

Turkish Journal of Botany

Volume 48 | Number 3

Article 4

5-14-2024

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Recommended Citation

SEMİZ, Gürkan; BOZKURT, Meryem; GÜNAL, Batıkan; and UYSAL, Tuna (2024) "Cephalaria gokturkii (Caprifoliaceae), a new species from southwestern Anatolia, Türkiye," Turkish Journal of Botany: Vol. 48: No. 3, Article 4. https://doi.org/10.55730/1300-008X.2804 Available at: https://journals.tubitak.gov.tr/botany/vol48/iss3/4

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Turkish Journal of Botany

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Research Article

Turk J Bot (2024) 48: 155-164 © TÜBİTAK doi:10.55730/1300-008X.2804

Cephalaria gokturkii (Caprifoliaceae), a new species from southwestern Anatolia, Türkiye

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Received: 25.12.2023 Accepted/Published Online: 30.04.2024 **Final Version:** 14.05.2024

Abstract: A new Cephalaria (Caprifoliaceae) species, Cephalaria gokturkii, is described and illustrated from Girdev Mountain (Muğla Province) for the Flora of Türkiye. Throughout this manuscript, the new species and its relatives were compared in terms of morphological, palynological and molecular features. The obtained findings indicated that the new species is similar to C. elmaliensis but is characterized by having larger capitula and receptacular bracts in flower (vs. 1-2 cm capitula and less than 3 mm in flower) and hairy trigonous-narrowly ovate involucral bracts (vs. glabrous and broadly ovate involucral bracts) and smaller involucels (vs. involucels longer than 10 mm in fruit). Although the new species is similar to C. elmaliensis in terms of pollen shape and general appearance, its pollens are larger in terms of mean equatorial diameter and polar axis. Following morphological data, molecular analyses (based on ITS and trnL introns) indicated that the phylogenetically closest relative of the species is also C. elmaliensis. Additionally, we report that taxonomic notes for this new species are provided, including distribution, ecology, and conservation status.

Key words: Anatolia, Cephalaria, endemic, palynology, phylogeny

1. Introduction

The genus Cephalaria Schrader ex Roemer & Schultes, long assumed to belong to the Dipsacaceae Juss., has been reclassified by the Angiosperm Phylogeny Group (APG III) to be a member of the Caprifoliaceae Juss. (Mabberley, 1997; Reveal and Chase 2011; Reveal 2012). The species of Cephalaria are found throughout the world, but they are mostly distributed in South Africa and the Holarctic Kingdom, which includes the Mediterranean region (Szabó, 1940). This genus, which includes approximately 100 species worldwide, is represented by 44 taxa in Türkiye (Matthews, 1972, 1988; Sümbül, 1991; Göktürk and Sümbül, 1997; Göktürk et al., 2003; Göktürk and Sümbül, 2003; Kuş and Göktürk, 2005; Aksoy et al., 2007; Parolly and Eren, 2007; Göktürk et al., 2012; Göktürk and Sümbül, 2014; Göktürk and Sümbül, 2016; Ranjbar and Ranjbar, 2018; Yıldırımlı, 2019; Göktürk et al., 2023).

It is widely recognized that morphological traits of pollen grains constitute additional diagnostic elements that are frequently employed in taxonomy and in reconstructing potential evolutionary patterns of different plant groups (Celenk et al., 2008; Jacobs et al., 2011; Yıldız et al., 2011; Tsymbalyuk et al., 2018, 2019a, 2019b, 2021; Cui et al., 2020). There have been few investigations of

Although morphological characterization studies are extremely beneficial in identifying species, they are occasionally influenced by environmental variables and do not always represent genetic variations. However, because molecular approaches directly identify the species' genetic traits, they can enable the identification of different species based primarily on physical similarities and differences (Kaya and Yılmaz Gökdoğan, 2016; Ahmed et al., 2017). The genetic code of the species determines the extent to which relationships between species with similar ancestry are passed down across generations. As a result, the methods used for the examination of genetic material are completely able to distinguish between genera, species, and even individuals from the same species (Göktürk et al., 2023).

