

## Morphological and anatomical investigation on endemic *Cota melanoloma* and *Cota antitaurica* (Asteraceae) in Turkey

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### Abstract

*Anthemis* section *Cota* was accepted as separate genus recently. Genus *Cota* is represented by 24 taxa in Turkey. Some of them have economic importance because of their uses for various purposes such as obtaining drugs, food and dye. etc. In this study, *Cota melanoloma* subsp. *melanoloma*, *C. melanoloma* subsp. *trapezuntica* and *C. antitaurica* were studied morphologically and anatomically. Three of them are endemic to Turkey. *C. antitaurica* is only known from the type locality and critically endangered species, while *C. melanoloma* is distributed in only northern Anatolia. As the results, the diagnostic characteristics, synonyms, flowering and fruiting times, habitat properties, distributions and threat categories of each taxon are presented. The most important morphological characteristics for more proper discrimination the species are determined as the shape of capitula and palea, indumentum of stem and leaf. The detailed anatomical studies indicate the diagnostic characteristics as both hair and vascular bundle length and pith of the stems, hair length and length/width ratio and the type of stomata of the leaves, the layer number of parenchymatic ray cells and the cortex length of the roots.

**Keywords:** Morphology; anatomy; *Cota*; endemic; Turkey.

**Abbreviations:** Excl\_exclude; inc\_include; cm\_centimeter; diam\_diameter; mm\_millimeter; µm\_micrometer; c\_about; euro-Sib\_Euro-Siberian; Ir-Tur\_Irano-Turanian; subsp\_subspecies.

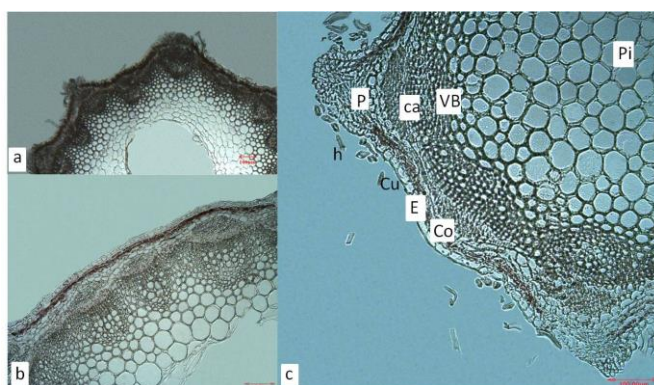
### Introduction

Asteraceae is represented by the greatest number of taxa in Angiospermae from all over the world. It has nearly 1600-1700 genera and 24000-30000 species (Funk et al., 2005). *Cota* J. Gay is comprised of 49 species (63 taxa). A total of 24 taxa are distributed in Turkey, 10 of which are endemic to Turkey (Özbek, 2012). *Cota* is distributed in Mediterranean and Iran-Turan phytogeographic regions. Generally, members of the genus occur mostly in Europe (excl. North side), northern Africa, Caucasus, southern Russia and Anatolia to Afghanistan (Davis et al. 1975; Oberprieler, 2007). Recently, *Anthemis* sect. *Cota* was accepted as a generic name, *Cota*, by Oberprieler (2001), Greuter et al. (2003), Oberprieler et al. (2007, 2009) and Lo Presti (2010). Some species of the genus *Cota* have economic importance because of their uses for various purposes such as obtaining drug, food and dye etc. (Ghafoor, 2010; Öztürk et al., 2013). Genus *Cota* morphologically resembles *Anthemis*, but it differs from *Anthemis* by obconical, dorsiventrally flattened achenes, with prominent lateral ribs that are smooth or with 3-10 ribs on each side. The achenes of *Anthemis* are obovoid to obconical, circular or quadrangular in cross-section, usually with c. 10 smooth or tubercular ribs. There are few reports about the anatomy, classification and seed morphology of *Cota* (Metcalf and Chalk, 1957; Zareh, 2009; Bani and Adıgüzel 2010; Ghafoor, 2010; Özbek et al., 2011). Moreover, there

