

The morphological intermediacy of *Erigeron ×huelsenii* (Asteraceae), a hybrid between *E. acris* and *E. canadensis*

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Received: 17.11.2017 • Accepted/Published Online: 09.04.2018 • Final Version: 24.07.2018

Abstract: Morphological intermediacy is considered the first evidence of hybridization in many plant studies. However, morphological intermediacy does not always provide hybrid origin and can be a result of divergence. In this study, the authors revised the morphological intermediacy of *Erigeron ×huelsenii*, a putative hybrid between the Eurasian *E. acris* s. str. and North American *E. canadensis*, using the character count procedure, a method that allows distinguishing hybridity from divergence. The measurements were made on herbarium specimens, including 100 specimens of *E. acris* s. str., 100 specimens of *E. canadensis*, and 50 specimens of *E. ×huelsenii*, which were collected in northeastern and southern Poland in 2013–2016. Among the 15 characters selected for the character count procedure, the hybrid showed intermediacy in 12 characters. The deviation was very significant ($P < 0.05$) and therefore the hypothesis of hybridity was fully accepted. Moreover, details of indumentum and receptacle characters of the hybrid and its parental species were visualized using scanning electron microscopy. The morphological characters of *E. ×huelsenii* presented in this study seem to be useful in the proper identification of all the hybrids between *E. canadensis* and *E. acris* s. l.

Key words: Character count procedure, *Erigeron*, hybrid, indumentum, scanning electron microscopy micrographs

1. Introduction

Spontaneous hybridization between alien and native plant species has been well documented worldwide, reflecting the ongoing human influence on the geographical distribution of plants and biotic homogenization (Daehler and Carino, 2001; Bleeker et al., 2007; Stace et al., 2015; Klonner et al., 2017). Interestingly, hybridization between alien and native plant species is understood as a source of new alien taxa (Pyšek et al., 2004), and the ability to hybridize with native congeners appears to be a very useful criterion in the determination of the invasiveness of alien plants (Blackburn et al., 2014; Klonner et al., 2017). In extreme cases, hybrids between alien and native plant species cause the local extinction of native parental species by introgressive hybridization (Blackburn et al., 2014). It is commonly known that plant hybrids are morphologically intermediate between their putative parental species (Stace, 1989; Wilson, 1992; Stace et al., 2015). However, morphological intermediacy does not always provide hybrid origin and can be a result of divergence (Wilson, 1992). Numerous methods have been developed to prove hybridity based on intermediate morphology (e.g., hybrid index, principal component analysis, and pictorialized

scatter diagrams); unfortunately, many of them are not useful in distinguishing hybridity from divergence (Wilson, 1992 and the literature cited therein). According to Wilson (1992), the best way to infer hybridity from morphological intermediacy is to specifically show character-by-character intermediacy, referred to as the character count procedure. This method has been successfully applied in many plant hybrid studies (e.g., Wilson and Valenzuela, 2002; Albaladejo et al., 2004; Tovar-Sánchez and Oyama, 2004).

Erigeron ×huelsenii Vatke (Asteraceae), a natural hybrid between the Eurasian *E. acris* L. s. str. and North American *E. canadensis* L., was described from northwestern Poland in 1871 (Vatke, 1871). Nowadays, aside from the Polish records (Pliszko, 2015; Pliszko and Jaźwa, 2017), it has only been rarely recorded in the United Kingdom, Belgium, Germany, the Czech Republic, Slovakia, and Russia (Roper, 1911; Wurzell, 1995; Šída, 2000; Bleeker et al., 2007; Danihelka et al., 2012; Seregin, 2012; Verloove and Lambinon, 2014; Stace et al., 2015). The hybrid occurs together with its parental species in disturbed habitats such as abandoned sand and gravel pits, disused ironworks, roadsides, railway areas, and arable fields with grass-legume mixtures, usually on sandy soils

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(Roper, 1911; Seregin, 2012; Stace et al., 2015; Pliszko and Jaźwa, 2017). It is able to survive during a few seasons in the wild, being more casual than established (Wurzell, 1995; Pliszko and Jaźwa, 2017) due to its sterility and lack of vegetative reproduction (Roper, 1911; Stace et al., 2015). According to Wurzell (1995) and Stace et al. (2015), *E. ×huelsenii* is conspicuously intermediate between its parental species, especially in the size of the capitula and achenes. Unfortunately, the known morphological characteristics of the hybrid (Vatke, 1871; Roper, 1911; Wurzell, 1995; Šída, 2004; Stace et al., 2015) lack some details on the indumentum and receptacle, the characters that are considered useful in the *Erigeron* L. and Asteraceae taxonomy (Small, 1918; Šída, 1998; Pliszko, 2015).

