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VARIABILITY OF LEAF MORPHOLOGY FROM GENERATIVE AND VEGETATIVE SHOOTS OF *RUBUS CAPITULATUS* AND *R. KULESZAE* (*CORYLIFOLII* SECTION, ROSACEAE)

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ABSTRACT. Leaves from generative and vegetative shoots of *Rubus capitulatus* and *R. kuleszae* of *Corylifolii* section were investigated. The study was to demonstrate the variability of leaves of both types of shoots, and at the same time an examination on the contents of polyphenols and phenolic acids in their blades carried out. Selected blade traits, such as was: length, width, elongation, perimeter, dissection index, area, rectangularity and circularity were measured using computer program and statistically analysed. Additionally, fresh and dry leaves were weighed and the percentage of preserved mass of particular samples was calculated. A statistical analysis revealed the area to be the most variable feature in both species and both types of shoots. Leaves from vegetative shoots were the most similar to each other and the length, width, perimeter and area were the most strongly correlated traits to each other. The leaves of generative shoots of both species contained less water than the leaves of vegetative ones.

KEY WORDS: brambles, leaves, variability, statistical methods

INTRODUCTION

Rubus L. (Rosaceae) is considered to be one of the most taxonomically problematic genera of flowering plants, since species distinction is complicated by hybridization, polyploidy and apomixis (Lawrence and Campbell 1999). Recently i.e. for 25 years, owing to Weber's (1996) species concept for the *Rubus* agamosperms, progress of the investigations on this genus has been observed in central Europe. Systematic revisions for majority of bramble flora of this part of the continent have been done, but some taxonomic problems still remain unsolved. For example, the ranges of morphological variation of particular species of *Rubus* are rather poorly recognised. On the other hand many closely related species of brambles differ only in very subtle morphological characters, while the phenotypic plasticity in *Rubus* is distinct (compare Weber 1996, 1997, 1999, 2000, 2007, Matzke-Hajek 1999, Zieliński 2004, Trávníček et al. 2005, Trávniček and Zázvorka 2005, Žila and Weber 2005, Lepší and Lepší 2006, 2009, Oklejewicz 2006 and others).

Bramble leaves have been used in medicine for centuries. They contain polyphenolic compounds and some vitamins, having broad spectrum of biological activity. A lot of studies for antioxidant activity of *Rubus* leaves have been carried out only into the most common species, mainly *R. ideaus* and *R. fruticosus* (in fact treated as aggregate species; Gudej and Rychlinska 1996, Wang and Lin 2000, Gudej and Tomczyk 2004, Venskutonis et al. 2007, Buřičova et al. 2011).

The goal of the present study was to compare morphological variation of leaves from annual and biennial shoots of two *Rubus* species: *Rubus capitulatus* Utsch and *R. kuleszae* Ziel. (*Corylifolii* section). *Rubus capitulatus* is endemic bramble to Poland, mainly noted in the Dolny Śląsk and south Wielkopolska, while *R. kuleszae* is a widespread species, occurring in the Czech Republic, Austria, south eastern Germany and southern part of Poland (Zieliński 1996, 2004). The latest investigations have showed, in the leaves of both species, a high content of polyphenols and phenolic acids (Gawron--Gzella et al. 2012). The project was carried out owing to the intercollegiate fund between Poznań University of Medical Sciences and Poznań University of Life Sciences no. 501-03-03309419-02030.

Further research will compare the contents of these biologically active compounds in the leaves of vegetative and generative shoots. Therefore, the present study has to demonstrate the differences in the morphology of these two types of leaves of both species.

METHODS

The studies on the leaf variability of two species of *Rubus* of *Corylifolii* section – *R. kuleszae* and *R. capitulatus* – were carried out in 2012. The material was gathered from the individual, massed clusters, growing in the Dendrological Garden of the Poznań University of Life Sciences on two dates. Trifoliate leaves from the middle part of generative shoots were taken at the end of flowering in the last days of June (sample 1 – *R. kuleszae* and 2 – *R. capitulatus*) and five-leaflet leaves from the middle part of vegetative shoots (sample 3 – *R. kuleszae* and 4 – *R. capitulatus*) were collected at the beginning of September, when the stem growth had been stopped. A total of 60 leaves, including 90 leaflets from generative shoots and 150 leaflets from vegetative ones, for each species were analysed.

