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Comparative morpho-anatomical analysis of *Gagea pratensis* (Pers.) Dumort. (Liliaceae) from Serbia and Montenegro

Original Article

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Abstract:

In this study, morphological and anatomical properties of species *Gagea pratensis* (Pers.) Dumort. were investigated and described. The comparative analysis included six populations from Serbia and Montenegro. This study aimed to examine potential morpho-anatomical differentiation and the characters that contribute most to the differences between populations. Statistical analyses were carried out for 19 morphological and 5 anatomical characters of 122 specimens using program STATISTICA 7, including the analysis of variance (ANOVA), principal component (PCA) and discriminant analysis (CDA). Analysis of variance showed that almost all investigated characters are statistically significant for differentiation of analysed populations. According to the principal component analysis characters that contributed the most to the separation of populations were height of the whole plant, bulb width, width and length of basal leaf, width and indumentum type of peduncle, length of first cauline leaf, width and length of second cauline leaf, and width of outer and inner segments of perigone. The results of thr discriminant analysis showed the existence of morpho-anatomical differentiation between analysed populations, separating the population from locality Gamzigradska banja from all other populations.

Key words:
Gagea pratensis, morphology, anatomy, variability, differentiation

Apstract:

Komparativna morfo-anatomska analiza vrste *Gagea pratensis* (Pers.) Dumort. (Liliaceae) iz Srbije i Crne Gore

U ovoj studiji ispitivane su i opisivane morfološke i anatomske karakteristike vrste *Gagea pratensis* (Pers.) Dumort. Komparativnom analizom obuhvaćeno je šest populacija sa prostora Srbije i Crne Gore. Cilj istraživanja bio je određivanje potencijalne morfo-anatomske diferencijacije, kao i definisanje onih karaktera koji najviše doprinose postojanju razlika između populacija. Sprovedene su statističke analize za 19 morfoloških i 5 anatomskih karaktera na 122 uzorka primenom programa STATISTICA 7, uključujući analizu varijanse (ANOVA), analizu glavnih komponenti (PCA) i diskriminantnu analizu (CDA). Analiza varijanse je pokazala da gotovo svi istraživani karakteri imaju statistički značaj u diferencijaciji analiziranih populacija. Na osnovu analize glavnih komponenti izdvojeni su karakteri koji su imali najveći uticaj na razdvajanje populacija i to su visina cele biljke, širina lukovice, širina i dužina bazalnog lista, širina i tip indumentuma stabla, dužina prvog pricvetnog lista, širina i dužina drugog pricvetnog lista i širina unutrašnjih i spoljašnjih segmenata perigona. Rezultati diskriminantne analize ukazali su na postojanje morfo-anatomske diferencijacije, pri čemu je populacija sa Gamzigradske banje bila jasno izdvojena od svih ostalih populacija.

Ključne reči:
Gagea pratensis, morfologija, anatomija, varijabilnost, diferencijacija

Introduction

The genus *Gagea* Salisb. (Liliaceae) is a taxonomically complicated group, comprising more than 320 species (Levichev, 2013; Peterson et al., 2016), distributed in temperate and subtropical regions of Europe and Asia (Levichev, 1999a). The primary cause of the taxonomic problems in the genus *Gagea* is the superficial similarity of most of the species (Rix & Woods, 1981). In the last few decades several new species of the genus *Gage*a Salisb. were described based on analyses of

morphological and anatomical characters (Zarrei and Zarre, 2003; Peruzzi et al., 2007; Hamzaoğlu et al., 2008, Zarrei et al., 2010a,b; Levichev et al., 2019).

Fig. 1. *Gagea pratensis*: A – habitus, B – underground organs, C – floral details

According to Levichev (in Peterson et al., 2008) *Gagea pratensis* (Pers.) Dumort. belongs to section *Gagea* Davlianidze, 1972, Not. Syst. Geogr. Inst. Bot. Thbilissiensis. 29: 73., genus *Gagea* Salisb., tribe Tulipeae and family Liliaceae (Peruzzi, 2011). Section *Gagea* forms a well-defined monophyletic clade which is marked by four morphological synapomorphies - angular peduncles and pedicels, second leaf concrescent with peduncle and seeds with distinct arillode (Peruzzi et al., 2008a). *Gagea pratensis* is distributed in most parts of Europe and Asia Minor (Diklić in Josifović, 1975). It is characterized by the bulb and bulblet being exserted (Peruzzi et al., 2007). Basal leaves have nine or more vascular bundles, first cauline leaves have five barely pronounced keels, pedicels are triangular with six to eight vascular bundles and peduncles are polyedric with a 'channel-like' structure inside (Peruzzi et al., 2007).