To date, the morphological intermediacy of *E. ×huelsenii* considered as a result of natural hybridization between *E. acris* and *E. canadensis* has not been a subject of detailed study. In this paper, therefore, the authors aimed to revise the hybrid nature of *E. ×huelsenii* using the character count procedure, a method recommended by Wilson (1992), and to contribute to the micromorphological description of its indumentum and receptacle characters using scanning electron microscopy (SEM) study (Bozzola and Russell, 1999).

2. Materials and methods

2.1. Plant material

In this study, the name *Erigeron ×huelsenii* was adapted instead of *×Conyzigeron huelsenii* (Vatke) Rauschert following the taxonomic treatment of the genera *Conyza* Less. and *Erigeron* proposed by Greuter (2003). The terminology of the indumentum and receptacle follow Werker (2000) and Small (1918), respectively. A total of 250 specimens were used in the morphometric study, including 100 specimens of *E. acris*, 100 specimens of *E. canadensis*, and 50 specimens of *E. ×huelsenii*. The specimens were randomly sampled during field surveys conducted in 14 localities in northeastern and southern Poland in 2013–2016 (Appendix). The measurements were made on dried herbarium specimens using a PZO Warszawa 18890 stereoscopic microscope for the flower, achene, indumentum, and receptacle characters. The voucher specimens are deposited in the Herbarium of the Institute of Botany of the Jagiellonian University in Kraków (KRA).

2.2. SEM study

The details of the indumentum and receptacle characters of *Erigeron ×huelsenii* and its parental species were visualized using the SEM method (Bozzola and Russell, 1999). Samples of the stems, cauline leaves, capitula, mature receptacles, and achenes were mounted on aluminum stubs coated with double-sided conductive carbon tabs. The stubs were then coated with gold in a sputter-coater

and examined with a Hitachi S-4700 scanning electron microscope at an accelerating voltage of 10 or 20 kV.

2.3. Character count procedure

As the first step of the character count procedure (Wilson 1992; Wilson and Valenzuela, 2002), we revised the diagnostic characters that separate the putative parental taxa. According to Šída (2000, 2004) and Pliszko (2015), the most useful morphological characters allowing *Erigeron acris* s. str. to be distinguished from *E. canadensis* are the number and size of the capitula, indumentum density of the involucre bracts, number and length of the ray and disc flowers, and length of the achenes and pappus. With regard to the data provided by abovementioned authors, we selected 12 quantitative and 3 qualitative characters for the analysis (Table 1). The length of the eligulate ray flowers was not considered since such flowers do not occur in *E. canadensis*. We also excluded from consideration the size of the achene, due to its subnormal nature in the hybrid (Roper, 1911; Stace et al., 2015); however, we included the length of the pappus, which is well developed. Measurements of IH, IBN, LRFN, LRFL, ERFN, DFN, DFL, IBI, RT, and RDS were made for a single capitulum per specimen; measurements of LR were made for a single middle cauline leaf per specimen; and measurements of PL were made for a single achene per specimen (abbreviations are explained in Table 1). For each specimen, the capitulum was sampled from the top part of the synflorescence. The mean values, standard deviations, and statistical tests (multiple comparisons) for each of the selected characters in the putative hybrid and its parental species were provided and tabulated. The normal distribution of the untransformed data of each morphological character in a particular taxon was tested using the Kolmogorov–Smirnov test, while the variance homogeneity was tested using the Levene test at a significance level of $P < 0.05$. If the values of the respective character in at least one taxon were not consistent with the normal distribution, and/or the variance was not homogeneous, the nonparametric Kruskal–Wallis H test for multiple comparisons was used to check the statistical significance of differences between the putative hybrid and its parental species. If the values of the respective character in all three taxa were consistent with the normal distribution and the variance was homogeneous, the Tukey honestly significant difference (HSD) test was used. Furthermore, following the character count procedure (Wilson, 1992; Wilson and Valenzuela, 2002), the value of each character in the putative hybrid taxon was determined as intermediate or nonintermediate between the parental taxa. Next, the intermediate and nonintermediate characters were counted. A one-sided sign test ($P < 0.05$) of intermediate versus nonintermediate characters was conducted to accept or reject the hypothesis of hybridity. The statistical analysis was performed using the Statistica 13 software package.

