



## Presence of *Teucrium microphyllum* in Turkey: Morpho-anatomical, karyological and ecological studies

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### Abstract

This study presents investigations on the morphological, anatomical, karyological and ecological features a perennial shrubby species *Teucrium microphyllum* Desf., belonging to Sect. *Chamaedrys* (Mill.) Schreb., endemic to Central and South Aegean Islands and Southwest Anatolia. Description of *T. microphyllum* was expanded. The morphological features indumentum of stem, leaf, calyx and corolla were determined using stereo-microscope and scanning electron microscope. Cross-sections of stem and leaf was taken using razor by manually. Stem is rectangular and has 4-5-layered collenchyma in the corners. Leaf is bifacial. There are 2(-3)-layered palisade parenchyma and spongy parenchyma has 2-4 layers. Chromosome number was determined with using squash preparation method and the somatic chromosome number was found as  $2n = 30$ . The basic chromosome number of the species was  $x = 15$ . The karyotype formula of this species consists of fifteen median chromosome pairs. The soil samples were collected from the localities at a depth of between 0–20 cm. The texture, total salt, inorganic matters (P, K, Cu, Fe, Zn, Mn) and the percentage of organic matter were determined. Besides, the associated species of *T. microphyllum* were determined for the ecological observation. *T. microphyllum* was studied morphologically, anatomically, karyologically and ecologically for the first time.

**Key words:** Anatomy, Morphology, Ecology, Chromosome number, *Teucrium microphyllum*, Turkey.

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### Özet

Bu çalışma, Merkez ve Güney Ege Adaları'na ve Güneybatı Anadolu'ya endemik olan, *Chamaedrys* (Mill.) Schreb. seksiyonu üyesi çok yıllık çalimsı *Teucrium microphyllum* Desf. türünün morfolojik, anatomik, karyolojik ve ekolojik özelliklerine dair araştırmaları sunmaktadır. *T. microphyllum*'un betimi genişletilmiştir. Morfolojik özellikler, gövde, yaprak, kaliks ve korollanın tüy örtüsü stereo-mikroskop ve taramalı elektron mikroskobu kullanılarak belirlenmiştir. Gövde ve yaprak enine kesitleri jilet kullanılarak elle alınmıştır. Gövde dört köşeli ve köşelerde 4-5 tabaka kollenkima içermektedir. Yaprak bifasiyal tiptedir. 2(-3) sıra palizat parankiması vardır ve sünger parankiması da 2-4 sıralıdır. Kromozom sayısı ezme preparat yöntemi kullanılarak belirlenmiştir ve somatik kromozom sayısı  $2n = 30$  olarak bulunmuştur. Türün temel kromozom sayısı ise  $x = 15$ 'tir. Türün karyotip formülü ise 15 medyan kromozom çiftinden meydana gelmektedir. Toprak örnekleri lokalitelerden 0-20 cm arasındaki derinliklerden alınmıştır. Toprak bünyesi, toplam tuz, inorganik maddeler (P, K, Cu, Fe, Zn, Mn) ve organik madde yüzdesi belirlenmiştir. Ayrıca, *T. microphyllum* türünün iştirakçi türleri ekolojik gözlemler için belirlenmiştir. *T. microphyllum* morfolojik, anatomik, karyolojik ve ekolojik açıdan ilk kez çalışılmıştır.

**Anahtar kelimeler:** Anatomi, Morfoloji, Ekoloji, Kromozom sayısı, *Teucrium microphyllum*, Türkiye.

### 1. Introduction

The genus *Teucrium* L. belonging to the family Lamiaceae is a large genus in the subfamily Ajugoideae (Harley et al., 2004). The polymorphic and cosmopolitan genus *Teucrium* comprises approximately more than 260 species (about 370 taxa) (Tutin and Wood, 1972; Govaerts et al., 2010). *Teucrium* is also widely distributed in Europe,

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Asia, America, Australia, but the major area of distribution for this genus is the Mediterranean area, containing about 96% of all taxa of the genus (Cantino, 1992; Navarro and El Oualidi, 2000). The flowers of this genus completely lack in the upper lip of the corolla and it is an unusual feature compared with the other members of Lamiaceae (De Martino et al., 2010). And also style is not gynobasic (Ekim, 1982; Navarro and El Oualidi, 2000).

