ANTHER AND POLLEN MORPHOLOGY OF NATIVE AND ALIEN SPECIES OF THE GENUS HERACLEUM L. (APIACEAE) IN POLAND

Małgorzata Klimko, Mariola Truchan, Zbigniew Sobisz

ABSTRACT. Morphological features of the anther and pollen grains of four taxa: *Heracleum sphondylium* subsp. *sphondylium* and subsp. *sibiricum*, *H. mantegazzianum* and *H. sosnowskyi* were examined using light, stereo and scanning electron microscopy. Anthers based on their mode of dehiscence belong to the longitudinal type and introrse subtypes. Significant differences are found in the size of anthers and pollen grains between native and alien taxa. Three types were recognised based on the anther sculpture; rugulate, rugulate-striate and tuberculate. The pollen grains were isopolar, prolate and perprolate in equatorial view, operculate and tricolporate, colpi with costae. On the basis of exine sculpturing one type was recognised rugulate at the equator and two types psilate-perforate and striate at the pole. The sculpturing of operculum is psilate. The results confirmed the stenopalyous characteristic of the family Apiaceae. On the basis of the distribution, biometrical and micro-morphological traits of anthers and pollen grains we conclude that *H. sphondylium* and *H. sibiricum* are not two distinct species, but *H. sphondylium* in Polish flora should be divided into two lowest taxa in the rank of subspecies. The study revealed that anther morphology and palynological characters display taxonomic significance in the genus.

KEY WORDS: *Heracleum*, Apiaceae, morphology, anther, pollen, statistical analyse, SEM

INTRODUCTION

The genus *Heracleum* L. (Apiaceae) includes biennial and perennial plants characteristic of the Northern hemisphere. Important features for the determination of *Heracleum* specimens are life forms, leaf-shape and leaf indumentum, as well as indumentum on the stem, pedicles and ovaries. The genus *Heracleum* L. in Europe is represented by nine species (Brummitt 1968). However, the relationships between them have not been thoroughly investigated to date. Among European hogweeds seven are endemic species from mountain ranges: *H. austriacum* L. and *H. minimum* Lam. found in the Austrian and French Alps, *H. ligusticfolium* Bieb., *H. pubescens* (Hoffm.) Bieb. and *H. steveni* Manden. on the Crimea, *H. orphanidis* Boiss. in the mountains of Macedonia and *H. carpathicum* Porc. in the eastern Carpathians. Another species, *H. mantegazzianum* Sommier & Levier, accidentally introduced from Asia, has been classified as an invasive species (Ochsman 1996, Pyšek et al. 1998). The seventh taxon is a common, polymorphic species *H. sphondylium* L., being a frequent meadow, scrub and ruderal species in many European countries (Sheppard 1991). Due to its considerable morphological variability, *H. sphondylium* is divided into several dozen subspecies, 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). Some of the taxa are sometimes treated as separate species. Among them two are found in Poland in the rank of species, i.e. *H. sphondylium* L. and *H. sibiricum* L. (Gawlowska 1961, Koczwar 1960, Mirek et al. 2002, Zając and Zając 2009) or subspecies *H. sphondylium* subsp. *sphondylium* and subsp. *sibiricum* (Brummitt 1968, Rutkowski 2006). The former has white flowers, of which most are characterised by bilateral symmetry. Umbels of hogweed are dense and form one surface. Flowers in Siberian cow parsnip are yellowish-green and with a radial structure, while its inflorescences have a loose structure (Gawlowska 1961). It needs to be added here that apart from the wild, *H. sphondylium* being a native taxa for our flora, there are also three other taxa: *H. pubescens*, *H. mantegazzianum* and *H. sosnowskyi* Manden. The first of the three, downy cow parsnip, is an eufemophyte, noted as an oekiohyphe in Wrocław (Rostański and Sowa 1986-1987) and in the area of Kwidzyn (Rutkowski 2006). The last of the three above mentioned, Sosnowsky’s hogweed, originating from Abkhazia, was described as a separate species in 1944 by Mandenova (1951). However, the name *H. sosnowskyi* for a long time was used as a synonym for *H. mantegazzianum* (Jahodová et al. 2007). Diagnostic traits 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 pedicels and peduncles of umbels, as well as characteristic traits of the leaf blade (HAEUPLER and MUE 2007). Pedicels 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).