are some studies about chemical composition and antimicrobial activity of the closely related genus *Anthemis* L. (Uzel et al., 2004; Çelik et al., 2005; Uysal et al., 2005; Albayrak and Aksoy, 2012). The aim of this study is to determine the morphological and anatomical properties of *Cota antitaurica* (Grierson) Holub and *C. melanoloma* (Trautv.) Holub (including both subsp. *melanoloma* and subsp. *trapezuntica* (Grierson) Oberpr. & Greuter). However this is the first study on anatomy of any members of genus *Cota* in Turkey. These three taxa are endemic to Turkey. *C. antitaurica* is local endemic species that had not been collected since 1952. It was re-collected for the second time from its type locality by the authors in 2007. It grows in *Cedrus libani* and *Abies cilicica* subsp. *cilicica* forest between 1600-1900 m. *C. melanoloma* subsp. *melanoloma* which occurs on volcanic and calcareous rocks, high mountain steppe, and edge of mixed forest and open area of *Quercus* forest is distributed in North and Northeast side of Turkey, between altitudes of 1050-2600 m. while *C. melanoloma* subsp. *trapezuntica* is scattered in Northeast Anatolia, between 800-3000 m. It prefers mountain steppes and edges of mixed forest as its habitat.

**Table 1.** Comparison of some morphological characters of *Cota antitaurica* in Flora of Turkey (Yavin and Grierson 1975) with the new findings.

Characteristics	Flora of Turkey	New findings
Stems	25-30 cm	17-33 cm
Primary segments of leaf lamina	4-paired	3-4-paired
Secondary segments of leaf lamina	3-5-paired	4-6-paired
Involucre	3-5-paired	4-6-paired
Outer phyllaries	ovate, 3.5-5 x 2 mm	ovate, 3.5-4 x 1-1.5 mm
Inner phyllaries	7-15 mm	6-7 mm
Disc flowers	4-5 mm	3-4 mm
Achenes	2.5-2.75 mm	3.05-3.25 mm
Corona	0.5-0.75 mm	0.5-0.7 mm



**Fig 1.** Stem transverse sections. a) *C. melanoloma* ssp. *melanoloma*, b) *C. melanoloma* ssp. *trapezuntica*, c) *C. antitaurica*, ca: cambium; Co: collenchyma; Cu: cuticle; E: epidermis; h: hair; P: parenchyma; Pi: pith; VB: vascular bundle.

## Results and Discussion

### Morphology

The morphological description of each taxon is given in Flora of Turkey (Grierson and Yavin, 1975). Almost all of the morphological characteristics were expanded with the detailed investigations on our recent collections (Table 1 and 2).

#### *C. melanoloma* (Trautv.) Holub

Description: Perennial, rhizome woody. Stem mostly simple or numerous and branched from base, erect or ascending-erect, 15-60 cm long, 1-3 mm diam, green or greyish-green, adpressed-pilose. Sterile shoots up to 15 cm. Basal leaves (at first 5 node) reduced, 2-pinnatisect; 3.5-15 x 2-10 mm, oblong or oblong-elliptic. Median leaves 2-3 pinnatisect; 15-40 x 5-30 mm, oblong or obovate-oblong; primary segments 3-6-paired, oblong-obovate, 2-15 x 0.5-7 mm; secondary segments 4-6-paired, lanceolate, 0.5-4 x 0.25-1 mm, acute at apex. Upper stem leaves 2-3 pinnatisect, 10-20 x 4-10 mm, ovate or obovate-oblong; primary segments 3-6-paired, oblong-obovate, 5-6 x 2-3 mm; secondary segments 3-4-paired, lanceolate, 0.5-2 x 0.25-1 mm, acute at apex. Peduncles 6-20 cm, thickened below capitula at fruiting time. Capitula heterogamous hemispherical; 22-43 mm diam. (inc. ligules). Phyllaries imbricate, 4-seriate, pale or dark brown margined; outer phyllaries 5-6 x 2-2.5 mm, triangular-lanceolate; median phyllaries 6-7 x 2.5-3 mm, oblong; inner phyllaries 6.5-7.5 x 2.5-3 mm, elliptic, obtus at apex. Ray flowers (14-)20-25(-35), white or sometimes cream; 3 toothed, lamina 10-20 x 4-7 mm, elliptic; ligula tube glabrous, 2 x 1.5 mm. Disc flowers tubular, yellow, hermaphrodite, 3-4 x 0.5-1 mm, 5-lobbed, shallowly at base; style 2-2.5 mm bifurcate. Paleae 6-7 x 1-2 mm; narrowly lanceolate,

