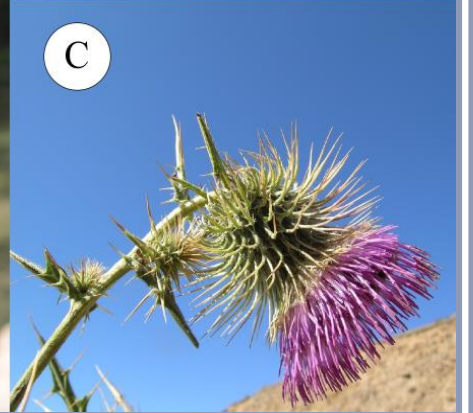
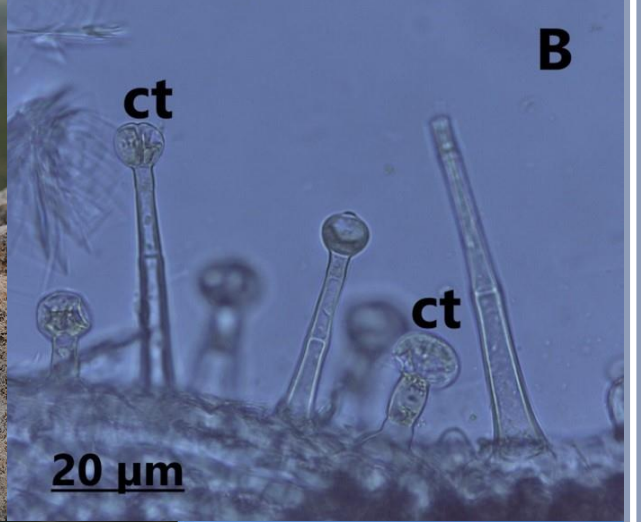


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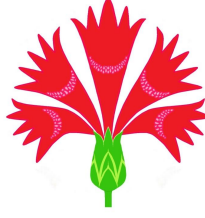
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Sahibi

Dr. Ergin Hamzaoğlu

Yazışma Adresi

Gazi Üniversitesi, Gazi Eğitim Fakültesi, Fen Bilgisi Eğitimi Anabilim Dalı, Hersek Binası
TR-06500, Emniyet Mahallesi, Yenimahalle, Ankara, Türkiye
Telefon: (+90) 535 404 29 49
E-posta: erginhamzaoglu@yahoo.com
Web: <https://dergipark.org.tr/tr/pub/turvehab>

Baş Editör

Dr. Ergin Hamzaoğlu

Editörler

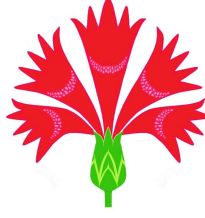
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Research Article

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***Campanula quercetorum* subsp. *densiflora*: A New Taxon of *Campanula* (Campanulaceae) from Eastern Anatolia (Türkiye)**

Hasan Yıldırım , Tuğkan Özdöl , Ademi Fahri Pirhan 

Department of Biology, Faculty of Science, Ege University, TR-35100, İzmir, Türkiye

*Correspondence: Hasan Yıldırım, hasanyldrm@gmail.com

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Abstract

Türkiye is a crucial country for the genus *Campanula* diversity in the Mediterranean Basin. Also, more than half of *Campanula* species in Türkiye are endemic. In this paper, *Campanula quercetorum* subsp. *densiflora* (Campanulaceae) is described as a new subspecies from Tunceli (Türkiye). According to its morphological features, it belongs to the *Campanula* subgen. *Campanula* sect. *Rupestres*. This section mainly consists of perennial polycarpic or perennial monocarpic and chasmophyte species. It is morphologically closely related to *C. quercetorum* subsp. *quercetorum*. It is easily distinguished from *C. quercetorum* subsp. *quercetorum* by its dense inflorescence, dense indumentum, densely hairy corolla surface, conspicuous purplish-blue corolla veins, and ascending to erect habitus. Diagnostic characteristics, a full description, and comprehensive photographs are given in this study.

Keywords: *Campanula*, sect. *Rupestres*, endemic, taxonomy, Türkiye

***Campanula quercetorum* subsp. *densiflora*: Doğu Anadolu'dan (Türkiye) Yeni Bir *Campanula* (Campanulaceae) Taksonu**

Özet

Türkiye, Akdeniz Havzasındaki *Campanula* cinsi çeşitliliği için anahtar ülkelerinden biridir. Ayrıca Türkiye'deki *Campanula* türlerinin yarısından fazlası endemiktir. Bu çalışmada, *Campanula quercetorum* subsp. *densiflora* (Campanulaceae), Tunceli'den (Türkiye) yeni bir takson olarak tanımlanmaktadır. Morfolojik özelliklerine göre, *Campanula* altcinsi *Campanula* sect. *Rupestres*'e aittir. Bu seksiyon çoğunlukla çok yıllık polikarpik veya çok yıllık monokarpik ve kazmofit türlerinden oluşur. Morfolojik olarak *C. quercetorum* subsp. *quercetorum* ile yakından ilişkilidir. *C. quercetorum* subsp. *quercetorum* 'dan yoğun çiçek durumu, yoğun tüy durumu, korolla üzerinde yoğun tüylenmesi ve korolla damarların bariz morumsu-mavi olması ve yükseliciden dik duruşluya kadar olan habitusu ile kolaylıkla ayırıt edilmektedir. Teşhis özellikleri, tam betimi ve kapsamlı fotoğrafları bu çalışmada verilmiştir.

Anahtar kelimeler: *Campanula*, endemik, sect. *Rupestres*, taksonomi, Türkiye

INTRODUCTION

Campanulaceae Juss. (Asterales) is a cosmopolitan family containing 84 genera and approximately 2400 species (Lammers 2007). Campanulaceae is divided into five main subfamilies (namely, Campanuloideae Burnett, Lobelioideae Burnett, Cyphioideae Walp. Nemacladoideae Lammers, Cyphocarpoideae Miers.) (Cosner et al. 1994; 2004; Lundberg & Bremer 2003; Tank & Donoghue 2010; Beaulieu et al. 2013; Crowl et al. 2016; Liveri et al. 2020).

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The genus *Campanula* L. belongs to the Campanulinae section of the subfamily Campanuloideae. *Campanula* contains about 450 species worldwide. The genus is distributed throughout the northern hemisphere, southern Asia, and central Africa (Eddie et al. 2003; Roquet et al. 2008; Cellinese et al. 2009; Haberle et al. 2009; Yoo et al. 2018; POWO 2023). *Campanula* is represented by approximately 140 taxa, more than 50% of which are endemic in Türkiye (Damboldt 1978; Davis et al. 1988; Güner 2000; Akçiçek et al. 2005; Kandemir 2007; İkinci 2012; Yıldırım 2013; 2018; Yıldırım & Şenol 2014; Mutlu & Karakuş 2015; Yıldırım et al. 2019; Yıldırım & Özdöl 2019; Özdöl et al. 2022a; 2022b; 2022c; 2022d; Fırat et al. 2022). Türkiye species of the genus *Campanula* are classified into six subgenera (namely, *Megalocalyx* Damboldt, *Rapunculus* (Fourr.) Kharadze, *Roucela* (Dumort.) Damboldt, *Brachycodonia* (Fed.) Damboldt, *Sicyodon* (Feer) Damboldt and *Campanula*). In “*Flora of Turkey and the East Aegean Islands*”, the subgenus *Campanula* is classified into 13 sections (Damboldt 1976; 1978). The section *Rupestres* (Boiss.) Charadze., which also includes *Campanula quercetorum* Hub.-Mor. & C.Simon, consists mainly of perennial polycarpic or perennial monocarpic and chasmophyte species. This situation causes the limitation of the distribution areas of the taxa in question and indirectly increases the endemism rates.

Campanula quercetorum is an endemic species known only from Tunceli (Türkiye). During field studies in the province of Tunceli, an exciting population of *Campanula*, closely related to *C. quercetorum*, was discovered by us, between the center of Tunceli and Ovacık district in the Munzur Valley (Figure 1). As a result of our detailed studies, we decided that this specimen is a new taxon of *C. quercetorum*.

MATERIAL AND METHOD

The material was collected from the Munzur Valley/Ovacık district of Tunceli province in June 2015. Samples of the new subspecies were compared with other *Campanula* collected from different localities and deposited in various herbaria, such as AEF, ANK, E, EGE, G, HUB, K, NGBB, VANF, and W (Thiers 2023). In addition, relevant literature (Boissier 1875; Fedorov 1957; Rechinger & Schimann-Czeika 1965; Fedorov & Kovanda 1976; Damboldt 1976; 1978; Davis et al. 1988; Güner 2000; IPNI 2023) was revised. The gross morphology of the new subspecies was examined under a stereo-binocular microscope, and measurements of these specimens were performed with a millimetric ruler. A total of 25 specimens of the new subspecies were examined. During the field studies, photographs of the living material of the new subspecies and its related taxa were taken with a Nikon D300 digital camera.

RESULTS AND DISCUSSION

Campanula quercetorum Hub.-Mor. & C.Simon subsp. *densiflora* Yıldırım, **subsp. nov.** (Figures 2 and 3)

Type. Türkiye. **Tunceli:** Between Tunceli and Ovacık, Munzur Valley, rocks, 1120 m a.s.l., 21.06.2015, *H. Yıldırım 3242* (**holotype:** EGE!, **isotype:** EGE!, ANK!, HUB!, NGBB!).

Paratypes. Türkiye. **Tunceli:** Between Tunceli and Ovacık, 15. km, rocks, 1150–1200 m a.s.l., 10.06.2002, *H. Altınözlü 3801* (ANK!); Ovacık road, 20 km from Ovacık, 1127 m a.s.l., 20.07.2015, *H. Yıldırım 3464* (EGE!).

Diagnosis. *Campanula quercetorum* subsp. *densiflora* is distinguished from *C. quercetorum* subsp. *quercetorum* by its fully dense setulose-hispidulous hairy corollas (not only on veins), conspicuously purplish-blue veins on corolla (not inconspicuous or concolour), ascending or erect and rigid stem (not fragile, flexuose and decumbent), and dense inflorescence (not mostly lax and sparse).

Description. Perennial, chasmophyte, retrorsely setulose-hispidulous. Stem 1 or many at base, 15–40 cm long, branched, erect or ascending, rigid. Rosette leaves 7–15 cm long (including petiole), with numerous small lobes on through the petiole 0.2–1 cm long, sometimes upper segments lyrate ca. 2.5×1 cm, both surface setulose-hispidulous hairy; lamina obovate-spathulate to oblong-elliptic, attenuate, irregularly and deeply dentate, dentate or entire, $2.5\text{--}6 \times 1.5\text{--}4$ cm; petiole 4.5–9 cm long, mostly winged to the base. Stem leaves elliptic-ovate to obovate-spathulate, $0.5\text{--}7 \times 0.2\text{--}4$ cm, irregularly dentate or regularly dentate, setulose-hispidulous hairy. Inflorescence raceme to paniculate, 25–80-flowered, mostly upturned; pedicel 1–9 mm long. Bract $4\text{--}12 \times 0.8\text{--}1.2$ mm, lanceolate, setulose-hispidulous. Calyx lobes linear-lanceolate, $5\text{--}6.5 \times 1\text{--}2.2$ mm, apex and margins retrorsely setulose-hispidulous, mid-vein conspicuous; appendage small, not more than 0.8 mm long. Corolla cylindrical-campanulate, densely retrorsely setulose-hispidulous hairy, $12\text{--}17 \times 4\text{--}6$ mm, whitish-cream, very lightly whitish-blue or light pinkish-white, veins conspicuously purplish-blue, divided into $1/5\text{--}1/4$; lobes triangular, $2.4\text{--}4 \times 1.7\text{--}3.5$ mm. Stamen 5–8 mm long; anther $4\text{--}5.5 \times 0.7\text{--}1$ mm; filament 1.8–2.6 mm long, triangular at base, 1–1.5 mm wide, margins ciliate. Style 8–13 mm long, including. Capsule $3\text{--}5 \times 2.5\text{--}4$ mm, semi-spheroidal, densely setulose-hispidulous hairy, conspicuously 9–10-keeled, opening by three basal pores. Seeds light brown, $0.5\text{--}0.7 \times 0.2\text{--}0.3$ mm.

Flowering time. June to August

Etymology and suggested Turkish name. The specific epithet indicates the dense inflorescence of the plant. The Turkish name of this species is given as “ovacıqçanı”, according to the guidelines of Menemen et al. (2016).

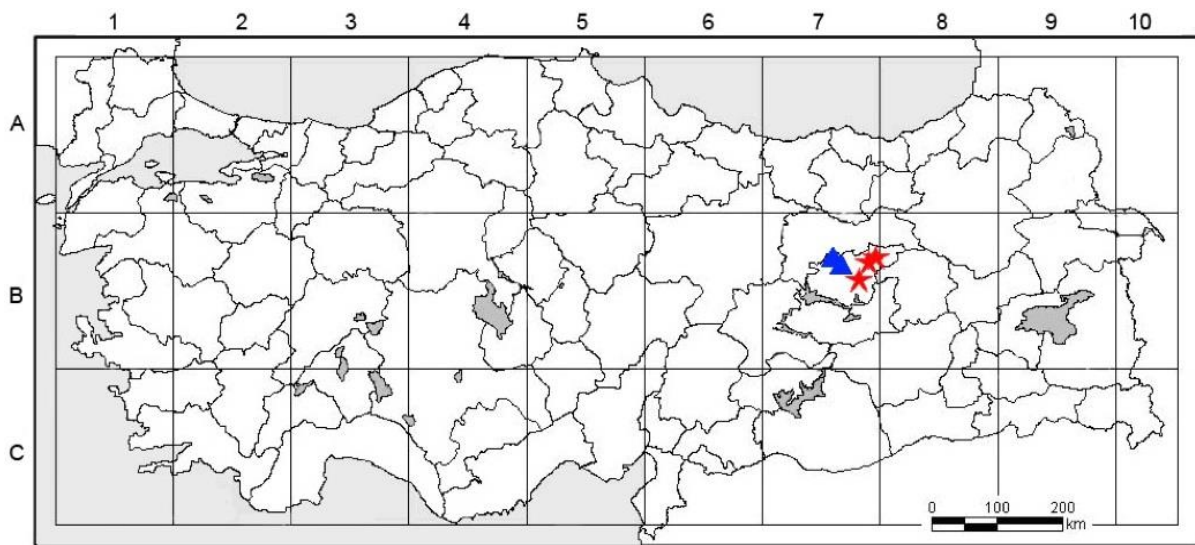


Figure 1. Distribution map of *Campanula quercetorum* subsp. *quercetorum* (★) and *C. quercetorum* subsp. *densiflora* (▲).



Figure 2. Holotype of *Campanula quercetorum* subsp. *densiflora*.



Figure 3. *Campanula quercetorum* subsp. *densiflora*. A-B: Habitus, C: Flowers.

Taxonomical notes

Campanula quercetorum is closely related to *C. hedgei* P.H.Davis. It is distinguished from *C. hedgei* by having prominent rosette leaves, long and generally paniculate inflorescences, and whitish-cream flowers. Basal leaves are very similar to *C. yildirimlii* Kit Tan & Sorger, *C. demirsoyi* Kandemir, and *C. dersimensis* Fırat & Yıldırım (Damboldt 1978; Davis et al. 1988; Kandemir 2007; Fırat et al. 2022). It can be mixed with these species when it is juvenile. But it can be easily distinguished from all of them by its capsule opening with three basal pores, corolla color and indumentum, calyx indumentum, calyx appendages, inflorescence type, and stem indumentum (Table 1). Damboldt (1978) stated that the flowers as of *C. quercetorum* are purplish-blue. However, this appears to be incorrect. Sometimes the flowers are slightly bluish when wilted, but the mature flowers are always whitish and sometimes a thin striped purplish-blue color on the veins, rarely quite a light pinkish-white. *Campanula quercetorum* subsp. *densiflora* is closely related to *C. quercetorum* subsp. *quercetorum* (Figure 4). It is easily distinguished from *C. quercetorum* subsp. *quercetorum* by its denser flowers and densely hairy flowering stems (not lesser flowers and slightly hairy); wholly densely hairy corolla (not hairy only on vein), and vein coloration on the corolla is generally conspicuous (not inconspicuous). Although the specimens of *C. quercetorum* subsp. *quercetorum* are habitus decumbent and stems are quite fragile and flexuous, the *C. quercetorum* subsp. *densiflora* has stiffer, coarser, and erect stems. In general, individuals of *C. quercetorum* subsp. *densiflora* show more branches. Though some of the characters slightly overlap, these specimens are considered a new subspecies of *C. quercetorum* (Table 1).

Identification key for subspecies of *Campanula quercetorum*

- 1a.** Corolla setulose-hispidulous only on veins; coloration on veins of corolla concolour; stems decumbent, flexuous, and fragile; inflorescence mostly lax and sparse **subsp. *quercetorum***
- 1b.** Corolla wholly densely setulose-hispidulous; veins conspicuous purplish-blue; stem ascending to erect and rigid; inflorescence dense.....**subsp. *densiflora***

Table 1. Morphological differences between *Campanula quercetorum* subsp. *densiflora*, *C. quercetorum* subsp. *quercetorum*, *C. dersimensis* and *C. yildirimlii*.

Characters	<i>C. quercetorum</i> subsp. <i>densiflora</i>	<i>C. quercetorum</i> subsp. <i>quercetorum</i>	<i>C. dersimensis</i>	<i>C. yildirimlii</i>
Plant colour	green	green	dark green	glaucous
Stem	erect or ascending, 15–40 cm long, branched, retrorsely setulose-hispidulous	prostrate to ascending, 10–40 cm long, distinctly branched, retrorsely hirsute to pubescent	erect, 15–85 cm long, unbranched or shortly branched in upper half, retrorsely hirtulous to pubescent, rarely subglabrous above	erect, 25–92 cm long, unbranched or very shortly branched, glabrous
Leaves	both surface setulose-hispidulous hairy	both surfaces densely retrorsely hirsute to pubescent, margin hispidulous and hirtulous	both surfaces densely retrorsely hirsute and pubescent, margin usually retrorsely hispidulous and hirtulous	both surfaces glabrous, margin retrorsely hispidulous

Inflorescence	raceme to paniculate flowers solitary or in clusters, dense; pedicel 1–9 mm	raceme to paniculate; flowers solitary, mostly lax and sparse; pedicel 1–9 mm long	spicate to branched spicate; flowers solitary or in clusters of 2 or 3, sessile or pedicel to 2 mm long	spicate; flowers solitary or in clusters of 2–7, sessile or pedicel to 2 mm long
Bracts	4–12 m long, lanceolate, setulose-hispidulous	4–12 mm long, both surfaces and margin hispidulous	4–20 mm long, both surfaces densely pubescent to puberulent, margin retrorsely hispidulous	2–4 mm long, glabrous
Calyx lobes	linear-lanceolate, 5–6.5 mm long, apex and margins retrorsely setulose-hispidulous	linear-lanceolate, 4.7–6.7 mm long, retrorsely hispidulous at apex	linear-lanceolate, 3–6 mm long, retrorsely hispidulous toward apex	triangular, 2.5–6 mm long, glabrous
Calyx appendages	to 0.8 mm long	to 0.8 mm long	to 1 mm long	absent
Corolla	whitish-cream, very lightly whitish-blue or light pinkish-white with conspicuously purplish-blue veins, 12–17 mm long, splitting to 1/5–1/4, wholly retrorsely densely setulose-hispidulous hairy	whitish blue, cream, or light greenish yellow to whitish pink with concolour veins, 10–17 mm long, splitting to 1/5–1/4, setulose-hispidulous only on veins	light greenish yellow to yellowish white with concolour veins, 9–17 mm long, splitting to 1/5–1/4, glabrous outside, midrib and apex of lobes sometimes sparsely hirtulous	whitish to pinkish purple with concolour veins, 8–15 mm long, splitting to 1/4–1/3, glabrous outside
Filaments	1.8–2.6 mm long, triangular at base, ciliate at the margin of the base	1.7–2.7 mm long, triangular at base, ciliate at the margin of the base	2–3 mm long, triangular at base, shortly hairy at the margin of the base	2–3 mm long, triangular at base, papillate-puberulent at the margin of the base
Anthers	4–5.5 mm long	4–5.5 mm long	3–6 mm long	5–7 mm long
Style	8–13 mm long	6–13 mm long	5–11 mm long	8–13 mm long
Capsule	semi-spheroidal, 3–5 × 2.5–4 mm densely setulose-hispidulous hairy, conspicuously 9–10 keeled, opening by three basal pores	semi globose when mature, 2.5–4.8 x 2–3.5 mm, densely hirsute, 9- or 10-ribbed, capsule opening with three basal pores	broadly ovoid-cylindric when mature, 2–6 x 1.5–5 mm, glabrous, (9 or) 10-ribbed, membranous structure between veins breaking down and capsule opening with (9 or)10 valves	globose when mature, 2–4 x 2–4 mm, glabrous, 10 ribbed, membranous structure between veins breaking down and capsule opening with 5–10 valves



Figure 4. *Campanula quercetorum* subsp. *quercetorum*. A: Isotype (E!), B-C: Habitus, D: Flowers.

Habitat, distribution and floristic regions

Campanula quercetorum subsp. *densiflora* is a local endemic taxon, restricted to Tunceli Province, eastern Anatolia (Figure 1). It is an element belonging to the Irano-Turanian phytogeographical region. The new taxa are distributed between 1150 and 1200 m. a.s.l. The area of occupancy (AOO) of *Campanula quercetorum* subsp. *densiflora* was calculated as 0.051 km², and about 350–400 individuals were estimated to occur. Overgrazing by sheep and goat herds was observed. There are also road works nearby *C. quercetorum* subsp. *densiflora* population. According to the criteria of the IUCN (2016), *C. quercetorum* subsp. *densiflora* is evaluated as “Critically Endangered” (CR) B2ab (ii,iii,v) in this paper.

Additional specimens examined

***Campanula quercetorum* subsp. *quercetorum*.** TÜRKİYE. **Tunceli:** Kutuderesi, Pülümür road, rocks, 21.07.2014, *H.Yıldırım 3103* (EGE!); Between Pülümür and Tunceli, 8. tunnel entry, rocks, 1200 m a.s.l., 20.07.2015, *H.Yıldırım 3461* (EGE!); Pülümür, Tunceli to Pülümür NE on Tunceli, open slopes, 1500 m a.s.l., 26.08.1986, *Archibald 8139* (148/88-48, E!); Tunceli-Pülümür, quercetum beim Karakol am Harçik suyu, 1100 m a.s.l., 28.06.1951, *Hub.Mor. 11072* (G!); Pülümür, kalkschlucht Tunceli-Pülümür, 46 km nördlich Tunceli, 1100 m a.s.l., 07.06.1959, *Hub.Mor. 15705* (G!).

