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SEM STUDIES REGARDING MICROMORPHOLOGY OF FRUIT, SEED COAT AND POLLEN IN GENUS *HESPERIS* L. (*BRASSICACEAE*)

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Abstract: The paper presents the micromorphological characteristics (SEM investigations) of fruits, seed coats and pollen grains of genus *Hesperis* in Austria. The siliquae are usually terete, glabrous and rarely with simple trichomes (subsp. *nivea*), straight or slightly curved. The seeds can present apical wings or not. The testa is reticulate and two main types were recognize based on seed coat surface, reticulate and ocellate-papillate. The pollen is tricolpate and exine is reticulate. The study revealed that palynological, fruit and seed micromorphological characteristics are significant in the taxonomy of the genus. An original dichotomic key for *Hesperis* species in Austria (*H. matronalis* subsp. *matronalis* and subsp. *nivea*, *H. sylvestris*, *H. tristis*) based on micromorphological characteristics is provided.
Keywords: SEM studies, micromorphology, fruit, seed, pollen, *Hesperis*, *Brassicaceae*

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Introduction

Hesperis L. (dame′s rocket) is native to Europe, to Marocco, and to temperate and tropical Asia, but centred to the temperate zones of Eurasia. This genus comprises 55-60 species, 49 of them occurring in Europe (Ball 1964) and three species, which we investigated, occur in Austria: *H. matronalis* (subsp. *matronalis* and subsp *nivea*) with eglandular trichomes on stem, leaves and pedicels, and *H. tristis* and *H. sylvestris* with glandular trichomes (Fischer & al. 2008). These species are characterized mainly by white to purplish and even violet petals, or yellow green with brown-purplish veins in *H. tristis*, an endangered rarity in Austria, growing only in the Pannonian area. *H. matronalis* subsp. *nivea* is growing only in Styria, very rare and potentially endangered (Fig. 1). This study presents micromorphological (SEM) characters of fruit, seed coat (testa) and pollen, there are significant in the taxonomy of the genus (Padure & al. 2016).

H. matronalis subsp. *matronalis* is a perennial herb, uppermost leaves with a short stalk, petals 20-25 mm long, mostly dark violet to pale violet or white, height: 40-80 (100) cm, flowering period between May and November, cultivated as an ornamental plant and often wild, naturalized in wet areas and on ruderal sites, collinar to montane level, scattered in all federal states.

H. matronalis subsp. *nivea* (Fig. 1) is a perennial plant, height: 40-80 cm petals always bright, circa 20 mm long, siliqua 3-4 cm long, stem below with long downward-pointing bristles, rarely very short glandular trichomes on the leaf margins, flowering period between June and July, in herbaceous areas, forests, along ravines and streams, nitrophilic perennial corridors, collinar to montane level, very rare in Styria (in

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the Northern Alps, in the Grazer Bergland, near Mixnitz and Vordernberg), endangered species, fully protected in Styria (Gutermann 2019).

Fig. 1. *Heperis matronalis* subsp. *nivea*, Raabklamm, Styria, June 2018 (orig.)

H. sylvestris is a perennial plant, always with glandular trichomes (especially in the upper part of stem and on the leaves), upper leaves ± sessile, petals 20 mm long, light purple, height 40-80 cm, flowering period between June and August stony, light forests, sunny forest cuts and edges, collinar to submontane level, very rare in Austria, highly endangered (Fischer & al. 2008, Gutermann 2019, Schratt-Ehrendorfer & al. 2022).

H. tristis is a biennial to perennial plant, petals yellowish green with purple veins, only in the lowest leaves have stalks, otherwise all are sessile, stem and floral pedicels with glandular trichomes, petals 20-32 mm long, fruit 4-14 cm long, horizontally protruding to overhanging. Height 35-50 (60) cm, flowering period May to June, semi-arid grassland, forest edges, on lime soil, collinear level, rare, only in the Pannonian region of Austria, endangered (Fischer & al. 2008, Schratt-Ehrendorfer & al. 2022).

Material and methods

Plant material: The investigation of fruits, seed coats and pollen are based on fresh and dry material collected from wild populations and specimens of *Hesperis* from GJO Herbarium (GJO0090192 / GJO0090188) and personal collections. The plants were collected from different parts of Austria. Dozens of herbarium specimens have been revised, updated, corrected or refuted in the light of new taxonomic knowledge (GJO / GZU). [*https://herbarium.univie.ac.at/database/collections.htm*, Virtual Herbaria Jacq.].

