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MORPHOLOGICAL AND GENOME SIZE VARIATIONS WITHIN POPULATIONS OF *EDRAIANTHUS GRAMINIFOLIUS "JUGOSLAVICUS"* (*CAMPANULACEAE*) FROM THE CENTRAL BALKAN PENINSULA

TAMARA RAKIĆ[1], SONIA SILJAK–YAKOVLEV[2], JASMINA ŠINŽAR-SEKULIĆ[1], MAJA LAZAREVIĆ[1], BRANKA STEVANOVIĆ[1], V. STEVANOVIĆ[1] and D. LAKUŠIĆ[1]

[1] *Chair for Plant Ecology and Phytogeography, Faculty of Biology, University of Belgrade,* 11000 Belgrade, Serbia
[2] *University Paris-Sud, CNRS-AgroParisTech-UPS, UMR 8079, Ecologie, Systématique, Evolution, Bât. 360,* 91450 Orsay, France

Abstract – The *E. graminifolius* complex is widely distributed in the continental part of the central and western Balkan Peninsula and is characterized by pronounced morphological variability. Plants grow on different geological substrates, span a wide altitudinal range and inhabit heterogeneous microclimatic conditions. The aim of this study was to compare morpho-anatomical and genome size variations among 31 populations of *E. graminifolius*, and to correlate morpho-anatomical characteristics of plants with the geomorphologic and bioclimatic characteristics of their habitats. For these purposes, multivariate statistical analyses were performed. Results showed that most of morphological variability could be explained as the adaptive responses of plants to diverse environmental conditions that accompany life at different altitudes. Populations from SE Serbia had larger genome size in respect to other investigated populations. Genome size was bigger in sympatric populations of *Edraianthus* then in allopatric ones. Apart from the general morphological variability, plants from the Ovčar-Kablar Gorge are particularly morphologically specific.

Key words: Balkans, differentiation, *Edraianthus graminifolius,* genome size, morphometry

INTRODUCTION

The genus *Edraianthus* represents one of the taxonomically and biogeographically most interesting and polymorphic genera of the Balkan flora. It has drawn the attention of some well-known botanists, which resulted in three published monographs related to the morphological characteristics and taxonomy of this genus (Wettstein, 1887; Janchen, 1910; Lakušić, 1974). Recently, the genus *Edraianthus* was the subject of extensive molecular phylogenetic and phylogeographic (Stefanović et al., 2008; Surina et al., 2011), cytogenetic (Medjedović, 1981; Medjedović et al., 2007; Siljak-Yakovlev et al., 2010), taxonomic (Lakušić et al., 2009; Surina et al., 2009; Surina and Lakušić, 2010), and morpho-anatomical studies (Rakić, 2010; Rakić et al., 2012). The overall results of these studies confirmed the existence of extreme complexity within this genus that mainly derives from its outstanding morphological plasticity and processes of hybridization and cryptospeciation.

Phenotypic plasticity is especially pronounced within the *E. graminifolius* complex, which accordingly represents the most taxonomically intriguing group of the genus *Edraianthus* (Rakić et al. 2012). The studies on molecular phylogeny (Stefanović et al., 2008), based on chloroplast DNA sequence analysis, recognized 17 different molecularly distinct groups within the *E. graminifolius* complex, which, along with the recent multivariate statistical analysis of morphological parameters (Rakić, 2010; Rakić et al., 2012), showed that several morphologically distinct groups of populations exist within the *E. graminifolius* complex from the central Balkans. They largely correspond to taxa proposed by R. Lakušić in 1974, such as the formally non-accepted *E. caricinus, E. jugoslavicus, E. vesovicii*, and *E. montenegrinus*.

Populations of *E. graminifolius* that inhabit the continental part of the central and western Balkan Peninsula (for a map of distribution of populations named *E. graminifolius "jugoslavicus"* see Fig. 7 in Stefanović et al., 2008) are also named *E. jugoslavicus* (= *Edraianthus jugoslavicus* Lakušić, des. inval. in God. Biol. Inst. u Sarajevu 26, suppl: 49. 1974, applied to: *Edraianthus graminifolius* (L.) A. DC., according to Castroviejo et al., 2010) or *E. graminifolius "jugoslavicus"* (according to Stefanović et al., 2008; Rakić et al., 2012). The informal name *E. graminifolius "jugoslavicus"* is used as a combination of formal nomenclature presented by several authors (Kuzmanov, 1976; Greuter et al., 1984; Lammers, 2007; Castroviejo et al., **2010)** and proposed by Lakušić (1974). This name is applied to the populations of *E. graminifolius* s.l. restricted to the canyons, gorges, and mountain and subalpine regions of the mountains of the central part of the Balkan Peninsula (for details see Lakušić, 1974 and Stefanović et al., 2008).

Its populations extend over a wide range of elevations from 100 to 1950 m, and inhabit microclimatically heterogeneous habitats; plants frequently grow on limestone, but can also be found on different geological substrates, such as silicate or dolomite. Published data on cpDNA sequence analysis (Stefanović et al., 2008) showed that within these populations, named *E. graminifolius "jugoslavicus"*, there are a few molecularly distinct groups. Among them, the most specific are those populations that grow exclusively on silicate bedrock on both acid and basic (serpentine) soils, such as the population from Nebeske Stolice (Kopaonik Mt.), populations that inhabit the limestone cliffs of the Ovčar-Kablar Gorge in western Serbia, and populations from subalpine grasslands on the Suva Planina Mt. and surrounding areas and the gorges of Sićevo and Jerma (SE Serbia).

Taking into account the pronounced morphological heterogeneity of *E. graminifolius "jugoslavicus"* on the central Balkan Peninsula, the aim of this study was: 1) to quantify morphological and anatomical variation between populations of *E. graminifolius "jugoslavicus"* on the basis of multivariate statistics; 2) to analyze the nuclear DNA content; 3) to describe the differentiation among populations, and 4) to correlate the morpho-anatomical characteristics of plants with the geomorphologic and bioclimatic characteristics of the habitats.

MATERIALS AND METHODS

Plant material

The plant material was sampled from 31 populations of *E. graminifolius "jugoslavicus"* from the continental part of the central Balkan Peninsula, from altitudes between 100 and 1920 m (Fig. 1, Table 1). The geographic position of each population was recorded using a hand-held Global Positioning System (GPS Garmin eTrex Vista® C). From each of the 31 populations, 15 plant samples were used for morphological and anatomical analysis. For genome size measurements, 3-5 individuals were analyzed from each of the 16 populations. Voucher specimens are deposited in the Herbarium of the Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade (BEOU).

Morpho-anatomical analysis

Morphometric analyses were performed on dissected plant organs (rosette and cauline leaves, involucral bracts, stem and flowers) previously well preserved in glycerol: 96% ethanol (50:50, v/v). The anatomical structure of rosette leaves was analyzed on leaf cross sections obtained on a cryostat Leica CM 1850

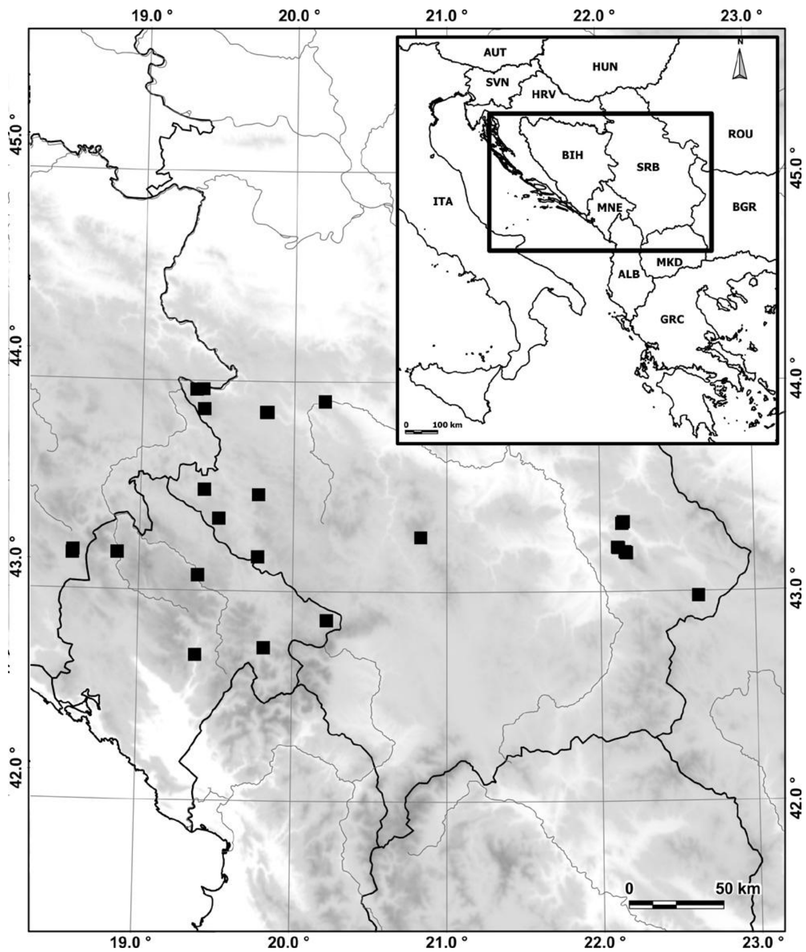

Fig. 1. Distribution of sampled populations of *E. graminifolius "jugoslavicus"* used in this study

at -21°C. Sections were cleared in parazone, washed in water and stained in safranin (1% w/v in 50% ethanol) and alcian blue (1% w/v, aqueous). Epidermal peels for analysis of stomata were obtained by warming the leaves in glacial acetic acid: 20% H_2O_2 (1:1, v/v); they were then thoroughly washed in water and mounted in glycerol (Jain 1976). All photographs were taken by digital camera Leica DFC295 and light microscope Leica DMLS. Leaf, stem and flower surface structures were analyzed with a Leica MZ75 stereomicroscope and JEOL JSM-6460LV scanning electron microscope. The measured morpho-anatomical characters are listed in Tables 2 and 3. All measurements were performed in Leica Q Win (Leica Microsystems). Meristic and qualitative characters used in the present analysis are as follows: *Stem.* Frequency of non-glandular trichomes, St_d. Trichome type, St_tp. Trichome direction, St_dir. *Rosette leaf.* Leaf shape, Lb_sh. Non-glandular trichomes: frequency of on the leaf upper surface, UpT_d, frequency on the leaf lower surface, LoT_d. *Involucral bracts*. Number of bracts, No_B. Shape of external bract, Br_sh. Leaf margins type, Br_c. Non-glandular trichomes: frequency of at the upper bract surface, Brup_d, direction on the upper bract surface, Brup_dir, frequency at the lower bract surface, Brlo_d, direction on the lower bract surface, Brlo_dir. *Inflorescence.* Number of flowers, No_F. *Cross section* - Shape, S_sh. Number of lateral vascular bundles, Blat_ no. Number of upper palisade tissue cell layers, Upt_NoL. Number of lower palisade tissue cell layers, Lpt_NoL. Number of spongy tissue cell layers, Spt_NoL.