Gagea pratensis is a highly variable, complex and critical taxon, which is poorly investigated in the Balkan Peninsula. Therefore, in this paper, the morphology and anatomy of *G. pratensis* from Serbia and Montenegro were studied in detail. The aim of the study was to examine potential morpho-anatomical differentiation of the studied populations, and to determine the characters that contribute most to the differences between populations.

Material and methods

Plant material

Plant samples were collected from 6 populations of *G. pratensis* from Serbia and Montenegro during the flowering time. The sampling localities with associated information are given in the **Tab. 1**. One part of the collected plant material was deposited in Herbarium Moesiacum Niš (HMN). The other part was fixed in 50% alcohol for anatomical study, which was done in the laboratory for Plant Systematics and Ecology (Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Serbia).

Table 1. The sampling localities with associated information (V.R. - V. Ranđelović, I.R. - I. Raca, J.J. - J. Jevtić, D.H. - D. Harpke)

Locality	N	E	Altitude (m)	Habitat	Date Collectors	Voucher
Gamzigradska banja	43° 92'38"	22° 17' 03"	164	Clearing in an oak forest	18.03.2017. V.R.	HMN-13289
The Jelašnička gorge	43° 28' 41"	22° 06' 61"	460	Arid subcontinental steppic grassland	23.03.2017. V.R, I.R., J.J.	HMN-13284
The hill Rgotski kamen	44° 02' 62"	22° 23' 32"	225	Arid subcontinental steppic grassland	18.03.2017. V.R.	HMN-13287
Mt. Seličevica	43° 18' 38"	21° 83' 09"	802	Oak forest	23.03.2017. V.R, I.R., J.J.	HMN-13285
Mt. Lovćen	42° 24' 02"	18º 49' 33"	1749	Subalpine pasture	07.04.2018. V.R, I.R., D.H.	HMN-13666
Mt. Vjetarnik	42° 36' 30"	19º 25' 49"	1075		10.04.2018. V.R, I.R., D.H.	HMN-13667

Morpho-anatomical analysis

19 morphological characters were defined from the fresh plant material. Manual microtome (Gligorijević & Pejčinović, 1983) was utilized in order to make cross-sections of the basal leaves, first cauline leaves, peduncles and pedicels at their widest part. Cross-sections were stained with Safranin - Alcian Blue, examined by Leica DM 2500 microscope and photographed with camera Leica DFC490. 5 anatomical characters were analysed. All investigated characters are represented in the **Tab. 3**.

Statistical analysis

Basic descriptive statistics was performed for each quantitative character. The significance of differences between the populations studied was established by analysis of variance (ANOVA). Principal component analysis (PCA) was conducted to determine which characters contributed most to the delimitation of populations, while discriminant analysis (CDA) was used to determine the morpho-anatomical differentiation of investigated populations. All statistical analyses were performed using STATISTICA 7.0 software (StatSoft. Inc., 2004).

Results

The general habitus is represented in **Fig. 1a**, including the details of the underground organs (**Fig. 1b**) and flower (**Fig. 1c**) (Photo by J. Stojanović). Tunic's colour in all flowering plants was either light or dark brown. In all investigated populations bulbils on peduncle and in the base of cauline leaves were absent. Middle stem leaves and indumentum of basal leaves were lacking in all investigated populations. First cauline leaves were sparse ciliate, except some individuals from population from the hill Rgotski kamen. Peduncles were hairless in all populations, excluding some specimens from the locality of Gamzigradska banja and Mt. Seličevica with sparse hairy peduncles. Pedicels, outer perigone and inner perigone segments were glabrous in all populations. Perigone segments were yellow on the inside (white colour was only present in the population from the hill Rgotski kamen) and green on the outside.

