A MICROMORPHOLOGICAL STUDY ON THE GENUS THYMUS LAMIACEAE IN TURKEY

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A MICROMORPHOLOGICAL STUDY ON THE GENUS THYMUS LAMIACEAE IN TURKEY

submitted by **DAMLA KÜTÜKALAN** in partial fulfillment of the requirements for the degree of **Master of Science in Biology Department, Middle East Technical University** by,

Prof. Dr. Halil Kalıpçılar
Dean, Graduate School of **Natural and Applied Sciences** ____________

Prof. Dr. Ayşegül Çetin Gözen
Head of Department, **Biology** ____________

Prof. Dr. Musa Doğan
Supervisor, **Biology, METU** ____________

Examining Committee Members:

Prof. Dr. Sertaç Önde
Biology, METU ____________

Prof. Dr. Musa Doğan
Biology, METU ____________

Dr. Öğr. Üy. Cahit Doğan
Biology, Hacettepe University ____________

Date: 06.09.2019

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Surname: DAMLA KÜTÜKALAN

Signature:

ABSTRACT

A MICROMORPHOLOGICAL STUDY ON THE GENUS THYMUS LAMIACEAE IN TURKEY

Kütükalan, Damla
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The genus *Thymus* L.(Lamiaceae) having its center of diversity in the Mediterranean distributed mainly in the Old World includes 45 species in Turkey. It belongs to the monophyletic subfamily *Nepetoideae*. In this study, epidermal micromorphological properties of 32 species of *Thymus* were examined by using scanning electron microscopy. Micromorphology of vegetative and floral organs were found to be providing good taxonomic features for understanding phylogeny of the genus.

Keywords: Thymus, Lamiaceae, Micromorphology, Turkey

ÖZ

TÜRKİYEDE YAYILIŞ GÖSTEREN THYMUS L LAMIACEAE CİNSİ ÜZERİNE MİKROMORFOLOJİK BİR ÇALIŞMA

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Gen merkezi Akdeniz Fitocoğrafik Bölgesi olan Asya ve Avrupa'da da yayılış gösteren *Thymus* L. cinsi Türkiye'de 45 tür içermektedir. Bu cins monofiletik altfamilya olan *Nepetoideae* içinde bulunmaktadır. Bu çalışmada taramalı elektron mikroskobu kullanılarak 32 türün epidermal mikromorfolojileri çalışılmıştır. Vegetatif ve floral organların mikromorfolojisinin filogeninin anlaşılması için iyi taksonomik özellikler oluşturduğu saptanmıştır.

Anahtar Kelimeler: Thymus, Lamiaceae, Mikromorfoloji, Türkiye

To my beloved grandfather

Forever by myside

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ABBREVIATIONS

ANK ……………………………………………….. Ankara University Herbarium

Bal. .... Balansa, Benedict

Boiss. .... Boissier, François de Sauvages de Lacroix

Celak. …………………………………………………… Čelakovský, Ladislav Josef

C. Koch. .... Koch, Karl (Carl) Heinrich Emil (Ludwig)

C. Presl .... Presl, Carl Borivoj

CR .... Critically Endangered

Des.-Shost .... Desjatova-Shostenko, Nathalie A.

E .... Endangered

Fischer ………………………………………… Fischer, Friedrich Ernst Ludwig von

et al. .... et alii (and others)

Griseb. .... Grisebach, August Heinrich Rudolf

Hal. ………………………………………………………... Halacsy, Eugen von

Hausskn. …………………………………………..…… Haussknecht, Heinrich Carl

Hohen. .... Hohenacker, Rudolph Friedrich

Hub.-Mor. ………………………………………………… Huber-Morath, Arthur

IUCN ……... International Union for Conservation of Nature and Natural Resources

Jalas .... Jalas, Jakko

Klokov .... Klokov, Michail Vasilijevich

Kotschy .... Kotschy, Carl Georg Theodor

km .... kilometer

L. .... Linnaeus, Carolus

m .... meter

Mey. .... Meyer, Carl Anton von

μm .... micrometer

Ponert .... Ponert, Jiri

Ronniger .... Ronniger, Karl (Carl)

sect. .... section

subsect. .... subsection

sp. .... species

subsp. .... subspecies

T. .... *Thymus*

Vahl .... Vahl, Martin

var. .... variety

Velen. .... Velenovsky, Josef

Vis. .... Visiani, Roberto de

CHAPTER 1

INTRODUCTION

1.1. The Family *Lamiaceae* (*Labiatae*)

The Family *Lamiaceae (Labiatae)* also known as the mint family contains numerous herb genera of economical and medicinal importance. The family is represented by 236 genera and about 7200 species distributed almost all around the world (Harley et al., 2004). *Thymus* is one of the largest genera with around 250 species represented globally (Raja, 2012).

Turkey is known to be an important gene center for *Lamiaceae* due to its unique geographical condition. The family contains 45 genera and 546 species with 44.2 % rate of endemism in Turkey (Kocabas and Karaman, 2001).

Regarding the taxonomic delimitation *Lamiaceae* is a family of Angiosperms (flowering plants) and classified under *Lamiales* order. One of the key characteristics of *Lamiaceae* is its flower architecture in which petals fused into an upper lip and a lower lip, which are the origins of family name (Figure 1.1, 1.2).

Most of the members of *Lamiaceae* family used for culinary and flavoring purposes are cultivated throughout the world (Davis, 1982).

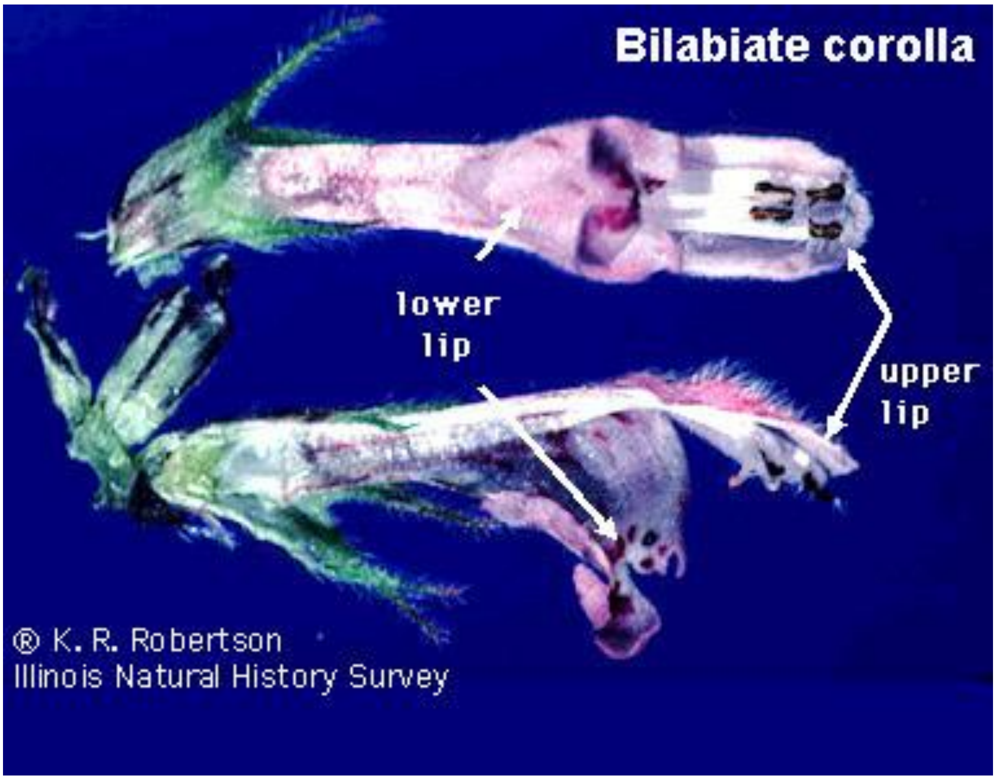

Figure 1.1. A front view and a section of a flower longitudinal of *Lamium purpureum* The upper lip is 2-lobed, while the lower lip is 3-lobed (K. R. Robertson and D. L. Nickrent of plants growing at the University of Illinois).

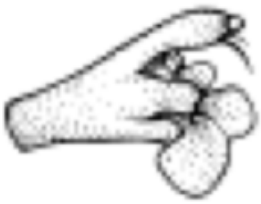

Figure 1.2. Bilabiate petal positions in *Lamiaceae* Family. After Frohne and Jensen (1998).

1.2. The Genus *Thymus* L.

In ancient Greece thyme was appreciated for its use as incense and considered as a sacred herb. 'Thyme' word is derived from Greek thymon or thumus meaning 'courage' or 'strength' which comes from Indo European word dheu meaning 'to smoke' (Cumo, 2013).

Thymus L. is one of the most important genera with nearly 250 taxa classified under *Lamiaceae* family, *Nepetoidea* subfamily and tribe *Mentha*. (Raja, 2012). *Thymus* species are generally bushy plants not more than 50 cm high and seen frequently in Mediterranean region but distributed almost all around the world that are adapted to hot and dry summers (Morales, 2012).

Genus *Thymus* L. has been studied for its antibiotic, anti-inflammatory, antioxidant and insecticidal properties. It has been widely used in folk medicine and for culinary purposes for ages (Reddy et al., 2014; Soare et al., 2009).

Thymus is a genus of aromatic plants, thus presence of various forms of glandular hairs (trichomes) which contain volatile essential oils is a common feature of these and many other aromatic plants. These essential oils have been used for industrial purposes for many years (Morales, 2012). Presence of different hair (trichome) forms on plant surfaces is also very helpful in taxonomy studies since micromorphology of trichomes is highly variable among different levels of taxa (Xiang et al., 2010; Atalay et al., 2016).

According to recent studies and revisions in flora, there are 43 *Thymus* species identified in Turkey (Jalas, 1982, 1988; Duman, 2000; Guner et al. 2012; Sevindik et al., 2016). The ratio of endemism is approximately 45% in Turkey (Başer, 2002).

1.2.1. Historical Background of Genus *Thymus* L.

Plant *Thymus* has been mentioned by botanists since very early ages. The term *Thymus* is found in the works of Dioscorides, Ipokrates, Plinio, Dodonaeus, Barrelier before Linné although it was also used to refer to many other aromatic plants of *Lamiaceae* (Katisotis and Iconomou, 1986; Morales, 2012).

In his Species Plantarum 3rd edition (1764, pp. 825-827) Linné names following 8 species of *Thymus*: 1. *T. serpyllum*, 2. *T. vulgaris*, 3. *T. zygis*, 4. *T. acinos*, 5 .*T. alpinus*, 6. *T. cephalotos*, 7. *T. villosus*, and 8. *T. mastichina* (Figure 1.3). Today we know some of Linné's *Thymus* species are classified in other genera.

After Linné, many new *Thymus* species were identified for the first time by botanists (Brotero, 1804; Hoffmannsegg and Link, 1809). In his *Flora Orientalis* (1888) Boissier described new *Thymus* species throughout the world including North Africa, Iberian Peninsula, Greece and Turkey.

Thymus was intensely studied in Spain and Iberian Peninsula but it is also one of the most studied genera in rest of the world. Some of the most famous botanists done research on *Thymus* are: Klokov, Lyka, Opiz, Ronniger and Jalas.

Klokov edited *Thymus* species in Flora of the U.S.S.R. (1954), 136 species and 5 sections as: *Goniothymus, Verticillati, Euserpyllum, Kotschyani, Subbrecteati* (Figure 1.4.).

Jalas edited *Thymus* species in **Flora of Turkey** (1982), Flora Europaea (1972) and Flora Iranica (1982). He grouped *Thymus* species in 8 sections: *Micantes, Mastichina, Piperella, Teucrioides, Pseudothymbra, Thymus, Hyphodromi,* and *Serpyllum.*

In Flora of Europaea (1972) Jalas described 66 *Thymus* species and grouped them in 8 aforementioned sections.

Jalas described 37 species and 75 taxa including subspecies and varieties in **Flora of Turkey** (1982). However, an infrageneric grouping was not given for *Thymus* in Turkey**.**

11. ORIGANUM foliis ovalibus obtusis, spicis subrotundis compactis pubescentibus. *Hort. cliff.* 304. *Hort. upf.* 161. *Mat. med.* 298. *Roy. lugdb.* 324. **Majorana.**
Majorana vulgaris. *Bauh. pin.* 224.
Amaracus vulgatior. *Lob. ic.* 498.
β. Majorana tenuifolia. *Bauh. pin.* 224.
Majorana hortensis odorata perennis. *Moris. hist.* 3. *p.* 359.
Habitat - - - - ⊙.

THYMUS.

1. THYMUS floribus capitatis, caulibus decumbentibus, foliis planis obtusis basi ciliatis. *Fl. svec.* 477; 535. *Mat. med.* 282. **Serpyllum.**
Thymus repens, foliis planis, floribus verticillato-spicatis. *Hort. cliff.* 306. *Roy. lugdb.* 325.
Serpyllum vulgare minus. *Bauh. pin.* 220.
Serpyllum vulgare. *Dod. pempt.* 277.
β. Serpyllum vulgare majus. *Bauh. pin.* 220.
γ. Serpyllum vulgare minus, capitulis lanuginosis. *Tournef. inst.* 197. *It. gotl.* 219.
δ. Serpyllum angustifolium hirsutum. *Bauh. pin.* 220.
ε. Serpyllum foliis citri odore *Bauh. pin.* 220.
Habitat in Europæ *aridis apricis.* ♄
Folia *basi ciliata.* Stamina *longitudine corollæ.*

2. THYMUS erectus, foliis revolutis ovatis, floribus verticillato-spicatis. *Hort. cliff.* 305. *Hort. upf.* 16[illegible]. *Mat. med.* 281. *Roy. lugdb.* 325. *Sauv. monsp.* 148. vulgaris.
Thymus vulgaris, folio tenuiore. *Bauh. pin.* 219.
β. Thymus vulgaris, folio latiore. *Bauh. pin.* 219.
Thymum durius. *Dod. pempt.* 276.
Fff 5 *Ha-*

Figure 1.3. Page where Linné describes *Thymus serpyllum* and *Thymus vulgaris* in Species Plantarum (1764).

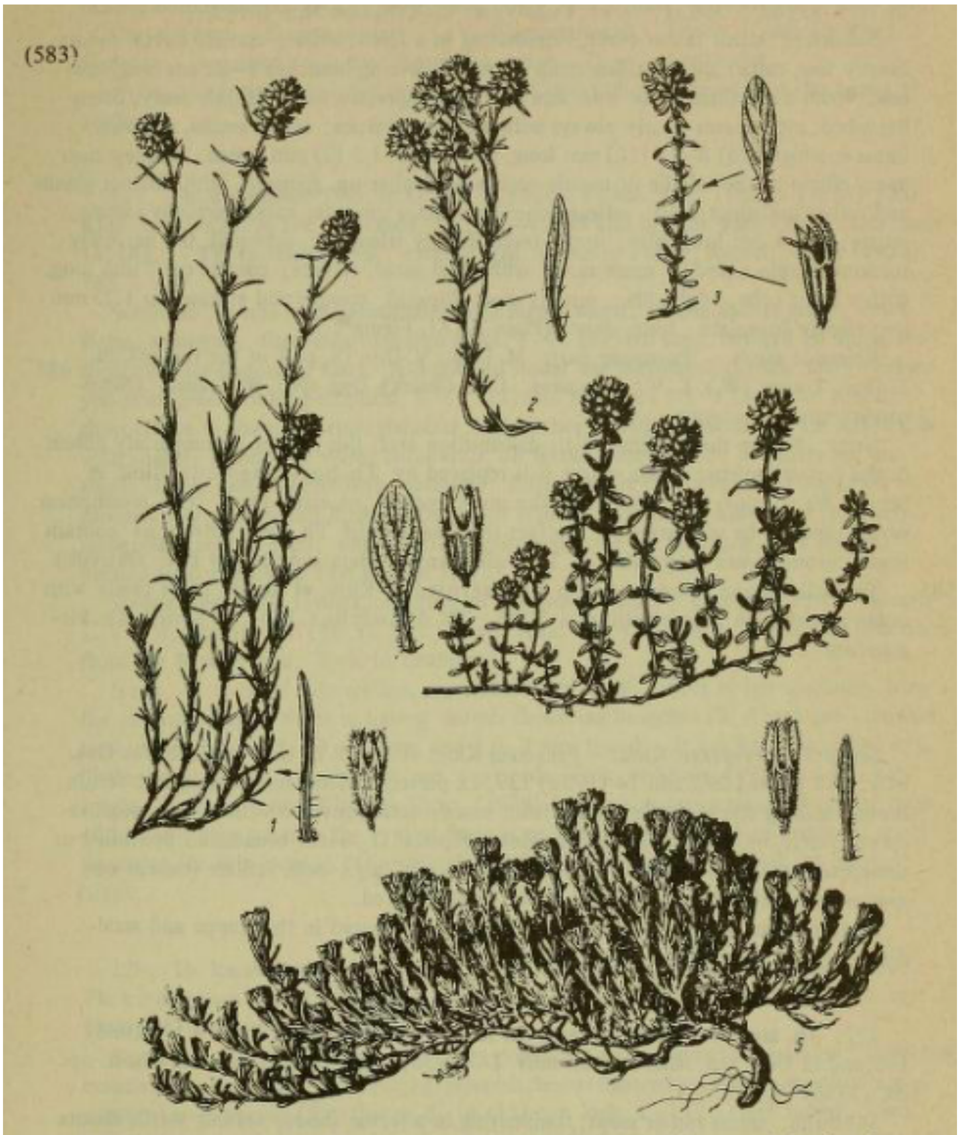

PLATE XXXI. 1 – Thymus borysthenicus Klok. et Shost., plant fragment, leaf, calyx; 2 – Th. pallasianus H. Braun, branchlet, leaf; 3 – Th. cretaceus Klok. et Shost., plant fragment, leaf, calyx; 4 – Th. dubjanskii Klok. et Shost., plant fragment, leaf, calyx; 5 – Th. helendzhicus Klok. et Shost., general aspect, leaf, calyx.