SEM techniques: For the SEM investigation of fruits, pollen grains and seeds, the specimens were coated with a thin layer (5-10 nm) Gold / Palladium for electrical conductivity. SEM analysis was performed using an SEM Zeiss Ultra 55 at 5.00 kV. Secondary electron imaging (SE) was performed using an Everhart-Thornly detector.

Results and discussion

Taxonomy. Gutermann (2019) agrees with *H. matronalis* subsp. *nivea* is the correct name of this subspecies instead *H. matronalis* subsp. *candida* in Notulae nomencl.:

H. matronalis subsp. *nivea* (Baumg.) E. P. Perrier, Cat. Pl. Vasc. Savoie **1**: 49 (1917); Kulcz. in Szafer [Red.], Fl. Polska **3**: 181 (1927), later isonym ≡ *H. nivea* Baumg., Enum. Stirp. Transsilv. 2: 278 (1816) ≡ *H. inodora* subsp. *nivea* (Baumg.) Simk., Enum. Fl. Transsilv.: 82 (1887) = *H. candida* Kit. [apud Schulzer & al. in Verh. K. K. Zool.-Bot. Ges. Wien **16**: 143 (1866) pro syn. *H. matronalis* var. *integrifolia* Neilr.] in Hayek, Sched. Fl. Stiriac.: no. 538 (1907), Fl. Steiermark **1**: 504 (1909) ≡ *H. matronalis* subsp. *candida* (Kit.) Hegi & E. Schmid in Hegi, Fl. Mittel-Eur. **4**: 467 (1919) = *H. moniliformis* Schur [in Oesterr. Bot. Z. **8**: 22 (1858), nom. nud.] in Verh. Mitth. Siebenbürg. Vereins Naturwiss. Hermannstadt **10**: 166 (1859), Enum. Fl. Transsilv.: 52 (1866) ≡ *H. matronalis* subsp. *moniliformis* (Schur) Borza, Consp. Fl. Romaniae: 123 (1947) ? incl. *H. inodora* var. *vrabelyiana* Schur in Verh. Naturf. Vereins Brünn **15**(2): 81 (1878) ≡ *H. vrabelyiana* (Schur) Borbás in Magyar Bot. Lapok **2**: 21 (1903) ≡ *H. matronalis* var. *vrabelyiana* (Schur) Soó in Tisia **3**: 90 (1939) [n. v.], Tisia **4**: 54 (1940).

The autochthonous, white-flowering populations of *H. matronalis* traditionally Notulae nomenclaturales 46–59 143 were classified as *H. candida* or as *H. matronalis* subsp. *candida* in Austria, where they are confined to two small areas in Upper Styria mountains with Palaeozoic limestones, near the village of Mixnitz, and north-east of the town of Trofaiach (Gitermann 2019). In respect to the stem leaves (the upper ones nearly sessile) and to the indumentum of stem and leaves (usually with only a few bifid hairs in addition to the prevailing simple bristles), there is no substantial difference from the Carpathian subsp. *nivea* (Ciocârlan 2000). This latter epithet holds priority at subspecific rank (by the combination under *H. inodora*) and is already in use for equivalent populations of northern Italy (Bartolucci et al. 2018). This indigenous mountain taxon was broadly conceived already by Dvořák (1968), a definition also accepted in the Flora of Slovakia (Zahradníková et al. 2002, as *H. matronalis* subsp. *candida*) in which country it is mainly known from the Malé Karpaty, the Fatra and Tatra mountains. Plants from the Bükk mountains in northern Hungary are separated, even as species: *H. vrabelyiana* (Tibor 2008) on account of the glandular indumentum, but glandular hairs may occur though sparsely also in otherwise typical populations of subsp. *nivea* (Gutermann 2019).

Macro- and micromorphology of fruit and fruiting pedicels. The trichome types on fruiting pedicels are various: capitate glands (short-stalked or with 2-celled stalk) in *H. sylvestris* and *H. tristis* or eglandular, which may be simple (uni- or multicellular), bifurcated (Y-type or with unequal arms). Sometimes the fruiting pedicels are glabrous (subsp. *matronalis*). The siliquae are usually terete or ± flattened-tetraquetros in *H. tristis*, glabrous and rarely with simple trichomes (subsp. *nivea*), straight or slightly curved, erect, or ascending to erect (Fig. 2). The pericarp of dehiscent siliquae is relatively thin in *H. matronalis* (both subspecies) and *H. sylvestris*. *H. tristis* present a ± thick pericarp. Internal part of fruit and septum is very similar to all species.