All collected leaves were divided into separate leaflets (in total 240 blades), weighed using the analytical balance (OHAUS, Analytical Plus Model AP 250 DE) and placed in the thermostat (ST 1200 B60 fotoperiod) in 30°C for 24 hours. Then they were weighed again and the percentage of preserved mass of particular sample was calculated.

The leaflets were scanned and their biometry was done using the DigiShape 1.9.220 computer program (Cortex Nova 2005, Poland). The following blade traits were measured: length (mm), width (mm), elongation (= length/width), perimeter (mm), dissection index [= perimeter/(π × length)], area (mm^2), rectangularity [= area/(length × width)] and circularity [= perimeter^2/(π × area)]. The arithmetical mean, minimum, maximum, median, standard deviation (SD) and coefficient of variation (CV) were calculated for each of the abovementioned traits. In order to determine statistical significance of average values of traits the factor variance ANOVA F-statistics was used. The Principal Component Analysis (PCA) enabled the examination of relations between the specimens samples belonging to different populations, not formulating any prior assumptions. Due to the cluster analysis performed on the basis of Euclidean distance, according to the single linkage method, the degree of similarity between samples could be determined (Sneath and Sokal 1973, Sokal and Rohlf 1997).

RESULTS

In respect to length, width, perimeter and area, the biggest blades were observed in *R. capitulatus* leaves (sample 2), and the smallest ones in *R. kuleszae* (sample 1), both from the generative stems (Table 1, Fig. 1). The highest values of elongation were noted in *R. capitulatus* leaflets from vegetative stems (sample 4) and the smallest in *R. kuleszae* leaflets from generative stems (sample 1). Rectangularity values were similar in all four samples. Dissection and circularity index reached the highest values in *R. kuleszae* blades from generative stems (1) and the smallest in *R. capitulatus* blades from vegetative stems (3). In general, the most variable trait was the area, and relatively the most constant – rectangularity. Four traits (elongation, dissection index, rectangularity, circularity) in four samples were more or less different from each other and their box diagrams did not overlap (Fig. 1). The leaflets from vegetative

Table 1. Basic statistical characteristics calculated for each sample. Samples: 1 – *R. kuleszae* leaflets from generative shoots, 2 – *R. capitulatus* leaflets from generative shoots, 3 – *R. kuleszae* leaflets from vegetative shoots, 4 – *R. capitulatus* leaflets from vegetative shoots

Trait	Sample	Mean	Median	Min.	Max.	SD	CV%
1	2	3	4	5	6	7	8
Length (mm)	1	75.81	75.96	55.95	92.79	8.34	11.00
	2	94.49	93.77	66.39	122.08	12.91	13.66
	3	82.84	80.11	66.38	120.59	11.27	13.60
	4	82.54	83.17	66.48	94.90	5.90	7.15
Width (mm)	1	57.45	57.24	39.83	71.79	7.40	12.88
	2	71.03	71.37	41.32	101.36	13.44	18.93
	3	62.82	61.25	46.06	87.39	9.00	14.33
	4	60.25	60.24	46.89	70.94	6.12	10.16
Elongation	1	1.33	1.33	1.19	1.48	0.08	5.63
	2	1.37	1.33	1.14	1.77	0.15	10.86
	3	1.35	1.35	1.18	1.49	0.08	5.81
	4	1.43	1.43	1.28	1.60	0.07	4.92

Table 1 – cont.

