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Molecular phylogeny of *Edraianthus* (Grassy Bells; Campanulaceae) based on non-coding plastid DNA sequences

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The Balkan Peninsula is known as an ice-age refugium and an area with high rates of speciation and diversification. Only a few genera have their centers of distribution in the Balkans and the endemic genus *Edraianthus* is one of its most prominent groups. As such, *Edraianthus* is an excellent model not only for studying speciation processes and genetic diversity but also for testing hypotheses regarding biogeography, identification and characterization of refugia, as well as post-glacial colonization and migration dynamics in SE Europe. The genus comprises 10 to 28 species and was traditionally divided into three sections: *E. sect. Edraianthus*, *E. sect. Uniflori*, and *E. sect. Spathulati*. We present here the first phylogenetic study of *Edraianthus* based on multiple plastid DNA sequences (*trnL-F* region and *rbcL-atpB* spacer) derived from a wide taxonomic sampling and geographic range. While the sister-group to *Edraianthus* could not be ascertained, the results strongly support its monophyly as currently circumscribed. The phylogenetic relationships among *Edraianthus* and its closely related genera *Halacsyella*, *Petkovia*, *Muehlbergella*, and *Protoedraianthus* are discussed and their respective taxonomical standings are reevaluated based on molecular evidence. Our study identified several distinct monophyletic groups within *Edraianthus*, some of which correspond closely to previously established taxonomic treatments and some of which are first identified here. Morphologic, taxonomic, and biogeographic implications of these relationships are also discussed.

KEYWORDS: Balkans, Campanulaceae, *Edraianthus*, *Halacsyella*, molecular phylogeny, *Muehlbergella*, *Petkovia*, *Protoedraianthus*, *rbcL-atpB* spacer, *trnL-F*

INTRODUCTION

The Balkan Peninsula is floristically one of the most diverse parts of Europe. There are more than 7,500 species of native plants in this region (Turill, 1929), and approximately one third of them are endemic (Stevanović, 1996). Within Europe, the floristic richness of the Balkans is comparable only to that of the Iberian Peninsula, Asia Minor, and the Caucasus. There are several reasons for this diversity. In addition to the long-lasting and far-reaching anthropogenic influences, the main factors are (1) the uninterrupted presence of an old floristic endemic element (so-called paleoendemics) predating the Quaternary ice ages, (2) a highly structured topography, with numerous isolated mountainous regions and diverse edaphic conditions, (3) high rates of speciation and diversification due to the refugial character of this region (resulting in so-called neoendemics), as well as (4) a geographical position on the crossroads of several major floras such as Central European, Mediterranean, Anatolian, and Pontic (Stevanović

& al., 1995). As a result, there are about 20 genera, such as *Amphoricarpos* Vis., *Degenia* Hayek, *Heliosperma* Rchb., *Halacsyella* Dörf., *Haberlea* Friv., *Jankaea* Boiss., *Pancicia* Vis., *Portenschlagiella* Tutin, etc., that are nearly or completely restricted to the Balkans in their distribution (Turill, 1929).

The genus *Edraianthus* A.DC. has a center of distribution in the Balkans (Fig. 1) and it is one of the most prominent groups of endemic plants in this region. Disjunct species are found in the Apennines, Sicily, and southern Carpathians. Also, according to some authors (e.g., Fedorov, 1957; Denisova, 1984; Radži, 1988), one isolated species, *E. owerinianus*, occurs in the Caucasus (Fig. 1).

Due to its distinctive mode of capsule dehiscence (irregular apical rupture as opposed to lateral porate dehiscence; compare Fig. 2D, F), a traditional placement for *Edraianthus* is in the wahlenbergioid group of Campanulaceae (de Candolle, 1839; Schönland, 1889; Kovanda, 1978; Takhtajan, 1987). In contrast, Fedorov (1972) and Kolakovskiy (1987, 1995) included this genus in the tribe

Campanuloideae based on its overall morphology. However, taking into account the morphological distinctiveness of *Edraianthus* within the Campanulaceae as a whole, Fedorov (1972) went a step further and introduced a new subtribe, *Edraiantheae*, to accommodate this genus. More recently, the position of *Edraianthus* within *Campanula* s.str. clade received a strong support from a series of family-wide studies including those on growth and seedling morphology (Shulkina & al., 2003), nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences (Eddie & al., 2003), and plastid DNA (ptDNA) structural rearrangements (Cosner & al., 2004).

For the most part, *Edraianthus* seems to be morphologically well defined. Besides the apical capsule dehiscence, typical members of the genus also have purplish-blue “campanuloid” flowers, 1–3(–4) cm long, either solitary or arranged in terminal clusters but always sessile and closely subtended by leaf-like bracts (Fig. 2A–C). The latter features (Fig. 2B–C) are reflected in the very name of the genus, derived from Greek words *hedraios* (sitting) and *anthos* (flower). Typical representatives also possess “graminoid” leaves (i.e., leaves sessile, linear, linear-lanceolate, to narrow-spatulate, long ciliate), and simple, unbranched main stems (Fig. 2A). However, uncertainties exist regarding both delimitation of the genus and infrageneric relationships. The precise circumscription has been controversial due to four lineages that do not share some of the above mentioned characteristics, but otherwise seem allied, to various degrees, with *Edraianthus*. These are the genera *Protoedraianthus*, *Halacsyella*, *Muehlbergella*, and *Petkovia*.

A strictly endemic population from the canyon of river Tara (Montenegro; Fig. 1) was recently discovered and described by Lakušić (1987) as a new species, *Edraianthus tarae*. Several morphological features (Fig. 2E–F) separate this taxon from typical members of *Edraianthus*. First, the

flowers are white and unusually large (3–5 cm long). More importantly, the flowers are stalked (not sessile) and are arranged in terminal dichasia or polychasia (Fig. 2E). This inflorescence type is unusual not only in comparison with other *Edraianthus* species but also represents a curiosity for the Campanulaceae as a whole. Finally, the fruit dehiscence represents perhaps the most distinct feature found in *E. tarae*. Its capsules open by 3–4 irregular pores at the calyx base (Fig. 2F), as opposed to the lateral openings found in campanuloid taxa or apical openings found in all other members of the genus (Fig. 2D). For these reasons, this taxon was segregated by Lakušić (1988) into its own genus, *Protoedraianthus*. However, by their general morphological appearance, these plants stand very closely to other species of *Edraianthus* (in particular to those of the *E. graminifolius*-complex), and the question of their phylogenetic relationships remains open.

Edraianthus parnassicus, an endemic species from Sterea Ellas and N Peloponnese (Greece), was originally described by Boissier (1846: 17) as *Campanula parnassica*. Even though this species clearly differs from all other species of *Edraianthus* by its branching pattern (branched main stem), general leaf morphology (wide, spatulate leaves with crenulate-serrulate margins), as well as leaf vestiture (short, curly hairs) it was nevertheless transferred to *Edraianthus* by Halácsy (1894), based primarily on superficial similarities. While Janchen (1910) pointed out these significant morphological differences and suggested this species be separated from *Edraianthus* and placed into its own genus, *Halacsyella* (*H. parnassica*), his view was not adopted in major contemporary floristic works covering the Balkans such as *Flora Europaea* (Kuzmanov, 1976), *Med-Checklist* (Greuter & al., 1984), and *Mountain Flora of Greece* (Hartvig, 1991).

In addition, a high-mountain endemic species from the Caucasus was described originally as *Edraianthus*

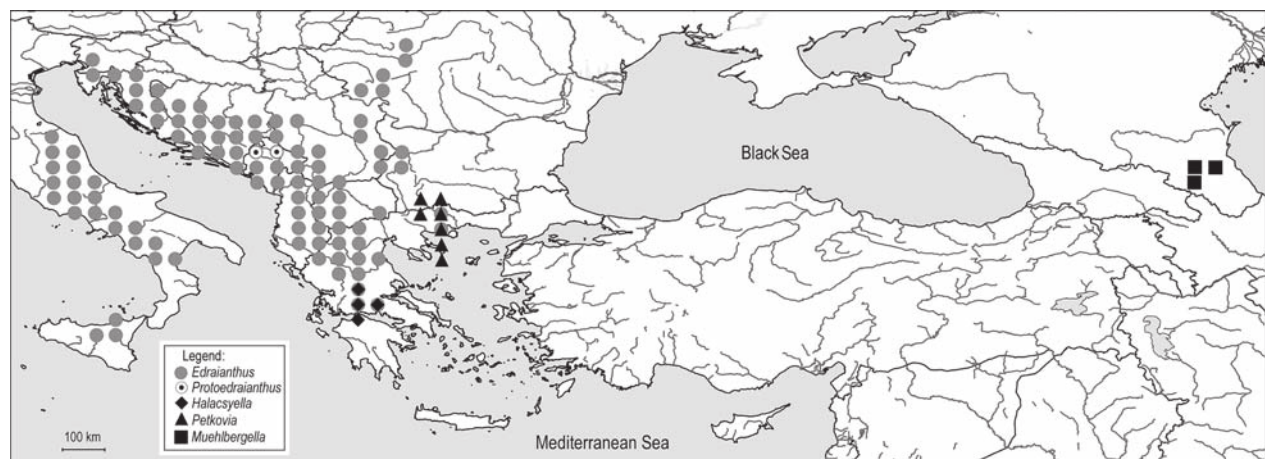


Fig. 1. Geographic distribution of *Edraianthus* and some of its putatively closely related genera. Distribution is mapped using an underlying 50 × 50 km UTM coordinate grid.

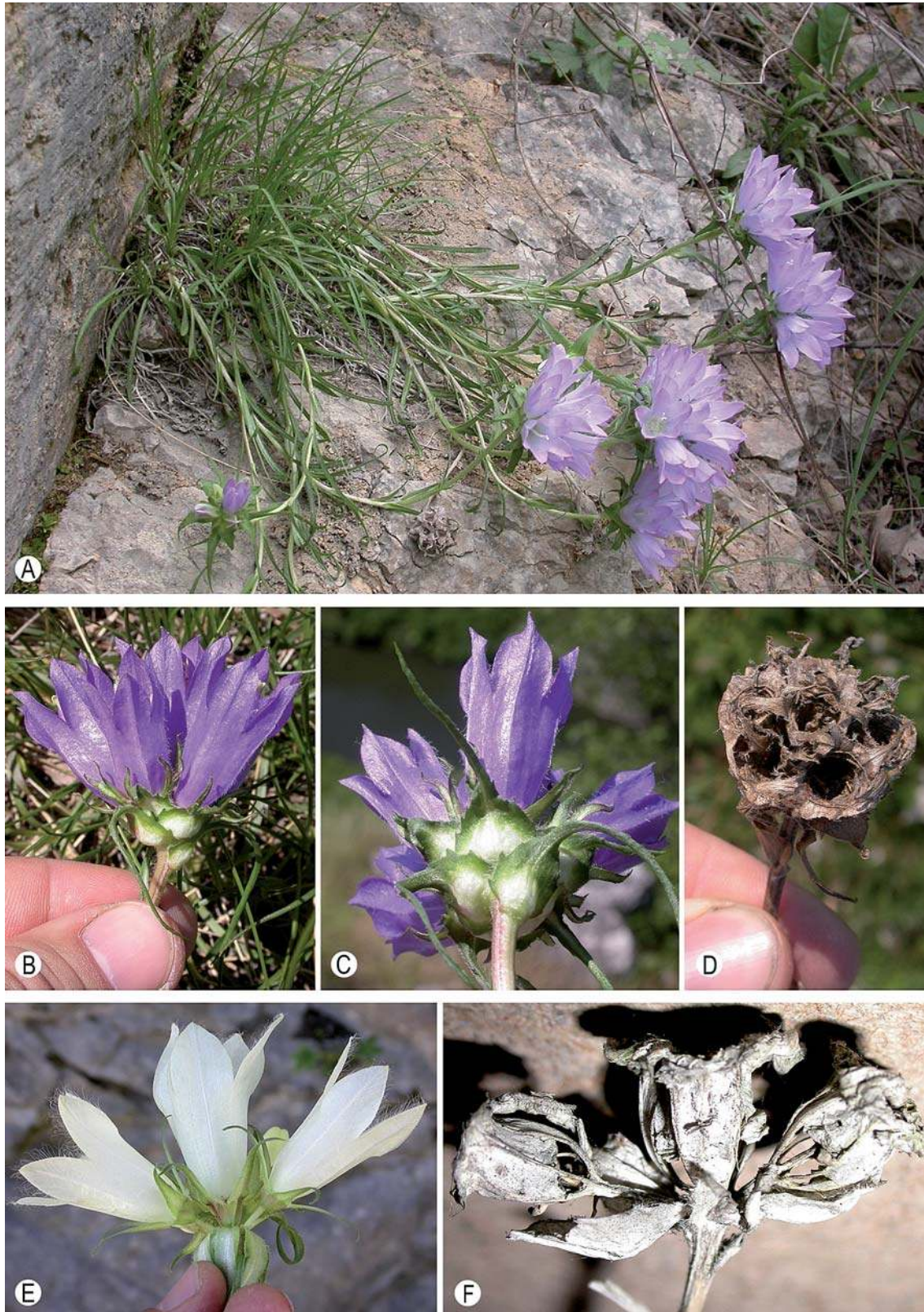


Fig. 2. Morphological variation within *Edraianthus*. A–D, *E. graminifolius* “jugoslavicus”; A, general habitat; B, typical inflorescence; C, bracts; D, dehiscent capsule. E–F, *E. tarae* (= *Protoedraianthus tarae*); E, inflorescence; F, infructescence. All photographs by Dmtar Lakušić.

owerinianus by Ruprecht (1867) based on the similarity of this species with *E. wettsteinii*. This relationship, however, would imply a major extension of the range of *Edraianthus* and would introduce a disjunction of approximately 2,000 km in its present distribution (Fig. 1). Feer (1890) removed this species from *Edraianthus*, based on its distinct fruit characteristics, among others, and transferred it to a monotypic genus *Muehlbergella* (*M. oweriniana*). As with *Halacsyella*, however, Feer's view was not adopted in subsequent floristic works (e.g., Fedorov, 1957; Denisova, 1984; Radži, 1988), which retained it within *Edraianthus*.

Finally, some authors, primarily because of similar modes of capsule dehiscence, include *Petkovia* (= *Cam-*

panula) *orphanidea* from S Bulgaria and NE Greece within *Edraianthus* under the name *E. calaminthifolius* (Degen in Lengyel, 1934: 63; Degen, 1934). Their close relationship was also suspected by Hartvig (1991).

