

Comparative analysis of seed morphology in genus *Scutellaria* L.

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Abstract

The genus *Scutellaria* L. comprises about 400 - 500 species, some of which are of practical value due to their biological activities. To prevent misidentification and contamination of plant material harvested from species of this genus, knowledge of the morphological and micromorphological characters of the seeds has proved useful. The present paper aims to perform a morphological and micromorphological characterization of seeds belonging to 12 taxa of the genus *Scutellaria* L., with the idea of completing the literature data and discussing the importance of these characters. The seeds of the analysed taxa showed different colours (yellow, black, brown) and ornamentations (generated by the arrangement and shape of the epidermal papillae). In some taxa, the seeds were glabrous (*Scutellaria baicalensis* Georgi, *S. resinosa* Torr., *S. suffrutescens* S. Watson., *S. incana* Spreng., *S. integrifolia* L.), others showed tector hairs concentrated in the apical region of the epidermal papillae (*S. altissima* L.) or distributed over the entire surface (*S. orientalis* L., *S. supina* L., *S. orientalis* L. var. *pinnatifida* Rchb.), covering the whole or part (*S. pontica* K. Koch.) of the seed surface. Sessile glands were observed in *S. galericulata* L., among the papillae, whereas *S. alpina* L., presented stellate hairs. Seed sizes also varied between the taxa. The results obtained confirm the taxonomic importance of the analysed features but indicate that the correct identification of *Scutellaria* species cannot be performed based only on seed characteristics, requiring additional research and observations.

Keywords: dispersals; morphology; micromorphology; seeds; *Scutellaria*; taxonomy

Introduction

Scutellaria L. is a cosmopolitan and morphologically isolated genus belonging to the family Lamiaceae (Hedge, 1992) subfamily Scutellarioideae (Harley and Heywood, 1992). Within this genus, the taxa are divided into two subgenera, namely the subgenus *Scutellaria* with five sections: 1. section *Scutellaria* (with 34 species groups classified according to geographical distribution and morphological characteristics of seeds); 2. section

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Anaspis (Rech. f.) Paton; 3. section *Salviifoliae* (Boiss.) Edmondson; 4. section *Salazaria* (Torrey) Paton; 5. section *Perilomia* (Kunth) Epling *emend.* Paton and subgenus *Apeltanthus* (Nevski ex Juz.) Juz. *emend.*, Paton, within which taxa are classified into two sections: 1. *Apeltanthus* and 2. *Lupulinaria* A. Hamilton, the latter section is divided into subsections *Lupulinaria* (with four species groups) and *Cystaspis* Juz. (Paton, 1990).

This natural and well-defined genus (Leonard, 1927) comprises probably 400 - 500 species (Salimov *et al.*, 2021), of which 13 species were described in “*Flora Europaea*”; for Romania Săvulescu (1961) described eight native species, while Borza (1968) mentioned three species, and Coldea and Ursu (2019) mentioned *Scutellaria altissima* L. and *S. galericulata* L., the latter exhibiting limited distribution. In the Republic of Moldova are reported exotic and autochthonous species (Pânzaru and Sirbu, 2014) as well as an endangered species, *S. supina* L. widespread on the Podolian Plateau (Hrușca) and included in the Red List of rare plant species of this country (Pânzaru *et al.*, 2002). In different geographical regions, new species of the genus are periodically discovered, for example *S. holguinensis* I.E. Méndez, *sp. nov.* (Santos and Tellez 2019), *S. anatolica* M. Cicek & O. Ketenoğlu, *sp. nov.* (Cicek and Ketenoğlu 2011), *S. × ketenoglui* M. Cicek & Yaprak (Cicek and Yaprak 2011), *S. tenasserimensis* A. J. Paton, *sp. nov.* and *S. khaoyaiensis* A. J. Paton, *sp. nov.* (Paton *et al.*, 2016).

The primary center of species diversity of *Scutellaria* L. is located in Central Asia (Hindukush and Pamir-Alay Mountains), while the secondary centers are considered Eastern Mediterranean and the Andes Mountains. Species of the genus migrated to Europe and North America (early mid-Tertiary) from Asia via Beringia (Paton, 1989). *Scutellaria* species are predominantly specific to the Northern Hemisphere (Salimov *et al.*, 2021), and they occur in different habitats, such as grasslands, open forests, scrublands (Lord, 2003), and even in high alpine habitats (Paton, 1989). American species occur in xerophytic or wet habitats, along roadsides, and in lowland forests (Felter and Lloyd, 2001), while North Chinese species occur in shrubland habitats, on sunny slopes, in forests and grasslands with a cold-dry climate (Minareci and Pekönür, 2017). Australian and New Zealand species occur in disturbed and flooded areas (Minareci and Pekönür 2017; Paton, 1990).

Some *Scutellaria* L. species exhibit therapeutic activity due to biologically active compounds that are secondary metabolites such as flavonoids, phenolic acids, phenylethanoid glycosides, and terpenes (neoclerodanes, iridoids) which act both individually and in combination (Georgieva *et al.*, 2021) being valuable in terms of possible practical uses; and due to this fact, they have been used in herbal medicine since ancient times in countries such as Nepal, India, Turkey, China, Japan, North America, Uzbekistan (Shen *et al.*, 2021). Thus, plants belonging to the species *S. baicalensis* Georgi considered the most studied species of the genus (Malikov and Yuldashev, 2002), have been used in the therapy of skin disorders, allergies, ulcers, herpes, bronchitis, asthma, pneumonia, intestinal and respiratory infections (Shang *et al.*, 2010; Hempen and Fischer, 2009). Biologically active compounds of these species exhibit anticarcinogenic, antibacterial, anxiolytic, anticonvulsant, neuroprotective (Shang *et al.*, 2010), anti-inflammatory, antiviral (Tutunchi *et al.*, 2020), and antifungal (Cole *et al.*, 1991) activities.

In addition, some biologically active compounds (baicalin, baicalein, and wogonin) of *S. baicalensis* exhibit inhibitory activity on plant pathogens such as *Phytophthora* sp., *Magnaporthe oryzae*, *Puccinia triticina*, *Ralstonia solanacearum* (Do *et al.*, 2021) and insecticidal activity on individuals of *Liposcelis bostrychophila* (a common pest of stored grain, widespread worldwide, highly prolific and resistant to chemical control) (Wang *et al.*, 2022).

Investigations on the insecticidal activity of the bioactive compounds present in *S. galericulata* on the moth *Spodoptera littoralis* have been carried out since the beginning of the last decade of the 20th century by Cole *et al.* (1990) and by Rodríguez *et al.* (1993). Later investigations aimed at the biological activity of bioactive compounds present in the taxa *S. brevibracteata* Stapf., *S. hastifolia* L., and *S. orientalis* L. ssp. *alpina*

(Boiss.) O. Schwarz were carried out by Formisano *et al.* (2013) on the same pest widespread in Africa, Mediterranean Europe, and Middle Eastern countries, labelled as an A2 quarantine pest according to the European and Mediterranean Plant Protection Organizations (EPPO). Later, Bozov and Georgieva (2017) investigated the insecticidal activity of compounds present in plant organs belonging to *S. altissima* species on the pest *Leptinotarsa decemlineata* Say and Zhu *et al.* (2016) investigated the possible inhibitory effect of compounds present in the aerial parts of *Scutellaria strigillosa* Hemsley plants on dicotyledonous (*Amaranthus retroflexus* L.) and monocotyledonous (*Poa annua* L.) weeds. While Yang *et al.* (2013) claimed that the volatile oil obtained from *Scutellaria barbata* D. Don., exhibits acaricidal activity, particularly on the mites *Dermatophagoides farinae* (Hughes) and *Dermatophagoides pteronyssinus* (Trouessart).

