

Campanula Skanderbegii: Molecular and Morphological Evidence of a New Campanula Species (*Campanulaceae*) Endemic to Albania

Author(s): Sandro Bogdanović, Salvatore Brullo, Ivana Rešetnik, Dmtar Lakušić, Zlatko Satovic, and Zlatko Liber

Source: Systematic Botany, 39(4):1250-1260. 2014.

Published By: The American Society of Plant Taxonomists

URL: <http://www.bioone.org/doi/full/10.1600/036364414X682571>

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

Campanula skanderbegii: Molecular and Morphological Evidence of a New *Campanula* Species (Campanulaceae) Endemic to Albania

Sandro Bogdanović,^{1,6} Salvatore Brullo,² Ivana Rešetnik,³ Dmtar Lakušić,⁴ Zlatko Satovic,⁵ and Zlatko Liber³

¹Department of Agricultural Botany, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia.

²Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università degli Studi di Catania, via A. Longo 19, 95125 Catania, Italy.

³Department of Botany and Botanical Garden, Faculty of Science, University of Zagreb, Marulićev trg 9a, 10000 Zagreb, Croatia.

⁴Botanical Institute and Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia.

⁵Department of Seed Science and Technology, Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia.

⁶Author for correspondence (sbogdanovic@agr.hr)

Communicating Editor: Min Feng

Abstract—A new species of *Campanula* from Central Albania, named *C. skanderbegii*, belonging to isophyllous *Campanula* ser. *Garganicae* is illustrated and described. Morphologically and phylogenetically it is well differentiated from other species of this series. It is morphologically similar to *C. portenschlagiana* from Central Dalmatia (Croatia) based on corolla shape. Phylogenetic analyses, maximum parsimony, maximum likelihood, and Bayesian inference, based on nuclear ITS and chloroplast *trnL-trnF* data support *C. skanderbegii* as a clearly distinct taxon within the isophyllous species of *Campanula*. A distribution map for *C. skanderbegii* and a key to all species of *Campanula* ser. *Garganicae* are provided. Because of its rarity and highly restricted distribution, *C. skanderbegii* should be included in the IUCN red list as a critically endangered species.

Keywords—Balkan Peninsula, endemism, isophyllous bellflowers, ITS, phylogeny, *trnL-trnF*.

Campanula L., comprising ca. 580–600 species (Mansion et al. 2012), is the largest genus of Campanulaceae. Its distribution is in the Northern Hemisphere and is represented by annual and perennial taxa growing in various habitats, such as meadows, grasslands, garrigues, woodlands, and often in rocky sites (Kovačić 2004; Roquet et al. 2008). Within the genus, there is high variability in morphology (Kolakovsky 1986; Roquet et al. 2008), carpology (Kolakovsky 1986), palynology (Dunbar 1975; Dunbar and Wallentinus 1976), karyology (Gadella 1964; Damboldt 1965, 1968; Kovanda 1970a, 1970b, 1977; Contandriopoulos 1966) and seed micromorphology (Geslot 1980; Akcin 2009; Alçitepe 2010). Based on literature, the Mediterranean basin is one of the main centres of diversity of the genus, with about 250 species (Damboldt 1965; Podlech 1965; Kovanda 1970a, 1970b, 1977; Geslot 1984; Park et al. 2006). A majority of these species are highly specialized chasmophytes, often with limited geographical distribution.

Since most morphological characters are highly plastic and not suitable to delineate natural groups (Roquet et al. 2008, 2009), the taxonomic treatment of *Campanula* is rather complex. There have been several attempts of classification (De Candolle 1830; Boissier 1875; Gadella 1966a, 1966b; Contandriopoulos 1984; Kolakovsky 1994), but none of them have been confirmed by any phylogenetic studies available to date (Eddie et al. 2003; Park et al. 2006; Roquet et al. 2008, 2009; Borsch et al. 2009; Cellinese et al. 2009; Haberle et al. 2009; Mansion et al. 2012).

In the genus *Campanula*, morphological and molecular results are often in conflict, but some small groups are morphologically and karyologically well differentiated, forming distinct and well supported clades in phylogenetic trees, suggesting their monophyletic origin (Carlström 1986; Runemark and Phitos 1996; Eddie and Ingrouille 1999; Sáez and Aldasoro 2003; Park et al. 2006; Mansion et al. 2012; Lakušić et al. 2013). One of these groups is series *Garganicae* Trinajstić, whose

species are found in the amphi-Adriatic and Ionian territories (see Fig. 1). Although previously defined as a part of the *Isophylla* group (Damboldt 1965), recent phylogenetic studies based on plastid and ITS sequences (Park et al. 2006; Liber et al. 2008; Frajman and Schneeweiss 2009; Bogdanović et al. 2014) have rejected the monophyly of the *Isophylla* group and revealed three well supported clades corresponding to series *Elatines* Trin., ser. *Fragiles* Trinajstić, and ser. *Garganicae* (Lovašen-Eberhardt and Trinajstić 1978). Ten taxa are currently included in ser. *Garganicae*: *C. garganica* Ten., *C. reatina* Lucchese, *C. fenestrellata* Feer, *C. fenestrellata* subsp. *istriaca* (Feer) Federov, *C. portenschlagiana* Roem. & Schult., *C. poscharskyana* Degen, *C. teutana* Bogdanović & Brullo, *C. cephallica* Feer, *C. acarnanica* Damboldt and *C. debarensis* Rech. f. The sister species of ser. *Garganicae* is Albanian endemic *C. comosiformis* (Hayek & Janch.) Frajman & Schneew. (Frajman and Schneeweiss 2009; Bogdanović et al. 2014). Morphologically, these species are characterized by a monopodial growth form, isophyllous and long petiolate leaves, with cordate to ovate blades, elongated and rather unilateral inflorescence, campanulate or rotate corolla, obtuse hairs at the base of filaments, and brown shiny seeds (Damboldt 1965). In contrast, *C. comosiformis* has a slender habit, densely clustered racemose inflorescences, ovate-rhombic leaves, and dentate calyx teeth (Hayek 1921; Frajman and Schneeweiss 2009). The phylogenetic relationships of these species were recently inferred with plastid and ITS sequence data (Park et al. 2006; Frajman and Schneeweiss 2009; Bogdanović et al. 2014), but they remain unclear due to low clade support and conflicting signals between plastid and nuclear data.

During field trips in 2011 and 2012, an unknown population of *Campanula* was found in the town of Krujë, in central Albania. Based on some morphological features (e.g. habit, leaves, inflorescence, and flowers), the specimens belong to ser. *Garganicae*, and appear closely related to *C. portenschlagiana*. On the other hand, they differ from *C. portenschlagiana* in some

