



MICROMORPHOLOGICAL STUDIES ON *HERACLEUM* L. (APIACEAE) IN POLAND WITH EMPHASIS ON OVARY ORNAMENTATIONS AND TRICHOMES

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ABSTRACT. The ovary surface ornamentation, pilosity and hair types were observed under SEM in Polish taxa of the genus *Heracleum*: *H. sphondylium* subsp. *sphondylium* and subsp. *sibiricum*, *H. pubescens* (ephemerophyte from two new localities), *H. mantegazzianum* and *H. sosnowskyi*. Differences of taxonomic significance were found in the pilosity and ornamentation of the ovary surface. Pilosity of ovaries supports intraspecific variation reported to date in *H. sphondylium*, but these features are not correlated with any other characters in either subspecies. The ovary surface was either rugose with secondary striations or smooth. The ovaries were glabrous (in *H. sphondylium* subsp. *sibiricum*) and more or less densely covered by hairs in the other taxa. Examined taxa exhibit a considerable homogeneity of trichome structure, they are nonglandular, but differ in their size and shape, with several different types distinguished for: macrohairs, setae, prickles and papillae. The microsculpturing of trichomes varies from smooth, striate to tuberculate. Results of this study provided data on new qualitative traits of ovary epidermal structure, which may be used in identification of *Heracleum* specimens.

KEY WORDS: *Heracleum*, ovary surface, trichomes, micromorphology, SEM

INTRODUCTION

According to literature nine species and 11 subspecies of *Heracleum* L. are found in Europe (BRUMMITT 1968). Among the European hogweeds seven are endemic species of different mountain ranges. In Poland two subspecies, i.e. *H. sphondylium* subsp. *sphondylium* [subsp. *australe* (Hart.) Ahlfv.] and *H. sphondylium* subsp. *sibiricum* (L.) Simonk., are commonly found (BRUMMITT 1968, ZYCH 2002, RUTKOWSKI 2004). The polymorphic species *H. sphondylium* in Poland is usually described as a common meadow species (GAWŁOWSKA 1956), growing also in thickets, on roadsides and forest margins (RUTKOWSKI 2004). Its geographical range includes most European countries (BRUMMITT 1968, SHEPPARD 1991). In *Heracleum* spp. the ovary may be smooth or with various types of pubescence. Pubescence is observed in the form of long or short hairs, soft or stiff. Hairs may be distributed randomly or relatively regularly and then all or almost all are pointed in one direction. The characters relating to ovary pilosity seem to be most constant. *Heracleum sphondylium*, a native taxon in our flora, occurs in Poland in numerous different forms, the taxonomy of which is fairly difficult to establish. The differences concern above all the pilosity of the ovary, the size of the outer petals in the marginal flowers of the umbellula, and the form of leaves. Flowers

are white, rarely slightly yellowish or pink, and most are characterised by bilateral symmetry. Outer petals are radiate, 2-4 mm long. The stylopodium is almost always whitish and the ovary is overgrown with soft, blunt and supple trichomes. Therefore, GAWŁOWSKA (1956) distinguished *H. sphondylium* var. *chaetocarpoides* Gawł., where the ovary is covered with extremely short, stiff hairs or sharp warts. In *H. sphondylium* subsp. *sibiricum*, the flowers are greenish or green-yellowish, never whitish and with a radial structure, while its inflorescences have a loose structure. The stylopodium is almost greenish and the ovary is glabrous. The plant closely resembles those of subsp. *sibiricum*, which differs by its ovary overgrown with tiny, stiff hairs or sharp warts, being classified to *H. sphondylium* subsp. *sibiricum* var. *chaetocarpum* H. Neumayer & Thell. (KOCZWARA 1960, GAWŁOWSKA 1961, BRUMMITT 1968). It needs to be added here that apart from the wild *H. sphondylium* there are also in Poland three other taxa: *H. pubescens* (Hoffm.) Bieb., *H. mantegazzianum* Sommier & Levier and *H. sosnowskyi* Manden. The first of the three, downy cow parsnip, is an ephemerophyte, noted as an oekiophyte in Wrocław (ROSTAŃSKI and SOWA 1986-1987) and in the area of Kwidzyn (RUTKOWSKI 2004). It is characterised by white flowers, elongated externally, with fruits being large and densely hairy (BRUMMITT 1968, KOWAL and PIC 1975, RUTKOWSKI 2004). The last two were

introduced species. Diagnostic traits and trichomes of *H. sosnowskyi* are ambiguous and this species is frequently mistaken with *H. mantegazzianum*. The traits differentiating *H. sosnowskyi* from *H. mantegazzianum* is the dense and coarse indumentum of pedicles and peduncles of umbels, as well as characteristic traits of the leaf blade (HAEUPLER and MUER 2007). Flowers are white with petals 9-10 mm long. Pedicles and peduncles in *H. mantegazzianum* are covered by scarce and soft hairs, while leaf blades are more deeply indented and more acuminate at the tip and serrate at the margin of the leaflet (STACE 1997). Flowers are white or pinkish with petals up to 12 mm long.

