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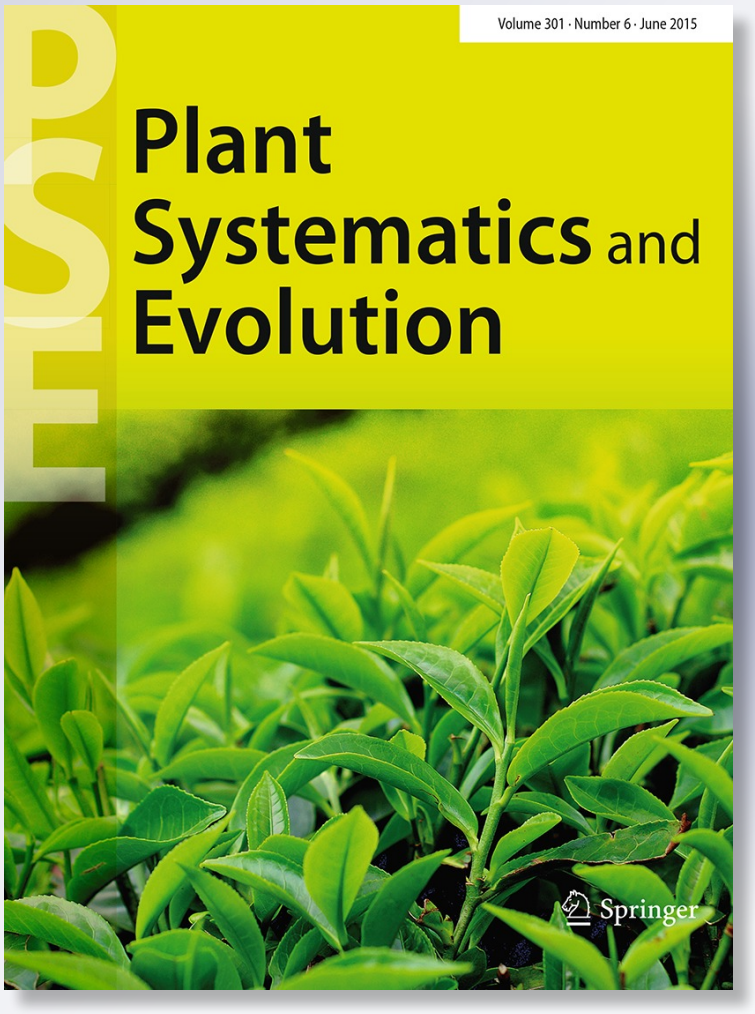

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ORIGINAL ARTICLE

Campanula aureliana (Campanulaceae), a new species from Albania

Sandro Bogdanović · Ivana Rešetnik · Salvatore Brullo · Lulëzim Shuka

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Abstract *Campanula* is a species-rich genus with high variability of the morphological traits, controversial taxonomic treatments within the Mediterranean basin as a species diversity center. One of the monophyletic groups in the genus is the *Campanula* series *Garganicae* Trinajstić distributed in the amphi-Adriatic and Ionian region. The group as currently delimited encompasses 11 taxa, mostly with highly restricted distributional ranges. In the present study plants previously named as *Campanula garganica* Ten. var. *albanica* Markgr. are described and illustrated as an isophyllous species from central Albania, for which name *Campanula aureliana* Bogdanović, Rešetnik, Brullo & Shuka is proposed. The results of phylogenetic analyses based on nuclear ITS and chloroplast *trnL–trnF* data support *C. aureliana* as a clearly distinct taxon within the *Campanula* ser. *Garganicae*. Its description is given, and diagnostic morphological and SEM seed micromorphological characters of closely related species are compared and discussed. Morphologically *C. aureliana* shows close affinity with *C. fenestrellata* Feer, mainly for the habit, shape and size of the corolla, but differs in a lot of significant features. Information on the ecology and conservation status of the newly described species is presented.

Keywords *Campanula* · Balkan Peninsula · Endemic · Isophyllous bellflowers · Phylogeny · Taxonomy

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Introduction

Campanula L. is the largest genus of the family Campanulaceae, and includes ca. 580–600 species (Mansion et al. 2012) distributed in the Northern Hemisphere, with the Mediterranean basin as a major center of species diversity, where about 250 species occur (Damboldt 1965; Podlech 1965; Kovanda 1970a, b, 1977; Geslot 1984; Park et al. 2006). The annual and perennial *Campanula* taxa grow in various habitats, such as meadows, grasslands, garrigues, woodlands, and mainly in rupestrian stands (Kovačić 2004; Roquet et al. 2008). The high variability of the morphological traits provided numerous classification proposals (De Candolle 1830; Boissier 1875; Gadella 1966a, b; Contandriopoulos 1984; Kolakovsky 1994), but none of them reflect the phylogenetic relationships recently obtained with molecular data, which showed that *Campanula* and many closely related genera are not monophyletic (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; Crowl et al. 2014). Nevertheless, molecular data identified several well-

supported monophyletic groups within *Campanula*, e.g., the garganica clade (Park et al. 2006; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b), the fragilis clade, the pyramidalis clade (Lakušić et al. 2013), and closely related lineages, e.g., *Phyteuma* (Schneeweiss et al. 2013). All of these monophyletic groups that were further explored revealed ambiguous interspecific relationships and discrepancies with traditional circumscription and taxonomy.

The Balkan Peninsula has long been known for its high level of biodiversity and endemic taxa, refugial character and importance for the European phytogeography. However, some parts of the Balkan Peninsula, e.g., Albania, are still insufficiently explored. That fact is manifested in many new taxa confirmed for the Albanian flora in recent years (e.g., Barina and Pifkó 2008a, b, 2011; Rakaj 2009; Ball 2011; Barina et al. 2009, 2011, 2013; Meyer 2011; Frajman et al. 2013) including numerous species described new for science (Shuka et al. 2010; Meyer 2011; Tan et al. 2011, 2013; Polatschek 2013; Bogdanović et al. 2014a). The combination of less explored geographical area with frequent cases of old, single and recently unconfirmed literature reports together with taxonomic and phylogenetic complexity of the studied genus indicates the necessity for cautious examination of the studied material.

