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Edraianthus stankovicii (Campanulaceae), an overlooked taxon from the Balkan Peninsula—Evidence from morphometric, molecular and genome size studies

DMITAR LAKUŠIĆ[1,*], SAŠA STEFANOVIĆ[2], SONIA SILJAK-YAKOVLEV[3], TAMARA RAKIĆ[1], NEVENA KUZMANOVIĆ[1] & BOŠTJAN SURINA[4,5]

[1]*Institute of Botany and Botanical Garden Jevremovac, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia. E-mail: dlakusic@bio.bg.ac.rs, tamaraz@bio.bg.ac.rs, nkuzmanovic@bio.bg.ac.rs*

[2]*Department of Biology, University of Toronto Mississauga, 3359 Mississauga Rd., Mississauga, Ontario L5L 1C6, Canada. E-mail: sasa.stefanovic@utoronto.ca*

[3]*Ecologie Systématique Evolution, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91400, Orsay, France. E-mail: sonia.yakovlev@u-psud.fr*

[4] *Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Glagoljaška 8, SI-6000 Koper, Slovenia, E-mail: bostjan.surina@prirodoslovni.com*

[5] *Natural History Museum Rijeka, Lorenzov prolaz 1, 51000 Rijeka, Croatia*

**Author for correspondence: Dmitar Lakušić, dlakusic@bio.bg.ac.rs*

Abstract

The taxonomically intricate *Edraianthus dalmaticus-serbicus* group within *E. tenuifolius*-complex in the Balkan Peninsula is reviewed using morphological, molecular and genome size data based on extensive sampling of populations across the species' range. The phylogenetic analyses based on Amplified Fragment Length Polymorphisms (AFLPs), plastid DNA (*trnL-F* region and *rbcL-atpB* spacer) and nuclear ribosomal external transcribed spacer (nrETS) sequences confirmed the monophyly of *E. serbicus* as traditionally defined but have also revealed the presence of two distinct and allopatrically distributed taxa. The genome size and morphological analyses, performed on the same widespread sample of populations, largely corresponded with molecular results, allowing us to raise the overlooked taxon *E. serbicus* subsp. stankovici, to the species level. The names *Edraianthus serbicus* and *E. serbicus* subsp. *stankovicii* (≡ *E. stankovicii*) are typified. Furthermore, a new differential diagnosis, description and illustration of *E. stankovicii* are provided, as well as its conservation status is assessed. *Edraianthus stankovicii* is a rare and critically endangered stenoendemic taxon, with the distribution limited only to Mts. Veliki Krš and Stol in NE Serbia.

Key words: AFLP, Balkan Peninsula, Edraianthus, endemic taxa, genome size, morphometry, nomenclature, plastid and nuclear sequences

INTRODUCTION

The genus *Edraianthus* Candolle (1839: 448) (Campanulaceae), or grassy bells, represents one of the taxonomically and biogeographically most interesting and polymorphic genera of the Balkan flora. This is corroborated by the fact that three monographs of the genus have already been published (Wettstein 1887, Janchen 1910, Lakušić 1974), as well as many recent extensive studies, spanning anatomy and morphology (Rakić 2010, Rakić *et al.* 2012), cytogenetic investigations (Međedović *et al.* 2007, Siljak-Yakovlev *et al.* 2010), molecular phylogeny and phylogeography (Stefanović *et al.* 2008, Surina *et al.* 2011, 2014) and taxonomy (Lakušić *et al.* 2009, 2013, Surina *et al.* 2009, Surina & Lakušić 2010).

A broadscale molecular phylogeny of *Edraianthus* identified several distinct monophyletic groups of taxa, some of which corresponded to those established in the previous taxonomic treatments (Stefanović *et al*. 2008). One such case is the taxonomically intricate *E. dalmaticus-serbicus* group (taxa with broadly triangular calyx-teeth that are shorter than ovary), traditionally assigned to *E.* ser. *Dalmaticii* Lakušić (Lakušić 1974: 7). This group consists of two species, *E. dalmaticus* (Candolle 1830: 134) Candolle (1839: 449) and *E. serbicus* Petrović (1882: 549), which together with *E. tenuifolius* (Waldstein & Kitaibel 1804: 168) Candolle (1839: 449) and *E. wettsteinii* Halácsy & Baldacci (in Halácsy 1891: 371) constitute a newly defined *E. tenuifolius*-complex (Stefanović *et al*. 2008). Despite the fact that the

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genus is taxonomically very complicated, all the taxa share the same chromosome number 2n=32 (Međedović 1981, Međedović *et al*. 2007).

While *E. dalmaticus* and *E. serbicus* are morphologically and molecularly very similar to each other, their distribution and ecology are quite distinct. *Edraianthus dalmaticus* occurs in the central Dinaric Alps of Croatia (Dalmatia), Bosnia and Herzegovina and Montenegro (Lakušić 1974, Bubanja *et al*. 2007), and contrary to other *Edraianthus* taxa inhabits frequently flooded karst meadows and is therefore ecologically the most distinct grassy bell. Its closest relative, *E. serbicus*, is a montane to subalpine species, distributed in E Serbia and W Bulgaria, and predominantly inhabits dry calcareous rocky grasslands and rock crevices. Within *E. serbicus*, Lakušić (1974) distinguished two groups of populations based on morphological traits; the typical subspecies covers the majority of the species' distribution range, while *E. serbicus* subsp. *stankovicii* is restricted only to Mts. Veliki Krš and Stol, at the north-easternmost distribution range of the species. The results of the molecular phylogeny of *Edraianthus* based on non-coding plastid DNA sequences (Stefanović *et al*. 2008, Surina *et al*. unpublished data), have also identified two separate lineages within *E. serbicus*, consistent with distinctions observed in its morphology and distribution.

The main aim of our present study is to conduct an in-depth investigation using a detailed population-level sampling from across the distribution span, in order to establish whether the haplotype distinctions observed initially between the two lineages of *E. serbicus* on a small sample hold more broadly, and whether this is congruent and further supported by evidence from other non-linked molecular sources (AFPL, nrETS), as well as morphological and genome size data.

MATERIALS AND METHODS

Two subspecies belonging to *Edraianthus serbicus* are reviewed using morphological, molecular and genome size data based on an extensive sampling of populations across the species range, along with several populations of *E. dalmaticus* used as outgroup.

Morphometric analyses

Morphological studies were carried out using herbarium specimens deposited in B, BEO, BEOU, NHMR, P, PAD, WU (abbreviations follow Thiers 2016). Morphometric analyses were done on the material collected in the field which was preserved in a solution of 96% ethanol and glycerol (1: 1). We included 140 specimens of *E. serbicus* subsp. *serbicus* from four populations from Serbia and Bulgaria, 17 specimens of *E. serbicus* subsp. *stankovicii* from Mts. Veliki Krš in NE Serbia, and 7 specimens of *E. dalmaticus* from Dalmatia in Croatia (Table 1, Fig. 1). The material used for morphometric analyses is deposited in BEOU and NHMR.

The 36 morphological character states measured in this study are listed in Table 3. Descriptive statistics were calculated for each character state and canonical discriminant analysis (CDA) was used to test the hypothesis of morphological segregation of three groups of individuals, *a priori* identified as separate taxa: *E. serbicus* subsp. *serbicus*, *E. serbicus* subsp. *stankovicii* and *E. dalmaticus*. Canonical scores for each case were calculated in order to estimate the distances between individuals that were used to visualize the relationship among *a priori* defined groups. Discriminant function analysis was done to estimate the contribution of individual characters to overall discrimination.

Morphological characters were measured using a Leica Qwin and ImageJ 1.38x programs, and a Leica DMLS stereomicroscope. Data were processed in the statistical package Statistica 5.1 for Windows (Statsoft 1996).

AFLP data analysis

Total genomic DNA was extracted from similar amounts of dried tissue (~10 mg) following the CTAB protocol (Doyle & Doyle 1987) with some modifications (Surina *et al*. 2011). Details on the plant material are provided in Table 1. The quality of the extracted DNA was checked on 1% TAE-agarose gels. The AFLP procedure followed Surina *et al*. (2014). One negative control sample was included to test for systematic contamination, and 20 samples were replicated to test for reproducibility (Bonin *et al*. 2004).

Raw AFLP data were collected and aligned with the internal size standard using ABI Prism GeneScan analysis software 3.7.1 (Applied Biosystems, Foster City, California). The GeneScan files were imported into Genographer v. 1.6.0 (available at http//hordeum.oscs.montana.edu/genographer) for scoring the fragments.

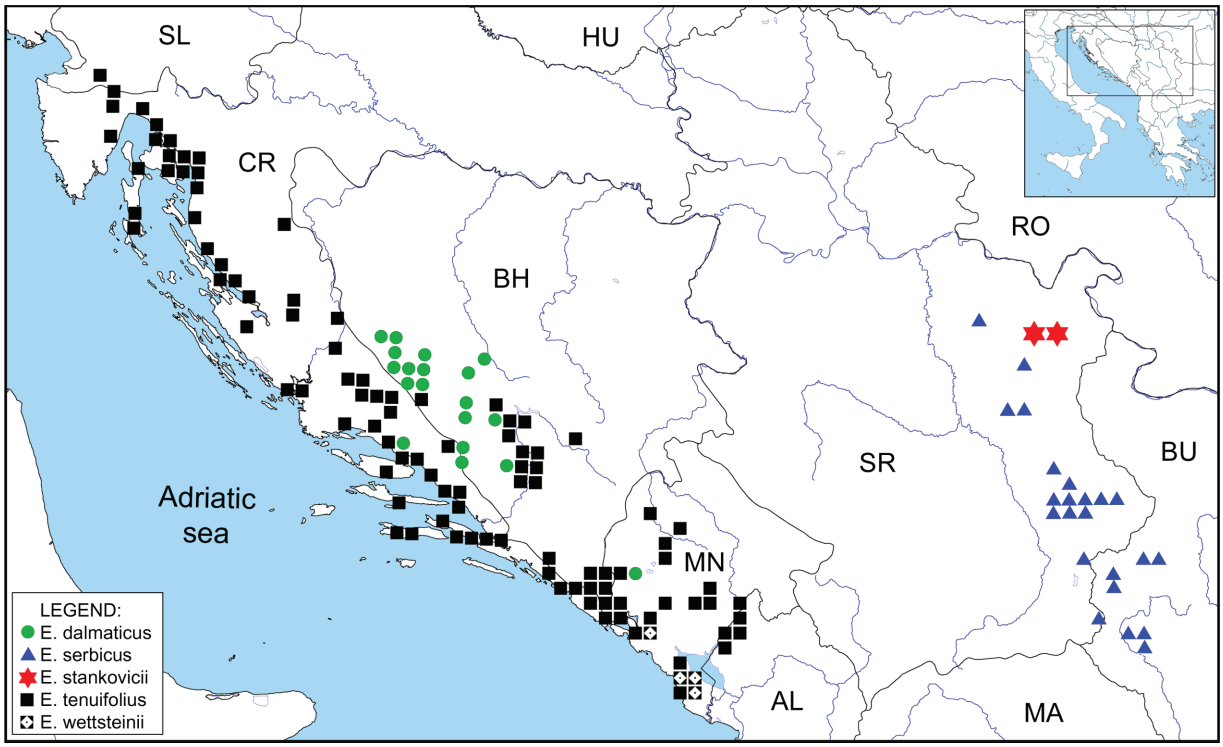

FIGURE 1. Distribution of taxa of the *Edraianthus tenuifolius*-complex in the Balkan Peninsula. State abbreviation: AL—Albania, BH—Bosnia and Hercegovina, BU—Bulgaria, CR —Croatia, HU—Hungary, MA—Macedonia, MN—Montenegro, RO—Romania, SL—Slovenia, SR—Serbia.