***Campanula dersimensis*.** TÜRKİYE. **Tunceli:** On way from Tunceli to Nazimiye, Kutu Stream, around Zülfikar Fountain, 1040 m a.s.l., limestone rock crevices, 01.08.2012, *M.Fırat 28888* (EGE!, 43200); ibid., 1104 m a.s.l., rocky areas, 05.08.2014, *M.Fırat 31538* (herb. M.Fırat!); ibid., 28.07.2012, *M.Fırat 28876* (herb. M.Fırat!); On way from Tunceli to Ovacık, Munzur Valley, 1087 m, rocky areas, 27.07.2012, *M.Fırat 28868* (herb. M.Fırat!); Tunceli-Ovacık road, Ovacık Canyon, approximately 20 km from Ovacık District, 1127 m a.s.l., 21.07.2015, *H.Yıldırım 3464a* (EGE!).

***Campanula yildirimlii*.** TÜRKİYE. **Erzincan:** Kemaliye, Sandıkbağı surroundings, 900 m a.s.l., rocky places, 17.11.1980, *Ş.Yıldırım 4174* (paratypes, ANK!, B!); Kemaliye, Salihli village, 1484 m a.s.l., serpentine area, 15.07.2014, *H.Yıldırım 3060* (EGE!); **Malatya:** Arguvan, Göldağı slopes, Yukarı Soğuksu, Perideresi Valley, 1484 m a.s.l., 15.06.2014 (sterile), *H.Yıldırım 2887* (EGE!); Arguvan, Divriği road, 3. km, 1609 m a.s.l., limestone cliffs, 21.07.2015, *H.Yıldırım 3469* (EGE!); Arapgir, Kayaarası Canyon, 1195 m a.s.l., cliffs, 08.09.2015, *H.Yıldırım 3690* (EGE!); Arapgir, 2–3 km after Divriği road junction, near fountain, 1451 m a.s.l., 08.09.2015, *H.Yıldırım 3691* (EGE!).

***Campanula hedgei*.** TÜRKİYE. **Erzincan:** Munzur Mountains, Çağlayan Village, Çağlayan (Girlevik) Waterfall side, calcareous rocks, 1372 m a.s.l., 22.07.2014, *H.Yıldırım 3109* (EGE!); Keşiş Dağ above Cimin, igneous rock crevices, 2700–2900 m a.s.l., 27.07.1957, *P.H.Davis 31634* (ANK!; K!); **Tunceli:** Above Ovacık, Munzur Mountains, below Karaçukur place, Sor Yaylası, calcareous rocks, 2259 m a.s.l., 20.07.2014, *H.Yıldırım 3100* (EGE!); Ovacık, Mercan place, 1450 m a.s.l., 22.06.2008, *M.Vural 15845* (AEF!); ibid., *M.Vural 15848* (AEF!); Laç Stream, rocks, 965 m a.s.l., 15.06.2002, *H.Duman 22033* (AEF!); Munzur Dağ above Ovacık, dry limestone cliff, 2100 m a.s.l., 19.07.1957, *P.H.Davis 31315 & I.Hedge* (E! [E00275093]; G! [G154487]; K! [2139/60-3, 2139/60-4, 2139/60-5]); Munzur gebirge NW Ovacık (Bekirdashe dağ), felsfluren, 1500–2900 m a.s.l., 17.08.1982, *F.Sorger 82-127-6* (W! [01469]); Ovacık, Munzur Mountains, above Aksudere, rocks, 1706 m a.s.l., 21.06.2015, *H.Yıldırım 3246* (EGE!); **Van:** Özalp, district center surroundings, 2300 m a.s.l., 09.08.1988, *F.Özgökçe 5480* (VANF!).

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AUTHOR CONTRIBUTION STATEMENT

In this study; the study idea and design, data collection, analysis and interpretation of the results, and drafting of the article were made by the authors.

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Research Article

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New Locality Records in Türkiye for Two Rare Members of Ascomycota

Abdullah Kaya ^{1,*}, Yasin Uzun ²

¹Biology Department, Science Faculty, Gazi University, TR-06560, Ankara, Türkiye

²Pharmacy Services Department, Ermenek Uysal and Hasan Kalan Health Services Vocational School, Karamanoğlu Mehmetbey University, TR-70100, Karaman, Türkiye

*Correspondence: Abdullah Kaya, kayaabd@hotmail.com

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Abstract

Apothecia of two previously reported rare members of Ascomycota, *Dumontinia tuberosa* and *Sarcopeziza sicula*, were collected from different localities and identified. *Dumontinia tuberosa* was collected from the Eastern Black Sea Region while *S. sicula* was collected from Central Anatolian and Southeastern Anatolian Regions of Türkiye. Brief descriptions and new distribution localities of the species were provided together with the photographs of their macro and micromorphologies.

Keywords: Biodiversity, rare fungi, Pezizaceae, Sclerotiniaceae

Nadir İki Ascomycota Üyesi İçin Türkiye’de Yeni Lokalite Kayıtları

Özet

Daha önceden rapor edilmiş olan iki nadir Ascomycota üyesi, *Dumontinia tuberosa* ve *Sarcopeziza sicula*, yeni lokalitelerden toplanarak teşhis edilmiştir. *Dumontinia tuberosa* Türkiye’nin Doğu Karadeniz Bölgesinden, *S. sicula* ise İç Anadolu ve Güneydoğu Anadolu Bölgelerinden toplanmıştır. Türlerin kısa betimlemeleri ve yeni yayılış lokaliteleri, makro ve mikromorfolojilerine ait fotoğrafları ile birlikte verilmiştir.

Anahtar kelimeler: Biyoçeşitlilik, nadir mantarlar, Pezizaceae, Sclerotiniaceae

INTRODUCTION

Fungi are important components of the ecosystem. Besides their vital roles in biogeochemical cycling, nutrient recycling, and decomposing the dead organic matter in soil, they play indispensable roles in many areas such as industry, agriculture, textile, bioremediation, etc. The most visible group, the macrofungi, are the most prominent members of the fungal kingdom, some of which have long been used primarily for nutritional and medicinal purposes. Utilization of naturally growing macrofungi can only be possible after their collection from nature. Though many of them are cosmopolitan, occurring in all aerobic ecosystems, and colonize a wide range of substrates, some are specialists and found only in restricted substrates or habitats. Since most species can be detected only when they produce reproductive structures, some macrofungi are often difficult to detect, especially the rare, sparsely-distributed, and seldom-fruiting species. Depending on species-specific relationships to nutrient availability and environmental conditions, different species may produce detectible fruit bodies at different times and seasons (Marcot 2017). In general, fungal fruiting records are used to understand the fungal distribution. The majority of fungal studies have taken place at localized scales, and to provide any assurance of detection, at

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least a five-year surveying at various times of the year, especially in spring and autumn, was recommended (O'dell et al. 1996).

Almost 2.700 species of macrofungi have been reported from Türkiye, more than 30% of which were recorded once and 18% twice. The overall list was created from the accumulated data, presented either as local lists or new records. A quick overview of the publications on macrofungal diversity of Türkiye also indicates that the majority of the local surveys were finished in two to three years. Therefore, additional data (regional lists, new distribution localities, etc.) need to be constantly presented in order to holistically reveal the diversity and regional distribution of Turkish macrofungi.

Here we present new localities for two rare (Mircea et al. 2016; Agnello et al. 2018; Fokshei 2022) and previously presented ascomycete species, *Dumontinia tuberosa* (Bull.) L.M. Kohn and *Sarcopeziza sicula* (Inzenga) Agnello, Loizides & P.Alvarado (Doğan & Kurt 2016; Sesli et al. 2020; Altuntaş et al. 2021; Akçay et al. 2023; Uzun 2023). The study aims to contribute to the knowledge of macrofungi of Türkiye.

MATERIAL AND METHOD

Apothecia of *Dumontinia tuberosa* was collected from Gümüşhane province in 2023, and *Sarcopeziza sicula* was collected from Gerger district of Adıyaman in 2009 and central district of Karaman province in 2021. During field surveys, the apothecia were photographed in their natural habitats, and ecological and some morphological characteristics were noted. Then the samples were transferred to the fungarium in paper boxes and dried in an air-conditioned room. A Nikon eclipse Ci-S trinocular light microscope, equipped with a DS-Fi2 digital camera was used for microscopic investigations. SEM images were obtained by a Hitachi SU5000 scanning electron microscope. By comparing the accumulated data with the relevant literature (Inzenga 1869; Doğan & Kurt 2016; Kim et al. 2009; Uzuhashi et al. 2010; Medardi 2012; Agnello et al. 2013; 2018; Thompson 2013; Beug et al. 2014; Mircea et al. 2016; Altuntaş et al. 2021) the samples were identified. The specimens are kept at the Biology Department, Kamil Özdağ Science Faculty, Karamanoğlu Mehmetbey University.

RESULTS AND DISCUSSION

Ascomycota Caval.-Sm.

Leotiomyces O.E.Erikss. & Winka

Helotiales Nannf.

Sclerotiniaceae Whetzel

Dumontinia tuberosa (Bull.) L.M.Kohn (Figures 1 and 2)

Synonyms. [*Aleuria rapulum* (Bull.) Gillet var. *tuberosa* (P.Karst.) Gillet, *Helotium tuberosum* (Hedw.) P.Karst., *Hymenoscyphus tuberosus* (Bull.) W.Phillips, *Macroscyphus tuberosus* (Hedw.) Gray, *Octospora tuberosa* Hedw., *Peziza tuberosa* Bull., *Peziza tuberosa* (Hedw.) Dicks., *Peziza tuberosa* Bull. f. *strobilina* Fr., *Peziza tuberosa* Bull. subsp. *radicata* (Reichard) N.Lund, *Peziza tuberosa* Bull. var. *communis* Alb. & Schwein., *Rutstroemia tuberosa* (Bull.) P.Karst., *Sclerotinia tuberosa* (Hedw.) Fuckel, *Sclerotinia tuberosa* (Hedw.) Fuckel f. *pallida* Henn., *Whetzelinia tuberosa* (Hedw.) Korf & Dumont].

Macroscopic and microscopic features. Apothecia 12–27 mm in diameter, stipitate, goblet-shaped with a small apical aperture at the beginning, later cup to funnel-shaped, some become plane at

maturity, hymenial surface smooth, some slightly wrinkled at the center, light brown to yellowish brown, outer surface smooth, concolorous with hymenial surface, flesh thin and brittle. Stipe 22–80 × 2–4 mm, arises from a globose to irregularly shaped black sclerotium, dark-brown to blackish-brown. Asci 150–170 × 9–12 µm, cylindrical, hyaline, 8-spored. Paraphyses cylindrical to filiform, aseptate, some slightly enlarged towards the apex. Ascospores 11.5–16.3 × 6.5–7.5 µm, ellipsoid to elongated-elliptical, hyaline, smooth, some with two drops at the poles.

Dumontinia tuberosa was reported to arise from the sclerotia, which develop on the rhizomes of an *Anemone* L. species, in deciduous and mixed forests (Uzhashi et al. 2010; Medardi 2012; Thompson 2013; Beug et al. 2014; Fokshei 2022).



Figure 1. Ascocarps of *Dumontinia tuberosa*.

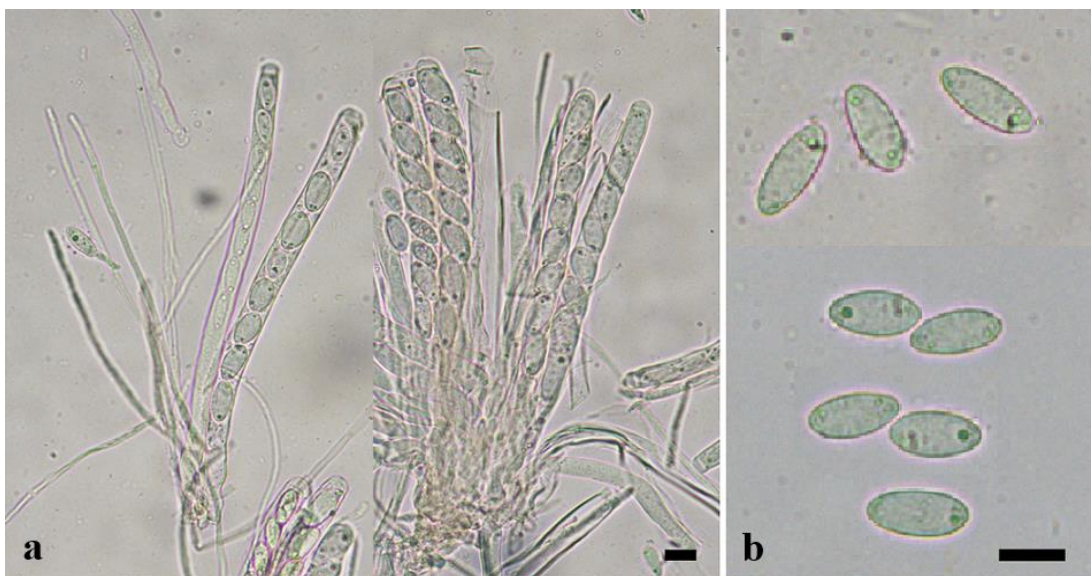


Figure 2. Asci and paraphyses (a) and ascospores (b) of *Dumontinia tuberosa* (Bars:10 µm).

Specimen examined

Türkiye. **Gümüşhane:** Torul, Zigana Village, *Pinus* L. and *Populus* L. containing mixed forest, on rhizomes of *Anemone* sp. under *Populus* sp., 17.05.2020, Yuzun 7321.

Chorological notes

The first Turkish record of *Dumontinia tuberosa* was reported by Doğan and Kurt (2016) from Pozantı district of Adana province on *Anemone* sp., in a mixed forest containing *Abies cilicica* (Antoine & Kotschy) Carrière subsp. *cilicica*, *Cedrus libani* G.Don, and *Quercus* L. sp. We found it on rhizomes of *Anemone* sp. in *Pinus* and *Populus* containing mixed forest, under *Populus* sp.

Pezizomycetes O.E.Erikss. & Winka

Pezizales J.Schröt.

Pezizaceae Dumort

Sarcopeziza sicula (Inzenga) Agnello, Loizides & P.Alvarado (Figures 3 and 4).

Synonyms. [*Peziza sicula* Inzenga, *Sarcosphaera sicula* (Inzenga) Pat.]

Macroscopic and microscopic features. Apothecia up to 50–75 in diam., 45–74 in height, hypogeous or semihypogeous when young, subglobose, globose to pear-shaped, some longitudinally ellipsoid, hollow, with a small apical opening, partially or fully emerging from the soil at maturity, and superiorly turn into several large, 4–5 irregular lobes, substipitate to indistinctly stipitate. Hymenium smooth, vinaceous-red to brownish-red. Outer surface smooth to covered with tiny warts, almost concolorous with the hymenial surface, somewhat paler. Flesh up to 5 mm thick, fragile, concolorous with the hymenial surface or somewhat brighter. Asci 340–370(–390) × 12.5–13.5(–15) µm, cylindrical, 8-spored, amyloid especially at the apex. Paraphyses cylindrical, the same length as the asci, some longer, often slightly enlarged towards the apex, rarely distinctly enlarged. Ascospores 13–15(–17) × 7.8–9.2(–9.7) µm, ellipsoid, uniseriate, hyaline to subhyaline, 1–2 to multiguttulate, visible as smooth under light microscope but clearly warty to irregularly ribbed under scanning electron microscope.



Figure 3. Ascocarps of *Sarcopeziza sicula*.

Sarcopeziza sicula was reported to grow on bare ground, among grasses and herbs, sometimes under Aleppo pine (*Pinus halepensis* Mill.), olive tree (*Olea europaea* L.) and other sclerophyllous vegetation of the Mediterranean basin (Agnello et al. 2013; 2018).

Specimen examined

Türkiye. **Adıyaman:** Gerger, Budaklı village, both sides of highway, in meadow, and among grass under *Pinus brutia* Ten., 07.03.2009, K. 5964; **Karaman:** KMU Yunus Emre Campus, on soil among grass, 21.04.2021, *Yuzun* 7325.

Chorological notes

Sarcopeziza sicula (Inzenga) Agnello, Loizides & P.Alvarado, also reported as *Peziza sicula* Inzenga and *Sarcosphaera sicula* (Inzenga) Pat., is the only member of the genus and has a Mediterranean distribution. It has so far been reported from Cyprus, Greece, Israel, Italy (Inzenga 1869; Agnello et al. 2018) and Tunisia (Patouillard 1904).

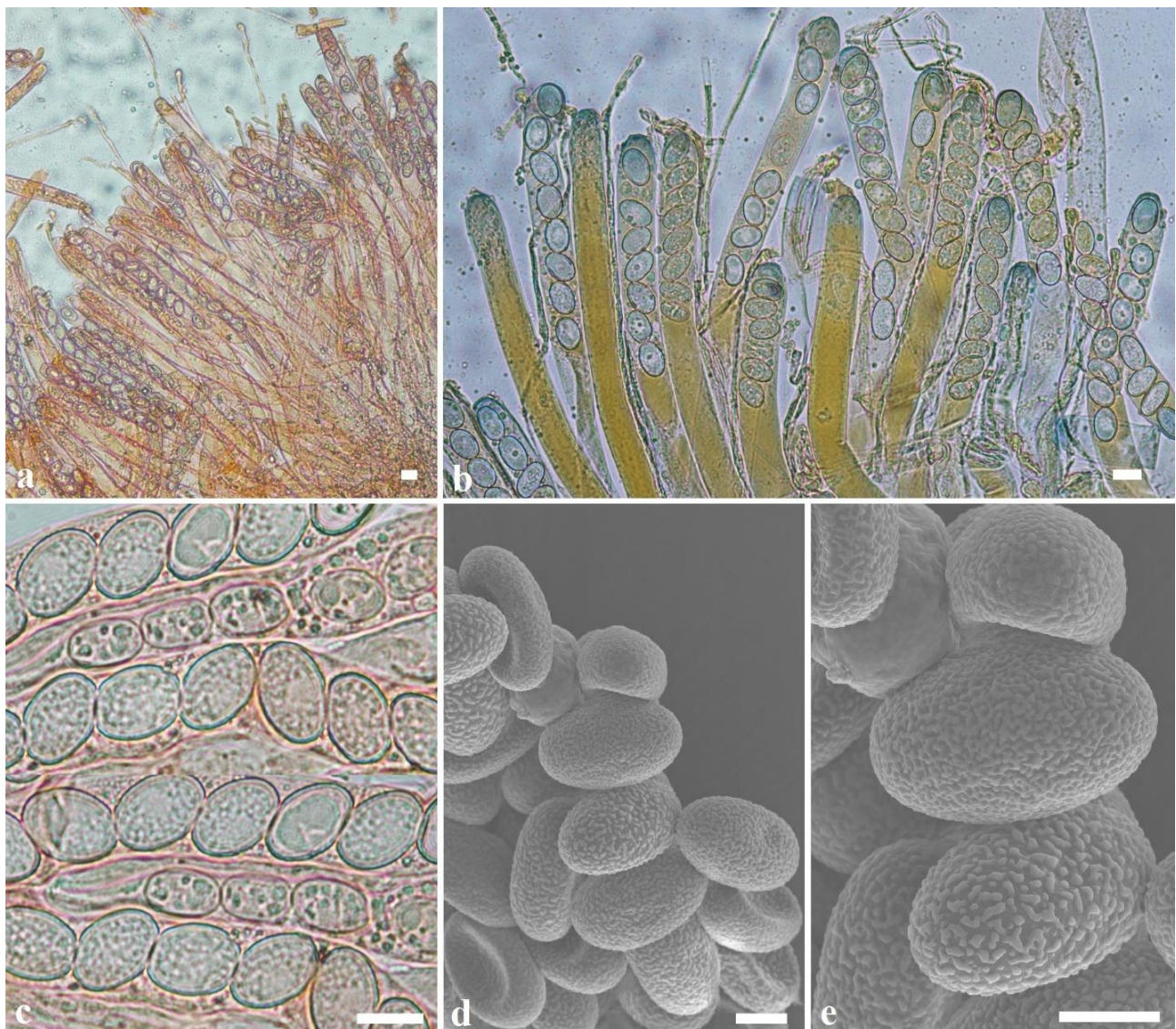


Figure 4. Asci and paraphyses (a,b) and ascospores (c-e) of *Sarcopeziza sicula* (Bars: a-c:10 μ m, d-e: 5 μ m) (a,c: Congo Red, b: Melzer) (a-c: light microscope, d-e: SEM).

Sarcopeziza sicula was reported previously from Türkiye only once by Altuntaş et al. (2021) from Tavşanlı district of Kütahya province. The sample were collected on bare ground in April. We collected two samples of *S. sicula*. The first one was collected in meadow and grassy soil under *Pinus brutia*, while the second sample was collected on bare ground among dry herb remains.

Though the ascocarp and ascospore dimensions of the investigated samples are generally in agreement with all of the reported data, the length/diameter ratio for some of our collections is considerably greater, especially for those collected from tight soil in meadow, compared to those reported before. The length/diameter ratio of the samples, collected from loose soil under *Pinus* sp. and bare ground among grass well fit with Altuntaş et al. (2021) and Agnello et al. (2018). On the other hand, we did not clearly observe the purple color or the lilac tinge in fresh material.

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AUTHOR CONTRIBUTION STATEMENT

In this study; the study idea and design, data collection, analysis and interpretation of the results, and drafting of the article were done by the authors.

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Research Article

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**A New Rupicolous Species from West of Türkiye: *Stachys cuhacioglui*
(Lamiaceae)**

Özal Güner ¹, Tuğkan Özdöl ², Hasan Yıldırım ^{2,*}

¹Harran District Directorate of National Education, TR-63510, Şanlıurfa, Türkiye

²Department of Biology, Faculty of Science, Ege University, TR-35100, İzmir, Türkiye

*Correspondence: Hasan Yıldırım, hasanyldrm@gmail.com

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Abstract

Stachys is one of the most diverse genera of the family Lamiaceae and currently consists of 360 species. Here, we describe *Stachys cuhacioglui* (Lamiaceae) as a new species based on morphological and micromorphological data. The new species was discovered in İzmir province in west of Türkiye. *Stachys cuhacioglui* differs from *S. annua* complex by its perennial, chasmophyte habitus, cordate basal leaves, shorter campanulate calyx with recurved teeth, and shorter corolla. The description, etymology, phenology, geographical distribution, IUCN threat category and ecology of the new species are given.

