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**Competing interests**

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## ORIGINAL RESEARCH PAPER

# A morphometric study on *Senecio erucifolius* (Asteraceae) from Poland and its taxonomic implications

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**Abstract**

Twenty-three populations of *Senecio erucifolius* were sampled to study morphological diversity of the species in Poland. A total of 690 shoots, leaves, capitula, flowers, and fruits were characterized in respect to 27 quantitative traits and were subjected to morphometric analyses. Principal component analysis made it possible to distinguish two groups of individuals, corresponding to two infraspecific taxa – *S. erucifolius* subsp. *tenuifolius* (19 populations) and *S. erucifolius* subsp. *erucifolius* (four populations). The characters of the greatest importance in differentiating these two subspecies included the length of the upper lobe of the middle leaf, the width of the upper lobe, the width of the longest lateral lobe, the width of the upper lobe at the base, and the length of the tubular flower. Six of the 27 morphological features significantly differentiated populations within subspecies *erucifolius* according to a stepwise discriminant analysis. The length of the middle leaf, length of the tubular flower and width of the upper lobe at the base contributed most to the discrimination between the investigated populations. The discriminant analysis also showed considerable morphological heterogeneity of the 19 populations classified as subspecies *tenuifolius*. Seventeen characters significantly differentiated the populations, with the length of the achene and the ratio between the length and the width of the achene as the most important ones.

**Keywords**

taxonomy; morphological variability; *Jacobaea erucifolia*; Asteraceae; Poland

**Introduction**

*Senecio* L. (Asteraceae; Senecioneae) is a large and taxonomically complex genus comprising between 1000 and 3000 species [1–4]. The genus is divided into approximately 150 sections, which are not clearly distinguished from one another. *Senecio erucifolius* L. belongs to sect. *Jacobaea*, which according to different authors comprises from three to 15 species, with *S. jacobaea* as the type species of the section [5]. Delimitation of sect. *Jacobaea*, its phylogenetic position and its relationships within and among the *Senecio* sections were recently examined by Pelsner et al. [5–8]. In recent years, some authors proposed to transfer some *Senecio* species to a separate genus *Jacobaea* Mill. [8–11]. According to this approach, the name of *Jacobaea erucifolia* (L.) P. Gaertn., B. Mey & Schreb. is used for *Senecio erucifolius* L. and currently both names are accepted [12] and used.

*Senecio erucifolius* is a thermophilic rhizomatous perennial plant. The general range of this species extends over a large part of Europe, as well as Western and Central Asia. The species is absent from the northern part of Scandinavia, the Iberian Peninsula, and the southeastern part of Europe. Its range extends in a belt through Siberia

and reaches up to Yakutia. In Poland it has a scattered distribution, and is mainly found in the southeastern part of the country [13–16]. In the system of geographical elements *S. erucifolius* represents a linking southeuro-siberian-northmediterranean-iranoturanian element [17].

Morphologically *S. erucifolius* is a highly variable species. In studies concerning the European flora, two subspecies are most commonly distinguished within this species: subsp. *erucifolius* and subsp. *tenuifolius* (J. Presl & K. Presl) Schübl. & G. Martens [18–23], while less frequently several varieties: var. *erucifolius*, var. *communis* Rouy, var. *tenuifolius*, var. *latilobus* Boiss, var. *viridulus* Martrin-Donos and the form f. *discoideus* DC are considered [24,25]. Subspecies *tenuifolius* is sometimes reduced to a variety *tenuifolius* (Schübl. et G. Martens) Steudel or elevated to full species status as *S. tenuifolius* Jacq. [26–32]. Soó [33] noticed different geographical ranges of the intra-specific taxa. The nominative subspecies is a Mediterranean–Atlantic taxon, whereas subsp. *tenuifolius* is a continental one. In the Polish literature there are no scientific publications on the intraspecific differentiation of the hoary ragwort *S. erucifolius* and only isolated papers [31,34,35] have reported the occurrence of var. *tenuifolius*. The first map presenting the distribution of this species in Poland [16] does not account

for the occurrence of intraspecific taxa, although the authors of the paper mention them. However, according to Greuter [36], only *S. erucifolius* subsp. *erucifolius* occurs in Poland.

The main aim of our study was to determine the morphological variability of *S. erucifolius* in Poland, taking into account the presumed intraspecific taxa: subsp. *erucifolius* and subsp. *tenuifolius*. Since to date the taxa have been distinguished based on selected features of the leaves [16,18,22,23,29–33], the aim of our study was also to make an attempt to find new diagnostic characteristics useful to distinguish the intraspecific taxa.

Another aim was to determine the interpopulational differentiation within the subspecies, including examination of the geographic dependence of morphological differentiation in the taxa in Poland.

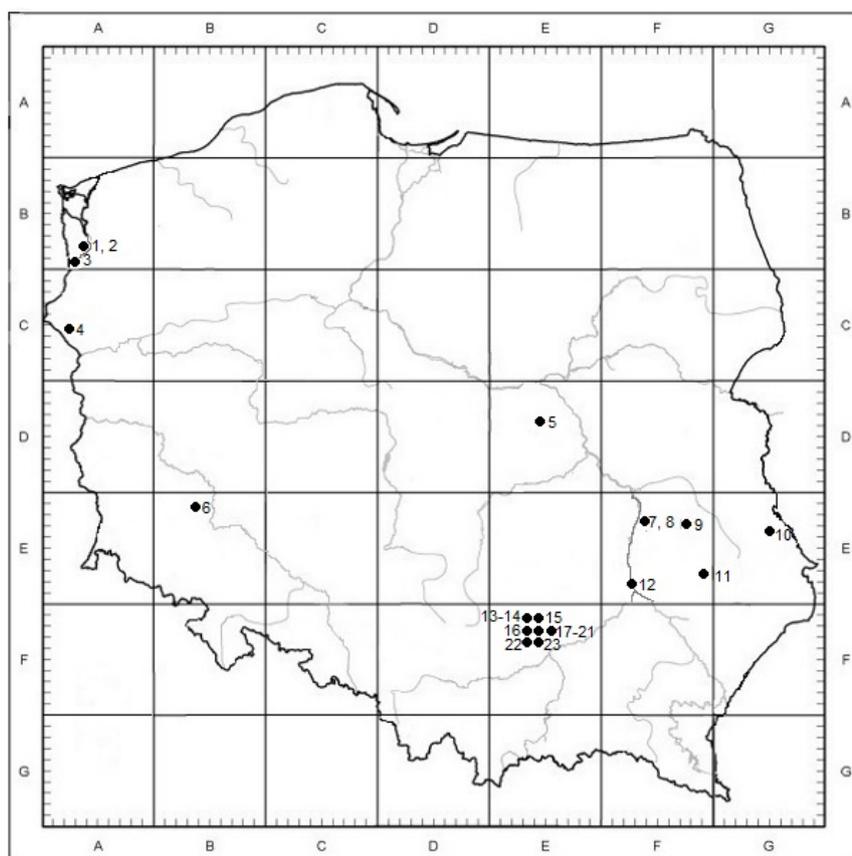
## Material and methods

### Material

Plant material was collected in late summer of the years 2011–2013. A total of 23 populations representing the whole range of distribution and variation of *Senecio erucifolius* in Poland were examined (Tab. 1, Fig. 1). In each population shoots with inflorescences and fruits of 30 randomly selected individuals were sampled.