Table 1. Sample provenances, habitats and herbarium vouchers

Sample provenances	Acronym	Habitat	Voucher
Montenegro: Canyon of Morača	MORAC	rocky crevices, limestone, *Asplenietea trichomanes,* 100 m altitude	BEOU-26619
Montenegro: Canyon of Morača	MORAC	rocky crevices, limestone, *Asplenietea trichomanes,* 230 m altitude	BEOU-24337
Montenegro: Canyon of Tara	KTARE	rocky crevices, limestone, *Asplenietea trichomanes,* 600 m altitude	BEOU-27013
Montenegro: Canyon of Piva	PIVA	rocky crevices, limestone, *Asplenietea trichomanes,* 690 m altitude	BEOU-27012
Montenegro: Canyon of Lim	LIM	rocky crevices, limestone, *Asplenietea trichomanes,* 750 m altitude	BEOU-24022
Montenegro: Canyon of Lim	LIM	rocky crevices, limestone, *Asplenietea trichomanes,* 550 m altitude	BEOU-26623
Montenegro: Canyon of Ibar	IBAR	rocky crevices, limestone, *Asplenietea trichomanes,* 1020 m altitude	BEOU-24023
Bosnia and Herzegovina: Canyon of Sutjeska	SUT	rocky crevices, limestone, *Asplenietea trichomanes,* 750 m altitude	BEOU-27027
Serbia: Canyon of Varoška river	VAR	rocky crevices, limestone, *Asplenietea trichomanes,* 700 m altitude	BEOU-26618
Serbia: Canyon of Đetina	DJET	rocky crevices, limestone, *Asplenietea trichomanes,* 625 m altitude	BEOU-26615
Serbia: Canyon of Đetina	DJET	rocky crevices, limestone, *Asplenietea trichomanes,* 515 m altitude	BEOU-26616
Serbia: Canyon of Derventa	DERV	rocky crevices, limestone, *Asplenietea trichomanes,* 300 m altitude	BEOU-20891
Serbia: Canyon of Derventa	DERV	rocky crevices, limestone, *Asplenietea trichomanes,* 300 m altitude	BEOU-26612
Serbia: Canyon of Derventa	DERV	rocky crevices, limestone, *Asplenietea trichomanes,* 400 m altitude	BEOU-26613
Serbia: Ovčarsko-Kablarska gorge	KABL	rocky crevices, limestone, *Asplenietea trichomanes,* 300 m altitude	BEOU-26617
Serbia: Ovčarsko-Kablarska gorge	KABL	rocky crevices, limestone, *Asplenietea trichomanes,* 300 m altitude	BEOU-24020
Serbia: Ovčarsko-Kablarska gorge	KABL	rocky crevices, limestone, *Asplenietea trichomanes,* 300 m altitude	BEOU-20960
Serbia: Sićevo gorge	SIC	rocky crevices, limestone, *Asplenietea trichomanes,* 245 m altitude	BEOU-26626
Serbia: Sićevo gorge	SIC	rocky crevices, limestone, *Asplenietea trichomanes,* 400 m altitude	BEOU-24070
Serbia: Canyon of Jerma	JER	rocky crevices, limestone, *Asplenietea trichomanes,* 600 m altitude	BEOU-20893
Bosnia and Herzegovina: Gacko	GAC	rocky grounds, limestone, 1140 m altitude	BEOU-27022
Bosnia and Herzegovina: Gacko	GAC	rocky grounds, limestone, 1070 m altitude	BEOU-27020
Serbia: Mt. Jabuka	JAB	rocky grounds, limestone, *Festuco-Brometea,* 1220 m altitude	BEOU-24295
Serbia: Mt. Tara	TARA	alpine grassland, limestone, *Festuco-Seslerietea,* 980 m altitude	BEOU-26614
Serbia: Mt. Tara	TARA	rocky crevices, limestone, *Asplenietea trichomanes,* 980 m altitude	BEOU-27008
Serbia: Mt. Kopaonik	KOPBS	rocky crevices, limestone, *Asplenietea trichomanes,* 1810 m altitude	BEOU-27612
Serbia: Mt. Kopaonik	KOPNS	rocky crevices, serpentine, *Asplenietea trichomanes,* 1920 m altitude	BEOU-27610
Serbia: Mt. Suva planina	SPDEV	alpine grassland, limestone, *Festuco-Seslerietea,* 1550 m altitude	BEOU-27438
Serbia: Mt. Suva planina	SPSOK	alpine grassland, limestone, *Festuco-Seslerietea,* 1300 m altitude	BEOU-27504
Serbia: Mt. Suva planina	SPSOK	alpine grassland, limestone, *Festuco-Seslerietea,* 1500 m altitude	BEOU-27503
Serbia: Mt. Suva planina	SPTR	alpine grassland, limestone, *Festuco-Seslerietea,* 1800 m altitude	BEOU-27439

Chromosome preparation

For mitotic chromosome analysis of the population from the Ovčar-Kablar Gorge, root-tip meristems of germinated seeds were pre-treated with 0.002 M 8-hydroxyquinoleine for 4 h 30 min at 16°C, and fixed in cold 3/1 (v/v) ethanol/acetic acid for 48 h. After hydrolysis in 1N HCL for 12 min at 60°C and staining in Schiff's reagent for 2 h, the squash was realized in a drop of acetic carmine. Chromosomes were observed under a Leica DMLS light microscope and photographed by DFC295 digital camera.

Flow cytometry for genome size assessment

DNA amounts were determined by flow cytometry following Marie and Brown (1993). Cell nuclei were isolated from young leaves of at least five individuals per population. Tomato *Lycopersicon esculentum* Mill. cv. Roma (2C=1.99 pg, Marie and Brown, 1993) was used as internal standard. The standard and investigated species leaf tissues were simultaneously chopped with a razor blade in a Petri dish in 600 µl of cold buffer (Galbraith et al., 1983), supplemented with 1% polyvinyl-pyrrolidonyl 10.000 and 5 mM sodium metabisulfite. Nuclei suspensions were filtered through 48 µm nylon mesh, treated with RNAse (2.5 U/mL) at 4°C and stained with 50 µg/mL propidium iodide (Sigma Chemical Co. St. Louis, USA). For each sample, at least 5 000 to 10 000 nuclei were measured. The 2C DNA value was calculated using the linear relationship between the fluorescent signals from stained nuclei of the investigated species and the internal standard.

A distribution map was produced with Manifold 5.50 software (Manifold System, CDA International Ltd.).

Statistical analysis

Descriptive statistics (minimal value, mean value, maximal value, variance, standard deviation (std. dev.) and coefficient of variation (CV)) were performed for each continuous character. A principal component analysis (PCA) was performed on the complete data set (comprising 465 individuals and 59 characters of which 35 morphological and 24 anatomical characters) to show the overall morphological and anatomical variation and relationships between individuals from all populations. The hypothesis of morphological and anatomical separation of analyzed populations was tested by a canonical discriminant analysis (CDA) based on the complete data set. CDA analyses were performed on individuals, and the obtained results are represented graphically in respect to *a priori* defined groups. Furthermore, classificatory discriminant analysis was used in order to obtain the percentage of correctly classified individuals, based on morphological and anatomical characters, respectively. Overall dissimilarity between populations was described by Mahalanobis distances, and for the clustering, the UPGMA method was used. Qualitative characters of all investigated populations were statistically analyzed by Multiple Correspondence Analysis (MCA) and presented on a scatterplot diagram and dendrogram constructed on the basis of the UPGMA cluster method. The regression analysis (linear regression) was performed to estimate the correlations between the variation of morpho-anatomical characters of *E. graminifolius "jugoslavicus"* and the basic bioclimatic habitat characteristics of each population, as well as between the variation genome size and the morpho-anatomical characters. The extraction of 19 bioclimatic parameters was done with DIVA-GIS 7.5 software (Hijmans et al., 2012). Prior to the regression analysis, morpho-anatomical characters were logarithmically transformed and habitat characteristics were tested for the multicollinearity using the variance inflation factor (VIF). Bioclimatic predictors that showed significant correlations with other predictors were not used in regression analysis of the anatomical characters. The statistical significance of the linear relationship between morpho-anatomical and bioclimatic habitat characteristics was adjusted by the Bonferroni correction of multiple comparisons. Tukey's HSD of Homogenous Groups for Unequal N post-hoc test was used to analyze differences in the genome size of 16 populations. All statistical analyses were performed using the package Statistica 5.1 (StatSoft 1996).

