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Research Article

Anatomy and Trichome Micromorphology of Endemic *Ballota pseudodictamnus* subsp. *lycia* (Lamiaceae)

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ABSTRACT

In this study, the anatomical features of *Ballota pseudodictamnus* (L.) Benth. subsp. *lycia* Hub.-Mor. (Lamiaceae), endemic taxa from Turkey are examined and evaluated by light (LM) and scanning electron microscopy (SEM). Different trichome types (glandular -including Lamiaceae type, capitate and peltate- and non-glandular trichomes) are observed. The densest trichome type is dendroid trichomes on the aerial part of the plant. The leaf is bifacial and amphistomatic. In the petiole, there are two collateral vascular bundles in the centre and one small vascular bundle in each corner. The prismatic crystals are present in the pith area of the stem. Results are compared with other studies in the literature. The anatomical characteristics of the stem, petiole, lamina and measurements are presented additionally trichome micromorphology is given for the first time.

Keywords: *Ballota pseudodictamnus subsp. lycia, Anatomy, Trichome micromorphology, Lamiaceae, Turkey*

Endemik *Ballota pseudodictamnus* subsp. *lycia* (Lamiaceae) Türünün Anatomisi ve Trikom Mikromorfolojisi

ÖZ

Bu çalışmada Türkiye için endemik olan *Ballota pseudodictamnus* (L.) Benth. subsp. *lycia* Hub.-Mor. (Lamiaceae) taksonunun anatomik özellikleri ışık ve taramalı elektron mikroskobu (SEM) ile incelenip değerlendirilmiştir. Farklı trikom tipleri (glandular -kapitat, peltat ve Lamiaceae tipi dahil- ve glandular olmayan trikomlar) gözlenmiştir. En yoğun trikom tipi, bitkinin toprak üstü kısımlarında bulunan dendroid trikomlardır. Yaprak mezofili bifasyal ve stomalar amfistomatiktir. Yaprak sapında, merkezde iki kollateral iletim demeti ve her köşede birer küçük iletim demeti vardır. Gövde öz bölgesinde prizmatik kristaller bulunmaktadır. Sonuçlar literatürdeki diğer çalışmalarla karşılaştırılmıştır. Gövde, petiyol ve lamina anatomik özellikleri, ölçüleri bunlara ilaveten trikom mikromorfolojisi ilk kez verilmiştir.

Anahtar Kelimeler: *Ballota pseudodictamnus subsp. lycia, Anatomi, Trikom mikromorfolojisi, Lamiaceae, Turkey*

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I. INTRODUCTION

The genus *Ballota* L. is represented by 35 species belonging to Lamiaceae (subfamily Lamioideae) in the world [1, 2]. It is represented by 17 taxa in the Turkish flora and 9 of them are endemic - endemism 53% [3, 4]. *Ballota pseudodictamnus* (L.) Benth. subsp. *lycia* Hub.-Mor., the subject of this study, is one of the endemic species in the South-west of Turkey. *Ballota* is a genus of low-growing perennial herbs and subshrubs, mainly distributed around the Mediterranean and Eurasia [5].

Ballota taxa are used in traditional folk medicine. These are noteworthy as herbal drugs. For instance, species are used for cold, flu, flatulence, hemorrhoids in Turkey [6-8] for hysteria in Bosnia and Herzegovina [9], as an antispasmodic, sedative and for jaundice, hemorrhoids in Italy [10, 11], to stimulate milk production in pregnant women in South Africa [12], for stomach and intestinal pain in Jordan [13].

Ballota taxa have different phytochemical classes such as terpenoids, flavonoids, phenolic acids, essential oils, iridoids, saponins, tannins and organic acids [5]. Saltan-Citoglu *et al.* [14] studied the chemotaxonomy of *Ballota* species in Turkey. For this aim they determined "the diterpenoid and flavonoid profiles of *Ballota* species". They found that there were some meaningful links but in general view no concord findings between phylogenetic orders and the sections.

Metcalfe and Chalk [15] demonstrated that the structure of trichomes has taxonomic significance in the Lamiaceae. The structure of the vascular bundles in petioles is also a useful characteristic in the Lamiaceae. In the literature there are a few anatomical, morphological and palynological studies on *Ballota* taxa [16-18]. This study is aimed to investigate detailed anatomy and trichome features of *B. pseudodictamnus* subsp. *lycia* and determine the distinctive feature.