Medicinal plants with a practical value, such as some *Scutellaria* L. species, are commonly associated with problems related to over-harvesting and taxon misidentification (Sherman *et al.*, 2021). For example, *Scutellaria lateriflora* L. is often confused with *Teucrium canadense* L. and *Teucrium chamaedrys* L. (Gafner *et al.*, 2003) due to morphological similarities, but the *Teucrium* species mentioned contain hepatotoxic diterpenes, which can induce hepatotoxicity (Sun and Chen, 2011). Currently, attempts are being made to develop molecular methods, such as the identification of molecular markers (Choi *et al.*, 2021) and specific chemical compounds (Cole *et al.*, 1991), which can be helpful in the classification and identification of the taxa of this genus. According to the information presented by Yildirim *et al.* (2021), the morphological and micromorphological characteristics of seeds of plants belonging to different *Scutellaria* species are also of taxonomic significance, and Al-Joboury (2017) argues that approaching the taxonomy of the genus *Scutellaria* through interdisciplinary geographical, morphological and biochemical studies is very useful, providing valuable information for taxonomic classification.

In this context, the present work aims to perform a brief morphological and micromorphological characterization of seeds belonging to 12 taxa of the genus *Scutellaria* L. to extend the literature data and to discuss the importance of different traits in taxonomy and seeds dispersals.

Materials and Methods

Biological material

The biological material analyzed consisted of seed samples harvested from 12 taxa of the genus *Scutellaria* L.: 11 samples purchased from Jelitto Perennial Seeds, Schwarmstedt, Germany (<https://www.jelitto.com/>) for each sample of seeds purchased, its identification code in the producer's database is specified in brackets: *Scutellaria alpina* L. (SA303), *Scutellaria altissima* L. (SA304), *Scutellaria baicalensis* Georgi (SA309), *Scutellaria galericulata* L. (SA311), *Scutellaria incana* Spreng. (SA312), *Scutellaria integrifolia* L. (SA313), *Scutellaria orientalis* L. (SA314), *Scutellaria orientalis*, var. *pinnatifida* Rchb. (SA316), *Scutellaria suffrutescens* S.Watson. (SA317), *Scutellaria resinosa* Torr. (SA318), *Scutellaria pontica* K.Koch. (SA319); the last sample of seeds analyzed, belonging to the species *Scutellaria supina* originates from the personal collection of Dr. in biology Pavel Pânzaru (district of Schinoasa - Chisinau, Republic of Moldova).

Research methods

To highlight morphological characters (appearance, size, colours), seeds belonging to the 12 *Scutellaria* taxa were observed under an Olympus stereo zoom microscope SZX7, bino, 0.8-5.6 x, and the photographs were taken with the professional lens Macro Lens 100 mm Apexel Technology Co., LTD.

The micromorphological features of the external surfaces were analysed according to the working protocol of the Electron Microscopy Laboratory of the Faculty of Biology of the "Alexandru Ioan Cuza" University of Iasi. The samples were dried with carbon dioxide in the EMS850 dryer, after which it was glued

on aluminum support using a double-adhesive carbon strip and subjected to metallization (gold coating $\pm 95\%$ purity) in the EMS 550x metallizer. The samples, thus coated with three gold layers with a thickness of 10 - 15 nm, were subsequently introduced into the analysis chamber of the Tescan Vega II scanning microscope and scanned with a 30 kV electron beam.

Micrographs of the samples were made at a magnification power between 500 and 20 μm using VegaTC Software.

Statistical analysis

To determine the seed dimension (length and width) for each taxon, batches of 50 randomly selected seeds were analysed, and measurements were made using ImageJ-win64 software on photographs taken with Macro Lens, seeds size reported in mm is the mean \pm SD of the 50 measurements, calculated using Microsoft Excel 365.

Results and Discussion

Fruits belonging to the taxa of the genus *Scutellaria* L. consistently have four seeds attached to the gynophores at mid-length or towards the base but with the same orientation to the hilum. They develop protected from external factors in the chamber formed by the fusion of the calyx lips during fruiting (Paton, 1989). Turner and Delprete (1996) claim that the first observations on seeds of *Scutellaria* L. using the scanning electron microscope (SEM) were made by Lane (1983). According to him (Lane, 1983; Lane, 1976), three aspects are of great importance in the study of seeds belonging to *Scutellaria* taxa, namely: 1. the seeds must be mature because their morphology (size and shape) can change significantly during the development process, particularly in the last days of the maturation stage. Thus, in *Scutellaria parvula* Michx., the band on the seed surface formed by the papillae before complete maturation is much extended outwards, looking like a wing, whereas, at the end of the maturation process, it becomes wavy; 2. the seeds must be fresh; and 3. sometimes the morphology of the seeds may change during the development stage, due to contact with other tissues (ovary wall, other seeds). Turner and Delprete (1996), following Olmstead (1990), argue that the following micromorphological characters of the seed surface are taxonomically important for *Scutellaria* L. taxa: color, size, shape, absence or presence of a circumferential band of papillae, presence or absence of sessile glands, size and shape of epidermal cells, and conformation of papillae. Lane (1983) claims that each species produces seeds with a unique morphological and micromorphological string of characters, of which micromorphology is a conservative character that appears to vary less within a species and is valuable in species-specific classifications. The taxonomic utility of observations on morphological and micromorphological characters of seeds of species of the genus *Scutellaria* L. is also supported by Badamtsetseg (2016) and Hasani-Nejad *et al.*, (2009), but sometimes the species of the genus cannot be taxonomically determined only by seed morphology. This is the case for the varieties *Scutellaria parvula* var. *leonardi* (Epling) Fernald and *S. parvula* var. *australis* Fassett. as well as the species *S. wrightii* sensu Epling, and *S. resinosa* Lane (1983) observed similarities between the seeds of *S. wrightii*, and *S. resinosa*, proposed that the two species should be considered synonyms, and Turner and Delprete (1996) argue that the seeds of *S. wrightii* A. Gray resemble those of *S. muzquiziana* B.L. Turner.

In our study, we have tried to observe all the aspects described above, in the following, we will present the results of the investigations carried out on the seeds of the plants belonging to the 12 taxa of *Scutellaria* L. in discussion with the data from specialized literature. The comparative variation of seed color, shapes, and sizes can be observed in Figure 1 and Figure 2. As can be seen in Figure 1 it is difficult to distinguish 4 taxa (*S. alpina*; *S. orientalis*; *S. orientalis* var. *pinnatifida* and *S. supina*) based on morphological characters alone.



Figure 1. Seed morphology of some *Scutellaria* L. taxa

1. *S. alpina*; 2. *S. altissima*; 3. *S. baicalensis*; 4. *S. galericulata*; 5. *S. incana*; 6. *S. integrifolia*; 7. *S. orientalis*; 8. *S. orientalis* var. *pinnatifida*; 9. *S. pontica*; 10. *S. resinosa*; 11. *S. suffrutescens*; 12. *S. supina*

