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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANATOMICAL, PALYNOLOGICAL AND MICROMORPHOLOGICAL INVESTIGATIONS ON ENDEMIC *THYMUS BRACHYCHILUS* JALAS (LAMIACEAE)

ENDEMİK THYMUS BRACHYCHILUS JALAS (LAMIACEAE) ÜZERİNDE ANATOMİK, PALİNOLOJİK VE MİKROMORFOLOJİK ARAŞTIRMALAR

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ABSTRACT

Objective: *In this study, it was aimed to investigate the anatomical and palynological features of endemic Thymus brachychilus Jalas.*
Material and Method: *The plant material was fixed in 70% alcohol for anatomical investigation. Cross sections of the stem, leaves, and leaf surface sections were stained with sartur solution, and the anatomical aspects were examined. The trichome architectures, mesophyll cells, stomatal index, and glandular and non-glandular trichomes features of leaves and stem have been investigated and compared with the other Thymus species anatomical findings. In addition, measurements of anatomical structures were made. Pollen, flower parts, epidermis and trichome structure of the leaves, were examined in SEM.*
Result and Discussion: *This is the first anatomical, palynological and micromorphological study on Thymus brachychilus. The epidermis of leaf consists of a single row of elongated shaped cells and is covered with a thin cuticle. There are nonglandular and glandular trichomes in the upper and lower epidermis. Two different types of nonglandular trichomes were found. Stomata are diacytic, and the epidermis is amphistomatic. The mesophyll layer is dorsiventral. The stem cross-section is square. The outermost single-row, elongated, square or rectangular epidermis is covered with a thin cuticle. The pollen symmetry is isopolar, shape is suboblate. It has hexacolpate and bireticulate properties.*

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ÖZ

Amaç: *Bu çalışmada, endemik Thymus brachychilus Jalas'ın anatomik ve palinolojik özelliklerinin araştırılması amaçlanmıştır.*
Gereç ve Yöntem: *Anatomik inceleme için bitki materyali %70 alkol içinde fikse edildi. Gövde ve yaprak enine kesitleri, yaprak yüzey kesitleri sartur solüsyonu ile boyandı ve anatomik açıdan incelendi. Yaprak ve gövdenin trikom yapıları, mezofil hücreleri, stoma indeksi, salgı ve örtü tüyleri incelendi, aynı zamanda diğer Thymus türlerinin anatomik bulguları ile karşılaştırıldı. Ayrıca anatomik yapıların ölçümleri yapıldı. SEM'de polen, çiçek kısımları, yaprakların epidermis ve trikom yapısı, kısımları incelenmiştir.*
Sonuç ve Tartışma: *Thymus brachychilus üzerinde yapılan ilk anatomik, palinolojik ve mikromorfolojik çalışmadır. Yaprağın epidermisi tek sıra uzun şekilli hücrelerden oluşur ve ince bir kütikül ile kaplanmıştır. Üst ve alt epidermiste glandüler olmayan ve glandüler trikomlar vardır. İki farklı tipte nonglandüler trikom bulundu. Stomalar diastiktir ve epidermis amfistomatiktir. Mezofil tabakası dorsiventraldir. Gövde enine kesiti karedir. En dıştaki tek sıralı, uzun, kare veya dikdörtgen epidermis ince bir kütikül ile kaplıdır. Polen simetrisi izopolardır ve şekli suboblate. Hexacolpate ve bireticulate özelliklere sahiptir.*
Anahtar Kelimeler: *Aladağlar, mikromorfoloji, palinoloji, bitki anatomisi, Thymus brachychilus*

INTRODUCTION

Thymus L. has 341 species in the world and 46 species in Türkiye, 19 of which are endemic [1-3]. *Thymus* species are generally woody at the base, small shrubs or cushion plants. Leaves are entire, sessile or petiolate, with flat or revolute edges, and frequently ciliate at base of lamina. Sessile, colorless to brilliant red glands are present on the bracts, calyces, and leaves. Verticillasters with two or more flowerings, subtending floral leaves, or clustered into a terminal head with distinct bracts. Calyx clearly bilabiate; tube cylindrical to campanulate, straight, with 10-13 veins; upper lip broad patent or recurved. Corolla is purple, pink, cream, or white; tube is straight; the top and lower lips are emarginate and straight. Nutlets are glabrous. The stems and leaves of *Thymus brachychilus* are similar to *T. leucotrichus* but have distinct coarse trichomes, more narrow leaves, weak differentiation between the inflorescence and the vegetative shoots, oil glands are primarily orange or red. Upper lip of the calyx is shorter than lower teeth; bracts are practically leaf-like, 0.8-1.5 mm broad, narrowly rhombic, and progressively narrow into a short petiole. Flowering time is between 6 and 8 months. Grows in 1800-3660 m., rocks and screes. *Thymus brachychilus* grows naturally in the provinces of Adana, Erzincan, İçel, Niğde, Tunceli, Van in the Southern and Central Anatolian regions of Türkiye [3].