In the Flora of Turkey (Ekim, 1982), the genus *Teucrium* is represented by 27 species and the total number has reached 34 species (46 taxa) by adding the new species and new records (Duman, 2000; Dönmez, 2006; Dönmez, 2010; Dinç, 2012; Dirmenci, 2012). Twelve of these taxa are endemic for Turkey (Ekim, 1982; Dönmez, 2006; Dönmez, 2010; Dinç, 2012; Dirmenci, 2012). *Teucrium* has been divided into eight sections distinguishable from the calyx shape and the inflorescence structure in the Flora of Turkey (Ekim, 1982). Among them, Sect. *Teucrium* Benth. has thirteen taxa, the number of taxa Sect. *Scordium* Boiss. is three, Sect. *Chamaedrys* (Mill.) Schreb. has thirteen taxa, Sect. *Polium* Benth. has two taxa, Sect. *Isotriodon* Boiss. has ten taxa, Sect. *Stachybotrys* Benth. has three taxa, Sect. *Scorodonia* Benth. and Sect. *Spinularia* Boiss. have one each taxon (Ekim, 1982; Duman, 2000; Dönmez, 2006; Dönmez, 2010; Dinç, 2012).

The members of *Teucrium* genus grow in open, dry, rocky places (especially limestone and serpentine), slopes and disturbed areas and tend to occupy exposed habitats. Most of the species are chamaephytes and under 50 cm, and show a flowering peak at the end of spring-summer, but the flowering season of some Mediterranean species may also extend into the autumn and winter (Kummerov, 1983).

The Aegean endemic species *T. microphyllum* belonging to Sect. *Chamaedrys*, including twelve taxa, distributed in South Aegean Islands and South West Turkey (Datça peninsula). *T. microphyllum* is known as Adayavşanı in Turkey (Dirmenci, 2012). Some specimens were collected from Datça Peninsula by Annette Carlström in 1987. The Anatolian finds were published in Carlström (Carlström, 1987) but it is too late to publish in Davis and Greuter et al. (Med-Checklist 24) (Greuter and Raus, 2006). Morpho-anatomical, karyological and ecological features of *T. microphyllum* were investigated for the first time in this study. At the same time, the species description in Flora of Turkey was expanded.

## 2. Materials and methods

The aerial parts of *Teucrium microphyllum* used in this study were collected in the flowering season (June, 2012) from Datça peninsula (around Knidos antique city) in Muğla province. The specimens were dried using standard herbarium techniques and deposited in the Necatibey Education Faculty Herbarium, Balıkesir University.

### 2.1 Anatomical methods

Anatomical observations were performed using specimens fixed in F.A.A (Formalin-Acetic acid-Alcohol) during twenty four hours and then stored in 70 % ethanol (Feder and O'Brien, 1968). Anatomical investigations were carried out on the cross-sections of the stems and the leaves. The cross-sections were dyed with phloroglucinol-HCl and cleared with using chloral hydrate for observing better (Yakar-Tan, 1982). The photographs of the sections were taken using Olympus BX51 microscope and Nikon Eclipse E600 microscope.

### 2.2 Morphological methods

Morphological features were determined on living and herbarium materials. About ten specimens were used to determine the morphological characteristics. A Olympus SZX14 stereomicroscope with a drawing tube was used for the morphological studies.

Also, trichome micromorphology was studied by Tabletop scanning electron microscopy (SEM). For SEM, small pieces of stem and leaves, calyx, corolla and nutlet were investigated and photographed using a NeoScope JCM. SEM studies were made in Basic Sciences Research and Applied Center, Balıkesir University.

### 2.3 Ecological methods

The soil samples were collected from the localities at a depth of between 0–20 cm. The texture, total salt, inorganic matters (P, K, Cu, Fe, Zn, Mn) and the percentage of organic matter were determined (Kaçar, 1972; Bayraklı, 1987; Tüzüner, 1990). On the other hand, the associated species of *T. microphyllum* were determined for the ecological observation

### 2.4 Karyological methods

All of the cytological observations were made using root tips (about 1-2 mm), germinated on wet filter paper in petri dishes. After germination, fresh root tips pretreated in  $\alpha$ -mono-bromonaphthalene at 4°C during 16 hours, and then fixed with glacial absolute alcohol:acetic acid (3:1) 4°C during 24 hours. After 24 hours, these were deposited in

70% ethanol at 4°C until analysis. The root tips were hydrolyzed in 1N HCl at room temperature for 8-8.5 minutes. Finally, they were squashed and stained in 2% aceto-orcein. Karyotypes were determined using Image Analysis System (BsPro200) on a personal computer (Martin, 2006). Cytological investigations were made in Necmettin Erbakan University, Ahmet Keleşoğlu Education Faculty, Plant Biology Research Laboratory.

### 3. Results

#### 3.1 Morphological Studies

**Teucrium microphyllum** Desf. (Syn: *T. quadratum* Sm.)

Shrubby, much branched from the woody base. **Stem** 5-40 cm, densely white pubescent with sessile glands.