Apiaceae pollen characters have been mentioned by several authors in general pollen keys (ERDTMAN 1952, PUNT 1984, FAEGRI and IVersen 1989, 1993, MOORE et al. 1991). PUNT (1984) divided the family into 50 pollen types and studied the pollen morphology of *H. sphondylium* subsp. *sphondylium*, subsp. *montanum* and *H. mantegazzianum*. The four Polish *Heracleum* taxa accepted here belong to two different groups. *Heracleum sphondylium* belongs to the section of *Heracleum*, while *H. mantegazzianum* and *H. sosnowskyi* belong to the section of *Pubescentia* Manch. Apart from morphological descriptions of Polish species there are several published studies on the distribution of *H. mantegazzianum* and *H. sosnowskyi* (SOBISZ 2007, SOBISZ and TRUCHAN 2008, PIWOWARSKI 2011 and cited literature) and pollination biology of *H. sphondylium* (ZYCH 2002, 2007) but the ultrastructure of the anther and pollen grain surface has not been given.

The purpose of this study was to describe and investigate anther and pollen grain morphology and micro morphology of *Heracleum* in Poland and to evaluate the diagnostic value of these characters in the taxonomic context.

**MATERIAL AND METHODS**

Anthers and pollen grains of four taxa belonging to the *Heracleum* genus were studied. Mature flowers prior and after dehiscence were collected from 64 different localities distributed all over Poland. Voucher specimens were deposited in the Herbarium of the Department of Botany, the Poznań University of Life Sciences (PZNB) and are available from the first author upon request. Observations of anther dehiscence were carried out first under a stereo microscope (SM), a pollen grain under light microscope (LM) and finally a scanning electron microscope (SEM). Anthers of *H. sphondylium* subsp. *sphondylium* came from specimens with white and pinkish flowers, while those of subsp. *sibiricum* from green flowers with glabrous and short setae on the ovary. Anther and pollen slides were prepared using the technique of GAWŁOWSKA (1961). Each specimen was represented by 30 anthers and pollen grains. Numbers of specimens from the studied taxa are presented in Tables 1 and 2. The following anther features were measured: length (mm), width (mm) and length/width ratio, while for pollen grains it was the length of polar axis (P), equatorial diameter (E) and P/E ratio. The arithmetical mean, standard deviation and coefficient of variation for each above-mentioned trait were calculated (Tables 1 and 2). The biometrical data analysed statistically. For each anther and pollen grains feature, one-factor analysis of variance (ANOVA) was used to examine differences in mean among taxa studied. If critical differences were noted, multiple comparisons were carried out bases on Tukey’s test for unequal sample sizes. Statistical analyses were performed using JMP 8.0 (SAS Institute, Inc. Cary, NC, USA: http://www.sas.com/).

**Table 1. Ranges (minimum-maximum), mean values (±SE), and coefficient of variation (CV) of anther morphological features of Heracleum**

<table>
<thead>
<tr>
<th>Taxa</th>
<th>No of specimens</th>
<th>Feature</th>
<th>min</th>
<th>max</th>
<th>mean (±SE)</th>
<th>CV (%)</th>
<th>min</th>
<th>max</th>
<th>mean (±SE)</th>
<th>CV (%)</th>
<th>min</th>
<th>max</th>
<th>mean (±SE)</th>
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<th>df</th>
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</tr>
</thead>
<tbody>
<tr>
<td><em>H. sphondylium</em> subsp. <em>sphondylium</em></td>
<td>133</td>
<td>anther length</td>
<td>0.70</td>
<td>1.20</td>
<td>0.88 (0.012)c</td>
<td>10.64</td>
<td>0.40</td>
<td>0.80</td>
<td>0.53 (0.012)b</td>
<td>18.52</td>
<td>1.28</td>
<td>2.11</td>
<td>1.70 (0.026)c</td>
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<td><em>H. sphondylium</em> subsp. <em>sibiricum</em></td>
<td>75</td>
<td>anther width</td>
<td>0.65</td>
<td>1.00</td>
<td>0.84 (0.015)c</td>
<td>11.20</td>
<td>0.40</td>
<td>0.70</td>
<td>0.51 (0.010)b</td>
<td>11.43</td>
<td>1.16</td>
<td>2.25</td>
<td>1.67 (0.039)c</td>
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<tr>
<td><em>H. mantegazzianum</em></td>
<td>50</td>
<td>length/width ratio</td>
<td>1.10</td>
<td>1.30</td>
<td>1.21 (0.011)b</td>
<td>10.02</td>
<td>0.50</td>
<td>0.90</td>
<td>0.64 (0.015)a</td>
<td>12.95</td>
<td>1.41</td>
<td>2.40</td>
<td>1.91 (0.043)b</td>
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<tr>
<td><em>H. sosnowskyi</em></td>
<td>50</td>
<td></td>
<td>1.25</td>
<td>1.45</td>
<td>1.33 (0.013)a</td>
<td>4.15</td>
<td>0.50</td>
<td>0.80</td>
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<td>1.75</td>
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<td>2.12 (0.057)a</td>
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<td>249.91</td>
<td>&lt; 0.0001</td>
<td></td>
<td>22.95</td>
<td>&lt; 0.0001</td>
<td>23.29</td>
<td>&lt; 0.0001</td>
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</tbody>
</table>

