Black Sea Journal of Engineering and Science
doi: 10.34248/bsengineering.1324086

Open Access Journal
e-ISSN: 2619 – 8991

Research Article
Volume 7 - Issue 1: 43-54 / January 2024

ANATOMICAL, ECOLOGICAL AND TRICHOME MICRO-MORPHOLOGICAL FEATURES OF TWO *MARRUBIUM* L.TAXA (LAMIACEAE)

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Abstract: The anatomical, ecological and trichome micro-morphological features of two taxa (*Marrubium amasiensis* and *Marrubium parviflorum* subsp. *parviflorum*) belonging to the genus *Marrubium* were determined and these features were compared. *M. amasiensis* is endemic and is distributed only around Amasya in Türkiye. In anatomical examinations, cross sections were taken from the root, stem, petiole, leaves and surface sections were taken from stem, petiole, and the lower and upper surfaces of the leaves. When the anatomical findings were examined, multi-layered lamellar collenchyma layer was encountered at the corners of the stem. The studied taxa have equifacial leaves in terms of mesophyll structure. Stomata of *M. amasiensis* are anomocytic and rarely anisocytic type, while stomata of *M. parviflorum* subsp. *parviflorum* are anomocytic type. The margins of the adjacent cells of the stomata in both taxa are very undulate. Eglandular and glandular trichomes were seen on vegetative organs of the two taxa. The eglandular trichomes are stellate, dendroid, marrubioid and simple unicellular types, while glandular trichomes are capitate and peltate types. Dense stellate trichomes were found in the vegetative organs of the studied taxa. Since the head and stalk cell numbers of capitate trichomes showed variation, these trichomes were divided into subtypes. Three subtypes of capitate trichomes were found on the vegetative organs of *M. parviflorum* subsp. *parviflorum*, while four subtypes of capitate trichomes were found on the vegetative organs of *M. amasiensis*. Soil samples were taken during flowering periods of taxa, physical and chemical properties were determined. Some similarities and differences were detected in the anatomical, ecological and trichome micro-morphological features of the studied two taxa. It has been emphasized that these different features will be used as valuable taxonomic characters in distinguishing of the two taxa.

Keywords: *Marrubium* taxa, Anatomy, Ecology, Trichome micro-morphology, Lamiaceae, Türkiye

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Received: July 07, 2023
Accepted: November 24, 2023
Published: January 15, 2024

Cite as: Koçak KV, Kandemir N. 2024. Anatomical, ecological and trichome micro-morphological features of two *Marrubium* L. taxa (Lamiaceae). BSJ Eng Sci, 7(1): 43-54.

1. Introduction

Marrubium L. genus belongs to the family Lamiaceae and includes annual and perennial plant species. The *Marrubium* taxa are mainly distributed in the Irano-Turanian and Mediterranean phytogeographic regions. The endemism rate of this genus in the Mediterranean phytogeographical region (88%) is higher than in the Irano-Turanian phytogeographical region (71%). The genus is represented by 27 taxa and 23 species in Türkiye and rate of endemism is 59% (Celep and Dirmenci, 2017).

Some members of the genus have medicinal and ethnobotanical importance. They are used to treat various diseases such as bronchitis, respiratory disorders, tuberculosis, asthma, skin damage, ulcers, boils, rheumatism, stomach disorders, cough, inflammation, myocardial infarction, edema, ear pain, appetizer, high blood pressure, spasm, flatulence, dyspepsia, and women infertility (Meyre-Silva and Cechinel-Filho, 2010; Yousefi et al., 2014; Okur et al., 2019; Benzidane et al., 2020). In addition, the above-ground parts of *Marrubium* species, known as "dağ çayı, bozotu, kalartopu, yabaniderme, çalba, şapla, kukasotu" in Anatolia, are used as a pain reliever, antipyretic, expectorant and menstrual-reducing (Sargın et al., 2015; Tuzlacı, 2016; Selvi et al., 2022).

The genus is characterized by flowers arranged densely on the stems, leaves with toothed margins, having many lateral branches, the presence of stamens in the corolla tube, the beard of the calyx throat, the number of teeth in the calyx, the presence of hairs inside the corolla tube and stellate trichomes covering all organs (Ahvazi et al., 2016; Çalı, 2017; Koçak and Kandemir, 2023). The stellate trichomes are multicellular and branched at the tip of the stalk cell to form star shaped (Xiang et al., 2010). The branched eglandular trichomes (dendroid) are branched along the stalk cell (El-Deen Osman, 2012). The eglandular trichomes were reported on the vegetative and generative organs of the Lamiaceae taxa. These trichomes were divided into four types (simple

unicellular, simple multicellular, branched unicellular and branched multicellular) by Cantino (1990). On the other hand, two main types of glandular trichomes-peltate and capitate-were reported in some species of *Marrubium* genus (Dmitruk and Haratym 2014; Haratym and Weryszko-Chmielewska, 2017; Gyuzeleva et al., 2022; Koçak and Kandemir, 2023). Some taxa of the Lamiaceae family have the peltate trichomes with four, eight and twelve celled head in a single circle (Kamatou et al., 2006). The peltate trichomes were abudantly seen on the vegetative organs of some taxa in the Lamiaceae family. However, these trichomes were rarely seen on the reproductive organs of some taxa in this family (Serrato-Valenti et al., 1997; Kandemir, 2011). In capitate trichomes, head and stalk cell numbers are highly variable and these trichomes are subdivided into according to stalk and head cell numbers and head shape. The capitate ones were frequently found in the Lamiaceae taxa (Corsi and Bottega, 1999; Kandemir, 2011; El-Deen Osman, 2012). Both eglandular and glandular trichomes are known as very significant taxonomic characters in the Lamiaceae family, since the structures of trichomes show variation in this family (El-Deen Osman, 2012; Seyedi and Salmaki, 2015; Haratym and Werszko-Chmielewska, 2017).

M. amasiensis Akgül and Ketenoglu is an endemic species limited distribution only around Amasya (Türkiye). This species is distributed in mixed forests and stony slopes. It has very important distinguishing characters such as stem is unbranched and dark green, the presence dense stellate trichomes on above of the stem, stem leaves being ovate to rounded, bracteoles being of equal in length to the calyx tube, calyx teeth having 10 equal or subequal. lengths and the corolla yellowish white, the pollen ornament reticulate, and the seeds oblong shaped (Akgül et al., 2017). In the areas where the species is distributed, activities such as road works and overgrazing have been encountered. This situation endangers the populations of the locally distributed and endemic species. Therefore, the species is placed in the CR category by Akgül et al. (2017). *M. parviflorum* Fisch. & Mey. subsp. *parviflorum* is a widely distributed taxon in Türkiye. The subspecies is distinguished from other *Marrubium* taxa with its morphological characters such as light green and grayish green color, dense lanate trichomes, protruding calyx teeth protruding outwards, teeth 1/3 spiny, 10 in number, cylindrical structure and no back-curving.

