NUTLET MICRO-MORPHOLOGY IN SCUTELLARIA L. (LAMIACEAE) IN IRAN

M. Hasani-Nejad, Z. Jamzad & M. Yousefi

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Nutlet micromorphology of 14 taxa of *Scutellaria* of subgenera *Scutellaria* (sects. *Scutellaria* and *Anaspis*) and *Apeltanthus* (sect. *Lupulinaria*) in Iran was studied by scanning electron microscopy (SEM). Nutlet surface sculpturing varies prominently among species within two subgenera and this is mostly congruent with the latest subgenric classification of the genus. However some differences were observed among species in some sections. Five nutlet types were identified: Type I, papillate with sessile glands among the papillae (subgenus *Scutellaria* sect. *Scutellaria*: *Sc. galericulata*). Type II. papillate, papillae circular with concave apex. (subgenus *Scutellaria* section *Scutellaria*: *Sc. tournefortii*). Type III. Nutlet surface papillate, papillae with obtuse to acute apex, more or less finger like (subgenus *Scutellaria*, section *Anaspis*: Scutellaria ariana). Type IV, nutlet surface with flattened or apical depressed papillae, with adpressed and patent hairs partially covering the surface, concentrated near the papillae apices. (subgenus *Scutellaria*: *Sc. condensata* subsp. *condensata* and subsp *pycnotricha* and *Sc. velenovskyi* subsp. *subsimilis*). Type V, surface densely hairy with stellate or long simple hairs (sect. *Lupulinaria*: *Sc. araxensis, Sc. farsistanica, Sc. litwinowii, Sc. luteo-coerulea, Sc. multicaulis, Sc. pinnatifida, Sc. platystegia, Sc. theobromina and Sc. tomentosa.*).

Ziba Jamzad (correspondence), Research Institute of Forests & Rangelands, P.O. Box 13185-116, Tehran, Iran; Email:jamzad@rifr-ac.ir- Maryam Hasaninejad, University of Payam Noor Najafabad, Talaghani Blv, Najafabad, I. R.IRAN. 8515643144.- Yousefi, M., University of Payam Noor Esfahan, Ashrafi Blv. Kohandezh, Esfahan

Key words. *Scutellaria*, Nutlet micromorphology, *Lamiaceae*, Iran , SEM.

مطالعه میکرو مورفوژیکی جنس Scutellaria (Lamiaceae) در ایران

مریم حسنی نژاد، دانشجوی کارشناسی ارشد گروه زیست شناس دانشگاه پیام نور، واحد نجف آباد.

زیبا جم زاد، دانشیار پژوهش مؤسسه تحقیقات جنگلها و مراتع کشور.

مهدی یوسفی، استادیار دانشگاه پیام نور اصفهان.

میکرومورفولوژی فندقه در ١٤ تاکسون از جنس *Scutellaria* با ٢ زیر جنس *Scutellaria* (بخش های *Scutellaria* و *Anaspis*) و *Apeltanthus* (بخش *Lupulinaria*) در ایران توسط میکروسکوپ الکترونی (SEM) مورد مطالعه قرار گرفت. تصاویر سطح فندقه به طور چشمگیری تفاوت در سطح گونه در ٢ زیر جنس نشان می دهد و با آخرین طبقه بندی درون جنسی در این جنس همخوان است. به هر حال تفاوتهای کوچکی در میان گونه ها در بعضی از بخش ها مشاهده می شود. ٥ تیپ فندقه مشخص شد. تیپ ١. فندقه با تزئینات سطحی زگیل مانند با غدههای بدون پایه که در بین زگیلها پراکنده شدهاند.(زیرجنس *Scutellaria* بخش *Scutellaria*: *Sc. galericulata*). تیپ ٢. فندقه با تزئینات سطحی زگیل مانند، زگیلهای گرد با نوک محدب. (زیرجنس *Scutellaria* بخش *Scutellaria*: *Sc. tournefortii*). تیپ ٣. فندقه با تزئینات سطحی زگیل مانند. زگیلها با نوک گرد تا نوک تیز و کم و بیش شبیه انگشت میباشند. (زیر جنس *Scutellaria*، بخش *Anaspis*: *Sc. ariana*). تیپ ٤. فندقه با تزئینات سطحی زگیل مانند مسطح شده یا در نوک فرو رفته با کرک های خوابیده یا گسترده که در رأس زگیلها متمرکز شده و قسمتی از فندقه را میپوشاند. (زیرجنس *Scutellaria* بخش *Scutellaria* با *Sc. velenovskyi* subsp. *subsimilis* و با دو زیر گونه subsp. *condensate* و subsp. *Pycnotricha*) تیپ ٥. فندقه با کرکهای متراکم ستارهای طویل یا کرکهای ساده. (زیر جنس *Apeltanthus* بخش *Lupulinaria* شامل گونههای زیر:

Sc. araxensis, Sc. farsistanica, Sc. litwinowii, Sc. luteo-coerulea, Sc. multicaulis, Sc. pinnatifida, Sc. platystegia, (*Sc. theobromina and Sc. tomentosa.*

Introduction

Scutellaria L. is a member of *Lamiaceae* which belongs to the subfamily *Scutellarioideae* (Cantino et al. 1992) and grows both in Old and New Worlds. It is typically characterized by its calyx shape, with two undivided lips and the presence of a scutellum on the upper lip. The calyx shows variable characters in different species, and the scutellum may be absent or calyx may be inflated in the upper lip.

Scutellaria is one of the large genera within *Lamaiceae* with 425 currently recognized species, but considering the possible synonyms the actual number of species is closer to 360 (Paton 1990a). The Irano-Turanian Region particularly Central Asia and Afghanistan is the centre of maximum diversity, for the genus. Eastern Mediterranean and the Ands is the second centre of speciation (Paton 1990a). The genus represents 40 species in Flora Iranica Region including 22 in Iran of which 10 taxa are endemics (Rechinger 1982). They are distributed all over the country mainly in mountainous area but a few in wet places at aquatic habitats and in forests.