Campanula series *Garganicae* Trinajstić represents a morphologically, karyologically and phylogenetically well-supported monophyletic group distributed in the amphi-Adriatic and Ionian region (Park et al. 2006; Liber et al. 2008; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b). The group as currently delimited includes 11 taxa, while the sister species of the group is the Albanian endemic *C. comosiformis* (Hayek & Janch.) Frajman & Schneew. Morphologically, the members of this group are characterized by a monopodial growth form, isophyllous and long petiolate leaves, with cordate to ovate blades, elongated and more or less unilateral inflorescence, campanulate or rotate corolla, obtuse hairs at the base of filaments, and brown shiny seeds (Damboldt 1965; Lovašen-Eberhardt and Trinajstić 1978). The phylogeny inferred with plastid and ITS sequence data (Park et al. 2006; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b) unambiguously separate all taxa in the group; however, their relationships remain unclear due to low clade support and conflicting signals between plastid and nuclear data. In the course of the current comprehensive study of the ser. *Garganicae*, detailed investigation on herbarium specimens and literature data indicated the occurrence of an endemic variety of *C. garganica* Ten., named var. *albanica* Markgr. in central Albania. In particular, *C. garganica* was described by Tenore (1827) from Gargano promontory (central Italy), while in Albania, its presence was unlikely and doubtful. This variety was described by Markgraf (1931) on material from a single locality in Albania (Tomori: Kapinova, Kalkfels im Dorf, 800 m, bl., 19.VI.1928) and no type specimens exist to confirm this finding, as the deposited material to Berlin Herbarium (B) was burned and destroyed during the Second World War (Damboldt 1965). We also carried out additional checks in B and BP herbaria, as well as in other herbaria, but neither the type nor any duplicates have been hitherto found. From the nomenclatural aspect, the name *Campanula garganica* var. *albanica* Markgr. was described and validly published by Markgraf (1931) and in the protologue Markgraf stated that it differs from *C. cephallenica* Feer in having denser inflorescence, shorter calyx teeth and denser hairs on the whole plant. Some authors (Damboldt 1968; Fedorov and Kovanda 1976; Geslot 1984) considered *C. cephallenica* and *C. acarnanica* Damboldt, respectively, occurring in the Ionian islands and West Greece, to be a subspecies of *C. garganica*, but this treatment is rejected by molecular data (Park et al. 2006; Frajman and Schneeweiss 2009; Bogdanović et al. 2014a, b). *Campanula garganica* var. *albanica* was synonymised with *C. debarensis* Rech.f. by Damboldt (1965) and not included in the Albanian flora by Qosja et al. (1996). More recently, Park et al. (2006) considered *C. debarensis* an Albanian–Macedonian endemic, phylogenetically distinct from the others species of this group, but they do not elucidate other distribution in Albania.

To test the taxonomic assignment and to determine the phylogenetic position of *C. garganica* var. *albanica* individuals from Mt. Tomori, within the ser. *Garganicae*, we investigated plastid and nuclear ribosomal ITS sequence data and evaluated morphological evidence. Accordingly, here we provide a taxonomic treatment of these individuals as a new species, *Campanula aureliana* Bogdanović, Rešetnik, Brullo & Shuka (see "Taxonomic treatment"), including a comprehensive description accompanied by an illustration plate and diagnostic characters. Finally, we provide information about its ecology and propose an IUCN conservation status.

Materials and methods

Plant material

Investigations were carried out on the specimens collected during field trips in Albania, in July 2012 and 2013. In total, only ten individuals were collected to avoid damage to the current small populations in the villages of Tomori and Kapinovë. 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

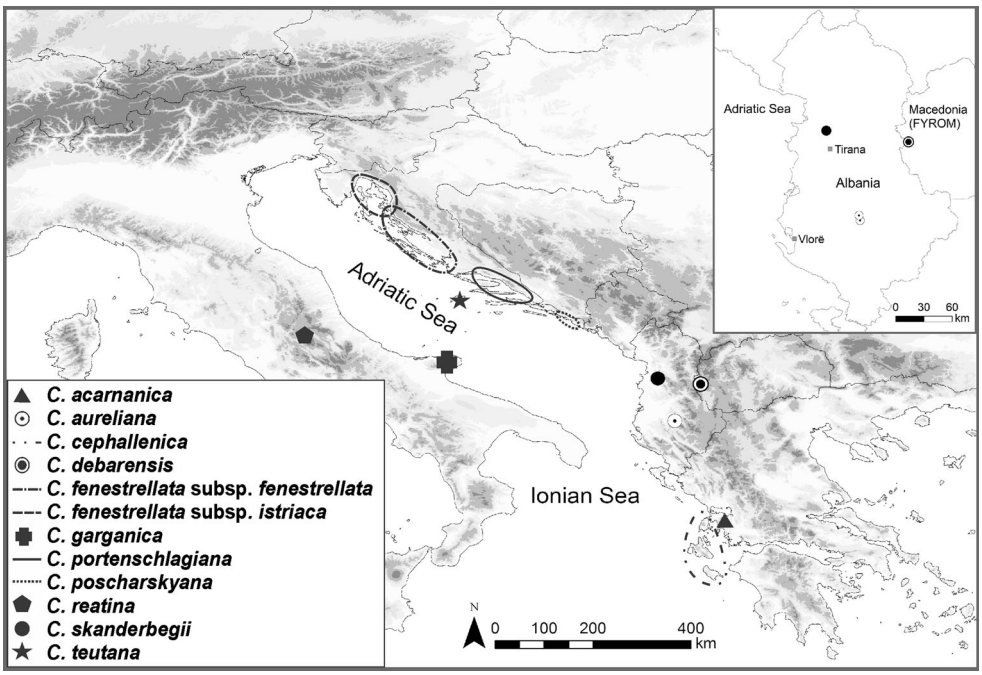

Fig. 1 Distribution of *Campanula* series *Garganicae*

were placed in 50 % glycerine-ethyl alcohol solution for further morphological assessment. To obtain a denser sampling in *Campanula* ser. *Garganicae* (Fig. 1), we used the data sets of Park et al. (2006), Frajman and Schneeweiss (2009), and Bogdanović et al. (2014a, b), extended with four new sequences from newly found taxon. Voucher data and GenBank accession numbers of the newly sequenced taxa, as well as GenBank accession numbers from previous studies are given in Table 1. Herbarium specimens of the isophyllous species of *Campanula* were studied from B, BEOU, BM, BP, CAT, CNHM, K, MKNH, NAP, NHMR, PAL, RO, TIR, W, WU, ZA, ZAGR, and ZAHO (abbreviations follow Thiers 2014).

Scanning electron microscopy (SEM)

The micromorphology of the testa of ten dried mature seeds collected in Tomori village (Albania) was studied using a scanning electron microscope (Zeiss EVO LS10). The preparation of the seeds of *C. aureliana* 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. Each 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 Inc., Shiga, Japan), 0.2 µM of each primer (17SE and 26SE of Sun et al. (1994) for the nuclear ITS; c and f of Taberlet et al. (1991) for the plastid *trnL–trnF*) and 1.25 U of TaKaRa Taq™ HS polymerase (TaKaRa Bio Inc., Shiga, Japan). The PCR conditions for ITS were the same as described in Park et al. (2006) and for *trnL–trnF*, the same as described in Bogdanović et al. (2014b). The PCR reactions were performed using a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, California). The PCR products were purified with the GenElute PCR clean-up kit (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), according to the manufacturer's protocol. The products were sequenced by the Macrogen Inc. (Seoul, Korea) using the BigDye™ terminator cycle sequencing kit (Applied Biosystems, Foster City, California) and analyzed on an ABI PRISM 3730XL automated sequencer (Applied Biosystems, Foster City, California). Sequences were edited and manually aligned using the Geneious Pro 5.3.6 (Drummond et al. 2011). Sequence alignments are available from TreeBASE (study number 16523).

