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A new bellflower, Campanula dersimensis (Campanulaceae), from E Anatolia, Turkev

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Abstract: Campanula dersimensis Fırat & Yıldırım, a new species endemic to the E Anatolian region of Turkey, is described and illustrated. It is currently known from two localities in Tunceli province (Dersim). Campanula dersimensis shows similarities to C. quercetorum Hub.-Mor. & C. Simon and C. yildirimlii Kit Tan & Sorger. A close relationship of the three taxa is also supported by phylogenetic analyses based on the nuclear-encoded ribosomal internal transcribed spacer (ITS) region. Diagnostic morphological characters are discussed and compared with those of closely related taxa. Campanula dersimensis is easily distinguished from related species, especially by its retrorsely hairy stem and leaf surface, to 1 mm long calyx appendages and glabrous and light greenish yellow to yellowish white corollas.

Keywords: Anatolia, endemic, *Campanula quercetorum*, *Campanula yildirimlii*, *Campanulaceae*, new species, novelty, taxonomy, Tunceli province, Turkey

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Introduction

Campanula L. is the largest genus of the family Campanulaceae and is represented by c. 420 species, distributed in temperate and subtropical regions of the N hemisphere (Federov 1957; Lammers 2007a, 2007b). The main centres of diversity for the genus are in the E Mediterranean region and the Caucasus, with approximately 150 species (Fedorov & Kovanda 1976; Contandriopoulos 1984; Jones & al. 2017). The majority of Campanula taxa are microclimatically and edaphically specialized rupicolous plants, and are often narrow endemics (Damboldt 1965; Kovanda 1970; Park & al. 2006; Pignatti 1982). The hotspots of endemic Campanula species are found in the E Mediterranean, the Balkans, the Caucasus and Turkey (Borsch & al. 2009; Haberle & al. 2009; Khansari & al. 2012).

Campanula is present in Turkey with c. 128 taxa, of which 61 are endemic, with an endemism rate of 47.7% (Damboldt 1965, 1978; Davis & al. 1988; Duman 1999; Güner 2000; Yıldız & Alçıtepe 2010; Alçıtepe 2011; İkinci 2012; Yıldırım 2013; Yıldırım & Şenol 2014; Mutlu & Karakuş 2015; Behçet & İlçim 2018; Yıldırım 2018; Yıldırım & al. 2019). The genus is represented in Turkey by six subgenera, namely C. subg. Brachycodonia (Fed.) Damboldt, C. subg. Campanula, C. subg.

Megalocalyx Damboldt, C. subg. Rapunculus (Fourr.) Charadze, C. subg. Roucela (Dumort.) Damboldt and C. subg. Sicyodon (Feer) Damboldt. Campanula in Turkey was further divided into 13 sections by Damboldt (1978).

Campanula sect. Rupestres is characterized as follows: plants perennial, suffruticose-caespitose; stems numerous, fragile, few-flowered, often low; basal leaves usually in dense rosettes; calyx appendages distinctly developed or inconspicuous; capsule opening by 3 basal pores, rarely dehiscing irregularly between ribs (Phitos 1965; Damboldt 1978; Yıldırım 2013).

In the summer of 2012, during a botanical trip by the first author to districts of Nazimiye and Ovacık in Tunceli province (Dersim), in E Anatolia, Turkey, an interesting specimen of *Campanula* was collected. As a result of a detailed literature survey and morphological studies with copious herbarium material along with phylogenetic analyses, it was concluded that the plants from Tunceli province differed from their close relatives.

Material and methods

The samples of new species were compared with herbarium specimens at the herbaria AEF, AIBU, ANK,

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B, E, EGE, G, GAZI, HUB, ISTE, ISTF, K, NGBB, P, VANF, W and WU (herbarium codes according to Thiers 2022+) as well as the personal herbarium of M. Fırat by Hasan Yıldırım. In addition, relevant literature (Phitos 1963, 1964, 2016; Boissier 1875; Fedorov 1957; Rechinger & Schimann-Czeika 1965 Damboldt 1976, 1978; Fedorov & Kovanda 1976; Pignatti 1982; Davis & al. 1988; Lammers 2007a, 2007b; Güner 2000; İkinci 2012) was reviewed. The gross morphology of the new species, *Campanula quercetorum* Hub.-Mor. & C. Simon and *C. yildirimlii* Kit Tan & Sorger were examined using a stereo-binocular microscope.

Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Internal Transcribed Spacer (ITS) regions (including ITS1 and ITS2) and the 5.8S gene of nuclear ribosomal DNA (hereafter ITS) were amplified using the primers ITS1 and ITS4 (White & al. 1990). Polymerase chain reaction (PCR) amplification was carried out in 50- μ L volumes containing 25 μ L of Dreamtag green PCR Mastermix (Thermo-Fisher Scientific, U.S.A.), 23 μ L of water, 0.5 μ L each of primer, and 1 μ L of DNA template. The PCR conditions were: 95°C for 3 min; 35 cycles at 95°C for 35 s, 48°C for 35 s, and 70°C for 1 min; and finally 72°C for 10 min. Purification

and sequencing were performed by ECGR Lab (Hacettepe University, Biology Department, Ankara, Turkey). Exonuclease (ExoI) and shrimp alkaline phosphatase (SAP) enzymes were used to purify the PCR products. Sequence purification was accomplished by adding sodium acetate to the samples and then centrifuging them at 13,000 rpm for 20 min with 100% alcohol and then for 5 min with 70% alcohol, respectively. An Applied Biosystem Genetic Analyzer 3500 was used to perform Sanger sequencing of the PCR products. The ITS sequences of two specimens of Campanula sp. nov., two specimens of C. quercetorum, and one specimen of C. yildirimlii were obtained in the present study for the first time, and the remaining sequences from the genus Campanula were taken from GenBank (Table 1). The Turkish members of the traditional C. sect. Rupestres and the CAM 17 clade described by Mansion & al. (2012), in addition to C. jacquinii (Sieber) A. DC. as an outgroup, were used in the phylogenetic analyses for 2 reasons: (1) considering the large number of species in C. sect. Rupestres, only Turkish members of the section were selected because the aim of this study was to reveal the relationship between the new species and its asumed close relatives, C. quercetorum and C. yildirimlii, rather than revealing the phylogeny of this section; (2) some species from outside

Table 1. Voucher information and GenBank accession numbers of the ITS sequences used in the phylogenetic analyses. Sequences newly generated for this study are marked with an asterisk (*).

