


Fig. 1. SEM micrograph of a hole seed of *Jovibarba heuffelii* (Schott) A&D Löve; scale bar approx. 100 µm.

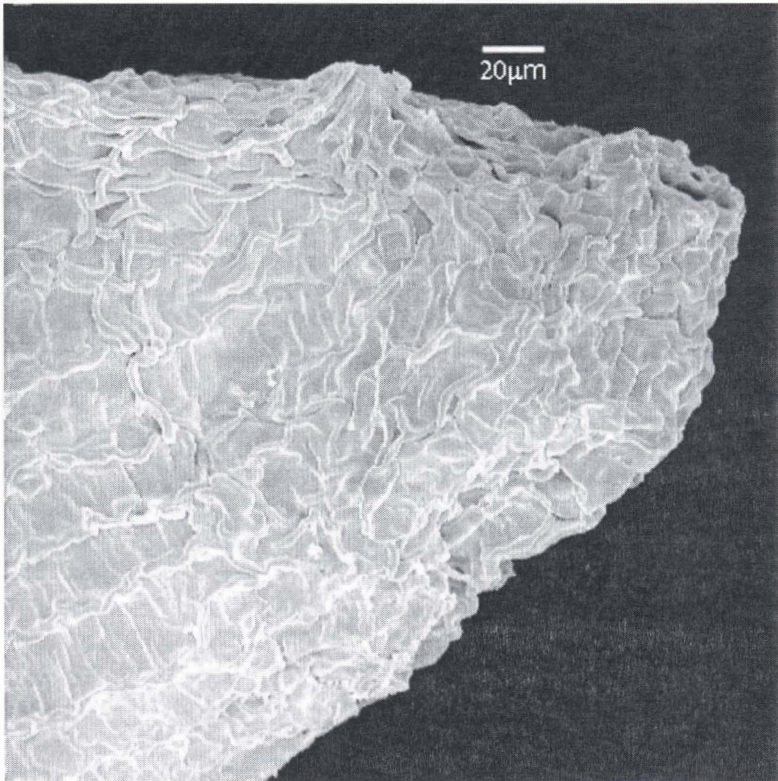


Fig. 2. SEM micrograph detail of a seed apex of *Jovibarba heuffelii* (Schott) A&D Löve lacking any corona; scale bar approx. 20 µm.