**Leaves** 5-10×2-5 mm, elliptic to narrowly lanceolate, crenate-dentate, slightly revolute-margined, acute at apex, attenuate at base, discolour, green and puberulens above, white tomentose beneath, midrib impressed above. Inflorescence raceme, verticillasters distant below, approximate above, 2-4-flowered.

**Calyx** 4-6(-7) × 2-3 mm, not bilabiate, green to purplish, tube green below, purplish towards to teeth, gibbous, pedicellate, pedicel 3-4 mm long, white tomentose with long hairy and glandular papillate with sessile glands; teeth almost equal, triangular-lanceolate, acute, almost 1/3 of calyx, glabrous inside, 10-nerved. **Corolla** 9-10 mm, pinkish-purple, tube inside or slightly exceeding the calyx, sparsely long hairy and glandular papillate on tube, bearded on both surface of lower lip. **Stamens** 4, didynamous, exerted from corolla tube, filament glabrous above, hairy below. **Stylus** exerted from corolla tube, 2 short subequal branches, not gynobasic. **Nutlets** 2-3 mm long, oblong-obovate, blackish-brown, glandular and eglandular hairy.

#### Examined Specimens

**Turkey.** C1 Muğla: Marmaris, around Knidos, 30 m, 13.06.2011, Özcan (184), Dirmenci & Akçiçek. From Knidos to Marmaris, 1-1.5. km, 58 m, 36°41.119' K, 27°23.161' D, 16.05.2012', Özcan (207), Dirmenci & Yıldırım.

**Greece.** Kreta, Namos Lassithiou, Eparchia Mirabellou Kalo Chorio (-Kalamafka), 35°06'29" N, 25°42'33" E, 200 m, NN orchideenreiche Phrygana auf NE-exp Kalkstein Terra fusca, 13.05.1998. (N. Böhring), (B-Photo !); Near Odigiritias, Roadside, 22.05.1984. (J.M. Shay Coll. No: 1335), (B-Photo !); Archeological site "Sedones" near Kamilari, Rounded Knoll, several km W. of Kamilari, 20.05.1987. (J.M. Shay Coll. No: 1817). (B-Photo !); South of Sivas on road to Listaros, steep slope below terracewith grazed shrubby vegetation. 31.05.1987. (J. M. Shay Coll. No: 1863), (B-Photo !); Kommos, 1.3 km from W from Pitsidia, Midslope, N. facing, shiny leaves and red flowers. (J. M. Shay Coll. No: 82-1247), (B-Photo !); Berg Kolas, NE-Flanke zwischen Aperi und Kato Lastos, 35°33'50" N, 27°10'20" E, Kalkgestein, 650-900 m, 14.05.1982. (WG 19060- TR 6372), (B-Photo !); Eparchie Kissamos: Kap Koutri (alt Phalasarna), 35°30'30" N, 23°34' E. Kalkfelsen, 5-30 m, 01.06.1982. (No: 19452). (B-Photo !); Ep. Ajos Vasillios, Hang zwischen Preveli-Bucht und Straße W der Schlucht 35°09'30" N, 24°28' E, Kalkfelstriften, z. T. Phrygana, 0-150 m, 22.05.1983. (No:889). (B-Photo !); Tristomon, 35°49'20" N, 27°13'50" E, Ruderalstellen im Dorf. 10 m, 22.05.1982. (Raus 6534 & Pleger), (B-Photo !); Griechenland, Kreta, Nomos Chania, Sfakia, unterster Teil der Imbros-Schlucht (Faragi imbrou) NE oberhalb Kommitades, ca. 200-300 m, Phrygana, 10.05.1998. (Leg E. Hörandl & F. Hadacek- Nr 8497 (B-Photo !); Griechenland, Kreta, Nomos Lassithiou, N Agios Nikolaos, Akrotiri Agios Ioannis NE Voruas, Weg von den Windmühlen S Voruas zum Kap, S-SE Hang des Berges Trouli, bei der Kapelle, 260 m, s. m. Ernst Vitek. 14.05.2002, (B-Photo !).

As it is seen from figures. 1, 2 and 3, leaves curve to the abaxial side. There are glandular and eglandular hairs both adaxial and abaxial sides but eglandular hairs are dense on the abaxialsides and mostly 1-2 celled. Calyx is almost same length of corolla tube and gibbous at base. Stem is mostly woody.