Apart from morphological descriptions of the Polish species, there are only scarce published studies on the micromorphology e.g. of the anther and pollen grain (KLIMKO et AL. 2012), and fruit and seed (KLIMKO et AL. 2013). In Poland the surface sculpture of *Heracleum* ovary has never been critically studied by SEM.

The aims of this study were: (1) to analyse in detail the sculpture and trichomes of the ovary surface in *Heracleum* taxa; (2) to identify traits that are of diagnostic value for the differentiation of species, subspecies and taxa of lower rank.

MATERIAL AND METHODS

Flowering specimens of five taxa belonging to the *Heracleum* genus were studied. *Heracleum sphondylium* subsp. *sphondylium* and subsp. *sibiricum*, *H. pubescens*, *H. mantegazzianum* and *H. sosnowskyi* material was collected during the years 2008-2012 from living plants growing in 15 natural localities (Appendix 1). In each locality flowers were collected from three to five individuals. Micromorphological features of ovary (pilosity and ornamentation) and trichome characters were studied. Observations of dried pistils were carried out under a scanning electron microscope (SEM). The SEM micrographs were taken with a Zeiss EVO 40 microscope at the Electron Microscopy Laboratory, Faculty of Biology, the Adam Mickiewicz University in Poznań. Prior to observations, the prepared material was sputtered with gold using an SCB 050 ion sputter. Microchemical test of trichomes (reactions: diluted H_2SO_4 dissolves $CaCO_3$ and next $CaSO_4$ needles appear) was conducted. SEM pictures of pistils were taken whole and in detail. The terminology of ovary sculpturing follows BARTHLOTT (1981, 1990) and CORNER (1976).

RESULTS AND DISCUSSION

The main micromorphological features of the investigated ovaries and trichomes are shown in Figures 1-4. Our results comprise a description and comparison of ovary pilosity, types of trichomes and their ovary and trichomes ornamentation.

Ovaries in specimens belonging to *H. sphondylium* subsp. *sphondylium* s.s. are densely covered with hairs. Only one type of trichomes, with macro-hairs, was identified. All hairs are thin-walled, long, straight, soft uni- or bicellular and with an almost smooth surface (with

only small protrusion), lobate margins, smooth or delicately striate tips (Fig. 1 A, B) and are not mineralized.

In subsp. *sphondylium* var. *chaetocarpoides* ovary surface was more or less densely covered with uniseriate trichomes (Fig. 1 C-F). Most frequently there are stiff trichomes with thick walls encrusted with $CaCO_3$, which was confirmed by microchemical tests. Setae type, of varied length and ornamentation with a swollen, oval base (Fig. 1 D). Three trichome categories in terms of their length were distinguished on the basis of biometric measurements. Cuticle of long setae (95-130 μm) and medium-long setae (50-70 μm) is tuberculate and the base striate or rugulate, while short setae (30-45 μm) are striate (Fig. 1 C, D). All trichomes are directed towards the ovary apex. Intermediate hairs (macro-hairs and setae) were found rarely and were only observed on the ovary surface at one locality (Koszalin; Fig. 1 C, arrows). Typical prickles are unicellular, broad at the base with a sharply pointed apex and are typically found on the margins of e.g. leaves, fruits or on veins (KLIMKO and TRUCHAN 2006, KLIMKO et AL. 2013). Sometimes prickles are longer than usual, and then it is difficult to differentiate between prickles and macro-hairs. The occurrence of prickle hairs in *H. sphondylium* subsp. *sphondylium* var. *chaetocarpoides* is a new finding.

Sparsely pubescent ovaries were found both in specimens of *H. sphondylium* with pink petals and in those with white, but with lobes of segments, especially terminal ones, strongly elongated, several times longer than broad. Observed differences concern their length, shape and ornamentation, striate or smooth-tuberculate, respectively (Fig. 1 G, H). Some variants of *H. sphondylium* having short setae on the ovary may also merit a subspecific rank (GAWŁOWSKA 1961, BRUMMITT 1968). The prickle type of what: is also found on ovary margins. They are short, relatively stiff with sharply pointed apices (Fig. 1 F, arrow).

Trichomes, defined in our study as setae, are described by some authors as the prickle type.

Surface of ovaries in *H. sphondylium* subsp. *sibiricum* s.s. is glabrous, rugose and distinctly striate (Fig. 2 A, B). On the surface in *H. sphondylium* subsp. *sibiricum* var. *chaetocarpum* there are stiff, rough, tuberculate setae (Fig. 2 D, F) and the base of the hair is in shape of a 'socket' (Fig. 2 E, arrow). They differ in the size and shape from hairs found on veins, where hair-like trichomes are found and the surface of hair walls is striate (Fig. 2 E).

