

## A new *Teucrium* L. (Lamiaceae) Species from South Anatolia (Turkey)

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**Abstract:** In this study, a member of sect. *Teucrium*, hitherto undescribed as a new species, was analyzed based on morphological and micromorphological features and molecular findings. The closest species of the new species were defined according to leaf morphology via morphological characters. Leaf, stem, and nutlet trichome types were obtained and compared with resembling species. Moreover, two different DNA region sequences were used to evaluate its proper phylogenetic position in the section. The new species was described as *Teucrium turcicum* Çeçen & Özcan based on the results of using different approaches. According to our observations, flower color, leaf structure, and shape were the main distinctive morphological characters, leaf and nutlet trichomes were separative and informative, and nrITS DNA data had more informative characters than chloroplast DNA region data (*trnL-F* and *rpl32*) to determine phylogenetic relationship of the new species and its close relatives in sect. *Teucrium*.

**Key words:** *Teucrium*, new species, nrITS, *trnL-F*, trichome, Turkey

### 1. Introduction

Lamiaceae is a very important plant family and includes many medicinal uses and is well-known throughout the world. According to Harley et al. (2004), the Lamiaceae family is subdivided into seven subfamilies: Ajugoideae, Lamioideae, Nepetoideae, Prostantheroideae, Scutellarioideae, Symphorematoideae and Viticoideae. Cymarioideae, Peronematoideae, Premnoideae, Callicarpoideae and Tectonoideae subfamilies have also been described in recent studies (Li et al., 2016; Li and Olmstead, 2017). Turkey is one of the important countries where this family is widely distributed. Furthermore, members of Ajugoideae, Lamioideae, Nepetoideae, Scutellarioideae and Viticoideae subfamilies also grow in Turkey (Harley et al., 2004; Li et al., 2016; Li and Olmstead, 2017). Southern Anatolia is considered one of the 10 main hot spots in the world (Medail and Diadema, 2009), and current studies of new species published in recent years support this richness (Bona, 2016; Deniz, et al., 2016; Tugay and Ulukuş, 2018; Akalın et al., 2020; Aksoy et al., 2020; Aytaç et al., 2020; Celep et al. 2020; Demirelma, 2020; Dinç and Doğu, 2020; Dirmenci et al., 2020; Şirin et al., 2020). Ajugoideae includes the genus *Teucrium* L., and *Teucrium* is a cosmopolitan genus with about 250–300 species worldwide. Although the main distributing area of the genus is the Mediterranean region, it also spreads

in the temperate regions of Europe, North Africa, and Asia (Cantino et al., 1992; Harley et al., 2004; Navarro and El Oualidi, 2000; Salmaki et al., 2016; Navarro, 2020). *Teucrium* is one of the characteristic genera in the Lamiaceae family with its corolla structure. This cosmopolitan genus is represented by 37 species (49 taxa) distributed throughout Turkey, and the endemism ratio is over 36% according to recent studies (Dirmenci, 2012; Özcan et al., 2015; Vural et al., 2015; Dinç and Doğu, 2012; Dinç and Doğu, 2016; Aksoy et al., 2020).

*Teucrium* is divided into 10 sections worldwide (Navarro and El Oualidi, 2000). On the other hand, there are eight sections distributed in Turkey except the sections *Pycnobotrys* Benth. and *Teucropsis* Benth. Calyx shape and structure, inflorescence, and verticillasters are the main distinctive and useful characters to identify the members of different sections (Bentham, 1834, 1876; Boissier, 1879; Briquet, 1895; Ekim, 1982). In addition to these older characters, trichome types of calyx, corolla, nutlet, leaf, stem and leaf fragmentation are used quite a lot in relevant studies to divide the genus *Teucrium* into the sections (Dinç et al., 2009, Dinç et al., 2011a, 2011b; Ecevit-Genç et al., 2015, 2017, 2018a, 2018b, Özcan, 2020). In addition, micromorphological characters of *Teucrium*, especially trichomes, have been used in some recent studies, and according to these studies trichome types and density are

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especially important in the infrageneric classification of the genus (Navarro and El Oualidi, 2000; Grubestic et al., 2007; Dinç et al., 2008, 2009, 2011a, 2011b; Eshratifar et al., 2011; Dinç and Doğu, 2012; Kremer et al., 2012; Doğu et al., 2013).

*Teucrium* sect. *Teucrium* is the largest section with 15 taxa (6 endemics) present in Turkey (Parolly and Eren, 2007; Dinç and Doğu, 2016; Aksoy et al., 2020), and all six endemic species are distributed in the western part of Mediterranean (Aksoy et al., 2020). Not gibbous calyces and almost equal calyx teeth, deeply dissected leaves (*Orientalis* Group) and revolute margins beneath can be used to distinguish the members of sect. *Teucrium* from others.

On a field trip in Anamur district, İçel province, southern of Turkey, some *Teucrium* specimens were collected to identify and noticed to be different in corolla and anther color, and general shape and trichome density of leaves from other specimens presented in the literature (Figure 1). After using different approaches (morphological, micromorphological, and molecular studies) in detail, these collected specimens were identified as a new species belonging to sect. *Teucrium*, the analyses revealed that the new species is more closely related to *Teucrium pseudaroanum* Parolly, Erdag & Nordt and *T. alyssifolium* Stapf.

The aim of the present study is to answer following research questions: What are the close relatives of the species that to be a potentially new one for the science?, What characters similar/difference are there between

the new species and its allies?, What information does morphological, micromorphological and molecular data give about the new species? The answers to these important questions enable the new species to be introduced to the science in the light of morphological, micromorphological and molecular data. Moreover, drawings and a distribution map of the new species is also presented.

