

Two new species of *Cephalaria* (Caprifoliaceae) from Turkey

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Abstract: Two new *Cephalaria* Schrad. ex Roem. et Schult. species (Caprifoliaceae) from Turkey, *Cephalaria davisiana* Göktürk & Sümbül and *Cephalaria sumbuliana* Göktürk, are described and illustrated. A map showing their distribution and other related taxa is given. The morphological differences of the 2 new species are discussed and comparisons are made with the similar *Cephalaria speciosa* Boiss. & Kotschy and *Cephalaria elmaliensis* Hub.-Mor. & V.A. Matthews. In addition to morphological observations, *C. davisiana*, *C. sumbuliana*, *C. elmaliensis*, and *C. speciosa* were analysed by applying RAPD-PCR techniques. RAPD methods clearly distinguished between the new species and the 2 species that they resemble.

Key words: Caprifoliaceae, *Cephalaria*, new species, RAPD-PCR, taxonomy

Türkiye *Cephalaria* (Caprifoliaceae) cinsinden iki yeni tür

Özet: Türkiye'den iki yeni *Cephalaria* Schrad. ex Roem. et Schult. (Caprifoliaceae) türü, *Cephalaria davisiana* Göktürk & Sümbül ve *Cephalaria sumbuliana* Göktürk tanımlandı ve çizildi. Bu yeni türlerin ve akraba türlerin yayılışını gösteren bir harita verildi. Yeni türlerin morfolojik farklılıkları, akraba olan *Cephalaria speciosa* Boiss. & Kotschy ve *C. elmaliensis* Hub.-Mor. & V.A. Matthews ile tartışıldı ve karşılaştırıldı. *C. davisiana*, *C. sumbuliana*, *C. elmaliensis* ve *C. speciosa* türlerinin morfolojik incelemelerine ilave olarak RAPD-PCR tekniği ile de analizleri yapılmıştır. RAPD methodları yeni türler ile ona benzeyen iki tür arasındaki farkı açıkça göstermektedir.

Anahtar sözcükler: Caprifoliaceae, *Cephalaria*, yeni tür, RAPD-PCR, taksonomi

Introduction

Turkey is one of the richest countries in the world in terms of flora. Different new taxa were described from Turkey in 2011 (Aytaç & Türkmen, 2011; Budak & Koç, 2011; Dönmez, 2011; Karavelioğulları et al., 2011). The genus *Cephalaria* Schrad. ex Roem. et

Schult. is included in the family Caprifoliaceae (Reveal & Chase 2011). The main centres of distribution of *Cephalaria* species are in South Africa and the Holarctic kingdom (Mediterranean area, Balkan peninsula, South Ukraine, Caucasia, Iran, West China, and Middle East) (Szabó, 1940). During fieldwork for revision of the genus *Cephalaria* in Turkey, materials

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of the studied taxa were collected by the senior author from Kahramanmaraş (*C. davisiana*) in 2001 and from Konya (*C. sumbuliana*) in 2000.

Molecular markers successfully developed in recent years have largely overcome the problems that are associated with phenotype-based classification of plants. PCR-based techniques such as random amplified polymorphic DNA (RAPDs) are used to identify polymorphism between and among species. Amplified fragment length polymorphisms (AFLP) provide DNA markers that are dispersed throughout plant genomes and are easier to reproduce and analyse (Awasthi et al., 2004). RAPD is a polymerase chain reaction derived marker with nonspecific random primers. This technique has become popular as researchers do not need sequence information of the genome for the target plan for animal species (Nybom, 2004). The RAPD method was introduced in 1990 by Williams et al. (1990). DNA bands produced by the RAPD technique indicate dominantly inherited markers, but it does not distinguish heterozygous from homozygous (a band is present or absent). Most researchers use polymorphic bands in the assessment of genetic diversity. However, in order to compare closely related taxa, monomorphic and polymorphic bands are included in this RAPD approach to plant systematics (Williams et al., 1990; Çelebi et al., 2009; Kavalcioğlu et al., 2010). The sampling strategies are also very important. The increase in number of plants per population had a significant positive effect on the calculation of genetic diversity as well as increasing the number of primers tested. The advantages of these techniques are their simplicity, rapidity, and

ability to detect extensive polymorphisms from small amounts of genomic DNA (Hu & Quiros, 1991).

Cephalaria davisiana Göktürk & Sümbül, *C. sumbuliana* Göktürk, *C. speciosa* Boiss. & Kotschy, and *C. elmaliensis* Hub.-Mor. & V.A. Matthews are more closely related to each other than to the remaining species. *Cephalaria speciosa* is an endemic species found in eastern Anatolia in Turkey. *Cephalaria elmaliensis* is a narrowly endemic species that is a constituent of the Mediterranean element and grows in small area in Çıglıkara (Elmalı, Antalya) in Turkey. The hypothesised new taxa *C. davisiana* and *C. sumbuliana* and the other related species *C. speciosa* and *C. elmaliensis* were studied morphologically and then using molecular techniques.

The RAPD-PCR technique was then employed to differentiate the 2 new species and their 2 related taxa. In order to evaluate whether the proposed taxa really do represent 2 new *Cephalaria* species, we analysed and compared samples of 2 new species with closely allied species *C. speciosa* and *C. elmaliensis* using 22 RAPD primers.

Materials and methods

In total, 22 specimens (27 individuals) of *C. davisiana* and 34 specimens (41 individuals) of *C. sumbuliana* were collected from the type localities and deposited in Akdeniz Univ. Herb., ANK, GAZI, and HUB.