TABLE 1. Samples used in molecular (AFLP—number of individuals; cpDNA, ETS—numbers for samples used in the study of Stefanović *et al.* 2008), genome size (GS—number of individuals) and morphometric (M—number of individuals) analyses. *Population from locus classicus.

Locality	cpDNA	ETS	AFLP	GS	M	Collectors	No	Herbarium
Edraianthus dalmaticus								
BH: Glamočko polje	1456					Studnička		SARA
BH: Kupresko polje	1457	1457				Studnička		SARA
BH: Livanjsko polje				5		Bogunić		
BH: Livanjsko polje	1570	1570	4			Modrić & Surina	418	NHMR
BH: Dugo polje			4			Modrić & Surina	417	NHMR
BH: Lagumovići	1569	1569				Modrić & Surina	417	NHMR
CR: Muc	1568	1568				Mihelj, Ževrnja & Vladović,	2053	NHMR
CR: Sinjsko Polje	1571	1571				Mihelj	595	NHMR
CR: Dalmatia, Kmeti-Krilove			4		7	Mihelj & al.	2053	NHMR
MN: Nikšić	1586	1586				Bubanja	26560	BEOU
Edraianthus serbicus								
BU: Mt. Chepan, Znepole	1359	1359				Petrova	19983	BEOU
BU: Mt. Konjevska planina					227	Lakušić & al.	30487	BEOU
BU: Staro Selo			5			Frajman & Schönswetter	11313	WU
SR: Belava	1481	1481				Jušković	20669	BEOU
SR: Mt. Rtanj						Lakušić, D.	27624	BEOU
SR: Mt. Rtanj					22	Rakić & Rakić	27216	BEOU
SR: Mt. Rtanj	1197					Stevanović	10503	BEOU
SR: Mt. Rtanj			4			Frajman & Schönswetter	413, 414	NHMR
SR: Mt. Rtanj - Zlot	1228					Stevanović	10379	BEOU
*SR: Mt. Suva planina				5		Lakušić, D.	27501	BEOU

...continued on the next page

TABLE 1. (Continued)

Locality	cpDNA	ETS	AFLP	GS	M	Collectors	No	Herbarium
*SR: Mt. Suva planina					31	Lakušić, D.	27502	BEOU
*SR: Mt. Suva planina					37	Lakušić, D.	30391	BEOU
SR: Mt. Suva planina	1361					Lakušić, D.	19812.1	BEOU
SR: Mt. Suva planina	1482					Tomović & Zlatković	20889	BEOU
SR: Mt. Suva planina	1182	1182				Vukojičić & Tomović	6605	BEOU
SR: Mt. Suva planina			2			Frajman & Schönswetter	415	NHMRs
SR: Mt. Vukan	1667AB				23	Lakušić, D.	30390	BEOU
SR: Mt. Vukan	1447					Niketić & Tomović	16458	BEOU
Edraianthus stankovicii								
*SR: Mt. Stol	1213	1213				Benić	1545/91	BEOU
*SR: Mt. Stol			5			Frajman & Schönswetter	412	NHMR
SR: Mt. Veliki krš	1666AB			5	17	Lakušić, D.	27217	BEOU
	1668AB							

In the 28 individuals successfully analyzed, we scored 209 AFLP fragments with the error rate (Bonin *et al*. 2004) of unreliable characters being 0.0%. The results of the scoring were exported as a presence/absence matrix. Using the program SplitsTree 4 (Huson & Bryant 2006), a neighbor-net diagram was produced from Nei-Li distances (Nei & Li 1979). To obtain bootstrap support values for branches, 1000 pseudoreplicates were employed. A Principal Co-ordinate Analysis (PCoA) based on a matrix of Jaccard distances was performed using Past (Hammer *et al.* 2001).

DNA sequencing and phylogenetic reconstruction

General protocols for DNA extractions, PCR conditions, amplicon purifications, as well as sequencing procedures are outlined in Stefanović *et al*. (2007, 2008). PCR primers described by Taberlet *et al*. (1991) and by Hoot *et al*. (1995) were used to target plastid *trnL-F* and *rbcL-atpB* spacer regions, respectively. To amplify a portion of the nuclear ribosomal external transcribed spacer (3'-ETS sensu Alonso *et al*. 2014), we used the universal 3' 18S-IGS primer of Baldwin & Markos (1998). The 5' primer, ETS-R4 (5' - GCCACCCGCGTGCCAAGCAC-3'), was designed using *Edraiantus* sequences as a reference. The ETS amplicon (ca. 1350 bp) was sequenced using the external primers; when necessary, two additional *Edraianthus*-specific internal primes were used: ETS-R3 (5' - ATTCCGAAGCATGCCATGAG-3') and ETS-F3 (5' - TCTTGGCATCAGGCGTGGGC-3'). Two closely-related species from the *E. tenuifolius* complex, *E. tenuifolius* and *E. wettsteinii*, were used as more distant outgroups, along with samples from *E. dalmaticus*. The chloroplast sequences were obtained from 23 *Edraianthus* individuals in total, and include multiple accessions of *E. serbicus* and *E. stankovicii*. The nuclear ETS sequences were obtained from 14 *Edraianthus* individuals in total, including all ingroup and outgroup taxa. Sequences obtained for this study are deposited in GenBank (see Table 1). Alignments were done manually, using Se-Al v.2.0a11 (Rambaut 2002). Gaps in the alignments were scored as missing data.

Parsimony searches, along with accompanying clade support estimations, were conducted for each region separately, as well as for the concatenated chloroplast sequences and all concatenated sequences (the "total evidence" approach). We run full heuristic search algorithm in PAUP* v.4.0b10 (Swofford 2002), with 1000 random addition, tree bisection and reconnection (TBR) branch swapping, ACCTRAN optimization, and MULTREES on. To assess clade support, bootstrap analyses (BS; Felsenstein 1985) were conducted with 500 replicates, 100 random addition per replicate, TBR branch swapping, and MULTREES off options.

Flow cytometry for genome size assessment

For genome size measurements, 15 individuals were analysed from three populations of *E. serbicus* subsp. *serbicus*, 5 individuals from one population of *E. serbicus* subsp. *stankovicii*, and 5 individuals from one population of *E. dalmaticus* (Table 1). DNA amounts were determined by flow cytometry following Marie & Brown (1993). Cell nuclei were isolated from young leaves of at least five individuals per population. Tomato *Solanum lycopersicum* Montfavet '63-5' (2C=1.99 pg, Lepers-Andrzejewski *et al*. 2011) 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 Gif Nuclear Buffer: 45 mM $MgCl_2$, 30 mM sodium citrate, 60 mM 4-morpholinepropane sulfonate pH 7, 0.1 % (w/v) Triton X-100, 1% polyvinylpyrrolidone (~10,000M_r, Sigma P6755), 5 mM sodium metabisulfite and 10 µg/ml RNase (Sigma Aldrich, Saint Quentin, France). The nuclei suspension was filtered through nylon mesh (pore size 30 µm) and kept at

4°C. Genome size was assessed using propidium iodid (50 µg/ml; Sigma). 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 unknown *Edraianthus* species and the known *Solanum lycopersicum* internal standard.

RESULTS

Morphometrics.—The Canonical Discriminant Analysis (CDA) conducted on three *a priori* defined groups has shown that all three groups are distinct and statistically well supported (Fig. 2). Plotted along the first and the second discriminant axis, all three groups were positioned in distinct parts of the DA1–DA2 space. Scores of *E. dalmaticus* are grouped at positive part of the first axis, while scores of *E. serbicus* subsp. *serbicus* and *E. serbicus* subsp. *stankovicii* are grouped at negative part of the first axis, and fully separated along the second axis.

TABLE 2. Discriminant function analysis of morphometric characters.

		Wilks' Lambda	Partial Lambda	F-remove (2, 62)	p-level
Stem					
1. Stem height	St_H0	0.029	0.972	0.881	0.419
Cauline leaf					
2. Maximal width	Le_W1	0.030	0.949	1.652	0.200
3. Width in the upper quarter	Le_W2	0.030	0.941	1.951	0.151
4. Total length	Le_L0	0.030	0.940	1.995	0.145
5. Distance between the largest leaf width point and the leaf base	Le_L1	**0.039**	**0.730**	**11.452**	**0.000**
Inner invol. bract					
6. Maximal width	B1_W1	**0.032**	**0.895**	**3.643**	**0.032**
7. Width in the upper quarter	B1_W2	0.029	0.993	0.231	0.795
8. Total length	B1_H0	0.030	0.944	1.823	0.170
9. Distance between the largest width point and the base	B1_H1	0.029	0.990	0.315	0.731
10. Distance between apex base and the bract base	B1_H2	0.030	0.951	1.583	0.214
Central invol. bract					
11. Maximal width	B2_W1	0.031	0.914	2.900	0.063
12. Width in the upper quarter	B2_W2	0.030	0.948	1.706	0.190
13. Total length	B2_H0	0.029	0.982	0.557	0.576
14. Distance between the largest width point and the base	B2_H1	**0.032**	**0.897**	**3.561**	**0.034**
15. Distance between apex base and the bract base	B2_H2	0.031	0.918	2.786	0.069
Outher invol. bract					
16. Maximal width	B3_W1	**0.033**	**0.859**	**5.084**	**0.009**
17. Width in the upper quarter	B3_W2	**0.036**	**0.798**	**7.842**	**0.001**
18. Total length	B3_H0	**0.036**	**0.793**	**8.100**	**0.001**
19. Distance between the largest width point and the base	B3_H1	0.031	0.921	2.661	0.078
20. Distance between apex base and the bract base	B3_H2	**0.035**	**0.822**	**6.710**	**0.002**
Calyx					
21. Diameter	Ca_W0	0.031	0.919	2.748	0.072
22. Width of lobe base	Ca_W1	0.029	0.987	0.414	0.663
23. Width of lobe base in the upper quarter	Ca_W2	0.031	0.925	2.522	0.089
24. Lobe length	Ca_H1	0.029	0.995	0.146	0.864
Corolla					
25. Maximal width	Co_W1	**0.035**	**0.813**	**7.111**	**0.002**
26. Width of lobe base	Co_W2	**0.032**	**0.884**	**4.087**	**0.022**
27. Total corolla height	Co_H1	**0.038**	**0.748**	**10.436**	**0.000**
28. Lobe height	Co_H2	0.031	0.919	2.726	0.073
Style					
29. Length	St_L	0.031	0.919	2.726	0.073

TABLE 3. Descriptive statistics and measurements (in mm) of 23 vegetative and 13 floral characters used in the present study. N—number of samples, min—minimal value, M—mean value, max—maximal value, SD—standard deviation.

