Molecular phylogeny of the *Campanula pyramidalis* species complex (Campanulaceae) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications

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Abstract　The *Campanula pyramidalis* complex is a group of closely related taxa with a distribution across the Balkans, from the Gulf of Trieste in the north to the Peloponnese Peninsula in the south, with small disjunct parts of the range in the south Apennines. Although 21 taxa were described within this complex, only three, *C. pyramidalis*, *C. versicolor*, and *C. secundiflora*, have been generally accepted in recent synoptical taxonomic treatments. Our molecular phylogenetic analyses based on sequences of three non-coding chloroplast regions (*psbA-trnH*, *psbZ-trnfM*, *trnG-trnS*) as well as of nuclear ribosomal internal transcribed spacers (nrITS), lend strong support to the recognition of several lineages which only partially correspond to generally accepted taxonomic concepts. Molecular data presented in this study showed that *C. pyramidalis* is a polyphyletic assemblage that segregates into three distinct lineages, one of which is described here as a new species, *C. austroadriatica* sp. nov. The lectotype of *C. pyramidalis*, redefined in a strict sense, is designated. Neither *C. versicolor* nor *C. secundiflora* were found to be strictly monophyletic, but their monophyly could not be rejected. Morphological and biogeographical implications are discussed.

■ INTRODUCTION

The Balkan Peninsula is floristically one of the most diverse parts of Europe (Turrill, 1929; Polunin, 1997; Stevanović, 2009). At the same time, the Balkan Peninsula is known as a major glacial refugium for temperate plant species, a region which was a source area for post-glacial northward range expansion (e.g., Taberlet & al., 1991; Ibrahim & al., 1996; Comes & Kadereit, 1998; Hewitt, 1999, 2000; Hampe & al., 2003; Petit & al., 2003; Eastwood, 2004; Tzedakis, 2004; Médail & Diadema, 2009; Surina & al., 2011). Nevertheless, detailed phylogeographic and molecular systematic studies in the Balkans are still rare compared to those of comparable European refugial regions, and the sampling is usually relatively sparse. Exceptions include studies on *Cardamine* L. (Brassicaceae; Perný & al., 2005; Lakušić & al., 2006; Kučera & al., 2008, 2010), *Heliosperma* (Rchb.) Rchb. (Caryophyllaceae; Frajman & Oxelman, 2007), isophyllous *Campanula* L. species (Campanulaceae; Kovačić, 2006; Park & al., 2006), *Edraianthus* DC. (Campanulaceae; Stefanović & al., 2008; Lakušić

& al., 2009; Surina & al., 2009, 2011), *Asyneuma* Griseb. & Schenk. (Campanulaceae; Frajman & Schneeweiss, 2009; Stefanović & Lakušić, 2009), *Androsace* L. sect. *Aretia* (L.) W.D.J. Koch (Primulaceae; Schönswetter & Schneeweiss, 2009), *Onosma* L. (Boraginaceae; Kolarčik & al., 2010) and *Limonium* Mill. (Plumbaginaceae; Bogdanović, 2009). As evident from these studies, molecular data, when available, contribute significantly to a better understanding of relationships of the Balkans flora and to a sounder assessment of species diversity in groups with poorly known taxa.

Several of those underinvestigated groups of species belong to *Campanula* L. ("bellflowers") lineages endemic to the Balkans. With more than 400 species distributed mainly in extra-tropical areas of the Northern Hemisphere, this genus is the largest within Campanulaceae (Meusel & Jäger, 1992; Shulkina & al., 2003). The Mediterranean region, where ca. 250 species occur, represents a major center of diversity of *Campanula* (Geslot, 1984), and there are at least 85 taxa described from the circum-Adriatic and west Balkans regions (Kovačić, 2004).

During the last two centuries, many authors tried to develop a workable classification of this large genus (e.g., Candolle, 1830; Boissier, 1875; Nyman, 1878–1882; Janchen, 1958; Gadella, 1964, 1966a, b; Contandriopoulos, 1984; Kolakovsky, 1994) but none of these systems seemed to be successfully predictive of phylogenetic relationships (Park & al., 2006). Such classifications were based on morphological characters partly refined by karyological data. However, the evolution of morphological and karyological characters, and possible problems with rampant homoplasy for both data types, are poorly understood in the Campanulaceae as a whole. Across the family, chromosome numbers (n) range from $n = 6$ to $n = 17$, with the latter being the most common (Lammers, 1992). Chromosome counts are available for 238 out of ca. 400 species of *Campanula*, and $n = 17$ has been found in 72% of the investigated cases (Lammers, 2007). For the species from the Balkans and circum-Adriatic regions, data are available only for isophyllous (sensu Damboldt, 1965) and isophylloid (sensu Eddie & al., 2003; Park & al., 2006) taxa, and they have all been found to be diploids, with $2n = 34$ (see Kovačić, 2004). More detailed cytological examinations revealed significant similarities in chromosome number and morphology between Central and East European bellflowers (Böcher, 1960; Merxmüller & Damboldt, 1962; Gadella, 1964; Podlech & Damboldt, 1964; Damboldt, 1965, 1968; Phitos & Kamari, 1988). The chromosome number for *Campanula pyramidalis* L. ($2n = 32$) was first provided by Marchal (1920), confirmed later by Sugiura (1942) and Gadella (1964), and for *C. versicolor* Sibth. & Sm. ($2n = 32$) by Contandriopoulos (1966), confirmed by Damboldt in Damboldt & Phitos (1971). No chromosome counts are currently available for *C. secundiflora* Vis. & Pančić.

Early molecular phylogenetic approaches revealed that although Campanulaceae as a family are monophyletic, the genus *Campanula* is polyphyletic (Cosner & al., 1994; Eddie & al., 2003). Subsequently, a number of contributions focusing primarily on regional or taxonomic subgroups appeared (e.g., Cosner & al., 2004; Cellinese & al., 2009; Haberle & al., 2009; Roquet & al., 2009; Prebble & al., 2011, 2012; Olesen & al., 2012; Zhou & al., 2012). Several molecular studies focused on a larger sample of *Campanula* species (e.g., Roquet & al., 2008; Wendling & al., 2011), including investigations conducted on the Balkans and on circum-Adriatic endemic isophyllous and isophylloid *Campanula* species (Kovačić & al., 2004; Liber & al., 2004, 2005, 2008; Park & al., 2006; Frajman & Schneeweiss, 2009; Stefanović & Lakušić, 2009).

Due to the large number and wide geographical distribution of taxa, an overall phylogenetic framework for *Campanula* has not yet been completed (Eddie & Kovačić, 2010). Despite these general problems, some smaller groups within *Campanula* are well-defined molecularly, morphologically, karyologically, and biogeographically (Carlström, 1986; Kovanda & Ančev, 1989; Frizzi & Tammaro, 1991; Runemark & Phitos, 1996; Eddie & Ingrouille, 1999; Oganesian, 2001; Eddie & al., 2003; Sáez & Aldasoro, 2003; Shulkina & al., 2003; Cosner & al., 2004; Akçiçek & al., 2005; Nikolov, 2005; Kovačić & Nikolić, 2006; Aghabeigi & Assadi, 2008; Cano-Maqueda & Talavera, 2011; Cupido & al., 2011), suggesting that they may be monophyletic. The *C. pyramidalis* complex is one such group. It comprises

Balkans species characterized by isophylly, long petiolate basal leaves with kidney-shaped to cordate blades, distinctly petiolate cauline leaves with cordate to ovate blades, ascending or erect stems (up to 300 cm), calyces without appendages between the lobes, lanceolate, triangular or oblong to ovate teeth, erect trilocular capsules with basal-median valves or pores, and orbicular to ovate seeds with a reticulate testa (Damboldt, 1965; Fedorov & Kovanda, 1976).

The *C. pyramidalis* complex, also informally referred to as the "Pyramidalis" aggregate (Geslot in Greuter & al., 1984) or subsection *Pyramidalis* (Kolakovsky, 1992), is a group that includes three closely related, morphologically polymorphic, and generally accepted species: *C. pyramidalis*, *C. versicolor*, and *C. secundiflora* (Fedorov & Kovanda, 1976; Greuter & al., 1984). Recently, the monophyly of the *C. pyramidalis* complex received strong support from molecular phylogenetic and phylogeographic studies (Park & al., 2006; Liber & al., 2008). The distribution of this complex is centered mostly in the Balkans, from the Gulf of Trieste in the north to the Peloponnese Peninsula in the south, and to Mt. Konjevska planina in Bulgaria in the east, while some small disjunct parts of the range lie in the southern Apennines (Fig. 1). According to our previous investigations, among the "isophylloids" (Kovačić & Nikolić, 2006; Park & al., 2006; Liber & al., 2008), the *C. pyramidalis* complex is most closely related to the *C. waldsteiniana* aggregate, which consists of only two species, the subendemic *C. waldsteiniana* Roem. & Schult. (Mt. Velebit, Croatia and adjacent areas) and the stenoendemic *C. tommasiniana* Koch ex F.W. Schultz (Istria, Croatia).

In order to capture the morphological diversity observed in this group, up to 17 taxa at specific and intraspecific levels as well as four hybrids were described over the years in the broadly circumscribed *C. pyramidalis* complex (Lammers, 2007). However, their taxonomic and geographical distinctiveness was neither clear nor generally accepted, to the point that the modern floristic literature does not recognize any of these taxa. Instead, they are either "lumped", i.e., reduced to synonymy with one of three accepted species, or are simply ignored.

The most prominent and morphologically diverse species of this complex, *C. pyramidalis*, is also one of the oldest documented horticultural species in cultivation, much older than renaissance gardens (Parkinson, 1629; Pignatti, 1982). Today, it is naturalized in England ("Chimney bellflower"), the Channel Islands (Guernsey), France, and northern Italy (Lammers, 2007; Pignatti, 1982; Greuter & al., 1984), and is well established in horticulture worldwide (Crook, 1951; Lewis & Lynch, 1998). Despite this, relationships of multiple intraspecific taxa (i.e., subspecies, varieties, forms) proposed within this broadly defined species in the wild are poorly known. The most comprehensive research ever performed on *C. pyramidalis* in a broad sense was carried out by Zimmer (1982a, b, c, 1983a, b). Relationships among the populations and "main species" of the *C. pyramidalis* complex—*C. pyramidalis*, *C. versicolor*, and *C. secundiflora*—have never been investigated thoroughly.

The main goals of our present study are: (1) to infer a molecular phylogenetic hypothesis for relationships among populations of *C. pyramidalis*, *C. versicolor* and *C. secundiflora*,

and (2) to find if there is any support from molecular data for the currently recognized or previously described taxa of this complex. To address these questions, a comprehensive dataset was constructed containing representatives from across the entire morphological and geographical range of these species (Fig. 1). We here present the results of molecular phylogenetic analyses based on sequences from three non-coding chloroplast regions (*psbA-trnH*, *psbZ-trnfM*, *trnG-trnS*) as well as from nuclear ribosomal internal transcribed spacers (nrITS).