Table 1. Morphological characters used in the study.

Acronym	Definition of the character
Quantitative characters	
LN	Number of cauline leaves
LR	Leaf length and width ratio (cm)
IL	Length of internode under the synflorescence (cm)
CN	Number of capitula
IH	Height of involucre (cm)
IBN	Number of involucre bracts in capitulum
LRFN	Number of ligulate ray flowers in capitulum
LRFL	Length of ligulate ray flower (cm)
ERFN	Number of eligulate ray flowers in capitulum
DFN	Number of disc flowers in capitulum
DFL	Length of disc flower (cm)
PL	Length of pappus (cm)
Qualitative characters	
IBI	Indumentum of involucre bracts: 1 – dense, 2 – sparse, 3 – almost glabrous
RT	Type of receptacle: 1 – foveolate, 2 – foveolate and areolate, 3 – areolate
RDS	Shape of receptacle depressions: 1 – circular, 2 – circular and polygonal, 3 – polygonal

3. Results

3.1. Morphological intermediacy of the hybrid

Erigeron ×huelsenii is morphologically intermediate between *E. acris* and *E. canadensis* in 12 characters and nonintermediate in three of them (Table 2). The deviation is very significant ($P < 0.05$). This pattern would not be expected from divergence; therefore, the hypothesis of hybridity is accepted. Two characters (LRFN and DFN) in *E. ×huelsenii* were different from those of both putative parental species but were not intermediate between them (Table 2). Three other characters (LN, CN, and LRFL) in the hybrid were very similar to those of *E. acris* but were significantly different from *E. canadensis* (Table 2), and one character (ERFN) in the hybrid was very similar to that of *E. canadensis* but was significantly different from *E. acris* (Table 2). Moreover, two characters (IL and LR) in the hybrid were significantly similar to those of the putative parental species (Table 2).

3.2. Indumentum and receptacle characteristics

The indumentum of *Erigeron ×huelsenii* and its parental species is represented by three types of trichomes (type 1: unbranched multicellular uniseriate nonglandular trichomes; type 2: unbranched short-stipitate multicellular biseriolate glandular trichomes; type 3: acroscopic twin hairs with distinctive bifid acute tips), which are found in different parts of the presented taxa. The apex of the type 1

trichomes is acute, whereas the apex of the type 2 trichomes is capitate or obtuse. The surface of the type 1 trichomes is characteristically bumpy. In contrast, the surfaces of the type 2 and type 3 trichomes are smooth. In *E. ×huelsenii* and its parental species, the type 1 trichomes occur on the stems, leaf surfaces and margins, and abaxial surfaces of the involucre bracts (Figure 1). In the hybrid and *E. acris*, they are also present on the disc flowers. In all three taxa, the type 2 trichomes occur on the upper parts of the stems and abaxial surfaces of the involucre bracts (Figure 1), as well as on the ray and disc flowers, being rarely found on the leaf surfaces and lower parts of the stems. In all three taxa, the type 3 trichomes are typically found on the achenes (Figure 2). The distribution of the type 1 trichomes on the abaxial surfaces of the involucre bracts in the hybrid is intermediate between a densely covered *E. acris* and almost glabrous *E. canadensis* (Figure 1). Furthermore, the hybrid resembles *E. acris* by the presence of the type 1 trichomes on the disc flowers, but its type 1 trichomes on the leaf margins are similar to those found in *E. canadensis*. The receptacle of *E. ×huelsenii* shows an intermediate character between the foveolate and areolate receptacle types occurring in its parental species (Figure 3). Moreover, the shape of the receptacle depressions is circular and polygonal in the hybrid, whereas it is circular and polygonal in *E. acris* and *E. canadensis*, respectively (Figure 3).

Table 2. Count of characters as intermediate (+) or not (-) for hybridity hypothesis. Means and standard errors are given. Abbreviations of characters are explained in Table 1. *: after the Kruskal–Wallis H test, **: after the Tukey HSD test.

