Campanula cichoracea (Campanulaceae), a neglected species from the Balkan-Carpathian C. lingulata complex as inferred from molecular and morphological characters

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Abstract

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The taxonomically intricate *Campanula lingulata* complex confined to the Balkan Peninsula is reviewed using molecular and morphological data. An extensive sample of 62 individuals for phylogenetic analyses and 402 individuals for morphometric analysis from 17 populations across the species range was used. The phylogenetic analyses based on two chloroplast intergenic spacers (*trnG*^{UCC}-*trnS*^{GCU} and *psbA-trnH*) and morphological analysis based on 50 characters revealed two allopatrically distributed lineages of the *C. lingulata* complex that comprise individuals from the C and S Balkans, respectively. Both molecular and morphological data allowed us to re-establish *C. cichoracea* Sm., a species endemic to Thessaly in Greece. This species can easily be distinguished from *C. lingulata* s.str. by its calyx appendages hairy on the margins and adaxial side, and ovary continuously downwards hairy all over the surface. Molecular characters that can be used to distinguish these two species comprise four parsimony-informative substitutions within *trnG*^{UCC}-*trnS*^{GCU}, and a microsatellite with a dinucleotide (AT) motif present only within the *psbA-trnH* region in *C. lingulata*. Further studies are required for resolving the taxonomic status of the remaining Macedonian and Rhodopean sub-lineages from the S Balkans.

Additional key words: morphometry, phylogeny, chloroplast, taxonomy, DNA barcodes

Introduction

The bellflower genus *Campanula* L. is one of the most prominent examples of a plant group with an exceptional diversity of species in the N hemisphere. It comprises some 420 species in its traditional circumscription (morphological evidence, Lammers 2007), but up to 600 species when including all lineages to which *Campanula* is paraphyletic (molecular evidence, Roquet & al. 2008; Borsch & al. 2009; Cellinese & al. 2009; Haberle & al. 2009; Mansion & al. 2012). However, given the high plasticity of many morphological characters in this genus (Eddie & Ingrouille 1999; Roquet & al. 2008), its taxonomic delimitation and infrageneric classification are unclear, incomplete and somewhat controversial (Fedorov 1957; Damboldt 1978; Oganesian 1995; Quézel 1953). Furthermore, available molecular phylogenies, which generally supported the polyphyly of *Campanula* and related taxa, failed to provide a comprehensive phylogenetic hypothesis for the genus because of a rather limited taxon sampling (e.g. Eddie & al. 2003; Park & al. 2006; Roquet & al. 2008; Borsch & al. 2009; Cellinese & al. 2009; Haberle & al. 2009). Recently, Mansion & al. (2012) generated the most comprehensive phylogenetic

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inference for the genus *Campanula* including more than 70 % of the described *Campanula* species. They used a short genomic region with a high phylogenetic signal and overall recovered 17 well-supported and circumscribed sub-lineages instrumental for developing more specific evolutionary hypotheses.

The most recent phylogeny of *Campanula* (Mansion & al. 2012), however, contained unresolved nodes at a shallow phylogenetic level. It was particularly the case for the clade "Cam17" comprising some 195 taxa occurring predominantly throughout the E Mediterranean Basin and the Middle East, which are regions well-known as biodiversity hot-spots (Myers & al. 2000) and centres of speciation and diversification (Griffiths & al. 2004). Mansion & al. (2012) further observed that within clade "Cam17", individuals from the same species from several morphologically polymorphic complexes, such as *C. barbata* L., *C. lingulata* Waldst. & Kit., *C. sibirica* L. or *C. spatulata* Sm., were generally grouped as sisters. The authors suggested the need for future phylogeographic and/or speciation studies in these complexes.

The morphologically and genetically diverse *Campanula lingulata* complex is sub-endemic to the Balkans. The species ranges from costal to subalpine zones of this region, i.e. at altitudes from sea level to 2000 m in the C and S Dinaric, Scardo-Pindic, Rhodope-Rila and Balkan mountain systems including the mountains of N Serbia, referred to as the Carpatho-Balkanides (Stevanović & al. 2009). The northernmost sites of this complex are at the S edge of the Pannonian Plain, at Fruška Gora and the Vršačke Mts (Serbia) and in Romanian Banat, while the southernmost localities are found within mountain ranges in S Peloponnese (Greece). Plants from the *C. lingulata* complex grow mostly in open sites but also in canyons and gorges. They occupy predominantly rocky habitats, crevices and screes, as well as grasslands on limestone, dolomite and serpentine bedrocks found within different phytogeographic regions and provinces such as the Carpathian region and the C and S Balkan, Adriatic and Aegean provinces (Meusel & Jäger 1992; Jäger & Welk 2003).

The described diversity of habitats in which *Campanula lingulata* is present is indicative of a species with high adaptability, plasticity and/or heterogeneity. Thus, it is not surprising that a considerable morphological polymorphism has been observed throughout the range of the species. That, however, has triggered delineation of several taxa within this complex overall resulting in a plethora of "taxonomic" synonyms for *C. lingulata* (Greuter & al. 1984; Conti & al. 2005; Lammers 2007). *Campanula tenuiflora* Ten., described by Tenore (1824–1829) based on only one finding in Cilento (Italy), has been considered as a synonym of *C. lingulata* by Nyman (1879). The former name has been suppressed from the floristic literature, and furthermore the record of *C. lingulata* in Italy, which would suggest an amphi-Adriatic distribution of this species, has been considered doubtful (Pignatti 1982) or represents a finding of a species that became extinct (Greuter & al. 1984) and is no longer present in Italy (Conti & al. 2005). Schultes (in Roemer & Schultes 1819) and Candolle (1830) supported delineation of *C. cichoracea* Sm., described initially by Smith (in Sibthorp & Smith 1806, 1819), but they included *C. capitata* Sims (Sims 1805) as a doubtful synonym. Nyman (1879), however, recognized only one species, *C. lingulata*, and considered both *C. cichoracea* and *C. capitata* as synonyms of *C. lingulata*. The latter view remained valid until the present day, and consequently the name *C. cichoracea* disappeared from modern floristic literature while *C. capitata* has been mentioned in a few floristic papers only regarding the flora of Dalmatia (de Visiani 1847; Degen 1938) and Montenegro (Pančić 1875). Infraspecific taxa have also been described within *C. lingulata* s.l., comprising *C. lingulata* var. *intybacea* Griseb. (Grisebach 1846), *C. lingulata* f. *gracilis* K. Malý and *C. lingulata* f. *grandiflora* K. Malý (Malý 1908).

Based on morphological grounds, *Campanula lingulata* is placed into the large *C.* sect. *Campanula*, characterized by dehiscent capsules with basal pores, 3-locular ovary and calyx with appendages between the lobes (Fedorov & Kovanda 1976). Further, *C. lingulata* is positioned into *C.* ser. *Involucratae* (Fomin) Kharadze (1949) (≡ *C.* [sect. *Medium*] subsect. *Involucratae* (Fomin) Fedorov (1957)), characterized by subsessile flowers, crowded in heads, whorls or clusters subtended and enveloped by large bracts (Damboldt 1978). The first, very short description of *C. cichoracea* based on plants collected in Thessaly (Greece) was published in Florae graecae prodromus (Sibthorp & Smith 1806: 140), while a more detailed account and illustration of this species can be found in Flora graeca (Sibthorp & Smith 1819: 7, t. 209). The authors, however, provided a diagnosis of this species but without explaining why *C. cichoracea* should be distinguished from *C. lingulata*. As already mentioned, recent floristic literature does not recognize *C. cichoracea* as a distinct taxon, and treats it as a synonym of *C. lingulata* (Greuter & al. 1984; Euro+Med 2006+; Lammers 2007).

Molecular data, on the other hand, can successfully be used not only for revealing organismic evolution but also for taxonomic work (e.g. Filipowicz & al. 2012). Furthermore, homologous DNA sequences of one or several genomic regions can be used for identifying species ("DNA barcoding", CBOL Plant Working group 2009). Although several markers have been recommended for barcoding in plants (CBOL Plant Working group 2009), growing evidence reveals that along with the standard barcoding fragments, such as *matK* and *rbcL*, additional genomic regions may be required to provide sufficient information to distinguish closely related species in a taxonomic context (e.g. Seberg & Petersen 2009; Ran & al. 2010; Korotkova & al. 2011; González-Gutiérrez & al. 2013).

Given the considerable morphological variability of the *Campanula lingulata* complex throughout its range, and the potential molecular polymorphism detected so far in this complex, the aim of the present study is to eluci-

date the controversy concerning the taxonomic status of *C. cichoracea*. We utilize two chloroplast intergenic spacers and 50 morphological characters to assess: (1) whether a taxonomic separation of *C. cichoracea* initially suggested by Sibthorp & Smith (1806, 1819) and supported by Roemer & Schultes (1819) and Candolle (1830) based on morphological data is supported by molecular data as well, and (2) whether morphological characters provide a consistent segregation of putative taxa within the *C. lingulata* complex. We provide morphological and molecular characterization of *C. cichoracea*, a neglected species from the *C. lingulata* complex confined to Thessaly (Greece).

Material and methods

Taxon sampling

Plant material from *Campanula lingulata* populations covering the entire range of the species was sampled from 2007 to 2012 (Table 1; Fig. 1). The samples were collected during summer, when plants were in full anthesis, and samples for both analyses (molecular and morphometric) were collected from each population, with six exceptions when populations could not provide sufficient material for both analyses. In those six cases, we used samples from populations SR-Kokin Brod, SR-Studenica and GR-Leptokaria for morphometric analyses, and samples from populations SR-Panjica, SR-Vujan and GR-Koromilles for molecular analyses. Nonetheless, population pairs from Serbia: SR-Kokin Brod/SR-Panjica and SR-Studenica/SR-Vujan, and from Mt Olympus, Greece: GR-Leptokaria/GR-Koromilles (henceforth referred to as GR-Olympus) represent three pairs of spatially neighbouring populations, between which significant morphological differences were not observed (S. Škondrić pers. obs.). Thus, plant material from 11 populations was used for both molecular and morphometric analyses, and each of these analyses was performed utilizing material from three additional populations. The number of populations analysed from both molecular and morphological aspects was 14, while the total number of sampled populations was 17 (Table 1).

For molecular analyses, leaves were collected from two individuals per population (SR-Panjica), four individuals per population (BH-Stolac; BU-Rhodopes; CR-Biokovo; GR-Ossa; MN-Herceg Novi) and five individuals per population (GR-Olympus; MA-Valandovo; MN-Ostrog; MN-Valdanos; SR-Fruška Gora; MA-Šara; SR-Stara; SR-Vujan) yielding a total sample of 62 (Table 1). For morphological analyses, one stem leaf and inflorescence per plant were collected at full anthesis from c. 30 individuals per population yielding a total sample of 402 (Table 1). Sampled individuals were evenly distributed throughout populations and at least 20 m distant from each other. Plant material for molecular analyses was put in silica gel, dried for 7 days, and kept in a dark and dry place prior to DNA isolation. For morphological analyses, plant material was fixed in 96 % ethanol-glycerol solution (1:1) and kept at room temperature prior to analyses.