In *H. pubescens* ovary surface is rugose and striate, in places very densely pubescent, with four types of hairs found. The ovary surface was covered by single, long, soft, almost smooth macro-hairs (Fig. 3 B, C) and short setae (35-75 μm) with tuberculate or smooth surface (Fig. 3 D, E) and at the base of the ovary straight, short and wide with a rounded apex, with a striate surface cuticle (Fig. 3 F). Hairs are distributed relatively regularly and almost all pointing in the same direction. All hair types can be found simultaneously on a single plant. Based on our observation the microsculpturing of trichomes and their density in *H. pubescens* are very important features that may be potentially informative characters of systematic importance.

A detailed description of macro- and micromorphological features of the ovary was based on specimens

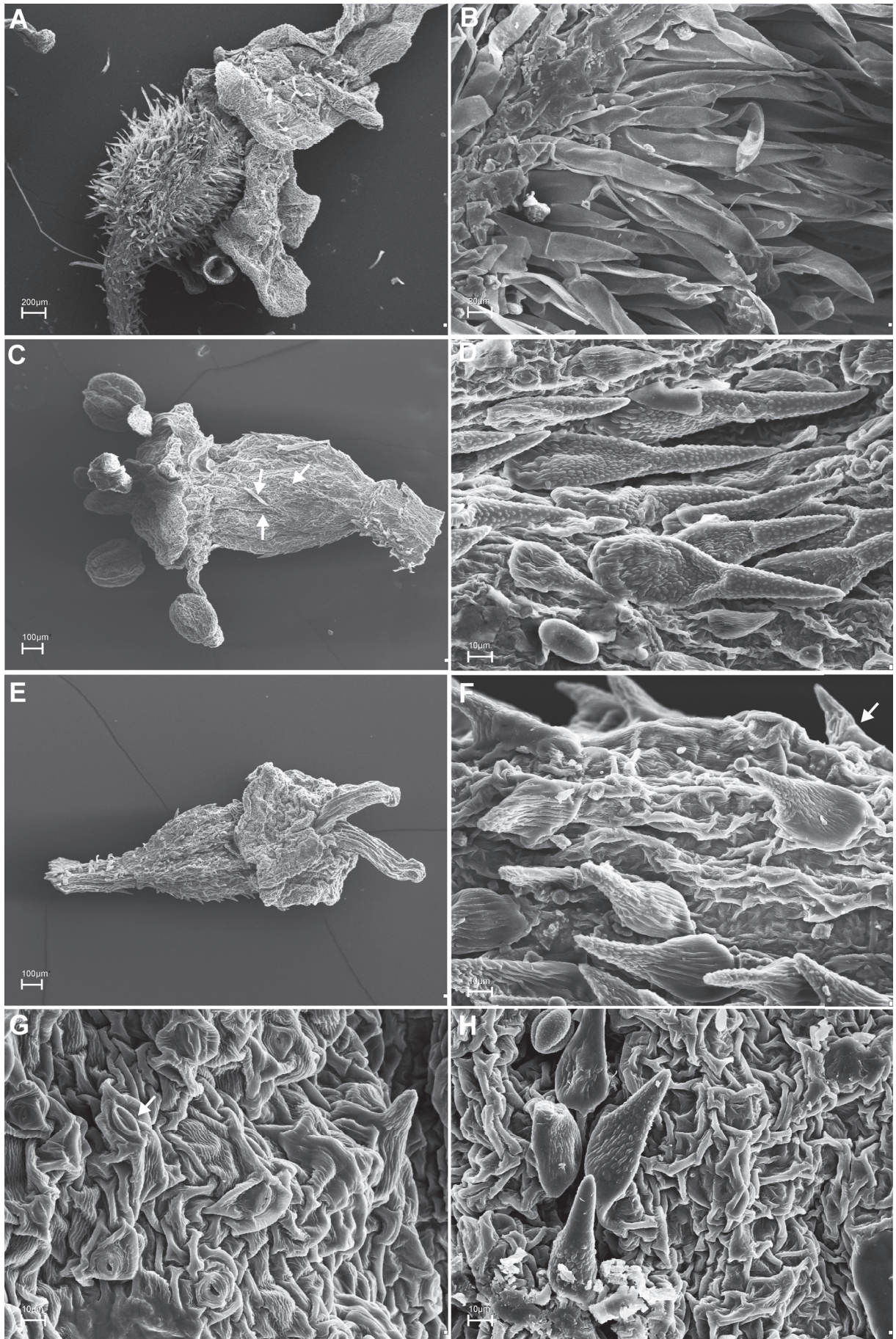


FIG. 1. SEM micrographs of pistil (A, C, E) and ovary surface of *Heracleum spondylium* subsp. *spondylium* (A, B - Bieszczady; C - note, the intermediate trichomes) and *H. spondylium* subsp. *spondylium* var. *chaetocarpoides* (C, D - Koszalin; E, F - Beskid Mały; G - Pogalewo Wielkie; H - Czarnogoździce; F - note, prickly type; G - note, stoma)