RESULTS

Morphological characteristics

Detailed morphological investigations showed that *E. graminifolius "jugoslavicus"* is characterized by exceptional morphological variability, especially related to stem height, shape and dimensions of involucral bracts and flowers, as well as to the general presence, density and orientation of hairs (Fig. 2, Table 2).

The stem is erect to ascending, longer in plants from canyons than in the high-mountain ones, and usually slightly to moderately covered by hairs directed towards the stem base. Rosette

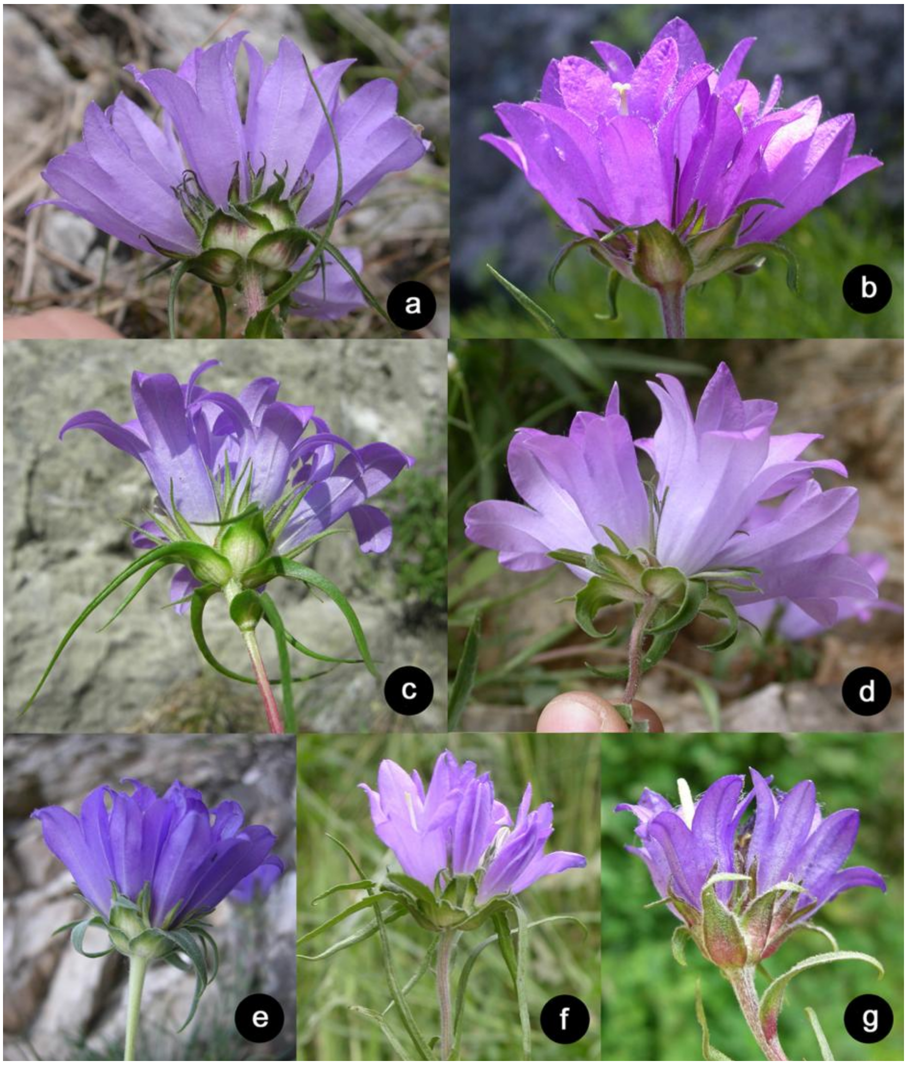

Fig. 2. Morphological variability within *E. graminifolius "jugoslavicus"*: (a) Serbia: Mt. Tara, alpine grassland, limestone, *Festuco-Seslerietea,* 980 m altitude, BEOU-26614; (b) Serbia: Mt. Kopaonik, rocky crevices, serpentine, *Asplenietea trichomanes,* 1 920 m altitude, BEOU-27610; (c) Montenegro: Canyon of Morača, rocky crevices, limestone, *Asplenietea trichomanes,* 100 m altitude, BEOU-26619; (d) Serbia: Canyon of Derventa, rocky crevices, limestone, *Asplenietea trichomanes,* 300 m altitude, BEOU-20891; (e) Serbia: Ovčar-Kablar Gorge, rocky crevices, limestone, *Asplenietea trichomanes,* 300 m altitude, BEOU-26617; (f) Serbia: Canyon of Jerma, rocky crevices, limestone, *Asplenietea trichomanes,* 600 m altitude, BEOU-20893; (g) Serbia: Mt. Suva planina, alpine grassland, limestone, *Festuco-Seslerietea,* 1 800 m altitude, BEOU-27439 (photography: D. Lakušić).

and cauline leaves are long, linear, canaliculated and pointed at the top. The lamina of the rosette leaves is usually glabrous, sometimes with rare and short non-glandular trichomes. The adaxial leaf side is mostly glabrous, except in plants from canyon populations in which the leaves are sparsely hairy with flattened trichomes oriented towards the leaf base. On the other hand, plants from the Ovčar-Kablar Gorge are densely covered with hairs that are especially pronounced on the adaxial leaf side and directed to the leaf apex, or orientated in all directions, except towards the leaf base. Cauline leaves are ovate to widely ovate in base and elongated at the top, linear and more

Table 2. Descriptive statistics (N – number of samples, min – minimum value, mean – mean value of the continuous variable, max – maximum value, std.dev. – standard deviation, CV – coefficient of variation) for analyzed morphological characters

Character	N	min	mean	max	std.dev.	CV (%)
Stem (mm)						
Stem height - St_H0	387	15.9	144.4	283	50.6	35.0
Rosette leaf (mm)						
Maximal width - Lb_W0	268	1.0	2.5	5	0.7	28.4
Width in the upper quarter - Lb_W1	269	0.7	1.5	4	0.4	28.9
Total length (mm) - Lb_L	264	17.8	105.8	231	37.7	35.6
Stem leaf (mm)						
Maximal width of the leaf - Le_W1	373	1.8	4.2	11	1.5	36.5
Width in the upper quarter - Le_W2	374	0.7	1.4	4	0.4	28.9
Total length - Le_L0	371	16.2	52.9	98	16.1	30.4
Distance between the largest leaf width point and the leaf base - Le_L1	373	1.1	3.2	10	1.3	40.3
Inner involucral bract (mm)						
Maximal width - B1_W1	409	1.9	6.8	15	2.1	30.7
Width in the upper quarter - B1_W2	407	0.6	1.8	5	0.7	39.6
Total length - B1_L0	407	7.6	18.3	47	5.3	29.0
Distance between the largest bract width point and the bract base - B1_L1	411	1.5	4.8	11	1.4	30.4
Base length - B1_L2	408	5.3	12.5	38	4.6	36.9
Central involucral bract (mm)						
Maximal width - B2_W1	411	2.7	8.4	19	2.5	30.0
Width in the upper quarter - B2_W2	407	0.4	1.5	4	0.5	36.1
Total length - B2_L0	407	9.1	24.7	78	8.0	32.3
Distance between the largest bract width point and the bract base - B2_L1	411	1.2	4.0	10	1.2	29.00
Base length - B2_L2	409	5.3	12.2	45	6.4	52.90
Outer involucral bract (mm)						
Maximal width - B3_W1	411	3.1	7.8	15	2.2	28.2
Width in the upper quarter - B3_W2	409	0.5	1.3	3	0.4	31.8
Total length - B3_L0	405	10.5	35.8	90	11.2	31.2
Distance between the largest bract width point and the bract base - B3_L1	410	1.2	3.0	7	1.0	32.3
Base length - B3_L2	407	3.1	13.6	57	11.4	83.8
Calyx (mm)						
Diameter - Ca_W0	377	3.4	6.2	12	1.4	21.7
Calyx lobe base width - Ca_W1	379	1.1	2.4	5	0.6	25.8
Calyx lobe width in the upper quarter - Ca_W2	379	0.4	0.9	2	0.2	26.8
Calyx lobe total length - Ca_L1	379	3.1	9.4	23	3.1	33.5
Corolla (mm)						
Maximal width - Co_W1	336	7.6	14.7	26	3.2	22.0
Width of the lobe base - Co_W2	340	3.4	6.6	13	1.5	22.2
Total height - Co_H1	338	11.7	23.7	39	5.0	21.1
Height of the lobe - Co_H2	340	5.4	10.5	18	2.3	21.7
Style (mm)						
Length - St_L	361	10.1	21.7	33	4.5	20.9
Anther (mm)						
Length - An1_L	364	3.8	7.5	13	1.6	21.3
Filamentum length - An2_L	298	0.5	1.6	4	0.6	35.5
Length of filamentum base - An3_L	198	0.9	2.4	5	0.8	31.7