#### 3.2 Anatomical Studies

##### 3.2.1 Stem

It is rectangle shaped just like the other members of the family. The epidermis consists of cubic or oval cells forming a single layer and is surrounded by a thinner cuticle layer. Eglandular hairs are mostly 2-3-celled and glandular hairs are densely one head and one stalk. There are 2-5 layers of collenchyma in the corner of the stem under the epidermis. In the cortex, 3-5-layered parenchymatous cells are located under the collenchyma tissue. And some of these cells include chlorophyll. The parenchymatic endodermis is 1(-2) layers. The vascular bundles at the corners are larger than between corners. Cambium is indistinguishable. The phloem and the xylem members are clear. Phloem is not only in the corners but also between the corners. But, it is more seriate in the corners. Xylem is 5-8-layered and phloem 4-5 layered in the corners. The pith is present at the middle of the stem, and it is completely filled up with large orbicular parenchymatic cells and some of these cells include lignin (Figure 4)

##### 3.2.2 Leaf

As it is seen Figure 5, the upper and lower epidermis cells comprise uniseriate, oblong or rectangular cells. The abaxial epidermis cells are smaller than the adaxial ones (Figure 5-A, B). Both epidermis cells are covered with a

cuticle. The adaxial cuticle layer is thick than the abaxial cuticle. There are eglandular and glandular trichomes on the entire epidermal surface, but trichome density of abaxial side is more than adaxial side. Midrib is orbicular shaped and has 5–6 layered collenchyma located above lower epidermis.



Figure 1. *T. microphyllum*. A-General appearance, B- Floral stem, C- Calyx and leaves, D- Flowers

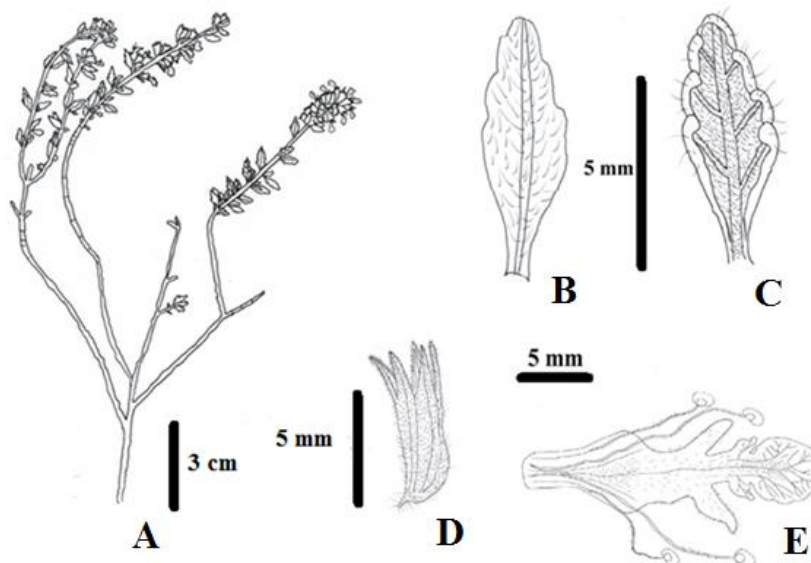


Figure 2. *T. microphyllum*. A- General appearance, B- Adaxial side of leaf, C- Abaxial side of leaf, D- Calyx, E-Corolla