The infrageneric classification of the genus has been done differently by different authors. Hamilton (1832) recognized three sections in the genus (sects. *Lupulinaria*, *Stachymacris* and *Galericularia)*. Bentham (1834) classified *Scutellaria* into five sections (sects. *Lupulinaria*, *Heteranthesia*, *Stachymacris*, *Galericularia* and *Maschalostachys*). Later, in 1876 he divided the genus into three sections (sects. *Lupulinaria*, *Heteranthesia* and *Vulgares*). Briquet (1896) considered two sections in his classification: *Euscutellaria* with three subsections (subsects. *Lupulinaria*, *Heteranthesia* and *Vulgares*) and *Scutellariopsis* with no subsectional division. Rechinger (1982) recognized four subgenera in his treatment of the genus: *Euscutellaria* (sects. *Lupulinaria, Stachymacris, Galericularia*); *Anaspis*; *Apeltanthus* and *Cystaspis*. He did not consider any sectional divisions for the last three subgenera. The New world taxa were classified by Epling (1942) into 18 sections noting that it is a provisional and suggestive. All these authors based their classifications on floral, inflorescence, nutlet and habit characters. The latest global taxonomic review and infrageneric classification of *Scutellaria* has been done by Paton (1990a). He did a comprehensive study on *Scutellaria* and the allied genera (Paton, 1990 b, 1992) and showed that the features of the inflorescence, calyx, corolla and nutlets were the most important and taxonomically reliable characters (Paton 1990a). He considered a wide concept for *Scutellaria*. The allied genera namely *Perilomia* Kunth, *Harlanlewisia* Epling and *Salazaria* Torrey were included in *Scutellaria.* (Paton, 1990 a). He divided *Scutellaria* into two subgenera: subgenus *Scutellaria* which is characterized with one- sided or rarely spiral inflorescence, flowers opposite or not subtended by leaves or leaf like bracts; subgenus *Apelthanthus* (Neveski ex Juz.) Juz. emend Paton, characterized by 4-sided inflorescence, flowers opposite and decussate subtended by cucullate bracts. Subgenus *Scutellaria* was divided into five sections (*Scutellaria, Anaspis, Salazaria, perilomia* and *Salviifoliae*). Subgenus *Apelthanthus* was divided to two sections (sects. *Apeltanthus* and *Lupulinaria*).

Based on this classification the Iranian *Scutellaria* species will be recognized in subgenus *Scutellaria* sections *Scutellaria* and *Anaspis* and subgenus *Apeltanthus*, section *Lupulinaria*.

Nutlet micromorphology is an important taxonomic character in flowering plants (Barthlott 1984) as well as in *Lamiaceae* and has been studied in many genera within eight subfamilies of *Lamiaceae*. In some genera it shows diagnostic character among species and in others at higher ranks. Nutlet showed to be a useful taxonomic character at species, sectional or subgeneric levels (Roth 1977; Barthlott 1981 & 1984; Stuessy 1990; Oran 1996; Ryding 1993 & 1994; Budantsev 1993; Marin 1994; Jamzad 2000; Salmaki 2008).

The nutlet micromorphology of New World *Scutellaria* species was studied by Billie et al. (1996). Paton (1992) studied nutlet and calyx character in *Scutellaria* as significant characteristics in seed dispersal and protection of immature fruits.

We studied nutlet micromorphology of Iranian *Scutellaria* species to evaluate its importance in delimiting the subgeneric and sectional boundaries as well as its taxonomic implication at species level.

Materials and Methods

Nutlets of 14 *Scutellaria* species were removed from the herbarium specimens in the Herbarium of Research Institute of Forests and Rangelands (TARI), table 1. The morphological characters such as size, shape and color was observed and measured by an Olympus SZH10 research stereo microscope. For SEM studies

Table1. Materials and collecting data for nutlet studies in the genus *Scutellaria*.

Subgenera, sections & species	Collecting data
Subgen. Scutellaria	
Sect. Anaspis	
Sc. ariana Hedge	Bandar–Abbas: Hajiabad, Shamile-Bala, Bokhan, 1700 m, Mozaffarian 58730 (TARI).
Sect. Scutellaria	
Sc. galericulata L.	Azerbaijan: Road of Talatapeh towards Urmieh, Gol- emarz village, Izadpanah and Taheri 68342 (TARI).
Sc. condensata Rech. f. subsp. *condensata*	Kermanshah: 18 km N. of Kerend gharb, Lotfeh village, Hamzehee and Hatami 1437 (TARI).
Sc. condensata Rech.f subsp. *pycnotricha* (Rech. f.) Rech. f.	Kurdestan: 20 km from Shevisheh to Marivan, Assadi 84968 (TARI).
Sc. tournefortii Benth.	Gorgan: Golestan forest, forest S. of Tang- e Gol, 700-1000 m, Wendelbo and Foroughi 12759 (TARI).
Sc. velenovskyi Rech. f. subsp. *subsimilis* Rech. f.	Kermanshah: 14 km N. of Kerend, 1800-2000 m, Assadi 60883 (TARI).
Subgen. Apeltanthus	
Sect. Lupulinaria	
Sc. araxensis Grossh.	Azerbaijjan: 35 km to Maku on road from Marand, 1900 m, Assadi and Mozaffarian 30084 (TARI).
Sc. farsistanica Rech. f.	Esfahan: Kashan, Ghamsar, Kah-e, Kargar above the village Barzok, 2081 m, Assadi 82736 (TARI).
Sc. litwinowii Bornm. & Sint. ex Bornm.	Khorassan: 18 km from Sabzevar to Esferayen, 1600 m, Mozaffarian 48396 (TARI).
Sc. luteo-coerulea Bornm. & Sint. ex Bornm.	Khorassan: 40 km of Ghuchan on the road to Darrehgas, 1820 m, Assadi and Massoumi 21451 (TARI).
Sc. multicaulis Boiss.	Baluchestan: Taftan. Mnt. Region Tamendan vally, 2330-2500 m, Mozaffarian 53166 (TARI).
Sc. pinnatifida A. Hamilt.	Kermanshah: Between Kermanshah and Paveh, before Shamshir, Mansour, Aghai, 2300-2800 m, Assadi and Mehregan 89158 (TARI).
Sc .platystegia Juz.	Azerbaijan: 6 km from Germi to Ani, W. of Easemar village, 900-1200m Mozaffarian and Nowrizi 34959(TARI).
Sc. theobromina Rech. f	Azerbaijan: Between Oshnovieh and Urmia, Sangar (NG2), 1700 m, Assadi 85171 (TARI).
Sc. tomentosa Bertol.	Esfahan: Meyameh to Muteh-Varkan environment station, 1920 m, Feyzi 13102 (TARI).

nutlets were mounted directly on aluminum stubs using double- sided adhesive and were sputter coated with gold. The SEM micrographs were taken in a VEGA// TESCAN at an accelerating voltage of 15 KV. The terminology used for nutlet sculpturing is based on the work by Billie et al. (1996) and Barthlott (1984).