Campanula species	Voucher information	GenBank accession no.	
C. argentea	Turkey, Erzincan: H. Yıldırım 3265 (EGE!)	MT774677	
C. bellidifolia	Armenia, Lori province: Mt Lalvar, <i>Fayvush, Tamanyan, Ter-Voskanian & Vitek 03-0453</i> (B 10 0259162)	MT774629	
C. bornmuelleri	Turkey, Van: Bahçesaray road, H. Yıldırım 3139 (EGE!)	MT774679	
C. calaminthifolia	Greece: Isl. Naxos, Mt Koronos, Runemark 51461 (LD 1377534)	MT774671	
C. choruhensis	Turkey, Erzurum: Erzurum to Artvin way to Morkayalar, H. Yıldırım 2931 (EGE!)	MT774619	
C. conferta	Turkey, Tunceli: Ovacık üzeri, Kepirtepe aşağısı, Kepir Yaylası, <i>H. Yıldırım 3096</i> (EGE!)	MT774675	
C. dersimensis	Turkey, Tunceli: on way from Tunceli to Nazimiye, Kutu Deresi, around Zülfikar OL764503*, OL764504 fountain, 1040 m, limestone rock crevices, 1 Aug 2012, <i>M. Fırat</i> 28888 (holotype: EGE 43200!)		
C. isaurica	Turkey, Antalya: Göndoğmuş, Cirlavik hill, H. Yıldırım 2989 (EGE!)	MT774626	
C. karakuschensis	Turkey, Van: Mt Erek, H. Yıldırım 3133 (EGE!)	MT774674	
C. massalskyi	Turkey, Kars: Digor, H. Yıldırım 3123 (EGE!)	MT774624	
C. pinnatifida	Turkey, Kayseri: Pınarbaşı, H. Yıldırım 3028 (EGE!)	MT774621	
C. pulvinaris	Turkey, Yozgat: Çayırhan to Büyük Toraman, H. Yıldırım 3671 (EGE!)	MT774620	
C. quercetorum	Turkey, Tunceli: Pülümür-Tunceli road in front of tunnel 8, 1200 m, on rock, 20 Jul 2015, <i>H. Yıldırım 3461</i> (EGE!)	OL764505*, OL764506*	
C. scoparia	Turkey, Malatya: Darende girişi, H. Yıldırım 3062 (EGE!)	MT774618	
C. stricta	Turkey, Malatya: Arapgir, summit of Mt Göldağı, H. Yıldırım 3465 (EGE!)	MT774617	
C. strigillosa	Turkey, Sivas: E Gök Pinar, Sorger 76-21-11 (B 10 0356910)	MT774702	
C. teucrioides	Turkey, Izmir: Boz Dağ, <i>Hein 4238a</i> (B 10 0209967)	MT774710	
C. tridentata	Turkey, Rize: Alpine pasture 800 m E of Ovitdagi pass, Svensson 6219 (LD 1312844)	MT774708	
C. yildirimlii	Turkey, Erzincan: Kemaliye, Salihli village, 1484 m, serpentine area, 15 Jul 2014, <i>H. Yıldırım 3060</i> (EGE!)	OL764507*	
C. yildirimlii	Turkey, Malatya: Arapgir to Kayaarası canyon, H. Yıldırım 2900 (EGE!)	MT774688	
C. jacquinii (outgroup)	Greece: Isl. Crete, Montes Lefka Ori, supra pagum Kampi in loco Chionotrypa, <i>Tzanoudakis 1520</i> (UPA)	MT774694	



Fig. 1. Holotype of Campanula dersimensis: M. Fırat 28888 (EGE 43200).

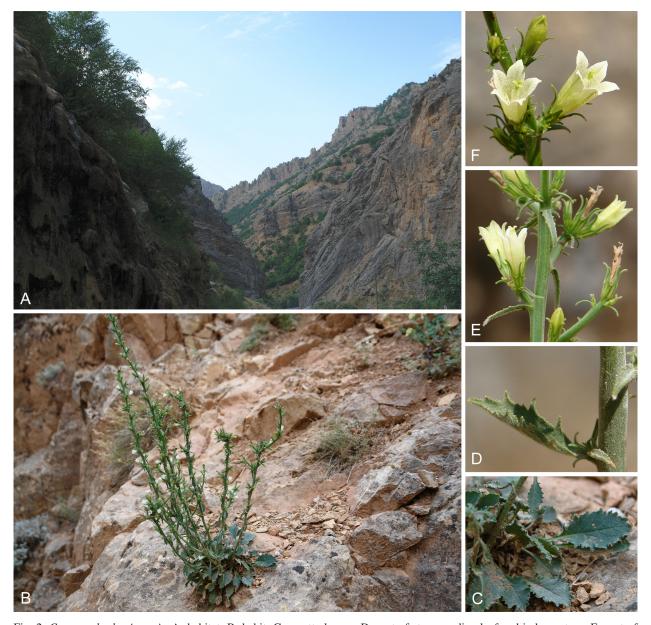


Fig. 2. Campanula dersimensis: A: habitat; B: habit; C: rosette leaves; D: part of stem, cauline leaf and indumentum; E: part of inflorescence; F: flowers. – Turkey, Tunceli, 2012, photographs by Mehmet Fırat.

of *C.* sect. *Rupestres* were also used to obtain a compatible tree with the CAM17 clade of Mansion & al. (2012).

The raw ITS sequences were edited with Codon code aligner (CodonCode Corporation) and aligned with MUSCLE v.3.6 (Edgar 2004). The alignment file is provided as Supplementary file S1. The GTR+G model was selected by MEGA X (Kumar & al. 2018). Maximum likelihood analysis was performed with RAxML (Stamatakis 2006), choosing the rapid bootstrapping option with 1000 bootstrap iterations. Bayesian analysis was performed using MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003). Two simultaneous runs of Metropolis-coupled Markov chain Monte Carlo (MCMC) were sampled for 10,000,000 generations, and one tree was sampled every 1000 generations. The phylogenetic trees were visualized using FigTree v1.4.4 (Rambaut 2018).

