



A comparative anatomical study on two endemic *Nepeta* L. species (*N. baytopii* and *N. sorgerae*)

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Abstract

Nepeta baytopii (sect. *Schizocalyx*) and *N. sorgerae* (sect. *Subinterruptae*) are the two endemic species of the genus *Nepeta* L. in Turkey. In this paper, this two *Nepeta* species which are in two different sections are examined anatomically. In this study, it was investigated on stem-leaf anatomy and trichomes micromorphology of these *Nepeta* species in order to improve our knowledge of its anatomy for systematics and to help separate the species. The anatomical characters of the species, such as stem and leaf are described in detail. The anatomical results were compared to the same investigations done before. In anatomical studies, cross-sections of stems and leaves were examined and are supported by illustrations and photographs. Also, trichomes in stems and leaves were investigated by LM and SEM. Anatomical characters of the species were observed to be similar to the usual features of Lamiaceae anatomy. Finally, these two species are different for serrate of pericycle, collenchyma, xylem and phloem. And the trichome covers of these two species are different from each other.

Key words: Anatomy, Endemic, *Nepeta baytopii*, *Nepeta sogerae*, Trichome

1. Introduction

Nepeta L. is one of the largest genera in Lamiaceae (subfam: Nepetoideae, tribe Mentheae). *Nepeta* has approximately 300 species are distributed in south-west and central Asia, Europe, North Africa and North America. The main diversity center of the genus *Nepeta* are South-west Asia and Western Himalayas (Pojarkova 1954; Hedge 1986; Jamzad et al. 2000; Harley et al 2004).

Turkish *Nepeta* is represented by 44 taxa of which 22 are endemic to Turkey. Most of the species and endemics are distributed in East Anatolia and the Taurus mountains in Turkey. Endemism rate of the genus is 50% (Hedge & Lamond, 1982; Guner et al., 2000; Dirmenci, 2005).

Many of these species are often perennial herbaceous and pleasantly aromatic, rich in essential oils, and of potential economic interest (Kaya & Dirmenci, 2008). These species are known as catnip or catmint because of their effect on cats. The main component is nepetalactone. Some species are used in the traditional medicine of many countries of the Mediterranean area as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue and sedative agents (Rapisarda et al., 2001). Also, *N. meyeri* and *N. racemosa* are used as herbal tea and spices in East Anatolia in Turkey.

There are many cytological, palynological and nutlet morphology studies about *Nepeta* species. Also, anatomical studies were encountered about *Nepeta* species in a few literatures, too. Anatomical and morphological studies were investigated with *Nepeta* species growing around Eskisehir, by Kaya et al.(1991). Morphological and anatomical investigations about *N. caesarea* Boiss. were studied by Kaya et al. (1997). Also, the genus *Nepeta* was studied palynologically in Turkey (Çelenk et al. 2008).

Also, there are new investigations about nutlet micromorphology of some species in the genus *Nepeta*. Nutlet surface micromorphology of *Nepeta* species growing in Turkey were investigated by Kaya and Dirmenci (2008). There are some investigations which were studied Budantsev and Lobova (1997) about fruit morphology and anatomy of *Nepeta* species.

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In this study, two local endemic species, *N. baytopii* Hedge & Lamond (sect. *Schizocalyx* Pojark.) and *N. sorgerae* Hedge & Lamond (sect. *Subinterruptae* Budantz.), were investigated and described in detail for anatomical characters. Turkish name of these species are known as *N. baytopii* “Pembe Kedinanesi”; *N. sorgerae* “Nemrut Kedinanesi”. These species are only known type locality and the vicinity. Also, *N. baytopii* and *N. sorgerae* were included in “Critically Endangered Category-(CR)” according to IUCN criteria (Dirmenci et al. 2004). There is not any study about their anatomical structure. We studied with light microscopy (LM) and scanning electron microscopy (SEM) for their trichomes cover.

2. Materials and methods

2.1 Plant material

The main materials of this investigation are *N. baytopii* (Figure 1) collected from between Lice (Diyarbakır) and Genç (Bingöl) in Turkey. Plant is perennial, stems arcuate ascending, 25-70 cm, shortly and retrorsely pilose with scattered longer spreading trichomes and sessile glands.



Figure 1: General appearance and inflorescence of *Nepeta baytopii* in Turkey

N. sorgerae (Figure 2) collected from Adiyaman-Nemrut mountain in Turkey. Plant is perennial, too. Stems ascending-erect, 25-60 cm, branched from the base, densely glandular-villous with long and short glandular trichomes on stem and leaves.

Voucher specimens belonging to two species (*N. baytopii*: Dirmenci (3712) & Akçiçek, *N. sorgerae*: Dirmenci (3705a) & Akçiçek) are deposited in Necatibey Faculty of Education Herbarium in Balikesir University, Turkey.

2.2 Anatomical analysis

The specimens collected from growing area put in private bottles which has 70 % alcohol and labeled for anatomical investigations. Midrib of leaves and stems from fully flowered plants were used for anatomical study. Cross-sections of leaves and stems were made manually by razor.

The sections were cleared with cloral hydrat and stained phloroglucinol-HCL solutions (Yakar-Tan, 1982). The photographs of fixed preparation had been taken with Olympus BX51 microscope and Nikon Eclipse E600 microscope. Leaf and stem cross-sections had been investigated on Nikon Alphaphot YS trinocular microscope with drawing attachment.

Trichome micromorphology was studied by Tabletop scanning electron microscopy (SEM). For SEM, small pieces of leaves and stem were fixed on aluminum stubs using double-sided adhesive. The SEM micrographs were taken in a NeoScope JCM-5000 at an accelerating voltage of 10 kV.



Figure 2: General appearance and inflorescence of *Nepeta sorgerae* in Turkey.

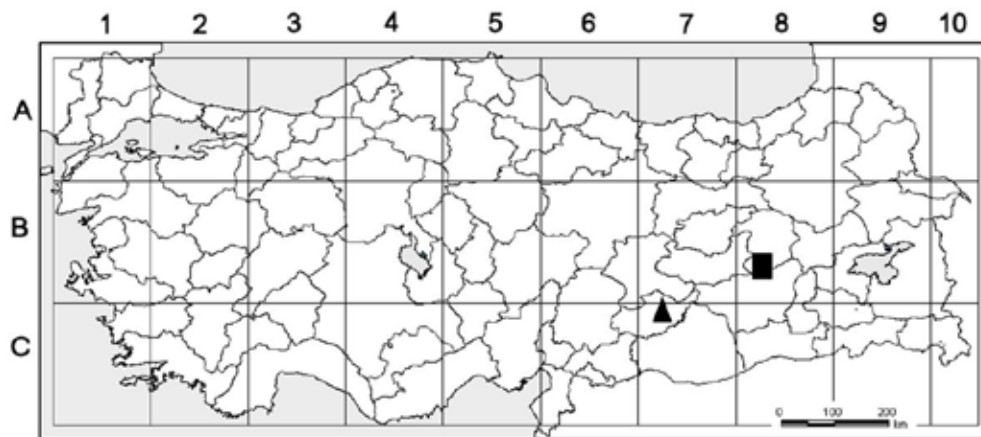


Figure 3: Distribution map of the *Nepeta baytopii* and *Nepeta sorgerae* (■ : *N. baytopii* ▲ : *N. sorgerae*).