	E. serbicus					*E. stankovicii*					*E. dalmaticus*				
	N	min	**M**	max	SD	N	min	**M**	max	SD	N	min	**M**	max	SD
Stem															
1. Stem height-St_H0	108	24.5	**104.3**	189.3	38.1	15	28.1	**65.3**	106.3	24.2	7	24.5	**41.9**	62.8	16.4
Rosette leaf															
2. Base width-Lb_W0	80	1.1	**1.8**	3.7	0.5	12	1.1	**1.9**	3.7	0.7	0	-	**-**	-	-
3. Maximal width-Lb_W1	80	0.8	**1.5**	3.6	0.5	12	1.1	**1.8**	3.6	0.6	0	-	**-**	-	-
4. Total length-Lb_L	80	12.8	**52.2**	93.7	16.9	12	12.8	**26.8**	35.2	6.9	0	-	**-**	-	-
Cauline leaf															
5. Maximal width-Le_W1	109	1.2	**2.9**	7.0	1.2	16	1.7	**2.1**	3.3	0.4	7	2.2	**2.6**	3.5	0.4
6. Width in the upper quarter-Le_W2	109	1.0	**1.7**	3.1	0.4	16	1.3	**1.8**	3.1	0.4	7	1.8	**2.2**	2.6	0.3
7. Total length-Le_L0	109	13.7	**30.3**	63.8	10.3	16	13.7	**18.8**	24.7	3.2	7	36.3	**47.3**	63.8	10.0
8. Distance between the largest leaf width point and the leaf base-Le_L1	106	1.0	**7.0**	44.2	8.9	16	1.3	**9.3**	19.1	6.0	7	24.6	**33.8**	44.2	7.0
Capitulum															
9. number of involucral bracts-No_B	101	5.0	**9.1**	13.0	1.6	17	6.0	**8.0**	10.0	1.3	0	-	**-**	-	-
10. Number of flowers in capitulum-No_F	100	2.0	**7.9**	14.0	2.5	17	2.0	**5.2**	8.0	2.0	0	-	**-**	-	-
Inner invol. bract															
11. Maximal width-B1_W1	110	2.5	**6.9**	12.8	2.4	17	4.1	**5.2**	6.6	0.7	7	2.5	**3.8**	5.5	1.0
12. Width in the upper quarter-B1_W2	110	0.7	**5.2**	9.9	2.1	17	2.0	**4.0**	6.0	1.0	7	0.7	**1.5**	2.5	0.6
13. Total length-B1_H0	110	6.0	**13.0**	24.1	3.4	17	6.0	**9.3**	11.9	1.8	7	10.9	**12.4**	14.3	1.2
14. Distance between the largest width point and the base-B1_H1	110	2.0	**6.7**	14.1	2.6	17	3.3	**5.0**	8.4	1.6	7	2.0	**3.2**	4.9	1.0
15. Distance between apex base and the bract base-B1_H2	110	1.2	**11.7**	19.2	2.9	17	1.2	**8.5**	11.9	2.5	7	9.3	**10.6**	12.4	1.2
Central invol. bract															
16. Maximal width-B2_W1	110	3.8	**8.8**	14.8	2.4	17	3.8	**6.0**	7.7	1.1	7	5.1	**7.0**	9.4	1.5
17. Width in the upper quarter-B2_W2	110	1.0	**2.5**	10.3	1.5	17	1.4	**2.5**	4.1	0.8	7	1.1	**1.6**	2.1	0.3
18. Total length-B2_H0	110	6.5	**15.2**	29.9	4.8	17	6.5	**9.9**	13.0	1.9	7	18.0	**20.2**	24.3	2.2
19. Distance between the largest width point and the base-B2_H1	110	1.5	**4.1**	11.0	1.7	17	1.5	**2.7**	5.4	0.9	7	1.7	**2.4**	3.3	0.7
20. Distance between apex base and the bract base-B2_H2	110	5.1	**9.0**	14.0	2.0	17	5.1	**7.7**	12.7	1.9	7	6.5	**7.4**	9.6	1.1
Outher invol. bract															
21. Maximal width-B3_W1	110	3.4	**6.3**	10.6	1.8	17	3.4	**4.7**	6.3	0.9	7	4.2	**5.5**	6.6	1.1
22. Width in the upper quarter-B3_W2	110	0.8	**1.7**	2.7	0.4	17	1.1	**1.8**	2.6	0.4	7	1.4	**2.0**	2.7	0.5
23. Total length-B3_H0	109	9.0	**20.6**	40.3	7.3	17	9.0	**12.4**	19.2	2.6	6	27.5	**32.4**	35.9	3.5
24. Distance between the largest width point and the base-B3_H1	110	0.8	**2.0**	4.1	0.6	17	1.3	**1.8**	2.5	0.3	7	1.1	**1.7**	2.7	0.5
25. Distance between apex base and the bract base-B3_H2	110	4.0	**6.5**	19.2	2.2	17	4.5	**8.6**	19.2	4.2	7	4.4	**5.9**	8.1	1.3
Calyx															
26. Diameter-Ca_W0	105	2.9	**4.3**	7.3	0.9	15	2.9	**3.3**	4.1	0.4	5	3.0	**3.6**	4.1	0.5
27. Width of lobe base-Ca_W1	107	0.9	**1.6**	2.6	0.4	15	0.9	**1.3**	1.6	0.2	7	1.3	**1.6**	1.9	0.2
28. Width of lobe base in the upper quarter-Ca_W2	106	0.3	**0.7**	1.5	0.2	15	0.5	**0.8**	1.0	0.2	7	0.5	**0.6**	0.7	0.1
29. Lobe length-Ca_H1	106	0.6	**1.7**	3.6	0.7	15	0.8	**1.4**	2.3	0.4	7	1.2	**1.4**	1.6	0.2

...continued on the next page

TABLE 3. (Continued)

		E. serbicus					*E. stankovicii*					*E. dalmaticus*				
		N	min	**M**	max	SD	N	min	**M**	max	SD	N	min	**M**	max	SD
Corolla																
30.	Maximal width-Co_W1	108	5.1	**9.6**	16.6	2.1	16	6.4	**9.2**	11.6	1.6	7	5.1	**5.5**	6.6	0.5
31.	Width of lobe base-Co_W2	108	2.3	**4.4**	6.7	0.9	16	3.2	**4.0**	5.3	0.6	7	2.3	**2.7**	3.2	0.3
32.	Total corolla height-Co_H1	108	9.8	**18.8**	32.7	4.3	16	12.6	**16.2**	20.9	2.4	7	9.8	**11.5**	13.1	1.3
33.	Lobe height-Co_H2	108	3.7	**7.2**	13.1	1.8	16	5.4	**6.8**	9.3	1.2	7	3.7	**4.7**	6.9	1.0
Style																
34.	Length-St_L	108	9.0	**16.8**	28.4	3.9	15	13.6	**15.9**	18.9	1.9	7	9.0	**12.3**	14.4	2.0
Anther																
35.	Anther length-An1_L	107	0.5	**5.6**	8.6	1.3	16	3.2	**5.0**	7.1	1.0	5	3.6	**4.0**	4.2	0.2
36.	Height of filamentum base-An3_L	103	1.1	**2.4**	4.4	0.6	15	1.5	**2.0**	2.7	0.3	4	1.1	**1.8**	2.2	0.5

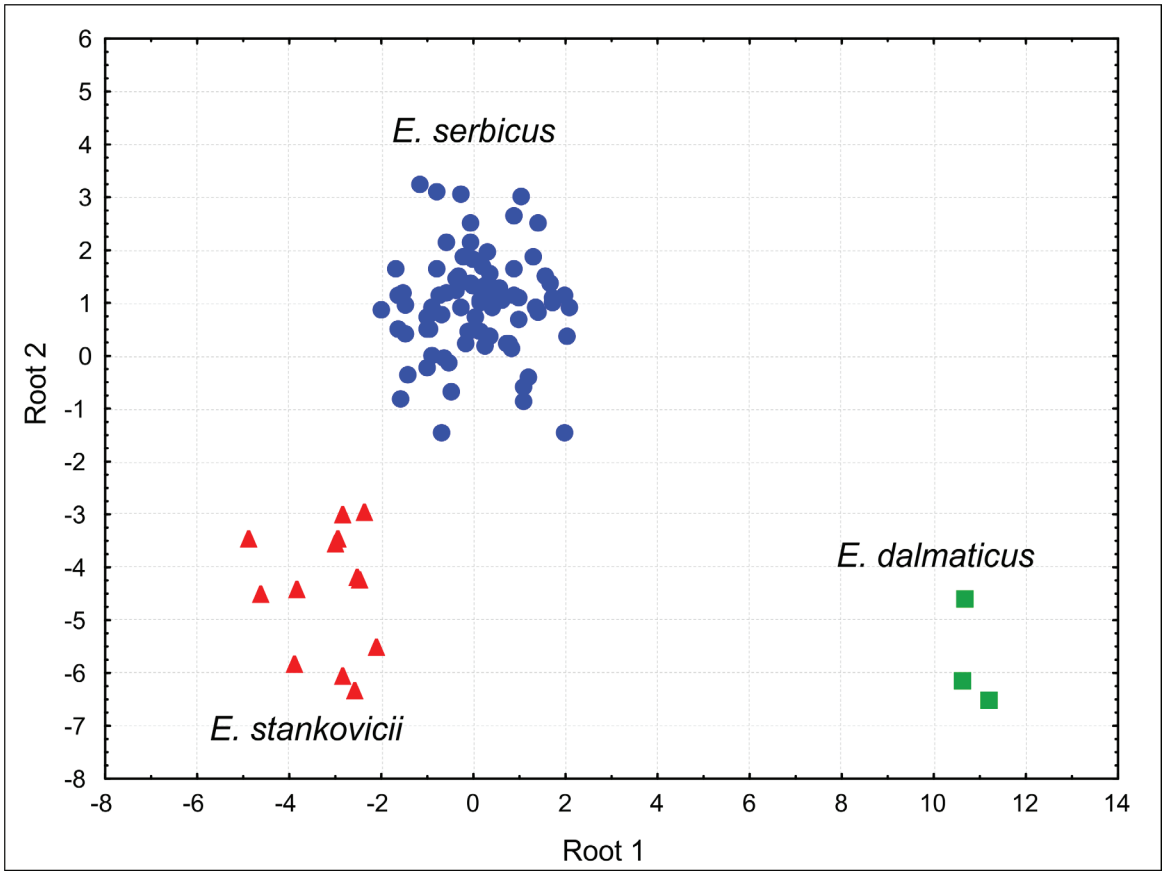

FIGURE 2. Discriminant function analysis (DCA) of morphometric data for three entities of the *Edraiantus dalmaticus-serbicus* group revealed in molecular and genome size analyses and traditionally recognized as different taxa.