The species with capitulate inflorescences, viz. *Campanula cervicaria* L., *C. foliosa* Ten., *C. moesiaca* Velen., *C. tymphaea* Hausskn. and *Edraianthus graminifolius* L., belonging to the clade "Cam17" of Mansion & al. (2012), were chosen as outgroups for molecular analyses. The nomenclature used follows Flora europaea (Fedorov & Kovanda 1976) and Med-Checklist (Greuter & al. 1984). Voucher specimens from all populations were deposited in the herbarium BEOU; herbarium codes follow Thiers (2013+).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from silica-gel-dried leaves using a modified hexadecyltrimethylammonium bromide (CTAB) technique from Doyle & Doyle (1987), and modification according to Aleksić & al. (2012). The polymerase chain reaction (PCR) was used to obtain the double-stranded DNA fragments of interest. Two plastid intergenic spacers were amplified using the published primers: *trnG*^{UCC}-*trnS*^{GCU} (Hamilton 1999), and *psbA-trnH* (Tate & Simpson 2003). For PCR amplification of both regions, PCR reactions were carried out in 25 µL volumes containing: 100 ng template DNA; 2.5 µl 10 × Taq Buffer with $(NH_4)_2SO_4$ (Invitrogen, Berlin, Germany); 2.5 mM $MgCl_2$; 0.2 mM dNTPs; 0.1 µM of each Forward (F) and Reverse (R) primer; 0.80 % BSA (Bovine Serum Albumin, Promega, St Louis, U.S.A.); and 0.025 U/µl of Platinum Taq DNA polymerase (Invitrogen, Berlin, Germany). PCR amplification profiles used for both loci were as follows: denaturation at 94 °C for 10 min; 35 cycles of denaturation at 94 °C for 45s; annealing for 1 min at 60 °C and at 53 ºC for *trnG*^{UCC}-*trnS*^{GCU} and *psbA-trnH*, respectively; extension at 72 °C for 1 min; and a final extension of 10 min at 72 °C. The presence of a specific PCR product was confirmed by gel electrophoresis on 2 % agarose gels, and PCR products were sequenced with the Forward primer by Macrogen Europe, Amsterdam, Netherlands (http://dna.macrogen.com/eng/) via Sanger sequencing using 96-capillary 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Inc., U.S.A.). For *psbA-trnH*, sequencing with Reverse primer was required as well, and consensus sequences for each individual were assembled using sequences of both DNA strands. Sequence chromatograms were proofed, edited, assembled and aligned manually using Muscle (Edgar 2004) in MEGA 5.04 (Tamura & al. 2011). All sequences generated in this study are deposited in GenBank (KJ146622 to KJ146679).

Phylogenetic analyses

Sequences were readily alignable among all accessions in both plastid matrices. Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian

Table 1. Sampling localities, sample sizes and voucher numbers of analysed species. – Population ID refers to the sampled populations presented in Fig. 1; longitude and latitude are according to the WGS84 system; sample sizes are numbers of individuals from a particular population used for morphological or molecular analysis.

Population ID	Origin of material	Population acronym	Latitude [°N]	Longitude [°E]	Altitude [m]	Habitat	Substrate	Sample size: morphol. / molec.	Voucher specimen in herbarium BEOU
	***Campanula lingulata* s.l.**								
1	Serbia, Fruška Gora	SR-Fruška Gora	45.10966	19.48681	225	rocky ground	limestone	31 / 5	BEOU-16754
2	Serbia, Vujan	SR-Vujan	44.010	20.610	500	rocky ground	serpentine	– / 5	BEOU-35425
3	Serbia, Panjica	SR-Panjica	43.662894	20.08283	400	rocky ground	limestone	– / 2	BEOU-30531
4	Serbia, Kokin Brod	SR-Kokin Brod	43.54959	19.78111	906	rocky ground	limestone	30 / –	BEOU-16747
5	Serbia, Stara planina, Balta Berilovac	SR-Stara	43.40616	22.51318	445	rocky ground	limestone	30 / 5	BEOU-16753
6	Serbia, Studenica	SR-Studenica	43.4758	20.54151	412	rocky ground	limestone	30 / –	BEOU-16751
7	Croatia, Biokovo, Živogošće	CR-Biokovo	43.182830	17.163013	22	rocky ground	limestone	26 / 4	BEOU-16736
8	Bosnia & Herzegovina, Stolac	BH-Stolac	43.072326	18.058730	179	rocky ground	limestone	28 / 4	BEOU-16733
9	Montenegro, Ostrog	MN-Ostrog	42.668557	19.030341	825	rocky ground	limestone	30 / 5	BEOU-16744
10	Montenegro, Herceg Novi	MN-Herceg Novi	42.465108	18.545214	167	rocky ground	limestone	30 / 4	BEOU-16745
11	Montenegro,Valdanos	MN-Valdanos	41.952291	19.166182	35	rocky ground	limestone	30 / 5	BEOU-16743
12	FYR Macedonia, Šar planina, Lešnica	MA-Šara	42.016770	20.496500	1803	rocky ground	limestone	31 / 5	BEOU-16740
13	FYR Macedonia, Valandovo	MA-Valandovo	41.229	22.22797	125	rocky ground	limestone	31 / 5	BEOU-16741
14	Bulgaria, Rhodopes, Despat	BU-Rhodopes	41.391250	24.132730	1263	rocky ground	limestone	30 / 4	BEOU-16734
15	Greece, Olympus, Leptokaria	GR-Olympus	40.025430	22.480370	1000	rocky ground	limestone	30 / –	BEOU-16738
16	Greece, Olympus, Koromilles	GR-Olympus	40.02899	22.49768	805	rocky ground	limestone	– / 5	BEOU-31934
17	Greece, Ossa, Spilia	GR-Ossa	39.783920	22.654540	799	rocky ground	limestone	15 / 4	BEOU-16739
	Campanula cervicaria								
18	Serbia, Kopaonik, Samokovka	SR-Kopaonik	43.298959	20.805758	1648	forest openings	limestone	– / 1	BEOU-16823
	Campanula foliosa								
19	FYR Macedonia, Galičica, Kazan	MA-Galičica	40.940567	20.827867	1925	alpine meadows	limestone	– / 1	BEOU-16824
	Campanula moesiaca								
20	Serbia, Besna, Kobila, Musulj	SR-Besna Kobila	42.51684	22.2123	1770	alpine meadows	limestone	– / 1	BEOU-16825
	Campanula tymphaea								
21	Greece, Pindus, Metsovo	GR-Pindus	39.778294	21.165621	1391	meadows	limestone	– / 1	BEOU-16826
	Edraianthus graminifolius								
22	Serbia, Zlatibor, Bela Zemlja	SR-Zlatibor	43.832449	19.808701	670	rock crevices	limestone	– / 1	BEOU-26615

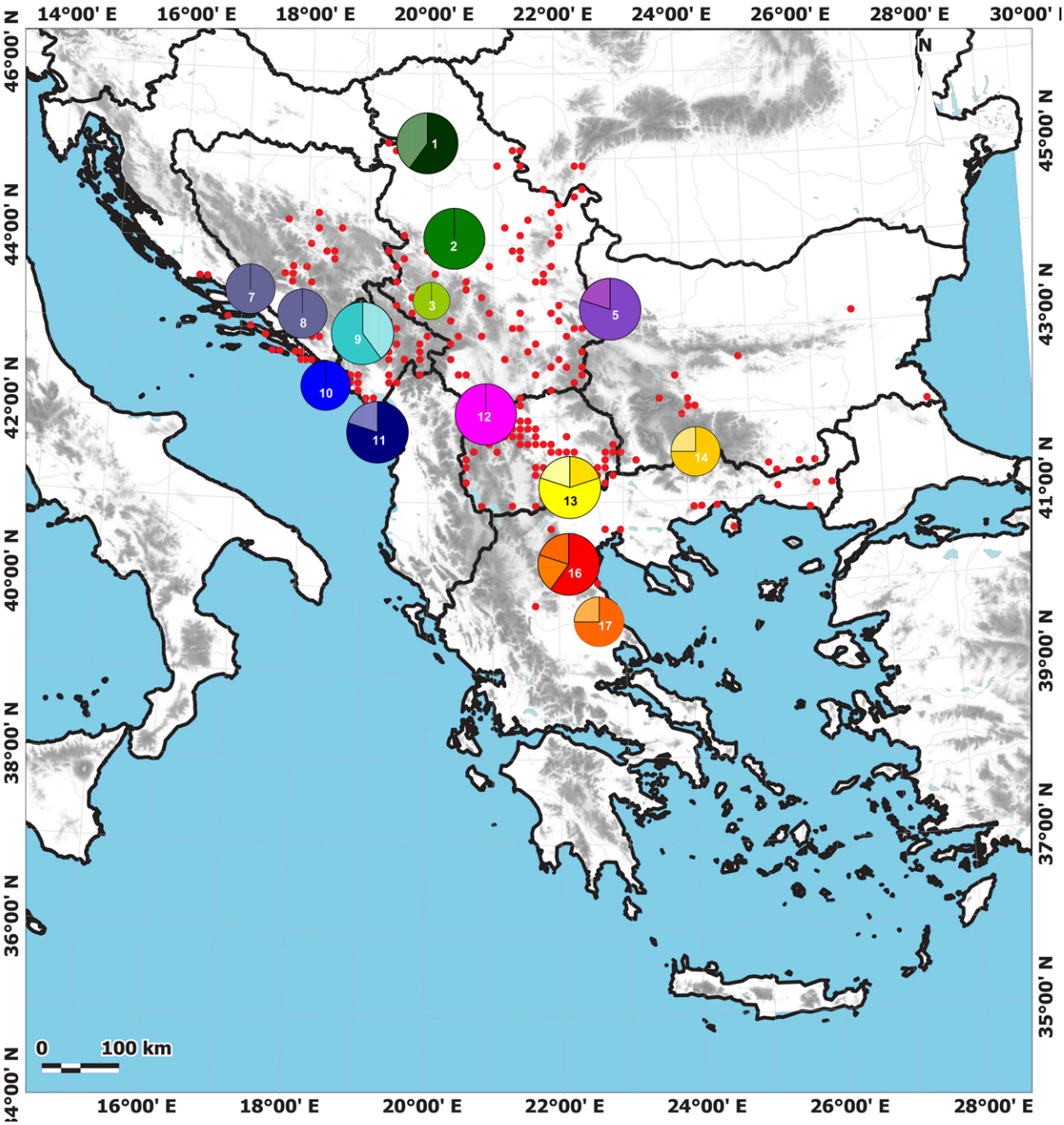

Fig. 1. Natural range of *Campanula lingulata* s.l., sampled populations used for molecular analyses, and spatial distribution of chloroplast haplotypes presented in different colours within pie charts. – The numbers within pie charts represent population identifiers listed in Table 1. The size of the pie chart is indicative of the sample size, i.e. it corresponds to the number of individuals from a particular population used for the molecular analyses.

inference (BI) algorithms applied to separate data sets and a concatenated matrix of both plastid loci. Gaps in the alignment were treated as missing data. The Akaike information criterion calculated in MEGA 5.04 (Tamura & al. 2011) used to evaluate models of evolution for both loci revealed that the general time reversible (GTR) model had the best fit to both plastid matrices. Trees were rooted on *Campanula cervicaria*, *C. foliosa*, *C. moesiaca*, *C. tymphaea* and *Edraianthus graminifolius*, which all belong to clade "Cam17" of Mansion & al. (2012).

The ML analyses were performed with RAxML (Stamatakis 2006) with the raxmlGUI v. 1.1 (Silvestro & Michalak 2011) using the default parameter settings (-f a function). Statistical support for nodes was based on 100 non-parametric bootstrap replicates (BS), with ≥ 75 % considered good support. Trees were visualized and edited using FigTree 1.0 (Rambaut 2006).

