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

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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 (Fedorov 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 & Alçetepe 2010; Alçetepe 2011; İkinci 2012; Yıldırım 2013; Yıldırım & Şenol 2014; Mutlu & Karakuş 2015; Behçet & Işık 2018; Yıldırım 2018; Yıldırım & al. 2019). The genus is represented in Turkey by six subgenera, namely C. subg. Brachycodon (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,
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 & Schimm in-Czéika 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. yıldirimlii 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 rRNA 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-μL volumes containing 25 μL of Dreamtag green PCR Mastermix (Thermo-Fisher Scientific) and 3 μL of DNA template. The PCR conditions were: 95°C for 1 min; 72°C for 10 min. Purification and sequencing were performed by ECGR Lab (Hacettepe University, Biology Department, Ankara, Turkey). Exonuclease (Exol) 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 Biosystems 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. yıldirimlii 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., 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 assumed close relatives, C. quercetorum and C. yıldirimlii, rather than revealing the phylogeny of this section; (2) some species from outside

| Campanula species                  | Voucher information                                                                 | GenBank accession no. |
|-----------------------------------|--------------------------------------------------------------------------------------|-----------------------|
| C. argentea                       | Turkey, Erzincan: H. Yıldırım 3265 (EGE!)                                           | MT774677              |
| C. bellidifolia                   | Armenia, Lori province: Mt Lalvar, Favyush, Tamanyan, Ter-Voskanian & Vitek 03-0453 | MT774629              |
| C. borrnmuelleri                  | Turkey, Van: Bahçeşaray road, H. Yıldırım 3139 (EGE!)                              | MT774679              |
| C. calaminthifolia                | Greece: Isl. Naxos, Mt Koronos, Runemark 51461 (LD 1377534)                        | MT774671              |
| C. chorudensis                    | Turkey, Erzurum: Erzurum to Artvin way to Morkayalar, H. Yıldırım 2931 (EGE!)      | MT774619              |
| C. conferta                       | Turkey, Tunceli: Ovacık işeri, Kepirtepe aşağısı, Kepir Yaylası, H. Yıldırım 3096   | MT774675              |
| C. desimsensis                    | Turkey, Tunceli: on way from Tunceli to Nazimije, Kutu Deresi, around Zülükfar fountain, 1040 m, limestone rock crevices, 1 Aug 2012, M. Fırat 28888 (holotype: EGE 43200)! | OL764503*, OL764504* |
| C. isaurica                       | Turkey, Antalya: Gündoğmuş, Cirilavik hill, H. Yıldırım 2989 (EGE!)                 | MT774626              |
| C. karakuschnica                  | Turkey, Van: Mt Erek, H. Yıldırım 3133 (EGE!)                                       | MT774674              |
| C. massalxkyi                     | Turkey, Kars: Digor, H. Yıldırım 3123 (EGE!)                                        | MT774624              |
| C. pinnatilda                     | Turkey, Kayseri: Pınarbaş, H. Yıldırım 3028 (EGE!)                                 | MT774621              |
| C. pulvinaris                      | Turkey, Yozgat: Çayırhan to Büyük Toraman, H. Yıldırım 3671 (EGE!)                 | MT774620              |
| C. quercetorum                    | Turkey, Tunceli: Pülimür-Tunceli road in front of tunnel 8, 1200 m, on rock, 20 Jul 2015, H. Yıldırım 3461 (EGE!) | OL764505*, OL764506* |
| C. scoparia                       | Turkey, Malatya: Darenê girîşî, H. Yıldırım 3062 (EGE!)                            | MT774618              |
| C. stricta                        | Turkey, Malatya: Arapgir, summit of Mt Goldaçi, H. Yıldırım 3465 (EGE!)             | MT774617              |
| C. strigillosa                    | Turkey, Sivas: E Gök Pınar, Sorger 76-21-11 (B 10 0356910)                          | MT774702              |
| C. tricrenoides                   | Turkey, Izmır: Boz Dağ, Hein 4238a (B 10 0299967)                                  | MT774710              |
| C. tridentata                     | Turkey, Rize: Alpine pasture 800m E of Ovitadi pass, Svensson 6219 (LD 1312844)      | MT774708              |
| C. yıldirimlii                     | Turkey, Erzincan: Kemaliye, Salihli village, 1484 m, serpentine area, 15 Jul 2014, H. Yıldırım 3060 (EGE!) | OL764507*              |
| C. yıldirimlii                     | Turkey, Malatya: Arapgir to Kayaarsı canyon, H. Yıldırım 2900 (EGE!)               | MT774688              |
| C. jacquinii (outgroup)           | Greece: Isl. Crete, Montes Lefka Ori, supra pagum Kampi in loco Chionotrypa, Tzanoudakis 1520 (UPA) | MT774694              |
Fig. 1. Holotype of *Campanula dersimensis*: M. Fırat 28888 (EGE 43200).
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** Firat & 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. querctorum*; D: habit; E: inflorescence; F: flower; Turkey, Tunceli, 2015. – G–I: *C. yildrimii*; G: habit; H: inflorescence; I: flower; Turkey, Erzincan, 2014. – Photographs: A–C, F by Mehmet Firat; D, E, G–I by Hasan Yıldırım.
pointed backward, linear-lanceolate, 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 short-ly branched in upper half, retrorsely hirtulous to pubescent-puberulent, rarely subglabrous above. Rosette leaves spatulate to oblanceolate, 1.5–8 × 0.5–2.3 cm, both surfaces densely retrorsely hisrute and pubescent, margin usually retrorsely hispidulous; petiole winged. Cauline leaves spatulate-ob lanceolate to elliptic, 1–8 × 0.4–2 cm, both surfaces hirsute-puberulent, 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 exserted; stigma 3-lobed. Capsule broadly ovoid-cylindric when mature, 2–6 × 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
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.