1	2	3	4	5	6	7	8
Perimeter (mm)	1	224.84	226.20	158.19	272.92	26.78	11.91
	2	272.98	272.61	176.70	379.62	41.58	15.23
	3	240.84	232.37	183.17	345.33	33.48	13.90
	4	232.37	234.15	185.58	264.35	18.97	8.16
Dissection index	1	0.95	0.94	0.88	1.06	0.04	4.22
	2	0.92	0.92	0.82	1.08	0.05	5.28
	3	0.92	0.92	0.88	1.01	0.03	3.46
	4	0.89	0.89	0.85	0.95	0.02	2.61
Area (mm^2)	1	2 903.14	2 899.10	1 509.93	4 309.27	649.12	22.36
	2	4 635.05	4 570.81	1 781.95	8 104.78	1 478.91	31.91
	3	3 689.62	3 409.34	2 012.61	7 103.77	1 031.98	27.97
	4	3 510.51	3 525.39	2 091.90	4 689.01	653.89	18.63
Rectangularity	1	0.66	0.65	0.62	0.71	0.02	3.11
	2	0.67	0.66	0.62	0.72	0.02	3.41
	3	0.66	0.66	0.64	0.71	0.02	2.57
	4	0.66	0.66	0.62	0.71	0.02	3.02
Circularity	1	1.42	1.42	1.29	1.67	0.07	4.94
	2	1.35	1.34	1.23	1.55	0.07	5.44
	3	1.35	1.34	1.27	1.42	0.04	2.58
	4	1.34	1.34	1.21	1.45	0.05	3.77

shoots of *R. kuleszae* and *R. capitulatus* (samples 3 and 4) showed the highest similarity to each other.

One-way analysis of variance ANOVA expressed by F-values (Table 2) showed that all the observed traits significantly differentiated all the investigated samples. The length of leaflets was the most differential trait while rectangularity the least. In general the vegetative leaves of *R. kuleszae* and *R. capitulatus* were the most similar to each other.

The scatter diagram, basing on the Principal Component Analysis, showed the common group in the middle of it, formed by the samples 1, 3 and 4 (Fig. 2). Sample 2 (consisting of leaflets from generative stems of *R. capitulatus*) was separated from the others. Majority of individuals of this sample had negative values of PC1, while some were marked out by high, positive values of PC2. Sample 2 was distinguished by higher values of length, width, perimeter and area. The dendrogram constructed on the basis of Euclidean distances for the analysed samples also proved a close relationship between sample 3 and 4 and distinction of sample 2 (Fig. 3).

The first principal component PC1 explained 56.71% of the observed variability. It was significantly correlated with the length, width, perimeter and area. On the other hand the second principal component PC2 elucidated 24.01% of the observed variability and was significantly correlated with elongation and dissection index (Table 3).

An impact of variables of the analysed traits on the principal components PC1 and PC2 was considered (Fig. 4). The length (1), width (2), perimeter (4) and area (6) were highly negatively related to PC1, while elongation (3) was highly positively correlated with PC2 and dissection index (5) – highly negatively correlated with PC2 (Table 3). Moreover, positive correlations between the length (1) and width (2), as well as between perimeter (4) and area, (6) were revealed. These features were negatively related with the circularity (8). Similarly elongation (3) was negatively correlated with dissection index (5) and rectangularity (7). Then length (1) and elongation (3), as well as length (1) and rectangularity (7), were not correlated with each other at all (Fig. 4).

The leaflets of both species from generative shoots contained more dry mass and hence a smaller percentage of loss of water was noted for them (Table 4). The leaflets collected from the vegetative shoots, as originated from 5-blades leaves, apparently had got higher fresh mass, but characterised by a higher water content. In general, the highest dry mass content was revealed in the blades from generative shoots of *R. kuleszae* and the biggest decrease of water was observed in the blades from vegetative shoots of the same species.