Due to the highly endemic character of many species of *Edraianthus*, its widespread distribution throughout the Balkans, and especially its high variability and differentiation along latitudinal and altitudinal range, this genus was the subject of multiple monographs early on (e.g., Wettstein, 1887; Beck, 1893; Janchen, 1910). The most prominent was the one by Janchen (1910; see Fig. 3A), who recognized eleven taxa within *Edraianthus* (ten species, one with two subspecies; Fig. 3A). In addition, he segregated two species previously included in *Edraian-*

A				B				
Genus	Section	Species/subspecies		Genus	Subgenus/Section	Series	Species/subspecies	
<i>Edraianthus</i>	Capitati	<i>E. dalmaticus</i>		<i>Edraianthus</i>	Capitati	Dalmaticii	<i>E. dalmaticus slavnicii</i>	
		<i>E. tenuifolius</i>					<i>E. dalmaticus horvaticii</i>	
		<i>E. serbicus</i>					<i>E. dalmaticus</i>	
		<i>E. graminifolius niveus</i>					<i>E. serbicus stankovicii</i>	
		<i>E. graminifolius coeruleus</i>					<i>E. serbicus</i>	
	Uniflori	<i>E. pumilio</i>				Tenuifolii	<i>E. hercegovinus</i>	
		<i>E. dinaricus</i>					<i>E. tenuifolius pivae</i>	
		<i>E. wettsteinii</i>					<i>E. tenuifolius</i>	
	Spathulati	<i>E. serpyllifolius</i>				Graminifolii	<i>E. graminifolius apenninus</i>	
		hybrids					<i>E. graminifolius siculus</i>	
<i>Halacsyella</i>	<i>H. parnassica</i>		Jugoslavicii			<i>E. graminifolius</i>		
	<i>Muehlbergella</i>	<i>M. oweriniana</i>		<i>E. jugoslavicus rugovae</i>				
				<i>E. jugoslavicus subalpinus</i>				
			<i>E. jugoslavicus</i>					
			<i>E. croaticus</i>					
			<i>E. niveus</i>					
			<i>E. murbeckii</i>					
			<i>E. montenegrinus polydentatus</i>					
			<i>E. montenegrinus durmitoreus</i>					
			<i>E. montenegrinus</i>					
C				D				
Genus	Species/subspecies			Genus	Species/subspecies			
<i>Edraianthus</i>	<i>E. dalmaticus</i>			<i>Edraianthus</i>			<i>E. caricinus ginzbergeri</i>	
	<i>E. tenuifolius</i>						Caricinii	<i>E. caricinus baldaccii</i>
	<i>E. serbicus</i>							<i>E. caricinus</i>
	<i>E. hercegovinus</i>							<i>E. horvatii</i>
	<i>E. australis</i>						Zogovicii	<i>E. australis subalpinus</i>
	<i>E. graminifolius</i>							<i>E. australis</i>
	<i>E. graminifolius siculus</i>							Kitaibelii
	<i>E. niveus</i>						<i>E. zogovicii</i>	
	<i>E. dinaricus</i>						hybrids	
	<i>E. pumilio</i>							<i>E. kitaibelii bhariense</i>
	<i>E. wettsteinii lovcenicus</i>							<i>E. kitaibelii</i>
	<i>E. wettsteinii</i>						Uniflori	<i>E. linifolius</i>
	<i>E. serpyllifolius</i>							<i>E. pumilio</i>
	<i>E. parnassicus</i>						<i>E. dinaricus</i>	
							<i>E. wettsteinii lovcenicus</i>	
			<i>E. wettsteinii</i>					
				<i>Protoedraianthus</i>	Protoedraianthus	<i>P. tarae</i>		
						<i>P. vesovicii</i>		
				<i>Halacsyella</i>		<i>P. pilosulus</i>		
						<i>Muehlbergella</i>		<i>P. serpyllifolius</i>
								<i>P. sutjeskiae maslesae</i>
								<i>P. sutjeskiae</i>
								<i>P. majae</i>
								<i>P. glisicii</i>
					<i>H. parnassica</i>			
					<i>M. oweriniana</i>			

Fig. 3. Synopsis of the most influential precladistic classifications and floristic treatments for *Edraianthus* and its closely related genera. A, classification scheme according to Janchen (1910); B, classification scheme according to Lakušić (1974; modified 1987, 1988); C, taxa accepted by the *Flora Europaea* (Kuzmanov, 1976) and *Med-Checklist* (Greuter & al., 1984); D, an additional species accepted by the *Flora of the USSR* and other floristic works covering the Caucasus (e.g., Fedorov, 1957; Denisova, 1984; Radži, 1988).

thus (*E. parnassicus*, *E. owerinianus*) into their own genera (*Halacsyella*, *Muehlbergella*). Despite some differences in their respective species concepts, all of these early monographs basically agreed that three groups could be separated within the genus. Traditionally, these are treated at sectional level and include: (1) *E. sect. Capitati* (= *Edraianthus*), with long, linear to linear-lanceolate, ciliate, basal leaves, sessile flowers, either solitary or in terminal cluster, closely subtended by large leaf-like bracts; (2) *E. sect. Uniflori* (= *Strigosi*), with short, linear, more or less densely hirsute and grayish above, glabrous beneath basal leaves, flowers solitary, closely subtended by small leaf-like bracts; and (3) *E. sect. Spathulati*, with short, spatulate, ciliate, basal leaves, flowers solitary, subtended by small leaf-like bracts.

The most recent and most comprehensive monograph was offered by Lakušić (1974). In this seminal work Lakušić conducted detailed systematic, phyto-geographic, and, especially, ecological investigations of *Edraianthus* and related species from the Balkans and adjacent regions. Lakušić built his classification scheme (Fig. 3B) on the basis of a more nuanced species concept, taking into account not only morphological differences but also distribution as well as ecological differentiation. As a consequence, the number of recognized taxa was increased to more than 45 (28 species plus a number of subspecies and/or varieties). In conjunction with two subsequent contributions (Lakušić, 1987, 1988), Lakušić proposed a new arrangement for edraianthoid campanulas, dividing them into two genera, *Edraianthus* and *Protoedraianthus*, followed by a very elaborated system of subgeneric classification (Fig. 3B). Under this scheme, *Halacsyella* and *Muehlbergella* remained separate genera as well, following Janchen (1910).

The most significant contemporary floristic works covering the SE Europe, *Flora Europaea* (Kuzmanov, 1976) and *Med-Checklist* (Greuter & al., 1984), basically accept Janchen's (1910) concept, with the number of taxa recognized within the genus ranging between 10–14, respectively (Fig. 3C). However, neither of these floras accepts Janchen's view regarding the status of *Halacsyella* as a separate genus. *Halacsyella parnassica* is instead included in *Edraianthus*. Similarly, *Muehlbergella oweriniana* is treated as congeneric with *Edraianthus* (Fig. 3D) in floristic works covering the Caucasus (Fedorov, 1957; Denisova, 1984; Radži, 1988).

Some additional recent treatments have focused on either a taxonomic subset of the genus (Mayer & Blečić, 1969; Međedović, 1981) or a particular geographic area (Šolić, 1981; Šoljan, 1987; Šoljan & Abadžić, 1988). Although very useful and information-rich, these studies did not provide either an overhaul of the genus or proposed alternative phylogenetic schemes. A molecular phylogenetic approach could help to resolve long-standing controversies

and nurture a greater understanding of the evolutionary processes that have shaped *Edraianthus*. However, this genus has not been the subject of broad molecular phylogenetic work to date.

Our research on *Edraianthus* was undertaken with several goals in mind: (1) to test the monophyly of *Edraianthus* and help place allied taxa that have ambiguous position in present classifications; (2) to circumscribe major lineages within the genus; (3) to develop a well-supported phylogenetic hypothesis for *Edraianthus* as a whole; (4) to investigate the scenarios of morphological character evolution; (5) to develop, in conjunction with reevaluation of traditional taxonomic characters, a comprehensive, phylogeny-based classification; and (6) to use this group of plants as a model to investigate biogeographical hypotheses regarding identification and characterization of refugia, as well as post-glacial colonization and migration dynamics in SE Europe. The present study focuses mainly on the first three goals. To address these questions, we generated a new molecular dataset consisting of DNA sequences from two non-coding regions of the plastid (pt) genome. The first region contains the *trnL* intron, 3' *trnL* exon, and the intergenic spacer between this exon and *trnF* (*trnL-F* region). Trees derived from the *trnL-F* region are well documented in their utility for resolving relationships among closely related taxa (e.g., Taberlet & al., 1991; Gielly & Taberlet, 1994; and references therein). The second region contains the intergenic spacer occurring between *rbcl* and *atpB* and the 5' end of the *atpB* gene (*rbcl-atpB* spacer). This region is comparatively less frequently used as source of data for phylogenetic inference (but see Kadereit & al., 2006; Walsh & Hoot, 2001). However, it proved to be a very useful phylogenetic marker in Lobeliaceae (Knox & al., 2006), the sister-family to Campanulaceae as well as in some other groups of plants (e.g., Chenopodiaceae; Solanaceae).

MATERIALS AND METHODS

Taxon sampling. — A total of 204 accessions were used in this study representing 39 species. Species names, sources, voucher information, and corresponding DNA extraction numbers are provided in the Appendix. Classification and formal nomenclature for *Edraianthus* follows the *Flora Europaea* (Kuzmanov, 1976) and *Med-Checklist* (Greuter & al., 1984) as the two most recognized and widely used references for this genus. In addition, informal names proposed by Lakušić (1974; modified 1987, 1988) were used wherever applicable. The 17 ingroup species (represented by 177 individuals), on which our analyses are primarily focused, include members of all three traditionally recognized sections within *Edraianthus* (sect. *Capitati*, sect. *Uniflori*, sect. *Spathulati*; Janchen, 1910)

plus the newly described species treated as a distinct genus, *Protoedraianthus* (Lakušić, 1987, 1988). Taking into account the difficulties in defining many of these species morphologically, and separating them from the neighboring ones, effort was made to sample multiple accessions to represent them. More than one individual was analyzed in 14 of the included ingroup species. Special attention was paid to morphologically variable species containing more than one subspecies/variety (e.g., numerous members of *E. graminifolius*-complex) and/or those with wide geographic range (e.g., *E. tenuifolius*, *E. serpyllifolius*, *E. australis*). These species were represented by 13 to >75 individuals from across their respective morphological/geographical range. The remaining three species are represented by a single individual mainly because they are rare, or locally abundant but known only from their type localities or otherwise restricted areas, or because they are underrepresented in collections. We relied on the only broad molecular systematic study of the bellflower family (Eddie & al., 2003) and our preliminary analyses to select the putative outgroups. According to this, *Edraianthus* belongs to the *Campanula* s.str. clade (Eddie & al., 2003). However, its relationships with the other taxa in that clade (e.g., *Campanula* spp. as well as some other segregate genera such as *Symphyandra* and *Petkovia*) are not resolved. We have chosen 13 diverse taxa from this group to represent more closely related outgroups. Ten additional species from the “rapunculoid” clade (Eddie & al., 2003) were selected as more distant outgroups.

Molecular techniques. — Total genomic DNA from silica-dried or herbarium material was extracted using a modified hexadecyltrimethylammonium bromide (CTAB) technique from Doyle & Doyle (1987) and purified using Wizard[®] minicolumns (Promega, Madison, Wisconsin, U.S.A.). Polymerase chain reaction (PCR) was used to obtain double-strand DNA fragments from *trnL-F* and *rbcL-atpB* spacer, two non-coding portions of ptDNA. The *trnL-F* region was amplified using C and F primers described by Taberlet & al. (1991). The *rbcL-atpB* spacer was amplified using S385R and RBCL1 primers described by Hoot & al. (1995). In addition, a set of internal primers (aB.rL-spac.F: 5'-CCAGACGTAGTGTGTTGATC-3'; aB.rL-spac.R: 5'-CYGCTCCTTGAGATTTGAG-3') was designed to facilitate PCR and sequencing for some difficult templates extracted from poor quality herbarium tissue. All PCRs were carried out in 50 µL volumes with annealing temperatures of 50°C–55°C. Amplified products were cleaned by polyethylene-glycol/NaCl precipitations. Cleaned products were sequenced directly, including both strands to ensure accuracy, using the DYEnamic[™] ET dye terminator sequencing kit (GE Healthcare, Baie-d'Urfé, Quebec, Canada) on an Applied Biosystems model 377 automated DNA sequencer (PE Biosystems, Foster City, California, U.S.A.). Sequence data were proofed, edited, and

contigs assembled using Sequencher[™] v.3.0 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). Sequences generated in this study are submitted to GenBank (accession numbers EF213141-EF213545; see Appendix).

Phylogenetic analyses. — Sequences were aligned manually using Se-Al v.2.0a11 (Rambaut, 2002). Although numerous gaps had to be introduced in the alignments, the sequences were readily aligned among all the taxa in both matrices. Gaps in the alignments were treated as missing data. However, the gaps were scored automatically using SeqState v.1.32 (Müller, 2005), coded as binary characters (Simmons & Ochoterena, 2000), and appended to the sequence matrix. Phylogenetic analyses were conducted under parsimony and Bayesian optimality criteria.

Parsimony searches, along with accompanying clade support estimations, were conducted for each matrix separately as well as for the combined dataset. Under this criterion, nucleotide characters were treated as unordered and all changes, including gap characters, were equally weighted. The heuristic searches for most parsimonious (MP) trees were performed using a two-stage strategy with PAUP* v.4.0b10 (Swofford, 2002). First, the analyses involved 1,000 replicates with stepwise random taxon addition, tree bisection-reconnection (TBR) branch swapping saving no more than 10 trees per replicate, and MULTREES option off. The second round of analyses was performed on all trees in memory with same settings except the MULTREES option on. Both stages were conducted to completion or until 100,000 trees were found. In addition, other searches were conducted using the parsimony ratchet analyses (Nixon, 1999) as implemented in NONA (Goloboff, 1999) with Winclada interface (Nixon, 2002). Ten consecutive tree searches were conducted using 200 iterations per search, one tree held for each iteration, 10% of total characters sampled, and amb-poly= (no swapping on ambiguously supported nodes), but they did not find shorter trees. Relative support for clades was inferred by nonparametric bootstrapping (Felsenstein, 1985) as implemented in PAUP* using 500 heuristic bootstrap replicates, each with 20 random addition cycles, TBR branch swapping, and MULTREES option off (DeBry & Olmstead, 2000). Nodes receiving bootstrap values < 70%, 70%–80%, and > 80% were considered weakly, moderately, and strongly supported, respectively. Conflict between datasets was evaluated by visual inspection, looking for the presence of strongly supported yet conflicting topologies from individual data partitions.