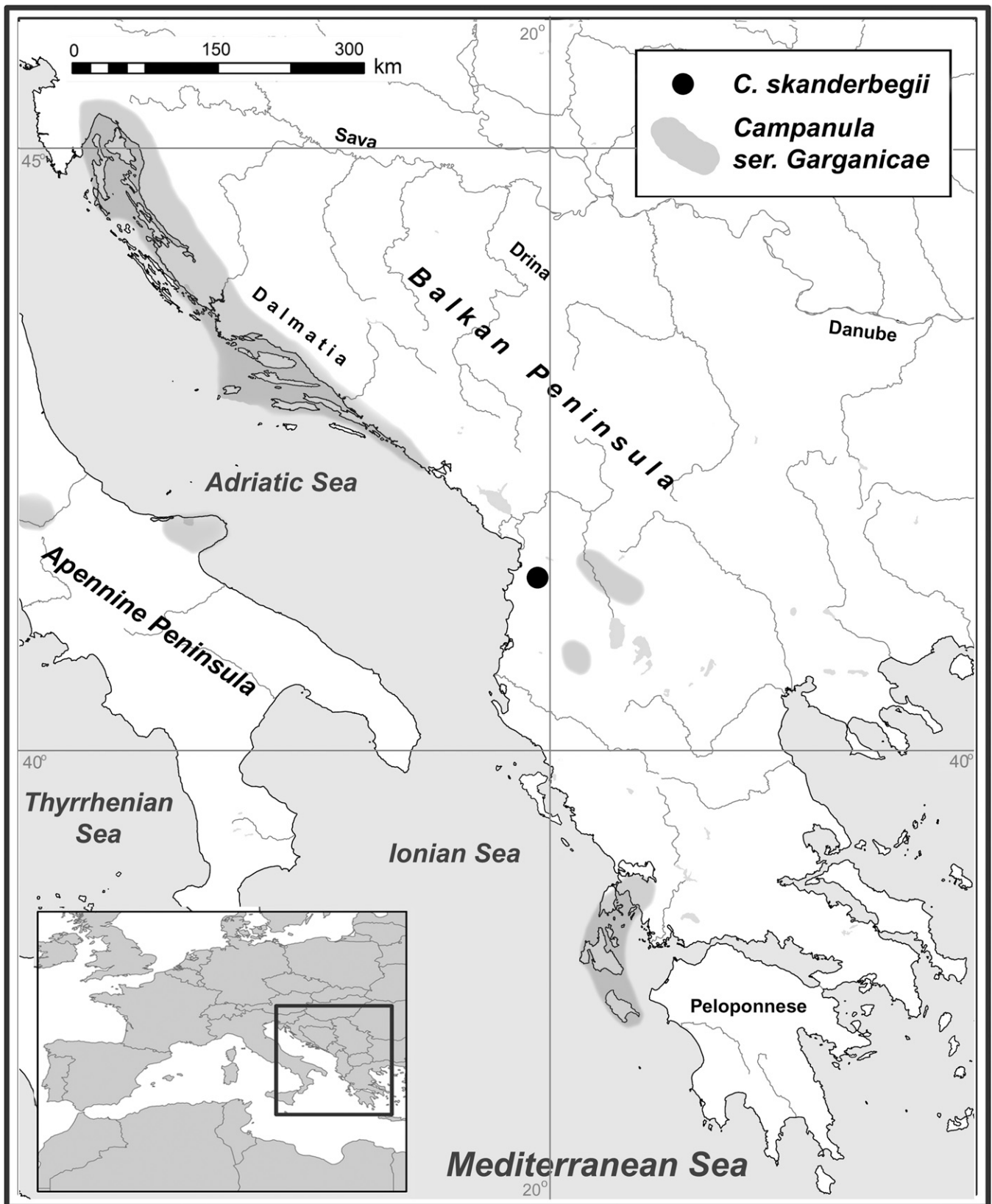


FIG. 1 Distribution map of *Campanula skanderbegii* from Albania, showing the area of distribution of the species of *Campanula* series *Garganicae*.

characters related to indumentum, leaf petiole, calyx, corolla, stamens, pollen colour, capsule, and seeds. In the Albanian flora (Markgraf 1931; Damboldt 1965; Qosja et al. 1996; Park et al. 2006; Frajman and Schneeweiss 2009), ser. *Garganicae* is

represented by *C. debarensis* and a species that is closely related to this group, *C. comosiformis*. However, these species are morphologically well differentiated from the *Campanula* specimens collected in Krujë. To verify the taxonomic position and

phylogenetic relationships of this newly discovered plant population from Albania, we carried out a molecular study using ITS and cpDNA sequences, as well as a morphological assessment of multiple species in *Campanula* ser. *Garganicae* and related groups.

MATERIALS AND METHODS

Plant Material—The investigations were carried out on specimens collected during field trips in Albania, in June 2011 and July 2012. In total, only ten individuals were collected to avoid damage to the current small population in the town of Krujë. The plants of these collections were used for herbarium exsiccata, while fresh leaves were conserved in silica-gel for DNA analysis. Floral and vegetative parts were placed in 50% glycerine-ethyl alcohol solution for further morphological assessment. To obtain a denser sampling in *Campanula* ser. *Garganicae*, we used the data sets of Park et al. (2006), Frajman and Schneeweiss (2009), and Bogdanović et al. (2014), extended with 22 new sequences from additional taxa. Voucher data and GenBank accession numbers of the newly sequenced taxa, as well as GenBank accession numbers from previous studies are given in Appendix 1. Herbarium specimens of isophyllous species of *Campanula* were studied from B, BEOU, BM, CAT, CNHM, MKNH, NHMR, RO, W, WU, ZA, ZAGR, and ZAHO (abbreviations follow Thiers 2013).

Scanning Electron Microscopy (SEM)—The micromorphology of the testa of dried mature seeds was studied using a scanning electron microscope (Zeiss EVO LS10). The preparation of the seeds of *C. skanderbegii* (collected in the wild from Krujë in Albania) and *C. portenschlagiana* (collected in the wild from Biokovo in Croatia) was done according to Huttunen and Laine (1983).

DNA Extraction, Amplification and Sequencing—Total genomic DNA was extracted from silica-gel dried leaves or herbarium specimens using the DNeasy plant mini kit (Qiagen GmbH, Hilden, Germany), following the manufacturer's instructions. The reaction mix for polymerase chain reaction (PCR) of 50 µL contained 25 ng of DNA, 1 × PCR Buffer (TaKaRa Bio Inc., Shiga, Japan), 0.2 mM each dNTP (TaKaRa Bio), 0.2 µM of each primer (17SE and 26SE of Sun et al. (1994) for nuclear ITS; *c* and *f* of Taberlet et al. (1991) for plastid *trnL-trnF*) and 1.25 U of TaKaRa Taq™ HS polymerase (TaKaRa Bio). The PCR conditions for ITS were as described in Park et al. (2006) and for *trnL-trnF* as described in Bogdanović et al. (2014). The PCR reactions were performed using a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, California). The PCR products were purified with GenElute PCR clean-up kit (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) according to the manufacturer's protocol. The products were sequenced by Macrogen Inc. (Seoul, Korea) using the BigDye™ terminator cycle sequencing kit (Applied Biosystems) and analyzed on an ABI PRISM 3730XL automated sequencer (Applied Biosystems). Sequences were edited and manually aligned using Geneious Pro 5.3.6 (Drummond et al. 2011). Sequence alignments are available from TreeBASE (study number S15431).

Phylogenetic Analyses—Three different datasets (ITS, *trnL-trnF*, ITS-*trnL-trnF* combined dataset) were analyzed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). The trees were rooted using *Trachelium caeruleum* L. as an outgroup. This species belongs to the *Campanula* s. s. clade while all other ingroup taxa belong to the *Rapunculus* clade (see Park et al. 2006).

To assess degree of phylogenetic congruence between the two different datasets, an incongruence length difference (ILD) test (Farris et al. 1994) implemented as partition-homogeneity test in PAUP* was performed using 1,000 partition replicates, each comprising 100 random sequence addition replicates, and TBR branch swapping. Invariant characters were removed from the data sets prior to performing the ILD test (Cunningham 1997).

Unweighted MP analyses were conducted using heuristic search, with 1,000 random addition sequence replicates, and tree bisection reconnection (TBR) branch swapping, as implemented in PAUP* 4.0b10 (Swofford 2003). Bootstrap support values (MPB; Felsenstein 1985) from 1,000 replicates were generated using the heuristic search options as above except for random addition sequence with 100 replicates. The scores between 50 and 74 bootstrap percentages were defined as weak support, scores between 75 and 89% MPB as moderate support, and scores above 90% MPB as strong support.

The best-fit substitution models for each region were determined using the Akaike Information Criterion as implemented in MrModeltest (Nylander 2004). MrModeltest retrieved General Time Reversible model

with a gamma distribution (GTR + G) as the most likely evolutionary model for the cpDNA and Symmetrical model with a gamma distribution (SYM + G) for the ITS region. For cpDNA region, GTR models with a gamma distribution (G) or with a proportion of invariable sites (I) or with both parameters (G + I) already had a cumulative Akaike weight of 1.00, while for ITS region, the best model, SYM + G, had an Akaike weight of 0.54 and three models (SYM + I + G, GTR + G, GTR + I + G) were included until the cumulative Akaike weight exceeded 0.95.

Maximum likelihood (ML) analyses were performed using RAxML 7.0.4 (Stamatakis 2006; Stamatakis et al. 2008) assuming the GTR + G model of substitution, GTR models (GTR + G, + I, + I + G) being the only ones implemented in RAxML. We did not use models including both invariable sites and gamma (I + G) due to the strong interaction between these parameters, making it impossible to estimate both parameters reliably (Ren et al. 2005; Yang 2006). The combined dataset was partitioned into two regions (ITS and *trnL-trnF*), with model parameters estimated and optimized individually for each partition. Bootstrap support (MLB) was estimated from 1,000 replicates.

BI was conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The analysis of the combined data set was carried out under partition-specific substitution models as selected for each partition separately using AIC scores in MrModeltest. Thus, all substitution model parameters were allowed to vary across partitions. The Markov Chain Monte Carlo (MCMC) settings consisted of two runs with four chains each for 10⁷ generations, with the sample frequency set to 1,000. The first 2,500 trees (prior to the 2.5 × 10⁶ generation), which was well after the chains had reached stationarity as judged from plots of the likelihood and from the average standard deviation of split frequencies being < 0.01, were discarded as burn-in. Convergence of the MCMC procedure was assessed further by calculating the effective sample sizes (ESS) with the program Tracer ver. 1.4 (Rambaut and Drummond 2007). A majority rule consensus tree was constructed from the posterior set of 15,000 trees.

RESULTS

The characteristics of the cpDNA, ITS and cpDNA-ITS combined datasets analysed using MP, ML and BI are summarized in Table 1. As the ILD test revealed no significant difference ($p = 0.23$) between the selected partitions (cpDNA and ITS), the phylogenetic tree of the combined data set obtained by ML and BI analyses is presented in Fig. 2.