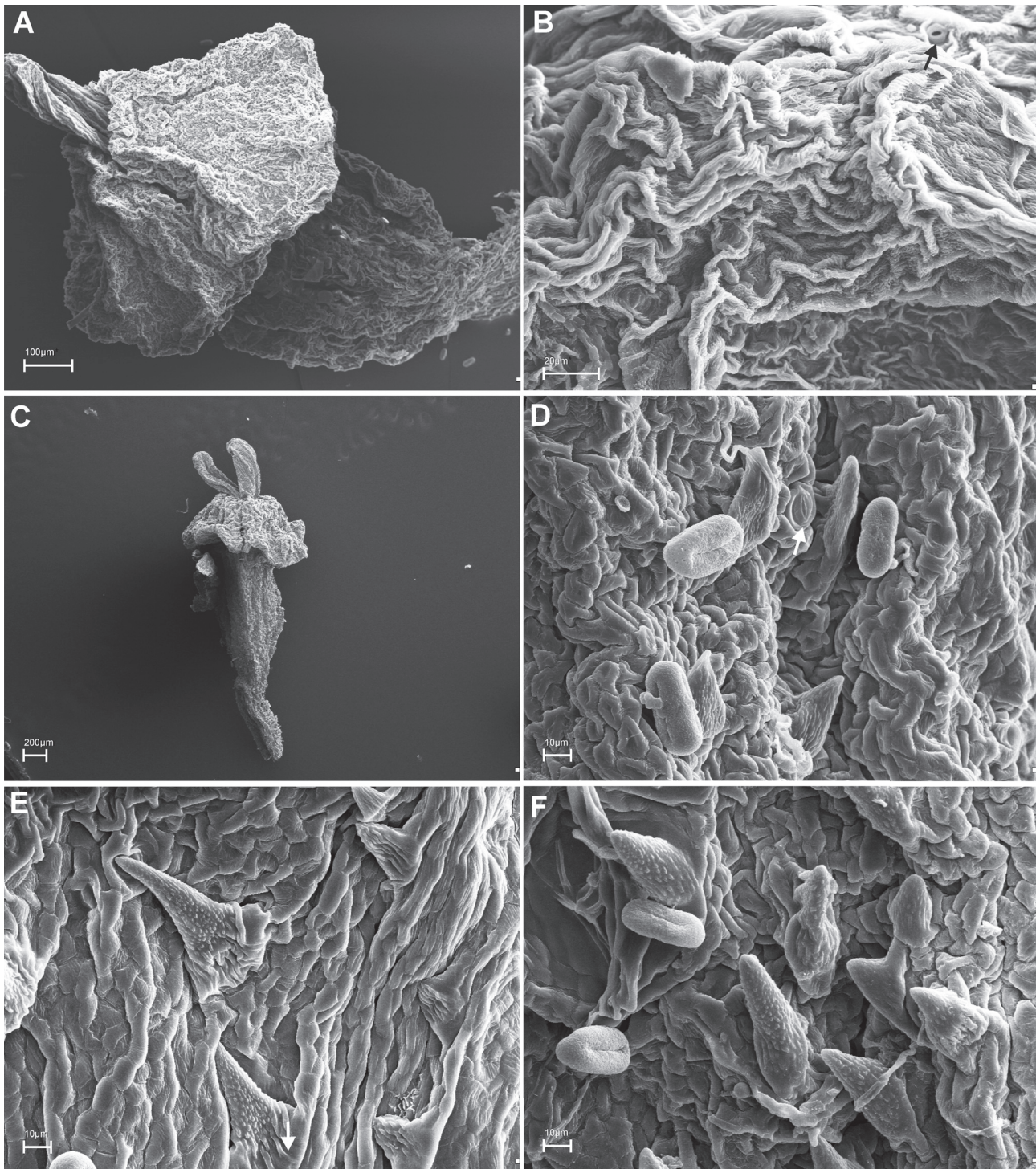


FIG. 2. SEM micrographs of pistil (A, C) and ovary surfaces of *Heracleum spondylium* subsp. *sibiricum* (A, B – Drawsko Pomorskie, B – note, stoma) and *H. spondylium* subsp. *sibiricum* var. *chaetocarpum* (C, D – Nowa Wieś Rzeczna, D – note, stoma; E – Zaniemyśl, note, the base of hair; F – Milicz)

identified as varieties of both subspecies of *H. spondylium* and *H. pubescens* (in selected localities with scarce pubescence). Epidermal cells of the ovary are usually irregular or polygonal in the shape from tetragonal to hexagonal. On veins the cells are rectangular in outline directed towards at the apex (Fig. 2 E). Anticlinal walls that form the boundary between epidermal cells are depressed below the outer tangential surfaces of cells (Figs 1 G, H; 2 D-F). Periclinal walls are protruding and

convex. The secondary sculpture is striate and smooth, in fragments striate (Figs 1 G, H; 2 B, D-F; 3 B, F). In terms of the primary sculpture the surface is similar in varieties of both subspecies of *H. spondylium*.