Discriminant function analysis showed that the floral (corolla and anthers) and bract characters (outer involucral bracts) had a greater contribution to the overall discrimination in respect to the characters of leaves (Table 2). Morphological characters which contributed mostly to the discrimination were the distance between the largest leaf width point and the leaf base of cauline leaf (Le_L1); maximal width of inner involucral bract (B1_W1); distance between the largest width point and the base of central involucral bract (B2_H1); maximal width (B3_W1), width in the upper quarter (B3_W2), total length (B3_H0) and distance between apex base and the bract base (B3_H2) of outer involucral bract; maximal corolla width (Co_W1), width of corolla lobe base (Co_W2) and total corolla height (Co_H1); anther length (An1_L) and height of filament base (An3_L).

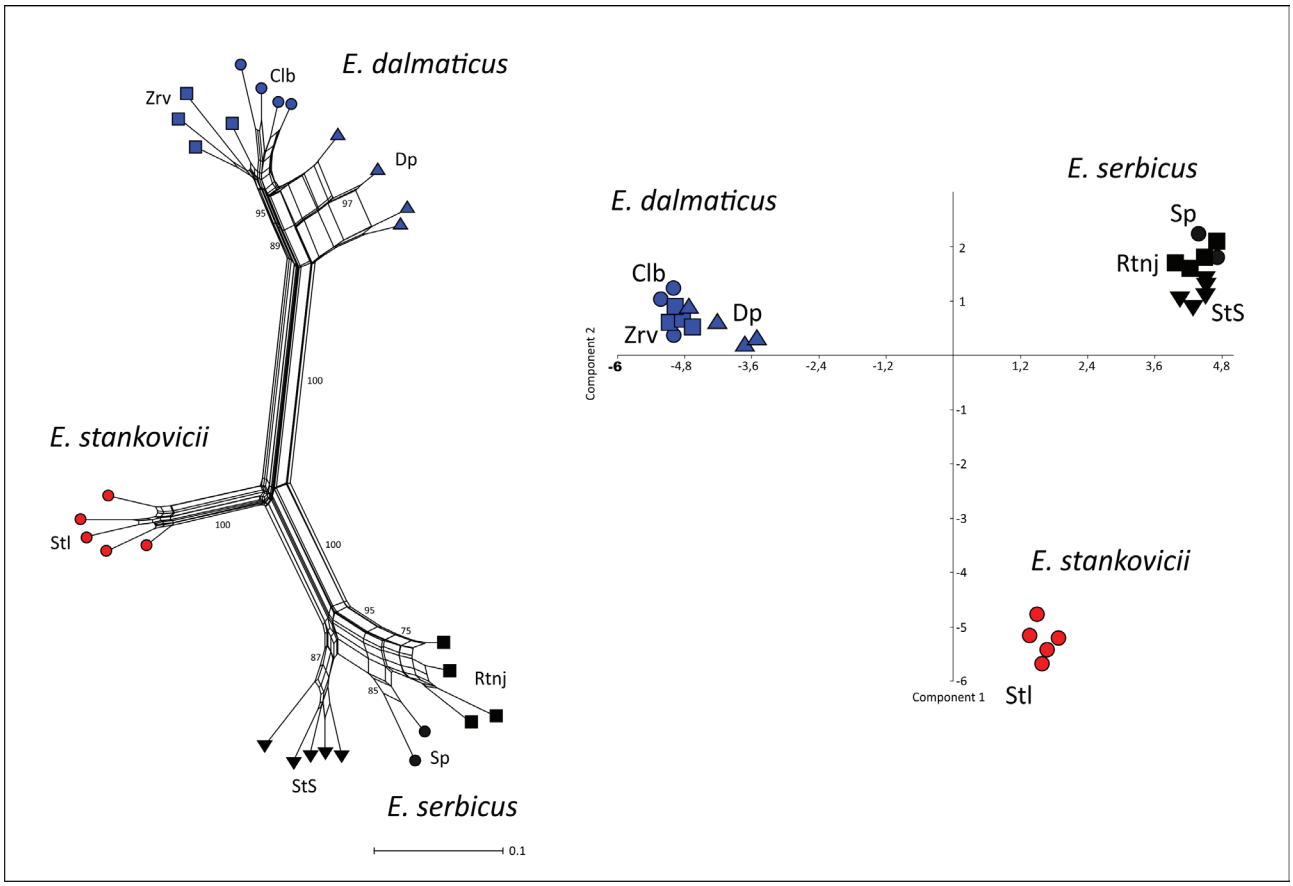

FIGURE 3. Amplified Fragment Length Polymorphisms (AFLPs) of *Edraianthus dalmaticus-serbicus* group: left) Neighbor-net diagram and right) PCoA scatterplot.

Amplified Fragment Length Polymorphisms (AFLPs).—The Neighbor-net diagram revealed three groups of populations with 100 % bootstrap support (Fig. 3A). The same result is obtained in the PCoA analysis (Fig. 3B), rendering the results of the morphometric and AFLP analyses highly congruent. Given that these three groups fully correspond to three morphological groups obtained in the morphometric analysis, we can conclude that molecular distinctiveness of previously described taxa *E. serbicus* subsp. *serbicus*, *E. serbicus* subsp. *stankovicii* and *E. dalmaticus* is confirmed by the AFLP analysis.

Chloroplast and nuclear sequences.—The phylogenetic analyses conducted on separate plastid DNA sequences (*trnL-F* region and *rbcL-atpB* spacer) and nuclear ribosomal external transcribed spacer (nrETS) data were completely congruent with each other (Fig. 4A, B). In full agreement with our morphological and AFLP findings, both have identified three lineages, each distinct (as evidenced by the branch lengths subtending them) and statistically well supported, including their backbone relationships. Not surprisingly, the analyses of the combined dataset reinforced the three ingroup lineages, with even higher overall supports (Fig. 4C). The first one is found throughout the range of *E. serbicus*, including the type locality (Mt. Suva Planina, Serbia), and hence it corresponds to the subspecies *E. serbicus* subsp. *serbicus*. The second lineage was found at Mt. Stol (NE Serbia) in a sample analysed from *E. serbicus* subsp. *stankovicii*, while the third lineage belonged to samples of the closely related *E. dalmaticus*, a species occurring in Dalmatia (Croatia), Bosnia and Herzegovina, and Montenegro.

Genome size.—Our studies showed that the nuclear DNA amount (2C value) ranged from 3.16 pg in a population of *E. serbicus* subsp. *serbicus* from Mt. Suva Planina to 3.63 pg in populations of *E. dalmaticus* from Livanjsko polje. The mean value of all investigated populations was 3.33 pg. Furthermore, all three entities revealed in morphometric and molecular analyses, and traditionally recognized as different taxa (*E. serbicus* subsp. *serbicus*, *E. serbicus* subsp. *stankovicii*, *E. dalmaticus*), have statistically significant different genome sizes (Fig. 5). 2C value in *E. dalmaticus* ranged from 3.57 to 3.63 with the mean value of 3.59 pg (data published previously in Međedović *et al*. 2007), in *E. serbicus* subsp. *serbicus* from 3.16 to 3.32 with the mean value of 3.23 pg, while *E. serbicus* subsp. *stankovicii* has intermediate position with 2C value from 3.32 to 3.41 with the mean value of 3.36 pg.

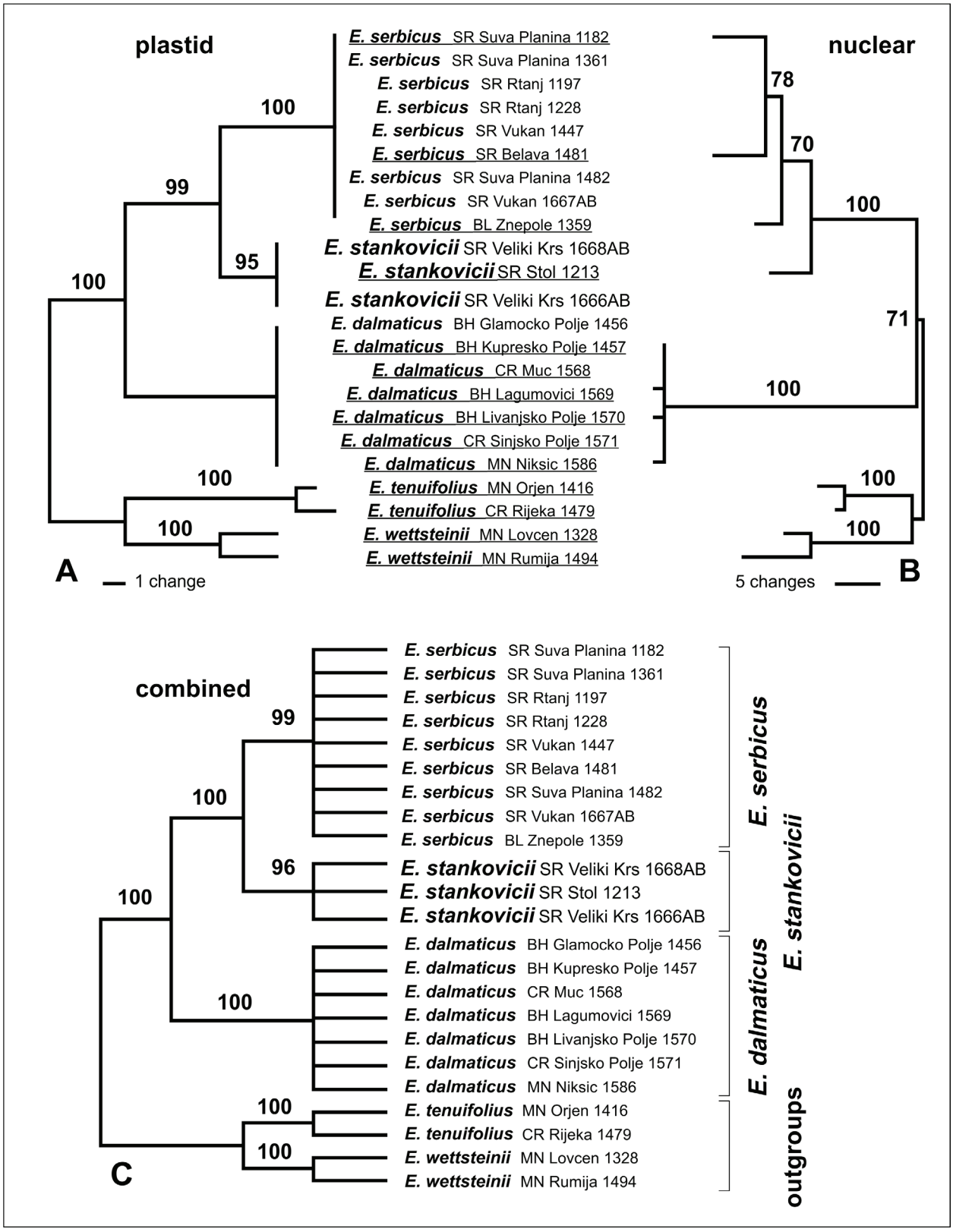

FIGURE 4. Phylogenetic relationships among species of the *Edraianthus dalmaticus-serbicus* group. **A.** The phylogram of a single MP tree (L=53; CI=0.981; RI=0.995) obtained from the concatenated plastid data (trnL-F region and rbcL-atpB spacer). **B.** The phylogram of one out of three MP trees (L=95; CI=0.947; RI=0.975) obtained from the nuclear data (3'-ETS). Underlined are individuals included in this analysis. **C.** The strict consensus of MP trees (L=148; CI=0.959; RI=0.985) from the combined dataset. In all analyses, closely related *E. tenuifolius* and *E. wettsteinii* are used as outgroup. Bootstrap values are indicated for nodes supported ≥50%. Names of species are followed by two-letter country abbreviation as well as the mountain/region from which the specimen originates. Numbers following species names correspond to DNA accessions (see Table 1). Abbreviations: BH—Bosnia and Herzegovina; BL—Bulgaria; CR—Croatia; MN—Montenegro; SR—Serbia.