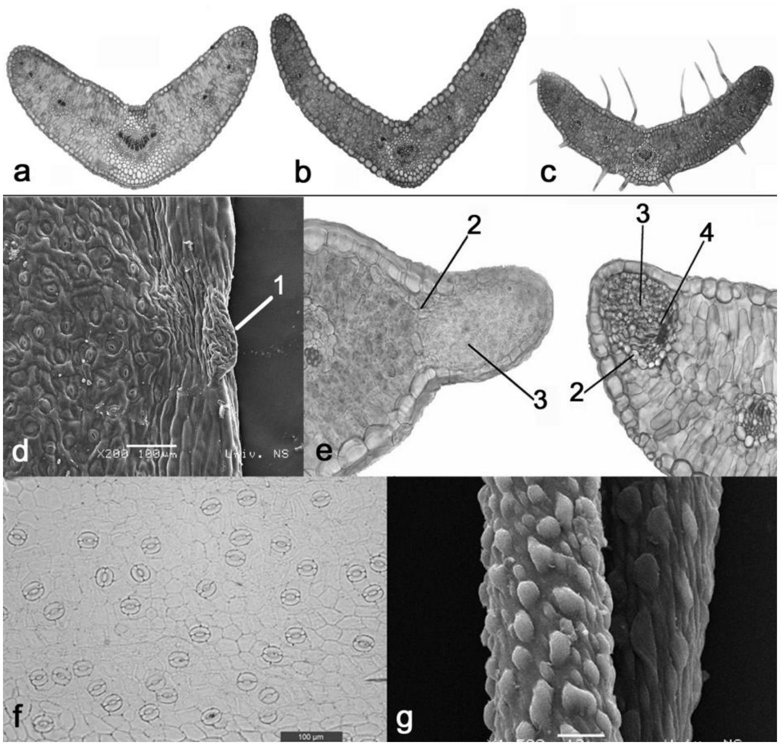

Fig. 3. Anatomical characteristics of *E. graminifolius "jugoslavicus"*: (a-c) Cross section of rosette leaves (a – Jabuka Mt.; b – Derventa Canyon; c – Ovčar-Kablar Gorge); (d) Leaf blade with hidatode-1; (e) Cross section of hydathode (2 – parenchyma cells of vascular bundle, 3 – epithem, 4 – tracheids); (f) Anomocytic stomata on the leaf surface; g) micropapillose structure of mechanical trichome (SEM).

or less narrow, glabrous to moderately hairy, in some cases with slightly and rarely toothed edges. Upper cauline leaves are similar to involucral bracts. The involucral bracts (3-) 5-9 (-19) are more or less wide, ovate to cordate in base. Bract margin can be entire, undulate or serrate. Outer involucral bract is longer than inflorescence. Their surfaces are usually glabrous, with the exception of plants from the Ovčar-Kablar Gorge in which they are densely covered with hairs directed towards the apex. The rare hydathodes are situated irregularly along the margins of leaves, involucral bracts, and calyx lobes. Flowers are sessile and organized in a terminal cluster, composed of (1-) 3-7 (-14) flowers, and closely subtended by numerous involucral bracts. Calyx lobes are subulate, often with revoluted appendix. The corolla is blue violet to purple, rarely light blue to white, glabrous or ciliate at veins and corolla lobes. Its lobes are slightly revolute and reflexed at the apex. Five anthers and style do not exceed the corolla length. Capsules open by irregular apical rupture, except in plants from the Ovčar-Kablar Gorge in which they open by basal lateral pores. Seeds are ovate, brownish and flattened.

Leaf anatomy

Rosette leaves are isolateral. The palisade tissue strongly dominates over the less pronounced spongy parenchyma, which in some leaves can even be absent. In most of the studied plants, there is no clear distinction between the palisade-like and spongy cells, but there are rather different intergrading cell shapes. Palisade tissue at the adaxial leaf side is constituted by 1 to 4 layers of palisade cells, usually 2, whereas at the abaxial leaf side it is formed by 1 to 3 cell layers, most often just by one. Only in shade-adapted plants from the Der-

Table 3. Descriptive statistics (min – minimum value, mean – mean value of the continuous variable, max – maximum value, std.dev. – standard deviation, CV – coefficient of variation) for analyzed morpho-anatomical characters

Character	N	min	mean	max	std.dev.	CV (%)
Cross section (μm)						
Width - L_w	372	857.6	1506.8	4744	393.5	26.1
Thickness - S_th	372	60.3	301.0	681	71.2	23.7
Area - S_a	372	179881.7	426181.1	1517553	177126.3	41.6
Perimeter - S_c	372	1963.9	3291.4	9867	804.2	24.4
Area of the central vascular bundle - Cb_a	372	7855.4	24076.1	122702	12497.6	51.9
Total vascular bundles area - Tb_a	372	16541.6	46240.4	169811	19327.6	41.8
Cuticle thickness - C_th	374	2.9	10.7	31	3.6	33.2
Upper epidermal cell: Height - Uec_h	374	11.4	22.8	51	5.8	25.3
Width - Uec_w	374	14.5	28.5	59	6.6	23.3
Lower epidermal cell: Height - Lec_h	372	12.2	23.4	59	5.7	24.3
Width - Lec_w	374	17.4	27.9	81	6.3	22.6
Upper palisade tissue: Thickness - Upt_th	370	26.2	104.6	263	37.6	36.0
Cell height - Upc_h	370	17.0	44.4	113	10.5	23.7
Lower palisade tissue: Thickness - Lpt_th	365	0.0	66.8	203	37.5	56.2
Cell height - Lpc_h	365	0.0	39.8	110	17.4	43.7
Spongy tissue: Thickness - St_th	357	15.0	68.5	185	30.3	44.3
Cell hight - Sc_h	358	12.5	24.7	54	5.9	23.8
Adaxial stomata: Perimeter - StC_Uep	320	433.7	728.6	1419	131.8	18.1
Length, StL_Uep	320	23.8	32.8	47	4.0	12.2
Width, StW_Uep	320	20.5	26.5	41	2.5	9.5
Abaksial stomata: Perimeter- StC_Lep	318	364.4	756.7	1042	111.0	14.7
Length, StL_Lep	318	23.2	34.0	45	3.3	9.6
Width, StW_Lep	318	19.1	26.5	34	2.4	8.9

venta Canyon are leaves dorsiventral, and the spongy tissue strongly dominates over the palisade one (Figs. 3 a-c).

Vascular tissue is well developed and represented by 1 central and 4-13 lateral ones, the number of which directly corresponds to the leaf width. Hydathodes are situated in shallow depressions along the margins of leaves, involucral bracts and calyx lobes, and they more or less protrude above the margin (Figs. 3d-e). Anomocytic stomata are more numerous at the upper than at the lower leaf side, and they are rare or absent along leaf veins and leaf margin (Fig. 3f). The highest frequency of stomata is found in leaves of high mountain populations, but also in plants from the Ovčar-Kablar Gorge, whose stomata are in addition particularly small. The largest stomata, present with low frequency, are found in leaves of plants from canyons (Morača, Jerma, Sutjeska and Derventa) as well as in plants from Kopaonik Mt. (Nebeske Stolice, approximately 2 000 m a.s.l.).

Unicellular non-glandular trichomes occur on the stem surface and along the margins of leaves,

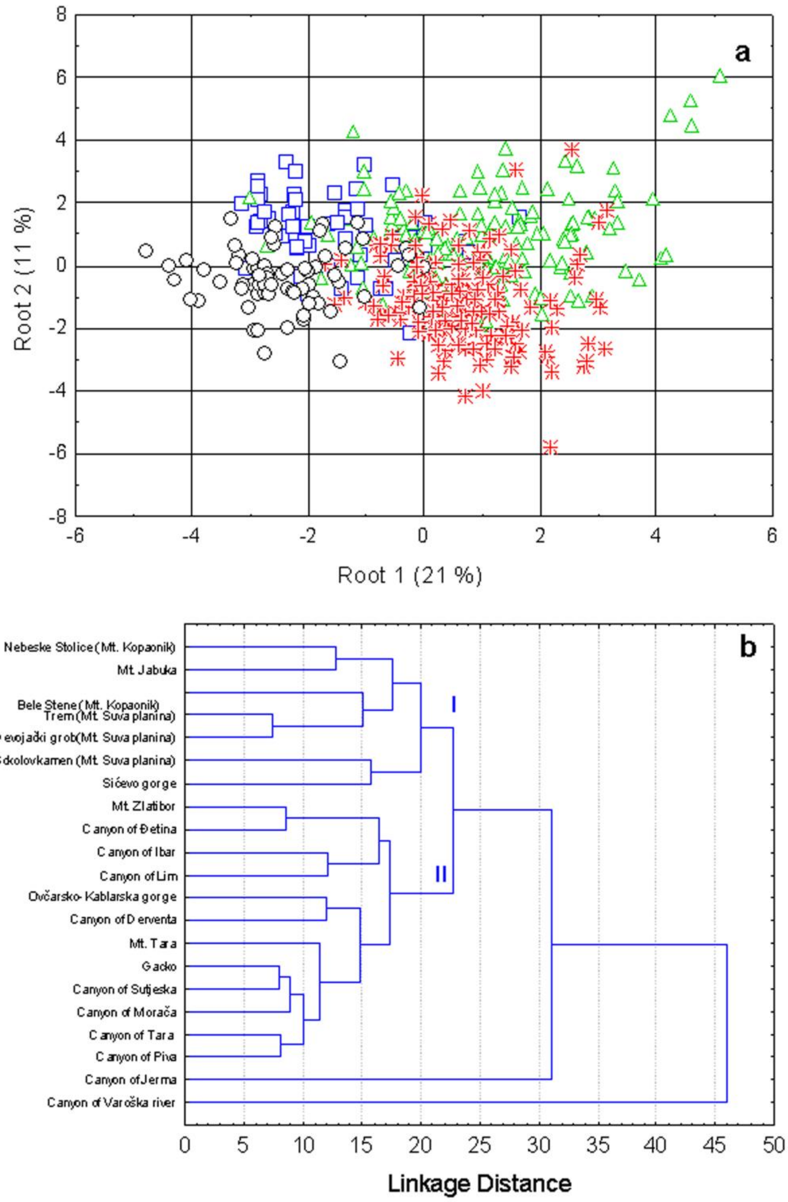

Fig. 4. Results of: (a) Canonical Discriminant Analysis (CDA) of morphological characters plotted along the first two discriminant axes (□ high-mountain populations from central and SW Serbia (Kopaonik, Jabuka); △populations from western Serbia (Zlatibor, Tara, Djetina, Derventa, Kablar, Varoška Reka); ✳ canyon populations from Montenegro and western Bosnia, (Morača, Tara, Lim, Piva, Ibar, Gacko, Sutjeska); ○ SE Serbia (Suva planina, Sićevo); ✚ SE Serbia (Jerma); (b) Cluster Analysis of morphological characters based on Mahalanobis distances.