The BI analyses were performed with BEAST v1.7.2 (Drummond & al. 2012). In cases when intra-population variability of concatenated plastid sequences (haplotypes) was not observed, the BI analyses were performed

with only one sequence as a representative of that population in order to reduce the number of very short branches that do not add information. The input file for the BEAST analyses was constructed using the BEAUti interface of the BEAST package and the file with parameter settings was executed in BEAST. We used a GTR + Γ model with four categories of rate heterogeneity for the final analysis and a demographic model of constant population size as a tree prior for modelling changes in population size through time. Following a burn-in of 1 million steps, all parameters were sampled once every 1000 steps from 5 million MCMC steps. TRACER v.1.4.1 (Rambaut & Drummond 2007) was used to confirm acceptable mixing, likelihood stationarity of the MCMC chain and adequate effective sample sizes for each parameter (200). TreeAnnotator 1.4.2 (part of the BEAST package) was used to construct a majority-rule consensus tree using the trees remaining after the burn-in, and also to summarize the posterior distributions of nodes. For BI analyses, posterior probabilities (PP) ≥ 0.98 were considered good support.

Morphometric analysis

A total of 402 *Campanula lingulata* specimens from 14 populations covering the entire range of this species were used for morphological analyses (Table 1; Fig. 1). Vegetative (leaves and bracts) and generative (inflorescence) dissected organs from each plant were scanned at high resolution using standard PC tools and adjusted transparency scale. We analysed a total of 50 morphological characters using Digimizer Image Analysis Software Version 4.0.0.0. (MedCalc Software 2005–2011; http://www.digimizer.com), and a Leica DMLS stereomicroscope.

Canonical discriminant analysis (CDA) was used to test the hypothesis of morphological segregation of groups of populations obtained from molecular analyses. CDA was performed utilizing the reduced data matrix of morphological characters after standardization to zero mean and unit variance. Character reduction was envisaged by computing pairwise Spearman correlations and retaining only one out of character pairs with absolute values of correlation coefficients exceeding 0.8. 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 performed to estimate the contribution of individual characters to overall discrimination.

Descriptive statistics of morphological characters (i.e. mean, minimum and maximum values, standard deviations, standard errors and coefficients of variation) were computed for each out of 50 morphological characters analysed in this study. They were calculated separately for each out of two groups of populations obtained in molecular analyses and were used for the delineation of morphological diagnostic characters of *Campanula cichoracea.*

All calculations were performed in Statistica 5.1 (StatSoft 1996).

Results

DNA regions and alignments

Sequences for two chloroplast regions were obtained from all 62 accessions of the *Campanula lingulata* complex as well as for five individuals used as outgroups. They were straightforward to align within the ingroup as well as between ingroup and outgroup taxa.

The characteristics of the sequenced regions with positions of variable sites are presented in Table 2. The aligned sequence length of the *trnG*^{UCC}*-trnS*^{GCU} intergenic spacer was 746 base pairs (bp) with 27 point mutations (18 transitions and nine transversions), three microsatellites and eight insertions/deletions (indels) of variable length. The aligned length of the *psbA-trnH* spacer was 461 bp with 33 point mutations (15 transitions and 18 transversions), seven microsatellites and five indels. A hypervariable region within this spacer spanned from position 938 to position 996 and harboured five consecutive microsatellites of which one harboured several additional transitions/transversions (see Table 2). The length of the concatenated matrix was 1207 bp with 60 point mutations (4.97 %). However, only 16 out of 60 point mutations represent substitutions among ingroup sequences (1.33 %), which harboured also ten microsatellites and indels in 12 positions.

Intra-population variability of the *trnG*^{UCC}*-trnS*^{GCU} intergenic spacer was not observed, as all individuals belonging to the same population harboured identical sequences in this region. However, mutations (nucleotide substitutions and/or indels) present in all individuals from a single population, which apparently represent synapomorphic molecular characters of distinct populations, were found in five populations (e.g. nucleotide character state "T" at position 69 within the *trnG*^{UCC}*-trnS*^{GCU} region was present only in individuals from population MA-Šara, see Table 2). The variability of the *psbA-trnH* sequences within populations, which came from microsatellite length mutations, was observed in nine out of 14 studied populations.

In order to assemble chloroplast haplotypes of all individuals, we combined the *trnG*^{UCC}*-trnS*^{GCU} and *psbA-trnH* sequences of each individual, and provided the number of individuals harbouring a particular haplotype in Table 2. In total, 23 distinct haplotypes were detected among 62 ingroup accessions, and only individuals from populations BH-Stolac and CR-Biokovo harboured an identical haplotype 2. All five outgroup species were characterized by distinct chloroplast types (haplotypes 24 to 28).

Phylogenetic analyses

The ML tree based on a combined plastid data set is presented in Fig. 2. It revealed the monophyly of the

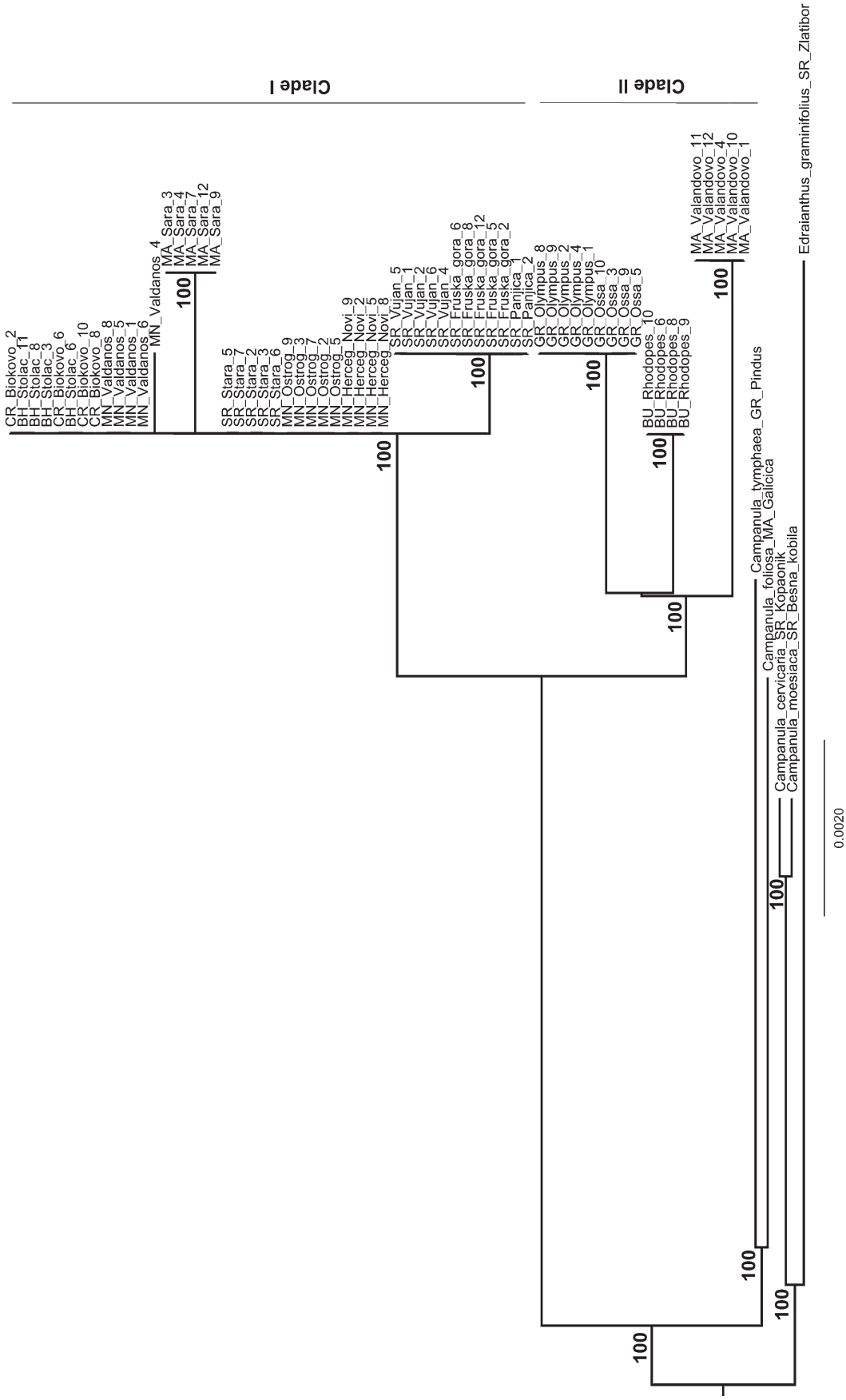

Fig. 2. Maximum Likelihood tree based on 1207 nucleotides of the *trnG*^{UCC}-*trnS*^{GCU} and *psbA*-*trnH* plastid DNA regions with 62 *Campanula lingulata* s.l. accessions and five outgroups. – Numbers at nodes refer to bootstrap values ≥ 75 %.

Table 2. Annotation of plastid DNA intergenic spacers *trnG*^{UCC}-*trnS*^{GCU} (p. 84) and *psbA-trnH* (p. 85). – Asterisk (*) indicates a mononucleotide microsatellite (A)n spanning from position 949 to 970 comprising several transitions and transversions: transversions into C in positions 949 and 954, and three and four transitions into G in four *Campanula* species used as outgroups and in *Edraianthus graminifolius*, respectively. – NA = not available.