For many years, people in Anatolia have used several *Thymus* species, with a variety of outcomes. *Thymus brachychilus* is known as "Mor kekik" in the Niğde [4]. According to ethnobotanical studies carried out in various regions of Türkiye, it is used for abdominal pain, anxiety, anorexia, asthma, backache, breathing problems, bronchitis, cancer, colds, diabetes, edema, enteralgia, flu, gastritis, halitosis, hemorrhoids, high cholesterol, hypertension, kidney diseases, renal inflammations, prostrate, stomachache, and tonsillitis [5-15]. *Thymus* species grown in Türkiye are especially important with their rich essential oil content. The essential oil contents of *Thymus* species grown in Türkiye contain borneol, carvacrol, carvacrol methyl etherl, 1,8-cineole, cymene, farnesol, limonene, linalool, γ-terpinen, thymol, α-pinene [16-20]. It is important to determine the anatomical, palynological and micromorphological characteristics of *Thymus* species which are so important in terms of medicine and widely used in traditional medicine of Anatolia. These findings are also valuable in helping to accurately identify *Thymus* species. This study includes anatomical and palynological studies on the endemic *Thymus brachychilus* (TB). It is the first research on the anatomy and palynology of this species.

MATERIAL AND METHOD

Plant Material

Samples of the *Thymus brachychilus* were collected in the Maden Valleys of the Aladağlar

mountain range (Niğde-Türkiye) between 2500 and 3500 meters in July 2022 (Figure 1). After identification of the plant species by authors, voucher specimens were prepared. These voucher specimens were kept in the Herbarium of the Pharmacy Faculty, Altınbaş University (HERA 1074).

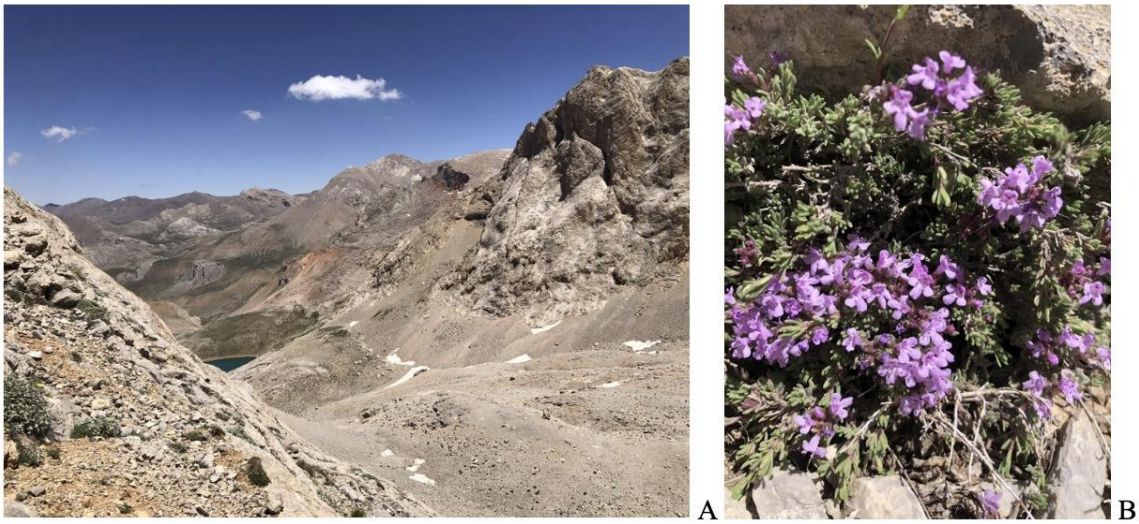

Figure 1. A- Habitus of *Thymus brachychilus* in Aladağlar (Niğde, Türkiye), B- General view of *Thymus brachychilus*

Anatomical Studies

The material was fixed in 70% alcohol for anatomical investigation. Parts of the stem, leaves, and leaf surface were stained with sartur solution. The well-stained slices were photographed using an Soif Optical Instruments Trinocular Stereo microscope and Zeiss light microscope. Investigations have been done in the anatomical characteristics of stems, leaves and leaf surfaces. Anatomical studies were conducted on cuticle, epidermis cells, mesophyll cells, stomatal index, stomata structures, non glandular and glandular trichomes of the leaf; cuticle, epidermis cells, cortex layer, collenchyma, parenchyma endodermis cells, trachea of the stem. Additionally, calculations have been made for the micro-anatomic measurements of cells and tissues.

Palynological and Micromorphological Studies

Pollens were removed with the help of arrowheads. Pollens and epidermis, trichome structure of the leaves, flos, calix parts adhered to the sample holder (stab) with double-sided tape. The prepared samples were coated with gold and examined in detail under a table-top scanning electron microscope (FEI Versa 3D dual beam) and photographed. In pollen morphology, Walker and Doyle [21] terminology was used.

RESULT AND DISCUSSION

This is the first anatomical, palynological and micromorphological study on *Thymus brachychilus*.

