



## *Campanula teutana*, a new isophyllous *Campanula* (Campanulaceae) from the Adriatic region

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### Abstract

The Balkan Peninsula and the amphi-Adriatic region are biodiversity centres with a high number of *Campanula* species, some of them with restricted and isolated distributions. Among those is the morphologically, karyologically and genetically well-defined lineage of isophyllous *Campanula* species of the *garganica* clade, taxonomically treated as *Campanula* ser. *Garganicae*. In the central Adriatic, on the Island of Vis (Croatia), an isophyllous *Campanula* was found growing in rocky crevices of calcareous sea cliffs among chasmophytic vegetation with extremely challenging ecological conditions. Based on morphological characters (monopodial growth form, cordate to ovate basal leaves, campanulate corolla, presence of obtuse hairs at the base of filaments, capsule opening by basal pores and brown shiny seeds) the unidentified plant from the Island of Vis falls into ser. *Garganicae*. To establish the phylogenetic position and relationships of the unknown *Campanula* among other species of the *garganica* clade, nuclear ITS and chloroplast *trnL-F* sequences as well as comparative morphology, karyology and scanning electron microscopy of seeds were used. Due to its campanulate corolla the plants from Vis are morphologically close to *C. portenschlagiana* from the eastern Adriatic, from which they differ in several quantitative floral characters. The karyological analysis revealed a diploid chromosome number ( $2n = 34$ ), while SEM analysis showed that seed coat of new taxon has weakly striate testa, thin wavy striped radial walls and slightly marked lumen. Additionally, molecular data show that the Vis taxon is clearly separated as a distinct species from other species of ser. *Garganicae*. Therefore, it is described and illustrated as a new species, *Campanula teutana* Bogdanović & Brullo. Because of the small number of individuals within the single population, *C. teutana* should be included on the Red List of the vascular flora of Croatia as an endangered species (EN).

**Key words:** *Campanula*, Croatia, narrow endemic species, morphology, phylogeny, taxonomy

### Introduction

The genus *Campanula* Linnaeus (1753: 163) (Campanulaceae) is distributed in extra-tropical territories of the northern hemisphere and includes from 420 to 600 species (Shulkina *et al.* 2003, Lammers 2007, Mansion *et al.* 2012). Many species (ca. 250) are localized in the Mediterranean area (Greuter *et al.* 1984), usually linked to rupestrian habitats and characterized by very specialized edaphic or microclimatic conditions. Molecular phylogenetic studies have revealed that *Campanula*, as currently delimited: (1) is not monophyletic, (2) has unresolved phylogenetic relationships with several closely related genera, and (3) that the majority of *Campanula* species constitute two main groups named the *Campanula* s.str. clade and the *Rapunculus* clade (Eddie *et al.* 2003, Park *et al.* 2006, Roquet *et al.* 2008, Mansion *et al.* 2012, Lakušić *et al.* 2013).

The Western Balkan and amphi-Adriatic regions are rich in endemics (Kryštufek & Reed 2004, Park *et al.* 2006, Kučera *et al.* 2008, 2010, Stefanović *et al.* 2008, Frajman & Schneeweiss 2009, Kolarčik *et al.* 2010, Lakušić *et al.* 2013). These include several endemic *Campanula* species from the Apennine and the Balkan Peninsula,

which were included by Damboldt (1965, 1968) in the *Isophylla* group. Recent phylogenetic studies have shown that all species of the *Isophylla* group are included within the *Rapunculus* clade, but do not constitute a monophyletic group (Park *et al.* 2006, Liber *et al.* 2008, Frajman & Schneeweiss 2009). They are divided into three clades: *fragilis*, *elatines* and *garganica*. These clades consist of species with similar morphology, mainly allopatric distribution and two diploid chromosome numbers:  $2n = 32$  in the *fragilis* clade and  $2n = 34$  in the *garganica* and in the *elatines* clades (Damboldt 1965, 1968, Lucchese 1993).

Phylogenetic and biogeographic studies based on nuclear ITS and chloroplast *trnL-F* sequences (Park *et al.* 2006, Liber *et al.* 2008, Frajman & Schneeweiss 2009) revealed that the *garganica* clade is phylogenetically more distinct and comprises taxa with amphi-Adriatic and Ionian distribution. The *garganica* clade (=ser. *Garganicae*) includes *C. reatina* Lucchese (1993: 265) and *C. garganica* Tenore (1827: 203) from central Italy, *C. fenestrellata* Feer subsp. *fenestrellata* (1890: 272), *C. fenestrellata* Feer subsp. *istriaca* (Feer) Damboldt (1965: 349), *C. portenschlagiana* Roemer & Schultes (1819: 93) and *C. poscharskyana* Degen (1908: 103) from the east Adriatic coast and islands, *C. cephalenica* Feer (1890: 273) and *C. acarnanica* Damboldt (1965: 341) from the Ionian islands and West Greece, *C. debarensis* Rechinger f. (1939: 175) from Macedonia and a sister species *C. comosiformis* (Hayek & Janch.) Frajman & Schneeweiss (2009: 599) from Albania. The phylogenetic relationships within the *garganica* clade, with low support for some clades and conflicting relations between the nuclear and plastid data, suggest that vicariance as well as incomplete lineage sorting and hybridization are present in this group (Park *et al.* 2006). Molecular dating and diversification analyses of the isophyllous *Campanula* species, i.e. representatives of the *garganica* clade, reveal a late Pliocene – Pleistocene origin for the group (Park *et al.* 2006, Frajman & Schneeweiss 2009).

The isolated central Adriatic islands and islets of the Vis Archipelago (Vis, Biševo, Svetac, Brusnik, Jabuka, Kamik, Sušac and Palagruža) have suitable conditions for specific morphological and genetic variation as well as differentiation from other plant populations (Nikolić *et al.* 2008), e.g. from the Croatian continental island system (Kučera *et al.* 2010, Surina *et al.* 2011). Although all Adriatic islands have ca. 40 narrow endemic taxa and compared to the rest of the Mediterranean have a lower level of endemism (Nikolić *et al.* 2008, Nikolić 2013) the number of endemic taxa is increasing and new species belonging to genera *Allium* Linnaeus (1753: 294), *Puccinellia* Parlato (1850: 366), *Campanula* Linnaeus (1753: 163), *Viola* Linnaeus (1753: 933) and *Cardamine* Linnaeus (1753: 654) were recently described from the Adriatic islands and the adjacent costal parts (Bogdanović *et al.* 2008, 2009, 2012, Kučera *et al.* 2010, Mered'á 2011, Lakušić *et al.* 2013). Overall the highest number of endemic species is found on remote central Adriatic islands and islets.