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.

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.
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 hirsute and pubescent, 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 hirsute | both surfaces densely retrorsely hirsute to pubescent, margin hispidulous and hirsute | 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 hirsute | 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 | whith 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 × 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 Nazimiyé, Kutu Deresi, 39°12'N, 39°39'E, 1104 m, rocky areas, 5 Aug 2014, M. Fırat!; same locality, 28 Jul 2012, M. Fırat 28876 (herb. M. Fırat!); on way from Tunceli to Ovacık, Munzur valley, 39°15'N, 39°28'E, 1087 m, rocky areas, 27 Jul 2012, M. Fırat 28868 (herb. M. Fırat!).

— Turkey: Erzincan: Kemaliye, Sandıkbağı surroundings, 900 m, rocky places, 17 Nov 1980, Ş. Yıldırımlı 4174 (paratypes: ANK!, B!);

— Malatya: Arguvan, Göldağı slopes, Yukarı Soğuksu, serpentine area, 15 Jul 2014, H. Yıldırım 3060 (EGE!).

— Malatya: Arguvan, Divriği road junction, near fountain, 39°02'50''N, 38°26'31''E, 1195 m, 8 Sep 2015, H. Yıldırım 3690 (EGE!).

*Campanula quercetorum* — TURKEY: Tunceli: on way from Tunceli to Nazimiyé, Kutu Deresi, 39°14'N, 38°39'E, 1500 m, on steep, open slopes, 26 Aug 1986, Archibald 8139 (E 148/88-48!); Tunceli-Püllümür, Quercetum beim Karakol am Harçik suyu, 1100 m, 28 Jun 1951, Huber-Morath 11072 (G!); Püllümür, Kalkschlucht [limestone gorge] Tunecilik-Püllümür, 46 km nördlich Tunceli, 1100 m, 7 Jun 1959, Huber-Morath 15705 (G!).

— Malatya: Arguvan, Göldağı slopes, Yukarı Soğuksu, serpentine area, 15 Jul 2014, H. Yıldırım 3060 (EGE!).

*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, serpentine area, 39°02'50''N, 38°25'50''E, 1484 m, 15 Jun 2014 (sterile), H. Yıldırım 2887 (EGE!).

— Malatya: Arguvan, Divriği road junction, near fountain, 39°02'50''N, 38°26'31''E, 1195 m, 8 Sep 2015, H. Yıldırım 3690 (EGE!).

— Malatya: Arguvan, 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 Nazımie. 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 & Alçetepe 2010; Alçetepe 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. BO conducted the laboratory studies and phylogenetic analyses. MF, BO, and HY wrote the manuscript and approved the final version.

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Supplemental content online

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