The Felsenstein (1981) model of DNA substitution, with rate variation among nucleotides following a discrete gamma distribution (F81+G), was selected as the best-fit by both the hierarchical likelihood ratio test (hLRT) and Akaike Information Criterion (AIC), as implemented in ModelTest v.3.7 (Posada & Crandall, 1998). Bayesian

phylogenetic inferences were performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) on the combined dataset only. Two runs starting from random trees were carried out using the F81 + G substitution model. All model parameters were treated as unknown variables with uniform prior probabilities and were estimated as part of the analysis together with tree topologies. Metropolis-coupled Markov chain Monte Carlo algorithm was used with four simultaneous chains, set at two million generations, and sampled every 100 generations. To determine the burn-in cut-off point, we plotted the $-\ln$ likelihood scores against generation time for both runs. After discarding all preasymptotic samples, remaining data points were first analyzed separately using PAUP* to compute the 50% majority-rule consensus tree. Because no significant differences between two runs were detected, the reported topologies and posterior probabilities (PP) are based on trees pooled from both independent Bayesian analyses. Only the nodes receiving $PP \geq 0.95$ were considered statistically significantly supported, given the assumptions of DNA sequence evolution (Rannala & Yang, 1996).

Alternative hypotheses testing. — Alternative topologies, mainly designed to investigate the monophyly of traditionally delimited sections and genera, were constructed using MacClade v.4.06 (Maddison & Maddison, 2003). Their cost in parsimony, excluding the gap characters, was assessed using PAUP*. To statistically compare resulting alternative phylogenetic hypotheses, the one-tailed Shimodaira-Hasegawa tests (SH tests;

Shimodaira & Hasegawa, 1999; Goldman & al., 2000) were conducted, using the aforementioned substitution model and likelihood settings. The SH tests were conducted with PAUP* using 1,000 replicates and full parameter optimization of the model.

Phytogeographical analyses. — A total of 1,995 single chorological records were scored. Chorological information was obtained from two main sources, bibliographical references and herbarium specimens. Published literature data provided 1,375 data points. Whenever possible, different monographs were consulted and cross-referenced to verify the accuracy of the collected data. Herbarium specimens deposited in two major regional herbaria (BEO, BEOU) provided the additional 620 data points. All of these data were mapped using the universal transverse mercator (UTM) coordinate system with the 50×50 km or 10×10 km grid.

RESULTS

Sequences and alignments. — Characteristics of the sequences obtained from *trnL-F* and *rbcL-atpB* spacer are summarized in Table 1. Although these two non-coding plastid regions exhibited length variation, the alignments were straightforward and the assessment of primary homology was unambiguous throughout the entire length of both of these matrices. No significant heterogeneity in base composition was detected within any of

Table 1. Summary descriptions for sequences included in, and maximum parsimony trees derived from, individual and combined analyses of *Edraianthus* and its outgroups selected from Campanulaceae s.str.

	<i>trnL-F</i>	<i>rbcL-atpB</i> spacer	Combined data
Number of individuals sequenced	202	203	204
Number of OTUs analyzed ^a	93	94	95
Sequence characteristics			
Aligned length	1,021	1,297	2,318
Number of gaps coded as binary characters	51	66	117
Variable sites ^b	280	363	643
Parsimony informative sites ^b	152	215	367
Mean AT content (%)	65.4	64.3	63.9 ^c
Base frequency homogeneity ($\chi^2/df/P$)	29.1/276/1.0	29.2/279/1.0	25.3/273/1.0 ^c
Tree characteristics			
Number of trees	>100,000	>100,000	>100,000
Length	370	490	868
CI/RI	0.84/0.94	0.82/0.94	0.82/0.94

^aAfter individuals with identical sequence for both regions were aggregated into a single terminal taxon.

^bIncluding coded gaps.

^cIncluding only OTUs for which both sequences are available.

CI, consistency index; *df*, degrees of freedom; OTU, operational taxonomic unit; RI, retention index.

these data matrices across all taxa. Due to the poor quality of the DNA extracted from some herbarium specimens, sequences could not be obtained for three individuals. *Edraianthus graminifolius* “*croaticus*” (accession no. 1345) and *E. hercegovinus* (accession no. 1459) are unavailable for the *trnL-F* region, and *E. owerinianus* (accession no. 1452) for the *rbcl-atpB* spacer. Alignments in Nexus format are available on request from the first author.

For phylogenies aimed at resolving species-level relationships it is of paramount importance to incorporate within-species variability and take into account possible biological phenomena that can confound results (such as lineage sorting, deep coalescence, etc.). For these reasons, most of the species in the study were represented by multiple individuals, sampled from geographically distinct areas and encompassing morphological variability. However, addition of terminal taxa results in a sharp increase of computational burden (Felsenstein, 1978). Therefore, in order to facilitate the phylogenetic analyses, individuals of the same species having the same haplotype (i.e., both plastid regions identical to each other in sequences as well as gaps) were grouped into a single operational taxonomic unit (OTU). Following this procedure, the 202 individuals from *trnL-F* matrix were aggregated into 93 OTUs, 203 from *rbcl-atpB* spacer matrix into 94, and 204 individuals used in the combined dataset were aggregated into 95 OTUs (Table 1).

Unconstrained and alternative tree topologies.

— A number of distinct preliminary phylogenetic analyses were conducted using parsimony to explore the distribution of phylogenetic signal in the different matrices, with and without coded gaps. Statistics of MP trees derived from separate and combined analyses are summarized in Table 1. As expected from the sequenced regions that co-occur in the haploid plastid genome, the independent analyses gave remarkably congruent results, albeit quite unresolved (results not shown). Hence, we combined all data following a total-evidence approach. Two sets of analyses were conducted on this combined data matrix, one using Bayesian inference (on sequences alone) and the other using parsimony (on all available data, including coded gaps).

The trees produced by the combined analysis had better resolution and overall support relative to those produced by independent analyses. Therefore we base our discussion on the analysis of the combined dataset. Both Bayesian runs, each initiated from a random starting tree, converged on similar log-likelihood scores and reached stationarity at no later than 200,000 generations. The burn-in of data points accumulated before asymptotic plateaus were reached left a total of 36,000 combined samples ($2 \times 18,000$). A majority-rule consensus of 36,000 trees resulted in a phylogenetic hypothesis depicted in Fig. 4. The parsimony analysis using the same sequence

matrix, but with addition of gaps coded as binary characters, resulted in > 100,000 MP trees, each 868 steps in length. Strict consensus of equally parsimonious trees resulted in relationships topologically almost identical to results derived under the Bayesian criterion (Fig. 4). The only differences were due to the characters derived from gaps, which offered some additional resolution and support (depicted with solid bars; Fig. 4). One of the MP trees, randomly selected, was chosen to illustrate the branch lengths (Fig. 4; inset).

According to our results, *Edraianthus*, as traditionally defined (sensu Janchen, 1910; Lakušić, 1974), is monophyletic and nested within *Campanula* s.str. clade (Eddie & al., 2003). Both of these results received very high overall support (BS 100%, PP 1.0; Fig. 4). Out of four lineages with questionable positions with regard to the delimitation of *Edraianthus*, only one, *Protoedraianthus* (Lakušić, 1987, 1988), makes a natural group with *Edraianthus* s.str. The other two taxa frequently allied with *Edraianthus*, *E. parnassicus* and *E. owerinianus*, were not found in a monophyletic group with this genus (whether circumscribed strictly, or more broadly, to include *Protoedraianthus*). Instead, the individuals belonging to *E. parnassicus* were found on the optimal trees forming a clade together with *Campanula* spp. from Greece. Similarly, *E. owerinianus* is in a clade with other *Campanula* spp. primarily from the Caucasus and Pontic region. To confine both of these species in a clade with *Edraianthus*, several nodes, some of them at BS 100% and PP ≥ 0.95 , would have to be collapsed. Not surprisingly, the enforcement of this monophyly resulted in trees 22 steps longer than the MP trees and was rejected as significantly worse solution by the SH test (Table 2). Lastly, *Petkovia orphanidea* forms an isolated lineage within the *Campanula* s.str. clade and is not closer related to any particular group within this polytomy, including *Edraianthus*.

Within the broadly defined *Edraianthus* clade (to include *Protoedraianthus*), a total of four major species complexes were resolved (labeled in Roman numerals in the inset of Fig. 4). One of these groups corresponds loosely to *E. sect. Spathulati* and is labeled as “*E. serpyllifolius*-complex.” This complex consists of two clades, and is not monophyletic according to our current estimates (Fig. 4). One of these clades, containing two distinct haplotypes of *E. serpyllifolius*, is resolved as a sister group to *E. graminifolius*-complex on the optimal trees. However, this relationship was supported only weakly (PP < 0.95, BS < 70%). Its potentially closer connection with the second clade of *E. serpyllifolius*-complex involves only a slight topological distortion (a nearest-neighbor interchange) and requires one extra step. Hence, it cannot be rejected as an alternative (Table 2). The remainder of *E. serpyllifolius*-complex consists of four additional lineages of *E. serpyllifolius*, arranged paraphyletically into two clades, with in-

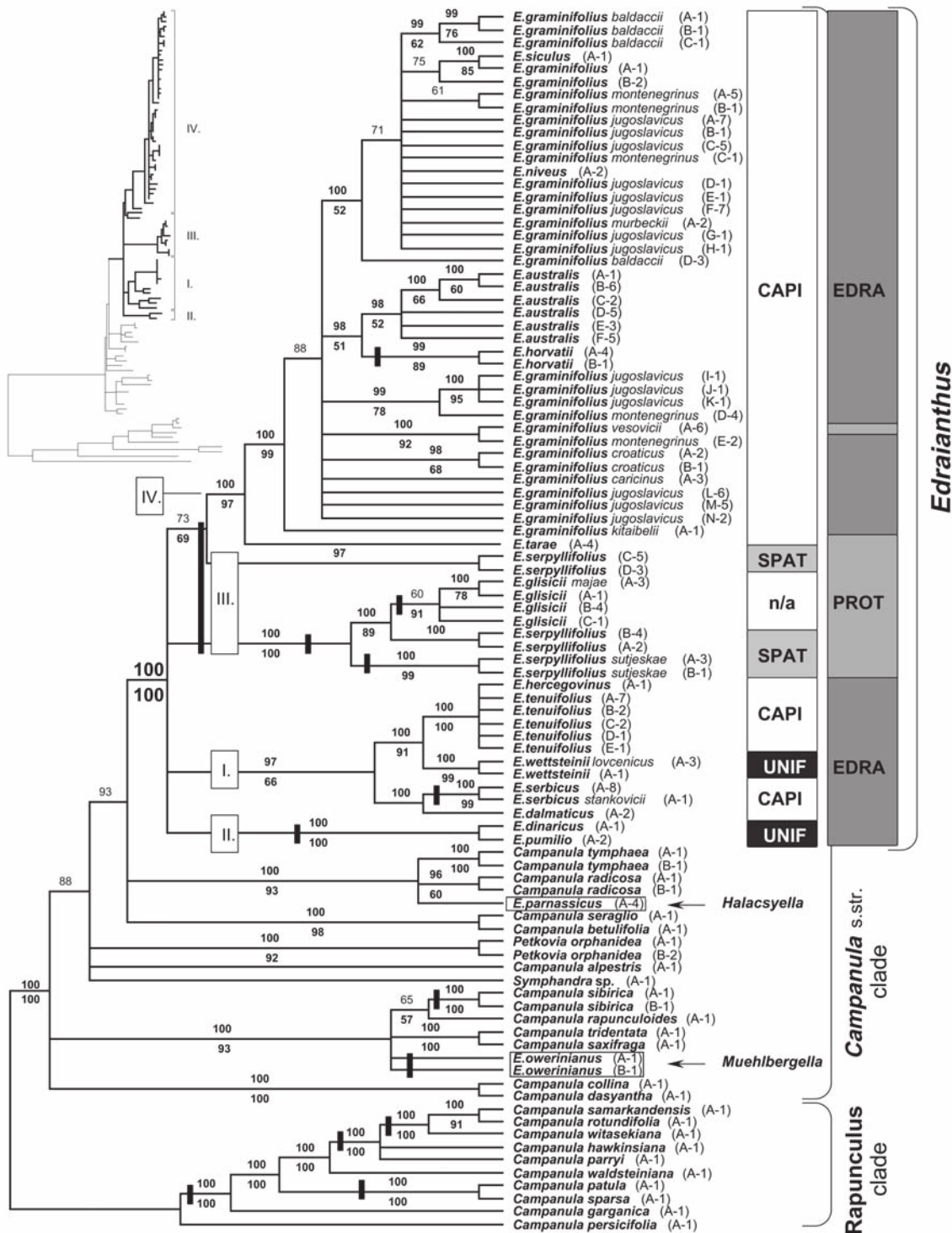


Fig. 4. Bayesian analysis tree derived from combined *trnL-F* and *rbcL-atpB* spacer sequences. MP search resulted in a strict consensus with nearly identical topology (L = 868). Solid bars depict additional resolution and/or support derived from the gaps in MP search. Inset shows one of equally parsimonious trees chosen to illustrate the branch lengths. Four major species complexes of *Edraianthus* are labeled with Roman numerals. Bayesian posterior probabilities are indicated above branches. Parsimony bootstrap supports ($\geq 50\%$) are indicated below branches. Arrows indicate phylogenetic placements of taxa believed to be closely allied with *Edraianthus*. Labeling in parentheses following species names corresponds to a haplotype of a given species and the number of individuals with that haplotype (compare with DNA accessions in the Appendix). Traditional infrageneric classifications according to Janchen (1910) and Lakušić (1988) are indicated in the left and right column, respectively (n/a = not applicable).

dividuals belonging to *E. glisicii*, a species traditionally classified in *E. sect. Capitati*, nested within it (Fig. 4). The other three major groups, *E. pumilio*-, *E. tenuifolius*- and *E. graminifolius*-complex, are all monophyletic and strongly supported as such, each receiving PP \geq 0.95 and BS > 80% BS. The *E. pumilio*-complex consists of two morphologically similar stenoendemic species, *E. pumilio* and *E. dinaricus*, both belonging to the *E. sect. Uniflori*. Members of the *E. tenuifolius*-complex are split into two strongly supported subclades. The first consists of two species, *E. dalmaticus* and *E. serbicus*. Albeit geographically distant from each other at present, these two species are morphologically quite similar. The second clade contains three species. Two of them, *E. tenuifolius* and *E. hercegovinus*, are morphologically similar and are indeed found to be very closely allied. Surprisingly, the third member of this clade is *E. wettsteinii*, a species traditionally assigned to *E. sect. Uniflori*. Finally, the fourth major group is the *E. graminifolius*-complex, the largest and most diverse group of *Edraianthus*. Within this group, *E. tarae* (= *Protoedraianthus tarae*) forms an isolated lineage, positioned as a sister group to the rest of the *E. graminifolius*-complex. This well-differentiated position of *E. tarae* is further underlined by the strong branch support for the clade comprising the remainder of this complex (PP 1.0, BS 99%). In contrast, the backbone relationships within the rest of the *E. graminifolius*-complex remain largely unresolved. Nevertheless, several moderately to strongly supported clades and distinct lineages (i.e., groups of individuals with identical or very similar haplotypes) can be identified within this complex (Fig. 4).