II. MATERIALS and METHODS

Ballota pseudodictamnus subsp. *lycia* were sold in Fethiye (South-west part of Turkey) public market by local people and collected from Taşyaka village, Fethiye, Turkey. The collected plant specimens were identified using "Flora of Turkey and the East Aegean Islands" [3] and compared the plant with the specimens deposited in the ISTE (the Herbarium of the Faculty of Pharmacy of Istanbul University). We kept the studied plant materials as a herbarium specimen in ISTE Herbarium (ISTE no: 117092). The materials were fixed in 70% alcohol - used for anatomical studies. Cross-sections of the plant petiole, stem, and surface sections of leaves taken by free-hand in the middle part using a razor blade. The sections were stained with Sartur solution (a compound reagent of lactic acid, Sudan III, aniline, iodine, potassium iodide, alcohol, and water) [20]. The measurements were made on 20 sections taken from five different leaves and stems. The well-staining sections were photographed with Canon A 640 digital camera and Olympus BH-2 light microscopy (LM).

For scanning electron microscopy (SEM) analysis, the samples (petiole, lamina, calyx, and stem) were mounted on stubs and then coated with gold. The FEI Quanta 450 FEG-EDS SEM was used for trichome micromorphology. The general classification and the terminology follow Metcalfe & Chalk [15], as well as Osman [18].

The stomatal index was calculated according to the method of Meidner and Mansfield [21]. Anatomical measurements were made by using the image analysis systems KAMERAM (ARGENİT Microsystems). The trichomes were investigated by LM and SEM.

III. RESULTS

Ballota pseudodictamnus subsp. *lycia* is an endemic taxon in Turkey. The aerial part of the taxon is used as a sedative and sold in Fethiye (Southwest of Turkey) public market by local people. In this study detailed the anatomy of *B. pseudodictamnus* subsp. *lycia* is investigated. Anatomical measurements are summarized in Table 1 and photos are shown in Figure 1.

Table 1. *Anatomical measurements of B. pseudodictamnus subsp. lycia. Avr: average, Sd: standard deviation.*

Lamina	**Measurements**
Upper epidermis - Stomata index	20.02
Lower epidermis - Stomata index	23.21
Stomata index ratio	0.86
Stomata length (µm) (Avr. ± Sd)	21.58±1.04
Stomata width (µm) (Avr. ± Sd)	17.04±0.59
Stem	
Epidermis cell length (µm) (Avr. ± Sd)	8.11±0.73
Epidermis cell width (µm) (Avr. ± Sd)	11.45±1.67
Diameter of pith ray cell (µm) (Avr. ± Sd)	47.90±6.51
Diameter of trachea (µm) (Avr. ± Sd)	26.96±3.71

A. 1. Stem Anatomy

In the transverse section the stem is quadrangular. The epidermis is covered by thick cuticles (c. 0.7 µm) with non-glandular and glandular trichomes. The surface densely bears dendroid trichomes. The epidermis is composed of a single layer of oval-rectangular cells. The collenchyma tissue is located under the epidermis which is 7–10 layer at the corners and 3–4 layered is found between the corners. The cortex, consisting of 1–3 layered parenchymatous cells, is located under the collenchyma. The endodermis is distinct and composed of 1–2 layers of rectangular cells. The phloem is partly surrounded by 1–3 layers of sclerenchyma cell. The pith is present at the middle of the stem and is filled up with large parenchymatous cells. Prismatic crystals are observed in the pith area (Figure 1A).

A. 2. Petiole Anatomy

In transverse section, the petiole shows that the adaxial surface is convex and has small protrusions at the abaxial side. There are glandular and densely dendroid trichomes on both surfaces. The epidermis is composed of rectangular to oval cells and covered by a cuticle. The 3–5 layered collenchyma is located under the epidermis. There are two collateral vascular bundles in the centre and one small vascular bundle in each corner. The vascular bundle type is collateral (Figure 1B).

A. 3. Lamina Anatomy

In the transverse section of the lamina, the upper and the lower epidermis are covered with a cuticle layer (c. 0.5 µm). There are glandular and non-glandular trichomes on both sides. Both epidermis layers consist of a single layer, rectangular or squarish cells. The stomata cells are present on both sides of epidermis; amphistomatic (Figure 1C). The leaf is of the bifacial. The palisade cells are elongated rectangularly, (1–) 2 layered and the spongy parenchyma cells are 2–3 layered with large intercellular spaces. The vascular bundle is the collateral type and surrounded by sclerenchyma cells (Figure 1D).