C. davisiana, *C. sumbuliana*, *C. elmaliensis*, and *C. speciosa* were compared using the RAPD-PCR technique (Table 1). Total genomic DNA was isolated

Table 1. The voucher specimens of the *Cephalaria* species used in RAPD analyses and their localities.

Species	Locality
<i>C. davisiana</i>	C6 Kahramanmaraş : between Göksun and Sarız, east of Doğankonak village, Binboğa mountain, mountain slopes, 1700-2000 m, 3.8.2001, R.S.Göktürk 4733 & M.Göktürk (Akdeniz Univ. Herb.).
<i>C. sumbuliana</i>	C4 Konya : Seydişehir, 21 km from Seydişehir to Akseki, rocky places, under and clearings in <i>Abies</i> forest, 1435 m, 8.8.2000, R.S.Göktürk 4466 & F.Göktürk (Akdeniz Univ. Herb.).
<i>C. elmaliensis</i>	C2 Antalya : Elmalı, Çıglıkara, near security building, rocky places and opening <i>C. libani</i> forest, 1900 m, 12.8.1995, R.S.Göktürk 3532 (Akdeniz Univ. Herb.).
<i>C. speciosa</i>	B8 Erzincan : 30 km from Erzincan to Sivas, roadside, 1500 m, 17.viii.2000, R.S.Göktürk 4531 & F.Göktürk (Akdeniz Univ. Herb.).

from the fresh leaf tissue of plants using the methods of Waugh (1997), with minor modifications. DNA was isolated from liquid nitrogen frozen tissue. Fresh young leaves (0.05 g) or dried leaves were ground using a mortar. The grindate was added to 1 mL of extraction buffer (2% (w/v) CTAB; 100 mM Tris-Cl buffer (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, (1% 8w/v) PVP-40) and incubated at 65 °C for 90 min. The extract was mixed with 500 (24:1) µL chloroform:isoamyl alcohol (v/v) and mixed well by gentle inversion. Following centrifugation at 10,000 rpm for 10 min, the upper aqueous layer was transferred to a fresh tube containing 600 µL of isopropanol; the mixture was then left to sit at room temperature for 40 min. After being centrifuged for 20 min the pellets were left to sit overnight at room temperature and were resuspended in TE buffer (10 mM Tris-Cl, pH 8.0, 1 mM EDTA pH 8.0). The selected 22 out of 40 arbitrary primers were used (purchased from Operon Technologies, Alameda, CA, USA) (Table 2). Amplification reactions were performed using the protocol reported by Williams et al. (1990). At least 3 or 4 samples representing each taxon were used for amplification; amplification was performed twice for each sample. For the DNA amplification, a Biometra thermocycler (T Personel Germany) was programmed for 45 cycles at 96 °C for 30 s, 35 °C for 30 s, and 72 °C for 30 s, for denaturing, annealing, and primer extension, respectively. Following the procedure, 20 µL of the samples were loaded in 2% agarose gels in 1X TAE buffer and run

at 75 V for about 4 h. A 1 kb DNA ladder was used as molecular weight markers. The gel was stained with ethidium bromide and photographed under UV light (Maniatis et al., 1982). The images were used for the analysis of the amplification products. Only the reproducible bands in multiple runs were considered in this study. Out of 22, only 11 primers that showed the best readability were chosen for calculation.

Bands on RAPD gels were scored as either present (1) or absent (0) for all species studied. Common band analysis was conducted using the computer software POPGEN (Dendrogram Based Nei's (1978). UPGMA-Modified from NEIGHBOR procedure of PHYLIP Version 3) to determine the genetic distance between them. The values for genetic distance were then used in cluster analysis to generate dendrograms (Nei, 1978).

Cephalaria davisiana Göktürk & Sümbül, sp. nov. (Figure 1).

Type: Turkey, C6 Kahramanmaraş: between Göksun and Sarız, east of Doğankonak village, Binboğa mountain, mountain slopes, 1700-2000 m, 3.8.2001, R.S.Göktürk 4733 & M.Göktürk (holotype: Akdeniz Univ. Herb., isotypes: ANK, GAZI, HUB).

Paratype: Turkey, C6 Kahramanmaraş: between Göksun and Sarız, east of Doğankonak village, Binboğa mountain, mountain slopes, 1700-2000 m, 9.8.1995, R.S.Göktürk 3508 & M.R.Tunç (Akdeniz Univ. Herb., ANK, GAZI, HUB).

Table 2. Primers used in this study.

Primer no.	Primer sequence (5→3')	Primer no.	Primer sequence (5→3')
A1	CAGGCCCTTC	OPB 05	TGCGCCCTTC
A2	TGCCGAGCTG	OPB 08	GTCCACACGG
A7	GAAACGGGTG	OPO 04	AAGTCCGCTC
LA12	ACGACCCACG	OPO 06	CCACGGGAAG
LA13	CACCACGCCT	OPW 06	AGGCCGATG
SC1023	GGCTCGTACC	OPW 10	TCGCATCCCT
SC1079	CGCCACGTTC	OPC 02	GGTCTACACC
B4	GGACTGGAGT	OPR 03	ACACAGAGGGT
B6	TGCTCTGCCC	OPB 10	CTGCTGGGAC
B7	GGTGACGCAG	OPU 16	CTGCGCTGGA
B18	CCACAGCAGT	M13	GAGGGTGGCGTTCT