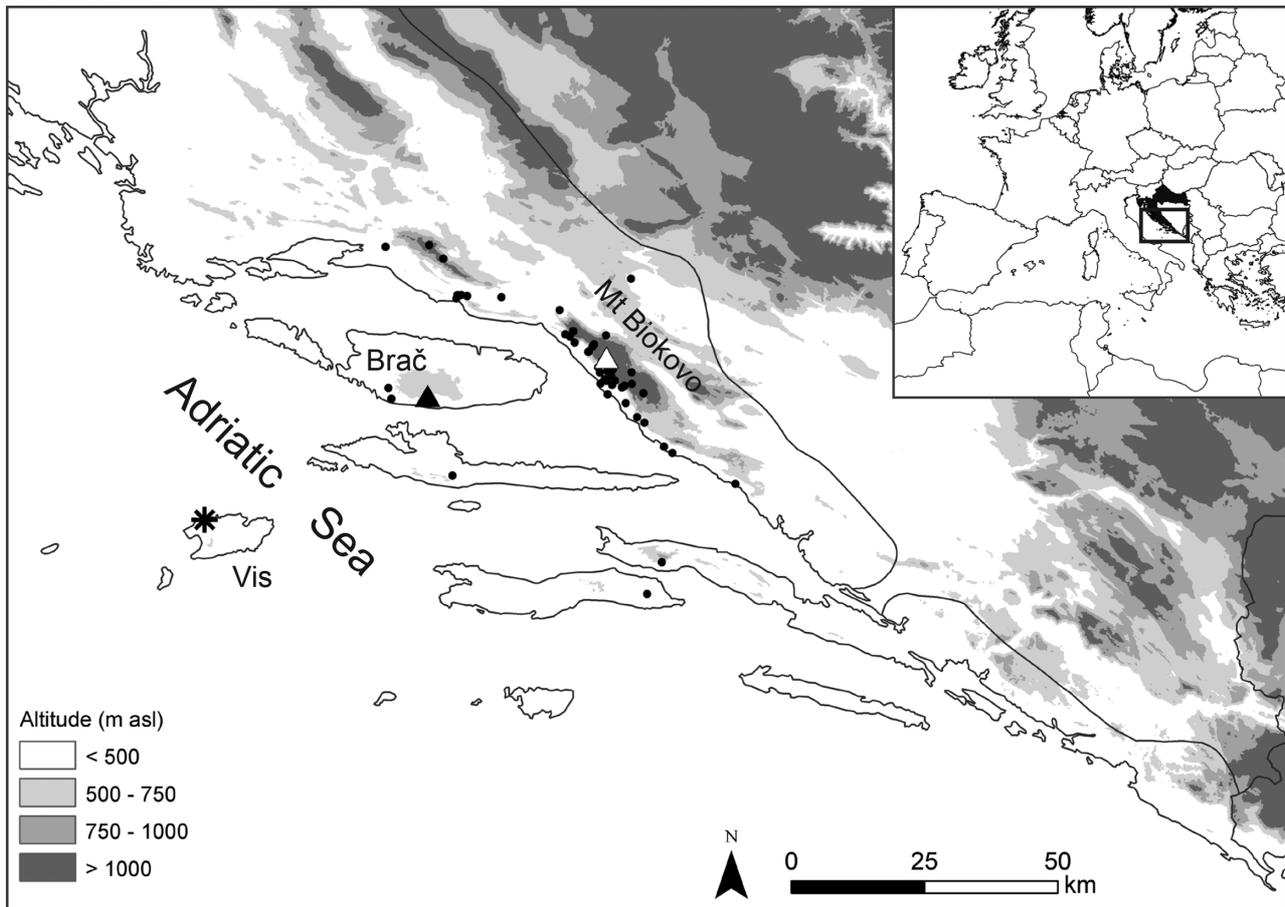
During an autumnal floristic survey on the Island of Vis, a population of *Campanula* sp. with approximately 250 individuals was found, which were morphologically similar to, but still distinct from, the Eastern Adriatic endemic *C. portenschlagiana*, belonging to the amphi-Adriatic isophyllous *Campanula* ser. *Garganicae*. In order to assess its phylogenetic position and relationships, we included two individuals from the Vis population in the broad datasets of nuclear ITS and plastid *trnL-F* sequences of the *garganica* clade and performed phylogenetic analyses. In addition, we compared its morphological features and chromosome number to other isophyllous *Campanulas*.

## Materials and methods

**Plant material:**—Plant material of the unidentified *Campanula* species was collected in October 2009, and April to May 2010 (Fig. 1). Five living plants were cultivated in the Botanical Garden in Zagreb for karyological studies. For morphological analyses only 10 specimens from the wild were used to avoid any negative impact on the species' small population size. To obtain a denser sampling in the *garganica* clade (ser. *Garganicae*) we used the published sequences of Park *et al.* (2006) and Frajman & Schneeweiss (2009) extended with 14 new sequences. Voucher information and GenBank accession numbers of the newly sequenced taxa as well as the GenBank accession numbers from previous studies are given in Appendix 1. Herbarium specimens of isophyllous *Campanula* species were studied from B, BEOU, BM, CAT, CNHM, MKNH, NHMR, RO, W, WU, ZA, ZAGR and ZAHO.

**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  $\mu$ L contained 25 ng of DNA, 1 x PCR Buffer (TaKaRa Bio Inc., Shiga, Japan), 0.2 mM each dNTP (TaKaRa Bio Inc., Shiga, Japan), 0.2

$\mu\text{M}$  of each primer (17SE and 26SE of Sun *et al.* (1994) for nuclear ITS; c and f of Taberlet *et al.* (1991) for chloroplast *trnL-F*) and 1.25 U of Tag<sup>TM</sup> HS polymerase (TaKaRa Bio Inc., Shiga, Japan). PCR conditions for amplification of ITS were as described in Park *et al.* (2006), and for *trnL-F*, they were: 3 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, 2 min at 72°C, followed by a final 10 min extension period at 72°C. The PCR reactions were performed using a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). The PCR products were purified with GenElute PCR Clean-up Kit (Sigma-Aldrich, Steinheim, Germany) according to the manufacturer's protocol. The products were sequenced by Macrogen Inc. (Seoul, Korea) using an ABI PRISM 3730XL automated sequencer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned using ClustalX 2.0.12 (Larkin *et al.* 2007) and adjusted manually in BioEdit 7.0.5.3 (Hall 1999).



**FIGURE 1.** Distribution map and sample sites. Distribution of *Campanula portenschlagiana* (●), Sample sites of *C. portenschlagiana* var. *pumila* (▲), *C. portenschlagiana* var. *hirsuta* (△) and of *C. teutana* (\*) used in this study.

**Phylogenetic Analyses:**—Three different datasets (ITS, *trnL-F* and ITS-*trnL-F* combined dataset) were analyzed using Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian Inference (BI). The trees were rooted using *Trachelium caeruleum* Linnaeus (1753: 171) as outgroup. This species belongs to the *Campanula* s.str. clade while all other ingroup taxa belong to the *Rapunculus* clade (see Park *et al.* 2006).

To assess the degree of phylogenetic congruence between the two different datasets, an Incongruence Length Difference (ILD) test (Farris *et al.* 1994) implemented in PAUP\* was performed using 1,000 partition replicates, each comprising 100 random sequence additions, 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 the heuristic search options with 1,000 random addition sequence replicates and tree bisection reconnection (TBR) branch swapping algorithm, 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 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-fitting substitution models for each data partition were determined using the Akaike Information Criterion as implemented in MrModelTest (Nylander 2004). The resulting best fitting models were a symmetrical model with gamma distribution accounting for rate heterogeneity (SYM+G) for ITS and the general time reversible model with a gamma distribution (GTR+G) for *trnL-F*. While for *trnL-F*, general time reversible models with a gamma distribution or with a proportion of invariable sites or with both parameters had a cumulative Akaike weight of 1.00, model uncertainty for ITS region was somewhat higher and included four models (SYM+G, SIM+I+G, GTR+G, GTR+I+G) until the cumulative Akaike weight exceeded 0.95. Therefore, we used the GTR+G for both data partitions, subsuming the proportion of invariable sites under the gamma distribution accounting for rate heterogeneity (Ren et al., 2005; Yang, 2006).

ML analyses were performed using RAxML 7.0.4 (Stamatakis *et al.* 2006) assuming the GTR+G model of substitution. The combined dataset was partitioned into two regions (ITS and *trnL-F*), 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 & Huelsenbeck 2003). The analysis of the combined data set was carried out using GTR+G models for both partitions with parameter values being estimated separately for each partition during the analyses.

The Markov Chain Monte Carlo settings consisted of four runs with four chains each (three heated ones using the default heating scheme) for  $6 \times 10^6$  generations sampling every 1,000<sup>th</sup> generation, using default priors and estimating all parameters during the analysis. The first 25% trees were discarded as burn in, which was well after the chains had reached stationarity. A majority rule consensus tree was constructed from the posterior set of 18,000 trees showing the posterior probabilities (PP) of all observed bi-partitions. We considered PP values below 0.95 to be weak, values between 0.95 and 0.98 to be moderate, and values above 0.99 to be strong (Alfaro and Holder, 2006).