Cephalaria pollen morphology using light microscopy and scanning electron microscopy. The results of the investigations can be summarized as follows: pollen grains are triporate, suboblate to subprolate, large-sized, circular or eliptic in equatrial view, circular to triangular in polar view, and have lolongate to circular pores (Erdtman, 1952; Vinokurova, 1959; Halbritter and Svojtka, 2016; Mostafa et al., 2017; Halbritter and Weis, 2018; Tsymbalyuk et al., 2021).

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During floristic fieldwork for plant biodiversity, the first author collected some interesting specimens from Girdev Mountain in 2020. All samples were compared to specimens from related Cephalaria taxa that have been deposited in different herbaria. In addition, the relevant literature was searched (Matthews, 1972, 1988; Sümbül, 1991; Göktürk and Sümbül, 1997; Göktürk et al., 2003; Göktürk and Sümbül, 2003; Kuş and Göktürk, 2005; Aksoy et al., 2007; Parolly and Eren, 2007; Göktürk et al., 2012; Göktürk and Sümbül, 2014; Göktürk and Sümbül, 2016; Ranjbar and Ranjbar, 2018; Yıldırımlı, 2019; Göktürk et al., 2023). Our samples were determined to belong to a previously unidentified species after extensive comparisons based on morphology, palynology, and genetic investigations. In this context, we compared these specimens to the closely related Cephalaria elmaliensis Hub.-Mor. & Matthews via morphological, palynological, and molecular assessments. Hereby, we report the morphological, palynological, and molecular differences between the Cephalaria gokturkii and its allied Cephalaria elmaliensis.

2. Materials and methods

2.1. Plant materials

Plant samples were collected by the first author from Girdev Mountain in Muğla of the southwest Turkish province (Figure 1). Using the most recent research (Göktürk et al., 2012, 2023); the specimens were recognized and compared to herbaria materials. The IPNI database (2012) was used to check plant names, Brummitt and Powell (1992) used to check plant author names, and Punt et al. (2007) utilized pollen morphology terminology.

2.2. Morphological and palynological studies

The morphological data was gathered through microand macromorphological analyses using light binocular and scanning electron microscopy (SEM) methods. The pollen was cleaned with 70% ethanol for the SEM analysis, then dried and mounted with gold-palladium stubs. The samples were covered with Quarum 150 RS Au-Pd, and SEM photomicrographs were captured using a Zeiss Supra 40 VP Electron Microscope.

2.3. Molecular studies

DNA extraction was carried out based on the leaves of herbarium samples of the new species and C. elmaliensis. For the determination of phylogenetical positions of targeted species, the ITS and trnL intron regions were selected and amplified (Table 1). And then, the sequences used here were downloaded from the Genbank (NCBI). Total DNA extraction was performed via 2X CTAB method described by Doyle and Doyle (1987) and modified by Soltis et al. (1991) and Cullings (1992). The universal primers (ITS1 and ITS4) were selected for the amplification of ITS regions (White et al., 1990). ITS-PCR conditions were enforced according to Garcia-Jacas et al. (2006). For the trnL, trnc-d primers were used and previous protocols were preferred (Taberlet et al., 1991). Purification and reading of DNA intron sequences were accomplished through service procurement (Macrogen, Netherlands). Alignments were performed through the use of Bioedit v.7.0.5.3 version (Hall, 1999). Typical phylogenetic trees based on both intron regions were created separately for the targeted species. Because of observed similar and common topology, ITS and trnL introns data were combined and, finally the phylogenetical



Figure 1. Distribution map of *C. gokturkii* (\blacktriangle) and *C. elmaliensis* (\bullet).

Table 1. Genbank assessions numbers of Cephalaria and its related taxa.

