THE USE OF QUANTITATIVE CHARACTERS IN DETERMINATION OF FREQUENTLY MISDIAGNOSED SPECIES WITHIN *LEPIDIUM* L. SECT. *DILEPTIUM* (BRASSICACEAE)

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(Received: March 2, 2009. Accepted: June 10, 2009)

ABSTRACT

A morphometric study of the three species of *Lepidium* L. sect. *Dileptium* DC. is presented. Multivariate methods (cluster analysis and canonical discriminant analysis) were employed to investigate phenetic relations between examined individuals. As a result a set of quantitative characters useful in species determination was proposed and discussed.

KEY WORDS: *Lepidium*, *Dileptium*, determination, cluster analysis, canonical discriminant analysis.

INTRODUCTION

The question of the *Lepidium* species taxonomy has been in the centre of attention of many scholars for almost two centuries (De Candolle 1821; Thellung 1906; Marais 1971; Hewson 1981; Al-Shehbaz 1986). Among their works the Karol Latowski's monograph of the Eurasian species deserves special attention (Latowski 1982). The work is an important source of information about the taxonomy and distribution of the pepperweeds species. Among the recent works on taxonomy the most prominent are the works based on the research on cpDNA (Mummenhoff et al. 1995, 2001), fragments of nuclear rDNA (Mummenhoff et al. 2004), fragments of non-coding cpDNA (Bowman et al. 1999) and the genome size (Johnston et al. 2005). The research cast new light on the complicated relations of the species within the *Lepidium*.

In spite of this, the correct determination of the species within the *Dileptium* DC. section remains questionable even for experienced botanists. According to us, the question of determination of the taxa in this problematic section may not be neglected since the correct determination of species is the first and the most important step in any work, whether in ecology, genetics, cytogenetics, physiology, ecotoxicology or any other field of the plant biology.

In the present study, using the classical morphological analysis as well as multivariate methods of statistical analysis, we were searching for characters, which may serve more precise and objective determination of the species within the section *Dileptium* DC. We posed these questions:

1. How many taxa may be distinguished in the sample material on the basis of the morphological characters?
2. Are the diagnostic quantitative characters used hitherto functional and efficient in the diagnostics of the species belonging to the examined section?
3. Can we point out new, functional, diagnostic quantitative characters?

MATERIAL AND METHODS

The section Dileptium DC

There are 8 species of the *Lepidium* L. section *Dileptium* DC. in Europe. The occurence of three species is restricted to saline soils in Spain and SE Russia, and another two are naturalised only in Western Europe and their distribution is not vast (De Carvalho 1993). The examined species are the three remaining representatives of the section *Dileptium* DC. The greatest diagnostic problems occur with *Lepidium ruderale, Lepidium densiflorum* and *Lepidium virginicum*, since they are extended across the continent (Meusel et al. 1965) and morphologically similar.

Morphological research

The sample material consisted of 205 plant specimens (*L. ruderale* – 95 plants, *L. densiflorum* – 65 plants, *L. virginicum* – 45 plants) from several herbaria in Poland: KTU, KRAM, KRA and WRSL.

Morphological research was carried out on the basis of quantitative and qualitative characters. They were carefully chosen on the basis of the existing taxonomy literature

TABLE 1. Quantitative characters of the *Lepidium* used in the present study. Name of the character, acronym and measure units were given.

	Character	Acronym	Unit
1.	Plant height	H	cm
2.	Heigth of the first branch	HB	cm
3.	Number of branches	BN	–
4.	Density of the pedicels	PD	x/cm
5.	Length of the stem hair	SHL	mm
6.	Length of the pedicel hair	PHL	mm
7.	Length of the leaf hair	LHL	mm
8.	Length of the pedicel	PL	mm
9.	Length of the silicle	SL	mm
10.	Width of the silicle	SW	mm
11.	Width of the upper part of the apical notch	WUAN	mm
12.	Width of the lower part of the apical notch	WLAN	mm
13.	Depth of the apical notch	DAN	mm
14.	Length of the beak	BL	mm
15.	Length of the seed	SDL	mm
16.	Width of the seed	SDW	mm
17.	Width of the seed wing in the middle part of the seed	FSW	mm
18.	Width of the seed wing in the basal part of the seed	BFW	mm

(Thellung 1906; Kobendza 1950; Latowski 1982, 1985; Al-Shehbaz 1986; Szafer et al. 1986; De Carvalho 1993; Shultze-Motel 1986). Apart from the characters used so far, the significance of the characters not used previously was also tested.