Character	<i>Erigeron acris</i>	<i>Erigeron ×huelsenii</i>	<i>Erigeron canadensis</i>	<i>Erigeron ×huelsenii</i> is different from	Intermediate ?
LN	14.95 ± 3.80	12.94 ± 4.81	25.49 ± 16.45	<i>E. canadensis</i> **	-
LR	10.28 ± 1.88	10.65 ± 1.80	10.71 ± 4.14	neither*	+
IL	1.32 ± 0.48	1.21 ± 0.60	1.09 ± 0.49	neither**	+
CN	19.58 ± 9.75	36.88 ± 26.30	118.23 ± 99.32	<i>E. canadensis</i> **	+
IH	0.53 ± 0.05	0.45 ± 0.05	0.33 ± 0.04	both*	+
IBN	38.38 ± 4.81	36.12 ± 3.74	33.68 ± 3.59	both*	+
LRFN	44.19 ± 7.15	72.46 ± 17.69	34.85 ± 9.38	both**	-
LRFL	0.47 ± 0.04	0.46 ± 0.04	0.30 ± 0.02	<i>E. canadensis</i> *	+
ERFN	78.03 ± 15.40	0.12 ± 0.33	0.00 ± 0.00	<i>E. acris</i> *	+
DFN	20.19 ± 5.87	23.40 ± 7.42	14.73 ± 4.35	both**	-
DFL	0.40 ± 0.04	0.31 ± 0.02	0.26 ± 0.02	both*	+
PL	0.53 ± 0.05	0.39 ± 0.04	0.27 ± 0.02	both**	+
IBI	dense 1.01 ± 0.10	sparse 2.00 ± 0.00	almost glabrous 3.00 ± 0.00	both*	+
RT	foveolate 1.00 ± 0.00	foveolate and areolate 2.00 ± 0.00	areolate 3.00 ± 0.00	both*	+
RDS	circular 1.00 ± 0.00	circular and polygonal 2.00 ± 0.00	polygonal 3.00 ± 0.00	both*	+
Scores of intermediate and nonintermediate characters	12:3				

4. Discussion

In this study, we evidenced that the character count procedure (Wilson, 1992) supports the hybridization hypothesis between *Erigeron acris* and *E. canadensis*. The morphological intermediacy of *E. ×huelsenii* between its putative parental species is clearly noticeable in many characters (Table 2), as suggested by Wurzell (1995) and Stace et al. (2015). However, some of these characters are pointed out as intermediate for the first time, namely the number of involucre bracts and eligulate ray flowers in the capitulum, the types and arrangement of the trichomes, and the type of receptacle. What is interesting is that only in a few hybrid specimens were eligulate ray flowers found in the capitula. The inner female eligulate ray flowers, together with outer female ligulate ray flowers and inner bisexual disc flowers, occur typically in the capitula of *Erigeron* sect. *Trimorpha* (Cass.) DC., to which *E. acris* s. str. belongs (Pliszko, 2015).

The types of trichomes present on the various organs of *E. acris* and *E. canadensis* have been well documented (e.g., Kothari et al., 2012; Pliszko, 2015). In this paper, optical and SEM studies revealed that the types of trichomes in *E.*

×huelsenii are identical to those found in its putative parental species. The differences in the indumentum between the presented taxa concern mainly the arrangement of the respective types of trichomes. The intermediate character of the hybrid, which was emphasized by other authors (Wurzell, 1995; Šída, 2000, 2004; Stace et al., 2015), is also noticeable in the distribution of the type 1 trichomes (multicellular uniseriate nonglandular trichomes) on the abaxial surfaces of the involucre bracts (Figure 1), as well as in the type of the receptacle (Figure 3). Interestingly, type 1 trichomes seem to be totally absent on the disc flowers in *E. canadensis*. However, to clarify this character a further study on more specimens is required.