3. Results

3.1 Anatomical results

3.1.1 Stem anatomy

Cross-sections taken from the middle part of the stem were observed as follows.

Nepeta baytopii

The epidermis is composed of a single layer of almost square, compactly arranged cells. The upper surface is covered with a curly cuticle and contains glandular and eglandular trichomes (Figure 4, 5). 1-4-celled (1 and 2 celled are more densely) had been found as eglandular trichomes. Two types glandular trichomes were encountered: capitat and peltat types. There were three types of capitat glandular trichomes: 1) Unicellular head and bicellular stalk, 2) Bicellular head and unicellular stalk, 3) unicellular head and unicellular stalk (Figure-16A-a, b, c). The third type was more densely. The collenchyma tissue, which is located immediately under the epidermis, is 5-8-layered on the corners. Parenchyma tissue, which is 1-3-layered in the corners and 4-5-layered in between the corners, is composed of irregular shape cells under collenchyma tissue. Singled-layer endodermis under parenchyma tissue, is composed of usually rectangeled. Scleranchymatic pericycle tissue is 1-2-layered and is only area under alignment of stem's corner. The phloem is 6-8-layered which is under the pericycle. There is xylem under the phloem tissue. The pith consists of large orbicular or polyhedral parenchymatic cells. Those cells underlying the xylem are thin-walled (Figure 6 and Table 1).

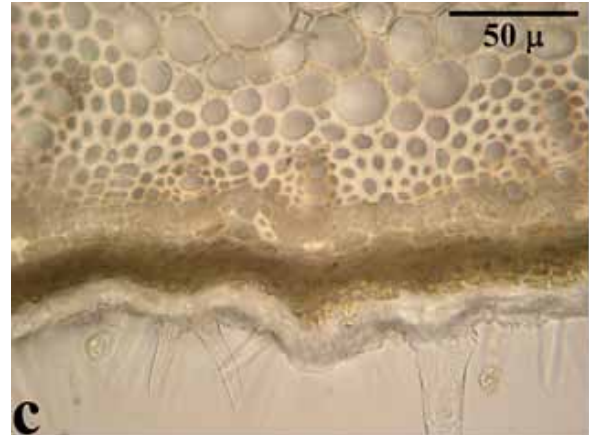
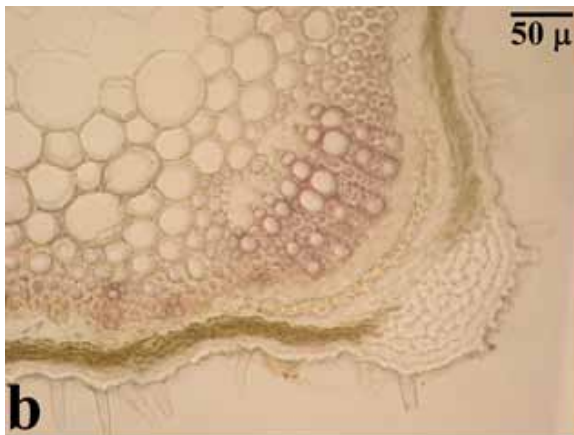


Figure 4: *Nepeta baytopii*. Cross-section of stem. a: General appearance of stem, b: The corner of stem, c: The region of between the corners.

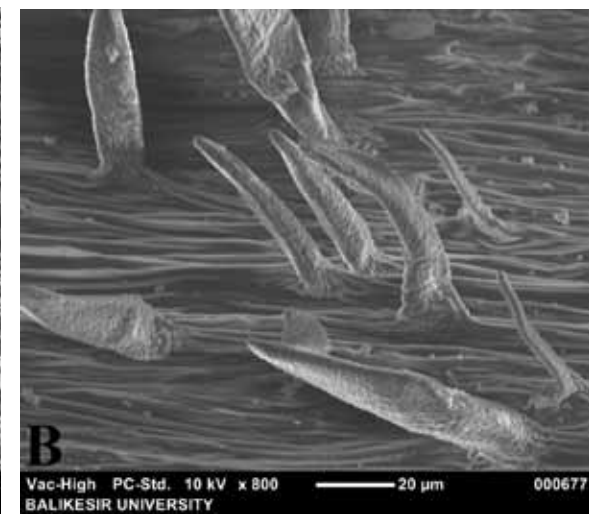
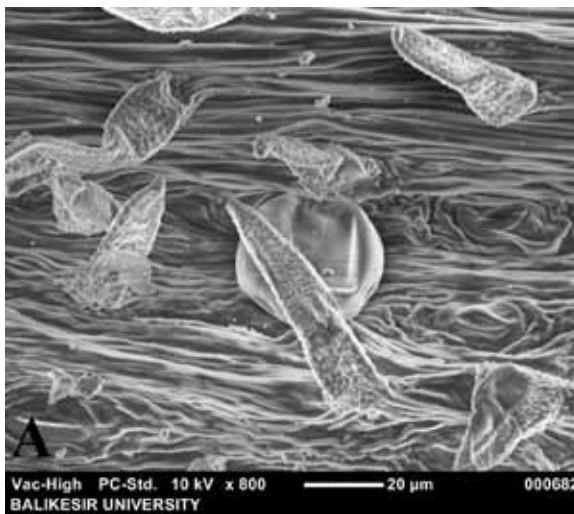


Figure 5: SEM photomicrographs of *Nepeta baytopii*'s stem. A: Glandular and eglandular trichomes, B: Eglandular trichomes.