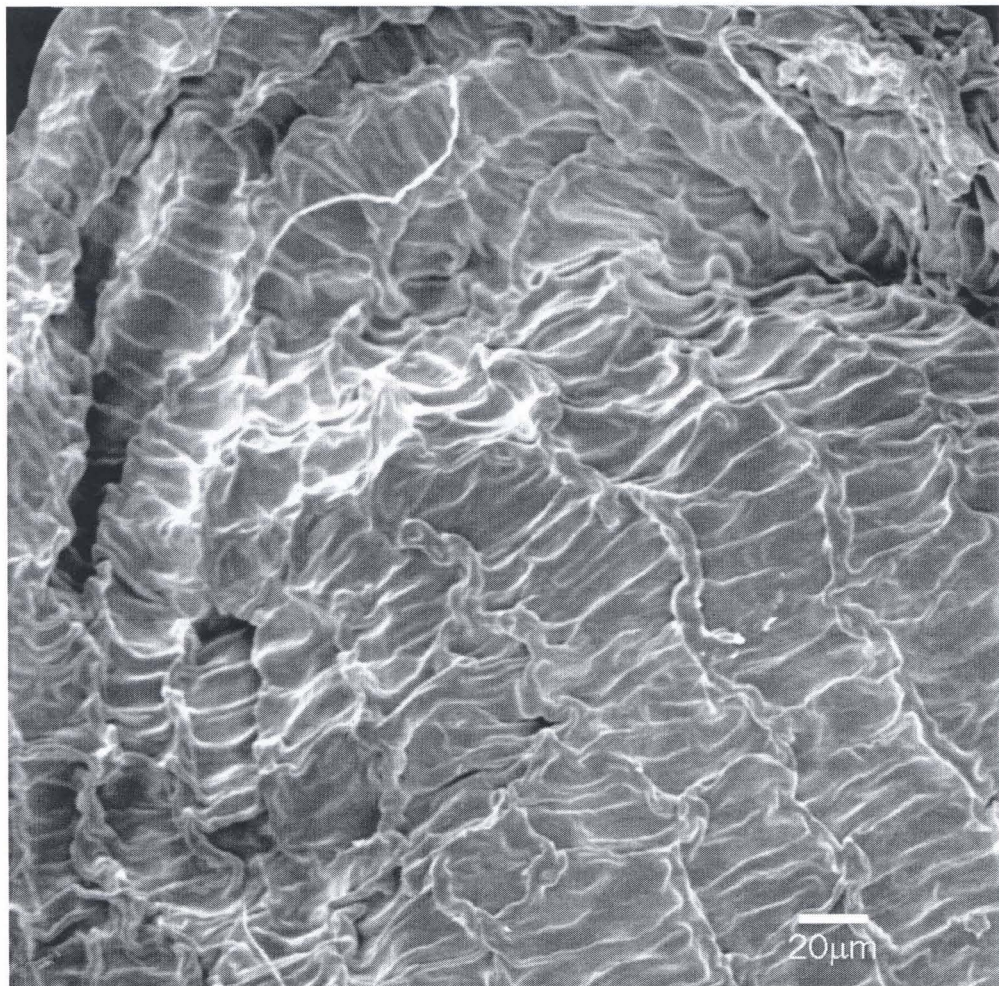


Fig. 3. SEM micrograph detail of a seed surface of *Jovibarba heuffelii* (Schott) A&D Löve showing longitudinal costa and transversal ridges; scale bar approx. 20 µm.

The seed coat in the *Jovibaba heuffelii* (Schott) A&D Löve material examined in this paper presents a more or less irregular network of longitudinal broken sinuous ridges intersected by transversal ones, without constant cell pattern or any wart-like protuberance (see Figs. 1, 2 and 3 for SEM micrographs of representative seed coats). This ornamentation pattern fits well the general characters of the "Leitermodel" described by Knapp U. (1994).

Morphometric data are presented in Table 1, showing the variability of some parameters measured for the seed coat ornamentation.

Table 1

Morphometric data describing the variability of some parameters measured for the seed coat ornamentation in *Jovibarba heuffelii* (Schott) A&D Löve material from 9 Romanian populations
n = 413

Parameter No.	Parameter measured *	Average value (μm)	Standard error; n = 413
1	total seed width	487	1.67
2	total seed length	989	1.18
3	width of the central (medial) longitudinal fold (costa)	2.2	0.014
4	width of the space between the 2 paramedial longitudinal costae	50	0.94
5	total number of costae on both sides of the seed	22	0.443

*brief descriptions of parameters are given in the Material and Methods section under the appropriate parameter number

The width of the space between the 2 paramedial longitudinal folds (costae) measured at the midpoint of the distance between the 2 apices of the seed, proved to be very difficult to measure reliably due to the sinuosity and irregularity of the longitudinal costae which were not strictly parallel as seen in Figs. 2 and 3. This parameter was the only one that showed such problems and variation in the same individual that make us believe it should be disregarded. The total seed width was also quite variable but measurable, further affecting the space between two consecutive costae.

Nevertheless, the pattern found in the Romanian material, although quite homogeneous within the populations examined in this paper, departs from the "Jovibarba Type" a.3. pattern reported by Knapp (11) p164 & 172. The pattern described in this paper is rather similar to the a.3. "Net structure" described by Knapp in *Orostachys japonicus*, but with sinuous transversal and longitudinal ridges of dissimilar height and the longitudinal ones being more numerous than 20 per seed (see Figures 1, 2 and 3). The dissimilarity with Knapp's "Jovibarba" type, found in the Romanian populations in this paper, suggests that the seed coat morphology has a considerable intraspecific variation between distant populations,

a fact that cannot be neglected when considering its taxonomic value even at infraspecific level.

CONCLUSIONS

The pattern found in the Romanian material, although quite homogeneous within the populations examined in this paper, departs from the "Jovibarba Type" a.3. pattern reported by Knapp (11), being rather similar to the a.3. "Net structure" described by him in *Orostachys japonicus*. The dissimilarity with Knapp's "Jovibarba" type found in this paper in the Romanian populations suggests that the seed coat morphology has a considerable intraspecific variation between distant populations, which cannot be neglected when considering its taxonomic value even at infraspecific level.

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