Keywords: Anatolia, İzmir, Labiatae, new species, *Stachys*, Türkiye

**Türkiye'nin Batısından Yeni Bir Rupikol Tür: *Stachys cuhacioglui*
(Lamiaceae)**

Özet

Stachys, Lamiaceae ailesinin en zengin cinslerinden biridir ve hâlihazırda 360 tür içerir. Burada, *Stachys cuhacioglui* (Lamiaceae) morfolojik ve mikromorfolojik verilere dayanarak yeni bir tür olarak tanımlanmaktadır. Yeni tür, Türkiye'nin batısından İzmir ilinden keşfedilmiştir. *Stachys cuhacioglui*, *S. annua* kompleksine ait türlerden, çok yıllık oluşu, kazmofit görünümü, kalpsi taban yaprakları, kıvrık dişli ve çan şeklindeki kaliksi ve daha kısa korollası ile kolaylıkla ayrılır. Bu çalışmada yeni türün tanımı, etimolojisi, fenolojisi, coğrafi dağılımı, IUCN tehdit kategorisi ve ekolojisi verilmiştir.

Anahtar kelimeler: Anadolu, İzmir, Labiatae, *Stachys*, Türkiye, yeni tür

INTRODUCTION

Stachys L., is the largest genus of the subfamily Lamioideae Harley (Harley et al. 2004) and it is one of the largest genera in the family Lamiaceae Martinov (Scheen et al. 2010). The genus *Stachys* is mainly distributed in the Mediterranean region and also in southwestern Asia and North and South America (Bhattacharjee 1980). However, it is not distributed in Australia and New Zealand. The genus comprises 362 species (435 taxa) (POWO 2022). Most of its species grow in forests on rocky places, on limestones, and banks of streams (Bhattacharjee 1980). In addition, endemic taxa are mostly intensified in the warm temperate regions of the Mediterranean Basin (Bhattacharjee 1982).

Bhattacharjee (1980), the most comprehensive revision of *Stachys* to date, revised the Old World species and categorized them into two subgenera *Betonica* L. and *Stachys*. Then, based on

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the current molecular phylogeny, as Lindqvist & Albert (2002) suggested, Scheen et al. (2010) clearly corroborate the need to reestablish the genus *Betonica*. Recent phylogenetic analyses of chloroplast DNA (cpDNA) and nuclear ribosomal showed that the genus *Stachys* is subdivided into two distinct clades (Scheen et al. 2010; Roy et al. 2013). The first clade forms the center of diversity in the eastern part of the Mediterranean region then its distribution expanded over time to Western Asia, Western Europe and Macaronesia, and sub-Saharan Africa. Whereas the second clade includes the Hawaiian mints, *Suzukia* Kudô, all New-World *Stachys* species and some Old-World species. Phylogenetic findings of studies over the last decade reveal that *Stachys* is polyphyletic (Scheen et al. 2010; Roy et al. 2013).

Stachys is composed of 96 species (123 taxa) and 67 of these taxa are endemic to Türkiye (Güner 2022a; 2022b; Fırat & Güner 2022). *Stachys* species exhibit considerable diversity in floral, nutlet, and habit features. For example, *Stachys annua* L., with three subspecies, of which one is endemic to Türkiye (Figure 1), and *S. iberica* M.Bieb., with 4 infraspecific taxa (of which one is endemic to Türkiye), are distributed throughout the country and display a remarkable diversity in floral, leaves and calyx shape features (Akçiçek 2020). *Stachys* species are mainly identified using indumentum, calyx and corolla length, tooth-to-calyx ratio and verticillaster arrangement (Bhattacharjee 1982; Güner et al. 2021). Based on the morphological characters, some new *Stachys* species have recently been identified (González-Gallegos 2015; Akçiçek et al. 2016; Doğu & Bağcı 2021; Fırat 2021; Akçiçek & Güner 2022) and revision studies (Akçiçek 2020) on *Stachys* sections have been carried out. Furthermore, phylogenetic studies based on nuclear ribosomal and plastid DNA data have supported the morphological distinction between *Stachys* species (Dündar et al. 2013; Bendiksby et al. 2014; Berumen-Cornejo et al. 2017).

In 2012, during fieldwork in İzmir, the third author and Cem Çuhacıoğlu, an amateur botanist and nature lover, collected some interesting *Stachys* specimens on volcanic rocks (Figures 1 and 2). As a result of our detailed macro- and micromorphological studies, we have concluded that those specimens collected in İzmir province differ from other *Stachys* species and after consulting the relevant literature, we concluded the specimens represent a new species.

MATERIAL AND METHOD

The specimens of the new *Stachys* species were compared with other *Stachys* specimens deposited in various herbaria such as ANK, HUB, E, EGE, G, GAZI, ISTE, ISTF, ISTO, K and W (Thiers 2022). In addition, the available relevant literature (Dumortier 1827; Ball 1972; Bhattacharjee 1982; Davis et al. 1988; Strid & Tan 1991; Akçiçek 2020) was reviewed. The gross morphology of the new species was examined under a stereo-binocular microscope and measurements of these specimens were performed with a millimetric ruler. A total of 30 specimens of the new species were examined. At least 50 pollen grains and 30 mature seeds were measured using a light microscope and a scanning electron microscope (SEM). For SEM observations, seed and pollen grains were placed on aluminium stubs with double-sided adhesive tape, sputter coated with gold using a Emiteck K550 and then examined using an FEI Quanta 250 FEG SEM. During field studies, photographs of living material of the new species and its related taxa were taken with a Nikon D300 digital camera. The conservation status of the new species was evaluated based on field observations in accordance with the IUCN (2022) guidelines.

RESULTS AND DISCUSSION

Stachys cuhacioglu Yıldırım & Ö.Güner, **sp. nov.** (Figures 2 and 3)

Type. Türkiye. **İzmir:** Menderes-Gümüldür road, across of Tahtalı Dam, on volcanic rock cracks, 156 m a.s.l., 24.05.2012, *H.Yıldırım 2334* & *C.Çuhacıoğlu* (**holotype:** EGE 43772!, **isotype:** EGE 43773!, NGBB!).

Paratype. Türkiye. **İzmir:** Menderes-Gümüldür road, 1 km to Şaşal, 143 m a.s.l., 04.05.2018, *T.Özdöl 982* & *H.Yıldırım* (EGE 43774!); Menderes, Tahtalı Dam road, before to Şaşal, right of the road, valley road, 231 m a.s.l., 05.08.2018, *T.Özdöl 1260* (EGE 43775!); *ibid.*, 09.05.2019, *T.Özdöl 1612* (EGE 43776!); Menderes, between Deliömerli and Şaşal, 494 m a.s.l., 26.07.2019, *T.Özdöl 2253* & *H.Yıldırım* (EGE 43777!).

Diagnosis. *Stachys cuhacioglu* is morphologically similar to *S. annua*, *sensu lato*, but differs from it by the perennial habitus, cordate basal leaves, campanulate calyx, recurved calyx teeth, and shorter calyx with 6–7 mm long and corolla with 8–9 mm long.

Description. Suffrutescent, caespitose perennial with basal sterile rosettes. Flowering stems 15–45 cm long, erect, simple or branched, densely to sparsely retrorsely pilose at base or throughout and sometimes retrorsely pubescent, upper parts with sessile glands. Leaves sparsely adpressed pilose with sessile glands. Basal leaves, 1.3–3 × 0.9–2.3 cm, crenate at margin, cordate at base; petiole 1.2–3.4 cm long. Cauline leaves 1.3–4 × 0.5–1.4 cm, ovate to ovate-lanceolate, crenate-dentate at margin, attenuate at base; petiole to 2 cm long. Floral leaves broadly lanceolate to linear-lanceolate, margin entire, longer than lower verticillasters and gradually shorter than upper verticillasters, obtuse to acute at apex, sessile. Verticillasters usually remote, 2–4-flowered, 0.3–3.5 cm distant. Bracteoles few, linear, 1–2.5 mm long. Pedicels c. 1 mm long. Calyx regular, campanulate, 6–7 mm long, densely pilose with sessile glands, ± gibbous at base in fruit; teeth ± equal, triangular, 1.5–3 mm long, slightly recurved, with aristate tips; aristae c. 1 mm long, glabrous. Corolla white, lips streaked and spotted with pink inside, 8–9 mm long, densely short pilose hairy with sessile glands outside; tube 6–7 mm long, shortly exerted from calyx, exannulate; limb bilabiate, upper lip c. 3 mm long, retuse; the lower lip 3-lobed, middle lobe longer than 2 lateral lobes, 4 mm long, emarginate. Style c. 7 mm long, not exceeding the upper lip, glabrous, apex equally bifid; stigmas subulate. Stamens 4, within corolla tube; anthers dithecous, thecae divaricate; filaments flattened and with short swollen hairs near point of attachment to corolla tube. Nutlets 2.5–3 × 1.7–2 mm, oblong–obovoid to oblong–discoid, faintly trigonous, winged near base, blackish brown.

Phenology. Flowering in May and fruiting in mid–May and June.

Eponym. This new species was named in honor of the amateur botanist “Cem Çuhacıoğlu” who first discovered the new species together with the third author. The Turkish name of this species is suggested as “efeçayı” according to the guidelines of Menemen et al. (2016).

Habitat and ecology. *Stachys cuhacioglu* is currently known to be locally endemic to İzmir Province, West Anatolia. It is an element belonging to the Mediterranean floristic region. The new species grows in the maquis vegetation and opening of *Pinus brutia* Ten. forests in volcanic rock cracks, between 143 and 494 m a.s.l. in the triangle of Seferihisar, Menderes, and Gümüldür Districts in İzmir (Figure 1). It’s an obligate rupicolous plant (Figure 2). It is never seen on soil. Species growing in the near vicinity include *Arbutus unedo* L., *Asparagus acutifolius* L., *Ceratonia siliqua* L., *Lavandula stoechas* L. subsp. *stoechas*, *Lonicera caprifolium* L., *Origanum onites* L.,

Osyris alba L., *Peucedanum guvenianum* Yıldırım & H.Duman, *Phillyrea latifolia* L., *Pistacia lentiscus* L., *Satureja thymbra* L., and *Verbascum rupicola* (Hayek & Siehe) Hub.-Mor.

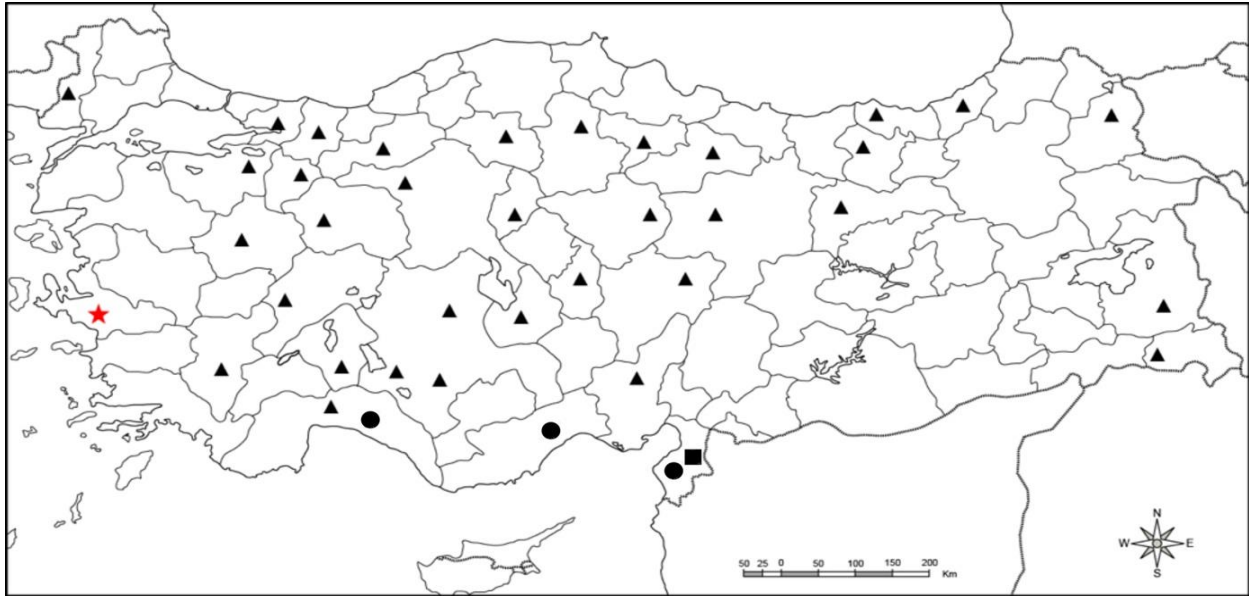


Figure 1. Distribution of *Stachys cuhacioglu* (★), *S. annua* subsp. *annua* (▲), *S. annua* subsp. *ammophila* (●) and *S. annua* subsp. *cilicica* (■) (data of subsp. *annua*, subsp. *ammophila* and subsp. *cilicica* from Akçiçek 2020; map obtained from CoğrafyaHarita 2022).

Taxonomical notes

Stachys cuhacioglu belongs to section *Olisia* Spach subsection *Annuae* Bhattacharjee. This subsection includes some closely related but remarkably variable species (notably *S. annua*), which could be interpreted as a species complex (Bhattacharjee 1982). *Stachys* subsect. *Annuae* is represented by only two species (*S. maritima* Gouan and *S. annua*) in Türkiye. *Stachys maritima* is closely related to *S. annua* but differs from it in having shorter calyx teeth and congested spicate inflorescence. While *S. maritima* is well adapted to live in the maritime sands of the Black Sea shore, *S. annua* prefers to grow in more variable habitats, such as the edges of cultivated and fallow fields, stream sides, forests, and limestone slopes in several regions of Türkiye (Bhattacharjee 1982). Moreover, *Stachys annua*, with its three recognized subspecies, is a widely distributed and morphologically variable taxon with a wide distribution from Europe to Siberia and Iran (POWO 2022). Much confusion has arisen regarding the taxonomic circumscription of the widely distributed and variable *S. annua*. Individuals of this species could be perennial, biennial, or annual with or without basal sterile rosettes (Bhattacharjee 1982; Akçiçek 2020).

Stachys annua contains the following infraspecific taxa: subsp. *annua*, subsp. *ammophila* (Boiss. & Blanche) Sam and subsp. *cilicica* (Boiss.) R.Bhattacharjee. While subsp. *annua* grows in various habitats and is widely distributed almost throughout Türkiye, subsp. *ammophila* and subsp. *cilicica* are locally found in South Anatolia. Moreover, subsp. *ammophila* is endemic to Türkiye (Bhattacharjee 1982; Akçiçek 2020). Although there is no problem among the subspecies of *Stachys annua* based on available data, these subspecies need to be re-evaluated in the light of molecular data. Our studies on the section *Olisia* continue at levels of molecular and micromorphological as well as morphological (Akçiçek 2020; Güner 2022b).

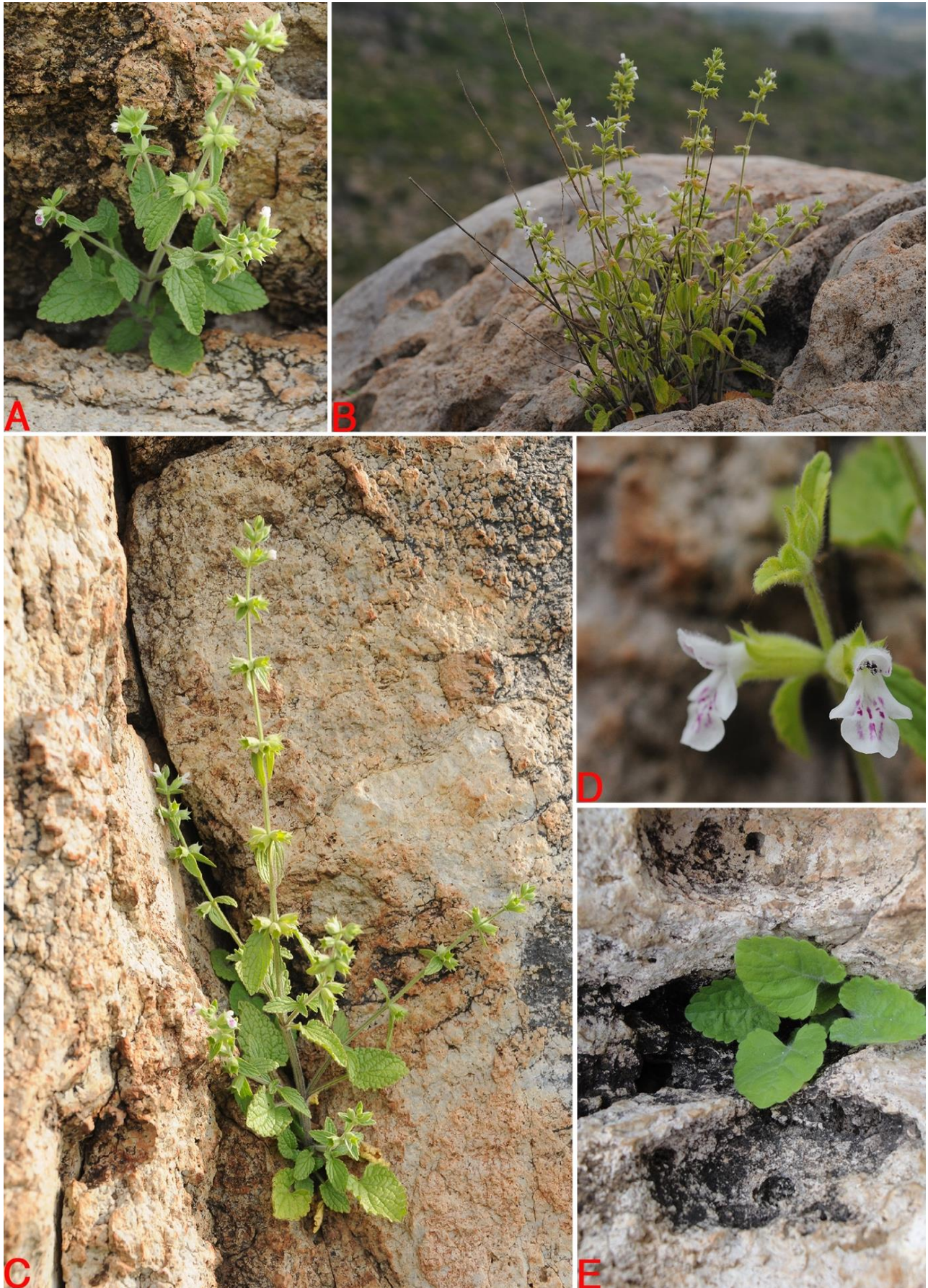


Figure 2. *Stachys cuhacioglu*. A, B and C: Habitus and habitat, D: Flowers, E: Leaf rosette.

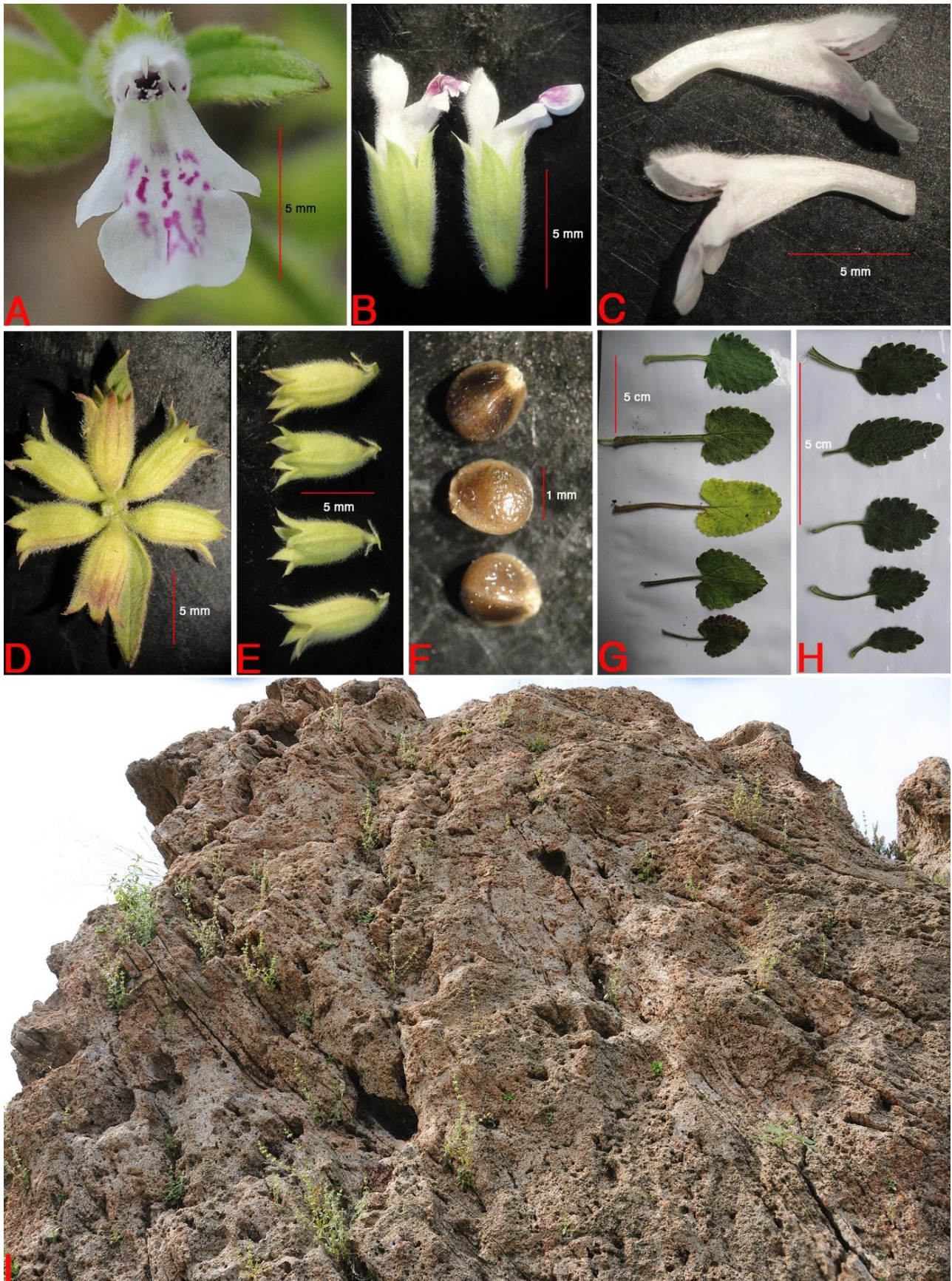


Figure 3. *Stachys cuhacioglu*. A and B: Flowers, C: Corolla, D and E: Calyx, F: Nutlets, G: Basal leaves, H: Cauline leaves, I: Habitat.

Stachys cuhacioglu only grows in volcanic rock crevices at elevations between 143 to 494 m in southwestern Türkiye. One of the most important differences of the new species from *S. annua* is that it lives in rocky habitats. It is an obligate rupicolous plant. Although our detailed research on the type locality of the new species, we could not see it in the soil or in any other habitat. On the other hand, according to previous studies (Bhattacharjee 1982; Akçiçek 2020) and observations, there are no specimens of *S. annua* complex living on rocks.