The anatomical characteristics of the basal leaf are presented in **Fig. 2**. Cross-sections of basal leaves of the species *G. pratensis* had recognisable V-shape in all investigated populations. Both adaxial and abaxial epidermis were made of square, rectangular to oval-shaped cells all arranged in one layer. Leaves were amphystomatic and stomata were at the same level as epidermal cells. Some indications of mesophyll differentiation were noticed in all investigated populations. Palisade tissue was present below both adaxial and abaxial epidermis in all populations, except that from Mt. Vjetarnik, where some individuals had palisade tissue only below their abaxial epidermis. Big and small vascular bundles were alternately lined in leaf mesophyll. Every vascular bundle was surrounded by parenchymatous tissue organised in one or two layers. Indumentum was absent from basal leaves.

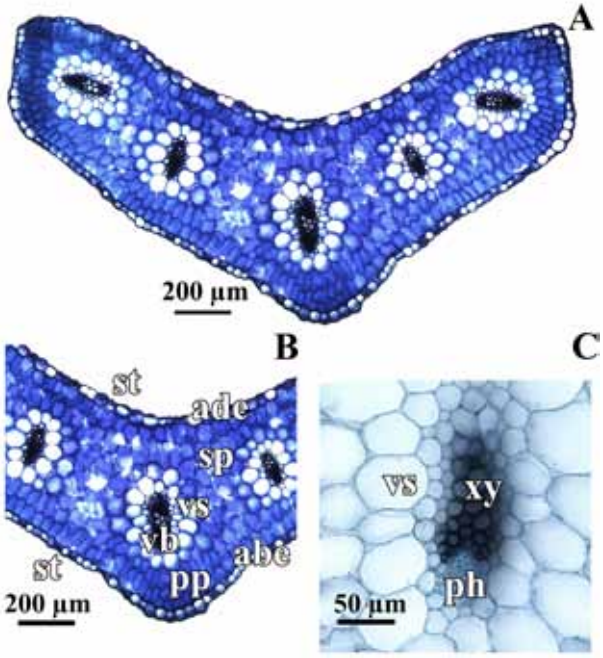

Figure 2. **A** – V-shaped cross-section of basal leaf of *G. pratensis*; **B** – central part of basal leaf cross-section; **C** – central vacular bundle (**ade** – adaxial epidermis, **abe** – abaxial epidermis, **ph** – phloem, **pp** – palisade parenchyma, **sp** – spongy parenchyma, **st** – stomata, **vb** – vascular bundle, **vs** – vascular sheath, **xy** – xylem)

The anatomical characteristics of the first cauline leaf are presented in **Fig. 3**. Cross-sections of first cauline leaves of the species *G. pratensis* had a crescent shape. The anatomy of first cauline leaves is similar to the anatomy of basal leaves. The epidermis was made of square, rectangular to oval-shaped cells all arranged in one layer. Leaves were amphystomatic. Mesophyll was usually made of spongy parenchyma and one or two layers of palisade parenchyma. Palisade tissue can be located only below abaxial (populations from Mt. Seličevica, Mt. Vjetarnik and Mt. Lovćen, and some individuals from the hill Rgotski kamen and the Jelašnička gorge), below both abaxial and adaxial epidermis (population from Gamzigradska banja and some individuals from the hill Rgotski kamen) or it can be absent (some individuals from the Jelašnička gorge had mesophyll made only of spongy tissue). Big and small vascular bundles were alternately lined in leaf

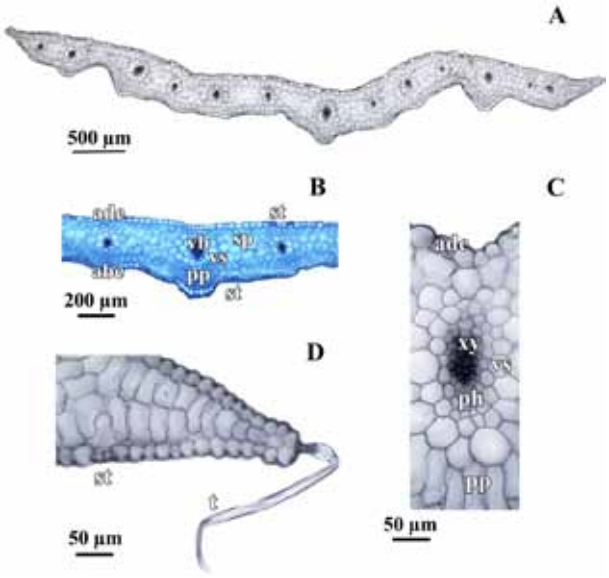

Fig. 3. **A** – cross-section of first cauline leaf of *G. pratensis*; **B** – central part of cross-section; **C** – central vascular bundle; **D** – leaf edge (**ade** – adaxial epidermis, **abe** – abaxial epidermis, **ph** – phloem, **pp** – palisade parenchyma, **sp** – spongy parenchyma, **st** – stomata, **t** – trichome, **vb** – vascular bundle, **vs** – vascular sheath, **xy** – xylem)