Figure 1.4. Five *Thymus* species illustrated in Flora of U.S.S.R. (1954)

1.2.2. Morphology and Distribution of Genus *Thymus* L.

The genus *Thymus* contains numerous species distributed nearly whole Eurasia, North Africa, Canary Islands and reaches to Greenland (Klokov, 1954). This vast geographical distribution pattern is one of the key components of morphological diversity of *Thymus* species.

Thymus species are generally differentiated in 2 groups; small bushy plants usually below 50 cm -sometimes sporadically to 1 m or creeping forms with rooting twigs. However, they are depicted as small shrubs, cushion plants or perennial herbs, woody at least at base.

Thymus plants usually have quadrangular stems similar to other genera classified under *Lamiaceae* family. The hairs on flowering stems can be on all sides (holotrichus), on two sides (alelotrichous) or only on the angles (goniotrichous) (Jalas, 1972) (Figure 1.5.).

Thymus leaves vary greatly in shape, size and venation. Morphology, anatomy, frequency and distribution of glands, stomata and trichomes are each, one of the most attention-grabbed topics in leaf epidermis studies of aromatic plants (Economou-Amilli et al., 1982).

Leaves of genus *Thymus* can be petiolate or rarely sessile, entire- sometimes denticulate, leaf margins are flat or revolute and/or marginal thickening (keeled or subtriquetrous) and often ciliate towards base of lamina (Jalas, 1982).

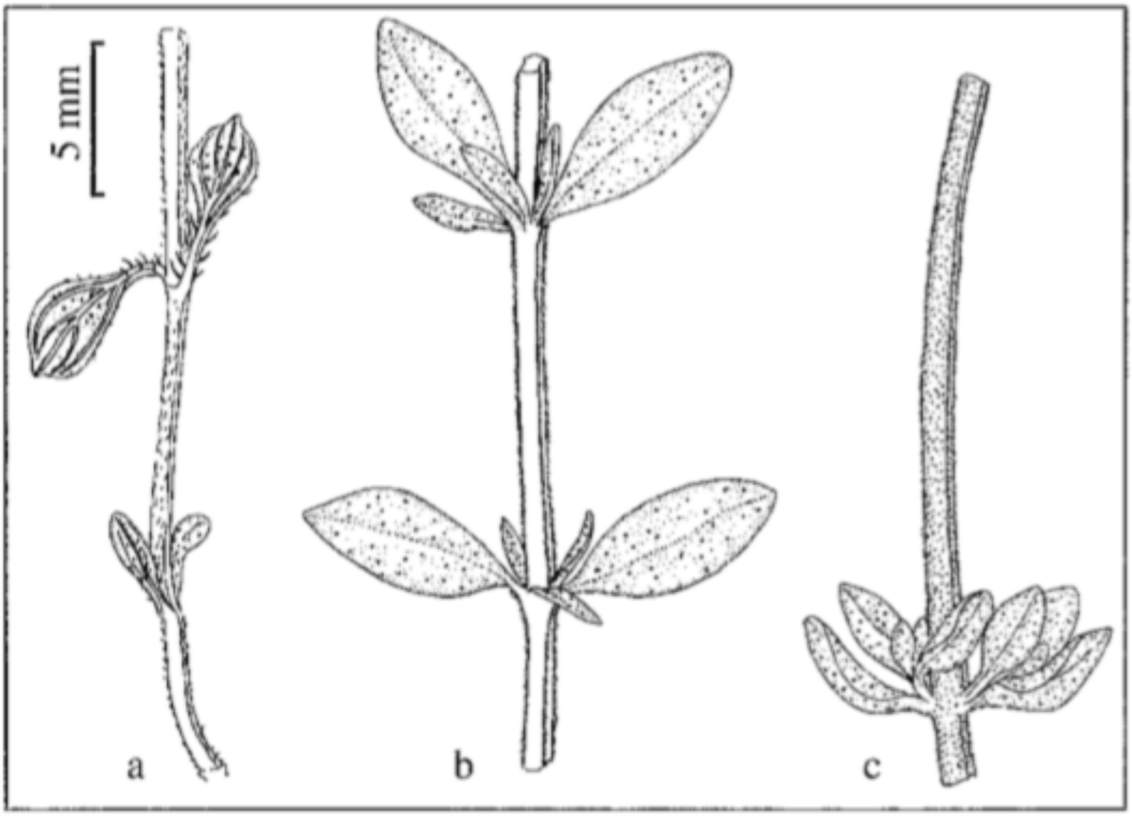

Figure 1.5. Various stem types in *Thymus*: a. alelotrichous (*Thymus praecox*), b. goniotrichous (*Thymus pulegioides*), c. holotrichous (*Thymus piperella*). (Morales, 2012).

Leaf indumentum is also highly variable through *Thymus* species. Some species have glabrous leaves, where some *Thymus* species have leaves usually ciliate either at the whole margin or only at the base or on the leaf stalk.

Glandular trichomes, which belongs to external secretory structures, secrete essential oils that are not only important for growth and development in *Thymus* species but for economical and taxonomical purposes.

As in most genera of *Labiate* family, glandular trichomes of *Thymus* are grouped as capitate or peltate glandular trichomes. Capitate glandular trichomes are composed of a cell or two cells that sit atop a stalk of one to several cells. On the other hand, peltate trichomes usually consist of several secretory head cells (up to 16), a wide short stalk, and a basal epidermal cell (Werker et al., 1985) (Figure 1.6.).

Thyme species have verticillasters 1- to many-flowered, often crowded into a terminal with clearly differentiated bracts or subtending leaves with capitate inflorescence. Calyx tube is usually cylindrical to campanulate with 10-13 veines and straight. Calyx throat has a setiform straight hairy row that plays an important role in dispersion of the nuculas by the wind. In *Thymus* genus, calyx shape is clearly bilabiate with three upper and two lower teeth. Upper lip broad, spreading, patent or recurved with three laneceolate or triangular teeth and lower lip with two narrow ovate/subulate, ciliate teeth curved upwards. Most of the time three upper teeth is shorter than the lower (Figure 1.7.).

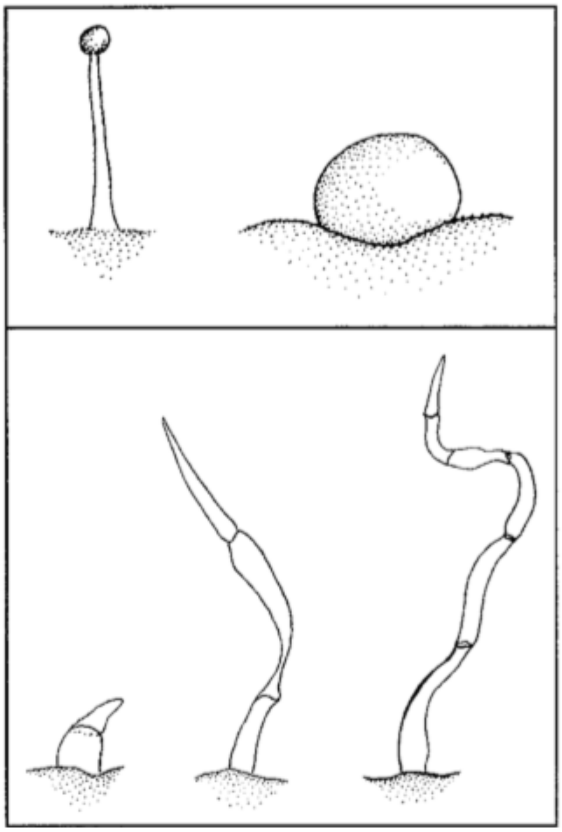

Figure 1.6. Morphology of essential oil glands (up) and hairs (down). (Morales, 2012)

Corolla of *Thymus* species is two lipped that is made of one emarginated upper lobe and three lower lobes with a straight tube. Corolla usually in color purple, cream or white. Four straight, exserted and divergent stamens are seemed to be poor in pollen production. Gynodioecy is very common among *Thymus* species. Female flowers often have smaller calyx and corolla. It is seen that female flowers are pollinated faster than the hermaphrodites by pollinators. Fruits are glabrous nutlets. (Britton and Brown, 1913; Klokov, 1954; Jalas 1972, 1982; Morales, 2012)

Figure 1.7. An illustration of *Thymus serpyllum* clearly depicting floral characteristics (Britton and Brown, 1913).

In terms of distribution and ecology genus *Thymus* shows a vast variation among species. They are usually found on rocky and gravelly ground. Some species live in very cold places while some of the species live in Mediterranean climate.

Soil requirements are also highly variable. For example, species *Thymus cappadocicus* needs calcareous grounds to grow while species *Thymus spathulifolius* lives on gypsaceous steppe slopes (Figure 1.8.).

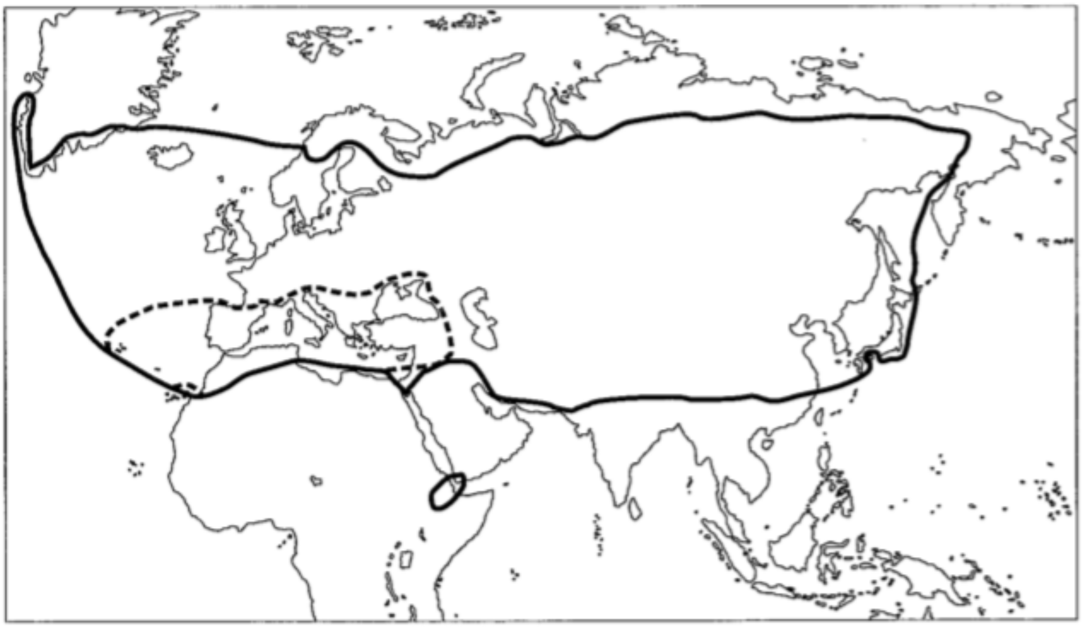

Figure 1.8. General distribution of *Thymus* species in the world. Dotted line represents all sections except section *Serpyllum* and section *Hyphodromi* subsection *Serpyllastrum* (Morales, 2012).

1.3. Micromorphology Studies and Their Importance

Due to environmental factors, geographical distribution, genetic variations, and phylogenetics, plants exhibit numerous forms, structures, and developmental features (Yigit, 2016). Study of these developmental features, forms, and structures or *exomorphic* features of a plant is called "plant morphology" or "phytomorphology" (Bold et al., 1987; Stuessy, 2009).

Morphology is basically classified in two groups as micromorphology and macromorphology. Macromorphological data is obtained with unaided naked eye or binocular microscope. Micromorphological data are those obtained only with light microscopes or with the scanning electron microscope (SEM)/transmission electron microscope (TEM) (Stuessy, 2009).

Morphology is one of the most frequently accepted and appreciated data used in plant taxonomy. The science of morphology is important for the definition and classification of taxa and their relation to certain groups. However solely macromorphological approach cannot effectively solve taxonomic problems. Modern taxonomic parameters are employed to solve these problems by means of using micromorphological features (Iceli, 2011; Bano et al., 2015).

Vegetative morphological characters and reproductive morphological characters are two main branches of morphological data in plants.

Vegetative characters are prone to environmental conditions, whereas, reproductive features are concerned to be more constant and conservative against fluctuating environmental pressures. However morphological features and their usefulness in classification varies from taxon to taxon (Mazaheri, 2016).

1.3.1. 1. Vegetative Morphological Characters and Micromorphology Studies

The leaf, stem, and root were regarded as the principal types of vegetative organs, the size, form, proportions, and arrangement of which are subject to the most varied development or modification (Bold et al., 1987).

Vegetative parts of the plants have many varied functions, such as support, food production, water transport, and storage, in contrast to the narrower (but apparently most important) role of floral features in reproduction.

Despite the fact that plasticity and variability, many features dealing with leaves, stems, and, roots have been used for taxonomic purposes with most attention being given to leaves. Other vegetative characters that are used less often include phyllotaxy, serial buds, and syllepsis (early growth from axillary buds) (Stuessy, 2009).

Leaves play a vital role in photosynthesis, thus observed modifications in leaves help explain how structure, function, and metabolism are related in the plants. Besides photosynthesis leaf morphology plays an important role for heat propagation and light intake. Different metabolic needs give rise to different arrangements of epidermal cells, stomata, and cuticle. Thus, leaf cuticular wax layer, the structures of leaf trichomes and glandular hairs, and stoma structures differ from one another both between and within species (Yigit, 2016).

If the preservation is good cuticular features have superior use in fossil plants (Stuessy, 2009; Mazaheri, 2016). Cuticle ornamentation and epicuticular wax composition may also serve as additional taxonomic characters by using TEM and SEM. The chemistry of the waxes can also be compared, which helps to avoid misinterpretations of relationship based only on micromorphology of the wax crystalloids. Cuticular waxes can show useful patterns of variation at different taxonomic levels, particularly infraspecific, but they can be adaptations to different moisture stresses, and, hence, may be most useful in suggesting ecotypic differentiation rather than formal taxa (Ditsch and Barthlott, 1997; Bredenkamp and Van Wyk, 2000; Ma et al., 2004).

Due to different climatic conditions, such as variations of moisture, and genetic factors micromophology studies on stomata reveals valuable patterns at numerous levels of hierarchy. Stomatal characters have been utilized taxonomically such as in *Passerina* (*Thymelaeaceae*) genus, at species level the sunken stomata and stomatal crypts of *Passerina sp.* are used in the delineation of the new taxon. (Bredenkamp and Van Wyk, 2000; Stuessy, 2009; Sonibare et al., 2014; Sharaibi and Afolayan, 2017).

Trichomes have been useful for centuries in morphology studies and now that they can be revealed more clearly and dramatically by SEM. Not only comparison of the basic structural types, which occur in numerous forms, but also the surface variations of the hairs themselves can be compared.

Trichomes have been mostly employed taxonomically to compare species within a genus, however higher levels of hierarchy can be compared by means of their trichomes (Stuessy, 2009; Sonibare et al., 2014; Bano et al., 2015; Sharaibi and Afolayan, 2017; Zareh et al., 2017). In a recent study Atalay et al. showed the systematic significance of trichomes in *Lamium* genus for taxonomical purposes (Atalay et al., 2016).

Celep et al. (2011) investigated stem, leaf and calyx anatomy and micromorphology of *Lamium truntacum* Boiss including indumentum. As a result, they suggested that these traits are significantly useful in identification of species in genus.

Although not as popular as foliar indumentum, trichomes on stem surfaces have been studied for their taxonomic implications for many years (Maleci et al. 1999; Marin et al., 2008a; Stevanovic et al., 2008; Boz et al., 2009; Jia et al., 2013; Atalay, 2016; Atalay et al., 2016; Zareh, et al., 2017).

Many studies indicate that presence, density/number and types of glandular and non-glandular trichomes, also essential oil composition of glandular trichomes are very variable among *Thymus* species (Economou-Amilli et al., 1982; Werker et al.; 1985, Maleci et al., 1999; Marin et al., 2008a; Stevanovic et al., 2008; Boz et al., 2009; Jia et al., 2012, 2013).

1.3.2. Reproductive Morphological Characters and Micromorphology Studies

Although impressive contributions of vegetative characters to angiosperm taxonomy, the data from reproductive characters have had far more impact. The various features have come primarily from the flowers, fruits, seeds, and variations in symmetry (Stuessy, 2009).