The relationships among the four major groups of *Edraianthus* are also unresolved when sequence data alone are used (under Bayesian or parsimony criterion). The MP trees using coded gaps in addition to sequence data place the *E. graminifolius*- and *E. serpyllifolius*-complexes in a single clade (trees not shown). This assemblage is sup-

ported by two gaps depicted by a long solid bar in Fig. 4. In a context of a rooted phylogenetic hypothesis, one of those gaps is an insertion found in the *trnL-F* spacer while the other is a deletion located in *rbcL-atpB* spacer. However, despite the presence of these two characters, the internal support for this relationship remained relatively low (BS 69%). Even though the exact relationships among major groups are not known presently, the taxonomic composition of these four complexes clearly contradicts the precladistic views of relationships as well as corresponding systematic arrangements (Janchen, 1910; Lakušić, 1988). None of the three sections proposed by Janchen (1910), *E. sect. Capitati*, *E. sect. Uniflori*, and *E. sect. Spathulati*, were found to be natural (as indicated in Fig. 4, left column). For example, *E. wettsteinii*, a species placed in *sect. Uniflori* is not in a close relationship with other putative members of this section. Instead, it is nested, with high support, within a group of species belonging to *sect. Capitati* (*E. tenuifolius*-complex). Similarly, *E. glisicii*, traditionally a member of *sect. Capitati*, is nested within *sect. Spathulati*. Also, albeit most of the species classified in *sect. Capitati* are found in the *E. graminifolius*-complex, several other species from this section are found elsewhere on the tree. In aggregate, a topological enforcement compatible with the three-sections hypothesis following Janchen's (1910) original classification resulted in trees 25 steps longer than the MP trees, due to the multiple well-supported nodes that would have to be collapsed, and were rejected by the SH test (Table 2). In addition to being nested within *Edraianthus* s.str., the genus *Protoedraianthus* itself, as circumscribed by Lakušić (1987, 1988), is not found to be monophyletic as well (Fig. 4, right column). In part, this is due to the fact that *E. serpyllifolius*-complex, species of which make the bulk of the proposed genus, is not monophyletic as already described. More importantly, *E. graminifolius* "vesovicii", a lineage segregated from *E. graminifolius*

Table 2. Comparison of unconstrained (most parsimonious) topologies with specific alternative hypotheses and results of the corresponding Shimodaira-Hasegawa tests derived from combined analyses of *Edraianthus* and its outgroups selected from Campanulaceae s.str.

Topology	Length ^a	Length penalty	Rejected ^b
Unconstrained: monophyly of <i>Edraianthus</i> s.l., including <i>Protoedraianthus</i> (Fig. 4)	710	–	Best
Monophyly of <i>Edraianthus</i> with <i>Halacsyella</i> and <i>Muehlbergella</i> (Fig. 3C, D)	732	22	Yes
Three-sections hypothesis (sensu Janchen, 1910): <i>Capitati</i> vs. <i>Uniflori</i> vs. <i>Spathulati</i> (Fig. 3A)	735	25	Yes
Two-genera hypothesis (sensu Lakušić 1987, 1988): <i>Edraianthus</i> vs. <i>Protoedraianthus</i> (Fig. 3B)	726	16	Yes
Monophyly of <i>E. serpyllifolius</i> -complex (Fig. 4)	711	1	No
Single- vs. multi-flower inflorescence	712	2	No

^aBased on combined dataset with exclusion of gap characters.

^bYes, rejected as significantly worse topology by the one-sided SH test using full optimization ($P < 0.05$); No, not rejected by the SH test.

into its own species (*E. vesovicii*) by Lakušić (1974) and subsequently transferred to *Protoedraianthus* (Lakušić, 1988), bears no close relationships to the other members of *E. serpyllifolius*-complex. It is mostly due to the position of this particular species, nested deeply and with high support within *E. graminifolius*-complex, that the enforced monophyly of *Protoedraianthus* resulted in trees 16 steps longer than the MP trees and was rejected by the SH test (Table 2).

DISCUSSION

Monophyly of *Edraianthus*. — Our results lend strong support for the single origin of *Edraianthus*, as defined traditionally by its early monographs (e.g., Janchen, 1910; Lakušić, 1974) and with the inclusion of the species that has been more recently segregated into a separate genus, *Protoedraianthus* (Lakušić, 1987, 1988). This taxon, characterized by unusual flower size and color (white), inflorescence type (dichasia or polychasia), and especially by its fruit dehiscence (irregular pores at the calyx base), is morphologically very distinct from the rest of members of *Edraianthus* (Fig. 2). Hence, *Edraianthus* as newly delimited here requires an expanded and more elaborated definition: plants perennial; rhizome stout and branched; basal leaves sessile, linear, linear-lanceolate to angustispatulate, (0.5–)2–4(–5) mm wide (“graminoid”), flat, entire, ciliate; stems simple, unbranched; flowers blue, lavender, or white, 1–3(–5) cm long, sessile, in terminal cluster or solitary, or pedicellate in terminal dichasium (polychasium), closely subtended by leaf-like bracts; fruits short-conical capsules, splitting irregularly at apex, or splitting by 3–4 irregular pores at calyx base. Despite the lack of an obvious, unique, and unreversed morphological synapomorphy for this more inclusive circumscription, *Protoedraianthus* is clearly nested within *Edraianthus* and should be treated as congeneric with it. This inclusion is supported by high statistical supports (BS 100%; PP 1.00; Fig. 4) as well as by the rejection of the two-genera hypothesis (i.e., *Edraianthus* vs. *Protoedraianthus*; Lakušić, 1987, 1988) by the SH test (Table 2).

The other three taxa suspected in the past to have close relationships with *Edraianthus* (*E. parnassicus*, *E. owerinianus*, *Petkovia orphanidea*) are found to have stronger ties with other members of the campanuloid clade. *Edraianthus parnassicus* from N Peloponnese and Sterea Ellas in Greece forms a well-supported clade with other Greek endemics such as *Campanula radicata* and *C. tymphaea*. Morphologically also this does not come as a surprise because these species, and in particular the latter one, are remarkably similar to *E. parnassicus* with whom they have been often confused in the past (Tan & Iatrou, 2001). *Edraianthus owerinianus* from Caucasus

also forms a well-supported clade separate from *Edraianthus*. It is found together with the other Caucasian bells such as *Campanula tridentata*, *C. saxifraga*, *C. tridens*, and *C. autraniana* as well as with the widely distributed *C. sibirica* and *C. rapunculoides*. Hence, *E. parnassicus* and *E. owerinianus* should be treated as closely related yet clearly distinct from *Edraianthus* and are perhaps best kept at present in their own monotypic genera, *Halacsyella* and *Muehlbergella*, respectively. In contrast to the previous two cases, the exact position of *Petkovia orphanidea*, a species from S Bulgaria and NE Greece, is not ascertained. Therefore, it cannot be excluded that this species could be in a closer association with *Edraianthus*. However, given the general morphological differences between the two as well as the lack of any evidence for the strong support between them, we also believe that the best taxonomical solution for *Petkovia* is to be kept segregated.

***Edraianthus tenuifolius*-complex.** — This group consists of five species, all of them endemic to the Balkans. Within the complex, two major and strongly supported clades can be distinguished (Figs. 4, 5E). The first contains *E. dalmaticus* and *E. serbicus* while the second places together *E. tenuifolius*, *E. hercegovinus*, and *E. wettsteinii*. Our plastid-based phylogenetic estimate shows high congruence with the delimitation of species and subspecies as well as the relationships among them previously inferred based on morphology.

The close connection between *E. dalmaticus* and *E. serbicus* is not surprising. Even though their distribution (Fig. 5A) and ecology are quite distinct, they are morphologically very similar to each other and were hence always placed closely together in different taxonomic treatments (Fig. 3). *Edraianthus dalmaticus* occurs in the central Dinaric Alps of Croatia (Dalmatia) and Bosnia-Herzegovina (Fig. 5A). This species inhabits frequently flooded karst meadows and is therefore ecologically the most striking grassy bell (Lakušić, 1974). Its closest relative, *E. serbicus*, is a montane to subalpine species, distributed in E Serbia and W Bulgaria (Fig. 5B). *Edraianthus serbicus* inhabits chiefly limestone rocky grounds and rock crevices. Within *E. dalmaticus* Lakušić (1974) distinguished three groups based primarily on their distribution. He proposed three subspecies, *dalmaticus*, *horvaticii*, and *slavnicii*, for populations occurring in eu-Mediterranean karst fields of Dalmatia, and sub-Mediterranean or deeper continental karst of Bosnia-Herzegovina, respectively. We sampled and analyzed two distinct populations of *E. dalmaticus*, from Kupres and Glamoč karst fields (both in Bosnia-Herzegovina), but only one plastid haplotype was recovered. The sampling of *E. serbicus*, with nine populations covering almost the entire range of this species, was more extensive. In this case, two distinct haplotypes were identified. The first one is found

throughout the species range, including the type locality (Mt. Suva Planina, Serbia), and hence it corresponds to the typical subspecies (i.e., *E. serbicus* subsp. *serbicus*). The second haplotype was found at Mt. Stol and surroundings (NE Serbia) in the single sample analyzed from *E. s.* subsp. *stankovicii*, described from Mt. Veliki Krš by Lakušić (1974). *Edraianthus serbicus* subsp. *stankovicii* is relatively rare and there are very few herbarium specimens. Therefore, further, more intensive investigations are necessary in order to establish whether the molecular distinctions observed between these two subspecies are consistent and whether they translate into two morphologically distinct and fully differentiated taxa.

Edraianthus tenuifolius is one of the most widespread species in this genus. It is distributed along the Adriatic coast, including the islands and a narrow inland strip, from Istria (N Croatia) southwards all the way to N Albania (Fig. 5C). It inhabits predominantly rocky grasslands but it also can be encountered, albeit rarely, in limestone crevices and screes. Also, some isolated populations of *E. tenuifolius* are rarely found in high-mountain regions where several subalpine forms and ecotypes have been recorded. Particularly interesting is *E. hercegovinus* found only on the highest peaks of Mt. Čvrsnica in Herzegovina (~2,100 m). In our study, *E. tenuifolius* is represented by 13 populations sampled from throughout the 600 km transect spanning the entire range of this species (Fig. 5). Five distinct haplotypes, labeled A–E in Figs. 4 and 5C, can be recognized. The haplotype labeled as “*tenuifolius* A” (with seven sampled populations) is widespread in southern parts of species range in Herzegovina, S Dalmatia, and Montenegro. The second haplotype, “*tenuifolius* B” with two sampled individuals, is restricted to Mt. Lovćen (Montenegro) where it grows in sympatry with *E. wettsteinii* (see below). Haplotypes labeled as “*tenuifolius* C” and “*tenuifolius* D” represent populations from the extreme southern and northern parts of the range (i.e., the foothills of Mt. Rumija in Montenegro and Tamara-Rapsa in the north-western Albania and Rijeka in Croatia, respectively). The last haplotype (“*tenuifolius* E”) belongs to the single sample from Mt. Biokovo (Croatia). While distinct plastid types within this species with large geographical and ecological amplitude can be clearly distinguished, relationships among them remain unresolved. Additional, faster evolving data will be necessary to approach this problem. Also, part of the *E. tenuifolius* clade is a representative of the sole population attributed to the enigmatic *E. hercegovinus* (Fig. 5C). This species was first described as distinct from *E. tenuifolius* by Malý (1906). Janchen (1910) placed it in synonymy with *E. tenuifolius* indicating that its distinct appearance is most likely due to the extreme environmental conditions of the habitat this population occupies as compared to the rest of populations of *E. tenuifolius*. Malý’s opinion, however, was followed

by Lakušić (1974), whose species definitions were generally more narrow and ecologically grounded, as well as by most of the recent floristic treatments (Kuzmanov, 1976; Greuter & al., 1984). Our data are consistent with both hypotheses and it is only through the future biosystematic studies that these questions will be settled.

The last and the most surprising member of *E. tenuifolius*-complex is *E. wettsteinii*. According to all traditional taxonomic concepts (Fig. 3), this rare species was placed into *E. sect. Uniflori* together with *E. pumilio* and *E. dinaricus* (Janchen, 1910; Mayer & Blečić, 1969; Lakušić, 1974). According to our results, however, it is most closely related to the *E. tenuifolius*/*E. hercegovinus* clade, with strong supports for this unexpected sister-group relationship (Figs. 4, 5E). *Edraianthus wettsteinii* has a very narrow distribution, restricted to the mountains of S Montenegro along the Adriatic coast (Fig. 5D). This species is divided into two geographically, morphologically, and karyologically distinct groups. The typical subspecies occurs on Mts. Sutorman and Rumija in Montenegro (with one population reaching into Albania, above Shkodra), while *E. w.* subsp. *lovcenicus* is restricted in its distribution only to southern slopes of Mt. Lovćen (Fig. 5D). Both subspecies inhabit calcareous rocky outcrops and grasslands and rock crevices in the upper montane zones (1,100–1,300 m), reaching the highest altitudes of ~1,600 m. Compared to *E. w.* subsp. *lovcenicus*, the typical subspecies is characterized morphologically by shorter stems, more compact habit, glabrous leaves, and the inflorescence with only one (rarely few) flower (Mayer & Blečić, 1969). In addition, Međedović (1981) documented karyological differences between two groups involving a series of translocations and secondary constrictions in their chromosomes. Presently, the evidence derived from molecular data lends further support for existence of two clearly distinct yet closely related entities. Within our samples of *E. wettsteinii*, two haplotypes are recovered, one from Mt. Lovćen and the other from Mt. Rumija, corresponding entirely to the traditional taxonomic concepts for this species (Mayer & Blečić, 1969). Taking all of these well-established differences between these two groups, we hypothesize that additional investigations will lend further support for their segregation into two well-differentiated species.