acuminate at apex. Achenes obconical, brown, sometimes purple, 2.5-3 x 0.75-1 mm, prominent 10 ribbed; corona symmetric, 0.25-0.6 mm, crenulate.

#### *C. melanoloma* subsp. *melanoloma*

≡ *Anthemis melanoloma* Trautv., Bull. Soc. Imp. Naturalistes Moscou 41(1): 461 (1868).

≡ *Anthemis extrarosularis* Freyn & Sint., Öst. Bot. Zeitschr. 44: 146 (1894).

Diagnostic characters: Stem and basal leaves greyish-green; primary segments 3-4-paired, upper stem leaves obovate-oblong.

Habitat: Volcanic and calcareous rocks, high mountain steppe, edge of mixed forest, open area of *Quercus* forest, 1050- 2600 m.

Flowering and fruiting time: Fl. 7-8, fr. 8-9.

Phytogeographic region: Euro-Sib. element

Distribution: Endemic (North and Northeast Anatolia: Kastamonu, Amasya, Sivas, Erzincan, Erzurum, Tunceli Trabzon, Bayburt, Ardahan).

Threat category: LC

*C. melanoloma* subsp. *trapezuntica* (Grierson) Oberpr. & Greuter, Willdenowia 33: 40 (2003).

≡ *Anthemis melanoloma* Trautv. subsp. *trapezuntica* Grierson, Notes R.B.G. Edinb. 33: 411 (1975).

Diagnostic characters: Stem and basal leaves green; primary segments 5-6 -paired, upper stem leaves ovate.

Habitat: High mountain steppe, edge of mixed forest, 800-3000 m.

Flowering and fruiting time: Fl. 7-8, fr. 8-9.

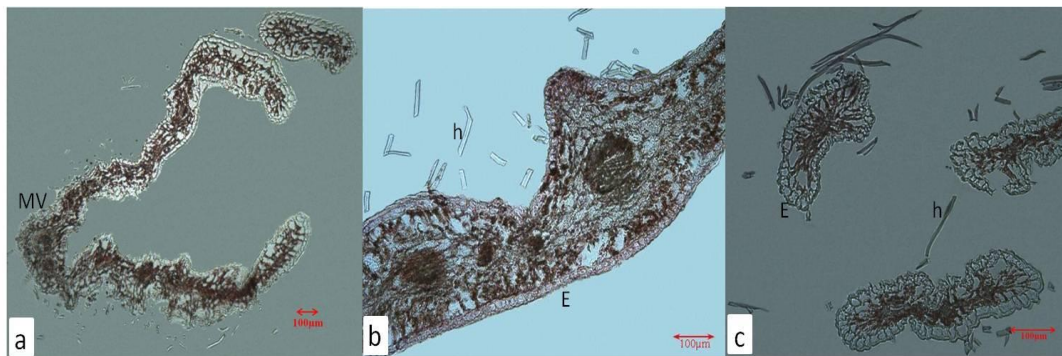
Phytogeographic region: Euro-Sib. element

Distribution: Endemic (Northeast Anatolia: Trabzon, Rize, Bayburt, Ardahan).