Many studies were shown that not only foliar but floral trichomes were highly valuable taxonomic sources at different levels of hierarchy (Maleci et al., 1999; Marin et al., 2008b; Jia et al., 2013; Seyedi and Salmaki, 2015; Atalay, 2016; Atalay et al., 2016; Mazaheri, 2016; Zareh et al., 2017). Giuliani et al. (2017) studied floral macro and micro traits on a complex genus in order to gain additional data for infrageneric grouping. They examined floral glandular indumentum characteristics of 23 *Sedum* species by using Scanning Electron Microscopy.

By using SEM epidermal cell patterns on corolla or tepals, fruit characters and seed coats provide tremendous taxonomical data at all levels of hierarchy (Karcz, 1996; Zareh et al., 2017).

In a study Gabr investigated seed shape, dimensions, surface texture and sculpture, hilum shape and position of seven species of each of the Apocynaceae and Asclepiadaceae by using light microscope and SEM. As a result, seven patterns were recognized based on surface sculpturing pattern (Gabr, 2014).

Taxonomy and systematics form the basis to collect valuable information regarding to variety of viability, and classification of this information within itself, and reorganize it (Yigit, 2016).

Morphological data especially micromorphology is helpful at all levels of taxonomic hierarchy from the variety to division. Morphology may be regarded as old-fashioned by some, but both vegetative and floral morphology are the most useful data for nearly all taxonomic problems in almost all groups. It is a great mirror of genetic structures and environmental adaptations of organisms (Stuessy, 2009).

The bottom line is if a researcher has to pick only one set of data for taxonomical purposes, he should at least start with micromorphology.

1.4. Economic Use of *Thymus* L.

Most of the countries in the world are dependent on agriculture. Thyme is one of the most valuable plant that is cultivated for culinary, cosmetic, decorative and medicinal purposes.

Unfortunately, the economic importance of *Thymus* has been underestimated for decades. Among 350 species, very few of them are considered as economically important: *Thymus capitatus, T. mastichina, T. serpyllum, T. vulgaris* and *T. zygis* (Lawrence and Tucker, 2012). However, studies are continued to unreveal implications of economic importance of other *Thymus* species.

Thyme production is carried out mostly by Mediterranean countries and Mexico in the world. Worldwide annual Thyme production is approximately 12000-13000 tons. Turkey is one of the leading countries in the world of *Thymus* exportation (Gul, et al., 2014).

In Turkey 121112 ha of land was planted with Thyme in 2016 and the production volume was 14724 tons. Denizli, Manisa, Kütahya, Uşak, Hatay, Aydın and Muğla are the most important production provinces in Turkey. Production volumes and changes are given in Table 1.1. for years 2004-2016. (Aslan and Gul, 2017).

Thymus species are advantageous plants to be cultivated since they are known to grow easily. They are resistant to drought and prefer dry and stony soils that would not be suitable for other plants to grow. Although they don’t like heavily moisturized climates, they can grow in wet soils with variations in their essential oil production (DAFF, 2012; Kachoie et al., 2013; Kleinwächter et al., 2015; Tatrai et al., 2016).

Table 1.1. *Thyme Production in Turkey (2004-2016) (Source: TUIK) (Aslan and Gul, 2017).*

Table 2. The Thyme Production in Turkey (2004-2016)

Years	Production (ton)	Index (2004=100)
2004	7000	100
2005	6400	91
2010	11190	160
2013	13658	195
2014	11752	168
2015	12992	186
2016	14724	210

Although for most species thymol and carvacrol are the main components of Thyme oil, it was shown that essential oil composition is highly variable among *Thymus* species (Kasumov, 1987; Fachini-Queiroz et al., 2012; Venskutonis, 2012; Khanavi et al., 2013; Gavaric et al., 2015).

Due to unique neurotoxic activity of their essential oil on agricultural pests, genus *Thymus* is considered as a natural source of industrial pesticides (Isman, et al., 2010). Thanks to its antiseptic and odor absorbant activity *Thymus* essential oil is also used in the preparation of cosmetics such as deodorant, cream and toothpaste (Nakatani et al., 1989; Gonçalves et al., 2011; Kiani et al., 2017).

Medicinal use of Thyme plant dates back to ancient times. In the couple of last decades numerous studies have been conducted to reveal pharmacological activity of *Thymus* L. extract and essential oil. It has been shown that various species of Thyme plant oil exhibits antibacterial, antifungal, antiinflammatory, antioxidant, antitumor and antiviral activity (Kandil et al., 1994; Sokmen et al., 1999; Tuzlacı and Tolon, 2000; Karaman et al., 2001; Al-Bayati, 2007; Nikolic et al., 2014).

Karaman et al. (2001) showed that, an endemic *Thymus* species of Turkey, *Thymus revolutus* exhibited significant antifungal and antibacterial activity against 11 bacteria and 4 fungi species at different levels.

It has been suggested that these medicinal properties provided by rich phenolic components of the essential oil of Thyme plants such as thymol and carvacrol (Linhart and Thompson, 1999; Karaman et al., 2001; Xu et al., 2008; Pirbalouti et al., 2011).

1.5. Conservation Status of Genus Thymus in Turkey

Due to overexploitation of the plant, demolishing effects of industrialization and urbanisation on wild, genus *Thymus* is facing serious problems in Turkey.

According to the 1997 IUCN Red List of Threatened Plants, there are 14 ‘Rare’, 2 ‘Vulnerable’ and 1 ‘Endangered’ *Thymus* species in Turkey. Almost all of the species listed from Turkey are endemic and this means extinction of *Thymus* species in Turkey would be a global problem in terms of biodiversity.

It was reported that 3 endemic *Thymus* species from Kazdagi mountain were categorized as ‘Not Threatened’, ‘Lower Risk’ and ‘Critically Endangered’. It is worth noting that *Thymus pulvinatus* was categorized as ‘Rare’ in 1997 and in 2011 it was moved into category ‘Critically Endangered’ (Ozturk et al., 2011).

Thymus aznavourii Velen. is another endemic species struggling to survive in Turkey. In 1997 it was categorized as ‘Endangered’ and by the Berne Convention (1998) it was determined that:

“Special protection (`appropriate and necessary legislative and administrative measures`) for the plant taxa listed, including prohibition of deliberate picking, collecting, cutting, uprooting and, as appropriate, possession or sale.”

In IUCN Red List of Threatened Species 2011 *Thymus aznavourii* Velen. is categorized as ‘Data Deficient’ and it was reported that ‘It is unknown whether the species still exists’ (Bilz, 2011). In 2018-1 IUCN Red List Online Database *Thymus* species from Turkey and Turkey as a location are not even present.

As a conclusion it should be stated that more biodiversity and conservation studies are needed in the field and governments should take more serious measures to sustain plant biodiversity.

1.6. Infrageneric Grouping of Genus *Thymus*

Bentham's grouping of *Thymus* species under two following sections for the first time was one of the most remarkable taxonomic studies in botanics history: *Mastichina* and *Sepryllum* (Bentham, 1834).

In 1845 Boisseri added a new section to genus and named it as *Pseudothymbra*. Furthermore, with new studies Klokov (1954) identified 5 distinct sections of *Thymus* as: *Goniothymus, Verticillati, Euserpyllum, Kotschyani, Subbrecteati* in Flora of U.S.S.R..

One of the most accepted infrageneric classification today is the one based on Jalas' studies in 1971 and 1972:

I. Sect: *Micantes,*

II. Sect: *Mastichina,*

III. Sect: *Piperella,*

IV. Sect: *Teucrioides,*

V. Sect: *Pseudothymbra*

- a. Subsect: *Pseudothymbra,*
- b. Subsect: *Anomalae*,

VI. Sect: *Thymus*

- a. Subsect: *Thymastra,*
- b. Subsect *Thymus*,

VII. Sect: *Hyphodromi*

- a. Subsect: *Subbracteati,*
- b. Subsect: *Serpyllastrum*,
- c. Subsect: *Thymbropsis*,

VIII. Sect: *Serpyllum*

- a. Subsect: *Insulares*,
- b. Subsect: *Kotschyani*,
- c. Subsect: *Pseudopiperallae*,
- d. Subsect: *Isolepides*,
- e. Subsect: *Alternantes*,
- f. Subsect: *Pseudomarginati*,
- g. Subsect: *Serpyllum*

In 1997, Morales investigated distribution patterns of sections and subsections of genus *Thymus* in Mediterranean area based on Jalas' infrageneric grouping.

However, infrageneric grouping is not available for *Thymus* species in **Flora of Turkey**. *Thymus* species that were collected different localities of Turkey used in this study are grouped according to different systems of infrageneric classification in Table 1.2.

Table 1.2. *Infrageneric classification of Thymus species examined in this study according to various systems of grouping through the years. (Endemic species are marked with *)*

Species	**Flora of the U.S.S.R (Klokov,1954)**	**Jalas, 1971**	**Flora of Europaea (Jalas, 1972)**	**Morales, 1997; 2012**
**T. cilicicus*	-	Hyphodromi	-	Hyphodromi
**T. revolutus*	-	Hyphodromi	-	Hyphodromi
**T. cherlerioides* var. *isauricus*	Goniothymus	Hyphodromi	Pseudothymbra	Hyphodromi
**T. leucotrichus.*	-	Hyphodromi	Pseudothymbra	Hyphodromi
**T. convolutus*	-	Hyphodromi	-	Hyphodromi
**T. argaeus*	-	Hyphodromi	-	Hyphodromi
* *T. cappadocicus*	-	Hyphodromi	-	Hyphodromi
**T. haussknechtii*	-	Hyphodromi	-	Hyphodromi
**T. spathulifolius*	-	Hyphodromi	-	Hyphodromi
**T. cariensis*	-	Hyphodromi	-	Hyphodromi

Table 1.2. (Continued)

Species	**Flora of the U.S.S.R (Klokov,1954)**	**Jalas, 1971**	**Flora of Europaea (Jalas, 1972)**	**Morales, 1997; 2012**
T. striatus var. interruptus	Subbrecteati	Hyphodromi	Hyphodromi	Hyphodromi
T. zygioides var. *zygioides*	-	Hyphodromi	Hyphodromi	Hyphodromi
T. roegneri	Verticillati	-	Serpyllum	-
T. fallax	-	Serpyllum	-	Serpyllum
T. transcaucasicus	Kotschyani	Serpyllum	-	Serpyllum
T. kotschyanus var. *glabrescens*	Kotschyani	Serpyllum	-	-
T. eriocalyx	-	Serpyllum	-	Serpyllum
T. migricus	Kotschyani	Serpyllum	-	-
T. fedtschenkoi var. *handelii*	Kotschyani	Serpyllum	-	-
**T. sipyleus* subsp. *sipyleus* var. *sipyleus*	-	Hyphodromi	Serpyllum	Hyphodromi
**T. leucostomus* var. *gypsaceus*	-	Hyphodromi	-	Hyphodromi
**T. pubescens* var. *cratericola*	-	Serpyllum	-	-
**T. bornmuelleri*	-	Serpyllum	-	Serpyllum
T. praecox subsp. *jankae* var. *jankae*	-	Serpyllum	Serpyllum	Serpyllum
T. thracicus	-	Serpyllum	Serpyllum	Serpyllum

Table 1.2. (Continued)

T. longicaulis subsp. *longicaulis* var. *longicaulis*	-	Serpyllum	Serpyllum	Serpyllum
T.pseudopulegioides	Goniothymus	Serpyllum	-	-
T. boissieri	-	Hyphodromi	Pseudothymbra	Hyphodromi
T. ararati-minoris	Kotschyani	Serpyllum	-	-
T. pruinosus var. *globifer*	-	Hyphodromi	-	Hyphodromi
* *T. artvinicus*	-	-	-	-
T eriophorus	Kotschyani	Serpyllum	-	-

Morales' infrageneric grouping was largely based on Jalas' previous works however Morales provided subsections within the sections of *Thymus* species.

According to the infrageneric classification of Morales (2012) the 32 species of *Thymus* examined in this study is placed in 2 sections which includes 7 subsections (Table 1.3., 1.4.). The results of this study were evaluated based on aforementioned infrageneric classification.

Table 1.3. *Study Taxa Grouped in Section Hyphodromi*

Section *Hyphodromi*		
Subsect. *Subbructeati*	Subsect. *Serpyllastrum*	Subsect. *Thymbropsis*
T. revolutus	*T. haussknechtii*	*T. cilicicus*
T. cherlerioides var. *isauricus*	*T. sphatulifolius*	*T. cariensis*
T. leucotrichus	*T. zygioides* var. *zygioides*	*T. sipyleus* subsp. *sipyleus* var. *sipyleus*
T. convolutus		*T. leucostomus* var. *gypsaceus*
T. argaeus		
T. cappadocicus		
T. striatus var. *interruptus*		
T. boissieri		
T. pruinosus var. *globifer*		

Table 1.4. *Study Taxa Grouped in Section Serpyllum*

Section Serpyllum			
Subsect. *Insulares*	**Subsect. *Kotschyani***	**Subsect. *Alternates***	**Subsect. *Pseudomarginati***
T. bornmuelleri	*T. fallax*	*T. pseudoplegioides*	*T. praecox* subsp. *jankae* var. *jankae*
	T. transcausicus		*T. tharacicus*
	T. kotschyanus var. *glabrescens*		*T. longicaulis* subsp. *longicaulis* var. *longicaulis*
	T. eriocalyx		
	T. migricus		
	T. fedtschenkoi var. *handelii*		
	T. pubescens var. *cratericola*		
	T. ararati-minori		
	T. roegneri		
	T. artvinicus		
	T. eriophorus		

1.7. Aim of The Study

Infrageneric classification of genus *Thymus* is not available in Flora of Turkey and there is limited information in the world. Also, micromorphological traits of *Thymus* species and their taxonomic implications has not been studied sufficiently. Therefore, biodiversity, distribution and taxonomy of genus *Thymus* in Turkey has not been fully understood.

The aim of the study was to reveal, compare and describe taxonomic implications of micromorphological characters of genus *Thymus* within species from Turkey using Scanning Electron Microscopy (SEM).

For this purpose, micromorphology of leaf, calyx and stems of 32 *Thymus* species gathered from Turkey were examined via Scanning Electron Microscopy (SEM).

CHAPTER 2

MATERIALS AND METHOD

2.1. Plant Material

32 species of *Thymus* given in appendix A and their varieties were collected from all over Turkey by Prof.Dr. Bayram Yıldız and his reseach partners as part of a project funded by The Scientific and Research Council of Turkey (TUBITAK) between 1997-2002. All the species were pressed and dried using standard preservation techniques under laboratory conditions. The specimens were cross-checked with taxonomic keys provided in *Flora Orientalis* (Bentham, 1848), *Flora of the U.S.S.R* (Klokov, 1954), *Flora of Europaea* (Jalas, 1972), *Flora of Turkey* (Jalas, 1982) and other various floras. Numerous additional specimens stored in ANK herbarium were also investigated. All the specimens (appendix A) used in this study are kept at the Plant Systematics Laboratory, Department of Biological Sciences, Middle East Technical University, Ankara.

2.2. Scanning Electron Microscopy Method

Small pieces of dried adaxial and abaxial leaf, calyx, and stem samples of 32 *Thymus* species from Turkey fixed directly on aluminum stubs with double sided carbon tapes. Fixed samples were coated with gold particles and imaged with QUANTA 400F Field Emission Scanning Electron Microscope (SEM) with the range of 30X-5000X magnification to observe micromorphological characters at METU Central Laboratory (Kahraman et al., 2009; Dmitruk and Weryszko-Chmielewska, 2010; Kim et al., 2012).

Qualitative and quantitative leaf, calyx and stem microtraits were analyzed with Image Tool software. The type of indumentum and epidermal micromorphological properties were described and classified following Singh et al. (1974), Bredenkamp and Van Wyk (2000); Celep et al. (2011), Sonibare et al., (2014), Eiji and Salmaki (2016), Atalay et al. (2016).

Briefly, qualitative characters refer to non-numerical properties such as shape, type, definition, position and categorization of a certain trait. On the other hand, quantitative characters refer to numerical, measured traits such as size, length and width of a certain character (Earl et al., 2014).

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Epidermal Cells and Stomata

Adaxial (upper) leaf surfaces of 32 and abaxial (lower) leaf surface of 28 *Thymus* species are examined in the study with SEM. Qualitative microtraits analyzed in the study are Epidermal Cell Shape, Cell Wall Pattern, Presence of Stomata, Stomata Position. Quantitative trait analyzed in the study is Size of Epidermal Cells (Table 2.3.). These foliar traits are considered as taxonomically key features by many researchers (Bredenkamp and Van Wyk, 2000; Sonibare et al., 2014; Bano et al., 2015; Seyedi and Salmaki, 2015; Mazaheri, 2016; Sharaibi and Afolayan, 2017). Shaha et al. (2018) showed that micromorphological traits such as epidermal cell shape/size and stomatal position are valuable taxonomic sources even for complex genera like *Dryopteris* and *Polystichum*.