In the first part of the research each specimen was described with the set of 97 qualitative characters. They were chosen in such a way so that they could describe each morphological detail of the examined organisms: general habit of the plant, its' smell, properties of the flower and raceme, fruit and infrutescense, basal leaves and cauline leaves, as well as the pubescense properties of all the vegetative and generative organs. The qualitative characters were coded binary. Analysis carried out on qualitative characters enabled us to recognise objectively three investigated species in sample material and thus allowing us to test the taxonomical significance of investigated quantitative characters.

18 quantitative characters were chosen to the analysis. Table 1 presents the quantitative characters used in the analysis as well as their measure units.

State of all the characters used in the present study was assessed on mature plants. Length of the stem hair (SHL) was assessed in the middle part of the stem, usually just below the fruit raceme. Length of the leaf hair was assessed on mature leaf, usually (when accessible) from the lower part of the stem. Density of the pedicels (PD) was assessed in the middle part of the fruit raceme. Other characters associated with pedicels (PHL, PL) were assessed on one, randomly chosen pedicel from the middle part of the raceme. Characters associated with silicles (SL, SW, WUAN, WLAN, DAN, BL) were assessed on randomly chosen, mature silicle form the middle part of the raceme. Length and width of the seed (SDL, SDW) was assessed on randomly chosen seed taken from mature silicle in the lower part of the raceme. All the other characters associated with seeds (FSW, BFW) were assessed in a similar way.

The basic unit of numerical taxonomy is the operational taxonomic unit (OTU) refering to taxons of lower rank used in the research and they are classified according to numerical methods (Stace 1992b). Each examined specimen was considered as one OTU.

During the present study the stereoscopic microscope Olympus SZX-9 was used together with image analysis software Olympus DP-Soft 3.0.

Data analysis

The data obtained in the morphological analysis were gathered in two matrices, first of them containing qualitative characters, and the other containing quantitative characters. Both matrices were analysed by means of multivariate statistical analysis (MSA) using the Q technique, which enables to follow the relations between OTU in the space of characters (Falniowski 2003). All analitic operations were carried out by means of integrated system of statistical analysis Statistica (Statsoft 1997).

The cluster analysis was carried out in order to separate OTU groups on the basis of the previously published mor-

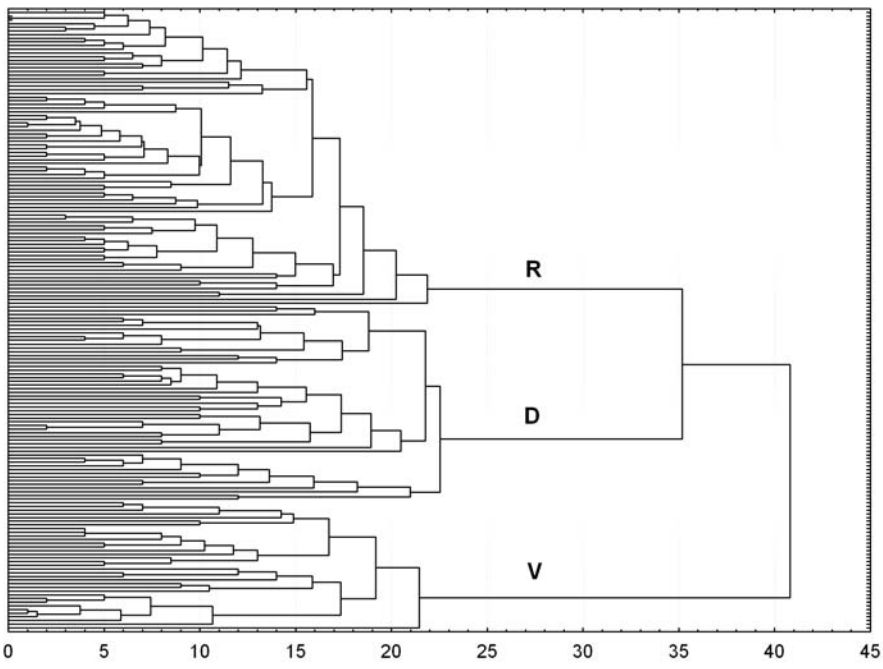

Fig. 1. Phenogram made on the basis of cluster analysis of qualitative characters matrix. Main clusters were marked with letters R, D and V corresponding to three different species: *L. ruderale*, *L. densiflorum* and *L. viginicum* (Manhattan, UPGMA).

phological qualitative characters. The distance between OTU was determined by means of the Manhattan (city-block) distance, whereas the distance between particular clusters was determined by means of unweighted pair-group method using arithmetic averages (UPGMA) (Sneath and Sokal 1973).