***Edraianthus pumilio*-complex.** — This small clade includes two very closely related species, *E. pumilio* and *E. dinaricus* (Figs. 4, 6C). Each of them is narrowly restricted in distribution to Mt. Biokovo and Mt. Mosor, respectively, the two neighboring mountains found along the mid-Dalmatian coast of Croatia (Fig. 6A). In comparison to the other representatives of *Edraianthus* these two species are quite distinct due to a number of shared morphological features, such as leaf and bract morphology/shape, inflorescence, and habit. The mor-

phological distinctiveness was used as grounds for their segregation, along with *E. wettsteinii*, into sect. *Uniflori*, a group originally proposed by Wettstein (1887) and followed by Janchen (1910) and Lakušić (1974, 1988). However, according to the molecular data, the latter species is found elsewhere on the tree (*E. tenuifolius*-complex; see above), rendering the section, as traditionally defined,

polyphyletic. Međedović (1981) was the first to point out the artificial nature of sect. *Uniflori* based on karyological data. The division of sect. *Uniflori* into two groups based on distinct karyotypes is implicit in his treatment of the section. However, because the study was conducted with the premise of a single origin for the section, the polyphyly of *Uniflori* could not be inferred.

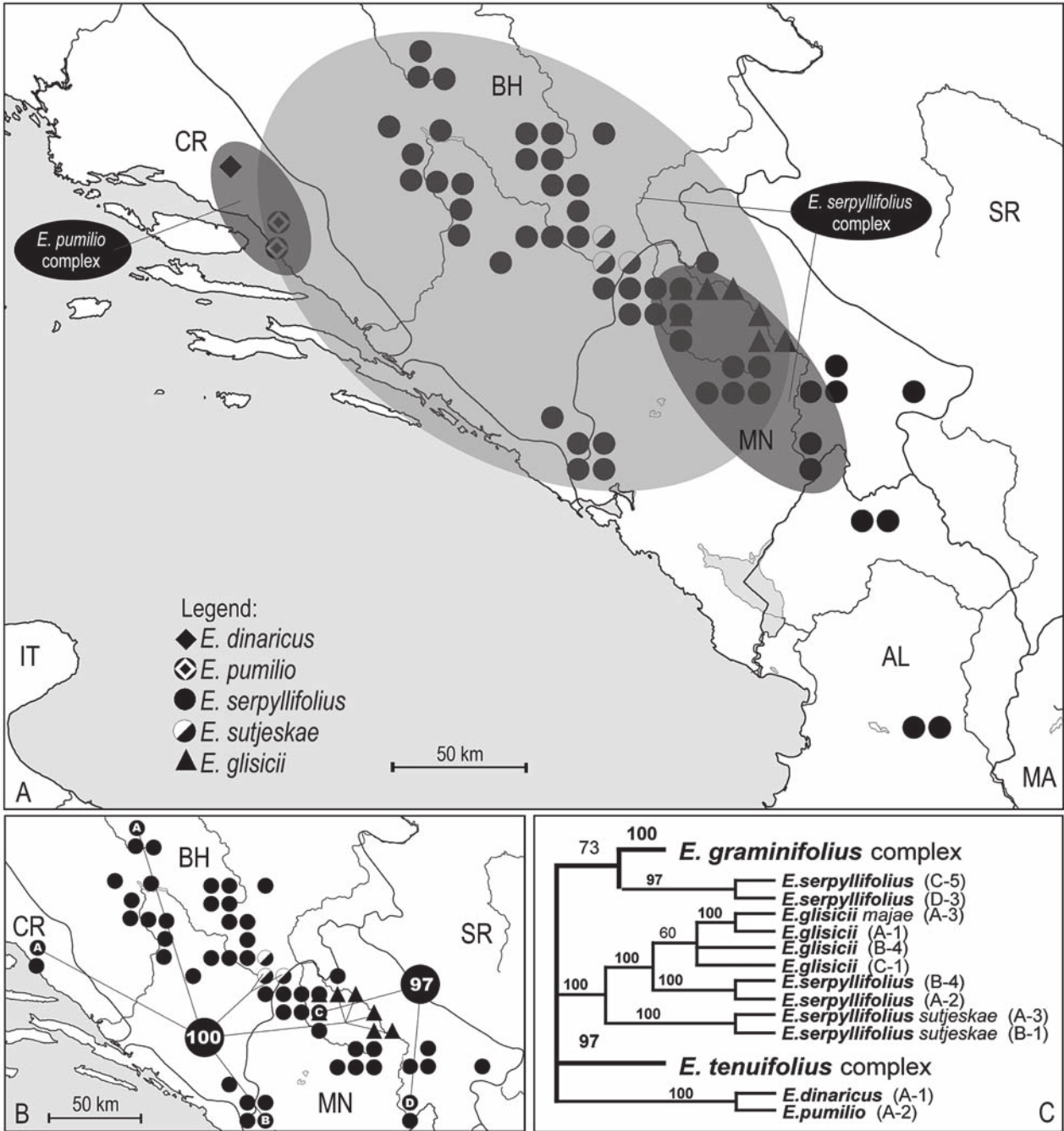


Fig. 6. Geographic distribution and phylogeny of *Edraianthus pumilio*-complex and “*E. serpyllifolius*-complex.” A, an overview of distribution of species belonging to *E. pumilio*-complex and “*E. serpyllifolius*-complex”; B, detailed distribution and sampling of *E. serpyllifolius*; C, phylogenetic relationships among species of *E. pumilio*-complex and “*E. serpyllifolius*-complex”. The UTM grid and two-letter country abbreviations are the same as in Fig. 5.

“*Edraianthus serpyllifolius*-complex”. — According to the molecular data and taking into account our current taxon sampling, this assemblage is found not to be monophyletic (Fig. 4). Nevertheless, we accept it here tentatively as a species complex for two primary reasons: (1) this group, consisting of two distinct clades, corresponds largely to a traditionally recognized taxon, *E. sect. Spathulati* sensu Janchen (1910) or *E. subg. Visiania* sensu Lakušić (1988), and (2) the paraphyly of this group is weakly supported and only one additional step is needed to enforce its monophyly (Table 2).

The bulk of populations belonging to this complex are identified as *E. serpyllifolius* s.l. This species is easily distinguished from other members of the genus by its short and spatulate basal leaves, with rounded leaf tips and blades gradually tapering to the base. Because this morphological feature stands in sharp contrast with long, narrow, grass-like leaves found in other members of *Edraianthus*, this species was singled out into a separate section (*E. sect. Spathulati*). *Edraianthus serpyllifolius* is primarily a high-mountain species distributed from the central Dinaric Alps in Croatia, via Bosnia-Herzegovina and Montenegro, reaching eastward into Albania (Fig. 6A–B). It inhabits mainly the north-facing limestone crevices and cold rocky mountaintops around snow patches, with some populations descending to the numerous river canyons in the area.

Among the 18 samples attributed to *E. serpyllifolius* collected from throughout the range of this species, four molecularly very distinct lineages were recovered, as evidenced by their respective branch lengths as well as relative relationships (Fig. 4). The first of these four lineages corresponds to the typical taxon (Fig. 6B–C). One of its haplotypes, labeled as “*serpyllifolius* A” (with two sampled populations) originates from Mt. Biokovo (Croatia), the type locality from which this species was first described (Visiani, 1829). Its closely related haplotype, “*serpyllifolius* B”, is restricted to Mt. Orjen, situated above Boka Kotorska bay (Montenegro). The second lineage comprises populations belonging to *E. serpyllifolius* “*sutjeskae*”, a taxon proposed by Lakušić (1974) as a separate species (*E. sutjeskae*) but never validly published. This lineage is narrowly endemic and restricted in its distribution only to the Sutjeska canyon and tributaries of this river running through the Maglić-Volujak-Zelengora mountain chain (Bosnia-Herzegovina). Two closely related haplotypes are identified within this lineage (Fig. 6B–C). One of them is distributed along an altitudinal gradient in Mt. Maglić, from the mountain pass Prijedor to the canyon of Sutjeska river, while the other inhabits mostly Vratar, the deepest parts of the same canyon. Besides the typical subspecies, Lakušić (1974) also proposed an additional taxon, *E. sutjeskae* subsp. *maslesae*, to accommodate some of the morphological and phenologi-

cal specificities encountered in mountaintop specimens, but this particular differentiation of populations is not reflected in our plastid-derived data.

The phylogenetic distinctiveness of the remaining two lineages of *E. serpyllifolius*, first identified here, emerges as one of the biggest surprises in this study. In their distribution, these two lineages, labeled in Fig. 6B as “*serpyllifolius* C” and “*serpyllifolius* D”, are known only from the highest peaks of Mt. Durmitor and Mt. Komovi, respectively (both in Montenegro). The latter group, from Mt. Komovi, morphologically and ecologically approaches, to some extent, the concept of a poorly known taxon treated by Lakušić (1974) as *E. serpyllifolius* subsp. *pilosulus*, but the clear correspondence, if any, is yet to be established. When compared to the other members of *Edraianthus*, the extent of this striking differentiation within *E. serpyllifolius* goes well beyond of what can be explained by a simple within-species polymorphism. We hypothesize, therefore, that this degree of distinctiveness is indicative of cryptic speciation, ostensibly driven by isolation of populations on high-mountain islands (Jordal & al., 2004; Pfenninger & Schwenk 2007). In order to further elucidate the relationships within this surprisingly diverse group, additional and more comprehensive work is needed, to include both molecular studies as well as fine-scale morphological and ecological analyses. Also, these findings underscore again the importance of sampling and sequencing multiple individuals/populations per species for detecting genetic differentiation at lower phylogenetic levels (Emerson, 2002).

The only other species found in “*E. serpyllifolius*-complex” is *E. glisicii*, a strict endemic, found only in the canyon of river Tara and its tributaries from Mt. Durmitor region (Montenegro; Fig. 6A–B). This species was not treated by Janchen (1910) because it was described subsequently to his monograph by Černjavski & Soška (1937). Lakušić (1974) placed it originally in *E. sect. Capitati*, but after realizing its closer connections with *E. serpyllifolius* s.l., he placed them together into *E. subg. Visiania* (Lakušić, 1988; Fig. 3). We sampled nine populations belonging to *E. glisicii* and recovered four closely related haplotypes. Three of these haplotypes can be taken as the typical subspecies, while the fourth corresponds closely to the subspecies described by Lakušić (1988) as *E. glisicii* subsp. *majae*.

***Edraianthus graminifolius*-complex.** — The deepest split within this large species complex occurs between two morphologically and geographically very unequal lineages, *E. tarae* and *E. graminifolius* s.l. The sister-group relationship of these two well-differentiated groups, as well as their respective monophyly, is strongly supported by molecular data (Fig. 4). Morphologically, *Edraianthus tarae* (= *Protoedraianthus tarae*) represents a curiosity, not only within the genus but also in *Campanula* s.str. as

