
Araştırma Makalesi / Research Article

Investigation of Anatomical, Ecological and Trichome Morphological Features of Two Endemic *Symphytum* Species

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Abstract

In this study, anatomical, ecological and trichome morphology features of *Symphytum bornmuelleri* and *Symphytum aintabicum* were compared and identified their valuable features taxonomically. *S. bornmuelleri* and *S. aintabicum* are endemic Euxine and East Mediterranean element of Turkey respectively, and have limited distribution. Anatomical investigations of species were made using light and stereo-microscopes. In anatomical investigations, the cross-sections were taken from root, stem and leaves of species, and the surface-sections were taken from upper and lower surfaces of leaves. *S. bornmuelleri* and *S. aintabicum* have bifacial leaves in terms of mesophyll structure. Stomata were observed on both upper and lower surfaces of the leaves. Stomata are anomocytic type in *S. bornmuelleri*, while stomata were anomocytic and anisocytic types in *S. aintabicum*. Straight and hooked glandular trichomes in different sizes and capitate glandular trichomes with different number of stalk cells and head part were seen on vegetative and generative organs. The number of layers of collenchyma and parenchyma in the stem cortex, the number of layer of sclerenchyma between the vascular bundles, the status of the vascular bundles, the arrangement of collenchyma and paranchyma layers in stem cortex, the density of micropapilla in the cuticle, the density of papillae in the epiderma, stoma index, stoma types, the margin structure of epiderma cells and number of stalk cell and head structure of capitate trichomes were detected very valuable as distinguishing anatomical characters. Soil samples were taken during flowering periods of these species and the physical and chemical properties of soil samples were determined. The species depict a distribution on the soil containing clayey-loamy, loamy, saltless, slightly alkaline, medium degree calcareous, rich in organic matter, nitrogen, phosphorus and potassium.

Keywords: *Symphytum* species, endemic, anatomy, ecology, trichome morphology.

İki Endemik *Symphytum* Türünün Anatomik, Ekolojik ve Tüy Morfolojik Özelliklerinin İncelenmesi

Öz

Bu çalışmada, *Symphytum bornmuelleri* ve *Symphytum aintabicum*'un anatomik, ekolojik ve tüy morfolojik özellikleri karşılaştırıldı ve onların taksonomik olarak değerli olan özellikleri tespit edildi. *S. bornmuelleri* and *S. aintabicum* sırasıyla Türkiye'nin endemik Öksin ve Doğu Akdeniz elementidir ve limitli yayılışa sahiptirler. Türlerin anatomik incelemeleri ışık ve stero mikroskoplar kullanılarak yapıldı. Anatomik incelemelerde, türlerin kök, gövde ve yaprağından enine kesitler alındı ve yaprakların alt ve üst yüzeylerinden yüzelsel kesitler alındı. *S. bornmuelleri* ve *S. aintabicum* mezofil yapı bakımından bifasiyel yapraklara sahiptir. Stomalar yaprakların hem alt hemde üst yüzeylerinde gözlemlendi. *S. aintabicum* da stomalar anomositik ve anisositik tipte iken, *S. bornmuelleri* de stomalar anomositik tiptedir. Vejetatif ve generatif organlar üzerinde farklı büyüklükte düz ve çengelli örtü tüyleri ve sap hücre sayıları ve baş kısımları farklı kapitat salgı tüyleri görüldü. Gövde korteksindeki kollenkima ve parankimanın tabaka sayısı, iletim demetleri arasındaki sklerenkimanın tabaka sayısı, iletim demetlerinin durumu, gövde korteksindeki kollenkima ve parankima tabakalarının düzeni, kutikuladaki mikropapilla yoğunluğu, epidermadaki papillaların yoğunluğu, stoma indeksi, stoma tipleri, epiderma hücrelerinin kenar yapısı ve kapitat salgı tüylerinin sap hücre sayısı ve baş yapısı çok değerli ayırtecdici anatomik karakterler olarak

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belirlendi. Toprak örnekleri bu türlerin çiçeklenme peryotlarında alındı ve toprak örneklerinin fiziksel ve kimyasal özellikleri tespit edildi. Türler killi-tınlı ve tınlı, tuzsuz, hafif alkali, orta derecede kireçli, zengin organik madde, azot, fosfor ve potasyum içeren topraklarda yayılış göstermektedir.

Anahtar Kelimeler: *Symphytum* türleri, endemik, anatomi, ekoloji, tüy morfolojisi.

1. Introduction

Symphytum L. genus is in the Boragineae Bencht & J. Presl (= Anchusae DC.) tribe of the Boraginaceae Juss. family [1]. Boraginaceae family consists of single, biennial and perennial herbaceous, shrubs and trees. There are 135 genera and 2600 species in the world [2, 3]. Boraginaceae family is of great importance in terms of ornamental and medicinal plants. In molecular systematic studies, Boragineae tribe and *Symphytum* genus were found to be monophyletic [2, 4]. *Symphytum* genus is represented by 40 species in the world and 18 species in the Turkey Flora [5, 6]. This genus is a mesophytic genus. Most of the species in Turkey have limited distribution and endemism ratio of the species is 39 %. In Turkey, *Symphytum* genus is mostly distributed in the Euro-Siberian phytogeographic region. Moreover, *Symphytum* species are distributed in forest and shady areas in Marmara Region, in the mountainous areas of the Mediterranean Region and in the mountainous areas and coasts of the Black Sea Region [6].

Some species with large stems and showy flowers of the genus are grown as ornamental plants in parks and gardens [7, 8]. Proteins, gum, phenolic acids, saponins, tannins, pyrrolizidine alkaloids, vitamins, especially vitamin B12, carotene, starch and high amounts of mineral substances were detected in different organs of some species [9-13]. Also, pyrrolizidine alkaloid (Echimidine) was determined in roots of *S. aintabicum* Hub.-Mor. & Wickens by Kurucu et al. [10]. Therefore, roots and leaves of *Symphytum* species are used by the public, herbalists, and physicians to treat bone fractures, tendon damage, gastrointestinal ulcers, rheumatism, lung obstruction [11, 14]. *Symphytum* taxa are also used as mush for the treatment of burns, eczema and wounds [8, 15-18]. Different *Symphytum* extracts were reported to have antifungal, antioxidant and inflammatory effects [18-22].

According to the morphological and molecular data, Hacıoğlu and Erik [4] were revised the sections of *Symphytum* species in Turkey and were indicated the necessity to take place in *Suborientalia* Buckn. section studied species (*S. aintabicum* and *S. bornmuelleri* Bucknall). Previously, *S. bornmuelleri* and *S. aintabicum* were placed to same section (*Suborientalia*) by Bucnall [23] and Sandrink et al. [24], respectively. Branched roots and thin stems are important characteristic features of this section. Also, species of this section are generally distributed in the Mediterranean and Mediterranean-like habitats. *S. bornmuelleri* is an endemic species, distributed in the north of Central Anatolia and morphologically related to *S. aintabicum*. *S. aintabicum* is an endemic species and has a small distribution area only to the east of the Mediterranean Region.

So far there are not sufficient data concerning anatomical and ecological features of *Symphytum* taxa in Turkey. Hence, the aim of this study was to focus on the anatomical and ecological features of endemic *S. bornmuelleri* and *S. aintabicum* in detail and to reveal the taxonomic characters that can be used to distinguishing species.