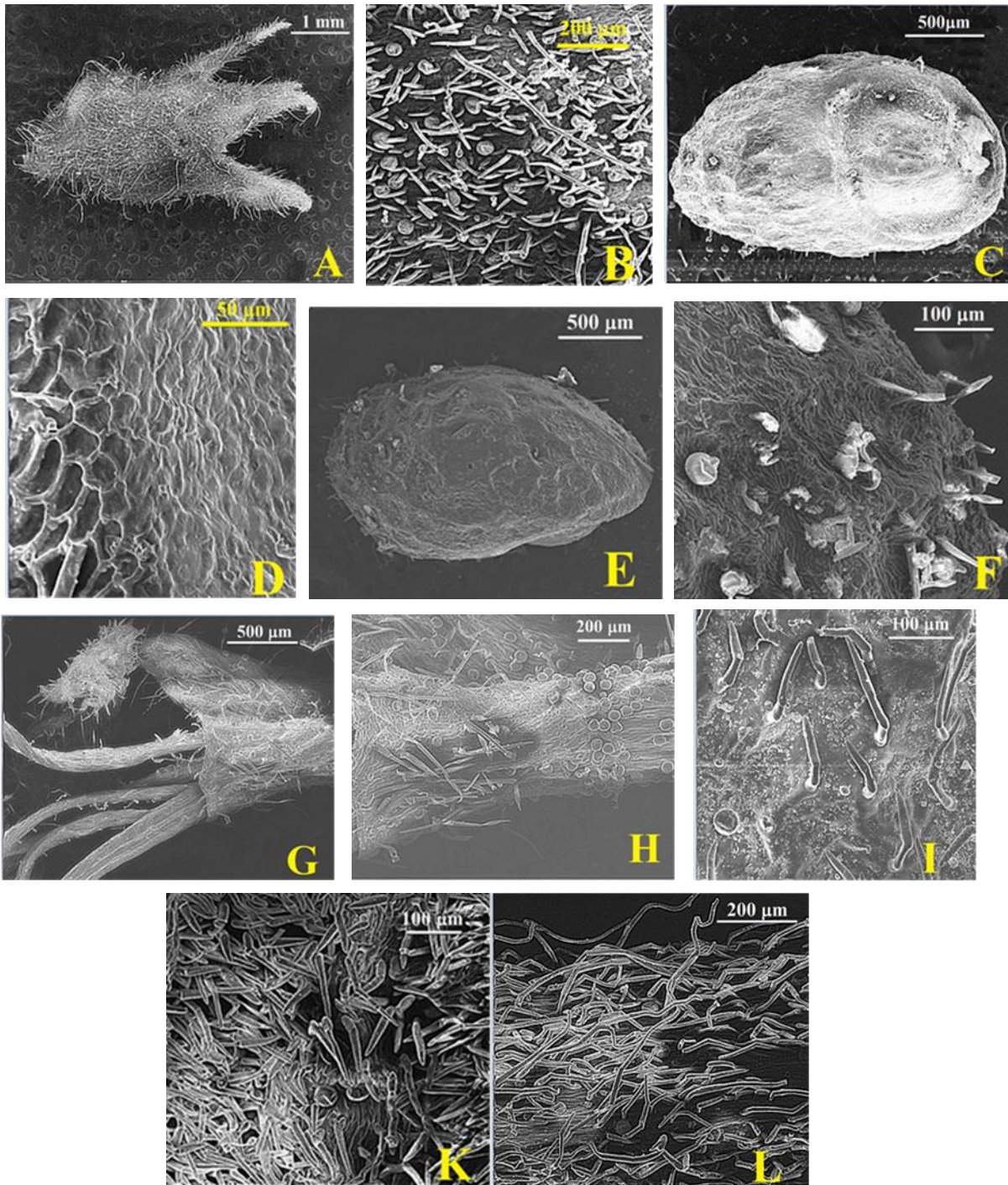


Figure 3. SEM photographs of *Teucrium microphyllum*. A, B- Calyx, C, D- Upside of nutlet, E, F- Underside of nutlet, G, H-Corolla, I-Adaxial side of leaf, K-Abaxial side of leaf, L-Stem.

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Phloem is 4-10 layers and xylem is 3-8 layers. Leaves are bifacial (dorsiventral). Palisade parenchyma cells are usually 2, sometimes 3-layered under the upper epidermis. Spongy parenchyma cells are 2-4-layered under the palisade tissue.



