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Original paper

Limonium gmelinii (Willd.) O. Kuntze in Serbia and Republic of Macedonia: Analysis of morphological variability

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Summary. *Limonium gmelinii* is distributed from Central and Southeast Europe, through Central Asia to Siberia. It is characterized by variable morphological characters, and many infraspecific taxa are described. As a consequence, characters that contribute to efficient identification of taxa are scarce. In order to analyse morphological variability among different samples, compare obtained data with existing keys, and revise morphological characters, four populations of *L. gmelinii* from Serbia and the Republic of Macedonia were examined. Plant materials were collected at different saline habitats, along the gradient of latitude in the western part of the taxa distribution area. Infraspecific taxa (f. *acuminatum*, f. *obtusum* i f. *hungaricum)* were determined within the analyzed plant material according to regional Floras. To present variability of morphological characters, multivariate analyses (PCA, DA, CA) were performed. Results confirmed high variability of the characters which are key characters in many literature sources, and also are important for discrimination of our samples. Finally, additional characters that contributed to sample discrimination were identified.

Keywords: Balkans, morphometry, multivariate statistical methods, Pannonian Plain, Plumbaginaceae.

INTRODUCTION

Limonium gmelinii (Willd.) O. Kuntze 1891 is distributed from Central and Southeast Europe (Pignatti 1972), through Central Asia to Siberia (Lynchevsky 1952). This taxon is characterized by variable morphological characters. Many infraspecific taxa were described within the distribution area, and it is impossible to define characters that contribute to clear differentiation of the taxa (Lynchevsky 1952; Moysiyenko 2008). Three subspecies are described within the areal: *L. gmelinii* subsp. *gmelinii*, *L. gmelinii* subsp. *hypanicum* (Klokov) Soó 1969 and *L. gmelinii* subsp. *hungaricum* (Klokov) Soó 1963. According to Klokov (1957), *L. gmelinii* subsp. *gmelinii* inhabits southwestern Siberia, from where it spread into Eastern Europe, but not beyond the Volga River. *Limonium gmelinii* subsp. *hypanicum* can be found in Ukraine and Moldova (Soó 1970; Pignatti 1972; Moysiyenko 2008). The third subspecies, *L. gmelinii* subsp. *hungaricum* is widespread in the western part of the distribution area, on the territory of the Panonian Plain; and grows in the saline steppes of lower Austria (Meusel et al. 1978), Hungary, Romania (Soó 1970; Pignatti 1972) and the southern part of Slovakia (Řehořek and Maglocký 1999). It should be noted that Klokov (1957) considered these three subspecies to be ranked on the species level. The characters that differentiate these three subspecies are petiole and calyx length (Soó 1970). *Limonium gmelinii* subsp. *gmelinii* has a petiole 1/5-1/3 as long as lamina and a calyx length of 3-4 mm (Pignatti 1972). The other two subspecies have subsessile leaves (Pawłowski 1963; Soó 1970; Pignatti 1972) and a calyx length of 4-5 mm (Soó 1970). The characters that differentiate *L. gmelinii* subsp. *hungaricum* and *L. gmelinii* subsp. *hypanicum* are calyx hair length, where the former has slightly longer

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calyx hairs (Klokov 1957; Pignatti 1972). According to Soó (1970), four forms are recorded within the *L. gmelinii* subsp. *hungaricum* in the Panonian Plain: f. *hungaricum*, f. *emarginatum* (Schur) Soó 1968, f. *acuminatum* (Schur) Soó 1968 and f. *obtusum* (Schur) Soó 1968. These forms differ on the basis of leaf character, f. *hungaricum* has obovate leaves with obtuse apex, f. *emarginatum* obcordate leaves, f. *acuminatum* posess a mucronate leaf apex and f. *obtusum* has obtuse and oval leaves.

Although *L. gmelinii* is a highly variable taxon and there are indications of the existence of several forms throughout the distribution area, regional floristic literature (Răvărut 1960; Gajić 1972; Anchev 1982; Micevski and Matevski 1995) treat this taxon as *L. gmelinii*, including Flora Europaea (Pignatti 1972), whereas the other two taxa, ranked as species *L. hungaricum* and *L. hypanicum*, are included within *L. gmelinii*, with a remark that their status requires further investigation.