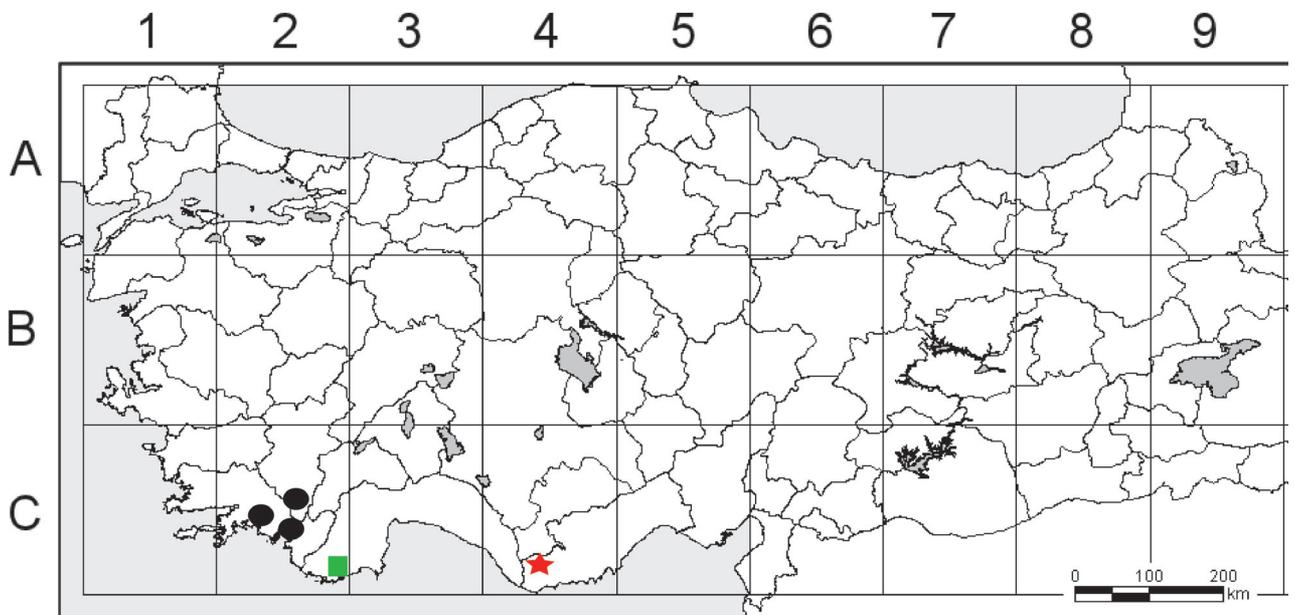
## 2. Materials and methods

### 2.1. Plant materials

Specimens were collected from the Anamur district (around Çukurabanoz village), Mersin province in the south of Turkey by Ömer ÇEÇEN (Figure 1, 2). The specimens were identified using the relevant literature (Ekim, 1982; Duman, 2000; Dirmenci, 2012; Seregin et al., 2018; Özhatay et al., 2019, Aksoy et al., 2020) and compared with materials stored in herbaria ANK, B, BM, E, EGE, GAZI, HUB, ISTE, ISTO, K, LE, MA, W, and WU (see <http://sweetgum.nybg.org/science/ih/> for the indicated acronyms), as well as with our own herbarium specimens.

### 2.1. Micromorphological studies

Trichome morphology of *Teucrium* species is useful to identify the differences of the new species and their allies according to some recent studies (Ecevit-Genç et al., 2015; Dinç and Doğu, 2016; Aksoy et al., 2020). Trichome types presented by Navarro and El Oualidi (2000) were used in this study, and trichome structure of the new species and its closest allies *T. pseudaroanum* and *T. alyssifolium* was



**Figure 1.** Distribution map of the new species *Teucrium turcicum* (★) and its allied species, *T. pseudaroanum* (■) and *T. alyssifolium* (●), in Turkey.



**Figure 2.** Habitat of *Teucrium turcicum* (indicated by arrows) (photo by Ömer ÇEÇEN).

studied using a tabletop SEM. Stems, leaves and nutlets were investigated and photographed using a scanning electron microscope for SEM analysis. For this purpose, individuals with distinguishing characters that represent the species were studied (at least 3 different samples were prepared for analysis). All the samples were mounted on stubs and coated with gold particles. Hence, they were made ready to study with FEI Quanta 450 FEG-EDS scanning electron microscope.

## 2.2. DNA extraction

DNA plant mini kit (Qiagen, Germany) was used to extract total genomic DNAs (nucleus, chloroplast, and mitochondria). Dried leaf pieces on silica drains were firstly preferred for extractions. Manufacturer's instructions recommended for the kit were conducted with some small modifications such as more incubation time and more RNase addition. Two different *T. turcicum* specimens, one *T. pseudaroanum*, and one *T. alyssifolium* specimens were extracted for the first time in this study (marked with "\*" on phylogenetic tree).

## 2.3. Polymerase chain reaction (PCR) amplification

The internal transcribed spacer (ITS) region from nuclear ribosomal DNA (nrDNA) and *trnL-F* and *rpl32-trnL* regions from chloroplast DNA genome were amplified to see the phylogenetic relationship of the new species and its closest species. Universal primers were used to perform PCR amplifications. For this purpose, ITS5A (5'-CCTTATCATTTAGAGGAAGGAG-3') (Stanford et al., 2000) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) primers for nrITS region, *trnL-c* (5'-CGAAATCGGTAGACGCTACG-3') (Taberlet et al., 1991) and *trnL-f* (5'-ATTTGAAGTGGTGACACGAG-3')

(Taberlet et al., 1991) primers for *trnL-F* region, and *rpl32-F* (5'-CAGTTCCAAAAAACGTACTTC-3') (Shaw et al., 2007) and *trnL*<sup>(UAG)</sup> (5'-CTGCTTCCTAAGAGCAGCGT-3') (Shaw et al., 2007) primers for *rpl32-trnL* region were used. All PCR amplifications were conducted using the PCR program presented by Shaw et al. (2007).

## 2.4. DNA sequencing and phylogenetic analysis

PCR products that have been properly reproduced were sent to Genoks Company (Turkey) for purifying and sequencing DNA nucleotides. Raw DNA sequences files taken from the company were edited to remove the low-quality parts using BioEdit 7.2.5 (Hall, 1999). After cutting some ambiguous nucleotides from the 5' end and the 3' end, DNA sequences of different species were aligned using BioEdit and CLUSTAL W2 online software (Larkin et al., 2007). The aligned sequences were saved in nexus format using Mesquite 3.61 (Maddison & Maddison, 2018) applying default options. The best substitution model was analyzed in jModelTest 2.1.7 (Darriba et al., 2012). The nexus files were edited to obtain the best phylograms based on jModelTest outputs. PAUP\* software (4.0a165) (Swofford, 2003) were used to construct ITS and *trnL-F* cladograms with uploading the edited nexus file. Parsimony and likelihood analysis of nrITS and *trnL-F* sequences were examined via Bootstrap/Jackknife search. Bootstrap analysis were adjusted to 20,000 replications. Moreover, the sequences saturation of *rpl32* data was analyzed and multiple correspondence analysis (MCA) scatter plot was obtained using DAMBE7 (Xia, 2018). All the DNA sequences edited in this study were deposited in the GenBank with their accession numbers (Appendix).