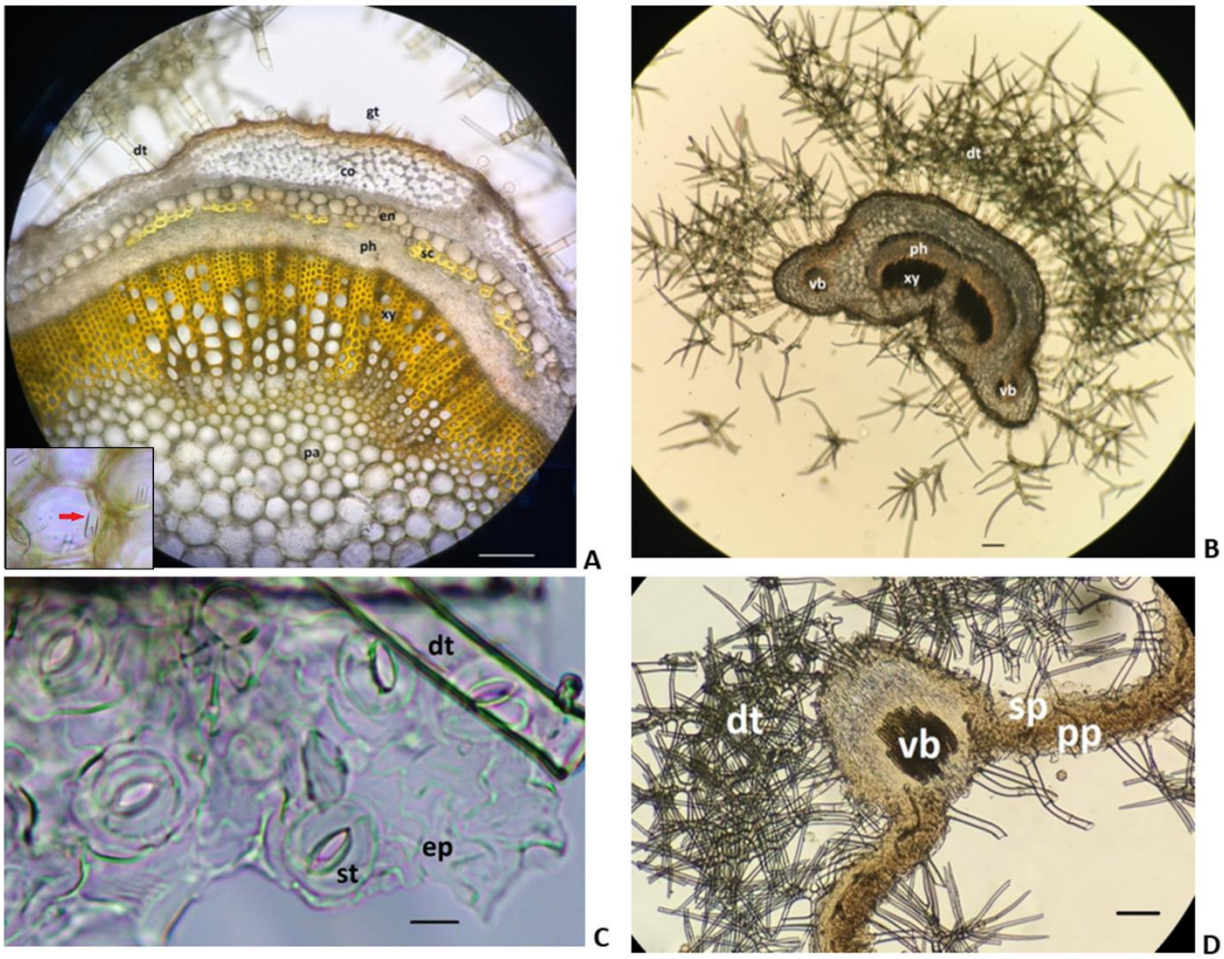

Figure 1. ***A****- Cross-sections of the stem (Scale: 100 µm),* ***B****- Cross-section of the petiole (Scale: 100 µm)* ***C****- Lower epidermis (Scale: 10 µm),* ***D****- Cross-section of the leaf blade (Scale: 100 µm) dt: dendroid trichomes; gt: glandular trichomes; co: collenchyma; sc: sclerenchyma; xy: xylem; ph: phloem; en: endodermis; pa: parenchymatic cells; vb: vascular bundle; st: stomata; ep: epidermis; sp: spongy parenchyma; pp: palisade parenchyma*

A. 4. Trichome Micromorphology

Different trichome types are observed. LM and SEM micrographs of trichomes of *B. pseudodictamnus* subsp. *lycia* are given in Figures 2 and 3. The densest trichome type is dendroid trichomes on the whole part of the plant. It is branched longitudinally at the nodes of the uniseriate long stalk cells. Multicellular non-glandular trichomes are found on the petiole. Different glandular trichomes are identified on the whole parts: unicellular stalked with unicellular head trichome, unicellular stalked with bicellular head trichome, unicellular stalked with 8 cells head trichome (Lamiaceae type glandular trichome).