Serbia and Republic of Macedonia represent the western limits of the *L. gmelinii* distribution area. In northern Serbia (Vojvodina province), which is a part of the Pannonian Plain, three forms were recorded within the *L. gmelinii* subsp. *hungaricum*: f. *hungaricum*, f. *acuminatum* and f. *obtusum* (Boža et al. 1987; Knežević 1994; Budak 1998; Šturc 2014). Nevertheless, no infraspecific taxa have been observed outside the Pannonian part of Serbia (Gajić 1972) or in the flora of the Republic of Macedonia (Micevski and Matevski 1995).

The aim of this study was to analyze the morphological variability of *L. gmelinii*, by comparing morphological features among populations from different saline habitats. Localities distributed along the gradient of latitude are chosen, from the Pannonian Plain to the central part of the Balkan Peninsula. As a result, additional characters which contribute to sample discrimination will be identified. Finally, according to published identification keys all specimens will be determined to the rank of forma. This will be the first results of this kind in a region outside of the Pannonian Plain.

MATERIALS AND METHODS

Plant material was collected from three saline habitats in Serbia and one saline habitat in the Republic of Macedonia (Fig. 1, Table 1). The type of salt marshes in Okanj (oknj) and Lalinačka slatina (lals) correspond to solonetz soils, whereas Slano Kopovo salt marsh (skop) and Ovče Pole (ople) correspond to solonchak soils. Collected materials were deposited in the Herbarium of the Department of Biology and Ecology, at the Faculty of Sciences, University of Novi Sad (BUNS) (Table 1). During processing of the material, standard OTU (Original Taxonomic Units) labels were used to label the exsiccated specimens. Prior to morphometric processing of the samples, the herbarized material was rehydrated via thermal treatment in an ethanol solution. Measurements were performed using a calibrated caliper (accuracy 0.001 mm).

Based on the relevant floristic literature (Linchevsky 1952; Klokov 1957; Răvărut 1960; Soó 1970; Gajić 1972; Pignatti 1972; Anchev 1982; Skrypnyck 1987; Micevski and Matevski 1995), a total of 32 characters were selected for morphometric analyses, 19 of which were quantitative and 13 qualitative (Table 2). The analyses were performed on a total of 60 specimens. Data obtained from morphometric analyses were processed using Statistica for Windows version 12.1 (StatSoft, Inc. 2012). Statistical analyses included descriptive statistics and multivariate analyses. In order to observe the function of the characters in the total variability of the samples and to observe the analyzed specimens spatially in terms of the given variables, principal component analysis (PCA) was performed. Discriminant analysis (DA) obtained the combination of characters that mostly contributed to the separation of groups. Based on data from DA UPGA, cluster analysis was performed for visualization of the similarities among population specimens. Correspondence analysis (CA) defined characters, character states or the combination of states that best define each group. Of the total number, statistical analyses included 17 quantitative characters (14 morphometric and 3 meristic) in 49 specimens and 8 qualitative characters in 53 specimens. The statistical analyses excluded characters not identified in all

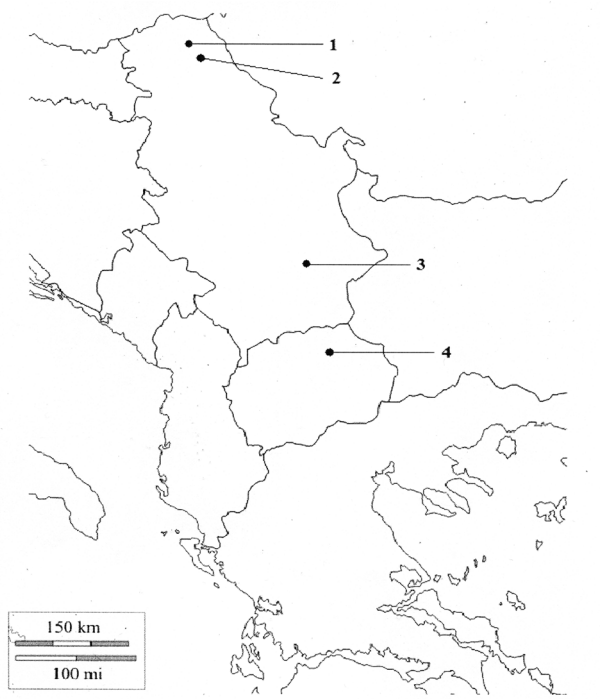

Fig. 1. Distribution of the sampled populations of *Limonium gmelinii* from Serbia and Republic of Macedonia. Localities: 1 - Okanj bara; 2 - Slano Kopovo; 3 - Lalinačka slatina; 4 - Ovče pole.