Threat category: LC

**Table 2.** Comparison of some morphological characters of *Cota melanoloma* in Flora of Turkey (Yavin and Grierson 1975) with the new findings.

Characteristics	Flora of Turkey	New findings
Stems	25-45 cm	15-60 cm
Primary segments of leaf lamina	3-6-paired	3-6-paired
Secondary segments of leaf lamina	4-6-paired	4-6-paired
Involucre	1.5-2 cm	1.2-2.3 cm
Outer phyllaries	lanceolate, 4-5 mm	lanceolate-triangular, 5-6 mm
Inner phyllaries	oblong-elliptic, 5-7 mm	elliptic, 6.5-7.5 mm
Disc flowers	4-5 mm	3-4 mm
Achenes	2-3 mm	2.5-3mm
Corona	0.5-1 mm	0.2-0.3 mm



**Fig 2.** Leaf transverse sections. a) *C. melanoloma* ssp. *melanoloma*, b) *C. melanoloma* ssp. *trapezuntica*, c) *C. antitaurica*, E: epidermis; h: hair; MV: midvein.

*C. antitaurica* (Grierson) Holub  
 ≡ *Anthemis antitaurica* Grierson, Notes R.B.G. Edinb. 33: 211 (1974). Description: Perennial, rhizomatous plants. Stem generally simple or branched at base, erect or erect-ascending, 17-33 cm long, 1-2 mm diam, lanate hairy, sparsely leafy at upwards. Steril shoots up to 7 cm. Basal leaves 2-pinnatisect, 17-40 x 5-15 mm, ovate-elliptic, petiole 7-30 (-40) mm; primary segments 3-4 paired, 3-7 x 1-2 mm, elliptic; secondary segments 4-6 paired, 1.5-2.5 x 0.5-1 mm, lanceolate, acute at apex. Median and upper cauline leaves similar, 2-pinnatisect; 8-25 x 4-15 mm, ovate-elliptic; primary segments 3-4 paired, 3-6 x 1-1.5 mm, elliptic; secondary segments 4-6 paired, 1.5-2.5 x 0.5-1 x 1 mm, lanceolate, acute at apex. Peduncles 5-17 cm long, slightly thickened below capitula at fruiting time. Capitula heterogamous, campanulate; 17-30 mm diam (inc. ligules). Phyllaries imbricate, 4-seriate, sparsely lanate, scarious margined, pale or dark brown margined; outer phyllaries 3.5-4 x 1-1.5 mm, ovate; median phyllaries 6-7 x 2 mm, oblong-lanceolate; inner phyllaries 6-7 x 2-2.5 mm, oblong-lanceolate, ciliate at apex. Ray flowers 18-22, white, 3 toothed, lamina 11-13 x 4-6 mm, elliptic; ligula tube glabrous, 1.5-2.5 x 1-1.5 mm. Disc flowers tubular, yellow, hermaphrodite, 3-4 x 0.5-1 mm, 5-lobed, shallowly at base; style 2-2.5 mm, bifurcate. Paleae 6-7 x 1-1.5 mm; oblanceolate, acuminate at apex, outer palea straw coloured, inner yellow. Achenes obconical, straw coloured to pale brown, 3.05-3.25 x 1-1.19 mm, 7 ribbed on both surfaces; corona symmetric, 0.5-0.7 mm, crenulate. Diagnostic characters: Capitula campanulate, stem lanate. Habitat: *Cedrus libani* and *Abies cilicica* subsp. *cilicica* forest, 1600-1900 m. Flowering and fruiting time: Fl. 6-7, fr. 7-8. Phytogeographic region: Ir-Tur. Element. Distribution: Endemic. Threat Category: CR