				trnG^{UCC}-*trnS*^{GCU}																																					
				6	28	69	71	**ΔΔ182**	218	**ΔΔ224**	240-297		305	312-328	328-342	348	362	**Δ368**	374	379-386	396	398	**ΔΔ413**	414-417	447	452	465	477	489	513	533	535-541	571-599		625	631	675	686-689	**Δ697**	703	**ΔΔ739**
Taxon	Population acronym	No. of individuals	Haplotype	A	A	C	A	G	G	T	58 bp gap (240-297)	(A)n (257-266)	C	17 bp gap	15 bp gap	T	G	A	A	(T)n	1 bp (A) insertion	T	A	4 bp gap	T	C	G	A	A	C	T	(T)n	29 bp gap	G (589)	G	G	1 bp (A) insertion	4 bp gap	G	A	A
Campanula lingulata	MA-Šara	5	1	.	.	T	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	A	.		.	.	.	G
Campanula lingulata	BH-Stolac; CR-Biokovo	4; 4	2	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	.	G
Campanula lingulata	MN-Valdanos	4	3	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.	A	.	.	.	G
Campanula lingulata	MN-Valdanos	1	4	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.	A	.	.	.	G
Campanula lingulata	SR-Vujan	5	5	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	C	G
Campanula lingulata	SR-Fruška Gora	2	6	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	C	G
Campanula lingulata	SR-Fruška Gora	3	7	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	C	G
Campanula lingulata	SR-Panjica	2	8	.	.	.	.	A	.	.		9	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	C	G
Campanula lingulata	SR-Stara	4	9	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	.	G
Campanula lingulata	SR-Stara	1	10	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	.	G
Campanula lingulata	MN-Herceg Novi	4	11	.	.	.	.	A	.	.		8	.		.	.	.	.	.	7		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	.	G
Campanula lingulata	MN-Ostrog	2	12	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	.	G
Campanula lingulata	MN-Ostrog	3	13	.	.	.	.	A	.	.		8	.		.	.	.	.	.	8		.	C		.	.	.	.	.	.	.	7		.	.	.		.	.	.	G
Campanula cichoracea	MA-Valandovo	1	14	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8		.	.		.	.	.	.	.	.	.	7		.	.	C		.	.	.	.
Campanula cichoracea	MA-Valandovo	3	15	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8		.	.		.	.	.	.	.	.	.	7		.	.	C		.	.	.	.
Campanula cichoracea	MA-Valandovo	1	16	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8		.	.		.	.	.	.	.	.	.	7		.	.	C		.	.	.	.
Campanula cichoracea	GR-Olympus	3	17	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8	A	.	.		.	.	A	.	.	.	.	7		.	.	.		.	.	.	.
Campanula cichoracea	GR-Olympus	1	18	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8	A	.	.		.	.	A	.	.	.	.	7		.	.	.		.	.	.	.
Campanula cichoracea	GR-Olympus	1	19	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8	A	.	.		.	.	A	.	.	.	.	7		.	.	.		.	.	.	.
Campanula cichoracea	GR-Ossa	3	20	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8	A	.	.		.	.	A	.	.	.	.	7		.	.	.		.	.	.	.
Campanula cichoracea	GR-Ossa	1	21	.	.	.	.	.	.	G		8	.		.	.	.	.	.	8	A	.	.		.	.	A	.	.	.	.	7		.	.	.		.	.	.	.
Campanula cichoracea	BU-Rhodopes	3	22	G	.	.	C	.	.	G		8	.		.	.	.	.	.	8		.	.		.	.	.	.	.	.	.	6		.	.	.		.	.	.	.
Campanula cichoracea	BU-Rhodopes	1	23	G	.	.	C	.	.	G		8	.		.	.	.	.	.	8		.	.		.	.	.	.	.	.	.	6		.	.	.		.	.	.	.
Campanula cervicaria	SR-Kopaonik	1	24	.	.	.	.	.	.	.		8	T		.	C	.	G	C	8		.	.	.	.	.	.	.	.	.	.	6		.	.	.		.	T	.	.
Campanula foliosa	MA-Galičica	1	25	.	.	.	.	.	.	.		10	.		.	.	A	G	.	8		.	.		C	.	.	.	.	T	.	7		.	.	.		.	T	.	.
Campanula moesiaca	SR-Besna Kobila	1	26	.	.	.	.	.	.	.		8	.		.	C	.	G	C	8		.	.	.	.	.	.	.	.	.	.	6		.	.	.		.	T	.	.
Campanula tymphaea	GR-Pindus	1	27	.	.	.	.	.	A	.		8	.	.		.	.	G	.	8		G	.		.	T	.	.	.	.	.	6	.	.	.	.		.	T	.	.
Edraianthus graminifolius	SR-Zlatibor	1	28	.	G	.	.	.	.	.	.		.			.	.	G	.	5		.	.		.	.	.	G	C	.	C	5		T	.	.			T	.	.

Δ Molecular diagnostic character to distinguish *Campanula lingulata* s.l. from other species **ΔΔ** Molecular diagnostic character to distinguish *Campanula lingulata* from *C. cichoracea*

psbA-trnH

Taxon	Population acronym	No. of individuals	Haplotype	767	786	802	814	835	836	**Δ863**	869	876-883	886-917		938-948	949-970				**ΔΔ971-984**	985-991	992-996	1019	**Δ1027**	1035	1037	1040	1043	1051-1055	1056	1058	1061	1062	**Δ1065**	1073	1079-1092	1093-1099	1109	1116	1127	1135	1136	1140	1163-1168	1186
				A	C	A	A	T	T	T	C	(T)n	32 bp insertion	31 bp insertion	(T)n	(A)n	C at position 949*	Transversion into C*	**ΔTransitions into G***	**(AT)n**	(T)n	(G)n	C	A	C	A	T	T	4 bp insertion	C	A	A	G	G	C	14 bp insertion	7 bp insertion	C	C	G	C	T	T	(G)n	A
Campanula lingulata	MA-Šara	5	1	.	.	.	.	.	.	.	.	7			6	10	.			6	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula lingulata	BH-Stolac; CR-Biokovo	4; 4	2	.	.	.	.	.	.	.	.	7			5	10	.			6	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	5	.
Campanula lingulata	MN-Valdanos	4	3	.	.	.	.	.	.	.	.	7			5	9	.			6	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	5	.
Campanula lingulata	MN-Valdanos	1	4	.	.	.	.	.	.	.	.	7			5	9	.			6	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	A	5	.
Campanula lingulata	SR-Vujan	5	5	.	.	.	.	.	.	.	.	7			6	12	.			6	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula lingulata	SR-Fruška Gora	2	6	.	.	.	.	.	.	.	.	7			6	11	.			6	7	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula lingulata	SR-Fruška Gora	3	7	.	.	.	.	.	.	.	.	7			6	11	.			6	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula lingulata	SR-Panjica	2	8	.	.	.	.	.	.	.	.	7			6	14	.			5	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula lingulata	SR-Stara	4	9	.	.	.	.	.	.	.	.	7			6	8	.			7	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula lingulata	SR-Stara	1	10	.	.	.	.	.	.	.	.	7			6	10	.			6	5	4	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula lingulata	MN-Herceg Novi	4	11	.	.	.	.	.	.	.	.	7			5	12	.			4	6	5	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	5	.
Campanula lingulata	MN-Ostrog	2	12	.	.	.	.	.	.	.	.	7			5	8	.			6	6	5	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	5	.
Campanula lingulata	MN-Ostrog	3	13	.	.	.	.	.	.	.	.	7			5	10	.			5	6	5	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	5	.
Campanula cichoracea	MA-Valandovo	1	14	.	.	.	.	.	.	.	.	6			2	18					2	5	.	.	.	.	.	.		.	.	.	.	.	A			.	.	.	.	.	A	6	NA
Campanula cichoracea	MA-Valandovo	3	15	.	.	.	.	.	.	.	.	7			2	22					2	5	.	.	.	.	.	.		.	.	.	.	.	A			.	.	.	.	.	A	6	NA
Campanula cichoracea	MA-Valandovo	1	16	.	.	.	.	.	.	.	.	7			2	20					2	5	.	.	.	.	.	.		.	.	.	.	.	A			.	.	.	.	.	A	6	NA
Campanula cichoracea	GR-Olympus	3	17	.	.	.	C	.	.	.	.	7			11	11	.				3	5	.	.	.	.	.	.		.	.	.	.	.	.			.	T	.	.	.	.	6	.
Campanula cichoracea	GR-Olympus	1	18	.	.	.	C	.	.	.	.	7			10	11	.				3	5	.	.	.	.	.	.		.	.	.	.	.	.			.	T	.	.	.	.	6	NA
Campanula cichoracea	GR-Olympus	1	19	.	.	.	C	.	.	.	.	7			9	11	.				3	5	.	.	.	.	.	.		.	.	.	.	.	.			.	T	.	.	.	.	6	NA
Campanula cichoracea	GR-Ossa	3	20	.	.	.	C	.	.	.	.	7			9	12	.				5	5	.	.	.	.	.	.		.	.	.	.	.	.			.	T	.	.	.	.	6	.
Campanula cichoracea	GR-Ossa	1	21	.	.	.	C	.	.	.	.	7			10	11	.				5	5	.	.	.	.	.	.		.	.	.	.	.	.			.	T	.	.	.	.	6	.
Campanula cichoracea	BU-Rhodopes	3	22	.	.	.	.	.	.	.	.	7			7	11	.				2	5	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula cichoracea	BU-Rhodopes	1	23	.	.	.	.	.	.	.	.	7			7	12	.				2	5	.	.	.	.	.	.		.	.	.	.	.	.			.	.	.	.	.	.	6	.
Campanula cervicaria	SR-Kopaonik	1	24	G	T	.	.	.	.	C	.	8	.		7	12	.		3		4	5	.	C	T	.	.	.	.	.	.	.	.	T	.			.	.	.	A	.	A	6	.
Campanula foliosa	MA-Galičica	1	25	.	.	.	.	.	.	C	.	7			6	10	.	.	3		4	5	.	C	.	T	.	A		A	.	.	.	T	.	.		.	.	.	.	G	.	6	.
Campanula moesiaca	SR-Besna Kobila	1	26	G	T	C	.	.	.	C	.	8		.	7	12	.		3		4	5	.	C	T	.	.	.	.	.	.	.	.	T	.			.	.	.	A	.	A	6	.
Campanula tymphaea	GR-Pindus	1	27	.	.	.	.	.	.	C	T	8			6	12	.		3		4	5	A	C	.	.	.	.		.	G	.	T	T	.			.	.	.	.	G	.	6	G
Edraianthus graminifolius	SR-Zlatibor	1	28	.	T	.	.	C	C	C	.	7			5	13	.		4		4	5	.	C	.	.	A	.		.	.	T	.	T	.		.	T	.	T	.	.	A	5	.

Δ Molecular diagnostic character to distinguish *Campanula lingulata* s.l. from other species **ΔΔ** Molecular diagnostic character to distinguish *Campanula lingulata* from *C. cichoracea*

Campanula lingulata complex and the split of this complex into two strongly supported clades (100 % BS). The clade I comprised individuals from the C Balkans (SR-Fruška Gora, SR-Vujan, SR-Panjica, MA-Šara, SR-Stara, BH-Stolac, CR-Biokovo, MN-Valdanos, MN-Ostrog and MN-Herceg Novi), while the clade II comprised individuals from the S Balkans (MA-Valandovo, BU-Rhodopes, GR-Olympus and GR-Ossa). Within the clade I, sub-clades Ia and Ib comprising individuals from SR-Fruška Gora/SR-Vujan/SR-Panjica and MA-Šara, respectively, were strongly supported. The relations among the individuals from the remaining C Balkan populations, however, were unresolved. Within the clade II, individuals from MA-Valandovo were first to diverge (sub-clade IIa), and individuals from BU-Rhodopes (sub-clade IIb) and GR-Olympus/GR-Ossa (sub-clade IIc) were sisters. The support for all sub-clades within clade II was strong (100 % BS).

Two molecular characters within the *trnG*^{UCC}-*trnS*^{GCU} region, nucleotide character states "A" and "G" in positions 368 and 697, respectively, were synapomorphic for the *Campanula lingulata* complex, while four molecular characters within this region were synapomorphic either for clade I or clade II. Nucleotide character states "A" in position 182, "C" in position 413 and "G" in position 739 were synapomorphic for clade I, while nucleotide character state "G" in position 224 was synapomorphic for clade II (Table 2). The *psbA-trnH* intergenic spacer also harboured molecular characters synapomorphic for the *C. lingulata* complex: nucleotide character states "T", "A" and "G" in positions 863, 1027 and 1065, respectively, but lacked mutations that could distinguish the two clades described above. Nonetheless, an (AT) microsatellite spanning from position 971 to 984 was present only in individuals from the C Balkans and may be considered as synapomorphic for this group of populations. However, since the majority of the variability of *psbA-trnH* was associated with the microsatellite length mutations, the resolution of this region for phylogenetic inference was rather limited and the unfolding of the *C. lingulata* complex based solely on the variability of this region was grossly unresolved (Fig. 5). Concordantly, the ML tree based on the *trnG*^{UCC}-*trnS*^{GCU} region (Fig. 6) displayed a topological similarity with the ML tree based on a combined plastid data set.