Species	ITS	trnL intron	
Cephalaria leucantha	KF993465	AJ427376	
Cephalaria gokturkii	PP573510	PP566122	
<u> </u>	(In this study)	(In this study)	
Cephalaria elmaliensis	PP573511	PP566123	
Cephaiaria eimanensis	(In this study)	(In this study)	
Cephalaria squamiflora subsp. mediterranea	FJ379654	FJ379629	
Cephaiaria squamijiora suosp. meaiterranea	FJ379702	F)3/9029	
Cephalaria microcephala	FJ640759	FJ640657	
Cephalaria paphlagonica	FJ640758	FJ640659	
Cephalaria aristata	FJ640757	FJ640656	
Cephalaria tenella	FJ640756	FJ640653	
Cephalaria zeyheriana	FJ640755	FJ640654	
Cephalaria natalensis	FJ640754	FJ640655	
Caphalaria hirouta	FJ640760	FJ640658	
Cephalaria hirsuta	FJ640790	FJ040038	
Caphalaria amiaca	AJ426525	A1427277	
Cephalaria syriaca	AJ426526	AJ427377	
Dipsacus mitis	AY236187	AF446977	

trees were built by PAUP and MrBayes. The parsimony and Heuristic searches were performed by using of PAUP v.4.0b10 version (Swofford, 2002). By means of PAUP, the homoplasy index (HI), consistency index (CI), and retention index (RI) were determined for the strict consensus tree, with the exclusion of the uninformative characters. The MrModelTest v2.2 was useful in identifying the best-fit model regarding DNA evolution and it was determined according to Akaike information criterion (AIC) (Nylander, 2004). In Bayesian analyses, two independent runs of four Metropolis-coupled chains each were operated for 72 × 10³ generations, sampling every 1000 generations. Almost, 20% of all generations sampled (20%) were discarded after they were visually examined with regard to the likelihood score plots in Tracer v.1.7.0 version (Rambaut et al., 2018). Finally, the graphical views of phylogenetic trees were created in the FigTree v1.4.0 program.

3. Results

3.1. Taxonomic treatment

Cephalaria gokturkii Semiz & Uysal sp. nov. (Figures 2 and 3)

Type:—TÜRKİYE. Muğla: Seydiler, Girdev Mountain, Girdev Plateau, on limestone, 1800–1900 m, 19 July 2020, G.Semiz GSE 2182 (holotype: PAUB!; isotype: KNYA!, AKDU!).

Diagnosis: Cephalaria gokturkii is closely related to C. elmaliensis. The distinctive characteristics of these species are: a) The capitula size of C. gokturkii is 2.0-3.2

cm diameter (not smaller than 2.0 cm); b) while the involucel is less than 10 mm long in fruit in *C. gokturkii*, it is longer than 10 mm long in *C. elmaliensis*; c) other typical characters: the lower leaves simple or lyrate; lyrate cauline leaves broadly lanceolate; lyrate leaf segments lanceolate to triangular; corolla cream; receptacular bracts oblanceolate.

Description: Slender, erect, or ascending perennial herbs. Stem 49.10-95.50 (89.50 \pm 4.42) cm, simple or 2-7 branched in the upper part, longitudinally striate, minute puberulent and densely stellate (one of the hairs conspicuously longer) in stem and sericeous in nodes. Leaves coriaceous, densely stellate (one of the hairs conspicuously longer on rachis), acute at apex; lower leaves simple or lyrate; simple leaves entire or denticulate, lanceolate or oblanceolate, 7.20-47.50 (28.96 \pm 2.21) \times 1.70–4.60 (3.00 ± 0.19) cm; lyrate leaves broadly lanceolate in outline, 15.80-36.90 (27.96 \pm 1.87) cm, with 2-7 segments, lateral segments lanceolate to triangular, entire, 0.50-5.40 (2.50 ± 0.34) × 0.2-1.8 (0.98 ± 0.13) cm, terminal segments larger than laterals, lanceolate or narrowlylanceolate, $11.30-22.70 \ (15.54 \pm 0.74) \times 1.60-3.70 \ (2.6$ ± 0.15) cm; cauline leaves simple or lyrate, simple leaves lanceolate or linear-lanceolate, 6.60–30.30 (19.28 \pm 2.30) \times 1.10-3.40 (2.19 ± 0.24) cm, lyrate leaves broadly lanceolate in outline, 6.20-32.10 (18.00 ± 1.77) cm, with 3-7 segments, lateral segments lanceolate to triangular, 0.40-6.40 (2.09 ± 0.35) × 0.10-1.70 (0.57 ± 0.10) cm, terminal segments larger than lateral ones, lanceolate or broadly lanceolate, $4.90-27.30 \ (13.29 \pm 1.21) \times 0.60-4.00 \ (2.63 \pm 0.26) \ cm;$ upper leaves simple, sessile, linear, 1.00-8.60 (4.45 ± $0.50) \times 0.10$ –1.10 (0.57 ± 0.07) cm. Capitula subglobose,