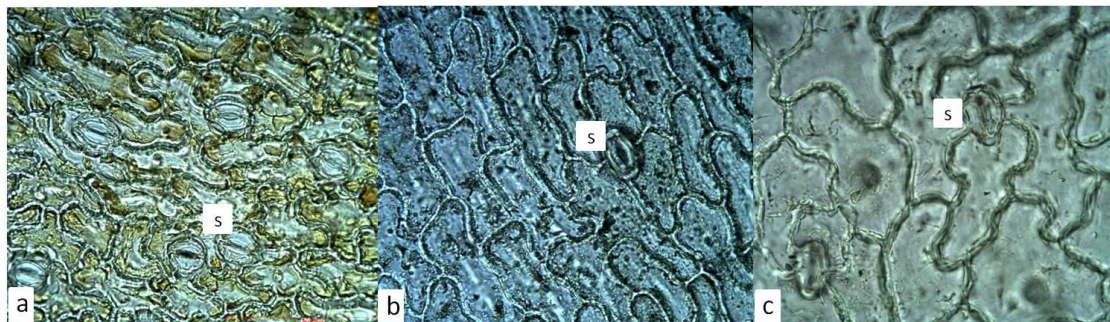
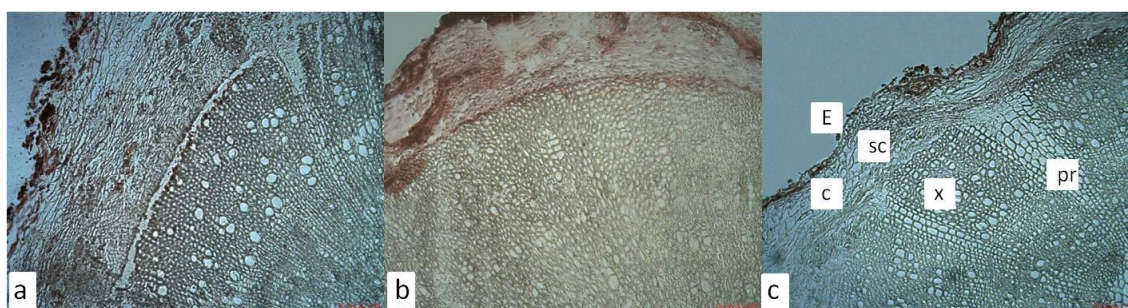
#### Anatomy

**Stem Anatomy:** Generally, all the stem sections seem to have the same structure (Fig. 1). In this structure, a thin layer of cuticle covers epidermis, which makes a single layer consisting regularly arranged, rectangular cells. Four or five layers of collenchyma cells are surrounded by epidermis. Under the collenchyma cells, there is a narrow ring of parenchyma cells. Parenchyma cells cover the vascular bundles and the pith. The cambium seems to have 4 or 5 cells in all the stem's transverse sections. The vascular bundles are all connected to each other and cover the parenchymatic cells in the pith. The main prominent difference between these three stem structures is that the piths of *C. melanoloma* subsp. *melanoloma* and *C. melanoloma* subsp. *trapezuntica* are hollowed. However, the stem pith of *C. antitaurica*, seems to be solidly coated with parenchymatic cells. In the stem sections of *C. melanoloma* subsp. *melanoloma*, the epidermal layer consists of single celled hairs with the average length of  $50.47 \pm 7.125 \mu\text{m}$ . However, in *C. antitaurica*, the length of single celled hairs were measured as  $35.96 \pm 1.935 \mu\text{m}$ . The length/diameter ratios of vascular bundles in stems show that, in *C. antitaurica*, the vascular bundles have nearly the same length and diameters as  $148.75 \pm 10.287 \mu\text{m}$  and  $148.23 \pm 4.191 \mu\text{m}$  respectively. In *C. melanoloma* subsp. *melanoloma*, the average length of vascular bundles is  $140.91 \pm 8.550 \mu\text{m}$  and the average diameter is  $106.75 \pm 8.149 \mu\text{m}$ . However, *C. melanoloma* subsp. *trapezuntica* has vascular bundles with an average length as  $253.36 \pm 9.893 \mu\text{m}$  and the average diameter as  $155.76 \pm 13.872 \mu\text{m}$ .