Different types of epidermal cell shapes, cell wall pattern, stomata position, presence of stomata and epidermal cell sizes in different taxa of *Thymus* are given in Table 3.1-3.2 and Figure 3.1-3.2.

According to the research finding 3 types of epidermal cell shapes were observed among the studied taxa; elongated (e.g. *T. boissieri, T. thracicus, T. pubescens* var. *cratericola*), slightly elongated (e.g *T. convolutus*, *T. leucostomus* var. *gypsaceus, T. ararati-minoris*), and isodiametric (*T. pseudoplegioides* and *T. migricus*) on adaxial and abaxial leaf surfaces.

In most cases epidermal cell shapes did not differ on adaxial and abaxial surfaces. Epidermal cell type differed only in 3 species of *Thymus* on adaxial and abaxial surfaces. On adaxial surface *T. cherlerioides* var. *isauricus* and *T. migricus* had Slightly Elongated, *T. cappadocicus* had Elongated epidermal cell shapes, on abaxial surface they had Elongated, Isodiametric, and Slightly Elongated cell shapes, respectively.

The most common epidermal cell shape observed in the taxa was slightly elongated shape. 18 out of 32 species had slightly elongated shaped epidermal cells whereas only 1 of them had isodiametric shaped epidermal cells on adaxial leaf surface. 20 out of 28 species had slightly elongated shaped epidermal cells whereas only 2 of them had Isodiametric shaped epidermal cells on abaxial leaf surface.

3 types of cell wall pattern were observed among the taxa: undulated/wavy (e.g. *T. pseudoplegioides*, *T. migricus, T. convolutus*), straight to curved (e.g. *T. boissieri*, *T. pubescens* var. *cratericola*, *T. artvinicus*) and straight (*T. fedtschenkoi* var. *handelii*) on adaxial and abaxial leaf surfaces. Most common cell wall pattern was undulated/wavy on both adaxial and abaxial sides. Only species *T. fedtschenkoi* var. *handelii* had Straight cell wall pattern in the taxa. Slightly elongated epidermal cell shape with Undulated/Wavy cell wall pattern (9 taxa out of 11 had the same) in subsection *Kotschyani*, Slightly Elongated epidermal cell shape with Straight to Curved cell wall pattern in subsection *Serpyllastrum* was a characteristic feature on adaxial leaf surface. Isodiametric epidermal cell shape was only observed in subsection *Kotschyani* and *Alternates* of section *Serpyllum*.

T. pseudoplegioides was unique with its isodiametric epidermal cell shape with undulated/wavy cell wall pattern on both adaxial and abaxial leaf surfaces among other *Thymus* species. *T. pseudoplegioides* is the only representative of subsection *Alternates* in Turkey therefore, isodiametric shape with undulated/wavy cell wall pattern on both leaf surfaces was also valuable in subsectional delimitation.

Among all taxa only *T. cherlerioides* var. *isauricus* had elongated cell shape observed with Undulated/Wavy cell wall pattern. In other species Elongated cell shape accompanied by Straight to Curved cell wall pattern except *T. fedtschenkoi* var. *handelii* with Straight cell wall pattern. Slightly elongated epidermal cell with Undulated/Wavy cell wall pattern was the most common type on both adaxial and abaxial leaf surfaces in taxa.

Adaxial leaf epidermal cell length ranged between 18.4 µm in *T. pseudoplegioides* and 190.9 µm in *T. tharacicus,* epidermal cell width ranged between 7.4 µm in *T. ararati-minoris* and 33.4 µm in *T. migricus*. Abaxial leaf epidermal cell length ranged between 19.7 µm in *T. kotschyanus* var. *glabrescens* and 95.7 µm in *T. leucotrichus*, epidermal cell width ranged between 6.8 µm in *T. cilicicus* and 57.8 µm *T. cappadocicus*.

Table 3.1. *Adaxial and Abaxial Leaf Epidermal Cell Sizes*

	Adaxial		Abaxial	
Taxon	Length	Width	Length	Width
T. revolutus	N/A	N/A	44.2±11.4 (28.4-60.5)	18±4.7 (9.5-21.7)
T. cherlerioides var. *isauricus*	54.4±13.1 (31.9-67.2)	20.4±4.9 (12.4-27.2)	N/A	N/A
T. leucotrichus	45.8±14.8 (26.4-67.7)	25.7±4.4 (19.9-32.1)	73.8±14.7 (60.7-95.7)	21.7±2.9 (17.9-24.9)
T. convolutus	56.9±7.9 (44-62.5)	19.5±5 (13.2-26)	62.6±13.5 (47.2-75.6)	22.5±8.2 (17-28.3)
T. cappadocicus	N/A	N/A	83±9.1 (73.6-94.2)	33.7±17.3 (16.8-57.8)
T. striatus var. *interruptus*	59.3±16.4 (41-78.6)	21.3±5.1 (15.4-28.3)	54.5±18 (38.5-76.8)	18±5.2 (9.7-23.3)
T. haussknechtii	52±17.5 (27.6-76.2)	18.6±3.5 (14.4-23.3)	38.2±14.1 (20.2-54.2)	20.3±5.5 (13-22.8)
T. zygioides var zygioides	47.2±17.6 (28.7-71)	30.6±6.4 (24.3-40)	53.1 ± 10.9 (41-68.5)	18.6±5.5 (12.2-25.2)
T. cilicicus	48.9 ± 9.7 (36.5-65.4)	18.9 ± 4.9 (8.2-28.8)	31.4 ± 6.8 (20.9-41.5)	17.1 ± 4.9 (6.8-24.5)
T. leucostomus var. *gypsaceus*	43.9 ± 6.5 (33.9-51.5)	15.7 ± 3.4 (10.2-18.4)	64.2 ± 16.5 (42.4-88)	18.6 ± 4.5 (12-24.7)

Table 3.1. *(Continued)*

T. bornmuelleri	55.8±3.3 (51.8-59.6)	21.7±3.7 (17.2-27.6)	54.5 ± 3.3 (49.8-58)	21.6±12 (13.4-42.3)
T. fallax	45.9±9.9 (34.6-61.7)	17.4±3.8 (14.1-23.8)	39±5.2 (35.8-48.3)	18.2±7.5 (10.5-27)
T. transcausicus	46.1±9.9 (33.7-60.7)	18.9±5.5 (13-26.3)	37.3±2.3 (33.2-38.7)	20±2.9 (17.6-24.7)
T. kotschyanus var glabrescens	39.7±9.8 (28-54.3)	17.3±3.2 (12.7-21.1)	28.2±6.3 (29.7-36.1)	13.5±3.6 (8.8-16.7)
T. migricus	52.2±10.9 (44.5-64.7)	28.5±4.4 (24.6-33.4)	27.9 ± 7.4 (20.1-35)	24.3±9.7 (14.6-34)
T. ararati-minoris	24.6±3.4 (19.7-28.6)	14.2±4.7 (7.4-18.7)	49.9±7.9 (44.2-61.5)	28.5±6.7 (23.6-40.4)
T. roegneri	N/A	N/A	45.2±15.1 (29.1-62.9)	27.4±9 (13.3-35.7)
T. artvinicus	58.7±19.5 (36.8-89.8)	25.7±4.7 (18.6-30.5)	47.3±10.5 (32.5-56.6)	22.7±5.3 (14.4-28.7)
T. eriophorus	49.6±15.1 (29.5-64.8)	18.2±6.8 (10.6-26.6)	46±15 (30-69.1)	15.6±4.2 (11.6-22.3)
T. pseudoplegioides	22.1±2.7 (18.4-26)	14.8±5.5 (8.4-23)	34.8±12.8 (24.5-56.6)	26.9±5.3 (21.1-34.3)
T. praecox subsp. *jankae* var. *jankae*	36.4±6 (30.6-45.2)	19.7±4.8 (12.5-23.5)	49.6±16.9 (31.6-77.5)	18±3.9 (14.1-23.5)

Table 3.1. *(Continued)*

T. tharacicus	120.6±40.1 (90.7-190.9)	19.9±4 (15.5-25.7)	(N/A)	(N/A)
T.longicaulis	39.3±7.6 (28.8-49.9)	18.4±4.6 (14.1-24.1)	34.1±5.1 (29.3-40.5)	17.9±3.5 (13.2-22.6)

Table 3.2. *Adaxial and Abaxial Leaf Epidermal Cell Qualitative Features*

	Adaxial Leaf				Abaxial Leaf			
Taxon	EPCS	CWP	PoS	SP	EPCS	CWP	PoS	SP
T. revolutus	N/A	StC	P	E	SEl	U/W	P	E
T. cherlerioides var. *isauricus*	SEl	U/W	P	S	El	U/W	P	S
T. leucotrichus	SEl	U/W	P	E	SEl	U/W	P	E
T. convolutus	SEl	U/W	P	S	SEl	U/W	P	S
T. argaeus	N/A	StC	P	S	El	StC	P	S
T. cappadocicus	El	StC	P	S	SEl	U/W	P	S
T. striatus var. *interruptus*	SEl	StC	P	S	SEl	StC	P	S
T. boissieri	El	StC	P	S	El	StC	P	S
T. pruinosus var. *globifer*	N/A	U/W	P	E	N/A	U/W	P	E
T. haussknechtii	SEl	StC	P	E	SEl	StC	P	E
T. spahulifolius	N/A	N/A	N	N/A	N/A	N/A	N/A	N/A

Table 3.2. *(Continued)*

T. zygioides var. *zygioides*	SEl	StC	P	S	SEl	U/W	P	S
T. cilicicus	SEl	StC	P	S	SEl	U/W	P	S
T. cariensis	N/A	StC	P	S	N/A	StC	P	S
T. sipyleus subsp. *sipyleus* var. *sipyleus*	N/A	StC	P	S	SEl	StC	P	S
T. leucostomus var. *gypsaceus*	SEl	U/W	P	S	SEl	U/W	P	S
T. bornmuelleri	SEl	U/W	P	S	SEl	U/W	P	S
T. fallax	SEl	U/W	P	E	SEl	U/W	P	E
T. transcausicus	SEl	U/W	P	S	SEl	U/W	P	S
T. kotschyanus var. *glabrescens*	SEl	U/W	P	S	SEl	U/W	P	S
T. eriocalyx	N/A	U/W	P	S	N/A	U/W	P	S
T. migricus	SEl	U/W	P	S	Iso	U/W	P	S
T. fedtschenkoi var. *handelii*	El	St	P	E	N/A	N/A	N/A	N/A
T. pubescens var. *cratericola*	El	StC	P	S	N/A	StC	P	S
T. ararati-minoris	SEl	U/W	P	S	SEl	U/W	P	S
T. roegneri	N/A	StC	P	E	SEl	StC	P	E

Table 3.2. *(Continued)*

T. artvinicus	SEl	U/W	P	E	SEl	U/W	P	E
T. eriophorus	SEl	U/W	P	S	SEl	U/W	P	S
T. pseudoplegioides	Iso	U/W	P	E	Iso	U/W	N	N/A
T. praecox subsp. *jankae* var. *jankae*	SEl	StC	P	S	SEl	U/W	P	S
T. tharacicus	El	StC	P	S	N/A	N/A	N/A	N/A
T. longicaulis subsp.*longicaulis* var. *longicaulis*	SEl	U/W	P	S	SEl	U/W	P	S

EPCS: Epidermal Cell Shape; CWP: Cell Wall Pattern; PoS: Presence of Stomata; SP: Stomata Position; SEl: Slightly Elongated; El: Elongated; Iso: Isodiametric; StC: Straight to Curved; St: StraightU/W: Undulated/Wavy; P: Positive; N: Negative; S: Sunken; E: Even

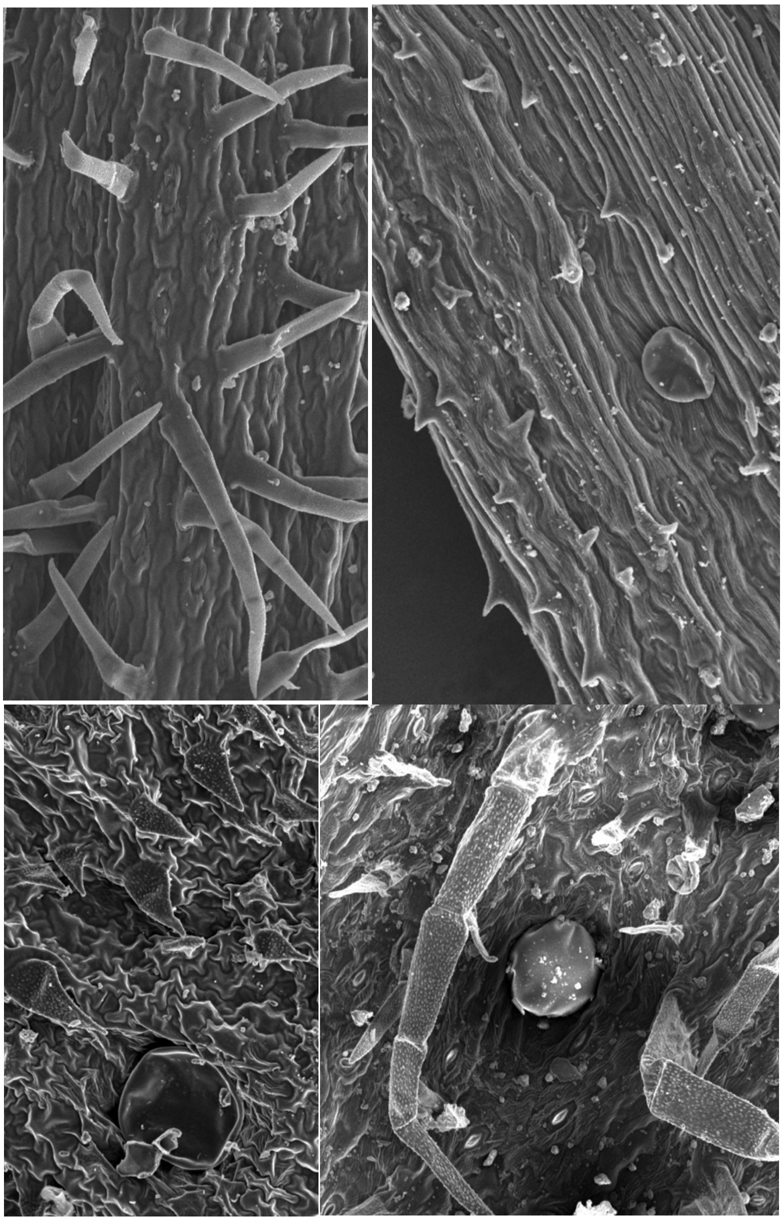

Figure 3.1. Slightly Elongated shape with Undulated/Wavy cell wall pattern on *T. convolutus* adaxial leaf surface (scale 200 µm). (Top Left) .Elongated epidermal cells with Straight to Curved cell wall pattern on *T. boissieri* adaxial leaf surface. (scale 200 µm).(Top Right). Isodiametric shape with Undulated/Wavy Cell Wall Pattern on *T. pseudoplegioides* adaxial leaf surface. (scale 100 µm). (Bottom Left). Slightly Elongated shape with Undulated/Wavy Cell Wall Pattern on *T. migricus* adaxial leaf surface. (scale 200 µm) (Bottom Right)

All of *Thymus* species except 2 had stomata present on their adaxial and abaxial leaf surfaces. Stomata was not visible only in *T. spahulifolius*, however this might be the result of very dense indumentum structure on leaf surface. *T. pseudoplegioides* did not have any stomata on its abaxial leaf surface. This feature of *T. pseudoplegioides* provides delimitation of both in species and subsectional level as it is the only representative of subsection *Alternates* in Turkey. 2 types of stomata position even (eg. *T. artvinicus, T. fallax*, *T. haussknechtii*) and sunken (eg. *T. cilicicus, T. praecox* subsp. *jankae* var. *jankae, T. eriophorus*) were observed among the taxa. Most of the species has sunken stomata on their leaf surfaces. In section *Hyphodromi,* subsection *Thymbropsis* all stomata were positioned as sunken on both leaf surfaces. Sunken stomata position was also a characteristic feature in section *Serpyllum,* subsection *Pseudomarginati*