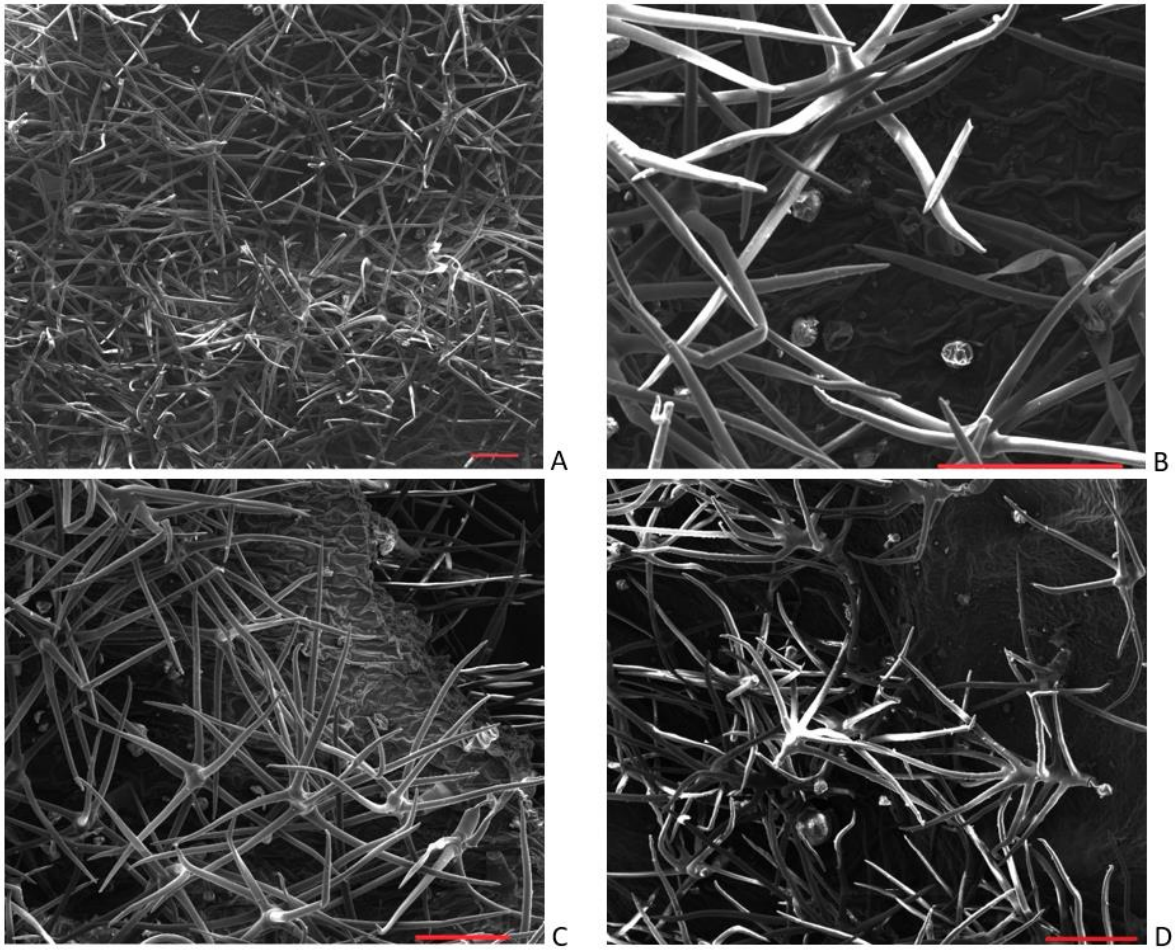

Figure 2. *SEM micrographs of trichomes of Ballota pseudodictamnus subsp. lycia. Stem* ***(A)****, petiole* ***(B)****, lamina* ***(C)****, calyx* ***(D)****. Scale bars: 100 µm*