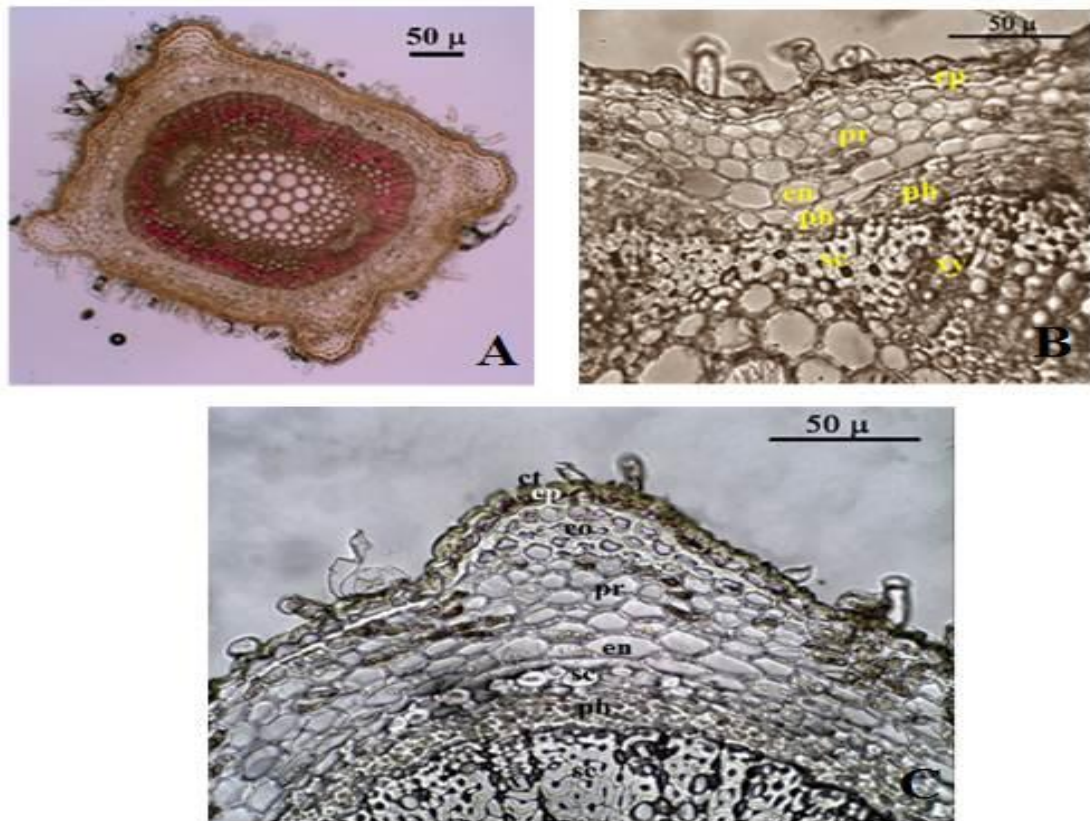


Figure 4. *T. microphyllum*. Cross-sections of stem. A- General appearance, B- The region of between the corners, C- One corner of stem (ct: cuticle, ep: epidermis, co: collenchyma, pr: parenchyma, en: endodermis, sc: sclerenchyma, ph: phloem, xy: xylem).

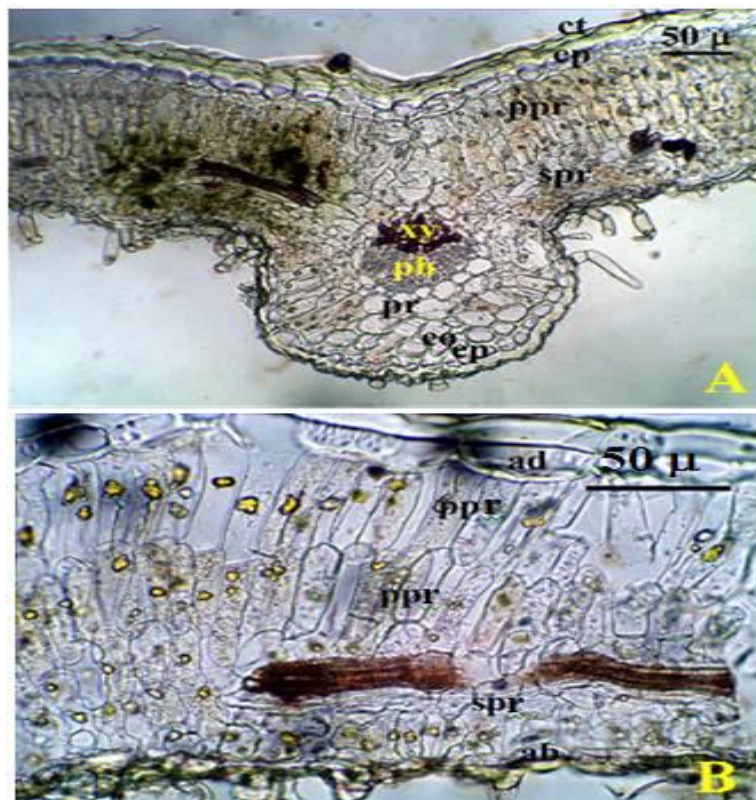


Figure 5. *T. microphyllum*. Cross-sections of leaf. A- Midrib, B- Mesophyll (ct: cuticle, ep: epidermis, ppr: palisade parenchyma, spr: spongy parenchyma, xy: xylem, ph: phloem, co: collenchyma, pr: parenchyma, ad: adaxial epidermis, ab: abaxial epidermis).

3.3 Karyological Studies

The somatic chromosome numbers of *T. microphyllum* are  $2n = 30$  (Figure 6). Besides, the basic chromosome number in this species are  $x = 15$ . In addition to chromosome numbers, homolog chromosome pairs were determined by the study of their total and relative length. Centromeres and the type of chromosomes could be determined. The chromosome morphology of the mitotic metaphase chromosomes of species are investigated according to their total length, relative size and total haploid chromosome length. Detailed morphological characters are given in Table 1 and the idiogram is shown in Figure 6-B.