Results

Nutlets of the examined species are brown or black. Their length varies from 1.3 to 1.9 mm and their width from 0.7 to 1.6 mm (table 2). They are ovate-triangular, ovate, oblong elliptic or orbicular. They are usually convex on the dorsal side. On the ventral side an approximately columnar attachment scar is observed. The surface around hilum is smooth (table 2, figs.1-5).

The nutlet surface ornamentation is usually identical in both dorsal and ventral views and shows two general patterns: papillate and hairy.

The nutlet features of the studied species in each of the above mentioned groups are described bellow.

The species with papillate surface are *Sc. galericulata*, *Sc. ariana* and *Sc. tournefortii*. In *Sc. galericulata* (fig. 1 a-d) the nutlet is more or less orbicular, the surface is papillate with sessile glands interspersed among papillae, the papillae are rounded, with small rounded projections at their apices and

Table 2. Seed characters in *Scutellaria* species.

Subgenera, sections & species	Nutlets size (mm)	Ornamentation in dorsal view
Subgen. Scutellaria **Sect. Anaspis** *Sc. ariana* Hedge	0.8 ×1.3	Papillate, ± finger like
Sect. Scutellaria *Sc. galericulata*	1.4-1.5× 1.1- 1.4	papillate- glandular
Sc. condensata subsp. *condensata*	1.3-1.6 × 0.8-1.3	papillate, scattered hairs
Sc. condensata subsp. *pycnotricha*	1.3-1.5 × 1.1-1.3	papillate, scattered hairs
Sc. tournefortii	1.3-1.7 × 0.8-1.1	papillate
Sc. velenovskyi subsp. *subsimilis*	1.7-1.8 × 1.1-1.4	papillate, scattered hairs
Subgen. Apeltanthus **Sect. Lupulinaria** *Sc. araxensis*	1.3-1.6 × 0.8-1	densely stellate
Sc. farsistanica	1.5-2.4 × 1-1.5	densely stellate
Sc. *litwinowii*	1. 6 × 1.1-1.2	densely long villouse
Sc. luteo-coerulea	1.7 × 1	densely stellate
Sc. multicaulis	1.7-1.8 × 0.9-1	densely stellate
Sc. pinnatifida	1.8 × 1.1	densely stellate
Sc. platystegia	1.9-2.1 × 1.4-1.6	densely stellate
Sc. theobromina	1.9 × 1.6	densely stellate
Sc. tomentosa	1.4-1.5 × 0.7-1	densely stellate

convex in the centre (fig. 1b). The surface around hilum is not papillate and consists of a soft tissue. In *Sc. ariana* (fig. 1 e-h) the nutlet is elliptic to orbicular, the surface is papillate-tuberculate, ± finger like, the papillae apices are obtuse to ± acute. In *Sc. tournefortii*, the nutlet is oblong, the surface is papillate and the papillae have dark concave part at their apices. The tissue around the hilum is smooth with flat surface cells (Fig. 2 a-d).

The group with hairy nutlet surface is divided into two subgroups: the species with adpressed and patent hairs partially covering the surface, concentrated near the papillae apices. *Scutellaria condensata* and *Sc. velenovskyi* belong to this subgroup. In *Sc. velenovskyii* subsp. *subsimilis* nutlet is ovate-rounded, surface with flattened polygonal papillae, covered with scattered hairs. These hairs are also present around the hilum (fig. 2 e-h). In *Sc. condensata* subsp. *condensata* and subsp. *pycnotricha* the nutlets are ovate triangular, the surface is papillate, the papillae are rounded and concave at the apices, and the long hairs are concentrated in the concave part of papillae apices. The hairs are also present close to the hilum. The two subspecies are very similar in their nutlet sculpturing, however subsp. *pycnotricha* is covered by denser hairs. In species with very dense adpressed stellate or simple lairs the nutlet surface is completely covered with hairs and their sculpturing can not be observed. The examined species of this group are *Sc. araxensis* (fig. 4-1 a-d), *Sc. farsistanica* (fig. 4 i-l), *Sc. litwinowii*, *Sc. luteo-coerulea* (fig. 5 a-d), *Sc. multicaulis*, *Sc. pinnatifida* (fig. 4 q-t), *Sc. platystegia* (fig. 4 u-x), *Sc. theobromina* (fig. 5 e-h) and *Sc. tomentosa* (fig. 4 m-p). In *Sc. multiaculis* the nutlet is elliptic to obovate and densely covered with distinctly stellate hairs. A short conical appendix is observed in the centre of each stella. This is possibly the long apex of the surface papilla. This structure was not seen in the other species with stellate hairs (fig. 4 e-g). *Scutellaria litwinowii* is another species with hairy nutlet. We only had a few nutlets to examine. The specimen that we studied had long dense villous hairs and the stellate pattern could not be identified (fig. 6 a-b). All the other species of this group have identical hair covering on their surface