Anatomical Studies

Leaves Anatomy:

The outermost layer, the epidermis, consists of a single row of elongated shaped cells and is covered with a thin cuticle. The upper cuticle thickness is 2.81 µm, lower side cuticle thickness is 3 µm. The upper epidermis cell length is 11.02 µm and the width is 17.31 µm. The lower epidermis cell length is 12.74 µm and the width is 11.21 µm. The epidermis cells on the upper side are wider. There are

nonglandular and glandular trichomes in the upper and lower epidermis. Two different types of nonglandular trichomes were found. Non-glandular trichomes are simple, erect mostly 3-5 cells and the other type nonglandular trichomes are simple with one cell (Figure 2). Along the leaf surface, two different types of glandular trichomes were found. One of them has a base, a stalk, and a unicellular head, and is called a capitate glandular trichome (Figure 3). Peltate glandular trichomes are the other type; they feature a base, a short sessile stalk, and a multicellular head. Stomata are diacytic, and they were identified as being at the same level as the epidermis (amphistomatic). The upper stomata length is 26 µm and the width is 17 µm. The lower stomata length is 18 µm and the width is 14 µm. It was determined that the stomata on the upper side were larger. The upper side stomata index is 17.78 while the lower side stomata index is 18.10 (Table 1). The stomatas are more dense on the lower side (Figure 4). The mesophyll layer is dorsiventral. It consists of 1-2 rows of palisade and 3-4 rows of sponge parenchyma. Vascular bundles are collateral. On the midrib region, the xylem is directed towards the upper epidermis and the phloem towards the lower epidermis. Just below the xylem, multilayered (10-15) sclerenchymatic bundles are seen. Phloem has 2-3 rows. Oval collenchyma is located under the upper and lower epidermis on the middle vein region. Round parenchyma seen below the collenchyma (Figure 5).

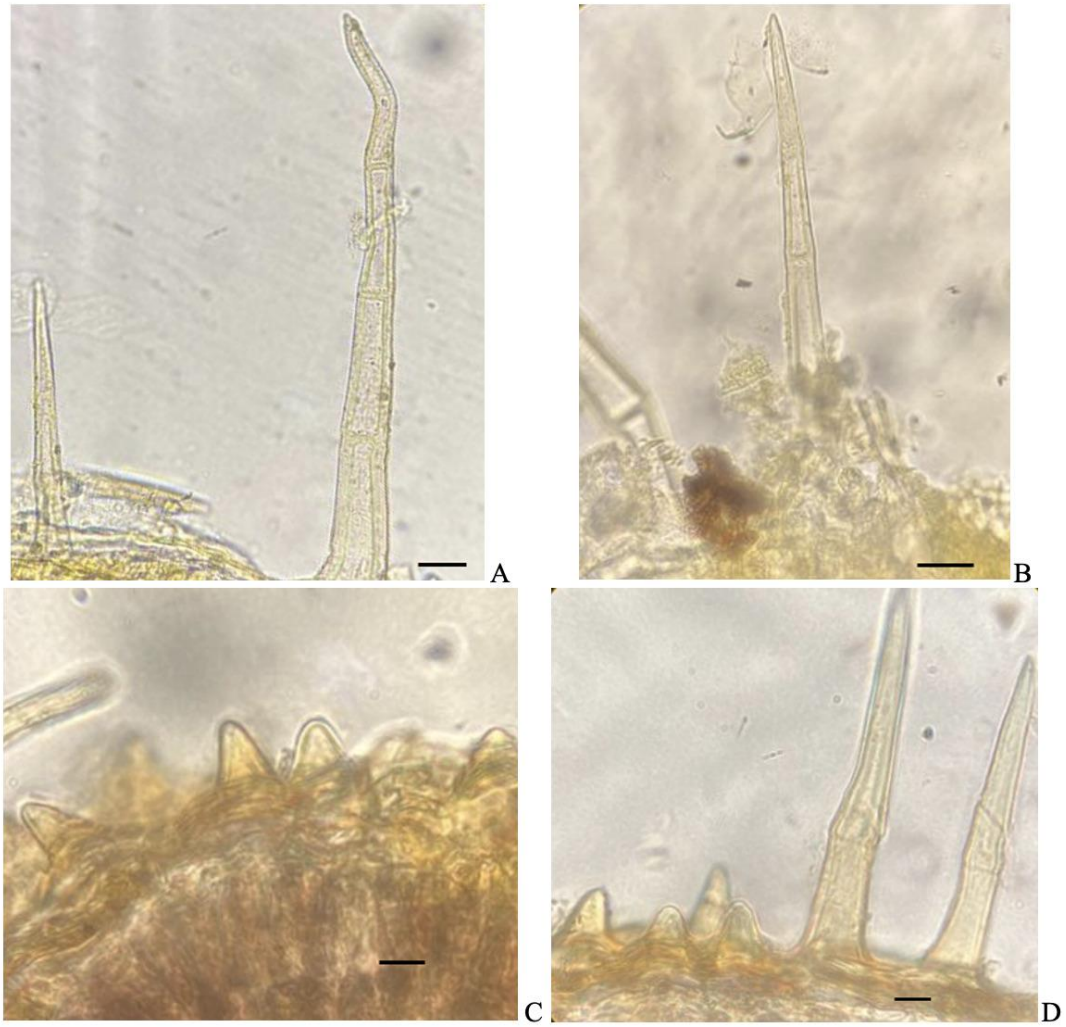

Figure 2. Nonglandular trichomes observed on studied TB. A-4 celled nonglandular trichomes on abaxial surface (Scale bars: 25 µm); B-4 celled nonglandular trichomes on adaxial surface; C-Single celled nonglandular trichomes (Scale bars: 100 µm); D-Single celled and 2-celled nonglandular trichomes (Scale bars: 100 µm)