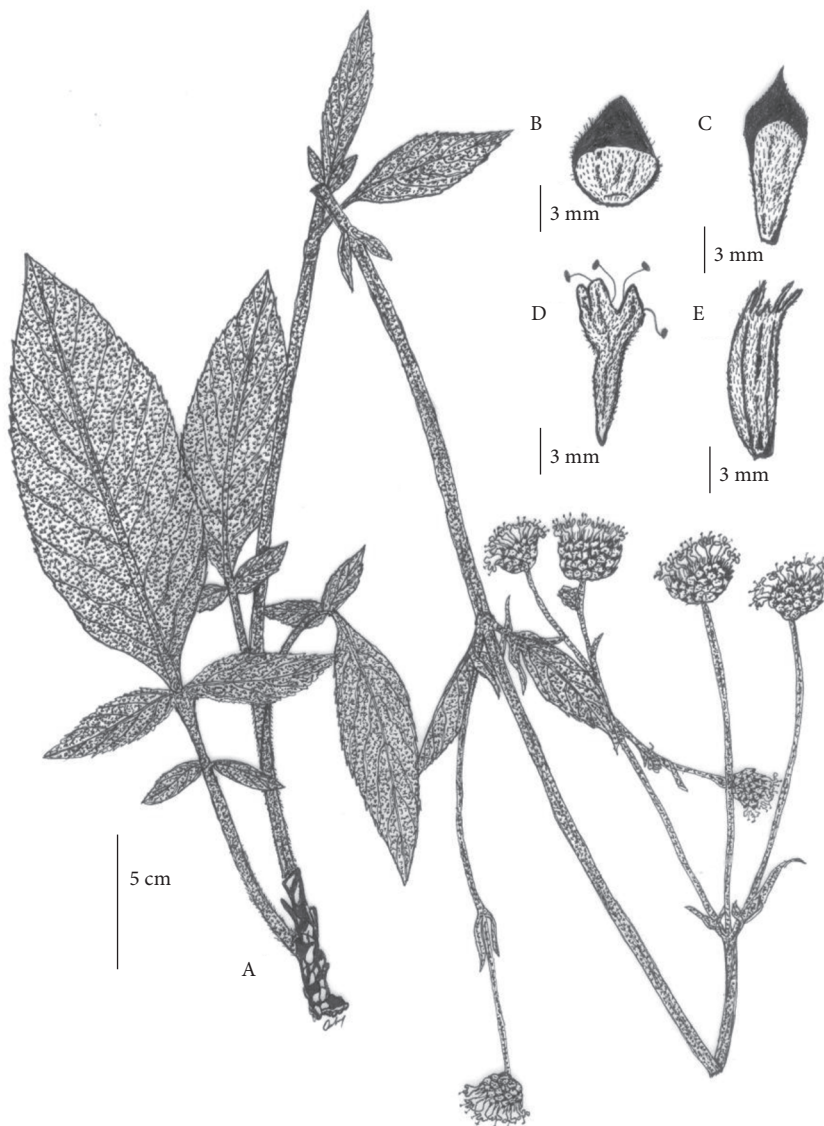


Figure 1. *Cephalaria davisiana* (from the holotype). A- Habit, B- involucrel bract, C- receptacular bract, D- corolla, E- involucrel.

Diagnosis: *Affinis Cephalariae speciosa sed foliis inferioribus simplicibus vel lyratis (non solum simplicibus), bracteis involucrelialibus ovatis-orbicularis vel triangulis-ovatis omnino nigris vel atris in dorsalis et ad apicem, acutibus vel subacutibus ad apicem (non ovatis triangularibus-lanceolatis, omnino stramineis, acuminatis vel subacuminatis ad apicem), bracteis receptacularibus ovatis-oblongis, atris ad apicem (non triangularibus-lanceolatis, omnino stramineis) differt.*

Stout, erect perennial herbs. Stem up to 1.5 m, simple, striate, covered with densely stellate hairs,

and densely retrorse stellate hairs in lower part and beneath capitula. Leaves coriaceous, hairy; lower leaves simple or lyrate, simple leaves and segments of lyrate leaves margin entire or crenate-serrate, acute or acuminate at apex, oblong-lanceolate or oblong, 10-34 × 4-7 cm, lyrate leaves oblong-lanceolate in outline, 12-50 × 3.5-12 cm with 2-6 lanceolate segment; lateral segments, 1-5.5 × 0.8-2.5 cm, terminal segment larger than lateral ones, oblong-lanceolate, 8-28 × 4-8 cm; stem leaves lyrate, lanceolate or oblong-lanceolate in outline, 8-25 × 2-8 cm, with 2-6 lanceolate segments, 1-3 × 0.2-1.2 cm, entire, acute or subacuminate at