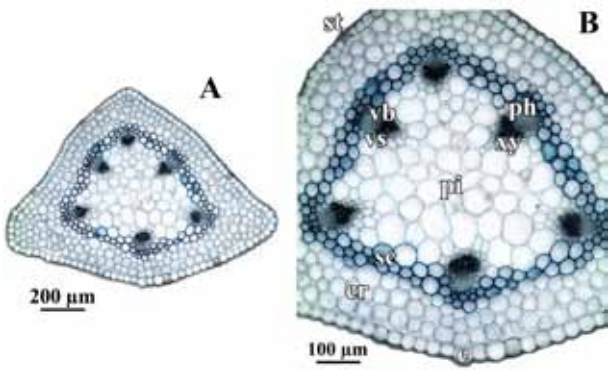

Fig. 4. **A** – general outlook and **B** – anatomical features of triangular cross-section of pedicel of *G. pratensis* (**cr** – cortex, **e** - epidermis, **ph** – phloem, **pi** – pith, **sc** – sclerenchyma, **st** – stomata, **vb** – vascular bundle, **vs** – vascular sheath, **xy** - xylem)

mesophyll. Every vascular bundle was surrounded by parenchymatous tissue organized in one or two layers. Indumentum was present on first cauline leaves in all investigated populations.

The anatomical characteristics of pedicels are presented in **Fig. 4**. Cross-sections of pedicels of the species *G. pratensis* had recognizable triangular shape. The epidermis was made of square to oval cells organised in one layer. Stomata were at the same level as epidermal cells. Both cortex and pith, were made of parenchymatous cells with thin walls. The cortex was made of 3-5 layers of cells. Vascular bundles were arranged in a circle, with xylem oriented toward the pith and surrounded by its cells. Each vascular bundle was surrounded by a parenchymatous vascular sheath. Sclerenchymatous tissue was also present in the form of a ring, made of 2-3 layers of cells, located beneath the cortex and covering only the phloem. Indumentum was absent from pedicels.

Microphotographs of peduncle cross-sections for all investigated populations are presented in **Fig. 5.** This organ was very variable in shape. Peduncles with square shape without channel-like structures were noticed in populations from the hill Rgotski kamen, Mt. Lovćen and Mt. Vjetarnik; deltoid shape without channel-like structures from Mt. Lovćen and