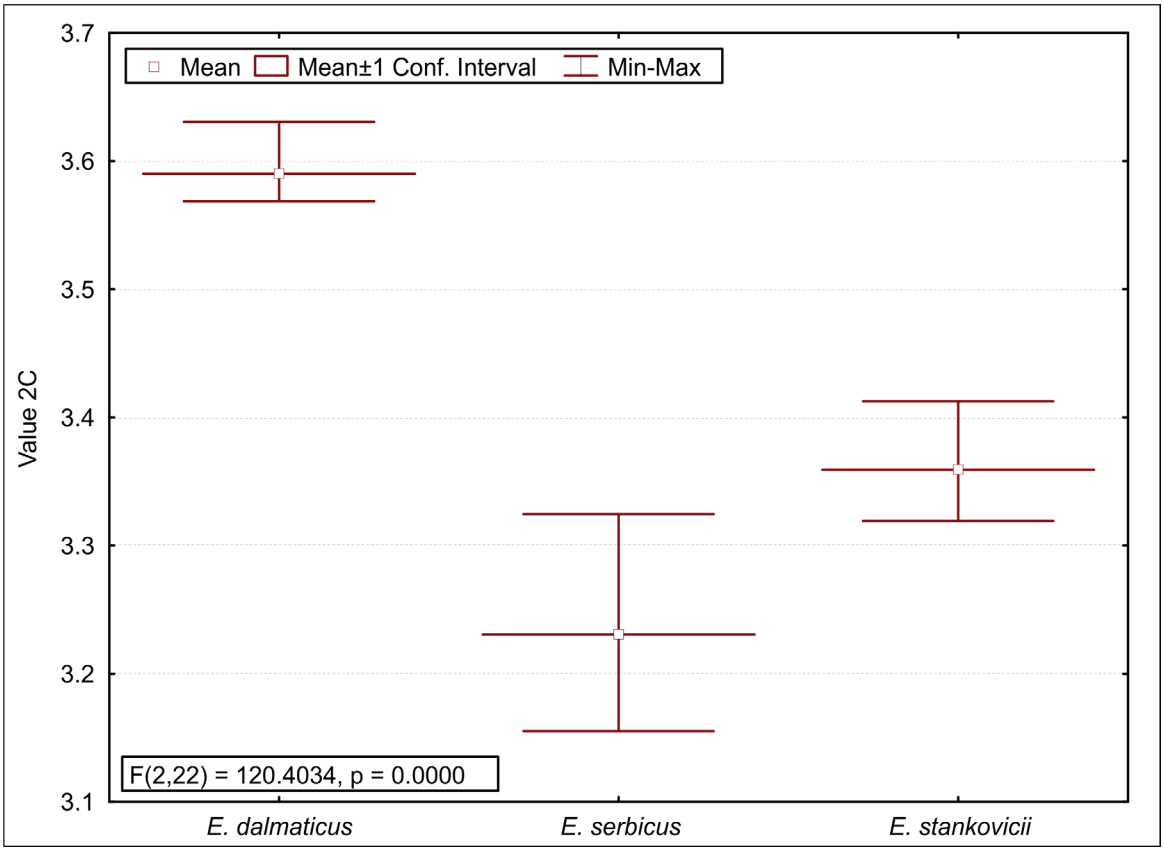

FIGURE 5. Genome size of *Edraianthus dalmaticus-serbicus* group.

The congruence between morphometric, molecular and genome size data that revealed significant morphological differences as well as distinctiveness based on molecular and genome size data indicate that all three groups merit the species rank.

TAXONOMIC TREATMENT

Edraianthus serbicus Petrović (1882: 549) ≡ *Wahlenbergia serbica* (Petrović) Beck (1893: 298).

Type:—SERBIA. Eastern: Suha plan. [Mt. Suva planina], [1]878, *[Pančić, J.] s.n.* (BEOU Herb. Panc. 9538!, lectotype designated here) (Fig. 6).

− *Hedraeanthus tenuifolius* A. DC. sensu Pančić (1856: 564).

− *Campanula serbica* Kerner (1872: LXXI) (*nom. nud.*)

− *Campanula caudata* Vis. pro parte sensu Pančić (1874: 478) - Rtanj, Stol, Pleš.

− *Campanula serbica* Kern. in Pančić (1884: 178) (*nom. nud.*)

− *Campanula kerneriana* Pančić in sched. (unpublished name).

Note:—The peculiarity of this taxon was noticed already by Anton Joseph Kerner (1831–1898), who published the name *Campanula serbica* (Kerner 1872: 71) most probably on the basis of the material collected by Josif Pančić (1814–1888) on Mt. Rtanj in 1870 (WU-Kerner 0069977, image available at http://herbarium.univie.ac.at/database/detail.php?ID=376608). However, he did not provide a description or diagnosis, leaving this name invalidly published (*nomen nudum*).

Two years later, Pančić (1874: 478) published the records of this species in "Flora Principatus Serbiae" under the name "*Campanula caudata* Vis.". In fact, even much earlier, the records from Mt. Rtanj were published by Pančić under the name "*Hedraeanthus tenuifolius* A. DC" (Pančić 1856: 564), supported by the herbarium material he collected in

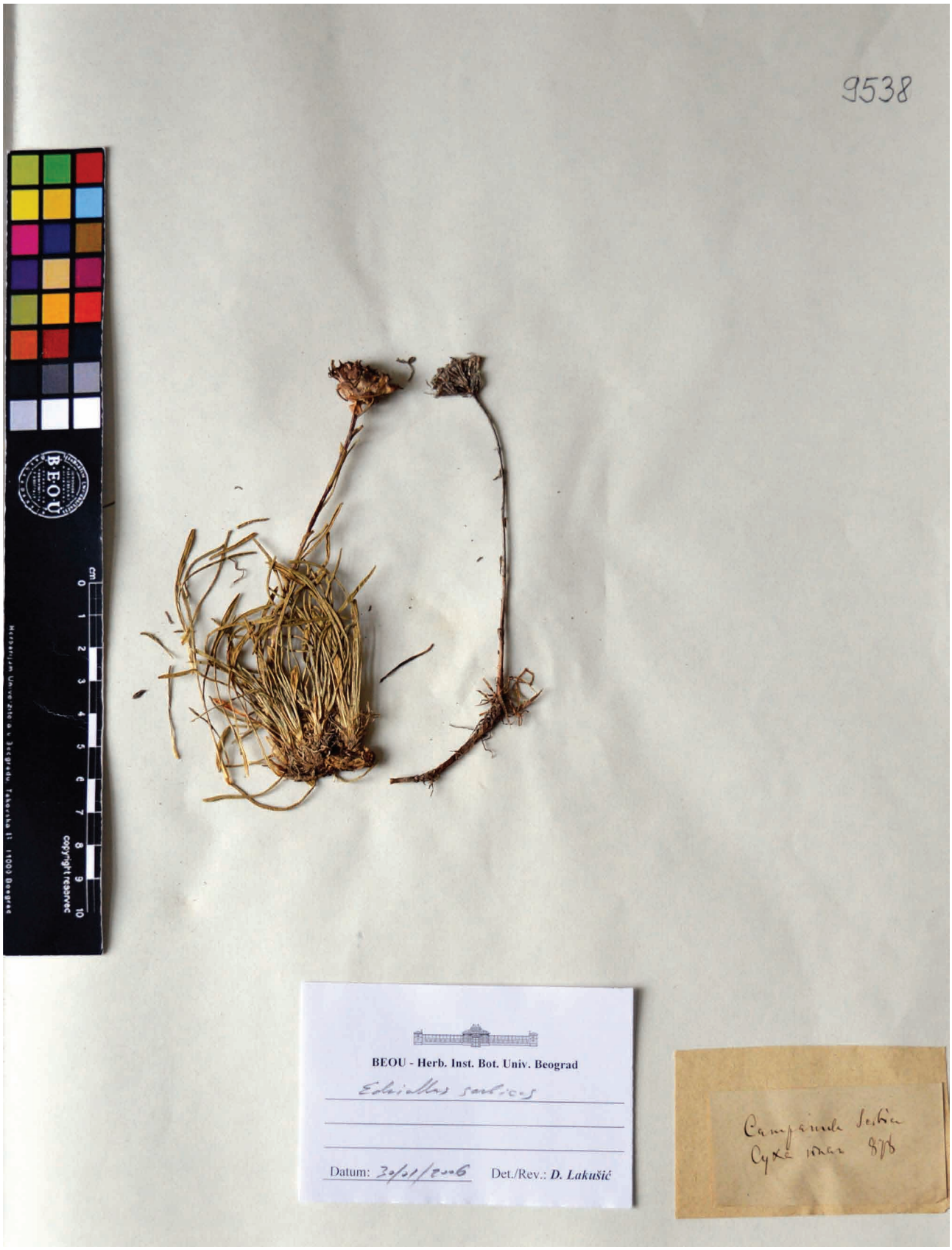

FIGURE 6. Lectotype of the name *Edraianthus serbicus* Petrović (BEOU Herb. Panc. 9538!).