In all three data sets, the *Campanula* individuals from Albania are inferred as distinct members of the Garganica clade (Fig. 2). Specifically, *C. skanderbegii* is resolved as sister to *C. portenschlagiana* with the cpDNA and combined cpDNA-ITS datasets, with low to high support (cpDNA: 0.96 PP, 71 MLB, 54 MPB; cpDNA-ITS: 0.92 PP, no support in ML and MP analyses), or as sister to a clade comprising *C. reatina*, *C. poscharskyana*, *C. acarnanica*, and *C. garganica* (0.88 PP, 62 MLB, 50 MPB) in the ITS phylogeny. Within the Garganica clade all species with the exception of *C. acarnanica* are congruently inferred as monophyletic albeit with partly conflicting relationships between plastid and ITS data. This is not only evident in the position of *C. skanderbegii*, but is also noticeable regarding the position of *C. debarensis*, *C. reatina* and *C. teutana* (Suppl. Figs. 1, 2).

TAXONOMIC TREATMENT

Campanula skanderbegii Bogdanović, Brullo & D. Lakušić, sp. nov. — TYPE: ALBANIA. Krujë, calcareous rocky cliff under the Skanderbeg's castle, N 41.50787°, E 019.79527°, 14 July 2012, S. Bogdanović & M. Jug-Dujaković s. n. (holotype: ZAGR!; isotypes: BEOU! CAT!, ZAGR!).

Campanula portenschlagiana Roem. et Schult. *similaris* sed scapis semper pilosis, folii petiolo usque ad 8 cm longo, dentibus calycinis integris, corolla brevior, extus pilosa,

TABLE 1. Characteristics of cpDNA, ITS and cpDNA-ITS combined datasets analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). CI-consistency index (excluding uninformative characters); RI-retention index.

Region	cpDNA	ITS	Combined
Alignment length	979	731	1,710
Number / % of parsimony-informative characters	118 / 12.05	208 / 28.45	326 / 19.06
Number / length of MP trees	5460 / 276	6 / 583	66 / 864
CI / RI	0.773 / 0.937	0.612 / 0.842	0.652 / 0.875
Maximum likelihood score (ln L) of the ML tree	-3,113,34	-4,120,19	-7,315,90
Substitution model used in BI	GTR + G	SYM + G	GTR + G (cpDNA), SYM + G (ITS)
Harmonic mean of the posterior likelihood scores (ln L) of BI trees	-3,184,43	-4,187,68	-7,379,60
Effective sample size (ESS)	14,260,67	15,373,08	14,961,47

stylo brevior, lamina basali staminorum semicirculari, 0.6–0.8 mm longa, filamentibus staminorum 1.2–1.3 mm longis, anthera albida, brevior, polline albo, capsula obovoidea, dentibus calicis omnino reflexis, seminibus ellipsoideis, minoribus, lucidis, differt.

Plant perennial, densely hairy, with rigid and erect-patent hairs. **Rootstock** woody, branched, covered by leaf remains, with numerous ascending stems, simple or branched at the base. **Stems** herbaceous, 10–30 cm long, leafy, ending in many flowered racemes. **Leaves** arranged in basal rosettes,

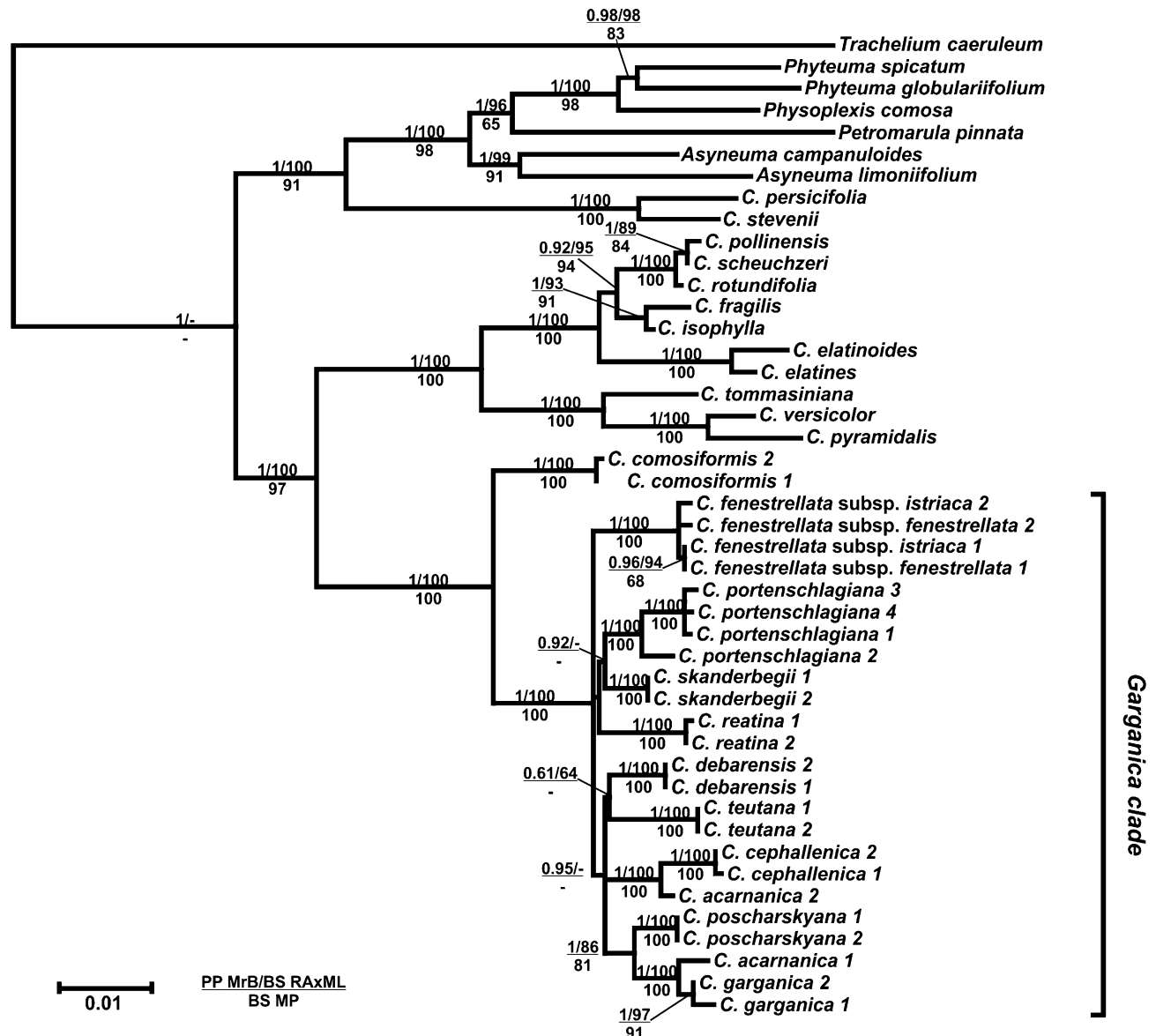


FIG. 2. Maximum likelihood tree based on combined data (ITS + *trnL-trnF*). Values above branches are Bayesian posterior probabilities (PP) / maximum likelihood (MLB) bootstrap percentages and values below branches are maximum parsimony (MPB) bootstrap percentages (only shown if at least 50%).