### 3. Results

#### 3.1. Taxonomic information

*Teucrium turcicum* Çeçen & Özcan **sp. nov.** (Figure 2, 3, 4C, 4H, 4I, 5C, 5F, 5I, 5L)

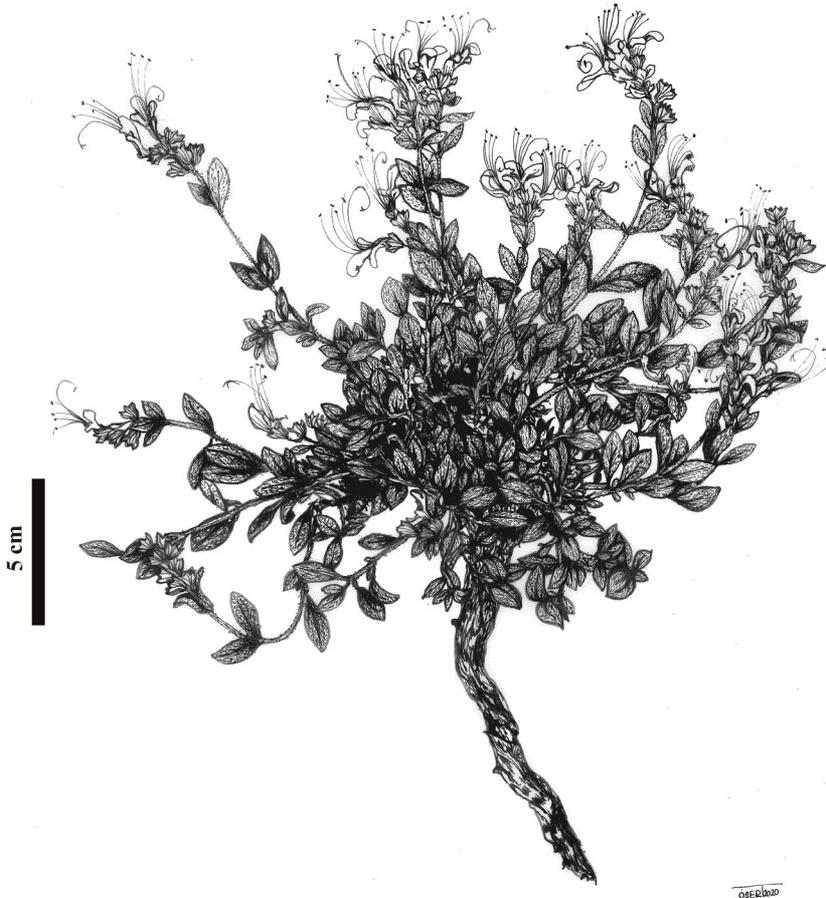
**Type:** Turkey. Mersin: Anamur, 13th km from Abanoz village to Boğuntu village, around Çukurabanoz village, eastern slopes of calcareous rocky areas, 650–905 m, 12 May 2020, Çeçen 6012. (holo. GAZI, iso. ANK, HUB, KNYA).

**Paratype:** Turkey. Mersin: Anamur, 13th km from Abanoz village to Boğuntu village, around Çukurabanoz village, eastern slopes of calcareous rocky areas, 650–905 m, 16 June 2020, Çeçen 6349. (GAZI, ANK, HUB, KNYA).

**Diagnosis:** *Teucrium turcicum* is a characteristic member of sect. *Teucrium*. It has a close relationship with two endemic species (*T. alyssifolium* and *T. pseudaroanum*) growing in Turkey. *Teucrium turcicum* differs from *T. alyssifolium* with its stems 10–30 cm long (not 3.5–9 cm long), leaves villous above and woolly below, discolorous and obovate (not tomentose at both sides, concolorous and narrowly elliptic to lanceolate), inflorescences

6–10-flowered (not 2–6-flowered), calyx teeth broadly triangular and shorter than calyx tube (not triangular and spine-tipped and almost equal to tube), corollas whitish to creamy-yellow (not white to pale pinkish mauve), anthers purplish (not yellowish). It is similar to *T. pseudaroanum* but differs in its leaves, which are more hairy on upper side (not pilose) and obovate (not elliptic-broadly to lanceolate), calyx teeth broadly triangular, (narrowly triangular), corollas whitish to creamy-yellow (not light pinkish-lavender), anthers purplish (not yellowish-orange) (Table 1).

**Description:** Saxatile shrubs, 10–30 cm long and branched from the base, ascending, lower part of stem densely woolly and sparse villous from base to inflorescence. Leaves distinctly petiolate, petiole 2–7 mm long; leaves increase in size from base to inflorescence, lamina 10–30 × 4–14 mm, obovate, cuneate at base, crenate and slightly revolute at margins, acute to obtuse at apex, villous green above and woolly grey beneath; veins are visible in both sides. Inflorescence 5–8 cm long, 6–10-flowered, verticillasters 2-flowered, stalked



**Figure 3.** General appearance of *Teucrium turcicum* (collected by Ömer ÇEÇEN (ÖÇ 6012), drawn by Özer TÜRKÖĞLU).



**Figure 4.** General morphological characters of stem, flowers, and leaves of *Teucrium alyssifolium* (A,D,E), *T. pseudaroanum* (B, F, G), and *T. turcicum* (C, H, I).

glandular hairs. Bracts are similar to leaves in shape and hairy, 4–6 mm long. Pedicel 3–5 mm long. Calyx 5–8 mm long, campanulate, densely villous with sessile glands, with glandular papillae; teeth are 2.5–4mm long, broadly triangular, acute apex, ±reflexed. Corolla whitish to creamy-yellow, rarely with indistinct purple-striped, 18–22 mm long, longer than calyx; middle lobe of lower lip is bearded outside; style and filaments are exerted from corolla, filaments 20–24 mm long, glandular pubescent at the base of both, style equally stamens, bilobed. Nutlets obovoid, 1.5–2.5 mm long, brown, tuberculate, sessile glandular and villous in 1/3 upper part.