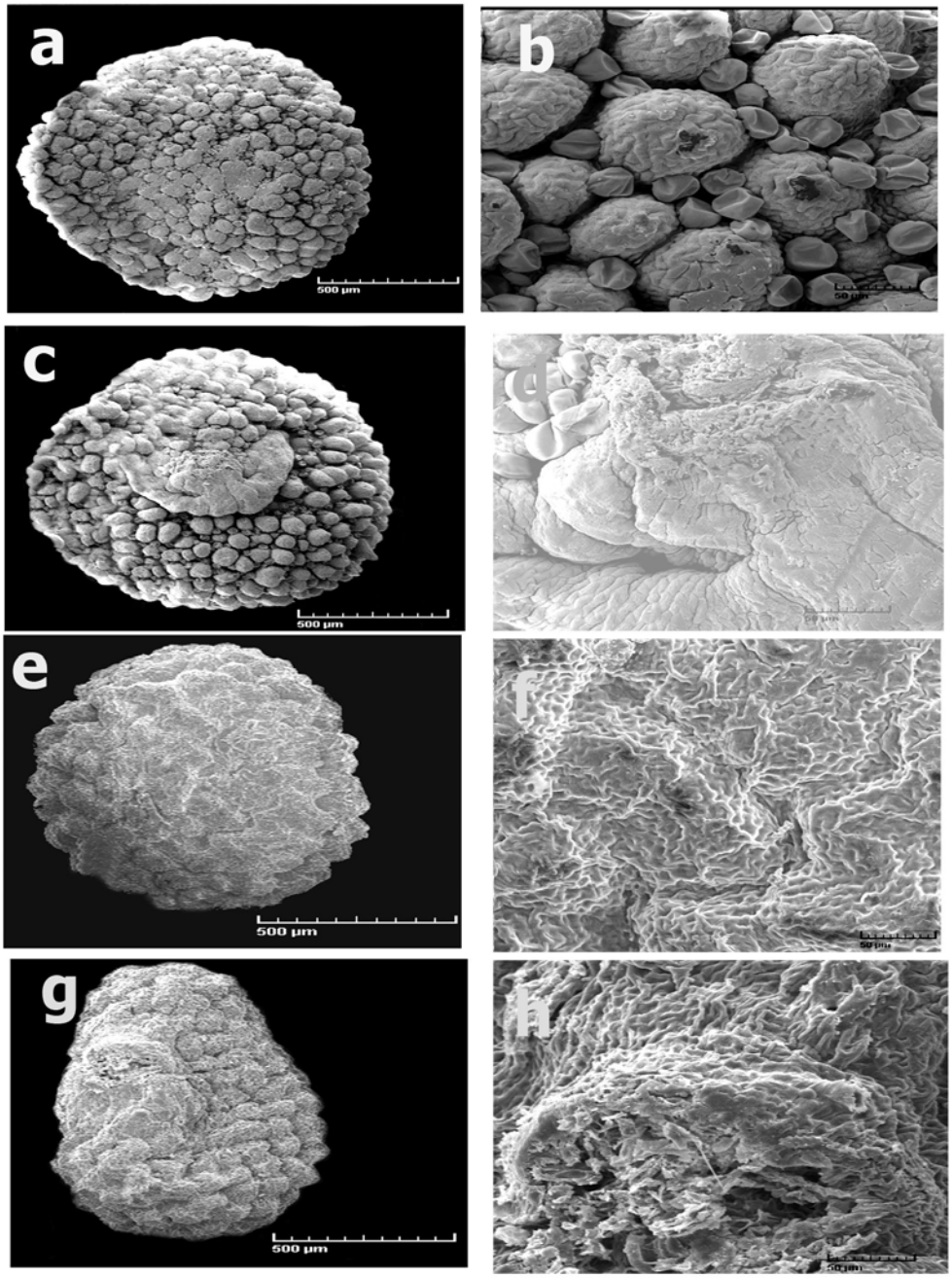

Fig. 1, a-d. *Scutellaria galericulata*: a. dorsal view, c. ventral view of nutlet, b. nutlet sculpturing, d. detail of hilum. e-h. *Sc. ariana*: e. dorsal view, g. ventral view of nutlet, f. nutlet sculpturing, h. detail of hilum. scale bars: a, c, e, g 500 µm; b, d, f, h 50 µm.

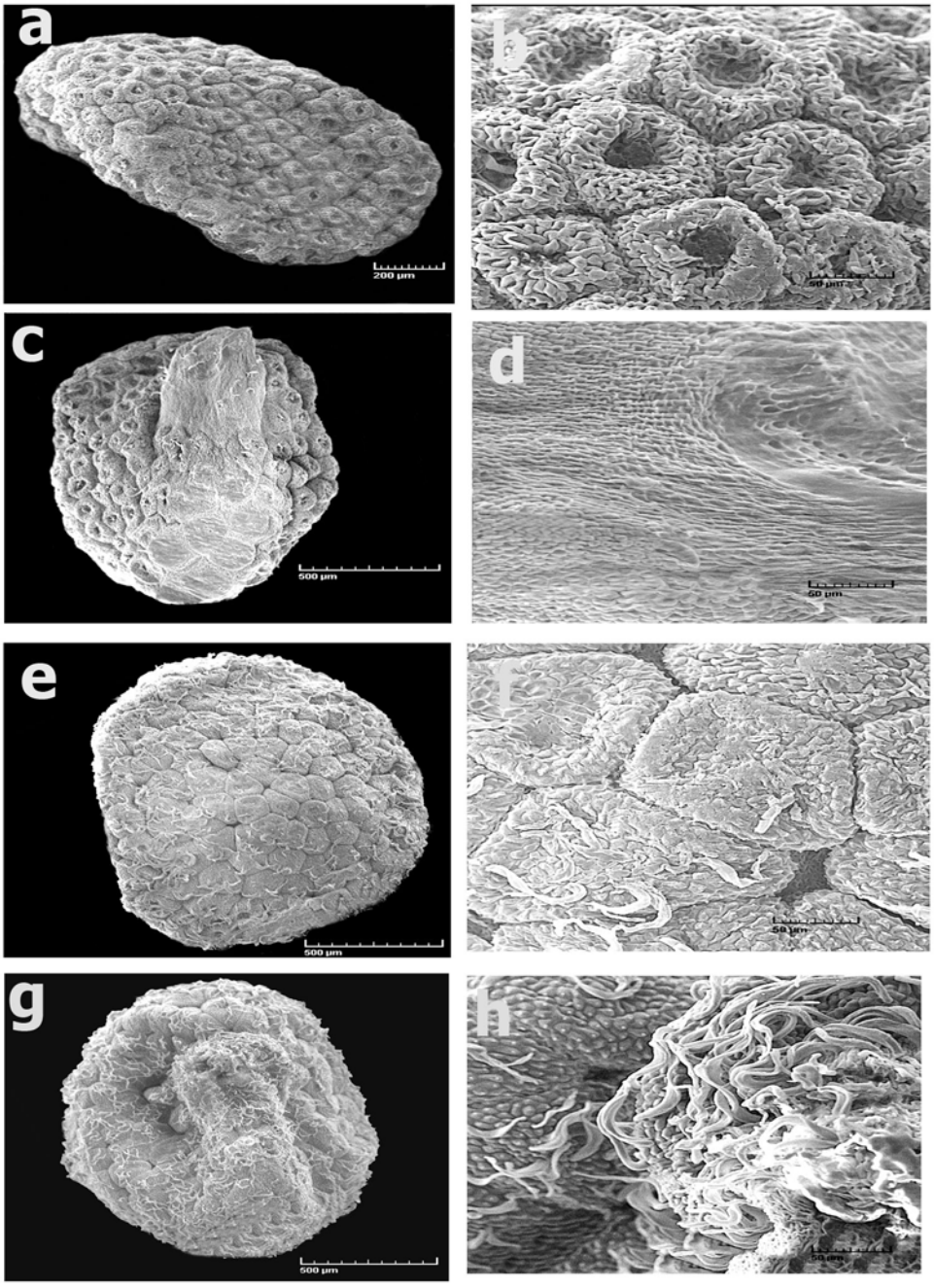

Fig. 2, a-d. *Scutellaria tournefortii*: a. dorsal view, c. ventral view of nutlet, b. nutlet sculpturing, d. detail of hilum. e-h. *Sc. velenovskyi* subsp. *subsimilis*: e. dorsal view, g. ventral view of nutlet, f. nutlet sculpturing, h. detail of hilum. scale bars: a 200 µm; c, e, g 500 µm; b, d, f, h 50 µm.