involucral bracts, calyx teeth and corolla lobes. The density of hairs and their orientation differ among populations. The surface of vegetative organs is mostly glabrous, but it can also be partially and slightly covered by hairs. Plant organs are rarely completely covered by trichomes, such as in plants from the Jerma Gorge, Sutjeska Canyon, Derventa Canyon and Suva Planina Mt.. The exceptionaly dense hair cover

Table 4. Results of PCA for morphological, anatomical and morpho-anatomical characters within *E. graminifolius "jugoslavicus"* (For explanation of character acronyms see Tables 2 and 3).

	morphology			anatomy			morpho-anatomy		
	PCA 1	PCA 2	PCA 3	PCA 1	PCA 2	PCA 3	PCA 1	PCA 2	PCA 3
Eigenvalue	11.89	3.02	2.80	6.82	3.63	3.58	12.73	6.96	4.40
% Total variance	33.96	8.64	8.01	24.36	12.95	12.80	19.90	10.87	6.87
Cumulative Eigenvalue	11.89	14.91	17.71	6.82	10.45	14.03	12.73	19.69	24.09
Cumulative (%)	33.96	42.61	50.61	24.36	37.31	50.10	19.90	30.76	37.63
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
St_H0	-0.59076	-0.094937	-0.434620				0.58597	0.061884	0.160832
LbW0	-0.39321	0.037872	-0.109623				0.38985	-0.027301	0.006929
Lb_W1	-0.27750	0.334161	-0.062416				0.28550	-0.037068	-0.226178
Lb_L	-0.36126	-0.263359	-0.289008				0.37367	0.074595	0.270192
Le_W1	-0.71866	0.117747	0.098863				0.70279	-0.095830	-0.071492
Le_W2	-0.32738	0.496068	-0.080072				0.33234	-0.100311	-0.402738
Le_L0	-0.41285	-0.375166	-0.553186				0.39794	0.093683	0.384613
Le_L1	-0.39807	0.086203	-0.300105				0.38803	0.011726	0.018692
B1_W1	-0.69062	-0.209399	0.178459				0.67300	-0.117479	0.117651
B1_W2	-0.55945	0.373040	0.303985				0.56876	-0.045552	-0.302018
B1_L0	-0.71096	-0.077548	-0.430299				0.68917	0.060988	0.159373
B1_L1	-0.66254	0.309556	0.013061				0.65526	-0.053186	-0.175085
B1_L2	-0.58114	0.389312	-0.311321				0.58185	0.035384	-0.120719
B2_W1	-0.76928	-0.116026	0.190305				0.75271	-0.111508	0.054503
B2_W2	-0.52915	0.562825	0.175695				0.53125	-0.064913	-0.468994
B2_L0	-0.67363	-0.284150	-0.486778				0.65636	0.086913	0.326180
B2_L1	-0.58598	0.387377	-0.010984				0.57510	-0.038285	-0.170638
B2_L2	-0.40360	0.412578	-0.381520				0.40254	0.063150	-0.076922
B3_W1	-0.77708	0.031409	0.174583				0.75904	-0.087422	-0.041817
B3_W2	-0.54862	0.563348	-0.031585				0.54796	-0.071546	-0.446175
B3_L0	-0.60578	-0.366424	-0.523454				0.58441	0.120682	0.394910
B3_L1	-0.53156	0.348740	0.007066				0.52027	-0.044609	-0.067961
B3_L2	-0.21147	0.391967	-0.485080				0.21197	0.051442	-0.079562
Ca_W0	-0.67217	-0.136816	0.349619				0.65827	-0.042027	0.057468
Ca_W1	-0.64446	-0.053643	0.296667				0.64957	0.038595	-0.031242
Ca_W2	-0.32976	0.211961	0.357430				0.33815	0.026154	-0.201715
Ca_L1	-0.73865	-0.165800	-0.036695				0.73714	0.050694	0.114252
Co_W1	-0.76466	-0.166525	0.324479				0.76454	-0.036178	0.061263
Co_W2	-0.71980	-0.218448	0.314411				0.71803	0.036789	0.089554
Co_H1	-0.79055	-0.327689	0.103668				0.79435	0.079350	0.164167
Co_H2	-0.60949	-0.292170	0.008933				0.62740	0.211637	0.207031
St_L	-0.69832	-0.308429	0.221531				0.70022	0.019146	0.109579
An1_L	-0.64456	-0.210928	0.155252				0.63647	0.031562	0.059393
An2_L	-0.22399	-0.150766	0.167305				0.19822	0.067008	0.034542
An3_L	-0.38343	-0.072101	0.221661				0.38082	-0.075364	-0.099770
C_th				0.426276	0.156204	0.297691	0.16524	-0.401735	0.439297
Uec_h				0.545373	-0.224671	0.328679	0.24704	-0.512899	0.278993
Uec_w				0.540443	-0.249684	0.270083	0.23516	-0.513843	0.188737
Lec_h				0.400931	0.149060	0.277970	-0.00617	-0.376723	0.352278
Lec_w				0.514398	0.184141	0.259916	0.02320	-0.490178	0.378798
Upt_h				0.501472	0.710439	-0.040106	-0.36860	-0.502612	0.250289
Upc_h				0.453232	0.425728	0.209113	-0.23772	-0.431708	0.331930
Lpt_h				0.336553	0.781371	-0.062225	-0.39027	-0.343100	0.280398
Lpc_h				0.344578	0.685828	0.001705	-0.35432	-0.345270	0.257771
St_h				0.147038	-0.356914	0.098382	0.09934	-0.140497	-0.090786
Sc_h				0.438541	0.119489	0.171517	-0.09017	-0.426354	0.168433
L_w				0.639756	0.640225	0.066706	-0.28525	-0.634138	0.340932
S_a				0.956659	-0.066054	0.012926	0.00819	-0.953905	0.030876
S_c				0.815476	-0.437610	-0.089512	0.17667	-0.819595	-0.211319
S_w				0.773351	-0.492871	-0.103696	0.19470	-0.777955	-0.251849
LB_l				0.733283	-0.476876	-0.141465	0.17731	-0.738337	-0.284002
LPrT_h				0.048269	-0.049103	0.231768	0.05369	-0.037596	0.159844
LB_d				0.607074	-0.441854	-0.152755	0.22365	-0.619618	-0.306738
CB_a				0.593901	-0.061752	0.245473	0.05666	-0.583162	0.238799
TB_a				0.792364	-0.046655	0.114702	0.02840	-0.787534	0.144530
StC_Uep				-0.179609	-0.012544	0.807380	0.12618	0.221152	0.597113
StL_Uep				-0.137904	-0.059912	0.716514	0.13823	0.173004	0.506755
StW_Uep				-0.223505	-0.026554	0.729201	0.15352	0.263724	0.540691
StC_Lep				-0.209222	-0.051222	0.686158	0.05194	0.239932	0.449450
StL_Lep				-0.270195	-0.165673	0.554773	0.08938	0.293005	0.295302
StW_Lep				-0.157429	0.011739	0.622212	0.06128	0.191361	0.465615
StA_Uep				-0.010206	0.393568	-0.134700	-0.27567	0.008300	0.064514
StA_Lep				0.032019	0.232985	0.073003	-0.19934	-0.028307	0.089809

characterizes plants from the Ovčar-Kablar Gorge, resulting in grayish reflection of its stem, leaves, involucral bracts and calyx. The surface of the cuticle of non-glandular trichomes has a distinct micropapillose structure (Fig. 3g).

Multivariate analysis of morpho-anatomical characters

The analysis of the coefficient of variation (CV) shows that there is a certain hierarchy in variability of the analyzed morphological and anatomical traits. Involucral bracts, leaves and photosynthetic tissues exhibit higher variability, whereas other plant organs and structures, such as flowers or stomata, exhibit a more conservative character (Tables 2 and 3). The most variable characters (CV>50%) are length of the base of the central and the outer involucral bracts (B2_L2, B3_L2), thickness of the lower palisade tissue (Lpt_h) and central vascular bundle area (CB_a). A coefficient of variation lower than 20% characterizes only the dimensions of stomata, while all other investigated characters are moderately variable (CV 20-50%).

The principal component analysis (PCA) of morphological, anatomical and morpho-anatomical characters together indicate that the structural variability of the sample is complex, since the first three derived PCA components accounted for 50.6%, 50.1% and 37.6% of the total variability, respectively (Table 4). Most of the variation of morphological parameters within the first component is due to the maximal width of the cauline leaf (Le_W1), total length of the inner involucral bract (B1_L0), maximal width of the central and outer involucral bracts (B2_W1, B3_W1), length of the calyx lobe (Ca_L1), corolla height (Co_H1) and width (Co_W1), and width of the corolla lobe (Co_W2). Among anatomical characters, most of the variation within the first component is due to the thickness of the upper and lower palisade tissues (Upt_h, Lpt_h), thickness (L_w), area (S_a), perimeter (S_c), and width of the cross section (S_w), as well as to dimensions of the stomata in the upper epidermis – perimeter (StC_Uep), length (StL_Uep) and width (StW_Uep). The PCA of both morphological and anatomical characters together showed that characters with the strongest influence on sample variability described on the first three axes are maximal width of the cauline leaf (Le_W1), maximal width of the central and outer involucral bracts (B2_W1, B3_W1), length of the calyx lobe (Ca_L1), all corolla dimensions, as well as cross section area (S_a), perimeter (S_c) and width (S_w) (Table 4).