■ MATERIALS AND METHODS

Taxon sampling. — A total of 96 specimens representing the ingroup members of the *C. pyramidalis* species complex were sampled in this "top-down" study. A complete list, along with voucher information, DNA extraction numbers,

and approximate locality of sampled populations is provided in Appendix 1. These accessions represent the entire geographic range of this group (Fig. 1) and all three species that have been generally accepted in recent taxonomic treatments: *C. pyramidalis*, *C. secundiflora*, and *C. versicolor*. The names initially applied to these accessions follow *Flora Europaea* (Fedorov & Kovanda, 1976) and *Med-Checklist* (Greuter & al., 1984). Our sample includes material unambiguously corresponding to 9 of 18 additional taxa proposed in the past but not recognized by modern floristic literature. These are *C. kapelae* Topić & Ilijanić, *C. plasonii* Formánek, *C. tenorii* Moretti, *C. mrkvickana* Velen., *C. versicolor* var. *tomentella* Hal., *C. versicolor* var. *thessala* Boiss., *C. versicolor* f. *matkae* Nikolov, *C. secundiflora* subsp. *limensis* R. Lakušić, and *C. secundiflora* subsp. *montenegrina* R. Lakušić. These taxa have either been completely neglected or reduced to synonyms of one of three broadly accepted species (Appendix 1).
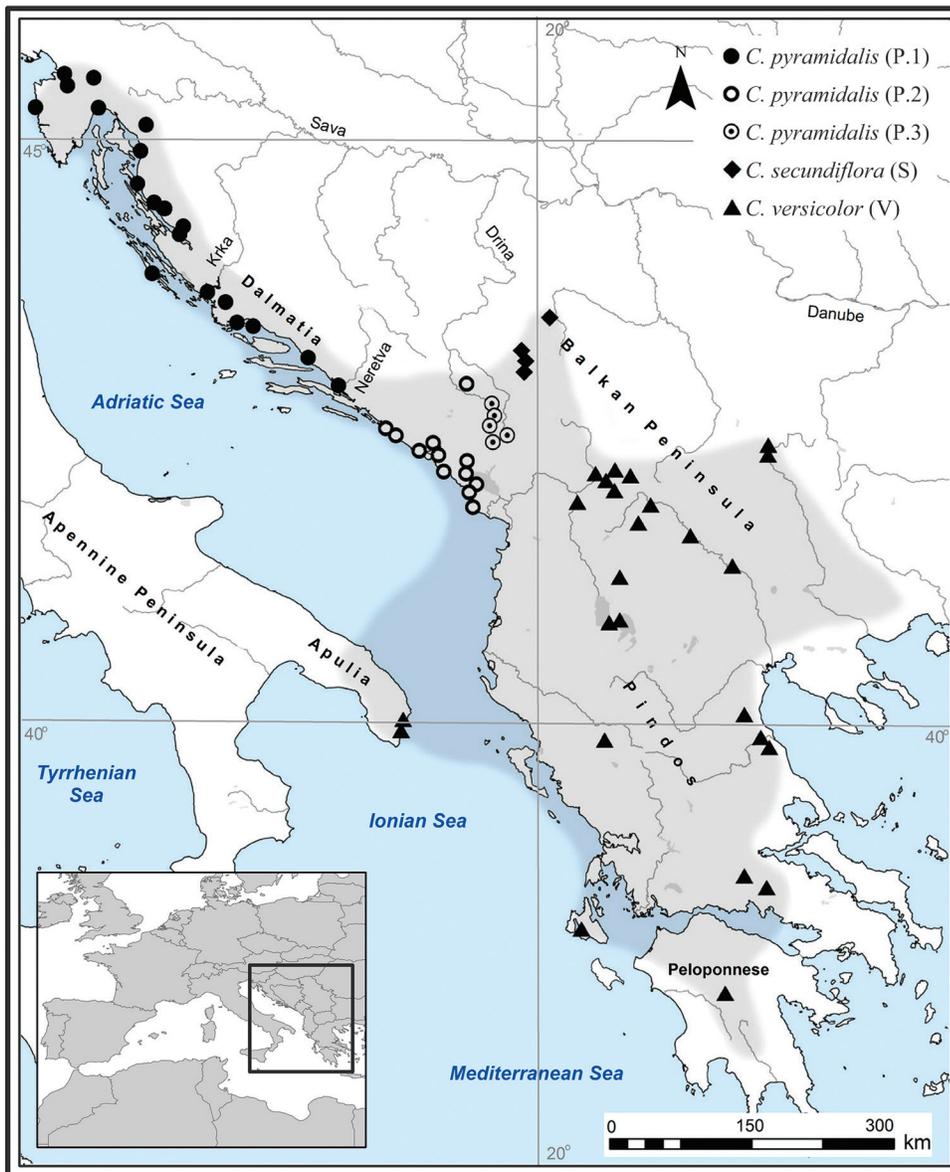

Fig. 1. Distribution range of the *Campanula pyramidalis* species complex (shaded) across its geographic range in the Balkans and portions of the Apennine peninsula. Approximate positions of sampling sites used in this study are indicated (for details, see Appendix 1).

In addition, based on their well-supported sister-group relationship to the *C. pyramidalis* complex, two species belonging to the "Rapunculus" clade of isophyllous *Campanula*, *C. tommasiniana* and *C. waldsteiniana*, were chosen as outgroups (Eddie & al., 2003; Park & al., 2006).

DNA extraction, amplification, and sequencing. — Total genomic DNA was extracted from silica-dried or herbarium material using the GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, Missouri, U.S.A.) or a modified hexadecyltrimethylammonium bromide (CTAB) technique (Doyle & Doyle, 1987), and purified using Wizard minicolumns (Promega, Madison, Wisconsin, U.S.A.). The polymerase chain reaction (PCR) was used to obtain double-stranded DNA fragments of interest. Three plastid intergenic spacer regions were amplified using published primers: psbA-trnH^{GUG} (Sang & al., 1997), psbZ-trnfM^{CAU} (Demesure & al., 1995), and trnG^{UCC}-trnS^{GCU} (Hamilton, 1999). The nuclear ribosomal region encompassing ITS-1, 5.8S rDNA, and ITS-2 (nrITS) was amplified using primers ITS5 and ITS4 (White & al., 1990). For all regions, PCR reactions were carried out in 50 μL volumes with annealing temperature ranging from 50°C to 55°C, following protocols detailed in Lo & al. (2007). Amplified products were cleaned by polyethylene glycol/NaCl precipitations. To ensure accuracy, we sequenced both strands of PCR products. Cleaned fragments were sequenced using the DYEnamic ET dye terminator sequencing kit (GE Healthcare, Baie-d'Urfe, Quebec, Canada) on an Applied Biosystems model 377 automated DNA sequencer (PE Biosystems, Foster City, California, U.S.A.). Initial sequencing of plastid and nuclear amplicons was done directly. No sign of additive polymorphic sites (APS) or electropherogram displacements (indicating the presence of indels in the amplicon) was detected in the plastid sequences. However, variation in both sequence length and APS was detected in some nrITS amplicons. In cases where more than two polymorphic sites or electropherogram displacements were detected, PCR products were cloned into the pSTBlue-1 Acceptor vector (EMD Biosciences, San Diego, California, U.S.A.) and two to five clones were sequenced. Sequence chromatograms were proofed and edited, and contigs assembled using Sequencher v.4.8. (Gene Codes Corp., Ann Arbor, Michigan, U.S.A.). All sequences generated in this study have been deposited in GenBank (Appendix 1).

Phylogenetic analyses. — Sequences were aligned manually with Se-Al v.2.0a11 (Rambaut, 2002). Albeit sequences of all accessions were readily alignable in both the plastid and nuclear matrices, a number of gaps had to be introduced in the alignments. Preliminary phylogenetic analyses were conducted to explore the distribution of phylogenetic signal in the individual matrices with and without coded gaps. Neither resolution nor support was affected in a substantial way by inclusion of gaps (results not shown), and therefore gaps in the alignments were treated as missing data in subsequent analyses. Phylogenetic analyses were conducted under a variety of distance- and character-based methods.

To start exploring relationships among and within species and populations of the *C. pyramidalis* complex, we initially constructed phylogenetic networks for each individual dataset. The networks were constructed using a neighbor-net (NN) algorithm (Bryant & Moulton, 2004) as implemented in SplitsTree v.4.11.3 (Huson & Bryant, 2006). Prior to network analyses, sequences were corrected by imposing corresponding models of DNA evolution. The program ModelTest v.3.7 (Posada & Crandall, 1998) was used to determine the model of sequence evolution that fits best each of the four individual datasets as well as the combined chloroplast data (Table 1).

Parsimony searches, along with clade support estimates, were conducted for each chloroplast matrix separately as well as for the cpDNA, nrITS, and combined datasets. Nucleotide characters were treated as unordered and all changes were equally weighted. In all of these analyses, heuristic searches for most parsimonious trees were performed with PAUP* v.4.0b10 (Swofford, 2002), using a two-stage strategy. First, the analyses involved 10,000 replicates with stepwise random taxon addition, tree bisection-reconnection (TBR) branch swapping saving no more than ten trees per replicate, and MULTREES off. The second round of analyses was performed on all trees in memory with the same settings except with MULTREES on. Both stages were conducted to completion or until one million trees were found. Support for relationships was inferred from nonparametric bootstrapping (Felsenstein, 1985) implemented in PAUP* by using 500 pseudoreplicates, each with 20 random sequence addition cycles, TBR branch swapping, and MULTREES off (DeBry & Olmstead, 2000). Conflict between datasets was evaluated by visual inspection, looking for strongly supported yet conflicting tree topologies resulting from individual data matrices.

Bayesian phylogenetic inference was performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) on the cpDNA, nrITS, and combined datasets. This combined dataset was split into two partitions prior to analysis. The models of sequence evolution as determined before (Table 1) were used for each sequence partition. For each search, two runs starting from random trees were carried out. The Metropolis-coupled Markov chain Monte Carlo algorithm was used with four simultaneous chains set initially to five million generations and sampled every 500 generations. The likelihoods of the independent runs were considered indistinguishable when the average standard deviation of split frequencies was less than 0.01%, as suggested by Ronquist & Huelsenbeck (2003). To determine the burn-in cut-off point, we plotted the −ln likelihood values against generation time and discarded pre-asymptotic samples. The remaining data were analyzed in PAUP* where the 50% majority-rule consensus tree was constructed. With no significant difference between the two runs observed, we only report topologies and posterior probabilities based on pooled trees from the separate Bayesian analyses.

Topological incongruence and alternative hypothesis testing. — Two sets of alternative topologies were constructed using PAUP* (Swofford, 2002). One set was designed to evaluate the conflict between the chloroplast and nuclear datasets, by constraining reciprocal topologies. For each observed incongruence between the two datasets, one randomly chosen most parsimonius tree representing the results obtained from the chloroplast data was imposed on the nuclear data and vice versa.

The other set was constrained to investigate the monophyly of the three species of the complex as traditionally circumscribed. To evaluate the significance between alternative phylogenetic hypotheses, we conducted the Approximately Unbiased test (AU test; Shimodaira, 2002). This test is recommended for general tree comparison because it is considered less biased than other methods employed for these purposes, and is less conservative than the frequently employed Shimodaira-Hasegawa test (Shimodaira, 2002). The *P*-values for the AU test were calculated in CONSEL v.0.1j (Shimodaira & Hasegawa, 2001), using ten repetitions of multiscale bootstrapping, each consisting of ten sets with 10,000 bootstrap replicates.

■ RESULTS

DNA regions and alignments. — The characteristics of the sequenced regions as well as the statistics of the trees derived from the separate and combined analyses are described in Table 1.

Sequences for the three chloroplast regions were obtained from all 96 accessions of the *C. pyramidalis* complex as well as from the two outgroups used in this study. Levels of variation, measured as either percentage of parsimony-informative sites or percentage of variable sites, were overall very low (Table 1). The sequences were straightforward to align within the ingroup as well as between the ingroup and outgroup taxa. The *psbA-trnH* spacer, a marker suggested as universal for plant DNA barcoding purposes (Hollingsworth & al., 2011), showed the highest levels of variation among the three assayed regions.