2. Material and Method

Plant and soil samples were taken from the areas where the species naturally spread during flowering periods. The taxonomic descriptions of these species were made according to Wickens [25]. Some of the plant specimens were made into herbarium specimens and some of them were fixed in 70% alcohol for anatomical investigations. In anatomical examination, the cross-sections of root, stem and leaf and surface-sections of leaves were taken by hand and sarture reagent was applied to cross and surface sections [26]. Photographs were taken on 10 and 40X lenses of the Leica ICC50 HD microscope. Anatomical measurements were made with millimeter ocular of light microscope and measurement results are shown in table 1. The width and length measurements of the stomata on the upper and lower surfaces of the leaves are given in Table 2. Stoma index was calculated according to Meidner and Mansfield [27]. Determining the morphology of trichomes, trichomes on the vegetative and generative organs of studied species were examined using 40X magnification of the Leica ICC50 HD microscope. Trichomes were classified according to Weker et al. [28], Tarikahya [29] and given in Table 3. 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 [30]. Mean and standard deviation values of soil analysis results are shown in Table 4. The localities where the species was collected are listed below;

Symphytum bornmuelleri

A5 Amasya: Merzifon-Derealan Village, roadside and over water canal, near *Fagus* forests, open areas, 1585 m., 05.06.2018. H. Yakupoğlu (A1 locality).

A6 Amasya: Taşova-Borabay Lake around, 1000 m., 29.04.2018, A. Cansaran (A2 locality).

A6 Tokat: Erbaa-Boğalı Village, *Fagus* forests 950 m., 05.06.2018, H. Yakupoğlu (A3 locality).

B6 Sivas: Şarkışla-Gülören Village, *Pinus sylvestris* forests, shady areas, 1560 m., 10.06.2018, H. Yakupoğlu (A4 locality).

Symphytum aintabicum

C6 Gaziantep: Acaroba Village, damaged scrub and stony areas, 1070 m., 7.05.2007, B.T. Hacıoğlu (B1 locality).

C6 Gaziantep: Kabasakız Village, open stony areas, 850 m., 10.05.2018, H. Yakupoğlu (B2 locality).

C6 Gaziantep: Sof Mountain, TV transmitter surroundings, open steppe areas, 1200 m., 11.05.2018, H. Yakupoğlu (B3 locality).

Table 1. Comparison of anatomical characteristics between studied *Symphytum* species

Anatomical characters		<i>S. bornmuelleri</i>		<i>S. aintabicum</i>	
		Length (µm)	Width (µm)	Length (µm)	Width (µm)
R O O T	Peridermis cell	22-30	10-14	20-28	10-16
	Cortex cell	30-70	20-30	30-75	22-32
	Phloem	16-18	14-18	14-18	12-16
	Trachea diameter	-	30-40	-	30-35
	Primary xylem diameter	-	20-25	-	18-24
S T E M	Cuticle	3-5	-	4-6	-
	Epidermis cell	16-22	10-14	14-22	12-16
	Parenchyma cell	20-28	18-24	20-30	16-24
	Collenchyma cell	25-30	24-28	24-32	20-26
	Parenchyma cell	50-70	50-65	-	-
	Sclerenchyma cell	12-18	12-16	10-16	10-16
	Phloem	12-20	10-14	10-16	8-16
	Trachea diameter	-	18-30	-	20-30
	Endodermis cell	20-22	18-22	20-22	16-18
L E A F	Cuticle	5-7	-	6-8	
	Upper epidermis cell	28-40	20-25	26-38	18-25
	Palisade parenchyma	45-60	20-30	42-58	20-35
	Spongy parenchyma	30-45	20-30	28-40	20-28
	Collenchyma cell	20-25	22-28	20-24	20-28
	Lower epidermis cell	22-26	18-24	20-28	20-24

Table 2. Stomata measurements and stomata index for *Symphytum* species (upper and lower surfaces of leaf)

Species Name	Leaf surface	Number of epidermis cells	Number of stomata cells	Stomata index (%)	Stomata length (µm)	Stomata width (µm)	Stomata type
<i>S. bornmuelleri</i>	Upper surface	173	55	23.9 %	20-28	18-24	Anomocytic
<i>S. aintabicum</i>	Upper surface	178	49	21.58 %	22-28	20-26	Anomocytic and rarely anisocytic
<i>S. bornmuelleri</i>	Lower surface	147	70	32.26 %	26-36	20-24	Anomocytic
<i>S. aintabicum</i>	Lower surface	150	74	33 %	24-34	20-24	Anomocytic and rarely anisocytic

3. Results

3.1. The Anatomical Features of *Symphytum bornmuelleri*

Root: In root cross sections, periderma is 5-6 layered, cortex is multilayered and parenchymatic (Figures 1 A and B). Phloem elements are obvious. Cambium is 1-3 layered, flat celled and distinguishable. Secondary xylem is composed of dense trachea and sclerenchyma cells (Figure 1 C). Trachea cells are quite large. In the pith region, there are primary xylem elements (Figure 1 D).

Stem: Epiderma consists of single layered, small and square shaped cells. Cuticle is medium thickness and with micropapillae (Figures 1 E and F). Cortex is composed of parenchyma, collenchyma and parenchyma layers. Parenchyma cells located under the epiderma are single layered, dense with chloroplast and oval shaped. Collenchyma is 2-3 layered, oval or hexagonal shaped (Figure 1 F). Parenchyma under the collenchyma is 2 layered, sometimes 3 layered, large, oval or hexagonal shaped and without chloroplast. Endoderma is between cortex and vascular bundles and single layered (Figure 1 G). Vascular bundles are collateral type and have same sizes. Phloem and xylem elements are clear. Cambium is 2-3 layered and parenchymatic. In the xylem, there are dense sclerenchyma cells. Sclerenchyma layer between vascular bundles is 3-4 layered. In the pith region, there are large, oval or hexagonal shaped parenchyma cells (Figure 1 H). Sand crystals are observed rare in these parenchyma cells. Strigose, long or short unicellular, thick walled, straight or hooked eglandular and capitate glandular trichomes are seen on the epiderma of stem (Figure 1 T, Table 3). On the stem and petiole, hooked and strigose trichomes are dense seen. The stalk part of capitate glandular trichomes are uni or multicellular, the head part is large and unicellular.

Leaf: Cuticle is medium thickness and with micropapillae. Upper and lower epidermis cells are rectangular shaped. The upper epiderma cells are larger than the lower epiderma cells. Both epiderma are with dense papillae. Mesophyll is bifacial type (Figure 1 K). Palisade parenchyma cells are 1 layered, long and cylindrical shaped, spongy palisade cells are 2-3 layered and oval shaped. Stomata are anomocytic type and observed in both epiderma (Figures 1 M and N). Stomata are large and have same level with epidermis cells. In the surface-sections, margins of both epidermis cells are dense undulated. In the cross-section of main vein, 1-2 layered of collenchyma and 4-7 layered of large, oval or hexagonal parenchyma cells were found in the lower epiderma part. In the median region of the leaf, there is a large and collateral vascular bundle (Figure L). Phloem and xylem elements are apparent. On the upper and lower epiderma present eglandular and capitate glandular trichomes (Table 3). Capitate glandular trichomes have uni-multicellular stalk part and unicellular, large head part (Figures 1 P, R and S). Eglandular trichomes are swollen based, thin or thick walled, hooked or straight, long or short. On the veins and margins, there are dense long, hooked or straight eglandular trichomes (Figures 1 S and V). But, on the lamina, there are rare short, hooked eglandular trichomes. Especially, capitate glandular trichomes with unicellular stalk and head part were observed on the middle veins and margins (Figure 1 V). Peltate glandular trichomes were not seen on the vegetative and generative organs of *S. bornmuelleri*. On the calyx, strigose trichomes were obtained (Figure 1 Y). There are prominent papillae in the corolla (Figure 1 Z).