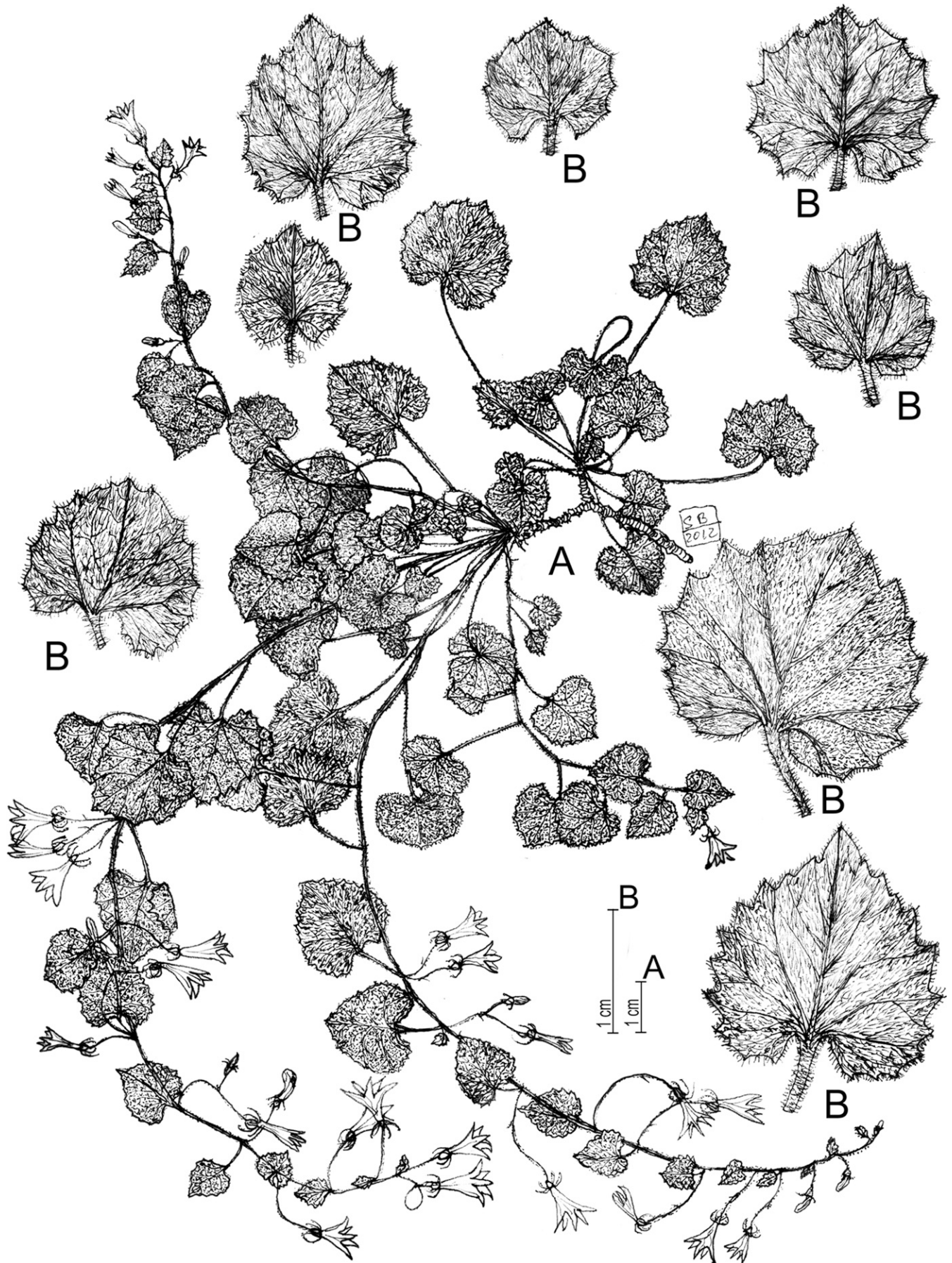


FIG. 3. *Campanula skanderbegii*. A. Habit (CAT!, isotype). B. Leaves. Drawing by Salvatore Brullo.

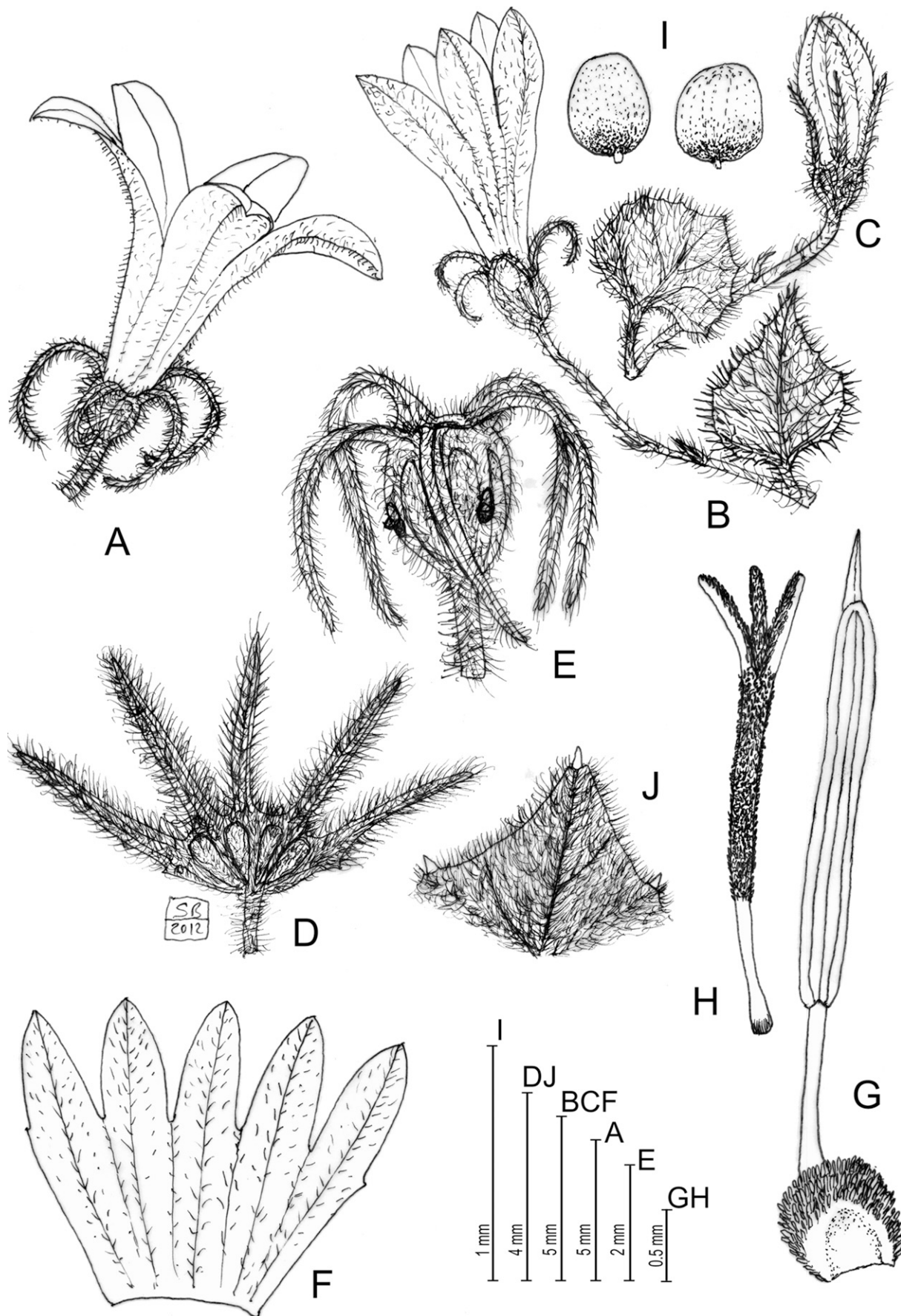


FIG. 4. *Campanula skanderbegii*. A. Flower. B. Flower and bracts. C. Bud and bracts. D. Calyx open (dorsal view). E. Fructiferous calyx and capsule. F. Corolla open (dorsal view). G. Stamen. H. Style and stigma. I. Seeds. J. Leaf apex (CAT!, isotype). Drawing by Salvatore Brullo.

densely covered by rigid hairs, 0.1–0.8 mm long; petiole 1–8 cm long; blade cordate to reniform, pale gray-green, 4–25 × 4–27 mm, cordate at the base, irregularly dentate at the margin (10–22 acute teeth), with palmate to palmate-pinnate venation; cauline leaves similar to the basal, gradually decreasing in size upwards, with petioles 3–30 mm long, blade 4–22 × 4–22 mm. **Inflorescence** a raceme. **Flowers** usually solitary or verticillate (2–3 at leaf axil); pedicel 5–22 mm long, densely hairy, with 1, rarely 2 flowers, with 1–2 bracteoles. **Calyx** green, densely hairy, with teeth entire, linear, 1-nerved, 3–4 × 0.3–0.4 mm, patent to reflexed, acute at the apex. **Corolla** lilac, turning blue-violet by drying, campanulate, 9–11 mm long, 12–14 mm in diameter, glabrous inside, outside densely hairy on the principal veins and sparsely hairy on the tube and lobes; tube subconic, 4.5–6 mm long; lobes 4.5–5 × 2–2.8 mm, ovate-lanceolate, patent-reflexed to completely reflexed, with 1 midrib and various secondary veins, apex subobtusely. **Style** with stigma exerted from corolla tube, 7.5–9 mm long, white on upper and lower parts, pale blue in the middle part, slightly verrucose above, with 3 stigmas, each 1.5–2 mm long. **Stamens** 5; filaments widened at base into a semi-circular blade (or disk), 0.6–0.8 × 0.6–0.8 mm, densely ciliate at the upper and marginal parts; filaments glabrous, slightly violaceous, 1.2–1.3 mm long; anthers white, 3.4–3.5 mm long, apiculate at the apex; pollen white. **Capsule** obovoid, 2.5 mm long, 5-ribbed, densely hairy (hairs 0.2–0.6 mm long), opening by basal pores, with completely deflexed calyx teeth, 5 mm long.

Seeds elliptical-rounded in outline, 0.40–0.45 × 0.35–0.38 mm, yellow-brownish to brown, shiny. Figures 3, 4.

Additional Specimens Examined—ALBANIA. Krujë tundra, stene (*Asplenietea trichomanes*), krečnjak, 574 m n/v, 24 June 2011, *Lakušić et al.* 32470 (BEOU!, ZAGR!).