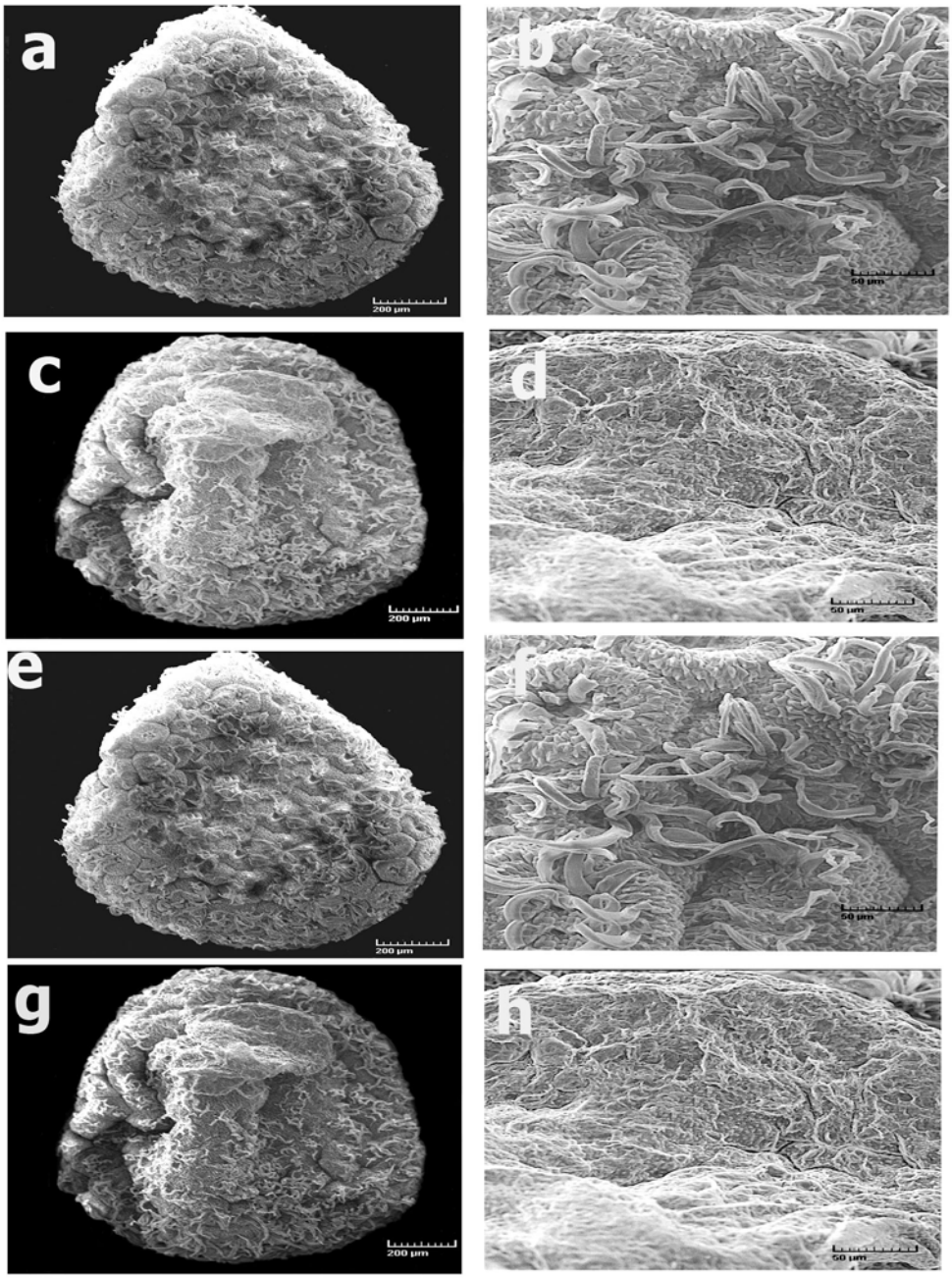

Fig. 3 a-d. *Scutellaria condensata* subsp. *condensata*: a. dorsal view, c. ventral view of nutlet, b. nutlet sculpturing, d. detail of hilum. e-h. *Sc. condensata* subsp. *pycnotricha*, e. dorsal view, g. ventral view of nutlet, f. nutlet sculpturing, h. detail of hilum. scale bars: a, c, e, g 200 µm; b, d, f, h 50 µm.