Small arm length of the smallest chromosome is  $0.53 \mu\text{m}$  and the long arm length is  $0.62 \mu\text{m}$ . The largest chromosome length ranges between  $1.09\text{-}1.29 \mu\text{m}$ . Whole chromosomes are metacentric. Total chromosome length range between  $1.15\text{-}2.39 \mu\text{m}$ . In this study, chromosome number and chromosome morphology of *T. microphyllum* were identified for the first time.

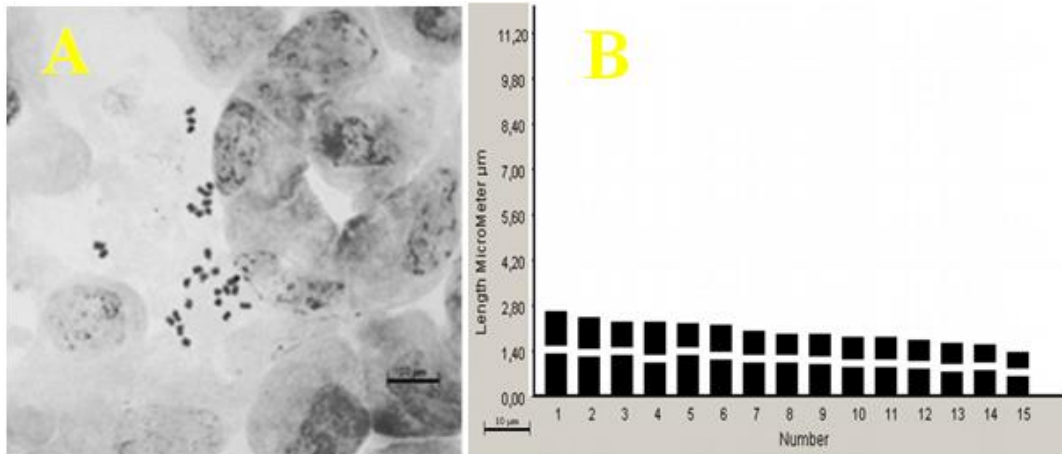


Figure 6. *T. microphyllum*. A- Chromosome number. B- Idiogram

Table 1. Measuring of the somatic chromosomes in *T. microphyllum* ( $\mu\text{m}$ ) (M and m:Metacentric)

Chr. Pair	Chromosome arms ( $\mu\text{m}$ )		Total Length ( $\mu\text{m}$ )	Arm Ratio (L/S)	Total Length (%)	Centromer Index	Chr. Type
	Long arm (L)	Small arm (S)					
1	1,29	1,09	2,39	1,18	4,16	9,07	m
2	1,21	0,97	2,17	1,25	3,67	8,25	m
3	1,24	0,80	2,03	1,55	3,03	7,72	m
4	1,01	1,01	2,02	1,00	3,83	7,67	M
5	1,24	0,77	2,01	1,60	2,93	7,63	m
6	1,10	0,83	1,94	1,33	3,16	7,35	m
7	1,04	0,77	1,82	1,35	2,93	6,90	m
8	1,01	0,67	1,68	1,51	2,54	6,38	m
9	0,97	0,70	1,67	1,37	2,68	6,35	m
10	0,88	0,71	1,60	1,23	2,71	6,06	m
11	0,90	0,68	1,58	1,32	2,58	6,00	m
12	0,85	0,65	1,50	1,32	2,45	5,67	m
13	0,74	0,67	1,41	1,10	2,54	5,35	m
14	0,70	0,58	1,38	1,35	2,22	5,22	m
15	0,62	0,53	1,15	1,16	2,03	4,38	m

3.4 Ecological Studies

*T. microphyllum*, an East Mediterranean element, is distributed in Aegean Islands and SW Anatolia. The species is element of the garig formation and has been determined to grow on clay-loamy soils, with pH 7.17, with 4.01% of organic matter, very little ( $<1.0$ ) phosphorus and 162 mg/kg potassium. The saltness is brackish and limeness is lower. The results obtained from ecological studies are shown in Table 2.

During the field trips, some associated species that share their habitats are detected. The associated taxa of the *T. microphyllum* are *Inula heterolepis* Boiss., *Olea europaea* L., *Origanum onites* L., *Phillyrea latifolia* L.,

*Sarcopterium spinosum* (L.) Spach, *Tymbra capitata* (L.) Cav., *Achillea cretica*, *Amygdalus* sp., *Arbutus* sp., *Genista* sp., *Helichrysum* sp.

#### 4. Discussion

In this study, Aegean endemic *Teucrium microphyllum* was studied morphological, anatomical, karyological and ecologically for the first time. Morphologic data obtained in this study was compared with Ekim's (1982) data. Description of the species was expended.