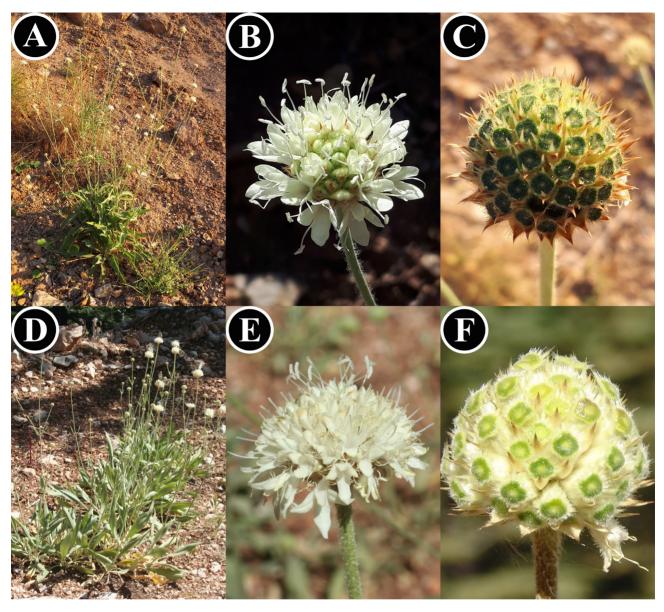


Figure 2. General habitus (A), capitula (B) and involucel (C) of *C. gokturkii*. General habitus (D), capitula (E) and involucel (F) of *C. elmaliensis*.



Figure 3. Involucels (A), Receptacular bracts (B), and Involucral bracts (C) of *C. gokturkii* (1) and *C. elmaliensis* (2).

50-80-flowered, 2.00-3.20 (2.68 \pm 0.06) cm in diameter in flower, 1.70-2.80 (2.34 \pm 0.06) cm in diameter in fruit. Involucral bracts hairy trigonous-narrowly ovate, $4.24-6.67 (5.25 \pm 0.17) \times 4.78-7.04 (5.97 \pm 0.16) \text{ mm},$ straw-coloured, brownish in dorsal and apex, pilose, margins ciliate, obtuse at apex; receptacular bracts oblanceolate, 7.43-11.81 (10.21 \pm 0.28) \times 2.68-6.40 (3.87 ± 0.23) mm, straw-coloured, brownish in dorsal and apex, pilose, margin ciliate, pilose at apex, acute at apex. Calyx cupuliform, 3.08-6.52 (5.17 ± 0.21) mm, with irregular teeth. Corolla cream, $10.59-15.65 (13.62 \pm 0.36)$ mm long, densely ± adpressed pilose outside. Involucel 4-angled, 6.82-8.80 (7.79 ± 0.12) mm in fruit, pilose, 4 long and 4 short teeth at apex; long teeth 2.89-4.27 (3.36 \pm 0.07) mm long, short teeth 0.95-2.19 (1.62 \pm 0.08) mm long.

Distribution and ecology: Cephalaria gokturkii is endemic to south-west Anatolia, Türkiye. This species grows in calcareous grasslands at altitudes between 1800 and 1900 m. This endemic new species is associated with other endemics such as Centaurea inexpectata Wagenitz, Salvia chrysophylla Stapf, and Echinops emiliae P.H.Davis.

Phenology: Flowering from June to July. Fruiting from July to August.

A modified Identification Key updated from Göktürk and Sümbül Revision (2014) for the taxa growing in Türkiye.

- 1. Perennials
- 8. Plant with stellate hairs
- 9. Stem and leaves with dense stellate hairs, stem not hollow
 - 10. Plant stout, taller than 1 m high
 - 10. Plant slender, up to 1 m high
- - 14. Lower and cauline leaves lanceolate
- 15. Capitula subglobose; involucral bracts ovate; involucel pilose

Etymology: The specific epithet was dedicated to Prof. Dr. Ramazan Süleyman GÖKTÜRK (Akdeniz University, Türkiye), a Turkish botanist who made an outstanding contribution to the *Cephalaria* genus in Türkiye.