The results of the cluster analysis enabled introducing another analytical procedure: canonical discriminat analysis. This method, widely used in taxonomy (e.g. Marhold 1996; Ekrt and Stech 2008), enables deciding on which characters best discriminate groups within the sample material. Another advantage of this technique is the fact, that, according to many authors, it is, to the great extend, robust to the disturbance of the multidimensional normality of the analysed data distribution (Sneath and Sokal 1973; Thorpe 1976). Discriminant analysis was carried out on the matrix of the quantitative characters in groups separated in the course of the cluster analysis of qualitative characters matrix. This made possible to define which quantitative characters can serve best in determination of the examined species of *Lepidium*. Quantitative data were standardized before the analysis.

RESULTS

Analysis of qualitative data carried by means of cluster analysis resulted in a phenogram presenting three distinct clusters (Fig. 1). The next step was checking to what extend the division of the sample materials into clusters reflects the species determination of the analysed plants. It appeared that the clusters on the phenogram agree to a great extend with the species diagnoses from the herbarium specimens. For that reason, particular clusters were marked with letters R, D and V depending on specimens of which species were grouped in the given cluster. Cluster *L. ruderale* was marked with letter R, cluster *L. densiflorum* with letter D and cluster *L. virginicum* with letter V. Within cluster V there were all plants previously determined in herbarium material as *L. virginicum*. Within clusters D and R the situation was a bit more complicated. Although the plants of both species were grouped here, it was dominated by plants determined initially as *L. ruderale* in cluster R and *L. densiflorum* in cluster D.

Canonical discriminant analysis carried out in groups separated in cluster analysis enabled better inspection of the relations between quantitative characters and OTU groups. The result of chi-square test for both canonical variables generated for the analysed data matrix proved their statistical significance.

Table 2 presents standarised coefficients of discriminant function for canonical variables. Characters for which coefficient value was higher then 0.4 were treated as potentially useful in practical determination of analysed species. The highest values for the first canonical root were for SHL, SW, BL and BFW, whereas for the second canonical root for PD and WLAN.

Table 3 presents means of canonical variables. As we can see, the first discriminant function discriminates mostly between the V group and the other two groups. The second discriminant function seems to distinguish between D group and the other two groups of OTUs. As can be seen from Table 3 and eigenvalues from Table 2, the magnitude of this discrimination is a bit smaller.

TABLE 2. Results of canonical discriminant analysis. Standardized coefficients for canonical variables. Eigenvalues for each canonical variable and cumulative proportion of explained variance were given as well. Values for which discrimant functions are most weighted (coefficient value >0.4) were given in bold.

Character	Root 1	Root 2
H	0.10689	0.031425
HB	0.01002	0.190278
BN	-0.09106	-0.341637
PD	-0.05718	**0.772244**
SHL	**-0.42866**	-0.124865
PHL	0.05597	-0.138400
LHL	-0.27614	-0.347053
PL	-0.34042	-0.259224
SL	0.37732	0.163710
SW	**-0.52297**	0.289609
WUAN	-0.09015	0.049465
WLAN	0.26063	**-0.467600**
DAN	0.15294	0.356975
BL	**-0.47960**	-0.155185
SDL	0.27145	-0.057006
SDW	-0.32545	-0.049021
FSW	-0.15466	0.291278
BFW	**-0.53400**	-0.028123
Eigenvalue	25.83966	5.296585
Cumulative proportion	0.82989	1.000000

TABLE 3. Results of canonical discriminant analysis. Means of canonical variables.

Group	Root 1	Root 2
R	3.39765	-1.89127
D	1.37516	3.25602
V	-9.55721	-0.76975

Figure 2 presents the scatterplot of canonical scores for each OTU. Whereas OTU grouped previously (in course of clutering analysis) in cluster V are well marked, the border between the two remaining groups (R and D), although visible, is not marked so well.

The range of variation of the diagnostic characters separated in discriminant analysis was presented in Figure 3. In case of characters with the highest values of standardized coefficients of discrmininant function for the first canonical score, non-overlapping ranges of variation are visible.