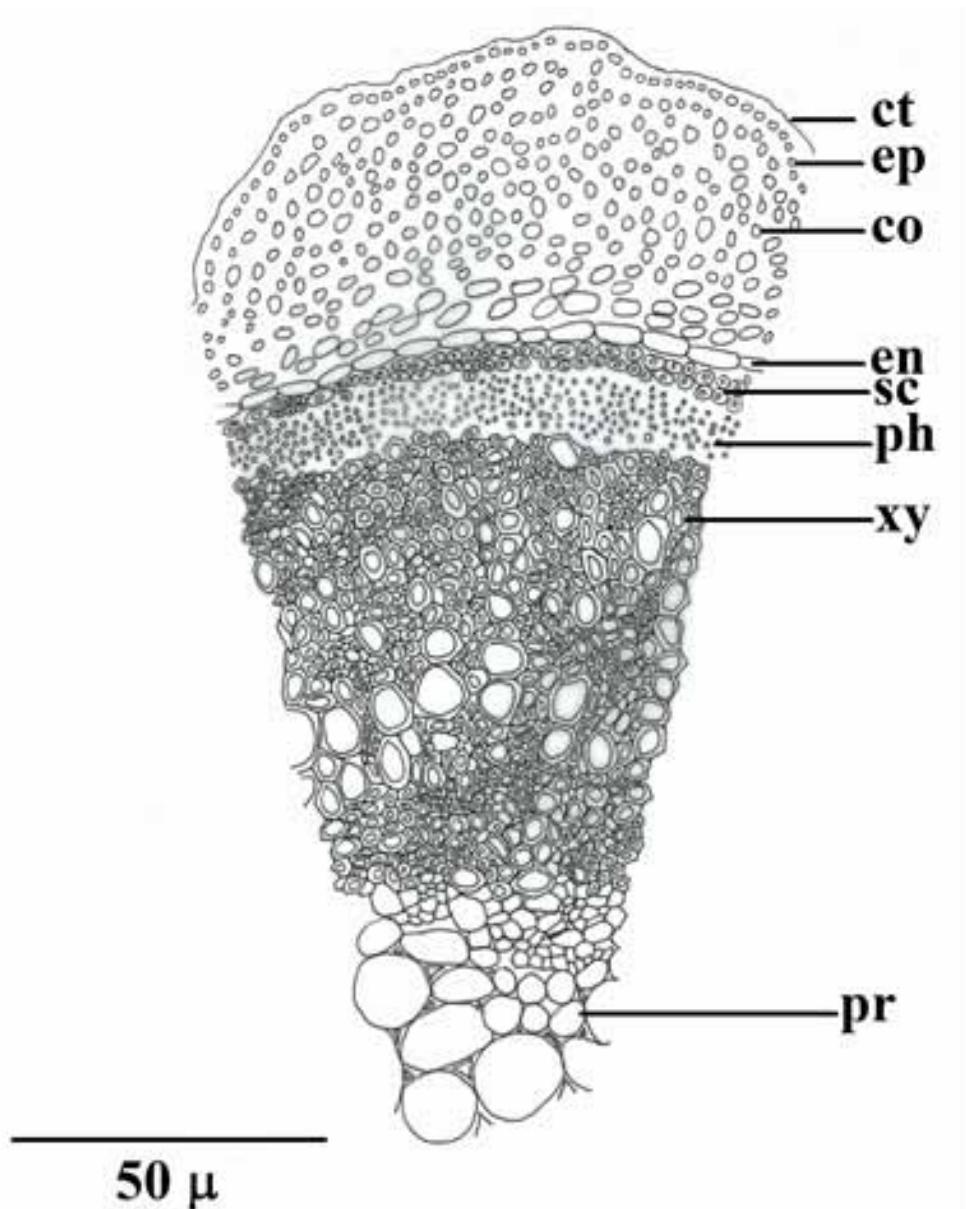


Figure 6: *Nepeta baytopii*. Cross-section of stem. (ct:cuticle, ep:epidermis, co:collenchyma, en:endoderma, sc: scleranchyma, ph: phloem, xy: xylem, pr:parenchyma).

Nepeta sorgerae

Epidermis is a single-layered with rough cuticle which is the most outer layer. There are two types trichomes (glandular and eglandular trichomes) (Figure 7, 8). 1-4-cellular eglandular trichomes (especially 1-2-cellular) are densely. Three types of capitate glandular trichomes: 1) Unicellular head and bicellular stalk, 2) Bicellular head and unicellular stalk, 3) Unicellular head and unicellular stalk (Figure 16B-b, c, d). And there are peltate type glandular trichomes. The collenchyma tissue, which is located immediately under the epidermis, is 5-8-layered on the corners. Under collenchyma, parenchyma tissue, which is 1-2-layered in the corners and 4-5-layered in between the corners, is composed of irregular shape cells. Singled-layer endoderma under parenchyma tissue is composed of usually rectangeled. Scleranchymatic pericycle tissue is 1-5-layered and is only under alignment of stem's corner. The phloem which is under the pericycle is 5-7-layered. There is xylem under the phloem tissue (Figure 9 and Table 1).

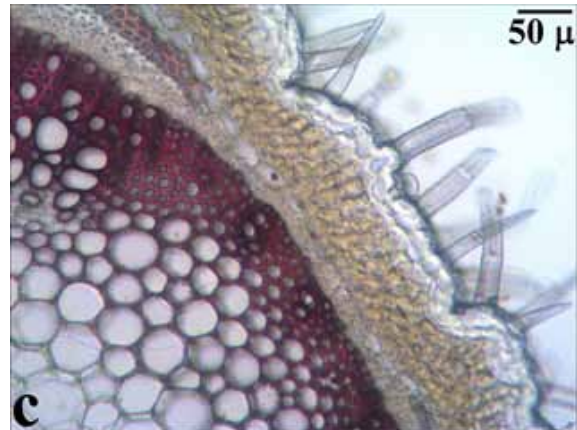
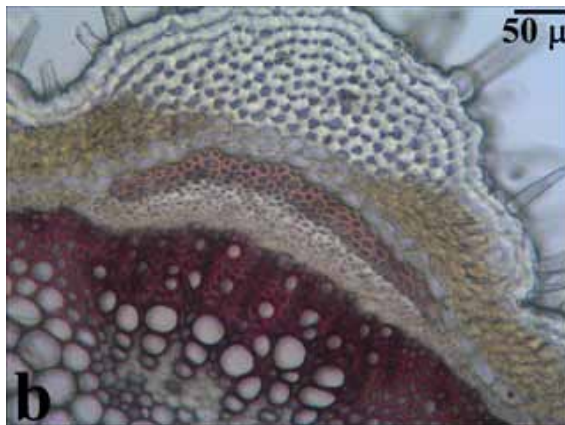
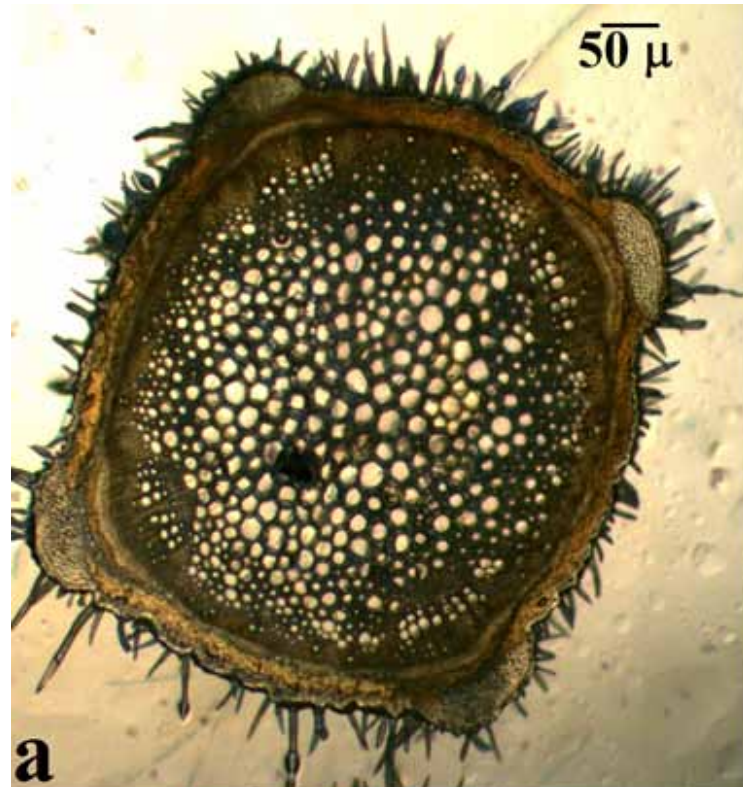