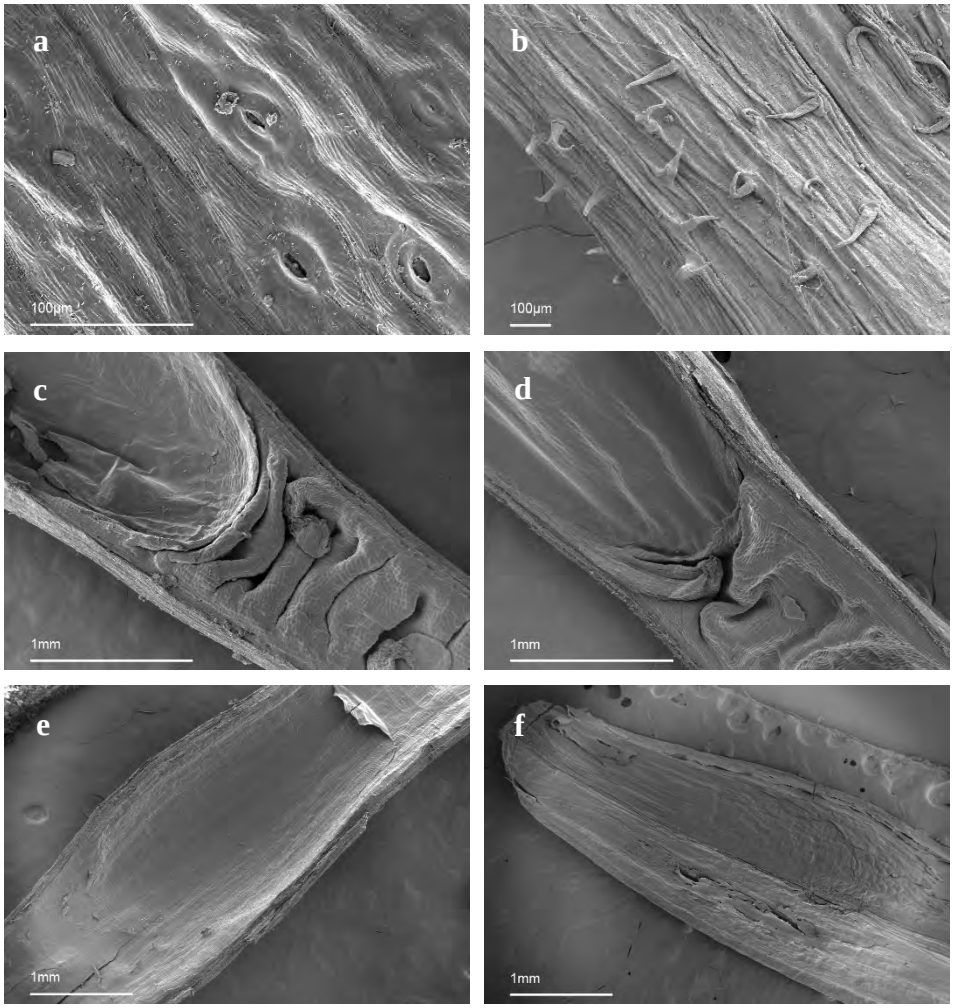

Fig. 2. SEM images of fruits in *Hesperis*: a. glabrous pericarp in *H. matronalis* subsp. *matronalis*, b. hairy pericarp with short-protective trichomes in *H. matronalis* subsp. *nivea*; internal part of siliqua with septum: c., e. subsp. *matronalis*, d., f. subsp. *nivea*

Macro- and micromorphology of seeds. The colour and shape of seeds from the upper and lower siliques are very similar. From brown to reddish- or dark brown, the seeds are oblong to elliptic, with a visible hilum. The surface is longitudinal pitted or with ± perceptible furrow on the ventral side, usually with apical wings or without it (*H. tristis*). The apical wings are rounded or ± truncated and obviously. *H. matronalis* subsp. *matronalis* and *H. sylvestris* present reticulate seed coat, while the testa from subsp. *nivea* and *H. tristis* is ocellate-papillate (Table 1). The wart position is in the middle of the lumen. Epidermal cells of seed coat showing tetra- to septagonal as well as ± isodiametric patterns that forms a network (Fig. 3-5).

Pollen grain micromorphology. The main palynological features of the examined Austrian taxa were summarized in Table 1 and the are shown in Fig. 6-7.