The canonical discriminant analysis (CDA) of morphological parameters showed that, within a relatively homogenous structure of analyzed samples, there is a fine differentiation of populations according to both their geographical distribution and distribution along the altitudinal range (Fig. 4a). The correct classification of individuals into their *a priori* defined groups, based on the morphological parameters, was 77.87%. Mountainous populations from central (Kopaonik Mt. – Nebeske Stolice, Bele Stene; Jabuka Mt.) and south-eastern Serbia (Suva Planina Mt.– Trem, Devojački Grob, Sokolov Kamen, Sićevo Gorge) are distributed on the negative part of the first discriminant axis (DA). Mountainous and canyon populations of western Serbia are grouped together with canyon populations from Montenegro at the positive part of the first DA, but they are separated on the second discriminant axis. The cluster analysis recognizes two main groups (Fig. 4b) and two isolated populations – from the Jerma Gorge and the Varoška River Gorge. Group I includes mountainous populations from central and SE Serbia, while group II includes mountainous and canyon populations of W Serbia and Montenegro.

The analyzed populations show a very homogenous structure at the anatomical level, without indication of their differentiation according to geographical distribution or distribution along the vertical profile (Fig. 5a). The correct classification of individuals into their *a priori* defined groups, based on anatomical parameters, was 66.59%. Only plants from the Derventa Canyon are anatomicaly specific due to the dorsiventral structure of their leaves, strongly developed spongy tissue and thinner palisade tissue, in respect to plants from other investigated popula-

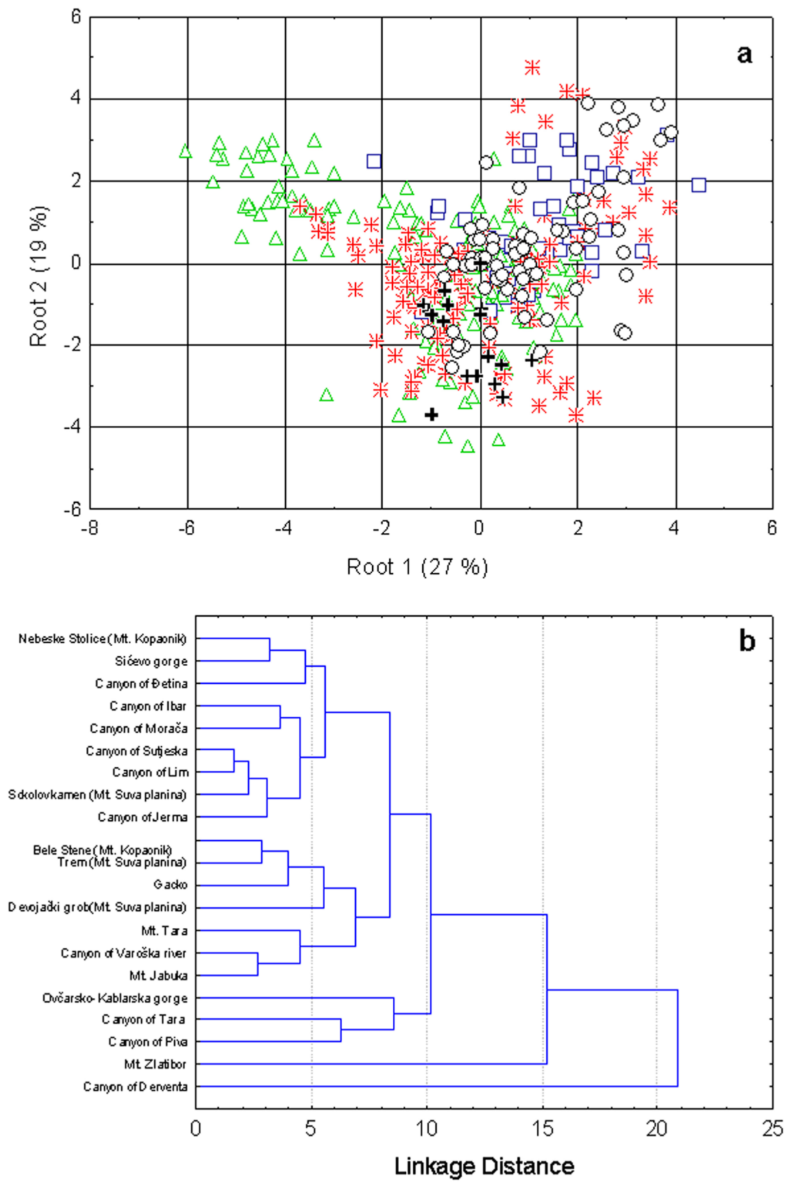

Fig. 5. Results of: (a) Canonical Discriminant Analysis (CDA) of anatomical characters plotted along the first two discriminant axes (☐ high-mountain populations from central and SW Serbia (Kopaonik, Jabuka); △ populations from western Serbia (Zlatibor, Tara, Djetina, Derventa, Kablar, Varoška reka); ✳ canyon populations from Montenegro and western Bosnia, (Morača, Tara, Lim, Piva, Ibar, Gacko, Sutjeska); ○ SE Serbia (Suva planina, Sićevo); ✚ SE Serbia (Jerma); (b) Cluster Analysis of anatomical characters based on Mahalanobis distances.

tions, which is particularly highlighted within the results of the cluster analysis (Fig. 5b).

The multiple correspondence analysis (MCA) shows that, in respect to the analyzed qualitative characters, the plants from the majority of the studied populations are similar (Fig. 6). The most frequent differences are related to the density of hair cover, shape of involucral bracts, shape of the leaf cross section and number of lateral vascular bundles.

Table 5. Summary statistics of regression (R^2) for independent bioclimatic variables
Abbreviations: BIO3 – Isothermality, BIO4 – Temperature seasonality, BIO6 – Min Temperature of Coldest Month, BIO7 – Temperature Annual Range, BIO8 – Mean temperature of the wettest quarter, BIO9 – Mean temperature of the driest quarter, BIO12 – Annual Precipitation, BIO14 – Precipitation of Driest Month, BIO15 – Precipitation seasonality, BIO18 – Precipitation of the warmest quarter. (For explanation of character acronyms see Tables 2 and 3).

	BIO3	BIO4	BIO6	BIO7	BIO8	BIO9	BIO12	BIO14	BIO15	BIO18
St_H0	0.30**	0.16	0.32***	0.27**	0.00	0.00	0.08	0.07	0.00	0.00
LbW0	0.07	0.07	0.04	0.09	0.03	0.06	0.01	0.01	0.08	0.01
Lb_W1	0.02	0.03	0.00	0.03	0.05	0.05	0.06	0.01	0.04	0.00
Lb_L	0.22*	0.14	0.35***	0.22*	0.01	0.03	0.12	0.06	0.01	0.00
Le_W1	0.07	0.20	0.02	0.18	0.08	0.18	0.09	0.00	0.20	0.00
Le_W2	0.04	0.01	0.00	0.03	0.06	0.06	0.06	0.01	0.03	0.00
Le_L0	0.11	0.09	0.20	0.14	0.02	0.01	0.12	0.04	0.02	0.00
Le_L1	0.04	0.05	0.02	0.06	0.00	0.00	0.00	0.00	0.00	0.01
B1_W1	0.20	0.20	0.16	0.26**	0.07	0.11	0.00	0.05	0.11	0.09
B1_W2	0.14	0.27**	0.09	0.26**	0.27**	0.13	0.06	0.01	0.12	0.01
B1_H0	0.02	0.08	0.03	0.08	0.00	0.07	0.02	0.08	0.05	0.07
B1_H1	0.14	0.15	0.07	0.17	0.10	0.06	0.01	0.01	0.14	0.01
B1_H2	0.14	0.24*	0.15	0.23*	0.06	0.00	0.00	0.01	0.03	0.00
B2_W1	0.25*	0.33***	0.18	0.38***	0.08	0.12	0.01	0.01	0.13	0.02
B2_W2	0.12	0.13	0.07	0.15	0.08	0.07	0.01	0.00	0.08	0.01
B2_H0	0.04	0.08	0.06	0.08	0.00	0.02	0.02	0.05	0.02	0.01
B2_H1	0.06	0.19	0.03	0.15	0.07	0.05	0.06	0.01	0.09	0.01
B2_H2	0.03	0.07	0.01	0.05	0.04	0.03	0.01	0.01	0.09	0.00
B3_W1	0.21	0.30**	0.14	0.33***	0.09	0.12	0.02	0.00	0.12	0.01
B3_W2	0.05	0.05	0.01	0.06	0.02	0.06	0.01	0.00	0.04	0.01
B3_H0	0.08	0.07	0.14	0.10	0.01	0.00	0.06	0.04	0.00	0.00
B3_H1	0.09	0.15	0.02	0.14	0.11	0.10	0.14	0.04	0.10	0.03
B3_H2	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.02
Ca_W0	0.07	0.28**	0.03	0.22*	0.15	0.23*	0.05	0.01	0.27**	0.08
Ca_W1	0.21*	0.27**	0.13	0.30**	0.13	0.10	0.01	0.02	0.16	0.02
Ca_W2	0.09	0.12	0.07	0.13	0.05	0.01	0.00	0.00	0.02	0.00
Ca_L1	0.28**	0.25**	0.19	0.32***	0.14	0.13	0.00	0.01	0.09	0.04
Co_W1	0.08	0.30**	0.11	0.26**	0.03	0.15	0.00	0.09	0.17	0.11
Co_W2	0.08	0.32***	0.12	0.27**	0.04	0.15	0.01	0.06	0.12	0.11
Co_H1	0.30**	0.37***	0.33***	0.43***	0.02	0.04	0.06	0.06	0.03	0.03
Co_H2	0.30**	0.20	0.32***	0.28**	0.01	0.00	0.07	0.03	0.00	0.00
St_L	0.17	0.38***	0.26**	0.37***	0.04	0.05	0.04	0.05	0.03	0.05
An1_L	0.16	0.40***	0.15	0.38***	0.05	0.08	0.00	0.00	0.05	0.01
An2_L	0.00	0.01	0.00	0.01	0.00	0.05	0.02	0.07	0.04	0.09
An3_L	0.17	0.08	0.13	0.13	0.04	0.01	0.01	0.07	0.06	0.03
L_w	0.28**	0.16	0.33***	0.24*	0.01	0.05	0.03	0.00	0.04	0.13
S_a	0.03	0.01	0.04	0.02	0.00	0.02	0.02	0.00	0.02	0.00
S_c	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.01
S_w	0.01	0.01	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.02
C_th	0.12	0.12	0.19	0.16	0.01	0.01	0.10	0.20	0.05	0.15
Uec_h	0.11	0.00	0.12	0.03	0.07	0.06	0.11	0.08	0.01	0.01