Figure 7: *Nepeta sorgerae*. Cross-section of stem. a: General appearance of stem, b: The corner of stem, c: The region of between the corners.

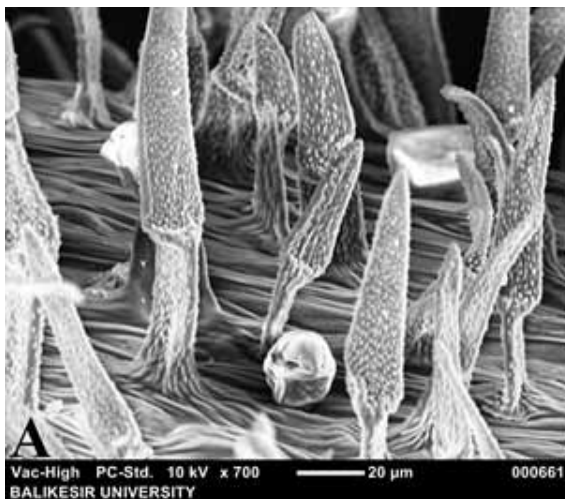


Figure 8: SEM photomicrographs of *Nepeta sorgerae*'s stem. A and B: Glandular and eglandular trichomes.

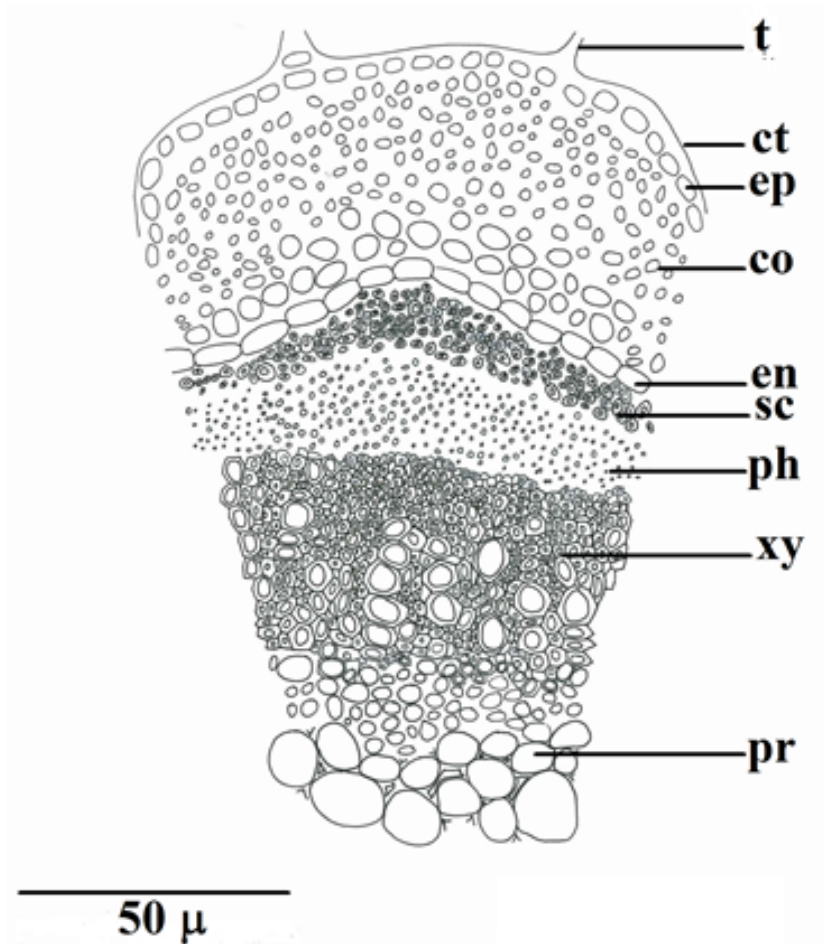


Figure 9: Cross-Section of stem in *Nepeta sorgerae* (t:trichome, ct:cuticle, ep:epidermis, co:collenchyma, en:endoderma, sc: scleranchyma, ph: phloem, xy: xylem, pr: parenchyma).

3.2.2 Leaf anatomy

Cross-sections taken from the leaves were observed as follows.

Nepeta baytopii

Both epidermises adaxial and abaxial of the leaf are covered with a thin cuticle. There are glandular and eglandular trichomes (Figure 10, 11). Eglandular trichomes are 1-5-cellular (Figure 16A). 2-3-cellular trichomes are more densely. Glandular trichomes are two types: Capitate and peltate. The capitate types are unicellular head and bicellular stalk or unicellular head and unicelleular stalk (Figure 16A- a, b, c). Stomata type is hygromorphic. In the midrib, there are 4-5-seriate collenchymatous cells under the upper and lower epidermis. The xylem faces towards the upper surface while the phloem faces the lower epidermis. Phloem tissue is under xylem and it is 5-7-seriate. 3-4-seriate scleranchymatic tissue is under phloem layer. Scleranchymatic tissue and ensuing 6-8-seriate collenchymatic tissue make up center vein of a leaf's denticulate region. The mesophyll is differentiated into an elongated 3-5-seriate spongy parenchyma and 2-seriate palisade tissue. Spongy parenchyma is under the palisade tissue (Figure 12 and Table 2).