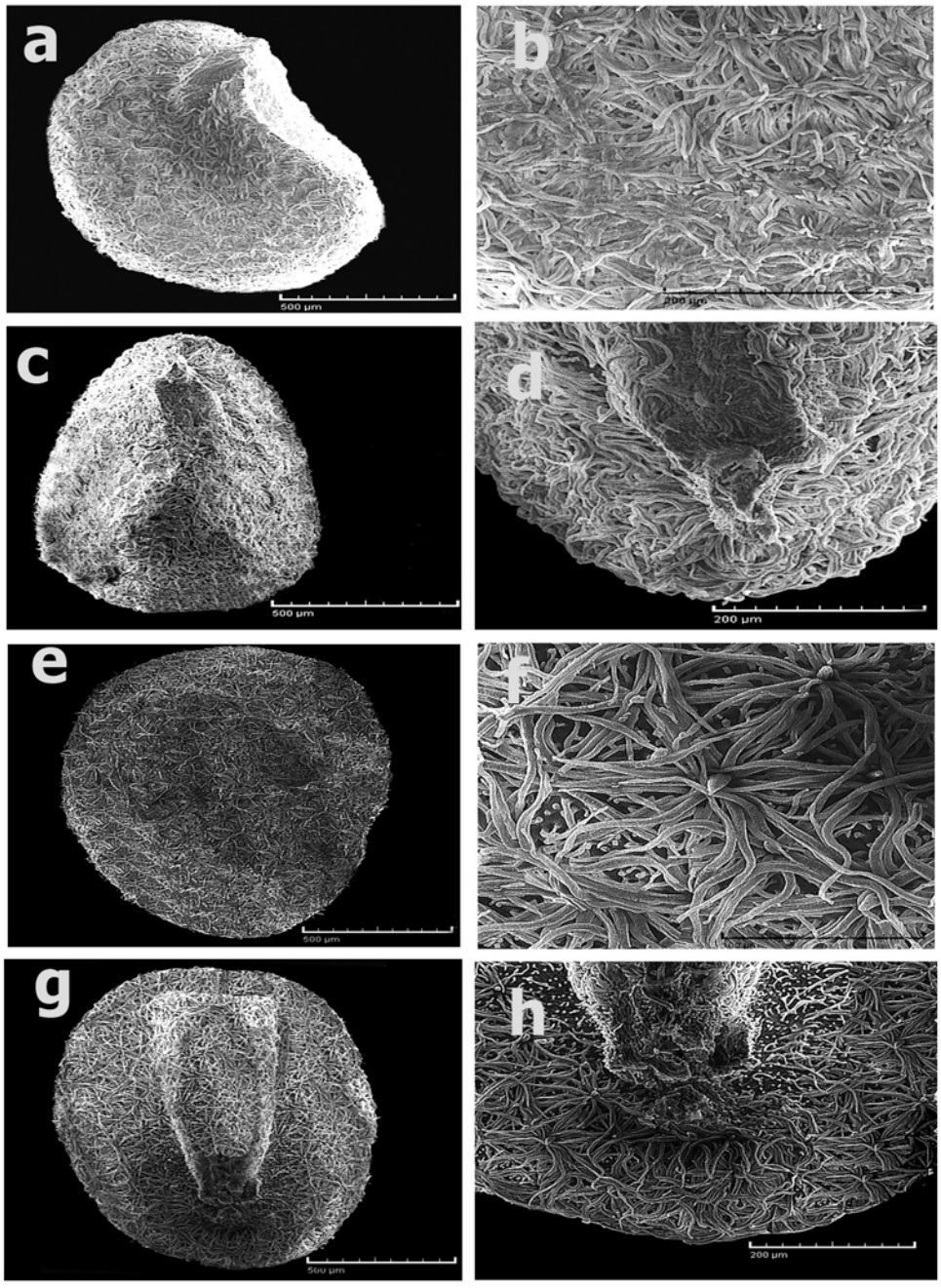

Fig. 4, a-d. *Scutellaria araxensis*: a. dorsal view, c. ventral view of nutlet, b. nutlet sculpturing, d. detail of hilum. e-h. *Sc. multicaulis*: e. dorsal view, g. ventral view of nutlet f. nutlet sculpturing, h. detail of hilum. scale bars: a, c, e, g 500 µm; b, d, f, h 200 µm.

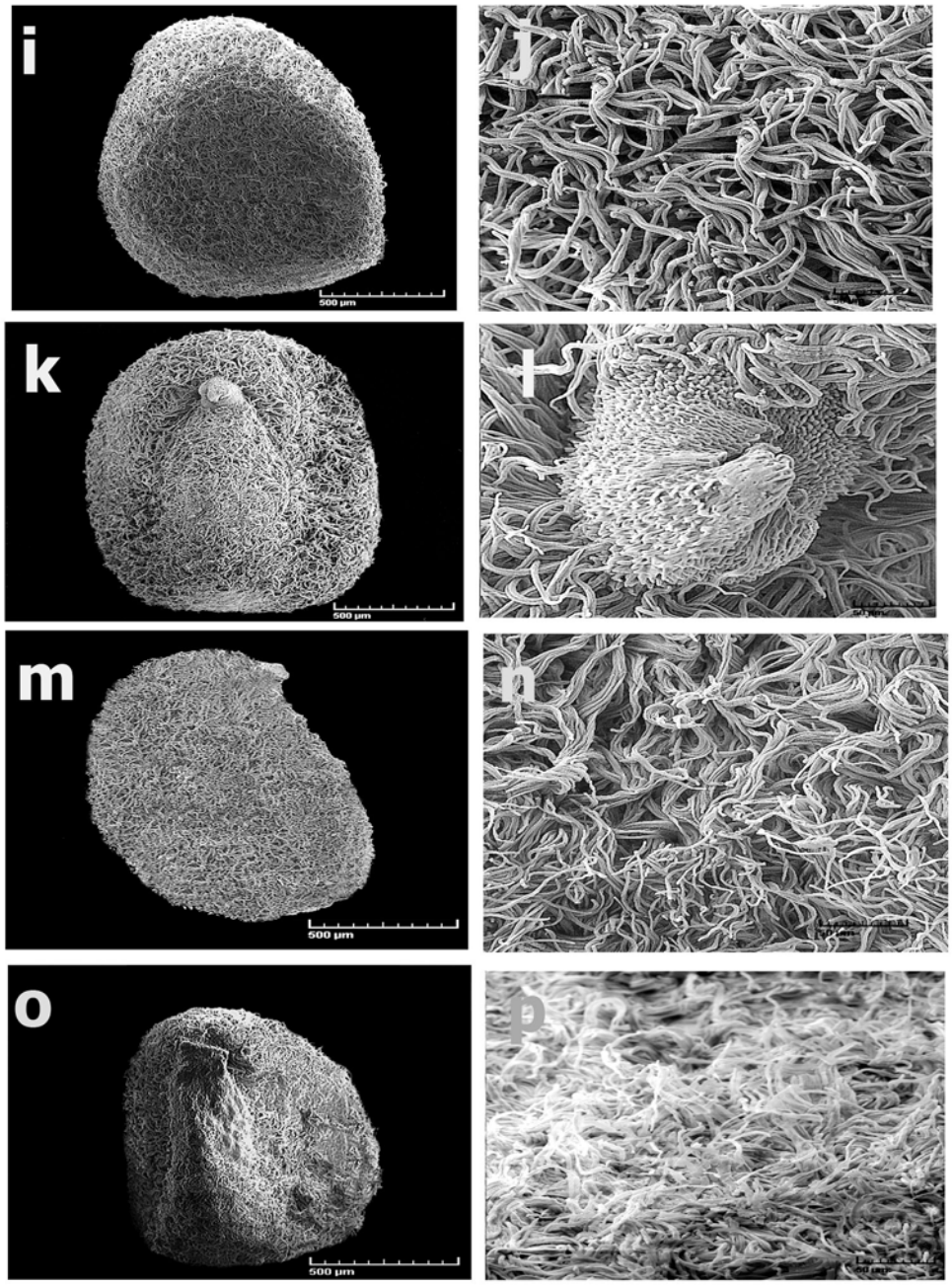

Fig. 4. continued. i-l. *Scutellaria farsistanica*: i. dorsal view k. ventral view of nutlet, j. nutlet sculpturing, l. detail of hilum. m-p. *Sc. tomentosa*: m. dorsal view o. ventral view of nutlet, n. nutlet sculpturing, p. detail of hilum. scale bars: i, k, m, o 500 µm; j, l, n, p 50 µm.