apex, terminal segment larger than lateral ones, oblong-lanceolate or lanceolate, 5-18 × 2-6 cm, margins entire or crenate-serrate, acute or acuminate at apex; upper leaves simple or lyrate, sessile; simple leaves and segments of lyrate leaves margins entire, acuminate or subacuminate at apex; simple leaves linear or linear-lanceolate, 0.7-4.2 × 0.2-0.8 cm; lyrate leaves lanceolate or oblong-lanceolate in outline, 1.2-6 × 0.2-0.8 cm, with 2-4 linear segment, 0.2-0.5 × 0.1-0.2 cm, terminal segment larger than lateral ones, linear-lanceolate, 1-5 × 0.2-0.7 cm. Peduncle 5-40 cm long. Capitula globose, 30-50-flowered, 1.5-2.5 cm in diam. in flower, 1.5-3 cm in diam. in fruit. Involucral bracts ovate-orbicular or triangular-ovate, 6-9 × 4-8 mm, completely blackish or blackish in dorsal side and at apex, pubescent or adpressed pilose, margins long ciliate, acute or subacute at apex; receptacular bracts ovate-oblong, 8-11 × 3-5 mm, straw-coloured in dorsal side and base, black at apex, pubescent or sparsely adpressed pilose, margins ciliate, acuminate or subacuminate at apex. Calyx cupuliform, 1-2 mm in diameter, with irregular teeth. Corolla 8-14 mm long, yellow or pale yellow, densely adpressed hairy outside. Involucel 4 angled, 7-14 mm long in fruit, pilose, 4 long and 4 short teeth at apex, long teeth 3-3.5 mm long, short teeth 1-1.5 mm long.

Distribution, ecology, and phenology. *Cephalaria davisiana* is endemic to South Anatolia (Figure 2). It is associated with endemics such as *Michauxia tchihatchewii* Fisch. & C.A.Mey., *Silene brevicaulis* Boiss., *Onosma polioxanthum* Rech.f., *Lamium garganicum* L. subsp. *nepetifolium* (Boiss.) R.R.Mill,

and some non-endemic plants such as *Cephalaria stellipilis* Boiss., *Acantholimon armenum* Boiss. & A.Huet. var. *balansae* Boiss. & A.Huet., *Pelargonium endlicherianum* Fenzl, *Hieracium pannosum* Boiss., and *Potentilla speciosa* Willd. var. *speciosa*. It flowers in July-September and fruits in August-September.

Specimens examined:

Cephalaria davisiana: Turkey, B6 Kahramanmaraş: Göksun, Fındık köyü, Kandil Dağı, kuzey yamaç, orman içi ve açıklığı, 1600-2100 m, 27.7.1981, *B.Yıldız* 3068 (HUB). B6 Kayseri: Sarız, Yeşilyurt, Binboğa Dağı, kayalık yerler, 1800 m, 9.8.1995, *R.S.Göktürk* 3502 & *M.R.Tunç* (Akdeniz Univ. Herb.); C6 Kahramanmaraş: Göksun, Binboğa Dağı, Yalak, ca. 2000 m, *Davis* 20159 (ANK); Ahır Dağı, Akdere mevki, Yalnız ardiç bağları çevresi, yüksek dağ stebi, 1400 m, 13.9.1991, *Z.Aytaç* 5552 & *H.Duman* (GAZI); Göksun, Değirmendere, Büyükçamurlu-Yaniktepe arası, taşlı yamaçlar, 1600 m, 20.8.1993, *M.Ekici* 1694 (GAZI); Göksun-Maraş arası, Püren geçidi, kayalık yerler, 1550 m, 5.8.2003, *R.S.Göktürk* 5153. (Akdeniz Univ. Herb.).

Cephalaria speciosa Boiss. & Kotschy: Turkey, B7 Erzincan: Keşiş Dağı, Cimin, kayalık yamaçlar, ca. 2300 m, 28.8.1957, *Davis* 31828 (ANK); Yaylabaşı Köyü, Kazankaya Dağı, 1500-2300 m, 7.8.1980, *Ş.Yıldırımhı* 3861 (HUB); Kemah, Kömürköy yukarısı, bozkır açıklığı, 1850 m, 31.7.1996, *A.A.Dönmez* 5367 (HUB). B7 Tunceli: Ovacık, Munzur Dağı, Aksu Deresi, ca. 1700 m, 21.7.1957, *Davis* 31462 & *Hedge* (ANK); B7 Tunceli: Ovacık, Munzur Dağı, Karagöl

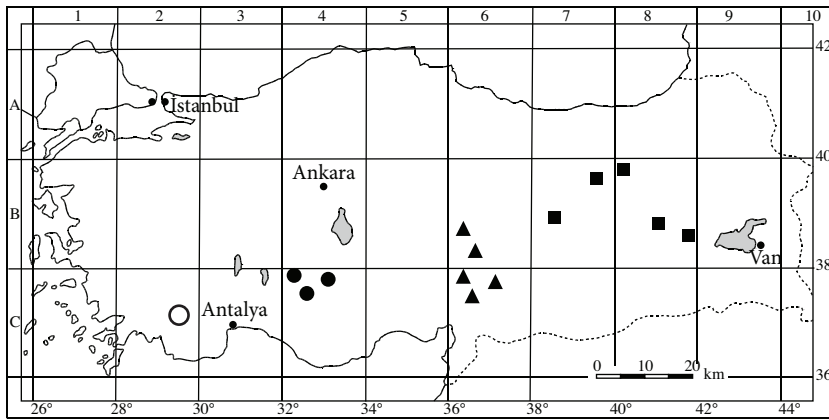


Figure 2. Distribution of *C. davisiana* (▲), *C. sumbuliana* (●), *C. speciosa* (■), and *C. elmaliensis* (○) in Turkey.