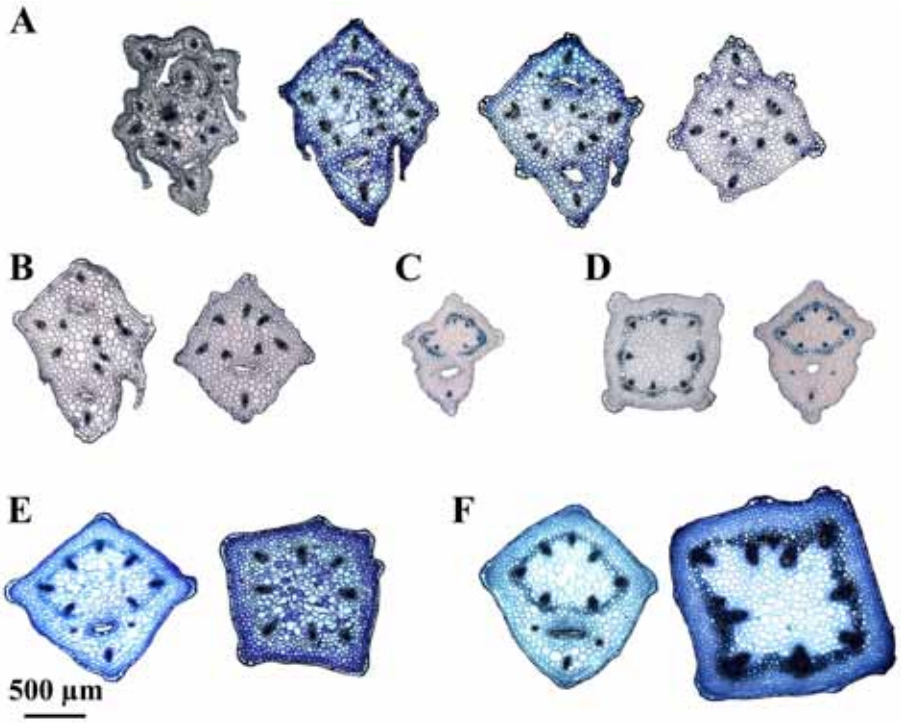

Fig. 5. Comparative overview of peduncle cross-sections of *G. pratensis* from localities: **A** – Gamzigradska banja, **B** – Seličevica, **C** – Jelašnička klisura, **D** – Rgotski kamen, **E** – Lovćen, **F** – Komovi.

Table 2. Descriptive statistics of quantitative morphological and anatomical characters of analysed populations of the species *G. pratensis.* Quantitative numerical values are expressed in mm, as mean±standard deviation

Locality	Gamzigradska banja	Jelašnička gorge	Rgotski kamen	Seličevica	Lovćen	Vjetarnik
Morphological characters			Mean±Std.Dev.			
Height of the whole plant	162.16±26.63	77.85±15.12	113.19±24.54	124.44±14.79	95.98±21.22	85.38±23.95
Number of bulbs in common tunic	3.00±0.00	1.00±0.00	1.00±0.00	3.00±1.00	1.00±0.00	1.00±0.00
Width of bulb	20.17±4.41	7.03±1.17	9.55±1.83	15.95±3.59	4.74±1.46	7.07±1.55
Width of basal leaf	4.51±0.74	2.30±0.63	2.71±0.53	3.54±0.48	2.19±0.63	2.97±0.74
Length of basal leaf	217.61±40.80	113.99±30.33	147.28±34.12	159.38±19.89	140.36±38.16	162.93±31.39
Width of peduncle	1.84±0.38	1.19±0.21	1.52±0.35	1.40±0.19	0.84±0.22	1.18±0.33
Heigh of peduncle from bulb to first cauline leaf	91.23±20.35	46.38±17.10	65.48±22.72	67.62±16.12	56.53±18.27	38.70±18.29
Width of first cauline leaf	6.79±1.30	4.73±6.19	5.68±1.20	5.07±0.91	5.70±1.34	6.48±1.72
Length of first cauline leaf	60.63±16.64	23.73±6.25	42.59±14.51	42.65±9.24	40.02±9.22	48.03±15.73
Width of second cauline leaf	5.07±1.12	1.64±0.57	2.60±0.61	2.88±0.63	2.67±0.59	2.84±0.85
Length of second cauline leaf	41.90±9.90	13.13±4.15	24.48±7.90	25.96±5.56	21.69±5.04	26.17±10.41
Width of pedicels	1.13±0.16	0.96±0.17	0.88±0.16	1.01±0.10	0.76±0.20	0.75±0.12
Number of flowers	3.00±1.00	1.00±1.00	4.00±2.00	2.00±1.00	2.00±1.00	3.00±2.00
Length of outer perigone segments	16.65±1.69	12.74±2.93	12.76±2.47	14.57±1.47	12.95±1.62	15.11±2.01
Length of inner perigone segments	15.21±1.59	11.63±2.06	11.37±2.25	13.55±1.39	12.21±1.66	14.26±1.95
Width of outer perigone segments	4.24±0.72	2.59±0.57	2.52±0.49	3.02±0.32	2.72±0.46	3.42±0.61
Width of inner perigone segments	3.23±0.56	2.00±0.49	2.10±0.43	2.47±0.21	2.41±0.34	2.72±0.36
Anatomical characters						
No. of vascular bundles in basal leaf	9.00±0.00	8.00±1.00	8.00±1.00	7.00±1.00	8.00±1.00	8.00±2.00
No. of vascular bundles in first cauline leaf	12.00±3.00	10.00±2.00	12.00±2.00	9.00±2.00	14.00±2.00	13.00±3.00
No. of vascular bundles in pedicel	6.00±1.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
No. of vascular bundles in peduncle	13.00±1.00	8.00±1.00	9.00±1.00	9.00±1.00	9.00±2.00	11.00±2.00
No. of channel-like structures in peduncle	2.00±0.00	1.00±0.00	0.00±1.00	1.00±1.00	0.00±1.00	1.00±0.00

Mt. Komovi; deltoid shape with one channel-like structure from all populations except the populaton from locality Gamzigradska banja. Deltoid shape with an arm and two channel-like structures was present only in populations from Gamzigradska banja and Mt. Seličevica. Deltoid shape without arms and with 2 channel-like structures, as well as, irregular shape with two arms and 2 or 3 channel-like structures was noticed only in population from Gamzigradska banja.