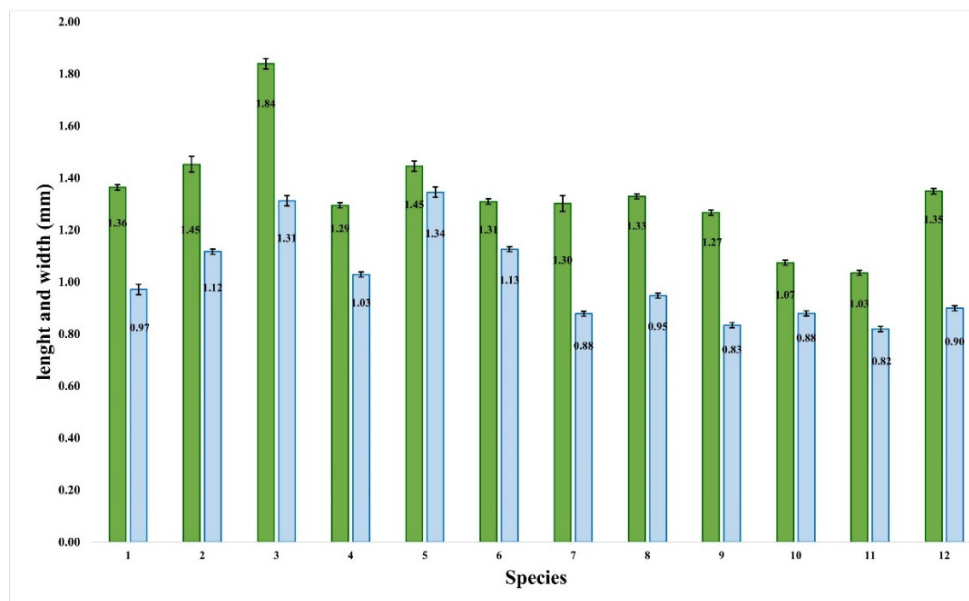


Figure 2. Seed size of some *Scutellaria* L. taxa

1. *S. alpina*; 2. *S. altissima*; 3. *S. baicalensis*; 4. *S. galericulata*; 5. *S. incana*; 6. *S. integrifolia*; 7. *S. orientalis*; 8. *S. orientalis* var. *pinnatifida*; 9. *S. pontica*; 10. *S. resinosa*; 11. *S. suffrutescens*; 12. *S. supina* (green columns: length; blue columns: width)

In the present study, the smallest seed sizes were recorded for *S. resinosa* and *S. suffrutescens*, while the largest seed sizes were recorded for *S. altissima*, *S. incana*, and *S. baicalensis* (Figure 2).

For the analyzed taxa, we observed the following morphological and micromorphological characters (species are described in alphabetical order):

S. alpina: black color; ovoid shape; rough surface covered with greyish - white, long, stellate, tector hairs pressed onto the outer surface, including the micropyle. In some areas of the dorsal surface (lateral view), the tector hairs are firmly pressed and merged along their length. Numerous apiculate epidermal cell extensions are visible between the tector hairs and in hairless regions (Figure 3). We observed distinct stellate tector hairs

exclusively in the seeds of this taxon, in the literature, their presence was observed in the case of *Scutellaria arakensis* Jamzad & Safikhani, *S. multicaulis* Boiss. subsp. *multicaulis*, var. *gandomanensis* Jamzad & Safikhani, var. *nov.*, *S. patonii* Jamzad & Safikhani (Safikhani *et al.*, 2017), *S. araxensis* Grossh., *S. farsistanica* Rech.f., *S. luteo-caerulea* Bornm. & Snit., *S. multicaulis* Boiss., *S. pinnatifida* A. Ham., *S. platystegia* Juz. (Hasani-Nejad *et al.*, 2009).

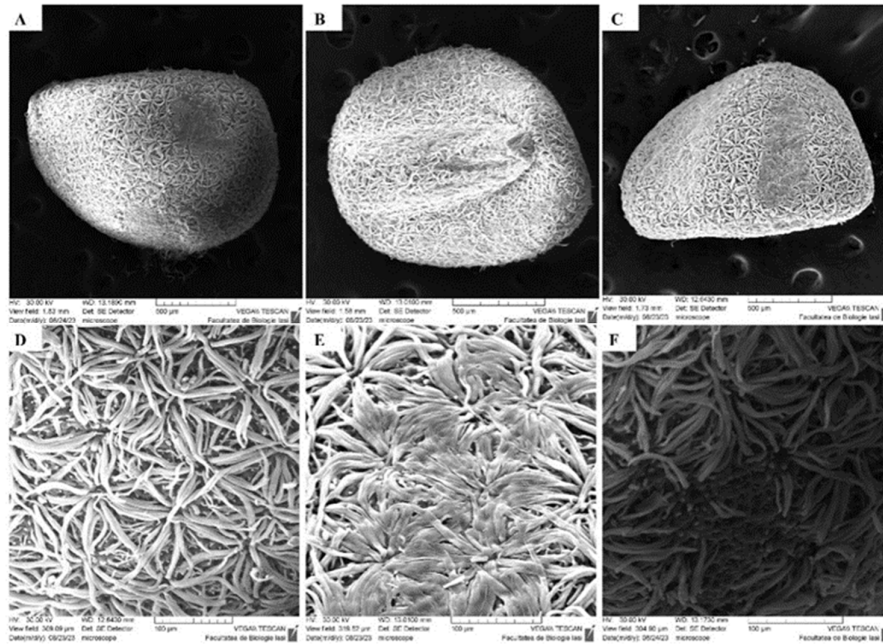


Figure 3. *S. alpina* micromorphology of seeds

A. dorsal; B. ventral; C. lateral; D-F. details

S. altissima: the color ranges from light to dark brown; with an ovoid shape and a papillate surface. Some papillae have well-defined outlines, while others merge in groups of two or three. In the apical region, they are concave, with long white tector hairs at the center of the apex. Papillae are absent on the surface and near the micropyle. The epidermal cells on the surface of the papillae are bulging and slightly apiculate, giving a papillate appearance. The epidermal cells between the papillae are flat, clearly delineating the papillae outlines (Figure 4). The morphology and micromorphology of the seeds of *S. altissima* plants were also studied by Toma *et al.*, (1998) and by Tunçkol and Haşayacak (2022), who also observed the presence of short, white tector hairs emerging from the apex of the papillae. Morphologically, the seeds of *S. altissima* observed in our study resemble those of *S. glaphyrostachys* Rech.f., as described by Yildirim *et al.* (2021), with some differences in epidermal cell shape and tector hair length, the latter being visibly longer in *S. glaphyrostachys* seeds.