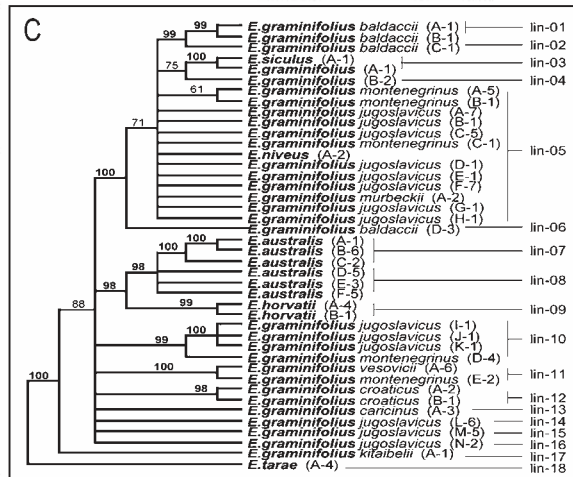
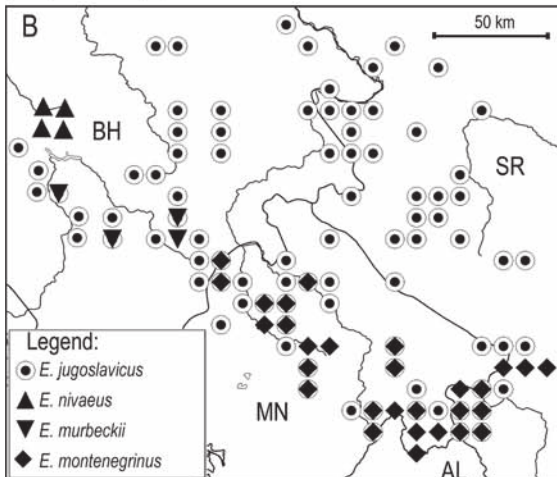
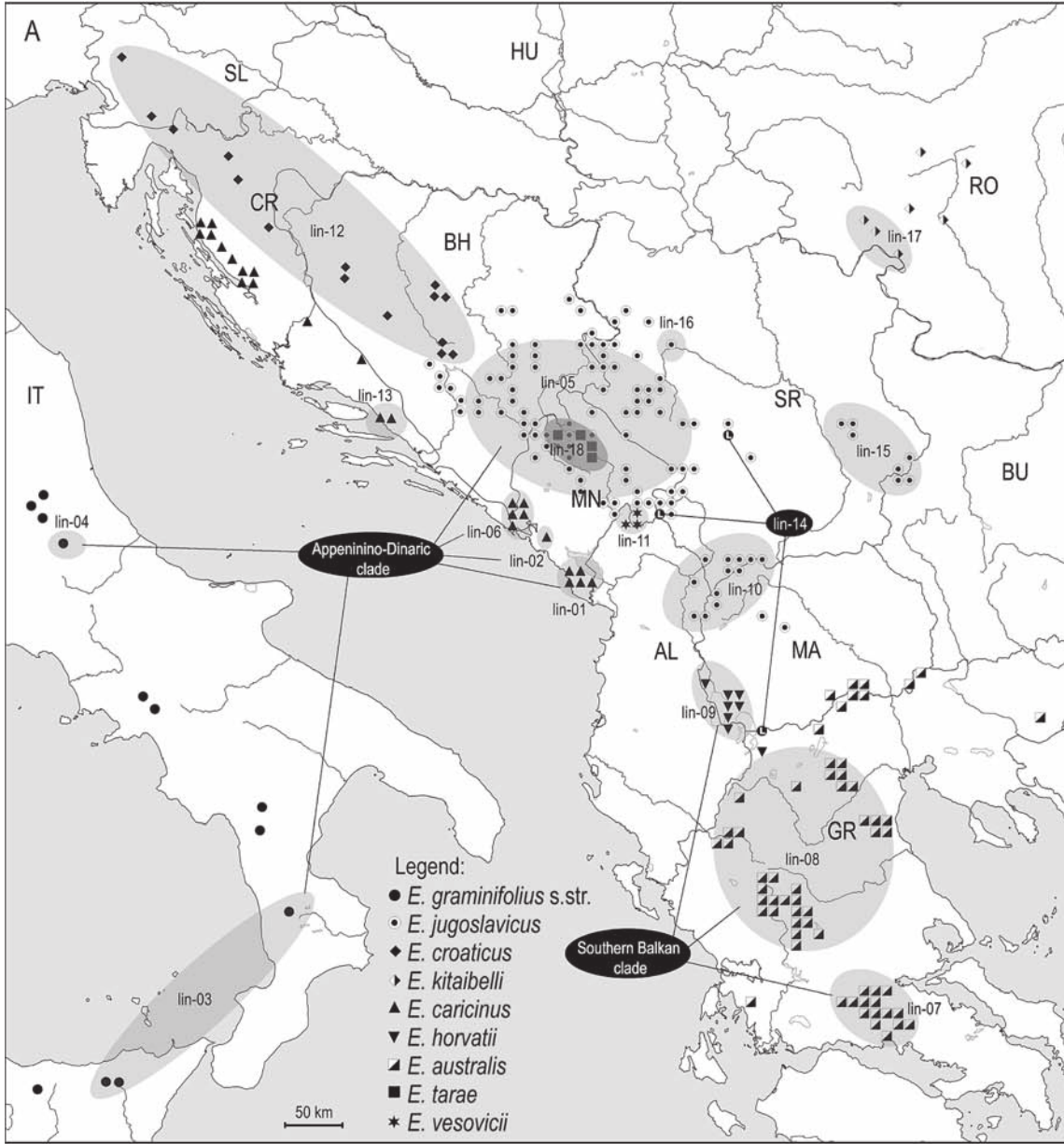
a whole, because of its large, white flowers, found at the tips of long pedicels and arranged in terminal dichasia/polychasia, as well as atypical fruit dehiscence (Fig. 2E–F; for more detail see below). Biogeographically, this steno-endemic species is restricted to the central part of the canyon of river Tara (Montenegro; Fig. 7A), where it inhabits vertical limestone cliffs. By defining its previously unsuspected relationships and pointing out its overall importance for understanding the systematics and biogeography of the genus, our present study provides new impetus to protect this extremely rare species known only from a handful of populations.

In contrast to this, *E. graminifolius* s.l., if circumscribed as a single species, occurs from Italy (Sicily and the Apennines) to the West, to Romania (Carpathian mountains) to the East, to Greece (Pindos mountains) to the South, covering almost completely the entire range of the genus (Figs. 1, 7A). Its populations occur predominantly in various limestone and dolomitic habitats, from rocky ground, to rocks and cliffs, to montane rocky grasslands and screes, to mountaintop snow-patches, spanning elevations from 300 to 2,900 m. Very rarely, some populations also inhabit silicate soils. Morphological variability of this group parallels to a large extent its vast geographic and ecological range. Hence, it does not come as a surprise that this diverse clade is at the same time taxonomically the most controversial group of *Edraianthus*. While the differences between two most influential classifications of *Edraianthus*, that of Janchen (1910) and Lakušić (1974; modified 1987, 1988) seem to be substantial (11 vs. 45 recognized taxa, respectively; compare Fig. 3A and 3B), the bulk of the difference stems from differential treatments of variation observed in *E. graminifolius* aggregate. Janchen (1910) treated this diversity within a single, broadly defined and intergrading species (with two subspecies) while Lakušić (1974) recognized not less than eleven species and a number of subspecies/varieties within this complex. Due to their relatively widespread distribution throughout the central Dinaric Alps (Fig. 7B), accompanied by high levels of localized morphological differentiation, *E. jugoslavicus* and *E. montenegrinus* emerge as especially worth emphasizing among the newly described taxa. Lakušić (1974) viewed these two taxa (and in particular *E. jugoslavicus*) as the morphological and ecological “counterparts” of *E. graminifolius* s.str., restricted to the Apennine peninsula. In addition, the evolutionary importance attributed to *E. jugoslavicus* by Lakušić is evident from its central position in the evolutionary scheme of *Edraianthus*, from which most other species in this genus are presumed to be derived (Lakušić, 1974; his fig. 71).

Based on our extensive current taxonomic sampling for *E. graminifolius* s.l. (> 100 individuals), we recognized 17 lineages and labeled them accordingly in Fig. 7A, C. While certain of these lineages show stronger affinities

toward each other (e.g., clades comprising lineages 1–6 and 7–9 have substantial support), in most cases the overall support is weak or non-existent. As in the above-mentioned case of *E. tenuifolius*, additional and more rapidly evolving data will be necessary to address these issues in detail. Hence, we will focus our discussion at present primarily to the composition and description of those 17 lineages as well as their potential taxonomical implications.

As already mentioned, the first six lineages form a well-supported clade (PP 100%; Fig. 7C). We refer to this clade informally as the “Apennino-Dinaric clade” (Fig. 7A). Altogether, it includes 45 sampled populations and accounts for the largest amount of geographical and morphological diversity encountered within the *E. graminifolius*-complex. Five of its constitutive lineages are further geographically differentiated and have received moderate to strong support (PP 75%–99%), while the sixth lineage is recovered as a large polytomy. Populations growing on the mountains along the Adriatic coast in Montenegro and attributed originally to *E. caricinus* subsp. *baldaccii* by Lakušić (1974) are found in two distinct lineages. Samples from Mts. Lovćen and Rumija are strongly supported as a distinct group (Fig. 7; lineage 1), with differentiation of several haplotypes within it. Three populations from Mt. Orjen are identical to each other (Fig. 7; lineage 6) but do not form a clade with the former lineage. However, due to generally unresolved relationships in this portion of the phylogeny, the closer relationships among these two lineages cannot be excluded at present. The grouping of plants from the Apennine peninsula and Sicily is also relatively well supported molecularly (PP 75%) and is further subdivided into two lineages. The first group (Fig. 7; lineage 3) includes populations from southern Apennines (Mts. Sirino, Basilicata) as well as Sicily (Mt. Scuderi) while the second group (Fig. 7; lineage 4) includes those from central Apennines (Mt. Majella). Taking into account their distribution and taxonomical history, these populations correspond to three subspecies of *E. graminifolius* described by Lakušić (1974) as *E. g.* subsp. *siculus*, subsp. *graminifolius*, and subsp. *apenninus*, respectively, with an overlapping zone between subspecies *graminifolius/siculus* occurring in S Apennines. Undoubtedly, the most diverse and taxonomically most complicated group of the *E. graminifolius*-complex is distributed in the mountains and canyons of C and E Bosnia, W Serbia and N Montenegro (Fig. 7B). From this part of the Balkans 35 populations were sampled for molecular analyses. From those samples we recovered 13 haplotypes but the phylogenetic relationships among them as well as with the other lineages remain unresolved (Fig. 7; lineage 5). Five of those haplotypes belong to high-mountain populations and mainly correspond to taxa *E. niveus* (Mt. Vranica; C Bosnia), *E. graminifolius* “*murbeckii*” (Mts. Prenj and Čvrstica; Herzegovina), and *E. graminifolius* “*montenegrinus*” (Mts. Durmitor and



Komovi; Montenegro). From the biogeographical and ecological point of view, the remaining eight haplotypes correspond to canyon populations included into *E. graminifolius* “*jugoslavicus*”. Five haplotypes are restricted to a single canyon or gorge each. For example, those include individuals from canyon of Derventa river (the right tributary of Drina), individuals from gorge of Rzav-Vranjak rivers, gorge of Uvac river, as well as canyon of Morača. Two haplotypes are recorded from individuals inhabiting two distant localities (gorge of Rzav-Vranjak rivers and canyon of Sutjeska), while one identical haplotype was recovered from individuals found in several microdisjunct localities (e.g., canyons of Derventa, Sutjeska, and Tara rivers, as well as gorge of Sutjeska near Ustibar and near Biogradska Gora). These populations, centrally located with respect to the distribution of the whole genus, were recognized by Lakušić (1974) as a separate species, *E. jugoslavicus*, parallel in its morphological and ecological characteristics to those populations of *E. graminifolius* found on the vertical profile of the Apennines.

The next three lineages (Fig. 7; lineages 7–9) are also consistently recovered as a clade, albeit only with moderate support. Because the populations from this group are found in the southernmost range of *Edraianthus*, covering the area from Macedonia to the Pindos Mts. in Greece, we refer informally to it as the “Southern Balkans clade” (Fig. 7A). These populations correspond to two traditionally recognized taxa, *E. australis* and *E. horvatii* (Lakušić, 1974), whose reciprocal monophyly is strongly supported by molecular data. Within *E. australis* two lineages are distinguished. Populations from the southernmost part of the genus range occurring in Mt. Parnassos, Mt. Giona, and Mt. Vardousia (Greece) constitute one of those lineages (Fig. 7; lineage 7). Despite its restricted distribution, this lineage shows a minimum of three haplotypes, with populations from Mt. Parnassos and Mt. Giona being more closely related to each other than either is to those from Mt. Vardousia. The genetic and geographical distinctiveness of this lineage is accompanied also by some morphological features, such as leaf shape and vestiture, indicative of a potentially new taxon, separate from the second lineage. The second lineage (Fig. 7; lineage 8) consists of *E. australis* populations from central and northern Greek mountains (e.g., Mts. Mazur, Katafidi, Rouista, Trapezitsa, Astraka, Kozuf-Tzena, Dudica, Loutra Arideas, Olimbos, etc.). Among these populations, covering a relatively large area, three haplotypes were recovered as well, but without any particular relation-

ships among them. The nomenclatural and taxonomical status of these two lineages within *E. australis* is further complicated because it is not clear where exactly is the type locality for this species. The third lineage from the “Southern Balkans clade” (Fig. 7; lineage 9) corresponds by its morphology and distribution to *E. horvatii*. This species, proposed by Lakušić (1974) includes populations from mountains in S Macedonia (Mts. Galičica and Jablanica) and N Greece (Mt. Boutsí).

Populations occurring on the Šara-Korab massive, attributed traditionally to *E. graminifolius* “*jugoslavicus*” and “*montenegrinus*”, form the strongly supported lineage 10. This lineage, referred informally to as the “Šara-Korab clade,” consists of two distinct groups, both strongly supported as monophyletic. The first group includes populations from Mts. Paštrik, Ljuboten, and Ošljak, while the second brings together representatives from several closely distributed populations found on the adjacent peaks of the Šara Mountain (Popova Šapka, Turčin, Kobilica). The taxonomical status of populations from this clade is uncertain. In some regards, they could be seen as belonging to *E. albanicus*, a poorly known taxon proposed originally by Degen & Kümmerle (in Jávorka, 1921) as a variety/subspecies of *E. graminifolius* and elevated to the species rank by Lakušić (1974). However, this taxon is known only from few herbarium specimens collected in the mountains of SE Albania and remains insufficiently defined at present.

The populations falling into the next three groups (Fig. 7; lineages 11–13) correspond, with high levels of support, to three distinct and traditionally defined species: *E. vesovicii*, *E. croaticus*, and *E. caricinus*. Lakušić (1974) separated populations of *E. graminifolius* s.l. from the Mt. Prokletije massive, a border region between Montenegro and Albania, into a new species, *E. vesovicii*. Two closely related haplotypes were recovered in our sampling from this region, consisting of five populations from peaks Bjelič and Karanfili (Mt. Prokletije) and two samples from Mt. Visitor (Fig. 7; lineage 11). Despite being characterized as the “weakest of the weak species” within the *E. graminifolius*-complex by Janchen (1910: 25), the populations attributed to *E. croaticus*, a taxon first recognized by Kerner (1872) and adopted by Lakušić (1974), form a strongly supported and distinct clade (Fig. 7; lineage 12). In a well-supported clade, we recovered two very similar haplotypes of *E. croaticus*, from Notranjski Snežnik in SW Slovenia and Mt. Vitorog in W Bosnia, two localities spanning almost the entire range of this species. The type local-

Fig. 7. Geographic distribution and phylogeny of *Edraianthus graminifolius*-complex. A, an overview of distribution of species belonging to *E. graminifolius*-complex; B, detailed distribution of several key morphologically variable species of this complex; C, phylogenetic relationships among species of *E. graminifolius*-complex (distribution of lineages enumerated 1–17 is shown in A). The UTM grid and two-letter country abbreviations are the same as in Fig. 5 (in addition, GR = Greece; HU = Hungary).

ity of *E. caricinus* is in Mt. Biokovo (central Dalmatia). All three of its samples collected on the vertical profile of this mountain (peaks Sv. Jure, Vošac, and Troglav) yielded one identical haplotype. However, this haplotype originating from mountains along the Croatian coast is significantly different than the haplotypes belonging to putatively the same species but distributed on the mountains along the Adriatic coast in Montenegro (Mts. Orjen, Lovćen, Rumija), and already discussed (lineages 1 and 6).