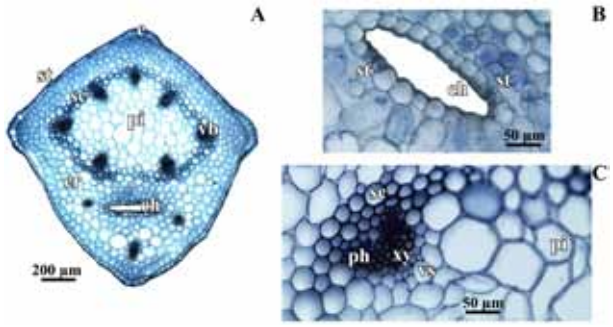

Fig. 6. **A** – cross-section of peduncle of *G. pratensis*, **B** – channel-like structure, **C** – fusion of two vascular bundles in the region of xylem (**ch** – channel-like structure, **cr** – cortex, **e** - epidermis, **ph** – phloem, **pi** – pith, **sc** – sclerenchyma, **st** – stomata, **vb** – vascular bundle, **vs** – vascular sheath, **xy** - xylem)

The anatomical structure was the same regardless the shape of peduncle (**Fig. 6**). The epidermis was made of cubed to oval-shaped cells arranged in one layer. Channel-like structures were limited by the cells which are similar to epidermal cells regarding their shape and size. Stomata were present in epidermis, as well as in the layer of cells surrounding channel-like structures. Cortex was made of 4-5 layers of oval-shaped parenchymal cells with thin walls. Sclerenchymatous tissue was present in the form of a ring, made of 2-3 layers of cells, located beneath the cortex and covering only the phloem. Vascular bundles were also arranged in the form of a ring. In the case of individuals with channel-like structures, there were additional vascular bundles (1-3) in the area surrounding this structure. It was common that two vascular bundles fuse in the region of xylem, while the phloem stays separated. Pith was surrounding xylem, and it was made of parenchymal cells. Indumentum was absent from the peduncle, except populations from localities Gamzigradska banja and Mt. Seličevica, where some plants with hairy peduncles were recorded.

Descriptive statistics is represented in the **Tab. 2**. The results of One-way Analysis of Variance (**Tab. 3**) showed that all analysed characters had statistical

Table 3. Results of One-way Analysis of Variance (* p<0.05) and Principal Component Analysis (PCA) of morphological and anatomical characters of six populations of the species *G. pratensis*. The first three principal components accounted for 62.69% of the variance

Characters	**ANOVA** p-values	**PCA 1**	**PCA 2**	**PCA 3**
Height of the whole plant	**0.00***	**-0.81**	-0.04	-0.31
Number of bulbs in common tunic	0.00*	**-0.69**	-0.43	0.15
Width of bulb	0.00*	**-0.85**	-0.35	-0.01
Width of basal leaf	0.00*	**-0.89**	-0.02	-0.06
Length of basal leaf	0.00*	**-0.79**	0.22	-0.18
Width of peduncle	0.00*	**-0.72**	-0.11	-0.39
Height of peduncle from bulb to first cauline leaf	0.00*	**-0.61**	-0.10	-0.36
Indumentum of peduncle	0.00*	**-0.82**	-0.18	0.11
First cauline leaf width	0.28	-0.31	0.36	-0.09
Length of first cauline leaf	0.00*	**-0.78**	0.41	-0.15
Width of second cauline leaf	0.00*	**-0.90**	0.21	-0.05
Length of second cauline leaf	0.00*	**-0.88**	0.26	-0.12
Width of pedicel	0.00*	**-0.55**	-0.42	-0.06
Number of flowers	0.00*	-0.36	0.45	**-0.58**
Length of outer perigone segments	0.00*	**-0.64**	0.13	0.35
Length of inner perigone segments	0.00*	**-0.64**	0.16	0.36
Width of outer perigone segments	0.00*	**-0.80**	0.18	0.30
Width of inner perigone segments	0.00*	**-0.73**	0.27	0.23
Color of the inner side of perigone segments	0.00*	-0.07	-0.01	**0.53**
No. of vascular bundles in basal leaf	0.00*	-0.30	0.18	0.18
No. of vascular bundles in first cauline leaf	0.00*	0.00	**0.68**	0.16
No. of vascular bundles in pedicel	0.00*	-0.27	-0.22	0.43
No. of vascular bundles in peduncle	0.00*	**-0.66**	0.25	0.32
Number of channel-like structures in peduncle	0.00*	**-0.68**	-0.46	0.17