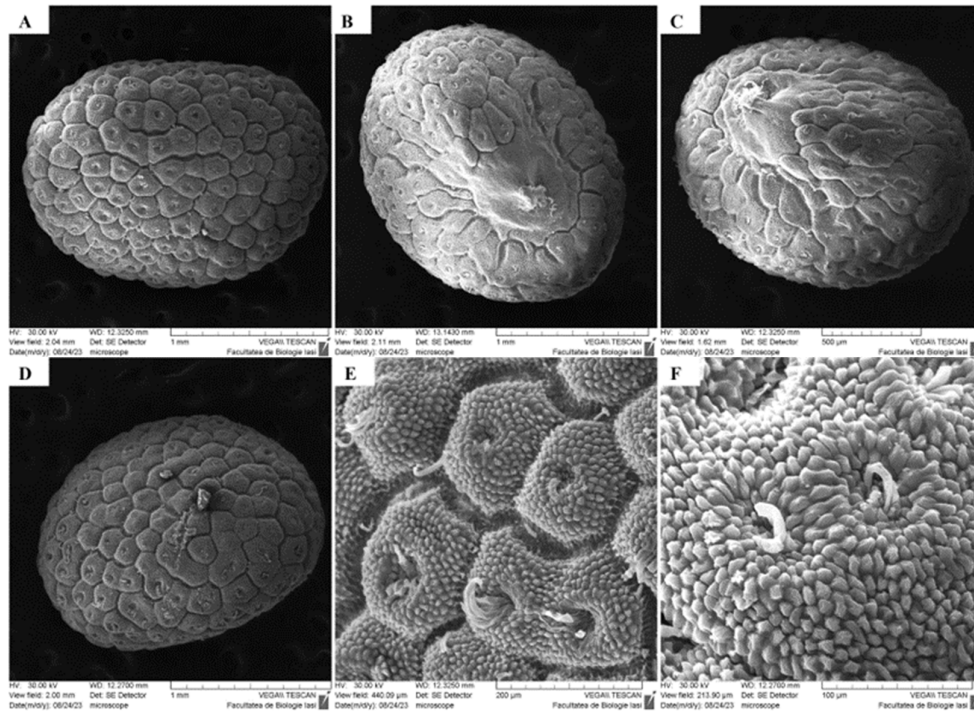


Figure 4. *S. altissima* micromorphology of seeds

A. lateral; B. dorsal; C. ventral; D-F. details

S. baicalensis: matte black color; ovoid shape with some deformations. The surface is glabrous and papillate, with conical papillae featuring truncate apices. In certain areas, the papillae merge into groups of two or three. The micropyle surface lacks papillae. The epidermal cells are conical and possess appendices with rounded apices (Figure 5). As can be seen in Figure 1 only *S. baicalensis* species presented black, glabrous seeds. This color of seeds was reported in the literature also in the case of plants belonging to other species of the *Scutellaria* genus, such as *Scutellaria bartlettii* B.L. Turner *sp. nov.*, *S. mulleri* B.L. Turner (Turner, 1944), *S. brittonii* Porter (Lane, 1976; Lane, 1983), *S. cuevasiana* J. G. González et A. Vázquez *sp. nov.* (Guadalupe González - Gallegos and Antonio Vázquez - García, 2013), *S. hintoniorum* Henrickson *sp. nov.*, *S. carmenensis* Henrickson *sp. nov.* (Henrickson, 1989), *S. indica* L. var. *satokoae* Wakasugi et Naruhashi var. *nov.* (Naruhashi *et al.*, 2004), *S. lactea* A. Pool, *S. oblongifolia* A. Pool (Pool, 2006), *S. potosina* var. *kaibabensis* S. Rhodes & T. Ayers var. *nov.* (Rhodes and Ayers, 2010), *S. hsiehi* T.H.Hsieh (Hsieh, 2013) and *S. tuvensis* Juz. (Badamtsetseg, 2016). Morphological and micromorphological characteristics of the seeds of *S. baicalensis* were also described by Lane, (1976); Lane, (1983); Sangtae and Sangtae (1995); Toma *et al.* (1998), and Badamtsetseg (2016), with the latter recording seed dimensions (mm) of 1.28 length and 1.05 width.

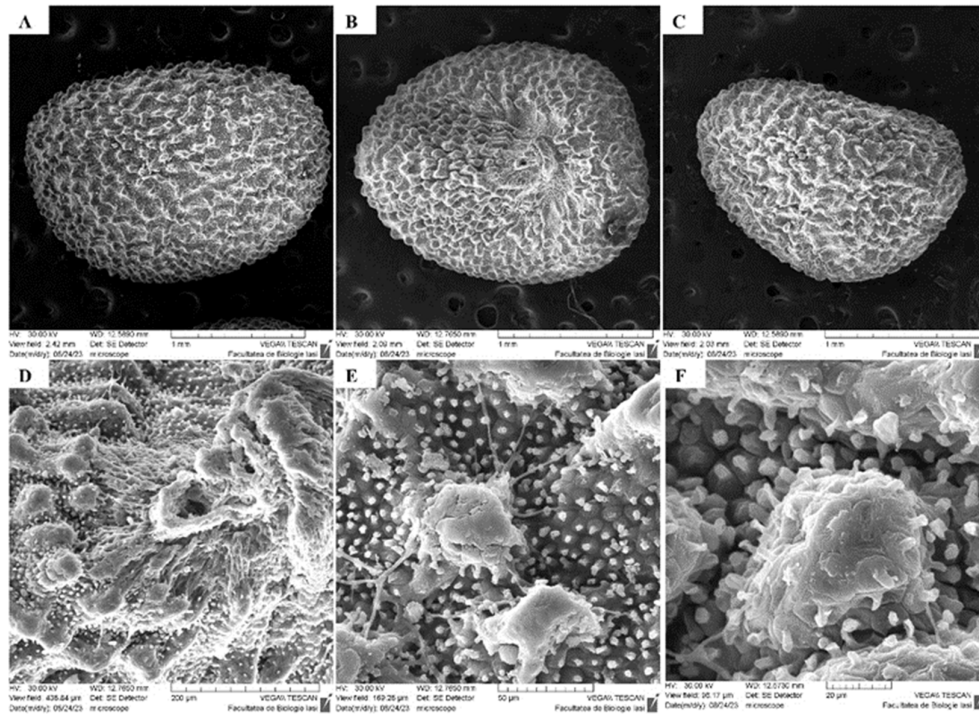


Figure 5. *S. baicalensis* micromorphology of seeds

A. dorsal; B. ventral; C. lateral; D - F. details

S. galericulata: dark pale yellow to orange-yellow color; ovoid shape; the surface is glabrous and papillate (Figure 1). The papillae are digitiform with rounded or slightly flattened apices, occasionally merging in groups. They have well-defined outlines, interspersed with numerous sessile glands that appear shiny under an Olympus stereo zoom microscope and more or less spherical and deformed under SEM. In the ventral view of the micropylar region, sessile glands, and a few papillae, some strongly flattened, are visible. The epidermal cells varying in shape are slightly domed and exhibit well-defined outlines (Figure 6). For this taxon, Hasani-Nejad *et al.*, (2009) report seed sizes (mm) equal to 1.4 - 1.5 length and 1.1 - 1.4 mm width, while Bazarraghaa *et al.*, (2019) claim that the seeds of these species closely resemble those of *Scutellaria krasevii* Kom. & I.Schischk. ex. Juz. The presence of sessile glands on the seed surface of *S. galericulata* was also observed by Badamtsetseg (2016) and Paton (1989). These sessile glands on the surface of mature seeds are non-functional relict structures left over from the early stages of development that were present on the ovary of the plant and functioned as auxiliary nectaries during early development before the corolla falls (Paton 1992).

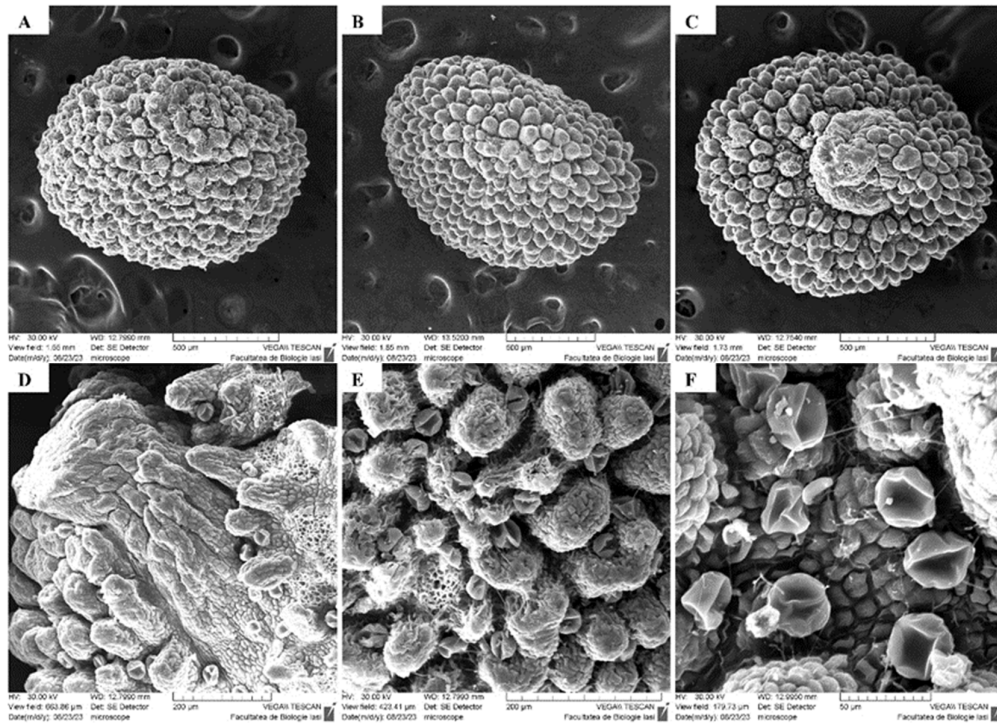


Figure 6. *S. galericulata* micromorphology of seeds
A. dorsal; B. lateral; C. ventral; D-F. details

S. incana: color varies from light to dark brown, greenish - brown; spherical shape; glabrous, papillate surface, size and shape of papillae vary to some, the apex appears strongly flattened; in some places, the papillae merge into groups of two or three, but in general, the outline between papillae is well defined. They are absent on the micropylar surface. The epidermal cells have a well-defined outline and different shapes (Figure 7). The morphological and micromorphological characteristics of the seeds of this taxon were very similar to those described by Lane (1976) and Lane (1983).