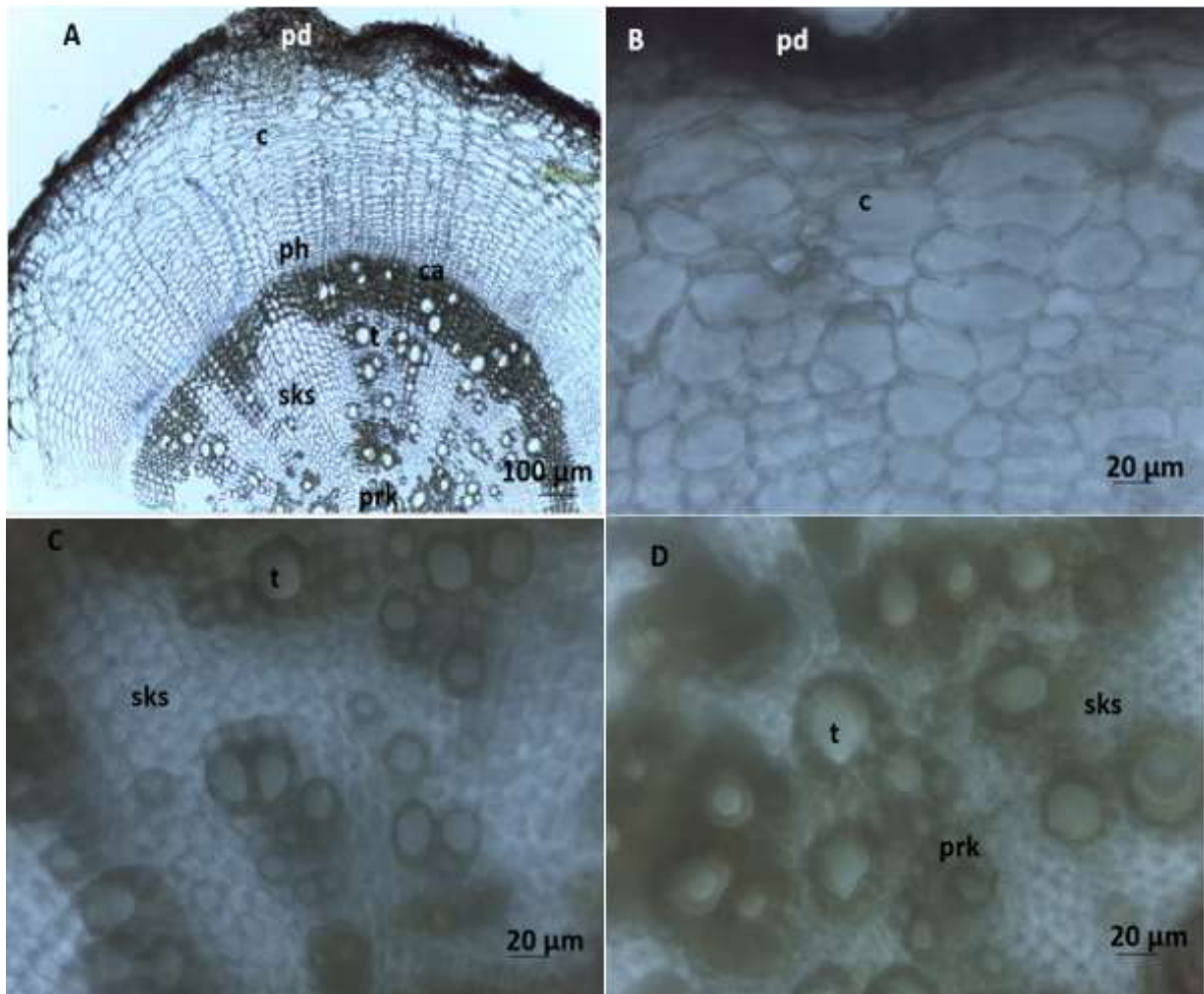


Figure 1. Root, stem and leaf anatomical structures and trichome types of *S. bornmuelleri*. (A) Cross section of root, (B) Cortex region of root, (C) Secondary xylem region of root, (D) Secondary and primary xylem regions of root, (E) Cross section of stem, (F) Cortex region of stem, (G) Vascular bundles of stem, (H) Pith region of stem, (K) Cross section of leaf, (L) Cross section of the main vein of the leaf, (M) Surface section of leaf (lower surface), (N) Surface section of leaf (upper surface), (P) Type II capitate glandular trichome in the leaf, (R) Type I capitate glandular trichome in the leaf, (S) Types II, III hooked eglandular and type I capitate glandular trichomes, (T) Type I hooked eglandular trichome in the stem, (V) Type II hooked eglandular trichome in the leaf, (Y) Strigose trichomes in the calyx, (Z) Papillae in the corolla, (pd) Periderma, (c) Cortex, (ph) Phloem, (ca) Cambium, (sks) Secondary xylem, (t) Trachea, (prk) Primary xylem, (e) Epidermis cell, (cu) Cuticle, (cl) Collenchyma, (p) Parenchyma, (en) Endoderma, (ks) Xylem, (sk) Sclerenchyma, (pr) Pith region, (ue) Upper epiderma, (pp) Palisade parenchyma, (sp) Spongy parenchyma, (le) Lower epiderma, (mp) Micropapillae, (pa) Papillae, (s) Stoma cell, (sg) Capitate glandular trichome, (st) Hooked trichome, (ut) Strigose trichome