DISCUSSION

Qualitative characters are undoubtedly significant for the taxonomy of Brassicaceae and *Lepidium* (De Carvalho 1993; Concert et al. 1986; Stace 1992a; Tutin et al. 1993). In spite of this, the use of them is in certain cases difficult and may cause many problems with the proper diagnostics of species. Unclear statements refering to morphology of different plant organs may be misleading. What is more, many authors are liable to use in keys sophisticated descriptions of characters that might be expressed with numbers. This practice raises doubts.

As shown in Table 4, quantitative characters within the sample section, are neither most often used in taxonomy and diagnostics of species, nor well known. The exception

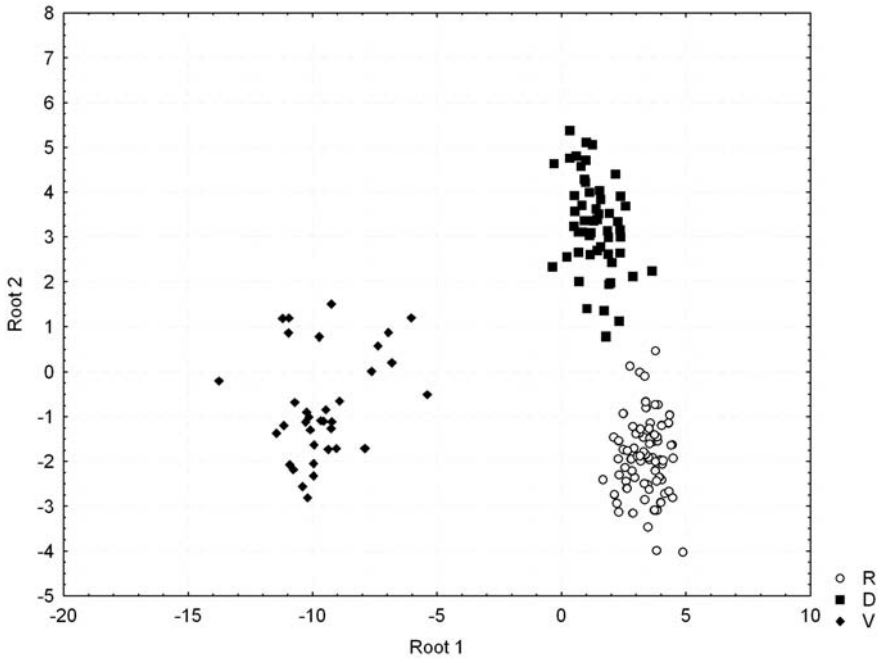

Fig. 2. Results of canonical discriminant analysis carried out on the matrix of quantitative data. Scatterplot of canonical scores. Groups of OTU were marked with letters R, D and V corresponding to three different species: *L. ruderale*, *L. densiflorum* and *L. virginicum*.

is Latowski's work (1982) containing most information about quantitative characters, but only refering to carpology. Other works contain only the information on the silicle length and width and individual characters like the beak length, which are rather rare (see Table 4).

Thorough biometric research was carried out in order to obtain possibly exact information on quantitative characters. In future this may replace such unclear and unprecise terms like "long hairs" or "loose fruit racemes" in keys and species descriptions with concrete numeral values. The authors perceive this procedure as more proper than formulating descriptive terms of which the practical value in species diagnostics remains controversial.

The analysis of results brought a set of six quantitative charaters helpful in the diagnostics of the examined species. Among them carpological characters are particularly important. Their significance in taxonomy and diagnostics of species within Brassicaceae has already been claimed by numerous authors (Haeupler and Muer 2000; Hitchcock 1936; Mirek 1981; Latowski 1975). The person who paid attention to the enormous use of carpological characters in the diagnostics of species in *Lepidium* was Latowski. In his monograph on Eurasian species of *Lepidium* (Latowski 1982) he enumerates the silicle length, the width of seeds' wing in the basal part, the beak length, seed length and the density of fruit racemes as importatnt quantitative characters. According to the results of the present research, only some items from the list are useful for determination of the examined species.

According to Latowski (1982), the density of fruit racemes distinguishes between *L. densiflorum* and the other two examined species. Although this character was in the group of the separated in canonical discriminant analysis (see Table 1), its variability (see Fig. 4) does not allow to use it as a diagnostic character. The variability range of this character ascertained by us is wider than it is known from literature. The present research confirms also the diagnostic significance of the lower part of the apical notch width, however, similarly as in case of fruit racemes density, the use of this character may cause diagnostic mistakes. It is different with characters like silicle width, beak length or seed's wing width in the basal part. Despite being ignored by many authors, these characters are of enormous diagnostic significance.