Tab. 1 Details of sampled Polish populations of *Senecio erucifolius*.

No. of population	Population	Latitude N	Longitude E	Altitude (m a.s.l.)	Aspect
1	Szczecin-Skolwin	53°30'	14°36'	58	SE
2	Szczecin-Stołczyn	53°30'	14°36'	3	-
3	Moczyły	53°19'	14°28'	3	E
4	Kłósów	52°44'	14°27'	35	-
5	Brwinów	52°08'	20°42'	88	SE
6	Sitno	51°22'	16°22'	58	-
7	Męcierz I	51°18'	21°54'	150	-
8	Męcierz II	51°18'	21°54'	150	-
9	Lublin	51°16'	22°32'	190	S
10	Brzeźno	51°09'	23°36'	178	-
11	Guzówka	51°51'	20°44'	296	SE
12	Dziurów	50°48'	21°48'	85	NW
13	Welecz	50°28'	20°38'	241	S
14	Kowala	50°28'	20°33'	203	W
15	Zbrodziejce	50°28'	20°45'	296	NW
16	Żurawniki	50°20'	20°41'	238	NE
17	Olganów	50°26'	20°45'	215	-
18	Łatanice	50°24'	20°41'	215	SE
19	Owczary	50°26'	20°45'	216	SE
20	Pęczelice	50°26'	20°47'	242	S
21	Zborów	50°22'	20°53'	185	-
22	Sielec	50°21'	20°40'	180	NW
23	Szczerbaków	50°20'	20°42'	175	SW



**Fig. 1** Location of sampled Polish populations of *Senecio erucifolius* (as in Tab. 1).

## Measurements

A total of 690 shoots, leaves, capitula, flowers and fruits were characterized in terms of 27 quantitative traits, including three ones describing ratios between traits (17, 19, 23) and two qualitative traits (11, 29) coded as binary or multistate (Tab. 2, Fig. 2). These traits were marked out on the basis of previous studies of Pelsler et al. [7], Oberprieler et al. [37], and Sell and Murrell [24]. Leaf measurements were made on well-developed leaves from the middle of the stem, while capitulum characters were recorded for terminal capitula of the main inflorescence axis.

## Data analysis

The biometric data of 27 quantitative characters were subjected to multivariate statistical analysis using the Statistica, ver. 10 (Stat-Soft, Poland). Each specimen studied was regarded as an operational taxonomic unit (OTU).

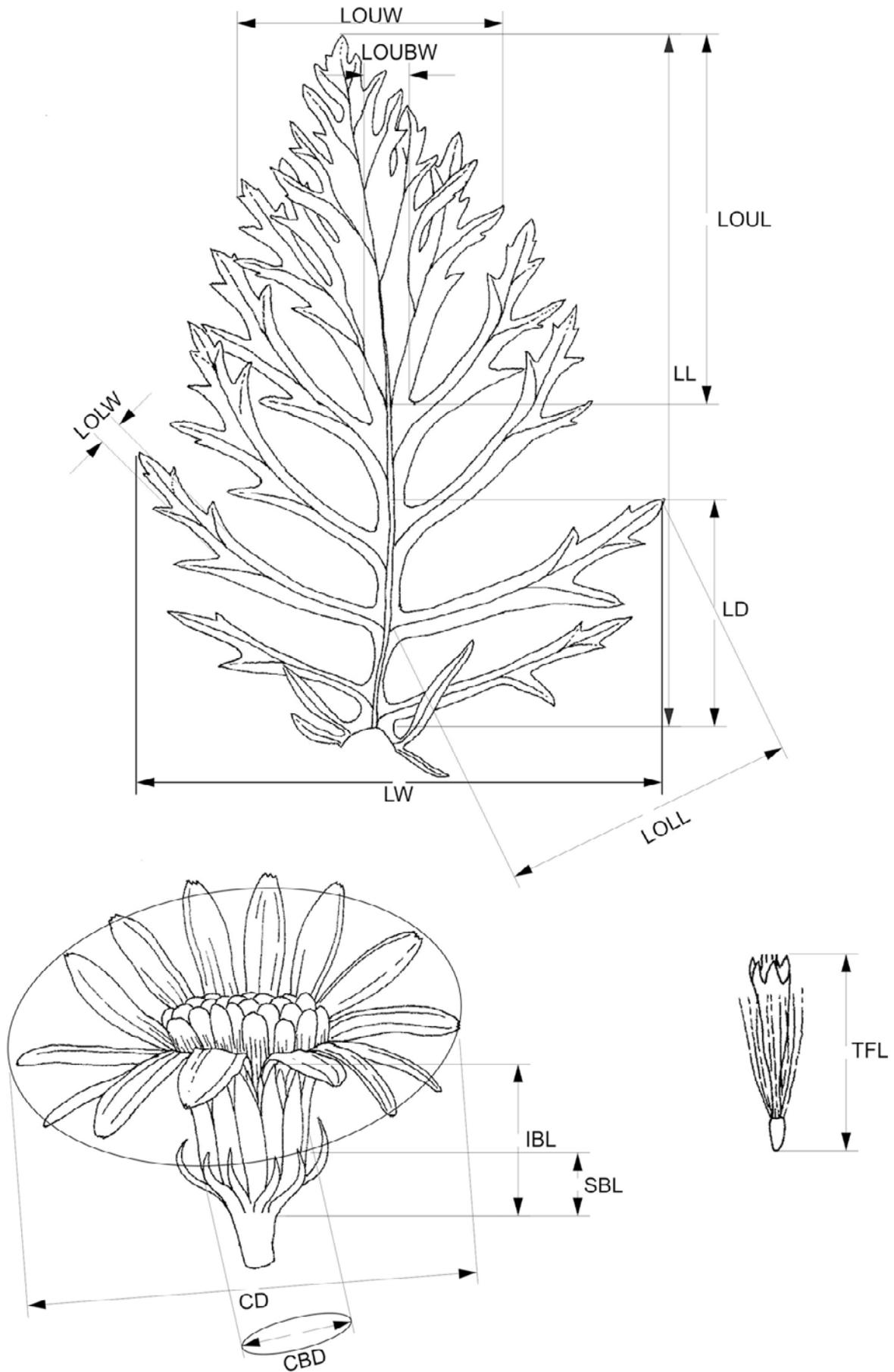
Prior to multivariate statistical analyses the quantitative variables were transformed to provide a better approximate normality, while they were also standardized to compare characters that were previously recorded in different units. Principal component analysis (PCA) on the correlation matrix and varimax rotation were used to examine the general variation, as well as to reduce the set of characters that were correlated the strongest with the principal components. In PCA the species was tested with no a priori assumptions. Factors were extracted by the scree test [38]. The characters with the highest factor loadings on the first three components ( $r > 0.60$ ) were determined. Discriminant analysis (DA) was used to determine which characters most effectively differentiate populations within the two subspecies *erucifolius* and *tenuifolius*. The models were built with a backward stepwise procedure. The contribution of particular characters to the discrimination between groups were interpreted based on standardized coefficients.