The pollen is tricolpate, usually symmetrical, isopolar and inoperculate. The pollen grains are prolate to subprolate, rare perprolate in ssp. *matronalis* or spheroidal in ssp. *nivea*, with a reticulate exina. The outline is elliptic in the equatorial optical section and circular in the meridional optical section. The distal and proximal pole are rounded in all investigated species and ± truncate in *H. matronalis* subsp. *matronalis*. The biggest pollen grains are founded in *H. tristis* (P×E: 34.9-35.7 × 20.9-22.1 µm). The lumen shape is irregular-amorphous, sinuous muri in ssp. *nivea*, and polyedrical 3-7 sided in subsp. *matronalis*, *H. sylvestris* and *H. tristis*.

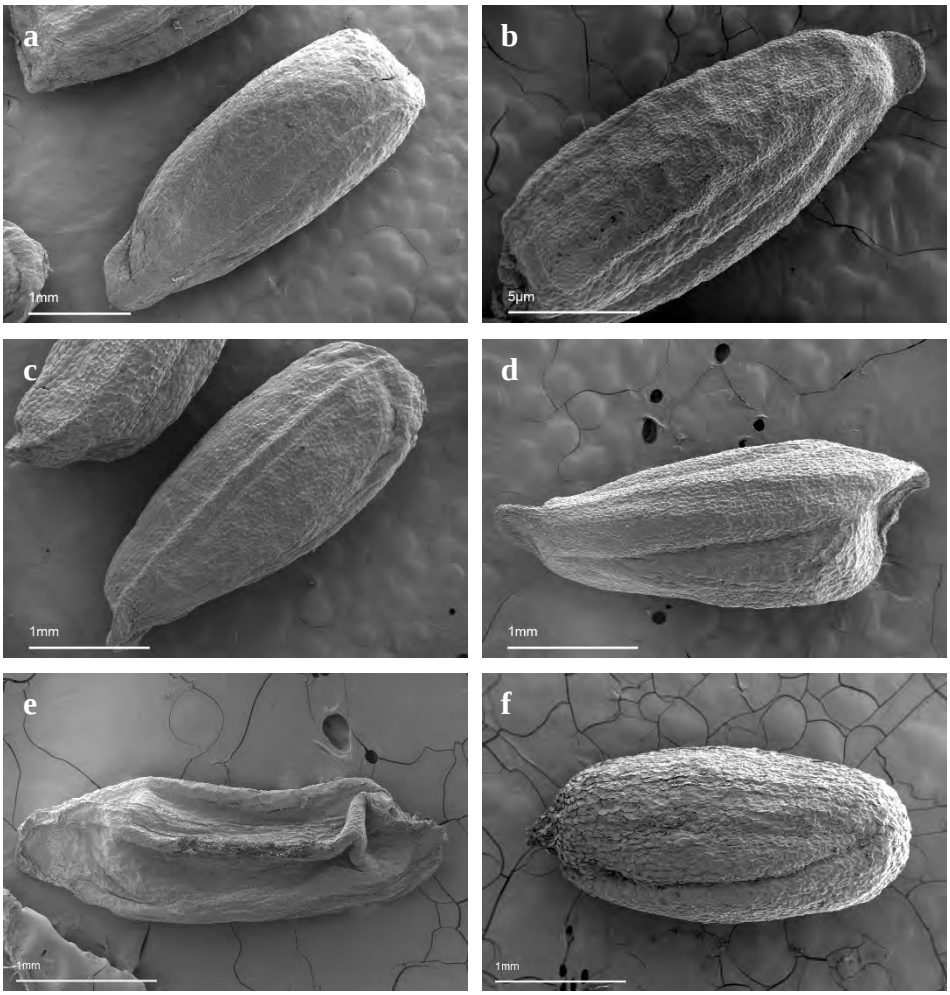

Fig. 3. SEM images of seed coat at in *Hesperis*: a., c. *H. matronalis* subsp. *matronalis*, b., d. *H. matronalis* subsp. *nivea*, e. *H. sylvestris* (a-e with apical wings), f. *H. tristis* (without apical wing), c-d lateral view of seed