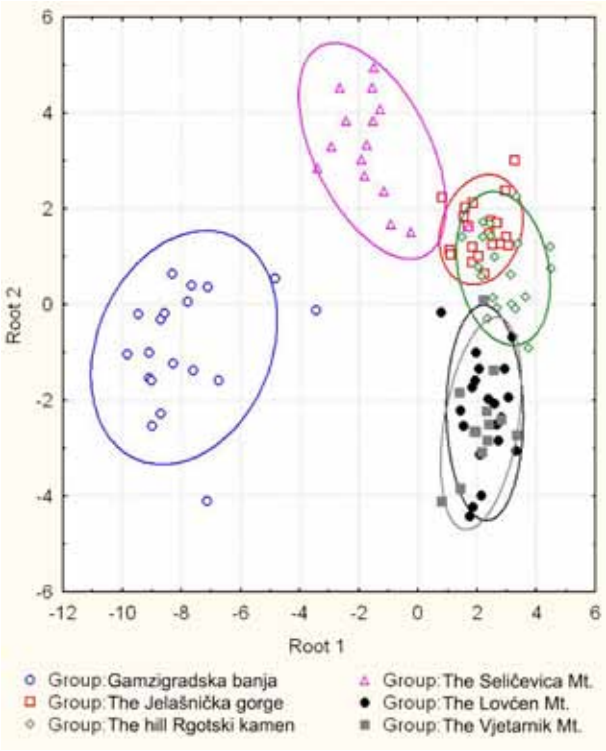

Fig. 7. Canonical discriminant analysis of the six *G. pratensis* populations from Serbia and Montenegro

significance for the formation of differences between analysed populations of species *G. pratensis*, except the width of the first cauline leaf. The results of Principal Component Analysis (PCA) showed that the three principal components contained 62.69% of the total variability. The most significant contribution to the formation of variability was provided by the first principal component with 44.77%, followed by the second one with 9.96% and the third one with 7.96% of variation. According to PCA analysis (**Tab. 3**) characters which contributed most to the differentiation of populations (factor loadings > 0.7) were height of the whole plant, width of bulb, width and length of basal leaf, width and type of indumentum of peduncle, length of first cauline leaf, width and length of second cauline leaf and width of outer and inner perigone segments. The results of Discriminant analysis (CDA) showed the existence of morpho-anatomical differentiation of analysed populations. Populations from the Jelašnička gorge, the hill Rgotski kamen, Mt. Lovćen and Mt. Komovi are on the positive side of the first canonical root, while populations from Mt. Seličevica and Gamzigradska banja are on the negative side. Separation is also present on the second canonical root, where populations from the Jelašnička gorge and Mt. Seličevica are on the positive, while populations from Mt. Lovćen and Mt. Vjetarnik are on the negative side (**Fig. 7**).

Discussion

In this study, the detailed morpho-anatomical comparison of six populations of *G. pratensis* from Serbia and Montenegro was conducted. The primary goal was to examine potential morpho-anatomical differentiation and to determine the characters that contribute most to the differences between populations.