Seed Micromorphology—Different types of seed micromorphology have been described in Campanulaceae (Geslot 1980; Murata 1992, 1995; Toniuc 1999; Buss et al. 2001; Akcin 2009; Alçitepe 2010). In *Campanula*, the seed coat patterns are either reticulate or striate. Striate testa, of which cells may vary in shape, size and arrangement, are usually characteristic for isophyllus bellflowers (Bogdanović et al. 2014). The seeds of *C. skanderbegii* are well-differentiated from those of *C. portenschlagiana*, its most closely related species: the seeds of the former are ellipsoid with a striate testa characterized by very elongate fibriform cells, slightly raised and anastomosed radial walls, with distinct, wide and rugose lumen, which is essentially linear with fused anticlinal walls (Fig. 5A, B), whereas in the latter, seeds are elongated and flattened, with a markedly striate testa characterized by ever elongate fibriform cells, but well prominent radial walls and deeply incise linear lumen, giving to the surface a cerebriform aspect (Fig. 5C, D).

Etymology—The specific epithet honours George Kastrioti Skanderbeg (1405–1468), national hero of Albania, whose castle stands on the hill where the new species was found.

Phenology—Flowering and fruiting late June to early July.

Distribution and Ecology—*Campanula skanderbegii* is only known from the type locality, inside the town of Krujë in

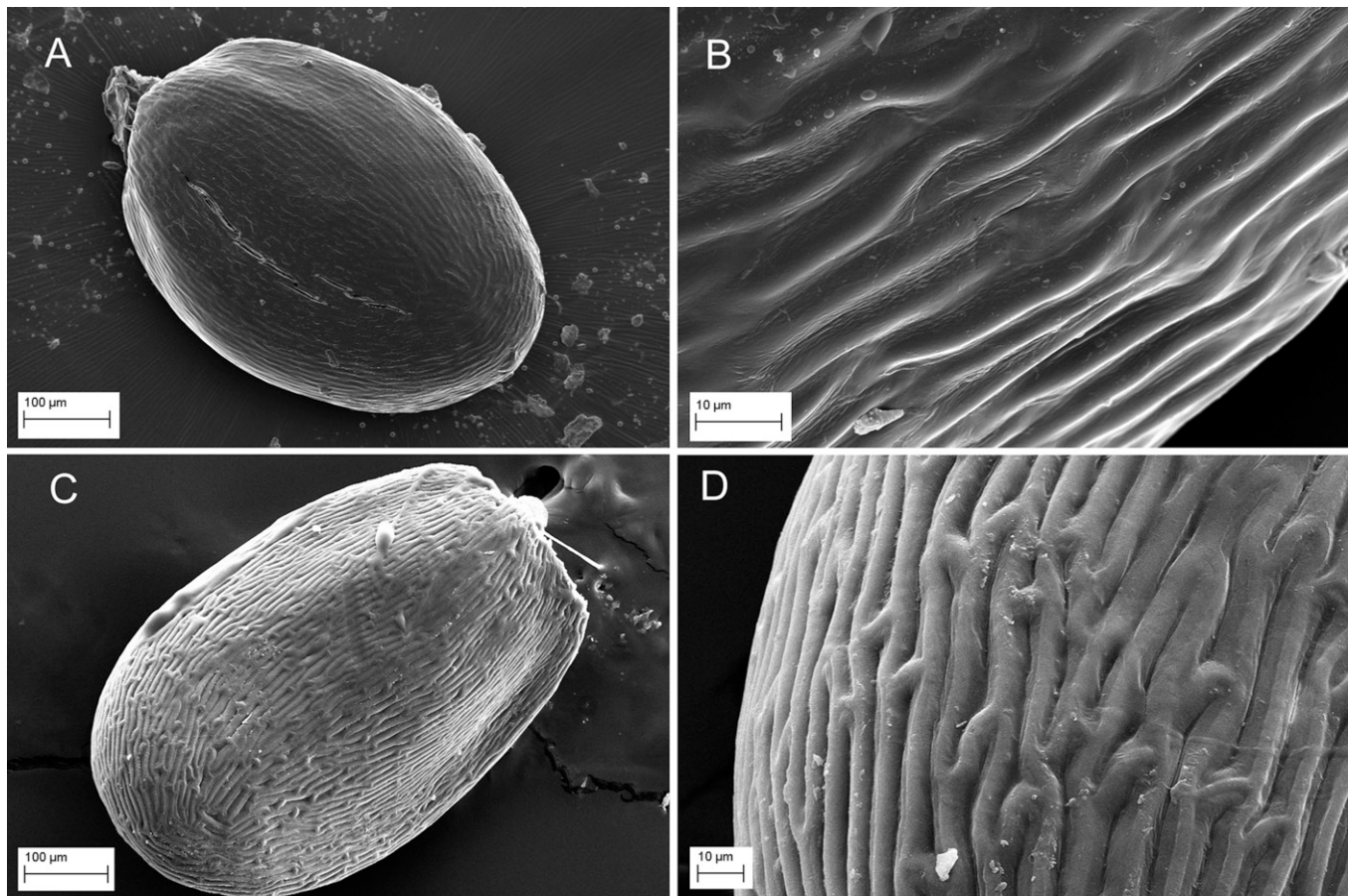


FIG. 5. Scanning electron micrographs of seed surface of *Campanula skanderbegii* (A, B) from CAT! isotype, and *C. portenschlagiana* (C, D) from Dalmatia, Biokovo mountain ZAGR!. A, C – seed (full view). B, D – testa detail.

Central Albania (Fig. 1). It grows in the shade, in small rock cavities with northern exposure, in a calcareous cliff under the Skanderbeg's castle (Fig. 6). It is a typical chasmophyte exclusive of fresher niches of rupestrian community charac-

terized by some rare or endemic species, such as *Campanula versicolor* Sibth. & Sm., *C. ramosissima* Sibth. & Sm., *Moltkia petraea* (Tratt.) Griseb., *Ramonda serbica* Pančić, *Portenschlagiella ramosissima* (Port.) Tutin, *Asperula scutellaris* Vis.,

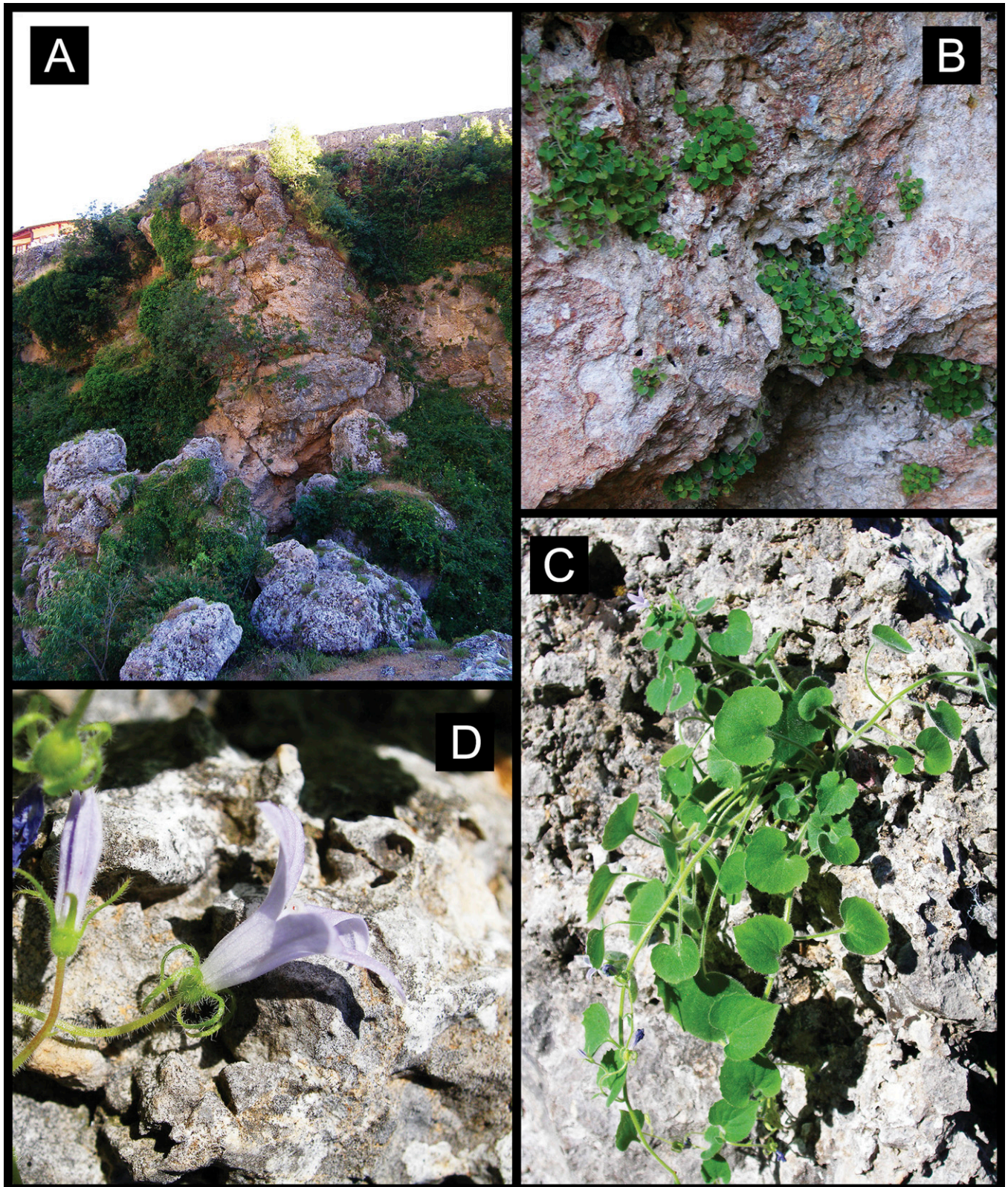


FIG. 6. *Campanula skanderbegii*. A. Habitat. B. Natural population. C. Habit. D. Flower detail. (photos from type locality by Sandro Bogdanović).