The cloned sequences confirmed the presence of additive polymorphic sites that were evident in the electropherograms obtained via direct sequencing of certain individuals, particularly those belonging to *C. versicolor.* Based on the preliminary evidence for nrITS locus polymorphism, our sampling strategy for nrITS was adjusted. By cloning and sequencing multiple clones (2–5) of those individuals where more than two polymorphisms were evident based on initial direct sequencing, we sought to capture as much as possible of the allelic variation. Following this approach, we obtained sequences for 56 individuals (out of 96 sampled) from all three traditionally recognized species, but with multiple clones per individual included where needed. In addition, we included nrITS sequences for three individuals from this complex as well as for both outgroup species obtained in a previous study (Park & al., 2006) and deposited in Genbank (as indicated in Appendix 1). Albeit the variation in ITS was comparable to that of the combined chloroplast sequences (Table 1), the primary homology was easy to assess and alignment was straightforward as well.

Chloroplast genealogies. — To explore the chloroplast data, a number of phylogenetic analyses were initially conducted on individual matrices using distance and parsimony approaches. The phylogenetic networks based on sequences from the three regions showed substantial topological agreement among each other and revealed three clusters with moderate to strong support (Electr. Suppl.: Fig. S1). The group informally labeled P.1 contains all accessions of *C. pyramidalis* found along the northern and central Adriatic coast, from the Gulf of Trieste, across the Slovenian and Croatian

Table 1. Summary descriptions for sequences included in and trees derived from individual and combined datasets of the *Campanula pyramidalis* complex.

	psbA-trnH	*psbZ-trnfM*	*trnG-trnS*	cpDNA	nrITS	Combined
Number of OTUs included	96	96	96	96	94[a]	98[b]
Sequence characteristics						
Aligned length	365	601	1016	1982	771	2753
Variable sites (%)	22 (6.0)	11 (1.8)	37 (3.6)	70 (3.5)	136 (17.6)	176 (6.4)
Parsimony-informative sites (%)	13 (3.6)	6 (1.0)	24 (2.4)	43 (2.2)	57 (7.4)	107 (3.9)
Mean AT content [%]	68	60	67	65	47	59
Base frequency homogeneity (χ^2/df/*P*)	5.1/285/1.0	9.9/285/1.0	31.4/285/1.0	20.0/285/1.0	11.3/279/1.0	10.6/171/1.0[c]
MP tree characteristics						
Length	24	13	40	77	171	216
CI/RI	0.96/0.99	1.0/1.0	0.98/0.99	0.97/0.99	0.85/0.96	0.88/0.82
Model of DNA evolution	F81	F81+I	F81	F81+G	SYM+G	Combined
Mean ln*L*	—	—	—	−3479.386	−2628.105	−5655.393

[a] Including multiple clones for certain individuals.
[b] Including two outgroup species.
[c] Including only OTUs for which both chloroplast and nuclear sequences are available.
CI = consistency index (excluding parsimony-uninformative characters); df = degrees of freedom; F81 = Felsenstein (1981) model of DNA evolution; G = rate variation among nucleotides following a discrete gamma distribution; I = proportion of invariable sites; OTU = operational taxonomic unit; RI = retention index; SYM = symmetrical model of DNA evolution (Zharkikh, 1994).

coastal range, south to the Neretva canyon, and is referred to here as *C. pyramidalis* in a strict sense. Cluster P.2 (described here as a new species, *C. austroadriatica*) contains samples of *C. pyramidalis* from the southern Adriatic coast, distributed from the Neretva canyon, along the coasts of southern Croatia and Montenegro to northern Albania. Accessions of *C. pyramidalis* from continental Montenegro (labeled as P.3) displayed strong similarity with the samples of *C. secundiflora* (S), together forming a distinct and well-supported cluster, *C. secundiflora* in a broad sense (Electr. Suppl.: Fig. S1). Individuals belonging to *C. versicolor* (V) failed to cluster together and showed very little resolution within this species. The MP tree characteristics for individual parsimony searches are indicated in Table 1. Topological agreements were found among the three separate analyses (trees not shown). Parsimony analyses of the individual chloroplast datasets produced clades identical (trees not shown) to their respective phylogenetic network (Electr. Suppl.: Fig. S1).

Taking the results from all these separate analyses into account, we concluded that the three matrices show no significant topological incongruence, and thus combined them into one cpDNA dataset. The majority-rule consensus tree resulting from the Bayesian analysis is depicted in Fig. 2, while the neighbor-net (NN) splits graph based on this cpDNA matrix is shown in Fig. S2A (Electr. Suppl.). Parsimony analysis of the combined chloroplast data (Table 1) resulted in essentially the same topology (tree not shown). The overall relationships are consistent with the results from the separate datasets. Clades identical to those described above were recovered from the combined analyses (Electr. Suppl.: Fig. S2A), all with greater than or equal to 95% bootstrap support (BS) in parsimony and with 1.0 posterior probability (PP) in the Bayesian analysis (Fig. 2). In addition, a segregation of two distinct haplotypes within clade P.1 of *C. pyramidalis* became more pronounced, one grouping populations from northern Italy to the northern Croatian coast, and the other restricted to the central Croatian coast, with the Krka river as boundary between the two. In contrast to the marked increase in support for the three major groups, the backbone relationships among them remained relatively weekly supported. Also, the relationships of individuals and populations of *C. versicolor* with the rest of the *C. pyramidalis* species complex remained largely unresolved. However, within *C. versicolor* four lineages emerged with moderate to strong support. The main split occurred between the northern (Bulgaria, southern Serbia, Macedonia) and southern (Greece, southern Italy) populations. These allopatric lineages were supported as distinct, with BS 84% and 86%, respectively, and both receiving 1.0 PP (Fig. 2). A morphologically peculiar population of *C. versicolor* from Kosovo (Prizren) as well as accessions from Demir Kapija in Macedonia form moderately supported independent lineages.

Nuclear genealogy. — The majority-rule consensus tree resulting from the Bayesian analysis is depicted in Fig. 3 (the parsimony consensus tree, resulting in a similar topology, is not shown). The phylogenetic network based on nuclear data is shown in Fig. S2B (Electr. Suppl.). The relationships inferred from nrITS were generally less resolved and less supported

than those of the combined cpDNA data despite overall similar levels of variation observed in these two matrices (Table 1). Nevertheless, two of three major clades found with plastid data were recovered here as well, with moderate to strong support. The first includes *C. pyramidalis* s.str. from the northern and central Adriatic coast (P.1) and fully corresponds to that found in the cpDNA data. However, the subdivision of this group into two, north and south of the Krka river as detected with plastid data, is not observed here. The second main cluster includes populations of *C. pyramidalis* from southern Dalmatia and Montenegro, corresponding entirely to group P.2 (*C. austroadriatica*). Also, as with the cpDNA data, the populations of *C. versicolor* (V) failed to cluster together, and their relationships among each other and with other species of this complex are generally unsupported. The north–south subdivision within *C. versicolor* seen in the chloroplast matrix is not recovered with nrITS data. Instead, a very weakly supported west–east subdivision is observed, and circum-Ionian populations (Otranto, Ionian Islands, western Greece) of *C. versicolor* are more closely related to populations of *C. secundiflora* and some populations of *C. pyramidalis* (P.2, P.3) than to the remainder of *C. versicolor* from eastern Greece, Macedonia, Serbia, and Bulgaria. Also in contrast to plastid-derived phylogenies, continental Montenegrin populations of *C. pyramidalis* (P.3) and populations of *C. secundiflora* (S) do not form a clade (i.e., *C. secundiflora* s.l.), albeit their close proximity in nrITS is evident (Fig. 3; Electr. Suppl.: Fig. S2B).

The most striking topological conflict between the chloroplast and nuclear data appears to involve the relative position of *C. austroadriatica* (P.2) with respect to *C. pyramidalis* s.str. (P.1) and *C. secundiflora* s.l. (S+P.3). Chloroplast data place *C. austroadriatica* and *C. pyramidalis* s.str. together while nuclear data place *C. austroadriatica* with *C. secundiflora* s.l. (compare unrooted phylograms in Figs. 2 and 3 as well as networks in Fig. S2, Electr. Suppl.). However, support for these relationships, as well as for other intervening backbone relationships, is only weak to moderate (e.g., 66% vs. 70% BS; Electr. Suppl.: Fig. S2). Despite the lack of strong support for these incongruences, we tested alternative hypotheses in a reciprocal fashion (Table 2). Using chloroplast data yet enforcing the nrITS topology resulted in a difference that failed to be rejected by the AU test. Similarly, constraining the cpDNA topology on a nuclear dataset did not result in a significant difference (Table 2). Taking this into account, we considered the two datasets congruent and combined them.