The two alien species, *H. mantegazzianum* and *H. sosnowskyi*, are very similar in terms of pubescence density (Fig. 4 A, B, D, G). On their surface we observed three types of trichomes: (1) long, soft, undulating uni- or bicellular with an almost smooth

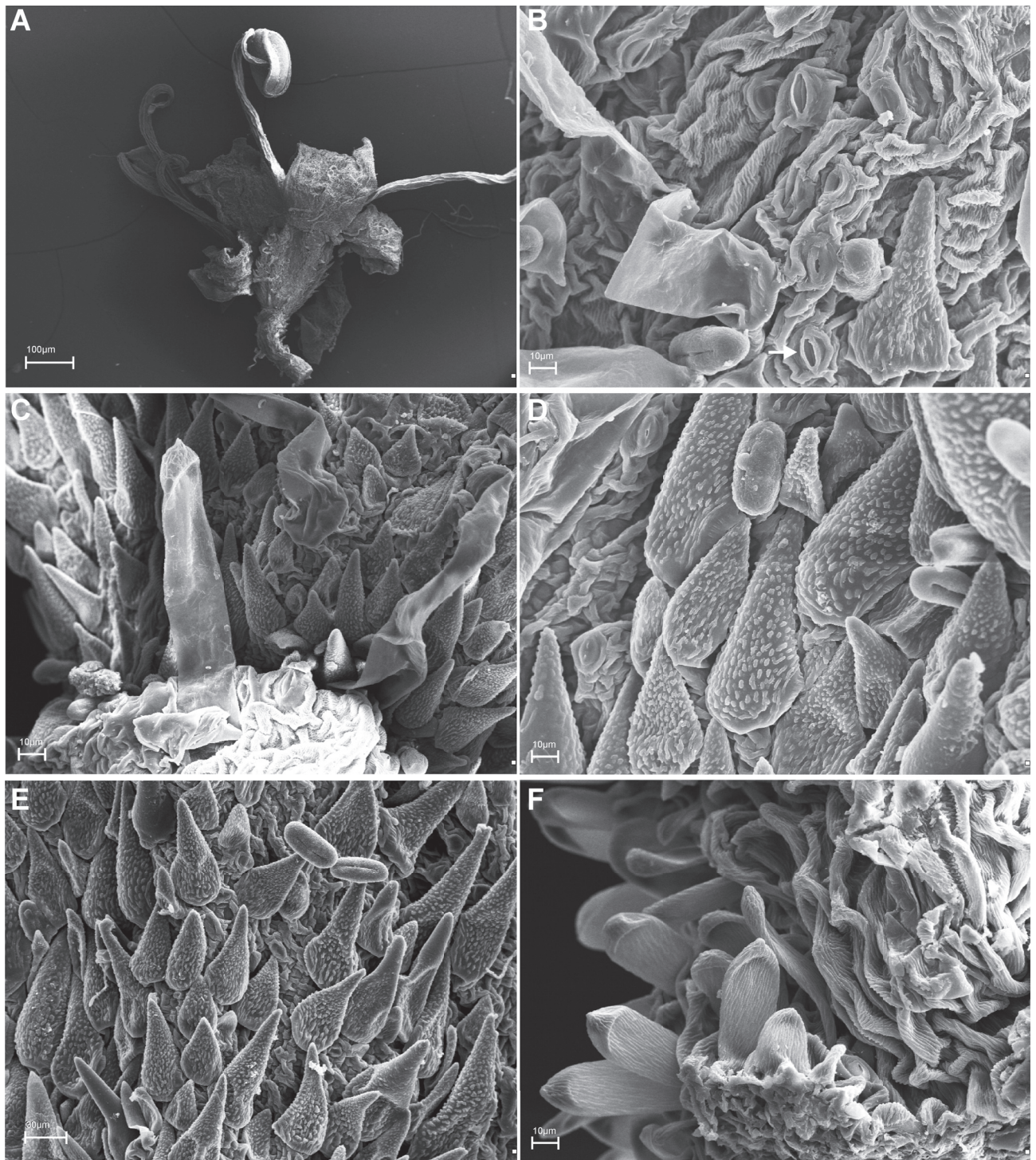


FIG. 3. SEM micrographs of pistil (A) and ovary surface of *Heracleum pubescens* (A, B – Pogalewo Wielkie, B – note stoma; C-F – Cząstkowice)

surface (Fig. 4 C, E, G); (2) short, smooth setae with a broader base in *H. mantegazzianum* (Fig. 4 C) and triangular in the outline in *H. sosnowskyi* (Fig. 4 F, arrow), and (3) short, pointed papillae in *H. mantegazzianum* and either pointed or rounded papillae in *H. sosnowskyi* (Fig. 4 F, arrows). Due to very dense pubescence in the above mentioned species macro- and micromorphological traits of the ovary could not be described.