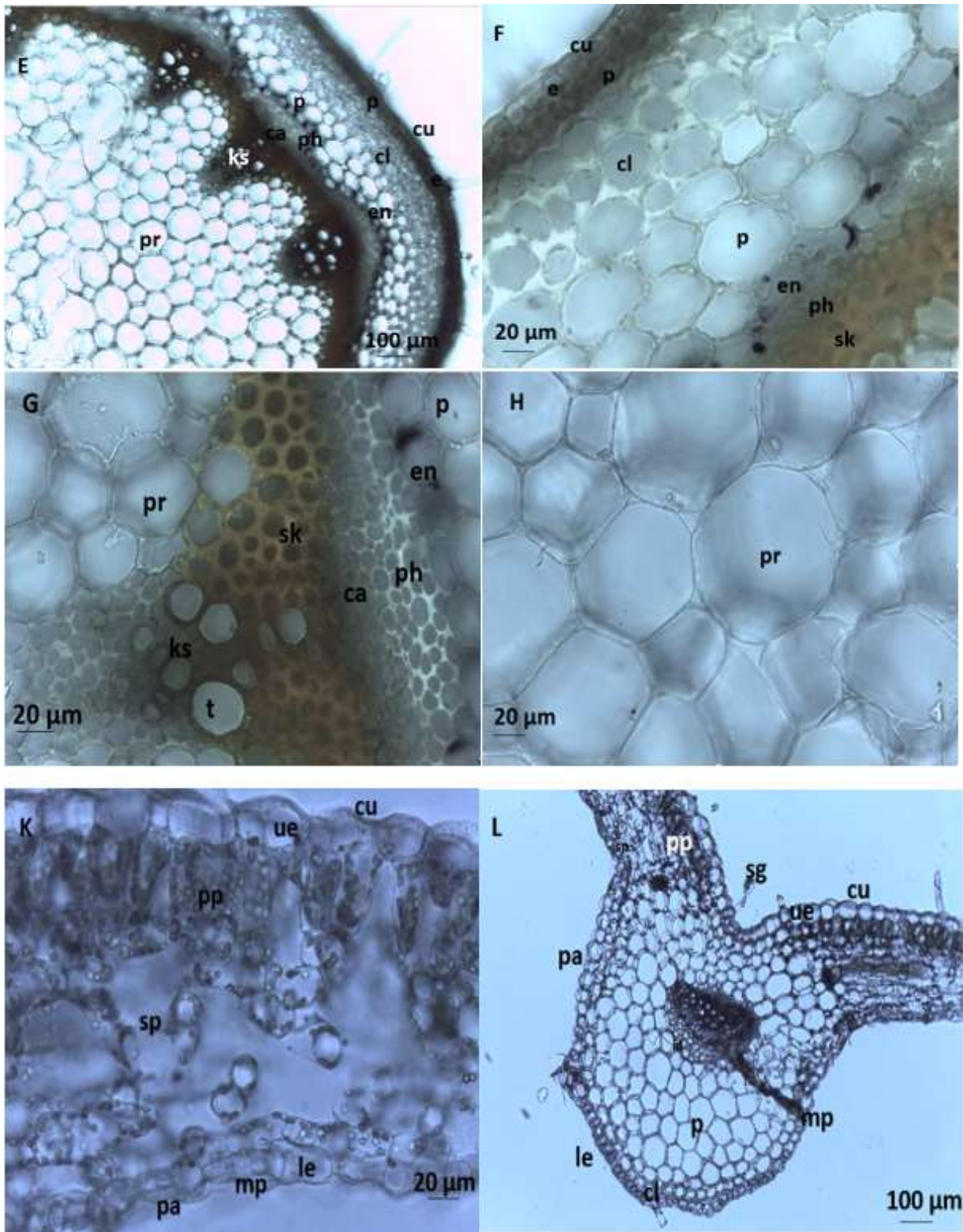


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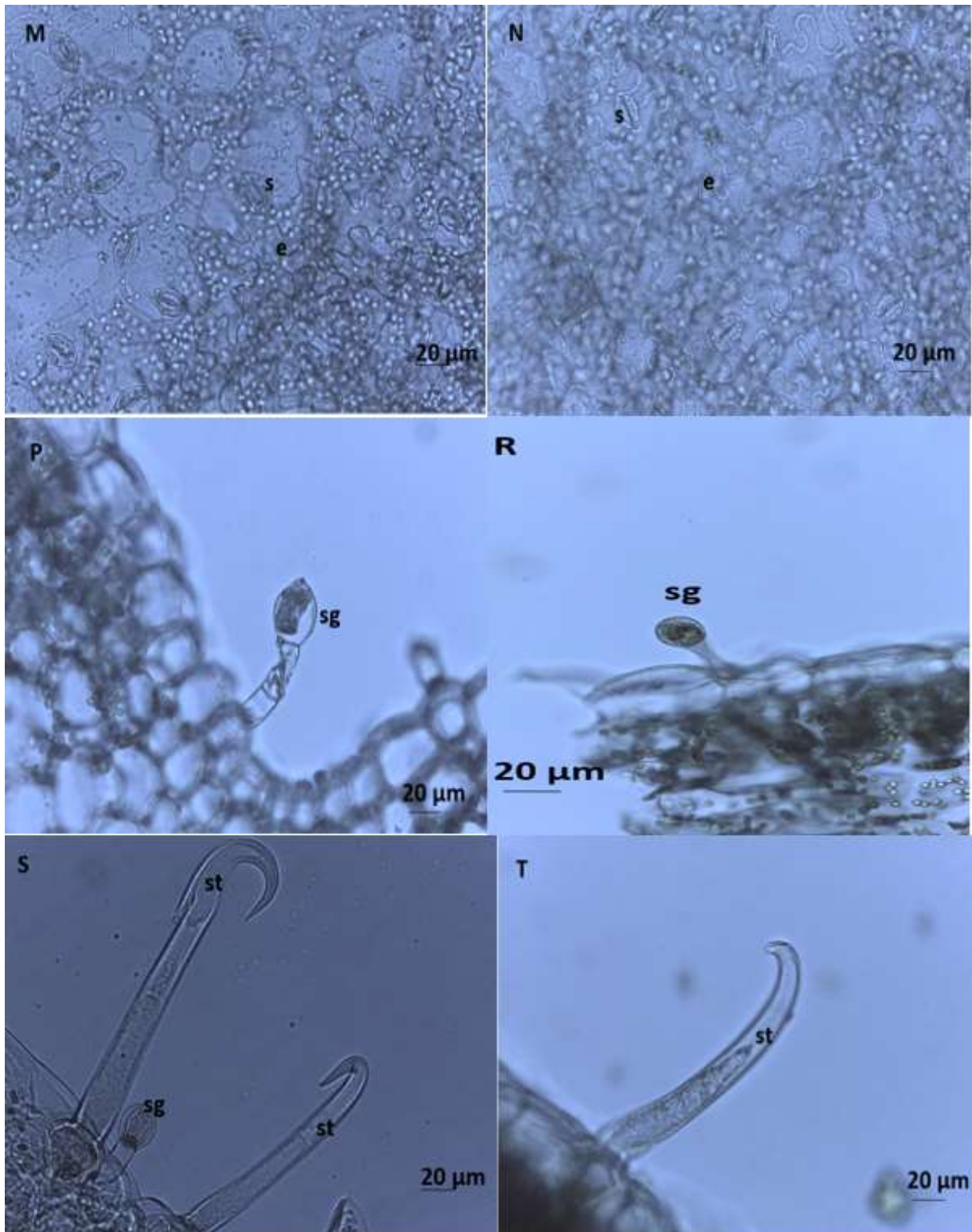


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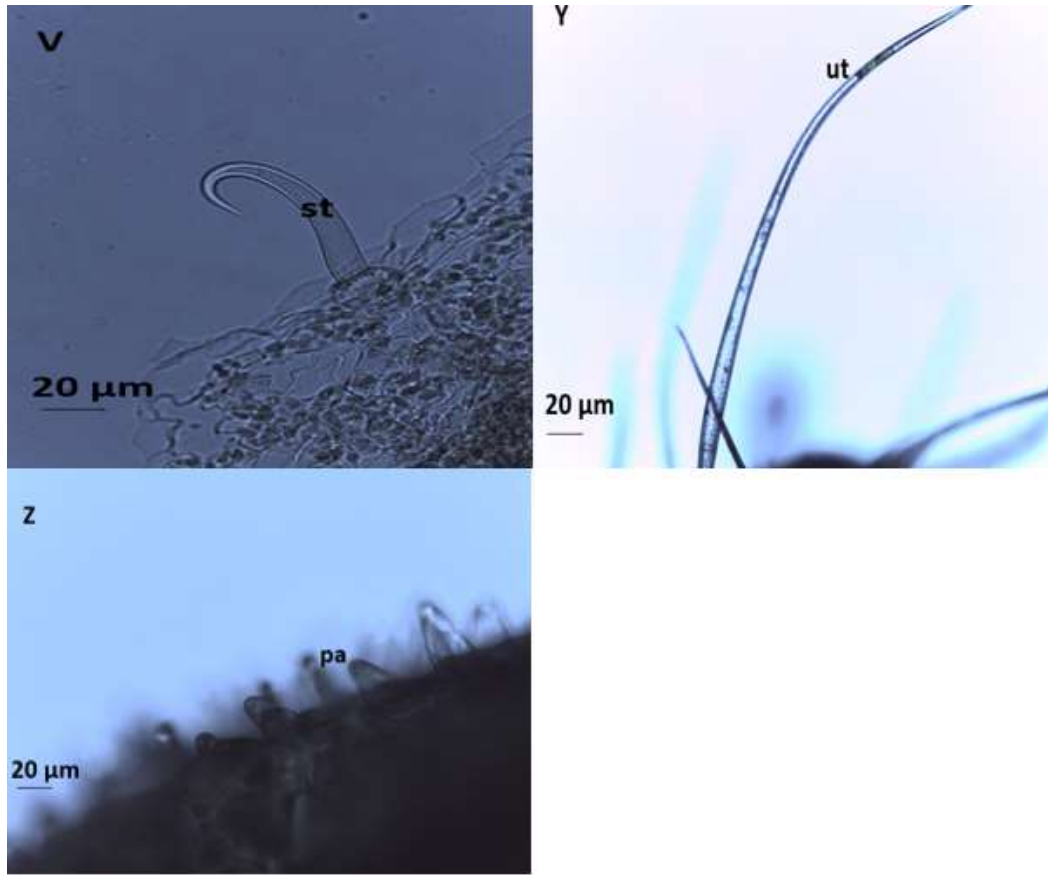


Figure 1. (Continue)

Table 3. Trichome types and distribution on organs of studied *Symphytum* species