Sesleria robusta Schott, Nyman & Kotschy subsp. *skanderbegii* (Ujhelyi) Deyl, and other more or less typical species of plant associations of rocky crevices (*Asplenietea trichomanes* Braun-Blanq.), including *Puttoria calabrica* (L.f.) DC., *Asplenium trichomanes* L., *A. ceterach* L., *Micromeria juliana* (L.) Benth. ex Rechb., *Satureja montana* L., *Aethionema saxatile* (L.) R. Br., *Leontodon crispus* Vill., *Globularia cordifolia* L., and *Hedera helix* L., among others.

Conservation Status—*C. skanderbegii* is only known from a small population (ca. 12,000 sq m, ca. 500 individuals), in peculiar habitat within an urban area. The species was not found during the field investigations of the surrounding mountains and similar limestone cliffs in the region. Therefore, this species is threatened of extinction by human activities and should be included in the IUCN red list of threatened plants as critically endangered – CR B2a(ii, v) (IUCN 2013).

KEY TO THE SPECIES OF *CAMPANULA* SER. *GARGANICAE* (PLUS *C. COMOSIFORMIS*)

1. Corolla campanulate, lobed for 1/2–3/4 its length 2
2. Corolla 9–11 mm long, 12–14 mm in diameter, outside totally hairy; calyx teeth in fruit deflexed; basal blade of stamen filament semi-circular, 0.6–0.8 mm long *C. skanderbegii*
2. Corolla 14–25 mm long; 20–24 mm in diameter, outside glabrous or hairy along the midrib; calyx teeth in fruit erect to patent; basal blade of stamen filament ovate to elliptical, 1.2–1.7 mm long 3
3. Pedicel 5–12 mm long; calyx teeth 5–7 mm long; corolla lobed for 1/2 its length; stamen filaments 2–2.5 mm long; anthers white tinged with pale blue, pollen whitish *C. teutana*
3. Pedicels 12–25 mm long; calyx teeth 3–4 mm long; corolla lobed for 2/3–3/4 its length; stamen filaments 0.8–1 mm long; anthers and pollen yellowish *C. portenschlagiana*
1. Corolla rotate, more or less flattened or infundibular, lobed for 1/6–1/2 its length 4
4. Corolla infundibular, 30–40 mm in diameter; calyx teeth ca. 7 × 3 mm, twice as long as the capsule *C. poscharskyana*
4. Corolla rotate to infundibular, 7–20 mm in diameter; calyx teeth max. 6 × 2 mm, subequal or shorter than capsule 5
5. Pollen yellow; leaves serrate, rarely 2-serrate to dentate 6
6. Corolla lobes linear, 1.5 mm wide, fused near the base *C. comosiformis*
6. Corolla lobes never linear, more than 3 mm wide, with tube 1/4–1/2 as long as corolla 7
7. Leaves reniform to cordate, usually rounded, with margin subentire to weakly crenate; capsule not dehiscent by pores *C. reatina*
7. Leaves ovate to ovate-cordate, acute to obtuse, with margin serrate to dentate, sometimes deeply crenate; capsule dehiscent by pores 8
8. Calyx teeth erect to patent; corolla infundibular, lobed for 1/3–1/2 its length; style hairy up to 2/3 its length *C. acarnanica*
8. Calyx teeth deflexed; corolla rotate to infundibular, lobed for 1/4–1/3 its length; style hairy up to 1/2 its length 9
9. Base of filaments (stamens) with long and acute hairs; anther 3 mm long; calyx teeth up to 1 mm wide; capsule globose, 3 × 2.5 mm, opening by basal pores *C. cephallica*
9. Base of filaments with short and rounded hairs; anther 4 mm long; calyx teeth up to 2 mm wide; capsule flattened, 3 × 4 mm, without pores *C. garganica*
5. Pollen blue; leaves markedly 2-serrate to dentate 10
10. Inflorescence loose; corolla dark blue, glabrous below, with lobes 3 mm wide at the base; calyx teeth deflexed, 0.5 mm wide, longer than capsule *C. debarensis*
10. Inflorescence compact; corolla pale blue, ciliate below, with lobes 3–4 mm long at the base; calyx teeth usually erect, 1.5 mm wide, exceeding a little the capsule 11
11. Plant glabrous, rarely pubescent above, corolla max. 15 mm in diameter; basal leaves with blade max. 4 × 3 cm, 2-serrate or dentate; capsule 2.5 × 3.5 mm *C. fenestrellata* subsp. *fenestrellata*
11. Plant densely tomentose-velvety, corolla up to 20 mm in diameter; basal leaves with blade up to 7 × 4 cm, serrate; capsule 4 × 4.5 mm *C. fenestrellata* subsp. *istriaca*

ACKNOWLEDGMENTS. We thank the curators of the following herbaria B, BEOU, BM, CAT, CNHM, MKNH, NHMR, RO, W, WU, ZA, ZAGR, and ZAHO for the examination of *Campanula* specimens. We also thank Theophanis Constantinidis (Greece) for sending the material of *Campanula acarnanica*, and Marija Jug Dujaković (Croatia), Lulëzim Shuka and Besnik Hallaci (Albania) for assistance in the field. This study was financially supported by project no. 119-1191193-1232 of the Ministry of Science, Education and Sports (Zagreb, Croatia), by the HAZU project “Genetic diversity of Croatian endemic *Campanula*”, and by project no. 173030 of the Serbian Ministry of Science and Technological Development.

LITERATURE CITED

- Akcin, T. A. 2009. Seed coat morphology of some Turkish *Campanula* (Campanulaceae) species and its systematics implications. *Biologia* 64: 1089–1094.
- Alçitepe, E. 2010. Studies on seed morphology of *Campanula* L. section *Quinqueloculares* (Boiss.) Phitos (Campanulaceae) in Turkey. *Pakistan Journal of Botany* 42: 1075–1082.
- Bogdanović, S., S. Brullo, I. Rešetnik, Z. Šatović, and Z. Liber. 2014. *Campanula teutana*, a new isophyllous *Campanula* (Campanulaceae) from the Adriatic region. *Phytotaxa* 162: 1–17.
- Boissier, E. 1875. *Flora Orientalis* vol. 3. Genève and Basel: H. Georg.
- Borsch, T., N. Korotkotova, T. Raus, W. Lobim, and C. Löhne. 2009. The *petD* group II intron as a species level marker: utility for tree inference and species identification in the diverse genus *Campanula* (Campanulaceae). *Willdenowia* 39: 7–33.
- Buss, C. C., T. G. Lammers, and R. R. Wise. 2001. Seed coat morphology and its systematic implications in *Cyanea* and other genera of Lobelioideae (Campanulaceae). *American Journal of Botany* 88: 1301–1308.
- Carlström, A. 1986. A revision of the *Campanula drabifolia* complex (Campanulaceae). *Willdenowia* 15: 375–387.
- Cellinese, N., S. A. Smith, E. J. Edwards, S.-T. Kim, R. C. Haberle, M. Avramakis, and M. J. Donoghue. 2009. Historical biogeography of the endemic Campanulaceae of Crete. *Journal of Biogeography* 36: 1253–1269.
- Contandriopoulos, J. 1966. Contribution à l'étude cytotoxonomique des Campanulacées de Grèce. II. *Bulletin de la Société Botanique de France* 113: 453–474.
- Contandriopoulos, J. 1984. Differentiation and evolution of the genus *Campanula* in the Mediterranean region. Pp. 141–156 in *Plant biosystematics*, ed. W. F. Grant. Toronto: Academic Press.
- Cunningham, C. W. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733–740.
- Damboldt, J. 1965. Zytotaxonomische revision der isophyllen Campanulae in Europa. *Botanische Jahrbucher für Systematik, Pflanzengeschichte und Pflanzengeographie* 84: 302–358.
- Damboldt, J. 1968. Kurzer Nachtrag zur “Zytotaxonomischen Revision der isophyllen Campanulae in Europa”. *Botanische Jahrbucher für Systematik, Pflanzengeschichte und Pflanzengeographie* 88: 200–203.