Furthermore, the *psbA-trnH* intergenic spacer comprised one homoplastic site, a transversion in position 1140. In this position, nucleotide character state "A" was present in sequences of three out five outgroup taxa (*Campanula cervicaria*, *C. moesiaca* and *Edraianthus graminifolius*), in all individuals from MA-Valandovo and in one individual from MN-Valdanos (MN-Valdanos_4), while nucleotide character state "T" was present in sequences of the remaining individuals (Table 2). Interestingly, this homoplastic mutation within the *psbA-trnH* region actually contributed towards the increased support of sub-clades within clade II because, when this mutation was excluded, the relations within clade II remained unresolved in the ML tree based on both regions (tree not shown).

A majority-rule consensus tree from Bayesian analyses constructed utilizing 23 ingroup and five outgroup haplotypes is presented in Fig. 3. It showed a topological agreement with a combined ML tree with one exception only, because within clade II the first diverging group comprised individuals from Thessaly in Greece (GR-Olympus/GR-Ossa) and not from MA-Valandovo, as obtained in the ML tree. In this analysis, the monophyly of the *Campanula lingulata* complex and the divergence into two strongly supported clades (clade I, 1 PP; clade II, 0.99 PP) was also observed. The support for sub-clades comprising individuals from MA-Valandovo, BU-Rhodopes and GR-Olympus/GR-Ossa within clade II (1 PP for all sub-clades), as well as for the sub-clade comprising individuals from SR-Fruška Gora/SR-Vujan/SR-Panjica within clade I (0.99 PP) was strong, similarly as in the ML tree.

Morphometric analysis

The character reduction procedure, in which correlations exceeding the threshold of 0.8 were not found, revealed that the data matrix should be reduced to 30 characters. These characters, which were used for CDA analysis, are listed in Table 3. The number of a priori defined groups inferred from molecular analyses was six (three sub-clades within clade I and three sub-clades within clade II). Thus, CDA was performed with six a priori defined groups × 402 individuals × 30 characters. The first three discriminant axes explained 88.04 % of variation between groups. For the graphical presentation of CDA, the scores of all specimens were plotted within a two-dimensional space defined by discriminant axis 1 (explaining 43.77 % of variation) and discriminant axis 2 (explaining 24.27 % of variation), and specimens belonging to each out of two groups of populations obtained in phylogenetic analysis (i.e. clade I and clade II) were marked with different symbols (Fig. 4A). The CDA largely supported the hypothesis of morphological separation of two genetic groups identified by phylogenetic analysis. The scores of individuals from populations from the C Balkans (clade I) were continuous but separated from scores of individuals from populations from the S Balkans (clade II; Fig. 4A). Interestingly, in a scatterplot defined by the second and the third discriminant axes (explaining 20 % of variation), further separation of individuals from the central Balkans was rather limited revealing homogeneity of this group (Fig. 4B), while S Balkans populations were more heterogeneous because scores of individuals from Thessaly, Greece (GR-Olympus and GR-Ossa), the river Vardar valley (MA-Valandovo) and Bulgaria (BU-Rhodopes) were separated (Fig. 4C).

Discriminant function analysis revealed that eight out of 30 tested characters: stem height (Ca_H), outer involu-

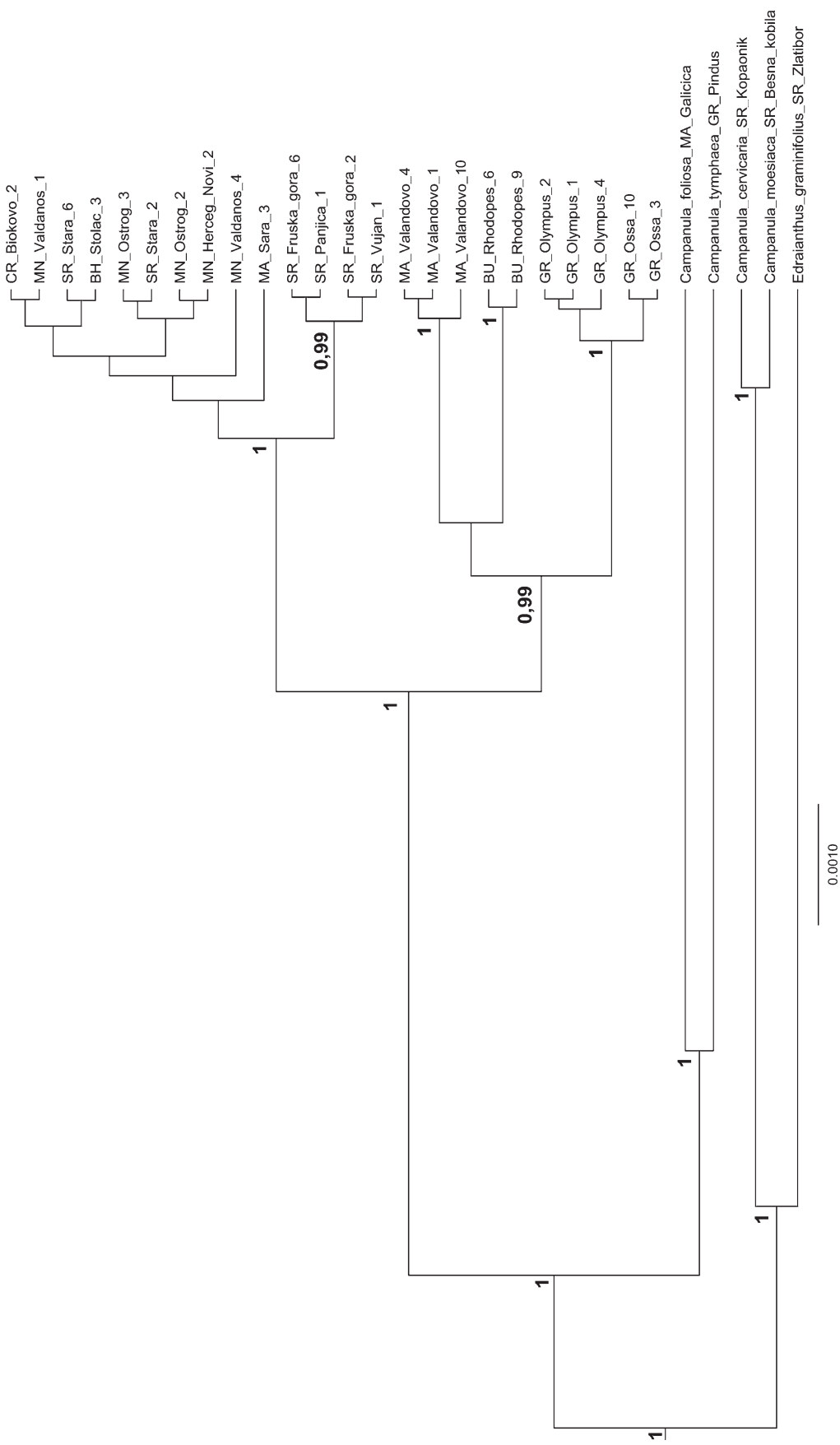

Fig. 3. Majority-rule consensus tree from Bayesian analysis with 23 *Campanula lingulata* s.l. accessions and five outgroups. – Numbers at nodes refer to posterior probabilities ≥ 98.

cral bracts number (Bc_No), outer involucral bract maximum width (Bc_Wm), calyx lobe total length (CaD_H), calyx appendix total length (CaA_H), corolla total length (Co_H), stamen total length (St_L) and distance between filament base and widest point (StB_h), had a dominant contribution to the overall discrimination (Table 3). This would suggest that the discrimination into two groups in CDA (Fig. 4) was predominantly based on characteristics of inflorescence and flowers.

Discussion

The genus *Campanula* comprising up to 600 taxa (Roquet & al. 2008; Borsch & al. 2009; Cellinese & al. 2009; Haberle & al. 2009; Mansion & al. 2012) is represented in Europe by more than 250 species. Flora europaea lists 93 *Campanula* species/subspecies from the Balkan Peninsula, among which 63 are endemic to this region (Fedorov & Kovanda 1976). A substantial morphological diversity observed in many *Campanula* taxa from the Balkans, which may be indicative of accelerated diversification and speciation expected in such refugial regions (Griffiths & al. 2004; Stewart & al. 2010), has burdened traditional morphological circumscription of these taxa with a considerable synonymy. Nonetheless, over the past years, more light has been shed on some *Campanula* taxa from the Balkans (e.g. Lakušić & Conti 2004; Kovačić 2006; Kovačić & Nikolić 2006; Park & al. 2006; Frajman & Schneeweis 2009; Stefanović & Lakušić 2009; Lakušić & al. 2013).

Campanula lingulata represents another taxonomically intricate complex of bellflowers confined to the Balkans. Although the monophyly of this complex has recently been supported by molecular data (Mansion & al. 2012), these authors also observed a molecular diversity in this complex and suggested further phylogeographic and/or speciation studies. Our work, based on both molecular and morphological data surveyed in an extensive sample of populations across the species range, provides new insights into the systematics, biogeography and evolution of the *C. lingulata* complex.

The phylogenetic analyses based on two chloroplast intergenic spacers (*trnG*^{UCC}-*trnS*^{GCU} and *psbA-trnH*) supported the monophyly of the *Campanula lingulata* complex as indicated in Mansion & al. (2012). We further observed the divergence of an ancestral *C. lingulata* gene pool into two lineages whose descendants are currently allopatrically distributed and confined to the C Balkans (clade I) and the S Balkans (clade II). Although the diversification of the lineage from the S Balkans apparently occurred earlier than that from the C Balkans, further studies utilizing the phylogenetic signal from additional genomic