Phylogenetic analyses of combined data. — Given the discrepancy between sampling strategies employed for chloroplast and nuclear sequencing efforts, two approaches to concatenating datasets were initially employed, differing in the amount of missing data. In the "broad" dataset, we used all individuals sampled for cpDNA (98 terminal units including outgroups) along with nrITS data for all sequenced individuals (58 terminals; ~60%). In the other dataset, we performed analyses using only 56 ingroup plus two outgroup individuals for which all data (chloroplast and nuclear) were available. Where multiple clones were available per individual, we used one randomly chosen sequence for both of these approaches. Preliminary
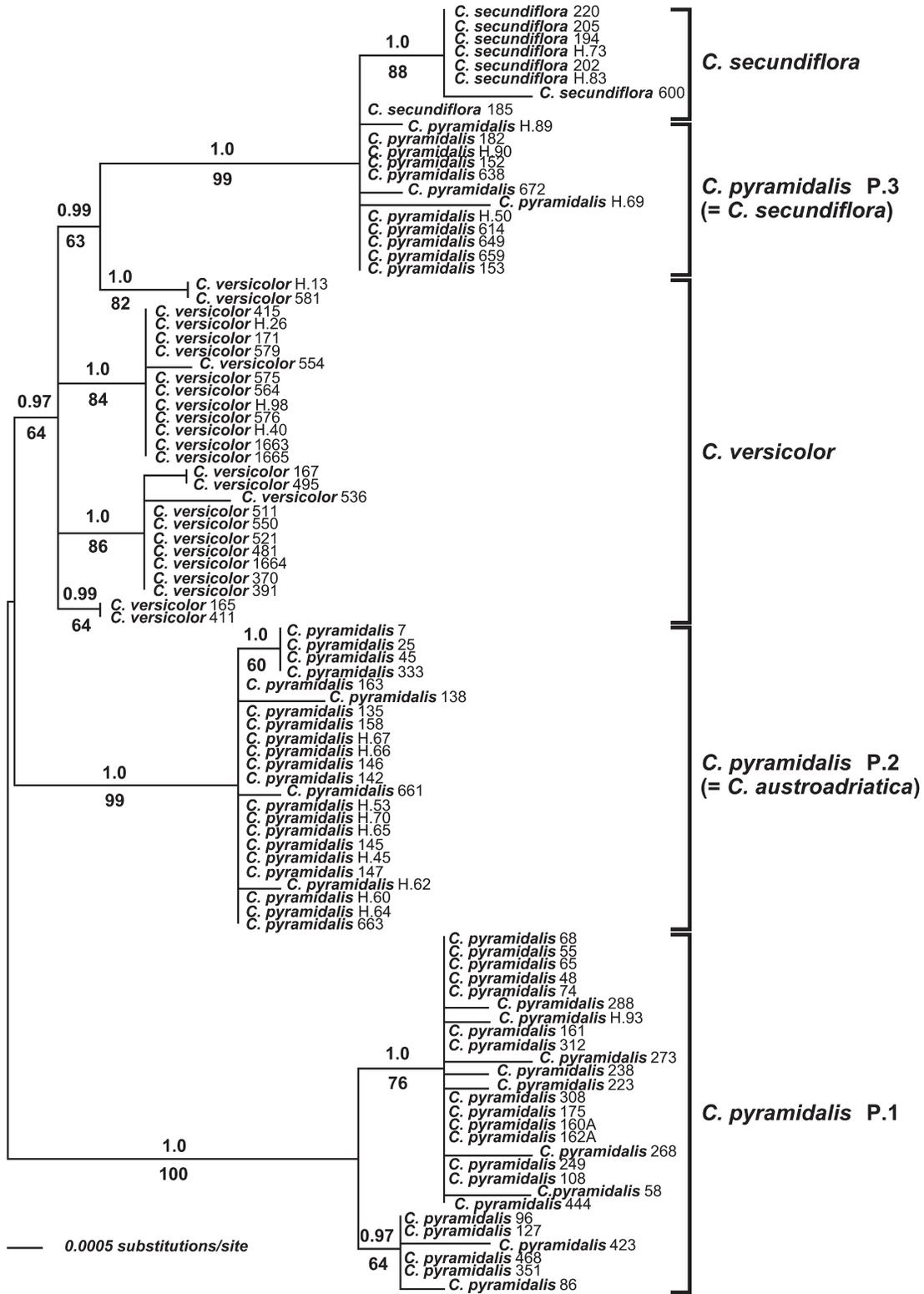
Fig. 2. Majority-rule consensus tree with mean branch lengths resulting from the Bayesian analysis of the combined plastid (*psbA-trnH, psbZ-trnfM, trnG-trnS*) sequence data. The tree was rooted using individuals of *C. pyramidalis* clade P.1 (= *C. pyramidalis* s.str.) as functional outgroup. The MP search resulted in a strict consensus tree with almost identical topology (77 steps in length). Bayesian posterior probabilities (≥0.95) are indicated above branches; parsimony bootstrap values (≥60%) are indicated below branches. Major clades discussed are labeled. Numbers following species names correspond to DNA accessions (see Appendix 1).
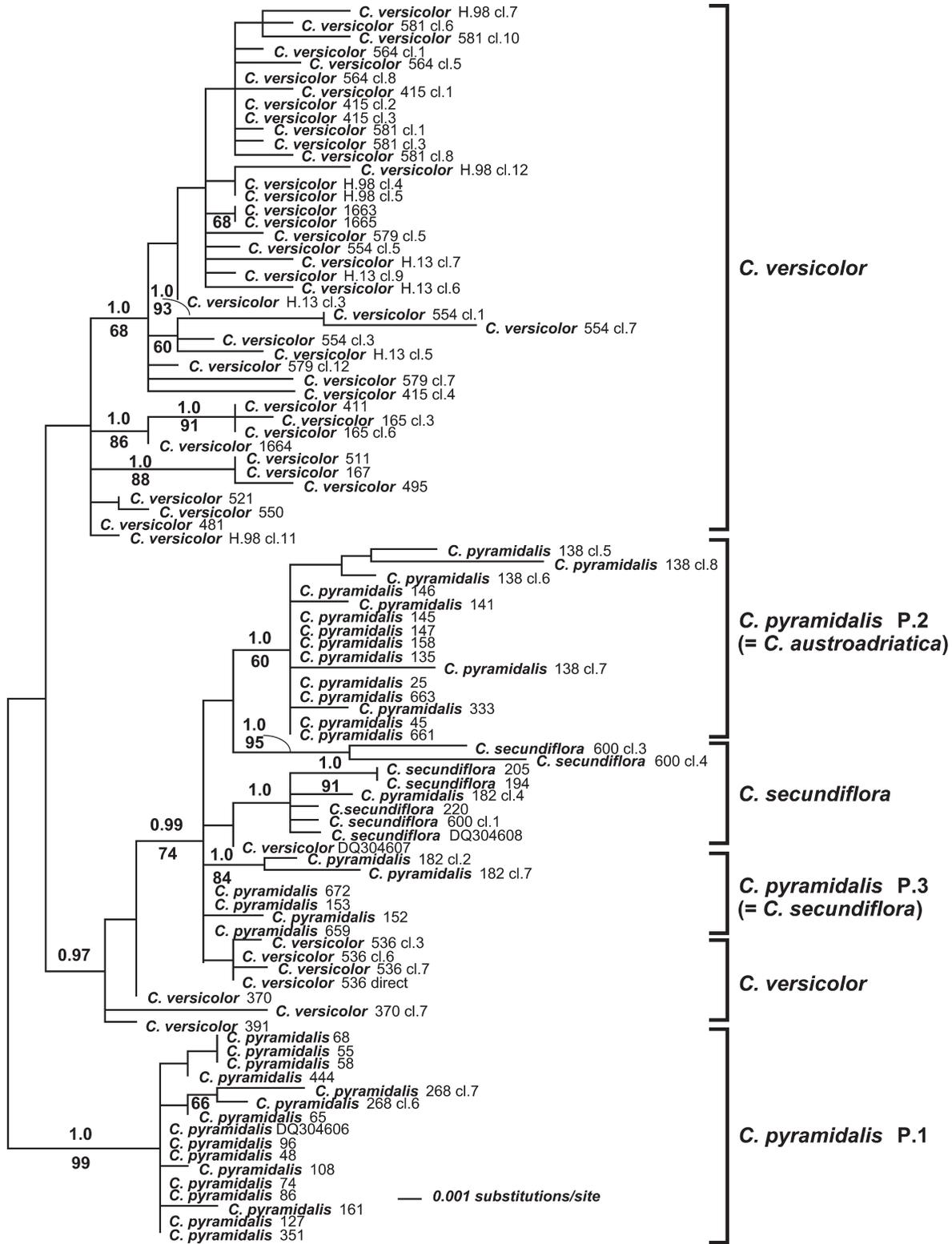
Fig. 3. Majority-rule consensus tree with mean branch lengths resulting from the Bayesian analysis of the nuclear ribosomal ITS sequence data. The tree was rooted using individuals of *C. pyramidalis* clade P.1 (= *C. pyramidalis* s.str.) as functional outgroup. The MP search resulted in a strict consensus tree with almost identical topology (171 steps in length). Bayesian posterior probabilities (≥0.95) are indicated above branches; parsimony bootstrap values (≥60%) are indicated below branches. Major clades discussed are labeled. Numbers following species names correspond to DNA accessions (see Appendix 1).

parsimony searches conducted using these two alternatives resulted in trees of compatible topology and similar levels of support. Hence, for further in-depth analyses we have chosen to use the "broad" dataset and we report only results based on this concatenated matrix.

Trees produced by the total-evidence approach had better resolution and overall support compared to those produced from individual analyses. Therefore, we have based our discussion on the analyses of the combined dataset. The Bayesian analyses from each of the two runs starting from a random tree reached an asymptotic plateau no later than one million generations, and all trees obtained prior to the plateau were excluded from the assemblage of a consensus tree. Figure 4 shows the majority-rule consensus phylogram resulting from the Bayesian analysis. The topology is consistent with the results from the separate dataset analyses using Bayesian inference (Figs. 2, 3) and distance (Electr. Suppl.: Fig. S2). All analyses confirm the *C. pyramidalis* species complex to be a strongly supported monophyletic group (100% BS; 1.00 PP). Within this complex, three major clades identical to those previously described can be delimited based on a combination of their strong individual support (≥95% BS; 1.00 PP) and molecular distinctiveness, as evidenced by the long branches subtending them (Fig. 4): *C. pyramidalis* s.str. (P.1), *C. austroadriatica* (P.2), and *C. secundiflora* s.l. (P.3+S). The combined results also confirmed that monophyly of *C. versicolor* has not yet been achieved, with a clade consisting of representatives of *C. austroadriatica* and *C. secundiflora* s.l. nested among individuals of *C. versicolor* in a phylogeny rooted using outgroups (Fig. 4).

As one of the most surprising results of this study, *Campanula pyramidalis* was not found to be monophyletic on the optimal (unconstrained) trees in any of the individual or combined datasets. When the topology was constrained so that *C. pyramidalis* was monophyletic, this produced suboptimal topologies with both the cpDNA and combined chloroplast/nuclear datasets that were rejected as significantly worse solutions by the AU tests (Table 2). This was not the case with the nrITS-only matrix, where imposing monophyly of *C. pyramidalis* s.l. could not be rejected as suboptimal (Table 2). This marginally non-significant result ($P = 0.058$) is most likely due to lower resolution and overall support for the backbone relationships found with the nuclear data. Finally, neither *C. versicolor* nor *C. secundiflora* were found to be strictly monophyletic, but their monophyly could not be rejected with confidence in any of the three data partitions tested (Table 2).

■ DISCUSSION

This work represents the first fine-scale molecular phylogenetic study of the *C. pyramidalis* species complex. It is based on a combination of plastid and nuclear DNA sequences obtained from individuals sampled across the entire geographic range of the three currently recognized species. The resulting phylogenetic inferences are robust and show significant support for the composition and relationships between major clades in the tree. Figure 4 summarizes our current best understanding of phylogenetic relationships among populations of the *C. pyramidalis* complex, and of the relationship between the traditional taxonomy and a putative phylogenetic classification suggested here. Our results also further confirm a newly emerging biogeographic pattern, recently detected based on a limited number of plant and animal taxa.

Up to 21 taxa were described within the *C. pyramidalis* complex from the Balkans and Apennine Peninsulas. However, according to *Flora Europaea* (Fedorov & Kovanda, 1976), *Med-Checklist* (Greuter & al., 1984), *World Checklist and Bibliography of Campanulaceae* (Lammers, 2007), and more recent national Floras and floristic papers covering these areas (Hartvig, 1991; Lovašen-Eberhardt, 2000; Kovačić, 2004; Nikolov, 2005; Nikolić, 2012), only *C. pyramidalis*, *C. versicolor* and *C. secundiflora* were recognised, and all other names were considered to be synonyms. Our results lend strong support to the recognition of several taxa, corresponding only partially to this generally accepted taxonomic concept.

Table 3 compares and contrasts the main diagnostic features, allowing for distinction among four different taxa newly circumscribed here. Morphological differences are seen in the general habit, life span, and overall dimension of plants, in leaf petiolation, inflorescence features, corolla color, as well as in the shape of seeds, calyx teeth, and ovaries. Given the morphological variation known to occur within this species complex, we use the phylogenetic species concept (PSC) approach. Unlike morphological or various species concepts emphasizing mechanisms of reproductive isolation, the PSC is historically based (Baum & Donoghue, 1995) and uses the criteria of monophyly and exclusivity to define species (Queiroz & Donoghue, 1990; Baum, 1992; Baum & Shaw, 1995).

Species delimitation within *C. pyramidalis*. — *Campanula pyramidalis* in the broad sense, as defined by currently accepted floristic treatments, is distributed along the Adriatic coast, including the islands and a narrow inland strip, from

Table 2. Results of the Approximately Unbiased (AU) tests for alternative hypothesis testing in the *Campanula pyramidalis* species complex.

