FRUIT AND SEED MORPHOLOGY OF THE GENUS HERACLEUM L. (APIACEAE) IN POLAND

MAŁGORZATA KLIJKO, MARIOLA TRUCHAN, ILONA WYSAKOWSKA

M. Klímko, I. Wysakowska, Department of Botany, Poznań University of Life Sciences, Wojska Polskiego 71 C, 60-623 Poznań, Poland, e-mail: klim@up.poznan.pl
M. Truchan, Department of Botany and Nature Conservation, Pomeranian Academy in Slupsk, Arciszewskiego 22 B, 76-200 Slupsk, Poland, e-mail: truchan@apsl.edu.pl

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ABSTRACT. The paper presents the results of a study on mericarps and seed morphology of four taxa occurring in Poland: Heracleum spondylium subsp. spondylium and subsp. sibiricum, H. mantegazzianum and H. sosnowskyi. Apart from selected biometric traits we analysed pericarps and seed-coat patterns using a stereoscopic microscope (SM) and a scanning microscope (SEM). Considerable differences in the dorsal surface of mericarps were observed, but major structural similarities were found on the ventral surface of mericarps. Pericarp surface differs significantly between native and alien taxa in terms of secondary sculpture. The examined seeds were characterised by reticulate primary sculpture with different size and shape of testa cells. The macro- and micromorphological characteristics of the mericarp and seeds may provide valuable additional diagnostic criteria for delimitation of Heracleum and can be used in the identification of fruits and seeds.

KEY WORDS: Heracleum, fruit, seed, morphology, trichome, SEM

INTRODUCTION

Important features for the determination of Heracleum specimens include life form, leaf (shape and leaf indumentum), as well as indumentums on the stem, rays, pedicles, ovaries, fruits and the width of vittae at the apex and width of the wing. Fruit characteristics play a crucial role in the taxonomy of the genus Heracleum both as auxiliary features and as the sole criteria on which the division into sections and other taxa is based (Mandeno 1951, Hegi 1975, Kowal 1975). Heracleum spondylium L. s. lat. forms a morphologically variable complex. Due to its considerable morphological variability, H. spondylium is divided into several dozen subspecies (Brummitt 1968), varieties and forms, of which most differ from one another in their flower and inflorescence structure (Hegi 1975, Meusel et al. 1978, Hultén and Fries 1986). Among them two are found in Poland in the rank of species, i.e. H. spondylium and H. sibiricum (Koczwar 1960, Gawłowska 1961, Kowal 1975, Mirek et al. 2002, Zając and Zając 2009), or subspecies: H. spondylium subsp. spondylium and subsp. sibiricum (Brummitt 1968, Zych 2003, 2007, Rutkowski 2004). It needs to be added here that apart from the wild H. spondylium being a native taxon in our flora, there are also 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 oekiphyte in Wrocław (Rostański and Sowa 1986-1987) and in the area of Kwidzyn (Rutkowski 2004). The last of the three above mentioned, Sosnovsky’s hogweed, originating from Abkhazia, was described as a separate species in 1944 by Mandeno (1951). Heracleum mantegazzianum Sommier & Levier, accidentally introduced from Asia, has been classified as an invasive species (Ochsman 1996, Pyšek et al. 1998). Diagnostic traits (primarily of vegetative organs) in H. sosnowskyi are ambiguous and this species is frequently mistaken with H. mantegazzianum. Data on fruit morphology and anatomy of some representatives of Heracleum are rather limited (Mandeno 1951, Kulpa 1958, Koczwar 1960, Gawłowska 1961, Kowal 1975, Kowal and Pic 1975, Sheppard 1991). The macro- and micromorphology features of pericarps and seed-coats of Heracleum in Poland were examined under SEM for the first time. Fruit and seed morphology provides a number of characters potentially useful in species identification, phylogenetic inference and character state evolution. Observations in many plant groups have shown that fruit and seed morphology features are rather conservative, which makes them taxonomically important (Bartlott 1984).