Phylogenetic analyses

Three different datasets (ITS, *trnL–trnF*, ITS–*trnL–trnF* combined dataset) were analyzed using maximum parsimony (MP) and Bayesian inference (BI). The trees were rooted using *Trachelium caeruleum* L. as an outgroup.

To assess the 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* 4.0b10 (Swofford

Table 1 Alphabetical list of taxa (numbers 1–4 indicate different populations of the same taxon), collection details, voucher information and GenBank accession numbers of Campanulaceae species analyzed in the present study

No	Taxon	Collection details	Voucher information	ITS GenBank number	trnL-F GenBank number
1	*Asyneuma campanuloides* Bornm.	Georgia, Greater Caucasus	Schönswetter & Tribsch 4469 (WU)	DQ304586	FJ426570
2	*Asyneuma limoniifolium* Bornm.	Greece, Ionian Islands, Lefkada	Gutermann 35549 (WU)	DQ304587	FJ426571
3	*Campanula acarnanica* Damboldt	Greece, Acarnania, Mt. Akarnanika Ori	Karamplianis Th. 1692 (ATHU)	KF957752	KF957763
4	*Campanula aureliana* Bogdanović, Rešetnik, Brullo & Shuka 1	Albania, Tomori Mt, western part of village Kapinovë	Bogdanović & Jug Dujaković (ZAGR)	KM215787[a]	KM215789[a]
5	*Campanula aureliana* Bogdanović, Rešetnik, Brullo & Shuka 2	Albania, Tomori Mt, Tomori village	Bogdanović, Rešetnik & Temunović s.n. (ZAGR)	KM215788[a]	KM215790[a]
6	*Campanula cephallenica* Feer 1	Greece, Ionian Islands, Kefallinía	Gutermann 28945 (WU)	DQ304597	FJ426576
7	*Campanula cephallenica* Feer 2	Greece, Isola Cephalonia, Mt. Aivos	Brullo, S. & Giacalone G. s.n. (CAT)	KF957753	KF957764
8	*Campanula comosiformis* (Hayek & Janch.) Frajman & Schneew. 1	Albania, Gjalica, Mustafe	Bogdanović & Jug-Dujaković s.n. (ZAGR)	KF957754	KF957765
9	*Campanula comosiformis* (Hayek & Janch.) Frajman & Schneew. 2	Albania, Šija gorge E of Bicaj	Frajman 11089 (WU)	FJ426592	FJ426572
10	*Campanula debarensis* Rech.f.	FYR Macedonia, Crni Drin	Kovačić 1097 (ZA)	DQ304595	FJ426575
11	*Campanula debarensis* Rech.f.	FYR Macedonia, Crni Drim	K. Micevski s.n. (MKNH 031830)	KF957738	KF957745
12	*Campanula elatines* L.	Italy, Alpi Cozie	Schönswetter & Tribsch 6349 (WU)	DQ304624	FJ426577
13	*Campanula elatinoides* Moretti	Italy, Southern Alps, Lago d'Iseo	Gutermann 1879 (WU)	DQ304625	FJ426578
14	*Campanula fenestrellata* Feer subsp. *fenestrellata* 1	Croatia, Velebit, Velika Paklenica	Kovačić 920 (ZA)	DQ304592	FJ426579
15	*Campanula fenestrellata* Feer subsp. *fenestrellata* 2	Croatia, NP Krka, Roški slap	Šegota & Hršak s.n.(ZAGR)	KF957755	KF957766
16	*Campanula fenestrellata* subsp. *istriaca* (Feer) Damboldt 1	Croatia, Krk, Uvala Oprna	Schönswetter & Tribsch 6272 (WU)	DQ304594	FJ426584
17	*Campanula fenestrellata* subsp. *istriaca* (Feer) Damboldt 2	Croatia, Istra, Plomin	Bogdanović & Ljubičić s.n. (ZAGR)	KF957756	KF957767
18	*Campanula fragilis* Cirillo	Italy, Calabria, city of Scalea	Gutermann 36164 (WU)	DQ304626	FJ426580
19	*Campanula garganica* Ten. 1	Cult. in Botanical Garden Zagreb (material from Italy); Italy, Foggia	Kovačić 1012 (ZA); Aldobrandi 12-VII-96 et al. (MA 625685)	DQ304596	EF088725
20	*Campanula garganica* Ten. 2	Italy, Gargano, Vieste	Brullo & Signorello s.n. (CAT 037.237/7)	KF957739	KF957746
21	*Campanula isophylla* Moretti	Cult. in Botanical Garden Zagreb (material from Italy)	Kovačić 1013 (ZA)	DQ304630	FJ426583
22	*Campanula persicifolia* L.	Austria, Northeastern Alps	Schönswetter & Tribsch 6288 (WU)	DQ304590	FJ426573
23	*Campanula pollinensis* Podlech	Italy, Monte Pollino	Brullo, Signorello, Spampinato s.n. (CAT 037.066/30)	KF957740	KF957747
24	*Campanula portenschlagiana* Roem. & Schult. 1	Croatia, Biokovo	Kovačić 692 (ZA)	DQ304600	FJ426587
25	*Campanula portenschlagiana* Roem. & Schult. 2	Croatia, otok Brač, Vidova gora	M. Ruščić s.n. (ZAGR 26291)	KF957741	KF957748
26	*Campanula portenschlagiana* Roem. & Schult. 3	Bosnia and Herzegovina, Ljubuški	Šiljeg s.n. (ZAGR)	KF957757	KF957768
27	*Campanula portenschlagiana* Roem. & Schult. 4	Croatia, Island Hvar, Pitve	Rimac s.n. (ZAGR)	KF957758	KF957769