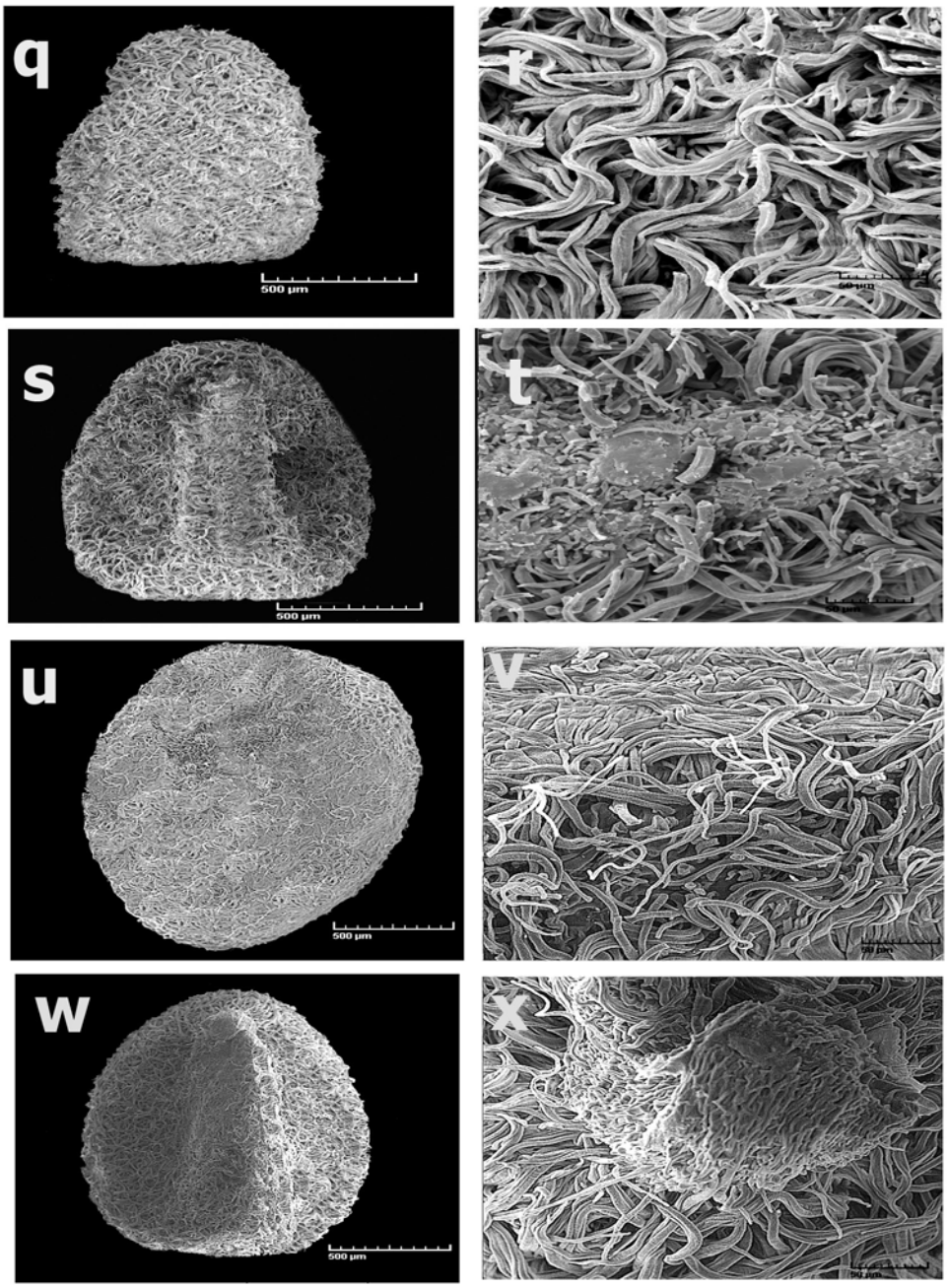

Fig. 4. continued, q-t. *Scutellaria pinnatifida*: q. dorsal view, s. ventral view of nutlet, r. nutlet sculpturing, t. detail of hilum. u-x. *Sc. platystegia*: u. dorsal view, w. ventral view of nutlet, v. nutlet sculpturing, x. detail of hilum. scale bars: q, s, u, w 500 µm; r, t, v, x 50 µm.