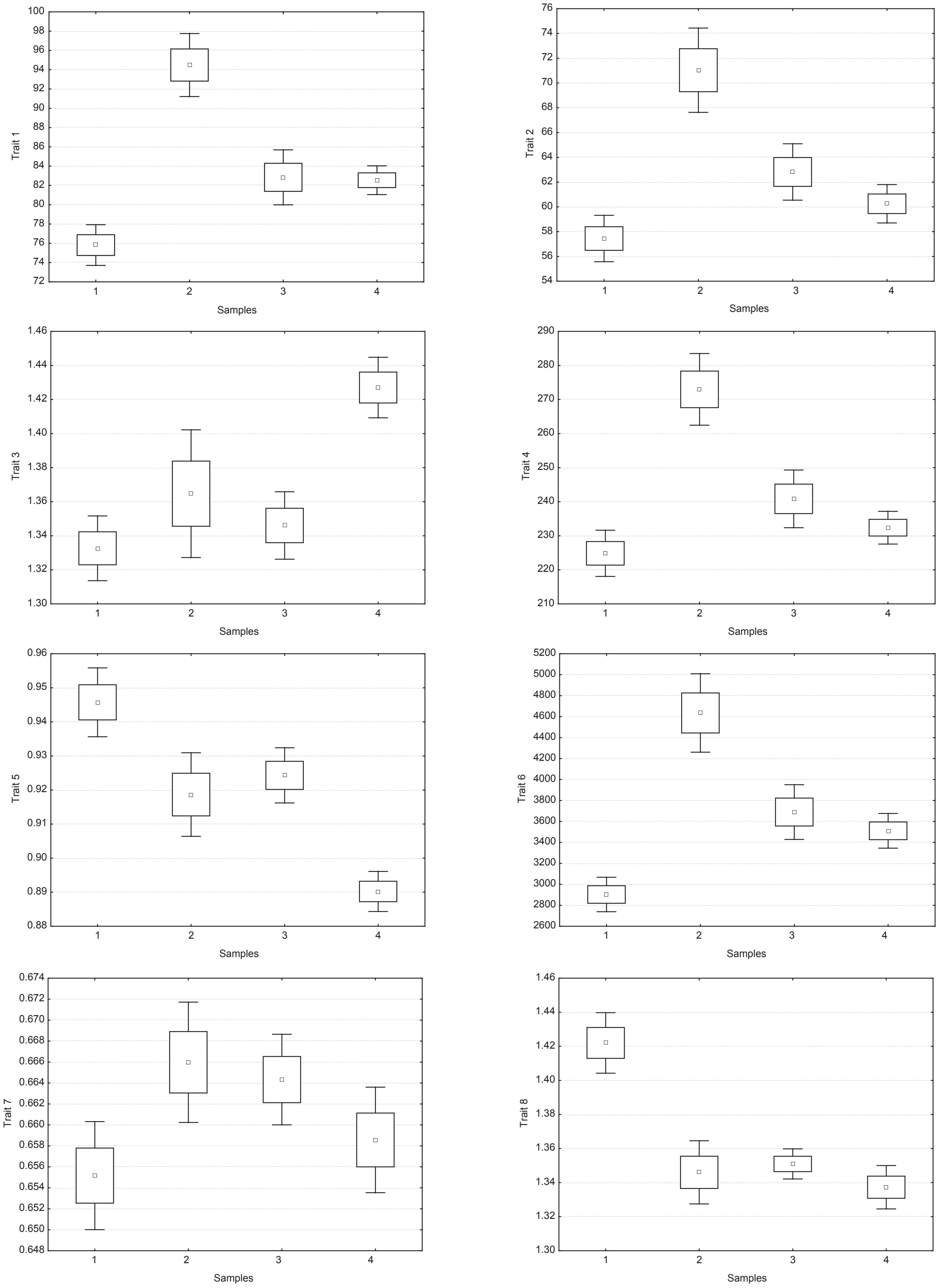

Fig. 1. Diagrams show the range of variation of eight traits of four studied samples: 1 – *R. kuleszae* leaflets from generative shoots, 2 – *R. capitulatus* leaflets from generative shoots, 3 – *R. kuleszae* leaflets from vegetative shoots, 4 – *R. capitulatus* leaflets from vegetative shoots; ▫ mean, □ ± standard deviation, ⊤ mean ± 1.96* standard deviation