Morphological intermediacy is considered the first evidence of hybridization in many plant studies (Stace, 1989; Albaladejo et al., 2004 and the literature cited therein). Furthermore, there are other criteria used for proving plant hybridity, such as reduced fertility, segregation of characters in the F₂ generation, geographical distribution, and recreation of hybrids in experimental conditions, as well as methods involving cytogenetic and molecular analyses (Stace, 1989; Tovar-Sánchez and

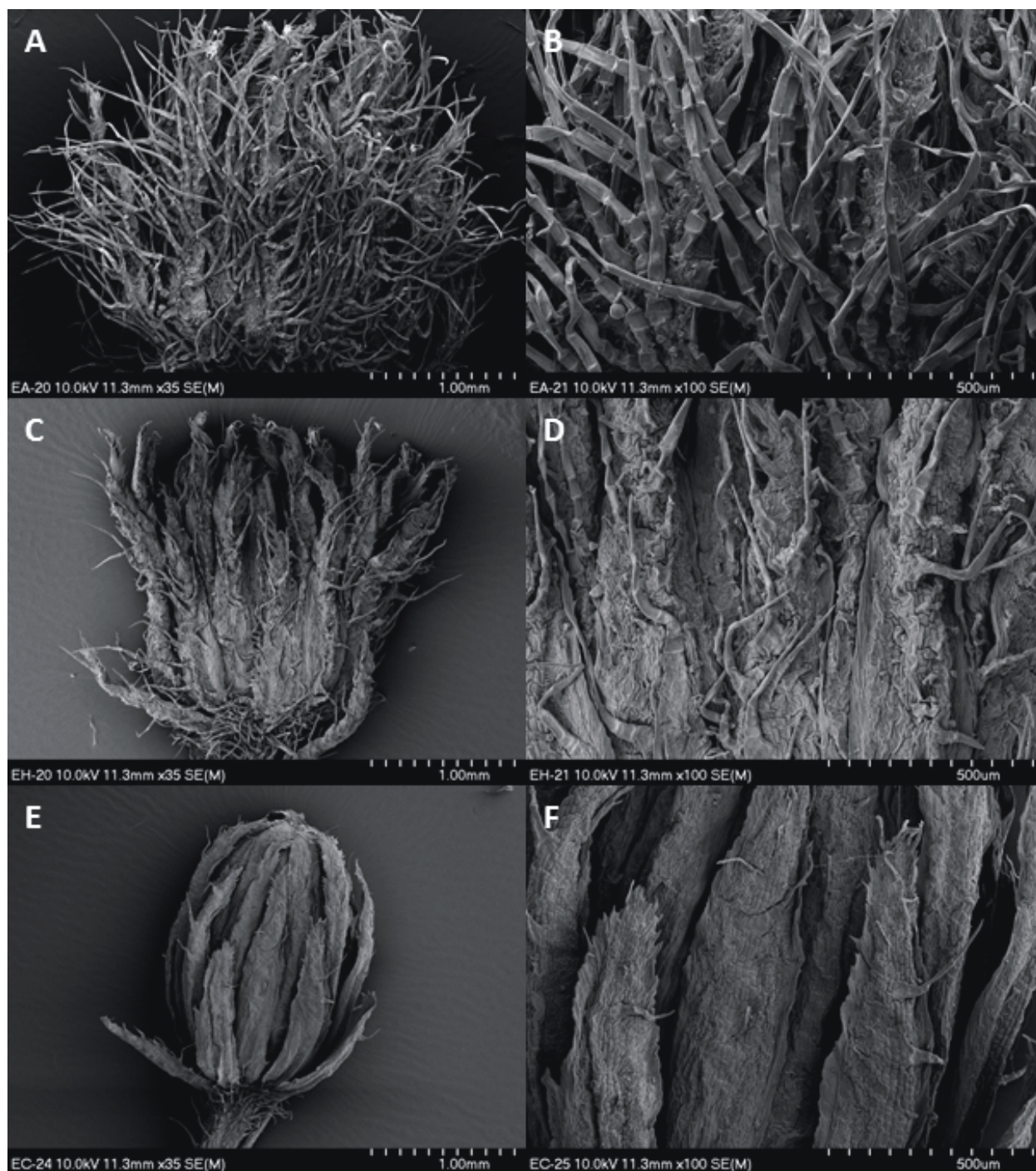


Figure 1. Indumentum details of the involucre bracts in *Erigeron acris* (A, B), *E. xhuelsenii* (C, D), and *E. canadensis* (E, F): young involucre (A, C, E), involucre bracts (B, D, F).

Oyama, 2004; Migdalek et al., 2014; Pliszko and Zalewska-Gałosz, 2016). Nevertheless, revising the hybrid identity of *E. xhuelsenii* during field surveys, we recommend looking at the intermediate size of the capitula and pappus, which are easy to notice without a microscope.