**Leaf Anatomy:** Transverse sections of mature leaves show similar structural patterns (Fig. 2). The general appearance of leaf transverse sections show that, they seem to be isobilateral leaves. Both adaxial and abaxial epidermises have regularly arranged into irregularly shaped cells. Moreover both upper and lower sides of the leaves have stomata and

**Table 3.** The measurements of stomata.

Taxa	Stoma type	Stoma length	Stoma width	Length/width ratio	Stoma density
<i>C. melanoloma</i> ssp <i>melanoloma</i>	Anomocytic, Actinocytic	22.17 ± 0.769	14.93 ± 0.899	1.50	7.60 ± 2.073
<i>C. melanoloma</i> ssp <i>trapezuntica</i>	Anomocytic, Actinocytic	27.39 ± 0.746	17.65 ± 0.989	1.55	3.00 ± 1.632
<i>C. antitaurica</i>	Actinocytic	23.48 ± 0.763	16.36 ± 0.871	1.43	3.00 ± 1.001

**Fig 3.** Surface views of leaves. a) *C. melanoloma* ssp *melanoloma*, b) *C. melanoloma* ssp *trapezuntica*, c) *C. antitaurica*, s: stoma.**Fig 4.** Transverse sections of roots. a) *C. melanoloma* ssp *melanoloma*, b) *C. melanoloma* ssp *trapezuntica*, c) *C. antitaurica*, c: cortex; E: epidermis; pr: parenchymatic ray; sc: sclerenchyma; x: xylem.

hairs. Maximum hair length of *C. melanoloma* subsp. *melanoloma* was measured as 66.32 µm. However, in *C. melanoloma* subsp. *trapezuntica* and *C. antitaurica* these measurements are 149.61 µm and 296.2 µm respectively. The midrib of all these leaves are triangle shaped. The abaxial surfaces of the leaves are shown in Fig. 3, and the stomatal properties with the measurements are given in Table 2. According to Table 2, the types and the length/width ratios of stomata seem to be important differences between the taxa. With the help of these characteristics, *C. antitaurica* can be separated from the others. However, the density of stomata seems to be the most in *C. melanoloma* subsp. *melanoloma*.

**Root Anatomy:** Fig. 4 shows the transverse sections of roots. General anatomical characteristics of the roots are mainly the same with some minor differences. The outermost layer, epidermis, consists of thin walled and single layered parenchymatous cells. The thick walled cells of the cortex are flat and the cortex includes groups of sclerenchymatic cells. The length of the cortex tissue changes according to the taxa. The cortex length was measured as 296.43 ± 21.595 and 276.87 ± 30.521 in *C. melanoloma* subsp. *melanoloma* and *C. melanoloma* subsp. *trapezuntica* respectively. However, in *C. antitaurica*, the average length of the cortex is 141.178 ± 25.787. The major part of the roots is reserved by xylem elements. There are thin walled ray parenchyma cells between these xylem elements. The layer numbers of these cells are maximum 2 in both *C. melanoloma* subsp. *melanoloma* and *C. melanoloma* subsp. *trapezuntica*. However, in *C. antitaurica*, there are minimum 3 layers of

wide parenchymatic ray cells between the vessels and tracheids. The central parts consist of thin walled, small sized parenchyma cells, surrounded by the portion of xylem tissue.