Table 2. F-statistic values of one-way analysis of variance (ANOVA) calculated for the eight studied traits of *R. kuleszae* and *R. capitulatus* leaflets

Trait	F
1. Length	36.25***
2. Width	23.26***
3. Elongation	10.81***
4. Perimeter	27.41***
5. Dissection index	22.80***
6. Area	30.12***
7. Rectangularity	3.75***
8. Circularity	26.02***

***Significant level p = 0.01.

Table 3. Correlation coefficients between the two first principal components (PC1 and PC2) and morphological traits

Trait	PC1	PC2
1. Length	**–0.85**	0.49
2. Width	**–0.98**	0.11
3. Elongation	0.57	**0.75**
4. Perimeter	**–0.94**	0.23
5. Dissection index	–0.40	**–0.84**
6. Area	**–0.95**	0.26
7. Rectangularity	–0.55	–0.49
8. Circularity	0.49	–0.08

The bold type marks the high correlation (r ≥ 0.60) of characters with principal components

Table 4. Comparison of the mass of fresh and dried leaflets

Sample	Mass of fresh leaflets (g)	Mass of dried leaflets (g)	Content of preserved mass (%)
1. *R. kuleszae* leaflets – generative shoots	36.80	18.10	49.18
2. *R. capitulatus* leaflets – generative shoots	54.35	21.45	39.47
3. *R. kuleszae* leaflets – vegetative shoots	86.05	31.75	36.90
4. *R. capitulatus* leaflets – vegetative shoots	82.85	31.35	37.84

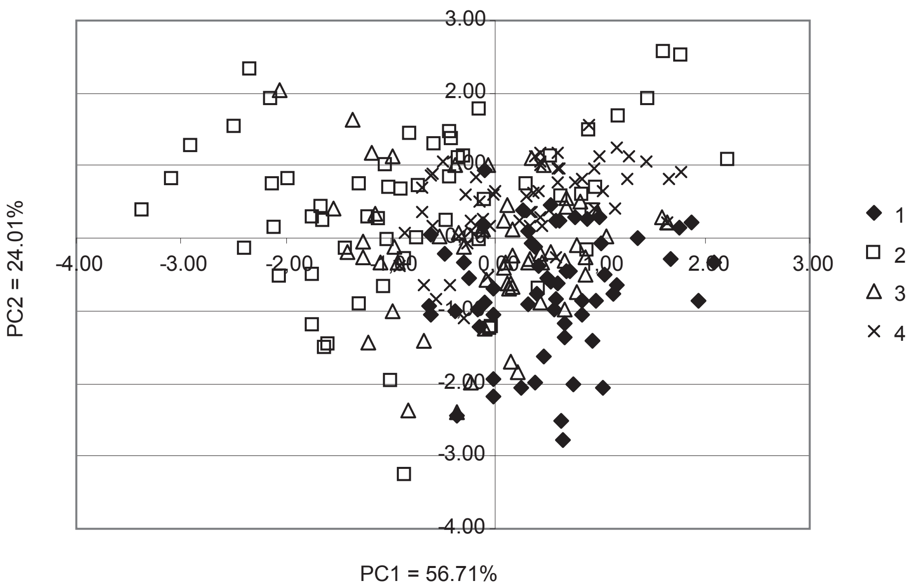

Fig. 2. Principal Components Analysis (PCA) – scatter diagram of specimens from four samples: 1 – *R. kuleszae* leaflets from generative shoots, 2 – *R. capitulatus* leaflets from generative shoots, 3 – *R. kuleszae* leaflets from vegetative shoots, 4 – *R. capitulatus* leaflets from vegetative shoots