Table 5. Continued

	BIO3	BIO4	BIO6	BIO7	BIO8	BIO9	BIO12	BIO14	BIO15	BIO18
St_H0	0.30**	0.16	0.32***	0.27**	0.00	0.00	0.08	0.07	0.00	0.00
Uec_w	0.10	0.01	0.10	0.04	0.00	0.00	0.07	0.09	0.00	0.00
Lec_h	0.01	0.01	0.08	0.00	0.18	0.13	0.22*	0.16	0.03	0.01
Lec_w	0.01	0.00	0.04	0.00	0.02	0.01	0.07	0.11	0.00	0.00
Upt_h	0.34***	0.20	0.29**	0.28**	0.05	0.00	0.00	0.00	0.00	0.07
Upc_h	0.01	0.08	0.02	0.06	0.01	0.04	0.00	0.00	0.00	0.03
Lpt_h	0.21	0.12	0.14	0.15	0.12	0.01	0.00	0.00	0.03	0.01
Lpc_h	0.16	0.09	0.08	0.11	0.12	0.02	0.01	0.00	0.06	0.00
St_h	0.03	0.02	0.02	0.02	0.21*	0.00	0.07	0.09	0.00	0.03
Sc_h	0.00	0.00	0.00	0.00	0.02	0.06	0.00	0.00	0.00	0.11
CB_a	0.00	0.03	0.02	0.02	0.01	0.01	0.00	0.02	0.06	0.02
TB_a	0.01	0.01	0.00	0.00	0.00	0.01	0.00	0.04	0.03	0.05
StC_Uep	0.12	0.07	0.14	0.08	0.01	0.02	0.00	0.01	0.00	0.14
StL_Uep	0.16	0.10	0.15	0.13	0.01	0.00	0.00	0.00	0.00	0.11
StW_Uep	0.16	0.07	0.12	0.10	0.06	0.00	0.01	0.04	0.00	0.12
StC_Lep	0.00	0.02	0.00	0.00	0.02	0.01	0.06	0.09	0.00	0.09
StL_Lep	0.00	0.05	0.03	0.02	0.05	0.01	0.05	0.05	0.00	0.05
StW_Lep	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.03
StA_Uep	0.27**	0.26**	0.20	0.29**	0.13	0.00	0.01	0.01	0.01	0.05
StA_Lep	0.00	0.12	0.07	0.09	0.01	0.04	0.01	0.00	0.02	0.02

* – $P<0.05$ (210/0.05); ** – $P<0.01$ (210/0.01), *** – $P<0.001$ (210/0.001) (after Bonferroni correction).

Plants that are most significantly different from other analyzed populations are: a) plants from the Ovčar-Kablar Gorge, which are characterized by a large number of involucral bracts with the short and wide apex, exceptionaly well developed and compact hair cover on the surface of all vegetative organs resulting in a glaucous color of plants stem, leaves, involucral bracts and calyx, as well as by hairs on the leaf surface oriented towards the leaf apex, or orientated in all directions except towards the leaf base; b) plants from the Derventa Canyon, which are strongly shade-adapted, their leaves are dorsiventral with 1-2 palisade cell layers, 3-7 spongy cell layers (mostly five) and moderately dense leaf hair cover, and c) plants from the Jerma Canyon, which are characterized by large linear and thick rosette leaves, wide and undulate bract apex, as well as by serrate margin of the bract base.

The results of linear regression analysis are presented in Table 5. These results show that 8 of 19 bioclimatic parameters (isothermality, temperature seasonality, minimal temperature of the coldest month, temperature annual range, mean temperature of the wettest quarter, mean temperature of the driest quarter, annual precipitation, precipitation seasonality) have demonstrated statistically significant correlation with some of the analyzed morpho-anatomical characters after Bonferroni correction. Moreover, these results have shown that the orographic characteristics and bioclimatic factors related to the temperature of the habitat represent the most significant abiotic factors correlated with the morpho-anatomical differentiation of the investigated populations. The most significant correlations were found for the annual temperature range and the temperature seasonality. The morpho-anatomical variability, however, is not significantly correlated with the bioclimatic factors referring to the precipitation and humidity. According to the regression analysis, the following factors have the lowest influence on the variation of morpho-an-

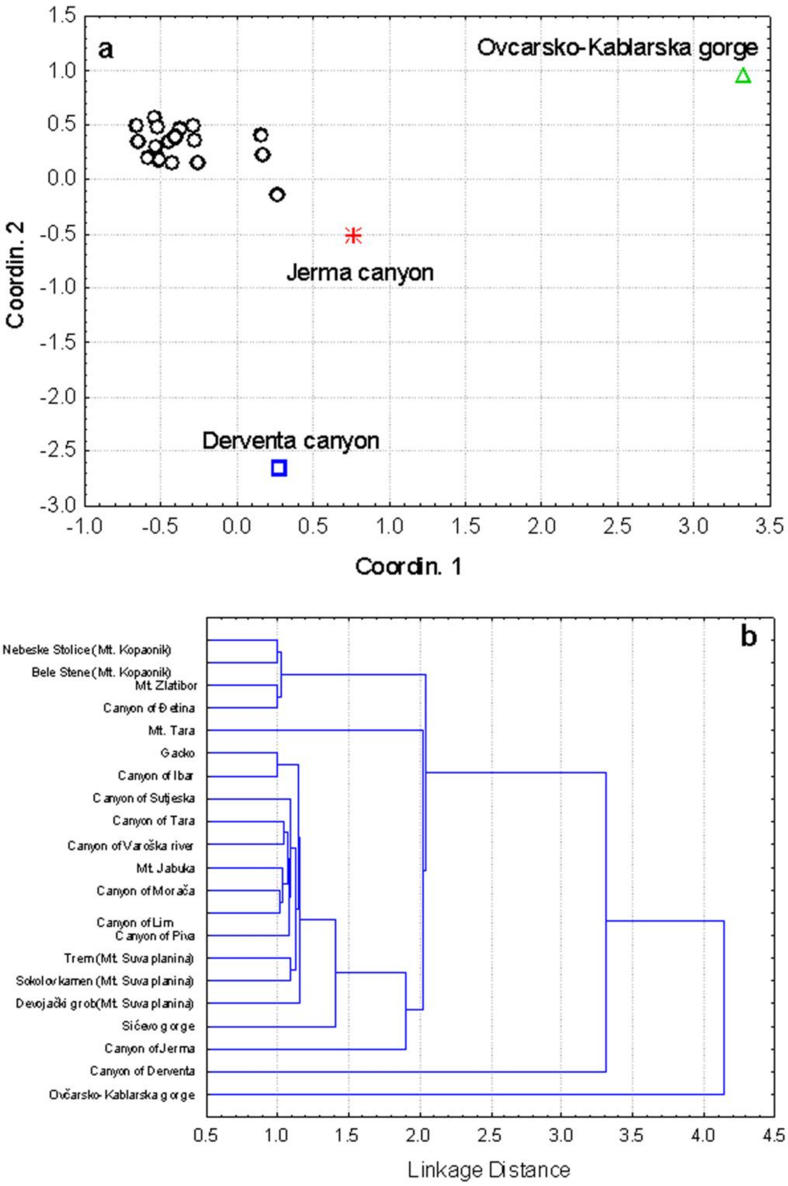

Fig. 6. Results of: (a) Multiple Correspondence Analysis (MCA) of qualitative characters plotted along the first two axes; (b) Cluster Analysis of qualitative characters.

atomical characters of the investigated populations: precipitation of driest month and precipitation of the warmest quarter.

As can be inferred from the regression analysis, 21 of 63 analyzed morpho-anatomical characters displayed a statistically significant correlation with some environmental factors after the Bonferroni correction. The greatest dependency in regard to the environmental factors were shown for corolla total height (Co_H1), maximal width of the central involucral bract (B2_W1), style length (St_L), calyx lobe total length (Ca_L1) and maximal width of outer involucral bract (B3_W1).

Table 6. Nuclear DNA content (2C in pg and 1C in Mbp) in 16 populations of *E. jugoslavicus*. Legend: B&H – Bosnia and Herzegovina, MNE – Montenegro, SRB – Serbia.