## Material and Methods

### Plant materials

*C. melanoloma* subsp. *melanoloma* were collected from Kastamonu (A4 Kastamonu: Ilgaz Mts., Küçük Hacet hills, high mountain steppe, 2500 m, 02.08.2005, *U.Özbek* 1918) (Fig. 5), *C. melanoloma* subsp. *trapezuntica* were collected from Rize (A8 Rize: İkizdere, Başköy, Çermaniman hills, high mountain steppe, 2610 m, 29.07.2007, *U.Özbek* 2687) (Fig. 6) and *C. antitaurica* were collected from Adana (B6 Adana: Saimbeyli-Tufanbeyli, Bozoğlan Mts., above Obruk upland, around Karlıktepe, edge of *Cedrus libani* and *Abies cilicica* subsp. *cilicica* forest, 1650-1900 m, 20.06.2008, *B. Bani* 6120) (Fig. 7). A distribution map of *C. antitaurica*, *C. melanoloma* subsp. *melanoloma* and *C. melanoloma* subsp. *trapezuntica* is given in Fig. 8.

### Anatomical methods

The investigated materials comprise our collections and deposited specimens at GAZI (Ankara). For anatomical investigations, herbarium samples were firstly boiled in distilled water to be softened. Then, these samples were placed in 70% ethyl alcohol and dehydrated with ethyl



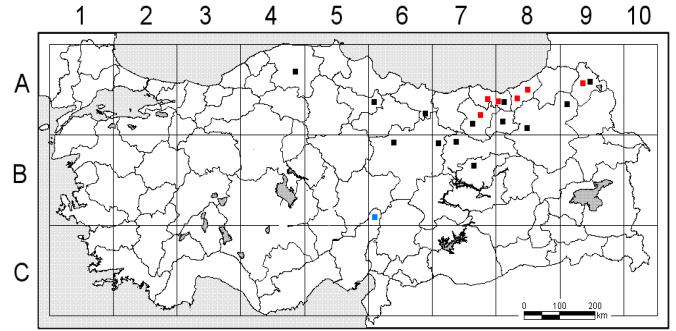
**Fig 5.** General appearance *Cota melanoloma* subsp.



**Fig 6.** General appearance *Cota melanoloma* subsp. *trapezuntica*.



**Fig 7.** General appearance of *Cota antitaurica*.



**Fig 8.** ■ *Cota melanoloma* subsp. *melanoloma*; ■ *Cota melanoloma* subsp. *trapezuntica*; ■ *Cota antitaurica*.

alcohol solutions of increasing strength. The samples were embedded and sectioned with the help of paraffin sectioning method (Johansen, 1944). Safranin was used for staining and Entellan was used for fixing. The slices were observed by ‘Euromex FE 2025’ microscope and photographed by using a ‘Euromex CMEX DC.1300’ camera. Moreover, the leaf surfaces of each taxon were examined by 10 slides. The numbers of stomata were counted from both adaxial and abaxial epidermis and the average measurements were calculated. For measurements of epidermal architecture, 10 slides for each taxon were prepared. Average values were measured from 30 different  $234 \times 186 \mu\text{m}^2$  samples of 4 leaves of each taxon.

### Conclusion

Consequently, these three taxa can be easily distinguishable with some morphological and the anatomical characteristics. The most accurate diagnostics of them are given as follows; *C. antitaurica* differs from *C. melanoloma* by its campanulate capitula (not hemispherical), lanate stem and leaves (not adpressed-pilose) and oblanceolate paleae (not narrowly lanceolate). *C. melanoloma* subsp. *melanoloma*, differs from *C. melanoloma* subsp. *trapezuntica* by its stem and basal leaves greyish-green (not green), primary segments 3-4-paired (not 5-6 paired) and upper stem leaves obovate-oblong (not ovate). Moreover, it was clearly observed from the stem sections that, *C. antitaurica* seems to have solid pith, while the two subspecies of *C. melanoloma* have hollowed pith. Furthermore, the types and the length/width ratios of stomata of the leaves and the length of the cortex tissue of the roots seem to separate *C. antitaurica* from the subspecies of *C. melanoloma*. Finally, in root anatomy, the layer numbers of parenchymatic ray cells are more the 3 in *C. antitaurica*, while there are a maximum of 2 layers of parenchymatic cells in *C. melanoloma* subsp. *melanoloma* and *C. melanoloma* subsp. *trapezuntica*.

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