Table 2. The soil of *T. microphyllum* analysis results and comment

Analysis Values			
Parameter	Module	Analysis Result	Comment
Structure	%	66	Clay loam
pH		7,17	Neutral
E.C.	µS/cm	278	Brackish
Lime	%	0,78	Lower lime
OrganicSubst.	%	4,01	Good
P(Phosphor)	mg/kg	<1,0	Very little
K (Potasium)	mg/kg	162	Enough
Cu (Copper)	mg/kg	1,0(±0,1)	Enough
Fe (Iron)	mg/kg	7,5(±0,8)	Enough
Zn (Zinc)	mg/kg	1,4(±0,1)	Enough
Mn (Manganese)	mg/kg	27,9(±2,5)	Enough

Stems of Lamiaceae species are rectangular and there are collenchymatic tissue at the corners. Sclerenchymatic tissue covers the vascular tissue (Metcalf and Chalk, 1983). The lower part of stem of *T. sandrasicum* O.Schwarz is orbicular shaped and parenchymatic cells are 4-5-layered. Schlerenchymatic cells do not cover all over the vascular bundle and they are like bouquets (Dinç et al., 2008). The stems of *T. polium* L. and *T. montanum* L. have one-layered collenchyma between the corners and 6-7-layered collenchyma in the corners. There are 4-5-layered parenchymatic cells under the collenchymatic tissue. Endodermis consists of 1-2 layers (Dinç et al., 2011). *T. polium* has a rectangle shaped stem and epidermis consists of rectangular cells and this tissue is single layered (Dehshiri and Azadbakht, 2012). The floral stem of *T. microphyllum* is rectangular and has thick cuticle (Figure 4). There are glandular and eglandular hairs (Figure 3-L and Figure 4). Collenchymatic tissue has 2-5 layers in the corners. Vascular bundle has phloem (4-5-layered), Xylem (5-8-layered) and schlerenchymatic cells. Sclerenchymatic cells forming bundles are between endodermis and phloem. Dinç et al. (2011) reported that abaxial and adaxial epidermises of *T. sandrasicum* consist of uniseriate, oval and rectangular cells. *T. microphyllum* is almost similar *T. sandrasicum*. Epidermis cells of the adaxial side are larger than abaxial side in *T. sandrasicum* and *T. microphyllum*. It is the same for *T. polium* and *T. montanum* (Dinç et al., 2011). The walls of abaxial epidermis cells are folded but adaxial epidermises are almost smooth and thicker. This feature is same for *T. polium*, *T. montanum* and *T. sandrasicum* (Dinç et al., 2008; Dinç et al., 2011). *T. microphyllum* has 1(-2)-layered collenchymatic tissue below the midrib, but *T. sandrasicum*, *T. polium* and *T. montanum* have collenchyma both below and above midrib (Dinç et al., 2008; Dinç et al., 2011). As it was reported by Lakusic et al. (2007), *T. arduini* L. has collenchymatic cells both adaxial and abaxial sides, too. Leaf is bifacial and the mesophyll is clearly differentiated into palisade and spongy parenchyma (Figure 5-B). The palisade parenchyma consists of usually 2 rarely 3 layers and spongy parenchyma is 2-4-layered. *T. arduini*, *T. sandrasicum*, *T. polium* and *T. montanum* are also bifacial. *T. arduini* had 1-3-layered palisade and 2-3-layered spongy parenchyma (Lakusic et al., 2007). *T. sandrasicum* are 2-layered palisade and 2-3-layered spongy parenchyma and it is same for *T. polium* and *T. montanum* (Dinç et al., 2008; Dinç et al., 2011).

The basic chromosome numbers for the genus *Teucrium* have a big variation as  $x = 5, 8, 13, 16$  (Darlington and Wylie, 1955; Valde'S-Bermejo and Sanchez-Crespo; 1978). In Flora of Turkey (Güner et al., 2000), the somatic chromosome number of *T. alyssifolium* Stapf and *T. divaricatum* Sieb. subsp. *divaricatum* have been reported as  $2n = 30$  and  $2n = 64$ , respectively. According to the results of Martin et al. (2006) studies, somatic chromosome number of *T. lamiifolium* d'Urv. subsp. *lamiifolium* was observed as  $2n = 32$ . In this study, somatic chromosome number of *T. microphyllum* was found as  $2n = 30$  and has showed idiogram and chromosome morphology of the species for the first time.

After analyzing soil structures of *T. microphyllum*, the soil has a neutral pH and it is lower limy. Inorganic matters (except phosphor) are enough. And associated species of the species were determined with this study.

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