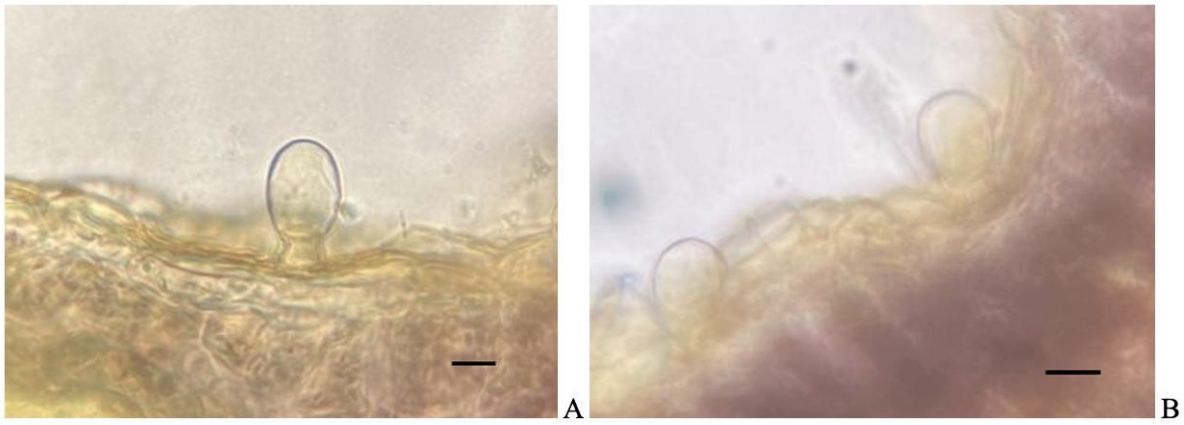

Figure 3. Glandular trichomes types observed on studied TB. A-abaxial surface; B-adaxial surface (Scale bars: 10 µm)

Table 1. Anatomical measurements of leaf

Anatomical Structures	**TB**
Upper epidermis	
Stomata type	diacytic
Stomata index	17.78
Stomata length (µm) (Avr. ± Sd)	26±2.23
Stomata width (µm) (Avr. ± Sd)	17±2.09
Cuticle thickness (µm) (Avr. ± Sd)	2.81±0.74
Epidermis cell length (µm) (Avr. ± Sd)	11.02±1.65
Epidermis cell width (µm) (Avr. ± Sd)	17.31±0.88
Lower epidermis	
Stomata type	diacytic
Stomata index	18.10
Stomata length (µm) (Avr. ± Sd)	18±3.26
Stomata width (µm) (Avr. ± Sd)	14±2.85
Cuticle thickness (µm) (Avr. ± Sd)	3±1.22
Epidermis cell length (µm) (Avr. ± Sd)	12.74±1.17
Epidermis cell width (µm) (Avr. ± Sd)	11.21±1.85

Avr: average, Sd: standard deviation

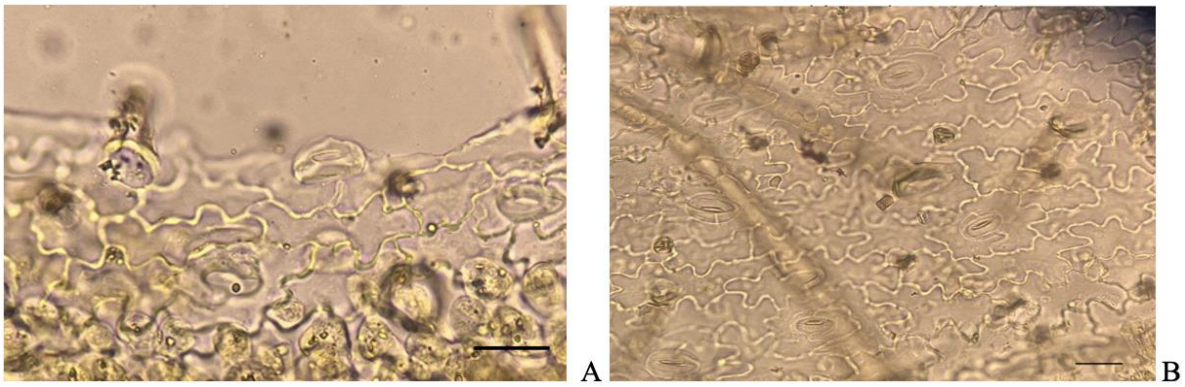

Figure 4. Lamina epidermal surface of TB. A- abaxial surface; B-adaxial surface (Scale bars: 25 µm)