Table 1 continued

No	Taxon	Collection details	Voucher information	ITS GenBank number	trnL-F GenBank number
28	*Campanula poscharskyana* Degen 1	Croatia, Dubrovnik region	Kovačić 690 (ZA)	DQ304601	FJ426588
29	*Campanula poscharskyana* Degen 2	Croatia, Radovčići	Kovačić (ZAGR)	KF957759	KF957770
30	*Campanula pyramidalis* L.	Croatia, Vratnik pass; Croatia, Rijeka	Schönswetter & Tribsch 6243 (WU); Vitek 99440 (MA 641379)	DQ304606	EF088754
31	*Campanula reatina* Lucchese 1	Italy, Turano Valley	Kovačić 768 (ZA)	DQ304599	FJ426589
32	*Campanula reatina* Lucchese 2	Italy, Valle del Salto, Ponte Figureto	Kirin s.n. (ZAGR)	KF957760	KF957771
33	*Campanula rotundifolia* L.	Croatia, Platak—Rijeka region; Andorra	Kovačić 784 (ZA); Sáez 6134 (BCB)	DQ304615	EF088759
34	*Campanula scheuchzeri* Vill.	Croatia, North Velebit	Kovačić 807 (ZA)	DQ304614	KF957749
35	*Campanula skanderbegii* Bogdanović, Brullo & D. Lakušić 1	Albania, Kruje	Lakušić, Kuzmanović, Lazarević & Alegro s.n. (ZAGR)	KF957761	KF957772
36	*Campanula skanderbegii* Bogdanović, Brullo & D. Lakušić 2	Albania, Kruje	Lakušić, Kuzmanović, Lazarević & Alegro s.n. (ZAGR)	KF957762	KF957773
37	*Campanula stevenii* Bieb.	Georgia, Minor Caucasus; Armenia, Vayk	Schönswetter & Tribsch 6976 (WU); Oganessian s.n. (ERE 154865)	DQ304591	EF088770
38	*Campanula teutana* Bogdanović & Brullo 1	Croatia, Island of Vis, Oključina, calcareous cliffs near Kraljičina špilja	S. Bogdanović s.n. (ZAGR 32628)	KF957742	KF957750
39	*Campanula teutana* Bogdanović & Brullo 2	Croatia, otok Vis, Oključina	S. Bogdanović s.n. (ZAGR)	KF957743	KF957751
40	*Campanula tommasiniana* Koch	Croatia, Učka	Kovačić 775 (ZA)	DQ304611	FJ426590
41	*Campanula* versicolor Andrews	Greece, Ionian Islands, Kefallinía	Gutermann 30067 (WU)	DQ304607	FJ426591
42	*Petromarula pinnata* DC.	Greece, Crete	Schönswetter & Tribsch 7821 (WU)	DQ304582	FJ426585
43	*Physoplexis comosa* Schur	Italy, Southern Alps	Schönswetter & Tribsch 3902 (WU)	DQ304585	FJ426586
44	*Phyteuma globulariifolium* Sternb. & Hoppe	Austria, Niedere Tauern	Schönswetter & Tribsch 4551 (WU)	DQ304583	FJ426582
45	*Phyteuma spicatum* L.	Croatia, Gorski Kotar; Spain, Barcelona, Aiguafreda	Schönswetter & Tribsch 6233 (WU); Roquet 8-V-05 (BC)	DQ304584	EF088787
46	*Trachelium caeruleum* L.	Spain, N of Malaga; Spain, Santander, Liencres	Schönswetter & Tribsch 8736 (WU); Aldasoro 3503 (MA)	DQ304570	EF088791

[a] New sequences produced in this study

2003) 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. 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 (Nylander 2004) 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 10^7 generations, with the sample frequency set to 1,000. The first 2,500 trees (prior to the 2.5×10^6 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

Morphology

Morphological features of *C. aureliana* are presented in Figs. 2, 3, and a detailed description is provided in the taxonomic treatment section.

Seed micromorphology

Campanula aureliana shows a seed coat morphologically well differentiated from the closely related *C. fenestrellata*. The seeds of the former are subglobose-ovoid with weakly striate testa characterized by very elongate fibriform cells, imperceptibly anastomosed, with smooth and slightly raised periclinal walls, fused with the anticlinal walls showing a chain of minute papillae (Fig. 4a, b). The seeds in the latter are ovoid, with a markedly striate testa characterized by shorter cells, evidently anastomosed, with periclinal walls showing a deeply incise linear lumen, while the anticlinal walls are quite prominent and slightly channeled on the back (Fig. 4c, d).

Phylogenetic analyses

The characteristics of the ITS, *trnL–trnF*, and ITS–*trnL–trnF* combined datasets analyzed using MP and BI are summarized in Table 2. As the ILD test revealed no significant difference ($p = 0.25$) between the selected partitions (*trnL–trnF* and ITS), the phylogenetic tree of the combined data set obtained by BI analysis is presented in Fig. 5. In all analyzed data sets, the *C. aureliana* individuals are inferred as distinct members of the garganica clade (Fig. 5) without resolved sister taxa. The individuals of *C. fenestrellata* also formed a separate clade, while all other members of the group formed a clade supported only with BI analysis (0.71 PP). Within this clade *C. reatina* formed one maximally supported subclade (100 BS, 1 PP), the second BI supported subclade (0.93 PP) included *C. skanderbegii* and *C. portenschlagiana*, and the third subclade also supported only with BI analysis (0.95 PP) included the remaining species.

Discussion

Phylogenetic analyses (Fig. 5) and comparison of morphological characters confirm *C. aureliana* as a distinct member of the *Campanula* ser. *Garganicae*. Furthermore, none of the other members of the garganica clade is resolved as a supported sister taxa of *C. aureliana*, according to the separate (data not shown) or combined phylogenetic analysis (Fig. 5). According to the herbarium material and literature (Reichenbach 1860; Feer 1890; Damboldt 1965), *Campanula aureliana* is morphologically closely related to *C. fenestrellata* Feer, mainly for the habit and shape and size of the corolla, but differs in a lot of significant traits. In particular, *C. aureliana* is characterized by stems and leaves always densely hairy, stems slender, leaves with petiole max. 7 cm long, blade smaller (max 30 mm long), dentate, calyx teeth linear–triangular, shorter than corolla tube (sometime subequal), adherent to the corolla, which is outside ciliate along the midribs, with lobes 2.5–3 mm wide al the base, stamen filaments 1.5–2 mm long, with basal blade subcircular, anthers shorter (3–3.2 mm), capsule subglobose, 2.6–3 mm in diameter, with calyx teeth suberect, seeds subcircular–ovoid, smaller (0.5 × 0.4 mm). Conversely, *C. fenestrellata* is glabrous (rarely tomentose above), more robust, with petiole up to 9 cm long, leaf blade up to 40 mm long, biserrate, calyx teeth linear-lanceolate, thin, longer than corolla tube, detached from the corolla or deflexed, corolla glabrous or ciliate at the base, with lobes 3–4 mm wide al the base, stamen filaments 3.5 mm long, with basal blade long ovate, anthers longer (4 mm), capsule ovoid, 3.5 mm long, with calyx teeth patent to deflexed, seeds elliptical–ovoid, bigger (0.65–0.8 × 0.4 mm). The features of seed micromorphology are also used in the genus *Campanula* to differentiate species, and two main seed coat patterns, the reticulate and striate types, are present (Geslot 1980; Murata 1992; Toniuc 1999; Buss et al. 2001; Akcin 2009). Each type shows a specific variability when the seeds of a taxon are analyzed by SEM, and specifically in the *Campanula* ser. *Garganicae*, including only isophyllous taxa, the seed coat is usually striate, with elongated cells and lumen essentially linear, but quite variable in shape, size and arrangement (Bogdanović et al. 2014a, b). These differences are also present between *C. aureliana* and *C. fenestrellata* (Fig. 4) providing further evidence for the separation of the two taxa. Furthermore, the morphological