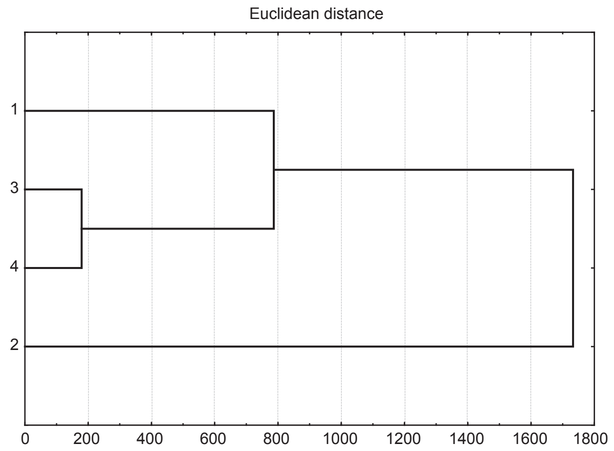

Fig. 3. Dendrogram constructed on the basis of the shortest Euclidean distances according to the single linkage method using eight morphological traits. Samples: 1 – *R. kuleszae* leaflets from generative shoots, 2 – *R. capitulatus* leaflets from generative shoots, 3 – *R. kuleszae* leaflets from vegetative shoots, 4 – *R. capitulatus* leaflets from vegetative shoots

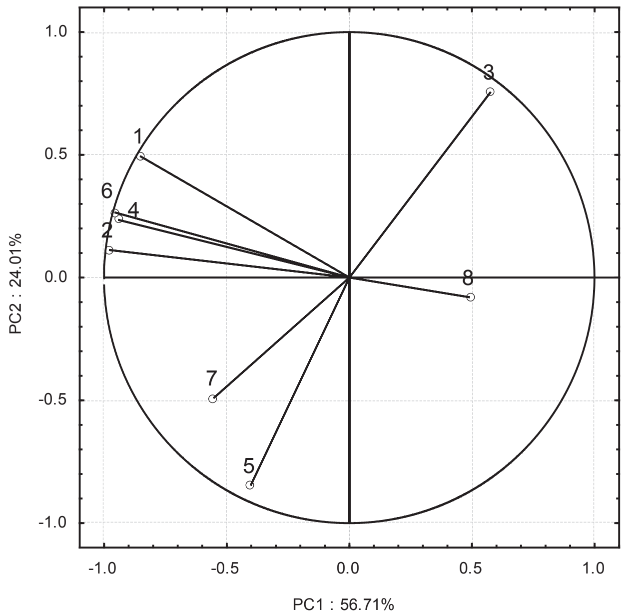

Fig. 4. Position of vectors in reference to the two first principal components (PC)

DISCUSSION AND CONCLUSIONS

The bramble species classified into the section *Corylifolii* are still poorly known and are usually treated marginally. They have arisen through the hybridization between various, unidentified species of the section *Rubus* and *R. caesius* L. From the previous ones they have taken the ability to form seeds without fertilization (apogamy) and many closely related forms have developed (Zieliński 2004, Ryde 2011). Consequently, taking into account the great morphological variation within all *Rubus* species, it is often difficult to decide in practice, whether the given populations belong to the same species.

According to our knowledge, the detailed studies on the morphological variability of *Rubus* leaves have not yet been carried out. The current research did not show unequivocal differences in leaf morphology between *R. kuleszae* and *R. capitulatus*. In turn, Gawron-Gzella et al. (2012) proved significantly greater total content of phenolic compounds and phenolic acids in the leaves of the first species than in the second one.

In the current study the analysed leaves of two closely related *Rubus* species of the section *Corylifolii* (however, belonging to the different series: *Subthyrsoidei* with *R. kuleszae* and *Hystricopses* with *R. capitulatus*) were obtained from the plants growing in nearly the same site conditions. Undoubtedly, it allowed to assume that the impact of external conditions on bramble phenotypic variation was minimized. A statistical analysis revealed the highest similarity between the leaves from vegetative shoots and significant differences between the leaves from generative shoots. At the same time the morphological similarity between the blades from vegetative and generative shoots within the same species was not obvious, especially in relation to *R. capitulatus*. The most variable feature in both species and both types of shoots was area and relatively most constant was rectangularity. In turn, length, width, perimeter and area were most strongly correlated with each other, irrespective of species and stem origin. The leaves of generative shoots of both species contained less water than the leaves of vegetative ones and they were also characterised by a higher content of the preserved mass.

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