The significance of differences between groups of specimens for characters provided by PCA as well as DA were subsequently tested with univariate analyses. Calculation were performed on untransformed data. For characters selected by PCA, the Student's *t*-test (for traits with normal distribution and equal variance) and the Mann-Whitney *U* test (for other traits) were used. For characters selected by DA, the one-way analysis of variance ANOVA (for traits with a normal distribution and equal variance) and the Kruskal-Wallis test (for the other traits) were performed. Descriptive statistics of the characters that differentiate the studied groups the best were presented as the box-plot diagrams.

The Mantel test (Arlequin, ver. 3.11; Laurent Excoffier, Berne) was used to examine whether populations of *S. erucifolius* subsp. *tenuifolius* are more similar morphologically if they are geographically close. As a measure of the morphological distance between each pair of the populations we used an Euclidean distance that was computed from the row data. The significance of the relationship between morphological and geographical distances was tested with 999 randomly permutations.

Tab. 2 Morphological characters recorded.

No.	Character	Description	Type of characters: continuous (C), discrete (D)	Units and accuracy/coding
1	SL	Length of stem	C	1 cm
2	SIH	Height of stem at the lowest branch of the inflorescence	C	1 cm
3	BIN	Number of branches of inflorescence	C	-
4	CAN	Number of capitula	C	-
5	CBD	Diameter of base of capitulum	C	1 mm
6	CD	Diameter of capitulum	C	1 mm
7	IBN	Number of involucre bracts per capitulum	C	-
8	IBL	Length of involucre bracts	C	1 mm
9	SBN	Number of supplementary bracts per capitulum	C	-
10	SBL	Length of supplementary bracts of capitulum	C	1 mm
11	SBH	Presence of hairs on supplementary bracts of capitulum	D	0–1
12	LFL	Length of ligulate flower	C	1 mm
13	LFW	Width of ligulate flower	C	1 mm
14	TFL	Length of tubular flower	C	1 mm
15	AL	Length of achene	C	0.01 mm
16	AW	Width of achene	C	0.01 mm
17	AL/AW	Ratio between length and width of achene		-
18	PHL	Length of pappus hairs of tubular flower	C	0.01 mm
19	PHL/AL	Ratio between length of pappus hairs of tubular flower and length of achene		-
20	LL	Length of middle leaf	C	1 cm
21	LW	Width of middle leaf	C	1 cm
22	LD	Distance from the leaf base to its widest part	C	1 mm
23	LD/LL	Ratio between the distance from the leaf base to its widest part and length of leaf		-
24	LOUL	Length of upper lobe	C	1 mm
25	LOUW	Width of upper lobe	C	1 mm
26	LOLL	Length of longest lateral lobe	C	1 mm
27	LOLW	Width of longest lateral lobe	C	1 mm
28	LOUBW	Width of upper lobe at the base	C	1 mm
29	LS	Shape of middle leaf	D	0–2



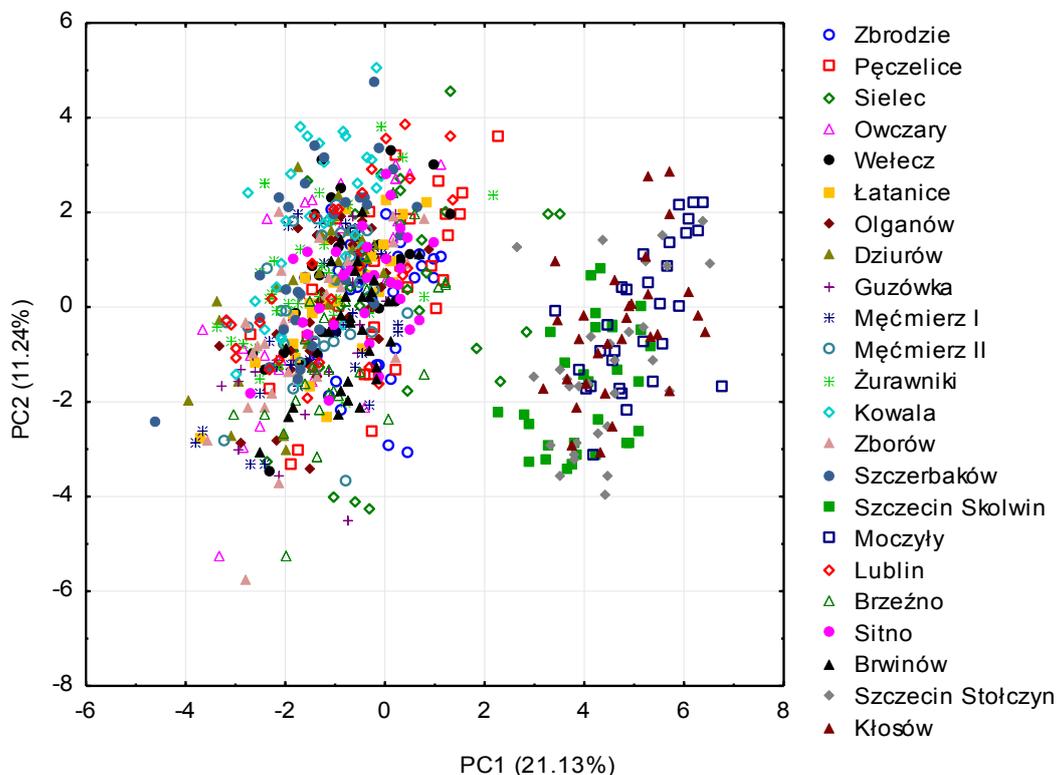
**Fig. 2** Leaf, flower, and capitulum measurements (characters as in Tab. 2).