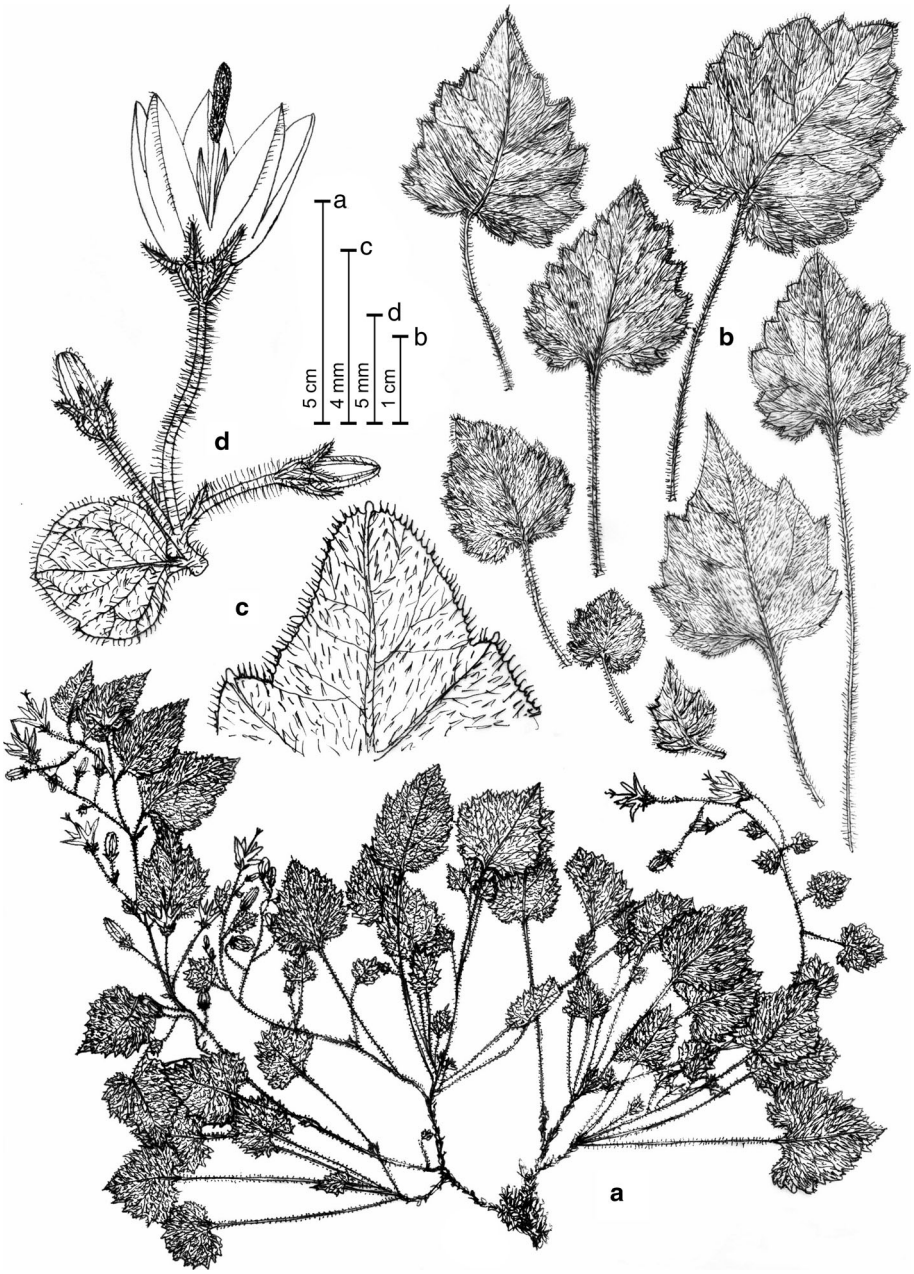

Fig. 2 *Campanula aureliana*. **a** Habit, **b** leaves, **c** leaf apex, **d** flower and buds in verticillaster. Drawing by Salvatore Brullo

resemblance with *C. fenestrellata* or with *C. garganica* and *C. debarensis* as postulated in the past (Markgraf 1931; Damboldt 1965), is not supported by molecular data. Although the overall phylogenetic relationships between taxa in the group are not fully resolved, some indications are provided. The morphologically similar Croatian endemic *C. portenschlagiana* and the Albanian endemic *C. skanderbegii* are resolved as sister taxa according to the BI (0.93 PP, Fig. 5). The rest of the southern Balkan taxa and a coastal Apennine taxon form a weakly supported group (0.71 PP) inferred in the Bayesian analysis. In that group maximally supported grouping is present among the Greek endemics *C. cephallenica* and *C. acarnanica,* indicating a very close relationship which is not surprising considering the close geographic proximity of the two species. More interesting is the moderately to strongly supported (81 MPB, 0.95 PP) grouping between the southern Adriatic endemics *C. poscharskyana* and *C. garganica* distributed on the opposite coasts of the Adriatic Sea (Fig. 5). As the other Apennine member of the garganica clade, *C. reatina*, is phylogenetically distinct, this provides further evidence for two dispersal events across the Adriatic as already suggested by Park et al. (2006) and Frajman and Schneeweiss (2009). Such amphi-Adriatic distributions are well known in plants (Turrill 1929), and interestingly, there is increasing evidence showing such independent dispersals