The purpose of this study was to describe and investigate mericarps, seed morphology and macro- and micromorphology of Heracleum in Poland and to evaluate the diagnostic value of these characters in the taxonomic context.
MATERIAL AND METHODS

Morphological fruit and seed characters of *H. spondylium* subsp. *spondylium* and *sibircum, H. mantegeazzianum* and *H. sosnowskyi* were studied using material collected in 2008-2012 from living plants growing in 24 natural localities (Appendix 1). From six localities, and from each locality from five plants, and from each plant, 10 fruits and 10 seeds were collected for each taxon material. Only mature mericarps were measured. The list of the studied features of mericarps and seeds is presented in Table 1. Measurements and optical observation of fruit and seed were carried out under a Zeiss, Lumar, V 12, stereomicroscope. The SEM micrographs were taken with a Zeiss EVO 40 microscope at the Electron Microscopy Laboratory, Faculty of Biology, the Adam Mickiewicz University of Poznań. Prior to observations, the prepared material was sputtered with gold using an SCB 050 ion sputter. Morphological features of mericarps and seeds were observed on the dorsal and ventral surfaces. The study was documented with photographs taken during observations. In this paper we followed KOWAL (1975) for the description of fruit morphology, while the terminology of pericarps and seed coat sculpturing follows BARTHLOTT (1981, 1984) and CORNER (1976). Sensu BARTHLOTT (1981) we distinguish primary and secondary sculptures. The primary sculpture is identified by several characters. The most important characters include (1) the outline of epidermal cells, (2) curvature of the outer periclinal wall, (3) the shape and relief on anticlinal walls, and (4) relief of cell boundaries. The secondary sculpture characterises fruit and seed micromorphology and is determined by the cuticle sculpture (BARTHLOTT 1981). Voucher specimens were deposited at the Herbarium of the Department of Botany, the Poznań University of Life Sciences (PZNB).

RESULTS

A description of the mericarp and seed morphology of the studied taxa is given below and illustrated with SM and SEM photographs (Figs 1-6).

**Detailed description of mericarps and seeds:**

*Heracleum spondylium* subsp. *spondylium*

Mericarps in outline ovate, widely ovate, flat, pale yellow to brown, matt. Mericarps 5.5-9.0 \( \times \) 4.2-7.4 mm, length/width ratio (1.06-)1.13-1.41(-1.73); wings 0.3-0.8 mm wide; dorsal vittae of equal width, four or sometimes five on the same specimen (Fig. 1 A, B), or three distinct and one invisible, not expanded at the apex; median ones 2.5-4.5 \( \times \) (0.13-)0.19-0.25 mm; lateral ones (2.3-)2.5-4.95 \( \times \) (0.13-)0.19-0.25 mm; two ventral vittae, slender at the base 1.5-3.7 \( \times \) (0.13-)0.19-0.38 mm and 1.5-3.7 \( \times \) 0.19-0.38 mm, frequently of identical length and width at the apex (Fig. 1 F). The vittae on the dorsal side of mericarps narrower than those on the ventral side. SEM analyses showed reticulate mericarps on the dorsal surface. The exocarp cells were polygonal or hexagonal (Fig. 1 C). The anticlinal walls were raised, straight and slightly undulate, while the outer, periclinal walls were slightly depressed (Fig. 1 C). Cuticle on the pericarp surface of the dorsal side was smooth or fragmented, striate and micropapillate (Fig. 1 C), or entirely striate (Fig. 1 D, I, J). Anomocytic stomata were present on the dorsal surface (Fig. 1 C, D, G, H, I, arrow). The length of stomata ranges from 16.2 to 297 \( \mu \)m (mean 21.98 \( \mu \)m). Ripe fruits were glabrous (Fig. 1 A, B, C), in younger specimens on the dorsal side two types of hairs were found on different specimens: long 133 to 180 \( \mu \)m macrohairs uni-and multicellular, at the apex pointed and smooth (Fig. 1 G) and short-subulate ca. 65 \( \mu \)m long, irregularly arranged with tuberculate surface (Fig. 1 H, I). Moreover, on young fruits wings were found, short-subulate (ca. 44.3 \( \mu \)m long) at the margin (Fig. 1 J). The ventral surface was glabrous (Fig. 1 E). The exocarp cells were polygonal. Anticlinal walls raised, smooth and slightly sinuate. Cuticular ornamentation was irregularly striate (Fig. 1 F). SEM observations showed