**Flowering and fruiting time:** May–June.

**Etymology:** The epithet of the new species refers to Turkey where the species is located.

**Proposed Turkish name:** “Türk acıyavşanı” (Menemen et al. 2016).

**Habitat and ecology:** The new species grows in calcareous rock crevices at the type locality include *Pinus brutia* Ten. var. *brutia* f. *brutia*, *Cedrus libani* A.Rich. var. *libani*, *Quercus trojana* P.B.Webb subsp. *trojana*, *Asynuema isauricum* Contandr., Quezel & Pamukç., *Hypericum pallens* Banks & Sol., *Iberis carnosus* Willd., *Teucrium polium* L. subsp. *polium*, *Onosma frutescens* Lam., *Lactuca tuberosa* Jacq., *Scutellaria diffusa* Benth., *Phlomis leucophracta* P.H.Davis & Hub.-Mor., *Convolvulus cantabrica* L., *Micromeria myrtifolia* Boiss. & Hohen., *Gladiolus atroviolaceus* Boiss., *Mixhauxia thyrsoides* Boiss. & Heldr., *Sideritis congesta* P.H.Davis & Hub.-Mor., *Centaureum erythraea* Rafn subsp. *turcicum* (Velen.) Melderis, *Dorycnium pentaphyllum* Scop. subsp. *haussknechtii* (Boiss.) Gams, *Helichrysum pamphylicum* P.H.Davis & Kubicha, *Thesium bergeri* Zucc., *Velezia rigida* L., *Cotinus coggygia* Scop.

**Distribution and conservation status:** *Teucrium turcicum* is an East Mediterranean element known only

from around the type location in south Turkey where its distribution area is less than 10 km<sup>2</sup>, and the number of mature individuals are 500. The new species was collected from a single location and recommended in CR: B2ab (i,iii) category with available data (IUCN, 2016).

**Species identification key of Sect. *Teucrium* in Turkey.** The latest identification key presented by Aksoy et al. (2020) is revised with addition of *T. turcicum*.

1. Leaves 2–3 pinnatisect; flowers born in spreading panicles
  2. Calyx teeth recurved to hooked at apex .... **pruinatum**
  2. Calyx teeth straight
    3. Corolla lavender-blue, 7.5–10 mm, clearly longer than calyx, to 2 × calyx ..... **orientale**
    3. Corolla dark blue, 6–6.5 mm, slightly longer than calyx ..... **parviflorum**
1. Leaves entire to 3-lobed; flowers in raceme
  4. All leaves and bracts entire
    5. Inflorescence in long lax bracteate raceme, more than 5 cm
      6. Plant shrub, 50–150 cm long; pedicel equal or shorter than calyx; corolla pinkish ..... **creticum**
      6. Plant suffruticose, 10–50 cm long; pedicel 1–3 × calyx; corolla lavender-blue ..... **sandrasicum**
    5. Inflorescence short raceme, shorter than 5 cm, flowers axillary in upper leaves
      7. Plant many woody branched and cushion forming, 30–110 cm long, calyx teeth obtuse, corolla whitish to whitish-blue ..... **brevifolium**
      7. Plants woody at base, not cushion forming, calyx teeth triangular
        8. Leaves concolorous, flat, adpressed-tomentose on both surfaces, almost sessile or short petiolate, calyx teeth narrowly triangular, corolla pink to pinkish-white .. ..... **alyssifolium**

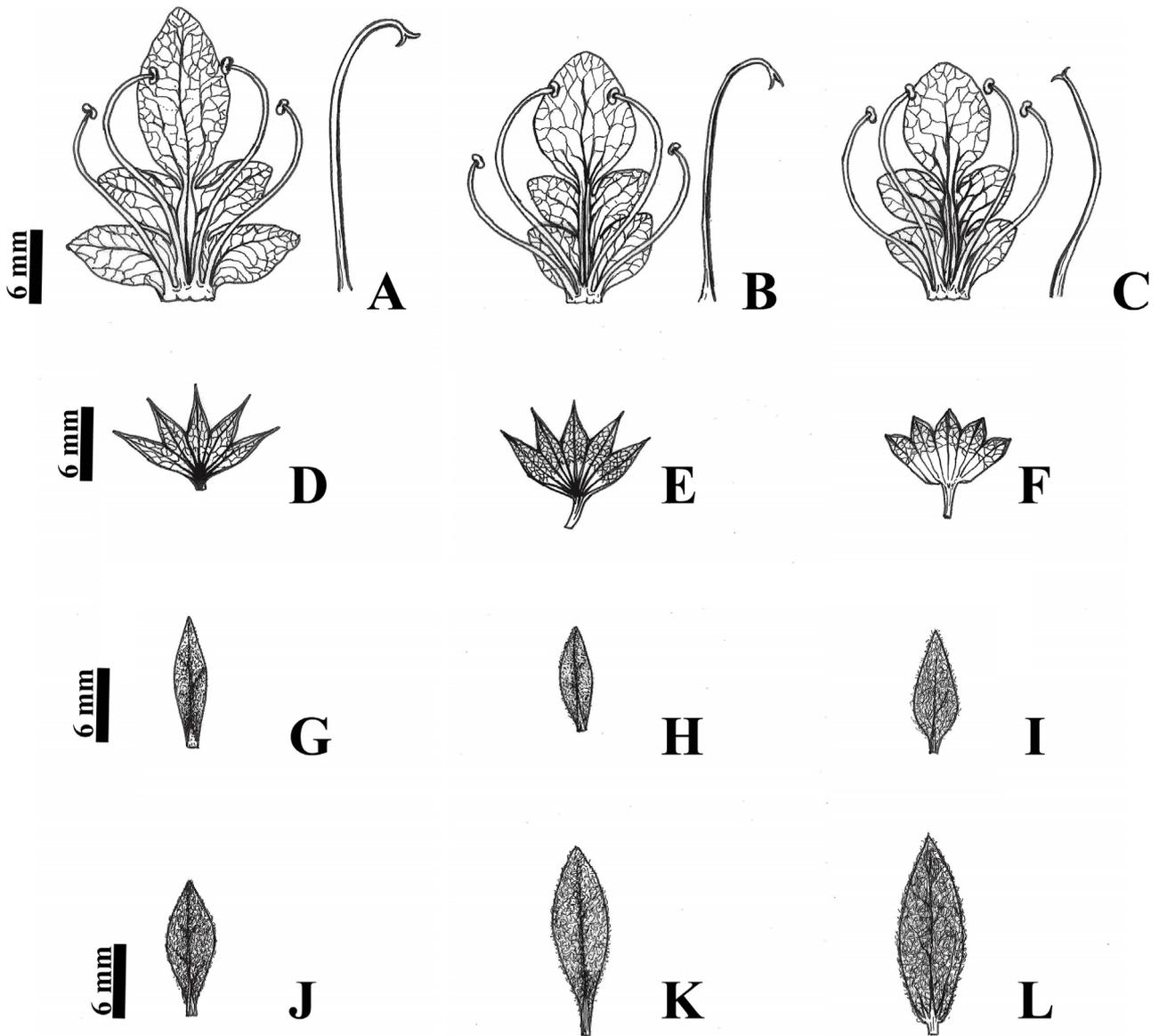


Figure 5. Flower characteristics (corolla: A, B, C; calyx: D, E, F), bract (G, H, I) and leaf shapes (J, K, L) of *Teucrium alyssifolium* (A, D, G, J), *T. pseudaroanum* (B, E, H, K), and *T. turcicum* (C, F, I, L).