1847 (BEOU 9653!). In the following years, after publishing "Flora Principatus Serbiae", Pančić collected several specimens of this species from the mountains in north-eastern Serbia, identified as "Campanula serbica" (BEOU 9537!, BEOU 9539!, BEOU 9541!, BEOU 9633!). He added notes in "Additamenta ad Floram Principatus Serbiae"

(Pančić 1884: 177) that, instead of the name *Campanula caudata* he used in Fl. Serbiae (Pančić 1874: 478), the name "*Campanula serbica* Kern." was used more often in recent times for these plants. Nevertheless, he was aware that the name of Kerner was published without a description (Pančić 1884: 178). Pančić's intention to validly describe it as a new species was not accomplished at the time, since he did not have any material of *Campanula caudata* (= *Edraianthus dalmaticus*) for a comparative study (Pančić 1884: 178). According to details on the labels of some specimens collected by Pančić and deposited in BEOU (BEOU 9447!, BEOU 9448!, BEOU 9540!), he intended to name it "*Campanula kerneriana*", in honour of Anton J. Kerner.

Between the two editions of *Flora of Serbia* (Pančić 1874, 1884), Sava Petrović (1839–1889) published *Flora of surroundings of Niš* ("Flora Agry Nyssani", Petrović 1882), and this was the first place in which conditions for valid publication of the name *Edraianthus serbicus* were fulfilled. In the preface of his book, he listed the names of the species described as new to science (Petrović 1882: VI), where no *Campanula* or *Edraianthus spp*. appeared. Further in the book, he presented some interesting rare species and their localities, listing the species "*Edraianthus serbicus* Kern." as occurring in Mt. Suva planina (Petrović 1882: XXV). His ascription of the name *Edraianthus serbicus* to Kerner was apparently a reference to Kerner (1872), in which the species was first mentioned under *Campanula*, as evident from Petrović's writing "*Campanula serbica* Kern. herb." (Petrović 1882: 549).

Therefore, the first effectively published description of *Edraianthus serbicus* was given by Petrović (1882: 549), who provided a diagnosis in the Serbian language. As he stated in the introductory remarks of the book, he studied the material collected by himself in 1879, as well as all the material collected by Pančić and Pelivanović in the years 1878, 1879, 1880 and 1881 (Petrović 1882: VIII). Following this statement, we designated the specimen collected by Pančić on Mt. Suva planina in 1878 as the lectotype.

Edraianthus serbicus subsp. ***stankovicii*** Lakušić, **subsp. nov.**

Validating description:—under '*Edraianthus serbicus* subsp. *stankovicii*' in Lakušić (1974: 27).

Type:—SERBIA. NE Serbia: Mt. Stol, kamenjar po vrhu [rocky grounds at summit], 25 June 1955, *Nikolić, V. s.n.* (BEO!, holotype) (Fig. 8).

Notes:—Radomir Lakušić (1933–2005) was the first who recognized that populations of *Edraianthus serbicus* from NE Serbia are different in regard to other populations of the species. Within *E. serbicus* Lakušić (1974) distinguished two subspecies: the typical *E. serbicus* subsp. *serbicus* found throughout the species range, and the newly described *E. serbicus* subsp. *stankovicii* (Lakušić 1974: 27) found only at Mts. Veliki Krš and Stol in NE Serbia. However, the name *E. serbicus* subsp. *stankovicii* was not validly published, since in the type citation neither a collector's name nor collecting number nor date were cited, as required by Art. 40.3 Note 2 of the ICBN (McNeill *et al*. 2012). Even if we consider the complete protologue, with introductory remarks and citation of specimens, no specimen deposited in IBUS was listed (Lakušić 1974: 23). Therefore, the name is validly published here by providing a type citation and a direct reference to the description published by Lakušić (1974: 27). The name is published here as accepted by R. Lakušić, not by us because we recognise the taxon as a species; for this reason, Art. 36.2 does not apply here.

During the siege of Sarajevo from 1991–1995, the herbarium at the Institute of Botany, University of Sarajevo (IBUS) was completely destroyed. Consequently, we could not trace the type specimen deposited in IBUS that was indicated by Lakušić (1974: 28). The specimen we choose as the holotype was seen and determined by R. Lakušić in 1969 (Fig. 7), as well as cited in his monograph (Lakušić 1974: 23), being therefore part of the original material and suitable for typification. Although this specimen was determined as *E. serbicus*, probably after more detailed investigations Lakušić (1974) decided to describe a new subspecies later on.

The original spelling "stankovićii" is correctable according to Art. 60.6 (McNeill *et al*. 2012).

Our phylogenetic analyses based on Amplified Fragment Length Polymorphisms (AFLPs), plastid DNA (trnL-F region and rbcL-atpB spacer) and nuclear ribosomal ETS sequences confirmed the monophyly of *E. serbicus* and revealed two distinct and allopatrically distributed taxa. The genome size and morphological analyses, performed on the same widespread sample of populations largely corresponded with molecular results, allowing us to raise this neglected taxon *E. serbicus* subsp. *stankovicii* to the species level.

Edraianthus stankovicii (Lakušić) D. Lakušić & Surina, **stat. nov.** ≡ *Edraianthus serbicus* subsp. *stankovicii* Lakušić in D. Lakušić *et al.*, *Phytotaxa* 269: 80. 2016 (validated in this paper). Figs. 7 and 9.

Type:—SERBIA. NE Serbia: Mt. Stol, kamenjar po vrhu [rocky grounds at summit], 25 June 1955, *Nikolić, V. s.n.* (BEO!, holotype) (Fig. 8).

− *Campanula caudata* Vis. pro parte sensu Pančić (1874: 478).

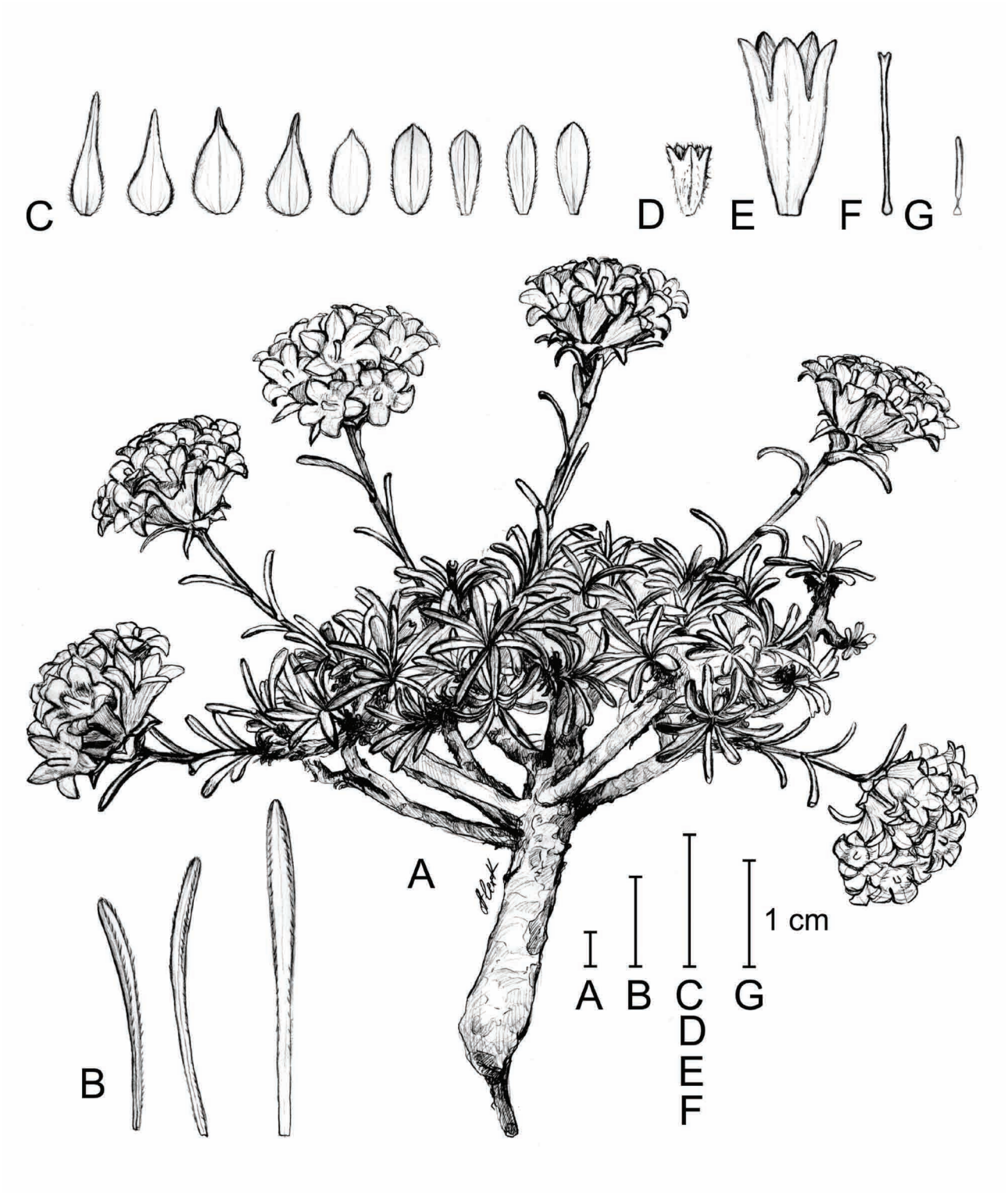

FIGURE 7. *Edraianthus stankovicii* (Lakušić) D. Lakušić & Surina, stat. nov. A. Habitus. B. Rosette leaf. Bracts of flowering capitula. D. Calyx. E. Corolla. F. Style. G. Anther. (Line drawing Aleksandra Kovačević).

The identity of the name *Edraianthus lemsii* subsp. *stankovicii* attributed to Lakušić, listed in e.g. Global Biodiversity Information Facility (GBIF) or Global Names Index (GNI), is not clear. By studying the work of Radomir Lakušić, we could not find any confirmation that he published this name. The species epithet exists in some species described

FIGURE 8. Holotype of the name *Edraianthus stankovicii* (Lakušić) D. Lakušić & Surina, stat. nov. (BEO!).

from Canary Islands (e.g. *Argyranthemum lemsii* Humphries 1976: 190, *Descurainia lemsii* Bramwell 1973: 24), and was given in honour of botanist Kornelius Lems (1931–1968) who contributed to the knowledge of the flora of

Canary Islands. This epithet exists also in some cultivars—*Dianthus* "lemsii" (*Dianthus plumarius* "lemsii"). Since *Edraianthus serbicus* is also cultivated in rock gardens throughout Europe, we assume that the mistake has occurred while taking the epithet "lemsii" from a *Dianthus* cultivar, which was wrongly included in the name *Edraianthus serbicus* as *E. serbicus* "lemsii" (later on as *E. lemsii* and/or *E. lemsii* subsp. *stankovicii*).