## Results

### Variability of *Senecio erucifolius* in Poland

Principal component analysis of 27 quantitative characters yielded three components, accounting for 40.7% of the total variance in the data set. The first principal component (PC1) represented 21.13% of the total variance, while the second axis (PC2) accounted for 11.24% and the third (PC3) for 8.32%. The scatterplot according to the first two components showed two separated groups of specimens, which correspond to two subspecies of *S. erucifolius* (Fig. 3). The smaller group comprised specimens from four populations: Szczecin-Skowlin, Szczecin-Stołczyn, Moczyły, and Kłosów, and represented *S. erucifolius* subsp. *erucifolius*. The bigger group was composed of specimens from 19 populations that represented *S. erucifolius* subsp. *tenuifolius*. The characters, which showed the greatest variability on the first component, included length of the upper lobe (LOUL), width of the upper lobe (LOUW), width of the longest lateral lobe (LOLW), width of the upper lobe at the base (LOUBW), as well as length of the tubular flower (TFL). These characters weighted the most in distinguishing between the subspecies (Tab. 2). Length of the middle leaf (LL), width of the middle leaf (LW), distance from the leaf base to its widest part (LD) and length of the longest lateral lobe (LOLL) were highly and positively correlated with the second component. The characters, which showed the greatest influence in the third component, included the number of involucre bracts per capitulum (IBL) and the number of supplementary bracts per capitulum (SBN; see Tab. 3, Fig. 3).

The results of the *t*-test and the Mann–Whitney *U* test revealed differences in 9 out of 11 characters that were distinguished by PCA (Tab. 2). The highest values of *t* and *Z* statistics were obtained for all features that had high loadings with the first principal component, which confirmed their very high importance for discriminating between the two subspecies. Characters that had significant loadings with the second component did not differentiate the subspecies (i.e., width of the middle leaf as well as length of the longest lateral lobe) or differentiated them very

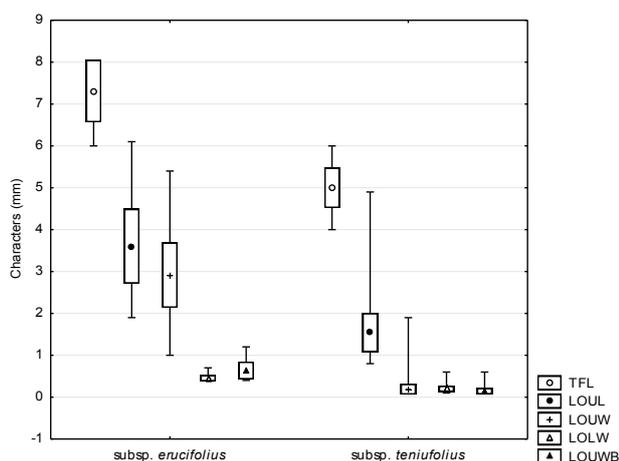


**Fig. 3** Two-dimensional ordination diagram of PCA (on the correlation matrix) based on 27 morphological characters of 960 *Senecio erucifolius* specimens.

**Tab. 3** Results of the principal component analysis (PCA) for *Senecio erucifolius* and a comparison of two subspecies provided by PCA by means of Student's *t*-test (for characters with normal distributions) and the Mann–Whitney *U* test (for characters with non-normal distributions).

Variable	Factor loadings <sup>T</sup>			Student's <i>t</i> -test (*) <sup>R</sup> / Mann–Whitney <i>U</i> test <sup>R</sup>	
	PC1	PC2	PC3	<i>t</i> */ <i>Z</i> values	<i>p</i> -value
LOUL	<b>0.89</b>	−0.17	−0.01	<b>−33.25*</b>	<0.0000
LOUW	<b>0.88</b>	0.06	0.00	<b>−17.23</b>	<0.0000
LOUBW	<b>0.86</b>	−0.08	−0.02	<b>−17.03</b>	<0.0000
LOLW	<b>0.80</b>	0.10	0.01	<b>−16.97</b>	<0.0000
TFL	<b>0.78</b>	−0.20	−0.09	<b>−16.90</b>	<0.0000
IBN	−0.44	0.14	0.62	8.21	<0.0000
SBN	−0.44	0.14	0.69	8.47	<0.0000
LL	0.49	0.74	0.07	<b>−6.28*</b>	<0.0000
LD	0.30	0.79	0.06	<b>−2.14*</b>	0.03
LW	0.28	0.70	0.10	−0.86	0.39
LOLL	0.25	0.71	0.12	<b>−0.35*</b>	0.73
Eigenvalues	5.71	3.03	2.25	-	-
Explained variance (%)	21.13	11.24	8.32	-	-
Cumulative explained variance (%)	21.13	32.37	40.69	-	-

Eleven morphological characters that are significantly correlated (>0.6) with the first tree principal components are presented. Values with the highest factor loadings on the first component (PC1) and the highest *t*/*Z* values are given in bold. For trait abbreviations, see Tab. 2. <sup>T</sup> Calculated for transformed data. <sup>R</sup> Calculated for row data.



**Fig. 4** Arithmetic means (squares), means ±SD (boxes) as well as minimum and maximum values (whiskers) for five characters that best distinguished between two subspecies of *Senecio erucifolius*. For trait abbreviations, see Tab. 2.

weakly (i.e., the distance from the leaf base to its widest part). These characters took part in the differentiation of particular populations within the subspecies. Descriptive statistics of all quantitative characters in the two subspecies are given in Appendix S1. The variability of five features that were most correlated with the first component are shown in Fig. 4.

#### Variability of *Senecio erucifolius* subsp. *erucifolius* in Poland

On the basis of field observations supported by PCA results four of the studied populations were classified as *S. erucifolius* subsp. *erucifolius*. Six of 27 morphological features significantly differentiated populations within subspecies according to the stepwise DA [Wilks' lambda = 0.54;  $F(18, 314) = 4.31$ ;  $p < 0.0000$ ]. Only the first canonical root provided to the discrimination between groups (chi-square = 71.04;  $df = 18$ ;  $p < 0.000$ ). Length of the middle leaf (LL), length of the tubular flower (TFL)

and width of the upper lobe at the base (LOUBW) had the lowest partial lambda and the largest standardized coefficients with the first canonical root, so they contributed most to the discrimination between populations (Tab. 4). A scatterplot showed one group of points with the most distant positions of specimens from the Szczecin

**Tab. 4** Results of discriminant analysis (DA) for population of *S. erucifolius* subsp. *erucifolius* and a comparison of populations by ANOVA (for characters with normal distributions) and the Kruskal–Wallis test (for characters with non-normal distributions).