- De Candolle, A. L. P. 1830. *Monographie des Campanulacées*. Paris: Veuve Desroy.
- Drummond, A. J., B. Ashton, S. Buxton, M. Cheung, A. Cooper, C. Duran, M. Field, J. Heled, M. Kearse, S. Markowitz, R. Moir, S. Stones-Havas, S. Sturrock, T. Thierer, and A. Wilson. 2011. Geneious v5.4. Available from <http://www.geneious.com/>.
- Dunbar, A. 1975. On pollen of Campanulaceae and related families with special reference to the surface ultrastructure. I. Campanulaceae subfam. Campanuloideae. *Botaniska Notiser* 128: 73–101.
- Dunbar, A. and H. G. Wallentinus. 1976. On pollen of Campanulaceae III. A numerical taxonomic investigation. *Botaniska Notiser* 129: 69–72.
- Eddie, W. M. M. and M. J. Ingrouille. 1999. Polymorphism in the Aegean “five-lobuled” species of the genus *Campanula*, section *Quinqueloculares* (Campanulaceae). *Nordic Journal of Botany* 19: 153–169.
- Eddie, W. M. M., T. Shulkina, J. Gaskin, R. C. Haberle, and R. K. Jansen. 2003. Phylogeny of Campanulaceae s.str. inferred from ITS sequences of nuclear ribosomal DNA. *Annals of the Missouri Botanical Garden* 90: 554–575.
- Farris, S. J., M. Källersjö, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. *Cladistics* 10: 315–319.
- Felsenstein, J. 1985. Confidence-limits on phylogenies—an approach using the bootstrap. *Evolution* 39: 783–791.
- Frajman, B. and G. M. Schneeweiss. 2009. A campanulaceous fate: The Albanian stenoendemic *Asyneuma comosiforme* in fact belongs to isophyllous *Campanula*. *Systematic Botany* 34: 595–601.
- Gadella, T. W. J. 1964. Cytotaxonomic studies in the genus *Campanula*. *Wentia* 11: 1–104.
- Gadella, T. W. J. 1966a. Some notes on the delimitation of genera in the Campanulaceae. I. *Proceedings of the Koninklijke Nederlandse Academie van Wetenschappen Series C: Biological and Medical Sciences* 69: 502–508.
- Gadella, T. W. J. 1966b. Some notes on the delimitation of genera in the Campanulaceae. II. *Proceedings of the Koninklijke Nederlandse Academie van Wetenschappen Series C: Biological and Medical Sciences* 69: 509–521.
- Geslot, A. 1980. Le tégument séminal de quelques Campanulacées: étude au microscope électronique à balayage. *Adansonia, ser. 2* 19: 307–318.
- Geslot, A. 1984. *Campanula* L. Pp 123–145 in: *Med-Checklist: A critical inventory of vascular plants of the circum-Mediterranean countries* vol. 1., eds. W. Greuter, H. M. Burdet, and G. Long. Geneva: Conservatoire et Jardin Botanique Ville de Geneve.
- Haberle, R. C., A. Dang, T. Lee, C. Peñafior, H. Cortes-Burns, A. Oestreich, L. Raubeson, N. Cellinese, E. J. Edwards, S.-T. Kim, W. M. M. Eddie, and R. K. Jansen. 2009. Taxonomic and biogeographic implications of a phylogenetic analysis of the Campanulaceae based on three chloroplast genes. *Taxon* 58: 715–734.
- Hayek, A. 1921. Diagnosen neuer von J. Dörfner und H. Zerny in den Jahren 1916 und 1918 in Albanien gesammelter Pflanzenformen. *Österreichische Botanische Zeitschrift* 70: 12–22.
- Huttunen, S. and K. Laine. 1983. Effects of air-borne pollutants on the surface wax structure of *Pinus sylvestris* needles. *Annales Botanici Fennici* 20: 79–86.
- IUCN. 2013. IUCN Standards and Petitions Subcommittee. Guidelines for using the IUCN red list categories and criteria. Version 10.1. Prepared by the Standards and Petitions Subcommittee. Downloadable from <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>.
- Kolakovsky, A. A. 1986. Carpology of the Campanulaceae and problems in their taxonomy. *Botaničeskij Žurnal* 71: 1155–1166.
- Kolakovsky, A. A. 1994. The conspectus of the system of the Old World Campanulaceae. *Botaničeskij Žurnal* 79: 109–124.
- Kovačić, S. 2004. The genus *Campanula* L. (Campanulaceae) in Croatia, circum-Adriatic and west Balkan region. *Acta Botanica Croatica* 63: 171–202.
- Kovanda, M. 1970a. Polyploidy and variation in the *Campanula rotundifolia* complex. Part 1 (General). *Rozprawy Československé Akademie věd a Umeni* 80: 1–95.
- Kovanda, M. 1970b. Polyploidy and variation in the *Campanula rotundifolia* complex. Part 2 (Taxonomic). 1. Revision of the groups *Saxicolae*, *Lanceolatae* and *Alpicolae* in Czechoslovakia and adjacent regions. *Folia Geobotanica et Phytotaxonomica* 5: 171–208.
- Kovanda, M. 1977. Polyploidy and variation in the *Campanula rotundifolia* complex. Part 2 (Taxonomic). 2. Revision of the groups *Vulgares* and *Scheuchzerianae* in Czechoslovakia and adjacent regions. *Folia Geobotanica et Phytotaxonomica* 12: 23–89.
- Lakušić, D., Z. Liber, T. Nikolić, B. Surina, S. Kovačić, S. Bogdanović, and S. Stefanović. 2013. Molecular phylogeny of *Campanula pyramidalis* species complex (Campanulaceae) inferred from chloroplast and nuclear non-coding sequences and its taxonomic implications. *Taxon* 63: 505–524.
- Liber, Z., S. Kovačić, T. Nikolić, S. Likić, and G. Rusak. 2008. Relations between western Balkan endemic *Campanula* L. (Campanulaceae) lineages: Evidence from chloroplast DNA. *Plant Biosystems* 142: 40–50.
- Lovašen-Eberhardt, Ž. and I. Trinajstić. 1978. O geografskoj distribuciji morfoloških karakteristika vrsta serije *Garganicae* roda *Campanula* L. u flori Jugoslavije. *Biosistematika* 4: 273–280.
- Mansion, G., G. Parolly, A. A. Crowl, E. Mavrodiev, N. Cellinese, M. Oganessian, K. Fraunhofer, G. Kamari, D. Phitos, R. Haberle, G. Akaydin, N. İkinci, T. Raus, and T. Borsch. 2012. How to handle speciose clades? Mass taxon-sampling as a strategy towards illuminating the natural history of *Campanula* (Campanuloideae). *PLoS ONE* 7: 1–23.
- Markgraf, F. 1931. Pflanzen aus Albanien (1928). *Denkschriften der Kaiserlichen Akademie der Wissenschaften, Wien. Mathematisch-Naturwissenschaftliche Klasse. Vienna* 102: 317–360.
- Murata, J. 1992. Systematic implications of seed coat morphology in *Lobelia* (Campanulaceae-Lobelioideae). *Journal of the Faculty of Science, University of Tokyo, Section III. Botany* 15: 155–172.
- Murata, J. 1995. A revision of infrageneric classification of *Lobelia* (Campanulaceae – Lobelioideae) with special reference to seed coat morphology. *Journal of the Faculty of Science, University of Tokyo, Section III. Botany* 15: 349–371.
- Nylander, J. A. A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. (Available at: www.abc.se/~nylander/).
- Park, J.-M., S. Kovačić, Z. Liber, W. M. Eddie, and G. M. Schneeweiss. 2006. Phylogeny and biogeography of isophyllous species of *Campanula* (Campanulaceae) in the Mediterranean area. *Systematic Botany* 31: 862–880.
- Podlech, D. 1965. Revision der europäischen und nordafrikanischen Vertreter der Subsect. *Heterophylla* (Wit.) Fed. der Gattung *Campanula* L. *Feddes Repertorium* 71: 50–187.
- Qosja, X., K. Papanisto, J. Vangjeli, and B. Ruci. 1996. *Flore de l’Albanie*. vol. 3. Tirane: Academie des Sciences de la Republique d’Albanie.
- Rambaut, A. and A. J. Drummond. 2007. Tracer v1.4 Available from <http://beast.bio.ed.ac.uk/tracer>.
- Ren, F., H. Tanaka, and Z. Yang. 2005. An empirical examination of the utility of codon substitution models in phylogeny reconstruction. *Systematic Biology* 54: 808–818.
- Ronquist, F. and J. P. Huelsenbeck. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
- Roquet, C., L. Sáez, J. J. Aldasoro, A. Susanna, M. L. Alarcón, and N. Garcia-Jacas. 2008. Natural delineation, molecular phylogeny and floral evolution in *Campanula*. *Systematic Botany* 33: 203–217.
- Roquet, C., I. Sanmartín, N. Garcia-Jacas, L. Sáez, A. Susanna, N. Wikström, and J. J. Aldasoro. 2009. Reconstructing the history of Campanulaceae with a Bayesian approach to molecular dating and dispersal–vicariance analyses. *Molecular Phylogenetics and Evolution* 52: 575–587.
- Runemark, H. and D. Phitos. 1996. A contribution to the distribution of the genus *Campanula* sectio *Quinqueloculares* in Greece. *Botanika Chronika* 12: 5–8.
- Sáez, L. and J. J. Aldasoro. 2003. A taxonomic revision of *Campanula* L. subgenus *Sicyocodon* (Feer) Damboldt and subgenus *Megalocalyx* Damboldt (Campanulaceae). *Botanical Journal of the Linnean Society* 141: 215–241.
- Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771.
- Sun, Y., D. Z. Skinner, G. H. Liang, and S. H. Hulbert. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32.
- Swofford, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates.
- Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Thiers, B. 2013. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. Available from: <http://sweetgum.nybg.org/ih/> (accessed 15 February 2013).