The aim of this study is to reveal the taxonomic importance of the anatomical, ecological and trichome micro-morphological features of two *Marrubium* taxa, which are morphologically similar to each other and to compare these features.

2. Materials and Methods

Plant and soil samples were taken from the areas where the taxa naturally spread. The taxonomic descriptions of these taxa were made according to Akgül et al. (2017) and Cullen (1982). Some of the plant samples were dried up according to standard herbarium techniques and stored, some of them were fixed in 70 % alcohol for anatomical investigations. In anatomical examinations, the cross sections of root, stem, petiole and leaf and surface sections of stem, petiole and leaves were taken by hand. Sarture reagent was applied to cross and surface sections (Çelebioğlu and Baytop, 1949). Anatomical investigations were performed using an average of 15 plant specimens which were kept in 70% alcohol. Photographs were viewed using the 10X and 40X lenses of the Leica ICC50 HD microscope. Determining the micro-morphology of trichomes on the vegetative organs of studied taxa were made using 40X magnification of the Leica ICC50 HD microscope. Trichomes were classified according to Navarro and El Oualidi (2000), El-Deen Osman (2012) and given in Tables 1 and 2. Soil samples were taken 1-2 kg from 0-20 cm depth and brought to the laboratory in polyethylene bags. These samples were dried in the laboratory and passed through a 2 mm sieve and made ready for physical and chemical analysis. Physical and chemical analyzes of soil samples were performed in Amasya University Central Research and Application Laboratory Application and Research Center. Soil texture, total salinity, calcium carbonate, pH, nitrogen, phosphorus, potassium and organic matter contents of the soil samples were determined according to standard methods (Kaçar, 1995). Mean and standard deviation values of soil analysis results were shown in Tables 3 and 4. The localities where the taxa were collected were listed below;

Marrubium amasiensis;

A5 Amasya: Direkli Village, Northern slopes of Sakarat Mountain, Karanlıkönü location, clearings in mixed and *Quercus* forest slopes, 1100-1400 m., 09.07.2020, Koçak and Kandemir.

A5 Amasya: Roadsides in Direkli Village, 1225m., 05.07.2020, Koçak and Kandemir.

A5 Amasya: Surroundings of Direkli Village, calcareous rock slopes, 1200m., 05.07.2020, Koçak and Kandemir.

A5 Amasya; Open areas between Direkli Village and Sulugöl, 1400 m., 05.07.2020, Koçak and Kandemir.

Marrubium parviflorum subsp. *parviflorum;*

A5 Amasya: Next to the TV tower, above Ormanözü Village, open areas and roadsides, 1500 m., 14.07.2020, Koçak and Kandemir.

A5 Amasya: Ormanözü Village, stony slopes by the roadside, 1250 m., 14.07.2020, Koçak and Kandemir.

A5 Amasya: Roadsides in Direkli Village, 1225m., 05.07.2020, Koçak and Kandemir.

A5 Amasya: Above Direkli Village, open areas, 1300 m., 05.07.2020, Koçak and Kandemir.

A5 Amasya: Aydınca Şehzade Village open spaces, 1100 m., 09.07.2020, Koçak and Kandemir.

A5 Amasya: Between Sultançayırı Gümüş Town, roadsides Sultançayırı, 1000 m., 14.07.2020, Koçak and Kandemir.

2.1. Statistical Analysis

The Mann Whitney U test was used to determine whether there was a difference between the results of the soil analysis of the studied taxa (Büyüköztürk, 2001). However, no statistical difference was found between the soil analysis results of the two taxa (P>0.05) (Table 5).

3. Results

3.1. Anatomical Features of *Marrubium amasiensis*

3.1.1. Root

It is observed that the root of the studied taxon has a secondary thickening. The periderm layer on the outermost surface is 6-7 layered (Figure 1 A). The cortex is beneath the periderma and consists of 9-11 layered, oval or rectangular shaped parenchyma cells. Phloem elements are obvious. Small groups of phloem sclerenchyma cells were located above the phloem (Figure 1 B). Cambium is distinguishable and 2-4 layered. The secondary xylem consisted mostly of sclerenchymatic cells and tracheids (Figure 1 C). The pith region is completely filled with primary xylem elements.

3.1.2. Stem

Cross-section of the stem is obviously quadrangular in shape. The epiderma consists of uniseriate, square shaped and small cells. The cuticle is thick and with dense micropapillae. Lamellar collenchyma is located immediately beneath the epiderma. The lamellar collenchyma at the corners is 8-10 layered (Figures 1 D, E). In the other parts of the stem, the lamellar collenchyma is 3 layered. Parenchyma is 3-4 layered, oval and elongated shaped, thin walled (Figure 1 F). Beneath of the cortex parenchyma, small groups of phloem sclerenchyma cells are located. The sclerenchyma is 3 layered. Phloem is in narrow region and its elements are clearly seen. Vascular cambium is 4-5 layered. The xylem is in large region and the tracheas are chain-shaped and quite large. Xylem elements are thick-walled. The pith region is composed of large, hexagonal and round shaped cells (Figure 1 G). Needles and solitary crystals were found in pith region.

3.1.3. Petiole

The cuticle is thick and with dense micropapillae. The epidermis cells are rectangular shaped and large. Collenchyma is 2-3 layered and parenchyma is 5-6 layered, large and hexagonal shaped (Figures 1 K, L). Both crystal granules and solitary crystals were obtained in the cortex (Figure 1 L). The endoderma layer around the vascular bundles contains of one layered, large and flattened cells. Phloem and xylem elements are clearly visible. The tracheas of the xylem are large and chain shaped. There are four vascular bundles in the petiole. Two of the vascular bundles are in the middle of petiole and quite large (Figures 1 H, K). The other two vascular bundles are at the margins of the petiole and smaller.