The new quantitative character with great diagnostic significance is the stem hairs length. Several authors paid attention to the great significance of the characters connected with hair morphology, but it referred mainly to qualitative characters (Hitchcock 1936; Landolt 2001; Rutkowski 2004). The use of characters connected with the hair morphology has got several practical advantages. The fact, that the hairs are less liable to destruction than the delicate (especially when ripe) fruits and easily falling off seeds, makes the marking of the characters state a much less problematic. It seems that the significance of quantitative characters connected with plants' hairs has been underrated so far (see Table 4).

The analysis of variability ranges shows one serious problem. Only some of the quantitative characters are useful for clear distinction between the three examined species. On that basis, the morphological distinction of *L. virginicum* is clearly visible, whereas the other two species have overlapping variability ranges. For that reason the necessity of use of a wide range of quantitave and qualitative characters in order to minimalise the possiblity of a diagnostic mistake seems to be inevitable.

Our results show also that *L. densiflorum* and *L. ruderale* are more similar to each other than to *L. virginicum*. This similarity has been also claimed by numerous authors (e.g. Shultze-Motel 1986; Clapham et al. 1990; De Carvalho 1993; Rothmaler 2000; Landolt 2001; Rutkowski 2004). In light of our knowledge on evolution of species within *Lepidium* (Lee et al. 2002) it can be concluded that morphological similarity is not a good indicator of evolutionary relationship within the genus.