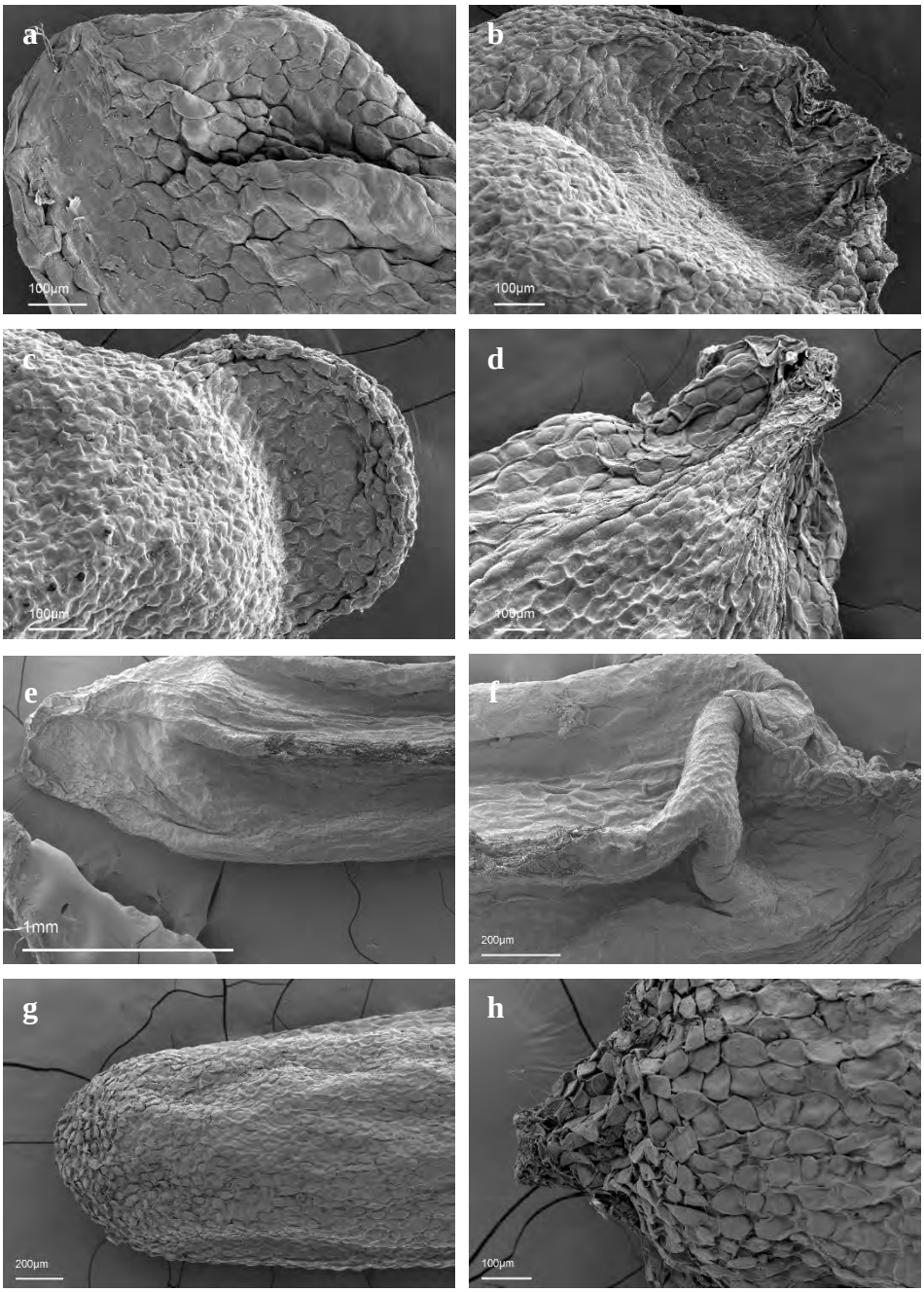

Fig. 4. SEM images of apical and basal part of seed in *Hesperis*: a-b. *H. matronalis* subsp. *matronalis*, c-d. *H. matronalis* subsp. *nivea*, e-f. *H. sylvestris* (a, c, e with apical wings), g-h. *H. tristis* (without apical wing)