Table 1. List of collecting sites with number of analyzed specimens and voucher data of the examined material of *Limonium gmelinii*.

Collecting sites					
Country	Locality	Acronym	GPS coordinate	Number of analyzed specimens	Voucher number
Serbia	Okanj bara (pond)	oknj	N 45°29′11.71″ E 20°18′19.08″	15	2-2136
	Slano Kopovo (pond)	skop	N 45°36′35.34″ E 20°13′1.26″	18	2-2138
	Lalinačka slatina	lals	N 43°20′20.64″ E 21°44′30.25″	13	2-2135
Republic of Macedonia	Ovče pole	ople	N 41°51′54″ E 21°56′34″	14	2-2144

specimens, as well as the specimens without all characters. Also, qualitative characters present in only one state were excluded from statistical analyses.

RESULTS

According to descriptive statistics, coefficients of variation (CV) can be grouped into three zones: low variability (CV < 10%), moderate variability (CV = 10-30%) and high variability (CV > 30%). In the present study, coefficients of variation for the characters were observed to belong to two zones – moderate and high variability; no characters were observed in the zone of low variability. Seven characters observed in the zone of high variability are: start of branching (SB), number of leaves (NL), leaf length (LL), leaf plate width (LPW), inflorescence length (IL), distance between the first two spikelets (DBSp) and number of spikelets per spike (NSSp). Two of those characters, leaf length (LL) and leaf plate width (LPW), are considered to be important in determining infraspecific taxa (Table 3).

As indicated by the first PCA axis, the morphometric characters that contribute most to sample variability are: plant height (PH), leaf length (LL), inflorescence length (IL), first inner bract length (FBL) and calyx length (ClL), whereas start of branching (SB) and anter length (AntL) are the major contributors in relation to the second axis (Table 4; Fig. 2A). The meristic characters with the strongest influence on the variability of the sampled populations are number of spikelets per spike (NSSp) and number of leaves (NL), as indicated by the first axis, and number of flowers per spikelet (NFSp), according to the second axis (Table 4). In the space delimited by the first and second axes, there is no clear grouping of populations based on morphometric and meristic characters (Fig. 2B).

Characters with the greatest impact on sample group formation along the first discriminant axis (DA1) are plant height (PH), start of branching (SB) and distance between the first two spikelets (DBSp), whereas stem width (SW) and anter length (AntL) were found to be most important along the second axis (DA2). Inflorescence length (IL) contributed to grouping along both axes (Table 4). Analysis of morphometric characters revealed that all four investigated populations have different positions in the space of the first two axes. That is, the morphometric characters contribute to the separation of the four groups. These groups correspond to analysed populations: Lalinačka slatina and Ovče Pole are located on one side of the first discriminant axis and show small overlap, whereas the populations from Slano Kopovo and Okanj are placed on the other. In relation to the second discriminant axis, all four groups are clearly separated (Fig. 3A). According to analysis of meristic characters, the highest impact to group discrimination along the first axis are number of spikelets per spike (NSSp) and number of flowers in the spike (NFSp), while number of leaves (NL) is the most important for delimitation along the second axis (Table 4). In the space defined by the first two discriminant axes, analysis of meristic characters discriminated the populations of Lalinačka slatina and Slano Kopovo from the populations of Ovče Pole and Okanj. In relation to the second discriminant axis, the highest discrimination was recorded for the population from Lalinačka slatina, whereas the populations of Okanj and Ovče pole are on the other side. The population from Slano Kopovo is located between these two groups (Fig. 3B).

Cluster analysis of morphometric characters indicates that the highest similarity level is between the Pannonian populations, which form a clearly separated group. Other groups comprise populations from Lalinačka slatina and Ovče Pole (Fig. 4A). Cluster analysis of meristic characters show that the population from Lalinačka slatina is clearly separated from the other group, which can be further divided into two subgroups. One of these subgroups consists of the population from Okanj, whereas the other subgroup contains populations from Ovče Pole and Slano Kopovo, which show a considerable level of similarity (Fig. 4B).

Correspondence analysis included seven qualitative characters. Character leaf shape (LS) was present in a total five states, while characters leaf base (LB) and calyx hairiness (CHC) occured in four states, and characters leaf apex (LA), calyx hair (CH) and corolla colour (CC) in three states. The character calyx lobe shape (CLS) occured in only two states (Table 2).

The qualitative characters that proved to be specific for each population are related with the characters of the

Table 2. Analyzed morphological characters of *Limonium gmelinii* (statistically processed characters are shown in bold).