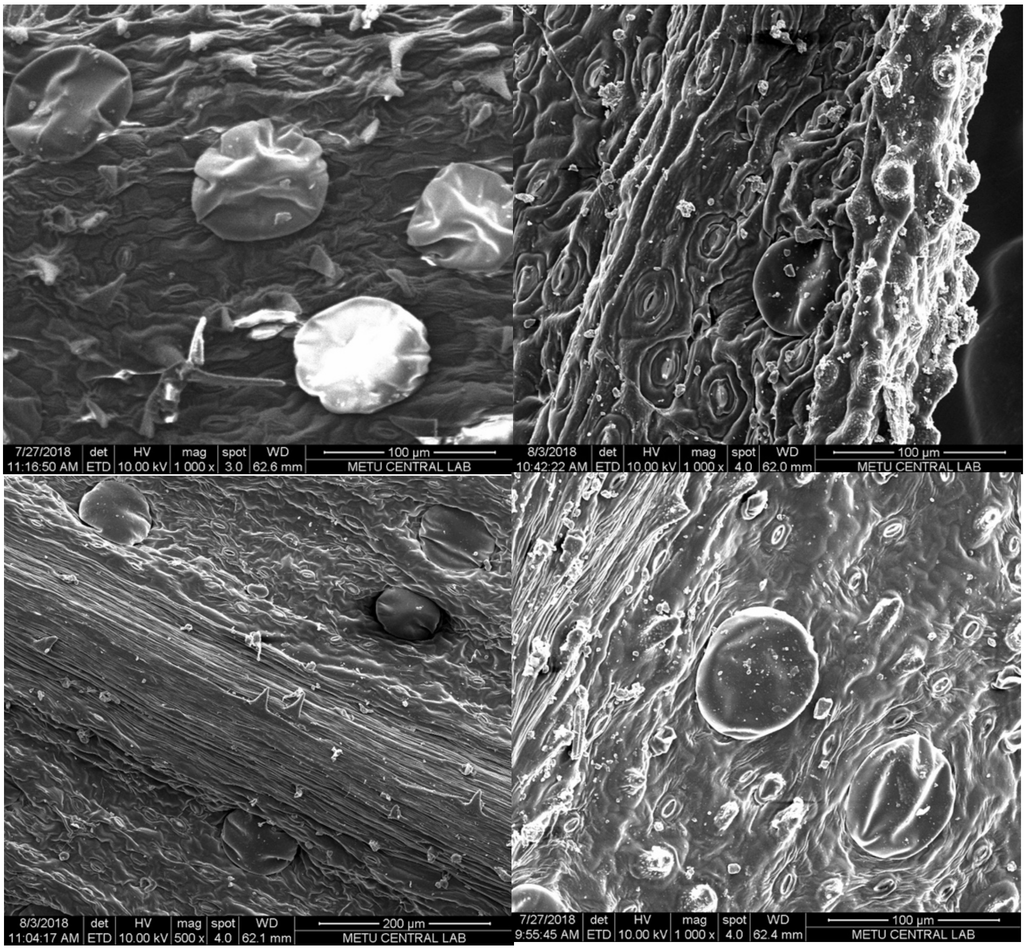

Figure 3.2. Sunken stomata on abaxial leaf surface of *T cilicius* (scale 100 µm) (Top Left) Sunken stomata on abaxial leaf surface of *T. praecox* subsp. *jankae* var. *jankae*. (scale 100 µm) (Top Right) Even stoma on abaxial leaf surface of *T. artvinicus* (scale 200 µm) (Bottom Left) Even stoma on abaxial leaf surface of *T. haussknechtii* (scale 100 µm) (Bottom Right)

3.2. Trichome Micromorphology

Non-glandular and glandular trichome types of plants were considered as highly valuable taxonomic sources of information. Indumentum of plant parts such as leaf, calyx and stem was studied and used by many scientists in infrageneric classification and distinguish species from one another. Trichome size, cell number, shape, density on plant surface are all key features in taxonomic studies (Economou-Amilli et al., 1982; Werker et al., 1985; Marin et al., 2008b; Stevanovic et al., 2008; Boz et al., 2009; Xiang et al., 2010; Jia et al., 2012, 2013; Eiji and Salmaki, 2016; Atalay, 2016; Atalay et al., 2016; Zareh et al., 2017).

According to the study published in 2018 by Sajna and Sunojkumar, non-glandular and glandular trichomes show variations in size, structure and distribution among *Leucas (Lamiaceae)* species therefore, trichome micromorphology can be used in section and species delimitation.

In this study different types of leaf, calyx and stem non-glandular and glandular trichomes and their distribution among 32 *Thymus* species are compared and categorized. The terminology used in this study follows Singh et al. (1974), Celep et al. (2011), Eiji and Salmaki (2016), Atalay et al. (2016).

Different types of non-glandular and glandular trichomes, their distribution and sizes in different *Thymus* species are given in Table 3.3.-3.8. Selected scanning electron micrographs of various trichome types are illustrated in Fig. 3.3-3.10. In general, basically 2 types of trichomes are observed as non-glandular and glandular trichomes. Non-glandular trichomes are categorized in 2 groups short (NGI) [with 3 subtypes (NGIA), (NGIB) and (NGIC)] and long (NGII). Glandular trichomes are either capitate (GI-GIII) or peltate (GIV). Capitate glandular trichomes are further divided into 3 subtypes (GI) subssessile or sessile, short-stalked (GII) or long stalked (GIII).

3.2.1. Non-Glandular Trichomes

In non-glandular trichomes size, cell number, shape, presence of papillae on trichome surface are considered as valuable taxonomic characters. In terms of size simple non-glandular trichomes of type (NGI) might be as short as 9 µm to 360 µm.

The type (NGI) is further divided into 3 three subtypes. (NGIA) are unicellular, unbranched, papillary non-glandular short trichomes (e.g. *T. leucostomus* var. *gypsaceus*, *T. cherlerioides* var. *isauricus*, *T. argaeus, T. eriophorus, T. migricus*). (Figure 3.2, 3.3). The type (NGIB) are bicellular, unbranched, papillary non-glandular short trichomes (eg. *T. longicaulis subsp. longicaulis* var. *longicaulis*, *T. migricus*, *T. fallax, T. fedtschenkoi* var. handelii) (Figure 3.3, 3.4.). The type (NGIC) are multicellular, unbranched, papillary non-glandular short trichomes (eg. *T. migricus, T. kotschyanus* var. *glabrescens, T. fedtschenkoi* var. *handelii, T. pseudoplegioides*) (Figure 3.3, 3.4.) (Table 3.3-5).

Non-glandular trichomes of the type (NGII) are all multicellular, simple (unbranched), papillary non-glandular long trichomes. In terms of size they range from 400 µm to 1000 µm (eg. *T. migricus, T. thracicus, T. argaeus*) (Figure 3.4.) (Table 3.3).

In terms of their shape nonglandular trichomes can be erect (eg. *T. leucostomus* var. *gypsaceus, T. cherlerioides* var. *isauricus*) (Figure 3.2), curved (eg. *T. longicaulis* subsp. *longicaulis* var. *longicaulis, T. kotschyanus* var. *glabrescens, T. argaeus*) (Figure 3.3, 3.4.) or appressed (eg. *T. migricus, T. fedtschenkoi* var. *handelii*) (Figure 3.4.).

All non-glandular trichomes of *Thymus spp.* were simple (unbranched) and papillary on surfaces. No glabrous calyx and stem were observed among the taxa. Trichome properties are mostly alike on both adaxial and abaxial leaf surface. Except *T. striatus* var. *interruptus, T. zygioides* var. *zygioides, T. bornmuelleri, T. kotschyanus* var. *glabrescens, T. roegneri, T. pseudoplegioides, T. praecox* subsp. *jankae* var. *jankae,* *T. thracicus, T. cariensis* all species had non-glandular hair on their adaxial and abaxial leaf surface.

It was observed that most of the taxa bears more than one non-glandular hair type on their leaf, calyx or stem surface. For example, species *T. revolutus* had all the 4 types of non-glandular hair on their adaxial and abaxial leaf surfaces. The taxon had trichomes with sizes ranged from 18 µm to 1000 µm on their leaf. Longest foliar and stem nonglandular trichomes are observed in subsection *Kotschyani* of section *Serpyllum* and subsection *Subbructeati* of section *Hyphodromi*. Longest calyx nonglandular trichomes are observed in subsection *Kotschyani* of section *Serpyllum.* The taxa had trichomes with sizes ranged from 31 µm to 883 µm on their stem (Table 3.3, 3.4, 3.5).

Unicellular short trichomes of (NGI) are the most common type of non-glandular hair on adaxial and abaxial leaf surfaces among the taxa. Most of the time they are accompanied by other types of trichomes. Although generally presence of non-glandular hair and trichomes types do not change in adaxial and abaxial leaf surfaces, in *T. pseudoplegioides* non-glandular trichomes present only on abaxial leaf surface and adaxial leaf surface is glabrous. This feature is unique to subsection *Alternates* and its only member in Turkey *T. pseudoplegioides*.

Type NGII long non-glandular trichomes are mostly found on calyx surface among the taxa. The longest non-glandular trichome (around 1000 µm) is found on *T. roegneri* calyx. Except *T. leucotrichus* all calyx (NGII) type trichomes observed in section *Serpyllum.* Calyx had also the most trichome density among other organs through *Thymus* taxa. The taxa had trichomes with sizes ranged from 13 µm to 1006 µm on their calyx. Type (NGIC) multicellular, short, unbranched trichomes are mostly found on calyx and stems of taxa.

Multicellular long trichomes (NGII) are the least observed trichome type on all leaf surfaces. Presence of (NGII) trichomes on leaf surface is unique to only *T. revolutus, T. argaeus, T. migricus, T. ararati-minoris* from subsection *Subbructeati* of section

Hyphodromi and subsection *Kotschyani* of section *Serpyllum,* respectively. Leaf NGII trichomes from subsection *Kotschyani* of section *Serpyllum* is distinguished by their appressed shape and prominent articulation at their nodes.

T. striatus var. *interruptus* is the only glabrous taxon on their leaf in subsection *Subbructeati* of section *Hyphodromi*. In the same subsection *T. cherlerioides* var. *isauricus* and *T. boissieri* are the only taxa covered with unicellular non-glandular trichomes densely without other non-glandular trichome types. *T. cherlerioides* var. *isauricus* is also unique with its lacking multicellular, short, unbranched (NGIC) type nonglandular trichomes on its calyx. *T. leucotrichus* is distinguishable with sparse unicellular/bicellular indumentum on the leaf and long multicellular (NGII) type trichomes on calyx from other taxa in subsection *Subbructeati.* Almost half of the members subsection *Subbructeati* lacks of unicellular NGI type trichomes on the calyx. This property is unique to this subsection. *T. pruinosus* var. *globifer* is peculiar among the taxa because of its foliar indumentum. It was the only species covered with only very dense (NGIC) type trichomes (Figure 3.5).

Species of subsection *Serpyllastrum* of section *Hyphodromi* are not much alike in terms of foliar indumentum structure. *T. spahulifolius* is very densely covered only with curved bicellular non-glandular trichomes (NGIB) and *T. haussknechtii* is covered only with sparse unicellular non-glandular trichomes (NGIA) where *T. zygioides* var. *zygioides* has no non-glandular trichomes at all on leaf surface. All of the plant parts of *T. spahulifolius* lacks unicellular nonglandular trichomes and foliar nonglandular trichome profile is also repeated on their stem. Indumentum on *T. spahulifolius* is unique among the taxa therefore it also provides delimitation at species level (Figure 3.5).

In subsection *Thymbropsis* of section *Hyphodromi* species *T. cariensis* is the only one with glabrous and *T. cilicicus* is the only one with bicellular short non-glandular trichome (NGIB) leaf surface. Their calyx and stem indumentum had uniformal profile in terms of their sizes and cell numbers however indumentum density and shape of trichomes varies among the subcsection. Unicellular, bicellular and multicellular short, unbranched nonglandular trcihomes on calyx and stem is a characteristic feature of subsection *Thymbropsis.*

Represented only one species in Turkey subsection *Insulares* of section *Serpyllum* is characterized by its glabrous leaf and sparse short uni/bi/multicellular trichomes on calyx and stem.

Subsection *Kotschyani* of section *Serpyllum* had the most member in Turkey. All members except *T. roegneri* had trichomes on their leaf surface and also all of them had unicellular nonglandular trichomes on their adaxial and abaxial leaf surfaces. Also all observed taxa except *T. roegneri* had multicellular, short, unbranched (NGIC) type nonglandular trichomes on their calyx. *T. roegneri* had only very dense (NGII) type trichomes on their calyx. *T. kotschyanus* var. *glabrescens* and *T. artvinicus* are almost glabrous had unicellular unbranched short trichomes sparsely scattered all over the leaf surfaces especially on midevein and margins. Most of the species calyx (NGII) type trichomes are observed in this subsection.

In subsection *Pseudomarginati* of section *Serpyllum* all adaxial and abaxial leaf surfaces are glabrous except sparse indumentum in *T. longicaulis* subsp. *longicaulis* var. *longicaulis*. As it was reported by previous studies sparse indumentum of *T. longicaulis* subsp. *longicaulis* var. *longicaulis* is observed on midvein and margins. (Marin et al., 2008a, Marin et al., 2008b). *T. praecox* subsp. *jankae* var. *jankae* is distinguished by its lacking type (NGII) trichome on calyx and only presence of short unicellular type (NGI) trichomes on stem.

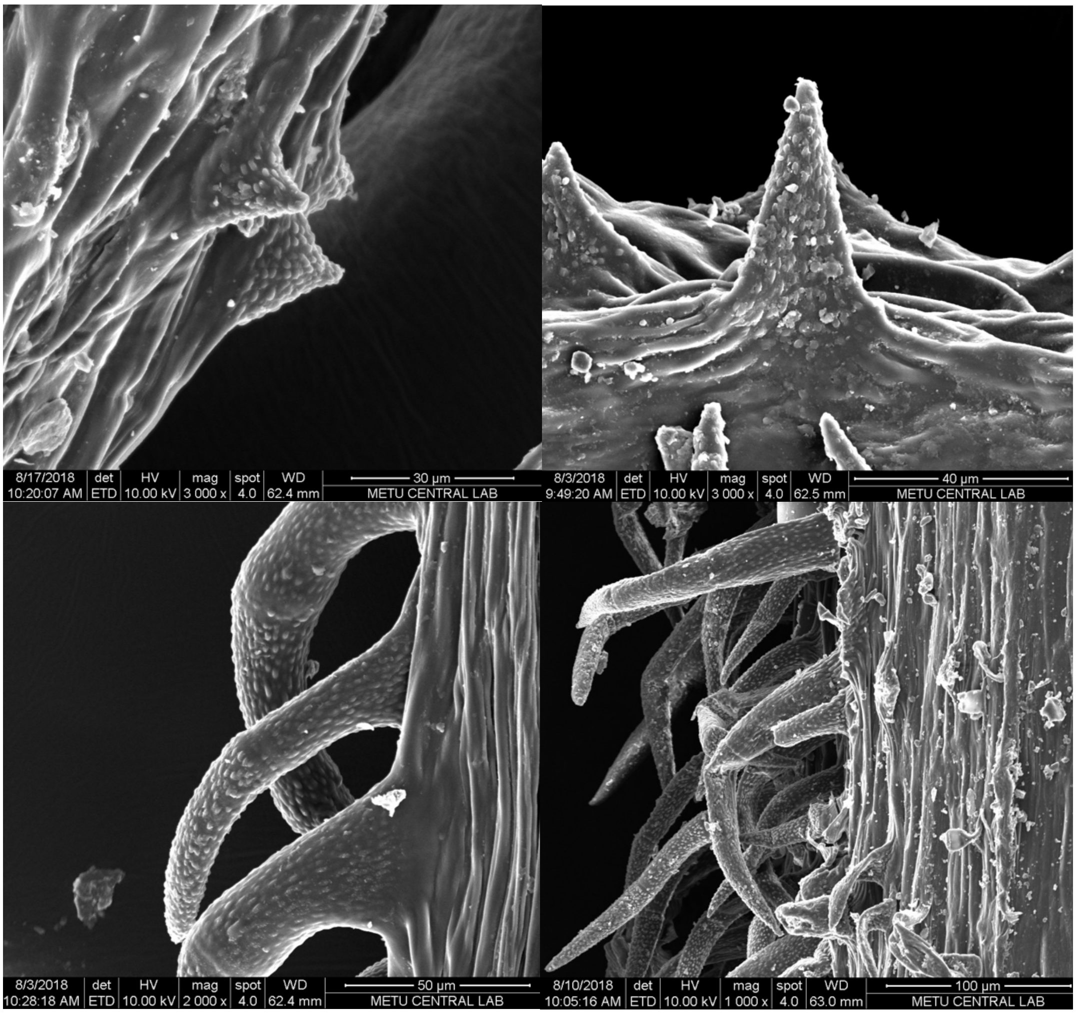

Figure 3.3. Scanning electron micrographs of short (NGI) nonglandular trichomes of selected *Thymus* taxa. (Top Left), *T. leucostomus* var. *gypsaceus*. Simple short nonglandular unicellular (NGIA) erect trichomes on abaxial leaf (scale 30 µm). (Top Right), *T. cherlerioides* var. *isauricus*. Simple short nonglandular unicellular (NGIA) erect trichomes on adaxial leaf (scale 40 µm). (Bottom Left), *T. longicaulis* subsp. *longicaulis* var. *longicaulis*. Simple short nonglandular bicellular (NGIB) curved trichomes on stem (scale 50 µm). (Bottom Right), T. *kotschyanus* var. *glabrescens*. Simple short nonglandular multicellular (NGIC) curved trichomes on stem (scale 100 µm).