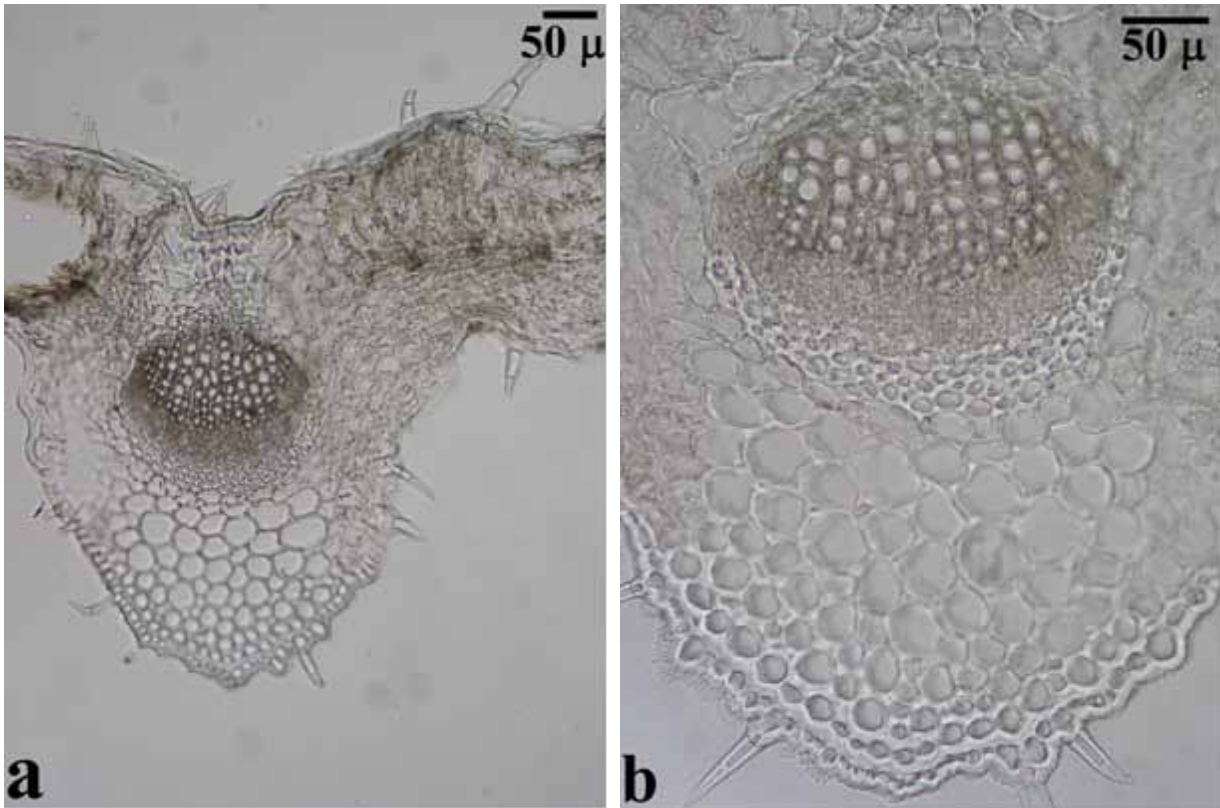


Figure 10: *Nepeta baytopii*. Cross-section of leaf. a: General appearance of leaf, b: Midrib region.

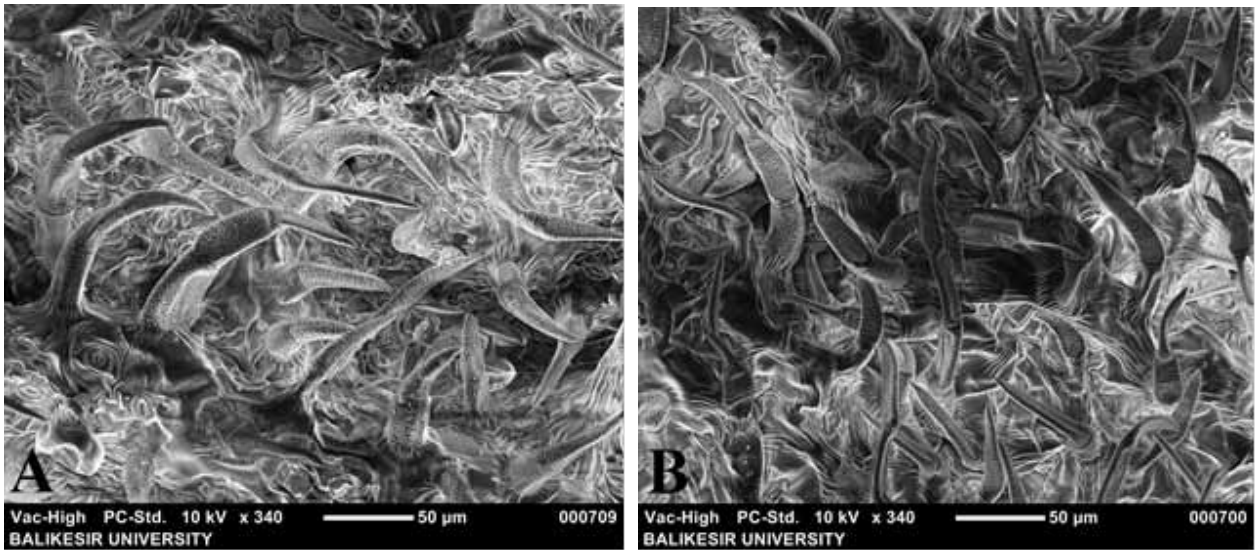


Figure 11: SEM photomicrographs of *Nepeta baytopii*'s leaf. A: The abaxial of leaf, B: The adaxial of leaf.