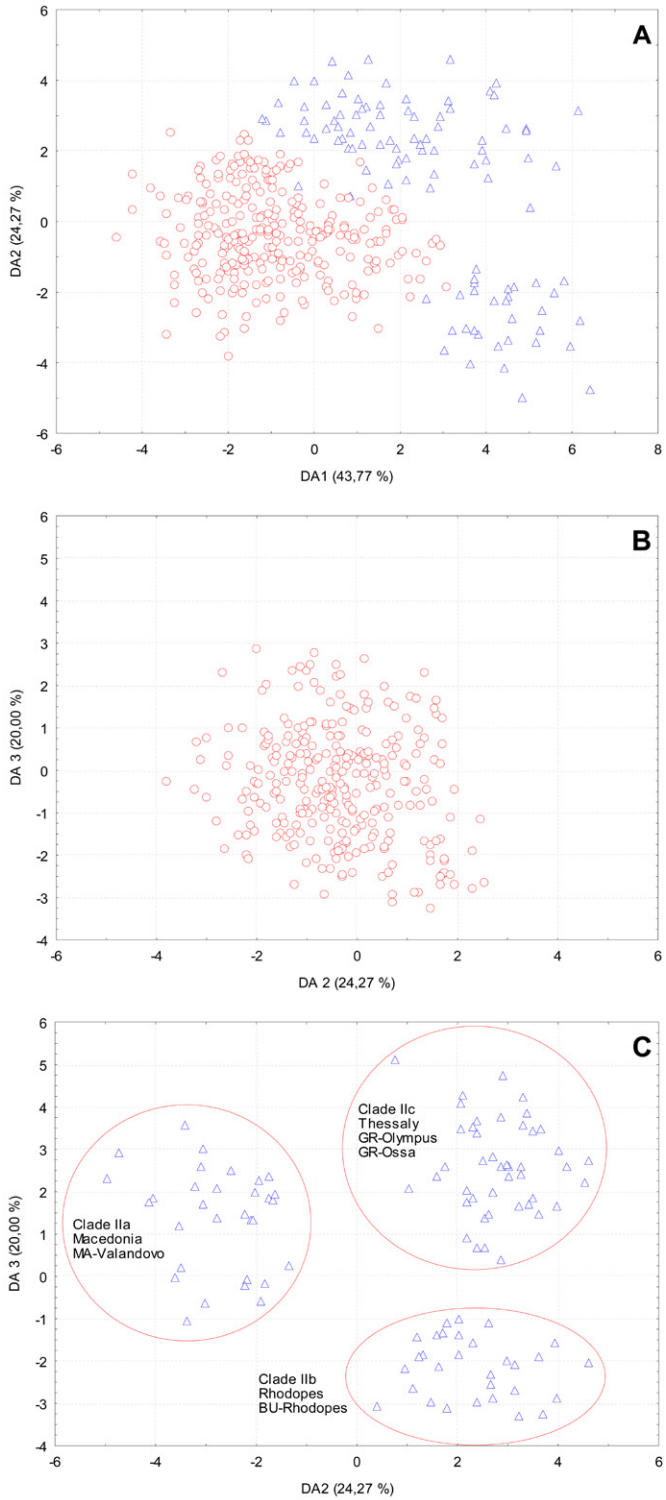

Fig. 4. Canonical discriminant analysis (CDA) of morphometric data performed with six a priori defined groups delineated from molecular analyses. – Individuals of *Campanula lingulata* s.l. belonging to clade I (C Balkans) are marked with a red circle (○) and those belonging to clade II (S Balkans) are marked with a blue triangle (△). – A: the scores of all specimens plotted within a two-dimensional space defined by discriminant axes 1 and 2; B: the scores of specimens from the C Balkans plotted within a two-dimensional space defined by discriminant axes 2 and 3; C: the scores of specimens from the S Balkans plotted within a two-dimensional space defined by discriminant axes 2 and 3.

Table 3. Characters used for morphometric analysis of *Campanula lingulata* s.l. and a summary of Discriminant function analysis. – Wilks's lambda is a multivariate generalization of the univariate F-distribution; F-remove represents a measure of the extent to which a variable makes a unique contribution to the prediction of a group membership; p-level values < 0.05 are shown in boldface.

	Character	Abbreviation	Wilks's lambda	F-remove	p-level
	Stem				
1	Height	Ca_H mm	0.011	26.227	**0.000**
2	Number per plant	Ca_No	0.009	3.330	**0.006**
3	Number of branches per 1 stem	Ra_No	0.008	1.189	0.314
4	Length of longest branch	Ra_H	0.009	3.236	**0.007**
	Rosette leaves				
5	Number	Fr_No	0.008	0.336	0.891
6	Total length	Fr_L	0.008	1.213	0.302
7	Base width	Fr_Wb	0.008	2.341	**0.041**
8	Maximum width	Fr_Wm	0.008	1.318	0.255
	Stem leaves				
9	Number	Fc_No	0.009	3.201	**0.008**
10	Total length	Fc_L	0.009	7.080	**0.000**
11	Base width	Fc_Wb	0.008	2.367	**0.039**
12	Maximum width	Fc_Wm	0.009	3.240	**0.007**
13	Distance between base and widest point	Fc_h	0.008	1.061	0.382
	Outer involucral bracts				
14	Number	Bc_No	0.009	10.257	**0.000**
15	Total length	Bc_L	0.009	6.936	**0.003**
16	Base width	Bc_Wb	0.009	3.432	**0.005**
17	Maximum width	Bc_Wm	0.010	19.537	**0.000**
	Calyx				
18	Calyx lobe total length	CaD_H	0.011	29.550	**0.000**
19	Calyx lobe base width	CaD_W	0.009	3.675	**0.000**
20	Distance between calyx lobe base and widest point	CaD_h	0.009	5.666	**0.000**
21	Calyx appendix total length	CaA_H	0.010	17.846	**0.000**
	Corolla				
22	Corolla total length	Co_H	0.010	20.086	**0.000**
23	Perimeter	Co_Per	0.009	7.106	**0.000**
	Stamens				
24	Stamen total length	St_L	0.010	18.527	**0.000**
25	Filament length	StF_L	0.009	7.127	**0.000**
26	Filament base total length	StB_H	0.009	5.596	**0.000**
27	Filament base maximum width	StB_W	0.009	4.607	**0.000**
28	Distance between filament base and widest point	StB_h	0.009	5.517	**0.000**
29	Filament base area	StB_Ar	0.009	2.833	**0.016**
	Style				
30	Total length	Pu_L	0.009	3.138	**0.009**

regions are required to enable inferences on the unfolding and diversification of the two lineages obtained in the present study. Nonetheless, a strong support for all three sub-clades within clade II (S Balkans) and sub-clades Ia and Ib comprising individuals from SR-Fruška Gora/SR-Vujan/SR-Panjica and MA-Šara, respectively, suggests that all these groups of populations are distinct entities. Furthermore, the occurrence of synapomorphic molecular characters in these populations is indicative of ancient divergence of populations and genetic drift effects.

Our morphological analysis, based on 50 characters, largely corroborated molecular results. It is worth noting that eight out of 50 surveyed characters had a dominant contribution to the overall discrimination in CDA analysis, which distinguished individuals from the C Balkans (clade I) from those found in the S Balkans (clade II). These characters were predominantly associated with the inflorescence and flowers. Furthermore, morphological data revealed a significant heterogeneity of clade II, which was divided into three sub-groups, the

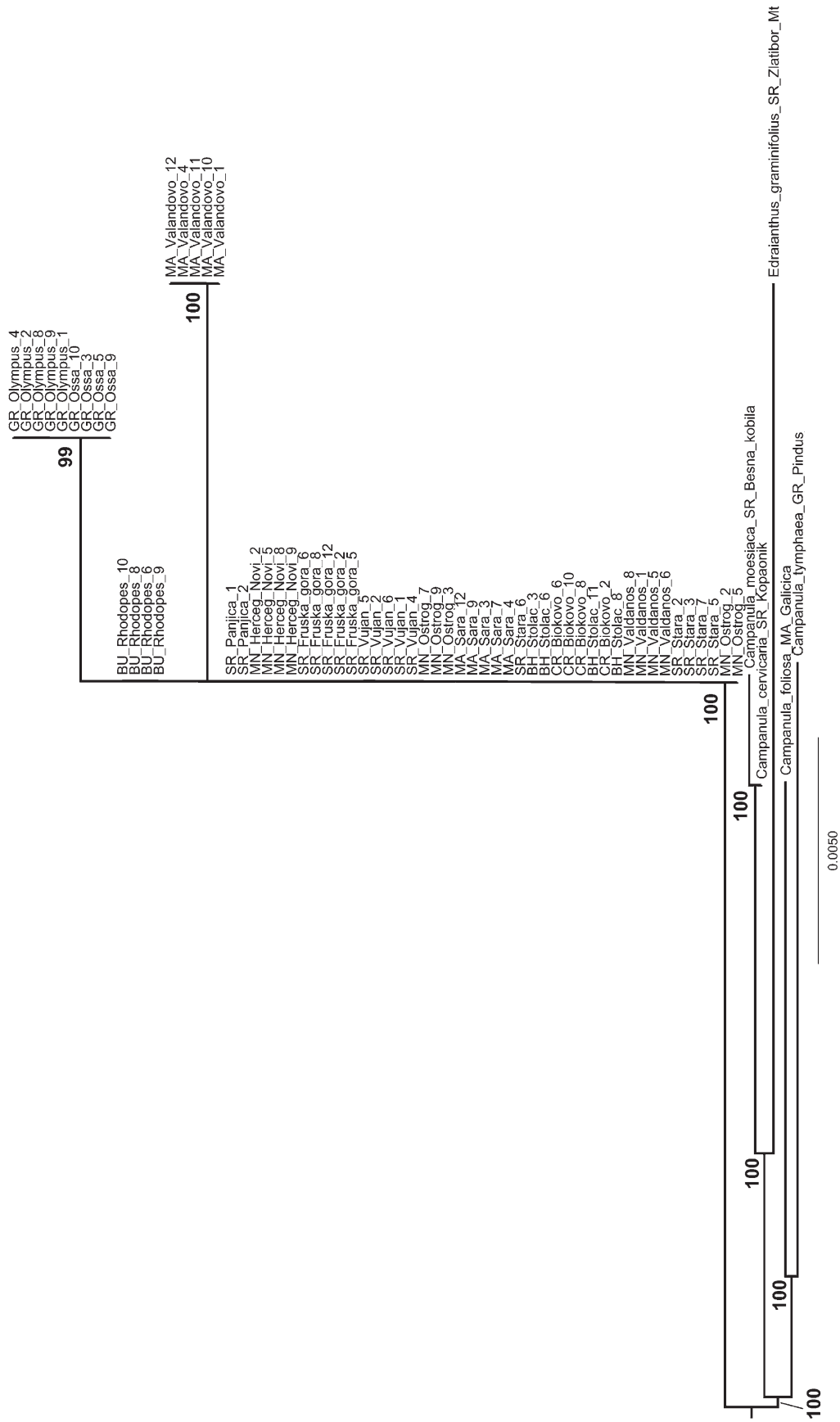

Fig. 5. Maximum Likelihood tree based on 461 nucleotides of the *psbA-trnH* plastid DNA intergenic spacer with 62 *Campanula lingulata* s.l. accessions and five outgroups. – Numbers at nodes refer to bootstrap values.