3.1.4. Leaf

In leaf cross sections, the upper and the lower epidermis cells are uniseriate, quadrangular shaped. However, upper epidermis cells are larger than lower epidermis cells. Papillae were observed in both epidermis cells. On the epiderma, cuticle is thick and with micropapillae. Mesophyll is equifacial type (Figure 1 M). Palisade parenchyma beneath the upper epiderma is 1-2 layered (usually 1-layered), long, cylindrical shaped and with abundant chloroplast. The palisade parenchyma in the lower epiderma is 1-2 layered, cylindrical and shorter. Spongy parenchyma is composed of 2-3 layered, round or irregular shaped and with large intercellular spaces. Vascular bundles in the middle of the mesophyll are uniseriate and collateral type. The walls of the adjacent epidermis cells around the stomata are clearly undulate. Stomata are generally anomocytic type and rarely anisocytic type (Figure 1 O). The numbers of the adjacent epidermis cells are 3-4, usually 4. A large vascular bundle was observed in cross-sections taken from the leaf midrib (Figure 1 N).

3.1.5. Micro-morphology and localizations of eglandular and glandular trichomes on the vegetative organs of *Marrubium amasiensis*

Both eglandular and glandular trichomes were seen on the epiderma of the stem, petiole and leaves of *M. amasiensis* (Table 1). The eglandular trichomes are simple unicellular, stellate, dendroid and marrubioid types. The simple unicellular trichomes were obtained only in the petiole. This kind of trichomes has only one cell and is unbranched structure. The marrubioid trichomes are generally 3 celled, elongated and curved. This kind of the eglandular trichomes was obtained on the stem and leaves (Figure 1 P). The dendroid trichomes are multicellular branched along the stalk cell. Dense dendroid trichomes were observed on the upper and lower surfaces of the leaves of this species (Figures 1 R, X, Y, Z). However, dendroid trichomes were not found on the stem and petiole of *M. amasiensis.* The third type of eglandular trichomes is stellate ones. Dense stellate trichomes were seen on the stem, petiole, both the lower and upper surfaces of the leaves of *M. amasiensis* (Figures 1 S, T). Some of the stellate trichomes are less branched, the middle branch is two-celled, long and curved, other branches are approximately equal in length, unicellular, thin and curved. The middle branches of the stellate trichomes on the upper epiderma of leaves are quite long and multicellular, the lateral branches are thin, usually unicellular. On the lower epiderma, the stellate trichomes are few and finely branched, the middle branch is longer and all unicellular. Also, dense stellate trichomes were detected on calyx teeth, calyx surface and corolla of this species. Glandular trichomes are peltate and capitate types. The peltate trichomes have a mainly with short stalk and a large four or eight-celled head. This kind of glandular trichomes was dense detected on both upper and lower of leaves (Figures 1 V, Y). But, 4-celled head kind of peltate trichomes was observed on the stem and petiole. The capitate trichomes were divided into four subtypes: I. unicellular short-stalked and with unicellular or bicellular head, II. unicellular long-stalked and with unicellular or bicellular head, III. unicellular long-stalked,

with bicellular head and unicellular short neck. IV. unicellular short-stalked and with a cup-shape unicellular head. The unicellular short stalked and with unicellular or bicellular head capitate trichomes (type I) were rare observed on the stem and petiole, while these trichomes were dense observed on the upper and lower surfaces of leaves (Figures 1 W, X, Y, Z). On the other hand, type II capitate trichomes were only found on the petiole and upper and lower surfaces of leaves. The types III and IV capitate trichomes were dense found on the upper and lower surfaces of leaves.