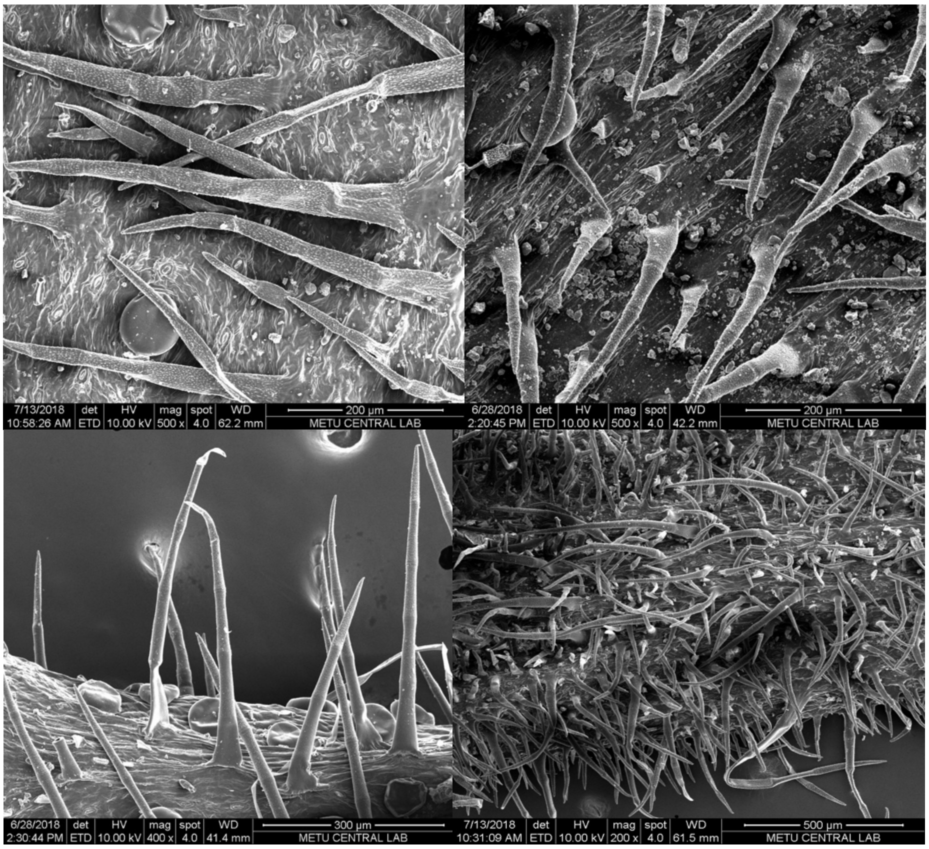

Figure 3.4. T. migricus. Simple short nonglandular unicellular (NGIA), bicellular (NGIB), multicellular (NGIC) and long (NGII) appressed trichomes on calyx (scale 200 µm). (Top Left); *T. fedtschenkoi* var. *handelii*. Simple short nonglandular bicellular (NGIB) and multicellular (NGIC) appressed trichomes on adaxial leaf (scale 200 µm). (Top Right); *T. thracicus*. Simple long nonglandular (NGII) trichomes on calyx. (scale 300 µm). (Bottom Left); *T. argaeus*. Simple long nonglandular (NGII) trichomes on adaxial leaf. *(*scale 500 µm*).* (Bottom Right).

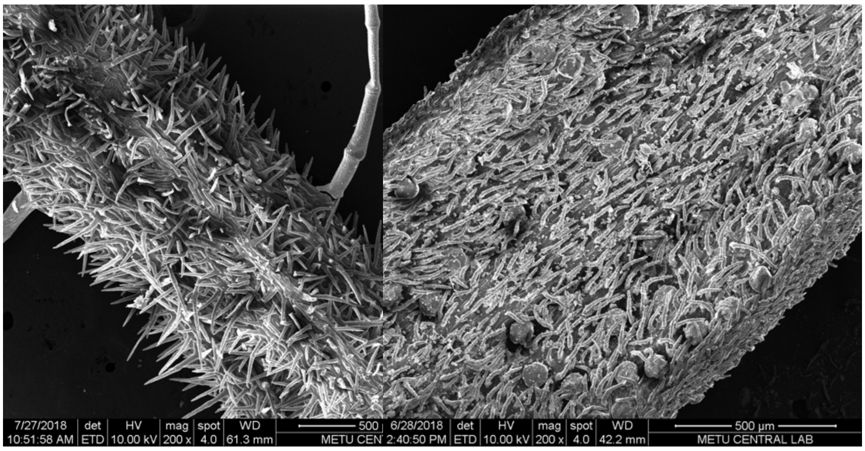

Figure 3.5. T. pruinosus var. *globifer*. Simple short multicellular nonglandular (NGIC) trichomes on adaxial leaf. (scale 500 µm).(Left); *T. spahulifolius*. Simple short bicellular nonglandular (NGIB) trichomes on adaxial leaf. (scale 500 µm). (Right).

Table 3.3. *Adaxial, Abaxial, Calyx and Stem Nonglandular Trichome Sizes*

Taxon	Adaxial Leaf	Abaxial Leaf	Calyx	Stem
T. revolutus	29.3±7.4 (17.8-38.5) 261.1±51.5 (181.3-307.5) 842.5±134.6 (774.16-996.2)	32.6±9.1 (28.3-45) 261.7±41.9 (209.8-302) 666.8±85.2 (601.5-815)	54.8±11.2 (44-71) 234.9±101.6 (191.6-225.2)	707.6±211.9 (467.5-883.4)
T. cherlerioides var. *isauricus*	61.49±27.13 (31.9-67.2)	34.5±15.38 (21.9-53.4)	47.7±7.2 (39-55.4) 95.4±18.5 (69.2 -116.8)	47.6±12.5 (35.3-66.6)
T. leucotrichus	61.8±31.1 (28.2-97.9)	47±13.3 (37.3-69.8)	197.4±57.1 (157-297.9)	52.9±13.14 (39.8-71) 196.5±68.2. (127.5-270.8)
T. convolutus	185.8±59.4 (124.4-258.5)	154.9±48 (98.1-214.7)	298.2±55.2 (222.7-352.3) 417.4±28 (393.9-461.9)	119.7±27.4 (99.8-163.3) 278.9±49.9 (200.5-329.1) 442.8±45.9 (395.9-508.8)
T. argaeus	N/A	N/A	69.1±11.8 (54.4-79.6) 260.4 ± 85.7 (154.6-333.4)	204.7±38.3 (161.9-256.7)
T. cappadocicus	N/A	N/A	211.5±28.9 (177-249.7)	186.7±36 (132.5-236)

Table 3.3. *(Continued)*

T. striatus var. *interruptus*	59.3±16.4 (41-78.6)	21.3±5.1 (15.4-28.3)	34.4±7.6 (25.9-40.9) 209.8±78.9 (142.6-317.1)	49.5±6.6. (42.5-61) 170.6±22.5 (131.5-189.4)
T. boissieri	N/A	N/A	96.6±55.7 (53.6-188.6) 245.2±69.3 (144.1-330.9)	90.7±38.3 (60.1-147) 293.2±42.9 (237-322.3)
T. pruinosus var *globifer*	N/A	N/A	318.5±47 (263.4-385.1)	149.7±23.1 (112.7-170.4)
T. haussknechtii	52±17.5 (27.6-76.2)	18.6±3.5 (14.4-23.3)	39.7 ± 6.4 (31-46.5) 110.9±25 (82.8-134.7)	57.4±13.5 (40.1-70.1)
T. spahulifolius	N/A	N/A	95.6±7.3 (87.4-103.7)	61.5±7.4 (51.1-69.6)
T. zygioides var. *zygioides*	47.2±17.6 (28.7-71)	30.6±6.4 (24.3-40)	24.5±8.2 (15.2-36.8) 155.3±29.8 (128.8-204.9)	143.9±51.3 (94.7-214.8)
T. cilicicus	48.9±9.7 (36.5-65.4)	18.9 ± 4.9 (8.2-28.8)	54.4±12.6 (37.7-68.4)	55.8±13.6 (34.4-72.6)
T. cariensis	N/A	N/A	68.4±11.2. (30.5-60.2) 179.9±38.2 (126.1-219)	54.1±3.8 (50.5-58.7) 127.9±22.4 (103.156.6)

Table 3.3. *(Continued)*

T. sipyleus subsp. sipyleus var. sipyleus	N/A	N/A	37.6±12.4 (31.4-59.8) 146.5±41.9 (95.6-208.9)	42.6±6.9 (31.9-49.2) 79.8±12.2 (62.6-91.4)
T. leucostomus var. *gypsaceus*	43.9±6.5 (33.9-51.5)	15.7±3.4 (10.2-18.4)	132.5±46.7 (87.6-197.2)	81.6±18 (55.4-96.6) 149.2±34.4 (103.5-192)
T. bornmuelleri	55.8±3.3 (51.8-59.6)	21.7±3.7 (17.2-27.6)	32±8 (26-45.7) 284.4±72.8 (194.8-380.8)	100.7 ± 30.2 (64.4-133.8) 437.5 ± 36 (395-486.7)
T. fallax	45.9±9.9 (34.6-61.7)	17.4±3.8 (14.1-23.8)	46.5±11.5 (36.8-66.4) 119.5±20 (102.4-148.5)	18.2±7.5 (10.5-27)
T. transcausicus	46.1±9.9 (33.7-60.7)	18.9 ± 5.5 (13-26.3)	175.9±29.1 (149.1-222.5) 423.7±65.3 (351-521.1)	142.8 ± 27.3 (105.2-170.4) 267.7±24.1 (245.6-300.5)
T. kotschyanus var. *glabrescens*	39.7±9.8 (28-54.3)	17.3±3.2 (12.7-21.1)	21.5±6.2 (12.9-29.2) 72.4±17.1 (54.9-100.7)	69.9±16.1 (51.4-93.1) 175.4±17.8 (155.3-198.5)
T. eriocalyx	N/A	N/A	57.8±27.3 (32.8-95.8) 260.9±20.6 (235.4-288) 469±63.7 (402.7-569.2)	43.7±27 (25.6-90.7) 164.2±26.4 (142.8-195.2)

Table 3.3. *(Continued)*

T. migricus	52.2±10.9 (44.5-64.7)	28.5±4.4 (24.6-33.4)	47.4±11.7 (31.7-58.6) 325.9±66.7 (224.6-389.1) 430.1±25 (398.7-455.3)	603.5±162 (467-862.8)
T. fedtschenkoi var. *handelii*	N/A	N/A	46.4 ± 22.5 (29.2-84.9) 334.9±28.6 (303.2-359.3) 437.1± 6.6 (432.7-447)	287.5±56.4 (202.4-361.2)
T. pubescens var. *cratericola*	N/A	N/A	47.7±16.5 (21.2-58.4) 266.4±56.9 (191.9-323)	64.1±10.8 (51.1-71.6)
T. ararati-minoris	24.6±3.4 (19.7-28.6)	14.2±4.7 (7.4-18.7)	50.7±30.8 (26.6-87.9) 279.7±62.3 (229.1-369.4)	118.9±42.5 (94.8-194.4) 575.1±121.4 (433.4-746.1)
T. roegneri	N/A	N/A	710±220.1 (428.9-1005.9)	69.7±22 (42.3-93.2) 236.2±34.7 (196.4-273.9)
T. artvinicus	58.7±19.5 (36.8-89.8)	25.7±4.7 (18.6-30.5)	227.2±65.9 (146.5-299.5)	40.3±10.7 (24.8-52.4)

Table 3.3. *(Continued)*

T. eriophorus	49.6±15.1 (29.5-64.8)	18.2±6.8 (10.6-26.6)	142.3±60.5 (68.7-225.8)	101.6±38.5 (52.8-152.4) 304.5±20.6. (280.1-328.6)
T. pseudoplegioides	22.1±2.7 (18.4-26)	14.8±5.5 (8.4-23)	46±26.2 (25.9-92.1) 313.9±37.7 (283.7-379.2) 616.8±92.4 (531.9-758.8)	261.6±59.6 (176.3-330.4)
T. praecox subsp. *jankae* var. *jankae*	36.4±6 (30.6-45.2)	19.7±4.8 (12.5-23.5)	27.2±9 (16.1-41.2) 208.2±33.2 (158.1-238.2)	34.1±2.8 (30.7-38.4)
T. tharacicus	120.6±40.1 (90.7-190.9)	19.9±4 (15.5-25.7)	540.8±37.1 (503.2-587.2)	49.6±15.3 (38.2-75.4) 114.2±40.3 (82.5-179.8)
T. longicaulis longicaulis var. *longicaulis*	39.3±7.6 (28.8-49.9)	18.4±4.6 (14.1-24.1)	33.4±11.4 (27.2-53.9) 298.5±30 (268.4-344.5) 433.6±35 (395.5-467.4)	66±8.1 (55.1-77.8) 158.8±32.2 (113.6-189.9)

Table 3.4. *Adaxial an Abaxial Leaf Nonglandular Trichome Types and Density*

	Adaxial Leaf					Abaxial Leaf				
Species	NGI			NGII	Density	NGI			NGII	Density
	NGIA	NGIB	NGIC	NGI/NG2		NGIA	NGIB	NGIC	NGI/NG2	
T. revolutus	+	+	+	+	D	+	+	+	+	D
T. cherlerioides var. *isauricus*	+	-	-	-	D	+	-	-	-	D
T. leucotrichus	+	+	-	-	S	+	-	-	-	S
T. convolutus	-	+	+	-	D	-	+	+	-	D
T. argaeus	+	+	+	+	VD	+	+	+	+	VD
T. cappadocicus	+	+	-	-	D	+	+	-	-	D
T. striatus var. *interruptus*	-	-	-	-	N/A	-	-	-	-	N/A
T. boissieri	+	-	-	-	D	+	-	-	-	D
T. pruinosus var. *globifer*	-	-	+	-	VD	-	-	+	-	VD
T. haussknechtii	+	-	-	-	S	+		-	-	S
T. spahulifolius	-	+	-	-	VD	N/A	N/A	N/A	N/A	N/A
T. zygioides var. *zygioides*	-	-	-	-	N/A	-	-	-	-	N/A
T. cilicicus	+	+	-	-	D	+	+	-	-	D
T. cariensis	-	-	-	-	N/A	-	-	-	-	N/A

Table 3.4. *(Continued)*

T. sipyleus subsp. *sipyleus* var. *sipyleus*	+	-	-	-	D	+	-	-	-	D
T. leucostomus var. *gypsaceus*	+	-	-	-	S	+	-	-	-	S
T. bornmuelleri	-	-	-	-	N/A	-	-	-	-	N/A
T. fallax	+	+	-	-	VD	+	+	-	-	VD
T. transcausicus	+	+	+	-	D	+	+	+	-	D
T. kotschyanus var. *glabrescens*	+	-	-	-	S	+	-	-	-	S
T. eriocalyx	+	-	-	-	VS	+	-	-	-	VS
T. migricus	+	+	+	+	D	+	+	+	+	D
T. fedtschenkoi var. *handelii*	+	+	+	-	D	N/A	N/A	N/A	N/A	N/A
T. pubescens var. *cratericola*	+	-	-	-	D	+	+	-	-	D
T. ararati-minoris	+	+	+	+	S	+	+	+	+	S
T. roegneri	-	-	-	-	N/A	-	-	-	-	N/A
T. artvinicus	+	-	-	-	VS	+	-	-	-	VS
T. eriophorus	+	+	+	-	D	+	+	+	-	D
T. pseudoplegioides	-	-	-	-	N/A	+	-	-	-	VS
T. praecox subsp. *jankae* var. *jankae*	-	-	-	-	N/A	-	-	-	-	N/A
T. tharacicus	-	-	-	-	N/A	N/A	N/A	N/A	N/A	N/A
T. longicaulis subsp. *longicaulis* var. *longicaulis*	+	-	-	-	VS	+	-	-	-	S

VS: Very Sparse; S: Sparse; VD: Very Dense, D: Dense

Table 3.5. *Calyx and Stem Nonglandular Trichome Types and Density*

	Calyx					Stem				
Species	NGI			NGII	Density	NGI			NGII	Density
	NGIA	NGIB	NGIC	NGI/NG2		NGIA	NGIB	NGIC	NGI/NG2	
T. revolutus	+	+	+	-	D	-	-	-	+	VD
T. cherlerioides var. *isauricus*	+	+	-	-	D	+	-	-	-	D
T. leucotrichus	-	-	+	-	VD	+	+	+	-	D
T. convolutus	-	-	+	+	D	-	+	+	+	D
T. argaeus	+	+	+	-	D	-	+	+	-	VD
T. cappadocicus	-	-	+	-	D	-	+	+	-	D
T. striatus var. *interruptus*	+	+	+	-	VD	+	+	+	-	D
T. boissieri	-	-	+	-	D	+	+	+	-	D
T. pruinosus var. *globifer*	+	+	+	-	VD	-	-	+	-	VD
T. haussknechtii	+	+	+	-	D	+	+	-	-	D
T. spahulifolius	-	+	+	-	VD	-	+	-	-	VD
T. zygioides var. *zygioides*	+	+	+	-	D	-	-	+	-	VS
T. cilicicus	+	+	+	-	D	+	+	+	-	D

Table 3.5. *(Continued)*

T. cariensis	+	+	+	-	S	+	+	+	-	S
T. sipyleus subsp. *sipyleus* var. *sipyleus*	+	+	+	-	D	+	+	+	-	D
T. leucostomus var. *gypsaceus*	+	+	+	-	S	+	+	+	-	D
T. bornmuelleri	+	+	+	-	S	+	+	+	-	S
T. fallax	+	+	+	-	VD	+	+	-	-	VD
T. transcausicus	-	+	+	+	VD	-	+	+	-	D
T. kotschyanus var. *glabrescens*	+	+	+	-	S	+	+	+	-	S
T. eriocalyx	+	+	+	+	S	+	+	+	-	S
T. migricus	+	+	+	+	D	-	-	-	+	VD
T. fedtschenkoi var. *handelii*	+	+	+	+	S	+	+	+	-	S
T. pubescens var. *cratericola*	+	+	+	-	V	+	-	-	-	S
T. ararati-minoris	+	+	+	-	S	+	+	-	+	S
T. roegneri	-	-	-	+	VD	+	+	+	-	VD
T. artvinicus	-	-	+	-	VS	+	+	-	-	S
T. eriophorus	+	+	+	-	D	-	+	+	-	D
T. pseudoplegioides	+	+	+	+	S	-	-	+	-	S
T. praecox subsp. jankae var. *jankae*	+	+	+	-	D	+	-	-	-	S
T. tharacicus	+	+	+	+	S	+	+	+	-	D
T. longicaulis subsp. *longicaulis* var. *longicaulis*	+	+	+	+	D	+	+	+	-	S

VS: Very Sparse; S: Sparse; VD: Very Dense, D: Dense

3.2.2. Glandular Trichomes

In glandular trichomes size and shape on trichome surface are considered as valuable taxonomic characters. Glandular trichomes are either capitate (GI-GIII) or peltate (GIV). Capitate glandular trichomes are further categorized in 3 subtypes (GI) subssessile or sessile, short-stalked (GII) or long stalked (GIII).