Organ	Quantitative characters (with acronyms)	Qualitative characters (with acronyms)	Character states
Stem	**Plant height (PH)**		
	Stem width (SW)		
	Start of branching(SB)	Scale shape (ScS)	triangular shape
	Scale length (ScL)		
	Scale width (ScW)		
Leaves	**Number of leaves (NL)** **Leaf length (LL)** **Leaf plate width (LPW)**	**Leaf base (LB)**	0 - petiole gradually transitions into the leaf 1 - short petiole 2 - long petiole 3 - very long petiole
		Leaf shape (LS)	0 - ovate 1 - obovate 2 - narrow ovate 3 - wide ovate 4 - narrow obovate
		Leaf apex (LA)	0 - transition between tapering to rounded 1 - tapering 2 - rounded
		Leaf margins (LM)	revolute margin
Inflorescence	**Inflorescence length (IL)** **Number of flowers per spikelet (NFSp)** **Spike length (SL)** **Distance between the first two spikelets (DBSp)** **Number of spikelets per spike (NSSp)**		
		Outer bracts shape (OBS)	ovate with acute apex
		The first inner bracts shape (FBS)	ovate with bifid apex
		The second inner bracts shape (BSS)	ovate with apiculate apex
Flower	**Outer bracts length (OBL)** **The first inner bract length (FBL)** **The second inner bract length (SBL)** **Calyx length (CIL)** **Corolla length (CrL)** **Anther length (AntL)**	**Calyx lobe shape (CLS)**	0 - deeper 1 - shallower
		Calyx hair coverage (CHC)	0 - 1/2 of the calyx 1 - 1/3 of the calyx 2 - 1/4 of the calyx 3 - combination of 1/2 and 1/4
		Calyx hairs (CH)	0 - hairs length equal to the width of the nerves in the lower part of the calyx 1 - hairs shorter than the width of the calyx nerves 2 - hairs longer than the width of the calyx nerves
		Hair distribution on the calyx (HDC)	0 - hairs on one nerve 1 - hairs on two nerves 2 - hairs on three nerves 3 - hairs on four nerves
		Corolla colour (CC)	0 - purple 1 - reddish 2 - pale purple

Table 3. Basic statistical parameters for quantitative characters of the tested populations of *Limonium gmelinii*.

Quantitative characters	Mean	Min	Max	SD	CV(%)
Vegetative part					
Plant height (PH)	408.18	212	650	100.01	24.50
Stem width (SW)	2.22	1.26	4.18	0.59	26.94
Start of branching (SB)	111.37	4	390	65.55	**58.86**
Number of leaves (NL)	10.51	6	26	4.19	**39.90**
Leaf length (LL)	96.18	38	195	39.65	**41.22**
Leaf plate width (LPW)	29.59	11	107	19.23	**65.00**
Generative part					
Inflorescence length (IL)	293.57	104	480	97.19	**33.10**
Spike length (SL)	12.15	3.62	19.56	3.20	26.34
Distance between the first two spikelets (DBSp)	1.84	0.6	4.14	0.87	**47.42**
Number of spikelets in the spike (NSSp)	10.71	4	24	3.86	**35.99**
Number of flowers in the spike (NFSp)	2.35	2	3	0.48	20.49
Outer bract length (OBL)	1.72	1.02	2.3	0.29	17.28
The first inner bract length (FBL)	3.08	1.8	4.2	0.50	16.26
The second inner bract length (SBL)	2.45	1.52	3.74	0.52	21.26
Calyx length (CIL)	4.24	2.78	5.8	0.58	12.81
Corolla length (CrL)	3.11	1.44	6.34	0.90	28.91
Anthers length (AntL)	0.79	0.52	1.2	0.17	21.16

Mean, Min, Max and SD values in mm.
Bold values are characters observed in the region of high variability.

Table 4. Results of principal component analysis (PCA) and discriminant analysis (DA) for morphometric and meristic characters of the studied populations of *Limonium gmelinii*.