The stomata in *Heracleum* are always anomocytic, oval in shape. In *H. sphondylium* they are located on the same level of the epidermis (Fig. 2 B, D, arrow) or slightly higher than the epidermal level (Fig. 1 G, arrow). In *H. pubescens* they are located slightly higher than the epidermal level (Fig. 3 B, arrow), while in *H. mantegazzianum* they are elevated in a chimney-like form as well (Fig. 4 C, arrow). In *H. sosnowskyi* stomata were not observed because their ovaries were very densely haired.

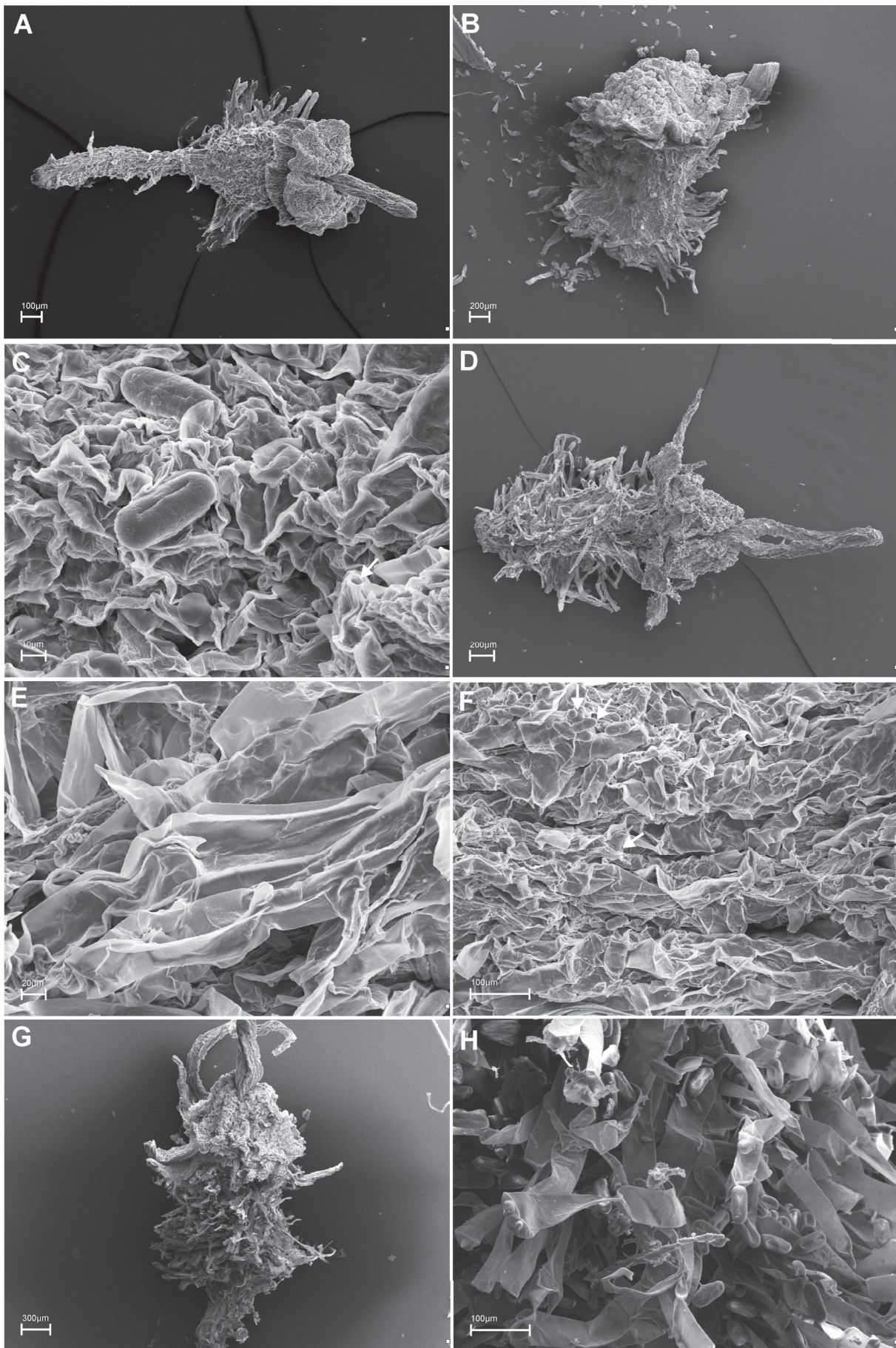


FIG. 4. SEM micrographs of pistil (A, B) and ovary surface of *Heracleum mantegazzianum* (A, C – Syrkowice, C – note, stoma; B – Głobino) and *H. sosnowskyi* (D-F – Ciecholub, F – note, acute and rounded papillae; G, H – Charbrowo, note D and G pistil)