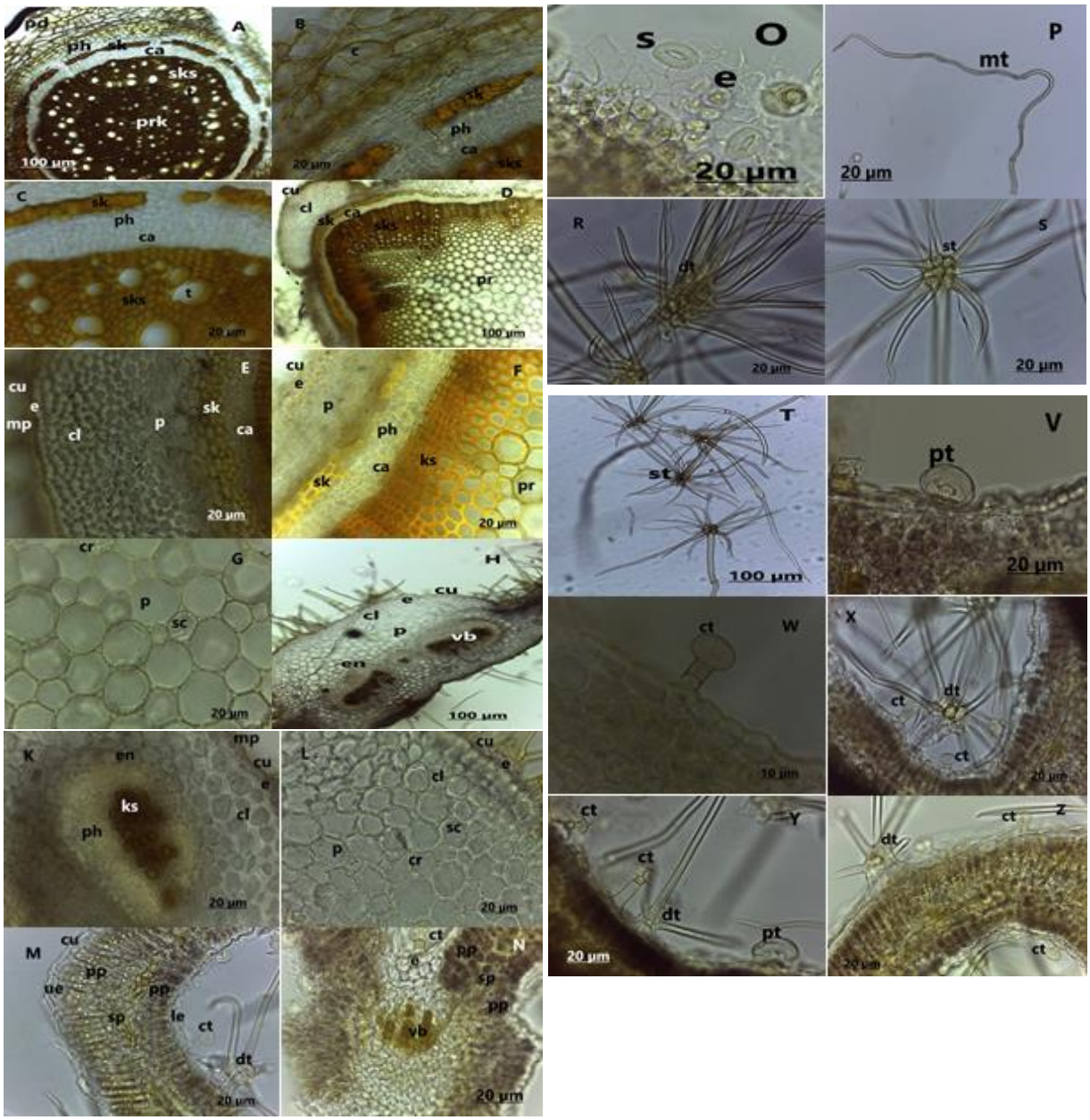

Figure 1. Root, stem and leaf anatomical structures and trichome types of *Marrubium amasiensis*. A=General structure of root, B=Cortex and phloem regions of root, C=Secondary xylem and phloem regions of root, D=General structure of stem, E=Cortex region of stem, F=Cortex, xylem and phloem regions of stem, G=Pith region of stem, H=General structure of petiole, K=Vascular bundle of petiole, L=Crystals in the petiole, M=General structure of leaf, N=Cross section of the leaf midrib, O=Stomata in surface section of leaf (lower surface), P=Marrubioid trichome in the leaf, R=Dendroid trichome in the leaf, S=Stellate trichome in the leaf, T=Stellate trichome in the stem, V=Peltate trichome in the leaf, W=Capitate trichome in the petiole, X=Dendroid and capitate trichomes in the leaf, Y=Dendroid, capitate and peltate trichomes in the leaf, Z=Dendroid and capitate trichomes in the leaf. pd= periderma, ph=pholem, t=trachea, sk=sclerenchyma, ca=cambium, sks=secondary xylem, prk=primary xylem, c=cortex, cu=cuticle, cl=collenchyma, p=parenchyma, pr=pith region, e=epiderma, mp= micropapillae, ks=xylem, cr=crystal granules, sc=solitary crystal, en=endoderma, ue=upper epiderma, pp=palisade parenchyma, sp=spongy parenchyma, le=lower epiderma, vb=vascular bundle, s=stoma, ct=capitate trichome, dt=dendroid trichome, mt=marrubioid trichome, st=stellate trichome, pt=peltate trichome.

Table 1. Trichome types and distributions on organs of *Marrubium amasiensis*

Organs of *M. amasiensis*	Type I capitate	Type II capitate	Type III capitate	Type IV capitate	Peltate trichomes	Simple eglandular	Stellate eglandular	Dendroid eglandular	Marrubioid eglandular
Stem	+	_	_	_	+	_	+++	_	+
Petiole	+	+	_	_	+	+	+++	+	_
Margin and veins of leaf	+++	++	++	++	++		+++	++	+
Lamina of leaf	+++	++	++	++	++		+++	++	+
Calyx teeth	++	_	_	_	+	+	++	+	_
Outer surface of calyx	++	+	_	_	+	+	++	_	_
Corolla	+	_	_	_	+	++	++	_	_

-absent, +scarce, ++ dense, +++ abudant

Type I capitate= unicellular short stalked and with unicellular or bicellular head, Type II capitate= unicellular long stalked and with unicellular or bicellular head, Type III capitate= unicellular long-stalked, with bicellular head and unicellular short neck, Type IV capitate= unicellular short-stalked and with a cup-shape unicellular head, Peltate trichomes= short stalked and a large four or eight-celled head, Stellate= star shaped trichome, Dendroid= multicellular branched trichome, Marrubioid= unicellular or multicellular and curly in the tip trichome

3.2. Anatomical Features of *Marrubium parviflorum* Subsp. *parviflorum*

3.2.1.Root

Periderma layer is located at the outermost part of the root (Figure 2 A). It is 5-6 layered. The cortex beneath the periderma layer is composed of large and ovoid parenchyma cells. Cambium consists of flat cells and it is 3 layered. Phloem is distinguishable and in a narrow region. It contains small sclerenchyma cell groups. Xylem is composed of tracheary elements. Tracheas are sparse and rather large (Figure 2 B). Dense sclerenchymatic cells were seen in the xylem. In the center of root, there are primary xylem elements.

3.2.2. Stem

Cuticle layer is thick and with dense micropapillae. The stem is apparent quadrangular shaped (Figure 2 C). Epiderma is uniseriate, small celled and rectangular shaped. Papillae were obtained in the epidermis cell. Lamellar collenchyma was observed at the corners of the stem. This collenchyma is 8-11 layered and covers a large area at the corners (Figure 2 D). Parenchyma cells beneath the epiderma are 5-6 layered, small and with chloroplasts. Crystal granules were observed in these parenchymatic cells. Other parenchyma cells are 3-4 layered (generally 4 layered), oval shaped, large and without chloroplast. Sclerenchyma is 4 layered (Figure 2 E). Phloem elements are conspicuous and consist of small cells. The cambium between the xylem and phloem is 1-3 layered and small celled. Xylem elements are evident and in a wide region. Dense sclerenchymatic cells were found in the xylem. There is a large pith region in the centre of the stem (Figures 2 C, F). The pith regions consist of large and orbiculate shaped parenchymatic cells. Pith parenchyma cells are smaller towards the xylem. In the pith region, there are needles and solitary crystals.

3.2.3. Petiole

The epiderma is uniseriate, quadrangular and with rare papillae. The cuticle is thick and with very dense micropapillae. On the xylem, 4-6 layered lamellar collenchyma and 4-5 layered parenchyma were seen (Figure 2 H). The parenchyma consists of hexagonal and round shaped cells. Chloroplasts do not have in the parenchyma cells. On the phloem, 4 layered lamellar collenchyma and 4 layered, oval shaped, large celled parenchyma were found. There are two large vascular bundles in the middle of petiole (Figure 2 G). In the margins of the petiole, smaller vascular bundles were observed. The parenchyma cells around the vascular bundles in the margins are small. Dense chloroplasts were observed in 3-4 layered of these parenchyma cells, while chloroplasts were not observed in 1-2 layered of parenchyma cells. The parenchyma cells around the large vascular bundles are rather large. These cells rarely contain crystal granules. Xylem and phloem elements were clearly seen. The endoderma layer around the vascular bundles consists of a single layered and large cell.