8. Leaves discolorous, flat, strongly or slightly revolute at margins, obviously petiolate, calyx teeth triangular to broadly triangular, corolla whitish to pale pinkish

9. Calyx teeth mucronulate, corolla whitish to pale pinkish mauve, filaments 14–18 mm, anthers yellowish brown in flowering ..... **pseudaroanum**

9. Calyx teeth not mucronulate, corolla whitish to yellow-creamy, filaments 20–24 mm, anthers purplish in flowering ..... **turcicum**

4. Leaves and bracts entire to 3-lobed

10. Leaves and bracts mostly 3-lobed,

11. Plants densely glandular; leaves lobes obovate to obovate ..... **semrae**

11. Plants eglandular; leaves lobes linear to linear-lanceolate ..... **multicaule**

10. Leaves and bracts rarely 2–3 lobed

12. Stems robust, retrorsely pubescent or long hairy above; leaves linear-oblongate to linear, discolorous; corolla lavender blue ..... **pestalozzae**

12. Stems fragile, long villose and glandular above; leaves obovate to linear, slightly discolorous, corolla whitish blue ..... **ekimii**

### 3.2. Micromorphological results

In this study, trichome type classification presented by Navarro and El Oualidi (2000) was used to discuss the indumentum on stem, leaf and nutlet of the new species and

**Table 1.** Comparison of *Teucrium alyssifolium*, *T. pseudaroanium*, *T. turcicum* morphologically and micromorphologically.

Characters		<i>T. alyssifolium</i>	<i>T. pseudaroanium</i>	<i>T. turcicum</i>
Stem	structure	quadrangular, mostly ascending, 3.5–9 cm	slender, ascending to erect, 2–18 cm	slender, ascending to erect, 10–30 cm
	indumentum	densely tomentose with sessile glands	puberulent to white tomentose	densely white tomentose
	trichome types*	H, B (very rare)	I, B	H
Leaf	color	concolorous	discolorous	discolorous
	shape	lanceolate, elliptic-ovate	broadly lanceolate	obovate
	margin	entire	entire (mostly) to sinuate-crenate	entire to rarely crenate- involute
	base	attenuate	attenuate	cuneate
	size	4–28 × 2.5–7 mm	(4–)10–20(–25) × (2–) 5–10(–12) mm	5–30 mm × 2–14 mm
	adaxial indumentum	white adpressed-tomentose	arachnoid-lanate and green	arachnoid and green
	abaxial indumentum		densely white-tomentose to canescent	densely white-tomentosa
trichome types*	H, B, A1( rare) (adaxial-abaxial)	I, B (adaxial)	H, B (abaxial)	
Petiole		subsessile or under 1 mm	distinctly petiolate, (1.5–)2–5 mm	distinctly petiolate, 2–7 mm
Inflorescence		2–6(–8)-flowered, verticillasters 1-2-flowered	(2–)6–8(–12)-flowered, verticillasters 2-flowered	6-10 flowered, verticillasters 2- flowered,
Bracts		leaf-like, 2.0–20 × 1.5–5.0 mm	leaf-like	leaf-like, 4–6 mm
Pedicel		2–4 mm, adpressed-white-tomentose	3–4 mm, adpressed-white-tomentose	3–5 mm, adpressedglandular pubescens
Calyx	teeth shape	narrowly triangular, acute, stronglyspine-tipped	triangular to broadly triangular, acute, mucronulate by the excurrentvein	Broadly triangular, acutecurrent vein
	teeth size	2–4 mm, adpressed-white-tomentose	3–4 mm, adpressed-white-tomentose	2,5–4 mm, adpressed glandular pubescens
	size	6–13 × 4–5 mm	3.5–5 (-6) × mm	8 × 2.5 mm
Corolla	colour	light pinkish-lavender	white to pale pinkish mauve	white to yellow-creamy rarely purple striped
	size	20–25 mm	12–17 mm	18–22 mm
	tube	2 mm	1.5–2.5 mm	5–6 mm
	Lower lips	12(–17) mm	10–15(–18) mm	11–14 mm
Filament	length	18–22 mm	filaments arcuate, c. 14–18 mm	20–24 mm
	color	whitish-pink	whitish-pink, somewhat darker than corolla	whitish
	indumentum	glandular (1–2-celled clavate and sessile) and puberulent (1–3-celled)	glandular (1–2-celled clavate and sessile) and puberulent (1–3-celled)	glandular (1- celled)
Anthers		yellowish-brown, 0.8–1 mm	yellowish-brown(pinkish in flowering), 1-1.2 mm	yellowish brown (purpish in flowering)1.2 -1.5
Nutlet	shape	light brown, broadly elliptic-obovate, 2–2.7 × 1.2–3.1 mm, alveolate	light brown, obovate-oblong, 2.5–3.0 × 1.8–2, alveolate, alveolishallow	light brown, obovoid, 2.5×1.3 mm, alveolate
	indumentum	densely glandular and hairy in upper part (Parolly & Eren, 2007)	glandular and hairy in upper part	sessil glandular and villous in upper part.
	trichome types*	B, F2, F5 (rarely)	B, F2, F5	B, F2, F5 (rarely)

\*according to Navarro and El Oualidi J (2000) (Figure 8).