Character	Discriminant function analysis (DFA) <sup>T</sup>				ANOVA (*) <sup>R</sup> / Kruskal–Wallis test <sup>R</sup>	
	Wilks' lambda	partial Wilks' lambda	<i>p</i> -value	Root 1 beta coefficients	<i>F</i> */ <i>H</i> values	<i>p</i> -value
LL	0.60	<b>0.90</b>	<b>0.006</b>	<b>-0.67</b>	25.87	<0.0000
TFL	0.61	<b>0.88</b>	<b>0.003</b>	<b>-0.47</b>	17.68	0.0005
LOUBW	0.58	<b>0.92</b>	<b>0.021</b>	<b>-0.44</b>	24.55	<0.0000
LOLL	0.56	0.95	0.133	-0.14	5.79*	0.001
LOUW	0.55	0.97	0.314	0.28	1.09*	0.36
LFW	0.55	0.97	0.358	-0.16	3.73	0.29
Eigenvalue	-	-	-	0.64	-	-
Proportion	-	-	-	0.83	-	-

For each characters and the first canonical function (Root 1) the standardized coefficients are given. Characters that contribute most to the discrimination between groups are in bold. For trait abbreviations, see Tab. 2. <sup>T</sup> Calculated for transformed data. <sup>R</sup> Calculated for row data.

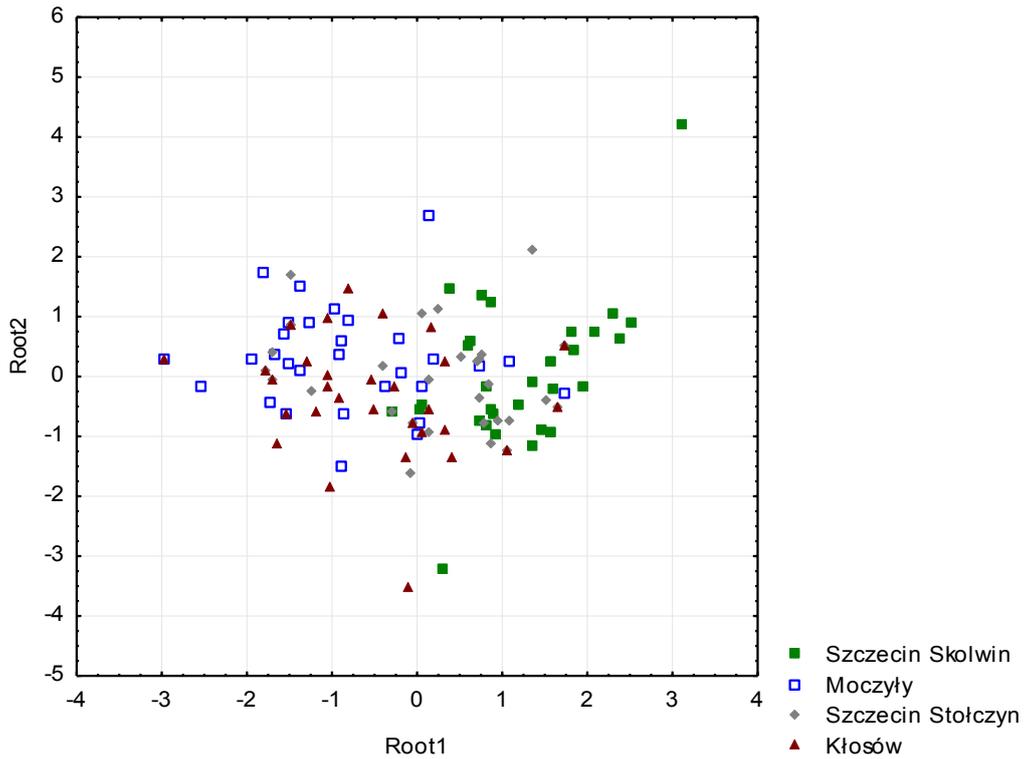
Skowlin and Moczyły populations (Fig. 5). ANOVA and the Kruskal–Wallis test confirmed that three selected characters significantly differentiated the examined populations ( $p < 0.0005$ ). Moreover, ANOVA indicated that also the length of the longest lateral lobe (LOLL) distinguished populations well (Tab. 4). The ranges and variability of the most important features are presented in Fig. 6.

#### Morphological description of *S. erucifolius* subsp. *erucifolius*

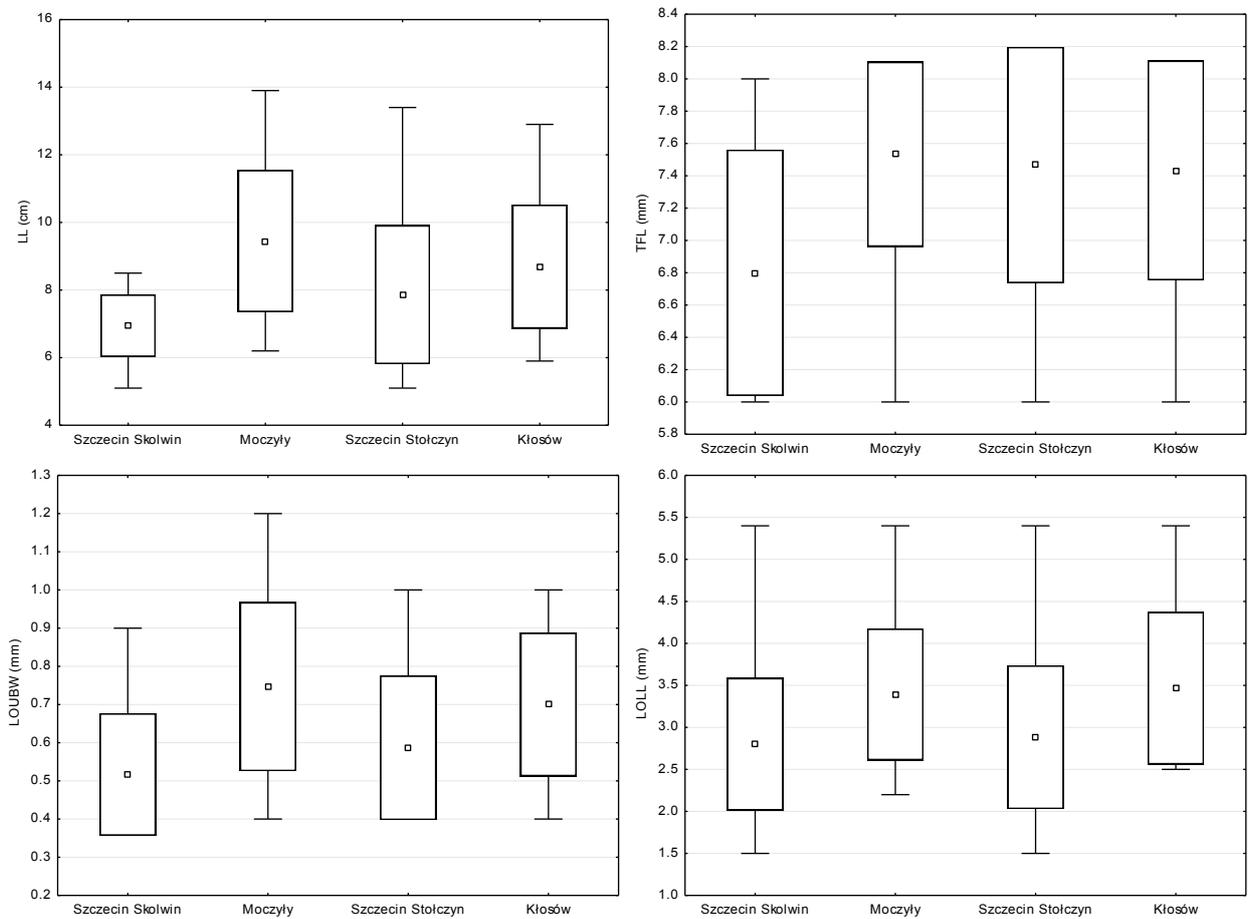
The stem of the specimens examined is 69–156 cm in height. The inflorescence is a compound, corymbose panicle with 2–15 branches terminating in capitula. The length of the middle leaves is 5.1–13.9 cm and the width is 2.9–8.3 cm. Single pinnate leaves, in general outline, are mostly ovate (55.83%) or obovate (41.66%), occasionally roundish (2.5%). The upper lobe is large, 1.9–6.1 cm long and 1–5.4 cm wide, and its width at the base is 0.4–1.2 cm. The length of the longest lateral lobe is 1.5–5.4 cm and its width is 0.4–0.7 cm. The number of capitula per inflorescence is 13–138; the diameter of the capitulum is 16–21 mm and the diameter of the base of the capitulum is 3–5 mm. The number of involucre bracts per capitulum is 12–13 and they are 3–4 mm long. Supplementary bracts are hairy, 4–6 in number and 2–3.5 mm long. Ligulate flowers are light yellow, 9–13 mm long and 1.5–2 mm wide. Tubular flowers are 4–8 mm long. Achenes are 1.33–2.22 mm long and 0.44–0.67 mm wide and the length of pappus is 3.33–5.67 mm.