Results

Campanula dersimensis Fırat & Yıldırım, **sp. nov.** – Fig. 1–3.

Holotype: Turkey, B7, Tunceli, on way from Tunceli to Nazimiye, Kutu Deresi, around Zülfikar fountain, 39°11'N, 39°42'E, 1040 m, limestone rock crevices, 1 Aug 2012, *M. Fırat* 28888 (EGE 43200!; isotypes: HUB!, VANF!, herb. M. Fırat!).

Diagnosis — Campanula dersimensis is similar to C. quercetorum and C. yildirimlii. It is easily distinguished from them mainly by having stems erect, dark green, unbranched or shortly branched in upper half, retrorsely hirtulous to pubescent-puberulent, rarely subglabrous above; bracts 4–20 mm long, distinctly hairy; calyx lobes



Fig. 3. A–C: *Campanula dersimensis*; A: habit; B: inflorescence; C: flower; Turkey, Tunceli, 2012. – D–F: *C. quercetorum*; D: habit; E: inflorescence; F: flower; Turkey, Tunceli, 2015. – G–I: *C. yildirimlii*; G: habit; H: inflorescence; I: flower; Turkey, Erzincan, 2014. – Photographs: A–C, F by Mehmet Fırat; D, E, G–I by Hasan Yıldırım.

pointed backward, linearlanceolate, retrorsely hispidulous toward apex; calyx appendages present, to 1 mm long; corolla light greenish yellow to yellowish white, mostly glabrous outside; capsule broadly ovoid-cylindric when mature, glabrous, membranous structure between veins breaking down and capsule opening with (9 or)10 valves.

Description — Herbs perennial, monocarpic, caespitose, with thick roots. Stems many, woody-based, erect, dark green, 15-85 cm long, unbranched or shortly branched in upper half, retrorsely hirtulous to pubescent-puberulent, rarely subglabrous above. Rosette leaves spatulate to oblanceolate, $1.5-8 \times 0.5-2.3$ cm. both surfaces densely retrorsely hirsute and pubescent, margin usually retrorsely hispidulous; petiole Cauline leaves winged. spatulate-oblanceolate to elliptic, $1-8 \times 0.4-2$ cm, both surfaces hirsute-pubescent, margin serrate to dentate, hispidulous or hirtulous; petiole absent to short and winged. Inflorescence spicate to branched spicate; flowers solitary or in clusters of 2 or 3, sessile or pedicel to 2 mm long. Bracts distinct, 1-3 at each node,

linear-lanceolate, 4–20 mm long, both surfaces densely pubescent to puberulent, margin retrorsely hispidulous. *Calyx* tube with pronounced dark green veins and light green membranous structure between veins; calyx lobes pointed backward, linear-lanceolate, 3–6 × 1–2 mm, retrorsely hispidulous toward apex; calyx appendages to 1 mm long. *Corolla* light greenish yellow to yellowish white, cylindric-campanulate, 9–17 × 4–6 mm, split to 1/5–1/4, glabrous outside, long hairy inside; corolla lobes triangular, 2–4 × 1–2.5 mm, midrib and apex sometimes sparsely hirtulous. *Stamens* 5–9 mm long; filament 2–3 mm long, triangular at base, base 1–1.7 mm wide, margin shortly hairy; anther 3–6 mm long. *Style* 5–11 mm long, as long as corolla or slightly