Taking into consideration that *E. canadensis* is naturalized in many European countries (Randall, 2017 and the literature cited therein) where *E. acris* s. str. is commonly distributed (Šída, 1998; Pliszko, 2015), *E. xhuelsenii* might be more widespread than previously

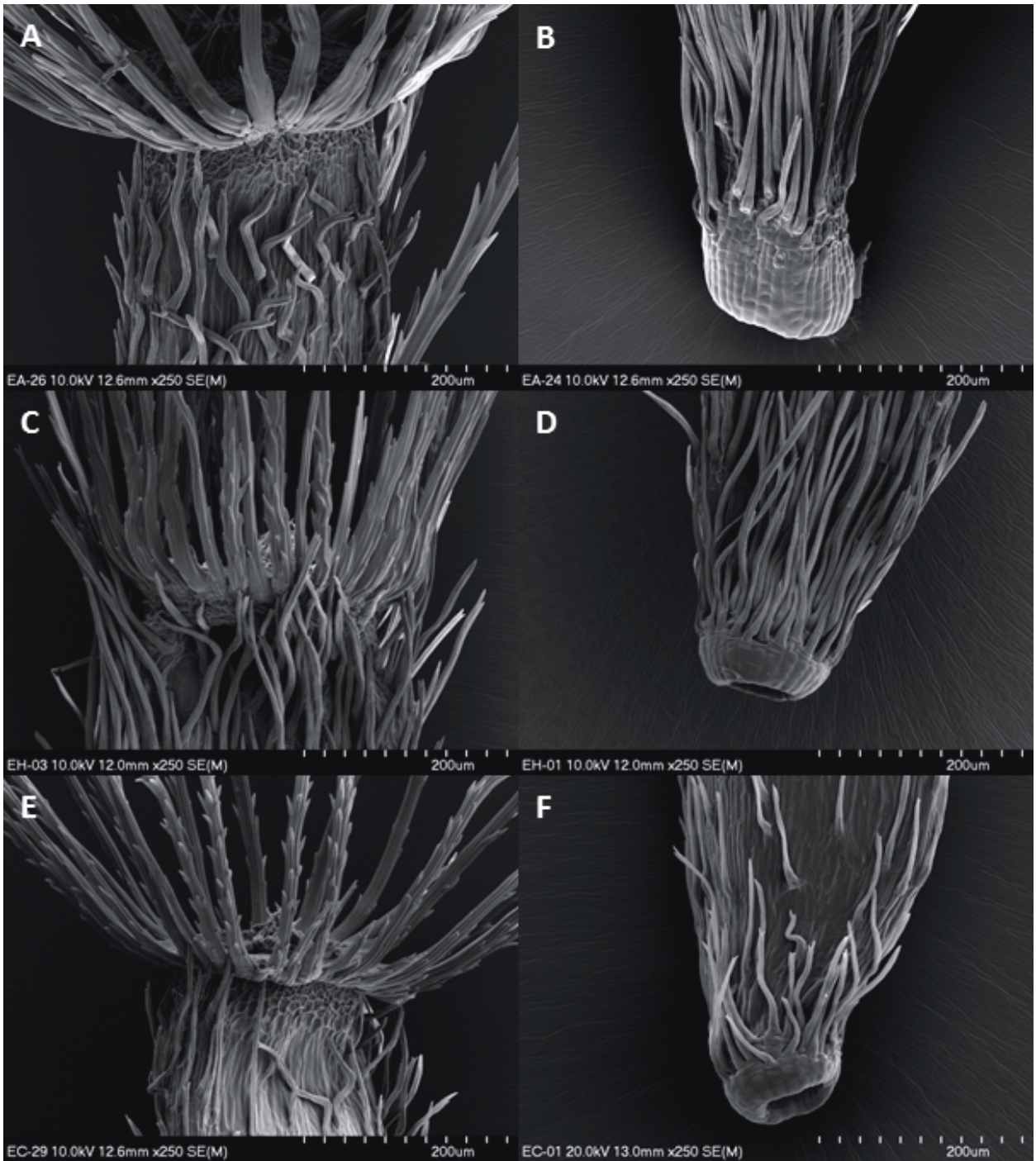


Figure 2. Indumentum details of the achenes in *Erigeron acris* (A, B), *E. xhuelsenii* (C, D), and *E. canadensis* (E, F): upper part of the achene (A, C, E), basal part of the achene (B, D, F).