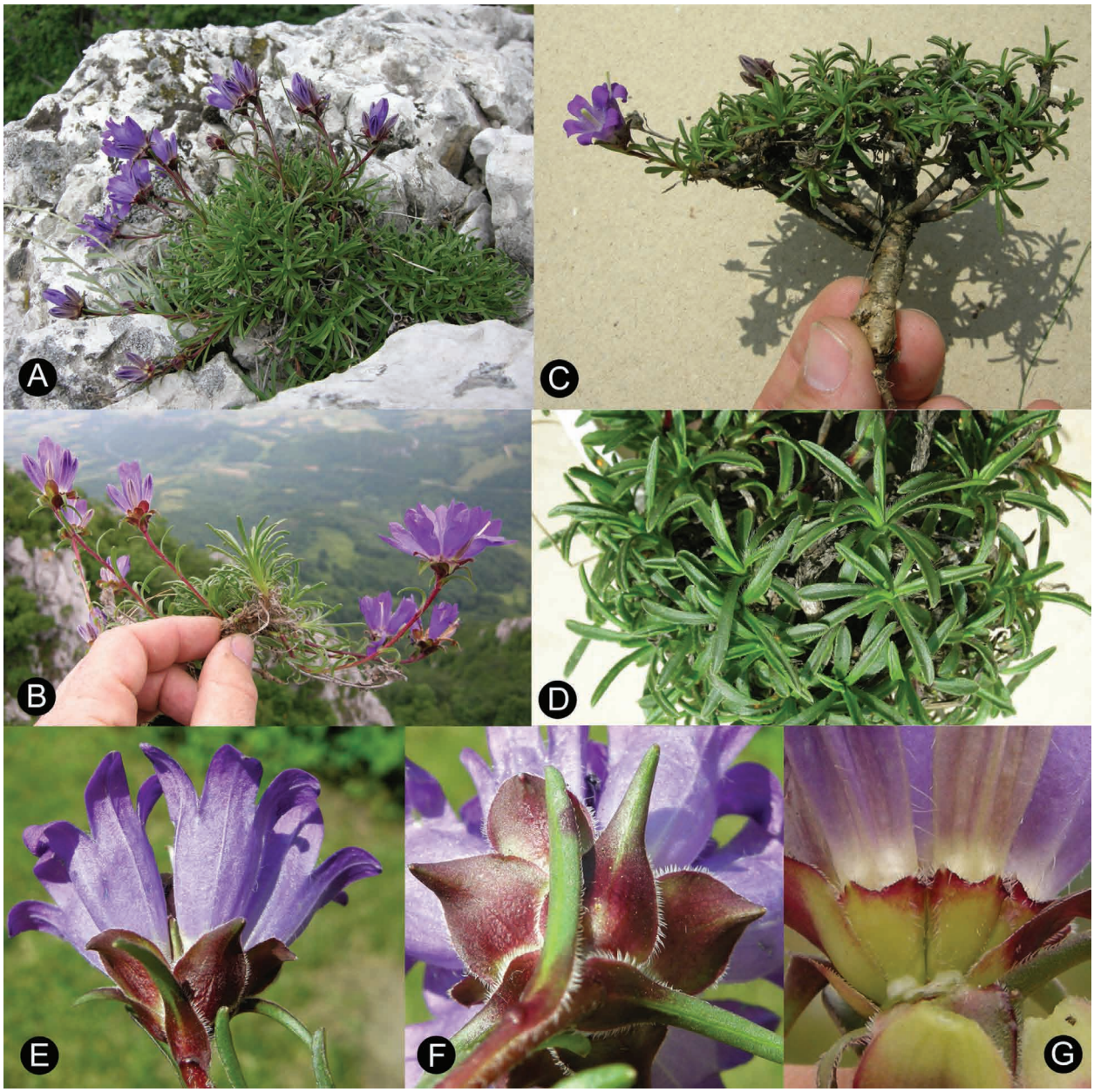

FIGURE 9. *Edraianthus stankovicii* from Mt. Veliki Krš in E Serbia. **A-B**. Habitus. **C**. Stout, woody, branched rhizome. **D**. Rosette leaf. **E**. Terminal cluster with subsessile flowers sparsely subtending by bracts. **F**. Bracts of flowering capitula, with white ciliate margin. **G**. Calyx with broadly triangular calyx-teeth that are shorter than ovary (Photos: D. Lakušić).

Given that in relevant recent floristic literature *E. stankovicii* is suppressed as synonym of *E. serbicus* (Castroviejo *et al.* 2010, Lammers 2007) or completely ignored (Kuzmanov 1976), as well as that recent research yielded new insights into the morphology, phylogeny and systematics of the *E. dalmaticus-serbicus* group, a new differential diagnosis, description and illustration of *E. stankovicii* are additionally provided (Tab. 3, Figs. 7, 9–10).

In the morphological descriptions value ranges correspond to the mean ± standard deviation, with the minimal and maximal values in brackets.

Diagnosis:—Closest to *E. serbicus*, from which it differs by leaf shape (spathulate to spathulate-ligulate *vs.* narrowly linear-lanceolate); length of basal leaves ((12.8–) 19.9–33.6 (–35.2) mm *vs.* (12.8–) 35.3–69.0 (–93.7) mm); shape of outer involucral bracts (slightly attenuate and shorter than the flower *vs.* abruptly long-attenuate and as long as or longer then flowers, Fig. 10).

FIGURE 10. Main morphological differences in characteristics of bracts and flowers. Left plant: *Edraianthus serbicus*—Mt. Rtanj. Right plant: *Edraianthus stankovicii*—Mt. Veliki Krš (Photo D. Lakušić).

Description:—Caespitose perennial. Rhizome stout, woody, branched. Stems numerous, simple, (2.8–) 4.1–8.5 (–10.6) cm, erect to ascending, more or less hairy. Leaves spathulate to spathulate-ligulate, in upper part sometimes slightly crenulate, glabrous, ciliate with ciliae oriented towards leaf apex; cauline leaves few or up to 10, (13.7–) 15.7–22.0 (–24.7) × (1.7–) 1.7–2.5 (–3.3) mm, subsessile; basal (rosette) leaves numerous, (12.8–) 19.9–33.6 (–35.2) × (1.1–) 1.3–2.6 (–3.7) mm. Inflorescence is a terminal cluster with (2–) 3–7 (–8) subsessile flowers, sparsely subtending by bracts. Bracts (6–) 7–9 (–10), entire, obtuse, slightly attenuate, shorter than the flower, glabrous, reddish to brownish, margin with white ciliae; outer broadly ovate to ovate-oblong, (9.0–) 9.8–15.0 (–19.2) × (3.4–) 3.8–5.6 (–6.3) mm, inner ovate to ovate-oblong, (6.0–) 7.5–11.1 (–11.9) × (4.1–) 4.5–5.9 (–6.6) mm. Calyx tube green, glabrous to sparsely hairy; calyx lobes widely triangular, (0.8–) 1.0–1.8 (–2.3) × (0.9–) 1.1–1.5 (–1.6) mm, much shorter than tube, reddish, white ciliate. Corolla campanulate, (12.6–) 13.8–18.5 (–20.9) × (6.4–) 7.6–10.8 (–11.6) mm, glabrous or hirsute on veins, violet; corolla lobes (5.4–) 5.5–7.9 (–9.2) × (3.2–) 3.4–4.5 (–5.3) mm. Style 1, (13.6–) 14.0–17.9 (–18.9) mm long, stigma 2–3 lobed; Stamens 5, inserted on disc, anthers (3.2–) 4.0–6.1 (–7.1) mm long, lower part distinctly dilated to deltoid shaped (1.5–) 1.7–2.3 (–2.7) mm long structure.

Chromosome Number:—*2n* = 32 (estimated based on the genome size).

Phenology:—Flowering specimens have been observed from June to July.

Etymology:—The species epithet is taken from Lakušić (1974). The new species is named in honour of Prof. Siniša Stanković, a Serbian biologist who significantly contributed to the development of zoological and botanical research in the former Yugoslav countries.

Distribution and ecology:—*Edraianthus stankovicii* is distributed in a very narrow area restricted to the Mts Veliki Krš and Stol (northeastern Serbia). The nearest populations of the *E. serbicus* complex are situated c. 50 km northwest (Mt. Vukan), and southwest (Mt Rtanj) from the type locality (Fig. 1). *Edraiantus stankovicii* is an eastern Moesian endemic species (Horvat *et al*. 1974).

The new species exclusively inhabits the calcareous north, east and south-east facing exposed rocky crevices (*Edraiantho graminifolii-Erysimion comati* Mucina *et al*. 1990, *Asplenietea trichomanis* Br.-Bl. 1934 corr. Oberd.

1977) at elevations between 900 and 1100 m. *Edraianthus stankovicii* is one of the accompanying rock dwellers growing together with *Achillea ageratifolia* subsp. *serbica* (Nyman) Heimerl., *Asplenium ruta-muraria* L., *A. trichomanes* L., *Aster alpinus* L. subsp. *dolomiticus* (Beck) Hayek, *Carex humilis* Leyss., *Carum graecum* Boiss. & Heldr., *Dianthus noeanus* Boiss., *Erysimum comatum* Pančić, *Euphrasia salisburgensis* Funck, *Festuca rupicola* Heuff. s.l., *Primula auricula* subsp. *serratifolia* (Rochel) Ják., *Saxifraga paniculata* Mill., *Sesleria filifolia* Hoppe, *Silene flavescens* Waldst. & Kit., *Thymus serbicus* Petrović, *Trinia glauca* (L.) Dumort., etc.

Conservation status:—*Edraianthus stankovicii* is found only at the Mts Veliki Krš and Stol that are only 5 km apart. Its population size is estimated to be less than 2000 mature individuals while the area of occupancy is smaller than 1 km^2. Therefore according to the IUCN (2001) Criteria it should be regarded as Critically Endangered (criteria CR B1 i, ii, iv; B2a).

Identification key to taxa with broadly triangular calyx-teeth which are shorter than ovary

1. Outer involucral bracts up to twice as long as flowers ..... *E. dalmaticus*
– Outer involucral bracts shorter or slightly longer than flowers 2
2. Basal leaves (12.8–) 35.3–69.0 (–93.7) mm long, narrowly linear-lanceolate; outer involucral bracts abruptly long-attenuate and as long as or slightly longer than flowers ..... *E. serbicus*
– Basal leaves (12.8–) 19.9–33.6 (–35.2) mm long, spathulate to spathulate-ligulate; outer involucral bracts slightly attenuate and shorter than flowers ..... *E. stankovicii*

SPECIMENS EXAMINED

Edraianthus serbicus

BULGARIA. **Znepole:** Mt. Chepan, near Dragoman, 42.9545 N, 23.0015 E, 1117 m, 19 July 2003, *Petrova, A. 19983* (BEOU!); **Pernik**: Staro Selo S from Pernik, E from the road Staro Selo-Studena, 42.498916 N, 23.138014E, 820 m, dry open meadow on limestone, 27 June 2006, *Frajman, B. & Schönswetter, P. 11313* (WU!); **Ćustendil**: Mt. Konjevska planina, Viden, 42.34408 N, 22.83953 E, 1487, rocky grasslands (*Festuco-Seslerietea*), limestone, 20 June 2010, *Lakušić, D., Tomović, G., Vukojičić, S., Uzunov, D., Gusev, Ch. 30487* (BEOU!), 454 (NHMR!).