#### Variability of *Senecio erucifolius* subsp. *tenuifolius* in Poland

The DA showed considerable morphological heterogeneity in the 19 populations, which were previously classified as subspecies *tenuifolius*. When 17 characters (see Tab. 5) were added to the model, the discrimination of populations were highly significant [Wilks' lambda = 0.005;  $F(306, 6685) = 11.93$ ;  $p < 0.0000$ ]. The first three roots contributed 91% of the total variance, while a separate contribution of the first root was 49%. Length of the achene (AL) and ratio between the length and the width of



**Fig. 5** Two-dimensional ordination diagram of DA based on six morphological characters of *Senecio erucifolius* subsp. *erucifolius* specimens.



**Fig. 6** Arithmetic means (squares), means  $\pm$ SD (boxes) as well as minimum and maximum values (whiskers) for four characters that varied most in *Senecio erucifolius* subsp. *erucifolius*. For trait abbreviations, see Tab. 2.

**Tab. 5** Results of discriminant analysis (DA) for population of *S. erucifolius* subsp. *tenuifolius* and a comparison of populations by ANOVA (for characters with normal distributions) and the Kruskal–Wallis test (for characters with non-normal distributions).

Characters	Discriminant function analysis (DFA) <sup>T</sup>						ANOVA (* <sup>R</sup> / Kruskal–Wallis test <sup>R</sup>	
	Wilks' lambda	partial Wilks' lambda	p-value	Root 1	Root 2	Root 3	F*/H values	p-value
SBN	0.01	0.60	0.00	-0.23	<b>0.65</b>	0.12	399.66	<0.000
AW	0.01	0.30	0.00	2.12	1.03	<b>-1.00</b>	214.88	<0.000
AL/AW	0.01	0.30	0.00	<b>3.93</b>	<b>1.71</b>	<b>-0.65</b>	11.60*	<0.0000
AL	0.01	0.30	0.00	<b>-3.24</b>	<b>-1.47</b>	0.40	38.13	<0.01
CAN	0.00	0.96	0.17	0.07	-0.06	0.23	10.79*	<0.0000
LL	0.01	0.81	0.00	-0.01	0.10	0.44	4.83*	<0.0000
SBL	0.01	0.83	0.00	-0.09	0.45	0.14	294.23	<0.000
IBN	0.01	0.85	0.00	-0.13	0.44	0.03	285.61	<0.000
LOUL	0.00	0.91	0.00	0.08	-0.01	-0.10	2.51*	<0.000
PHL/AL	0.01	0.60	0.00	-2.02	-0.47	<b>-1.24</b>	4.72*	<0.0000
PHL	0.01	0.70	0.00	1.93	0.40	<b>1.35</b>	2.34*	<0.01
LW	0.00	0.91	0.00	0.05	-0.04	-0.35	1.19*	0.26
SL	0.00	0.94	0.03	0.01	0.01	-0.25	1.08*	0.36
BIN	0.00	0.95	0.10	-0.04	0.16	0.44	134.67	<0.000
SIH	0.00	0.96	0.25	-0.02	0.02	0.19	26.13	0.10
LOLW	0.00	0.96	0.36	-0.04	-0.12	-0.03	24.31	0.14
LOLL	0.00	0.97	0.41	-0.08	0.06	0.06	2.04	<0.001
Eigenvalues	–	–	–	6.78	5.17	0.55	–	–
Cumulative proportions	–	–	–	0.49	0.87	0.91	–	–

For each characters and canonical functions (Roots) the standardized coefficients are given. Characters that contribute most to the discrimination between groups are in bold. For trait abbreviations, see Tab. 2. <sup>T</sup> Calculated for transformed data. <sup>R</sup> Calculated for row data.

the achene (AL/AW) had the strongest impact on the first and second roots (Tab. 5). As the ordination diagram shows (Fig. 7), the first root separated mainly populations from Brzeźno and Brwinów, whereas the other populations were intermediate. Apart from the previously mentioned features, the number of supplementary bracts per capitulum (SBN) weighted most on Root 2 and separated groups of populations (i.e., Wełecz, Łatanice, Olganów, Dziurów, Guzówka, Męcierz I and II, Żurawniki, Kowala, Szczerbaków). Length of the pappus hair of the tubular flower (PHL) and the ratio of the length of the pappus hairs of the tubular flower to the length of the achene (PHL/AL) showed high standardized coefficients for Root 3 and separated Lublin from six populations (i.e., Pęczelice, Sielec, Wełecz, Łatanice, Męcierz I, and Zborów). ANOVAs and Kruskal–Wallis tests confirmed the significance of the characters that contributed most to the discrimination between populations. These features are in bold in Tab. 5. Their descriptive statistics are presented in Fig. 8.