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Fruit and seed morphology of the genus Heracleum L. (Apiaceae) in Poland

F. M. SM and SEM micrographs of mature mericarps of *Heracleum sphondylium* subsp. *sphondylium* (A–D – dorsal side, E, F – ventral side) and young fruits (G–J – dorsal side), I – note, setae and stoma

Fig. 1. SM and SEM micrographs of mature mericarps of *Heracleum sphondylium* subsp. *sphondylium* (A–D – dorsal side, E, F – ventral side) and young fruits (G–J – dorsal side), I – note, setae and stoma
Fig. 2. SEM micrographs of seeds of *Heracleum sphondylium* subsp. *sphondylium* (A, B – dorsal side, C, D – ventral side) and *H. sphondylium* subsp. *sibiricum* (E, F – dorsal side, G, H – ventral side)
that the presence of different types of trichomes on the dorsal mericarp surface is a useful character for distinguishing taxa *H. spongylidium* subsp. *spongylidium* (long, weak, almost smooth), in var. *chaetocarpoides* (short, subulate, tuberculate). Plants having short setae on the fruit may be referred to as var. *chaetocarpoides* Gawl. Not only plants of *H. spongylidium* subsp. *spongylidium* (= var. *chaetocarpoides*) from the Western Carpathians have short seta [subsp. *trachycarpum* (Sojak) J. Holub]. These specimens were very frequent in different localities (Klimko et al. 2013).

**Seeds** elliptical, 3.5-5.25 × 2.5-3.35 mm; length to width ratio 1.21-1.81. SEM analyses indicate that the seed surface sculpture of the dorsal side was reticulate (Fig. 2 B). The testa cells varied from tetragonal to hexagonal. The anticlinal walls were straight, broad, raised, irregularly swollen. The outer, periclinal, walls were depressed and slightly papillate (Fig. 2 B). Seed surface sculpture on the ventral side was irregularly reticulate, anticlinal walls straight and delicately undulate, irregularly swollen, slightly raised. The outer, periclinal walls were concave and irregular projections were found. The secondary sculpture on the dorsal surface was smooth and on the ventral side it was smooth and delicately striate (Fig. 2 D).

*Heracleum spongylidium* subsp. *sibiricum*

The outline of mericarps lying on the ventral side approximately elliptic or elongated obovate. **Mericarps** 5.0-7.8 × 3.4-6.2 mm, length/width ratio 1.16-1.58(-1.72); wings (0.1-0.2-0.55-0.7) mm wide; dorsal vittae of equal width, four or sometimes six on the same specimen (Fig. 3 A, B) or slightly expanded at the apex (Fig. 3 A, B), median ones 2.2-4.5 × 0.13-0.25 mm; lateral ones (2.0-2.6-4.5 × 0.13-0.25 mm; two ventral vittae, 1.7-3.5 × (0.19-0.22-0.38(-0.44) mm and 1.6-3.2 × (0.19)-0.25-0.38 mm, frequently of identical length and width at the apex (Fig. 3 D). The vittae on the dorsal side of mericarps were longer and narrower than those on the ventral side.

SEM examination showed reticulate mericarps on the dorsal surface pattern. The exocarp cells were pentagonal or hexagonal, elongate in one direction. The anticlinal walls were slightly raised, thin, straight or delicately sinuous. The outer, periclinal walls were slightly depressed. The cuticular ornamentation was smooth. Anomocytic stomata were present on the dorsal surface (Fig. 4 C). Stomatal length ranged from 16.8 to 37.8 μm (mean 29.16 μm). Ripe fruits more or less pubescent, frequently glabrous (Fig. 4 C). Trichomes on fruits were usually found only on the dorsal side and were scarce, limp, unicellular of at least 150 μm in length, pointed at the apex, glabrous (Fig. 4 D) and on the edge of the wing in young and mature mericarps ‘slightly striate spiny’ – prickle type (to sometimes curved at the apex), of 116.6 to 133.3 μm in length (Fig. 4 E, G, arrows). Surface pattern of the ventral side was probably reticulate, hardly distinct and difficult to specify (Fig. 4 H).