Dataset	Reciprocal constraint		Constrained monophyly of		
			C. pyramidalis (P.1+P.2+P.3)	*C. versicolor* (V)	*C. secundiflora* (S)
cpDNA (Fig. 2; Electr. Suppl.: Fig. S2A)	0.139	nrITS: (P.2+P.3+S)	**0.007***	0.245	0.778
nrITS (Fig. 3; Electr. Suppl.: Fig. S2B)	0.178	cpDNA: (P.1+P.2)	0.057	0.138	0.272
Combined (Fig. 4)	n/a	–	**0.024***	0.427	0.671

Asterisks indicate probabilities below 0.05 (i.e., tree topology rejected as significantly worse compared to the optimal topology for the given dataset). "P" represents individuals traditionally identified as *C. pyramidalis* (sorting out into three lineages: P.1, P.2, and P.3), "V" represents those of *C. versicolor*, and "S" those of *C. secundiflora*.
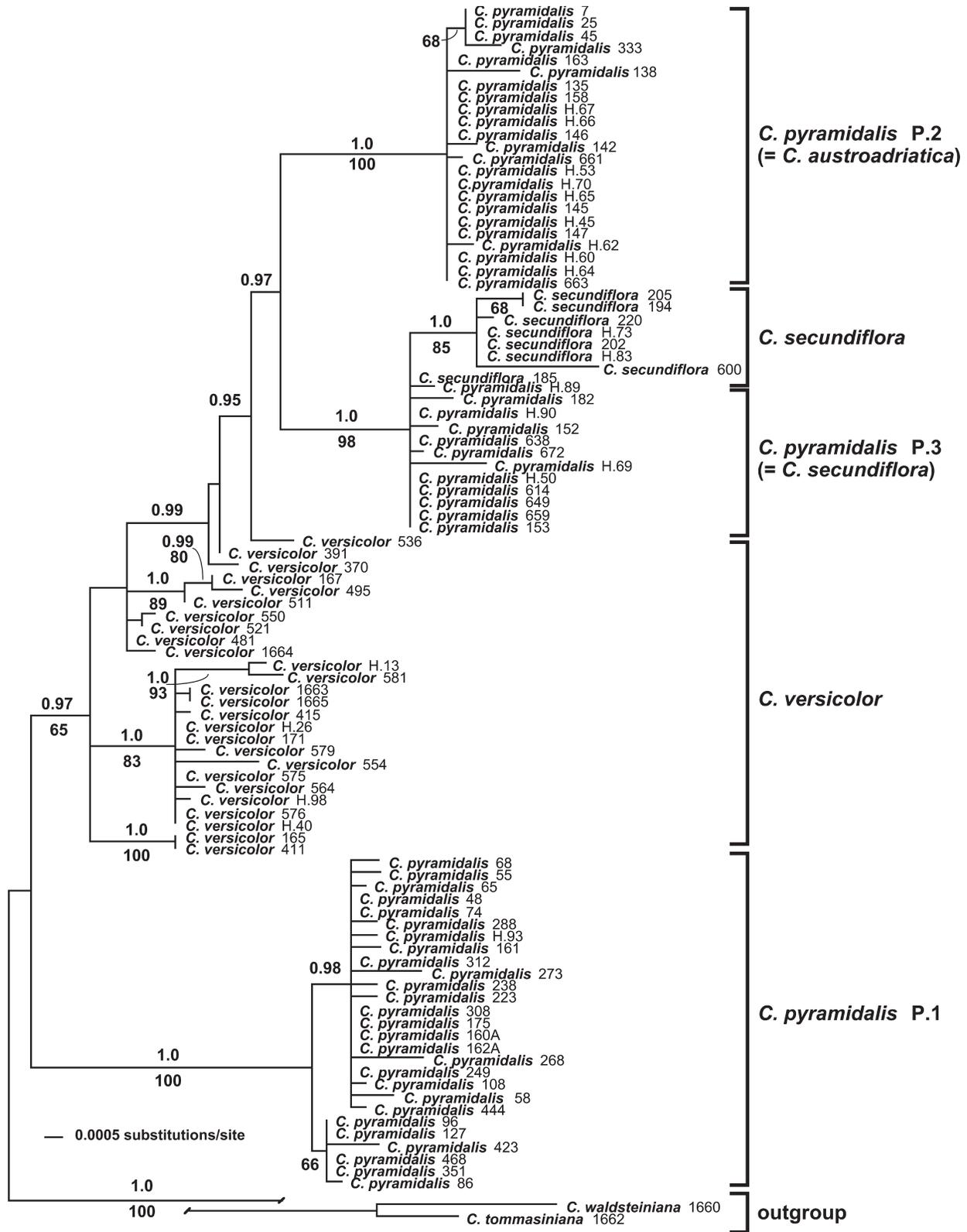
Fig. 4. Majority-rule consensus tree with mean branch lengths resulting from the partitioned Bayesian analysis of the combined plastid (*psbA-trnH, psbZ-trnfM, trnG-trnS*) and nuclear (rITS) non-coding sequence data. The tree was rooted using two sister species from isophyllous *Campanula* as outgroups. The MP search resulted in a strict consensus tree with almost identical topology (216 steps in length). Bayesian posterior probabilities (≥0.95) are indicated above branches; parsimony bootstrap values (≥60%) are indicated below branches. Major clades discussed are labeled. Numbers following species names correspond to DNA accessions (see Appendix 1).

the Gulf of Trieste (northeastern Italy) southwards to northern Albania (Fig. 1). According to our results, this species contains three quite distinct entities evident as three well-supported clades in the phylogenetic networks and trees (Figs. 2–4; Electr. Suppl.: Figs. S1, S2; clades labeled P.1–P.3). We regard these findings as sound arguments in favour of recognizing three separate taxa: *C. pyramidalis* s.str. (P.1), *C. austroadriatica* sp. nov. (P.2), and one additional group of populations (P.3) closely related to *C. secundiflora* but not yet taxonomically defined.

　　The split between allopatric northwestern Adriatic (*Campanula pyramidalis* s.str.; P.1) and southeastern Adriatic (*Campanula austroadriatica* sp. nov.; P.2) groups of populations of the *C. pyramidalis* species complex along the lower Neretva valley in central Dalmatia (Croatia) is remarkably congruent with phylogeographic patterns detected in a number of plant (e.g., Kučera & al., 2008, 2010; Surina & al., 2011) and animal (e.g., Podnar & al., 2004; Kryštufek & al., 2007; Sotiropoulos & al., 2007) taxa. For example, Kučera & al. (2008) identified a major phylogeographic and taxonomic split within the *Cardamine maritima* Port. ex DC. species aggregate (Brassicaceae) in the area of Neretva valley, a genetic pattern on which they based, along with morphological data, the segregation of *Cardamine maritima* and *C. fialae* Fritsch. Similarly, in *Edraianthus tenuifolius* A. DC. (Campanulaceae) both plastid and fingerprinting (AFLP) markers congruently identified a clear phylogeographic (but not taxonomic) split along the lower Neretva valley despite the lack of obvious dispersal barriers along the Adriatic coast (Surina & al., 2011). For the lizard *Podarcis melisellensis* (Podnar & al., 2004), this region has been suggested to be a secondary contact zone of lineages diversified in phases of geographic isolation (see also Kučera & al., 2010). In contrast to these studies, the lower Neretva valley does not seem to coincide with either phylogeographic or taxonomic splits in *Edraianthus serpyllifolius* A. DC. (Surina & al., 2011)

or in the nose-horned viper, *Vipera ammodytes* (Ursenbacher & al., 2008). This suggests that gene flow has occurred across this narrow but deep valley, and that the Neretva valley should perhaps be regarded as an important but not universal geographical barrier.

　　Geological evidence suggests greater overall aridity during cold intervals (e.g., Combourieu Nebout & al., 2002) and increased seasonality (Collier & al., 2000). At the end of the Last Glacial Maximum (LGM; ca. 18.000 years before present), the Adriatic Sea level at its northern part reached its lowest position at approximately 43th parallel N. This resulted in a very small, narrow (15–20 km), shallow, and semi-enclosed basin called Meso-Adriatic Depression (Correggiari & al., 1996; Asioli & al., 1996). This remained connected to the larger Otranto basin in the southern Adriatic, situated between the Pelješac and Gargano Peninsulas at the eastern and western Adriatic coast, respectively. The split between northwestern Adriatic *C. pyramidalis* s.str. (P.1) and southeastern Adriatic *C. austroadriatica* (P.2) coincides very well with the geographic position of the Adriatic Sea during the LGM. Therefore, we posit that this split, which geographically coincides with the lower Neretva valley, may not be caused by the valley itself but rather may mark a border between strongly different ecological conditions to the north and south at the time of the LGM, resulting in divergent evolution of populations of the *C. pyramidalis* aggregate in the two areas. An abrupt change in vegetation cover from coastal areas towards the inland is evident nowadays (e.g., Horvatić, 1964, 1967) and reflects well the importance of the proximity of the Mediterranean Sea for the vegetation cover and general ecological conditions even on a small spatial scale.

　　Campanula pyramidalis s.str. (P.1). — According to our examination of the original Linnean material, the name *C. pyramidalis* L. must be applied to the populations from the northern

Table 3. Main diagnostic features to distinguish the newly circumscribed species within the *Campanula pyramidalis* complex.

	C. pyramidalis s.str. (P.1)	*C. austroadriatica* sp. nov. (P.2)	*C. secundiflora* s.l. (S+P.3)	*C. versicolor* s.l. (V)
Habit	Biennial with strong, vertical, semi-woody rhizome	Perennial with stout, brittle woody stock	Perennial with stout, brittle woody stock	Perennial with stout, brittle woody stock
Stem	100–300 cm tall, erect	40–150 cm tall, erect to ascending	15–40 cm tall, pendent to ascending, rarely erect	10–50 cm tall, erect to ascending
Uppermost leaves	Short-petiolate	Short-petiolate	Sessile	Sessile
Inflorescence	Narrowly paniculate, elongate, 50–100(–150) cm long	Narrowly paniculate, elongate, 30–100(–120) cm long	Branched, 5–25 cm long	Narrowly paniculate, rarely subcapitate, 5–20(–30) cm long
Ovary	Shallowly sulcate	Shallowly sulcate	Deeply sulcate	Deeply sulcate
Calyx teeth	Shortly triangular, usually backward-bent, equal or shorter than ovary	Linear to narrowly triangular or subulate, erect to reflexed, 2–3 times as long as the ovary	Narrowly triangular to subulate, erect to reflexed, 1.5–2 times as long as the ovary	Narrowly triangular to subulate, erect to reflexed, 1.5–2 times as long as the ovary
Corolla	Violet to pale bluish-violet usually without dark blue center	Violet to pale bluish-violet usually with dark blue center	Violet to pale bluish-violet with a dark blue center	Pale bluish-violet with a dark blue center
Seeds	1.1–1.3 × 0.4–0.6 mm	0.9–1.0 × 0.4–0.5 mm.	0.5–0.9 × 0.3–0.4 mm	0.8 × 0.3 mm

and central Adriatic coast (lectotype is designated here; see Taxonomic Treatment). This species inhabits primarily islands and a narrow strip along the coast (the Mediterranean zone), and can rarely be found in the hinterland of this region (the
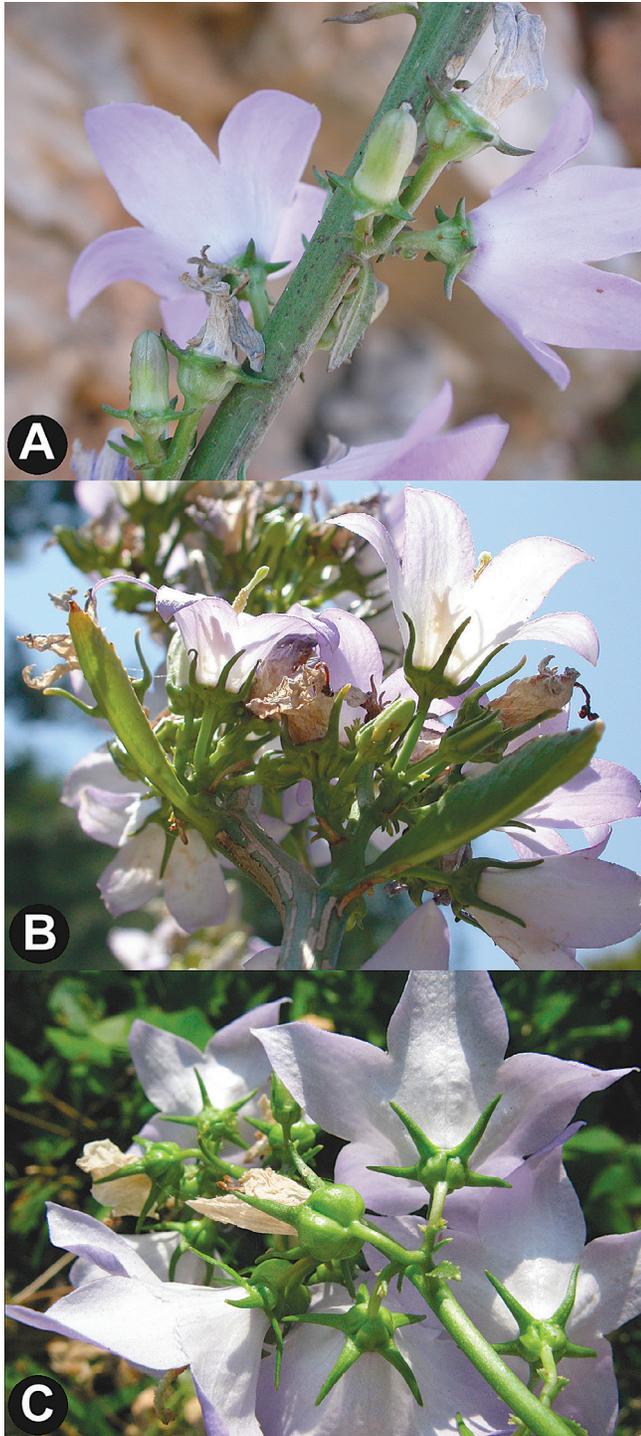