Stachys cuhacioglu is characterized by having cordate basal leaves, inflorescences with fewer verticillasters and shorter calyx. The new species is similar to *S. annua* (Figure 5) but can be clearly distinguished by some morphological characters (Table 1). These two species have especially significant differences in terms of calyx which is an important character in the discrimination of *Stachys* species (Güner et al. 2021). The new species has regular and campanulate calyx 6–7 mm long and recurved calyx teeth, while *S. annua* has sub-bilabiate and tubular to subcampanulate calyx 13–17 mm long and slightly recurved calyx teeth. Moreover, the new species has shorter corolla with white and 8–9 mm long, and tube subexserted. Based on morphological and micromorphological characters, the new species differs from all other members of the *S. annua* complex.

Table 1. Morphological comparison of *Stachys cuhacioglu* and *S. annua* subsp. *annua*.

Characters	<i>Stachys cuhacioglu</i>	<i>Stachys annua</i> subsp. <i>annua</i>
Life Form	perennial	annual
Habitat and altitude (a.s.l.)	on volcanic crevices, at altitudes of 143–494 m	dry igneous shady slopes, mixed forest, fallow fields; at altitudes of 90–2200 m
Basal leaves	cordate	ovate-oblong to ovate-lanceolate
Cauline leaves	ovate to ovate-lanceolate	ovate-rhomboid, broadly lanceolate to oblanceolate
Verticillasters	usually remote, 0.3–3.5 cm distant, 2–4(–6) flowered	usually remote, 1–4 cm distant, only the uppermost \pm approximate, 4–8 flowered
Bracteoles	linear, 1–2.5 mm long	linear to setaceous, 0.3–4.5 mm long
Calyx	regular, campanulate, 6–7 mm long, densely pilose with sessile glands, teeth \pm equal, triangular, 1.5–3 mm long, slightly recurved	sub-bilabiate, tubular to subcampanulate, 13–17 mm, patently villous or pilose with few sessile glands, teeth subequal, triangular-subulate to lanceolate, 2–5 mm, subpatent or recurved in fruit
Corolla	white, 8–9 mm long, tube shortly subexserted calyx	white or creamy-yellow, 10–17 mm, tube subexserted
Nutlets	obovoid, winged near base, 2.5–3 \times 1.7–2 mm	oblong-obovoid to obovoid, rarely subglobose, 1.5–2.2 \times 0.8–1.6 mm

Micromorphological Results. Pollen grains of the *S. cuhacioglu* are single, isopolar and tricolpate. The mean of a polar axis (P) and equatorial axis (E) is 41.16 $\mu\text{m} \pm 2.21 \mu\text{m}$ and 22.34 $\mu\text{m} \pm 2.55 \mu\text{m}$. The shape of pollen grains is prolate (Figure 4). The outline is elliptic in the

equatorial view. The sculpturing of the exine in *S. cuhacioglu* is micro-reticulate with perforations. Nutlets of *S. cuhacioglu* are oblong-obovoid to oblong-discoid, $1.7\text{--}2.1 \times 1.5\text{--}1.9$ mm, glabrous, reticulate on surface (Figure 4).

The pollen grains of *S. cuhacioglu* and *S. annua* subsp. *annua* are very similar. On the other hand, the seed morphology of the two species is different. Although the seeds of *S. cuhacioglu* are oblong-obovoid to oblong-discoid with reticulate surface, the seeds of *S. annua* L. subsp. *annua* are oblong to oblong-obovoid with reticulate-favolate surface.

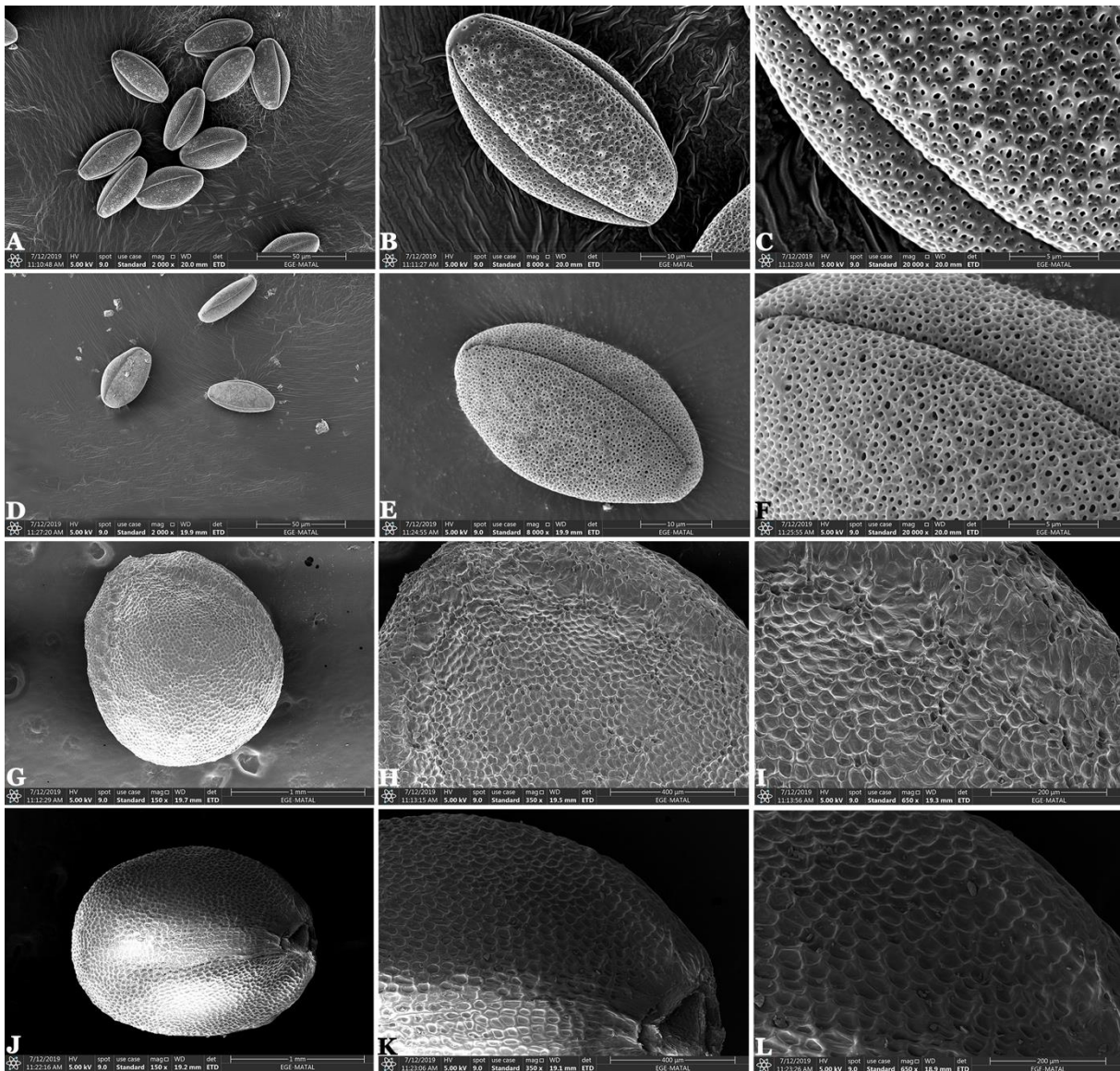


Figure 4. SEM photos of pollen grains. A, B and C: *Stachys cuhacioglu*, D, E and F: *S. annua* subsp. *annua*. SEM photos of nutlet. G, H and I: *S. cuhacioglu*, J, K and L: *S. annua* subsp. *annua*.

Distribution and Conservation status

The occupancy area (AOO) of *Stachys cuhacioglu* was calculated as 8 km^2 , in which about 700 individuals were estimated to occur. Forest fires occur frequently in the area. This situation affects the population of the new species more or less negatively. On the other hand, reforestation works and road works after forest fires have a very negative effect on *S. cuhacioglu*. Thus, according to

the criteria established by the IUCN (2022), *S. cuhacioglu* is hereby assessed as ‘Critically Endangered’ (CR) B2ab (iii,v), on account of its restricted distribution and anthropogenic effects on the population.



Figure 5. *Stachys annua*. A and B: subsp. *annua*, C and D: subsp. *ammophila*, E and F: subsp. *cilicica*.

Specimens Examined

***Stachys annua* subsp. *annua*.** Türkiye. **Hakkari:** Cilo Mountain, 11 km from Kırıkdağ to Cehennemdere, Dizderesi, 1760 m a.s.l., 09.06.2013, *Akçiçek 5561*, *Dirmenci & Ö.Güner* (Hb. Akçiçek!); **Van:** 110 km from Van to Başkale, streamsides, 2150 m a.s.l., 07.06.2013, *Akçiçek 5559*, *Dirmenci & Ö.Güner* (Hb. Akçiçek!); 60 km from Başkale to Yüksekova, stream sides, 1735 m a.s.l., 08.06.2013, *Akçiçek 5553*, *Dirmenci & Ö.Güner* (Hb. Akçiçek!).

***Stachys annua* subsp. *ammophila*.** Türkiye. **Hatay:** Dört Yol, Rabat, serpentine gravel, c. 100 m a.s.l., 25.04.2014, *Akçiçek 5611 & Ö.Güner* (Hb. Akçiçek!); **Antalya:** Aksu to Serik, moist fallow fields, 5 m a.s.l., 06.04.1956, *P.H. Davis 25672 & O.Polunin* (E!, K!).

***Stachys annua* subsp. *cilicica*.** Türkiye. **Hatay:** Antakya, St. Pierre church, rocky slopes, 120 m a.s.l., 27.04.2014, *Akçiçek 5619 & Ö.Güner* (Hb. Akçiçek!).

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AUTHOR CONTRIBUTION STATEMENT

In this study; study idea and design, data collection, analysis and interpretation of results, writing the article draft was done jointly by the authors.

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Research Article

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Glandular and E glandular Trichomes of Endemic *Ballota glandulosissima* (Lamiaceae)

Nezahat Kandemir 

Department of Mathematics and Science Education, Faculty of Education, Amasya University, TR-05100, Amasya, Türkiye

Correspondence: Nezahat Kandemir, nezahatkndmr@gmail.com

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Abstract

The aim of this study is to determine the taxonomic importance of the glandular and e glandular trichomes on the vegetative and reproductive organs of *Ballota glandulosissima*. This species is endemic to Türkiye and the Eastern Mediterranean element. It is among the medicinal plants of Türkiye. On the vegetative and reproductive organs of the studied species, both glandular and e glandular trichomes were seen. These trichomes were examined using light and stereo microscopes. The glandular trichomes were divided into peltate and capitate. The e glandular trichomes were grouped as branched (stellate, bicellular, and multicellular) and unbranched (simple unbranched e glandular trichomes). The stalk cell number of capitate glandular trichomes shows changes. According to the number of stalk cells, capitate glandular trichomes were grouped into uni, bi, tri, and multicellular trichomes. In the taxon, dendroid trichomes were not observed on the vegetative and reproductive organs. The stellate trichomes were dense on the upper and lower surfaces of the leaf, and calyx teeth, while capitate glandular trichomes were dense on the petiole, stem, leaf, and outside part of the calyx, and calyx teeth. Peltate glandular trichomes are dense on the calyx and corolla. Especially, the capitate glandular and stellate trichomes are found to have significant taxonomic characteristics in distinguishing this species.

Keywords: *Ballota glandulosissima*, endemic, trichome morphology

Endemik *Ballota glandulosissima* (Lamiaceae)'nın Salgı ve Örtü Tüyleri

Özet

Bu çalışmanın amacı, *Ballota glandulosissima*'nın vejetatif ve üreme organları üzerindeki örtü ve salgı tüylerinin taksonomik önemini belirlemektir. Bu tür Türkiye'de endemiktir ve Doğu Akdeniz elementidir. Türkiye'nin tıbbi bitkileri arasında yer almaktadır. İncelenen türün vejetatif ve üreme organları üzerinde hem örtü hemde salgı tüyleri görüldü. Bu tüyler ışık ve stero mikroskoplar kullanılarak incelendi. Salgı tüyleri peltat ve kapitat olarak ayrıldı. Örtü tüyleri dallanmış (yıldızsı, iki hücreli ve çok hücreli) ve dallanmamış (basit dallanmamış örtü tüyleri) olarak gruplandırıldı. Kapitat salgı tüylerinin sap hücre sayıları değişiklik gösterir. Sap hücre sayılarına göre kapitat salgı tüyleri bir, iki, üç ve çok hücreli tüyler olarak gruplandırıldı. Taksonda, dendroid tüyler vejetatif ve üreme organları üzerinde gözlenemedi. Kapitat salgı tüyleri gövde, petiyol, yaprak ve kaliksin dış kısmında ve kaliks dişlerinde yoğunken, yıldızsı tüyler ise yaprağın alt ve üst yüzeyinde ve kaliks dişleri üzerinde yoğundur. Peltat salgı tüyleri kaliks ve korolla üzerinde yoğundur. Özellikle yıldızsı ve kapitat salgı tüylerinin bu türün ayırt edilmesinde önemli taksonomik karakterlere sahip olduğu tespit edilmiştir.

Anahtar kelimeler: *Ballota glandulosissima*, endemik, tüy morfolojisi

Suggested Citation:

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INTRODUCTION

Ballota L. genus (Lamiaceae) is represented by 35 species in temperate and subtropical regions of the world and 12 species and 8 subspecies in Türkiye. Nine of these species are endemic to Türkiye, and the rate of endemism is quite high. This genus has high diversity in the Mediterranean Basin (Davis & Doroszenko 1982; Güner 2012; Morteza-Semnani & Ghanbarimasir 2019).

The fresh leaves, fruits, and seeds of *Ballota* species are very well known by the public. These species are frequently used for the treatment of wounds, burns, cough, insomnia, upper respiratory inflammation, diuretic, headache, nausea, eye infections, and hemorrhoids among people in Turkish folk medicine (Yeşilada et al. 1995; Baytop 1999; Dülger & Dülger 2012; 2017). Since *Ballota* species contain terpenoids, flavonoids, essential oils, saponins, tannins, and phenylpropanoids, these species have antiulcer, anticancer, antispasmodic, cholerectic, antihemorrhoidal, antioxidant, antibacterial, antiviral, antilisterial, diuretic, anti-inflammatory, anti-diabetic, sedative and astringent properties (Baytop 1999; Özbek et al. 2004; Saltan Çitoğlu et al. 2004; 2005; Sever Yılmaz et al. 2015; Riccobono et al. 2016; Rigano et al. 2017; Doğan & Duman 2019; Ağca et al. 2021). It has been reported that *B. glandulosissima* Hub.-Mor. & Patzak has strong antioxidant activity due to its high flavonoid content (Saltan Çitoğlu et al. 2004; Sever Yılmaz et al. 2015).

In many species of the Lamiaceae family, both glandular and eglandular trichomes were observed. Trichomes are used as significant taxonomic characters in distinguishing species and genera in the Lamiaceae family (Kandemir 2011; El-Deen Osman 2012; Seyedi & Salmaki 2015; Giuliani et al. 2021; Koçak & Kandemir 2023a; 2023b). Glandular trichomes are specialized secretory structures that vary in size, form, location and function in different plant species. These trichomes are generally separated into capitate and peltate (Werker et al. 1985; Navarro & El Oualidi 2000). The capitate trichomes have great importance in the systematic of the Lamiaceae family since these trichomes play an important role in the pollinization of family species (Kandemir 2011; Navarro & El Oualidi 2000). Generally, this type of trichomes has a longer stalk and a smaller head. However, peltate glandular trichomes have a short stalk or no stalk and a large secretory head which consists of 4–18 cells arranged in one or two concentric circles (Werker et al. 1985). Eglandular trichomes are also more common in the Lamiaceae family, and these trichomes were divided into four types: simple unicellular, simple multicellular, branched unicellular, and branched multicellular (Cantino 1990). Stellate trichomes are characteristic of some genera in the Lamiaceae family, such as *Marrubium* and *Ballota*.

It was aimed to determine taxonomic significance of eglandular and glandular trichomes on the vegetative and reproductive organs of *Ballota glandulosissima*. The other aim of this study is to separate the taxa *B. glandulosissima* and *B. saxatilis* Sieber ex C.Presl, which are very similar to each other according to their trichome micromorphology.

MATERIAL AND METHOD

The plant samples were collected from their habitat during the flowering period. The plant samples were described according to Davis and Doroszenko (1982). For the light microscopy observations (LM), some fresh plant samples were fixed in 70% alcohol solution for trichome investigations. The cross and surface sections of stem, leaf, petiole, calyx, and corolla were taken by hand using razor blades. Then preparations were made using Sartur reagent (Çelebioğlu & Baytop 1949). For the stereo microscopy observations, surface sections were taken from fresh plant and alcohol materials, and preparations were made. Preparations were photographed with a binocular light microscope,

with an equipped camera and 40X objectives (Leica ICC50 HD). The general classification of trichomes was done according to Cantino (1990) and Navarro & El-Qualidi (2000). The localities where the taxon was collected were listed below:

C3 Antalya: Finike Turuçoova-Elmalı road, machine areas, 175 m., 12.7.2021, N.Kandemir

C3 Antalya: Finike-Elmalı road, Arif Village, rocky areas, 700 m., 13.7.2021, N.Kandemir

C3 Antalya: Finike inner city, road to Finike Castle, 15 m., 13. 7.2021, N.Kandemir

RESULTS AND DISCUSSION

Morphology and localization of eglandular trichomes

Eglandular trichomes are common in the Lamiaceae family, where they may be unbranched and branched. On the vegetative and reproductive organs of this taxon, the two main different types of eglandular trichomes were seen: unbranched (simple unbranched eglandular trichomes) and branched (stellate, bicellular, and multicellular trichomes). In this study, simple unbranched trichomes were divided into four forms based on the shape and cell number: unicellular, bicellular, tricellular, and multicellular. While unbranched multicellular trichomes were rarely obtained on the stem, petiole, leaf, fruit, outside part of the calyx, calyx teeth, and corolla of this species, unbranched uni, bi and tricellular trichomes were densely obtained on the stem, petiole, calyx and corolla of *B. glandulosissima* (Figure 1A-D, Figure 2A-B and D, Figure 3A and 3E). There are unbranched unicellular trichomes between the vessels of the calyx (Figure 3A-E). These kinds of eglandular trichomes were reported in *B. kaiseri* Tachk., *B. undulata* (Sieber ex Fresen.) Benth., *B. saxatilis* and *B. pseudodictamnus* (L.) Benth (El-Deen Osman 2012) and on the surfaces of both vegetative and generative organs of *B. acetabulosa* (L.) Benth. (Giuliani et al. 2021).

In this species, three types of branched eglandular trichomes were recognized. The branched eglandular trichomes are bicellular, multicellular, and stellate trichomes. On the upper and lower leaves and the calyx teeth, multicellular and bicellular branched eglandular trichomes were obtained. These trichomes were reported in *B. kaiseri*, *B. undulata* and *B. saxatilis* (El-Deen Osman 2012).

Stellate trichomes are star-shaped and multicellular (Xiang et al., 2010). *Ballota* taxa were characterized by stellate trichomes on the upper and lower surfaces of leaves. El-Deen Osman (2012) reported the presence of stellate trichomes in *Ballota undulata*, *B. kaiseri* and *B. saxatilis*. These trichomes are densely seen on upper and lower surfaces, between the veins of the leaves, outside part of the calyx, and calyx teeth of this taxon (Figure 2C, Figure 3F and 3H). Especially, this type of trichomes is highly dense on the calyx and between the veins of the leaves of the studied taxon. The short stellate trichomes are densely seen only in the ridge parts of corolla's upper lip. However, long stellate trichomes were rare and seen only in the upper lip of the corolla. On the petiole and stem of the studied taxon, this type of trichome is not observed (Table 2). Stellate trichomes were reported in other *Ballota* taxa (*B. kaiseri*, *B. undulata* and *B. saxatilis*) (El-Deen Osman 2012). Generally, eglandular ones are mainly situated on the leaf and side of the calyx of the *B. glandulosissima*. In the studied taxon, stellate type of eglandular trichomes is abundance observed on the adaxial and abaxial surfaces of the leaf (Figure 2C).

In the studied taxon, dendroid trichomes were not seen on vegetative and reproductive organs. At the same time, there were no dendroid trichomes on vegetative and generative organs of *Ballota* taxa (excluding *B. acetabulosa*, *B. pseudodictamnus* (L.) Benth. ssp. *lycia* Hub-Mor and *B. cristata* P.H.Davis) distributed in Türkiye (Tezcan 2001). Also, dendroid trichomes were not observed in

some *Ballota* species (*B. kaiseri*, *B. saxatilis*, *B. undulata*) distributed in Egypt. But these trichomes were observed in *B. pseudodictamnus* and *B. damascens* growing in Egypt (El-Deen Osman 2012) and on the calyx and the corolla abaxial side of *B. acetabulosa* (Giuliani et al. 2021). The presence of both dendroid and stellate trichomes is of great importance in the separation of *Ballota* taxa (El-Deen Osman 2012). El-Deen Osman (2012) distinguished some *Ballota* taxa (*B. kaiseri*, *B. saxatilis*, *B. undulata* and *B. pseudodictamnus*) distributed in Egypt according to the presence or absence of dendroid and stellate trichomes.