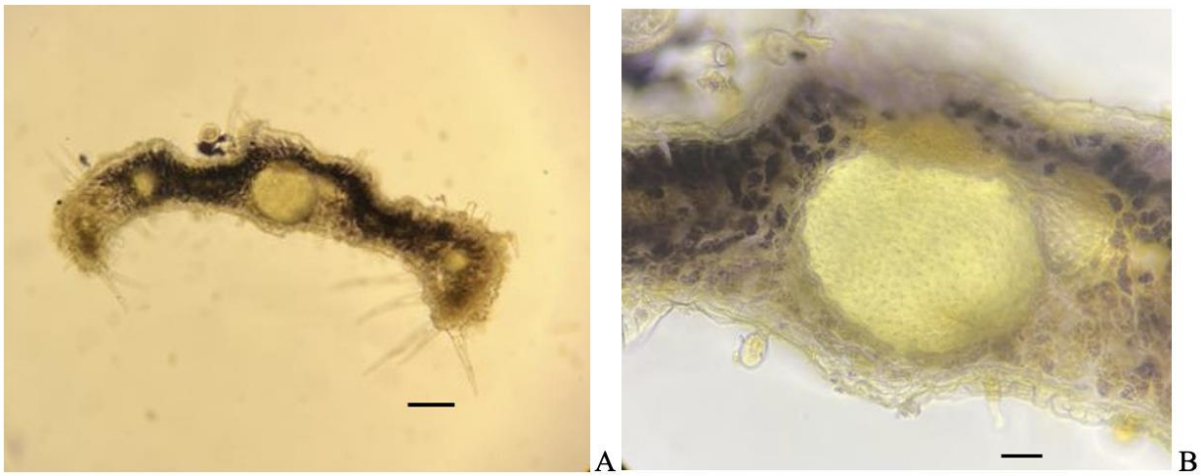

Figure 5. A- Cross section of leaves (Scale bars: 100 µm); B- Cross section of leaves (Scale bars: 25 µm)

Stem Anatomy:

The stem cross-section is square and hollow. The outermost single-row, elongated, square or rectangular epidermis is covered with a thin cuticle. The epidermis cell length is 16.90 µm and width is 16.87 µm. Glandular and nonglandular trichomes are observed on the epidermis. Nonglandular trichomes 1-6 cells, unbranched. The glandular trichomes are seen in the capitate type. Just below the epidermis is the cortex layer. This layer consists of collenchyma (3-5 rows), parenchyma (3-4 rows) and endodermis (1-2 rows). The collenchyma layer is 37.48 µm in the corner while it is 15.08 µm between corners. The parenchyma layer is 24.99 µm. The phloem is 3-6 rows and phloem layer is 20.81 µm. The xylem encircled the pith in an annular manner and the xylem layer is 31.22 µm (Table 2). The pith is composed of polygonal shaped, thin-walled parenchymatic cells (Figure 6).

Table 2. Anatomical measurements of stem

Anatomical Structures	**TB**
Epidermis cell length (µm) (Avr. ± Sd)	11.29±0.65
Epidermis cell width (µm) (Avr. ± Sd)	10.5±1.22
Pith ray cell	15.51±2.30
Trachea (µm) (Avr. ± Sd)	9.68±0.95
Endodermis cell length (µm) (Avr. ± Sd)	16.90±2
Endodermis cell width (µm) (Avr. ± Sd)	16.87±3.18
Collenchyma cell	12.52±0.59
Collenchyma layer (Corners)	37.48±3.19
Collenchyma layer (Between corners)	15.08±0.49
Parenchyma layer	24.99±6
Phloem layer	20.81±1.49
Xylem layer	31.22±2.26

Avr: average, Sd: standard deviation

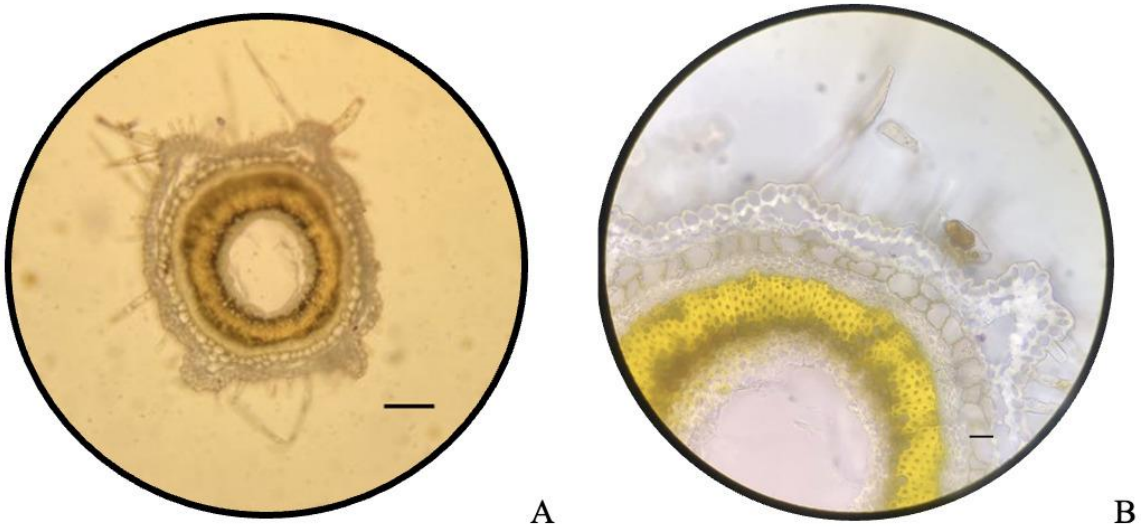

Figure 6. A- Cross section of stem (Scale bars: 100 µm); B- Cross section of stem (Scale bars: 25 µm)

Palynological and Micromorphological Studies:

In morphological pollen analysis, monad pollen is small-medium sized and its symmetry is isopolar (Figure 7). Pollen polar axis on average is 22.39 µm, equatorial axis on average is 26.45 µm. According to the P/E ratio, pollen shape is suboblate. Apertures are colpate, with an average colpus width of 1.7 µm and a length of 19.95 µm. 6 colpus makes pollen as a hexacolpate. Sculpture is a bireticulate (Table 3).