Fig. 5. Comparison of flowers and calices among the three distinct lineages in *Campanula pyramidalis* s.l. **A,** P.1 (= *C. pyramidalis* s.str.): Croatia, Velebit, Vratnik; **B,** P.2 (= *C. austroadriatica* sp. nov.): Montenegro, Rumija, Murići; **C,** P.3: Montenegro, canyon of river Morača, Platije. — Photos: D. Lakušić.

Submediterranean zone). The species has a strong, vertical, semi-woody rhizome, on top of which a very characteristic rosette of leaves develops. From its center, usually only one flowering stem grows, which is unbranched and leafy, and can reach over 300 cm in height. Multiple stems from one rhizome seem to be the consequence of mechanical injuries (caused mostly by grazing or by falling stones on screes and rocky banks), the damage caused by insects, or by phytoplasma infections. *Campanula pyramidalis* s.str. is easily recognizable in nature, even from the distance, due to its very specific habit, characterised by individual rod-like, narrow, and very tall simple or pyramidal stems. Based on our observation of plants in nature as well as plants cultivated in a common garden, these plants do not tend to live longer than two, rarely three years. Most plants die after flowering, although in some cases same individuals may form flowering stems the next year. For example, almost all individuals cultivated in the Botanical Garden "Jevremovac" in Belgrade (Serbia) died right after flowering. Only one formed a new rosette of leaves the following year, but did not flower and died at the end of the vegetation period. *Campanula pyramidalis*, as defined here in its narrow sense (P.1), differs from the southern Adriatic and continental Montenegrin populations (P.2, P.3) not only in its general habit but also in its mostly short, triangular, usually backward-bent calyx teeth (Fig. 5; Table 3).

***Campanula austroadriatica* sp. nov. (P.2). —** Populations of this new species have traditionally been recognised as members of *C. pyramidalis* L. by almost all classical botanical authorities and researchers of the Balkan flora, with the exception of Černjavski & al. (1949). In its color and general appearance of the flowers, and especially in its large dimensions, this plant resembles *C. pyramidalis* s.str. However, a number of general habit characteristics are shared with *C. versicolor*, a southern Balkan species (Fig. 1). For example, these plants have a stout, woody, branching rhizome on which a larger number of rosettes develop, along with many tall flowering stems (up to 150 cm) with many large and spirally arranged leaves, resulting in a clustered caespitose habit. Unlike *C. pyradmidalis* s.str., *C. austroadriatica* is a long-lived plant, a true perennial. Dense stem tufts of some individuals can reach over 50 cm in diameter, which indicates that the life span of these plants in nature is long, possibly a few decades. The plants cultivated in the Botanical Garden "Jevremovac" in Belgrade (Serbia) are now six years old and flower regularly and abundantly every year. A few of these plants have been cultivated in the Botanical Garden of the Faculty of Science in Zagreb (Croatia) since 1978, where one individual was planted in 1968. Results of preliminary morphometric analyses (I. Janković, pers. comm.) also suggest that individuals of this newly described species form a distinct entity, and only partly overlap with individuals from northern Adriatic (P.1) and continental Montenegrin (P.3) representatives of a broadly circumscribed *C. pyramidalis* (Janković & Lakušić, 2011). Apart for general habit (caespitose vs. scapose) and life span (perennial vs. biennial), *C. austroadriatica* is easily differentiated from *C. pyradmidalis* s.str. also by its long, backwards bent calyx teeth and usually very long flower pedicles. Also, these distinctive calyx characteristics

allow for easy differentiation of *C. austroadriatica* from the continental populations of *C. pyradmidalis* s.l. (P.3) found in Montenegro (Figs. 5, 6; Table 3).

Our discovery of *C. austroadriatica* confirms the opinion that the recognition of cryptic species, the presence of two or more distinct species that were previously classified as a single species due to morphological similarity (Bickford & al., 2007), is an important factor when assessing species diversity and conservation in this part of the Balkans. The example provided here adds to a growing list of cryptic species that have recently been described in plants (Surina & al., 2009; Kučera & al., 2010; Mereda & al., 2011) and animals (Ljubisavljević & al., 2007).

Campanula pyramidalis s.l. (P.3). — This lineage represents morphologically and geographically populations from continental areas of Montenegro (Morača canyon, Mt. Moračke planine, Mt. Sinjavina, Mt. Kučke Prokletije; Fig. 1). Albeit most botanical authorities included these populations in *C. pyramidalis*, these plants are closer to *C. versicolor* and especially *C. secundiflora* by their general habit. Our plastid- and nuclear-derived phylogenies both link these populations unequivocally and strongly with *C. secundiflora* s.str., found further inland in Serbia (Fig. 1). These molecular data provide the first evidence in support of the existence of fine-geographical structure along the Morača canyon–Lim canyon–Panjica Gorge transect. This concept was put forth originally by R. Lakušić (unpub. data), who informally recognized three subspecies of *C. secundiflora* in this area: (1) the type subspecies, confined to Panjica Gorge; (2) subsp. *limensis* found in the canyon of Lim river; and (3) subsp. *monetenegrina* in Sinjavina and the canyon of Morača river. However, to fully untangle the phylogeographic and taxonomic relationships of populations along this narrow transect and to decide whether their formal inclusion within *C. secundiflora* s.l. is warranted, much more detailed sampling is necessary than currently available. Until fine-scale molecular and morphological data become available, the question of the exact taxonomic status of this lineage and its intraspecific differentiation, remains open.

Campanula versicolor. — According to most recent national Floras, *C. versicolor* is distributed across southern parts of the Balkan peninsula, Greece, Albania and Macedonia, with its northern boundary in Kosovo and its northwestern boundary in Bulgaria. Small disjunct parts of the range are also known from the southern Apennines (Fig. 1). Several insufficiently differentiated lineages, with low support and somewhat different positions in cpDNA and nrITS trees were found in our study (Figs. 2, 3; Electr. Suppl.: Fig. S2). The cpDNA tree suggested a geographic structure in north–south direction, i.e., differentiation between northern (Bulgaria, southern Serbia, Macedonia) and southern populations (Greece, southern Italy). On the other hand, the nrITS data very weakly supported a west–east subdivision, i.e., between circum-Ionian populations from Otranto, the Ionian Islands, and western Greece and the remainder of the populations from eastern Greece, Macedonia, Serbia, and Bulgaria. In addition, the morphologically peculiar populations from Kosovo (Prizren) as well as accessions from Demir Kapija (Macedonia) form moderately to strongly supported lineages.

In contrast to *C. pyramidalis* s.l., our current molecular findings (Fig. 4; Table 2) can not be taken as arguments in favor of further taxon segregation within *C. versicolor.*

Taxa not accepted within the *C. pyramidalis* complex. — Our taxon sampling included material corresponding unambiguously to the following, previously proposed but generally not accepted taxa: *C. kapelae, C. mrkvickana, C. plasonii, C. tenorii, C. versicolor* var. *tomentella, C. versicolor* var. *thessala* and *C. versicolor* f. *matkae* (compare with Appendix 1). Except for individuals/populations putatively belonging to *C. plasonii* from Demir Kapija (Macedonia), which formed one of the well-supported lineages within the *C. versicolor* group (100% BS, 1.0 PP; Fig. 4), none of the other sampled and analyzed taxa were supported by our molecular results.

Unfortunately, our sampling did not include material corresponding to the following taxa: *C. pyramidalis* var. *calycina* A. DC., *C. pyramidalis* var. *compacta* auct., *C. pyramidalis* f. *alba* Voss, *C. staubii* Uechtr., *C. umbellulifera* Vuk., *C. × pyraversi* Cayeux, *C. × tymonsii* hort., *C. × fergusonii* hort. and *C. × hendersonii* hort. Hence, their taxonomic status could not be explicitly tested by molecular tools. Our ongoing research focuses on expanding the sampling to include these populations as well as on developing faster evolving markers (AFLP, SSR) capable of differentiating among closely related and recently diverged taxa. This approach will allow us to further address unsolved taxonomic and biogeographic questions in this species complex.

■ TAXONOMIC TREATMENT

Campanula pyramidalis L., Sp. Pl.: 164. 1753, cultivated, Uppsala Botanical Garden – **Lectotype** (**designated here** by D. Lakušić & S. Bogdanović): Herb. Linnaeus no. 221.12 (LINN!).

Note. – We found the original Linnaean specimen in the Herbarium of the Linnean Society of London (LINN) which corresponds to the original protologue and is a type of the name *Campanula pyramidalis*. LINN specimen no. 221.12 (image available at http://www.linnean-online.org/956/) clearly belongs to the original material as it bears not only the inscription "*pyramidalis*" written by Linnaeus's hand, but also the number "8" referring to the species number in *Species Plantarum* (Linnaeus, 1753: 164). This is strong evidence that the specimen was in Linnaeus's possession before 1753 and thus belongs to the original material (see Jarvis, 1992). Also, the label HU indicates that the plant was cultivated in the Uppsala Botanical Garden. In the original protologue there is no indication of the natural distribution of the species, and therefore the type locality of *C. pyramidalis* cannot be determined. There is another specimen of *C. pyramidalis* in UPS (Herb. Burser IV: 19, UPS; see Jarvis, 2007: 379) that belongs to the original material. This is from the Burser Herbarium, directly related to the entry in Bauhin's *Pinax* (Bauhin, 1623), and referred to in the protologue. Therefore, Art. 9.2 of the *Melbourne Code* (McNeill & al., 2012) permits us to select the Linnean specimen LINN no. 221.12 as a lectotype for *Campanula pyramidalis*.

Fig. 6. *Campanula austroadriatica* D. Lakušić & Kovačić sp. nov. **A,** habit; **B,** part of inflorescence; **C, D,** flowers with calyx; **E,** anther before anthesis; **F,** anther after anthesis; **G,** capsule; **H,** seed; **I,** rosette leaves, **J,** glandular-dentate margin of basal leaf. — Drawing: I. Janković.

Campanula austroadriatica D. Lakušić & Kovačić, **sp. nov.**
– Holotype: MONTENEGRO. Boka Kotorska, Ri-
san, Sopot, rock crevices, limestone, 42°30.832′N,
18°40.928′E, 30 m, 26 Sep 2010, *Lakušić, D., Tomović, G.,
Vukojičić, S. & Kuzmanović, N. 31510* (BEOU!; isotype:
ZA!). — Figure 6; Electr. Suppl.: Fig. S3.