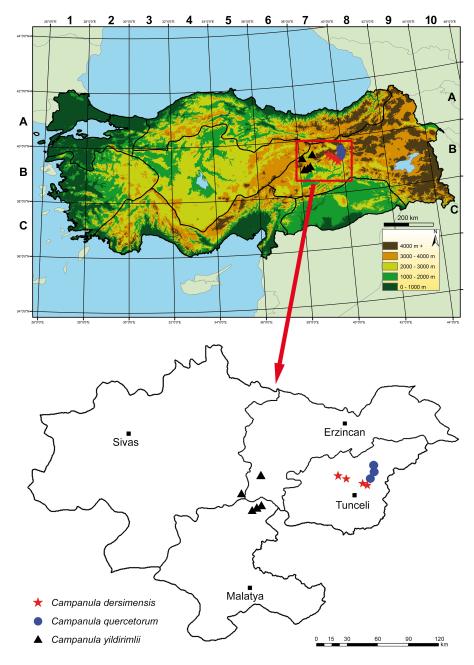


Fig. 4. Distribution map for *Campanula dersimensis*, *C. quercetorum* and *C. yildirimlii* in Turkev.

exserted; stigma 3-lobed. *Capsule* broadly ovoid-cylindric when mature, $2-6 \times 1.5-5$ mm, glabrous, prominently (9 or)10-ribbed; membranous structure between veins breaking down and capsule opening with (9 or)10 valves.

Phenology — Flowering in July and August.

Etymology — The specific epithet is derived from Dersim, which is the former name of Tunceli province.

Distribution and ecology — Campanula dersimensis is a local endemic to Tunceli province, E Anatolia, Turkey (Fig. 4), and belongs to the Irano-Turanian floristic

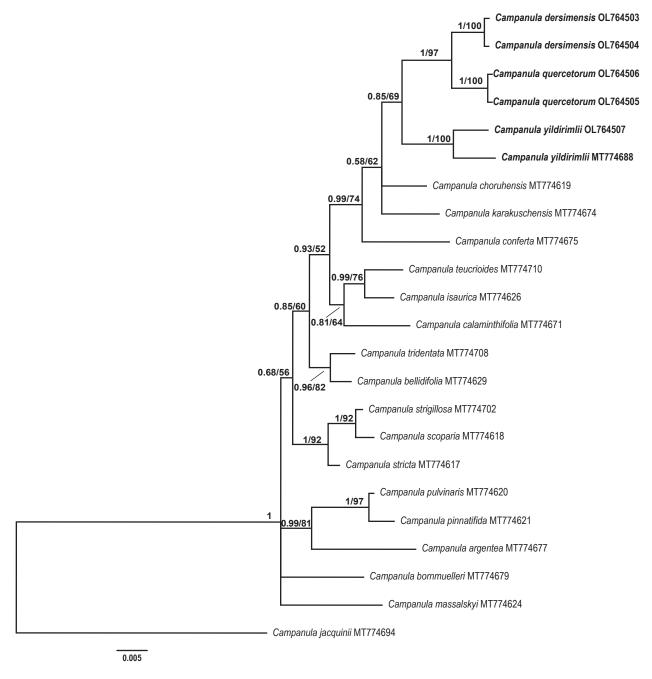


Fig. 5. Phylogenetic placement of *Campanula dersimensis* based on Internal Transcribed Spacer (ITS). Phylogram is derived from Bayesian analysis. Posterior probabilities (> 0.5) and bootstrap values (derived from maximum likelihood analysis) are given above the branches, respectively. *Campanula dersimensis* and its close relatives *C. quercetorum* and *C. yildirimlii* are indicated as boldface.

region element. This area has a continental, semiarid climate with hot, dry summers and cold, snowy winters. The species grows in rock crevices in calcareous rocky areas at altitudes of 1040–1130 m. Common species growing in the near vicinity include Asplenium haussknechtii Godet & Reut., Cerasus mahaleb (L.) Mill. var. mahaleb, Dianthus orientalis Adams, Ficus carica L. subsp. carica, Micromeria cremnophila subsp. anatolica P. H. Davis, Origanum munzurense Kit Tan & Sorger, Parietaria judaica L. and Teucrium polium L.

Phylogenetic placement — The aligned ITS dataset included 23 sequences belonging to 20 species and was 720 bp long, of which 50 were potentially parsimony informative and 83 were variable but not informative.