SERBIA. **Eastern**: Mt. Belava, Kardašica, from Suhodol through to the Belava top, rocky crevices, limestone, 18 May 2003, *Jušković, M. 20669* (BEOU!); Mt. Rtanj, 1870, *Pančić, J. s.n.* (BEOU 9640!), rocky grasslands, June 1870, *Pančić, J. s.n.* (WU069977!), 1872, *Pančić, J. s.n.* (BEOU 9426!), rocky crevices, June 1876, *Pančić, J. s.n.* (BEOU 9540!), June 1876, *Pančić, J. s.n.* (BEOU 9653!), 1880, *Pančić, J. s.n.* (BEOU 9448!), *Pančić, J. s.n.* (BEOU 9636!), east side of the main ridge, 1100–1550 m, pastures, 29 June 1971, *Nikolić, V. & Diklić, N. s.n.* (BEO!), east side of the main ridge, rocky terrain to the top, 1100–1550 m, pastures, 29 June 1971, *Nikolić, V. & Diklić, N. s.n.* (BEO!), southeastern slopes, rocky grasslands, limestone, 20 June 1972, *Nikolić, V., Diklić, N., Bogdanović, M. s.n.* (BEO!), to the top, rocky grasslands, 20 June 1972, *Nikolić, V., Diklić, N., Bogdanović, M. s.n.* (BEO!), southeastern slopes, 1450 m, rocky crevices, limestone, 29 June 1980, *Nikolić, V., Diklić, N., Mladenović, S. s.n.* (BEO!), 1000–1550 m, rocky grasslands, limestone, 29 June 1980, *Nikolić, V., Diklić, N., Mladenović, S. s.n.* (BEO!), 01 June 1983, *Stevanović, V., 9591* (BEOU!), rocky grasslands, limestone, 01 June 1983, *Stevanović, V. 10503* (BEOU!), top Šiljak, 43.77557 N, 21.89263 E, 1520, rocky grasslands (*Festuco-Seslerietea*), limestone, 06 August 2008, *Lakušić, D. 27624* (BEOU!), c. 1600 m, rocky grasslands, limestone, 15 June 2008, *Rakić, T. & Rakić, B. 27216* (BEOU!), c. 1400 m, 31 July 2013, *Jakovljević, K., Kuzmanović, N., Đurović, S., Buzurović, U. 38860* (BEOU!); Rtanj - Zlot, 30 June 1982, *Stevanović, V. 10379* (BEOU!), footpath from the village Rtanj to the summit, S of village Lukovo, 43.775186 N, 21.891624 E, 1300 m, rock fissures, limestone, 29 May 2006, *Frajman, B. & Schönswetter, P.413, 414* (NHMR!); Mt. Suva planina, June 1876, *Pančić, J. s.n.* (BEOU 9638!), 1878, *Pančić, J. s.n.* (BEOU 9538!), May 1884, *Petrović, S. s.n.* (BEOU 9541!), rocky grasslands, June 1884, *Petrović, S. s.n.* (P00276370!, P00276371!, P00276373!), in alpine region, 1500–1700 m, 28 July 1887, *Bornmüller, J. s.n.* (*B_10_0321656*!), 43.180262 N, 22.176100 E, 2005, *Graz 2003* (NHMR!), Trem, 1600 m, rocky crevices (*Asplenietea trichomanes*), limestone, 27 June 2005, *Lakušić, D. & Tomović, G. 19812.1* (BEOU!), Trem, rocky crevices (*Asplenietea trichomanes*), limestone, 31 May 2006, *Tomović, G. & Zlatković, B. 20889* (BEOU!), Sokolov kamen, 43.20236 N, 22.13316 E, 1430 m, rocky crevices (*Asplenietea trichomanes*), limestone, 08 May 2012, *Lakušić, D. & Zlatković, B. 34223* (BEOU!), Sokolov kamen, 43.21323 N, 22.10997 E, 1411.2 m, rocky grasslands (*Festuco-Seslerietea*), limestone, 12 July 2008, *Lakušić, D. 27502* (BEOU!), Sokolov kamen, top, 43.21033 N, 22.11493 E, 1531.6 m, rocky grasslands (*Festuco-Seslerietea*), limestone, 12 July 2008, *Lakušić, D. 27501* (BEOU!), Sokolov kamen, 43.21033 N, 22.11493 E, 1532.2 m, rocky grasslands (*Festuco-*

Seslerietea), limestone, 17 June 2010, *Lakušić, D. 30391* (BEOU!), Sokolov kamen, 1552 m, rocky grasslands, 17 July 1974, *Nikolić, V., Diklić, N., Bogdanović, M. s.n.* (BEO!), Mosor - Sokolov kamen, limestone, 06 July 1997, *Vukojičić, S.* & *Tomović, G. 6605, 6506* (BEOU!), Devojački grob - Trem, limestone, 08 July 1997, *Vukojičić, S.* & *Tomović, G. 6357.1* (BEOU!), Devojački grob - Trem, 1400–1800 m, limestone, 31 May 2006, *Tomović, G.* & *Zlatković, B. 21216* (BEOU!), Devojački grob - Trem, limestone, 03 July 2010, *Kuzmanović, N., Batanjski, V., Plećaš, M. 36166* (BEOU!); Svrljiške planine, Pleš, rocky grasslands, limestone, July 1880, *Pančić, J. s.n.* (BEOU 9539!), Zeleni vrh, foothill, rocky grasslands, 23 June 1957, *Nikolić, V. s.n.* (BEO!). **Northeastern:** Malinik, above the canyon Zlot, rock crevices, 26 June 1964, *Diklić, N.* & *Miladinović, Lj. s.n.* (BEO!), Zlot, 800 m, limestone, 13 July 1985, *Niketić, M. 27/85* (BEOU!). **Pomoravlje:** Vukan, Veliki Vukan, < 740 m, rocky crevices, limestone, 29 April 2003, *Niketić, M.* & *Tomović, G. 16458* (BEOU!), Veliki Vukan, top, 44.29928 N, 21.53833 E, 826 m, rocky grasslands (*Festuco-Brometea*), limestone, 17 June 2010, *Lakušić, D. 30390* (BEOU!). **Southeastern:** Rudina planina, 900–1200 m, limestone, 16 August 2006, *Niketić, M.* & *Tomović. G. 21842* (BEOU!).

Edraianthus stankovicii

SERBIA. **Northeastern**: Mt. Stol, rocky grasslands, limestone, 07.1871, *Pančić, J. s.n.* (PAD H0023192!), rocky grasslands, June 1876, *Pančić, J. s.n.* (BEOU 9537!), June 1876, *Pančić, J. s.n.* (BEOU 9633!), 1879, *Pančić, J. s.n.* (BEOU 9447!), rocky grasslands, *Pančić, J. s.n.* (BEOU 9639!), the ridge, 23 June 1955, *Nikolić, V. s.n.* (BEO!), top, rocky grasslands, 25 June 1955, *Nikolić, V. s.n.* (BEO!), below the top, 1100 m, pastures, 15 June 1979, *Nikolić, V., Diklić, N., Mladenović, S. s.n.* (BEO!), the ridge, 1000–1100 m, rocky grasslands, limestone, 27 June 1980, *Nikolić, V., Diklić, N., Mladenović, S. s.n.* (BEO!), 06 June 1991, *Benić, N. 1545/91* (BEOU!), 44.17606 N, 22.13036 E, 1036 m, rocky crevices, limestone, 30 July 2013, *Jakovljević, K., Kuzmanović, N., Đurović, S., Buzurović, U. 38849* (BEOU!), NNE slope, 44.175015 N, 22.126635 EE, 950 m, shady limestone rocks, 20 May 2006, *Frajman, B.* & *Schönswetter, P. 412* (NHMR!), rock fissures, 1100 m, 01 July 1961, *Blečić, V.415* (NHMR!), above the village of Krivelj, rock fissures, 800 m, 12 July 1985, *Mayer, M.* & *Mayer, E. 416* (NHMR!); Mt. Veliki krš, 1000 m, rocky crevices, limestone, 30 June 1960, *Diklić, N. s.n.* (BEO!), the ridge, 1000 m, rocky grasslands, limestone, 23 June 1961, *Diklić, N. s.n.* (BEO!), the ridge, 900–1100 m, rocky grasslands, 25 June 1971, *Nikolić, V.* & *Diklić, N.* (BEO!), the ridge, 1000–1150 m, 17 June 1972, *Nikolić, V., Diklić, N., Bogdanović, M. s.n.* (BEO!), the ridge, lilac thickets, limestone, 16 June 1979, *Nikolić, V., Diklić, N., Mladenović, S. s.n.* (BEO!), Strelinik, top, 1065 m, 16 June 1973, *Nikolić, V., Diklić, N., Bogdanović, M. s.n.* (BEO!), Vrat, rocky crevices, 09 July 1990, *Benić, N. 1783/90* (BEOU!), rocky grasslands, 07 July 1990, *Benić, N. 1664/90* (BEOU!), 07 June 1991, *Benić, N. 1512/91* (BEOU!), the ridge, 44.1711 N, 22.0874 E, 1155.2 m, rocky crevices (*Asplenietea trichomanes*), limestone, 15 June 2008, *Lakušić, D. 27217* (BEOU!).

Edraianthus dalmaticus

BOSNIA AND HERZEGOVINA. **Dinaric Mts.**: Čvrsnica mountain range, Dugo polje plain, Blidinje, between Mt. Vran and Mt. V. Čvrsnica at the entrance to polje from Doljani, 43.683349 N, 17.581934 E, 1212, m, moist grassland with prevailing *Deschampsia cespitosa*, dry river bed, 05 July 2006, *Modrić, Ž.* & *Surina, B 417* (NHMR!); Livanjsko polje, near Čelebić, 43.944583 N, 16.747222 E, 730 m, 04 July 1989, *Mayer, M.* & *Mayer E. 421* (NHMR!), between Kazani and Čelebić, 43.972500 N, 16.720002 E, 720 m, 12 June 1983, *Mayer, M.* & *Mayer E. 419* (NHMR!), between Čelebić and Donji Kazani, 43.972502 N, 16.720003 E, 714, m, dry grassland, 07 July 2006, *Modrić, Ž.* & *Surina, B. 418* (NHMR!); Glamočko polje, near Glamoč, 44.058611 N, 16.856667 E, 895 m, 13 June 1983, *Mayer, M.* & *Mayer E. 419, 420* (NHMR!).

CROATIA. **Dalmatia:** Zagora, 43.694167 N, 16.453878 E, 01 May 2005, *Mihelj et al.*, between Donje Postinje and Vrba on northeastern slopes of Gradina, 43.718056 N, 16.398056 E, 31 May 2006, *Mihelj & al. 2053* (NHMR!).

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