Diagnosis. – Affinis *C. pyramidalis* sed planta perennis
multicaulis (non biennis et solet unicaulis); caulis 40–150 cm
altus (non 50–300 cm); dentibus calycis longioribus (3.5–)5–
9(–14) mm (non (1.9–)3–5(–9) mm), recedit.

Description. – Caespitose perennial with stout, brittle
woody stock from which numerous glabrous herbaceous stout
stems arise. **Stems** simple, 40–150 cm, erect to ascending,
leafy, sometimes with short branches above. **Leaves** extremely
variable, broadly ovate-cordate to ovate-lanceolate, crenate to
deeply glandular-dentate, somewhat fleshy; basal and lower
cauline leaves long-petiolate: blade 5–10(–12) × 2.5–8(–
10) cm; uppermost leaves lanceolate, short-petiolate. **Inflores-
cence** narrowly paniculate, elongate, 30–100(–120) cm long,
with clusters of (1–)5–10(–15) flowers. Pedicels of flowers
(2–)4–18(–60) mm long. **Calyx** glabrous, shiny green, (3.8–)
4.4–5.8(–7.4) mm in diameter; calyx teeth (3.5–)5–9(–14) mm,
narrowly triangular to subulate, erect to reflexed, 2–3 times
as long as the ovary; calyx teeth base (1.5–)2–2.5(–3) mm
wide. **Corolla** campanulate, violet to pale bluish-violet, usu-
ally with dark blue center, (14–)20–28(–38) mm, divided
to 1/3 to 1/2, rarely to 3/4; corolla lobes (10–)13–19(–27) ×
(6–)9–13(–16) mm. **Style** 16.4–21.5(–24) mm long, usually
curved upwards. **Ovary** trilocular, glabrous, shiny green.
Stamens 5; anthers (5.5–)6.5–9(–10.5) mm long; filaments
(0.9–)1.5–2.3(–3.1) mm long, basal part of filaments has del-
toid shape, 2–5 mm long. **Capsule** pale brownish, broadly
obovoid, shallowly sulcate with six prominent ribs, dehisc-
ing by basal pores or irregularly rupturing laterally and api-
cally, (3.3–)4.1–5.4(–6.7) × (4.4–)5.2–6.6(–8.2) mm. **Seeds**
numerous, reticulate, elliptic-ovate, light brown, 0.9–1.0 ×
0.4–0.5 mm.

Eponymy. – The specific epithet is derived from the dis-
tribution range of the new species, the southern Adriatic coast.

Distribution and ecology. – According to available mo-
lecular and morphological data, it appears that the delta and
valley of the Neretva river (Fig. 1) represents the geographi-
cal boundary between the newly described *C. austroadriatica*
(P.2) and *C. pyramidalis* s.str. (P.1). Surprisingly, however, the
geographical borders between the Mediterranean-Submed-
iterranean (*C. austroadriatica*), the continental populations
attributed to the third lineage of *C. pyramidalis* (P.3) in Mon-
tenegro and *C. secundiflora* s.str. (S) found further along in
Serbia, are not clear. Based on the currently known distribu-
tion of the Montenegrin populations, it seems that the conti-
nental border of *C. austroadriatica* in Montenegro stretches
along the Plužine-Šavnik-Nikšić-Podgorica-Ulcinj line. Also,
it seems that the ranges of *C. austroadriatica* and the continen-
tal populations (P.3) converge somewhere towards the end of
the Morača canyon, near Podgorica. Plants with intermediate
morphological characteristics occur in this area, indicating a
possible hybrid swarm.

In urban areas, individuals of *C. austroadriatica* richly
overgrow fortresses and old walls, but in less disturbed areas
its populations occur exclusively in the vegetation of rocky
crevices (Asplenietea rupestris), and rarely in screes (Drypetea
spinosae) on limestone, at altitudes between 0 and ca. 1000 m
a.s.l. Even though we have observed many populations of this
species across its entire range, we did not notice it to enter
the Mediterranean and Submediterranean vegetation of rocky
grounds (Cymbopogono-Brachypodietea) and garrigue (Cisto-
Ericetea), the habitats where typical *C. pyradmidalis* s.str. is
usually found north of the Neretva river.

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Appendix 1. Taxa, authorities, labels for C. *pyramidalis* groups and taxa, DNA extraction numbers, locality from where specimens were collected, latitude/longitude/altitude, voucher information, and GenBank accession numbers for sequences used in this study. Synonyms are provided (in parantheses) when available. DNA extraction numbers are indicated on the phylogenetic networks and trees in the main text and supplemental figures following species names. Latitudes and longitudes are provided in decimal degrees; altitudes are in meters above sea level. GenBank accession numbers are given in the following order: *psbA-trnH*, *psbZ-trnfM*, *trnG-trnS*, ITS (if applicable, multiple clones are separated by forward slash). Asterisks indicate sequences that are not obtained.

Campanula pyramidalis **complex**

C. pyramidalis L.: **P.1** (= *C. pyramidalis* s.str.): **48,** Croatia, Senj, 44.9900/14.9044/25, *Surina & Modrić Surina 144* (NHMR), KC180906, KC192789, KC180808, KC181004; **55,** Slovenia, Ilirska Bistrica, 45.6064/14.2281/500, *Surina 1304* (NHMR), KC180907, KC192790, KC180809, KC181005; **58,** Croatia, Kostrena, 45.2910/14.5360/140, *Surina & Modrić Surina 143* (NHMR), KC180908, KC192791, KC180810, KC181006; **65,** Slovenia, Koper, 45.5231/13.9069/300, *Surina 235* (NHMR), KC180909, KC192792, KC180811, KC181007; **68,** Italy, Trieste, 45.6128/13.8569/60, *Surina 1303* (NHMR), KC180910, KC192793, KC180812, KC181008; **74,** Croatia, Senj, 44.9919/14.9036/30, *Šegota 30836* (ZA), KC180911, KC192794, KC180813, KC181009; **86,** Croatia, Split, 43.5087/16.3991/100, *Ruščić & Radosavljević 30819* (ZA), KC180912, KC192795, KC180814, KC181010; **96,** Croatia, Šibenik, 43.6957/16.0944/250, *Liber & Radosavljević 30830* (ZA), KC180913, KC192796, KC180815, KC181011; **108,** Croatia, Šibenik, 43.7651/15.8567/20, *Milović & Radosavljević 30826* (ZA), KC180914, KC192797, KC180816, KC181012; **127,** Croatia, Trogir, 43.5261/16.2238/5, *Ruščić & Radosavljević 30829* (ZA), KC180915, KC192798, KC180817, KC181013; **160A,** Croatia, Rijeka, 45.3594/14.3117/200, *Lakušić & Lakušić 24054* (BEOU), KC180966, KC192849, KC180868, *; **161,** Croatia, Mt. Velebit, 44.9824/14.9732/630, *Lakušić & Lakušić 24697* (BEOU), KC180916, KC192799, KC180818, KC181014; **162A,** Croatia, Mt. Velebit, 44.9788/14.9863/700, *Lakušić & Lakušić 24698* (BEOU), KC180967, KC192850, KC180869, *; **175,** Croatia, Novi Vinodolski, 45.1287/14.7879/50, *Alegro & al. 30847* (ZA), KC180965, KC192848, KC180867, *; **223,** Croatia, Jablanac, 44.7085/14.9047/100, *Alegro & al. 30854* (ZA), KC180963, KC192846, KC180865, *; **238,** Croatia, Rabac, 45.0821/14.1471/70, *Alegro & al. 30854* (ZA), KC180962, KC192845, KC180864, *; **249,** Croatia, Cres, 44.8748/14.3703/60, *Alegro & al. 30943* (ZA), KC180968, KC192851, KC180870, *; **268,** Croatia, Mošćenička Draga, 45.2412/14.2577/50, *Alegro & al. 30837* (ZA), KC180917, KC192800, KC180819, KC181015/KC181016; **273,** Croatia, Krk, 45.2160/14.5522/10, *Alegro & al. 30844* (ZA), KC180961, KC192844, KC180863, *; **288** (= *C. kapelae* Topić & Ilijanić), Croatia, Mt. Velika Kapela (type locality), 45.2256/14.9442/1200, *Nikolić 30848* (ZA), KC181002, KC192885, KC180904, *; **308,** Croatia, National Park Paklenica, 44.2971/15.4633/100, *Alegro & al. 30833* (ZA), KC180964, KC192847, KC180866, *; **312,** Croatia, Dugi Otok, 44.2971/14.9394/10, *Bogdanović 30843* (ZA), KC180959, KC192842, KC180861, *; Croatia, Mt. Biokovo, 43.2313/17.1091/500, *Liber 30835* (ZA), KC180918, KC192801, KC180820, KC181017; **423,** Croatia, Omiš, 43.4483/16.6902/5, *Surina*

Appendix 1. Continued.

& *Blokar 20* (NHMR), KC180969, KC192852, KC180871, *; **444,** Croatia, Bakar, 45.3073/14.5339/20, *Kovačić 30838* (ZA), KC180919, KC192802, KC180821, KC181018; **468,** Croatia, Klis, 43.5610/16.5346/250, *Ruščić 30824* (ZA), KC180970, KC192853, KC180872, *; **H.93** (= *C. kapelae* Topić & Ilijanić), Croatia, Mt. Velika Kapela (type locality), 45.2256/14.9442/1300, *Kovačić 30850* (ZA), KC181003, KC192886, KC180905, *; ***s.n.***, Mt. Velebit, Croatia, 44.9788/14.9863/700, *Schönswetter & Tribsch 6243* (WU), *, *, *, DQ304606.

P.2 (= ***C. austroadriatica*** D. Lakušić & Kovačić, sp. nov.): **7,** Croatia, Dubrovnik, 42.6403/18.1236/50, *Jasprica 30822* (ZA), KC180971, KC192854, KC180873, *; **25,** Croatia, Dubrovnik, 42.6410/18.1263/50, *Liber & Radosavljević 30822* (ZA), KC180920, KC192803, KC180822, KC181019; **45,** Croatia, Dubrovnik, 42.5933/18.2347/20, *Liber & Radosavljević 30820* (ZA), KC180921, KC192804, KC180823, KC181020; **135,** Montenegro, Mt. Rumija, 42.1552/19.2098/250, *Lakušić 24859* (BEOU), KC180922, KC192805, KC180824, KC181021; **138,** Montenegro, Mt. Rumija, 42.1552/19.2098/250, *Lakušić 24859* (BEOU), KC180923, KC180923, KC192806, KC180825, KC181022/KC181023/KC181024/KC181025; **141, 142,** Montenegro, Virpazar, 42.2592/19.0922/10, *Lakušić 25003* (BEOU), KC180975, KC192858, KC180877, *;**145,** Montenegro, Virpazar, 42.2592/19.0922/10, *Lakušić 25003* (BEOU), KC180924, KC180924, KC192807, KC180826, KC181027; **146,** Montenegro, Virpazar, 42.2592/19.0922/10, *Lakušić 25003* (BEOU), KC180925, KC192808, KC180827, KC181028; **147,** Montenegro, Virpazar, 42.2592/19.0922/10, *Lakušić 25003* (BEOU), KC180926, KC192809, KC180828, KC181029; **158,** Montenegro, Karuč, 42.3553/19.1053/10, *Lakušić 24995* (BEOU), KC180927, KC180927, KC192810, KC180829, KC181030; **163,** Montenegro, Mt. Orjen, 42.4642/18.5572/200, *Lakušić 24860* (BEOU), KC180972, KC192855, KC180874, *; **333,** Croatia, Klek, 42.9503/17.5655/50, *Liber 30834* (ZA), KC180928, KC192811, KC180830, KC181031; **661,** Montenegro, Ulcinj, 41.9541/19.1653/20, *Stevanović & al. 27176* (BEOU), KC180929, KC192812, KC180831, KC181032; **663,** Montenegro, Mt. Durmitor, 43.0093/19.0521/980, *Lakušić 27227* (BEOU), KC180930, KC192813, KC180832, KC181033; **H.45,** Montenegro, Virpazar, 42.2460/19.0926/10, *Lakušić 25002* (BEOU), KC180979, KC192862, KC180881, *; **H.53,** Montenegro, Herceg Novi, 42.4553/18.5339/100, *Jovanović 601/95* (BEOU), KC180976, KC192859, KC180878, *; **H.60,** Montenegro, Herceg Novi, 42.4519/18.5458/100, *Čepić & al. 144/94* (BEOU), KC180981, KC192864, KC180883, *; **H.62,** Montenegro, Kotor, 42.4259/18.7695/20, *Jovanović & Petanović 2331/90* (BEOU), KC180980, KC192863, KC180882, *; **H.64,** Montenegro, Risan (type locality), 42.5140/18.6825/50, *Stevanović 2101/96* (BEOU), KC180982, KC192865, KC180884, *; **H.65,** Montenegro, Budva, 42.2792/18.8358/10, *Jovanović & Petanović 2339/90* (BEOU), KC180978, KC192861, KC180880, *; **H.66,** Montenegro, Bar, 42.0941/19.1361/110, *Jovanović 430/95* (BEOU), KC180974, KC192857, KC180876, *; **H.67,** Montenegro, Bar, 42.0941/19.1361/110, *Jovanović 31/94* (BEOU), KC180973, KC192856, KC180875, *; **H.70,** Montenegro, Mt. Rumija, 42.1552/19.2098/250, *Lakušić 24591* (BEOU), KC180977, KC192860, KC180879, *.