Contrary to the previous three lineages whose distinctiveness was already recognized earlier (as evidenced by their acceptance at the species level by at least some of the monographers), the populations of the next three lineages were never proposed as separate species. One of the most unexpected and potentially the most intriguing such lineage within the *E. graminifolius*-complex is made up from populations found on several disjunct localities dispersed throughout the central Balkans (Fig. 7; lineage 14). An identical haplotype was recovered from two samples collected in central Serbia (Mt. Kopaonik), three samples from SW Serbia (Mt. Prokletije), as well as one sample from S Macedonia (Mt. Pelister). The unifying factor for these relatively distantly distributed populations (Fig. 7A) is edaphic in nature. The vast majority of the populations/species of *Edraianthus* are found on the calcareous soils (limestone bedrocks). However, the populations from lineage 14 occur exclusively on the silicate soils of both the acid and basic (serpentine) types. The importance of unusual soil conditions, such as high salt concentrations, presence of heavy metals, serpentinite outcrops, etc., for the diversification of plant species in general is well established (for a review, see Kruckenberg, 1986), and is well-documented for the Balkan flora in particular (Stefanović & al., 2003). The taxonomical status of populations found within this “siliceous clade” remains uncertain. Further in-depth investigations are needed to determine whether these edaphically differentiated plants represent polymorphism within a single species, an incipient species, or a well-established, albeit morphologically cryptic, species. The other two molecularly clearly distinct yet previously unrecognized lineages are found in E Serbia, growing from the subalpine grasslands on the top of Mt. Suva Planina and surrounding areas to the gorges of Sićevo and Jerma rivers (Fig. 7; lineage 15) as well as in W Serbia, with several populations sampled from the limestone cliffs of Ovčar-Kablar gorge (Fig. 7; lineage 16). Further analyses are also necessary to determine if these groups could represent new species as well.

Finally, as expected, one of the molecularly most distinct lineages of the *E. graminifolius*-complex corresponds to one of its geographically most isolated species, *E. kitaibelii* (Fig. 7; lineage 17). This species, distributed throughout the Transylvanian Alps (S Carpathians) in Romania, occupies the easternmost fringe of the genus range.

Unfortunately, as a placeholder for this entire group, only a single sample was available for our study. Nevertheless, given its distinctiveness, as evidenced by the long branch subtending it (Fig. 4; inset) and its clear separation from the members of the complex distributed in the Balkans, albeit weakly supported, we hypothesize that additional samples will result in a clearly differentiated and potentially quite diversified “Transylvanian clade.”

Fruit dehiscence in *Edraianthus* and its position within Campanulaceae. — In addition to the primary emphasis on the circumscription and relationships of major lineages within *Edraianthus*, our data are also pertinent to the questions of position of this genus within Campanulaceae and the value of the fruit dehiscence character as a major taxonomic and evolutionary feature for the family.

Dehiscence and other capsule characters vary considerably in Campanulaceae and provide the basis for most intrafamilial classification schemes. The most comprehensive treatment of the Campanulaceae remains the monograph of Alphonse de Candolle (1830), who recognized two groups. The first group, Campanuleae, typically include taxa with capsules dehiscing by lateral pores, whereas the second group, Wahlenbergieae usually include taxa with capsules dehiscing by apical valves. This view was adopted, with some modifications, by most subsequent synoptic works on the family (e.g., Schönland, 1889; Kovanda, 1978; Takhtajan, 1987). On the basis of its distinctive mode of capsule dehiscence used as the most important diagnostic feature, almost all monographs of Campanulaceae included *Edraianthus* in the wahlenbergioid suprageneric taxon (De Candolle, 1839; Schönland, 1889; Kovanda, 1978; Takhtajan, 1987).

The contrary opinion, that *Edraianthus* is not part of wahlenbergioid campanulas, was originally suggested by Fedorov (1972). This was further elaborated by Kolakovsky (1986, 1987, 1995) in a series of classical taxonomic treatments based on carpological data. Kolakovsky has studied intensively the internal fruit structure in Campanulaceae and has demonstrated that fruits of many genera, including *Edraianthus*, have a specialized organ involved in the capsule dehiscence. This organ, named axicorn by Kolakovsky (1986), opens a pore on the lateral wall of the fruit in *Campanula* (and other genera in this group), while in *Edraianthus* it irregularly tears apart the membranous top of the capsule. Thus, the capsules in *Edraianthus* and *Campanula* open in different places but the underlying mechanism is the same. An explanation for the observed differences regarding the position of dehiscence between *Edraianthus* and other campanuloids can be found in its correlation with the inflorescence types present in those taxa. *Edraianthus* exhibits a variety of dehiscence modes and in this respect it epitomizes the complexity of the family as a whole. Almost all of its species have sessile inflo-

rescences, tightly subtended by bracts. In these cases, the apical opening of the fruit is observed, ostensibly because this would facilitate seed dispersal more readily than a basal or lateral opening. However, some representatives of *Edraianthus* have pedicellated inflorescences surrounded by less dense bracts. This condition is best illustrated by *E. tarae* (= *Protoedraianthus tarae*). The inflorescences in these plants are dichasia (rarely polychasia) composed of one central (sub-)sessile flower and several lateral flowers found at tips of 1–3 cm long pedicels (Fig. 2E–F). As a consequence of their respective positions, the central fruits open apically, in a fashion similar to the other *Edraianthus* spp., while the pedicellate fruits open laterally, reminiscent of those found in *Campanula*. Taking into account that different states can be found even within a single species, it seems apparent that the fruit dehiscence is a highly homoplastic character, and thus, its value as a major taxonomic and evolutionary feature is compromised. This is an example of analogous structures having the same biological function—facilitation of seed dispersal.

Further indication that the morphology of capsules, and in particular the position of capsule dehiscence, is not of significant diagnostic value for the higher-level family classification in Campanulaceae stems from the phylogenetic position of *Edraianthus* in the family-wide context. Its relationships were first tested in an explicit phylogenetic framework by Eddie & al. (2003). Using the nuclear ribosomal ITS sequences these authors placed *Edraianthus* within *Campanula* s.str. clade with strong internal support. This placement was subsequently confirmed by works of Shulkina & al. (2003) using morphological data and Cosner & al. (2004) using data from ptDNA structural rearrangements. Our results, based on ptDNA sequences derived from a wide taxonomic and geographic sampling, are fully consistent with these previous findings and provide additional independent evidence for position of *Edraianthus* (and other closely allied genera) within the Campanuleae.

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Appendix. Taxa, DNA accession numbers, sources of plant material from which DNA was extracted, and GenBank accession numbers for sequences used in this study. Classification and formal nomenclature for *Edraianthus* follows its treatments in the *Flora Europaea* (Kuzmanov, 1976) and *Med-Checklist* (Greuter & al., 1984). Nomina nuda used in this work are indicated in quotation marks and follow those proposed by Lakušić (1974, 1987, 1988).

Species, haplotype: DNA accession number, voucher information, country of origin, *trnL-F*, *rbcL-atpB* spacer. A dash indicates missing data.

Campanula alpestris All., **A:** 1319, SS-05-167; TRTE, cultivated, EF213141, EF213343. *Campanula betulifolia* K. Koch, **A:** 1251, SS-04-09; TRTE, cultivated, EF213142, EF213344. *Campanula collina* M. Bieb., **A:** 1324, SS-05-185; TRTE, cultivated, EF213143, EF213345. *Campanula dasyantha* M. Bieb., **A:** 1320, SS-05-169; TRTE, cultivated, EF213144, EF213346. *Campanula garganica* Ten., **A:** 1012, SRD-485; IND, cultivated, EF213145, EF213347. *Campanula hawkinsiana* Hausskn. & Heldr. ex Hausskn., **A:** 1250, SS-04-08; TRTE, cultivated, EF213146, EF213348. *Campanula parryi* A. Gray, **A:** 1241, no voucher, cultivated, EF213147, EF213349. *Campanula patula* L., **A:** 1325, SS-05-174; TRTE, Serbia, EF213148, EF213350. *Campanula persicifolia* L., **A:** 1326, SS-05-171; TRTE, Serbia, EF213149, EF213351. *Campanula radicata* Bory & Chaub., **A:** 1432, *Strid & Papanicolaou 15286*; C, Greece, EF213150, EF213352. **B:** 1433, *Baden & al. 969*; C, Greece, EF213151, EF213353. *Campanula rapunculoides* L., **A:** 1323, SS-05-172; TRTE, Serbia, EF213152, EF213354. *Campanula rotundifolia* L., **A:** 1245, SS-01-99; TRTE, cultivated, EF213153, EF213355. *Campanula samarkandensis* J. Halda, **A:** 1258, SS-04-16; TRTE, cultivated, EF213154, EF213356. *Campanula saxifraga* M. Bieb., **A:** 1321, SS-05-166; TRTE, cultivated, EF213155, EF213357. *Campanula seraglio* Kit Tan & Sorger, **A:** 1259, SS-04-17; TRTE, cultivated, EF213156, EF213358. *Campanula sibirica* L., **A:** 1210, no voucher, Serbia, EF213157, EF213359. **B:** 1264, no voucher, cultivated, EF213158, EF213360. *Campanula sparsa* Friv., **A:** 1322, SS-05-175; TRTE, Serbia, EF213159, EF213361. *Campanula tridentata* Schreb., **A:** 1253, SS-04-11; TRTE, cultivated, EF213160, EF213362. *Campanula tymphaea* Hausskn., **A:** 1434, *Strid & al. 30314*; C, Greece, EF213161, EF213363. **B:** 1435, *Hartvig & al. 7246*; C, Greece, EF213162, EF213364. *Campanula waldsteiniana* Roem. & Schult., **A:** 1261, SS-04-19; TRTE, cultivated, EF213163, EF213365. *Campanula witasekiana* Vierh., **A:** 1257, SS-04-15; TRTE, cultivated, EF213164, EF213366. *Edraianthus australis* (Wettstein) R. Lakušić, **A:** 1337, *Baden & Franzén 831*; C, Greece, EF213165, EF213367. **B:** 1339, *Gustavsson 9693*; C, Greece, EF213167, EF213369; 1468, *Gustavsson 762*; C, Greece, EF213174, EF213376; 1469, *Gustavsson 3578*; C, Greece, EF213175, EF213377; 1470, *Gustavsson 993*; C, Greece, EF213176, EF213378; 1472, *Gustavsson 7088*; C, Greece, EF213178, EF213380; 1473, *Gustavsson 3544*; C, Greece, EF213179, EF213381. **C:** 1340, *Hagemann & al. 549*; C, Greece, EF213168, EF213370; 1475, *Gustavsson 1312*; C, Greece, EF213181, EF213383. **D:** 1356, *Hartvig & al. 7999*; C, Greece, EF213172, EF213374; 1535, *Kit Tan 29116*; C, Greece, EF213184, EF213386; 1536, *Kit Tan 29115*; C, Greece, EF213185, EF213387; 1537, *Kit Tan 29127*; C, Greece, EF213186, EF213388; 1471, *Aldén 719*; C, Greece, EF213177, EF213379. **E:** 1338, *Hartvig & al. 5506*, C, Greece, EF213166, EF213368; 1474, *Franzén & al. 166*; C, Greece, EF213180, EF213382; 1534, *Kit Tan 29060*; C, Greece, EF213183, EF213385. **F:** 1341, *Strid & al. 1407*; C 19963; BEOU, Greece, EF213169, EF213371; 1354, *Strid & al. 19029*; G 19966; BEOU, Greece, EF213170, EF213372; 1355, *Strid & Gustavsson 11879*; C, Greece, EF213171, EF213373; 1401, *Tomović & Zlatković 19149*; 20 Jul 2004, Macedonia, EF213173, EF213375; 1532, no voucher, Greece, EF213182, EF213384. *Edraianthus dalmaticus* A.DC., **A:** 1456, *Hilda Ritter s.n.*; SARA, Bosnia-Herzegovina, EF213187, EF213389; 1457, *Hilda Ritter s.n.*; SARA, Bosnia-Herzegovina, EF213188, EF213390. *Edraianthus dinaricus* (Kerner) Wettstein, **A:** 1333, *E. Janchen 3083*; BEOU, Croatia, EF213189, EF213391. *Edraianthus glisicii* Černjav.

Appendix. Continued.

Species, haplotype: DNA accession number, voucher information, country of origin, *trnL-F*, *rbcL-atpB* spacer. A dash indicates missing data.