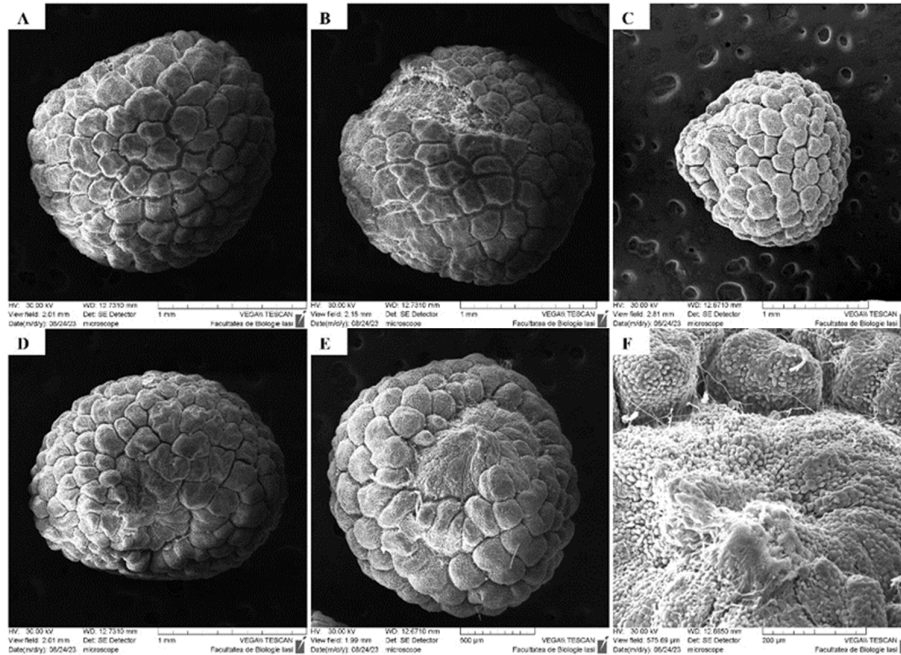


Figure 7. *S. incana* micromorphology of seeds
A-B. dorsal; C. lateral; D-E. ventral; F. detail

S. integrifolia: dark brown color, but also observed yellow and green seeds, probably immature, smaller in size; spherical shape, slightly flattened dorso - ventrally; surface glabrous, papillate, the papillae are well delimited, of different sizes and with a lamellate appearance, arranged in a circular pattern around the micropyle; some merge into groups of two or three, forming a larger lamella. The epidermal cells are convex and have different shapes (Figure 8).

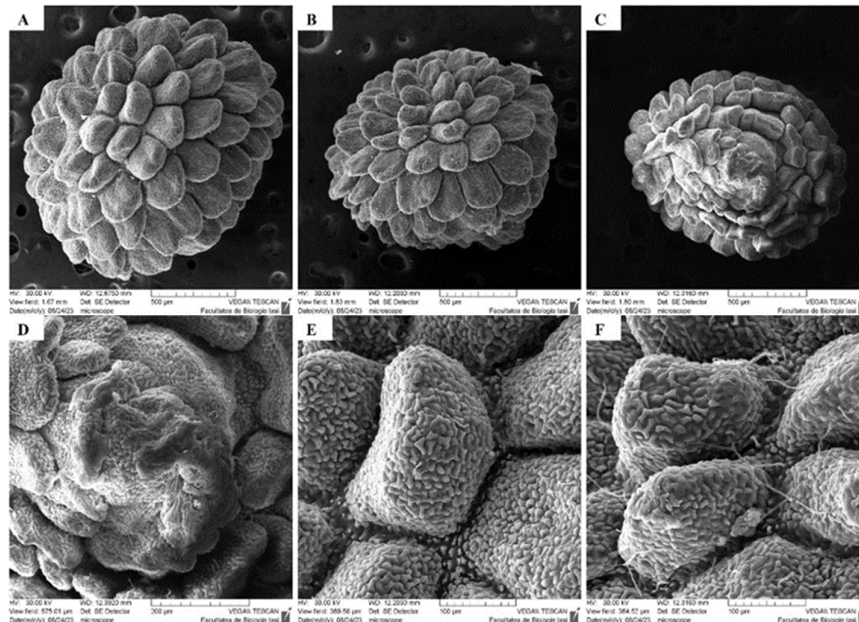


Figure 8. *S. integrifolia* micromorphology of seeds
A-B. lateral; C. ventral; D. micropyle detail; E-F. detail

S. orientalis: black color; ovoid shape; surface strongly pubescent, entirely covered with white, occasionally yellowish tector hairs which are not stellate. They are long, unicellular, and pressed against the seed surface, sometimes very firmly. The micropyle region is also densely coated with hairs (Figure 9).

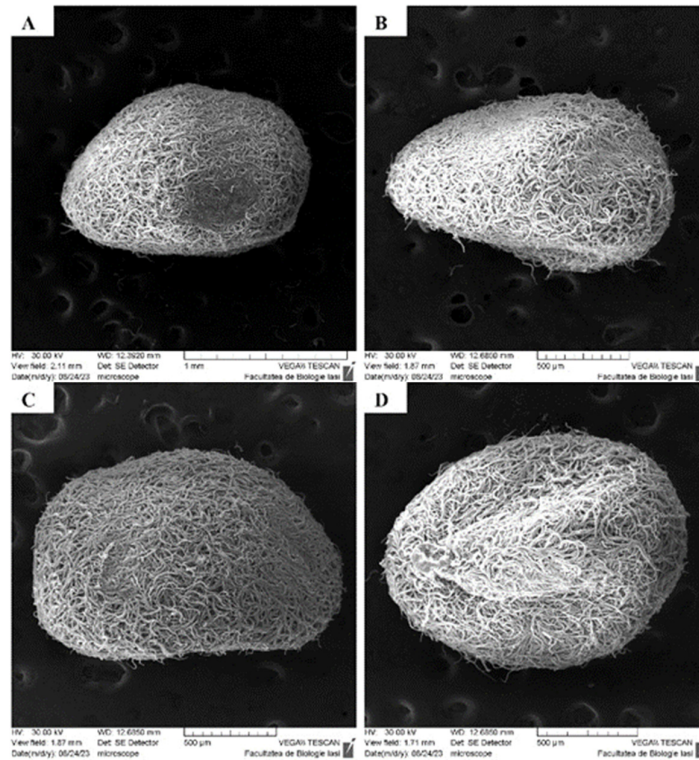


Figure 9. *S. orientalis* micromorphology of seeds
A-B. lateral; C. dorsal; D. ventral

S. orientalis var. *pinnatifida*: black color; ovoid shape; some seeds show a deformed shape. The surface is strongly pubescent and entirely covered with long, unicellular, non-stellate tector white hairs, which in some places are firmly pressed on the surface of the seeds. Between tector hairs can be seen convex epidermal cells with white apex (Figure 10).