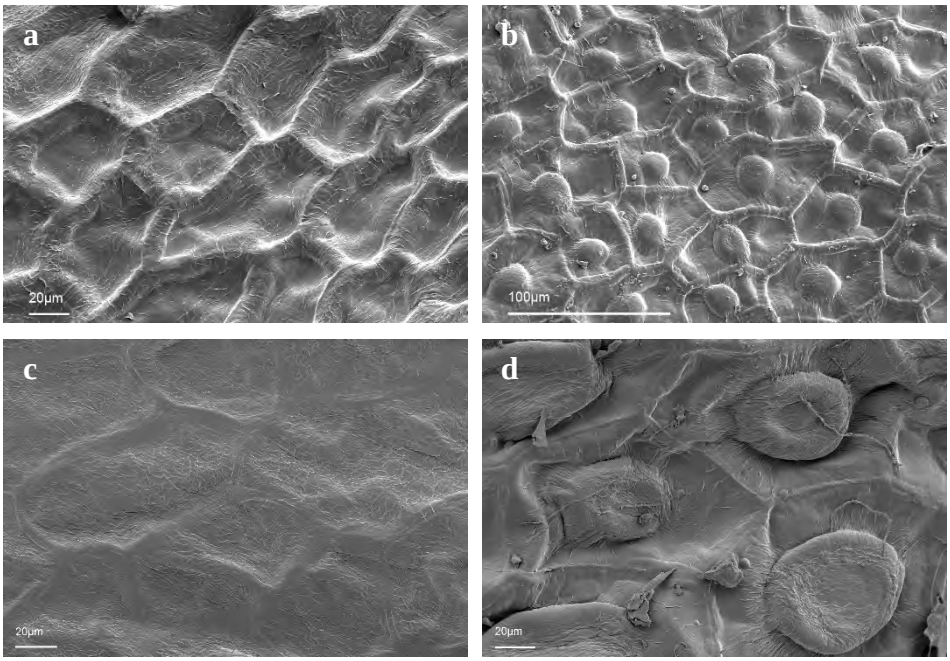

Fig. 5. SEM images of seed coat type in *Hesperis*: a., c. reticulate testa in *H. matronalis* subsp. *matronalis* and *H. sylvestris* (without warts), b., d. ocellate-papillate testa in *H. matronalis* subsp. *nivea* and *H. tristis*; b., d. wart position in the middle of lumen

Dichotomic key of *Hesperis* species based on micro- and macromorphological characters (SEM) of fruits, seeds and pollen grain:

1a Seed elliptic, L/W (based on mean values) 1.8-1.9 with a deep furrow on ventral side, apical wing of seed absent, P/E ratio 1.6 ….....…….…….…. *H. tristis*
1b Seed elongated, L/W between 2.4-2.7 longitudinal pitted, apical wings present, P/E ratio 1,3-1.5……... **2**
2a Seed surface pitted in longitudinal rows, apical wing absent …..……..… *H. sylvestris*
2b Apical wings present, truncated or rounded ……..……..…….... **3**
3a Fruct glabrous, seed coat ornamentations reticulate, the lumen shape of pollen polyedrical 4-7 sided …….……...…….…..… *H. matronalis* subsp. *matronalis*
3b Fruct hairy, testa ornamentation ocellate-papillate, the lumen shape of pollen irregular-amorphous, sinuous muri …….…… *H. matronalis* subsp. *nivea*