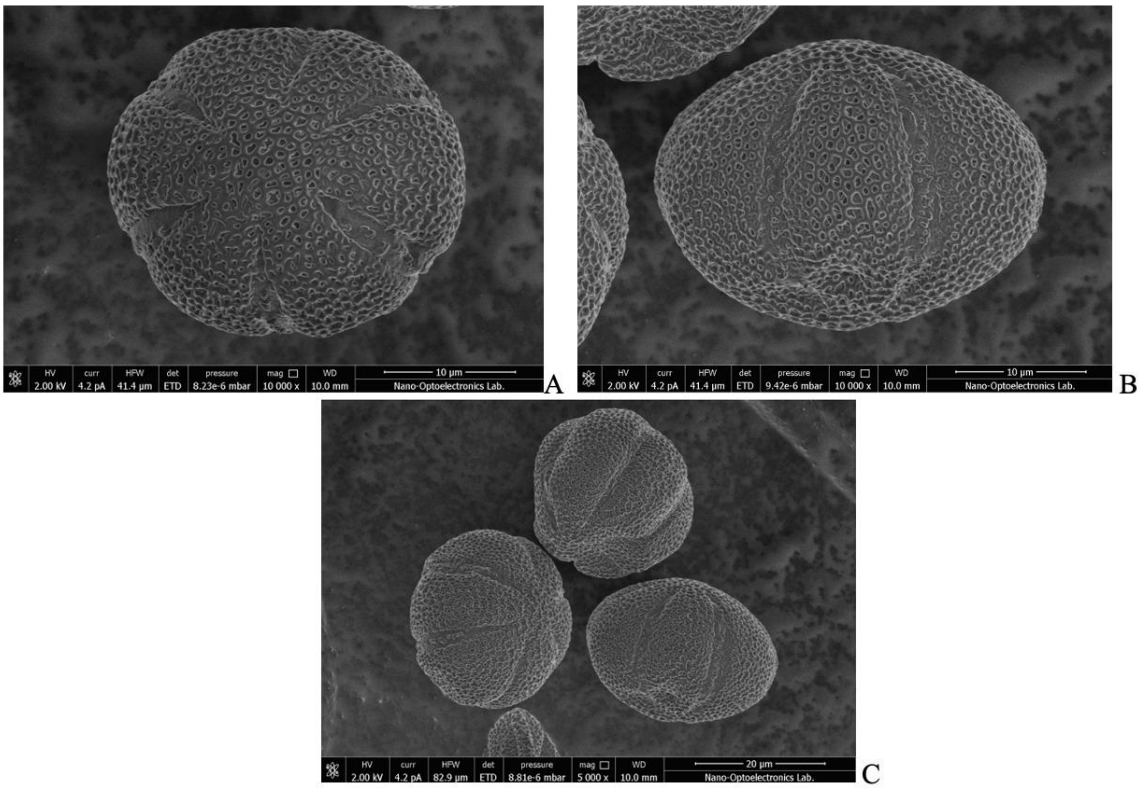

Figure 7. SEM images of TB, A- Polar view; B- Equatorial view; C- General view

Table 3. Palynological features and measurements

Ornamentation	**Bi-reticulate**
Aperture type	Colpus
Pollen shape	Suboblate
Aperture number	6
Colpus width	1.7±0.54 μm
Colpus length	19.95±0.64 μm
Polar axis (P)	22.39±0.24 μm
Equatorial axis (E)	26.45±1.26 μm
Pollen symmetry	isopolar
P/E	0.85

In leaf micromorphology, leaf arm tips are revolute. It was observed that the nonglandular trichomes on the lower and upper surfaces were similar to each other. The glandular trichomes were densely peltate and sparsely capitate type on both sides. Calyx has the nonglandular trichomes and peltate glandular trichomes (Figure 8-10).