Vernacular name: Girdev pelemiri (Menemen et al., 2016).

Proposed conservation status: Cephalaria gokturkii is known only from one locality with small

subpopulations in Girdev Mountain. It is suggested that this new species should be placed under the IUCN threat category "Critically Endangered (CR)" (IUCN 2019), because the estimated area of occupancy is less than $10~\rm km^2$, and there is only one known location. The population size of the new species is estimated to be less than 50 mature individuals in each subpopulation [CR B2ab(ii, iii, v) + C2a(i)]. The population size of the new species may decrease in the near future due to intense grazing pressure and anthropogenic effects.

3.2. Morphological comparison

The new species, *C. gokturkii*, is closely related to *C. elmaliensis*. These species share some common features, such as a slender stem, a flowering stem covered by stellate hairs, subglobose capitula, ovate involucral bracts, and pilose involucels. However, the distinctive characteristics of these species are a) Capitula 2.0-3.2 cm diameter in flower in *C. gokturkii* (vs. capitula 1-2 cm); b) while the involucel is less than 10 mm long in fruit in *C. gokturkii*, it is longer than 10 mm long in *C. elmaliensis*; c) other typical characters: the lower leaves simple or lyrate; lyrate cauline leaves broadly lanceolate; lyrate leaf segments lanceolate to triangular; corolla cream; receptacular bracts oblanceolate (Table 2).

3.3. Pollen morphology

Pollen grains are triporate, with lolongate pores, defined annulus and margins, and an indistinct, diffuse wide halo; aperture membranes have long, branched stick-shaped protrusions. Pollen grains are generally prolate-sphaeroidal, occasionally subprolate in equatorial view. Polar axis: 83.19-99.01 (90.92 ± 0.93) µm, equatorial axis: 66.31-87.68 (79.01 ± 1.05) µm, and P/E ratio: 1.09-1.30 (1.15 ± 0.01). AMB shape (polar view): circular, triangular to triangular, obtuse, convex. Pore length 18.26-25.43 (21.23 ± 0.41) µm and pore width 9.38-15.19 (12.50 ± 0.37) µm. Exine 5.29-6.75 (7.17 ± 0.08) µm, structure intectate. Sculpture echinulate. Detailed pollen morphological characters of the related species are presented in Figure 4 and Table 3.

3.4. Phylogenetic analyses

Phylogenetic analyses included 13 taxa, one of which was an outgroup. The aligned combined ITS+*trnL* intron data matrix consists of 1037 base pairs long, and 914 of them are constant. While the informative character number for parsimony was 42 and the uninformative was 81. The tree created by the PAUP program has reliable tree scores (CI: 0.860, RI: 0.589, and HI: 0.140). Bayesian analysis was executed with the TIM2+I+G model according to the chosen AIC criteria. Parsimony and Bayesian trees created with ITS and *trnL* intron data were combined because their topologies were similar (Figure 5).

Table 2. Morphological comparison of *C. gokturkii* and *C. elmaliensis*.

	C. gokturkii	C. elmaliensis		
Basal and lower leaves	simple or lyrate	simple		
Cauline leaves	simple or lyrate, lyrate leaves having lanceolate to triangular segments in outline	simple or lyrate, lyrate leaves having narrowly ovate-lanceolate outline		
Capitula	2–3.2 cm diameter in flower	1-2 cm diameter in flower		
Corolla	cream	yellow or cream		
Involucral bracts	trigonous-narrowly ovate, obtuse at the apex	ovate, acute at the apex		
Receptacular bracts	Oblanceolate to oblong, broader; up to 6.4 mm in wide, acute at the apex	narrowly ovate or lanceolate, narrower; up to 3 mm in wide, acuminate at the apex		
Involucel	Narrowly obovoid-oblong, 6–9 mm in fruit, long teeth 2.8–4.2 mm in long, short teeth 0.9–2.1 mm in long	Ovoid, 10–12 mm in fruit, long teeth 3 mm in long, short teeth 1 mm in long		