ANOVA was performed separately for each anther feature to determine the differences among taxa studied. Same letters indicate a lack of statistically significant differences between analysed taxa according to Tukey’s a posteriori test (P < 0.05).
The pollen grains in the equatorial view were used. 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. The study was documented by photograph taking during observations, primarily at a magnification of ×250 and ×2500 for anthers, and ×4000 and ×10000 for pollen grains. Micro-morphological features of anthers were observed on the dorsal and ventral surfaces and exine sculpturing at the equator and at the pole, while pollen grains were analysed in the proximal and distal view. The terminology used to describe anther dehiscence followed (RADFORD et al. 1974, ISLAM et al. 2008). With regard to the anther surface, mainly terminology of BARTHLOTT (1981) was applied. The pollen terminology was adopted from PUNT (1984), FAEGRI and IVERSEN (1989) and PUNT et al. (2007), while the shape classification followed that of ERDTMAN (1952) based on the P/E ratio (Table 2).

RESULTS AND DISCUSSION

The main morphological and micro-morphological features of the investigated anthers and pollen grains are summarized in Tables 1 and 2, while SEM micrographs are shown in Figures 1-33.

Anther type, shape and size

Typically the stamen consists of a filament and an anther. The anther is divided into two locular thecae, which are joined by a connective. The anthers generally open with the aid of mechanical tissue, the endothecium, by creating tension in the maturing anthers and helping in pollen dispersal (ABID and QAISER 2004). Based on the mode of anther dehiscence four anther types are distinguished, such as longitudinal, poricidal, valvular and transverse. The longitudinal type is further divided into three subtypes, i.e. extrorse, intorse and latrorse (RADFORD et al. 1974). Anthers of the studied taxa based on their mode of dehiscence belong to the longitudinal type (Figs 8, 10, 16) and intorse subtypes. In this subtype anthers dehisc longitudinally inward to the centre of flower. The mode of anther dehiscence is a diagnostic feature and can be utilized for taxonomic delimitation at the generic and family levels (BANO et al. 2008, ISLAM et al. 2008).

Generally, most anthers were ovate and elliptic (Figs 1 A, 4, 6, 8, 10 and 14), but other shapes were also observed, as shown in Figs 12 and 16. The colour varies from green, yellowish-green (subsp. sibiricum) to yellow and sparsely dark-brown in the rest. The entire surface is uniformly coloured and glabrous. The dorsal surface is slightly convex and ventral flat. The mean anthers range from 0.84 mm (subsp. sibiricum) to 1.33 mm (H. sosnowskyi) in length and 0.51 mm (subsp. sibiricum) to 0.64 mm (alien species) in width (Table 1). Two categories were identified on the basis of biometrical measurements, small-sized (H. sphondylum) and medium with lengths greater than 1.0 mm (Table 1). As it was demonstrated in this study, anther size in individual taxa varies slightly and moderately, as indicated by the calculated coefficients of variation (Table 1). In the analysed taxa coefficients of variations ranged from 4.15% to 11.2% for length and from 11.43% to 18.52% for width. The coefficient of variation for the length/width ratio ranged from

<table>
<thead>
<tr>
<th>Taxa</th>
<th>No of specimens</th>
<th>length of polar axis (±SE)</th>
<th>equatorial diameter (±SE)</th>
<th>P/E ratio (±SE)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>min</td>
<td>max</td>
<td>mean</td>
<td>CV (%)</td>
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<td>133</td>
<td>24.3</td>
<td>45.9</td>
<td>36.90</td>
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<td>75</td>
<td>27.0</td>
<td>43.2</td>
<td>37.66</td>
</tr>
<tr>
<td>H. mantegazzianum</td>
<td>50</td>
<td>51.3</td>
<td>70.2</td>
<td>62.45</td>
</tr>
<tr>
<td>H. sosnowskyi</td>
<td>50</td>
<td>54.0</td>
<td>70.2</td>
<td>61.86</td>
</tr>
</tbody>
</table>