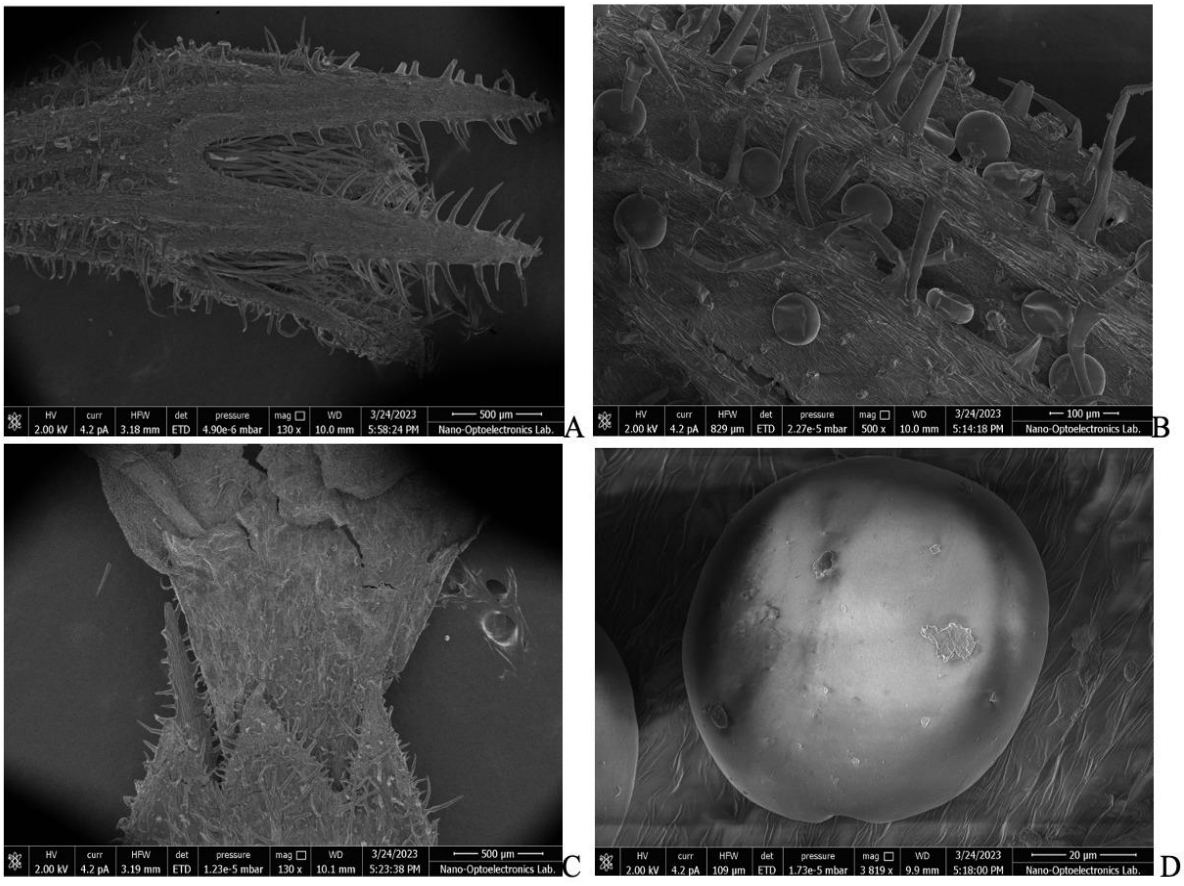

Figure 8. A, B- Calyx of TB; C- Calyx and Corolla of TB; D- Glandular trichomes of TB