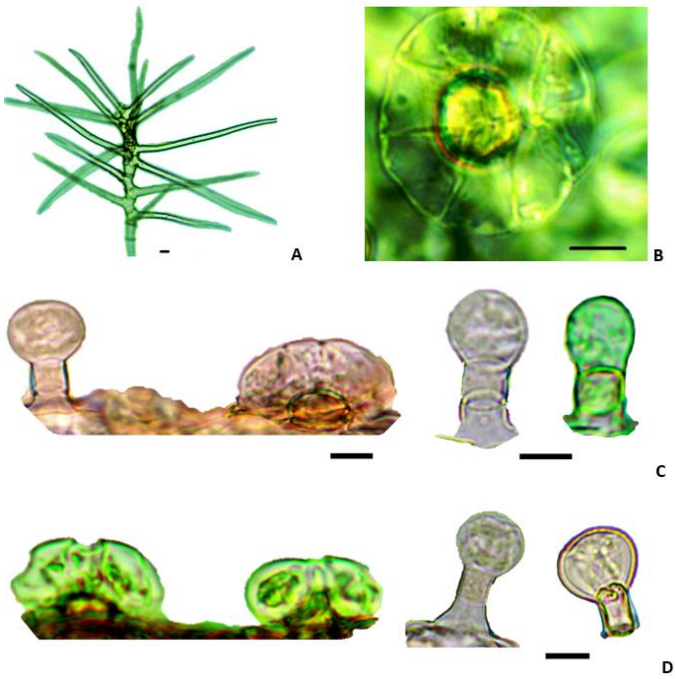

Figure 3. *Light micrographs of trichomes of Ballota pseudodictamnus subsp. lycia.* ***A****- Dendroid trichome* ***B****- unicellular stalked with 8 cells head trichome on leaves* ***C****- glandular trichomes (capitate and peltate) on petiole* ***D****- glandular trichomes (peltate and capitate) on stem. Scale bars: 10 µm*

IV. DISCUSSION

According to Metcalfe & Chalk [15], the arrangement of the collenchyma in the stem is diagnostic value and also the vascular structure of the petiole is taxonomic interest for the family Lamiaceae. A few anatomical studies have been conducted about some taxa of the genus *Ballota*. Some anatomical properties show differences between the taxa.

Trichomes micromorphology of Egyptian *Ballota* was examined by Osman [18]. He showed the significant variability of the indumentum among taxa and so that, provided valuable characters in the identification of species. He also presented an identification key for trichomes. According to his results *B. pseudodictamnus* (L.) Benth. subsp. *pseudodictamnus* has prickles, unicellular and dendritic trichomes as non-glandular. Besides that, it has unicellular to a multicellular head and unicellular stalked trichomes as glandular trichomes. In our study *B. pseudodictamnus* subsp. *lycia* has dendroid and multicellular non-glandular trichomes in addition to this same glandular trichomes types of *B. pseudodictamnus* subsp. *pseudodictamnus*. Trichomes differences between these two subspecies are prickles, unicellular and multicellular non-glandular trichomes. Trichomes are also a useful key characters in Flora of Turkey [3]. A group of taxa, including *B. pseudodictamnus* subsp. *lycia*, are distinguished from others by trichomes. So that, the presence of dendritic and stellate trichomes has a great systematic value for taxa division. Şahin *et al*. [16] studied the morphology and anatomy of *B. nigra* L. subsp. *nigra*. They identified different trichome types in the stem and leaves, and observed idioblasts in the pith region. Osman [17] studied anatomy and palynology of the genus *Ballota* from Eygpt. One of them was *B. pseudodictamnus* subsp. *pseudodictamnus*. The anatomy of stem and petiole was examined. The raphide crystals were identified in the pith of four studied taxa (*B. damascene* Boiss., *B. pseudodictamnus* (L.) Benth. subsp. *pseudodictamnus*, *B. kaiseri* Täckh. and *B. undulata* (Sieber ex Fresen.) Benth., except *B. saxatilis* Sieber ex C.Presl). Two types of collenchyma (annular and lamellar) were observed in the stem of *B. pseudodictamnus* subsp. *pseudodictamnus*, annular collenchyma at the

corners (6 – 12 layers), and lamellar collenchyma in between the corners (3 – 4 layers). In our study, prismatic crystals were present in the pith area of the stem. Angular collenchyma at the corners and lamellar collenchyma between the corners were observed in the stem. Differences in crystal types and collenchyma types can be useful in distinguishing subspecies of *B. pseudodictamnus*.

V. CONCLUSIONS

In conclusion, the stem, petiole, lamina anatomy, and trichome micromorphology of *B. pseudodictamnus* subsp. *lycia* are studied in detail. The presence of dendritic and stellate trichomes is a useful key character for this taxon in Flora of Turkey. Trichomes differences between the two subspecies of *B. pseudodictamnus* are prickles, unicellular, and multicellular non-glandular trichomes. According to our data and the literature, anatomy studies (presence of glandular and non-glandular trichomes, stomata character, vascular structure, etc.) and trichome micromorphology can be useful tools in the taxonomy of the genus *Ballota*. Anatomy of medicinal plants is also providing distinctive character to identify them and used in pharmacopeias and monographs.

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