Campanula dersimensis and its morphologically close relatives, C. quercetorum and C. yildirimlii, were grouped together with moderate posterior probability (0.85) and bootstrap support (69) in the phylogenetic analyses (Fig. 5), whereas the monophyly and sister relationship of C. dersimensis and C. quercetorum were strongly supported (PP = 1 and BS = 97, respectively).

Table 2. Morphological differences between Campanula dersimensis, C. quercetorum and C. yildirimlii.

Characters	C. dersimensis	C. quercetorum	C. yildirimlii
Plant colour	dark green	green	glaucous
Stem	erect, 15–85 cm long, unbranched or shortly branched in upper half, retrorsely hirtulous to pubescent- puberulent, rarely subglabrous above	prostrate to ascending, 10–40 cm long, distinctly branched, retrorsely hirsute to pubescent	erect, 25–92 cm long, unbranched or very shortly branched, glabrous
Leaves	both surfaces densely retrorsely hirsute and pubescent, margin usually retrorsely hispidulous and hirtulous	both surfaces densely retrorsely hirsute to pubescent, margin hispidulous and hirtulous	both surfaces glabrous, margin retrorsely hispidulous
Inflorescence	spicate to branched spicate; flowers solitary or in clusters of 2 or 3, sessile or pedicel to 2 mm long	racemose to paniculate; flowers solitary, pedicel 1–9 mm long	spicate; flowers solitary or in clusters of 2–7, sessile or pedicel to 2 mm long
Bracts	4–20 mm long, both surfaces densely pubescent to puberulent, margin retrorsely hispidulous	4–12 mm long, both surfaces and margin hispidulous	2–4 mm long, glabrous
Calyx lobes	linear-lanceolate, 3–6 mm long, retrorsely hispidulous toward apex	linear-lanceolate, 4.7–6.7 mm long, retrorsely hispidulous at apex	triangular, 2.5–6 mm long, glabrous
Calyx appendages	to 1 mm long	to 0.8 mm long	absent
Corolla	light greenish yellow to yellowish white, 9–17 mm long, splitting to 1/5–1/4, glabrous outside, midrib and apex of lobes sometimes sparsely hirtulous	whitish blue, cream or light greenish yellow to whitish pink, 10–17 mm long, splitting to 1/5–1/4, wholly retrorsely hirsute outside or sometimes only on midrib	whitish to pinkish purple, 8–15 mm long, splitting to 1/4–1/3, glabrous outside
Filaments	2–3 mm long, triangular at base, shortly hairy at margin of base	1.7–2.7 mm long, triangular at base, ciliate at margin of base	2–3 mm long, triangular at base, papillate-puberulent at margin of base
Anthers	3–6 mm long	4–5.5 mm long	5–7 mm long
Style	5–11 mm long	6–13 mm long	8–13 mm long
Capsule	broadly ovoid-cylindric when mature, $2-6 \times 1.5-5$ mm, glabrous, (9 or) 10-ribbed, membranous structure between veins breaking down and capsule opening with (9 or)10 valves	semiglobose when mature, 2.5 – 4.8×2 – 3.5 mm, densely hirsute, 9- or 10-ribbed, capsule opening with 3 basal pores	globose when mature, 2–4 × 2–4 mm, glabrous, 10-ribbed, membranous structure between veins breaking down and capsule opening with 5–10 valves

Additional specimens examined — Campanula dersimensis (paratypes) — TURKEY: TUNCELI: on way from Tunceli to Nazimiye, Kutu Deresi, 39°12'N, 39°39'E, 1104 m, rocky areas, 5 Aug 2014, M. Firat 31538 (herb. M. Firat!); same locality, 28 Jul 2012, M. Firat 28876 (herb. M. Firat!); on way from Tunceli to Ovacık, Munzur valley, 39°15'N, 39°28'E, 1087 m, rocky areas, 27 Jul 2012, M. Firat 28868 (herb. M. Firat!); Tunceli-Ovacik road, Ovacık canyon, approximately 20 km from Ovacik district, 39°17'N, 39°23'E, 1127 m, 21 Jul 2015, H. Yıldırım 3464a (EGE!).