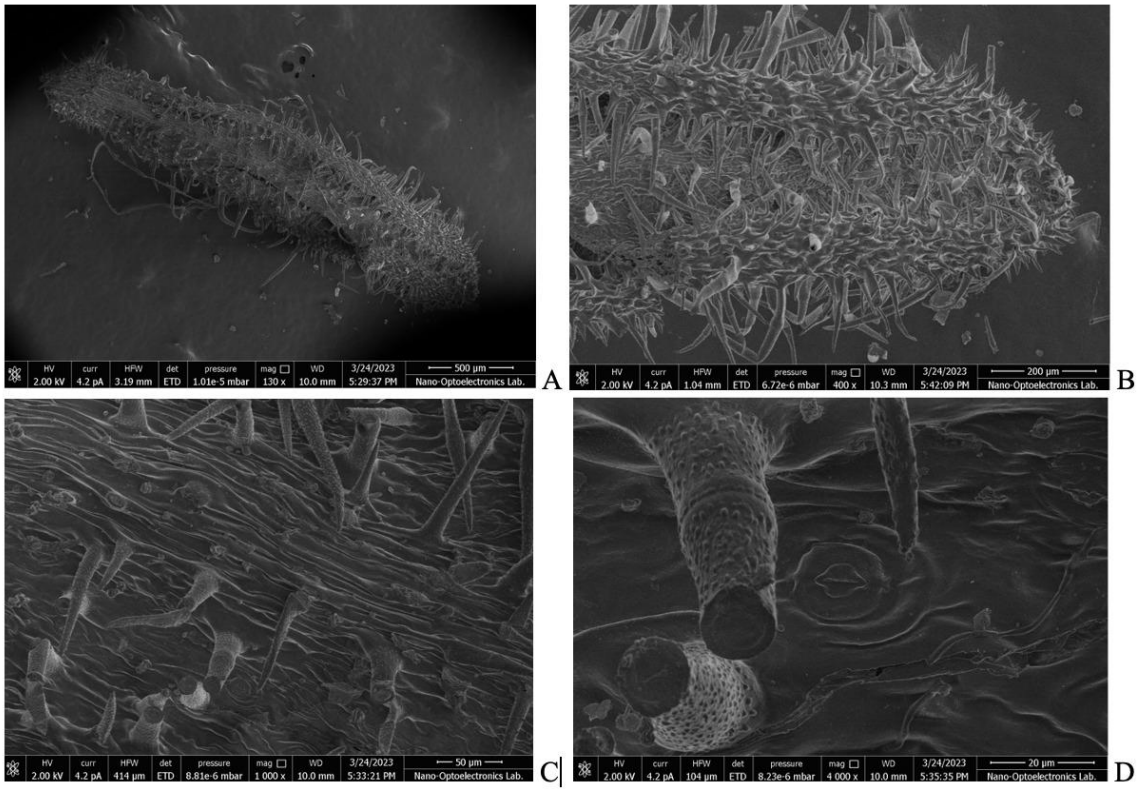

Figure 9. A, B, C, D- Lamina abaxial surface of TB

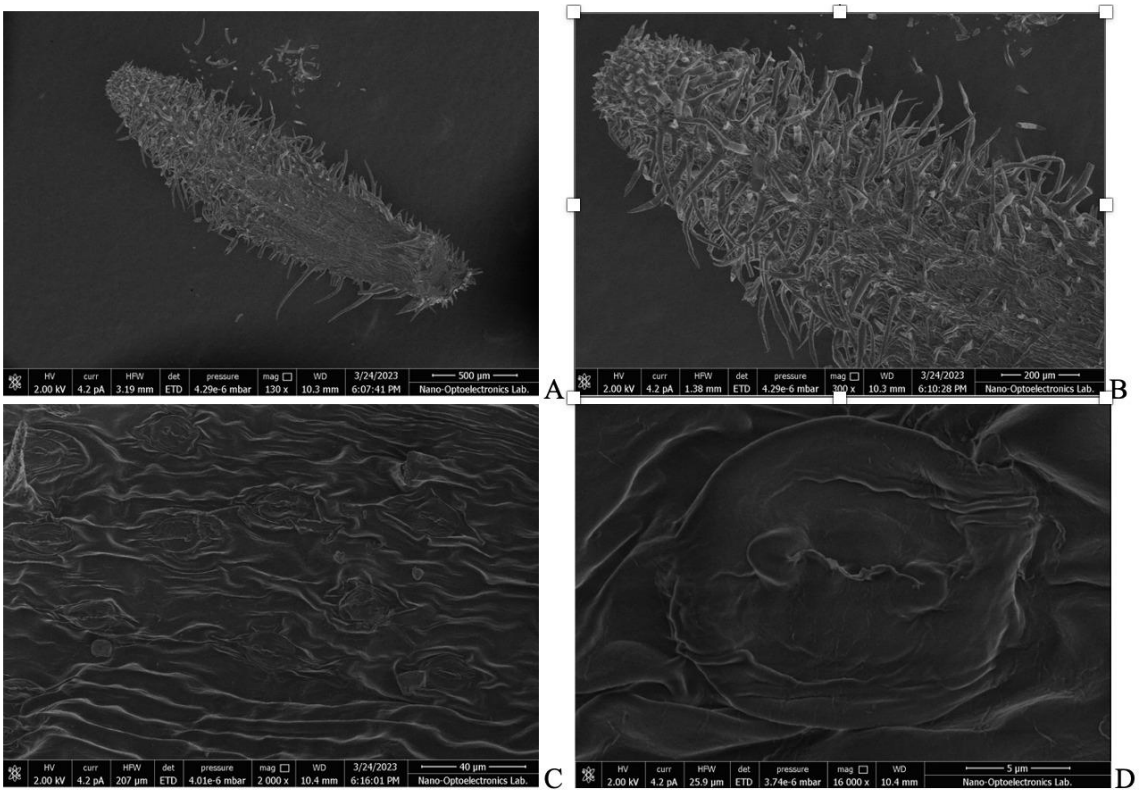

Figure 10. A, B, C, D- Lamina adaxial surface of TB

The results of the study were compared with previous anatomical, micromorphological and palynological results of *Thymus* species. According to an anatomical study on *Thymus cherlerioides* and T. pulvinatus, stomata index of *T. brachychilus* leaves is very similar to *T. cherlerioides* and less than *T. pulvinatus*. The stomata size of *T. brachychilus* is bigger than *T. cherlerioides* and *T. pulvinatus*. Glandular and nonglandular trichome types are very similar with this two species. The stem cross-section is of *T. brachychilus* is similar to *T. pulvinatus* [22]. In a micromorphological study of *Thymus* genus the most common epidermal cell shape was a slightly elongated form. Only one of the 32 species had isodiametric-shaped epidermal cells, while 18 of them had slightly elongated epidermal cells. The *T. brachychilus* epidermal cells of leaf and stem are elongated. Except for two species, all of the leaves of *Thymus* contained stomata on both the adaxial and abaxial surfaces. Only in *T. spahulifolius* were stomata visible, however this might be because of the extremely extensive indumentum structure on the leaf surface. *T. pseudopulegioides* abaxial leaf surface was devoid of stomata. *T. brachychilus* have stomata on both surface. The shape of nonglandular trichome is erect (*T. leucostomus* var. *gypsaceus, T. cherlerioides* var. *isauricus*), curved (*T. longicaulis* subsp. *longicaulis* var. *longicaulis, T. kotschyanus* var. *glabrescens, T. argaeus*) or appressed (*T. migricus, T. fedtschenkoi* var. *handelii*) and Glandular trichomes are either capitate or peltate [23]. *T. brachychilus* usually has erect nonglandular trichomes, capitate and peltate glandular trichomes. Pollen morphological features of five *Thymus* taxa examined by Kızılpınar et al (2009). According to this study *T. longicaulis* subsp. *longicaulis* var. *subisophyllus* has oblate-spheriodal, *T. sipyleus* subsp. *sipyleus* var. *sipyleus* has spheriodal, *T. sipyleus* subsp. *rosulans* and *T.cappadocicus* var. *globifer* suboblate, *T. leuchotricus* var. *leuchotricus* has prolate-spheriodal pollen shape. In our study, *T. brachychilus* has suboblate pollen shape [24].

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AUTHOR CONTRIBUTIONS

Concept: E.Ö.N., S.S.; Design: E.Ö.N., S.S.; Control: E.Ö.N, S.S.; Sources: E.Ö.N., S.S.; Materials: E.Ö.N., S.S.; Data Collection and/or Processing: E.Ö.N., S.S.; Analysis and/or Interpretation: E.Ö.N., S.S.; Literature Review: E.Ö.N., S.S.; Manuscript Writing: E.Ö.N., S.S.; Critical Review: E.Ö.N., S.S.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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