**Karyological analyses:**—Chromosomes were counted from mitotic divisions in root meristems from five plants cultivated in the Botanical Garden in Zagreb. Root tips were pre-treated with 0.002 M hydroxyquinoline for 4 h at 18°C, fixed overnight at 4°C in a freshly prepared mixture of ethanol and glacial acetic acid (3:1), stained in a drop of 1% acetocarmine and squashed in a drop of 45% acetic acid (Damboldt 1965, Kovanda 1970a, 1970b). Metaphase handling and chromosome counting were conducted with an Olympus BX 51 microscope with a Quick Photocamera 2.3.

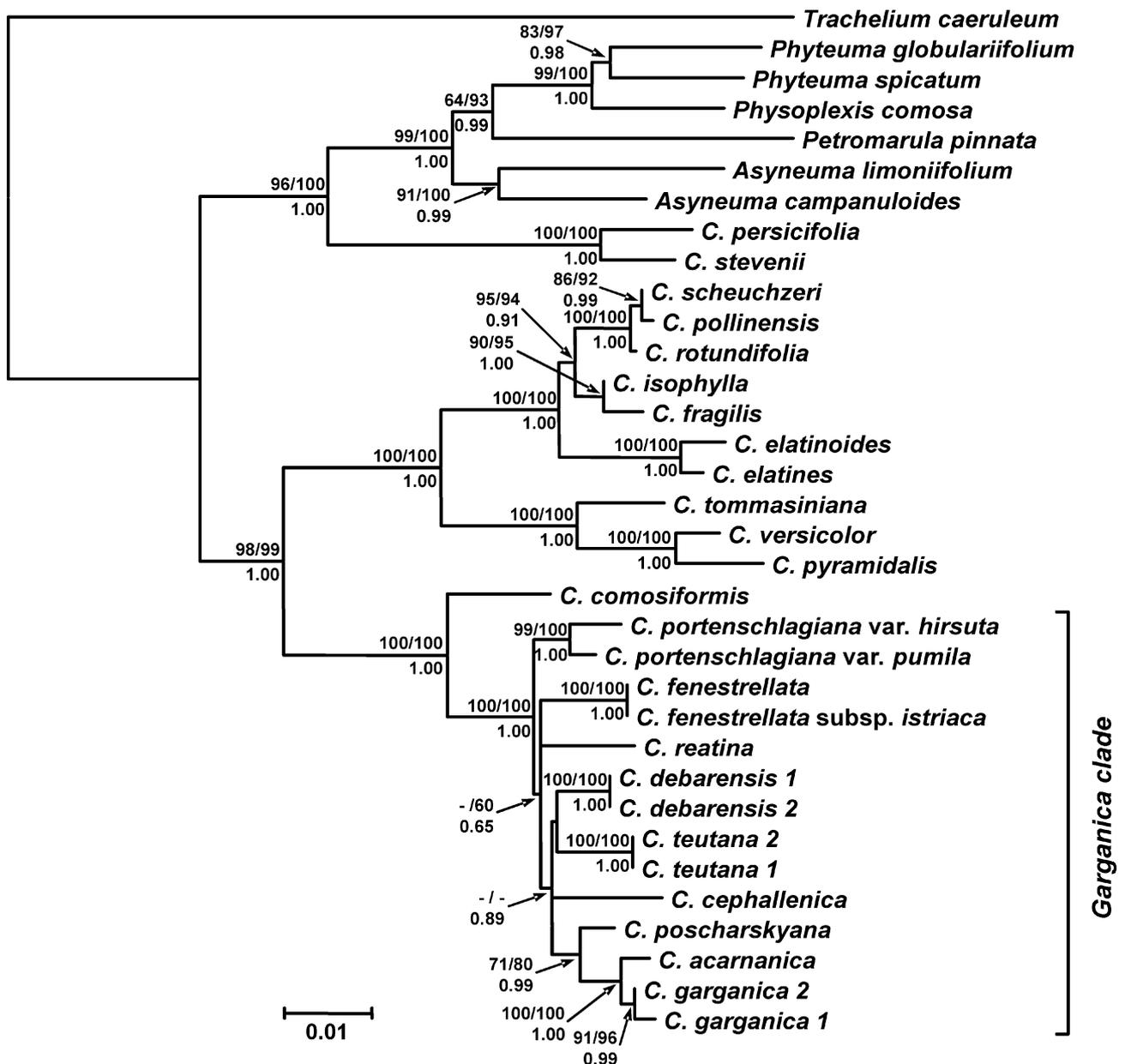
**Scanning electron microscopy:**—The micro-morphology of the seed coat was studied using 50 dried mature seed material from capsules collected in the wild, with a scanning electron microscope (Zeiss EVO LS10). The preparation of the seeds of *C. teutana* and *C. portenschlagiana* was done according to Huttunen & Laine (1983).

## Results

**Phylogeny:**—The descriptors of ITS, *trnL-F* and ITS-*trnL-F* combined datasets, MP tree statistics and the evolutionary models used are summarized in Table 1. The ILD test revealed no significant difference ( $P = 0.518$ ) between the partitions defined by the two regions. MP, ML and BI analyses recovered topologies that were mutually congruent and therefore only the ML topologies are presented in Figs. 2–4.

**TABLE 1.** Characteristics of ITS, *trnL-F* and ITS-*trnL-F* combined datasets and the results of phylogenetic analyses. CI—consistency index excluding uninformative characters; RI—retention index. Substitution model proposed by MrModelTest and used in Bayesian Inference.

Region	ITS	trnL-F	Combined
Alignment length	728	976	1704
Number / % of parsimony-informative characters	195 / 26.8	105 / 10.8	300 / 17.6
Number / length of MP trees	1 / 575	300 / 274	7 / 852
CI / RI	0.604 / 0.829	0.759 / 0.924	0.643 / 0.859
Substitution model	SYM+G	GTR+G	SYM+G (ITS), GTR+G ( <i>trnL-F</i> )



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

The *Campanula* individuals from the Island of Vis were clearly resolved as distinct members of the *garganica* clade (Figs. 2–4) albeit with unresolved relationships to other members of the clade. In all datasets, *C. comosiformis* is highly supported (MPB 98–100%, MLB 100%, PP 1.00, Figs. 2–4) as sister to the *garganica* clade (*sensu* Park *et al.* 2006). Within the *garganica* clade, all species form highly supported branches (MPB 71–100%, MLB 96–100%, PP 0.99–1.00, Fig. 2) but without support for internal clusters. Only *C. garganica* and *C. acarnanica* formed a maximally supported clade (MPB 100%, MLB 100%, PP 1.00, Fig. 2) that is sister to *C. poscharskyana* (MPB 71%, MLB 80%, PP 0.99, Fig. 2). Moreover, relationships of the species within the *garganica* clade are to some extent incongruent between the ITS and *trnL-F* tree (Figs. 3–4). For example, *C. reatina* is sister to the clade comprising *C. poscharskyana*, *C. garganica* and *C. acarnanica* in the ITS tree (MPB 53%, MLB 73%, PP 0.99; Fig. 3), while in the *trnL-F* tree its place is occupied by *C. cephallica*, *C. debarensis* and *C. teutana* (MPB 74%, MLB 87%, PP 0.99; Fig. 4).

**Karyology:**—The investigated *Campanula* from the Island of Vis has a diploid chromosome number ( $2n = 34$ ), which is in accordance with the previously published data for all other *Campanula* taxa of the ser. *Garganicae* (Damboldt 1965, 1968, Lucchese 1993).