Characters	PCA		DA	
	PCA 1	PCA 2	DA 1	DA 2
Morphometric characters				
Plant height (PH)	**-0.864**	0.020	**1.138**	0.115
Stem width (SW)	-0.574	0.474	-0.013	**-0.563**
Start of branching (SB)	-0.375	**-0.685**	**-0.868**	0.017
Leaf length (LL)	**-0.726**	0.049	-0.223	-0.296
Leaf plate width (LPW)	0.127	0.472	0.454	0.009
Inflorescence length (IL)	**-0.653**	0.451	**-1.170**	**-0.481**
Spike length (SL)	-0.473	-0.143	0.516	0.126
Distance between the first two spikelets (DBSp)	-0.514	-0.399	**-1.097**	0.146
Outer bract length (OBL)	-0.520	0.058	-0.142	-0.291
The first inner bract length (FBL)	**-0.635**	-0.048	-0.199	0.351
The second inner bract length (SBL)	-0.452	-0.406	0.293	-0.107
Calyx length (CIL)	**-0.679**	-0.136	0.299	-0.089
Corolla length (CrL)	-0.318	0.465	-0.135	0.206
Anther length (AntL)	-0.152	**0.646**	0.286	**-0.521**
The cumulative value (%)	29.55	44.73	64.18	93.89
Meristic characters				
Number of leaves (NL)	**0.721**	-0.389	-0.278	**-0.657**
Number of spikelets per spike (NSSp)	**-0.763**	-0.267	**0.751**	0.350
Number of flowers per spikelet (NFSp)	0.085	**0.903**	**-0.784**	0.553
The cumulative value (%)	36.95	71.55	83.40	98.05

Bold values are morphometric characters that contribute most to sample variability.

leaves, calyx hairiness and corolla colour, and have particular combinations in each analysed population. Within the Okanj population, most specimens have leaves with a medium length petiole (gradual transition of the petiole into the leaf), a hairy calyx base and four nerves. The population from Slano Kopovo is characterized by individuals with rounded leaf apexes, flowers with a purple corolla, and hairs on the calyx that are shorter than the width of the calyx

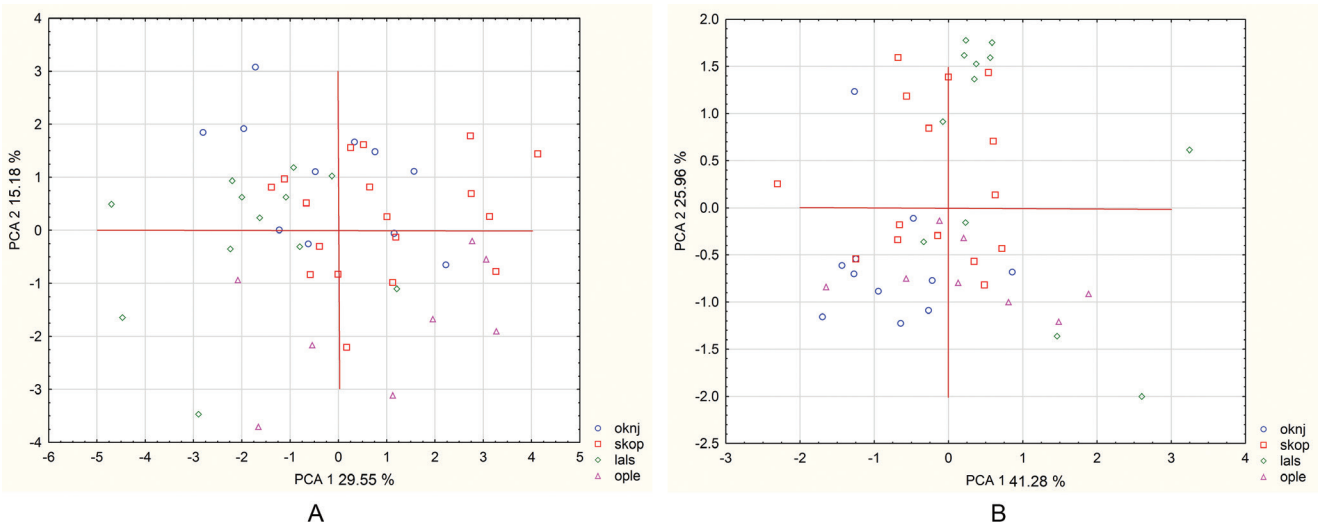

Fig. 2. Positions of analyzed specimens of *Limonium gmelinii* in the area of the first and the second PCA axes: **A**, morphometric characters; **B**, meristic characters