Campanula quercetorum — TURKEY: TUNCELI: on way from Tunceli to Nazimiye, Kutu Deresi, 39°14'N, 39°44'E, 1272 m, rocky areas, 28 Jul 2012, *M. Furat* 28892 (herb. M. Fırat); Kutu Deresi, Pülümür road, on rocks, 21 Jul 2014, *H. Yıldırım 3103* (EGE!); Pülümür-Tunceli road in front of tunnel 8, 39°20'N, 39°47'E, 1200 m, on rock, 20 Jul 2015, *H. Yıldırım 3461* (EGE!); Pülümür, Tunceli to Pülümür NE of Tunceli, 1500 m, on steep, open slopes, 26 Aug 1986, *Archibald 8139* (E

148/88-48!); Tunceli-Pülümür, Quercetum beim Karakol am Harçik suyu, 1100 m, 28 Jun 1951, *Huber-Morath 11072* (G!); Pülümür, Kalkschlucht [limestone gorge] Tunceli-Pülümür, 46 km nördlich Tunceli, 1100 m, 7 Jun 1959, *Huber-Morath 15705* (G!).

Campanula yildirimlii — Turkey: Erzincan: Kemaliye, Sandıkbağı surroundings, 900 m, rocky places, 17 Nov 1980, Ş. Yıldırımlı 4174 (paratypes: ANK!, B!); Kemaliye, Salihli village, 39°20'N, 38°30'E, 1484 m, serpentine area, 15 Jul 2014, H. Yıldırım 3060 (EGE!). — Malatya: Arguvan, Göldağı slopes, Yukarı Soğuksu, Perideresi valley, 39°02'N, 38°25'E, 1484 m, 15 Jun 2014 (sterile), H. Yıldırım 2887 (EGE!); Arguvan, Divriği road 3 km, 39°01'N, 38°23'E, 1609 m, limestone cliffs, 21 Jul 2015, H. Yıldırım 3469 (EGE!); Arapgir, Kayaarası Kanyonu, fish farm, 39°02'50"N, 38°26'31"E, 1195 m, cliff, 8 Sep 2015, H. Yıldırım 3690 (EGE!). — Malatya/Sıvas: Arapgir, 2–3 km after Divriği road junction, near fountain, 39°10'N, 38°17'E, 1451 m, 8 Sep 2015, H. Yıldırım 3691 (EGE!).

Discussion

Campanula dersimensis grows in rock crevices in the Ovacık canyon and on rocky roadsides along the road from Tunceli to Nazimiye. It is similar to *C. quercetorum* by its calyx structure and the presence of small appendages between the calyx lobes, and a close relationship was strongly supported by the Bayesian and maximum likelihood analyses (Fig. 5). It is also close to *C. yildirimlii* by its unbranched or shortly branched stems, corolla glabrous outside and capsules opening with 10 valves between the veins.

However, the new species is clearly distinguished from both *Campanula quercetorum* and *C. yildirimlii* by the features summarized in Table 2.

With the description of this new species, the total number of *Campanula* taxa in Turkey has increased to 129 (105 species), 62 of which are endemic to Turkey (Damboldt 1965, 1978; Davis & al. 1988; Duman 1999; Güner 2000; Yıldız & Alçıtepe 2010; Alçıtepe 2011; İkinci 2012; Yıldırım 2013; Yıldırım & Şenol 2014; Mutlu & Karakuş 2015; Behçet & İlçim 2018; Yıldırım 2018; Yıldırım & al. 2019).

The phylogenetic backbone of the tree was congruent with that of Liveri & al. (2020), and all of the species, assigned to the CAM17 clade of Mansion & al. (2012), except for *Campanula jacquinii* (outgroup), formed a monophyletic group. Additionally, the close relationship between *C. quercetorum* and *C. yildirimlii* was supported by the phylogenetic analyses, and those two species along with the newly described *C. dersimensis* comprised a clade.

Author contributions

MF collected the plant specimens and, together with HY, conducted the morphological studies. BÖ conducted the laboratory studies and phylogenetic analyses. MF, BÖ, and HY wrote the manuscript and approved the final version.

Acknowledgements

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Supplementary file S1. Alignment in Nexus format for the ITS dataset.

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