**Seeds** elliptical, relatively large (5.7-6.1-7.1 × (3.75-) 4.0-4.35(-4.6) mm; length to width ratio 1.41-1.73. SEM investigation indicates that the seed surface sculpture of the dorsal side was reticulate. The testa cells were tetragonal or pentagonal. The anticlinal walls were broad, raised, straight and slightly undulating, irregularly thickened. The outer, periclinal walls were concave. The cuticular ornamentation on both walls was smooth (Fig. 6 B). Seed surface sculpture on the ventral side was irregularly reticulate (Fig. 6 D). The testa cells were large, anticlinal walls irregularly swollen. The outer, periclinal walls were slightly depressed and irregularly folded (Fig. 6 D). The cuticular ornamentation of both walls was irregularly striate.

*Heracleum sosnowskyi*

The outline of mericarps lying on the ventral side from oval to elliptical. **Mericarps** 10.7-14.4 × 7.0-9.8 mm,
Fig. 3. SM and SEM micrographs of mature mericarps of *Heracleum sphondylium* subsp. *sibiricum* (A-C – dorsal side, D-F – ventral side) and young fruits (G, H – dorsal side)
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Fig. 4. SM and SEM micrographs of mature mericarps of Heracleum mantegazzianum (A-E – dorsal side, F-H – ventral side), E, G – note, prickle type
Fig. 5. SM and SEM micrographs of mericarps of *Heracleum sosnowskyi* (A-E – dorsal side, F-H – ventral side), G – note, prickle type.
Fig. 6. SEM micrographs of seeds of *Heracleum mantegazzianum* (A, B – dorsal side, C, D – ventral side) and *H. sosnowskyi* (E, F – dorsal side, G, H – ventral side)
between the veins four distinct stripes are found (oc-
casionally three, five or six, which may be considered
an anomaly). Stripes may be almost identical in width,
gradually expanding downwards, or markedly widened
in the lower part. The dorsal side of mature mericarps
may be smooth or variably pubescent. Pubescence is fre-
cently slightly undulating and over the ring-shaped
depression under the stylodium up to the base. Dorsally
between the veins four distinct stripes are found (oc-
casionally one, three, six, or more) which may be consid-
ered an anomaly. Stripes may be almost identical in width,
gradually expanding downwards, or markedly widened
in the lower part. The dorsal side of mature mericarps
may be smooth or variably pubescent. Pubescence is fre-
cently found only in young fruits in the form of long or
short hairs, soft or stiff. The wing margin sometimes has
small, stiff and sharp hairs (prickle; Figs 4 E, G and 5 G).
The ventral side typically smooth, slightly glossy, in the
central section slightly concave or flat, has two stripes.
Stripes most frequently reach half-length of the ventral
side. Fruit colour, varying depending on the degree of
maturity, is as a rule uniform. The dorsal side may be
yellowish-grey, greenish-grey or dark brown. The ven-
tral side of mericarps as a rule is lighter. Its colour may
range from whitish through smoky to yellowish-grey.
Stripes distinctly showing through the tissues covering
the mericarp are in different shades of brownish-grey.

Seeds are fused with the pericarp, but they are eas-
ily separated. Seeds do not fill the entire surface of the
mericarps, but free spaces are found in corners formed
by the wing. Seed colour is brownish. The surface of
the dorsal side of seeds in all taxa is slightly convex
with shallow depressions for stripes on the dorsal side
of mericarps (Figs 2 A, E and 6 A, E) and approximately
flattened or slightly concave on the ventral side with
distinct depressions for stripes (Figs 2 C, G and 6 C, G).