thought. Moreover, there is a high probability that *E. canadensis* hybridizes with other closely related taxa of *E. acris* s. l. such as *E. acris* subsp. *serotinus* (Weihe) Greuter, *E. acris* subsp. *droebachiensis* (O. F. Müll.) Arcang., and *E. acris* subsp. *angulosus* (Gaudin) Vacc., but as far as it

is known such hybrids are not evidenced. However, we believe that the morphological characters of *E. xhuelsenii* presented in this study (Table 2) will contribute further to the proper identification of all the hybrids between *E. canadensis* and *E. acris* s. l.

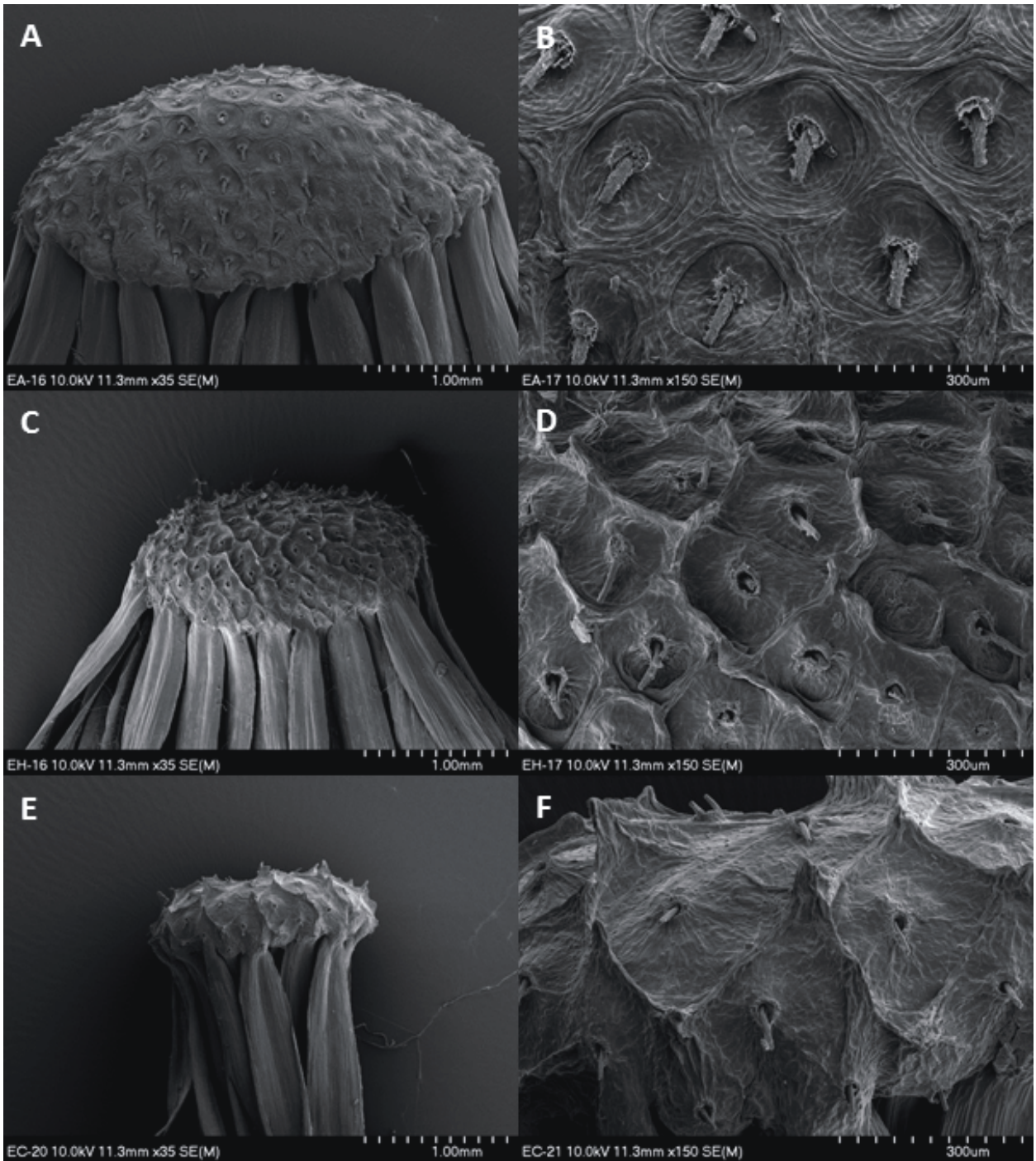


Figure 3. Receptacle details of *Erigeron acris* (A, B), *E. xhuelsenii* (C, D), and *E. canadensis* (E, F): receptacle types (A – foveolate, C – intermediate between foveolate and areolate, E – areolate), shape of receptacle depressions (B – circular, D – intermediate between circular and polygonal, F – polygonal).