Organs of <i>S. bornmuelleri</i>	Type I capitate	Type II capitate	Type III capitate	Strigose	Type I eglandular	Type II eglandular	Type III eglandular	Papillae
Stem	+	+	+	+++	+++	+++	++	-
Petiole	+	+	+	+++	+++	+++	++	-
Margin and veins of leaf	+++	++	+	+++	++	++	+++	-
Lamina of leaf	+	++	++	+	+	+	+	-
Calyx teeth	-	-	-	+++	+	+	+	-
Outer surface of calyx	+	+	-	+	++	++	++	+
Corolla	-	-	-	-	-	-	-	+++
Organs of <i>S. aintabicum</i>	Type I capitate	Type II capitate	Type III capitate	Strigose	Type I eglandular	Type II eglandular	Type III eglandular	Prominent papillae
Stem	+	+	-	++	++	++	++	-
Petiole	+	+	-	++	+	+	+	-
Margin and veins of leaf	+	+	-	++	+	++	++	-
Lamina of leaf	++	+++	-	+	+	+	+	-
Calyx teeth	-	+	-	++	++	++	+	-
Outer surface of calyx			-	+	+	+	-	+
Corolla	-	-	-	+	-	-	-	+++

-: absent, +: scarce, ++: dense, +++: abundant

type I capitate: stalk and head parts unicellular, type II capitate: stalk part bicellular, head parts unicellular, type III capitate: stalk part tri-multicellular, head part unicellular, type I eglandular: stalk part long, less hooked, type II eglandular: stalk part long, medium hooked, type III eglandular: stalk part long, very hooked.

3.2. The Anatomical Features of *Symphytum aintabicum*

Root: Root shows secondary growth. Periderma is 3-4 layered on the outer root surface. Cortex is composed of multilayered, large and longish parenchyma cells (Figure 2 A). Cambium is undistinguishable. Phloem is clearly seen. In the secondary xylem, there are sclerenchyma and trachea cells (Figure 2 B). Secondary xylem is in a wide region. The pith region consists of primary xylem elements.

Stem: Outside of the stem, there is an epiderma layer consisting of single layered, small and rectangular cells. The cuticle on the epiderma layer is of medium thickness. Rare micropapillae and papillae present in the cuticle and epiderma, respectively. Cortex is multilayered and consists of collenchyma and parenchyma cells. The collenchyma is located under the epiderma and is usually 3, sometimes 4 layered, large ovoid celled. Parenchyma is 4-5 layered, very large, hexagonal or oval shaped (Figures 2 C and D). Endoderma is between cortex and vascular bundles, single layered, large celled and with starch grains. Vascular bundles are collateral type and of different sizes. Cambium is 1-2 layered. Sclerenchyma between vascular bundles is 2-3 layered. Xylem consists of large trachea and sclerenchyma cells (Figure 2 E). Phloem elements are apparent. The pith region has large and hexagonal shaped parenchyma cells (Figure 2 F). On the epiderma, there are strigose, unicellular, thick walled, straight or hooked eglandular and capitate glandular trichomes (Figures 2 K and L, Table 3). Stalk part of capitate glandular trichomes is 1-2 celled, head part is one celled, large and cylindrical.

Leaf: Upper and lower epidermis cells are rectangular shaped and rare papillae. Cuticle is medium thickness and with rare micropapillae. The lower epidermis cells are smaller than the upper epidermis cells. Mesophyll is bifacial type, 1-2 layered palisade parenchyma located under upper epiderma (Figure 2 H). Spongy parenchyma is 3-4 layered. Anomocytic and anisocytic type stomata were observed in both upper and lower epiderma (Figure 2 I and J). Stomata are at same level with epidermis cells. Margins of lower and upper epidermis cells are undulated. In the cross-section of main vein, collenchyma is 1-2 layered, parenchyma is 4-5 layered, large, oval or hexagonal shaped. Vascular bundle in the median region is one, large and collateral type. Xylem and phloem elements were clearly seen. On the both epiderma, there are eglandular and capitate glandular trichomes (Figures 2 M, N, P and R, Table 3). The head part of capitate glandular trichomes are unicellular, large ve cylindrical shaped. The stalk part of these trichomes are uni-bicellular. Eglandular trichomes have swollen base, hooked or straight, thin wall, long or short. Long hooked of eglandular trichomes are dense on the margin, lamina and veins, whereas long and straight eglandular trichomes are rare on the margin, lamina and veins. Also, capitate glandular trichomes have a long neck part (Figure M). In the corolla, there are prominent papillae (Figure 2 O). Although capitate glandular trichomes were observed on the vegetative and generative organs of *S. aintabicum*, peltate glandular trichomes were not observed on the vegetative and generative organs of *S. aintabicum*.