Populations	Number of individuals studied	2C DNA in pg (sd) means	2C DNA in pg min-max	1C DNA in pg (Mbp) means	HSD	Sympatry
B&H: Bukovik (1)	5	2.92 (± 0.01)	2.91-2.94	1.46 (1428)	a/b	No
B&H: Kladanj (2)	5	3.03 (± 0.02)	3.01-3.06	1.52 (1483)	b/c	No
B&H: Canyon of Sutjeska (3)	5	3.03 (± 0.01)	3.01-3.04	1.51 (1479)	b/c	with *E. sutjeskae*
MNE: Canyon of Morača (4)	5	2.94 (± 0.04)	2.89-2.99	1.47 (1439)	a/b	with *E. tenuifolius*
MNE: Canyon of Piva (5)	5	2.98 (± 0.03)	2.95-3.01	1.49 (1456)	a/b	with *E. tenuifolius*
MNE: Canyon of Lim (6)	4	2.90 (± 0.02)	2.88-2.92	1.45 (1419)	a/b	No
SRB: Canyon of Varoška River (7)	5	2.84 (± 0.02)	2.82-2.88	1.42 (1391)	a	No
SRB: Mt. Zlatibor (8)	5	2.92 (± 0.04)	2.86-2.97	1.46 (1428)	a/b	No
SRB: Canyon of Đetina (9)	3	2.90 (± 0.03)	2.87-2.94	1.45 (1418)	a/b	No
SRB: Mt. Tara (10)	5	2.93 (± 0.02)	2.90-2.95	1.46 (1431)	a/b	No
SRB: Canyon of Derventa (11)	6	2.93 (± 0.02)	2.90-2.96	1.47 (1434)	a/b	No
SRB: Ovčarsko-Kablarska Gorge (12)	11	2.95 (± 0.05)	2.90-3.07	1.47 (1442)	a/b/c	No
SRB: Sićevo Gorge (13)	5	3.22 (± 0.03)	3.18-3.26	1.61 (1575)	d	with *E. serbicus*
SRB: Oblik (14)	9	3.25 (± 0.2)	3.07-3.65	1.83 (1672)	d	with *E. serbicus*
SRB: Canyon of Jerma (15)	5	3.00 (± 0.04)	2.96-3.03	1.50 (1468)	a/b/c	No
SRB: Mt. Suva planina (16)	5	3.15 (± 0.03)	3.11-3.19	1.6 (1564)	c/d	with *E. serbicus*
Allopatric Groups	54	2.95 (± 0.07)	2.82-3.13	1.47 (1442)		No
Sympatric Groups	24	3.11 (± 0.19)	2.89-3.65	1.55 (1518)		No
All Groups	88.00	3.01 (± 0.14)	2.82-3.65	1.50 (1465)		No

[*]1 pg = 978 Mbp (according to Doležel et al. 2003)

Chromosome number and genome size analyses

Because the individuals from the Ovčar-Kablar Gorge stood out from other individuals in several morpho-anatomical characters, the chromosome number was determined herewith for the first time for this population. It has 2n=32 chromosomes that are small, less than 2 µm long. The nuclear DNA amount (2C value) ranged from 2.82 (population from the Varoška River Gorge) to 3.65 pg (populations from Oblik and the Suva Planina Mt.) (Table 6). The mean value of all investigated populations was 3 pg ($\pm$0.14). Nevertheless, some populations were significantly different, especially populations from the Sićevo Gorge (13), Oblik (14), and Suva Planina Mt. (16). The statistical analyses provided some interesting facts: i) the intra-population variation was low, except in population 14 (Table 6), and ii) the genome size was bigger in sympatric populations at the eastern boundary of *E. graminifolius "jugoslavicus"* distribution range in which *E. jugoslavicus* grows together with *E. serbicus* (Table 6), than in allopatric populations. Significant correlation among the genome size and morphological characters was found only between 2C value and perimeter ($r = -0.68$, $p = 0.000$) and length ($r = -0.727$, $p = 0.000$) of adaxial stomata.

DISCUSSION

The mountainous nature of the central Balkan Peninsula and diversity of its geological substrates offer an abundance of ecologically diverse habitats, which results in morphological heterogeneity of some widely distributed taxa, such as the Balkan relic and subendemic genus *Edraianthus* DC (Schönswetter et al., 2005; Lakušić et al., 2009; Surina et al., 2009). Populations of *E. graminifolius "jugoslavicus"* successfully inhabit microclimatically and pedologically heterogeneous habitats, across a wide range of altitudes, from canyons to alpine belts, in the wide area of the continental part of the central Balkan Peninsula. Therefore, the variety of ecological conditions can be considered the factor that most effectively influences the morpho-anatomical adaptive response in plants from investigated populations, resulting in exceptional morphological and anatomical variability within *E. graminifolius "jugoslavicus"*.

Most of the observed morpho-anatomical variability within *E. graminifolius "jugoslavicus"* is related to the plant adaptive response to different altitudes, as can be seen in the dimensions of vegetative organs that are increasingly smaller at higher altitudes. The most significant morpho-anatomical differences noticed among the analyzed plant populations are related to the shape and dimensions of the involucral bracts, all dimensions of leaves (such as length, width, and thickness), thickness of palisade and spongy tissues, as well as density of hair cover on leaf and stem surfaces. This morpho-anatomical variability of the investigated populations is found to be influenced predominantly by the temperature in the habitat. This is consistent with the fact that at higher altitudes environmental conditions become more extreme and thereby affect plant growth and physiology, resulting in the development of different plant ecotypes. The observed morphological differentiation between *E. graminifolius "jugoslavicus"* populations that inhabit different altitudes has already been mentioned by R. Lakušić (1974). He described several subspecies and forms that inhabit specific altitudinal ranges, such as *E. jugoslavicus* subsp. *jugoslavicus* from the montane and lower alpine belt, and *E. jugoslavicus* subsp. *subalpinus* from the subalpine and upper alpine belt. To what extent the observed morphological differences between populations of *E. graminifolius "jugoslavicus"* arise from phenotypic plasticity that permitted the same genotype to be maintained in different environments (Novak et al., 1991; Dewitt and Scheiner, 2004), or from genetically fixed characters (West-Eberhard, 1989; Schlichting and Pigliucci, 1998; West-Eberhard, 2003; Sultan, 2004; Garland and Kelly, 2006), remains to be resolved.

Apart from the overall morphological variability, there are four populations (from the Derventa Canyon, Varoška River Gorge, Jerma Canyon, and Ovčar-Kablar Gorge) that are particularly morphologically and anatomically specific. (1) Plants from Derventa canyon are characterized by wide involucral bracts with strongly revoluted and reflexed apex, and pronouncedly wide but thin rosette leaves. Their leaves are pronouncedly mesomorphic, with a dorsiventral leaf structure that was detected only in this population, larger epidermal cells and the largest stomata in respect to plants from all other studied populations. (2) Plants from Varoška River Gorge have exceptionally large vegetative and generative organs, involucral bracts with pronouncedly wide cordate base, and large linear rosette leaves. (3) Plants from the Jerma Canyon are distinguished by wide-linear and thick rosette leaves, wide and undulated apex of the involucral bracts, and pronouncedly serrate margin of the bract base, as well as by some qualitative characters of inflorescence. The geographical position at the eastern boundary of *E. graminifolius "jugoslavicus"* distribution range emphasizes the specificity of this population. Molecular studies (Stefanović et al. 2008) as well as our studies related to genome size showed that these three populations do not differ from other investigated populations of *E. graminifolius "jugoslavicus"*. (4) The specific features of plants from the Ovčar-Kablar Gorge are the exceptionally dense hair cover on plant surfaces resulting in their grayish color, raised trichomes directed towards the leaf apex or orientated in all directions except towards the leaf base, involucral bracts with a short apex, the smallest stomata, and, in particular, the capsule with basal lateral porate dehiscence. The results of recent molecular analysis of cpDNA sequences also showed that plants from the Ovčar-Kablar Gorge are molecularly distinct from other populations investigated herein (Stefanović et al. 2008). Their geographical isolation (northern boundary of the distribution range of *E. graminifolius "jugoslavicus"*), morphological differentiation, distinct qualitative characters and significant differences at the molecular level show that plants from this population merit the rank of a new species, *E. canescens*, that arises in the process of allopatric speciation (Lakušić et al., 2013).

As regards the genome size, certain populations showed more or less important variations in 2C DNA values. Three populations from the eastern boundary of *E. graminifolius "jugoslavicus"* distribution range (13 – Sićevo Gorge, 14 – Oblik, and 16 –Suva Planina Mt.) are characterized by a larger genome size, which is significantly different from the other populations. The population from Oblik showed a more pronounced intrapopulation variation in the amount of DNA. The fact that a bigger genome size was observed in populations that live in sympatry with *Edraianthus serbicus* could be explained by the presence of hybridization, which was recently confirmed in sympatric populations of *E. wettsteinii* and *E. tenuifolius* (Lakušić et al., 2009). However, further investigations are necessary in order to explain the phenomenon of increasing genome size of these populations.

Edraianthus species are generally characterized by equal chromosome number of 2n=32, although their genome size varies from 2.82 to 4.16 pg (Medjedović, 1981; Siljak-Yakovlev et al., 2010). This could be explained by differences in the size of their chromosomes (Caceres et al. 1998), and indicates that the process of speciation occurs through chromosomal rearrangements, without changes in the chromosome number (Bou Dagher-Kharrat et al., 2001; Levin, 2002; Livingstone and Rieseberg, 2003; Bennetzen et al., 2005). Differences in the genome size among the investigated populations could be the consequence of different environmental conditions as well (Knight and Ackerly, 2002; Lavergne et al., 2010), but only moderate negative correlation was found between the genome size and precipitation, especially in warm and dry periods of the year ($r = -0.490$, $p = 0.000019$; $r = -0.592$, $p = 0.000$). Although many authors found positive correlations between genome size and some morphological characters of plants (Masterson, 1994; Chung et al., 1998; Beaulieu et al., 2008), in *E. graminifolius "jugoslavicus"* significant negative correlation was observed only between 2C value and perimeter ($r = -0.68$, $p = 0.000$) and length ($r = -0.727$, $p = 0.000$) of adaxial stomata.

It can be concluded that most of the observed morpho-anatomical variability of the widely distributed *E. graminifolius "jugoslavicus"* can be explained as a results of its potential to adapt effectively to different micro-environmental conditions. Our results indicate that there is a strong morphological differentiation within *E. graminifolius "jugoslavicus"* of those populations that are at the boundaries of its distribution range. These different populations, and potentially new taxa, which probably arose in the process of cryptic allopatric speciation and ancient and recent interspecific hybridization within *Edraianthus*, are generally followed by extensive morphological homoplasy that represents an additional problem in the resolution of opened taxonomic questions within the genus *Edraianthus*. Consequently, additional research related to phenotypic plasticity and inferences obtained from fast evolving DNA sequences is necessary to resolve the relationships between *E. graminifolius "jugoslavicus"* populations in detail and to interpret the mechanisms of their differentiation.

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