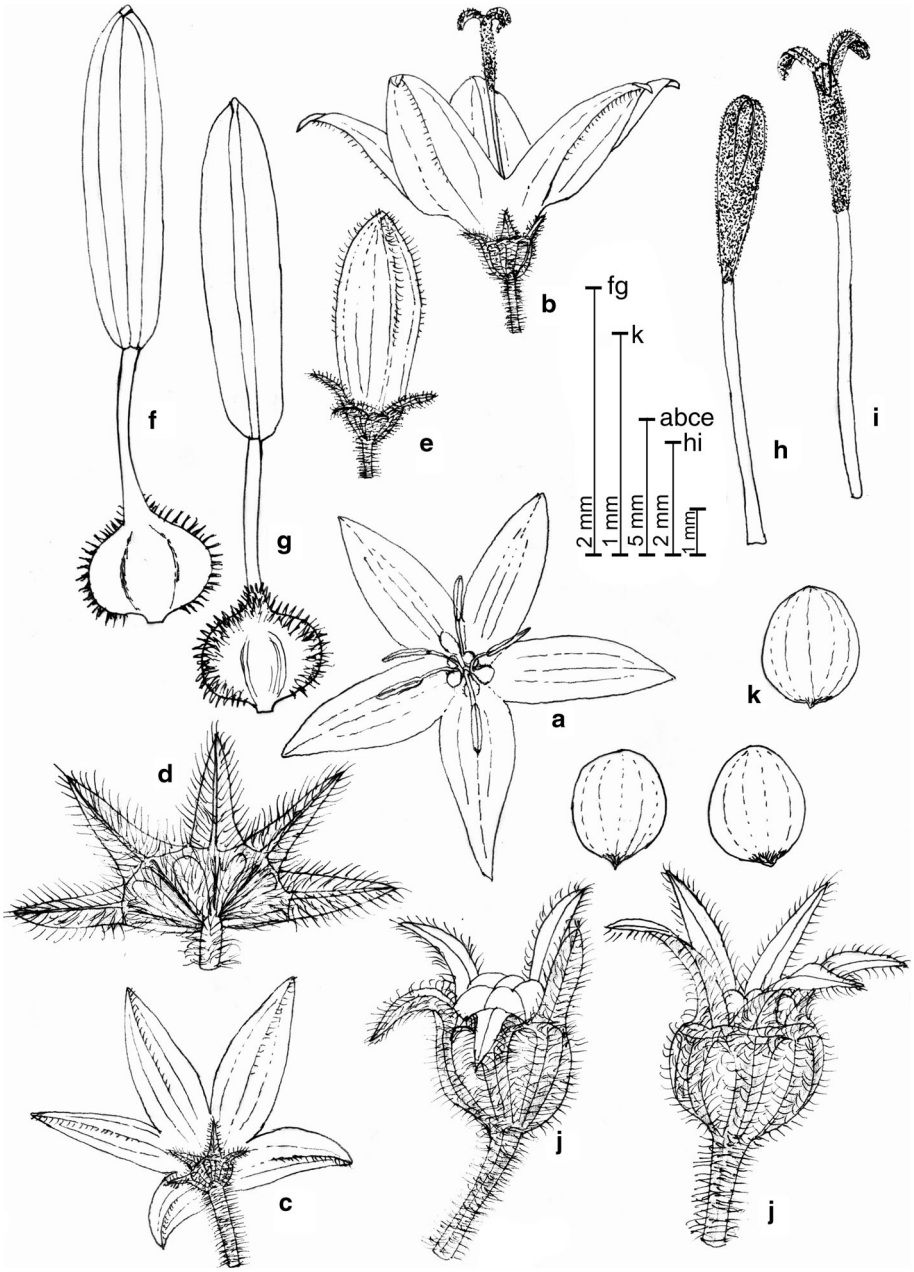

Fig. 3 *Campanula aureliana*. **a** Flower, **b** flower (lateral view), **c** flower (dorsal view), **d** corolla open (dorsal view), **e** bud, **f** and **g** stamens, **h** and **i** style and stigma, **j** fructiferous calyx and capsule, **k** seeds (ZAGR!, holotype). Drawing by Salvatore Brullo

within the same genera or groups (*Knautia*, Frajman et al. submitted; *Edraianthus*, Surina et al. 2014).

In conclusion, all lines of evidence presented in this paper strongly support the recognition of plants previously named as *C. garganica* var. *albanica* at species level, and without close relationship to Italian endemic *C. garganica.*

As the epithet "*albanica*" is unavailable in combination with *Campanula* at species level because of previously validly published name *Campanula albanica* Witasek, the name *C. aureliana* is proposed for this new species.

Taxonomic treatment

Campanula aureliana Bogdanović, Rešetnik, Brullo & Shuka, sp. nov. (Figs. 2, 3, 4)

=*Campanula garganica* var. *albanica* Markgr., *Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl.* 102: 356 (1931).

Campanula fenestrellata similaris sed scapis et foliis semper dense pilosis, folii petiolo usque ad 7 cm longo, lamina foliorum max. 30 × 25 mm, dentata, dentibus calycinis lineari-triangularibus, tubo corollino plerumque brevioribus et corolla adhaerentibus, corolla extus ciliata

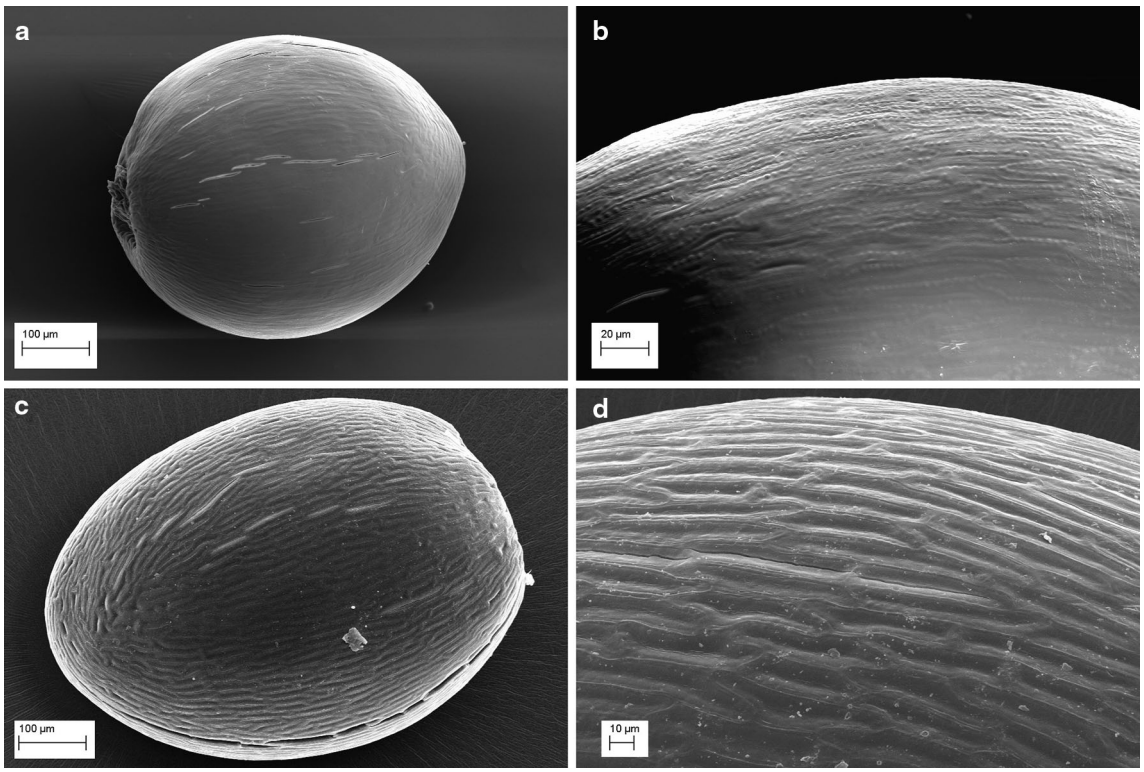

Fig. 4 Scanning electron micrographs of seed surface of *Campanula aureliana* (**a**, **b**) from holotype ZAGR! and *C. fenestrellata* (**c**, **d**) from Mt Velebit ZAGR!. **a**, **c** Seed (full view); **b**, **d** testa detail

Table 2 Characteristics of cpDNA, ITS and cpDNA–ITS combined datasets analyzed using maximum parsimony (MP) and Bayesian inference (BI)

Region	cpDNA	ITS	Combined
Alignment length	978	766	1744
Number/ % of parsimony-informative characters	118/12.06	208/27.15	326/18.69
Number/length of MP trees	13256/278	18/581	102/864
CI/RI	0.7644/0.935	0.613/0.842	0.651/0.874
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	−3201.98	−4227.94	−7433.22
Effective sample size (ESS)	14987.00	14247.89	13514.82

CI consistency index (excluding uninformative characters), *RI* retention index

secum costam, lobis 2.5–3 mm latis basi, filamentis staminorum 1.5–2 mm longis, lamina basali subcirculari, anthera breviore (3–3.2 mm), capsula subglobosa, dentibus calicis suberectis, seminibus subcirculari-ovoideis, minoribus, differt.