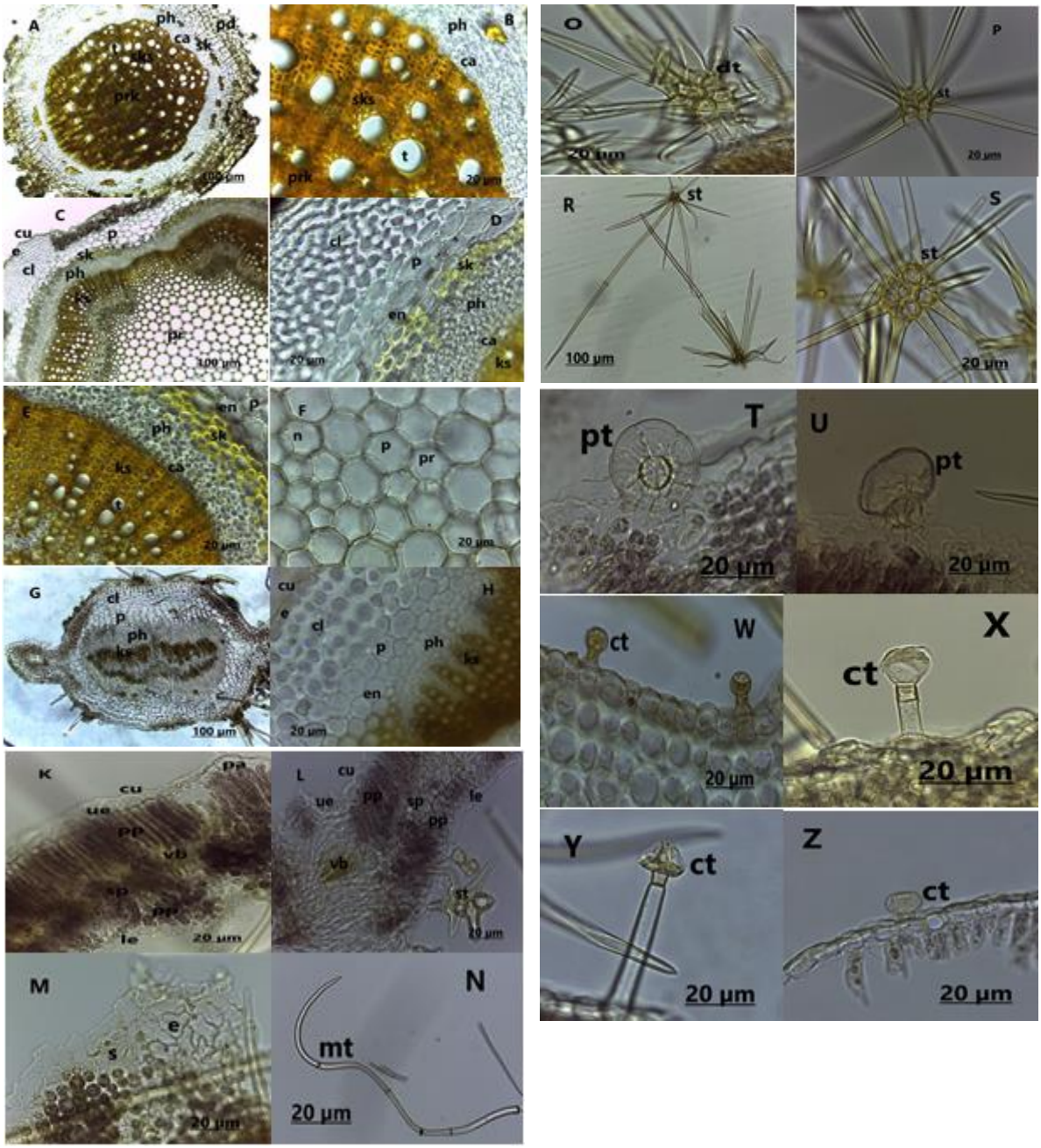

Figure 2. Root, stem and leaf anatomical structures and trichome types of *Marrubium parviflorum* subsp. *parviflorum*. A=General structure of root, B=Secondary xylem and phloem region of root, C=General structure of stem, D=Cortex and phloem regions of stem, E=Xylem and phloem regions of stem, F=Pith region of stem, G=General structure of petiole, H=Vascular bundle and cortex region of petiole, K=General structure of leaf, L=Cross section of the leaf midrib, M=Stomata in surface section of leaf (lower surface), N=Marrubioid trichome in the leaf, O=Dendroid trichome in the leaf, P=Stellate trichome in the stem, R,S=Stellate trichome in the leaf, T,U=Peltate trichomes in the leaf, W=Capitate trichome in the stem, X,Y,Z=Capitate trichome in the leaf. pd= periderma, ph= pholem, t=trachea, sk= sclerenchyma, ca=cambium, sks=secondary xylem, prk= primary xylem, c=cortex, cu=cuticle, cl=collenchyma, p=parenchyma, pr=pith region, e=epiderma, mp=micropapillae, ks=xylem, cr=crystal granules, sc=solitary crystal, en=endoderma, ue=upper epiderma, pp=palisade parenchyma, sp=spongy parenchyma, le=lower epiderma, vb=vascular bundle, pa=papillae, n=needles, s=stoma, ct=capitate trichome, dt=dendroid trichome, mt=marrubioid trichome, st=stellate trichome, pt=peltate trichome.

3.2.4. Leaf

In cross section of the leaf, the upper and lower epidermis cells are uniseriate and rectangular shaped. Upper epidermis cells are larger than the lower ones. The cuticle layer is very thick. Mesophyll layer of the leaf is of the equifacial type (Figure 2 K). The palisade parenchyma beneath the upper epiderma consists of 1 layered, very long cylindrical parenchyma cells. On the lower epiderma, palisade parenchyma is 1-2 layered (usually 1-layered) and short cylindrical shaped. Sponge

parenchyma is 2-3 layered and usually 2 layered. Vascular bundles are collateral type. The stomata are anomocytic type and they are mostly occur on the lower surface of leaf. The walls of the adjacent epidermis cells around the stomata are clearly undulate (Figure 2 M). The numbers of the adjacent epidermis cells are 4-5, usually 5. Crystal granules were seen in the epidermis cells. In the cross sections taken from the main vein, there are 3-4 layered of lamellar collenchyma layer on the phloem and xylem side. The vascular bundle in the leaf midrib is single and rather large (Figure 2 L).