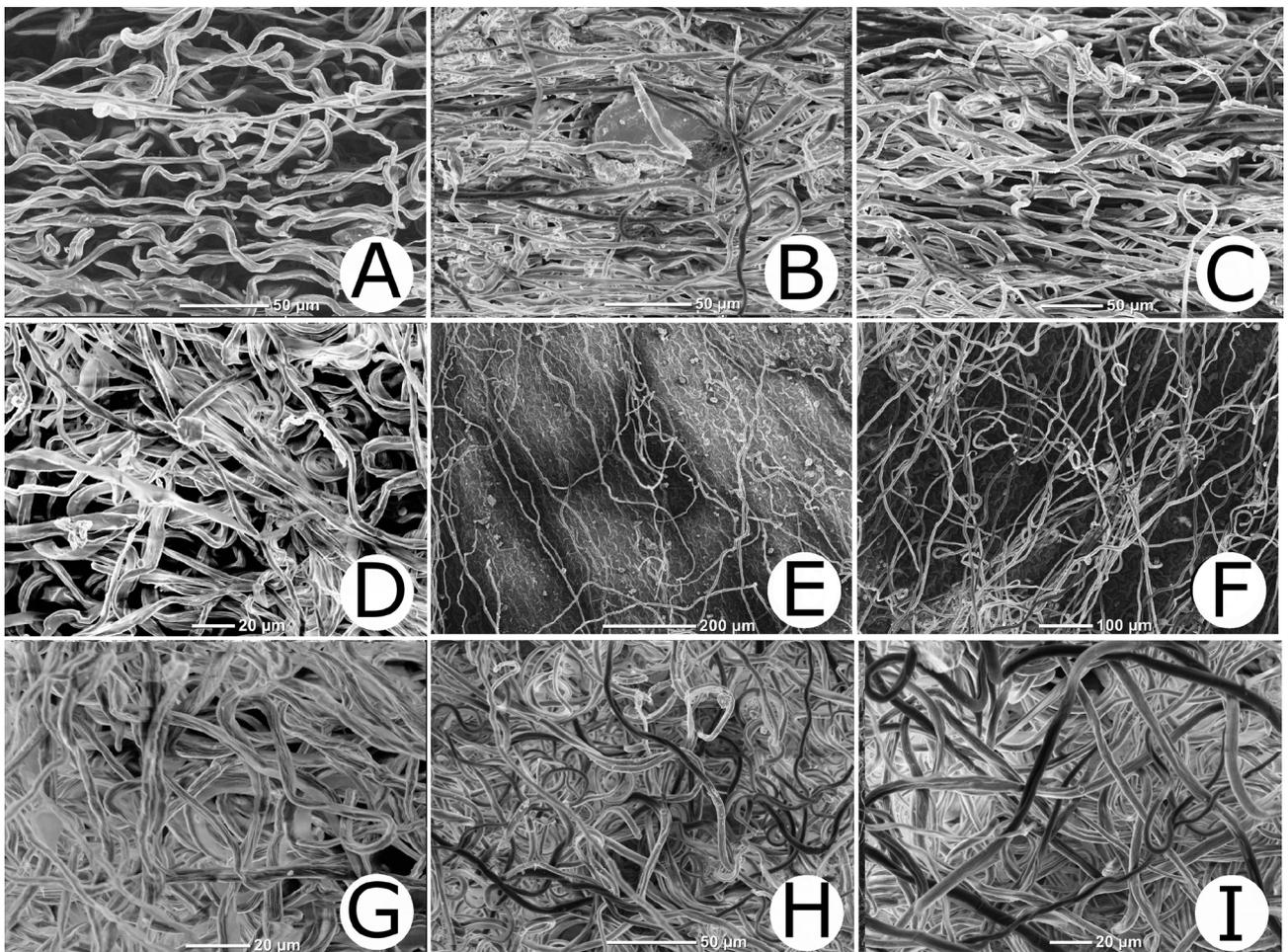
its allies. Since mature and proper nutlets of *T. alyssifolium* could not be obtained during the study, Ecevit-Genç et al.'s (2015) results were used to compare with the nutlet properties of *T. alyssifolium*, *T. pseudaroanium* and *T. turcicum*. On the other hand, stem and leaf trichomes were analyzed and compared with the previous literature (Parolly & Eren, 2007; Ecevit-Genç et al., 2015).

When stem indumentum types are analyzed, *T. alyssifolium* has the dense indumentum and *T. pseudaroanium* has the rarest. *T. alyssifolium* and *T. turcicum* have H type trichomes (Figures 6A, 6C, 6D, 6F, 8D) and *T. pseudaroanium* has I type trichomes (Figures 6B, 6E, 8C).

The most distinctive character is the indumentum types on adaxial and abaxial surfaces of the studied taxa. While the trichome density on both sides of *T. alyssifolium* is completely same (Figure 6A, 6D), adaxial and abaxial sides of *T. pseudaroanium* and *T. turcicum* are completely different (Figures 6B, 6C, 6E, 6F). H type

trichomes are densely located on adaxial and abaxial sides of *T. alyssifolium* (Figure 6A, 6D), and H type trichomes are also seen on both surfaces of *T. turcicum* (Figures 6C, 6F). On the other hand, I type trichomes are found on the both surfaces of *T. pseudaroanium* (Figures 6B, 6E). While adaxial surfaces are sparse, abaxial sides of *T. pseudaroanium* and *T. turcicum* are denser. So, leaves of *T. pseudaroanium* and *T. turcicum* are seen as discoloured unlike concolorous in *T. alyssifolium*. As seen in Figures 6B, 6E, 6C, 6F, *T. turcicum* has a denser indumentum than *T. pseudaroanium* on adaxial side of leaves.

Longitudinal ridges, which are a characteristic feature for distinguishing the species with entire leaves in the section, are also seen on nutlets of *T. turcicum*. B type (sessile glandular) trichomes distribute on the nutlets in the taxa studied (Figures 7A, 7B, 7C, 7D, 7E, 7F, 8F). Also, B type is the most general type present on the nutlets and leaves of three species. While B type trichomes can be observed more easily on the nutlets, these trichomes



**Figure 6.** Micromorphological characters of stem (A-C), and adaxial (D-F) and abaxial (G-I) sides of leaves of *T. alyssifolium* (A, D, G), *T. pseudaroanium* (B, E, H), and *T. turcicum* (C, F, I).