vadi, 1350-1500 m, 8.9.1979, Ş.Yıldırım 2443 (HUB). B8 Erzincan: Aşkale-Tercan, kuru kayalık yamaçlar, ca. 1700 m, 25.8.1957, Davis 32657 & Hedge (ANK); Tercan, Tercan-Aşkale arası, Erzincan-Erzurum il sınırı, tünele 250 m kala, yamaçlar, 1765 m, 17.8.2000, R.S.Göktürk 4528 & F.Göktürk (Akdeniz Univ. Herb.); Erzincan-Sivas karayolu 30. km'si, 3. tünelin ilerisi, yol kenarı, 1500 m, 17.8.2000, R.S.Göktürk 4531 & F.Göktürk (Akdeniz Univ. Herb.). B8 Muş: Muş-Solhan, hillsides, ca. 1350 m, 31.8.1954, Davis 24783 & Polunin (ANK); Varto, Muş-Varto arası, Varto'ya 10-12 km kala, kayalık yamaçlar, 1350 m, 2.8.2001, R.S.Göktürk 4727 & M.Göktürk (Akdeniz Univ. Herb.); Varto, Varto-Erzurum yolu 12 km, Seferek Geçidi, kayalık yamaçlar, 1800 m, 2.8.2001, R.S.Göktürk 4730 & M. Göktürk (Akdeniz Univ. Herb.); Muş-Solhan arası, Solhan'a 13 km kala, yol kenarı, 1600 m, 2.8.2001, R.S.Göktürk 4732 & M.Göktürk (Akdeniz Univ. Herb.).

Etymology. The new species is named in honour of Prof. Dr. P. H. Davis, late editor of *Flora of Turkey*.

Recommended IUCN threat category. It should be graded as Vulnerable (VU) (IUCN 2006), because the estimated area of occupancy is less than 20,000 km² (criterion B1) and it is known to exist at no more than 10 locations (criterion B1a).

Cephalaria sumbuliana Göktürk, sp. nov. (Figure 3).

Type: Turkey, C4 Konya: Seydişehir, 21 km from Seydişehir to Akseki, rocky places, under and clearings in *Abies* forest, 1435 m, 8.8.2000, R.S.Göktürk 4466 & F.Göktürk (holotype: Akdeniz Univ. Herb., isotypes: ANK, GAZI, HUB).

Paratypes: Turkey, C4 Konya: Seydişehir, Seydişehir-Akseki yolunun 21. km'si, kayalık yerler, *Abies* altı ve açıklıkları, 1435 m, 4.7.2002, R.S.Göktürk 4865 & F.Göktürk (Akdeniz Univ. Herb.); Seydişehir, Seydişehir-Akseki yolunun 21. km'si, kayalık yerler, *Abies* altı ve açıklıkları, 1435 m, 1.8.2002, R.S.Göktürk 5040 & M.Göktürk (Akdeniz Univ. Herb.).

Diagnosis. *Affinis Cephalariae elmaliense et Cephalariae speciosa, sed Cephalariae elmaliense caulibus crassis, rhizomatois (non gracilibus, non rhizomatois), bracteis involucribus late ovatis-orbicularibus vel orbicularibus, obtusibus ad apicem (non ovatis, acutibus ad apicem), bracteis receptacularibus oblongis vel angustis oblanceolatis*

(non angustis ovatis vel lanceolatis) et Cephalariae speciosa caulibus rhizomatois (non rhizomatois), bracteis involucribus late ovatis-orbicularibus vel orbicularibus solum longis pilosis, obtusibus ad apicem (non ovatis triangularibus-lanceolatis pubescentibus et adpressis pilosis, acuminatis vel subacuminatis ad apicem), bracteis receptacularibus oblongis vel anguste oblanceolatis, acutis vel brevis acuminatis ad apicem (non triangularibus-lanceolatis, acuminatis ad apicem) differt.

Stout, rhizomatous, erect perennial herbs. Stem up to 1.3 m, simple, striate, densely stellate hairy, densely retrorse stellate hairy in lower part and beneath capitula. Leaves coriaceous, densely stellate hairy; lower leaves lyrate, lanceolate or ovate-lanceolate in outline, 5-44 × 3.5-13 cm, with 2-6 oblong or oblong-lanceolate segments, 2-5 × 0.3-2.5 cm, entire or crenate-serrate, acute or obtuse at apex, terminal segment larger than lateral ones, oblong-lanceolate or lanceolate, 8-28 × 2-6 cm, crenate or crenate-serrate, acute at apex; stem leaves lyrate, lanceolate or oblong-lanceolate in outline, 9-20 × 3-10 cm, with 2-6 oblong-lanceolate segments, 2-6 × 0.5-2 cm, entire or crenate-serrate, acute at apex, terminal segment larger than lateral ones, oblong-lanceolate or lanceolate, 6.5-13 × 1.5-4.5 cm; upper leaves simple or lyrate, sessile; simple leaves and segments of lyrate leaves margins entire, subacuminate at apex; simple leaves linear or linear-lanceolate, 0.8-3 × 0.1-0.4 cm; lyrate leaves linear-lanceolate or lanceolate in outline, 2-8 × 1.2-2 cm, with 2-4 linear segments, 0.5-1.5 × 0.1-0.4 cm, terminal segment larger than lateral ones, linear-lanceolate, 2.5-7 × 0.3-0.9 cm. Peduncle 5-35 cm long. Capitula globose, 1-2 cm in diam. in flower, 1.5-2.5 cm in diam. in fruit. Involucral bracts broadly ovate-orbicular or orbicular, 6-9 × 5-9 mm, straw-coloured, long pilose hairy, margins long ciliate, obtuse at apex; receptacular bracts oblong or narrowly oblanceolate, 8-12 × 3-4 mm, straw-coloured, pubescent or adpressed pilose hairy, margins ciliate, acute or short acuminate at apex. Calyx cupuliform, 1.5-2.5 mm in diameter, subirregular teeth. Corolla 8-12 mm long, cream or pale yellow, densely adpressed hairy outside. Involucel 4 angled, 7-10 mm long in fruit, striate, densely adpressed pilose hairy, 4 long and 4 short teeth at apex, long teeth 3-3.5 mm long, short teeth 1-1.5 mm long.