Toniuc, A. 1999. Micromorphological considerations of the seeds surface of some *Campanula* species in Romania. *Revue Roumaine de Biologie. Serie de Biologie Vegetale* 44: 35–41.

Yang, Z. 2006. *Computational Molecular Evolution*. Oxford: Oxford Univ. Press.

APPENDIX 1. Alphabetical list of taxa, collection details, voucher information, and GenBank accession numbers of Campanulaceae species analysed in the present study. In cases where multiple collections were included for one species, they are listed in numerical order following the designations in Fig. 2. GenBank accession numbers are listed in a fixed order (ITS and trnL–trnF). Asterisks indicate sequences published previously in Park et al. (2006), Roquet et al. (2008), Frajman and Schneeweiss (2009), and Bogdanović et al. (2014).

Asyneuma campanuloides Bornm. GEORGIA. Greater Caucasus, *Schönschwetter & Tribsch* 4469 (WU) DQ304586*, FJ426570*. *A. limonifolium* Bornm. GREECE. Ionian Islands: Lefkada, *Gutermann* 35549 (WU) DQ304587*, FJ426571*. *Campanula acarnanica* Damboldt. GREECE. Acarnania, Mt. Boumistos, *Damboldt Ca1* 1058 (B) DQ304598*, KF957744*; GREECE. Acarnania, Mt. Akarnanika Ori, *Karamplianis Th.* 1692 (ATHU) KF957752, KF957763. *C. cephalenica* Feer. GREECE. Ionian Islands: Kefallinia, *Gutermann* 28945 (WU) DQ304597*, FJ426576*; GREECE. Isola Cephalonia, Mt Aivos, *Brullo, S. & Giacalone, G.* (CAT). KF957753, KF957764. *C. comosiformis* (Hayek & Janch.) Frajman & Schneew. ALBANIA. Gjalica, Mustafe, *Bogdanović & Jug Dujaković s. n.* (ZAGR) KF957754, KF957765; ALBANIA. Šija gorge E of Bicaj, *Frajman* 11089 (WU) FJ426592*, FJ426572*. *C. debarensis* Rech. f. FYR MACEDONIA. Crni Drin, *Kovačić* 1097 (ZA) DQ304595*, FJ426575*; FYR MACEDONIA. Crni Drim, *K. Micevski s. n.* (MKNH 031830) KF957738*, KF957745*. *C. elatines* L. ITALY. Alpi Cozie, *Schönschwetter & Tribsch* 6349 (WU) DQ304624*, FJ426577*. *C. elatinoides* Moretti. ITALY. Southern Alps, Lago d'Iseo, *Gutermann* 1879 (WU) DQ304625*, FJ426578*. *C. fenestrellata* Feer subsp. *fenestrellata*. CROATIA. Velebit, Velika Paklenica, *Kovačić* 920 (ZA) DQ304592*, FJ426579*; *C. fenestrellata* subsp. *fenestrellata*. CROATIA. NP Krka, Roški slap, *Šegota & Hršak s. n.* (ZAGR) KF957755, KF957766. *C. fenestrellata* subsp. *istriaca* (Feer) Damboldt. CROATIA. Krk, Uvala Oprna, *Schönschwetter & Tribsch* 6272 (WU) DQ304594*, FJ426584*; CROATIA. Istria: Plomin, *Bogdanović & Ljubičić s. n.* (ZAGR), KF957756, KF957767. *C. fragilis* Cirillo subsp. *fragilis*. ITALY. Calabria: city of Scalea, *Gutermann* 36164 (WU) DQ304626*, FJ426580*. *C. garganica* Ten. cult. in Botanical Garden Zagreb (material from Italy), *Kovačić* 1012

(ZA) DQ304596*, ITALY. Foggia, *Aldobrandi* 12-VII-96 et al. (MA 625685) EF088725*; ITALY. Gargano, Vieste, *Brullo & Signorello s. n.* (CAT 037.237/7) KF957739*, KF957746*. *C. isophylla* Moretti. cult. in Botanical Garden Zagreb (material from Italy), *Kovačić* 1013 (ZA) DQ304630*, FJ426583*. *C. persicifolia* L. AUSTRIA. Northeastern Alps, *Schönschwetter & Tribsch* 6288 (WU) DQ304590*, FJ426573*. *C. pollinensis* Podlech. ITALY. Monte Pollino, *Brullo, Signorello, Spampinato s. n.* (CAT 037.066/30) KF957740*, KF957747*. *C. portenschlagiana* Roem. & Schult. CROATIA. Biokovo, *Kovačić* 692 (ZA) DQ304600*, FJ426587*; CROATIA. otok Brač, Vidova gora, *M. Ruščić s. n.* (ZAGR 26291) KF957741*, KF957748*; BOSNIA AND HERZEGOVINA. Ljubuški, *Šiljeg s. n.* (ZAGR) KF957757, KF957768; CROATIA. Island Hvar, Pitve, *Rimac s. n.* (ZAGR) KF957758, KF957769. *C. poscharskyana* Degen. CROATIA. Dubrovnik region, *Kovačić* 690 (ZA) DQ304601*, FJ426588*; CROATIA. Radovčići, *Kovačić* (ZAGR) KF957759, KF957770. *C. pyramidalis* L. CROATIA. Vratnik pass, *Schönschwetter & Tribsch* 6243 (WU) DQ304606*, CROATIA. Rijeka, *Vitek* 99440 (MA 641379) EF088754*. *C. reatina* Lucchese. ITALY. Turano Valley, *Kovačić* 768 (ZA) DQ304599*, FJ426589*; ITALY. Valle del Salto, Ponte Figureto, *Kirin s. n.* (ZAGR) KF957760, KF957771. *C. rotundifolia* L. CROATIA. Platak – Rijeka region, *Kovačić* 784 (ZA) DQ304615*, ANDORRA. Sáez 6134 (BCB), EF088759*. *C. skanderbegii* Bogdanović, Brullo & D. Lakušić. ALBANIA. Krujë, *Lakušić, Kuzmanović, Lazarević, & Alegro s. n.* (ZAGR) KF957761, KF957772; ALBANIA. Krujë, *Lakušić, Kuzmanović, Lazarević, & Alegro s. n.* (ZAGR) KF957762, KF957773. *C. scheuchzeri* Vill. CROATIA. North Velebit, *Kovačić* 807 (ZA) DQ304614*, KF957749*. *C. stevenii* Bieb. GEORGIA. Minor Caucasus, *Schönschwetter & Tribsch* 6976 (WU), DQ304591*, ARMENIA. Vayk, *Oganessian s. n.* (ERE 154865), EF088770*. *C. teutana* Bogdanović & Brullo. CROATIA. Island of Vis, Oključina, calcareous cliffs near Kraljičina špilja, *S. Bogdanović s. n.* (ZAGR 32628) KF957742*, KF957750*; CROATIA. otok Vis, Oključina, *S. Bogdanović s. n.* (ZAGR) KF957743*, KF957751*. *C. tommasiniana* Koch. CROATIA. Učka, *Kovačić* 775 (ZA) DQ304611*, FJ426590*. *C. versicolor* Andrews. GREECE. Ionian Islands: Kefallinia, *Gutermann* 30067 (WU) DQ304607*, FJ426591*. *Petromarula pinnata* DC. GREECE. Crete, *Schönschwetter & Tribsch* 7821 (WU) DQ304582*, FJ426585*. *Physoplexis comosa* Schur. ITALY. Southern Alps, *Schönschwetter & Tribsch* 3902 (WU) DQ304585*, FJ426586*. *Phyteuma globulariifolium* Sternb. & Hoppe. AUSTRIA. Niedere Tauern, *Schönschwetter & Tribsch* 4551 (WU) DQ304583*, FJ426582*. *P. spicatum* L. CROATIA. Gorski Kotar, *Schönschwetter & Tribsch* 6233 (WU) DQ304584*, SPAIN. Barcelona: Aiguafreda, *Roquet* 8-V-05 (BC), EF088787*. *Trachelium caeruleum* L. SPAIN. N of Malaga, *Schönschwetter & Tribsch* 8736 (WU) DQ304570*, SPAIN. Santander, Liencres, *Aldasoro* 3503 (MA) EF088791*.