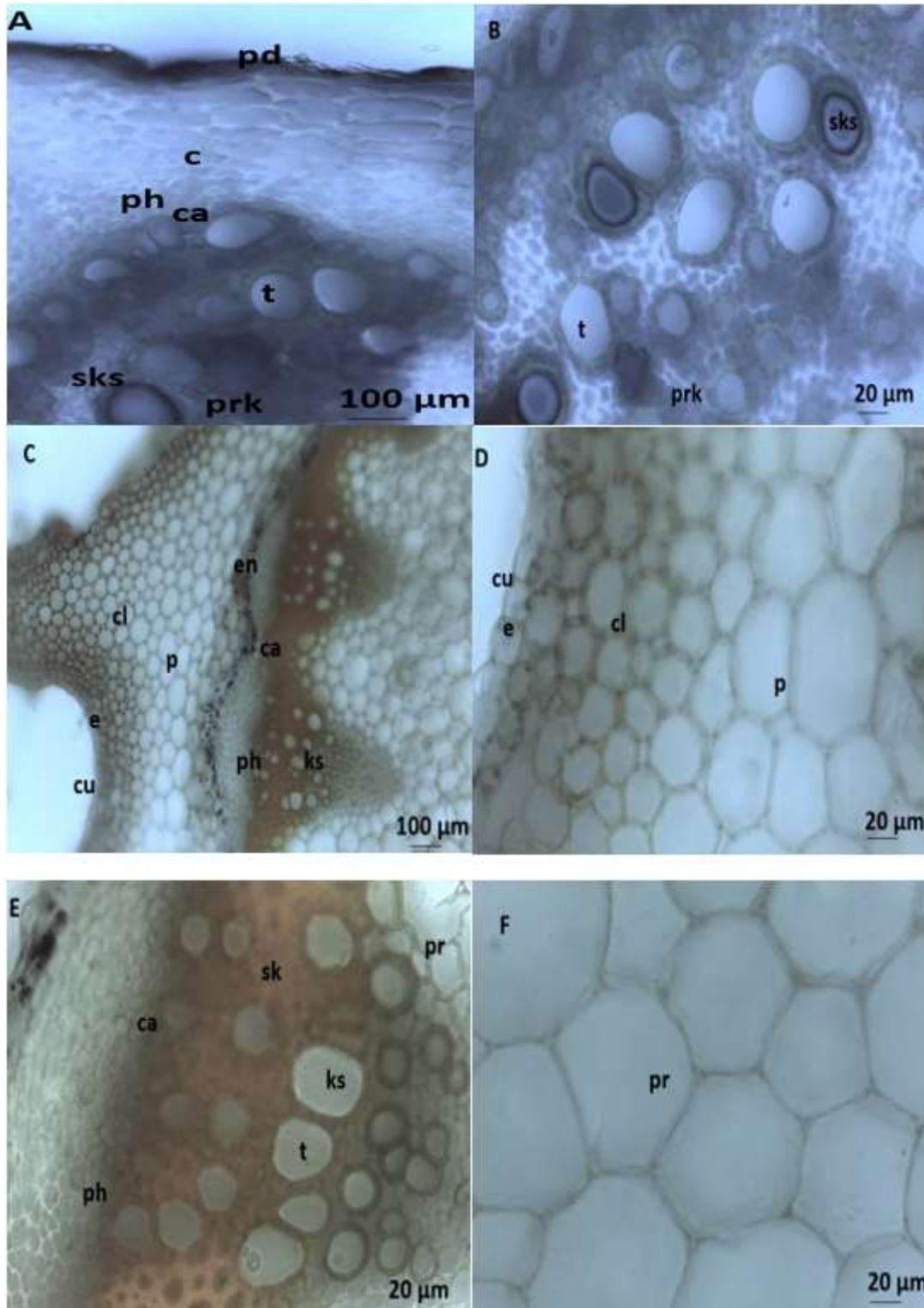


Figure 2. Root, stem and leaf anatomical structures and trichome types of *S. aintabicum*. (A) Cross section of root, (B) Secondary and primary xylem regions of root, (C) Cross section of stem, (D) Cortex region of stem, (E) Vascular bundles of stem, (F) Pith region of stem, (H) Cross section of leaf, (I) Surface section of leaf (upper surface), (J) Surface section of leaf (lower surface), (K) Type I capitate glandular trichome in stem, (L) Type II capitate glandular trichome in the petiole, (M) Type I capitate glandular trichome in the leaf, (N) Strigose trichome in the leaf, (O) Papillae in the corolla, (P) Straight eglandular trichome in the leaf, (R) Type III hooked eglandular trichome in the leaf, (pd) Periderma, (c) Cortex, (ph) Phloem, (ca) Cambium, (sks) Secondary xylem, (t) Trachea, (prk) Primary xylem, (e) Epidermis cell, (cu) Cuticle, (cl) Collenchyma, (p) Parenchyma, (en) Endoderma, (ks) Xylem, (sk) Sclerenchyma, (pr) Pith region, (ue) Upper epiderma, (pp) Palisade parenchyma, (sp) Spongy parenchyma, (le) Lower epiderma, (mp) Micropapillae, (pa) Papillae, (s) Stoma cell, (sg) Capitate glandular trichome, (st) Hooked trichome, (ut) Strigose trichome, (se) Straight eglandular trichome

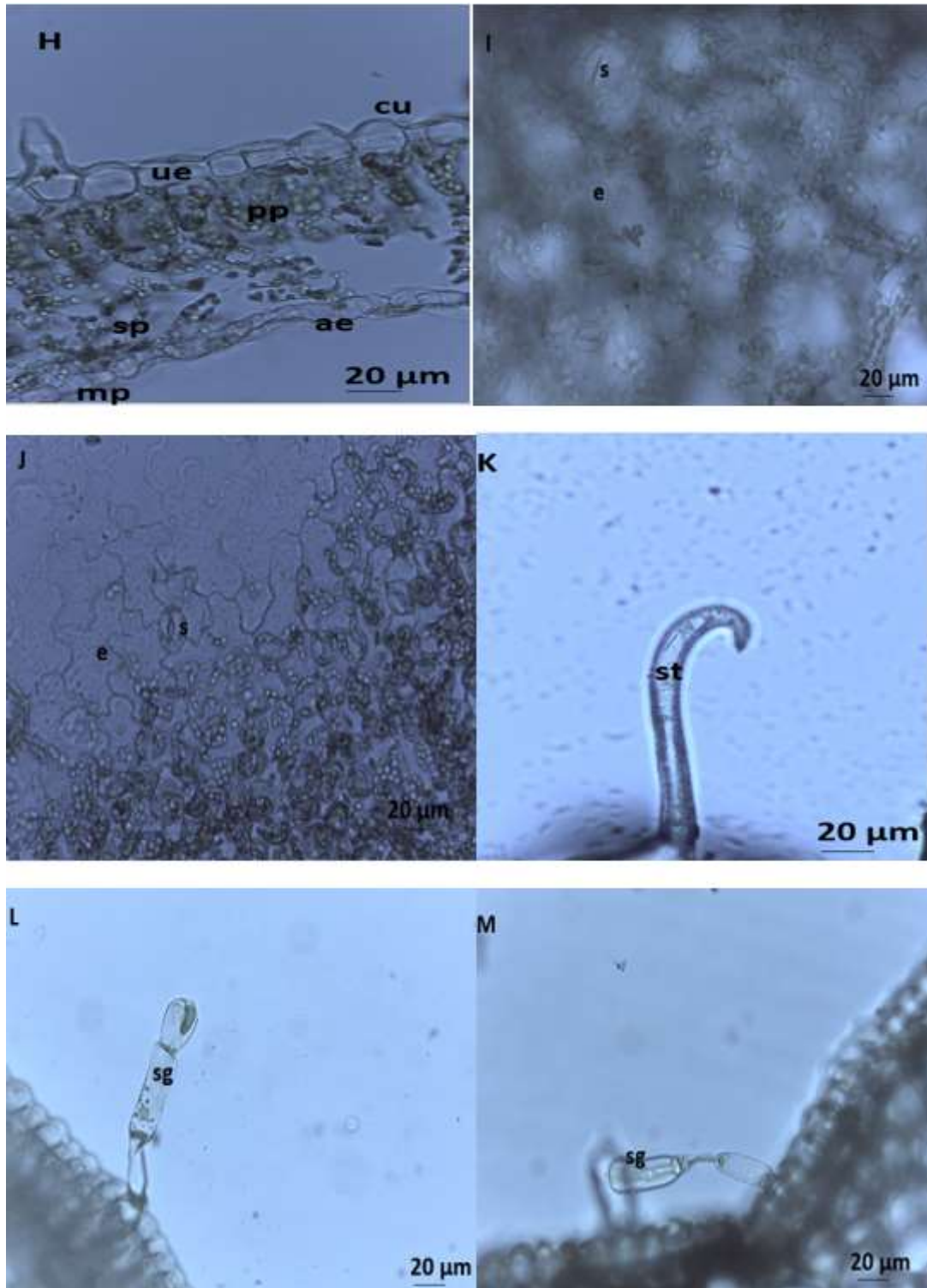


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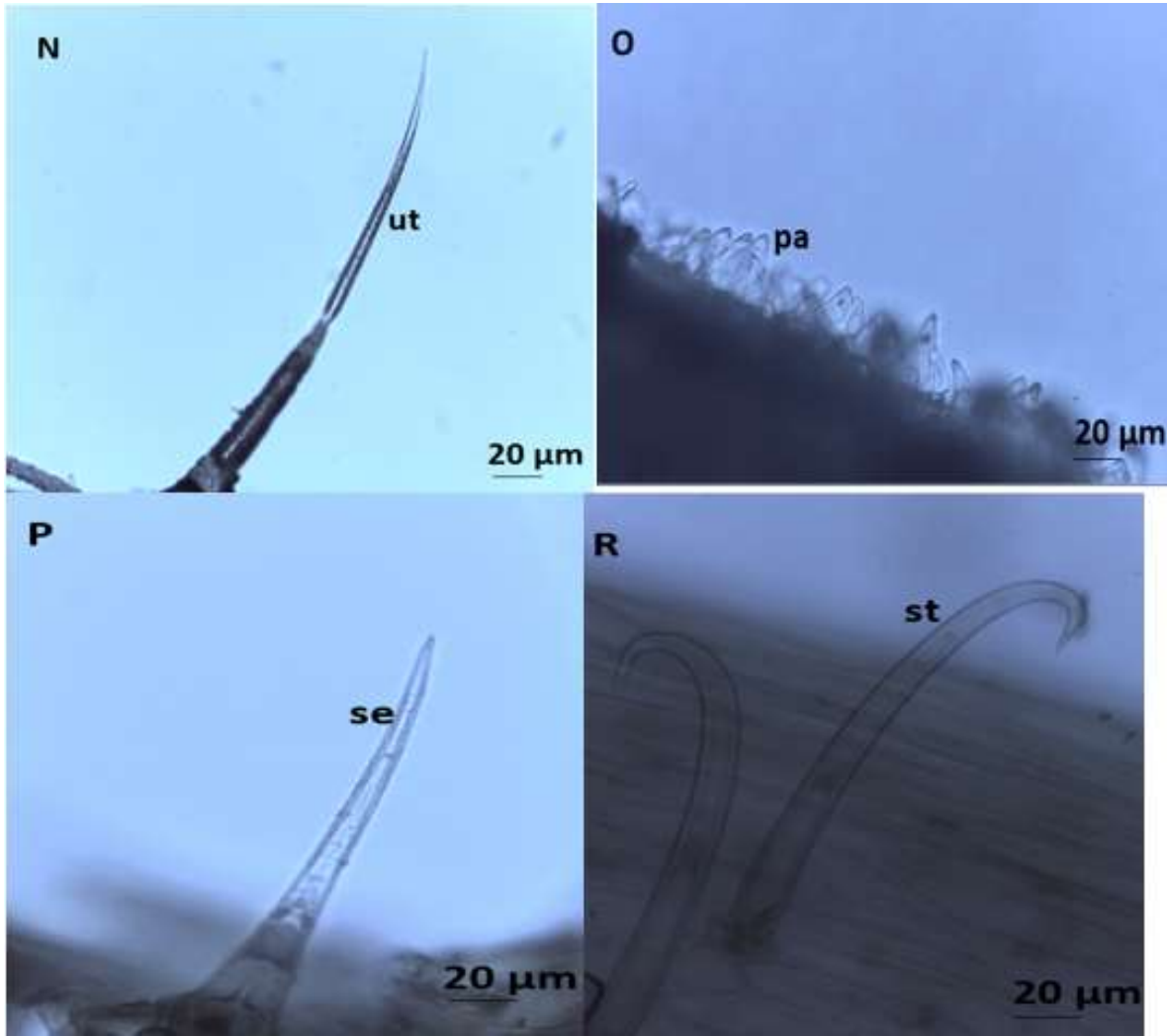