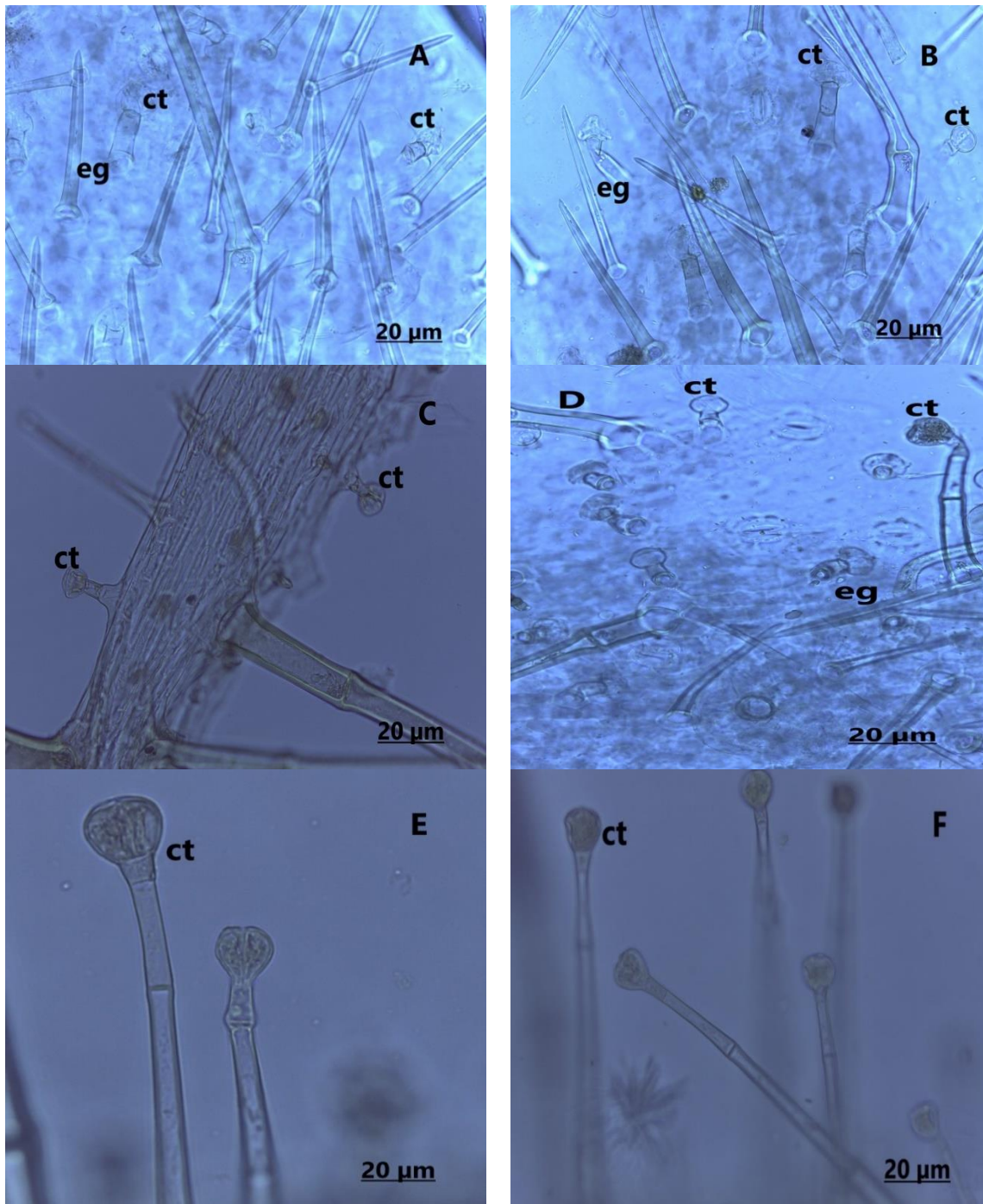


Figure 1. Continues.

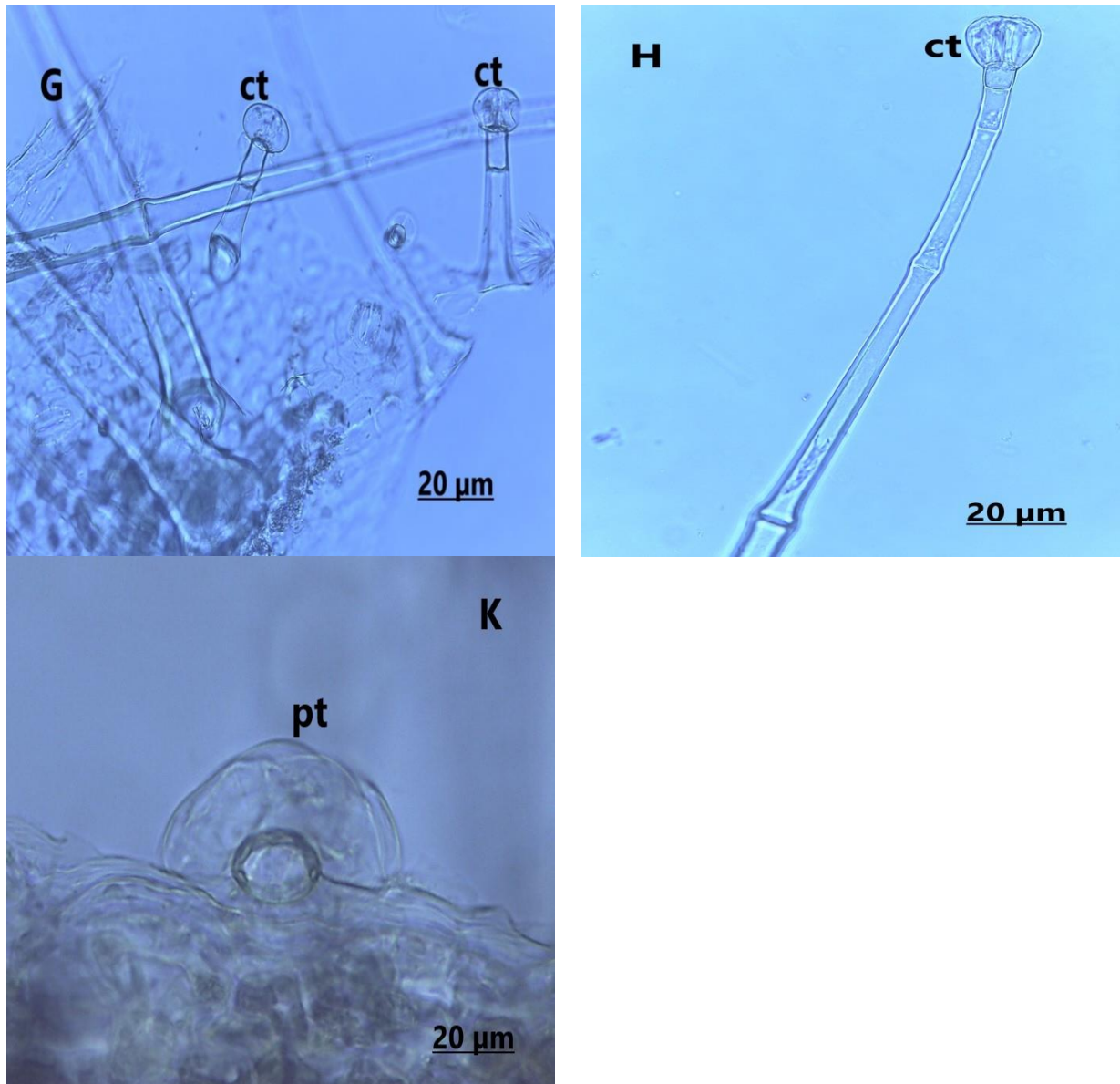


Figure 1. Glandular and eglandular trichome types on the stem. **A:** Unicellular eglandular and unicellular stalked with unicellular head capitate trichomes, **B:** Unicellular and bicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes, **C:** Multicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes, **D:** Bicellular eglandular and unicellular, tricellular stalked with unicellular, bicellular head capitate trichomes, **E:** Bicellular, tricellular stalked with unicellular, bicellular head capitate trichomes, **F:** Multicellular stalked with unicellular, bicellular head capitate trichomes, **G:** Bicellular stalked with bicellular head capitate trichomes, **H:** Multicellular stalked with bicellular head capitate trichomes, **K:** Peltate trichomes. **eg.** eglandular trichomes, **ct.** capitate trichomes, **pt.** peltate trichomes.

Morphology and localization of capitate glandular trichomes

Capitate glandular trichomes contain significant taxonomic characters since these trichomes play a significant role in the pollination of the Lamiaceae species (Navarro & El Oualidi 2000). These trichomes often vary in size, structure, abundance, and distribution among taxa. The capitate trichomes were detected abundantly on the vegetative and reproductive organs of *B.*

glandulosissima (Figure 1A-H, Figure 2A-B and 2D-H, Figure 3A-E and 3G-L). Specially, these trichomes were abundant on the stem, petiole, leaf, and calyx. In this study, capitate glandular trichomes were divided into four subtypes, which are unicellular stalked (subtype I), bicellular stalked (subtype II), tricellular stalked (subtype III), and multicellular stalked (subtype IV) stalked (Table 1). The number of head cells in the capitate trichomes can vary.

Unicellular stalked capitate glandular trichomes (Subtype I): The unicellular stalked capitate trichomes have a stalk cell and uni, bi, tri, and multicellular head. In this study, unicellular stalked capitate trichomes are uni and bicellular headed. The first form has a unicellular head and is present on the stem, petiol, leaf, calyx, and corolla of *B. glandulosissima* (Figures 1A-D, Figure 2A-B and 2D, Figure 3B, 3D-E). These trichomes are dense and present on the petiole and stem of this species. The second form has a bicellular head and is abundantly observed on the stem, petiole, and calyx of *B. glandulosissima* (Figure 1A-D; Figure 2A-B, Figure 3B and 3E). This type is rather rare on the leaf of *B. glandulosissima* (Figure 3D). The third form presents tricellular head and is seen on the stem, and petiole of *B. glandulosissima* (Figure 1B). The fourth form possesses a multicellular head, which is rarely observed on the stem of this species. The unicellular stalked capitate trichomes were found in *B. kaiseri*, *B. undulata*, *B. damascens* Boiss., *B. saxatilis*, *B. pseudodictamnus* (El-Deen Osman 2012). Moreover, these trichomes were detected on the vein system of the whole plant, especially on the leaf and corolla abaxial sides of *B. acetabulosa* (Giuliani et al. 2021). The unicellular stalked capitate trichomes were quite widespread in all Lamiaceae taxa (Giuliani & Maleci Bini 2008).

Bicellular stalked capitate trichomes (Subtype II): In this type of trichomes, stalk is two celled and the head has one, two and three cells. Unicellular, bicellular headed capitate trichomes are dense and observed on the stems, petiole, leaf, calyx, and calyx teeth of this species (Figure 1C, 1F-G, Figure 2A-B, 2D and 2H, Figure 3A-D and 3K). Tricellular headed capitate trichomes were obtained on the petiole of *B. glandulosissima*. Bicellular stalked capitate trichomes were observed in some *Ballota* taxa (*B. kaiseri*, *B. undulata*, *B. damascens*, *B. saxatilis*, and *B. pseudodictamnus*) (El-Deen Osman 2012) and on leaves and the calyx abaxial side of *B. acetabulosa* (Giuliani et al. 2021). These kinds of capitate trichomes were found in some members of the genera *Salvia*, *Stachys*, *Sideritis*, and *Scutellaria* (Giuliani & Maleci Bini 2008; Giuliani et al. 2020a; 2020b).

Tricellular stalked capitate trichomes (Subtype III): Subtype III capitate trichomes, which have three celled stalk and unicellular or bicellular head, are observed on the petiole, stem, calyx surface, calyx teeth, and leaf of *B. glandulosissima* (Figure 1D-F, Figure 2E-G, Figure 3A, 3D, 3H and 3L). In particular, these capitate trichomes are densely obtained on the calyx teeth, leaf margins, and leaf veins (Figure 3D and 3L). The kind of trichomes was reported in *B. kaiseri*, *B. undulata*, *B. damascens*, *B. saxatilis*, and *B. pseudodictamnus* (El-Deen Osman 2012).

Multicellular stalked capitate trichomes (Subtype IV): This type of trichomes has stalk multicellular celled (four, five, and six celled). The head part of this trichome is one or two celled. Multicellular stalked (subtype IV) capitate trichomes are densely seen on the stem, petiole, calyx teeth, and margin and veins of the leaf of *B. glandulosissima* (Figures 1F and 1H, Figure 2E-H, Figure 3D and 3G). But these trichomes were rarely found on the leaf lamina and corolla. This kind

of trichomes is found with stellate trichomes on the leaves of *B. glandulosissima*. Multicellular stalked capitate trichomes were recorded in only *B. saxatilis* and *B. undulata* (El-Deen Osman 2012). Also, these trichomes were obtained on the leaf adaxial side, on the sepal abaxial side and on floral peduncle *B. acetabulosa* (Giuliani et al. 2021) and on the reproductive organs of other Lamiaceae taxa (Giuliani & Maleci Bini 2008).

Morphology and localization peltate glandular trichomes

Peltate trichomes are also known as labiate type trichomes. In this study, peltate trichomes were scarcely seen on the stem, petiole and leaf while peltate trichomes were densely seen on the calyx and corolla (Figure 1K, Figure 2H and 2K, Figure 3M). These trichomes in the lower surface of the leaf of *B. glandulosissima* have 8 cells while these trichomes in the stem of *B. glandulosissima* have 4 celled. In the Lamiaceae family, the presence of peltate glandular trichomes was noted by some researchers (Metcalf & Chalk 1950; Cantino 1990; Werker et al. 1985; Salmaki et al. 2009; Kandemir 2011; Koçak & Kandemir 2023a; 2023b). Peltate trichomes were detected in *B. undulata*, *B. saxatilis*, and *B. kaiseri* (El-Deen Osman 2012). Eight-celled peltate trichomes were scarce on the leaves and abundant on calyx and corolla abaxial surfaces of *Scutellaria brevibracteata* subsp. *subvelutina* (Rech. f.) Greuter & Burdet (Giuliani et al. 2020a) and *B. acetabulosa* (L.) Benth (Giuliani et al. 2021).

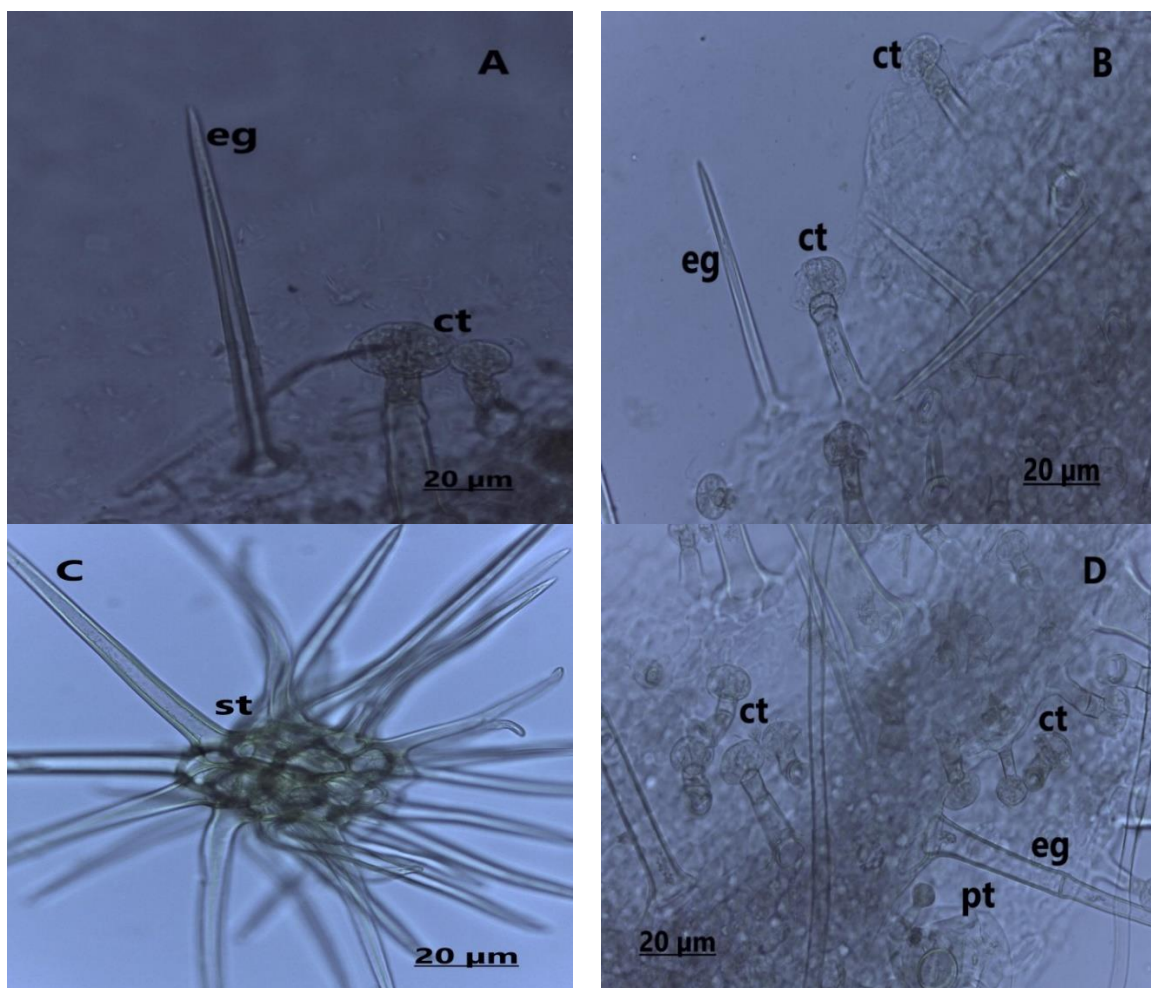


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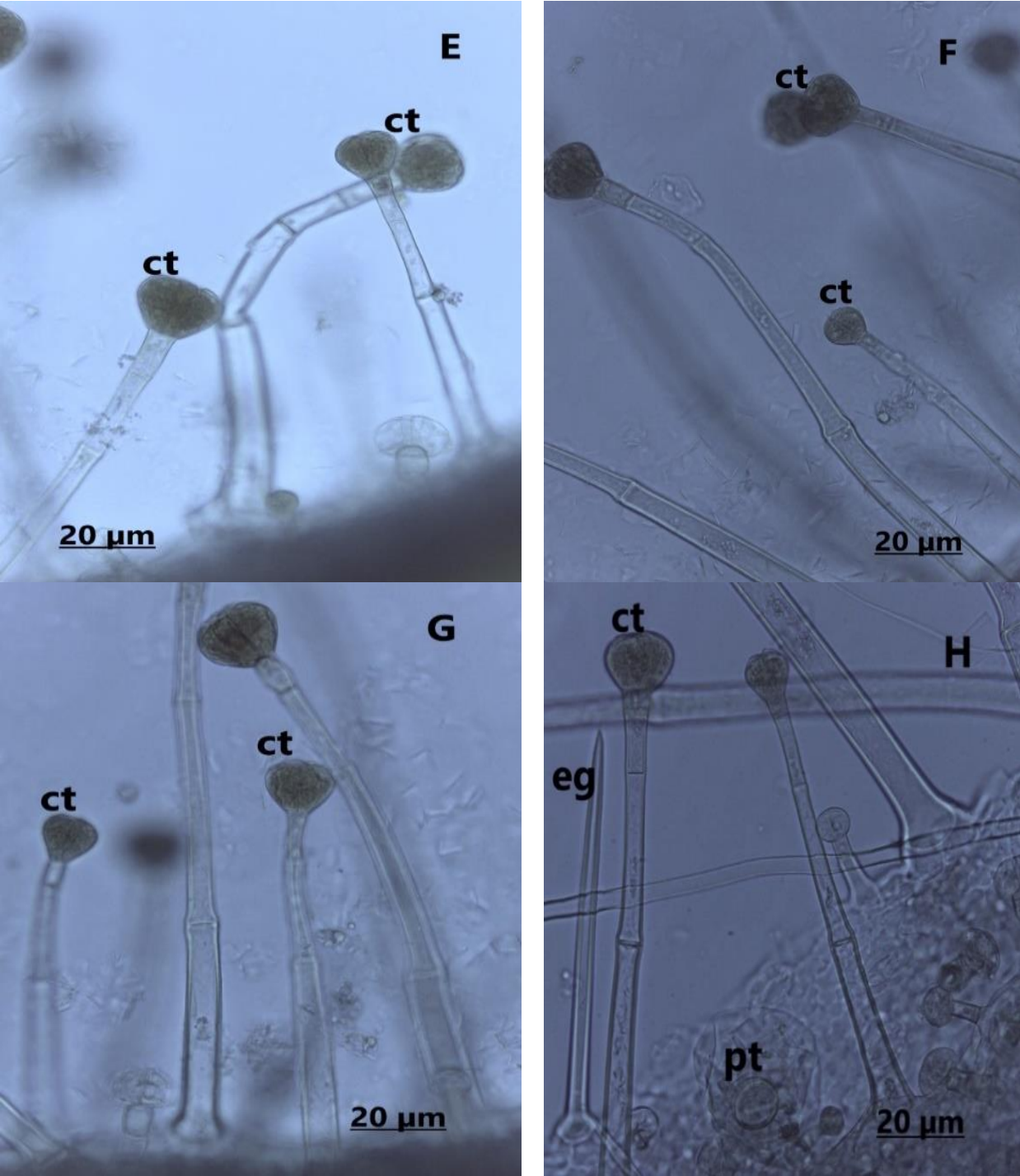


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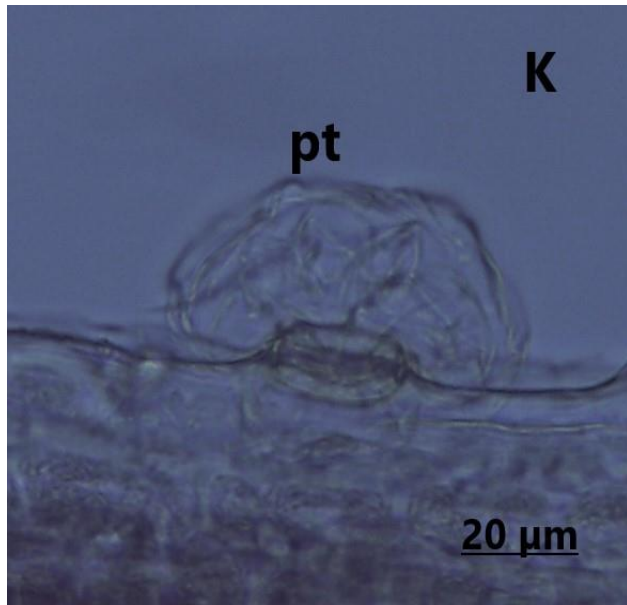


Figure 2. Glandular and eglandular trichome types on the petiole and leaves. **A:** Unicellular eglandular and unicellular long stalked with bicellular head capitate trichomes on the petiole, **B:** Unicellular eglandular and unicellular, bicellular stalked with unicellular head capitate trichomes on the petiole, **C:** Stellate trichomes on the leaf, **D:** Bicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes on the leaf, **E:** Multicellular stalked with bicellular head capitate trichomes on the petiole, **F:** Multicellular stalked with bicellular head capitate trichomes on the leaf, **G:** Tricellular stalked with bicellular head capitate trichomes on the leaf, **H:** Bicellular, tricellular, multicellular stalked with bicellular head capitate, peltate and unicellular trichomes on the leaf, **K:** Peltate trichomes on the petiole. **eg.** eglandular trichomes, **st.** stellate trichomes, **ct.** capitate trichomes, **pt.** peltate trichomes.

Trichome types on the vegetative and reproductive organs of the studied taxon are given in Tables 1 and 2.

Table 1. Glandular trichomes types and distribution on vegetative and reproductive organs of *Ballota glandulosissima*.