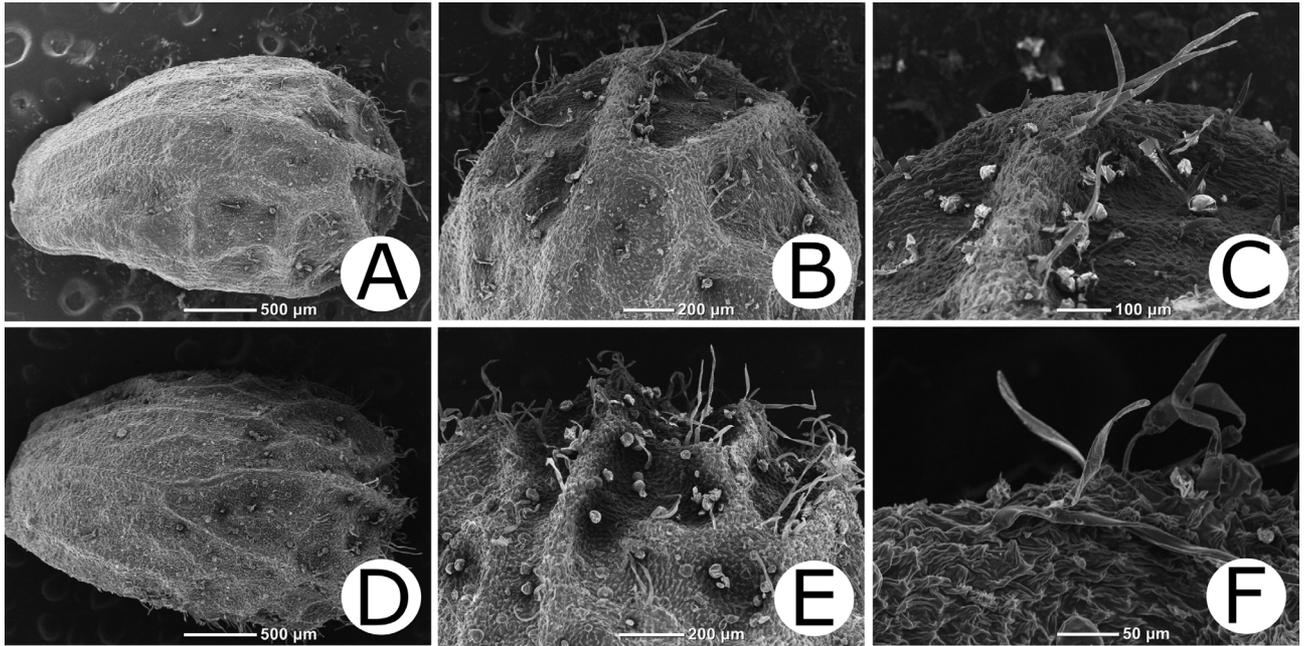


Figure 7. Micromorphological characters of nutlets of *Teucrium pseudaroanum* (A, B, C) and *T. turcicum* (D, E, F).

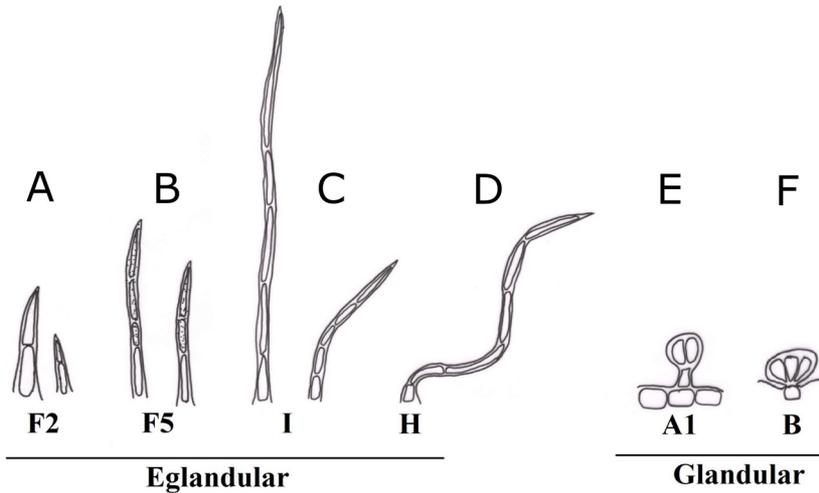


Figure 8. Trichome types of eglandular and glandular that observed in the studied taxa. Non glandular trichomes; A) F2. Large, very thin-walled, 2(-5)-celled trichomes with an acute apical cell, with ridges and marked internodes. B) F5. Elongated and flexuose, with thin-walled 3-7(-11)-celled trichomes, with distinct internodes, the apical cell acute with micropapillae, the basal cell smooth, each cell transverse to the preceding one. C) I: Vermiform hairs, thick-walled with elongated cells. D) H: Very long inter-twined trichomes, fibrous-like, sometimescoalescent. Glandular trichomes; E) A1-Short clavate glandular trichomes. Generally, with two, large and thin stalk cells. F) B: Subsessile glandular trichomes, peltate trichomes. (Navarro and El Oualidi, 2000).

are covered with eglandular trichomes due to the densely indumentum on abaxial sides of the leaves (Figures 6G, 6H, 6I). In addition, *T. turcicum* differs with its denser B type glandular and F5 type eglandular trichomes than *T. pseudaroanum* on their nutlets (Figure 8B, 8F).

Although *T. turcicum* is closer to *T. pseudaroanum* in terms of nutlet shape and trichome structure, it is more

similar to *T. allysifolium* in terms of leaf indumentum types (Figures 7A, 7B, 7C, 7D, 7E, 7F).

### 3.3. Molecular results

Three different regions were used to identify phylogenetic position of the new species in sect. *Teucrium*. Two different specimens from *T. turcicum*, and one specimen from *T. pseudaroanum* and *T. allysifolium* were examined

for the first time in this study (which marked with “\*” after their Latin names). Likelihood settings from best-fit model (GTR+I+G) selected by BIC (Bayesian information criterion) with jModeltest are based on nrITS sequences. nrITS results indicate that (Table 2), after alignment of 47 specimens, nrITS consisted of 675 characters, including 351 constant characters and 85 uninformative and 239 informative characters. After the analysis, tree scores were found to be as follows: Consistency index (CI): 0.541, retention index (RI): 0.696, and homoplasy index (HI): 0.475.

For *trnL-F*, likelihood settings from best-fit model (TVM+I) were selected by BIC with jModeltest. After alignment of *trnL-F* sequences belonging to 43 specimens, 942 characters were obtained. 735 characters were constant, 86 of the rest were uninformative, and 121 sequences were informative characters. These followings were the tree scores: Consistency index (CI): 0.805, retention index (RI): 0.805 and homoplasy index (HI): 195 (Table 2).

According to the DNA sequence analysis and phylograms, nrITS data had a better resolution and less polytomies to show phylogenetic relationship of the new species and its closest allies with other members of sect. *Teucrium*. As seen in Table 2, nrITS data is more useful with its more parsimony-informative characters than *trnL-F* data. Although *trnL-F* is not useful in distinguishing the intersectional species, it is very useful in separating the sections from each other (Figures 9–11).

*rpl32* sequences the data of third studied region in this study were not used to construct any phylogenetic tree due to lack of data (inner or outer group members). *rpl32* from chloroplast genome was also very useful to distinguish the new species and its allies (Table 3). Thirty-four nucleotide loci can be used to distinct *T. turcicum*, *T. pseudaroanium* and *T. alyssifolium* from some other sect. *Teucrium* members and outgroups. In addition, the nucleotides at 141., 330. and 440. positions differed between *T. turcicum* and *T. pseudaroanium*, which is the closest species to the new species.