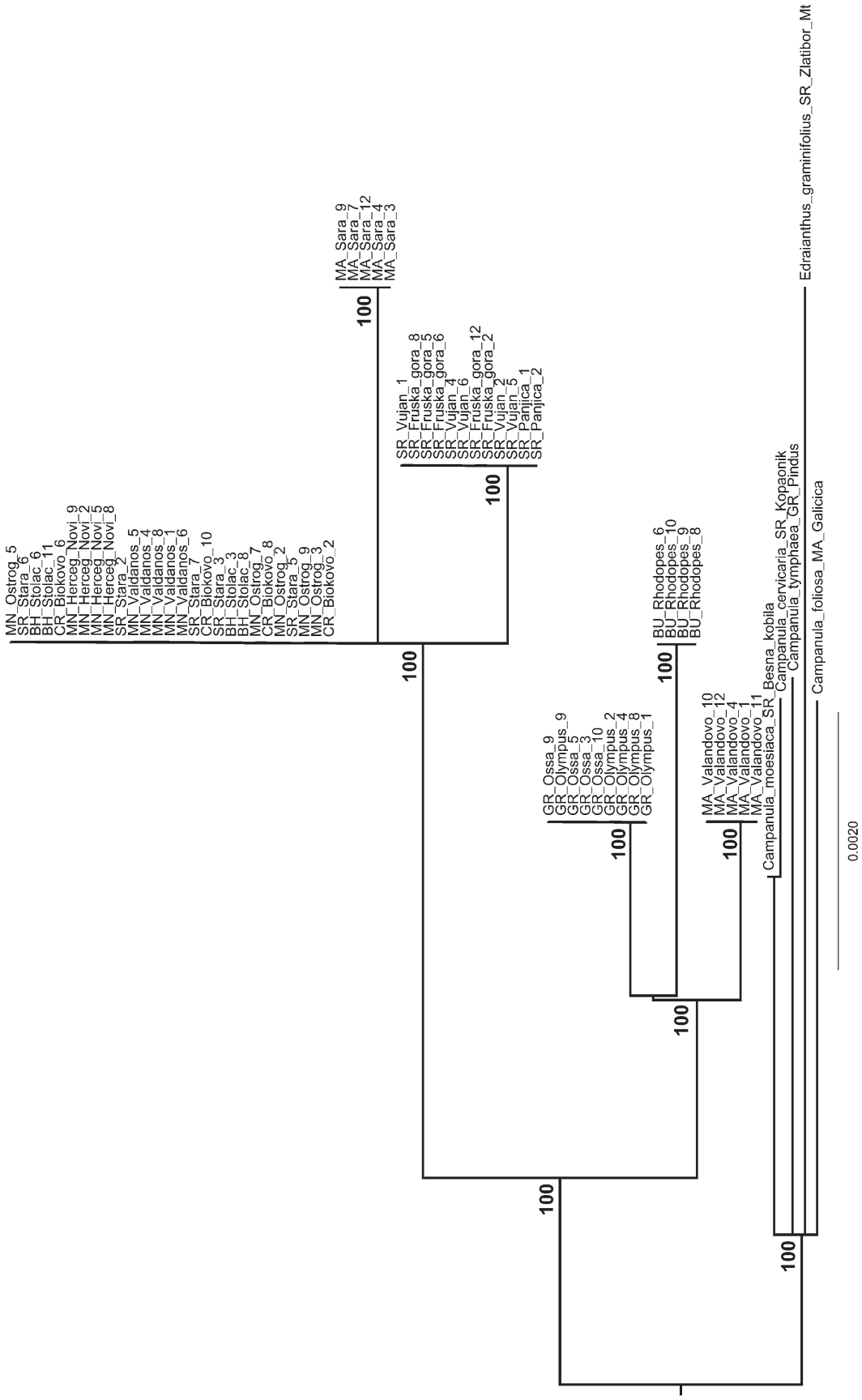

Fig. 6. Maximum Likelihood tree based on 746 nucleotides of the *trnG*^{UCC}-*trnS*^{GCU} plastid DNA intergenic spacer with 62 *Campanula lingulata* s.l. accessions and five outgroups. – Numbers at nodes refer to bootstrap values.

current ranges of which do not overlap (sub-group IIa is found in Macedonia, sub-group IIb in Rhodopes and sub-group IIc in Thessaly, Greece). These sub-groups were concordant with sub-clades obtained from molecular trees.

Altogether, our results have taxonomic implications because they reveal a clear pattern within the *Campanula lingulata* complex. As inferred from both molecular and morphological data, four allopatric units, each with a clearly delimited and rather restricted distribution range, can be easily recognized. In accordance with our molecular and morphological data and observations made on: (1) original descriptions of *C. lingulata* and *C. cichoracea* (Waldstein & Kitaibel 1801, Sibthorp & Smith 1806, 1819), (2) original specimens of these taxa deposited in Waldstein's collection in PG and BP and (3) Smith's collection in LINN-HS, the name *C. lingulata* should refer to individuals from the C Balkans and S Carpathians (clade I), while the name *C. cichoracea* should be applied to the individuals from Thessaly in Greece (sub-clade IIc). Unfortunately, due to the small amount and rather poor quality of *C. cichoracea* material in Smith's collection in LINN-HS (see Typification under Taxonomic treatment, below), we were able to observe only few morphological synapomorphies that support our extant material from Thessaly (sub-clade IIc) as *C. cichoracea* (calyx lobes ovate-lanceolate, hairy on adaxial surface and margins). On the other hand, individuals from Macedonia (IIa) and Rhodopes (IIb) require much more detailed sampling and additional analysis, and until fine-scale molecular and morphological data become available, the question of the exact taxonomic status of Macedonian and Rhodopean lineages remains open. This is the subject of an ongoing phylogenetic and phylogeographic study of the *C. lingulata* complex.

Recent work reveals that diagnosis and descriptions of new species based on morphological characters should be complemented by the DNA characters whenever possible (González-Gutiérrez & al. 2013). Although standard barcoding fragments, such as *matK* and *rbcL*, have commonly been used for identification of plant species (Chase & Fay 2009; CBOL Plant Working group 2009), growing evidence reveals that the resolution of these markers may be insufficient and that additional genomic regions may be required to provide sufficient information to distinguish closely related species in a taxonomic context (e.g. Seberg & Petersen 2009; Ran & al. 2010; Korotkova & al. 2011; González-Gutiérrez & al. 2013). Although we did not used standard barcoding fragments, both cpDNA regions used in this study have the resolution not only to distinguish the *Campanula lingulata* complex from related species, but also to distinguish closely related taxa within this complex. The *trnG*^{UCC}*-trnS*^{GCU} region harbours four diagnostic characters that can distinguish *C. lingulata* and S Balkan taxa, while within the *psbA-trnH* region a microsatellite with a dinucleotide (AT) motif was present only in *C. lingulata*. González-Gutiérrez & al. (2013) argued that the length variation of potentially homoplastic microsatellites should be avoided in DNA barcoding. Our data support this view but imply that the presence/absence of a particular microsatellite may be used for distinguishing closely related taxa.

Taxonomic treatment

Campanula cichoracea Sm. in Sibthorp & Smith, Fl. Graec. Prodr. 1: 140. 1806 – **Lectotype (designated here):** [unpublished watercolour illustration by Ferdinand Bauer] "*Campanula cichoracea*", MS Sherard 243: f. 174 (OXF) [viewable online at http://www.bodley.ox.ac.uk/users/millsr/isbes/FG/FGD3/ – later published in Sibthorp & Smith, Fl. Graeca 3: t. 209. 1819]. – Fig. 7.

Typification — In the protologue of *Campanula cichoracea* (Sibthorp & Smith 1806), Smith provided a validating diagnosis, a synonym cited with a question mark (and therefore not relevant nomenclaturally), a statement of provenance ("In Thessaliâ", i.e. Thessaly, Greece), the citation "Icon. Fl. Græc. t. 209." (see below) and the symbol "♂" meaning biennial. No specimens were cited. In the herbarium of Sir James Edward Smith held at the Linnean Society of London (LINN) we found one specimen under the number LINN-HS 309.56 that appears relevant to *C. cichoracea* (see http://linnean-online.org/30768/). This sheet, consisting of a stem bearing three branches, is annotated in Smith's handwriting: "*cichoracea*. Pr. Fl. Gr. 55*" and above: "Fl. Graec. t. 209.". Another annotation written on the sheet by Smith: "Mr. Evans's garden at Stepney [in London, U.K.] June 29. 1806." indicates that the specimen originated in cultivation in the same year as the publication of the protologue. It seems very unlikely that a specimen that was collected (or received) by Smith on 29 June 1806 could have been material on which was based the validating diagnosis in the protologue published in October–November of the same year. Therefore, it is very doubtful that this specimen is original material for the name, according to Art. 9.3 of the ICN (McNeill & al. 2012). Because we could trace no other specimens of *C. cichoracea* in the Sibthorpian Herbarium in Oxford (OXF) or in the herbarium of Sir James Edward Smith in Liverpool (LIV), we conclude that any specimen upon which the name was based, if it ever existed, is no longer extant. This leaves as the only extant original material, and therefore the obligate lectotype, an unpublished illustration: the watercolour by Ferdinand Bauer cited by Smith in the protologue. This watercolour is now conserved as MS Sherard 243: f. 174 in the Sherardian Library of Plant Taxonomy at the Oxford University Herbaria (OXF). It was available to Smith when he wrote the protologue and was annotated by him with "*Campanula cichoracea*" and "209", the latter being the relevant plate number for the subsequently published Flora graeca

Fig. 7. *Campanula cichoracea* – A: flowering plant in habitat; B: close-up of an inflorescence. – Greece, Mt Olympus, population ID 15 (see Table 1), 15 Jun 2011, photographs by S. Škondrić.

(Sibthorp & Smith 1819); see Lack (1999). We here designate Bauer's unpublished watercolour as the lectotype of the name *C. cichoracea*.

Morphological diagnostic characters — *Calyx lobes* ovate-lanceolate, hairy on adaxial surface and margins. *Calyx appendages* ovate-lanceolate to cordate, hairy on adaxial surface and margins. *Ovary* with downwards appressed hairs along ribs.

Molecular diagnostic characters — Nucleotide character state "G" in position 182, "G" in position 224, "A" in position 413, and "A" in position 739 of *trnG*^{UCC}-*trnS*^{GCU}, and the lack of a microsatellite with a dinucleotide motif (AT) downstream of three consecutive mononucleotide microsatellites spanning from position 938 to 970 and upstream of two mononucleotide microsatellites spanning from position 985 to 996 of *psbA-trnH* characterize individuals of the *Campanula lingulata* complex from the S Balkans, i.e. *C. cichoracea* s.l. An insertion of a single character (nucleotide character state "A") in position 396 and nucleotide character states "A", "C" and "T" in positions 465, 814 and 1116, respectively, characterize *C. cichoracea* from Thessaly (Greece). Sequences that describe individuals of *C. cichoracea* from Thessaly are available in GenBank under accession numbers KJ146639 to KJ146643 (*trnG*^{UCC}-*trnS*^{GCU}) and KJ146668 to KJ146672 (*psbA-trnH*). It is worth noting that although the length variation of potentially homoplastic microsatellites should be avoided in DNA barcoding (González-Gutiérrez & al. 2013), the number of repeats in a microsatellite with a mononucleotide motif (T) spanning from position 985 to 991 may be used to characterize individuals from different populations from Thessaly, because individuals from GR-Olympus are characterized by three repeats, whereas individuals from GR-Ossa are characterized by five repeats of a microsatellite repeat motif in this position.

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References

Aleksić J. M., Stojanović D., Banović B. & Jančić R. 2012: A simple and efficient DNA isolation method for *Salvia officinalis*. – Biochem. Genet. **50:** 881–892.

Borsch T., Korotkova N., Raus T., Lobin W. & Löhne C. 2009: The *petD* group II intron as a species level marker: utility for tree inference and species identification in the diverse genus *Campanula* (*Campanulaceae*). – Willdenowia **39:** 7–33.

Candolle A. L. P. P. de 1830: Monographie des Campanulacées. – Paris: Desray.

CBOL Plant Working Group 2009: A DNA barcode for land plants. – Proc. Natl. Acad. Sci. U.S.A. **106:** 12794–12797.

Cellinese N., Smith S. A., Edwards E. J., Kim S.-T., Haberle R. C., Avramakis M. & Donoghue M. J. 2009: Historical biogeography of the endemic *Campanulaceae* of Crete. – J. Biogeogr. **36:** 1253–1269.

Chase M. W. & Fay M. F. 2009: Barcoding of plants and fungi. – Science **325:** 682–683.

Conti F., Abbate G., Alessandrini A. & Blasi C. 2005: An annotated checklist of the Italian vascular flora. – Roma: Palombi.

Damboldt J. 1978: *Campanula* L. – Pp. 2–64 in: Davis P. H. (ed.), Flora of Turkey and the East Aegean Islands **6.** – Edinburgh: University Press.

Degen A. 1938: Flora velebitica **3.** – Budapest: Verlag der Ungar. Akademie der Wissenschaften.