3.2.5. Micro-morphology and localizations of eglandular and glandular trichomes on the vegetative organs of *Marrubium parviflorum* subsp. *parviflorum*

The eglandular trichomes on the vegetative organs of *M. parviflorum* subsp. *parviflorum* were simple, stellate, multicellular branched (dendroid) and marrubioid types (Table 2). The simple eglandular trichomes are unicellular or bicellular, long. These trichomes were observed on the stem, petiole and leaves. On the stem, the upper and lower surfaces of the leaves of this subspecies, rarely dendroid and marrubioid trichomes were detected (Figures 2 N, O). The marrubioid trichomes are usually bicellular and curved trichomes (Figure 2 N). There are dense stellate trichomes on the stem, petiole, both leaf surfaces (Figures 2 P, R, S). The middle and lateral branches of stellate trichomes on the lower and upper surfaces of the leaves are almost equal in length. However, the middle branches of the stellate trichomes on the stem and petiole are longer than the lateral branches. Also, there are abundant stellate trichomes on calyx teeth, calyx surface and corolla of this subspecies. The peltate trichomes in *M. parviflorum* subsp. *parviflorum* were rarely found on the stem and petiole. However, the peltate trichomes were densely observed on the both leaves (Figures T, U). The capitate trichomes in this taxon are of three subtypes: I. unicellular short stalked and with a unicellular or bicellular head and II. unicellular long stalked and with unicellular or bicellular head, III. unicellular long-stalked, with bicellular head and unicellular short neck. The unicellular short stalked and with unicellular or bicellular head capitate trichomes (type I) were densely seen on the petiole, upper and lower surfaces of leaves, while these capitate trichomes were rarely seen on the stem between eglandular trichomes (Figure 2 W, Z). The type II capitate trichomes were rarely encountered on the petiole and both surfaces of leaves of this taxon, whereas the type III capitate trichomes were encountered on both surfaces of leaves (Figure2 X, Y).

Table 2. Trichome types and distributions on organs of *Marrubium parviflorum* subsp. *parviflorum*

Organs of *M. parviflorum* subsp. *parviflorum*	Type I capitate	Type II capitate	Type III capitate	Peltate trichomes	Smiple eglandular	Stellate eglandular	Dendroid eglandular	Marrubiod eglandular
Stem	+	_	_	+	+	+++	+	+
Petiole	++	+	_	+	+	+++	+	+
Margin and veins of leaf	+++	++	+	++	+	+++	+	+
Lamina of leaf	+++	++	+	++	+	+++	+	+
Calyx teeth	+	_	_	_	_	++	_	_
Outer surface of calyx	+	–	–	–	–	++	_	+
Corolla	+	_	_	_	_	++	_	_

3.3. Physical and Chemical Characteristics of Soil Samples of *Marrubium* Taxa

3.3.1. *Marrubium amasiensis*

Soil samples of *M. amasiensis* were taken from 4 different localities (Amasya). The pH, salinity and $CaCO_3$ values of the soil samples varied between 7.54-7.94, 0.36-0.65 % and 12.60-15.50 %, respectively. The species is distributed in sandy and sandy-salty textured soils. The organic matter and nitrogen values of the soil samples were determined as 3.75-4.20 % and 0.18-0.98 %. Phosphorus and potassium values of soil samples were found to be 32.80-42.22 mg/kg and 102.4-195.3 mg/kg (Table 3).

3.3.2. *Marrubium parviflorum* subsp. *parviflorum*

M. parviflorum subsp. *parviflorum* were taken from 6 different localities (Amasya). The CaCO3, pH and salinity values of the soil samples were observed as 1.50-15-50 %, 6.50-8.06 and 0.46-0.69 %, respectively. The texture of the soil is sandy and sandy-salty. Nitrogen and organic matter values of soil samples were determined as 0.15-0.66 % and 3.30-4.95 %. Phosphorus and potassium values in soil samples were found as 32.65-48.59 mg/kg and 102.4-213.8 mg/kg (Table 4).

3.3.3. Statistical analysis of soil analysis results of the studied taxa

Mann Whitney U test was applied to soil analysis results of the these taxa. However, no statistical difference was found between the soil analysis results of the two taxa ($p>0.05$) (Table 5). This is due to the fact that the taxa grow in habitats with similar ecological conditions.

Table 3. Soil analysis results of *Marrubium amasiensis*

Localities	Texture	Salinity (%)	$CaCO_3$ (%)	pH	Organic matter (%)	N (%)	K (mg/kg)	P (mg/kg)
Sakarat Mountain	Sandy-salty	0.36	14.80	7.80	4.20	0.76	120.7	38.12
Direkli Village	Sandy-salty	0.56	15.50	7.54	3.93	0.18	102.4	42.22
Surroundings of Direkli Village	Sandy-salty	0.45	13.80	7.35	3.75	0.37	195.3	34.25
Between Direkli Village and Sulugöl	Sandy	0.65	12.60	7.90	4.01	0.98	182.5	32.80
Mean±Sd.	_	0.50±0.13	14.17±1.26	7.64± 0.25	3.97±0.18	0.57±0.36	150.2±45.57	36.8±4.22

Table 4. Soil analysis results of *Marrubium parviflorum* subsp. *parviflorum*

Localities	Texture	Salinity (%)	$CaCO_3$ (%)	pH	Organic matter (%)	N (%)	K (mg/kg)	P (mg/kg)
Ormanözü Village	Sandy-salty	0.69	14.35	6.70	4.25	0.66	187.9	32.65
Above Ormanözü Village	Sandy-salty	0.46	1.50	6.50	4.95	0.45	172.8	40,.2
Direkli Village	Sandy-salty	0.56	15.50	7.54	3.93	0.18	102.4	42.22
Above Direkli Village	Sandy-salty	0.58	13.80	7.95	3.30	0.34	156.6	36.82
Aydınca Şeyhsadi Village	sandy	0.64	12.73	7.37	3.46	0.15	132.67	41.80
Sultançayırı Gümüş Town	Sandy-salty	0,48	3.12	8.06	3.58	0.21	213.8	48.59
Mean±Sd.	_	0.57±0.09	10.2±6.17	7.35±0.64	3.91±0.61	0.33±0.19	161±397	40.4±5.39

Table 5. Mann Whitney U Test results of the studied taxa

	Salinity (%)	$CaCO_3$ (%)	pH	Organic matter (%)	N (%)	K (mg/kg)	P (mg/kg)
Mann-Whitney U	7.500	8.000	10.500	9.500	6.500	10.500	8.500
Z	-.962	-.858	-.321	-.535	-1.176	-.321	-.748
p	.336	.391	.748	.593	.240	.748	.454

4. Discussion

In the present study, the taxonomically valuable anatomical, ecological and trichome mic-morphological features of two *Marrubium* taxa were determined and these features were compared. It has been observed that these two taxa collected from the Amasya region are close to each other in terms of anatomical, ecological and trichome micro-morphological features. However, some anatomical, ecological and trichome micro-morphological features were appeared to be different in the studied taxa.