Figure 2. (Continue)

3.3. Physical and Chemical Features of Soil Samples

Soil Characteristics of *S. bornmuelleri*: Soil characteristics of the species were based on samples taken from four different locations (Amasya, Tokat ve Sivas). The pH, salinity, CaCO₃ values varied from 7.08-7.90, 0.03-0.07% and 2.25-5.75%, respectively. The soil samples had a clayey-loamy texture structure. The organic matter, N, P and K values varied between 3.80-4.75%, 1.98-2.97%, 9.12-14.50 and 183-365 mg/kg, respectively (Table 4).

Soil Characteristics of *S. aintabicum*: Soil samples of the *S. aintabicum* were taken from three different localities of Gaziantep. The pH values varied from 7.49-7.98 and the CaCO₃ values were between 2.20-5.65 %. The soil samples had a clayey-loamy and loamy texture structure. The salinity values were low (0.14-0.33 %). The organic matter values were found to be 1.95-3.84 %. The N, P and K values of the soil samples were between 2.16-2.57%, 5.32-8.30 and 134.77-192.9 mg/kg, respectively (Table 4).

Table 4. Soil characteristics of studied *Symphytum* species (sd: standard deviation)

Localities	Texture	Salinity (%)	CaCO ₃ (%)	pH	Organic matter (%)	N (%)	K (mg/kg)	P (mg/kg)
A1	clayey-loamy	0.03 saltless	2.5 moderately	7.08 slightly alkaline	4.20 high	2.76 high	120.7 high	9.12 high
A2	clayey-loamy	0.07 saltless	5.7 moderately	7.82 slightly alkaline	3.80 high	2.32 high	230.2 high	10.12 high
A3	clayey-loamy	0.05 saltless	4.2 moderately	7.41 slightly alkaline	4.75 high	2.97 high	365.1 high	14.20 high
A4	clayey-loamy	0.06 saltless	2.2 moderately	7.90 slightly alkaline	4.01 high	1.98 high	182.5 high	12.80 high
Mean±Sd	-	0.05±0.02	3.75±1.58	7.55± 0.38	4.19±0.407	2.50±0.44	251.3±78.81	11.63±2.46
B1	clayey-loamy	0.14 saltless	2.55 moderately	7.68 slightly alkaline	2.52 high	2.57 high	134.77 high	8.30 high
B2	loamy	0.33 light salty	5.56 moderately	7.98 moderate alkaline	3.84 high	2.21 high	157.8 high	6.70 high
B3	clayey-loamy	0.25 light salty	2.20 moderately	7.49 slightly alkaline	1.95 high	2.16 high	192.9 high	5.32 high
Mean±Sd	-	0.24±0.09	3.46±1.89	7.71±0.24	2.77±0.96	2.31±0.22	160.8±30.6	6.77±1.49

4. Discussion and Conclusion

In this study, anatomical, ecological characteristics and trichome morphology of two endemic *Symphytum* species were compared and valuable characters that may be useful in distinguishing of species were found. Although there are many similar morphological characters between these two species, some different morphological characters have been observed. Morphological characters such as the depth of the calyx teeth, ratio of limb length to the tube length in the corolla, corolla scales being long or short from stamens at the top, corolla colours, the number of flowers in chymos, the edge structures of calyx sections, the length of style were determined as important taxonomic characters between these species. In Flora of Turkey, the depth of the calyx teeth was recognized as an important taxonomic character and used in species identification key [25]. Also, Tarıkahya [29], Hacıoğlu and Erik [6] reported that morphological characters such as the depth of the calyx teeth, ratio the length of the tube to the length of the limb and scales are longer or shorter than stamens on the tip were taxonomically valuable.

When the anatomical characters of the species were compared, in the root cross-sections of *S. bornmuelleri*, the periderma is 5-6 layered, the cortex is multilayered, cambium is 1-3 layered, the secondary xylem is in the broad region and the primary xylem is in the narrow region. In the root cross-sections of *S. aintabicum*, the periderma is 3-4 layered, the cortex is multilayered, the cambium is undistinguishable and the secondary xylem are in a large region. When the root anatomical features of species are examined, it is seen that there is a difference only in the number of periderma layers. The studied species have secondary root structure. The secondary structure was identified in the perennial species of the Boraginaceae family [31-34].

In the stem of *S. bornmuelleri*, the epiderma is square shaped when the *S. aintabicum* is rectangular. The cuticle of both species are of medium thickness as they are distributed in damp areas. Cortex of *S. bornmuelleri* consists of parenchyma, collenchyma and parenchyma layers. However, cortex of *S. aintabicum* is composed of collenchyma and parenchyma layers. In *S. bornmuelleri*, the parenchyma under epiderma is 1 layered and with chloroplast, collenchyma is 2-3 layered and other parenchyma is 2-3 layered. In *S. aintabicum*, collenchyma is 3, sometimes 4 layered, parenchyma is 4-5 layered. Endoderma layer is found in both species. However, dense starch grains are seen in the endoderma of *S. aintabicum*. Vascular bundles of *S. bornmuelleri* have same sizes, while vascular bundles of *S. aintabicum* are of different sizes. The sclerenchyma layer between the vascular bundles is

3-4 layered in *S. bornmuelleri*. But, the sclerenchyma layer between the vascular bundles is 2-3 layered in *S. aintabicum*. In *S. bornmuelleri*, the stalk part of capitate glandular trichomes are 1 or multicellular, head part is one celled, large and without neck. In *S. aintabicum*, the stalk part of capitate glandular trichomes are 1 and 2 celled and the head part is very large, cylindrical shaped and has a long neck. Eglanular trichomes are short or long and hooked type in the stem of both species. When the stem anatomical findings of the species are considered, the anatomical characteristics such as the number of collenchyma and parenchyma layers, the status of the vascular bundles, the number of stalk cells of capitate glandular trichomes and the arrangement of the collenchyma and parenchyma layers in the cortex, the number of sclerenchyma layer between the vascular bundles can be used to distinguish of these endemic species. In particular, the number of stalk cells of capitate glandular trichomes and the arrangement of the parenchyma and collenchyma layers in the cortex are proposed as important taxonomic characters in distinguishing of these species.