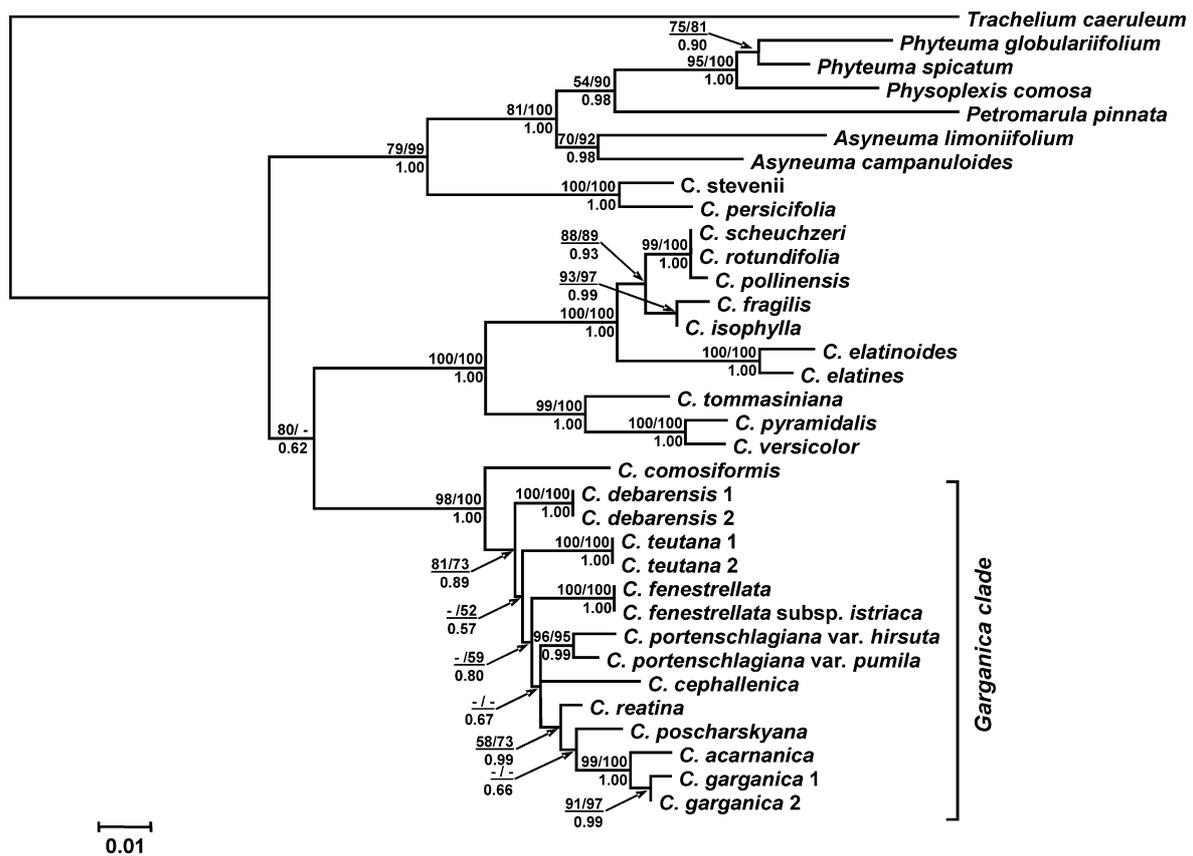


FIGURE 3. Maximum likelihood tree based on ITS data. Values above branches are maximum parsimony/maximum likelihood bootstrap percentages (only shown if at least 50%) and values below branches are Bayesian posterior probabilities.