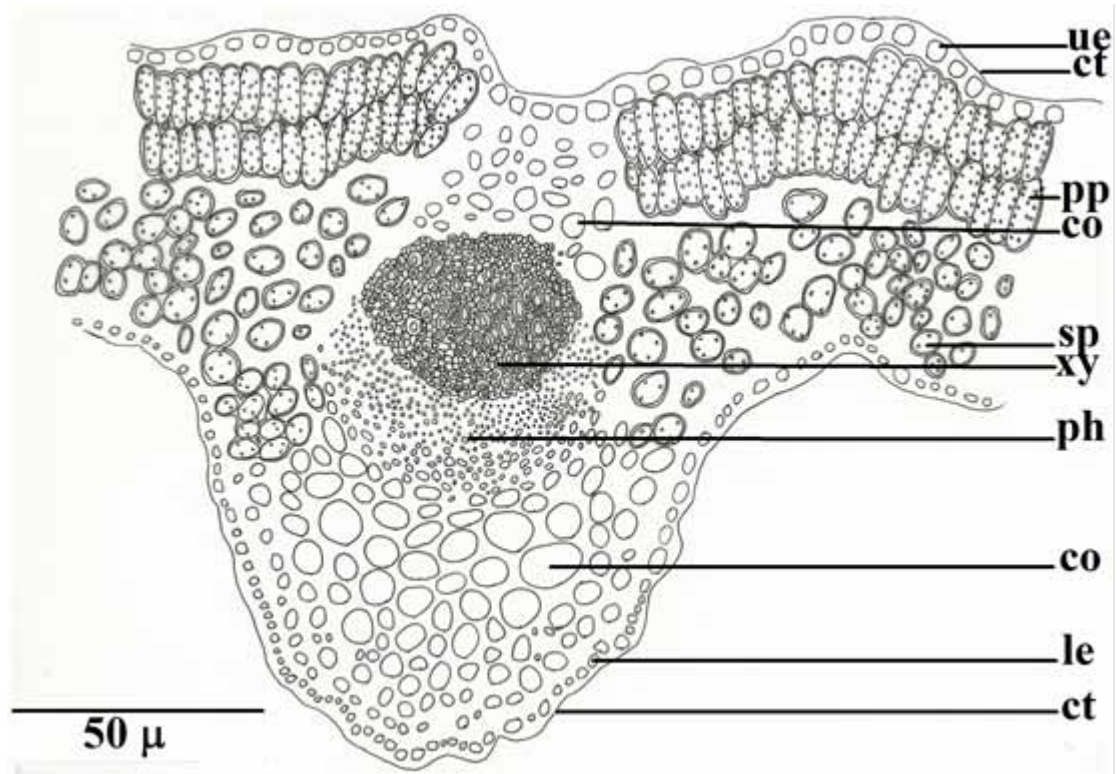


Figure 12: Cross-section of leaf in *Nepeta baytopii* (ue: upper epidermis, pp: palisate parenchyma, sp: spongy parenchyma, xy: xylem, ph: phloem, co: collenchyma, le: lower epidermis, ct: cuticle).

Nepeta sorgerae

Both epidermises adaxial and abaxial of the leaf are covered with a thin cuticle. Glandular and eglandular trichomes are seen between epidermis cells (Figure 13, 14). Eglandular trichomes are 1-5(-8)-cellular (Figure 16B). 1-2-cellular trichomes are more abundant. Glandular trichomes are two types: Capitata and peltate. The capitata trichomes are three types. These are unicellular head and bicellular stalk, unicellular head and unicellular stalk or bicellular head and unicellular stalk (16-B, b, c, d). 4-6-seriate collenchymatic tissue is in the midrib region upper and lower epidermises. Xylem tissue is under the collenchyma. Phloem tissue is under xylem and it is 5-7-seriate. 3-4-seriate Scleranchymatic tissue is under phloem layer. Scleranchymatic tissue and ensuing 6-8-seriate collenchymatic tissue make up center vein of a leaf's denticulate region. The mesophyll is differentiated into an elongated 3-5-seriate spongy parenchyma and 2-seriate palisade tissue. Spongy parenchyma is under the palisade tissue (Figure 15 and Table 2).

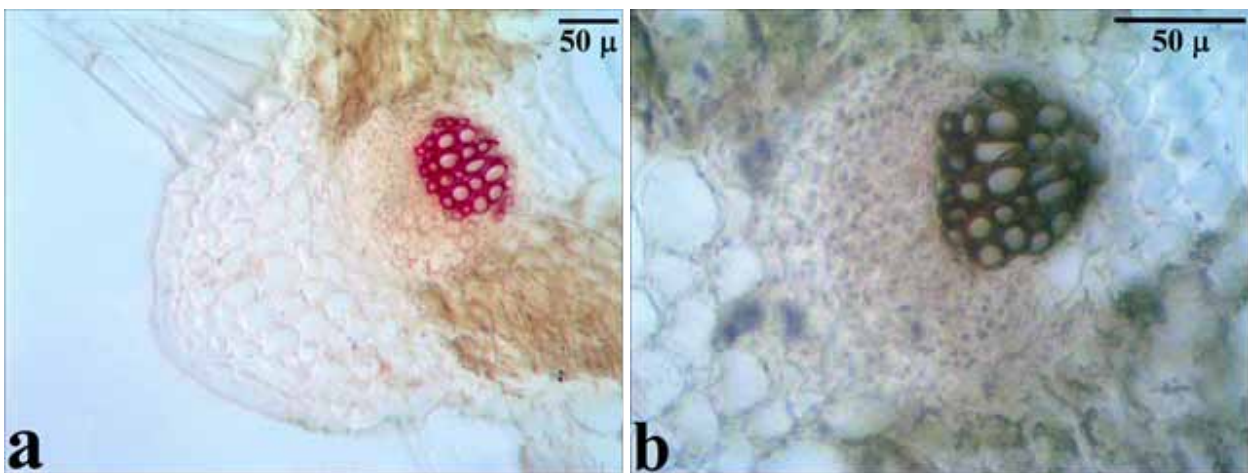


Figure 13: *Nepeta sorgerae*. Cross-section of leaf. a: General appearance of leaf, b: Midrib region.

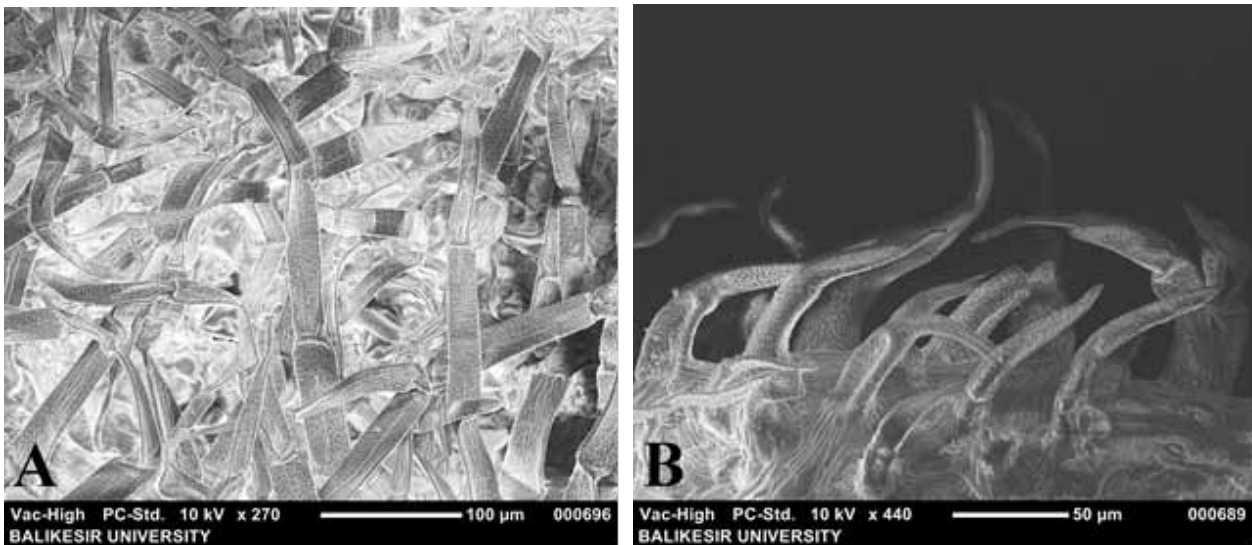


Figure 14: SEM photomicrographs of *Nepeta sorgerae*'s leaf. A: The abaxial of leaf, B: The adaxial of leaf.