ANOVA

<table>
<thead>
<tr>
<th>df</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
<th>F</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>408.21</td>
<td>&lt; 0.0001</td>
<td>242.42</td>
<td>&lt; 0.0001</td>
<td>3.97</td>
<td>0.0097</td>
<td></td>
</tr>
</tbody>
</table>

ANOVARs were performed separately for each pollen grains among taxa studied features to determine the differences among taxa studied. Same letters indicate a lack of statistically significant differences between analysed taxa according to Tukey’s a posteriori test (P < 0.05).
Figs 1-9. SEM. Ventral and dorsal surface of the anthers of *Heracleum*
Figs 1-5. *Heracleum sphondylium* subsp. *sphondylium*; Figs 6-9. *Heracleum sphondylium* subsp. *sibiricum* (Note, magnification Fig. 4× 1000; Fig. 8× 500)
Anther and pollen morphology of native and alien species of the genus Heracleum L. (Apiaceae) in Poland

Figs 10-17. SEM. Ventral and dorsal surface of the anthers of Heracleum
Figs 10-13. Heracleum mantegazzianum; Figs 14-17. Heracleum sosnowskyi (Note, magnification Fig. 10 × 200)
Figs 18-25. Pollen morphology in proximal and distal view of *Heracleum* in SEM
Anther and pollen morphology of native and alien species of the genus *Heracleum* L. (Apiaceae) in Poland

Figs 26-33. Pollen morphology in proximal and distal view of *Heracleum* in SEM
Figs 26-29. *Heracleum mantegazzianum*; Figs 30-33. *Heracleum sosnowskyi*
11.69% to 14.07%. Statistical analysis showed significant differences (P < 0.05) among taxa for all features of anther (Table 1). Multiple comparisons based on Tukey's test showed the presence of two uniform group of species based on mean value of anther. Anther size and shape in both subspecies of *H. spongrylium* and width in the giant species were similar and no statistically significant differences were noted between these taxa (Table 1). The significant differences were observed in length and length/width ratio between *H. mantegazzianum* and *H. sosnowskyi* (Table 1). There are only few data concerning the size of anthers. According to Nordic materials (INTERNET http://www.floranordica.org) another length of *H. spongrylium* were in the range of 0.7-0.9 mm and of *H. mantegazzianum* 0.9-1.4 mm. Thus, biometric data constitute a valuable supplementation of extremely scarce literature data.

**Ornamentation of anther**

To date the surface of anthers has not been studied in *Heracleum*. The micro-morphological features of anthers were observed on the dorsal (Figs 1 B, 4, 8, 10 and 16) and ventral surfaces (Figs 1 A, 6, 12, 14). The sculpturing varied from tuberculate (Figs 2, 11, 15), rugulate (Figs 9, 17) to rugulate-striate (Figs 3, 5, 7, 13). Micromorphological characters, especially those of the ventral surface, show greater variation between taxa and thus are more useful in the taxonomy of this genus.

The present study is the first to investigate the anther surface of *Heracleum* and detailed observations provided new data.

**Pollen**

**Symmetry, size and shape**

The pollen grains are radially symmetrical and isopolar with the polar axis of 24.3-70.2 μm and the equatorial diameter of 16.2-35.1 μm (Table 2). Two main types of pollen grains are present in *Heracleum*: one prolate in shape with a P/E ratio from 1.67 (subsp. *sibiricum*) to 1.91 in *H. mantegazzianum* and the other prolate with a P/E ratio of 2.12 in *H. sosnowskyi*. Perprolate pollen generally dominated in *H. sosnowskyi* (76.5%), *H. mantegazzianum* (69.6%), *H. spongrylium* subsp. *spongrylium* (58.06%) and was least abundant in *H. spongrylium* subsp. *sibiricum* (37.19%). CEREAU-LARRIVAL (1962) divided the pollen of Apiceae into five distinct types based on the P/E ratio. Our pollen grains of *Heracleum* taxa could be included in the oval and subrectangular pollen types as classified by CEREAU-LARRIVAL (1962). The outline is elliptic in the equatorial view, the inner of the mesocolpium side is straight, and the outer contour is straight or slightly concave in all the studied taxa.