TYPE: ALBANIA. Tomori village calcareous rocky places in the village, 23 June 2013, 807 m alt., *S. Bogdanović, I. Rešetnik & M. Temunović s.n.* (holotype: ZAGR!; isotypes: CAT!, ZA!, ZAGR! and TIR!).

Description

Plant perennial, densely hairy, with rigid and erect-patent hairs. Rootstock woody, branched, naked, with numerous prostrate or 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, densely covered by rigid hairs, 0.4–1 mm long; petiole 2–7 cm long, densely covered by patent hairs; blade cordate, dark green, 7–30 × 6–25 mm, cordate at the base, acute at the apex irregularly dentate at the margin (6–11 acute to obtuse teeth for side), with pinnate venations; cauline leaves similar to the basal, gradually decreasing in size upwards, with petioles 4–25 mm long, blade 4–25 × 3–20 mm. Flowers usually solitary or 2–4 arranged in raceme at leaf axil, 15–35 mm long; pedicel 5–20 mm long, densely hairy, with 0–1 bracteoles. Calyx green, densely hairy, with teeth entire, linear–triangular, 1-nerved, 1.5–2 (2.5) × 0.8–1 mm, patent, acute at the apex. Corolla blue–violet, rotate-infundibular, 8–10 mm long, 14–16 mm in diameter, glabrous inside, outside

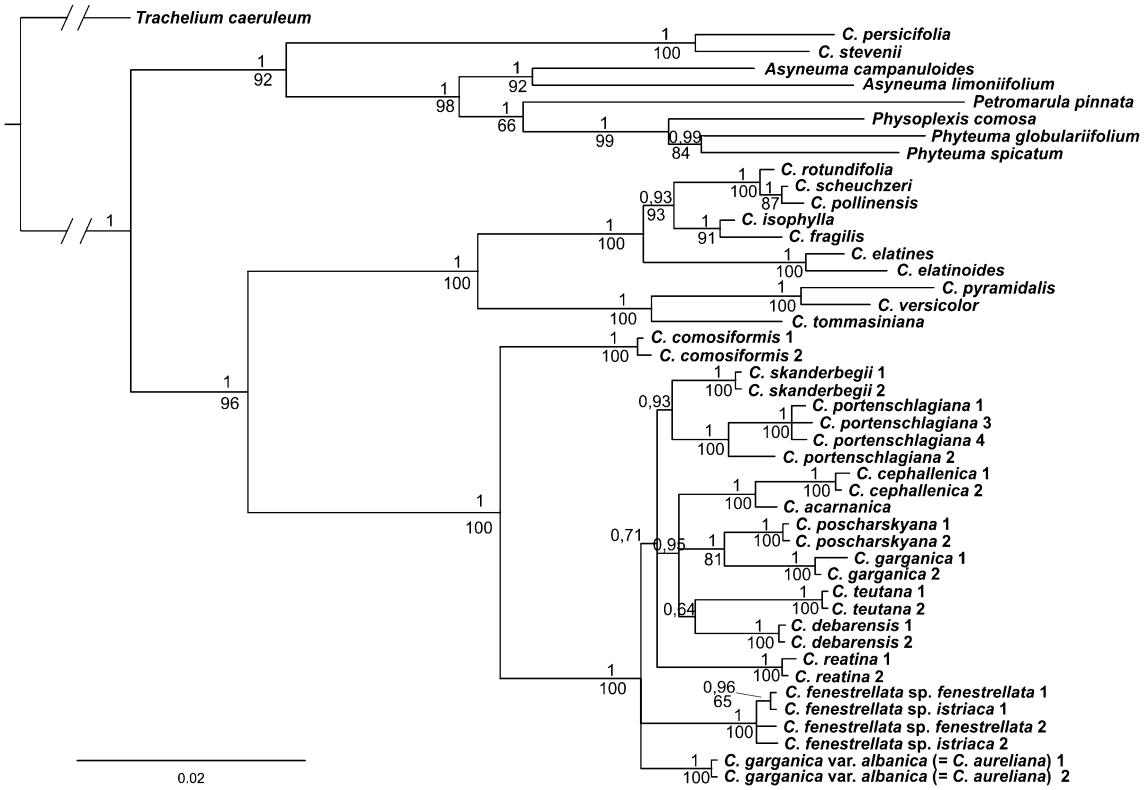

Fig. 5 Phylogenetic relationships of *Campanula* series *Garganicae* and relatives inferred from Bayesian analysis of combined nuclear ITS and plastid *trnL–trnF* data. Values above branches are Bayesian posterior probabilities (PP) and values below branches are maximum parsimony (MPB) bootstrap percentages (only shown if at least 50 %)

ciliate on the principal veins; tube cup-shaped, 1.5–2 mm long; lobes 6.5–8 × 2.5–3 mm, oblong-elliptical, divaricate to subpatent, with 1 midrib and various secondary veins, apex acute. Style with stigma exerted from corolla, 7.5–9 mm long, white and glabrous below, violet and papillose-hairy above, with 3 stigmas, ventrally whitish, each 1.5 mm long. Stamens 5, with filaments widened at base into a subcircular blade (or disk), 1 mm in diameter, densely ciliate in the upper part and margin; filaments glabrous, slightly violet, 1.5–2 mm long; anthers pale-blue to violet, 3–3.2 mm long, apiculate at the apex; pollen white to pale yellow. Capsule subglobose, 2.6–3 mm in diameter, 5-ribbed, densely hairy (hairs 0.2–0.3 mm long), without pores, with suberect calyx teeth, 3–3.5 mm long. Seeds subglobose-ovoid, 0.5 × 0.4 mm, brown, shiny (Figs. 2, 3, 4a, b, 6a, b).

Additional specimens examined

ALBANIA. Tomori Mt, western part of village Kapinovë, on limestone rocky crevices, in shady places. *Bogdanović S. & Jug-Dujaković M.,* 13 July 2012 (ZAGR32635!, ZAGR32634!, ZAGR32633!). District of Berat (Rrethi i Beratit), Tomori Mt (Mali i Tomorrit), western part of village Tomorr i Vogël, in the valley of River Tomorri (Lumi i Tomorrit); on limestone rock, near a spring, alt: 565 m, *Barin, Z. & Nemet, Cs.,* no. 6201, 24 May 2004 (BP746929!). North-western slopes of Tomori Mt, the north-eastern side of Tomori castle; on limestone rock crevices that occur between clearings of *Pinus heldreichii* and *Juniperus foetidissima* woods and in rocky calcareous cliff faces, partly shading by *Fagus sylvatica* woods, alt: 1200-1300 m, *Shuka* L. *& Xhulaj M.,* no. 5832–5836, 25 June 2013. Northern slopes of Tomori Mt, below the peak of Çuka e Partizanit; in shady crevices and alcoves of limestone cliffs with NW exposition and surrounded sparsely by *Pinus heldreichii* and *Juniperus communis subsp. alpina* woods, alt: 1950 m, *Shuka L. & Xhulaj M., Hoda P. & Mahmutaj E.,* no. 6238, 13 July 2014.