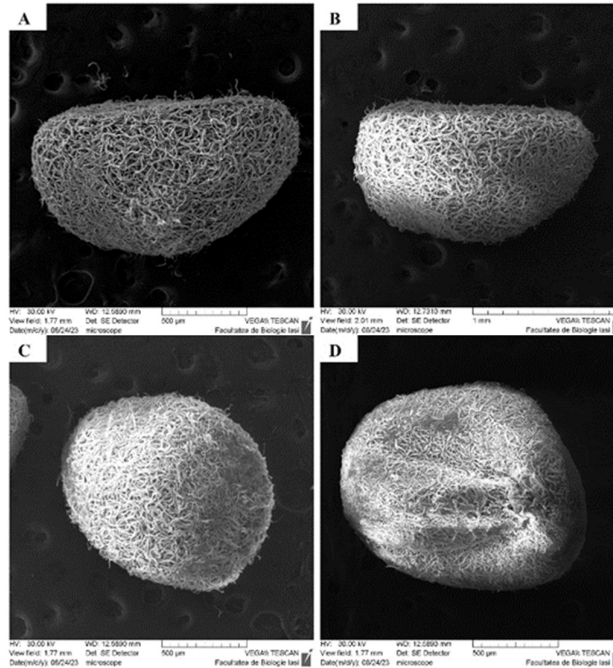


Figure 10. *S. orientalis* var. *pinnatifida* micromorphology of seeds
A-B. lateral; C. dorsal; D. ventral

S. pontica: brown color; ovoid shape; the surface is pubescent and entirely covered with long, unicellular, non-stellate white tector hairs, including the micropylar region. In regions lacking hairs (probably due to mechanical manipulation) can be observed approximately 15 elongated and convex epidermal cells arranged in a circular pattern, forming a structure from which very long hairs of varying thickness extend, pressed against the seed surface, and oriented in different directions (Figure 11).

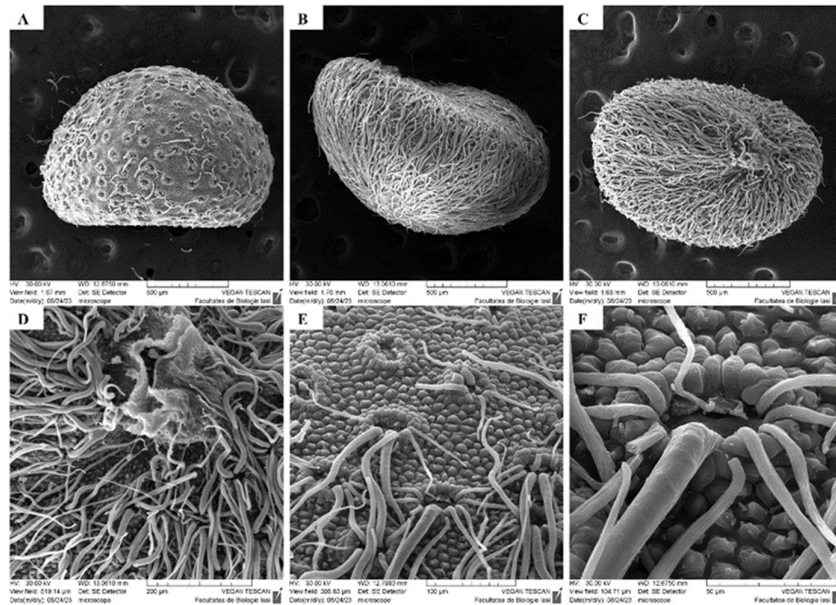


Figure 11. *S. pontica* micromorphology of seeds
A -B. lateral; C. ventral; D-F. details

S. resinosa: color varies from dark to light brown; shape spheroid to ovoid; surface glabrous, papillate; papillae are digitiform, small in size, and cover the whole surface of the seeds; in some seeds, the apex is slightly blunt, in others concave; in some areas, the papillae merge in pairs. The epidermal cells are strongly domed and apiculate (mastoid), particularly those forming the papillae; the outline of the cells is well-defined, and their shape is more or less uniform (Figure 12). In their studies, Lane (1976) and Lane (1983) reported in the case of *S. resinosa* seed dimensions (mm) of 1.31 axially, 1.03 tangentially, and 1.08 radially, while Turner and Delprete (1996) claim that the seed morphology of *S. resinosa* is similar to those of *S. fruticetorum* Epling, *S. texana* B.L.Turner and *S. carmenensis* Henrard.

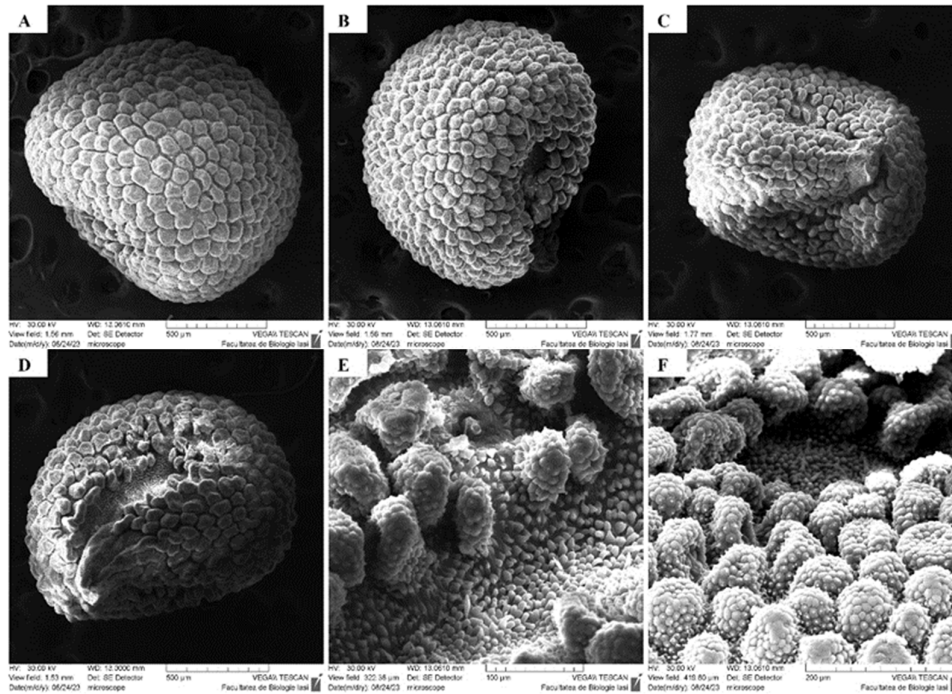


Figure 12. *S. resinosa* micromorphology of seeds
A. dorsal; B. lateral; C-D. ventral; E-F. details

S. suffrutescens: color varies from light to dark brown; spheroid to ovoid shape; surface glabrous, papillate, papillae are small, not well defined in some places, they are strongly flattened (dorsal surface); in the apical region of some papillae, a digitiform formation can be observed. The epidermal cells are large and convex, with a well-defined outline (Figure 13).