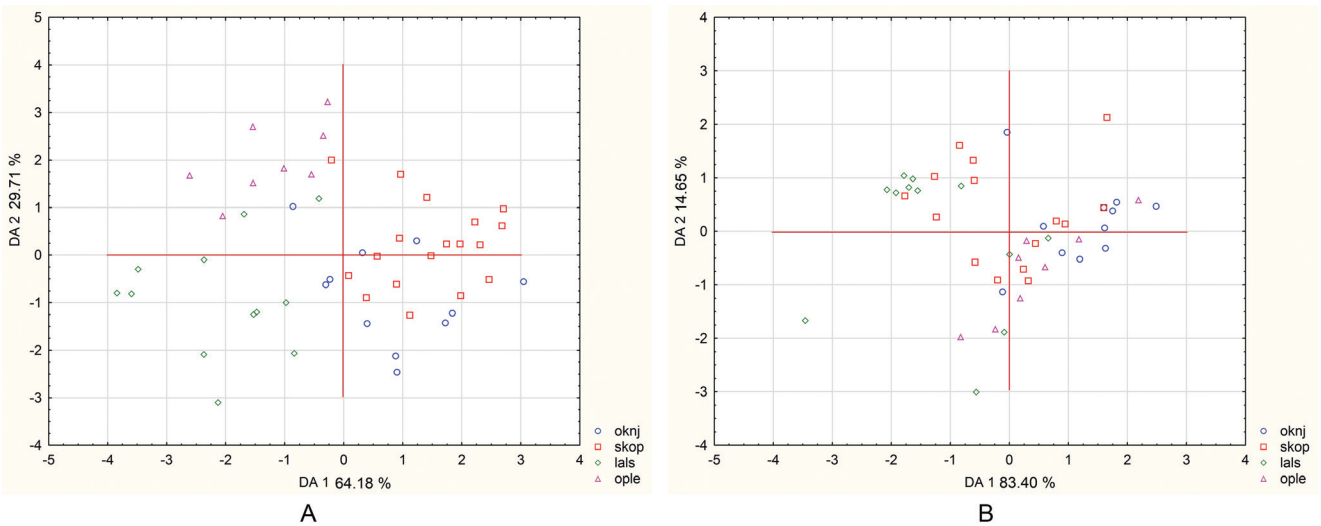

Fig. 3. Positions of analyzed specimens of *Limonium gmelinii* in the area of the first and the second DA axes: **A**, morphometric characters; **B**, meristic characters.

nerves. The characters that are dominant in specimens from the Lalinačka slatina locality are very long petioles and a reddish corolla, while a pale purple corolla is characteristic for specimens from the locality of Ovče Pole. The remaining qualitative character states are found at the intersection of the corresponding axis and uniformly occur in all four populations (Fig. 5).

According to previously analyzed morphological characters, and in accordance with published data, three forms within the analyzed populations (f. *acuminatum*, f. *hungaricum* and f. *obtusum*) are determined. Within populations from Okanj and Slano Kopovo all three forms have been identified, while in populations from Lalinačka slatina and Ovče pole only f. *acuminatum* is recorded.

DISCUSSION

The taxon *L. gmelinii* is characterized by high variability of morphological characters, which can be caused by hybridization and apomixis (Erben 2005; Moysiyenko 2008). For many forms registered within the distribution area (Klokov 1957; Moysiyenko 2008), it is difficult to define stable characters that could be used for separation.

According to Soó (1970), in the western part of the *L. gmelinii* distribution area, including Serbia and Republic of Macedonia, *L. gmelinii* subsp *hungaricum* should be expected. However, Pannonian populations correspond most to the subsp. *hungaricum*: they are characterized by a shorter petiole and longer calyx. Specimens from the Balkan Peninsula have a longer petiole (especially those from Lalinačka