Acknowledgments

We would like to thank Anna Łatkiewicz, M.Sc. Eng., Department of Mineralogy, Petrology, and Geochemistry of the Institute of Geological Sciences of the Jagiellonian

University in Kraków, for her help in taking the SEM micrographs. This work was financially supported by the Jagiellonian University in Kraków (DS/MND/WBiNoZ/IB/4/2013 and DS/MND/WBiNoZ/IB/2/2014).

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Appendix. Origin of *Erigeron* specimens sampled for the study (name of the species, location, GPS coordinates and altitude, habitat, collection date, number of collected specimens): *Erigeron acris* s. str.: Wólka, 54°12.603'N, 22°35.660'E, 185 m, abandoned field, 16 Jul 2016, 20; Garbas Pierwszy, 54°09.516'N, 22°36.896'E, 196 m, arable field with grass-legume mixture, 17 Jul 2016, 20; Filipów Czwarty, 54°10.142'N, 22°36.808'E, 186 m, arable field with grass-legume mixture, 18 Jul 2016, 20; Bakałarzewo, 54°05.129'N, 22°40.416'E, 171 m, abandoned field, 19 Jul 2016, 20; Sobolewo, 54°04.194'N, 22°58.103'E, 160 m, disused sand and gravel pit, 7 Aug 2014, 30 Jul 2016, 20; *Erigeron canadensis*: Filipów Czwarty, 54°10.166'N, 22°36.747'E, 190 m, roadside verge, 18 Jul 2016, 20; Matłak, 54°08.231'N, 22°37.518'E, 183 m, roadside verge, 21 Jul 2016, 20; Szafranki, 54°09.314'N, 22°37.678'E, 181 m, arable field with grass-legume mixture, 21 Jul 2016, 20; Suwałki (Żwirownia PKP), 54°06.379'N, 22°53.923'E, 171 m, disused sand and gravel pit, 26 Jul 2016, 20; Książ Wielki, 50°26.157'N, 20°07.858'E, 253 m, arable field with cereals, 8

Sep 2016, 20; *Erigeron xhuelsenii*: Sobolewo, 54°04.705'N, 22°57.505'E, 158 m, disused sand and gravel pit, 7 Aug 2013, 7 Aug 2014, 11; Suwałki (Żwirownia PKP), 54°06.379'N, 22°53.924'E, 171 m, disused sand and gravel pit, 26 Jul 2016, 1; Filipów Pierwszy, 54°09.836'N, 22°37.103'E, 187 m, arable field with grass-legume mixture, 16 Aug 2013, 9 Aug 2015, 26 Sep 2015, 6; Bakałarzewo, 54°07.011'N, 22°37.649'E, 179 m, arable field with grass-legume mixture, 10 Aug 2014, 1; Bakałarzewo, 54°06.580'N, 22°38.061'E, 187 m, arable field with grass-legume mixture, 2 Aug 2016, 2; Mieruniszki, 54°08.971'N, 22°34.356'E, 190 m, arable field with grass-legume mixture, 10 Aug 2014, 5; Kamionka Stara near Bakałarzewo, 54°04.770'N, 22°40.803'E, 164 m, roadside verge, 18 Aug 2013, 1; near Pluszkiejmy, 54°16.937'N, 22°27.206'E, 180 m, arable field with grass-legume mixture, 7 Sep 2014, 3; Filipów Czwarty, 54°09.904'N, 22°36.959'E, 190 m, roadside verge, 9 Aug 2013, 1; Filipów Czwarty, 54°10.094'N, 22°36.883'E, 194 m, arable field with grass-legume mixture, 8 Oct 2014, 26 Sep 2015, 31 Jul 2016, 23 Aug 2016, 19.