(GI) type capitate trichomes represents the subsessile and sessile, with a basal cell, a stalk cell and a unicellular head (e.g. *T. migricus, T. cilicicus, T. striatus* var. *interruptus*) (Figure 3.6). Most of the (GI) type glandular trichomes are observed on stem in section *Hyphodromi.* (GII) type capitate trichomes are short stalked glandular trichomes with sizes up to 15 µm. (GII) type trichome sizes ranged from 1.7 *µm* to 14 *µm* with unicellular, bicellular or multicellular head or stalk (e.g. *T. argaeus, T. cilicicus*, *T. cappadocicus*) (Figure 3.6, 3.7). (GII) is the most found capitate glandular trichomes through the taxa. Type (GIII) glandular trichomes are long stalked trichomes with sizes up to 150 *µm*. (GIII) type trichome sizes ranged from 16.3 to 157.5 *µm* with unicellular, bicellular or multicellular head or stalk. (e.g. *T. leucostomus* var. *gypsaceus, T. longicaulis* subsp. *longicaulis* var. *longicaulis, T. roegneri)* (Figure 3.8)*,* Type (GIV) trichomes are peltate glandular trichomes with a basal cell, a short stalk cell and a multicellular head (e.g. *T. pseudoplegioides*, *T. thracicus, T. eriocalyx, T. eriophorus, T. boissieri*) (Figure 3.8, 3.9) (Table 3.6, 3.7, 3.8). Cuticle rupture to secrete essential oil was observed in some species (Figure 3.10). Secretion of essential oil by cuticle rupture was observed in other *Thymus* species in previous studies (Boz et al., 2009, Jia et al., 2012).

Calyx is the richest plant part in terms of presence of glandular trichomes. Peltate trichomes are the most profoundly found glandular trichomes through the taxa on all plant surfaces.

Except *T. convolutus, T. praecox* subsp. *jankae* var. *jankae, T. tharacicus* all *Thymus* taxa had glandular trichomes on their adaxial or abaxial leaf. All of the *Thymus* species examined had (GIV) peltate glandular trichomes on their calyx. Almost half of them had glandular trichomes on their stem. Most of the time glandular trichome profile is similar on adaxial leaf surfaces however in *T. cherlerioides* var. *isauricus,* adaxial leaf had only (GIV) type glandular trichomes and abaxial leaf had only (GII) type glandular hair. *T. pruinosus* var. *globifer* is also peculiar with its (GIV) type glandular trichomes only on abaxial leaf surface.

In subsection *Subbructeati* of Section *Hyphodromi T. striatus* var. *interruptus* is distinguished by its (GI) type sessile-subsessile trichomes on stem. *T. argaeus* is the only taxon had (GIII) type long stalk glandular trichomes with unicellular head and multicellular stalk. Except *T. striatus* var. *interruptus*, *T. boissieri, T. pruinosus* var. *globifer* presence of (GII) type glandular trichomes accompanied by (GIV) type glandular trichomes on calyx is a characteristic feature of subsection *Subbructeati.* Except *T. sipyleus* subsp. *sipyleus* var. *sipyleus,* only taxa in subsection *Subbructeati* had glandular trichome types other than (GIV) on their leaf surfaces.

In subsection *Serpyllastrum* of Section *Hyphodromi T. haussknechtii* did not bear any capitate glandular trichomes. All the members of the subsection *Serpyllastrum* had peltate glandular trichomes on their adaxial leaf surface. *T. spahulifolius and T. zygioides* var. *zygioides* had sessile/subsessile glandular trichomes of type (GI) on stem.

In subsection *Thymbropsis* of Section *Hyphodromi T. cilicicus* is unique with its stem bearing (GI) sessile/subsessile glandular trichomes. *T. leucostomus* var. *gypsaceus* is peculiar with its having (GIII) long stalk capitate glandular trichomes on calyx. None of the group members had peltate type (GIV) glandular trichomes on their stem.

In section *Serpyllum* none of the taxa had capitate glandular trichomes on adaxial and abaxial leaf surfaces. Only *T. migricus* and *T. artvinicus* of subsection *Kotschyani* had (GI) sessile/subsessile glandular trichomes. Half of the members of this particular subsection had no glandular trichomes on their stem. Other half had type (GII) and

(GIV) on their stems. *T. eriocalyx* and *T. roegneri* are unique with (GIII) type of long stalk trichomes on calyx.

T. praecox subsp. *jankae* var. *jankae* of subsection *Pseudomarginati* of section *Serpyllum* did not have any glandular trichomes except (GIV) peltate trichomes on calyx.

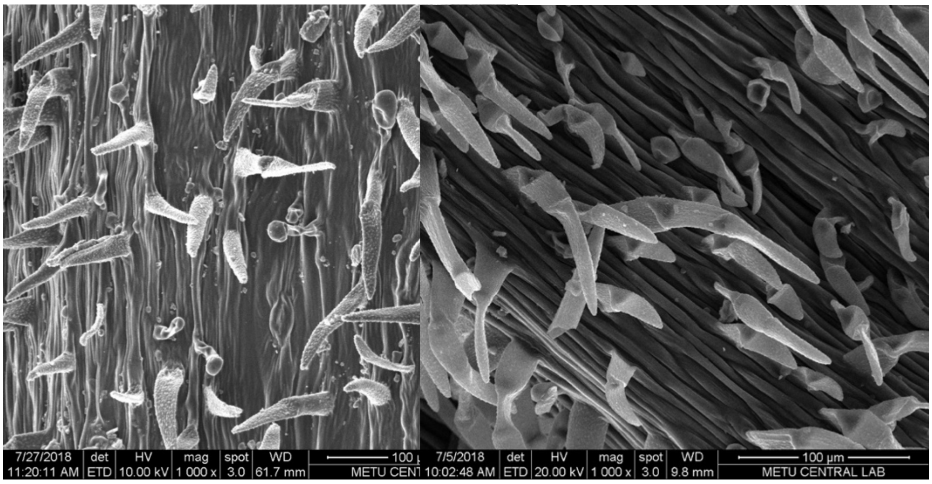

Figure 3.6. T. cilicicus (GI) Sessile/Subsessile and (GII) short stalk capitate glandular trichomes on stem. Left (scale 50 µm). *T. striatus* var. *interruptus.* (GI) Sessile/Subsessile capitate glandular trichome on stem. Right (scale 100 µm).

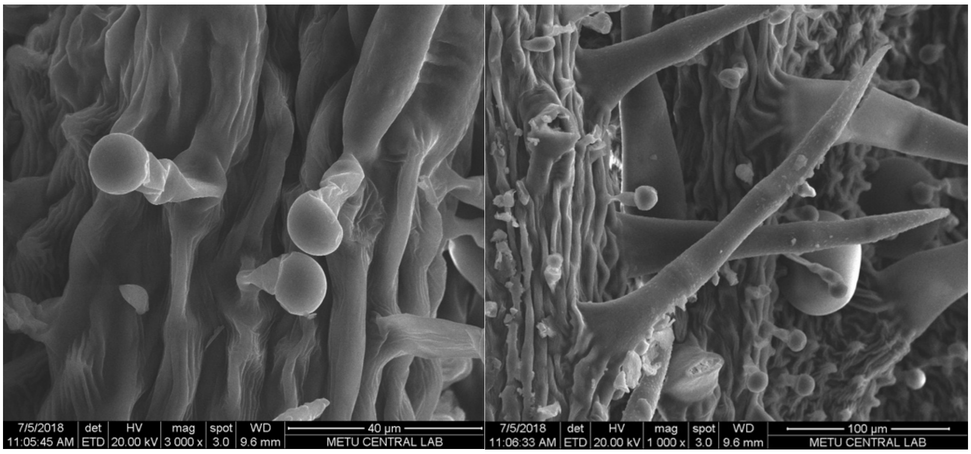

Figure 3.7. T. cappadocicus. (GII) short stalk capitate glandular trichomes on stem.
Left (scale 40 µm), Right (scale 100 µm)

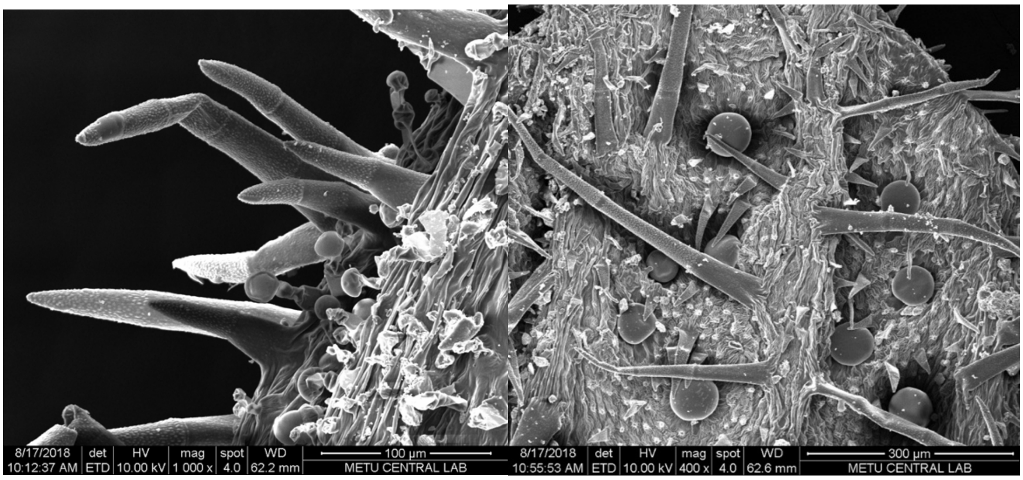

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Figure 3.8. *T. leucostomus* var. *gypsaceus*. (GIII) long stalk capitate glandular trichomes on calyx. Left (scale 100 µm) *T. eriocalyx*. (GIV) peltate glandular trichomes on calyx. Right (scale 300 µm).

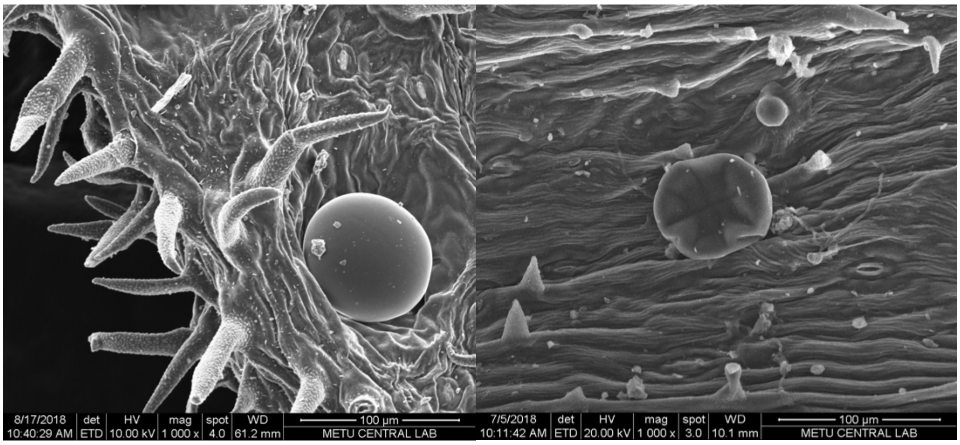

Figure 3.9. T. eriophorus. (GIV) peltate glandular trichomes on calyx. Left (scale 100 μm). *T. boissieri*. (GIV) peltate glandular trichomes on adaxial leaf. Right (scale 100 μm).

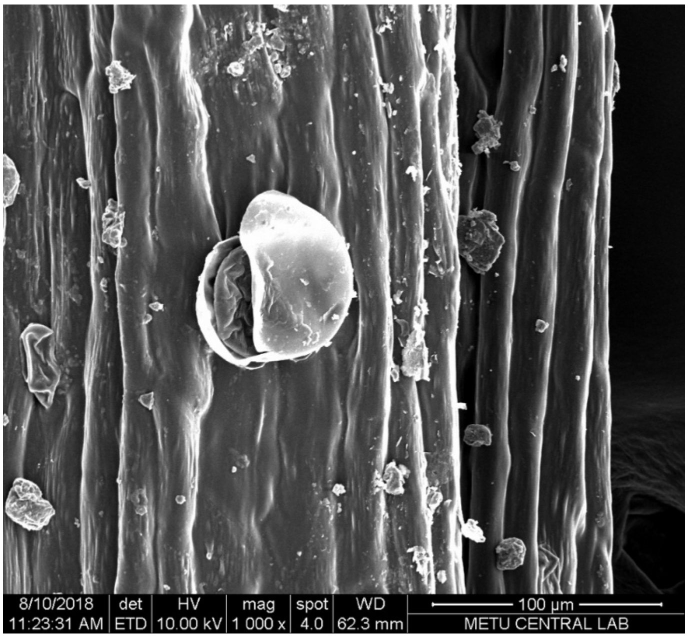

Figure 3.10. T. zygioides var. *zygioides.* Rupturing (GIV) peltate glandular trichome on calyx. (scale 100 μm).