Organs of <i>Ballota glandulosissima</i>	Subtype I capitate	Subtype II capitate	Subtype III capitate	Subtype IV capitate	Peltate trichomes
Stem	+++	–	+	++	+
Petiole	++++	+	+	++	+
Margin and veins of leaf	++	++	++	+++	+
Lamina of leaf	++	++	+	+	+
Calyx teeth	++	–	++	+++	+
Outer surface of calyx	++	–	+	++	++
Corolla	++	–	–	+	++

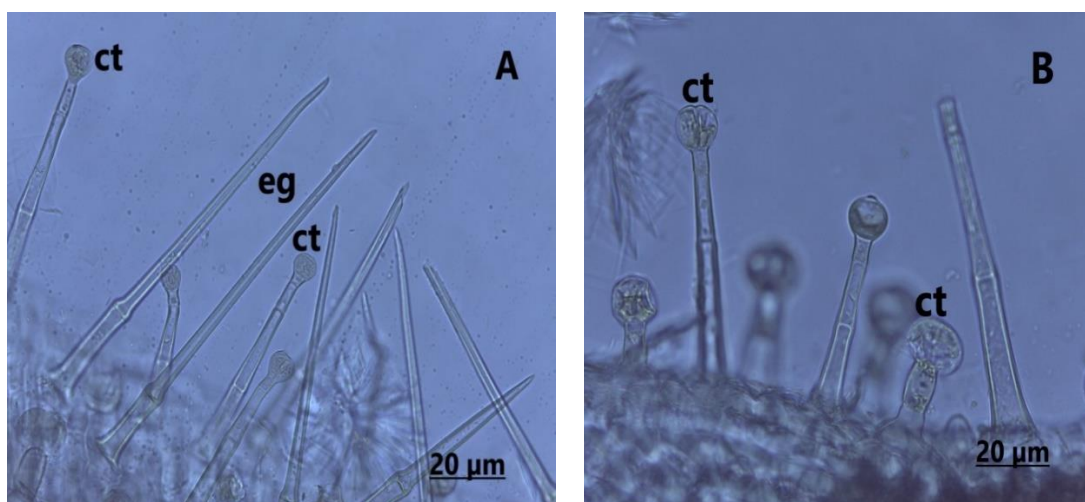
–: absent; +: scarce; ++: dense; +++: abundant

Table 2. Eglanular trichomes types and distribution on vegetative and reproductive organs of *Ballota glandulosissima*.

Organs of <i>Ballota glandulosissima</i>	Unbranched unicellular	Unbranched bicellular	Unbranched tricellular	Unbranched multicellular	Branched bicellular	Branched multi cellular	Stellate trichomes	Dendroid trichomes
Stem	++	++	++	+	-	-	-	-
Petiole	++	++	++	+	-	-	-	-
Margin and veins of leaf	-	-	-	-	+	+	+++	-
Lamina of leaf	-	-	-	+	+	+	+++	-
Calyx teeth	++	++	++	+	+	+	+++	-
Outer surface of calyx	++	++	++	+	-	-	+++	-
Corolla	++	++	++	+	-	-	++	-

In conclusion, on the vegetative and reproductive organs of the studied taxon, four main trichome types (peltate glandular, capitate glandular, unbranched and branched eglandular) were seen. Peltate glandular trichomes were rare on the vegetative and reproductive organs of the studied taxon, while capitate glandular trichomes (especially on the stem, petiole, leaf, and calyx surface, calyx teeth) and stellate trichomes (especially on the lower and upper surfaces of leaves, calyx and corolla) were dense on the vegetative and reproductive organs of studied taxon.

Trichome micromorphology provides significant data distinguishing sections and species. Our findings revealed that the density, type and distribution of trichomes provide important characters in distinguishing *B. glandulosissima* and *B. saxatilis*, which are morphologically very similar to each other. Glandular trichomes are similar in almost all *Ballota* species. However, glandular trichomes were denser in *B. glandulosissima* than in other *Ballota* species. The stellate trichomes are denser in *B. saxatilis* than in *B. glandulosissima*. On the other hand, stellate trichomes were not found in the petiole of *B. glandulosissima*, while stellate trichomes were found in the petiole of *B. saxatilis* (El-Deen Osman 2012). *B. glandulosissima* can be distinguished from *B. saxatilis* by the trichomes features mentioned above.

**Figure 3.** continues.

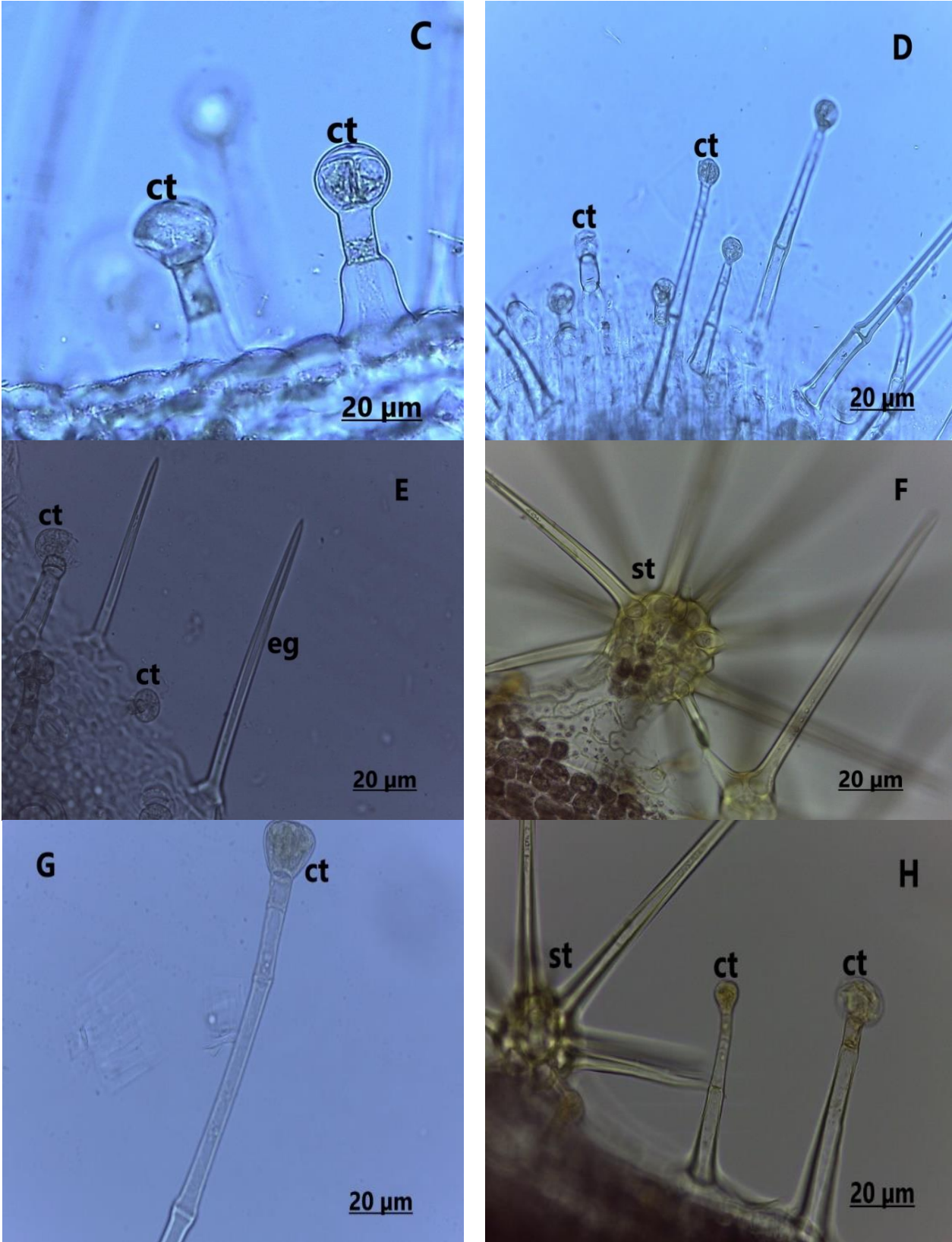


Figure 3. continues.

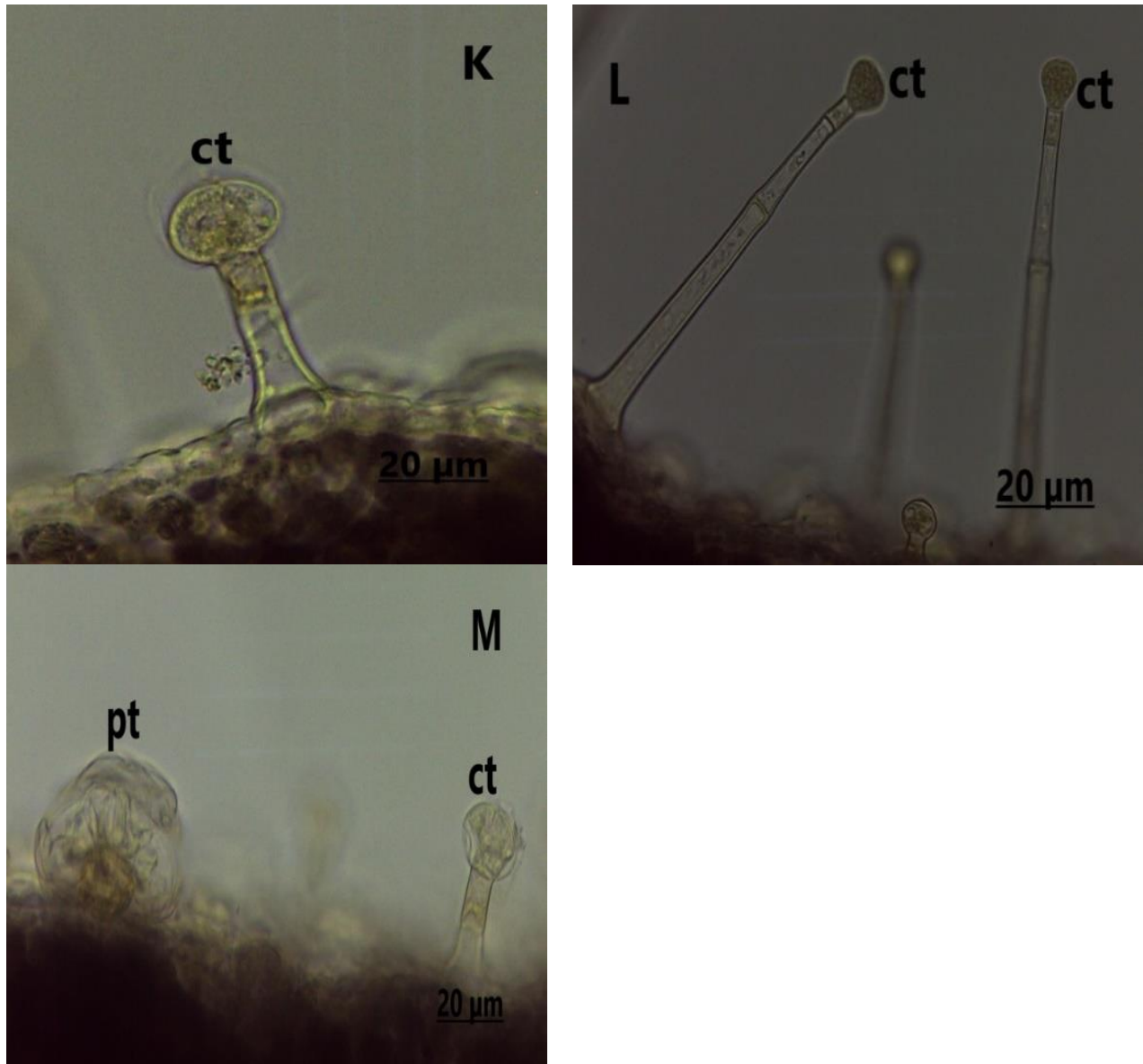


Figure 3. Glandular and eglandular trichome types on the calyx. **A:** Unicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes, **B:** Unicellular and bicellular stalked with unicellular, bicellular head capitate trichomes, **C:** Unicellular, bicellular stalked with uni,bicellular head capitate trichomes, **D:** Unicellular, bicellular, tricellular stalked with uni, bicellular head capitate trichomes, **E:** Unicellular eglandular and unicellular, bicellular stalked with unicellular head capitate trichomes, **F:** Stellate trichomes, **G:** Multicellular stalked with bicellular head capitate trichomes, **H:** Bicellular, tricellular stalked with unicellular head capitate, stellate trichomes, **K:** Bicellular stalked with bicellular head capitate, **L:** Unicellular, tricellular stalked with unicellular, bicellular head capitate trichomes, **M:** Peltate and unicellular stalked with unicellular head capitate trichomes. **eg.** eglandular trichomes, **st.** stellate trichomes, **ct.** capitate trichomes, **pt.** peltate trichomes.

AUTHOR CONTRIBUTION STATEMENT

In this study; the study idea and design, data collection, analysis and interpretation of the results, and drafting of the article were made by Nezahat Kandemir.

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Araştırma Makalesi

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Türkiye’den Yeni Bir *Cirsium* (Asteraceae) Hibriti, *Cirsium* × *giresunicum*

Veysel Uzun ¹, Tuncay Dirmenci ^{2,*}, Taner Özcan ²

Turan Arabacı ³, Bayram Yıldız ⁴

¹Biyoloji Bölümü, Fen Edebiyat Fakültesi, Balıkesir Üniversitesi, TR-10145, Balıkesir, Türkiye

²Matematik ve Fen Bilimleri Bölümü, Necatibey Eğitim Fakültesi, Balıkesir Üniversitesi, TR-10100, Balıkesir, Türkiye

³Eczacılık Meslek Bilimleri Bölümü, Eczacılık Fakültesi, İnönü Üniversitesi, TR-44210, Malatya, Türkiye

⁴İsmail Cem Sokak, Yenikale Mahallesi, TR-35320, Narlıdere, İzmir, Türkiye

*Yazışmadan sorumlu yazar: Tuncay Dirmenci, dirmenci@balikesir.edu.tr

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Özet

Bu çalışmada *Cirsium* cinsine ait yeni bir hibrit olan *Cirsium* × *giresunicum* tanımlanmaktadır. Cinsin *Epitrachys* seksiyonunda yer alan *C. poluninii* ve *C. trachylepis* türleri arasında oluşan bu hibrit, morfolojik ve moleküler açıdan analiz edilmiştir. Morfolojik incelemelerde yeni hibritin bazı özelliklerini ebeveynleri ile paylaştığı, bazı özelliklerinin iki ebeveynin özellikleri arasında geçiş formunda olduğu, bazı özelliklerinin ise ebeveynlerin özelliklerinden farklılık gösterdiği tespit edilmiştir. Hibrit bireyler, yaşam formu, gövde boyu, gövde rengi gibi özellikler ile *C. poluninii* türüne benzerken, dış fillarilerinin dikencikli ve orta fillarilerinin geriye kıvrık olması gibi özellikleri ile de *C. trachylepis*'e benzemektedir. Moleküler analizler kapsamında çekirdek genomuna ait ITS bölgesi kullanılmıştır. Moleküler sonuçlar, *Cirsium* × *giresunicum*'un nükleer ITS genomlarında bazı polimorfik nükleotid bölgelerine sahip olduğunu ve iki atasının arasında bir konumda olduğunu göstermektedir. Bu konumlanma da, aslında hibrit bireylerin moleküler açıdan da ara karakterlere sahip olduğunu göstermektedir.

Anahtar kelimeler: Asteraceae, *Cirsium*, Filogeni, Hibrit, Türkiye

A New *Cirsium* (Asteraceae) Hybrid from Türkiye, *Cirsium* × *giresunicum*

Abstract

In this study, a new hybrid of the *Cirsium* genus, *Cirsium* × *giresunicum*, is described. This hybrid formed between *C. poluninii* and *C. trachylepis* in the *Epitrachys* section was analyzed morphologically and molecularly. In morphological examinations, it was observed that the new hybrid showed transitional characters between its parents. While the hybrid individuals resemble *C. poluninii* species with features such as life form, stem length and color, they also resemble *C. trachylepis* species with features such as the outer phyllaries being spiny and the middle phyllaries being curved backward. Within the scope of molecular analyses, the ITS region of the nuclear genome was used. Molecular results show that *Cirsium* × *giresunicum* has some polymorphic nucleotide regions in its nuclear ITS genomes appears to be positioned between its two parents. This indicates that hybrid individuals also possess intermediate molecular characteristics.

Keywords: Asteraceae, *Cirsium*, Phylogeny, Hybrid, Türkiye

GİRİŞ

Cirsium Mill. cinsi hem Türkiye hem de Dünya genelinde Asteraceae familyasının en fazla türe sahip cinslerinden biridir. Cinsin başta Kuzey Amerika, Avrupa, Kuzey Afrika, Doğu, Orta ve Güneybatı

Önerilen Alıntı:

Uzun, V., Dirmenci, T., Özcan, T., Arabacı, T. & Yıldız, B. (2023). Türkiye’den Yeni Bir *Cirsium* (Asteraceae) Hibriti, *Cirsium* × *giresunicum*. *Türler ve Habitatlar* 4(2): 125–138.

Asya'da olmak üzere yaklaşık 300 türü bulunmaktadır (Kadereit & Jeffrey 2007, POWO 2023). Türkiye ve Türkiye ile bağlantılı Avrupa ve Kafkasya bölgesi tür sayısının en fazla olduğu bölgelerin başında gelmektedir (Charadze 1963; Davis & Parris 1975; Werner 1976; Yıldız 2012; Dirmenci vd. 2020). Türkiye, bulunduğu konum itibarıyla sahip olduğu yüksek biyolojik çeşitliliğini *Cirsium* cinsi için de göstermekte olup dünya genelinde cinsin en fazla türüne sahip ülkelerin başında gelmektedir. Cinsin, Türkiye'de 34'ü endemik olmak üzere toplam 81 taksonu (69 tür) yayılış göstermektedir. (Yıldız 2012; Duman vd. 2017; Dirmenci vd. 2019; 2020). Bu taksonlar üç seksiyon altında sınıflandırılmış olup seksiyonlara göre dağılım şöyledir: *Epitrachys* DC. 51 tür, (53 takson), *Cirsium* 17 tür, (27 takson) ve *Cephalonoplos* (Neck.) DC. (1 tür) (Davis & Parris 1975; Dirmenci vd. 2019; Dirmenci vd. 2020).

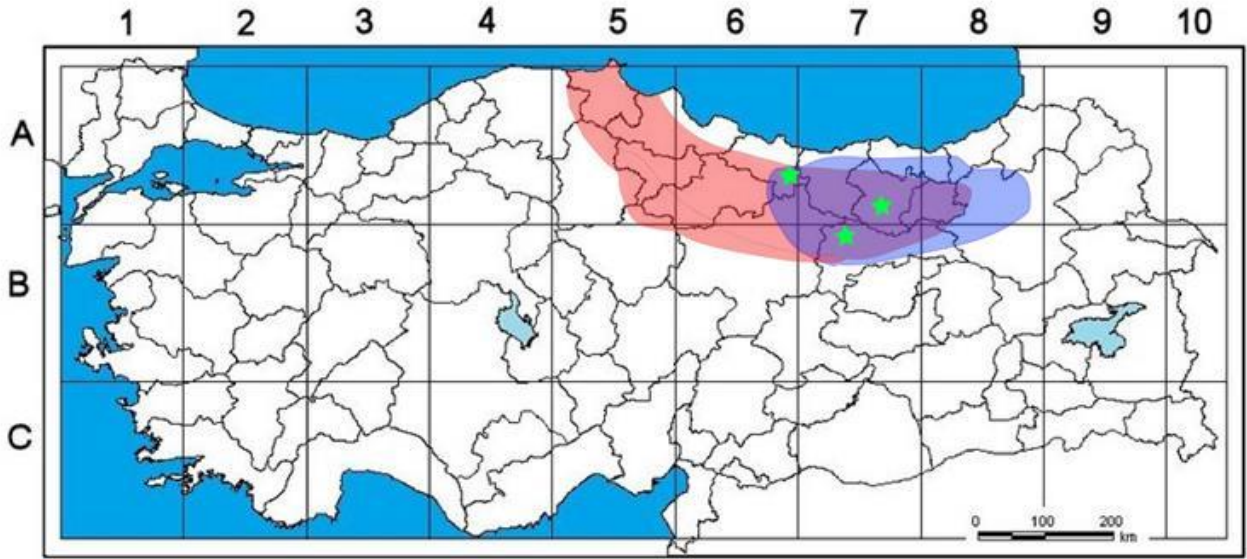
Cirsium cinsinin çeşitli floralarda hibritleri kayıtlı olsa da, *Flora of Turkey and the East Aegean Islands* adlı eserde herhangi bir hibriti kaydedilmemiştir. Ancak, sadece ebeveynleri verilen muhtemel iki (*Cirsium adjaricum* Sommier & Levier × *C. trachylepis* Boiss. ve *C. caucasicum* Petr. × *C. trachylepis*) ve bir ebeveyni bilinmeyen bir hibritten (*C. caucasicum* × *C. sp. ?*) bahsedilmiştir (Davis & Parris 1975). Türkiye'deki *Cirsium* hibritleri ile ilgili ilk çalışmalar ve tespitler Yıldız vd. (2016) tarafından yapılan çalışma ile ortaya konulmuştur. Bu çalışmada (Yıldız vd. 2016), Türkiye *Cirsium* seksiyonu için iki hibrit, *Cirsium* × *pratigagum* Petr. ve *C. woronowii* Petr., kaydı verilmiştir. Daha sonra ise *Epitrachys* seksiyonu türleri arasında meydana gelen 3 yeni hibrit, *Cirsium* × *erzincanicum* Yıldız, Dirmenci & Arabacı, *C. × kelkitense* Yıldız, Arabacı & Dirmenci ve *C. × nezaketiae* Yıldız, Dirmenci & Arabacı, tanımlanmıştır (Dirmenci vd. 2019). Bu çalışmalar sonucunda, Türkiye'den şimdiye kadar toplam 5 hibrit kaydı verilmiştir. Ancak, dünya genelinde cins için şimdiye kadar yaklaşık 100 hibrit tanımlandığı düşünülürse (POWO 2023), Türkiye'deki tür sayısına göre hibrit sayısının daha fazla olması beklenebilir. Nitekim *Cirsium* cinsi revizyonu esnasında ve daha sonrasında yapılan saha çalışmalarında hibrit sayısının daha fazla olabileceğini destekleyen morfolojik bulgular gözlenmiştir. Gözlemler sonucu, özellikle *Epitrachys* seksiyonu türleri arasında hibritleşmenin daha sık meydana geldiği sonucuna varılmıştır. Saha çalışmaları esnasında hibritlere ait toplanan örneklerin incelenmesi ile Türkiye'deki *Cirsium* cinsi türleri arasında meydana gelen hibritlerin tanımlanmasına Dirmenci vd. (2019)'nin çalışmasındaki üç hibritin tanımlanması ile başlanmış olup, bu çalışmada da 2006-2020 yılları arasında yapılan saha çalışmaları sırasında toplanan ve *Epitrachys* seksiyonunda yer alan *Cirsium poluninii* P.H. Davis & Parris ile *C. trachylepis* türleri arasında meydana gelen yeni bir hibritin tanıtımı yapılmaktadır.