The Mantel test showed that there was no significant relation between morphological similarity of populations and their geographical distance [ $F(1, 169) = 0.86; p = 0.36$ ].

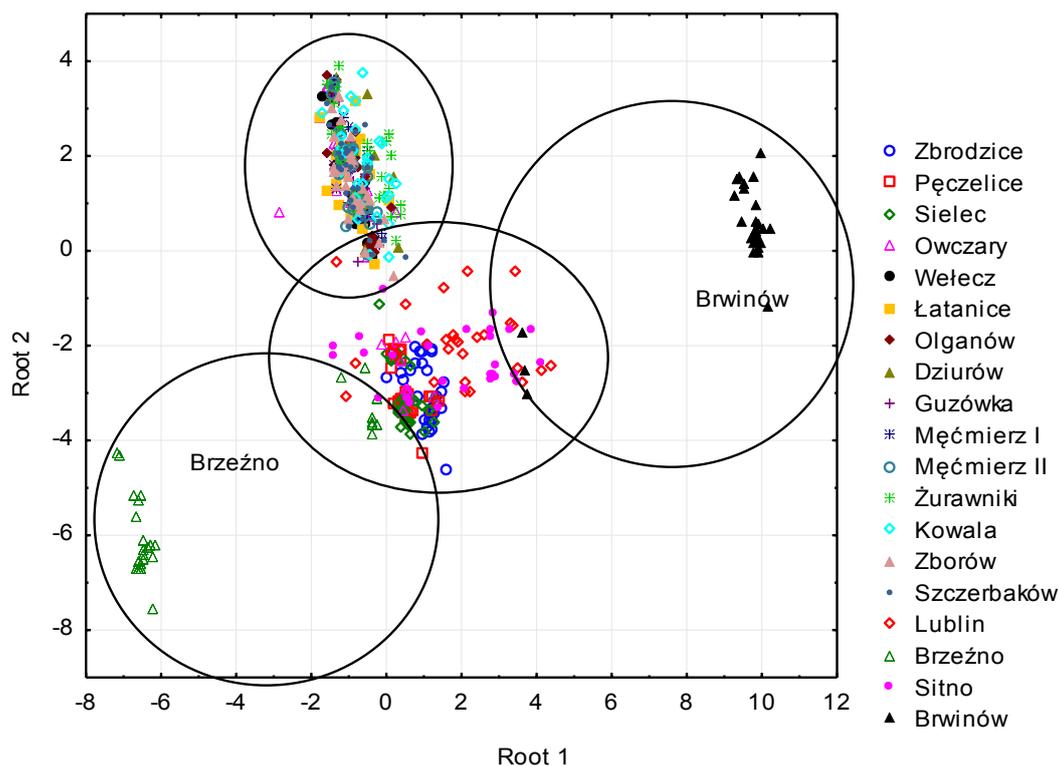


Fig. 7 Two-dimensional ordination diagram of DA based on 17 morphological characters of *Senecio erucifolius* subsp. *tenuifolius* specimens.

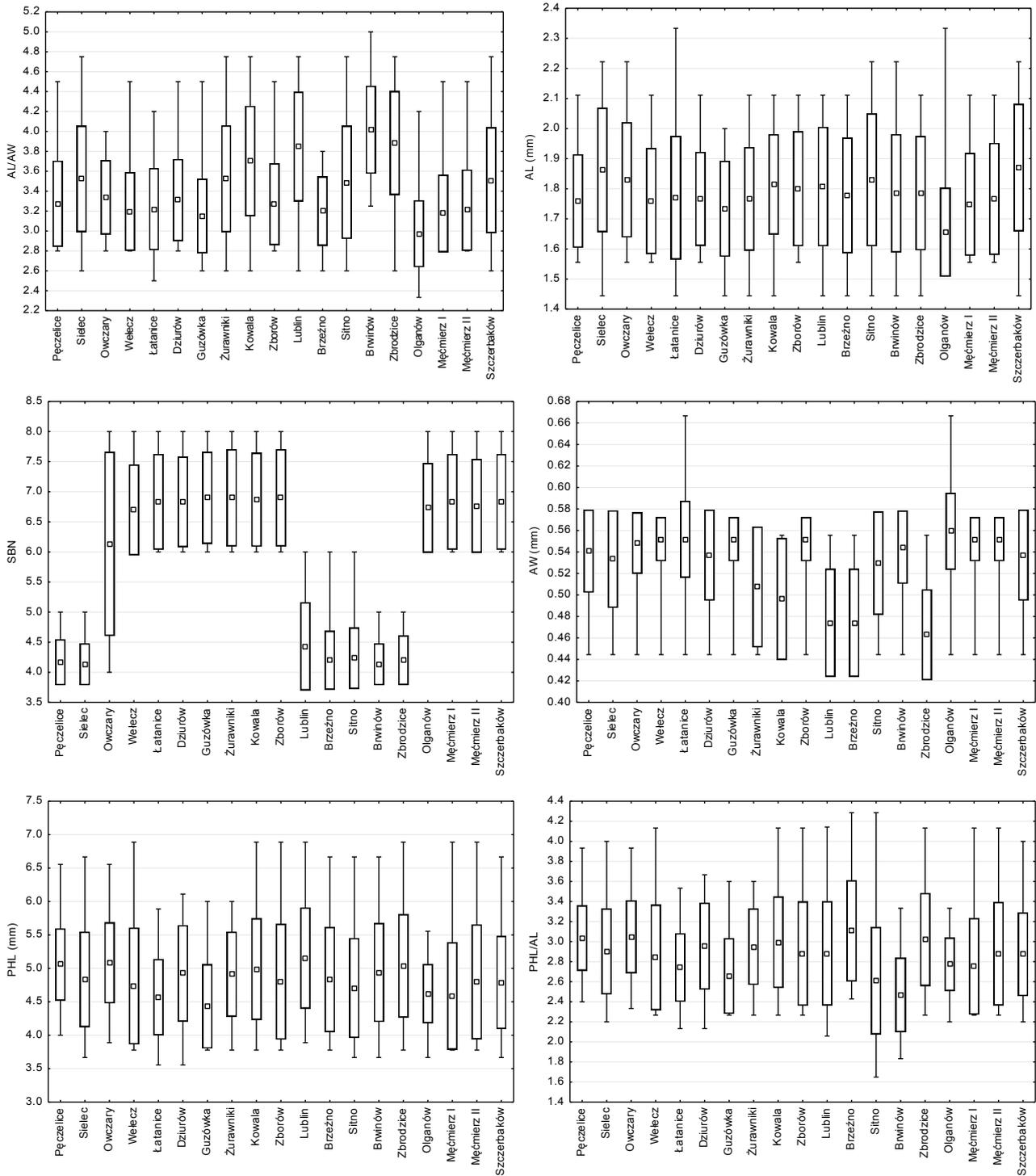
#### Morphological description of *S. erucifolius* subsp. *tenuifolius*