The outer stomatal rim was observed. Under SEM the inner margin of the outer stomatal rim is smooth or repand and the cuticular membrane is smooth in *H. pubescens* (Fig. 3 B) or striate in *H. sphondylium* (Fig. 1 G). The ring around stomata was sometimes observed on fruits (KLIMKO et AL. 2013). The outer epidermis of the ovary is identical as that of leaves (LIU et AL. 2009).

In relation to a study by GAWŁOWSKA (1961) our investigations did not confirm the presence of sharp warts and extremely short stiff hairs on ovary surface in *H. sphondylium* var. *chaetocarpoides* or in *H. sphondylium* subsp. *sibiricum* var. *chaetocarpum*. The length of hairs varies from 30 to 130 µm. Our micromorphological results show clear differences between *H. sphondylium* subsp. *sphondylium* and *H. sphondylium* subsp. *sibiricum*. The presence or absence of trichomes is a good diagnostic characteristic to distinguish the two subspecies, but the primary sculpture of ovary is similar and is not a good criterion for the identification of lower rank taxa.

Similarly as in the case of the other Apiaceae the taxonomy of *Heracleum* is relatively complicated and is based mainly on morphological traits. The application of modern methods based on the analysis of molecular markers (ITS rDNA sequences) showed that European hogweeds – endemic plants of mountain ranges are a closely related group (except for *H. stevenii* Mandel. and *H. orphanidis* Boiss.). In turn, most subspecies of *H. sphondylium* were characterised by an identical nucleotide sequence, which practically prevented the determination of phylogenetic interrelations. Similarly as subspecies of *H. sphondylium*, identical sequences were found for *H. mantegazzianum* (ZYCH 2004).

As it results from the studies on the ranges of the investigated *H. sphondylium* subspecies in Poland the occurrence of *H. sphondylium* subsp. *sphondylium* was limited to southern, mountainous regions of Poland, while *H. sphondylium* subsp. *sibiricum* was found in the central and northern parts (GAWŁOWSKA 1961). At present the ranges of both subspecies overlap and both taxa could be found in different regions of Poland (ZYCH 2002).

In terms of the occurrence of *H. sphondylium* subsp. *sphondylium* in Poland it is a rare species, found commonly as *H. sphondylium* subsp. *sphondylium* var. *chaetocarpoides*. Our results are consistent with the data reported by GAWŁOWSKA (1961). In turn, *H. sphondylium* subsp. *sibiricum* according to GAWŁOWSKA (1961) is commonly found both in the typical form, as well as *H. sphondylium* subsp. *sibiricum* var. *chaetocarpum*; however, our analyses did not confirm it. It is found much more frequently as the variety.

Our survey provides new information regarding the micromorphology of ovary epidermal structures in *Heracleum* and can be used in the identification of specimens. The addition of this suite of micromorphological features to other morphological, biochemical and molecular characters should provide more information about phylogenetic affinities between species.