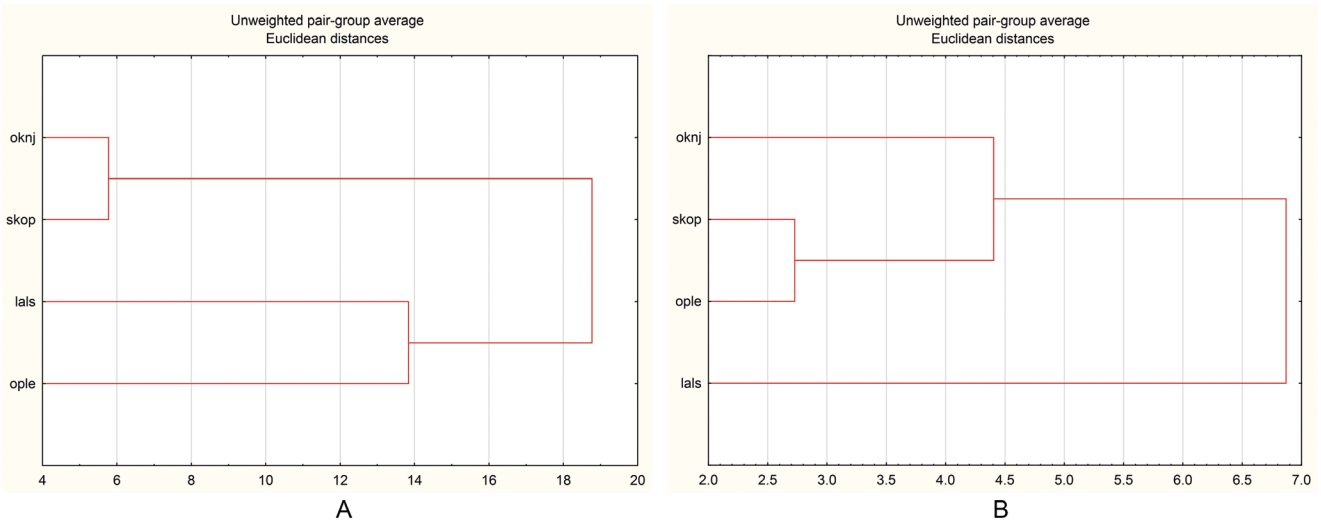

Fig. 4. UPGA cluster analysis of the *Limonium gmelinii* populations based on Mahalanobius distances: **A**, morphometric characters; **B**, meristic characters.