Doyle J. J. & Doyle J. L. 1987: A rapid isolation procedure for small quantities of fresh leaf tissue. – Phytochem. Bull. Bot. Soc. Amer. **19:** 11–15.

Drummond A. J., Suchard M. A., Xie D. & Rambaut A. 2012: Bayesian phylogenetics with BEAUti and the BEAST 1.7. – Molec. Biol. Evol. **29:** 1969–1973.

Eddie W. M. M. & Ingrouille M. J. 1999: Polymorphism in the Aegean "five-loculed" species of the genus *Campanula*, section *Quinqueloculares* (*Campanulaceae*). – Nordic J. Bot. **19:** 153–169.

Eddie W. M. M., Shulkina T., Gaskin J., Haberle R. C. & Jansen R. K. 2003: Phylogeny of *Campanulaceae* s.str. inferred from ITS sequences of nuclear ribosomal DNA. – Ann. Missouri Bot. Gard. **90:** 554–575.

Edgar R. C. 2004: MUSCLE: multiple sequence alignment with high accuracy and high throughput. – Nucl. Acids Res. **32:** 1792–1797.

Euro+Med 2006+ [continuously updated]: Euro+Med PlantBase – the information resource for Euro-Mediterranean plant diversity. – Published at http://ww2.bgbm.org/EuroPlusMed [accessed 4 Aug 2013].

Fedorov A. A. 1957: *Campanulaceae* Juss. – Pp. 126–450, 459–475 in: Shishkin B. K. (ed.), Flora URSS **24.** – Moscow: Akademia Nauk.

Fedorov A. A. & Kovanda M. 1976: *Campanula* L. – Pp. 4–93 in: Tutin T. G., Burges N. A., Chater A. O., Edmondson J. R., Heywood V. H., Moore D. M., Valentine D. H., Walters S. M. & Webb D. A. (ed.), Flora europaea **4.** – Cambridge: University Press.

Filipowicz N., Nee M. H. & Renner S. S: 2012: Description and molecular diagnosis of a new species of *Brunfelsia* (*Solanaceae*) from the Bolivian and Argentinean Andes. – PhytoKeys **10:** 83–94 [online at http://dx.doi.org/10.3897/phytokeys.10.2558].

Frajman B. & Schneeweiss G. M. 2009: A campanulaceous fate: the Albanian stenoendemic *Asyneuma comosiforme* in fact belongs to isophyllous *Campanula*. – Syst. Bot. **34:** 595–601.

González-Gutiérrez P. A., Köhler E. & Borsch T. 2013: New species of *Buxus* (*Buxaceae*) from northeastern Cuba based on morphological and molecular characters, including some comments on molecular diagnosis. – Willdenowia **43:** 125–137.

Greuter W., Burdet H. M. & Long G. (ed.) 1984: Med-Checklist **1.** – Genève: Conservatoire et Jardin botaniques & Med-Checklist Trust of OPTIMA.

Griffiths H. I., Kryštufek B. & Reed J. M. (ed.) 2004: Balkan biodiversity: pattern and process in the European hotspot. – Dordrecht: Kluwer.

Grisebach A. 1846: Spicilegium florae rumelicae et bithynicae **2.** – Brunsvigae: F. Vieweg et filium.

Haberle R. C., Dang A., Lee T., Peñaflor C., Cortes-Burns H., Oestreich A., Raubeson L., Cellinese N., Edwards E. J., Kim S.-T., Eddie W. M. M. & Jansen R. K. 2009: Taxonomic and biogeographic implications of a phylogenetic analysis of the *Campanulaceae* based on three chloroplast genes. – Taxon **58:** 715–734.

Hamilton M. B. 1999: Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. – Molec. Ecol. **8:** 521–523.

Jäger E. J. & Welk E. 2003: Pflanzengeographische Gliederung Europas. – Pp. 79–86 in: Bohn U. & Neuhäusl R. (ed.), Karte der natürlichen Vegetation Europas **1.** – Münster: Landwirtschaftsverlag.

Korotkova N., Borsch T., Quandt D., Taylor N. P., Müller K. F. & Barthlott W. 2011: What does it take to resolve

relationships and to identify species with molecular markers? An example from the epiphytic *Rhipsalideae* (*Cactaceae*). – Amer. J. Bot. **98:** 1549–1572.

Kovačić S. 2006: Relationships and chorology of isophyllous and heterophyllous bluebells (*Campanula* L., *Campanulaceae*) in Adriatic Dinarids. – Doctoral Thesis, Department of Biology, Faculty of Science, University of Zagreb [original in Croatian available from Central Botanical Library, Faculty of Science, Zagreb].

Kovačić S. & Nikolić T. 2006: Relations of the western Balkan endemic *Campanula* L. (*Campanulaceae*) lineages based on comparative floral morphometry. – Pl. Biosyst. **140:** 260–272.

Lack H. W. with Mabberley D. J. 1999: The flora graeca story: Sibthorp, Bauer, and Hawkins in the Levant. – Oxford: University Press.

Lakušić D. & Conti F. 2004: *Asyneuma pichleri* (*Campanulaceae*), a neglected species of the Balkan Peninsula. – Pl. Syst. Evol. **247:** 23–36.

Lakušić D., Liber Z., Nikolić T., Surina B., Kovačić S., Bogdanović S. & Stefanović, S. 2013: Molecular phylogeny of *Campanula pyramidalis* species complex (*Campanulaceae*) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications. – Taxon **62:** 505–524.

Lammers T. G. 2007: World checklist and bibliography of *Campanulaceae*. – Kew: Royal Botanic Gardens.

Malý K. 1908: Beiträge zur Kenntnis der illyrischen Flora. – Magyar Bot. Lapok **7:** 203–240.

Mansion G., Parolly G., Crowl A. A., Mavrodiev E., Cellinese N., Oganesian M., Fraunhofer K., Kamari G., Phitos D., Haberle R., Akaydin G., Ikinci N., Raus T. & Borsch T. 2012: How to handle speciose clades? Mass taxon-sampling as a strategy towards illuminating the natural history of *Campanula* (*Campanuloideae*). – PloS ONE **7:** e50076. – http://dx.doi.org/10.1371/journal.pone.0050076

McNeill J., Barrie F. R., Buck W. R. Demoulin V., Greuter W., Hawksworth D. L., Herendeen P. S., Knapp S., Marhold K., Prado J., Prud'homme van Reine W. F., Smith G. F., Wiersema J. H. & Turland N. J. (ed.) 2012: International Code of Nomenclature for algae, fungi and plants (Melbourne Code) adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011. – Königstein: Koeltz Scientific Books [Regnum Veg. **154**].

MedCalc Software 2005–2011: Digimizer Image Analysis Software Version 4.0.0.0. – Published at http://www.digimizer.com

Meusel H. & Jäger E. J. 1992: Vergleichende Chorologie der Zentraleuropäischen Flora **3.** – Jena: Fischer.

Myers N., Mittermeier R. A., Mittermeier C. G., da Fonseca G. A. B. & Kent J. 2000: Biodiversity hotspots for conservation priorities. – Nature **403:** 853–858.

Nyman C. F. 1879: Conspectus florae europaeae **2.** – Örebro Sueciae: Typis officinae bohlinianae.

Oganesian M. 1995: Synopsis of Caucasian *Campanulaceae*. – Candollea **50:** 275–308.

Pančić J. 1875: Elenchus plantarum vascularium quae eastate a. 1873. in Crna Gora legit Dr. J. Pančić. – Belgradi: Societas Erudita Serbica.

Park J.-M., Kovačić S., Liber Z., Eddie W. M. M. & Schneeweiss G. M. 2006: Phylogeny and biogeography of isophyllous species of *Campanula* (*Campanulaceae*) in the Mediterranean area. – Syst. Bot. **31:** 862–880.

Pignatti S. 1982: Flora d'Italia **2.** – Bologna: Edagricole.

Quézel P. 1953: Les Campanulacées d'Afrique du Nord. – Feddes Repert. Spec. Nov. Regni Veg. **56:** 1–65.

Rambaut A. 2006: FigTree v1.1.1: Tree figure drawing tool. – Published at http://tree.bio.ed.ac.uk/software/figtree/

Rambaut A. & Drummond A. J. 2007: Tracer v1.4 – Published at http://beast.bio.ed.ac.uk/Tracer

Ran J. H., Wang P. P., Zhao H. J. & Wang X. Q. 2010: A test of seven candidate barcode regions from the plastome in *Picea* (*Pinaceae*). – J. Integr. Pl. Biol. **52:** 1109–1126.

Roemer J. J. & Schultes J. A. 1819: Systema vegetabilium **5.** – Stuttgardtiae: Sumtibus J. G. Cottae.

Roquet C., Sáez L., Aldasoro J. J., Susanna A., Alarcón M. L. & Garcia-Jacas N. 2008: Natural delineation, molecular phylogeny and floral evolution in *Campanula*. – Syst. Bot. **33:** 203–217.

Seberg O. & Petersen G. 2009: How many loci does it take to DNA barcode a *Crocus*? – PLoS ONE **4:** e4598 [online at http://dx.doi.org/10.1371/journal.pone.0004598].

Sibthorp J. & Smith J. E. 1806: Florae graecae prodromus **1.** – Londini: Typis Richardi Taylor et socii, in vico Shoe Lane; Veneunt apud Johannem White, in vico Fleet street.

Sibthorp J. & Smith J. E. 1819: Flora graeca **3.** – Londini: Typis Richardi et Arthuri Taylor; Veneunt apud Payne et Foss, in vico Pal-Mal.

Silvestro D. & Michalak I. 2011: raxmlGUI: a graphical front-end for RAxML. – Organisms Diversity Evol. **12:** 335–337.

Sims J. 1805: *Campanula capitata*. – Bot. Mag. **21:** t. 811.

Stamatakis A. 2006: RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. – Bioinformatics **22:** 2688–2690.

StatSoft 1996: STATISTICA, Version 5.1 [data analysis software]. – Published at http://www.statsoft.com

Stefanović S. & Lakušić D. 2009: Molecular reappraisal confirms that the *Campanula trichocalycina-pichleri* complex belongs to *Asyneuma* (*Campanulaceae*). – Bot. Serbica **33:** 21–31.

Stevanović V., Vukojičić S., Šinžar-Sekulić J., Lazarević M., Tomović G. & Tan K. 2009: Distribution and diversity of Arctic-Alpine species in the Balkans. – Pl. Syst. Evol. **283:** 219–235.

Stewart J. R., Lister A. M., Barnes I. & Dalén L. 2010: Refugia revisited: individualistic responses of species in space and time. – Proc. R. Soc. B **277:** 661–671.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. 2011: MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. – Molec. Biol. Evol. **28:** 2731–2739.

Tate J. A. & Simpson B. B. 2003: Paraphyly of *Tarasa* (*Malvaceae*) and diverse origins of the polyploid species. – Syst. Bot. **28:** 723–737.

Tenore M. 1824–1829: Flora napolitana **3.** – Napoli: Stamperia Francese.

Thiers B. 2013+ [continuously updated]: Index herbariorum: a global directory of public herbaria and associated staff. – Published at http://sweetgum.nybg.org/ih/ [accessed 4 Mar 2014].

Visiani R. de 1847: Flora dalmatica **2.** – Lipsiae: Apud Fridericum Hofmeister.

Waldstein F. P. A. & Kitaibel P. 1801: Descriptiones et icones plantarum rariorum Hungariae **1.** – Viennae: Schmidt.