As previous studies showed, the morphological characters of significance for taxonomy within the genus *Gagea* Salisb. are number and dimensions of underground organs (Levichev, 1999b; Peruzzi & Caparelli, 2007; Zarrei et al., 2007; Hamzaoğlu et al., 2008; Peruzzi et al., 2008b; Peruzzi et al., 2011), presence/absence of bulbils (Levichev, 1999b; Peruzzi et al., 2011; Zarrei et al., 2011) and indumentum (Hamzaoğlu et al., 2008; Wörz et al., 2012), number and dimensions of basal and cauline leaves (Rix & Woods, 1981; Peruzzi & Caparelli, 2007; Peruzzi et al., 2011; Zarrei et al., 2011; Wörz et al., 2012), and number, dimensions and colour of flowers (Peruzzi & Caparelli, 2007; Zarrei et al., 2007; Hamzaoğlu et al., 2008; Peruzzi et al., 2008b; Peruzzi et al., 2011; Zarrei et al., 2011; Levichev et al., 2019). Our research confirmed the significance of previously mentioned morphological traits. *Gagea pratensis* is highly variable taxon, therefore wide ranges of values were obtained for almost all investigated characteristics. Maximal values of the morphological characters studied were mostly found in the population from Gamzigradska banja, which makes it easily distinguishable from all the other populations.

Also, significant role in the taxonomy of this genus plays the anatomy of basal leaves (Zarrei and Zarre, 2003; Peruzzi et al., 2007; Peruzzi et al., 2008a; Zarrei et al., 2010b; Zarrei et al., 2010c), first cauline leaves (Peruzzi et al., 2007), pedicels (Peruzzi et al., 2007; Peruzzi et al., 2008a, Zarrei et al., 2010b; Zarrei et al., 2010c) and peduncles (Peruzzi et al., 2007; Peruzzi et al., 2008a; Ajani et al., 2010; Zarrei et al., 2010b; Zarrei et al., 2010c). Relevant anatomical markers are the shape of cross-sections (Grossheim, 1935; Zarrei et al., 2010c) and the number of vascular bundles (Heyn and Dafni, 1977; Zarrei et al., 2010c). Investigated populations of *G. pratensis* had recognisable V-shaped basal leaves with inner part that was not fistulose, and rather, it was made up of mesophyll, which was in accordance to the study conducted by Peruzzi et al. (2008a). Mesophyll was not differentiated into palisade and spongy tissue, which was also confirmed by Akyol et al. (2015), but some indications of differentiation were noticed in all investigated populations. According to Grossheim (1935) presence/absence

of central parenchyma in basal leaves is one of the notable characters in terms of taxonomy. This tissue was absent from basal leaves in all investigated populations. The number of vascular bundles in basal leaves was less than nine in all populations, except in population from Gamzigradska banja. These results differ from the results obtained by Peruzzi et al. (2007). First cauline leaves had similar anatomical structure as basal leaves and presence of keels was noticed, as in the study carried out by Peruzzi et al. (2007). Cross-sections of pedicels of *G. pratensis* are triangular (Peruzzi et al., 2008a) and number of vascular bundles ranges from 6-8 (Peruzzi et al., 2007), which is also confirmed by our study. Of all the organs analysed, peduncles showed the highest variability in shape. According to Levichev (in Ajani et al., 2010) the form of a peduncle is particular, because the second basal leaf accretes with a peduncle from the basis up to an inflorescence and functions as the lower floral leaf. This accretion of basal leaf with a peduncle leads to a dissymmetric and the specific configuration of the transverse section of the peduncle. Peduncles are often polyhedric with a 'channel-like' structure inside (Peruzzi et al., 2007) and two circles of vascular bundles (Levichev, 2001). In our research, number of channel-like structures was one in all investigated populations except the populations from Seličevica (some plants had one, while others had two channel-like structures) and Gamzigradska banja (some plants had two, while others had three channel-like structures). These two populations are also distinguished by the presence of arms and hairs on their peduncles, which has not been recorded in the previous studies. We assume that the largest channel-like structure often followed by the presence of arms is a result of an incomplete concrescence between the first cauline leaf and peduncle.

Conclusion

Populations of *G. pratensis* analysed in this study are morpho-anatomically differentiated. Population from Gamzigradska banja is separated from all the other populations based on height of the whole plant, width of bulb, width and length of basal leaf, width and indumentum type of peduncle, length of first cauline leaf, width and length of second cauline leaf, and width of outer and inner segments of perigone. The values of the characters analysed in the population from Seličevica tend to be transit in between population from Gamzigradska banja and all the others populations. In order to determine the basis of morphological and anatomical differences, it is necessary to carry out analysis of the influence of ecological factors on investigated characters, perform experiments with cultivated plants, and conduct a molecular analysis.

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