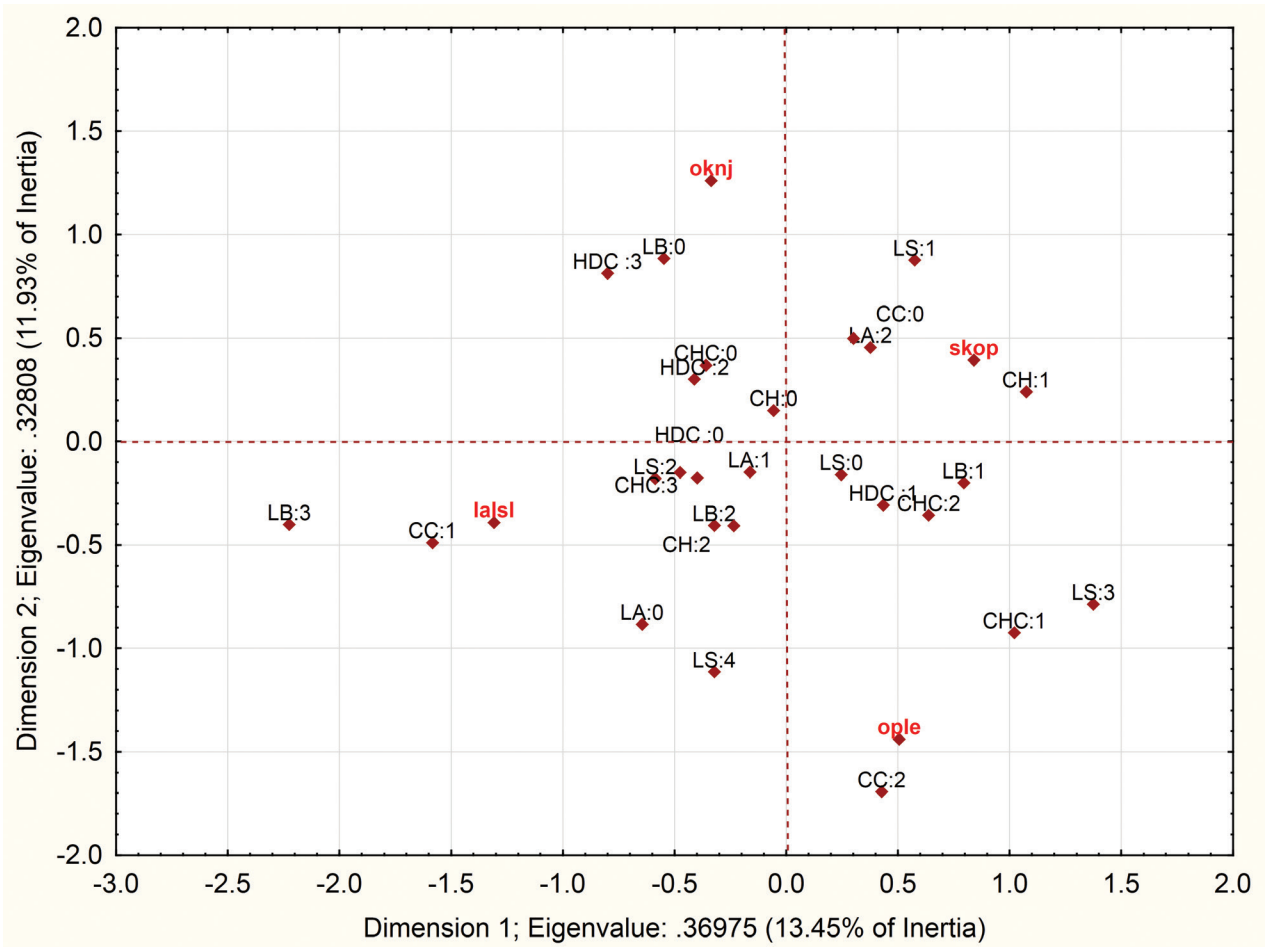

Fig. 5. Positions of characters states and populations of *Limonium gmelinii* in the space of I and II correspondence axes.
LB (leaf base): 0 - petiole gradually transitions into the leaf, 1 - short petiole, 2 - long petiole, 3 - very long petiole; LS (leaf shape): 0 - ovate, 1 - obovate, 2 - narrow ovate, 3 - wide ovate,4 - narrow obovate; LA (leaf apex): 0 - transition between tapering to rounded, 1 - tapering, 2 – rounded; CHC (calyx hair coverage): 0 - 1/2 of the calyx, 1 - 1/3 of the calyx, 2 - 1/4 of the calyx, 3 - combination of 1/2 and 1/4; CH (calyx hairs): 0 - hairs length equal to the width of the nerves in the lower part of the calyx, 1 - hairs shorter than the width of the calyx nerves, 2 - hairs longer than the width of the calyx nerves; HDC (hair distribution on the calyx: 0 - hairs on one nerve, 1 - hairs on two nerves, 2 - hairs on three nerves, 3 - hairs on four nerves; CC (corolla colour): 0 - purple, 2 - pale purple.

slatina), while calyx lengths were out of the range of values ascribed to subsp. *hungaricum*, and also include values typical for subsp. *gmelinii* (3-4 mm), which according to Klokov (1957) inhabits southwestern Siberia. According to PCA, the character calyx length has high variability and does not contribute to the discrimination of groups (Table 4).

Within Pannonian specimens, three forms were determined and confirmed (f. *acuminatum,* f. *obtusum* and f. *hungaricum*) that were previously published in the literature (Boža et al. 1987; Knežević 1994; Budak 1998; Šturc 2014). Specimens from the Balkan Peninsula populations have mucronate leaves that correspond to the f. *acuminatum*.

Discriminant analysis showed separation of the Pannonian and Balkan populations on the basis of: plant height (PH), start of branching (SB), inflorescence length (IL), stem width (SW), distance between the first two spikelets (DBSp) and anthers length (AntL) (Fig 3A). In relation to plant height, most distinguishable are individuals from Ovče Pole, which are considerably smaller than individuals from the other three populations. The specimens from Lalinačka slatina are higher and have shorter anthers. The characters with the greatest impact on the formation of sample groups are at the same time highly variable according to PCA: plant height (PH), start of branching (SB), inflorescence length (IL), distance between the first two spikelets (DPSp). Two most significant characters for group discrimination are stem width (SW) and distance between the first two spikelets (DBSp) (Table 4). Specimens from Lalinačka slatina have wider stems and the first two spikelets are more distant.

As indicated in previous studies of the genus *Limonium* (Luteyn 1976; Moysiyenko 2008), characters analysed in the present study are related to the ecological conditions of the habitat. Therefore, the geographical and geomorphological similarity of the Slano Kopovo and Okanj localities (Popov 2012) can be considered an important factor that contributes to the grouping of samples in the discriminant analysis of morphometric characters (Fig. 4A). In addition, both populations were collected near the salt lakes, which are formed in the abandoned channels of the Tisa River (Popov 2012). The populations in the Pannonian Plain are developing under the influence of a continental climate, whereas the populations from Lalinačka slatina and Ovče Pole are under the thermophylic conditions of a submediterranean climate.

Our results confirmed the high variability of characters that are, in most Floras, key for taxa separation, and also are important for sample discrimination. Moreover, additional characters were identified that have not been mentioned in the literature to date, but contribute to samples discrimination. In order to establish the importance of these characters it is necessary to conduct further research toward defining more stable characters. Similarly, sampling should include a wider area, and different types of saline habitats.

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