**DISCUSSION**

Fruits of the examined taxa from the genus *Herac-
leum*, despite significant and highly distinct differences,
constitute a highly uniform type of structure. Morpho-
logical variation to a considerable degree is limited
to quantitative traits. According to the literature data
mericarps of the examined taxa are similar in shape
and different in size. Mericarp dimensions vary greatly
among taxa, with the largest mericarps and seeds ob-
erved in *H. mantegazzianum* and *H. sosnowskyi*, while
the smallest in both subspecies of *H. sphondylium*.
Morphologically fruits in both subspecies of *H. sphon-
dylium* are similar and measurements of fruit length
and width are insufficient to distinguish the examined
taxa (Kulpa 1958, Gawłowska 1961). However, certain
differences in size are found e.g. in subsp. *sphondylium*
6.01-9.0 x 5.01-9.0 mm (Kowal 1975); 4.7-8.0 x 3.5-5.6
mm (Gawłowska 1961); (3-)5.0-10.0 mm (Kulpa 1958);
5.0-11.0 mm (Koczwara 1960). According to Nordic
materials (Internet http://www.floranordica.org) the size
of mericarp in *H. sphondylium* was 5.8-9.5 x 4.5-6.8 (77)
mm. Our measurements showed that fruit size in subsp.
sphondylium was 5.5-9.0 x 4.2-7.4 mm. Similar differ-
ces in mericarp size were observed in subsp. *sibiricum*
amounting to 4.9-9.4 x 3.5-6.3 mm (Gawłowska 1961);
6.0-7.0 x 4.01-5.0 mm (Kowal 1975); 7.0-8.0 x 5.0-6.0
mm (Koczwara 1960) and in our studies it was 5.0-7.8
x 3.4-6.2 mm. In relation to studies by Kowal (1975)
conducted on other diagnostic traits of fruits such as
width of vitte at their lower ends and width of wing,
several significant differences were found. Analyses pre-
sented by Kowal (1975) showed that in both subspecies
stripes on the ventral side are narrower than those on
the dorsal side. Our studies did not confirm that ob-
bservation and showed that the vitteae on the dorsal side
of mericarps were narrower than those on the ventral
side. According to the study by Kowal (1975) the width
of wing outside the vein in subsp. *sphondylium* was 0.5
mm, while in our studies it was 0.3-0.8 mm. Wing width
in subsp. *sibiricum* ranges from 0.25 to 0.50 mm (Kowal
1975) and it is very similar to our results of 0.20-0.55
mm. Comparable differences in these traits were ob-
erved in *H. mantegazzianum* and *H. sosnowskyi*. Ac-
cording to Kowal (1975) wing width in both species was
identical, amounting to 0.75 mm. According to Nordic
materials (Internet http://www.floranordica.org) wing
width in *H. mantegazzianum* was in range 0.6-1.2 (1.5)
mm. Our studies showed that this width is 1.0-1.2 mm,
and 1.0-1.6 mm, respectively. In *H. sphenphyllum* hairs were observed for the first time. In *H. mantegazzianum* and *H. sosnowskyi* hair types on the dorsal surface were described earlier by KOWAL (1975) and KOWAL and PIC (1975). The hair micromorphology of *Heracleum* was examined in our work for the first time. Based on anatomical examinations of fruits KOWAL and PIC (1975) stated that the exocarp on the dorsal side in *H. sphenphyllum* was smooth and in *H. sibiricum* smooth and striate. Our SEM examinations showed that the exocarp in subsp. *sphenphyllum* was smooth or fragmented striate, or entirely striate and micropapillate (Fig. 1 C), while in subsp. *sibiricum* it was only striate (Fig. 2 C). Additionally, a new characteristic of the *Heracleum* pericarp and seed coat pattern has been described in the present work. To date micromorphological features of the pericarp surface of *Heracleum* in China were examined under SEM (Internet http://en.cnki.com.cn). This study showed that the pericarp surfaces in cells of most species were of the pitting type and the ornamentation was classified into two types, i.e. reticular and strip ornamentation. Our conclusions are identical. The surface patterns on the dorsal surface of mericarps and seeds were reticulate in all the taxa, while the differentiation concerns the shape and size of the exocarp and testa cells and the structure of the outer periclinal and anticlinal cells walls – not only between taxa, but also between the dorsal and ventral sides. Macro-and micromorphological features, especially those on the dorsal side of mericarps show bigger variation and as such are more useful in the taxonomy of species. In conclusion, our study of the fruit and seed provided some important new data concerning macro- and micromorphology. A detailed analysis of the morphological features of mericarps and seeds greatly broadens our knowledge of individual taxa and may be helpful in providing more insight into the phylogeny of the taxa examined.