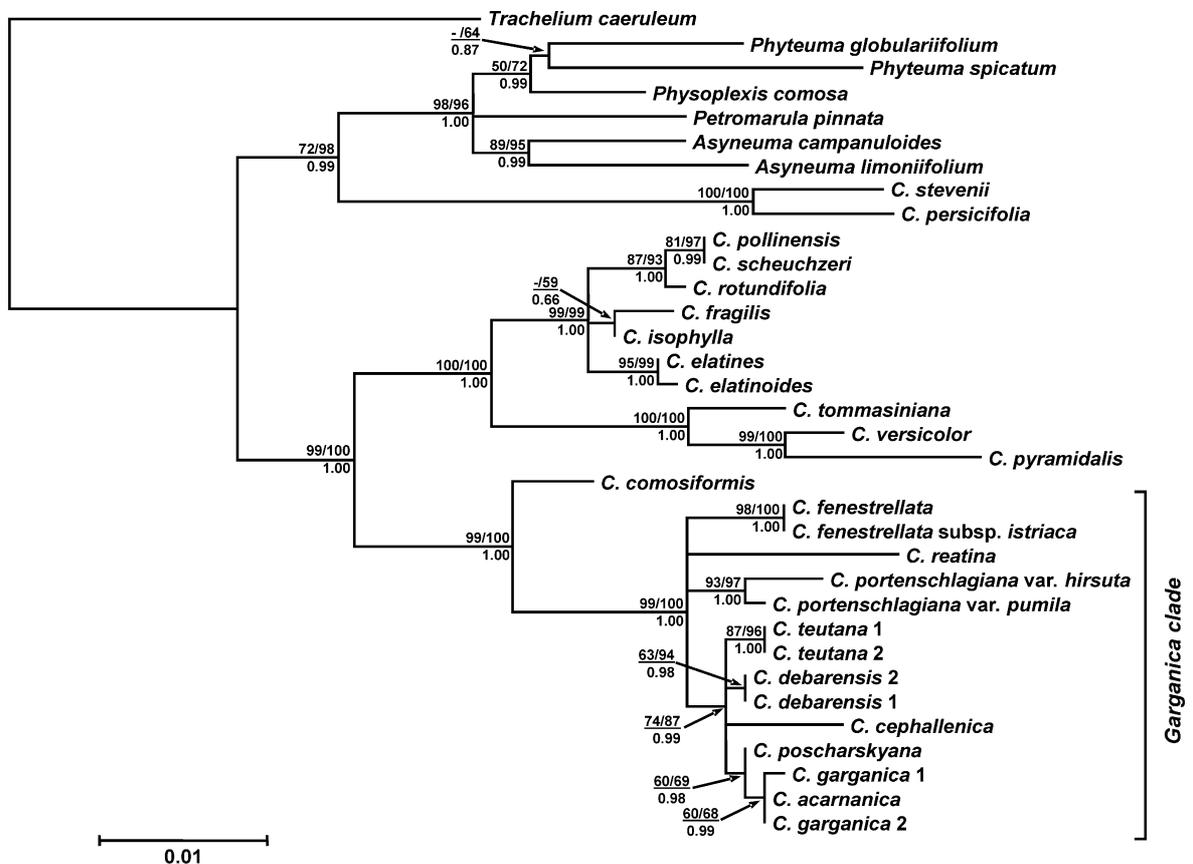
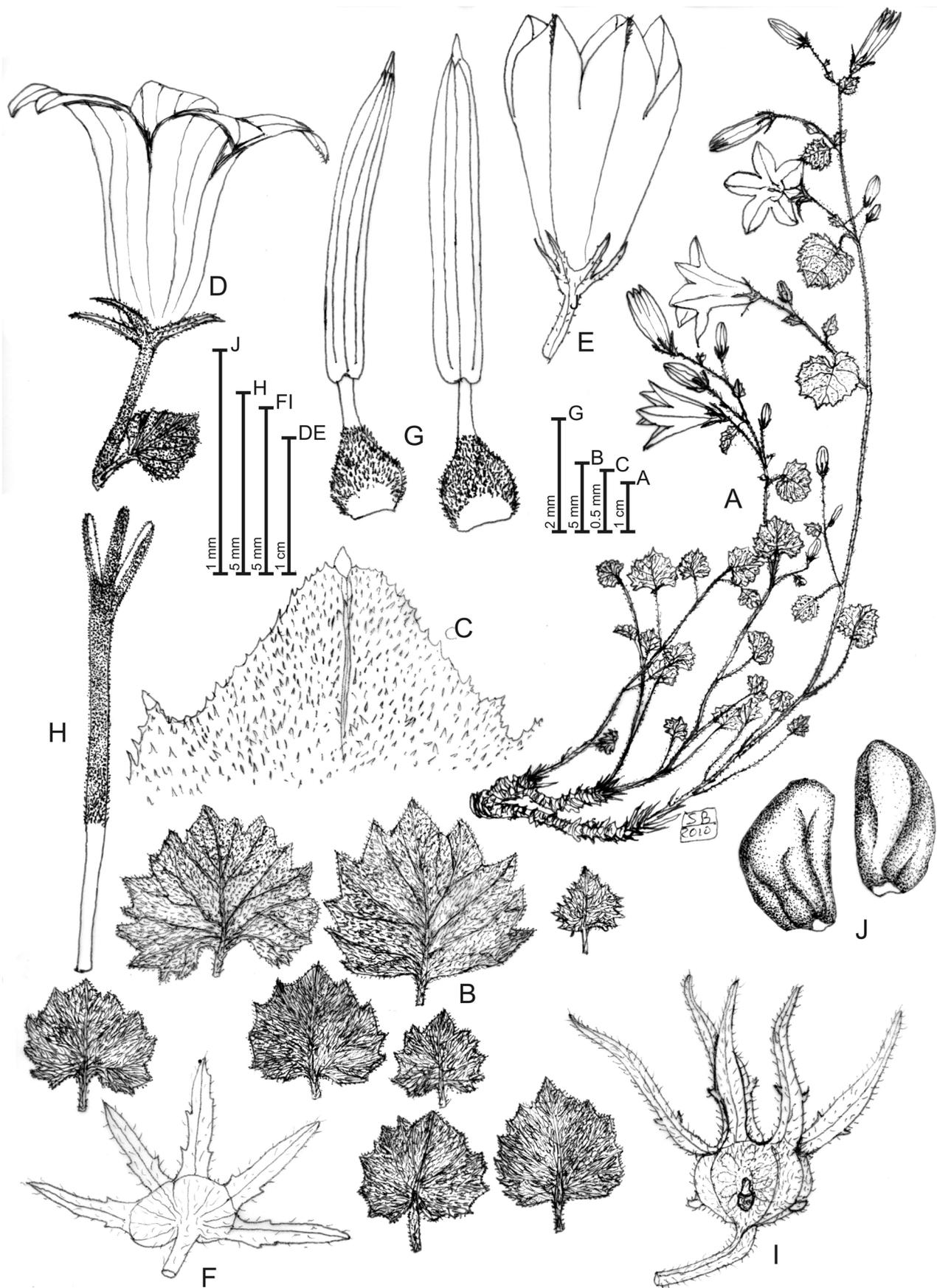
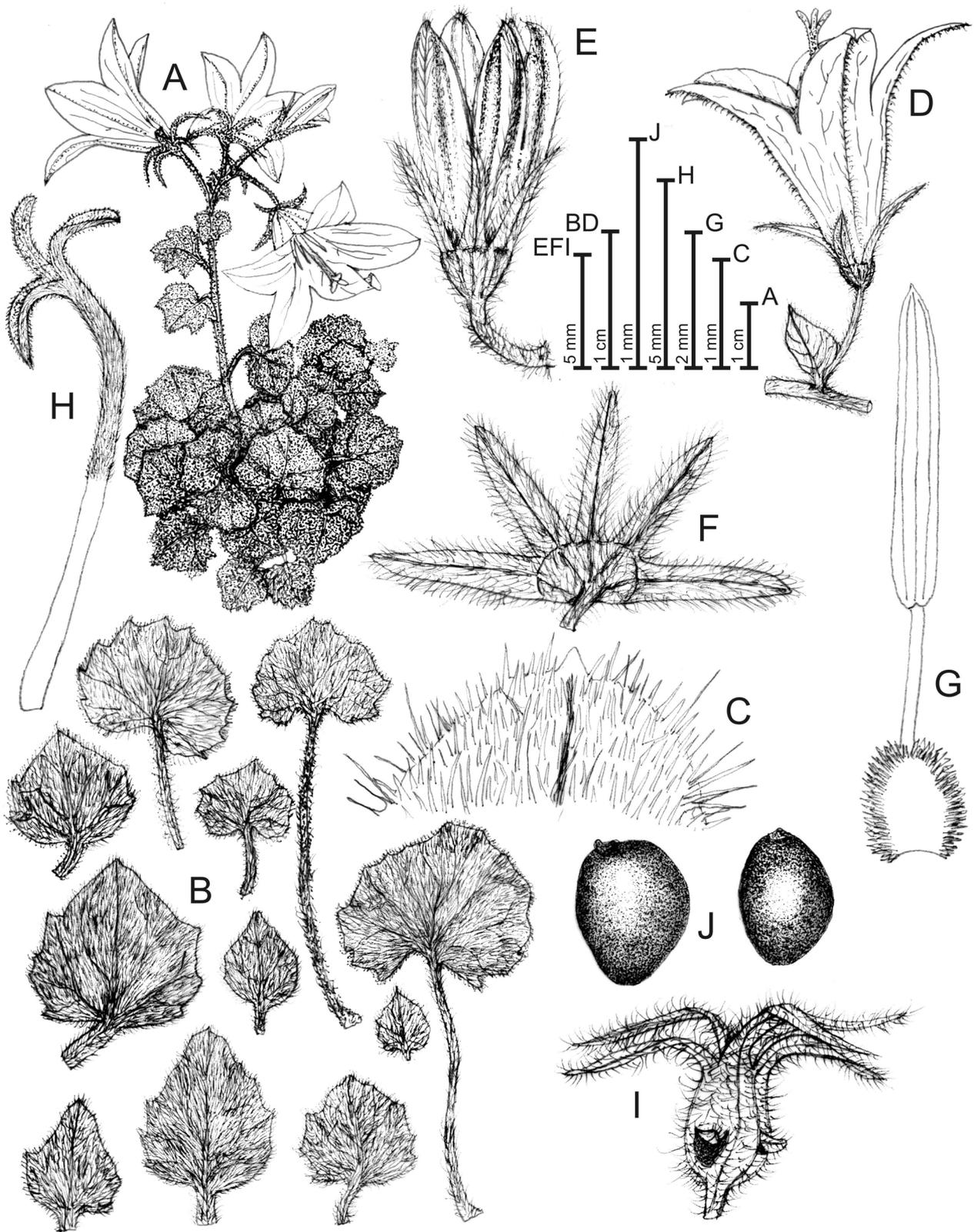


FIGURE 4. Maximum likelihood tree based on *trnL-F* data. Values above branches are maximum parsimony/maximum likelihood bootstrap percentages (only shown if at least 50%) and values below branches are Bayesian posterior probabilities.



**FIGURE 5.** *Campanula portenschlagiana* var. *hirsuta*. A, habit; B, cauline leaves; C, leaf apex; D–E, flower; F, calyx; G, stamen; H, stylus; I, capsule; J, seeds. Drawn by Salvatore Brullo.

**Seed micromorphology:**—The study of the seed coat revealed that *C. teutana* has ovoid seeds with a weakly striate testa, thin wavy striped radial walls and slightly marked lumen (Fig. 7 C–D), while *C. portenschlagiana* has irregular and flattened seeds, showing distinctly striate testa with prominent anastomosed radial walls and distinct linear lumen (Fig. 7 A–B).



**FIGURE 6.** *Campanula teutana*. A, habit; B, cauline leaves; C, leaf apex; D–E, flower; F, calyx; G, stamen; H, stylus; I, capsule; J, seeds. Drawn by Salvatore Brullo.