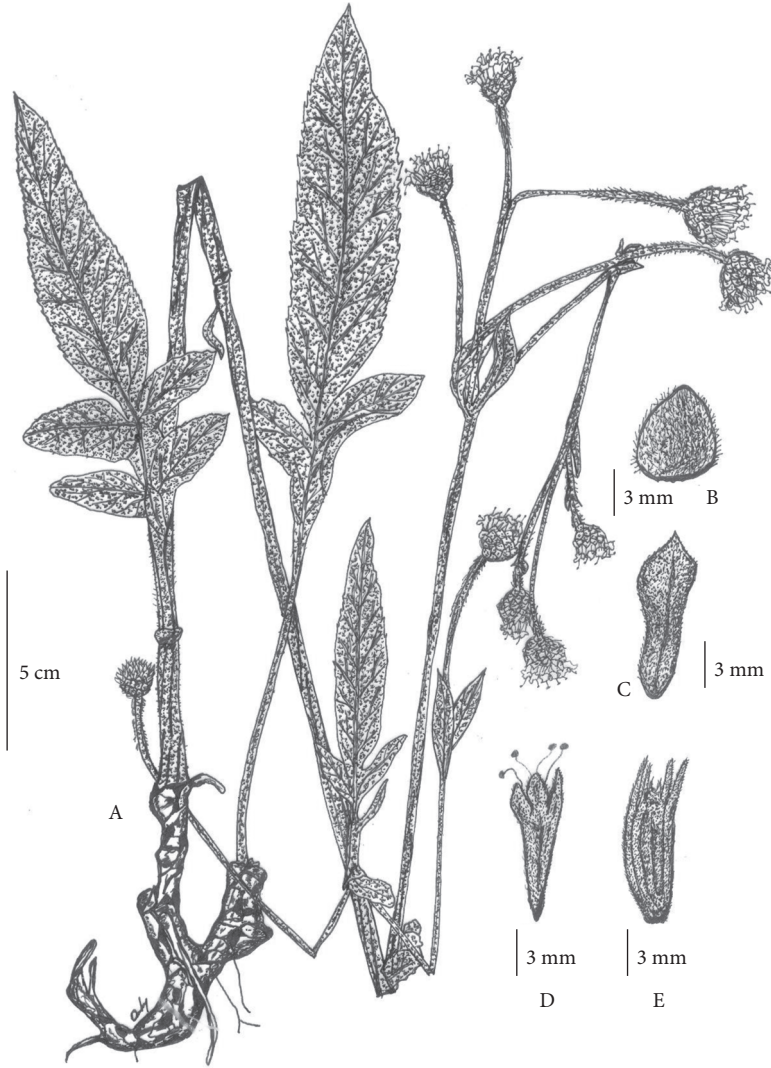


Figure 3. *Cephalaria sumbuliana* (from the holotype). A- Habit, B- involucral bract, C- receptacular bract, D- corolla, E- involucel.

Distribution, ecology, and phenology. *Cephalaria sumbuliana* is endemic to South Anatolia (Figure 2). It is associated with endemics such as *Abies cilicica* (Ant. & Kotschy) Carr. subsp. *isaurica* Coode & Cullen, *Centaurea isaurica* Hub.-Mor., *Iris schachtii* Markgr., *Geranium glaberrimum* Boiss. & Heldr., and some non-endemic plants such as *Allium orientale* Boiss., *Cephalaria dipsacoides* Boiss. & Balansa, *Pelargonium endlicherianum* Fenzl, *Xeranthemum annuum* L., *Silene compacta* Fisch., *Lonicera nummulariifolia* Jaub. & Spach subsp. *nummulariifolia*, *Rosa canina* L., and *Berberis crataegina* DC. It flowers in July-August and fruits in August-September.

Specimens examined:

Cephalaria sumbuliana: Turkey, C4 Konya: Ermenek, Kazancı Kasabası Yaylası, Kırk kuyu mevkii, 1800 m, 19.7.1984, H.Sümbül 3217 (HUB).

Cephalaria elmaliensis Hub.-Mor. & V.A.Matthews: Turkey, C2 Antalya: Elmalı, Çığlıkara, bekçi kulübesi civarı, *Cedrus libani* açıklıkları, 1700-1900 m, 25.8.1993, H.Duman 5345, Z.Aytaç & A.A.Dönmez (GAZI, Akdeniz Univ. Herb.); Elmalı, Çığlıkara, bekçi kulübesi civarı, kayalık yerler ve *C. libani* açıklıkları, 1900 m, 12.8.1995, R.S.Göktürk 3532 (Akdeniz Univ. Herb.).