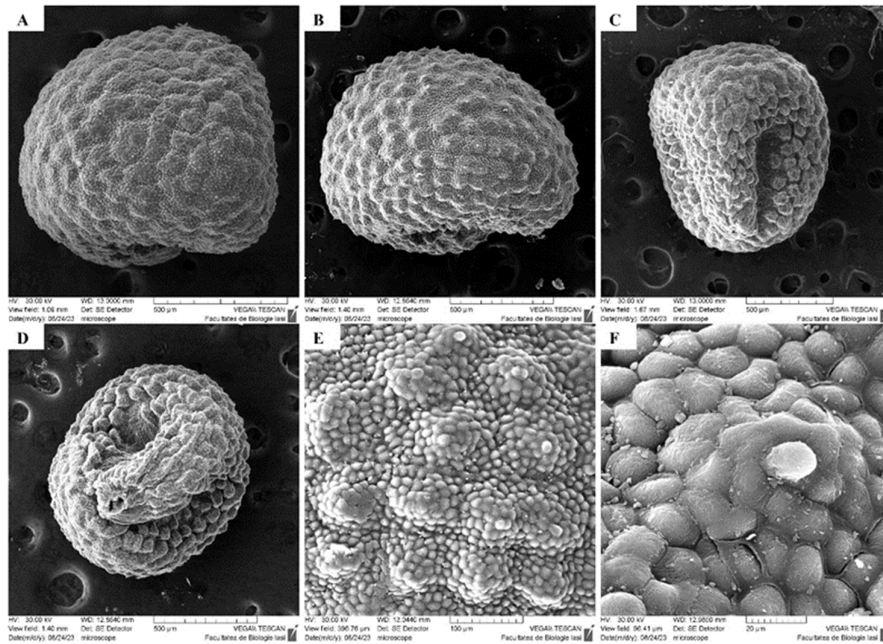


Figure 13. *S. suffrutescens* micromorphology of seeds
A. dorsal; B. lateral; C-D. ventral; E-F. details

S. supina: black color; ovoid shape; pubescent surface, entirely covered with white tector hairs; there are fewer hairs in the micropylar area; the hairs are not stellate, but unicellular, long, of different thicknesses, pressed on the seed surface; the long, white apex of the epidermal cells (mastoid) can be seen between them (Figure 14). The seed micromorphology of *S. supina* plants was also analyzed by Lane (1983) and Badamtsetseg (2016), the latter reporting seed dimensions (mm) of 1.240 long and 0.980 wide.

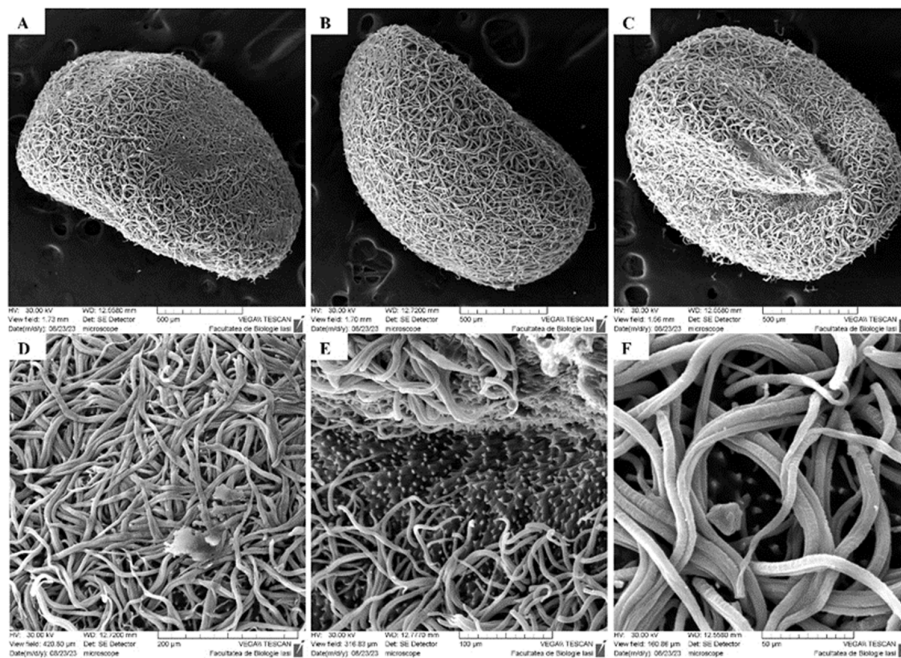


Figure 14. *S. supina* micromorphology of seeds
A - B. lateral; C. ventral; D - F. details

We have not found studies describing the morphology and micromorphology of seeds of plants belonging to the taxa *Scutellaria integrifolia*, *S. orientalis*, *S. orientalis* var. *pinnatifida* and *S. suffrutescens*. In this context, we consider that the results of morphological and micromorphological observations made on seeds of plants belonging to these taxa are presented for the first time as an integral part of the present work. But in addition to present work in Romania, Toma *et al.*, (1998) carried out morpho-anatomical, histo-anatomical, and micromorphological investigations of the seeds of plants belonging to some species of the genus *Scutellaria* L. (*S. altissima*; *S. albida*; *S. baicalensis* and *S. galericulata*), taking plant material from the “Anastasiu Fătu” Botanical Garden of Iasi, and their observations as in our case were consistent with data reported in the specialized literature. In addition to the morphological and micromorphological characters observed and described in the present study, in the scientific literature, for some taxa of the genus such as *Scutellaria indica* L., *S. violacea* B.Heyne ex Benthenth. and *S. tenax* W.W.Sm. of the *Scutellaria violacea* group (section *Scutellaria* L.) (Paton, 1989) and some species from Mesoamerica, was described the presence of acuminate papillae, which form hooks at the tip (Pool, 2006). In other species of the *Scutellaria parvula* group (*S. parvula* Michx., *S. nervosa* Pursh. and *S. leonardi* Epling) and *Scutellaria angustifolia* group (*S. angustifolia* Pursh., *S. austinae* Eastw., *S. californica* A.Gray., *S. antirrhinoides* Benth., *S. bolanderi* A.Gray. and *S. siphocampyloides* Vatke, except *Scutellaria brittonii*) a band in the basal or median region (*Scutellaria hastifolia*) of the seeds, formed by fusion of the digitiform papillae, was observed (Paton, 1989).

Sometimes, seed morphology can vary within species if samples have been taken from different populations. This has been observed by Lane (1976) and Lane (1983) in seeds of populations of *Scutellaria brittonii*: individuals from one population showed seeds with a median band, whereas individuals from other populations showed only a partial band or no band. In another paper, Lane (1983) recognized *Scutellaria parvula*, based on seed morphology as a species with three varieties rather than three separate species (*S. parvula*, *S. australis* (Fassett) Epling, *S. leonardi*). In the same context, according to research by Huang *et al.*, (2003), *Scutellaria* species from Taiwan (*S. barbata*, *S. indica*, *S. austrotaiwanensis* T.H.Hsieh & T.C.Huang, *S. tashiroi* Hayata, *S. taiwanensis* C.Y.Wu. and *S. playfairii* Kudô.) can be differentiated according to their seed morphology.

Sections through the seeds of some taxa of the genus *Scutellaria* L. have been made by Paton (1990), who claims that the seed papillae of plants belonging to the *Scutellaria albida* group, and those from the section *Anaspis* present large cavities with air, without sessile glands on the inner surface of the pericarp. In another paper, the same author (Paton, 1989) claims that the seed papillae of *Scutellaria albida* L. species are composed of large, thin-walled cells with air-filled cavities and lacking sessile glands on the inner wall of the pericarp. Whereas in *Scutellaria speciosa* Epling., these glands were observed on both surfaces of the seeds, in the seeds of *Scutellaria alpina* the sessile glands were present only on the inner surface of the pericarp. Papillae consisting of small, thin-walled cells and sessile glands on the inner surface of the pericarp was also observed in the seeds of *Scutellaria nummulariifolia* Hook.f., the seeds of *Scutellaria violascens* Gurke, morphologically and micromorphologically, are similar to those of *Scutellaria altissima* however, they lack glands on the inner surface of the pericarp in cross-section, and their papillae do not contain air-filled cavities.