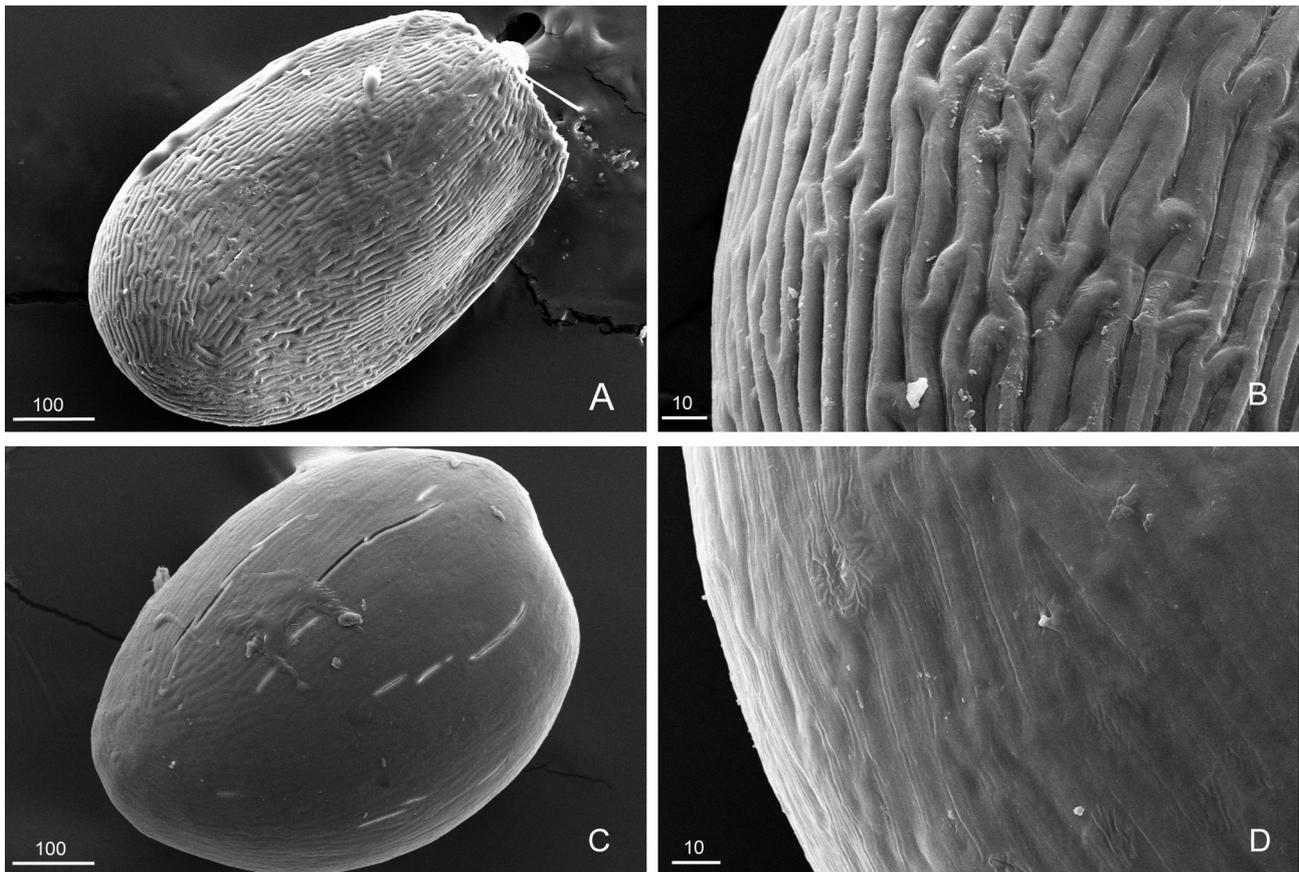


FIGURE 7. Scanning electron micrographs of *Campanula* seeds and detail of testa. A, B—*C. portenschlagiana*; C, D—*C. teutana*.

## Taxonomic Treatment

### *Campanula teutana* Bogdanović & Brullo, *sp. nov.* (Figs. 6, 8)

*A Campanula portenschlagiana* scapis usque ad 10 cm longis, semper pilosis, foliis basalibus crenato-dentatis margine, dentibus obtusis, petiole 1–5 cm longo, lamina cordata, 9–13 mm longa, 12–16 mm lata, inflorescentia semper simplice, plerumque 4-flora, pedicello florali 5–12 mm longo, dentibus calycinis integris, 5–7 mm longis, semper pilosis, corolla max. 20 mm longa, tubo aequanti lobis, extus pilosa in 5-nervis principalibus, stylo 12–15 mm longo, piloso in dimidio superiore, lamina basali staminorum elliptica, 1.5–1.7 mm longa, dense ciliata margine, ciliis 0.3 mm longis, filamentibus staminorum 2–2.5 mm longis, anthera ex coeruleo alba, breviter apiculata apice, polline albo differt.

**Type:**—CROATIA. Island of Vis, Oključina: calcareous cliffs near Kraljičina špilja, 23 May 2010, S. Bogdanović *s.n.* (holotype ZAGR!, isotypes CAT!, ZA!, ZAGR!).

Plant perennial, densely hairy, with patent and rigid hairs. Rootstock woody, shortly branched, covered by leaf remains, with numerous ascending stems, simple or branched at the base. *Stems* herbaceous, up to 10 cm long, leafy, ending in usually 4-flowered racemes. *Basal leaves* are arranged in rosettes, densely covered by rigid hairs 0.1–1 mm long, with 1–5 cm long petioles and cordate blade, dark green, 9–13 mm long, 12–16 mm wide, irregularly dentate-crenate at the margin (2–8 obtuse teeth), with venations pinnate to pinnate-radiate. *Cauline leaves* similar to the basal ones, gradually decreasing upwards, with petioles 1–10 mm long, and blade cordate to ovate, 4–12 mm long, 3–12 mm wide, irregularly dentate-crenate to subentire at the margin. *Floral pedicel* 5–12 mm long, densely hairy. *Calyx teeth* entire, linear-lanceolate, 1-nerved, green-purplish, densely hairy, 5–7 mm long, 1.2–1.5 mm wide, patent to reflexed, acute at the apex. *Corolla* lilac-blue, campanulate, 16–20 mm long, 22–24 mm in diameter, glabrous with hairs only outside along the 5 principal veins, tube subconic 9–10 mm long, lobes 8–10 mm long, 6–7 mm wide, ovate-lanceolate, reflexed, with 1 midrib and 2 secondary veins, apex obtuse. *Style* not exerted from corolla, 12–15 mm long, white below and pale blue above, hairy on the upper half, with 3 stigmas 2.5 mm long. *Stamens* 5; basal blade elliptical, 1.5–1.7 mm long, 1–1.2 mm wide, densely ciliate at the

margin, with cilia 0.3 mm long; filaments glabrous, 2–2.5 mm long, anthers white, tinged with pale blue, 4.5–5 mm long, apiculate at the apex; pollen whitish. *Capsule* ovoid, 5 mm long, 5-ribbed, densely hairy (hairs 0.5–1.5 mm long), opening by basal pores, with patent calyx teeth, 7–8 mm long. *Seeds* ovoid, 0.5–0.6 mm long, dark brown, shiny. Chromosome number  $2n = 34$ .

**Paratypes:**—CROATIA. Otok Vis, Oključina, stijene iznad Kraljičine špilje, 10.X.2009, S. Bogdanović s.n. (ZAGR!); Otok Vis, Oključina, Kraljičina špilja, okomite stijene kod ulaza u špilju, 26.IV.2010, S. Bogdanović s.n. (ZAGR!).

**Etymology:**—Named after Teuta, Illyrian queen who governed from 231–228 BC. Inhabitants of the Island of Vis dedicated a cave to queen Teuta. The new taxon was found in the vicinity of the cave.