Cephalaria speciosa Boiss. & Kotschy: Turkey, B7 Erzincan: Keşiş Dağı, Cimin, kayalık yamaçlar, ca. 2300 m, 28.8.1957, *Davis* 31828 (ANK); Yayla başı Köyü, Kazankaya Dağı, 1500-2300 m, 7.8.1980, Ş.Yıldırım 3861 (HUB); Kemah, Kömürköy yukarısı, bozkır açıklığı, 1850 m, 31.7.1996, A.A.Dönmez 5367 (HUB). B7 Tunceli: Ovacık, Munzur Dağı, Aksu Deresi, ca. 1700 m, 21.7.1957, *Davis* 31462 & *Hedge* (ANK); B7 Tunceli: Ovacık, Munzur Dağı, Karagöl vadi, 1350-1500 m, 8.9.1979, Ş.Yıldırım 2443 (HUB). B8 Erzincan: Aşkale-Tercan, kuru kayalık yamaçlar, ca. 1700 m, 25.8.1957, *Davis* 32657 & *Hedge* (ANK); Tercan, Tercan-Aşkale arası, Erzincan-Erzurum il sınırı, tünele 250 m kala, yamaçlar, 1765 m, 17.8.2000, R.S.Göktürk 4528 & F.Göktürk (Akdeniz Univ. Herb.); Erzincan-Sivas karayolu 30. km'si, 3. tünelin ilerisi, yol kenarı, 1500 m, 17.8.2000, R.S.Göktürk 4531 & F.Göktürk (Akdeniz Univ. Herb.). B8 Muş: Muş-Solhan, hillsides, ca. 1350 m, 31.8.1954, *Davis* 24783 & *Polunin* (ANK); Varto, Muş-Varto arası, Varto'ya 10-12 km kala, kayalık yamaçlar, 1350 m, 2.8.2001, R.S.Göktürk 4727 & M.Göktürk (Akdeniz Univ. Herb.); Varto, Varto-Erzurum yolu 12. km, Seferek Geçidi, kayalık yamaçlar, 1800 m, 2.8.2001, R.S.Göktürk 4730 & M.Göktürk (Akdeniz Univ. Herb.); Muş-Solhan arası, Solhan'a 13 km kala, yol kenarı, 1600 m, 2.8.2001, R.S.Göktürk 4732 & M.Göktürk (Akdeniz Univ. Herb.).

Etymology. The new species is named in honour of Prof. Dr. Hüseyin Sümbül, plant taxonomist at Akdeniz University, Faculty of Science, Antalya, Turkey.

Recommended IUCN threat category. It should be graded as Critically Endangered (CR) (IUCN 2006), because the estimated area of occupancy is less than 100 km² (criterion B1) and the population of the new species is estimated to number less than 250 mature individuals (criterion C).

A morphological key of *Cephalaria*

1. Plant stout, longer than 1 m high
 2. Lower leaves simple
 3. Lower leaves deflexed from petiolate; capitula globose, 2-2.5 cm in flower
.....*C. duzceënsis*
 3. Lower leaves not deflexed from petiolate; capitula ovate-globose, 2.5-4.5 cm in flower.....*C. speciosa*

2. Lower leaves lyrate
 4. All bracts blackish at apex; involucre bracts acute or subacute; receptacular bracts ovate-oblong.....*C. davisiana*
 4. All bracts straw-coloured at apex; involucre bracts obtuse; receptacular bracts oblong or narrowly oblanceolate.....*C. sumbuliana*
1. Plant slender, up to 1 m high
 5. Lower and stem leaves oblong-spathulate.....
.....*C. stellipilis*
 5. Lower and stem leaves lanceolate
 6. Capitula ovoid; involucre bracts ovate-orbicular or orbicular; involucre sericeous*C. elazigensis*
 6. Capitula subglobose; involucre bracts ovate; involucre pilose.....*C. elmaliensis*

Results and discussion

In Table 3, *C. davisiana*, *C. sumbuliana*, *C. speciosa*, and *C. elmaliensis* are compared on the basis of their vegetative organs (stem and leaves) and reproductive organs (capitula, involucre bracts, receptacular bracts, and corolla).

Cephalaria was previously revised by Matthews for the *Flora of Turkey* (29 species) (Matthews, 1972). Since then, 9 new species have been described from Turkey: (*Davis* et al., 1988; Sümbül, 1991; Göktürk & Sümbül, 1997; Göktürk et al., 2003; Göktürk & Sümbül, 2003; Kuş & Göktürk, 2005; Aksoy et al., 2007). In a revision of the genus *Cephalaria* in Turkey, the species *C. amana* Rech.f. was treated as a synonym of *C. taurica* Szabó species by the senior author (RSG). The total number of species of *Cephalaria* reported from Turkey is now 39.

An attempt was made to assess the relationships among 4 *Cephalaria* species using a molecular approach based on RAPD-PCR analysis. For the RAPD-PCR analysis 22 random primers were tested in the amplification reactions with *Cephalaria* species. Among them, 11 primers were chosen for further analysis. The 11 RAPD primers (M13, B7, B6, OPC 02, OPB 05, OPW 06, OPW 10, OPO 04, SC 1023, SC 1079, and A2) generated a total of 74 fragments from

Table 3. Morphological comparison of *Cephalaria davisiana* and *C. sumbuliana* with *C. speciosa*, and *C. elmaliensis*.