When the root cross-sections of the studied taxa were

examined, periderma were determined to have 6-7 layered in *M. amasiensis*, 5-7 layered in *M. parviflorum* subsp. *parviflorum*. The layer number of cambium is 2-4 in *M. amasiensis*, 3 in *M. parviflorum* subsp. *parviflorum*. The centers of roots of both taxa were filled with primary xylem elements. Similar anatomical features were also found in the roots of *Marrubium bourgaei* Boiss and *Marrubium heterodon* (Bentham) Boiss. Meat Ball. (Büyükkartal et al., 2016). Our results are in agreement with the data of the study mentioned above.

Stem anatomical examinations showed that the stem had well-defined collenchyma in the corners. This collenchyma is 8-10 layered in *M. amasiensis* and is 8-11 layered in *M. parviflorum* subsp*. parviflorum*. Metcalfe and Chalk (1972) stated that in each of the stem corners of many the Lamiaceae species have multilayered collenchyma as a characteristic features. The collenchyma between the corners is 2 layered in *M. amasiensis* and 2-3 layered in *M. parviflorum* subsp. *parviflorum*. The parenchyma in the cortex is 3-4 layered (generally 3 layered) in both taxa. Although these parenchyma cells are without chloroplasts in *M. amasiensis*, these cells are with chloroplasts in *M. parviflorum* subsp. *parviflorum*. Sclerenchyma is 2 layered in *M. amasiensis* and is 3-4 layered (generally 4 layered) in *M. parviflorum* subsp. *parviflorum.* The mentioned stem anatomical features (collenchyma, parenchyma and sclerenchyma layers in the cortex) were also found in some members of the Lamiaceae family distributed in Western Azerbaijan (Hatamneia et al., 2008), *M. bourgaei* and *M. heterodon* (Büyükkartal et al., 2016), *Marrubium lutescens* Boiss. and *M. cephalanthum* Boiss. & Noë subsp. *akdaghicum* (Tuylu et al.,2017), some *Marrubium* taxa distributed in Iranian (Talebi et al., 2019a), *Marrubium friwaldskyanum* Boiss. and *Marrubium peregrinum* L. (Gyuzeleva et al., 2022). However, the number of layers of collenchyma, parenchyma and sclerenchyma in the cortex differ both in other members of the Lamiaceae family and in the studied two *Marrubium* taxa. For this reason, we think that these anatomical features in the cortex can be used to distinguish these two taxa. The importance of these anatomical features were reported in some *Marrubium* taxa by Hatamneia et al., (2008), Büyükkartal et al. (2016), Talebi et al. (2019a). In addition, Kandemir et al. (2020) in *Heliotropium* L. taxa, Kandemir et al. (2022) in *Fritillaria* L. taxa suggested that the presence of different numbers of collenchyma, parenchyma and sclerenchyma layers in the cortex were important anatomical characters in the separation of taxa. In the studied taxa, there are sclerenchyma cells groups on the phloem of vascular bundles. The presence of sclerenchyma cell groups in phloem were detected in some taxa of the Lamiaceae family (Metcalfe and Chalk, 1979; Anhar et al., 2018; Koçak and Kandemir, 2023). Needles and solitary crystals were found in the pith region of stem of both taxa. These crystals were also seen in the stem of *M. vulgare* and other the Lamiaceae taxa (Anhar et al., 2018). In addition, Anhar et al. (2018) suggested that crystals are of great importance in identification and taxon delimitation. Ryding (2010) recorded that calcium oxalate crystals are in needle shaped or polyhedral, druses and prismatic forms in the vegetative and generative organs of the Lamiaceae family species. However, similar crystal types were found in stem of these two taxa. Therefore, the types of crystals in the stem were not an important feature in the separation of these taxa.

Outline aspects of the petiole is half circle with wings in both taxa. When the petiole cross-sections of these taxa are compared, the epidermis cells in *M. amasiensis* are rectangular shaped and without papillae, while epidermis cell in *M. parviflorum* subsp. *parviflorum* are quadrangular shaped and rarely papillae. In *M. amasiensis*, cuticle is thick and with dense micropapillae, the cuticle is thick and with very dense micropapillae in *M. parviflorum* subsp. *parviflorum*. The collenchyma in *M. amasiensis* is 2-3 layered, while the collenchyma in *M. parviflorum* subsp. *parviflorum* is 4-5 layered. The number of parenchyma layer is 5-6 in *M. amasiensis*. However, parenchyma is 4 layered in *M. parviflorum* subsp. *parviflorum.* In the parenchymatic cells of *M. parviflorum* subsp. *parviflorum,* crystal granules were determined. The ends of the petiole are found curved slightly upwards in *M. amasiensis* and *M. parviflorum* subsp. *parviflorum*. Metcalfe and Chalk (1979), Kandemir (2003), Shahri et al. (2016) emphasized that the petiole anatomical structure was value in species characterization in the Lamiaceae family. The two *Marrubium* taxa in this study can be distinguished according to the differences in the number of collenchyma and parenchyma layers in the petiole, whether or not there are micropapillae on the cuticle, the presence or absence of crystal granules in parenchyma cells and the epidermis cell shape.

Although leaf anatomical features are similar in both taxa, there are some differences in leaf anatomical features. When the leaf cross-section findings of the taxa are examined, the upper and lower epidermis cells of *M. amasiensis* are rectangular shaped. But, upper and lower epidermis cells in *M. parviflorum* subsp. *parviflorum* are quadrangular shape. The mesophyll is equifacial (isolateral) structure and almost similar in both taxa. In the surface sections of the leaves, anomocytic and rarely anisocytic stomata were observed in *M. amasiensis*. The numbers of adjacent cells of the stomata are 3-4 (usually 4) in *M. amasiensis*. Stomata are anomocytic structure in *M. parviflorum* subsp. *parviflorum* and the numbers of cells adjacent of the stomata are 4-5. Although crystal granules were seen in the epidermis cells of *M. parviflorum* subsp. *parviflorum*, the crystal granules were not seen in the epidermis cells of *M. amasiensis*. Kandemir et al. (2019), Kandemir (2019) reported that the presence of crystal granules in the epidermis cells of the leaves could be used as important characters in the taxonomy of plant species. There are not variable in

aspects of midrib region of two taxa. This region is characterized by presence of parenchyma cells. However, the midrib shape is half circular shaped in *M. parviflorum* subsp. *parviflorum* and midrib shape is circular shaped in *M. amasiensis*. Some differences were observed in vascular system aspects; rounded in *M. parviflorum* subsp. *parviflorum* and crescent shaped in *M. amasiensis.* In the midrib region, the numbers of vascular bundles are one in these taxa. The results of this study showed to be different in the studied taxa regarding in dermal system, midrib region and crystals of leaves. These parameters were used to identify the relationships among species of the Lamiaceae family (Inyama et al., 2016). Looking at the data of the cross-sections of the leaves, similar anatomical results were determined in the leaves of other *Marrubium* taxa (Anhar et al. 2018; Talebi et al., 2019 b; Gyuzeleva et al., 2022). The data obtained from the leaf cross sections in this study show parallelism with the results of the studies mentioned above.