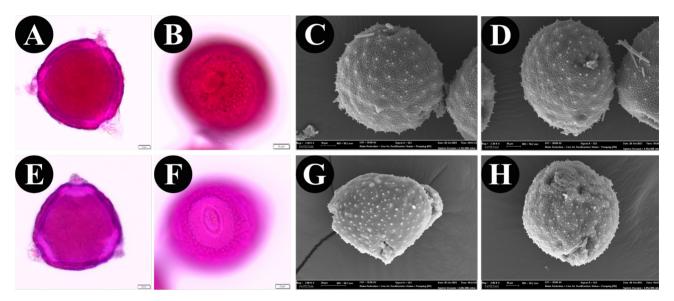


Figure 4. Pollen morphology of *C. gokturkii* and *C. elmaliensis*. A. Equatorial view of *C. elmaliensis* in LM, B. Polar view of *C. elmaliensis* in LM, C. Equatorial view of *C. elmaliensis* in SEM, D. Polar view of *C. elmaliensis* in SEM, E. Equatorial view of *C. gokturkii* in LM, F. Polar view of *C. gokturkii* in SEM.

4. Discussion

Although the genus *Cephalaria* is relatively complex in taxonomic terms, it has many macro- and micromorphological characters that can be used to solve the problems of the genus. In the revision of Göktürk and Sumbul (2014), a lot of characters were presented, such as habit, life form, indumentum, calyx, flower, involucel, involucral, and receptacular bracts, which are similar diagnoses in the Flora of Türkiye (Matthews, 1972), but more distinctive and comprehensive. When we looked

generally at *Cephalaria*, there are a few species with stellate hairs in the Flora of Türkiye, and among these, only *C. demirizii* has hollow stems. In this group, almost all the remaining species consist of endemic species (such as *C. davisiiana*, *C. duzceensis*, *C. elmaliensis*, *C. sumbuliana*, and *C. elazigensis*) with solid stems and simple or lyrate leaves. Among these, *C. elazigensis* and *C. elmaliensis* species are among the species whose stems are more slender and generally shorter than one m and whose leaves are mostly not divided (entire), and they are similar to the

Table 3. Measurements of pollen characters in *C. gokturkii* and *C. elmaliensis*.

	C. gokturkii			C. elmaliensis				
	Min.	Max.	Mean	SE	Min.	Max.	Mean	SE
P	83.19	99.01	90.92	0.93	78.31	89.39	82.83	0.72
E	66.31	87.68	79.01	1.05	69.09	81.39	74.89	0.72
P/E	1.09	1.30	1.15	0.01	1.04	1.17	1.11	0.01
Plg	18.26	25.43	21.23	0.41	12.10	18.95	15.67	0.39
Plt	9.38	15.19	12.50	0.37	10.14	15.47	12.35	0.36
Plg/Plt	1.33	2.51	1.73	0.07	1.13	1.51	1.27	0.02
Ex	5.29	6.75	6.17	0.08	5.22	7.51	6.60	0.12
Ann	2.33	3.14	2.79	0.05	2.13	2.93	1.52	0.05

All measurements given in μ m (P: Polar axis, E: Equatorial axis, P/E: Ratio of polar axis to equatorial axis-pollen shape, Plg: Pore length, Plt: Pore width, Plg/Plt: Ratio of pore length to pore width, Ex: Exine, Ann: Annulus, Min: Minimum value, Max: Maximum value, Mean: Average of measurements, SE: Standard Error).

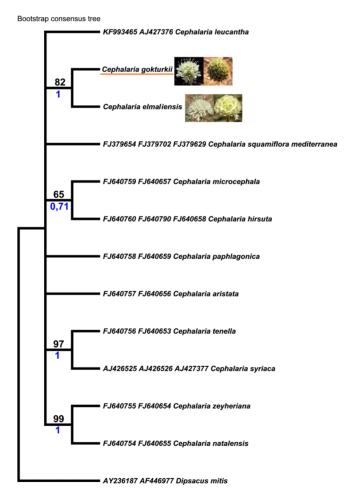


Figure 5. Phylogenetic tree obtained from the Parsimony and Bayesian analysis of combined (ITS and *trnL* intron) data. *Dipsacus mitis* was used as an outgroup. In branches, the upper value represents parsimony bootstrap, while the bottom value represents Bayesian posterior probability.

new species in terms of these features. The new species is seen as morphologically close to *C. elmaliensis*, and comprehensive comparisons are given in the diagnosis section and Table 2.