**Distribution and Ecology:**—*Campanula teutana* is known only from the Island of Vis in Central Dalmatia, where it grows on northern slopes near the village Oključina. In this locality it is very rare, occurring exclusively in the calcareous rocky crevices of sea cliffs and particularly in shadier and moister niches, usually covered by a dense moss layer. This species is a true chasmophyte and is a member of a rupestrian community characterized by endemic species such as *Limonium subanfractum* Trinajstić (1980: 6) and associated with *Brassica incana* Tenore (1811–1815: 39), *Adiantum capillus-veneris* Linnaeus (1753: 1096), *Erica multiflora* Linnaeus (1753: 355) and *Valantia muralis* Linnaeus (1753: 1051).

**Conservation:**—At present, *Campanula teutana* occurs only on one site of the northern slope of the Island Vis. This is a threatened species at risk of extinction with a small population which comprises a small number of individuals (less than 250) restricted to shady niches of cliffs close to the sea. Most individuals grow on inaccessible rock faces. Based on the criteria adopted by IUCN (2010), the species should be included in the Red List of the vascular flora of Croatia as endangered (EN).



**FIGURE 8.** Habit of *Campanula teutana* from type locality (photo: S. Bogdanović, May 23, 2010).

## Discussion

Based on morphological features (monopodial growth form, cordate to ovate blades of basal leaves, campanulate corolla, presence of obtuse hairs at the base of filaments and brown shiny seeds, Figs. 5–6) and on molecular data (Figs. 2–4), the individuals occurring on the Island of Vis clearly represent a distinct entity that belongs to the monophyletic *garganica* clade of Park *et al.* (2006). When comparing molecular data and morphology, the inferred relationships between the taxon from the Island of Vis and other species within the *garganica* clade are partially incongruent. Morphologically the Vis taxon mostly resembles *C. portenschlagiana*, a relationship not evident from molecular phylogenetic results (Figs. 2–4). Specifically, *C. portenschlagiana* (here represented with var. *hirsuta* and var. *pumila*) constitute a distinct phylogenetic lineage (MPB 100%, MLB 100%, PP 1.00, Fig. 2), while the Vis taxon is grouped with *C. debarensis*, *C. cephallica*, *C. poscharskyana*, *C. acarnanica* and *C. garganica* in a distinct lineage with unresolved internal relationships (MPB and MLB <50%, PP 0.89, Fig. 2). Recent phylogenetic analyses based on ITS and *trnL-F* data (Park *et al.* 2006, Frajman & Schneeweiss 2009) supported the recognition and taxonomic treatment of *C. cephallica*, *C. acarnanica* and *C. debarensis* at the specific level. Consequently, analogous to conclusions of Park *et al.* (2006) and Frajman & Schneeweiss (2009) as well as the distinct morphological features and the geographical isolation, the individuals from the Island of Vis may be treated taxonomically as an independent species. Dispersal-vicariance analysis and molecular dating of the ser. *Garganicae* (*garganica* clade *sensu* Park *et al.* 2006) suggests two dispersals across the Adriatic and the diversification of the clade probably in the late Miocene to Pleistocene (Park *et al.* 2006). The phylogenetic position of the Vis taxon suggests its evolution as an allopatric species possibly arising during the climatic fluctuations of the late Pliocene and the Pleistocene. The conflicting relationships between the ITS and plastid data together with mostly low support values may suggest incomplete lineage sorting or hybridization which further indicate the possible recent diversification of the *garganica* clade.

Morphologically most similar to the Vis taxon is *C. portenschlagiana* distributed on central Adriatic islands (Brač, Hvar and Korčula), the Pelješac Peninsula and the Croatian mainland facing Vis island (Fig. 1). The most important morphological character that both taxa have in common is the type of corolla. In fact, *C. portenschlagiana* was so far the only known species of ser. *Garganicae* possessing a campanulate corolla, as is found in the plants from Vis. *C. portenschlagiana* was described from Mt. Biokovo (Croatia) by Roemer & Schultes (1819) as glabrous plant with ascending stems. Later, several authors (Visiani 1826, 1847, Hayek 1931, Damboldt 1965, Lovašen-Eberhardt & Trinajstić 1978) reported about its strong hairiness, high morphological variability and variable degrees of branching, especially for plants from Mt. Biokovo. Ecological and morphological variability of *C. portenschlagiana* was studied by Šoljan (1978, 1990), who described the infraspecific taxa *C. portenschlagiana* var. *pumila* D. Šoljan (1990: 45) and *C. portenschlagiana* var. *hirsuta* D. Šoljan (1990: 46) from the Island of Brač and from Mt Biokovo, respectively.

Due to the lack of appropriate drawings and for better comparison to the newly described *Campanula* species from the Island of Vis, we provide a detailed iconography of *C. portenschlagiana* var. *hirsuta* in this paper (Fig. 5). *C. portenschlagiana* is morphologically well differentiated from the taxon occurring on the Island of Vis by stems 15–20 mm long, lax, pubescent to glabrous; leaves reniform to cordate-reniform, irregularly serrate with acute teeth, with petiole up to 10 cm long and blades up to 30 mm long and 25 mm wide; inflorescence simple or branched, 4–10 flowered; floral pedicels 12–25 mm long; calyx teeth 3–4 mm long, 1–2 denticulate at the margin, hairy to glabrous; corolla 14–25 mm long, with tube 2/3–3/4 the length of the corolla, glabrous with few hairs only on the outside of the lobe apex; style 10–12 mm long, hairy on the upper 2/3, stigmas 2 mm long; stamens with filaments 0.8–1 mm long and basal blade ovate, 1.2–1.4 mm long, densely ciliate at the margin and on the inner surface, with cilia 0.05 mm long, anthers yellowish, appendiculate, pollen yellowish; capsule sub-globose, 2–2.5 mm long, 5-ribbed, sparsely hairy (hairs 0.1 mm long), opening by basal pores, with suberect calyx teeth, 4–5 mm long; seeds irregular, flattened, 0.6–0.7 mm long, pale brown, opaque.

According to previous reports (Geslot 1980, Murata 1992, 1995, Toniuc 1999, Buss *et al.* 2001, Akcin 2009, Alçitepe 2010) various basic morphological seed coat patterns can be distinguished within Campanulaceae. In particular, in the genus *Campanula*, two types of testa are present: reticulate and striate, with certain variability among the taxa. *C. teutana* has ovoid seeds with a weakly striate testa, thin wavy striped radial walls and slightly marked lumen, giving the seed coat a smooth appearance (Fig. 7 C–D). This type of testa was hitherto observed only in *C. fenestrellata* s.l. (Kovačić 2006) and in *C. tomentosa* Lamarck (1785: 584) (Alçitepe 2010). In

morphologically closely related *C. portenschlagiana*, the seeds are irregular, flattened, showing distinctly striate testa with prominent anastomosed radial walls and distinct linear lumen, giving the seed coat a markedly striate appearance (Fig. 7 A–B). This type of testa is observed in many *Campanula* species (Geslot 1980, Alçitepe 2010). In conclusion, despite recent studies of isophyllous amphi-Adriatic *Campanula*, phylogenetic relationships among taxa still remain largely unresolved. The discovery of the new species belonging to this group indicates that a more complete sampling over the distribution range across the Balkan Peninsula, especially from poorly explored areas such as Albania, Peloponnese, Ionian and Croatian remote islands might reveal new hidden taxa. Further data employing other molecular markers, such as AFLPs, may be necessary to address the unresolved relationships and detailed questions regarding the spatiotemporal diversification of the *garganica* clade.