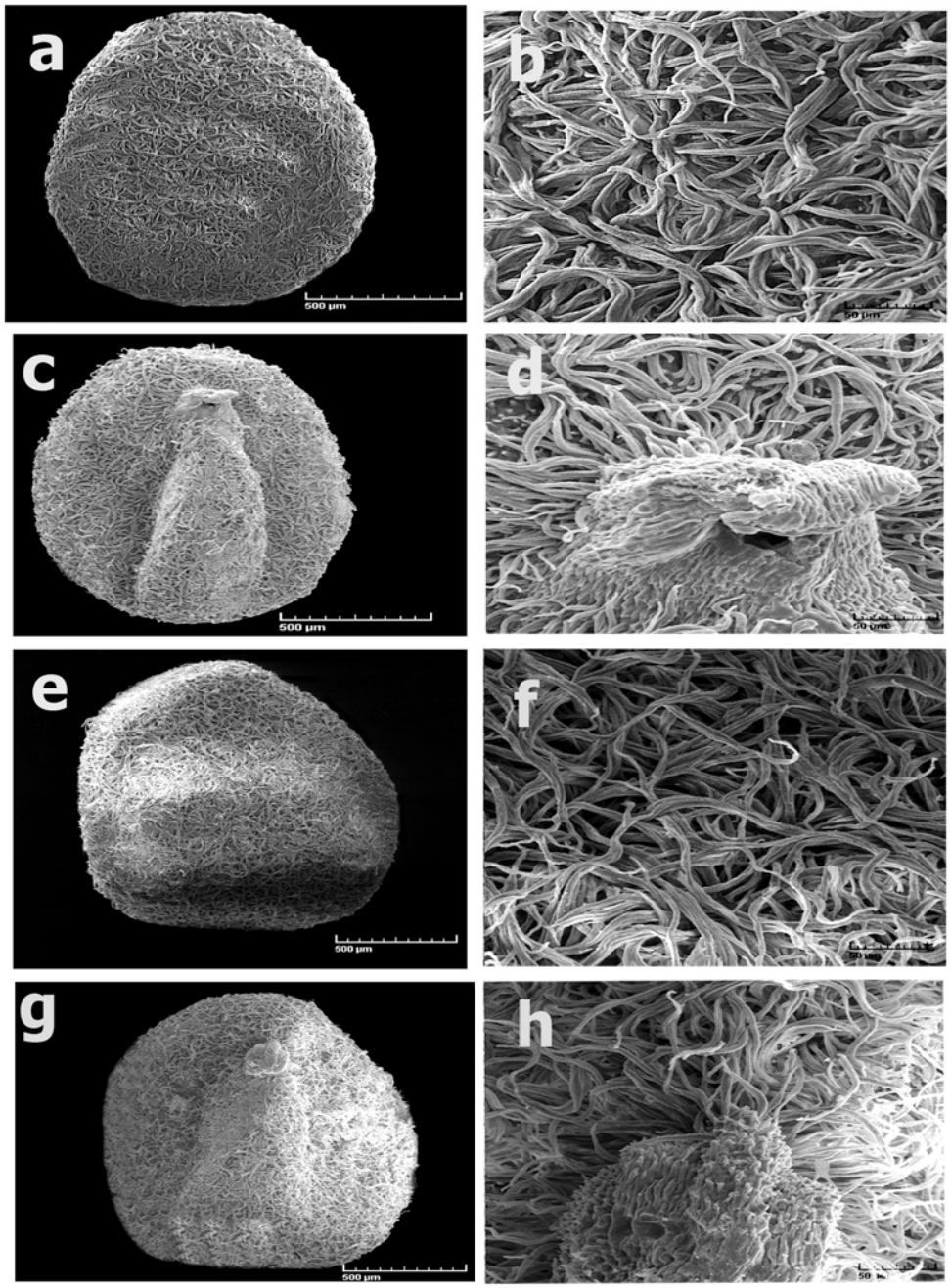

Fig. 5, a-d. *Scutellaria luteo-coerulea*: a. dorsal view, c. ventral view of nutlet, b. nutlet sculpturing, d. detail of hilum. e-h. *Sc. theobromina*: e. dorsal view, g. ventral view of nutlet, f. nutlet sculpturing, h. detail of hilum. scale bars: a, c, e, g 500 µm; b, d, f, h 50 µm.

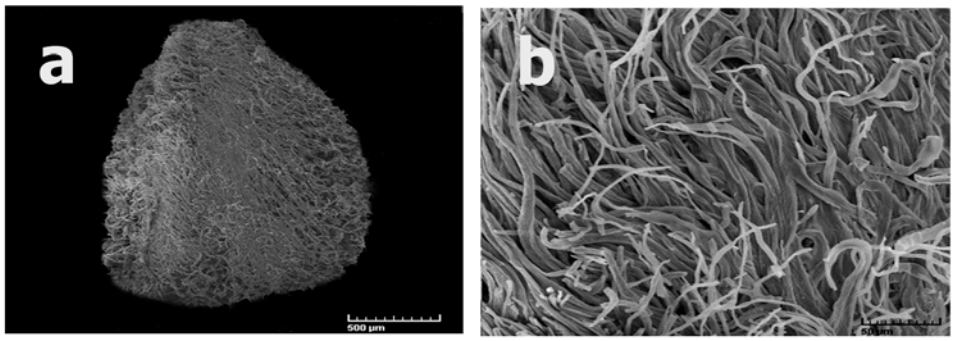

Fig. 6, a-b. *Scutellaria litwinowii*: a. dorsal view of nutlet, b. nutlet sculpturing. scale bars: a 500 µm; b 50 µm.

(fig. 4 a-d and h-x) but minor differences could be observed in other nutlet features summarized in table 2.

Discussion

The results of this study revealed a variety of sculpturing types which are classified as follows:

Type I. Nutlet surface papillate with sessile glands interspersed among papillae. This type is observed in *Sc. galericulata* of subgenus *Scutellaria* section *Scutellaria*.

Type II. Nutlet surface papillate, papillae circular with concave apex. This type is observed in *Sc. tournefortii* of subgenus *Scutellaria* section *Scutellaria*.

Type III. Nutlet surface papillate, papillae with obtuse to acute apex and more or less finger like. This type was observed in *Scutellaria ariana* of subgenus *Scutellaria*, section *Anaspis*.

Type IV. Nutlet surface with adpressed and patent hairs partially covering the surface, concentrated near the papillae apices. Papillae are either flattened polygonal or rounded, concave at the apices. This type is observed in *Sc. velenovskyi* and *Sc. condensata* of subgenus *Scutellaria*, section *Scutellaria*. These species were previously recognized in section *Stachymacris*.

Type V. Nutlet surface densely hairy with long stellate or simple hairs. This type is observed in *Sc. araxensis, Sc. farsistanica, Sc. litwinowii, Sc. luteo-coerulea, Sc. multicaulis, Sc. pinnatifida, Sc. platystegia, Sc. theobromina and Sc. tomentosa.* These species belong to subgenus *Apeltanthus* section *Lupulinaria*. The species within this category were included in subgenus *Scutellaria* section *Stachymacris* p.p. and subgenus *Scutellaria* sect. *Lupulinaria* p.p.

Our result is partly congruent with the classification system suggested by Paton (1990a), considering two subgenera for *Scutellaria* and including subgenus *Anaspis* in subgenus *Scutellaria*. Reviewing the data available on nutlet sculpturing shows that species of subgenus *Scutellaria* has papillate not hairy or sparsely hairy nutlets but those in subgenus *Apeltanthus* section Lupulinaria have densely hairy nutlets.

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