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REFERENCES

- BARTHLOTT W. (1981): Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nord. J. Bot.* 1: 345-354.
- BARTHLOTT W. (1990): Scanning electron microscopy of the epidermal surface. In: Scanning electron microscopy in taxonomy and functional morphology. Ed. D. Claugher. Systematic Association Special. Vol. 41. Clarendon Press, Oxford: 69-94.
- BRUMMITT R.K. (1968): *Heracleum L.* In: Flora Europaea. Vol. 2. Eds T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, D.A. Webb. Cambridge University Press, Cambridge: 364-366.
- CORNER E.J.H. (1976): The seeds of the Dicotyledons. Cambridge University Press, Cambridge.
- GAWŁOWSKA M. (1956): *Heracleum sphondylium L.* i *Heracleum sibiricum L.* na ziemiach polskich [*Heracleum sphondylium L.* and *Heracleum sibiricum L.* in Poland]. *Diss. Pharm.* 7: 141-164.
- GAWŁOWSKA M. (1961): Systematyka wewnątrzgatunkowa *Heracleum sphondylium L.* i *Heracleum sibiricum L.* występujących w Polsce i krajach sąsiednich. *Fragm. Florist. Geobot.* 7: 1-39.
- HAEUPLER H., MUER T. (2007): *Bildatlas der Farn- und Blütenpflanzen Deutschlands.* Eugen Ulmer Verlag KG, Stuttgart: 378-379.
- KLIMKO M., TRUCHAN M. (2006): Morphological variability of the leaf epidermis in selected taxa of the genus *Ficus L.* (Moraceae) and its taxonomic implications. *Acta Soc. Bot. Pol.* 75, 4: 309-324.
- KLIMKO M., TRUCHAN M., SOBISZ Z. (2012): Anther and pollen morphology of native and alien species of the genus *Heracleum L.* (Apiaceae) in Poland. *Rocz. AR Pozn. 391, Bot. Stec.* 16: 5-14.
- KLIMKO M., TRUCHAN M., WYSAKOWSKA I. (2013): Fruit and seed morphology of the genus *Heracleum* (Apiaceae) in Poland. *Rocz. AR Pozn. 392, Bot. Stec.* 17: 13-24.
- KOCZWARA M. (1960): *Heracleum L.* In: Dwuliścienne wolnopłatkowe – dwuokwiatkowe. Cz. 7. Instytut Botaniki, Polska Akademia Nauk, Kraków: 119-123.
- KOWAL T., PIC S. (1975): *Studia nad anatomią niektórych gatunków rodzaju Heracleum L.* *Monogr. Bot.* 49: 111-136.
- LIU H.Y., YU Y., DENG H.L., HE H.J. (2009): Leaf epidermal morphology and systematic significance of genus *Heracleum* in South west China. *Acta Bot. Boreali-Occidentalia Sin.* 29, 9: 1833-1845.
- ROSTAŃSKI K., SOWA R. (1986-1987): *Alfabetyczny wykaz efemerofitów Polski.* *Fragm. Florist. Geobot.* 31-32, 1-2: 151-205.
- RUTKOWSKI L. (2004): *Klucz do oznaczania roślin naczyniowych Polski niżowej.* Wyd. Nauk. PWN, Warszawa.

- SHEPPARD A.W. (1991): *Heracleum sphondylium* L. J. Ecol. 79, 1: 235-258.
- STACE C. (1997): New flora of the British Isles. Second edition. Cambridge University Press, The Bath Press, Bath.
- ZYCH M. (2002): Pollination biology of *Heracleum sphondylium* L. (Apiaceae). The advantages of being white and compact. Acta Soc. Bot. Pol. 71, 2: 163-170.
- ZYCH M. (2004): Ewolucja systemów zapylania na podstawie europejskich przedstawicieli *Heracleum* L. (Apiaceae). Roczn. Augustowsko-Suwalski 4: 91-98.

APPENDIX 1

Collection data of the investigated taxa

Heracleum sphondylium subsp. *sphondylium*

Tarnica, Bieszczady, Podkarpackie Province, 49°04'30"N / 22°43'40"E, 1308 m a.s.l.

H. sphondylium subsp. *sphondylium* var. *chaetocarpoides*

Koszalin, Pomorskie Province, 54°11'39"N / 16°10'17"E, 27 m a.s.l.

Czarnogózdzice, Dolnośląskie Province, 51°29'24"N / 17°18'16"E, 161 m a.s.l.

Pogalewo Wielkie, Dolnośląskie Province, 51°15'16"N / 16°38'07"E, 115 m a.s.l.

Beskid Mały, Śląskie Province, 49°40'00"N / 19°20'00"E, 595 m a.s.l.

Heracleum sphondylium subsp. *sibiricum*

Drawsko Pomorskie, Pomorskie Province, 53°20'00"N / 15°46'00"E, 116 m a.s.l.

H. sphondylium subsp. *sibiricum* var. *chaetocarpum*
Nowa Wieś Rzeczna, Pomorskie Province, 53°57'55"N / 18°30'09"E, 100 m a.s.l.

Zaniemyśl, Wielkopolskie Province, 52°08'58"N / 17°09'29"E, 72 m a.s.l.

Milicz, Dolnośląskie Province, 51°31'41"N / 17°16'12"E, 109 m a.s.l.

Heracleum pubescens

Pogalewo Wielkie, Dolnośląskie Province, 51°15'16"N / 16°38'07"E, 115 m a.s.l.

Cząstkowice, Dolnośląskie Province, 49°57'13"N / 22°33'24"E, 209 m a.s.l.

Heracleum mantegazzianum

Syrkowice, Zachodniopomorskie Province, 54°06'16"N / 15°51'28"E, 21 m a.s.l.

Głobino, Pomorskie Province, 54°26'21"N / 17°06'13"E, 51 m a.s.l.

Heracleum sosnowskyi

Ciecholub, Pomorskie Province, 54°10'32"N / 16°52'32"E, 116 m a.s.l.

Charbrowo, Pomorskie Province, 54°40'43"N / 17°35'47"E, 9 m a.s.l.

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