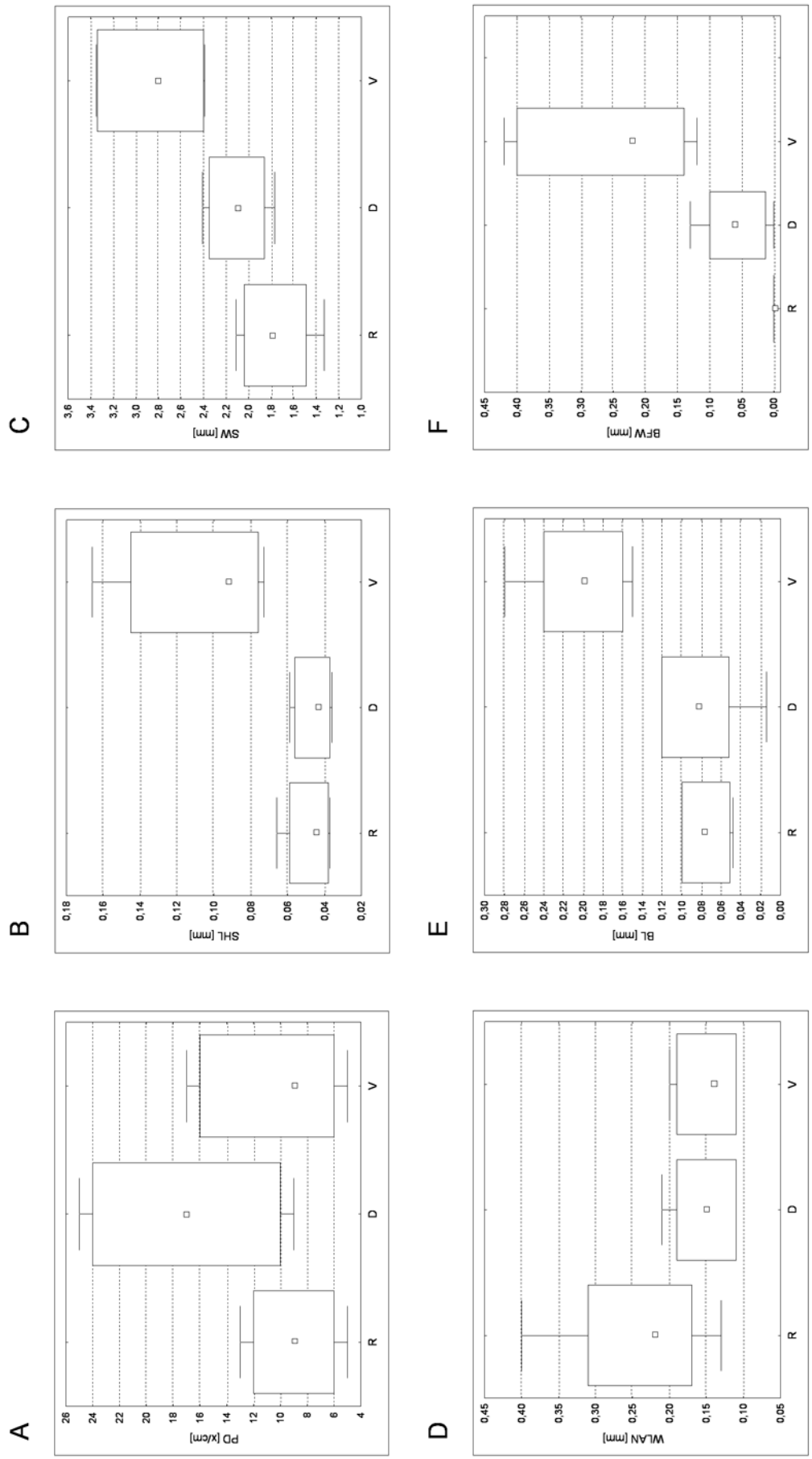

Fig. 3. Variability range for characters with the biggest values of standardized coefficient. Points indicate median value, boxes represent 5% and 95% percentile, whiskers around the box represent 1% and 99% percentile. A – density of the pedicels; B – length of the stem hair; C – width of the pedicel, D-width of the lower part of apical notch; E – length of the beak; F – width of the seed wing in the basal part of the seed.

TABLE 4. Variability range for selected characters according to different authors. Species were marked with letters R – *L.ruderale*, D – *L. densiflorum*, V – *L. virginicum*.

Character	Species	Latowski 1982	Fedorov 2001	Szafer et al. 1986	Rutkowski 2004	de Carvalho 1993	Rothmaler 2000
Density of the pedicels	R	8-11	–	–	–	–	–
PD	D	12-18	–	–	–	–	–
	V	9-14	–	–	–	–	–
Length of the steam hair	R	–	–	–	–	–	–
SHL	D	–	–	–	–	–	–
	V	–	–	–	–	–	–
Length of the leaf hair	R	–	–	–	–	–	–
LHL	D	–	–	–	–	–	–
	V	–	–	–	–	–	–
Length of the pedicel	R	–	–	–	–	–	–
PL	D	–	–	–	–	–	–
	V	–	–	–	–	–	–
Length of the silicle	R	2.10-2.60	2.00-2.50	2.00-2.50	2.00-2.50	1.50-2.50	2.00-2.50
SL	D	2.20-2.30	–	>3.00	2.00-3.00	2.50-4.00	2.50-4.00
	V	2.70-3.50	3.00-4.00	3.00-4.00	3.00-3.30	2.00-4.00	–
Width of the silicle	R	1.70-2.10	–	–	1.50-2.00	1.50-2.00	1.50-2.00
SW	D	2.00-2.70	–	2.00-2.50	2.00-2.50	2.00-3.00	2.50-3.00
	V	2.60-3.40	–	–	2.50-3.00	2.00-4.00	–
Depth of the apical notch	R	0.10-0.25	–	–	–	–	–
DAN	D	0.15-0.35	–	–	–	–	–
	V	0.25-0.50	–	–	–	–	–
Length of the beak	R	0.05-0.10	–	–	–	0.10	–
BL	D	0.10-0.20	–	–	–	–	–
	V	0.10-0.25	–	–	–	0.10-0.20	–
Length of the seed	R	1.00-1.50	–	–	–	–	–
SDL	D	1.20-1.60	–	–	–	–	–
	V	1.50-2.00	–	–	–	–	–
Width of the seed	R	0.55-0.80	–	–	–	–	–
SDW	D	0.60-0.90	–	–	–	–	–
	V	0.90-1.20	–	–	–	–	–
Width of the seed wing in the middle part of the seed	R	–	–	–	–	–	–
FSW	D	–	–	–	–	–	–
	V	–	–	–	–	–	–
Width of the seed wing in the basal part of the seed	R	0.00	–	–	–	–	–
BFW	D	0.05	–	–	–	–	–
	V	0.10-0.25	–	–	–	–	–

CONLUSIONS

1. The research proved that in the sample material the three species can be well separated on the basis of morphological characters.

2. On the basis of the present research, the quantitaive characters useful for diagnostics are: density of the pedicels (PD), length of the stem hair (SHL), width of the silicle (SW), width of the lower part of the apical notch (WLAN), length of the beak (BL), width of the wing in the basal part of the seed (BFW).

3. The length of the stem hairs (SHL) is a new and immensely useful character. It visibly distinguishes *L. virginicum*.

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