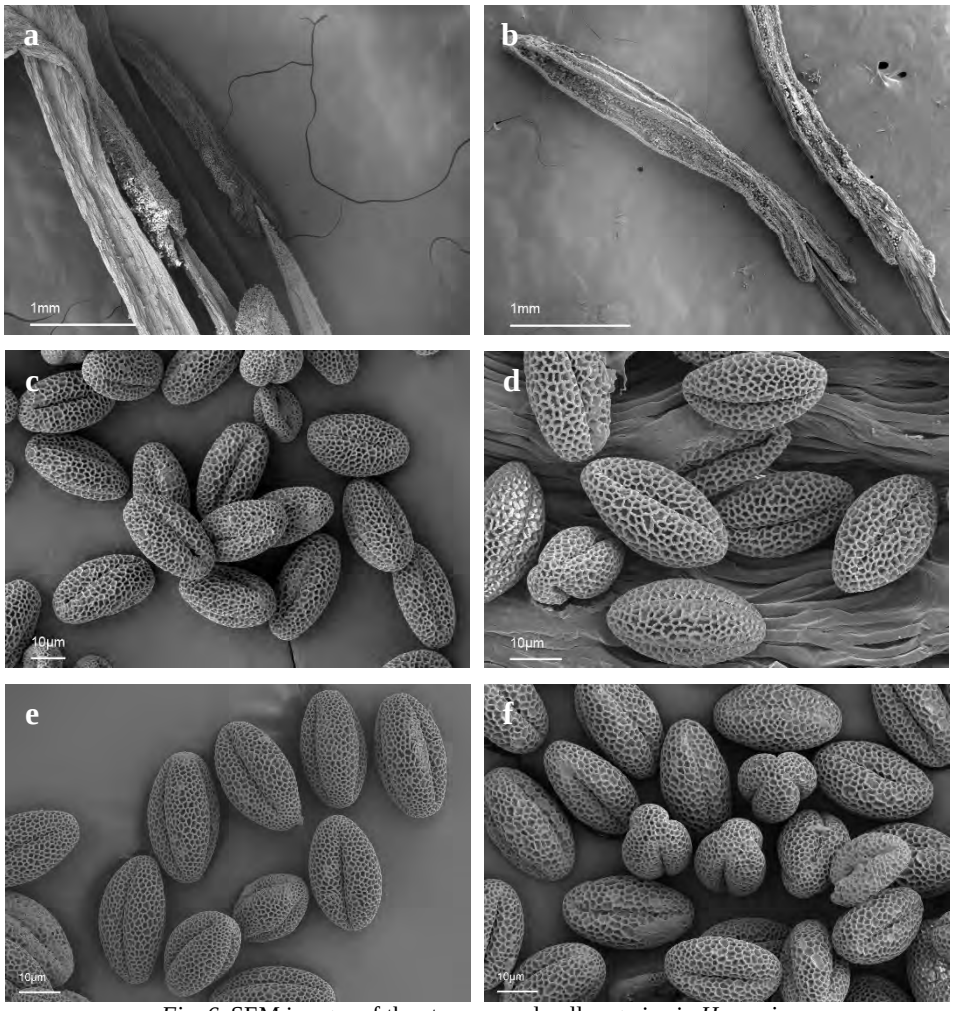

Fig. 6. SEM images of the stamens and pollen grains in *Hesperis*:
anthers with pollen – a. *H. matronalis* subsp. *matronalis*, b. *H. matronalis* subsp. *nivea*;
general view of pollen – c. *H. matronalis* subsp. *matronalis*, d. *H. matronalis* subsp. *nivea*,
e. *H. sylvestris*, f. *H. tristis*