Kandemir et al. [35] made comparative anatomical studies on seven *Heliotropium* L. species (Boraginaceae). In stem anatomical examinations, collenchyma is 2 layered in *H. bovei* Boiss. and *H. haussknechtii* Bunge, 3 layered in *H. dolosum* De Not., 3-4 layered in *H. suaveolens* Bieb., 2-3 layered in *H. samoliflorum* Bunge subsp. *erzurumicum* Dönmez and *H. myosotoides* Banks & Sol. and 4-5 layered in *H. thermophilum* Kit Tan, A. Çelik & Y. Gemici. These researchers reported that the number of collenchyma and hypoderma layers in the cortex can be used as a taxonomic character to distinguish of *Heliotropium* species. Similar cases were observed in *Heliotropium* species distributed in Sudan and Southwestern Saudi Arabia [36, 37]. In addition, Joubert et al. [38] suggested that the presence or absence and distribution of collenchyma is a very important taxonomic character in species identification. According to the above results, this feature can be used as a valuable anatomical character in the differentiation of the studied species because of the different number of collenchyma layers (2-3 layered in *S. bornmuelleri* and 3-4 layered in *S. aintabicum*).

Güven et al. [39] investigated the anatomical characteristics of the six *Onosma* L. taxa in Turkey (Boraginaceae). They reported the phloem / xylem ratio in the stem, the area covered of the cortex, the number of layers of collenchyma, the number of palisade and spongy parenchyma layers in the leaves and stoma index as useful characters in the separation of *Onosma* taxa.

In the leaf surface sections, stomata of *S. bornmuelleri* are anomocytic type and margin of epidermis cells are dense undulate. However, stomata of *S. aintabicum* are anomocytic and rarely anisocytic type and margin of epidermis cells are undulate. Also, stoma in the upper epidermis of *S. aintabicum* is less. In the studied species, stomata are same level with stomata epiderma cells. Micropapillae on the cuticle and papillae in the upper, lower epiderma are dense in *S. bornmuelleri*, while micropapillae on the cuticle and papillae in the upper, lower epiderma are rare in *S. aintabicum*. Eglanular and capitate glandular trichomes on the leaves of both species are similar to trichomes on the stem. The number of stoma, type of stoma, stoma index and marginal structures of epidermis cells can be used as important anatomical characters. Metcalfe and Chalk [40], Özörücü [41] reported that both anomocytic and anisocytic stomata present in Boraginaceae family. However, it has been reported that anomocytic stoma are dominant in this family. Similar results were determined in 31 species belonging to different genus and tribes of Boraginaceae family by Dasti et al. [42].

Mesophyll structure of the leaf is bifacial type in studied species. Palisade and spongy parenchyma are 1 and 2-3 layered in *S. bornmuelleri*, respectively. In *S. aintabicum*, palisade and spongy parenchyma are 1-2 and 3-4 layered, respectively. The intensity of the palisade parenchyma in the mesophyll directly depends on the high intensity of light [40]. Moreover, the number of layers of the palisade parenchyma is in agreement with the ecological factors in the areas where the studied species are distributed. Because *S. bornmuelleri* is spread under forests and in shady areas, and *S. aintabicum* in distorted maquis and open areas. In addition, Selvi and Bigazzi [43] stated that the layer number and arrangement of the palisade parenchyma changed between the species of the family.

Similar characteristics mentioned above were seen in the stem and leaf anatomic structures of other species of Boraginaceae family. Namely, Selvi and Bigazzi [43] were made studies on the leaf surface and leaf anatomical characters of some species belonging to the Boragineae tribe. They reported that the members of this tribe had similar anatomical characters and the hooked trichomes were found only in the species of the genus *Symphytum* of Boraginaceae family. The same researchers examined the pollen morphology of Boragineae tribe and according to the findings of pollen morphology collected *Symphytum* species in a single cluster and reported that the nearest genus *Trachystemon* (L) Don [44].

Yousefi [33] done morphological and anatomical studies on endemic *Heliocarya monandra* Bge (Boraginaceae). In anatomical studies, it was stated that there were 2-3 layered of collenchyma, 3-5 layered of parenchyma, and the one layered of endoderma layer in the stem. The leaves have isobilateral and anomocytic stomata type. Yousefi [33] reported that the anatomical results of *H. monandra* were in agreement with the anatomical results of the species of the Boraginaceae family. Yeşil [34] reported similar anatomical characters in the root, stem and leaf of *Nonea dumanii* Bilgili & Selvi (Boraginaceae). In the stem of this species, collenchyma is 4-5 layered, parenchyma is multilayered, endoderma is one layered, pith region is wide and xylem consists of dense sclerenchyma. On both surfaces of the leaves, there are anomocytic stomata. The mesophyll is isobilateral type. On the other hand, similar anatomical characters in the root, stem and leaf were identified in anatomical studies of species belonging to the genus *Anchusa* L. (Boraginaceae) [31, 32].

In anatomical studies, the mesophyll structure of different species of Boraginaceae family is generally determined as isobilateral [31-35, 43]. Mesophyll type is bifacial type in *S. bornmuelleri* and *S. aintabicum*. A similar mesophyll structure was also observed in *Trachystemon orientalis* [45]. Thus, the mesophyll structure shows variation between the genera of the Boraginaceae family and *Symphytum* genus is closer to *Trachystemon* genus in terms of mesophyll structure.

The hooked trichomes were seen only in taxa of *Symphytum* genus (excluding *S. savvalense* Kurtto and *S. ibericum* Steven), from taxa of Boraginaceae family. That's why Selvi and Bigazzi [43] noted systematically useful of hooked trichomes distinguishing *Symphytum* genus in Boraginaceae family. But, it has been reported that the distribution and shapes of these trichomes cannot be used as taxonomic characters in the identification of species of *Symphytum* genus [29]. On both the vegetative and generative organs of studied species are identified mentioned above trichome types and it has been concluded that these trichomes cannot be used as taxonomic characters to distinguish these species. However, it has been concluded that capitate glandular trichomes can be used as important taxonomic character in species separation, since capitate glandular trichomes of *S. bornmuelleri* and *S. aintabicum* have different head structure and stalk cell numbers. Kandemir et al. [35] noted capitate glandular trichomes only in *H. thermophilum* and *H. suaveolens* species from the 7 *Heliotropium* species. In *H. suaveolens*, stalk part of the capitate trichomes are multicellular (4-6 celled), while the stalk part of capitate trichomes in *H. thermophilum* is tricellular and rarely multicellular (4 celled). In addition, these trichomes were found in the stem, leaf, calyx and corolla in *H. thermophilum*. But, these trichomes were observed only on the lower and upper surfaces of the leaves in *H. suaveolens*. According to these results, Kandemir et al. [35] suggested that these two *Heliotropium* species could be separated according to their capitate trichomes characters. It is reported that the morphological structure and distribution of glandular trichomes are important characters in the separation of species and genera by many researchers [46-51].

The root, stem and leaf anatomical characteristics of *S. bornmuelleri* and *S. aintabicum* are in accordance with the anatomical characteristics observed in members of the Boraginaceae family. Also, our anatomical data may be useful for future studies about this genus.

S. bornmuelleri is distributed in soils with clayey-loam, saltless, slightly alkaline, moderately calcareous, sufficient organic matter, nitrogen, phosphorus and potassium-containing soils are distributed. *S. aintabicum* spreads in containing soils clayey-loam and loamy salty and slightly saline, moderately and slightly alkaline, moderately calcareous, rich organic matter, nitrogen, phosphorus and potassium. There is no lack of physical and chemical characters of the soil samples of these species. This situation is in arrangement with the ecological conditions in the distribution area of studied species. In the field observations, it was determined that studied *Symphytum* species generally prefer shaded forest bottoms and areas close to water. Moreover, it is reported that *Symphytum* taxa can only live in areas where there are special habitat types they can grow [29].

Authors' Contributions

Three authors contributed to the study.

Statement of Conflicts of Interest

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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