According to the classification of FAEGRI and IVERSEN (1989) based on the length of the polar (P) and equatorial diameter (E), two types of pollen grains can be distinguished in taxa of *Heracleum*. The first type, medium-sized of 36.90-37.66 × 18.9-19.11 μm are typical of *H. spongrylium*, while the other, larger of 61.86-62.40 × 29.46-29.93 μm are observed in *H. mantegazzianum* and *H. sosnowskyi*. Statistical analyses showed significant difference among taxa studied for all pollen features analysed. Multiple comparisons based on Tukey’s test showed the presence of two uniform groups of taxa (Table 2). It is well known that pollen grain dimensions in many genera are correlated with their chromosome number (KAPADIA and GOULD 1964, ANCHEV and DENEVA 1997, KATSIOTIS and FORSBERG 1995). All *Heracleum* taxa hitherto studied are diploid with 2n = 22. The range of P and E in pollen grains of *H. spongrylium* subsp. *spongrylium* and subsp. *sibiricum* is according to GAWŁOWSKA (1961). The dimension of pollen in *H. spongrylium* and *H. mantegazzianum* in Nordic materials is 38-47 × 19-26 μm and 50-74 × 28-42 μm, respectively, but there is no information whether the pollen was acetolised or not. The P, E, and P/E features for subsp. *sibiricum* and alien species showed low variability (P ~ 6.02-8.04%, E ~ 7.93-8.98%) and P/E ratio (5.83-9.28%). This features for subsp. *spongrylium* were characterized by moderate levels of variability (P ~ 12.79%, E ~ 11.86%) and P/E ~ 15.25% (Table 2).

Several studies on pollen length in *Heracleum* have been made and they all suggest that length alone is a useful diagnostic character e.g. in *H. platycarpum* Bois (pollen 65-67 × 30-34 μm), *H. persicum* Desf. ex Fisch. (51-66 × 24-33 μm). The ranges of pollen size in both subspecies of *H. spongrylium*, *H. mantegazzianum* and *H. sosnowskyi* overlap, but ranges in the length of polar axis (P) did not overlap between native and alien taxa.

**Aperture**

The pollen grains are operculate, tricolporate, colpi with costae. Endopores are equatorially elongated, forming an almost continuous coastal zone. Usually located in the middle of ectocolpi (Figs 28, 29, 32, 33), with a psilate operculum (Figs 28, 29). Margins of the endopore distinct in *H. spongrylium* subsp. *spongrylium* (Figs 20, 21, 24) and more or less distinct in *H. mantegazzianum* and *H. sosnowskyi* (Figs 28, 33). Ends of margins are more or less diffuse (Figs 21, 25, 28, 32).

**Exine sculpturing**

The exine sculpturing was observed on the proximal (Figs 18, 19, 21, 22, 23, 26, 27, 30) and distal view (Figs 20, 21, 24, 25, 28, 29, 32, 33) at the equator and at the pole. Various ornamentation types were observed: rugulate in the equatorial area was found in all the studied taxa, psilate-perforate at the pole (Figs 23, 27) in subsp. *sibiricum* and *H. mantegazzianum* and slightly striate (Figs 19, 31) in subsp. *spongrylium* and *H. sosnowskyi*. The results of the present paper demonstrated that sculpture is not a good criterion for the identification of taxa from the *Heracleum* sect. *Heracleum* and *Pubescents* and the pollen ornamentation was similar in all the specimens. Apiceae pollen grains are often rugulate, e.g. in the genus *Smyrnum* L. (MUNGAN et al. 2011), *Tetraenium* (DC.) Manden (YOUSEFZADI et al. 2006) and in *Bupleurum gilesii* Wolff (PERVEEN and QAISER 2006). In the genus *Seseli* L. differences can be found between the equator (rugulate, striate-recticulate) and at the pole (psilate-perforate, rugulate and striate) (GÜNER et al. 2011).

According to PUNT (1984) in the *Heracleum* type colpi are short or rather long with diffuse ends. In *H. spongrylium* columellae at poles are distinctly longer.
than those at the shoulders and usually longer than those at the equator; colllumelae in the mesocolpium in the cross-section are more or less circular, margins of the endoaperture distinct, P usually < 48 μm. In H. mantegazzianum colllumelae at the poles are slightly longer or not longer than at the shoulders, often shorter than the equator; colllumelae in the mesocolpium in the cross-section are more or less angular, margins of the endoaperture are less distinct, ends more or less diffuse, P usually > 48 μm. Our studies confirm literature data published to date. More detailed characteristics of pollen grains, such as the columnar structure of the tectum, exine thickness and the colpal membrane are of taxonomic importance, but for technical reasons are not practicable in pollination studies. On the basis of the distribution, biometrical and micro-morphological traits of anthers and pollen grains and ovary/fruits and seeds (Klimko and Truchan, unpubl. data, Kowal 1975) we conclude that H. spondylium and H. sibiricum are not two distinct species, but H. spondylium in the Polish flora should be divided into two lowest taxa in the rank of subspecies.

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