Etymology

The specific epithet refers to the Latin name *Aurelius* (in Croatian Zlatko), and it is dedicated to the botanists Prof. Zlatko Liber and Prof. Zlatko Šatović from University of Zagreb (Croatia).

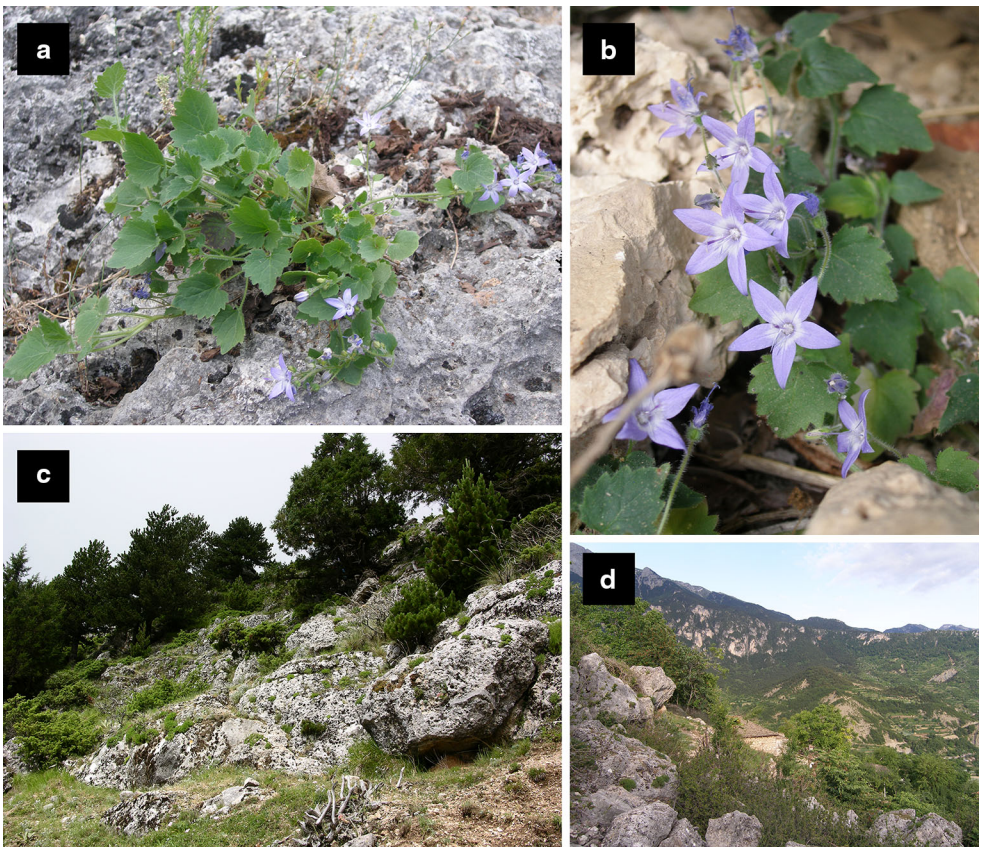

Fig. 6 *Campanula aureliana*. **a** Habit, **b** inflorescence, **c** and **d** habitat (photos by Sandro Bogdanović and Lulëzim Shuka)

Phenology

Flowering from May to July, depending on the altitude and fruiting late July to August.

Distribution and ecology

Campanula aureliana is only known from the single population in Tomori Mt that is fragmented into four subpopulations, Tomori and Kapinovë villages, Castle of Tomori and the northern peak of Tomori Mt in central Albania (Fig. 1). It grows on limestone in rocky crevices of shady places at an altitude of 500–1950 m asl (Fig. 6d). This species is a true chasmophyte exclusive of fresher niches, where it is a member of a rupestrian community characterized by *Aethionema saxatile* (L.) R.Br., *Ajuga chamaepytis* (L.) Schreb., *Asplenium ceterach* L., *A. ruta-muraria* L., *A. trichomanes* L., *Bupleurum veronense* Turra, *Campanula ramosissima* Sibth. & Sm., *Desmazeria rigida* (L.) Tutin, *Galium rubrum* L., *Geranium robertianum* L., *Leontodon crispus* Vill., *Micromeria juliana* (L.) Benth. ex Rchb., *Minuartia verna* (L.) Hiern, *Petrorhagia saxifraga* (L.) Link, *Putoria calabrica* (L.f.) DC., *Satureja montana* L., *Sedum acre* L., *Sedum ochroleucum* Chaix., and *Teucrium polium* L. among others. In the central part of distribution range (1200–1300 m asl), *C. aureliana* occurs in rocky cervices, within openings of *Fago-Pinetum leucodermis*, association of the order *Fagetalia sylvaticae* (Mahmutaj et al. 2013). In this habitat (Fig. 6c), it occurs in rupestrian communities dominated by *Juniperus foetidissima* Willd. and *J. oxycedrus* L., shrubs associated with *Acinos alpinus* (L.) Moench., *Alkanna pindicola* Hausskn., *Asplenium trichomanes* L., *Carex* sp., *Dactylis glomerata* L., *Globularia cordifolia* L., *Melica uniflora* Retz. *Micromeria cristata* (Hampe) Griseb., *M. juliana* (L.) Benth. ex Rchb., *Onosma echioides* L., *Parietaria officinalis* L., *Pterocephalus perennis* Coulter, *Ramonda serbica* Pančić, *Satureja montana* L., *Sedum acre* L., *Teucrium polium* L., and others. In the upper limit of distribution it is usually found in association with *Amphoricarpos autariatus* Blečić & E.Mayer, *Campanula spatulata* subsp. *spruneriana* (Hampe) Hayek, *Carex* sp., *Crepis baldaccii* Halácsy, *Festuca varia* Haenke, *Geranium macrorrhizum* L., *Heliosperma pusillum* (Waldst. & Kit.) Rchb., *Lamium garganicum* L., *Leontodon crispus* Vill., *Potentilla speciosa* Willd., *Saxifraga marginata* Sternb., *Saxifraga paniculata* Mill. and others.

Conservation status

Campanula aureliana is known from one population, distributed in four localities, all in western and north-western slopes of Mt. Tomori, in Berati district (central Albania), where approximately not more than 1000 mature individuals grow in a very small area of 8 km^2. Since the species is fragmented in four locations, half of which occurs in settled area, the habitat is under human influence and it could be threatened by human activities. Therefore, according to the IUCN Red list category (IUCN 2014), this species for its rarity, number of mature individuals and restricted population distribution, should be included on the list of threatened plants as Vulnerable—VU D1+2.

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