MATERYAL VE METOT

Morfolojik Çalışmalar

Bu çalışmada kullanılan bitkisel materyaller 2006–2020 yılları arasında Türkiye *Cirsium* cinsinin revizyonu sırasında ve sonrasında yapılan saha çalışmaları neticesinde Türkiye'nin çeşitli illerinden toplanmıştır (Şekil 1). Taksonların fotoğrafları doğal yaşam alanlarında çekilmiştir (Şekil 2–3). Türlerin tanımlanmasında *Flora of Turkey and the East Aegean Islands* (Davis & Parris 1975) başta olmak üzere, bölge floraları ile *Cirsium* cinsi üzerinde yapılan diğer çalışmalar kullanılmıştır (Charadze 1963; Werner 1976; Petrak 1979; Bures vd. 2004; 2018; 2023; Yıldız vd. 2009, 2016; Dirmenci vd. 2019). Ayrıca, saha çalışmalarında toplanan örnekler Türkiye'nin ve Avrupa'nın önemli herbaryumlarında (ANK, B, BM, E, FI, GAZI, K, LD, LE, W, WU) bulunan örnekler ile karşılaştırılmıştır. Morfolojik ölçümler Nikon SMZ-745T stereo mikroskop altında milimetrik cetvel

ile yapılmıştır. Yeni hibritin betimi yapılmış ve ebeveynleri ile benzerlik ve farklılıkları tablo şeklinde düzenlenerek karşılaştırılmıştır (Tablo 1).



Şekil 1. *Cirsium* × *giresunicum* (★), *C. trachylepis* (■) ve *C. poluninii* (■) taksonlarının yayılışı.

Moleküler Çalışmalar

Son yıllarda yapılan çalışmalar göstermiştir ki, hibritlerin belirlenmesinde moleküler düzeyde yapılan analizler büyük önem taşımaktadır. Bu çalışmadaki moleküler çalışmalar 3 ana basamak şeklinde gerçekleştirilmiştir. Bu basamaklar aşağıda sırayla verilmiştir.

DNA Ekstraksiyonu. Moleküler çalışmalar için ebeveyn ve olası hibritlerin doğal habitatlarından toplanmış ve küflenme belirtisi olmayan örnekleri seçildi. Ekstraksiyon çalışmaları için *Cirsium poluninii* türünden 7 örnek, *C. trachylepis* türünden ise 4 örnek kullanıldı. Atalara ait olan bu bireylere ek olarak, hibrit olduğu düşünülen ve bu çalışmada ilk kez tanımlanan *C. × giresunicum* taksonundan da 5 adet bireyden DNA ekstraksiyonu gerçekleştirildi. Arazi çalışmaları esnasında toplanan yukarıda bahsi geçen ve Tablo 2’de “*” sembolü ile 16 adet bireyin DNA ekstraksiyonları DNeasy Plant Mini Kit (QIAGEN, Germany) kullanılarak ve üreticinin talimatlarına uyularak gerçekleştirildi. Sadece inkübasyon süreci kullanıcı talimatlarından 10 dakika fazla uygulandı.

Polimeraz Zincir Reaksiyonu Çalışmaları. DNA ekstraksiyonu sonrasında polimeraz zincir reaksiyonu (PZR) için nrDNA ITS (Internal Transcribed Spacer) bölgesi kullanıldı. ITS bölgesinin amplifikasyonu için ITS5a (5’-CCT TAT CAT TTA GAG GAA GGA G-3’) (Stanford vd. 2000) ve ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’) (White vd. 1990) primerleri kullanıldı ve Shaw vd. (2007) tarafından önerilen PZR protokolü uygulandı. PZR çalışmaları üç basamak şeklinde gerçekleştirildi: Denatürasyon aşaması 94°C’de 1 dakika, bağlanma aşaması 52–54°C’de 30 saniye, uzama aşaması ise 72°C’de 40 saniye olarak 30 tekrar olacak şekilde gerçekleştirildi.

DNA dizilenmesi ve işlenmesi. Elde edilen PZR ürünleri, dizilemeye gönderilmeden önce %0,8’lik agaroz içeren jel elektroforezine tabi tutularak bantların oluşup oluşmadığı (yani PZR uzamasının

gerçekleşip gerçekleşmediği) kontrol edildi. Uygun bantlar gözlemlendikten sonra, elde edilen PZR örnekleri dizileme işlemleri için Genoks firmasına (Genetik Hastalıklar Değerlendirme Merkezi, Ankara, Türkiye) gönderildi. Ham DNA dizileri BioEdit (Hall 1999) programı kullanılarak incelendi ve 5' ve 3' uçlarından kirliliğe neden olan primer dizileri kesilerek çıkartıldı. Tüm elde edilen dizilerden tek tek kontigler (ortak karar dizileri) elde edildikten sonra tüm kontigler Clustal W (Thompson vd. 1994) kullanılarak alt alta hizalandı.

Filogenetik Analizler

Bir önceki aşamada yapılan hizalama işleminden sonra iki farklı analiz yapıldı. Birinci analiz için dış gruplarla beraber 47 takson çalışıldı (Tablo 2'de verilen ve gen bankasından alınan örnekler) ve toplamda 616 karakter elde edildi. İkinci analiz için ise hibritler çalışılmış fakat ataları çalışmaya dahil edilmedi. Bu analiz için ise yine dış gruplarla beraber 17 takson çalışılıp yine 616 karakter analiz edildi. Elde edilen bu dizi verileri Bayesian çıkarımı (BI) ve maksimum olasılık (ML) algoritmalarına göre analiz edildi. İki farklı analiz için de veriler online olarak işlem gören CIPRES (Miller vd. 2010) üzerinde değerlendirildi. Bayesian algoritması için filogenetik analizler CIPRES içerisindeki MrBayes on XSEDE programı ve maksimum olasılık analizleri için de RaxML-NG programları kullanıldı. MrBayes analizleri 5 milyon generasyon boyunca 1000 nesilde bir ağaç üretecek ve 4 kez koşturma olacak şekilde programlanmış olup bu analizler sırasında en uzak dış grup olarak da *Tyrimnus leucographus* Cass. (AY826343) seçildi. Yine RaxML analizleri için aynı dış grup seçilerek bootstrap döngüsü 1 milyon döngü olarak seçildi. Yapılan analizlere göre elde edilen filogenetik ağaçlar şekil 4 ve 5'te verilmiştir.

SONUÇLAR

Morfolojik Sonuçlar

Cirsium × giresunicum Yıldız, Arabacı & Dirmenci, **nothosp. nov.** (Şekil 2–3).

(*Cirsium poluninii* Davis & Parris × *C. trachylepis* Boiss.)

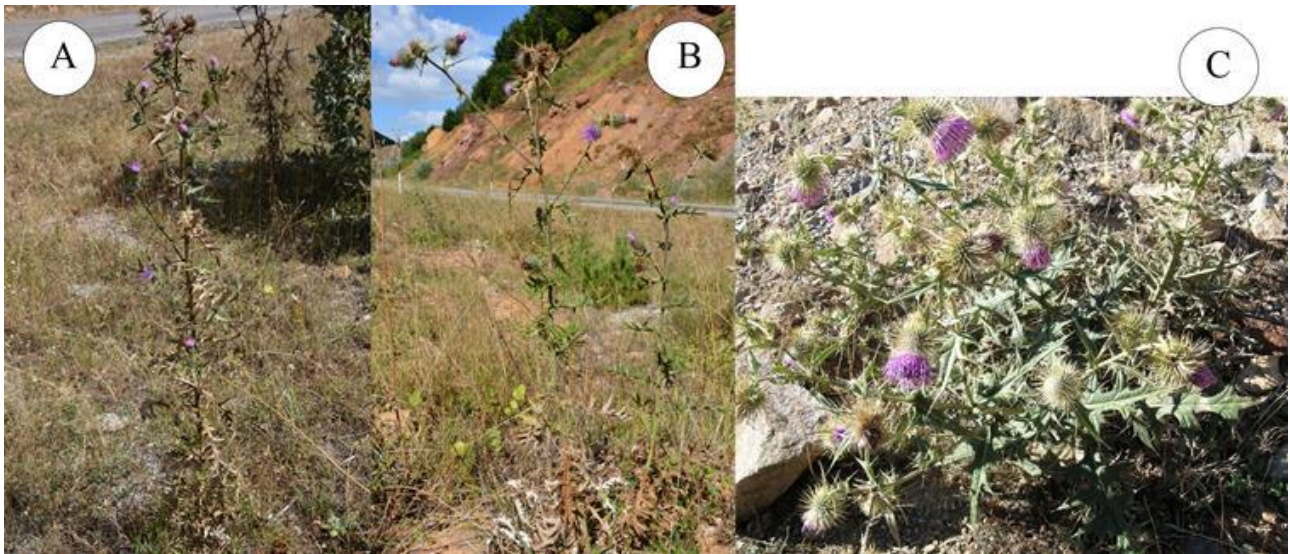
Tip. Türkiye. **Giresun:** Şebinkarahisar-Giresun arası 33. km, Eğribel geçidi kuzeyi, 1780 m a.s.l., alpinik çayırliklar, 06.08.2017, *Yıldız, Dirmenci 4841 & Arabacı (holotip: GAZI!; izotip: ANK!)*.

Paratip. Türkiye. **Sivas:** Hafik-Doğanşar arası, yol ayrımından 42–44. km, *P. sylvestrys* L. ormanı yakını yol kenarları, 1580 m a.s.l., 05.09.2016, *Yıldız, Dirmenci 4702 & Arabacı* (Hb. Dirmenci!); aynı yer, 05.08.2017, *Yıldız, Dirmenci 4830 & Arabacı* (Hb. Dirmenci!); aynı yer, *Yıldız 16399* (Hb. Dirmenci!); **Giresun:** Şebinkarahisar-Giresun arası 33. km, Eğribel geçidi kuzeyi, 1780 m a.s.l., alpinik çayırliklar, 03.09.2016, *Yıldız & Dirmenci 4666* (Hb. Dirmenci!).

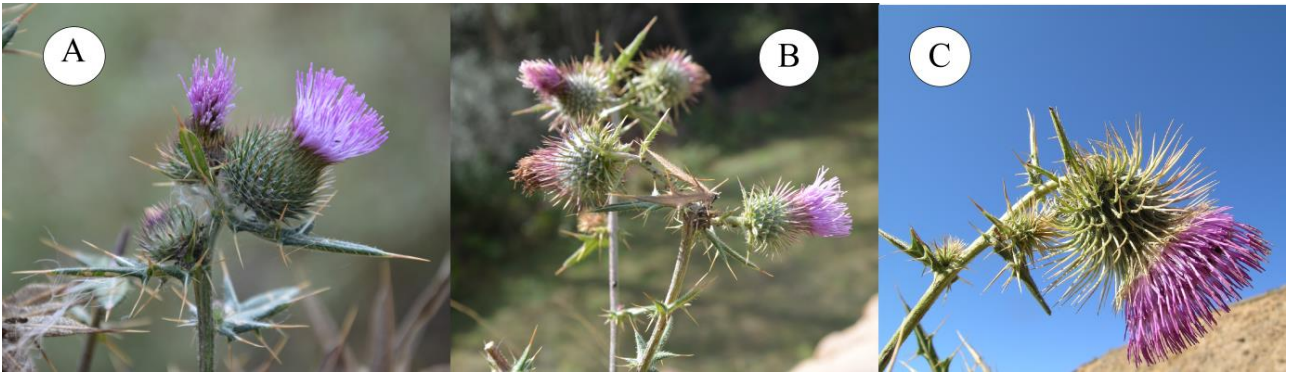
Diagnosis. *Cirsium × giresunicum* is similar to its parents, *C. poluninii* and *C. trachylepis*. It can be distinguished from *C. poluninii* as follows: its capitula nodding (not erect), its median stem leaves pinnatifid to pinnatisect (not pinnatisect), phyllaries are sparsely to densely arachnoid (not sparsely arachnoid), margins of outer phyllaries' spine are 1–3 mm long (not absent or shorter than 1 mm), median phyllaries are 15–20 mm long and recurved (not 8–15 mm and erect). It differs from *C. trachylepis* in its life form is biennial (not perennial), main stem is single and purple (not many from base and green), length of median phyllaries are 15–20 with c. 5 mm apical spine (not 25–30 mm with 5–13 mm apical spine), length of corolla and pappus are 10–15 mm and 10–15 mm long, respectively (not 25–30 mm long and 15–22 mm long, respectively) (Figure 2A–C and 3A–C).

Diyagnoz. *Cirsium* × *giresunicum*, ebeveynleri olan *C. poluninii* ve *C. trachylepis* ile bazı benzer özelliklere sahiptir. Ancak, onlardan farklı özellikleri de bulunmaktadır. *Cirsium poluninii*'den kapitulularının sarkık (dik değil), orta gövde yapraklarının az teleksiden derin teleksiye kadar parçalanması (derin teleksi değil), fillarilerinin seyrek ila yoğun tüylü olması (seyrek değil), dış fillarilerinin kenarlarının 1–3 mm uzunluğunda dikencikli (dikencik yok ya da varsa 1 mm'den kısa değil), orta fillarilerinin 15–20 mm uzunluğunda (8–15 mm uzunluğunda değil) olması ile ayırt edilir. *C. trachylepis*'ten ise, yaşam formunun iki yıllık (çok yıllık değil), ana gövdesinin tek ve morumsu renkli (tabandan çok sayıda ve yeşil değil), orta fillari uzunluğunun 5 mm uç dikenini dahil 15–20 mm (5–13 mm uç dikenini dahil 25–30 mm uzunluğunda değil), korolla uzunluğu 10–15 mm (25–20 mm uzunluğunda değil) ve pappus uzunluğunun 10–15 mm (15–22 mm uzunluğunda değil) olması ile ayrılır.

Betitleme. İki yıllık. Gövde 100–150 cm boyunda, tek ve yukarıda dallanmış, kırmızımsı, kanatsız, çizgili, seyrek örümcekağı tüylü. Yapraklar tabandan yukarı doğru azalan ölçülerde, üst yüzeyde 2 mm² deki seta sayısı 5'ten fazla, tüsüz, alt yüzeyde yoğun örümcekağı tüylü. Taban yaprakları 30–37 × 8–12 cm ölçülerde, derin teleksi parçalı, loblar iki parçalı ya da üç parçalı, şekli üçgensimzraksıdan mızraksıya kadar, uç dikenini 8–15 mm uzunluğunda, diken ince; alt gövde yaprakları yaklaşık 30 × 8 cm ölçülerde, derin teleksi parçalı, şekli dikdörtgensel; orta gövde yaprakları 17–22 × 10–13 cm ölçülerde, derin teleksi parçalı, şekli dikdörtgensel, alt yüzü azdan yoğuna kadar örümcekağı tüylü; üst gövde yaprakları 6–15 × 1–11 cm ölçülerde, az teleksiden derin teleksiye kadar parçalı, şekli dikdörtgenselden mızraksıya kadar, alt yüzü azdan yoğuna kadar örümcekağı tüylü. İnvolutrum yaprakları 3–5 adet, 3–6 cm uzunluğunda (1 cm uzunluğunda uç dikenini dahil), kapituluma eşit ya da daha uzun, şekli mızraksıdan şeritsiye kadar. Kapitulum belirgin veya hafif şekilde sarkık, ters küremsi, 2–3 × 1.5–2.5 cm. Fillariler 6–8 seri, yoğun örümcekağı tüylü, dıştakiler 12–17 mm uzunluğunda, 4 mm uzunluğunda uç dikenini dahil, şekli yumurtamsıdan mızraksıya kadar, kenar dikenini yok ya da 1–4 mm uzunluğunda dikencikli; ortadakiler 16–20 mm uzunluğunda, 5 mm uzunluğunda uç dikenini dahil, şekli mızraksı, hafifçe geriye kıvrık, seyrek ila yoğun örümcekağı; içtekiler 18–22 mm uzunluğunda, şekli şeritsi-mızraksı. Korolla mor, 10–15 mm uzunluğunda. Stamen 8–9 mm uzunluğunda, filament tüylü. Pappus 10–15 mm uzunluğunda. Aken bilinmiyor.



Şekil 2. Habitat ve genel görünüş. A: *Cirsium poluninii*, B: *C. × giresunicum*, C: *C. trachylepis*.



Şekil 3. Kapitulanın gövde üzerinde duruşu: A: *Cirsium poluninii*, B: *C. × giresunicum*, C: *C. trachylepis*.

Etimoloji. Hibrit ismi, hibritin yayılış alanı göz önüne alınarak “Giresun” iline ithaf edilmiştir.

Habitat ve Ekoloji. *Cirsium × giresunicum*, Giresun ve Sivas illerinden tespit edilmiştir. Giresun ilinde Şebinkarahisar-Tamdere arasında yer alan Eğribel geçidinin kuzey kısmında orman katı üzerinde 1800 m a.s.l. civarında, Sivas ilinde ise Hafik-Doğanşar ilçeleri arasında yaklaşık 1550–1600 m a.s.l. arasında yol kenarlarında ve *Pinus sylvestris* (sarıçam) ormanı yakınlarında yayılış göstermektedir.

Moleküler Sonuçlar

Bu çalışmada morfolojik verilerin desteğiyle ilk kez tanımlanan *C. × giresunicum* hibriti ve ebeveynleri (*C. poluninii* ve *C. trachylepis*) moleküler yaklaşımlar da kullanılarak incelenmiştir. Çalışmada 5 farklı *C. × giresunicum*, 7 farklı *C. poluninii* ve 4 farklı *C. trachylepis* örneği ilk kez çalışılarak analiz edilmiştir. Ayrıca, bir adet *C. trachylepis* dizisi de (MN918966) GenBank (N.C.B.I.)’tan temin edilmiştir. Dış grup olarak kullanılan türler ve daha iyi bir karşılaştırma için kullanılan diğer tür ve hibritler GenBank’ tan (N.C.B.I) alınmış olup, erişim numaraları ile verilmiştir. (Bkz. Ekler). Ayrıca bu çalışmada elde edilen ve düzenlenen çekirdek genomunun ITS bölgesine ait diziler GenBank’a yüklenmiştir.

Cirsium cinsinin farklı türleri ve hibritleri hakkında yapılan moleküler çalışmalara göre ITS bölgesinin türlerin ayrılmasında ve tanımlanmasında açıklayıcı bilgiler verdiği gösterilmiştir (Kelch & Baldwin 2003; Slotta vd. 2012; Dirmenci vd. 2019). Bu çalışmada da nrITS bölgesinin dizilenmesinden sonra 616 nükleotid uzunluğunda dizi elde edilmiş olup, ebeveyn ve hibritler arasındaki polimorfik bölgeler tablo 2’de verilmiştir. Bu verilere göre *C. × giresunicum* hibritine ait incelenen tüm bireylerde 429, 543, 547, 564 ve 571. nükleotidlerde polimorfik bölgeler olduğu tespit edilmiştir. Ayrıca TD4666, TD4830 ve TD4841 numaralı örnekler 605. bazda polimorfizm göstermiştir. Yine tablo 2’den görüleceği üzere ebeveyn türlerden bazılarının da farklı bazlarda polimorfik bölgeleri olduğu gösterilmiştir. Bilhassa, *C. trachylepis* atasına ait olan örneklerde bu polimorfik bölgeler daha dikkat çekicidir. Ek olarak, gen bankasından alınan *C. trachylepis* (MN918966) örneği ile bu çalışmada kullanılan atalardan olan TD4662 ve TD4840 kodlu *C. trachylepis* örneklerinde görülen polimorfik bölgeler benzer konumdadır.

Elde edilen filogenetik ağaç, yukarıda da bahsedildiği gibi Bayesian çıkarımı ve maksimum olasılık algoritmalarına göre yapılmıştır. İki farklı algoritma analizi ile elde edilen ağaçlara ait bayesian “posterior probability” (P.P.) değerleri ile maksimum olasılık “bootstrap” (B.S.) değerleri

tek bir ağaç üzerinde Şekil 4 ve 5'te gösterilmiştir. Dış gruplar ve hibritlerin atalarının da dahil edildiği 47 takson kullanılarak yapılan filogenetik analize göre 616 karakterin 517'si sabit, 45 karakter parsimonik açıdan bilgilendirici değilken, 54 karakter ise parsimonik açıdan bilgi verici olarak bulunmuştur. Türler ve hibritler arası ilişkiyi daha iyi açıklayabilmek adına iki farklı ağaç elde edilmiştir. 1'inci ağaçta (Şekil 4) dış gruplar ve hibritleri ile ataları verilmiştir. Bu ağaca göre, bu çalışmada tanımlanan *C. × giresunicum* iki atası ile ortak bir kladı paylaşmaktadır ve moleküler açıdan iki atasının arasında bir form göstermektedir. Ayrıca, 1'inci ağaca (Şekil 4) daha önce Dirmenci vd. (2019) tarafından tanımlanan ve moleküler açıdan nrITS dizilerinin özellikleri ortaya koyulan ve *Epitrachys* seksiyonuna ait olan üç farklı hibrit (*C. × kelkitense*, *C. × erzincanicum*, *C. × nezaketiae*) katılarak hibritlerin ve atalarının konumlanmaları daha net ortaya koyulmuştur. Buna göre, daha önce tanımlanan (Dirmenci vd. 2019) üç hibrit ve ataları bu çalışmada tanımlanan *C. × giresunicum* ve atalarından farklı bir moleküler doğaya sahiptir. Şekil 5'te yer alan diğer filogenetik ağaçta ise sadece hibritler ile dış gruplar ve *Cirsium* seksiyonuna ait bazı taksonlar kullanılmıştır. Bu verilere göre daha önce Dirmenci vd. (2019) tarafından tanımlanan hibritler bir grupta yer alırken, *C. × giresunicum* hibritleri ayrı bir grupta yer almıştır. Dış grup olan kullanılan türlerde ise *Tyrimnus* Cass. en dışta yer alırken *Carduus* L. üyeleri *Cirsium* türlerine daha yakın konumlanmıştır.

Tablo 1. Taksonların morfolojik olarak karşılaştırılması.