P.3 (= ***C. secundiflora*** Vis. & Pančić, p.p.): **152,** Montenegro, Morača, 42.7431/19.3864/200, *Lakušić 25015* (BEOU), KC180931, KC192814, KC180833, KC181034; **153,** Montenegro, Morača, 42.7431/19.3864/200, *Lakušić 25015* (BEOU), KC180932, KC192815, KC180834, KC181035; **182,** Montenegro, Morača, 42.8014/19.4237/900, *Lakušić 25017* (BEOU), KC180933, KC192816, KC180835, KC181036/KC181037/KC181038; **614,** Montenegro, Morača, 42.7994/19.4173/930, *Lakušić 26622* (BEOU), KC180991, KC192874, KC180893, *; **638,** Montenegro, Mt. Prokletije, 42.5969/19.5745/1550, *Lakušić 27256* (BEOU), KC180988, KC192871, KC180890, *; **649,** Montenegro, Morača, 42.6908/19.3745/100, *Lakušić 26620* (BEOU), KC180992, KC192875, KC180894, *; **659** (= *C. secundiflora* subsp. *montenegrina* R. Lakušić), Montenegro, Sinjajevina, 42.8996/19.2740/1500, *Lakušić 27243* (BEOU), KC180934, KC192817, KC180836, KC181039; **672,** Montenegro, Mt. Prokletije, 42.5820/19.5760/1900, *Lakušić 27259* (BEOU), KC180935, KC192818, KC180837, KC181040; **H.50,** Montenegro, Morača, 42.6943/19.3734/200, *Lakušić 24346* (BEOU), KC180990, KC192873, KC180892, *; **H.69,** Montenegro, Mala Rijeka, 42.5461/19.3778/300, *Stevanović & Bulić 99/86* (BEOU), KC180989, KC192872, KC180891, *; **H.89,** Montenegro, Mt. Prokletije, 42.6036/19.5344/1700, *Džukić 1968/96* (BEOU), KC180993, KC192876, KC180895, *; **H.90** (= *C. secundiflora* subsp. *montenegrina* R. Lakušić), Montenegro, Sinjajevina, 42.8631/19.3822/1900, *Mitrović & Lazarević 20211* (BEOU), KC180994, KC192877, KC180896, *.

C. secundiflora Vis. & Pančić: **S** (= ***C. secundiflora*** Vis. & Pančić s.str.): **185,** Serbia, Panjica (type locality), 43.6618/20.0812/470, *Lakušić 25034* (BEOU), KC180995, KC192878, KC180897, *; **194,** Serbia, Lim, 43.1561/19.7790/500, *Lakušić 25018* (BEOU), KC180936, KC192819, KC180838, KC181041; **202,** Serbia, Mileševka, 43.3591/19.7446/600, *Stevanović & Lakušić 20271* (BEOU), KC180997, KC192880, KC180899, *; **205,** Serbia, Jadovnik, 43.2653/19.7950/1500, *Lazarević 16192* (BEOU), KC180937, KC192820, KC180839, KC181042; **220,** Serbia, Jadovnik, 43.2470/19.7342/650, *Lazarević 16191* (BEOU), KC180938, KC192821, KC180840, KC181043; **600** (= *C. secundiflora* subsp. *limensis* R. Lakušić), Serbia, Lim, 43.1663/19.7704/550, *Lakušić 26624* (BEOU), KC180999, KC192882, KC180901, KC181044/KC181045/KC181046; **H.73,** Serbia, Mileševka, 43.3642/19.7299/700, *Niketić & Tomović 14021* (BEOU), KC180996, KC192879, KC180898, **H.83,** Serbia, Mileševka, 43.3591/19.7446/500, *Lakušić 20806* (BEOU), KC180998, KC192881, KC180900;*; ***s.n.***, Serbia, Mileševka, 43.6678/20.0956, *Zlatković 1109* (ZA), *, *, *, DQ304608.

C. versicolor Sibth. & Sm.: **V: 165** (= *C. plasonii* Formanek), Macedonia, Demir Kapija (type locality), 41.4068/22.2600/150, *Stevanović & al. 25040* (BEOU), KC180960, KC192843, KC180862, KC181047/KC181048; **167** (=*C. versicolor* var. *thessala* Boiss.), Greece, Mt. Olympus, 40.0931/22.3044/1150, *Lakušić & Lakušić 24729* (BEOU), KC180939, KC192822, KC180841, KC181049; **171** (= *C. versicolor* var. *tomentella* Hal.), Macedonia, Veles, 41.6890/21.7981/150, *Stevanović & al. 25035* (BEOU), KC180984, KC192867, KC180886, *; **370** (= *C. tenorii* Moretti), Italy, Tricase, 39.9116/18.3931/30, *Mikac 30840* (ZA), KC180940, KC192823, KC180842, KC181050/KC181051; **391** (= *C. tenorii* Moretti), Italy, Alessano, 39.8607/18.3923/10, *Mikac 30841* (ZA), KC180941, KC192824, KC180843, KC181052; **411** (= *C. plasonii* Formanek), Macedonia, Demir Kapija (type locality), 41.4068/22.2600/150, *Alegro & Cigić 30842* (ZA), KC180942, KC192825, KC180844, KC181053; **415,** Albania, Kukës, 41.9918/20.4233/500, *Rakaj & Surina 1094* (NHMR), KC180943, KC192826, KC180845, KC181054/KC181055/KC181056/KC181057; **481,** Greece, Mt. Giona, 38.6333/22.3768/850, *Lakušić & Lakušić 28090* (BEOU), KC180944, KC192827, KC180846, KC181058; **495,** Greece, Pineios, 39.8781/22.5849/10, *Lakušić & Lakušić 28150* (BEOU), KC180945, KC192828, KC180847, KC181059; **511,** Greece, Mt. Ossa, 39.7933/22.6666/1300, *Niketić & Tomović 27739* (BEOU), KC180946, KC192829, KC180848, KC181060; **521,** Greece, Peloponnese, Langadia, 37.6786/22.0175/850, *Lakušić & Lakušić 28142* (BEOU), KC180947, KC192830, KC180849, KC181061; **536,** Greece, Mt. Tymfi, 39.8621/20.7756/750, *Niketić & Tomović 27894* (BEOU), KC180948, KC192831, KC180850, KC181062/KC181063/KC181064/KC181065; **550,** Greece, Mt. Parnassus, 38.5555/22.5741/1500, *Niketić & Tomović 27700* (BEOU), KC180949, KC192832, KC180851, KC181066; **554** (= *C. versicolor* f. *matkae* Nikolov), Macedonia, Matka (type locality), 41.8808/21.1937/400, *Stevanović 27448* (BEOU), KC180950, KC192833, KC180852, KC181067/KC181068/KC181069/KC181070; **564,** Macedonia, Ohrid, 41.0109/20.8073/700, *Niketić & Tomović 27924* (BEOU), KC180951, KC192834, KC180853, KC181071/KC181072/KC181073; **575,** Macedonia, Veles, 41.6890/21.7981/170, *Stevanović 27450* (BEOU), KC180985, KC192868, KC180887, *; **576,** Macedonia, Mt. Galičica, 40.9639/20.8337/1700, *Lakušić & Lakušić 21848* (BEOU), KC180986, KC192869, KC180888, *; **579** (= *C. versicolor* var. *tomentella* Hal.), Macedonia, Veles, 41.6890/21.7981/170, *Stevanović 27451* (BEOU), KC180952, KC192835, KC180854, KC181074/KC181075/KC181076; **581,** Serbia, Prizren, 42.2007/20.7619/500, *Duraki 28087* (BEOU), KC180953, KC192836, KC180855, KC181077/KC181078/KC181079/KC181080/KC181081; **1663** (= *C. mrkvickana* Vel.), Bulgaria, Zemen (type locality), 42.4715/22.7347/750, *Zallikofov 75227* (SOM), KC180954, KC192837, KC180856, KC181082; **1664,** Greece, Kastoria, 40.4621/21.1379/700, *Papatsou 135684* (SOM), KC180955, KC192838, KC180857, KC181083; **1665** (= *C. mrkvickana* Vel.), Bulgaria, Zemen (type locality), 42.4715/22.7347/750, *Szelag 157675* (SOM), KC180956, KC192839, KC180858, KC181084; **H.13,** Serbia, Prizren, 42.1964/20.6997/450, *Niketić & al. 24520* (BEOU), KC180957, KC192840, KC180859, KC181085/KC181086/KC181087/KC181088/KC181089; **H.26,** Serbia, Mt. Šar Planina, 41.9653/20.7153/1700, *Niketić 2316/91* (BEOU), KC180983, KC192866, KC180885, *; **H.40,** Macedonia, Mt. Galičica, 40.9639/20.8337/1700, *Lakušić & Lakušić 21848* (BEOU), KC180987, KC192870, KC180889, *; **H.98,** Macedonia, Banjani, 42.1288/21.3878/600, *Nikolov 1999* (ZA), KC180958, KC192841, KC180860, KC181090/KC181091/KC181092/KC181093/KC181094; ***s.n.***, Greece, Ionian Islands, 38.7500/20.5900/100, *Gutermann 30067* (WU), *, *, *, DQ304607.

Outgroups: *C. tommasiniana* C. Koch: **1662,** Croatia, Mt. Učka, 45.2928/14.2077/1250, *Surina 1164* (NHMR), KC181001, KC192884, KC180903, *; ***s.n.***, Croatia, Mt. Učka, *Kovačić 775* (ZA), *, *, *, DQ304611. *C. waldsteiniana* Schultes: **1660,** Slovenia, Mt. Snežnik, 45.5675/14.4863/1350, *Surina 1165* (NHMR), KC181000, KC192883, KC180902, *; ***s.n.***, Croatia, Mt. Velebit, *Schönswetter & Tribsch 6202* (WU), DQ304610, *, *, *.