The stem of the specimens examined is 70–129 cm in height. The inflorescence is a compound, corymbose panicle with 2–15 branches terminating in capitula. The length of the middle leaves is 4.2–13.4 cm and the width is 2.1–8.6 cm. Single pinnate leaves, in a general outline, are mostly ovate (55.78%) or obovate (34.21%), rarely roundish (10%). The upper lobe is small – 0.8–4.9 cm long and 0.1–1.9 cm wide, and its width at the base is 0.1–0.6 cm. The length of the longest lateral lobe is 0.2–5 cm and its width is 0.1–0.6 cm. The number of capitula per inflorescence is 13–138; the diameter of the capitulum is 16–21 mm and the diameter of the base of the capitulum is 4–5 mm. The number of involucre bracts per capitulum is 12–14 and they are 3–5 mm long. Supplementary bracts are hairy, 4–8 in number and 2–4 mm long. Ligulate flowers are light yellow, 9–12 mm long and 1–2 mm wide. Tubular flowers are 4–6 mm long. Achenes are 1.44–2.33 mm long and 0.44–0.67 mm wide and the length of the pappus is 3.56–6.89 mm.

#### Discussion

The study confirmed the authors' earlier assumptions as to the occurrence in Poland of two subspecies of the hoary ragwort: subsp. *erucifolius* and subsp. *tenuifolius*. Populations representing subsp. *erucifolius* are found only in northwestern Poland, and subsp. *tenuifolius* in the other parts of the country, with a distinct center of their geographic distribution in southeastern Poland. According to the distribution maps of *S. erucifolius*, given by Greuter [36], only subsp. *erucifolius* occurs in Poland. This view must be corrected in view of our studies.

Although these subspecies are mentioned in numerous European floras, including floras of the neighboring countries [18,22,23], so far there has been no study which documents their differentiation on the basis of detailed morphometric studies and an advanced statistical analysis.



**Fig. 8** Arithmetic means (squares), means  $\pm$ SD (boxes) as well as minimum and maximum values (whiskers) for six characters that varied most in *Senecio erucifolius* subsp. *erucifolius*. For trait abbreviations, see Tab. 2.

Our research indicated the diagnostic value of the features which significantly distinguished the two subspecies. The major features providing differentiation between the two subspecies are in the leaves and flowers, such as the length and width of the end lobe of the leaf, the width of the upper lobe of the leaf at its base, the width of the longest lateral lobe, and the length of tubular flowers. The length of the tubular flowers, which was greater in subsp. *erucifolius*, proved to be a new feature useful in distinguishing the two subspecies.

Until now, such features of leaves as the size of the upper lobe and the size of the lateral lobes were used as the main characters to distinguish the subspecies. Most authors emphasized that subsp. *tenuifolius* showed narrower lobes as compared to

subsp. *erucifolius* [18,22,29–32]. According to Heupler and Muer [23], the upper lobe of specimens that represent subsp. *erucifolius* is lanceolate, peaked and usually narrow. Moreover, Kućowa [29] stated that the lower leaves in the subsp. *erucifolius* have a relatively large upper lobe. Our study confirmed that the major features facilitating identification of the two subspecies are such features of leaves as the length and width of the upper lobe of the leaf, width of the upper lobe at the base and the width of the longest lateral lobe. It is worth mentioning that Sell and Murrell [24], who distinguished four varieties instead of two subspecies, also emphasized the importance of lobe size in their diagnosis of the varieties. Apart from leaves, some characters of the inflorescence were also indicated as helpful in subspecies diagnosis. Prokudin [32] distinguished the two species, *S. erucifolius* and *S. tenuifolius*, on the basis of the number of supplementary bracts per capitulum; there are 5–6 in the former and 4–7 in the latter. Involucral bracts of the capitulum in *S. erucifolius* are 4.2–4.5 mm long, while in *S. tenuifolius* they are up to 4 mm. The diameter of capitula ranges from 2 to 2.5 cm and up to 2 cm, respectively. In contrast, Sell and Murrell [24] divided the whole species into varieties that differed in the diameter of the capitulum up to 15, 17, or 22 mm. The morphometric analysis of the Polish populations did not confirm the importance of these features when distinguishing the subspecies. However, according to our study a good diagnostic feature that has not previously been taken into consideration is the length of the tubular flowers, which are significantly bigger in subsp. *erucifolius* (ranged from 6 to 8 mm) as compared to subsp. *tenuifolius* (4–6 mm), and the ranges do not overlap.

The other recent studies [39] showed that these two subspecies could also be distinguished on the basis of some characters of the achenes. The achenes differed in several characters, but the most distinctive differences concerned their indumentum, especially length and morphology of hairs. In *S. erucifolius* subsp. *erucifolius* achenes were moderately hirsute with hairs occurring mainly on ribs, of medium length, slightly spirally twisted and flattened, with a sharp bifurcate or entire apex. In *S. erucifolius* subsp. *tenuifolius*, achenes were dense hirsute, with long hairs, distinctly spirally twisted and flattened, most often with a sharp entire apex.

Both literature data and our study show that the two subspecies are significantly differentiated. Grulich [22] gives a fairly accurate characterization of intraspecific differentiation in the Czech Republic, although he did not support it with any biometric research. He assumed that subsp. *tenuifolius* is a variable taxon, having three morphotypes. This variation is exemplified in leaf lobe width that decreases towards the east, as well as in the time of flowering which, in some parts of the population, coincides with the onset of the flowering period of *Senecio jacobaea*. He also stated that the variability of subsp. *tenuifolius* is likely to be clinal and that it requires further research. Our studies of 570 specimens from 19 populations showed that the size of achenes, the number of supplementary bracts per capitulum as well as the length of pappus of the tubular flowers contributed most to the discrimination of populations of subsp. *tenuifolius*. Also the length of the longest lateral lobe was diverse in the examined populations of subsp. *tenuifolius*, but we did not confirm the observations of Grulich [22] concerning leaf lobe width. Because of a wide range distribution of this taxon in Poland we checked the relationship between the morphological similarity of populations and their geographical distance. As the Mantel test showed no significant relation, we assume that the populations' heterogeneity probably resulted from environmental factors.

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### Supplementary material

The following supplementary material for this article is available at <http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3505/0>:

**Appendix S1** Statistical parameters for quantitative characters of examined subspecies of *Senecio erucifolius*.

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