The presence of four types of eglandular trichomes was detected on the vegetative organs of studied both taxa (Tables 1, 2). The simple eglandular trichomes in *M. parviflorum* subsp. *parviflorum* are unicellular or bicellular, long, while these trichomes in *M. amasiensis* are unicellular. This kind of trichomes in *M. parviflorum* subsp. *parviflorum* was observed on the stem, petiole and leaves. But, simple eglandular trichomes in *M. amasiensis* were obtained only in the petiole. Dense dendroid trichomes were observed on the upper and lower surfaces of the leaves of *M. amasiensis,* while rarely dendroid trichomes were observed on the stem, the upper and lower surfaces of the leaves of *M. parviflorum* subsp. *parviflorum*. However, the dendroid trichomes were not seen on the stem of *M. amasiensis.* Marrubioid trichomes were detected on the leaves and stems of both taxa. There are dense stellate trichomes on the stem, petiole, both leaf surfaces of the studied two taxa. In *M. amasiensis*, the middle branches of the stellate trichomes on the upper epiderma of leaves are quite long and multicellular, the lateral branches are thin, usually unicellular. On the lower epiderma, stellate trichomes are few and finely branched, the middle branch is longer and all unicellular. In *M. parviflorum* subsp. *parviflorum*, the middle and lateral branches of stellate trichomes on the lower and upper surfaces of the leaves are almost equal in length. However, the middle branches of the stellate trichomes on the stem and petiole of *M. parviflorum* subsp. *parviflorum* are longer than the lateral branches. Moreover, there are abundant stellate trichomes on calyx teeth, calyx surface and corolla of two taxa. The multicellular branched and unbranched eglandular trichomes were also recorded in other members of the genus *Marrubium* (Dmitruk and Haratym, 2014; Ahvazi et al., 2016; Anhar et al., 2018; Gyulezeva et al., 2022; Koçak and Kandemir 2023; Akbulut and Baykan, 2023).

The peltate and capitate glandular trichomes were determined on the leaves, petiole and stems of both taxa (Tables 1,2). Peltate trichomes were rarely found on the stem and petiole of *M. parviflorum* subsp. *parviflorum* and *M. amasiensis*. Whereas this kind of trichomes are dense on the leaves of both taxa. The capitate trichomes on the vegetative organs of *M. amasiensis* were seen four subtypes, whereas these trichomes on the vegetative organs *M. parviflorum* subsp. *parviflorum* were seen three subtypes. In *M. amasisensis*, the subtype I were rarely obtained on the stem and petiole, while this kind of capitate trichomes was dense obtained on the upper and lower surfaces of leaves. In *M. parviflorum* subsp. *parviflorum*, the subtype I was densely seen on the petiole, upper and lower surfaces of leaves, while this kind of capitate trichomes were rarely seen on the stem. The subtype II capitate trichomes are found only on the petiole and upper and lower surfaces of leaves in these taxa. On the other hand, the subtype III capitate trichomes were encountered on both surfaces of leaves of these two taxa. This kind of capiate trichomes (subtype III) is more dense on the leaves of *M. parviflorum* subsp. *parviflorum*. Although the subtype IV capitate trichomes are densely seen on the upper and lower surfaces of leaves of *M. amasiensis*, this kind of capitate trichomes was not seen in other organs and leaves of *M. parviflorum* subsp. *parviflorum*. This difference in the capiatate trichomes is seen as an important distinguishing feature between two taxa.

Recently, the presence of eglandular and glandular trichomes has been reported in trichome micro-morphology studies on *Marrubium* taxa (Dmitruk and Haratym, 2014; Ahvazi et al., 2016; Çalı, 2017; Haratym and Weryszko-Chmielewska, 2017; Anhar et al., 2018, Gyulezeva et al., 2022, Koçak and Kandemir 2023). Also, both eglandular and glandular trichomes were found to be common in the other members of the Lamiaceae family and these trichomes were recorded to be very value taxonomic characters (Navarro and El Qualidi, 2000; Kandemir, 2003; Kandemir, 2011; El-Deen Osman, 2012; Seyedi and Salmaki, 2015; Koçak and Kandemir, 2023). We think that these features mentioned can be used as important taxonomic characters in the differentiation of these two taxa, since there are variations in their features such as eglandular and glandular trichome types, branching shapes, localization on organs, long and short branch lengths in stellate trichomes, sparseness and density. On the other hand, it was revealed that environmental conditions, especially altitude and arid areas, were effective in the morphological variations and density of trichomes (Ahvazi et al. 2016).

Based on soil analysis results, we suggested that *M. amasiensis* and *M. parviflorum* subsp. *parviflorum* were independent taxa with close relationships. The similarities in ecological features of the taxa were supported by statistical analysis. Because no statistically a significant difference was found between the soil analysis results of the taxa. This may be due to the fact that taxa are distributed in habitats with similar ecological characteristics.

5. Conclusion

To sum up, two *Marrubium* taxa collected from Amasya vicinity (*M. amasiensis* is endemic and *M. parviflorum* subsp. *parviflorum* is common) appeared as closely related taxa in terms of anatomical, ecological and trichomes micro-morphological features, distinct taxa. However, some differences were seen such as the number of periderma layer in the root, the number of collenchyma layer in the corners of the stem, the number of collenchyma, parenchyma and sclerenchyma layers in the cortex, the presence or absence of dendroid trichomes on the stem, the number of collenchyma and parenchyma layers in the petiole, the presence of crystals in the leaves and the shape of the epidermis cells in the leaves, midrib region of leaves, differences in the capitate glandular trichomes, the number of cells adjacent to the stoma and stoma type. These characters were stated to be valuable taxonomic characters in distinguishing of these two taxa. Moreover, anatomical and trichome micro-morphological data obtained from this study may be useful for future taxonomic studies in *Marrubium* genus. No significant differences were found in the soil analysis results of these two *Marrubium* taxa.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	K.V.K.	N.K.
C	20	80
D	20	80
S	10	90
DCP	50	50
DAI	20	80
L	60	40
W	20	80
CR	30	70
SR	20	80
PM	20	80
FA	50	50

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

Acknowledgements

This study was produced from Kamer Volkan Koçak's master's thesis.

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