Characters	<i>C. davisiana</i>	<i>C. sumbuliana</i>	<i>C. speciosa</i>	<i>C. elmaliensis</i>	
Stem	non rhizomatous	rhizomatous	non rhizomatous	non rhizomatous	
Lower leaves	simple or lyrate, oblong-lanceolate	lyrate, lanceolate or ovate-lanceolate	simple, oblong-lanceolate	simple, lanceolate	
Stem leaves	lyrate	lyrate	lyrate	simple or lyrate	
Upper stem leaves	simple or lyrate	simple or lyrate	simple or lyrate	simple	
Capitula	globose, 1.5-2.5 cm in diam. in flower	globose, 1-2 cm in diam. in flower	ovate to globose, 2.5-4.5 cm in diam. in flower	subglobose, 1-2 cm in diam. in flower	
Involucral bracts	shape	ovate-orbicular or triangular-ovate	broadly ovate-orbicular or orbicular	ovate to triangular- lanceolate	ovate
	size	6-9 × 4-8 mm	6-9 × 5-9 mm	7-15 × 3-7 mm	4-6 × 2.5-3 mm
	colour	completely blackish or blackish in dorsal side and at apex	completely straw-coloured	completely straw-coloured	completely straw-coloured or only brown at apex
	apex shape	acute or subacute	obtuse	acuminate or subacuminate	acute
Receptacular bracts	shape	ovate-oblong	oblong or narrowly oblanceolate	triangular-lanceolate	ovate or lanceolate
	size	8-11 × 3-5 mm	8-12 × 3-4 mm	12-20 × 3.5-6 mm	8-12 × 2-3 mm
	colour	blackish at apex	completely straw-coloured	completely straw-coloured	straw-coloured at base and brown at apex
	apex shape	acuminate or subacuminate	acute or short acuminate	acuminate	acuminate
Corolla	yellow or pale yellow	cream or pale yellow	cream or pale yellow	yellow or cream	

patterns of the 4 *Cephalaria* species. The approximate size of the fragments ranged from 100 to 900 bp (Figure 4). The total number of amplified bands per primer varied from 3 (B7 and B6) to 13 (OPC 02) with an average of 6.7 fragments per primer.

For the data analysis, different fragments produced with each primer on the gel were numbered sequentially and presence and absence of fragments in each sample was scored (present 1, absent 0). Genetic distances were calculated using Nei's genetic distance: Method UPGMA. The results are given in Table 4. The dissimilarity coefficients were used to cluster the data using algorithm of unweighted pair group method with arithmetic averages (UPGMA)

in a dendrogram (Figure 5). *Cephalaria* species showed a clear separation from each other, and formed 2 main clusters. The first cluster includes *C. speciosa*, *C. sumbuliana* (putative new species), and *C. elmaliensis*. The second cluster includes only *C. davisiana*. *C. davisiana* would appear to be 59% different from *C. elmaliensis*, 72% different from *C. speciosa*, and 87% different from *C. sumbuliana*. Molecular and morphological analysis together indicate that *C. davisiana* appears to be genetically distant from *C. speciosa*.

RAPD analysis showed the capabilities of primer screened to determine the variability among 2 putative new species and 2 resembling species. The

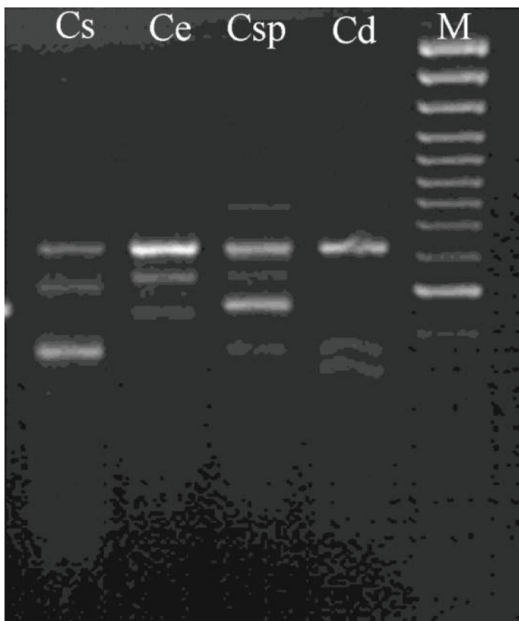


Figure 4. RAPD profiles of *Cephalaria* species with OPC02 primer. **M: Marker** (100 bp DNA Ladder), Cs: *C. sumbuliana*, Ce: *C. elmaliensis*, Csp: *C. speciosa*, Cd: *C. davisiana*.

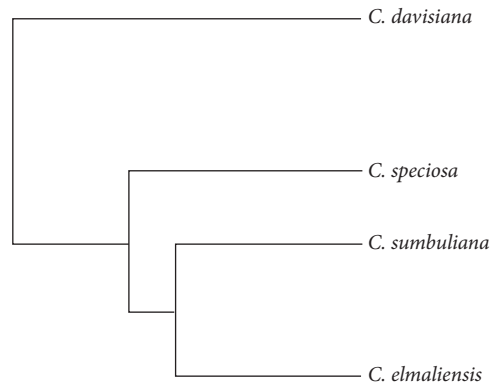


Figure 5. Dendrogram based on genetic distances between *Cephalaria* species.

Table 4. Genetic distances based on RAPD results of *Cephalaria* species.

	<i>C. sumbuliana</i>	<i>C. elmaliensis</i>	<i>C. speciosa</i>	<i>C. davisiana</i>
<i>C. sumbuliana</i>	***			
<i>C. elmaliensis</i>	0.4329	***		
<i>C. speciosa</i>	0.5429	0.4539	***	
<i>C. davisiana</i>	0.8701	0.5905	0.7205	***

interspecific genetic distances for each species vary from 43% to 87%. *C. davisiana* and *C. sumbuliana* were the most divergent species, with a distance of 0.87. The lowest genetic distance among the different species was observed between *C. elmaliensis* and *C. sumbuliana* (0.43). The results suggest that *Cephalaria* spp. (*C. davisiana* and *C. sumbuliana*) are very different from the other 2 related species based on RAPD results.

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