Research on seeds harvested from some *Scutellaria* species has also been carried out by Baskin and Baskin (1982) and Yoshimura *et al.*, (2019), who mainly investigated aspects of seed germination. Paton (1992) claims that the external morphology of seeds from taxa within the genus *Scutellaria* L. represents adaptations to their dispersal by animals, water, and wind. At the same time, seed morphology is not limited to this function but also exerts one of the most important functions for plants which is the protection of the embryo. The ornamentation present on the seed surface can decrease palatability and create a protective air barrier around the seed. This microclimate can shield the seeds from external factors, thereby prolonging embryo viability during periods of unfavorable environmental conditions. In some species, such as *S. albida* tector hairs on the

outer surface of seeds may help them to anchor in the soil. Depending on the way of dispersal, the seeds of *Scutellaria* L. taxa show certain morphological and micromorphological adaptations, which may be:

Adaptations of seeds for wind dispersal (anemochory) - in species inhabiting chasmophyte environments, tector hairs on the surface of seeds may favor their transport by air currents, thus compensating for the absence of a catapult dispersal mechanism. The seeds of some *Anaspis* species are well adapted to this type of dispersal, as they have tector hairs on the outer surface that can reach 1-1.5 mm in length. In species of the *Scutellaria albida* group and some species of section *Anaspis*, this mode of dispersal is favored by the presence of air-filled spaces localized at the papillae, which increase the surface area of action of the wind in relation to the weight of the seeds (Paton 1992). In *Scutellaria mexicana* (Torr.) A.J. Paton and *S. scutellarioides* (Kunth) Harley, dissemination by anemochory is favored by the presence of a winged mericarp (Bouman and Meeuse 1992).

Adaptations of seeds for water dispersal (hydrochory) or floating above water - have been observed in *S. galericulata* which has a water-shore habitat (Paton, 1989); seeds of this species can float above water (Paton, 1992) for up to 12 months without losing viability (Bouman and Meeuse, 1992). In this case, it was assumed that the floating of the seeds was due to the presence of air-filled cells in the seed's integument. This assumption does not fully explain the phenomenon of seed flotation, because the seeds of *S. altissima* and *S. albida* plants are similar in size to *S. galericulata* plants and have a pericarp consisting of cells with cavities and air spaces; within the papillae however, they do not float, the buoyancy of *S. galericulata* seeds being most likely due to the chemical composition of their cell walls (Paton, 1989; Paton, 1992). Williams (1992) claims that the seeds of *Scutellaria novae-zelandiae* Hook .f. plants can also float on water, the species being found in river margins where vegetation is periodically disturbed by flooding. In the American *S. drummondii* var. *edwardsiana* B.L. Turner and *S. wrightii*, Nelson and Goetze (2010) reported that seed dispersal is favored by the presence of the scutellum (the sail-like fold on the upper lip of the calyx) which becomes opened under the force of raindrops; after the calyx becomes dehiscent the seeds are dispersed by water currents, and the distances of dispersal vary with the velocity of water currents during rainfall.

Adaptations of seeds for dispersal by animals (zoochory) - are represented by the presence of hooks on the external surface of the seeds, which favor their attachment to the animal fur. Such ornamentation has been observed in *Scutellaria discolor* Wall. ex Benth., *S. violacea* and *S. ocymoides* (Kunth) Epling (Paton, 1992), the same author has previously stated that the seeds of *S. galericulata* are also dispersed by water birds (Paton, 1989).

Adaptations of seeds for autochory dispersal - the process is accomplished by active ballistic ejections due to the bilabiate calyx and the tension that accumulates between its two lips during seed maturation. In a dry environment, the wind breeze, a touch of animals, or raindrops are sufficient to catapult the seeds (Bouman and Meeuse, 1992). Although Paton (1992) believes that the force exerted by raindrops is not enough to remove the upper lip of the calyx, Nelson and Goetze (2010) claim that raindrops can generate sufficient mechanical force to promote scutellum removal in some species, such as *S. altissima*. In the seed dispersal of species of the genus *Scutellaria* L. calyx morphology also plays an important role, as the fusion of calyx lips generates two contradictory problems for plant survival: preventing the dissemination of immature seeds and facilitating the dissemination of mature seeds. Thus, species of the genus have adapted their calyx morphology according to the mechanisms of seed dispersal and the ecological characteristics of the habitat in which they grow. These adaptations have mainly concerned the preservation, loss, and reduction of the scutellum (Paton, 1990) and the modification of calyx morphology (Paton, 1992). The scutellum, having no function in seed dispersal in some habitats, was lost during evolution in some casmophyte species in subsection *Cystaspis* (Juz), section *Lupulinaria* (Paton, 1989) and some species included in sections *Salazaria* (Torrey), *Apeltanthus* Nevski ex Juz, and *Anaspis* (Rech.f.) Natural selection that has influenced the preservation or loss of the scutellum, as well as the modification of calyx morphology, can be observed in some species within the section *Perilomia* (Kunth.) Epling, which includes species with and without scutellum (Paton, 1992). In other species

of the genus, especially those from rocky habitats, the calyx is tight until seed maturation by cuculate bracteoles (Paton, 1989). Nelson and Goetze (2010), based on observations on seeds of *Scutellaria drummondii* var. *edwardsiana* and *S. wrightii*, argue that only brown and yellow calyx is dehiscent, whereas immature seedless calyx developed from unpollinated flowers, although turning brown, is not dehiscent.

Conclusions

Despite the practical importance of the taxa belonging to the genus *Scutellaria* L. little information on their classification can be found in the literature, and further investigations are needed to confirm the adaptive or non-adaptive importance of scutellum and their seed morphology, depending on habitat and dispersal type.

We agree with the statements in the literature that in some taxa of the genus *Scutellaria* L. the morphological and micromorphological characteristics of their seeds are not enough for a correct identification. We also agree that some taxa present seeds with numerous variations in morphology and micromorphology, which may be helpful in the classifications at species, subspecies, and variety levels. Following the observations made on the 12 taxa of the genus *Scutellaria* L. and after consulting the specialized literature, we have come up with two ideas for reflection, namely: seeds with a glabrous integument can also be spread by wind (just like pollen with smooth sporoderm can be spread by wind). Likewise, seeds with a tegument covered by tector hairs are more likely to be spread by animals, but also by wind (the hairs are dead at maturity, filled with air, and do not increase the weight of the seeds); in general, the dispersal of seeds of herbaceous species occurs in several ways, which is why the analysis of the micromorphology of the surface of the seed coat can provide indications of possible ways of dispersal related to the ecological habitat of the plant.

Results of morphological and micromorphological observations made on seeds of plants belonging to the taxa *S. integrifolia*, *S. orientalis*, *S. orientalis* var. *pinnatifida*, and *S. suffrutescens* presented for the first time in this paper, provide taxonomic specialists with additional information useful in the process of differentiation and classification of taxa and infra taxa of the genus *Scutellaria* L.

We also consider it necessary to extend the morphological and micromorphological research on more batches of seeds harvested from different plants belonging to a broad range of taxa of the genus *Scutellaria* L. The practical data thus obtained can become valuable clues in their accurate taxonomy identification.

Authors' Contributions

Conceptualization: TR, MEF, MMZ, MNG, and LCI; Data curation: TR; MMZ; LCI; Formal analysis: TR, MEF; Investigation: TR, MMZ, LCI, and MNG; Methodology: TR, ŞMO, MMZ; Software: TR, ŞMO, and MEF; Supervision: TR, MMZ, LCI and MNG; Writing - original draft: TR, MMZ, and LCI; Writing and editing: TR; Supervision: TR, MEF, MMZ, MNG, LCI and ŞMO; Validation: TR; MMZ, MNG and LCI. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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