Table 3.6. Adaxial and Abaxial Leaf, Calyx and Stem Glandular Trichome Sizes

Taxon	**Adaxial Leaf**	**Abaxial Leaf**	**Calyx**	**Stem**
T. revolutus	N/A	N/A	13.7 ± 5.1 (5.8-19.1)	N/A
T. cherlerioides var. *isauricus*	N/A	5.15±7 (4.2-5.9)	30.4±7 (26.6-41.4)	N/A
T. leucotrichus	N/A	N/A	35±21.5 (18.5-72.2)	20.5±8.1 (7.3-30.7)
T. convolutus	N/A	N/A	57.5±27.5 (28.9-95.1)	18.7±1 (18-19.4)
T. argaeus	17	11.6±1.9 (8.3-13.9)	3.4 ± 1.5 (1.4-5.8) 30.3±12.6 (16.3-45.8)	8.4±0.05 (8.4-8.5)
T. cappadocicus	8.3±2.2 (6.8-12.7)	10.5±4.9 (5.3-19.9)	12.3±5.6 (6.9-19.2)	14.5±7.4 (5.5-26.2)
T. zygioides var. *zygioides*	N/A	N/A	34.1±10.7 (22.8-52.6)	N/A
T. cilicicus	N/A	N/A	6.3±3.3 (3.7-10.1)	9.9±2.8 (7.5-14.8)
T. sipyleus subsp. *sipyleus* var. *sipyleus*	6.4	N/A	3.3 ± 1.2 (1.8-4.2)	N/A
T. leucostomus var. *gypsaceus*	N/A	N/A	18.4 ± 6.6 (11-27.6)	8.9 ± 1.7 (7.6-10.9)

Table 3.6. (Continued)

T. bornmuelleri	N/A	N/A	3.1±1.6 (1.7-5.3)	N/A
T. fallax	45.9 ± 9.9 (34.6-61.7)	N/A	N/A	N/A
T. transcausicus	46.1 ± 9.9 (33.7-60.7)	N/A	N/A	N/A
T. kotschyani var. *glabrescens*	39.7±9.8 (28-54.3)	N/A	3.7	11.3±5.7 (7.2-15.4)
T. pubescens var. *cratericola*	N/A	N/A	40.6±9.7 (27-50.3)	N/A
T. ararati-minoris	N/A	N/A	N/A	11.7±4 (7.6-15.6)
T. roegneri	N/A	N/A	106.1±50.9 (47.4-171.2)	N/A
T. artvinicus	N/A	N/A	15.1±5.3 (11.8-24.6)	N/A
T. pseudoplegioides	N/A	N/A	4.2±0.6 (3.7-4.7)	N/A

Table 3.7. Adaxial and Abaxial Leaf Glandular Trichome Types

	Adaxial Leaf				Abaxial Leaf			
Species	(GI)	(GII)	(GIII)	(GIV)	(GI)	(GII)	(GIII)	(GIV)
T. revolutus	-	-	-	+	-	+	-	+
T. cherlerioides var. *isauricus*	-	-	-	+	-	+	-	-
T. leucotrichus	-	-	-	+	-	-	-	+
T. convolutus	-	-	-	-	-	-	-	-
T. argaeus	-	+	-	+	-	+	-	+
T. cappadocicus	-	+	-	+	-	+	-	+
T. striatus var. *interruptus*	-	-	-	+	-	-	-	+
T. boissieri	-	-	-	+	-	-	-	+
T. pruinosus var. *globifer*	-	-	-	-	-	-	-	+
T. haussknechtii	-	-	-	+	-	-	-	+
T. spahulifolius	-	-	-	+	-	-	-	-

Table 3.7. (Continued)

T. zygioides var. *zygioides*	-	-	-	+	-	-	-	+
T. cilicicus	-	-	-	+	-	-	-	+
T. cariensis	-	-	-	+	-	-	-	-
T. sipyleus subsp. *sipyleus* var. *sipyleus*	-	+	-	+	-	-	-	+
T. leucostomus var. *gypsaceus*	-	-	-	+	-	-	-	+
T. bornmuelleri	-	-	-	+	-	-	-	+
T. fallax	-	-	-	+	-	-	-	+
T. transcausicus	-	-	-	+	-	-	-	+
T. kotschyanus var. *glabrescens*	-	-	-	+	-	-	-	+
T. eriocalyx	-	-	-	+	-	-	-	+
T. migricus	-	-	-	+	-	-	-	+
T. fedtschenkoi var. *handelii*	-	-	-	+	N/A	N/A	N/A	N/A
T. pubescens var. *cratericola*	-	-	-	+	-	-	-	+
T. ararati-minoris	-	-	-	+	-	-	-	+
T. roegneri	-	-	-	+	-	-	-	+
T. artvinicus	-	-	-	+	-	-	-	+
T. eriophorus	-	-	-	+	-	-	-	+
T. pseudoplegioides	-	-	-	+	-	-	-	+
T. praecox subsp. *jankae* var. *jankae*	-	-	-	-	-	-	-	-
T. tharacicus	-	-	-	-	N/A	N/A	N/A	N/A
T. longicaulis subsp. *longicaulis* var. *longicaulis*	-	-	-	+	-	-	-	+

Table 3.8. Calyx and Stem Glandular Trichome Types

	Calyx				Stem			
Species	(GI)	(GII)	(GIII)	(GIV)	(GI)	(GII)	(GIII)	(GIV)
T. revolutus	-	+	-	+	-	-	-	-
T. cherlerioides var. *isauricus*	-	+	-	+	-	-	-	-
T. leucotrichus	-	+	-	+	-	+	-	-
T. convolutus	-	+	-	+	-	+	-	-
T. argaeus	-	+	+	+	-	+	-	-
T. cappadocicus	-	+	-	+	-	+	-	+
T. striatus var. *interruptus*	-	-	-	+	+	-	-	-
T. boissieri	-	-	-	+	-	-	-	+
T. pruinosus var. *globifer*	-	-	-	+	-	-	-	-
T. haussknechtii	-	-	-	+	-	-	-	+
T. spahulifolius	-	-	-	+	+	-	-	-
T. zygioides var. *zygioides*	-	+	-	+	+	-	-	+
T. cilicicus	-	+	-	+	+	+	-	-
T. cariensis	-	-	-	+	-	-	-	-
T. sipyleus subsp. *sipyleus* var. *sipyleus*	-	+	-	+	-	-	-	-
T. leucostomus var. *gypsaceus*	-	-	+	+	-	+	-	-

Table 3.8. (Continued)

T. bornmuelleri	-	+	-	+	+	-	-	+
T. fallax	-	-	-	+	-	-	-	-
T. transcausicus	-	-	-	+	-	-	-	-
T. kotschyanus var. *glabrescens*	-	+	-	+	-	+	-	-
T. eriocalyx	-	-	+	+	-	+	-	+
T. migricus	+	-	-	+	-	+	-	-
T. fedtschenkoi var. *handelii*	-	-	-	+	-	-	-	-
T. pubescens var. *cratericola*	-	+	-	+	-	-	-	-
T. ararati-minoris	-	-	-	+	-	+	-	-
T. roegneri	-	-	+	+	-	-	-	-
T. artvinicus	+	+	-	+	-	-	-	+
T. eriophorus	-	-	-	+	-	-	-	+
T. pseudoplegioides	-	+	-	+	-	-	-	-
T. praecox sub jankae var jankae	-	-	-	+	-	-	-	-
T. tharacicus	-	-	-	+	-	-	-	+
T. longicaulis subsp. *longicaulis* var. *longicaulis*	-	-	+	+	-	-	-	+

CHAPTER 4

CONCLUSION

Thymus is a very complex genus in terms of its micromorphological properties. Therefore, its micromorphological features and their taxonomic implications are not studied sufficiently all around the world. Infrageneric classification of the genus *Thymus* was not given in Flora of Turkey. Hence understanding taxonomy, evolution and phylogeny of the genus *Thymus* in Turkey could be greatly improved by using micromorphological characters.

For this purpose, micromorphology of leaf, calyx and stems of 32 *Thymus* species gathered from Turkey were examined via Scanning Electron Microscopy (SEM) for the first time. The qualitative and quantitative traits found to have diagnostic value are Epidermal Cell Shape, Cell Wall Pattern, Nonglandular and Glandular Trichome Type and Size, Presence and Position of Stomata. (Table 4.1, 4.2, 4.3).

Although indumentum showed variations between sections and subsections, it was also meaningful at species level.

Table 4.1. *Qualitative and Quantitative Adaxial and Abaxial Leaf Micromorphological Characters Observed with SEM*

Leaf	
Qualitative Traits	Quantitative Traits
Epidermal Cell Shape Cell Wall Pattern Trichome Type Presence of Stomata Stomata Position	Size of Epidermal Cells Glandular Trichome Size Non- Glandular Trichome Size

Table 4.2. *Qualitative and Quantitative Calyx Micromorphological Characters Observed with SEM*

Calyx	
Qualitative Traits	Quantitative Traits
Trichome Type	Non-Glandular Trichome Size Glandular Trichome Size

Table 4.3. *Qualitative and Quantitative Stem Micromorphological Characters Observed with SEM*

Stem	
Qualitative Traits	Quantitative Traits
Trichome Type	Non-Glandular Trichome Size Glandular Trichome Size

The research finding of this study has been found to support the infrageneric delimitation of Morales' work (2012) which was based on the work of Jalas (1971, 1972). According to the findings of this micromorphological study there are 2 sections: *Hyphodromi* and *Serpyllum* and 7 subsections. It was observed that micromorphological traits and their taxonomic implications are consistent with aforementioned grouping. Micromorphological characters even implies further grouping under subsectional levels.

Due to its dense indumentum structure on leaf surfaces, it was tricky to study epidermal cell shape, cell wall pattern and stomata of the *Thymus* taxa. However, they were somehow informative in species and infrageneric levels.

3 types of epidermal cell shapes were observed among the studied taxa; elongated, slightly elongated, and isodiametric on adaxial and abaxial leaf surfaces. Slightly Elongated epidermal cell shape with Undulated/Wavy cell wall pattern in subsection *Kotschyani*, Slightly Elongated epidermal cell shape with Straight to Curved cell wall pattern in subsection *Serpyllastrum* was a characteristic feature on adaxial leaf surface. Isodiametric epidermal cell shape was only observed in section *Serpyllum*. *T. pseudoplegioides* was unique with its isodiametric epidermal cell shape with undulated/wavy cell wall pattern on both adaxial and abaxial leaf surfaces among other *Thymus* species. *T. pseudoplegioides* is the only representative of Subsection *Alternates* in Turkey therefore, isodiametric shape with undulated/wavy cell wall pattern on both leaf surfaces was also valuable in sectional and subsectional delimitation.

In section *Hyphodromi,* subsection *Thymbropsis* all stomata were positioned as sunken on both leaf surfaces. Sunken stomata position was also a characteristic feature in section *Serpyllum,* subsection *Pseudomarginati.*

In terms of their sizes there was no meaningful difference between 2 sections' nonglandular trichomes. Also leaf short type (NGI) trichome presence showed almost no difference between 2 sections. However, long type (NGII) trichomes were valuable diagnostic characters for the sections and through the taxa. Presence of type (NGII) trichomes on calyx was characteristic for Section *Serpyllum*. Presence of (NGII) trichomes on leaf surface is unique to only *T. revolutus* and *T. argaeus* from subsection *Subbructeati* of section *Hyphodromi* and *T. migricus* and *T. ararati-minoris* from subsection *Kotschyani* of section *Serpyllum.* Also leaf (NGII) trichomes from subsection *Kotschyani* of section *Serpyllum* is distinguished by their appressed shape and prominent articulation at their nodes form others.

Subsection *Alternates* of section *Serpyllum* and its only member in Turkey *T. pseudoplegioides* had a unique feature by having nonglandular trichomes only on abaxial leaf surface with a glabrous adaxial leaf surface.

Represented only one endemic species *T. bornmuelleri* in Turkey subsection *Insulares* of section *Serpyllum* is characterized by its glabrous leaf and sparse short uni/bi/multicellular trichomes on calyx and stem.

Another difference between the sections was the density of the trichomes. This difference was prominent especially on the stem and calyx surface. Stems and calyces of section *Hyhodromi* were more densely covered with nonglandular trichomes.

Glandular trichomes had valuable taxonomic implications in genus *Thymus*. Calyx is the richest plant part in terms of presence of glandular trichomes. Peltate trichomes are the most profoundly found glandular trichomes through the taxa on all plant surfaces. Presence of (GII) type short stalked glandular trichomes on leaf and subsessile and sessile glandular trichomes of (GI) on stem was a diagnostic feature for section *Hyphodromi.* Calyx type (GI) glandular trichome only observed in section *Serpyllum* and it was unique to only *T. migricus* and *T. artvinicus* of subsection *Kotschyani.*

Trichome micromorphology is very complex in the taxa and it is very informative at infrageneric and species levels. Following selected results supports that trichome micromorphology shows high levels of variability between different species even within the same subsections.

Unicellular, bicellular and multicellular short, unbranched nonglandular trichomes on calyx and stem is a characteristic feature of subsection *Thymbropsis*. However on leaf surface of subsection *Thymbropsis* the species can be glabrous or with only (NGIB) bicellular nonglandular trichomes or with only (NGIA) unicellular trichomes. *T. pruinosus* var. *globifer* is peculiar among the all taxa because of its foliar indumentum. It was the only species covered with only very dense (NGIC) type trichomes although it is considered to be grouped with other species under subsection *Subcructeati*. Another example to high levels of variability is, although all of the *Thymus* species examined had (GIV) peltate glandular trichomes on their calyx, in subsection *Subbructeati* all peltate trichomes are accompanied by different types of glandular and non-glandular trichomes on the calyx.

More detailed taxonomic surveys and statistical analysis with phenograms are needed for further grouping and diagnostic keys both in specific and infrageneric levels. In the sense of richness of species included in the study and complexity of micromorphological traits of genus *Thymus*, it can be concluded that this study can be regarded as a highly informative micromorphological study with taxonomic implications.

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APPENDICES

A. SPECIMEN INFORMATION OF EXAMINED *THYMUS* SPECIES

Species	Specimen Information (Voucher No, Locality, Date and Collector)
T. cilicicus Boiss. & Bal.	**14523**, C4 Antalya: Gundogmus, Geyik Mountain Road, Around Gelensandra, 1500 m, 22.07.1999, B. Yildiz & N. Adiguzel
T. revolutus Celak.	**14577,** C4 Icel: Anamur, Emirsah Village 50-100 m, 28.05.2000, B. Yildiz & Z. Bahcecioglu
T. cherlerioides Vis. var. *isauricus*	**15413**, C5 Mersin: Aslankoy, Bolkar Mountains, Gokkol Road, 2400-2500 m, 07.08.2002, B. Yildiz
T. leucotrichus Hal.	**14713**, B5 Kayseri: Yahyali, Aladag, 2500 m, 05.07.2000, B. Yildiz & T. Arabaci
T. convolutus Klokov	**14665,** B7 Erzincan: Kemaliye, Sirakonaklar Village, Saricicek Plateau Road, 1700-1800 m, 24.06.2000, B. Yildiz & Z. Bahcecioglu
T. argaeus Boiss. & Bal.	**14712**, B5 Kayseri: Erciyes Mountain, 1900 m, 05.07.2000, B. Yildiz & T. Arabaci
T. cappadocicus Boiss.	**14663**, B7 Erzincan: Kemaliye, Sırakonaklar Village, Saricicek Plateau Road, 1600 m, 26.06.2000, B. Yildiz & Z. Bahcecioglu
T. haussknechtii Velen	**16661**, B6 Malatya: Arapkir-Kemaliye Road, 1200 m, 14.06.2000, B. Yildiz & Z. Bahcecioglu
T. spathulifolius Hausskn. & Velen.	**15061,** E9 Erzincan: Around Ilic-Hasanova, 1400 m, 13.08.2001, B. Yildiz & T. Dirmenci

T. cariensis Hub.-Mor. & Jalas	**14490**, C2 Mugla: Koycegiz-Mugla Road, 150 m, 19.07.1999, B. Yildiz & N. Adiguzel
T. striatus Vahl. var. *interruptus* Jalas	**971,** A1 (E) Kırklareli: İnece, 200 m, 21.05.1999, T. Dirmenci
T. zygioides Griseb. var. *zygioides*	**14953,** A1 (E) Edirne: Kesan-Ipsala Road, 120 m., 30.05.2001, B, Yildiz & T. Dirmenci
T. roegneri C. Koch	**15085**, A2 Bilecik: Bilecik-Yenişehir Road, 500 m., 30.05.2002, B. Yildiz & T. Dirmenci
T. fallax Fischer & Mey.	**14720,** B5 Kayseri: Around Develi, 1700 m, 05.07.2000, B. Yildiz & T. Arabaci
T. transcaucasicus Ronniger	**1278**, B8 Erzurum: Patnos-Tutak Road, 1750 m, 06.06.2001, T. Dirmenci
T. kotschyanus Boiss. & Hohen. var. *glabrescens* Boiss.	**14762**, B9 Bitlis: Bitlis-Tatvan Road, 1600 km 16.07.2000, B. Yildiz & T. Arabaci
T. migricus Klokov	**1863**, B9 Van: Caldiran-Dogubeyazit Road, 2100 m, 12.06.2002, T. Dirmenci
T. fedtschenkoi var. *handelii*	**14827,** B9 Van: Van-Guzeldere (Cuh) Pass, 2300 m, 09.07.2000, B.Yildiz & N. Demirkus
T. sipyleus Boiss. subsp. *sipyleus* var. *sipyleus*	**14445**, B6 Kahramanmaras: Elbistan Yalak Village Road, 1200 m, 09.07.1999, B. Yildiz
T. leucostomus	**15432,** A5 Çankırı: 10 km North,

Hausskn. & Velen. var. *gypsaceus* Jalas	26.06.1997, B. Yildiz
T. praecox Opiz subsp. *jankae* (Velen) Jalas var. *jankae*	**15355**, A6 Ordu: Korgan-Persembe Road, 1500-1700 m, 26.07.2002, B. Yildiz
T. thracicus Velen	**14607**, A2 Yalova: Yalova Orhangazi Road, 250 m, 02.06.2000, B. Yildiz & & Z. Bahcecioglu
T. longicaulis C. Presl subsp. *longicaulis* var. *longicaulis*	**14615**, B2 Bilecik: Osmaneli-Iznik Road, 250-300 m, 02.05.2000, B. Yildiz & Z. Bahcecioglu
T. pseudopulegioides Klokov & Des.-Shost	**15069**, A8 Gumushane, Zigana Pass, 1700m, 13.08.2001, B. Yildiz & T. Dirmenci
T. boissieri	**14967,** A1 (E) Kırklareli: Dereköy Road, 600 m, 01.06.2001, B. Yildiz & T. Dirmenci
T. ararati-minoris Klokov	**2136**, B10 Igdir: Aralik, Little Ararat Mountain, Serdar-Bulak Road, 2200-2700 m, 02.08.2002, T. Dirmenci
T. pruinosus Boiss. var. *globifer* Jalas	**14612**, B6 Sivas: Gürün-Gökpınar, 1500 m 17.06.2000, B. Yildiz & Z. Bahcecioglu
T. artvinicus Ponert	**14870,** A9 Artvin: Savsat Exit, 1150 m, 21.07.2000, B. Yildiz & N. Demirkus
T. eriophorus	**14356,** B7 Malatya: Malatya-Arapkir Road, 1100 m., 26.06.1999, B. Yildiz