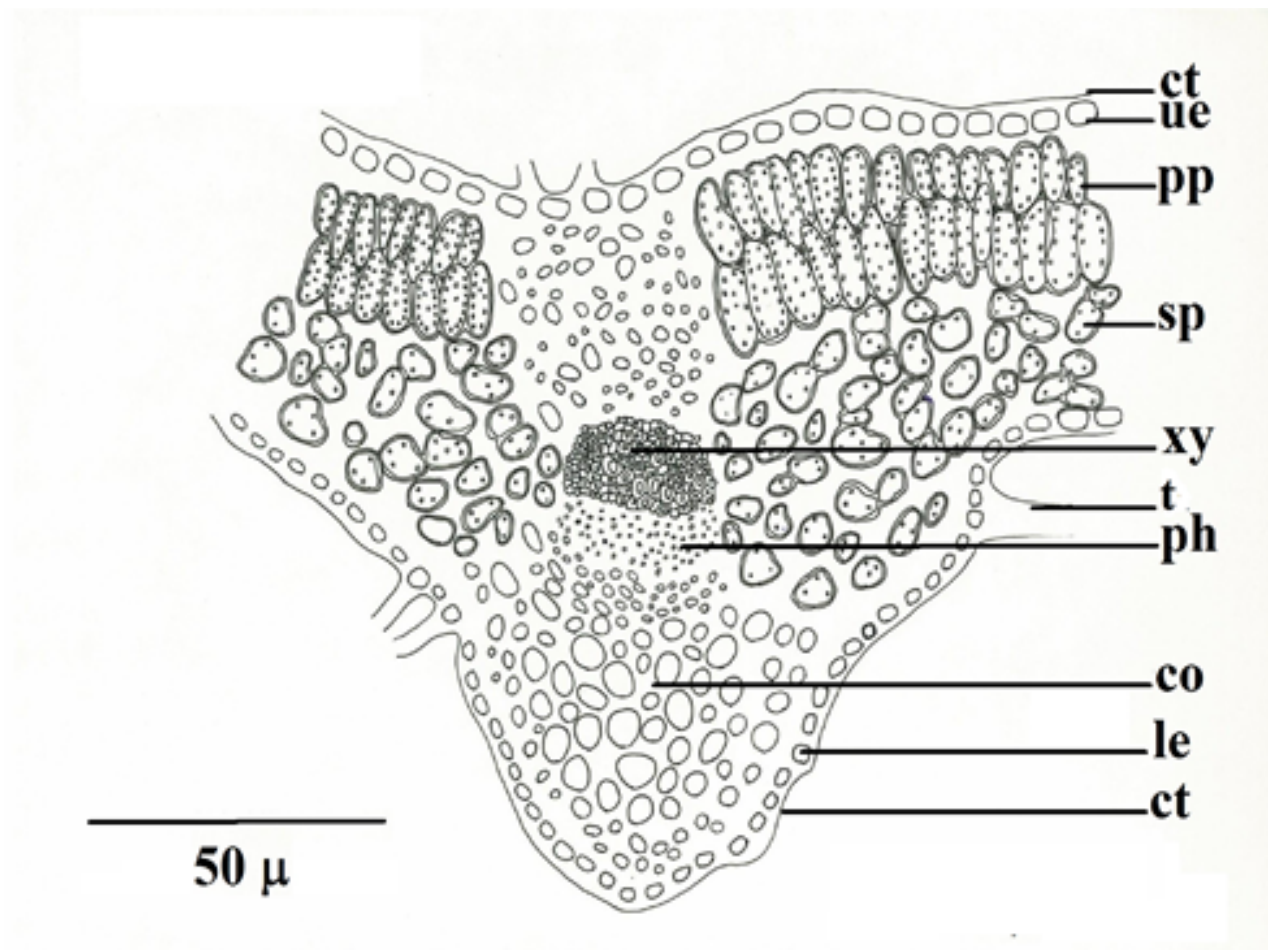


Figure 15: Cross-section of leaf in *Nepeta sorgerae* (ue: upper epidermis, pp: palisate parenchyma, sp: spongy parenchyma, xy: xylem, t: trichome, ph: phloem, co: collenchyma, le: lower epidermis, ct: cuticle).

4. Discussion

4.1 Stem

Stems anatomy of the investigated species were compared. And that is shown, The first ostensible difference is their trichomes cover. Also, scleranchymatic cells are 1-2-seriate in *N. baytopii* but 1-5-seriate in *N. sorgerae*.

Table 1. The comparison of stem anatomical characteristics of studied *Nepeta baytopii* and *Nepeta sorgerae*

Stem	Eglandular trichome	Glandular trichome		Schlerenchymatic pericycle	Phloem
		Capitate	Peltate		
<i>N. baytopii</i>	1-4 cellular 1-2 cellular denser	1. Unicellular head and 1-2 cellular stalk	+	1-2-seriate	6-8-seriate
<i>N. sorgerae</i>	1-6 cellular, 1-2 cellular denser	1. Unicellular head and bicellular stalk 2. Bicellular head and unicellular stalk 3. Unicellular head and unicellular stalk	+	1-5-seriate	5-8-seriate

Also, our investigation is compared with Kaya et al.'s (1991), 1-2-cellular eglandular trichomes of *N. baytopii* and *N. sorgerae* are densely but in *N. congesta* Fisch et Mey. var. *congesta* Fisch et Mey. and the others (*N. italica* L., *N. nuda* L. subsp. *nuda*, *N. stricta* (Banks et Sol.) Hedge et Lamond. var. *stricta* (Banks et Sol.) Hedge et Lamond) 1-3-cellular eglandular trichomes are denser. Glandular trichomes are almost same properties in all species.

Also, Kaya et al. (1991) reported that 1-3-seriate scleranchymatic cells and 7-11-seriate phloem tissue in their investigated species. Unlike the other species, scleranchymatic cells 1-2-seriate in *N. baytopii* and 1-5-seriate *N. sorgerae*. As phloem tissue is almost same each other of our species, it is more seriate layer in the other taxa. Kaya et al. (1991) found 9-12-seriate collenchyma layer on the corners in their investigations but in our examination it is shown that, *N. baytopii* and *N. sorgerae* have 5-8-seriate collenchyma layer.