According to Khalik (2010), pollen grains of the Dipsacaceae family are heterogeneous and eurypalynous, allowing for the division of the grains into multiple groups. These classifications mostly agree with the previously hypothesized Dipsacaceae groupings based on macromorphological and phylogenetic characteristics. Our findings confirm this basic assessment that there is an important correlation among these data. Besides this conclusion, Tsymbalyuk et al. (2021) mentioned that there are some additional diagnostic characters at the species level in Cephalaria and they can be used for taxonomy. Currently, these palynologic characters are the size of pollen grains and pores, the shape of pores, the width of the annulus, the structure of the exine, the dimension of echini and microechini, the location of microechini, and the presence/absence of nanoechini. In terms of exin sculpture, two main structures in Cephalaria that could be classified as echinate-microechinate and echinate-microechinatenanoechinates. The first sculpture type is seen commonly in the majority of Dipsacaceae (now Caprifoliaceae) species. It is clear that C. elmaliensis has a second pollen type in consideration of sculpture ornamentations, but the new species has the first type commonly seen in most of Honeysuckle family type. The facial ornamentation in C. gokturkii is fairly sparse compared to that in C. elmaliensis.

According to prior molecular phylogenetic analysis, *Cephalaria* is sister to *Dipsacus* L. (Avino et al., 2009). It differs from *Dipsacus* anatomically by having a more or less continuous subepidermal sclerenchyma layer in the epicalyx and morphologically by having softer indumentum on the vegetative parts and floral bracts that are less pungent and roughly as long as the flowers (Mayer and Ehrendorfer, 2013). The Mediterranean Basin and surrounding western Eurasia are home to the family's center of diversity, while species can also be found in Asia, and eastern and southern Africa. According to Rosselló et

al. (2009), there is a good reason for the recent publication of many new species in the Mediterranean basin: both cpDNA and ribosomal sequences have revealed a highly structured pattern of molecular variation made up of sister monophyletic lineages that resemble major biogeographic units. Our molecular analyses showed that the new species is not only morphologically but also phylogenetically related to *C. elmaliensis*. The closest positions to the monophyletic clade (*C. gokturkii* and *C. elmaliensis*) are other two Mediterranean species, *C. squamiflora* and *C. leucantha*.

Specimen examined:

Cephalaria elmaliensis:—TÜRKİYE. Antalya: Elmalı, Keçova, hillsides, vii 1964, Fahri Demirdöğen 2577 (E!). Antalya, Elmalı, Çığlıkara Cedar forests, 1110 m, Avlan lake north-eastern slopes, limestone, 02 vii 1974, R. Çetik 2197 (KNYA!). Antalya: Elmalı, Çığlıkara, around the guardhouse, Cedrus libani clearings,1700–1900 m, 25 viii 1993, H. Duman 5345, Z. Aytaç & A.A.Dönmez (GAZI!, AKDU!). Antalya, Elmalı, Çığlıkara, around the guardhouse, 1900 m, rocky places and Cedrus libani clearings, 12 viii 1995, R.S.Göktürk 3532 (AKDU!).

Acknowledgments

This research was financially supported by the Pamukkale University Scientific Research Coordination Unit (project no: 2022FEBE022). The authors are thankful to the curators GAZI, AKDU, and KNYA herbaria. The authors are thankful to the editor and the referees for their careful reading of the manuscript and for the useful remarks that allow us to improve the text and clarify some of the results.

Author contribution statement

G.S. and B.G.-Collection of plant material in the field, identification of plant material, preparation of the first draft of the manuscript; M.B and T.U.-Molecular and phylogenetic assays; G.S., M.B., B.G., and T.U. -Writing, review and editing of the manuscript. All authors contributed equally to the final version of the manuscript.

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