### Key to the Croatian taxa of *Campanula* ser. *Garganicae*

- 1 Corolla campanulate, with tube 1/2–3/4 its length ..... 2
- Corolla rotate, more or less flattened or infundibular, with tube 1/4–1/2 its length ..... 3
- 2 Floral pedicels 5–12 mm long; calyx teeth 5–7 mm long; corolla with tube 1/2 its length; stamen filaments 2–2.5 mm long; anthers white tinged with pale blue, pollen whitish ..... *C. teutana*
- Floral pedicels 12–25 mm long; calyx teeth 3–4 mm long; corolla with tube 2/3–3/4 its length; stamen filaments 0.8–1 mm long; anthers and pollen yellowish ..... *C. portenschlagiana* var. *portenschlagiana*
- 2a. Plant glabrous, habit lax ..... *C. portenschlagiana* var. *pumila*
- 2b. Plant hirsute, habit condensed ..... *C. portenschlagiana* var. *hirsuta*
- 3 Corolla infundibular, 30–40 mm in diameter; calyx teeth 7 mm long and 3 mm wide, twice as long as the capsule ..... *C. poscharskyana*
- Corolla rotate to infundibular, 7–20 mm in diameter; calyx teeth max. 6 mm long and max. 2 mm wide, subequal or shorter than capsule ..... 4
- 4 Plant glabrous, rarely pubescent above, corolla max. 15 mm in diameter; basal leaves with blade max. 4 x 3 cm, 2-serrate or dentate; capsule 2.5 x 3.5 mm ..... *C. fenestrellata* subsp. *fenestrellata*
- Plant densely tomentose-velvety, corolla up to 20 mm in diameter; basal leaves with blade up to 7 x 4 cm, serrate; capsule 4 x 4.5 mm ..... *C. fenestrellata* subsp. *istriaca*

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**APPENDIX 1.** List of taxa, collection details, voucher information and GenBank accession numbers of Campanulaceae species analysed in the present study. Asterisks indicate sequences published previously in Park *et al.* (2006), Roquet *et al.* (2008) and Frajman & Schneeweiss (2009).

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Species; origin; collector; collection number and herbarium; ITS GenBank number; *trnL-F* GenBank number

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- Asyneuma campanuloides* Bornm.; Georgia, Greater Caucasus; *Schönschwetter & Tribsch 4469* (WU); DQ304586\*, FJ426570\*
- Asyneuma limonifolium* Bornm.; Greece, Ionian Islands, Lefkada; *Gutermann 35549* (WU); DQ304587\*, FJ426571\*
- Campanula acarnanica* Damboldt; Greece, Acarnania, Mt. Boumistos; *Damboldt Cal 1058* (B); DQ304598\*, KF957744
- Campanula cephallica* Feer; Greece, Ionian Islands, Kefallinia; *Gutermann 28945* (WU); DQ304597\*, FJ426576\*
- Campanula comosiformis* (Hayek & Janch.) Frajman & Schneew.; Albania, Šija gorge E of Bicaj; *Frajman 11089* (WU); FJ426592\*, FJ426572\*
- Campanula debarensis* Rech. f., accession 1; FYR Macedonia, Crni Drin; *Kovačić 1097* (ZA); DQ304595\*, FJ426575\*
- Campanula debarensis* Rech. f., accession 2; FYR Macedonia, Crni Drim; *K. Micevski s.n.* (MKNH 031830); KF957738, KF957745
- Campanula elatines* L.; Italy, Alpi Cozie; *Schönschwetter & Tribsch 6349* (WU); DQ304624\*, FJ426577\*
- Campanula elatinoides* Moretti; Italy, Southern Alps, Lago d'Iseo; *Gutermann 1879* (WU); DQ304625\*, FJ426578\*
- Campanula fenestrellata* Feer subsp. *fenestrellata*; Croatia, Velebit, Velika Paklenica; *Kovačić 920* (ZA); DQ304592\*, FJ426579\*
- Campanula fenestrellata* Feer subsp. *istriaca* (Feer) Damboldt; Croatia, Krk, Uvala Oprna; *Schönschwetter & Tribsch 6272* (WU); DQ304594\*, FJ426584\*
- Campanula fragilis* Cirillo. subsp. *fragilis*; Italy, Calabria, city of Scalea; *Gutermann 36164* (WU); DQ304626\*, FJ426580\*
- Campanula garganica* Ten., accession 1; cult. in Botanical Garden Zagreb (material from Italy), *Kovačić 1012* (ZA); DQ304596\*; Italy, Foggia, *Aldobrandi 12-VII-96 et al.* (MA 625685); EF088725\*
- Campanula garganica* Ten., accession 2; Italy, Gargano, Vieste; *Brullo & Signorello s.n.* (CAT 037.237/7); KF957739, KF957746
- Campanula isophylla* Moretti; cult. in Botanical Garden Zagreb (material from Italy), *Kovačić 1013* (ZA); DQ304630\*, FJ426583\*
- Campanula persicifolia* L.; Austria, Northeastern Alps; *Schönschwetter & Tribsch 6288* (WU); DQ304590\*, FJ426573\*
- Campanula pollinensis* Podlech; Italy, Monte Pollino; *Brullo, Signorello, Spampinato s.n.* (CAT 037.066/30); KF957740, KF957747
- Campanula portenschlagiana* Roem. & Schult. var. *hirsuta* Šoljan, accession 1; Croatia, Biokovo; *Kovačić 692* (ZA); DQ304600\*, FJ426587\*
- Campanula portenschlagiana* Roem. & Schult. var. *pumila* Šoljan, accession 2; Croatia, otok Brač, Vidova gora; *M. Ruščić s.n.* (ZAGR 26291); KF957741, KF957748
- Campanula poscharskyana* Degen; Croatia, Dubrovnik region; *Kovačić 690* (ZA); DQ304601\*, FJ426588\*
- Campanula pyramidalis* L.; Croatia, Vratnik pass; *Schönschwetter & Tribsch 6243* (WU); DQ304606\*; Croatia, Rijeka; *Vitek 99440* (MA 641379); EF088754\*
- Campanula reatina* Lucchese; Italy, Turano Valley; *Kovačić 768* (ZA); DQ304599\*, FJ426589\*
- Campanula rotundifolia* L.; Croatia, Platak – Rijeka region; *Kovačić 784* (ZA); DQ304615\*; Andorra; *Sáez 6134* (BCB); EF088759\*
- Campanula scheuchzeri* Vill.; Croatia, North Velebit; *Kovačić 807* (ZA); DQ304614\*, KF957749
- Campanula stevenii* Bieb.; Georgia, Minor Caucasus; *Schönschwetter & Tribsch 6976* (WU); DQ304591\*; Armenia, Vayk; *Oganessian s.n.* (ERE 154865); EF088770\*
- Campanula teutana* Bogdanović & Brullo, accession 1; Croatia, Island of Vis, Oključina, calcareous cliffs near

Kraljičina špilja; *S. Bogdanović s.n.* (ZAGR 32628); KF957742, KF957750  
*Campanula teutana* Bogdanović & Brullo, accession 2; Croatia, otok Vis, Oključina, *S. Bogdanović s.n.* (ZAGR); KF957743, KF957751  
*Campanula tommasiniana* Koch; Croatia, Učka; *Kovačić 775* (ZA); DQ304611\*, FJ426590\*  
*Campanula versicolor* Andrews; Greece, Ionian Islands, Kefallinía; *Gutermann 30067* (WU); DQ304607\*, FJ426591\*  
*Petromarula pinnata* DC.; Greece, Crete; *Schönswetter & Tribsch 7821* (WU); DQ304582\*, FJ426585\*  
*Physoplexis comosa* Schur; Italy, Southern Alps; *Schönswetter & Tribsch 3902* (WU); DQ304585\*, FJ426586\*  
*Phyteuma globulariifolium* Sternb. & Hoppe; Austria, Niedere Tauern; *Schönswetter & Tribsch 4551* (WU); DQ304583\*, FJ426582\*  
*Phyteuma spicatum* L.; Croatia, Gorski Kotar; *Schönswetter & Tribsch 6233* (WU); DQ304584\*; Spain, Barcelona, Aiguafreda; *Roquet 8-V-05* (BC); EF088787\*  
*Trachelium caeruleum* L.; Spain, N of Malaga; *Schönswetter & Tribsch 8736* (WU); DQ304570\*; Spain, Santander, Liencres; *Aldasoro 3503* (MA); EF088791\*