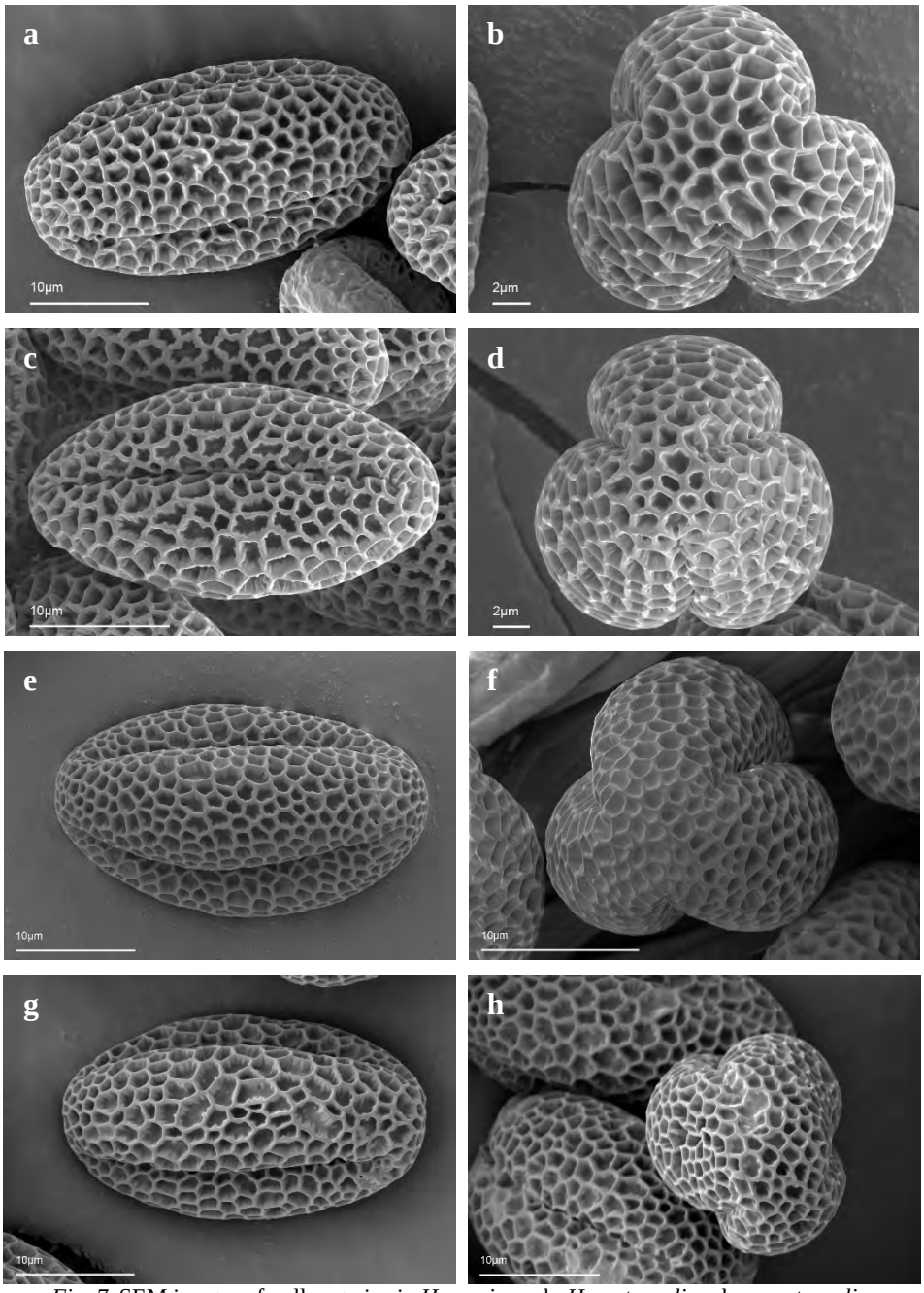

Fig. 7. SEM images of pollen grains in *Hesperis*: a., b. *H. matronalis* subsp. *matronalis*, c., d. *H. matronalis* subsp. *nivea*, e., f. *H. sylvestris*, g., h. *H. tristis* (left – equatorial view & aperture, right – polar view)

Table 1. Micromorphological characters of fruit, seed and pollen of *Hesperis* L. species

TAXON Features	***H. matronalis* subsp. *matronalis***	***H. matronalis* subsp. *nivea***	***Hesperis sylvestris***	***Hesperis tristis***
Pericarp	glabrous	hairy	glabrous	glabrous
Fruiting pedicels	hairy / glabrous, eglandular	capitate trichomes	capitate trichomes	capitate trichomes
Pollen type	tricolpate, isopolar	tricolpate, isopolar	tricolpate, isopolar	tricolpate,isopolar
Size **P x E** (µm)	18.3-33.3 × 17.2-19.4	12.3-31.9 × 15.2-17.3	29.5-32.5 × 16.8-18.5	34.9-35.7 × 20.9-22.1
Distal and proximal pole	rounded to truncate	rounded	rounded	rounded
Polar view (**P**)	circular	circular	circular	circular
Equatorial view (**E**)	prolate to sub prolate, rare perprolate	prolate to sub prolate, rare spheroidal	prolate to sub prolate	prolate to ± perprolate
Exine type	reticulate	reticulate	reticulate	reticulate
Lumen shape	polyedrical 4-7 sided	irregular-amorphous, sinuous muri	polyedrical 3-6 sided	polyedrical 3-5 sided
Seeds type	glabrous	glabrous	glabrous	glabrous
Shape of seed	oblong to elliptic or elliptic-elongate, surface longitudinal pitted	oblong-elliptic to ovate-elongate, surface longitudinal pitted	ovate to prolong-elliptic, surface pitted in longitudinal rows	Oblong to ovoid or ovate with a deep furrow on ventral side
Color	dark brown	reddish-brown	cinnamon-brown	brown
Apical wings	± truncated, yellowish	rounded, yellowish	± rounded / absent	absent
Length **L** (mm)	2.2-2.8	2.9-3.3	2.6-3	1.8-2.3
Width **W** (mm)	0.9-1.1	1.1-1.2	1.0-1.4	1.0-1.2
L/W mean val.	2.4	2.7	2.6	1.9
Seed coat	± smooth	sculptured	sculptured	sculptured
Ornamentations	reticulate	ocellate-papillate	reticulate	ocellate-papillate
Wart position	-	in the middle of the lumen	-	in the middle of the lumen

Conclusions

The micromorphological characters of fruits, seeds and pollen grains obtained by a SEM can be used successfully to differentiate the species. The hairiness of fruit pedicels and pericarp, the seed coat type and the presence of apical wings or furrows on ventral side of seed could be an important taxonomic character.

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