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**REFERENCES**


APPENDIX 1

The collection data of the investigated taxa

Heracleum sphyndylium subsp. sphyndylium
Manowo, Manowo Commune, Zachodniopomorskie prov., 54°07′32″N / 16°18′03″E, 34 m a.s.l.; Debrzno, Debrzno Commune, Pomorskie prov., 53°32′16″N / 17°14′11″E, 153 m a.s.l.; Dzierżyczowice, Żagań Commune, Lubuskie prov., 51°39′50″N / 15°21′18″E, 136 m a.s.l.; Dybów, Żagań Commune, 51°44′33″N / 15°17′19″E, 106 m a.s.l.; Beskid Mały, Bielsko-Biała Commune, Śląskie prov., 49°49′27″N / 19°04′11″E, 361 m a.s.l.; Zakopane-Olcza, Zakopane Commune, Małopolskie prov., 49°18′25″N / 19°59′31″E, 810 m a.s.l.

Heracleum sphyndylium subsp. sibiricum
Dygowo, Dygowo Commune, Zachodniopomorskie prov., 54°07′48″N / 15°43′12″E, 27 m a.s.l.; Konikowo, Świeszyna Commune, 54°08′54″N / 16°10′29″E, 35 m a.s.l.; Biała wieża, Białowieża Commune, Podlaskie prov., 52°41′59″N / 23°52′57″E, 164 m a.s.l.; Zaniemyśl, Zanie-}

myśl Commune, Wielkopolskie prov., 52°09′00″N / 17°09′58″E, 75 m a.s.l.; Mchy, Książ Commune, 52°16′33″N / 15°36′20″E, 70 m a.s.l.; Tymienice, Lubsko Commune, Lubuskie prov., 51°51′51″N / 14°56′42″E, 72 m a.s.l.

Heracleum mantegazzianum
Syrkowice, Karlino Commune, Zachodniopomorskie prov., 54°06′16″N / 15°51′28″E, 21 m a.s.l.; Globino, Słupsk Commune, Pomorskie prov., 54°26′21″N / 17°06′13″E, 51 m a.s.l.; Tursko, Miastko Commune, 53°55′40″N / 16°41′22″E, 92 m a.s.l.; Komoszewo, Wicko Commune, 54°41′19″N / 17°40′54″E, 31 m a.s.l.; Lędzichowo, Nowa Wieś Lęborska Commune, 54°38′47″N / 17°41′08″E, 32 m a.s.l.; Zalewo, Zalewo Commune, Warmińsko-Mazurskie prov., 53°51′03″N / 19°36′41″E, 100 m a.s.l.

Heracleum sosnowskyi
Rusinowo, Postomino Commune, Pomorskie prov., 54°30′38″N / 16°30′44″E, 20 m a.s.l.; Ciecholub, Kępice Commune, 54°10′32″N / 16°52′32″E, 116 m a.s.l.; Sycewie, Kobylnica Commune, 54°25′19″N / 16°51′45″E, 50 m a.s.l.; Charbrowo, Wicko Commune, 54°40′43″N / 17°35′47″E, 9 m a.s.l.; Słupsk, Słupsk Commune, 54°27′43″N / 17°01′39″E, 19 m a.s.l.; Barkowo, Czuchów Commune, 53°36′46″N / 17°11′09″E, 139 m a.s.l.