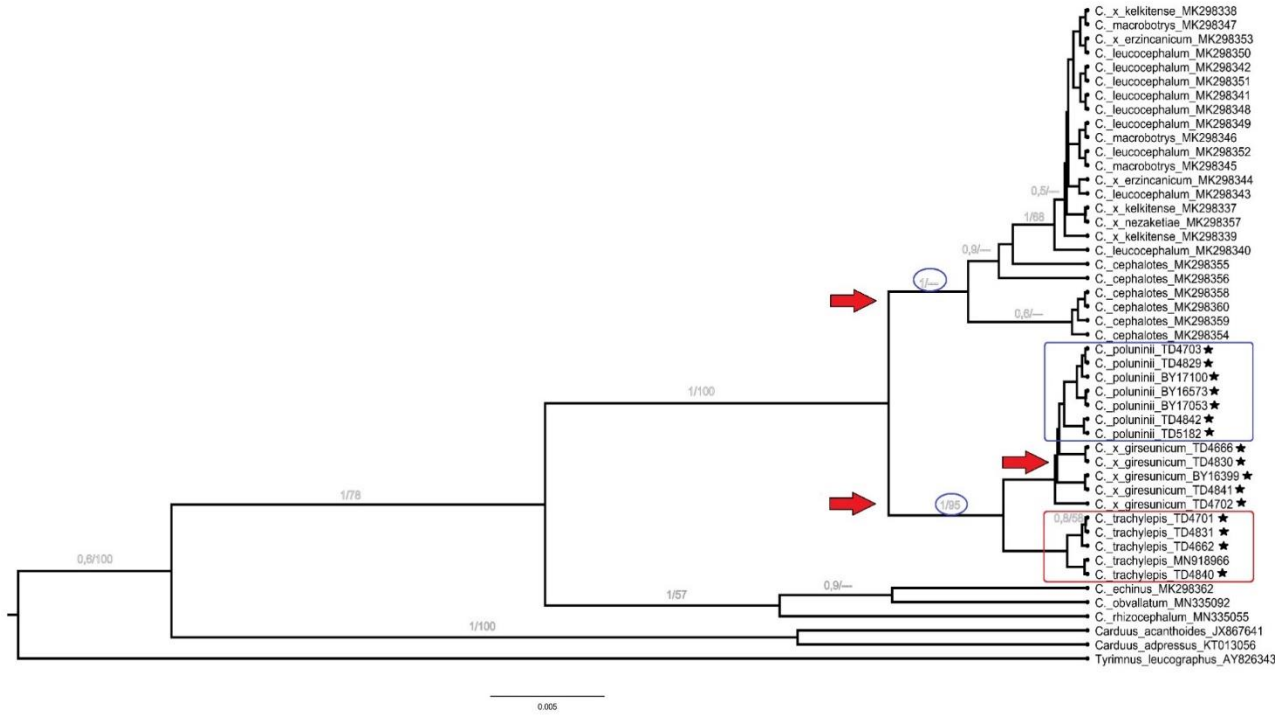
Karakterler	<i>C. poluninii</i>	<i>C. × giresunicum</i>	<i>C. trachylepis</i>
Yaşam formu	iki yıllık	iki yıllık	çok yıllık
Gövde özellikleri	tek, dik ve yukarıda dallanmış, morumsu renkli	tek, dik yukarıda dallanmış, morumsu renkli	tabanda çok gövdeli, yukarıda dallanmış, yeşil renkli
Orta gövde yaprak parçalanması	derin teleksi	az teleksiden derin teleksiye	az teleksiden derin teleksiye
İnvolutral yaprak özellikleri	3–4 adet, involukruma eşit veya daha uzun	3–5 adet, involukruma eşit veya daha uzun	2–10 adet, involukrumdan kısa, eşit veya nadiren daha uzun
Kapitulum özellikleri	saplı, dik, basık yumurtamsıdan ters küremsiye kadar, 15–25 × 15–30 mm	saplı, sarkık, ters küremsi, 20–30 × 15–30 mm	saplı, sarkık, basık küremsiden geniş ters yumurtamsıya, 25–35 × 30–35 mm,
Fillari tüylenmesi	seyrek örümcekağı tüylü	yoğun örümcekağı tüylü	seyrek ila yoğun örümcekağı tüylü
Dış fillari özellikleri	fillari kenarlarında diken yok ya da varsa 1 mm'den kısa	fillari kenarları 1–3 mm uzunluğunda dikencikli	fillari kenarları 1–5 mm uzunluğunda dikencikli
Orta fillari özellikleri	8–15 mm uzunluğunda ve 0.5–1.5 mm	15–20 mm uzunluğunda ve 5 mm uzunluğunda uç dikeni	25–30 mm uzunluğunda ve 5–13 mm uzunluğunda

	uzunluğunda uç dikenli dahil, dik	dahil, hafifçe geriye kıvrık	uç dikenli dahil, geriye kıvrık
Korolla uzunluğu	16–25 mm uzunluğunda	10–15 mm uzunluğunda	25–30 mm uzunluğunda
Pappus uzunluğu	13.5–20 mm uzunluğunda	10–15 mm uzunluğunda	15–22 mm uzunluğunda

Şekil 4'te verilen çekirdek genomundan ITS bölgesinin dizileri kullanılarak elde edilen filogenetik ağaçta görüldüğü üzere, her ne kadar *Cirsium* cinsinin üyeleri hariç dış grup olarak çok fazla örnek kullanılmamış olsa bile (*Carduus* ve *Tyrimnus* üyeleri) genel anlamda *Cirsium* cinsinin daha dar anlamda ise *Epitrachys* ve *Cirsium* seksiyonu üyelerinin kendi içlerinde monofiletik olduğu görülmektedir. Bu çalışmada analiz edilen ve *Epitrachys* seksiyonuna ait olan taksonların *Cirsium* seksiyonuna ait olan taksonlardan (*C. echinus* Hand. -Mazz., *C. obvallatum* M. Bieb. ve *C. rhizocephalum* C.A.Mey.) 1/100 (PP/BS) değerleri ile ayrıldığı görülmektedir. Diğer yandan, *Epitrachys* seksiyonu üyeleri de kendi içinde iki dala ayrılmıştır (bkz. sol taraftaki alt alta olan kırmızı oklar). Bu dallardan bir tanesi *C. cephalotes* Boiss., *C. leucocephalum* (Willd.) Spreng., *C. macrobotrys* (K. Koch) Boiss. ve bunların hibritlerini içermektedir. Bu dal kendi içinde incelendiğinde, *C. cephalotes* taksonun diğer gruplardan daha ayrı konumlandığı görülmektedir. Bu durum daha önce de Dirmenci vd. (2019) tarafından da gösterilmiştir. Bu açıdan bakıldığında, güncel çalışmamızda elde edilen verilerin bu ayrımı bozmadığı da net bir şekilde görülmektedir.

Tablo 2. *Cirsium × giresunicum* ve ebeveynlerinin polimorfik bölgeleri.

Taksonlar	Nükleotid						
	429	543	547	564	571	599	605
<i>Cirsium poluninii</i> TD4842*	G	C	C	G	G	G	T
<i>C. poluninii</i> BY16573*	G	C	C	G	G	G	G
<i>C. poluninii</i> TD4829*	G	C	C	G	G	G	G
<i>C. poluninii</i> TD5182*	G	C	C	G	G	G	G/T
<i>C. poluninii</i> TD4703*	G	C	C	G	G	G	G
<i>C. poluninii</i> BY17053*	G	C	C	G	G	G	G
<i>C. poluninii</i> BY17100*	G	C	C	G	G	G	G
<i>Cirsium × giresunicum</i> TD4702*	A/G	C/T	C/T	A/G	A/G	G	G
<i>C. × giresunicum</i> BY16399*	A/G	C/T	C/T	A/G	A/G	G	G
<i>C. × giresunicum</i> TD4666*	A/G	C/T	C/T	A/G	A/G	G	G/T
<i>C. × giresunicum</i> TD4841*	A/G	C/T	C/T	A/G	A/G	G	G/T
<i>C. × giresunicum</i> TD4830*	A/G	C/T	C/T	A/G	A/G	G	G/T
<i>Cirsium trachylepis</i> MN918966	A/G	C/T	T	A	A/G	G	G
<i>C. trachylepis</i> TD4701*	A	T	T	A	A	A	G
<i>C. trachylepis</i> TD4831*	A	T	T	A	A	A	G
<i>C. trachylepis</i> TD4662*	A/G	C/T	T	A	A	G	G
<i>C. trachylepis</i> TD4840*	A/G	C/T	T	A	A	G	G



Şekil 4. ITS verilerine göre elde edilmiş filogenetik ağaç (soldakiler PP, sağdakiler BS değerleridir).

İkinci dalı incelediğimizde ise ilk defa bu çalışmada kullanılan örnekleri belirtmek için yanlarına “★” sembolü eklenmiştir. Bu çalışmada tanımlanan olası hibrit bireylere ait *C. × giresunicum* dizilerinin ve atalarının toplandığı bu ikinci dalın 1/95 (PP/BS) değerleri ile ayrıldığı görülmektedir (mavi elips içine alınıp, dal kırmızı ok ile gösterilmiştir). Atasal olarak arazi çalışmalarında gözlemlenen veya herbaryum örnekleri olarak temin edilen *C. poluninii* ve *C. trachylepis* bireylerin kendi içlerinde gruplanmışlardır ve *C. × giresunicum* ise bu iki atasının arasında bir konumda fakat *C. poluninii* bireylerinden oluşan alt dala daha yakın konumdadır (kırmızı ok ile gösterilmiştir).

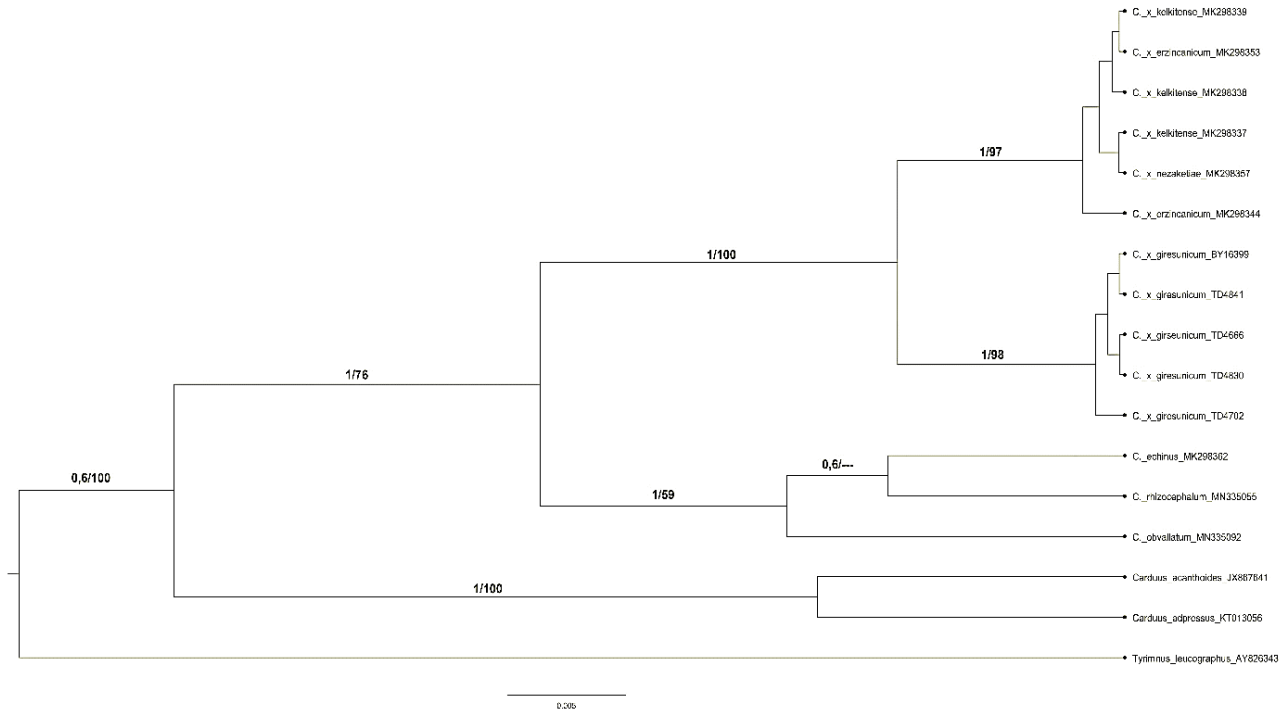
Şekil 5’te ise *C. × giresunicum* ve daha önceden Dirmenci vd. (2019) tarafından tanımlanan hibritleri ve bazı dış grupları içeren bir ağaç bulunmaktadır. Bu ağaç analiz edildiğinde görülmektedir ki, bu çalışmada ilk kez tanımlanan *C. × giresunicum* daha önce yine Türkiye’den aynı çalışma grubu tarafından tanımlanan *C. × nezakataiae*, *C. × kelkitense* ve *C. × erzincanicum* hibritlerinden moleküler açıdan (ITS bölgesi) bariz farklılık göstermektedir. *C. × nezakataiae*, *C. × kelkitense* ve *C. × erzincanicum* hibritlerinin dalı incelendiğinde (1/97 değerleri ile ayrılan) bu üç hibritin iç içe geçtiği görülmektedir. Bu gayet normaldir. Çünkü bu üç hibrit birbirine yakın alanlardan ve üçü de ortak atalarının genlerini içermektedir (Dirmenci vd. 2019). Şekil 4 ve 5 birlikte incelendiğinde açıkça görülmektedir ki, *C. × giresunicum* daha önce tanımlanan hibritlerden ebeveynlerinin farklı olmasından dolayı moleküler açıdan farklı konumdadır ve iki atasal taksona ait bireyler ile ortak dalları paylaşmakta ama iki atasının da DNA verilerine sahiptir (Tablo 2).

TARTIŞMA

Cirsium cinsinin revizyonu esnasında tespit edilen tanımlanmış veya tanımlanmamış hibritlerde olduğu gibi, Türkiye’de yapılan diğer hibrit tanımlama çalışmalarında da hibritlerin ölçülebilir veya ölçülemeyen bazı karakterlerinin ebeveynleri arasında geçiş özelliklere sahip olduğu, bazı karakterlerinin ise ebeveynlerden farklı olduğu rapor edilmiştir (Dirmenci vd. 2018; 2019; Arabacı vd. 2021; Cattaneo & Dirmenci 2022; Cattaneo vd. 2022). Burada tanımlanan *Cirsium × giresunicum* yaşam formu, gövde boyu ve rengi, gövde dallanması bakımından *C. poluninii*’ye benzerlik gösterirken, kapitulanın sarkık, dış fillarilerinin dikencikli, orta fillarilerinin geriye kıvrık olması ile

C. trachylepis'e benzerlik göstermektedir (Şekil 2 ve 3). Özellikle fillari boyu bakımından ara ölçülerde olmasına rağmen, yaprak parçalanması az teleksiden derin teleksiye kadar olması ile *C. trachylepis*'ten *C. poluninii*'ye doğru bir seri yaprak parçalanma özelliğine sahiptir. Korolla ve pappus ölçülerinin her iki ebeveynden daha kısa olması ile ayrı özellikler sergilemektedir (Tablo 1).

Bu çalışmadaki morfolojik verilerin yanı sıra, *Cirsium poluninii* ve *C. trachylepis* türleri arasında meydana gelen *C. × giresunicum* hibriti moleküler açıdan da detaylı olarak incelenmiştir. *C. trachylepis* türü daha önce farklı bir çalışmada moleküler açıdan çalışılsa da söz konusu çalışmada genel olarak genom boyutu ve GC içeriğine değinilmiştir (Ackerfield vd. 2020). *C. poluninii* türü ise moleküler veriler açısından ilk kez bu çalışmada incelenmiştir.



Şekil 5. Türkiye’de tanımlanan hibritlerin ITS verileri kullanılarak oluşturulmuş filogenetik ağaç.

Cirsium cinsi, yakın akraba türler arasında yaygın hibridizasyon ve poliploidizasyon nedeniyle yüksek morfolojik çeşitlilik ve polimorfizm sergilemektedir (Bureš vd. 2004; Nouroozi vd. 2012; Kang vd. 2021). *Cirsium* da dahil olmak üzere diğer cinslerin doğal melezlerinin ebeveyn türlerle karşılaştırıldığında ara DNA içeriğine sahip olduğu gösterilmiştir (Zhou vd. 2010; Dirmenci vd. 2019). nrITS bölgesi hem türler hem de hibritler arası ilişkileri açıklamada oldukça bilgi verici olduğundan filogenetik çalışmalarda sıklıkla tercih edilmektedir (Segarra-Moragues vd. 2007; Dirmenci vd. 2019; Ackerfield vd. 2020; Arabacı vd. 2021). Hibritlerin moleküler açıdan tanımlanmasında nrITS bölgesine ait nükleotidlerin polimorfizm göstermesi yararlı olmaktadır.

Türkiye her ne kadar *Cirsium* cinsinin en önemli gen merkezlerinden biri olsa da cinse ait hibritler ile ilgili moleküler açıdan sadece bir araştırma yapılmıştır. Dirmenci vd. (2019) tarafından yapılan bu çalışmada *Epitrachys* seksiyonuna ait üç hibrit morfolojik, palinolojik ve moleküler yönden araştırılarak tanımlanmıştır. Bunlar; *C. × nezaketiae* [*Cirsium cephalotes* Boiss. × *Cirsium macrobotrys* (K.Koch) Boiss.], *C. × kelkitense* [*Cirsium cephalotes* × *Cirsium leucocephalum* (Willd.) Spreng. subsp. *leucocephalum*] ve *C. × erzincanicum* [*Cirsium leucocephalum* subsp. *leucocephalum* × *Cirsium macrobotrys*] hibritleridir. Söz konusu çalışmaya göre hibritler morfolojik açıdan bazı özellikleri ile bir ebeveyne, bazı özellikleri ile de diğer ebeveyne benzemektedir. Aynı çalışmada polen tanelerinin boyutlarının hibritlerde ara değerlerde olduğu, hibrit polenlerinin düzensiz şekillerde olduğu, moleküler açıdan da hibritlerin polimorfik nükleotidlere sahip olduğu gösterilmiştir. Polimorfik bölge sayısı bazı hibritlerde fazla olabilirken, bazı hibritlerde daha az

sayıda olabilmektedir. Örneğin, Segarra-Moragues vd. (2007) tarafından yapılan çalışmada tanımlanan *Cirsium* × *vivantii* L. Villar, Segarra, J.López, Pérez-Coll. & Catalán hibriti ITS bölgesine ait 29 farklı polimorfik nükleotid içermektedir. Türkiye’de yapılan çalışmada ise (Dirmenci vd. 2019) *C. × nezaketiae* 10, *C. × kelkitense* 6 polimorfik nükleotid içermektedir. Ayrıca hibritler aynı ebeveynlere sahip olsalar da, farklı sayıda polimorfik bölge içerebilmektedir (Segarra-Moragues vd. 2007). Bu çalışmadaki bazı örneklerde (TD4702, BY16399) 5, bazı örneklerde ise (TD4666, TD4841, TD4830) 6 polimorfik bölge tespit edilmiştir. Polimorfik bölgelerin azlığı ya da çokluğu hibritlerin tanımlanmasında belirleyici karakter olmasa da, bazı bireylerde polimorfik bölgeler hibritin ayırt edilmesinde kullanışlı olabilmektedir. Polimorfik bölgeler, sadece hibritlerde değil aynı zamanda ebeveynlerde de görülmektedir. Bunun sebebi olarak ise ebeveyn türlerin ve hibritlerin çok küçük bir alanda kompleks içinde yaşamaları nedeniyle geri çaprazlamaların mümkün olabileceğidir.

Filogenetik ağaç incelendiğinde (Şekil 4) *C. × giresunicum* hibriti ebeveynleri ile aynı klad üzerinde ve ebeveynlerinin arasında ancak *C. poluninii*’ye daha yakın konumda yer almaktadır. Bu durum, hibritlerin ebeveynlerine yakın karakterler sergilese de aslında onlardan farklı taksonlar olduğunu da göstermektedir. Ayrıca bu sonuç morfolojik olarak ara karakterlere sahip olan hibritleri moleküler açıdan da desteklemektedir. Diğer filogenetik ağaç (Şekil 5) incelendiğinde farklı ebeveynlere sahip hibritlerin (*C. × nezaketiae*, *C. × kelkitense*, *C. × erzincanicum* ortak ebeveynlere sahiptir) farklı kladlar üzerinde yer aldığı görülmektedir. Ayrıca *C. × giresunicum* hibritinin Türkiye’de daha önce tanımlanan hibritlerden morfolojik özelliklerin yanı sıra moleküler açıdan da ayrı konumda olduğunu desteklemektedir. Sonuç olarak, hibritlerin tanımlanmasında DNA verileri tek başına yeterli olmasa da, bu veriler *Cirsium* cinsi içinde hibritleşmenin yaygın bir süreç olduğuna dair kanıtlar sunmaktadır.

Cirsium cinsinin bazı araştırmacılara göre dünya genelinde 450’den fazla türe sahip olduğu belirtilmektedir (Ackerfield vd. 2020; Moreyra vd. 2023). Cinsin büyüklüğü ve morfolojik açıdan çeşitliliği cinsin sınıflandırılması konusunda tartışmalara neden olmuştur. Del Guacchio vd. (2022) tarafından *Cirsium* dört farklı cinse ayrılmıştır (*Cirsium* Mill. s. str., *Lophiolepis* Cass., *Epitrachys* ve *Lophiocirsium* Del Guacchio, Bureš, Iamónico & P. Caputo). Bu çalışmanın da konusu olan ebeveyn türler Del Guacchio vd. (2022) tarafından *Lophiolepis* cinsi altında sınıflandırılmıştır. Söz konusu çalışmada kullanılan diziler gen bankasından iki çekirdek ve beş plastid DNA bölgesine dayanılarak alınmıştır. Bunların cins içindeki filogenetik çözümleme için yetersiz olduğu rapor edilmiştir (Moreyra vd. 2023). Yine Moreyra vd. (2023)’e göre *Cirsium*’un tek bir cins olarak korunması aynı zamanda en sağlam ve pratik yaklaşımdır. Bunun nedeni, *Cirsium*’un zaten yaygın olan eş anlamlılığını daha da arttıracak yüzlerce yeni isimlendirme kombinasyonunun ortaya çıkmasının engellenmesidir. Bu bağlamda yapmış olduğumuz çalışmada Moreyra vd. (2023)’nin önerisi üzerine burada tanımlanan hibritin (*Cirsium* × *giresunicum*) ve ebeveynleri olan *C. poluninii* ve *C. trachylepis* türlerinin *Cirsium* cinsi altında yer almasının daha uygun olduğu kanaatine varılmıştır.

Taksonların Gen Bankası Erişim Kodları

Carduus acanthoides (JX867641), *Carduus adpressus* (KT013056), *Cirsium cephalotes* (MK298354, MK298355, MK398356, MK298358, MK298359, MK298360), *C. echinus* (MK298362), *C. × erzincanicum* (MK298344, MK298353), *C. × kelkitense* (MK298337, MK298338, MK298339), *C. leucocephalum* (MK298340, MK298341, MK298342, MK298343, MK298348, MK298349, MK298350, MK298351, MK298352), *C. macrobotrys* (MK298345, MK298346, MK29834), *C. × nezaketiae* (MK298357), *C. obvallatum* (MN335092), *C. rhizocephalum* (MN335055), *C. trachylepis* (MN918966, OR678381, OR678382, OR678383, OR678384), *C. × giresunicum* (OR678385, OR678386, OR678387, OR678388, OR678389), *Tyrimnus leucographus* (AY826343), SUB13902323 *C. poluninii* (OR678374, OR678375, OR678376, OR678377, OR678378, OR678379).

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