4.2 Leaf

The adaxial epidermis cells are bigger than the abaxial epidermis cells. The stomata type is diacytic and stoma cells are hygromorphic. Eglandular trichomes density is different between two species. As 2-3-celled-trichomes are denser on *N. baytopii*, 1-2-celled-trichomes are denser on *N. sorgerae*. Parenchymatic tissue of these two species is almost same.

Table 2. The comparison of leaf anatomical characteristics of studied *Nepeta* species

Leaf	Eglandular trichome	Glandular trichome		Palisate parenchyma	Spongy parenchyma
		Capitate	Peltate		
<i>N. baytopii</i>	1-5 cellular 2-3 cellular denser	1. Unicellular head and unicellular stalk 2. unicellular head and bicellular stalk	+	2-seriate	3-5-seriate
<i>N. sorgerae</i>	1-5(-8) cellular, 2-3 cellular denser	1. Unicellular head and unicellular stalk 2. Unicellular head and bicellular stalk 3. Bicellular head and unicellular stalk.	+	2-seriate	3-5-seriate

Kaya et al. (1991) investigated *N. italica*, *N. nuda* subsp. *nuda*, *N. congesta* var. *congesta*, *N. stricta* var. *stricta* taxa and there were no differences between these taxa anatomically. In our investigation, there are some differences between *N. baytopii* and *N. sorgerae*'s anatomic structure. In point of number of palisate and spongy parenchyma layer, some differences were determined between our investigated species and Kaya et al. (1991) investigated taxa. As palisate parenchyma is 2-serried in *N. baytopii* and *N. sorgerae*, this tissue is 3-serried in *N. congesta* var. *congesta* and the other taxa. As seen that, spongy parenchyma is 3-5-serried in our investigated species, but uniserried in *N. congesta* and the other species. 1-2-celled eglandular trichomes are denser in *N. baytopii* and *N. sorgerae* when compared with *N. congesta* var. *congesta*. Stomata level is upper than epidermis level on all taxa (hygromorf type).

4.3 Stem and Leaf Trichomes

Glandular trichomes are important taxonomic characters in Lamiaceae (Cantino, 1990; Navarro and El Oualidi, 2000). The trichome types in the stems and leaves of each species are shown in Figure 16. They are generally more common on the abaxial than the adaxial surface leaves. On the stems of each species, 1-2-celled trichomes are the most frequent trichomes type. On the leaves of each species, 2-3-celled trichomes are the most frequent type.

Obviously, the density of trichome distribution in *N. baytopii* is less than in *N. sorgerae*. Also, only 8-celled eglandular trichomes (Figure 16-B,e) and 6-celled eglandular trichomes (Figure 16-B,t) were encountered in *N. sorgerae*. Both of the two species share the same types of glandular trichomes but different types of glandular trichomes were encountered in *N. sorgerae* (Table 1 and Table 2).

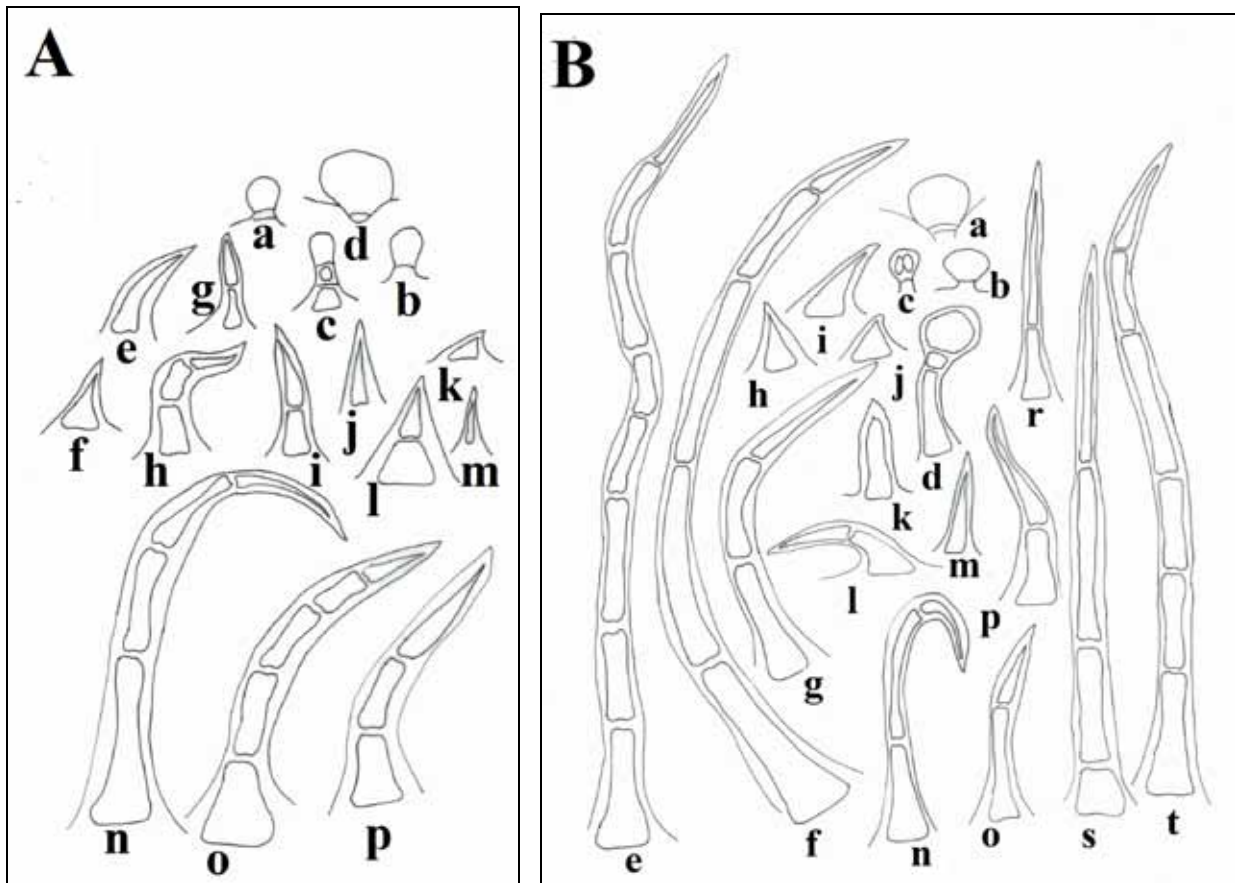


Figure 16: Stem and leaf eglandular and glandular trichomes. A: *Nepeta baytopii*, B: *Nepeta sorgerae*.

With this all observed properties, *N. baytopii* and *N. sorgerae* have general properties of Lamiaceae family and the genus, but parenchyma, phloem, scleranchyma layer and trichome structure and density in stems and leaves show some differences because of enviromental condition, climate, temperature, insolation and soil properties. Proximity degree and properties of the species will put forth better with more detailed investigations in the future.

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