& Soška, **A**: 1199, *Lakušić 447/92*; BEOU, Montenegro, EF213190, EF213392; **B**: 1225, *Stevanović & Jovanović 775/89*; BEOU, Montenegro, EF213191, EF213393; 1237, *Blecic K24256*; BEO, Montenegro, EF213192, EF213394; 1238, *Rajevski K24257*; BEO, Montenegro, EF213193, EF213395; 1396, *Lakušić & al. 703/96*; BEOU, Montenegro, EF213195, EF213397; **C**: 1395, *Lakušić & Bulić 437/94*; BEOU, Montenegro, EF213194, EF213396. *Edraianthus glisicii* Cernjav. & Soska “*majae*”, **A**: 1183, *Lakušić & al. 994/96*; BEOU, Montenegro, EF213196, EF213398; 1224, *Jovanović 2000/90*; BEOU, Montenegro, EF213197, EF213399; 1519, *Stevanović & Lakušić 20956*; BEOU, Montenegro, EF213198, EF213400. *Edraianthus graminifolius* A.DC., **A**: 1441, *Conti & al. 20624*; BEOU, Italy, EF213201, EF213403; **B**: 1192, *Lakušić & Conti 9631*; BEOU, Italy, EF213199, EF213401; 1193, *Lakušić & Conti 9637*; BEOU, Italy, EF213200, EF213402. *Edraianthus graminifolius* A.DC. “*baldaccii*”, **A**: 1522, *Stevanović 21841*; BEOU, Montenegro, EF213206, EF213408; **B**: 1495, *Međedović & Šiljak-Yakovlev s.n.*; SARA, Montenegro, EF213204, EF213406; **C**: 1530, *Stevanović & Lakušić 20950*; BEOU, Montenegro, EF213207, EF213409; **D**: 1190, *Stevanović 1018/89*; BEOU, Montenegro, EF213202, EF213404; 1218, *Stevanović s.n.*; BEO, Montenegro, EF213203, EF213405; 1521, *Stevanović & Lakušić 20944*, BEOU; 18 Jul 2006, Montenegro, EF213205, EF213407. *Edraianthus graminifolius* A.DC. “*caricinus*”, **A**: 1491, *Šolčić & Šiljak-Yakovlev s.n.*; MAKAR, Croatia, EF213208, EF213410; 1492, *Šolčić & Šiljak-Yakovlev s.n.*; MAKAR, Croatia, EF213209, EF213411; 1493, *Šolčić & Šiljak-Yakovlev s.n.*; MAKAR, Croatia, EF213210, EF213412. *Edraianthus graminifolius* A.DC. “*croaticus*”, **A**: 1343, *Franz Faltis 20007*; BEOU, Bosnia-Herzegovina, EF213211, EF213413; 1388, *Tomović 14169*; BEOU, Bosnia-Herzegovina, EF213212, EF213415; **B**: 1345, *Paulin 20010*; BEOU, Slovenia, –, EF213414. *Edraianthus graminifolius* A.DC. “*jugoslavicus*”, **A**: 1196, *Stevanović & al. 2840/91*; BEOU, Bosnia-Herzegovina, EF213215, EF213418; 1308, *Stevanović s.n.*; BEOU, Montenegro, EF213222, EF213425; 1312, *Stevanović s.n.*; BEOU, Serbia, EF213225, EF213428; 1387, *Tomović 15597*; BEOU, Montenegro, EF213235, EF213438; 1428, *Ivančević 2941/90*; BEOU, Montenegro, EF213242, EF213445; 1480, *Lakušić 20897*; BEOU, Montenegro, EF213244, EF213447; 1527, *Stevanović & Lakušić 20959*; BEOU, Serbia, EF213252, EF213455; **B**: 1311, *Stevanović s.n.*; BEOU, Serbia, EF213224, EF213427; **C**: 1217, *Stevanović & al. 15965*; BEOU, Serbia, EF213217, EF213420; 1313, *Stevanović s.n.*; BEOU, Bosnia-Herzegovina, EF213226, EF213429; 1330, SS-05-214; TRTE, Bosnia-Herzegovina, EF213227, EF213430; 1399, *Stevanović 19956*; BEOU, Bosnia-Herzegovina, EF213238, EF213441; 1498, *Međedović & Šiljak-Yakovlev s.n.*; SARA, Bosnia-Herzegovina, EF213249, EF213452; **D**: 1485, *Lakušić 20894*; BEOU, Serbia, EF213247, EF213450; **E**: 1349, *Stevanović & al. 703/90*; BEOU, Montenegro, EF213228, EF213431; **F**: 1189, no voucher, Serbia, EF213213, EF213416; 1216, *Stevanović & al. 645/90*; BEOU, Serbia, EF213216, EF213419; 1234, *Jurisić K246206*; BEO, Serbia, EF213220, EF213423; 1310, *Stevanović s.n.*; BEOU, Serbia, EF213223, EF213426; 1377, *Jovanović & Lakušić 137/92*; BEOU, Serbia, EF213231, EF213434; 1378, *Jovanović & Lakušić 2212/90*; BEOU, Serbia, EF213232, EF213435; 1499, *Međedović & Šiljak-Yakovlev s.n.*; SARA, Bosnia-Herzegovina, EF213250, EF213453; **G**: 1372, *Stevanović & al. 5158*; BEOU, Serbia, EF213230, EF213433; **H**: 1429, *Jovanović & al. 12597*; BEOU, Serbia, EF213243, EF213446; **I**: 1362, *Džukić & Milivojević 1129*; BEOU, Serbia, EF213229, EF213432; **J**: 1385, *Tomović 5640*; BEOU, Macedonia, EF213233, EF213436; **K**: 1194, *Nikolić & al. s.n.*; BEO, Serbia, EF213214, EF213417; **L**: 1231, *Nikolić & al. s.n.*; 17 Jul 1973; BEO, Serbia, EF213218, EF213421; 1239, *Nikolić & Diklić s.n.*; BEO, Serbia, EF213221, EF213424; 1386, *Niketić & Tomović 18928*; BEOU, Serbia, EF213234, EF213437; 1390, *Niketić & al. 19919*; BEOU, Serbia, EF213236, EF213439; 1400, *Džukić 20003*; BEOU, Macedonia, EF213239, EF213442; 1409, *Lakušić & al. 18039*; BEOU, Montenegro, EF213240, EF213443; **M**: 1232, *Nikolić & al. s.n.*; 18 Jun 1973; BEO, Serbia, EF213219, EF213422; 1391, *Tomović 19812.2*; BEOU, Serbia, EF213237, EF213440; 1486, *Lakušić 20893*; BEOU, Serbia, EF213248, EF213451; 1483, *Stevanović & Tomašević 20934*; BEOU, Serbia, EF213245, EF213448; 1484, *Stevanović & al. 20905*; BEOU, Serbia, EF213246, EF213449; **N**: 1423, *Niketić 19958*; BEOU, Serbia, EF213241, EF213444; 1517, *Stevanović & Lakušić 20960*; BEOU, Serbia, EF213251, EF213454. *Edraianthus graminifolius* A.DC. “*kitabelii*”, 1327, *Ciocarlan 3450*; BUAG, Romania, EF213253, EF213456. *Edraianthus graminifolius* A.DC. “*montenegrinus*”, **A**: 1185, *Lakušić 2161/96*; BEOU, Montenegro, EF213254, EF213457; 1235, *Jovanović 1063/92*; BEOU, Montenegro, EF213259, EF213462; 1236, *Lakušić 216/92*; BEOU, Montenegro, EF213260, EF213463; 1403, *Stevanović & Lakušić 19954*; BEOU, Montenegro, EF213261, EF213464; 1478, *Lakušić 20898*; BEOU, Montenegro, EF213265, EF213468; **B**: 1191, *Lakušić & Tomović 6013*; BEOU, Montenegro, EF213255, EF213458; **C**: 1518, *Stevanović & Lakušić 20954*; BEOU, Montenegro, EF213266, EF213469; **D**: 1230, *Nikolić & al. s.n.*; BEO, Macedonia, EF213258, EF213461; 1444, *Durkai 20630*; BEOU, Serbia, EF213262, EF213465; 1445, *Durkai 20631*; BEOU, Serbia, EF213263, EF213466; 1446, *Durkai 20632*; BEOU, Serbia, EF213264, EF213467; **E**: 1222, *Lakušić s.n.*; BEOU, Montenegro, EF213256, EF213459; 1223, *Lakušić s.n.*; BEOU, Montenegro, EF213257, EF213460. *Edraianthus graminifolius* A.DC. “*murbeckii*”, **A**: 1347, *Bucalović 20012*; BEOU, Bosnia-Herzegovina, EF213267, EF213470; 1496, *Međedović & Šiljak-Yakovlev s.n.*; SARA, Bosnia-Herzegovina, EF213268, EF213471. *Edraianthus graminifolius* A.DC. “*vesovicii*”, **A**: 1188, *Lakušić & Niketić 275/94*; BEOU, Montenegro, EF213269, EF213472; 1211, *Lakušić s.n.*; BEOU, Montenegro, EF213270, EF213473; 1221, *Lakušić s.n.*; BEOU, Montenegro, EF213271, EF213474; 1392, *Stevanović & al. 836/95*; BEOU, Montenegro, EF213272, EF213475; 1393, *Lakušić & Niketić 302/94*; BEOU, Montenegro, EF213273, EF213476; 1524, *Duraki 21837*; BEOU, Montenegro, EF213274, EF213477. *Edraianthus hercegovinus* Maly, **A**: 1459, *Santarius s.n.*; SARA, Bosnia-Herzegovina, –, EF213478. *Edraianthus horvatii* R. Lakušić, **A**: 1186, *Lakušić 2067/91*; BEOU, Macedonia, EF213275, EF213479; 1219, *Nikolić & al. s.n.*; BEO, Macedonia, EF213276, EF213480; 1352, *Strid & al. 18754*; G 19964; BEOU, Greece, EF213277, EF213481; 1514, *Lakušić 21838*; BEOU, Macedonia, EF213278, EF213482; **B**: 1515, *Tomović & Niketić 20963*; BEOU, Macedonia, EF213279, EF213483. *Edraianthus niveus* Beck, **A**: 1453, *Šilić s.n.*; SARA, Bosnia-Herzegovina, EF213280, EF213484; 1454, *Šilić s.n.*; SARA, Bosnia-Herzegovina, EF213281, EF213485. *Edraianthus owerinianus* Boiss., **A**: 1452, *Alexeenko s.n.*; LE, Russia, EF213282, –; **B**: 1477, *Radzki s.n.*; LENUD, Russia, EF213283, EF213486. *Edraianthus parnassicus* (Boiss. & Spruner) Halácsy, **A**: 1334, *Hörandl & al. 7584*; C, Greece, EF213284, EF213487; 1335, *Gustavsson 9536*; C, Greece, EF213285, EF213488; 1336, *Gustavsson 9862*; C, Greece, EF213286, EF213489; 1476, G.1319; C, Greece, EF213287, EF213490. *Edraianthus pumilio* A.DC.,

Appendix. Continued.

Species, haplotype: DNA accession number, voucher information, country of origin, *trnL-F*, *rbcL-atpB* spacer. A dash indicates missing data.

A: 1255, SS-04-13; TRTE, cultivated, EF213288, EF213491; 1488, Šolić & Šiljak-Yakovlev *s.n.*; MAKAR, Croatia, EF213289, EF213492. *Edraianthus serbicus* Petrovic, **A:** 1182, Vukojčić & Tomović 6685; BEOU, Serbia, EF213290, EF213493; 1197, Stevanović *s.n.*; BEOU, Serbia, EF213291, EF213494; 1228, Stevanović 10379; BEOU, Serbia, EF213292, EF213495; 1359, Petrova 19983; BEOU, Bulgaria, EF213293, EF213496; 1361, Tomović 19812.1; BEOU, Serbia, EF213294, EF213497; 1447, Niketić & Tomović 16458; BEOU, Serbia, EF213295, EF213498; 1481, Jusković 20669; BEOU, Serbia, EF213296, EF213499; 1482, Tomović & Zlatković 20889; BEOU, Serbia, EF213297, EF213500. *Edraianthus serbicus* Petrović “*stankovicii*”, **A:** 1213, Benić 1545/91; BEOU, Serbia, EF213298, EF213501. *Edraianthus serpyllifolius* (Vis.) A.DC., **A:** 1489, Šolić & Šiljak-Yakovlev *s.n.*; MAKAR, Croatia, EF213308, EF213511; 1497, Međedović & Šiljak-Yakovlev *s.n.*; SARA, Bosnia-Herzegovina, EF213309, EF213512; **B:** 1240, Stevanović *s.n.*; BEO, Montenegro, EF213303, EF213506; 1448, Niketić & Tomović 17646; BEOU, Montenegro, EF213306, EF213509; 1449, Niketić & Tomović 17611; BEOU, Montenegro, EF213307, EF213510; 1523, Stevanović & Lakušić 20945; BEOU, Montenegro, EF213311, EF213514; **C:** 1187, Lakušić & Tomović 6025; BEOU, Montenegro, EF213299, EF213502; 1198, Lakušić & Tomović 6104; BEOU, Montenegro, EF213300, EF213503; 1229, Stevanović & Jovanović 1168/89; BEOU, Montenegro, EF213301, EF213504; 1364, Lakušić & Conti 909/96; BEOU, Montenegro, EF213304, EF213507; 1233, Stevanović 496/92; BEOU, Montenegro, EF213302, EF213505; **D:** 1365, Stevanović & al. 2765/91; BEOU, Montenegro, EF213305, EF213508; 1513, Stevanović & Lakušić 20953; BEOU, Montenegro, EF213310, EF213513; 1529, Stevanović & Lakušić 20953b; BEOU, Montenegro, EF213312, EF213515. *Edraianthus serpyllifolius* (Vis.) A.DC. “*sutjeskae*”, **A:** 1329, SS-05-213; TRTE, Bosnia-Herzegovina, EF213313, EF213516; 1331, SS-05-215; TRTE, Bosnia-Herzegovina, EF213314, EF213517; 1528, Stevanović & Lakušić 20938; BEOU, Bosnia-Herzegovina, EF213316, EF213519; **B:** 1397A, Stevanović 19955; BEOU, Bosnia-Herzegovina, EF213315, EF213518. *Edraianthus siculus* Strobl, **A:** 1351, Raimondo & al. (III Iter Mediterraneum 2055); BEOU, Italy, EF213317, EF213520. *Edraianthus tarae* R. Lakušić, **A:** 1309, Stevanović *s.n.*; BEOU, Montenegro, EF213318, EF213521; 1418, Stevanović 19951; BEOU, Montenegro, EF213319, EF213522; 1419, Stevanović & Lakušić 19953; BEOU, Montenegro, EF213320, EF213523; 1487, Lakušić 20896; BEOU, Montenegro, EF213321, EF213524. *Edraianthus tenuifolius* A.DC., **A:** 1184, Stevanović & Jovanović 489/90; BEOU, Croatia, EF213322, EF213525; 1195, Lakušić & al. 443/89; BEOU, Montenegro, EF213323, EF213526; 1226, Lakušić & al. 456/89; BEOU, Montenegro, EF213324, EF213527; 1227, Grebenscikov & Janković K24205; BEO, Montenegro, EF213325, EF213528; 1398A, Stevanović & Lakušić 19957.1; BEOU, Montenegro, EF213326, EF213529; 1416, Stevanović & Lakušić 19949; BEOU, Montenegro, EF213328, EF213531; 1465, Ritter *s.n.*; SARA, Bosnia-Herzegovina, EF213329, EF213532; **B:** 1520, Stevanović & Lakušić 20947; BEOU, Montenegro, EF213333, EF213536; 1525, Stevanović & Lakušić 20948; BEOU, Montenegro, EF213334, EF213537; **C:** 1411, Lakušić & al. 17812; BEOU, Albania, EF213327, EF213530; 1516, Stevanović & Lakušić 20951; BEOU, Montenegro, EF213332, EF213535; **D:** 1479, Lakušić 20657; BEOU, Croatia, EF213330, EF213533; **E:** 1490, Šolić & Šiljak-Yakovlev *s.n.*; MAKAR, Croatia, EF213331, EF213534. *Edraianthus wettsteinii* Halácsy & Baldacci ex Halácsy, **A:** 1494, Međedović & Šiljak-Yakovlev *s.n.*; SARA, Montenegro, EF213335, EF213538. *Edraianthus wettsteinii* Halácsy & Baldacci ex Halácsy “*lovcenticus*”, **A:** 1328, SS-05-212; TRTE, Montenegro, EF213336, EF213539; 1363, Bulić 20094; BEOU, Montenegro, EF213337, EF213540; 1512, Stevanović & Lakušić 20946; BEOU, Montenegro, EF213338, EF213541. *Petkovia orphanidea* (Boiss.) Stef., **A:** 1332, Pashaliev SOM151369 19982; SOM, Bulgaria, EF213339, EF213542; **B:** 1368, Papanicolaou 134; C, Greece, EF213340, EF213543; 1369, Franzen & al. 18623; C, Greece, EF213341, EF213544. *Symphandra sp.*, **A:** 1254, SS-04-12; TRTE, cultivated, EF213342, EF213545.