**4. Discussion**

Leaf fragmentation properties and calyx/corolla characters (especially size and shape of calyx teeth or corolla lobes) are very useful general morphological characters to distinguish different *Teucrium* sections from each other. According to Flora of Turkey and East Eagean Islands (Ekim, 1982), section *Teucrium* is a complex group with two different main groups with one of them with 2-3-pinnatisect leaves and the others entire leaves. In addition, members of this section can be easily distinguished by the other sections with these followings: campanulate and not gibbous calyx, almost equal and triangular calyx teeth. According to quite recent study (Aksoy et al., 2020), section *Teucrium* was divided into three main groups: with 2–3 pinnatisect leaves

**Table 2.** Comparison of two different DNA regions.

	nrITS	trnL-F	
Examined taxa	47	43	
Total characters	675	942	
Constant	351	735	
Parsimony-uninformative	85	86	
Parsimony-informative	239	121	
Retention index	0.696	0.864	
Consistency index	0.541	0.805	
Homoplasy index	0.475	0.195	
BIC	Best Model	GTR+I+G	TVM+I
	f(a)	0.19	0.36
	f(c)	0.35	0.18
	f(g)	0.30	0.16
	f(t)	0.16	0.30
	kappa	0.00	0.00
	titv	0.00	0.00
	Ra	0.678	0.817
	Rb	0.954	1.926
	Rc	1.397	0.229
	Rd	0.284	1.092
	Re	3.452	1.926
	Rf	1.000	1.000
	plnv	0.33	0.46
gamma	0.85	N/A	

(*T. orientale* L., *T. pruinosum* Boiss. and *T. parviflorum* L.), with entire leaves (*T. alyssifolium*, *T. pseudaroanium*, *T. brevifolium*, *T.creticum* L., *T. sandrasicum* O.Schwarz), and with entire to partly 3-partite leaves on same species (*T. semrae* Aksoy, Dirmenci & Özcan, *T. ekimii* H.Duman, *T. pestalozzae* Boiss. and *T. multicaule* Montbret & Aucher ex Benth.). According to field observations and findings carried out in this study, the new collected specimen is located in sect. *Teucrium* because of its characteristic properties such as campanulate calyx, almost equal calyx teeth, entire leaves, large corolla and stamens. This new species named as *T.turcicum* belongs to second group (with entire leaves) presented by Aksoy et al. (2020), and *T. pseudaroanium* and *T. alyssifolium* are the closest species of *T. turcicum*.

*T. turcicum* is distinguished from *T. pseudaroanium* and *T. alyssifolium* with several features. First of all, these three species are endemics to Turkey and distributed in different limited areas. Although, all three species are east Mediterranean elements, *T. alyssifolium* has a wider

Bootstrap consensus tree

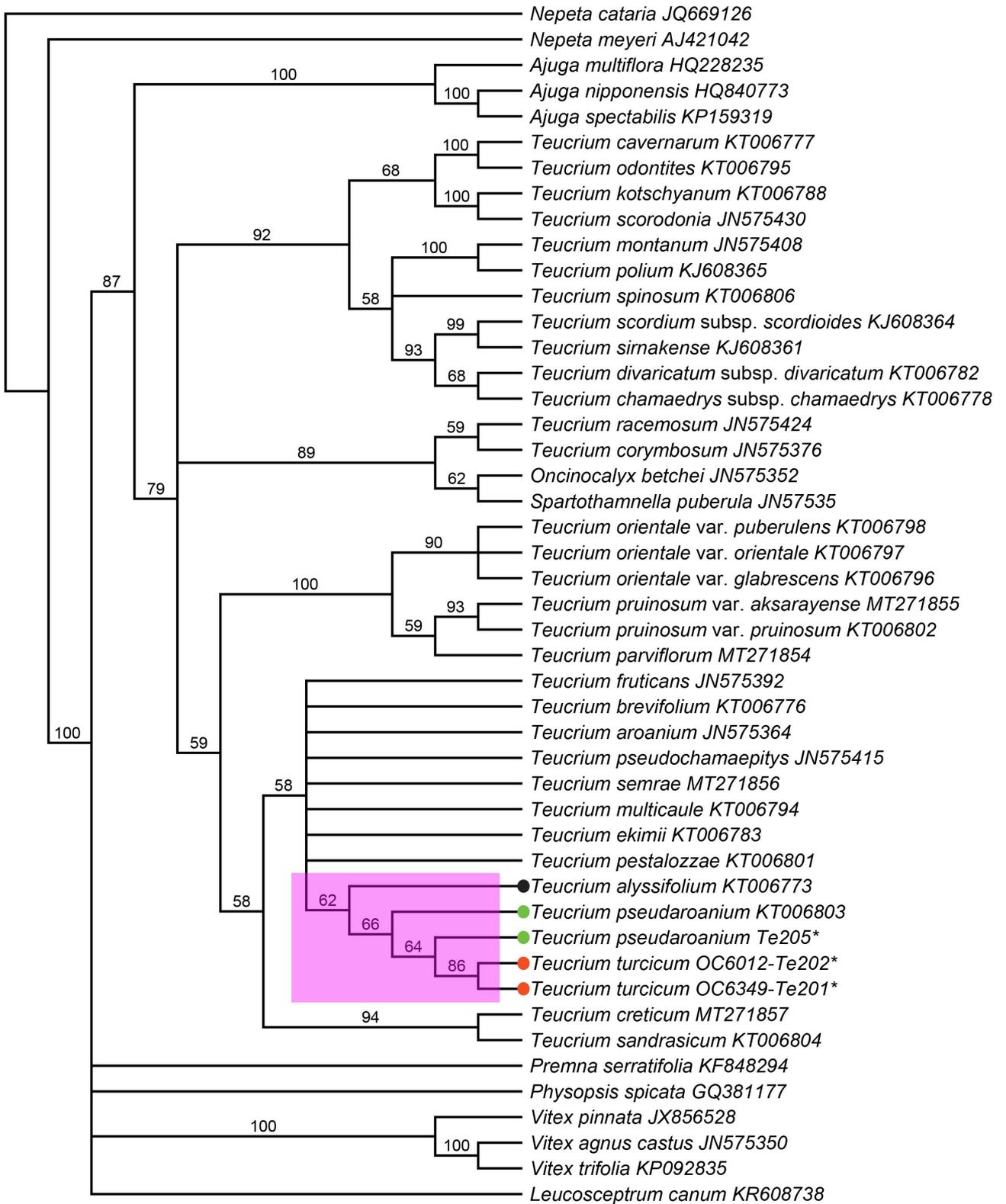


Figure 9. Phylogenetic cladogram of nrITS sequences based on MP analysis.

Bootstrap consensus tree

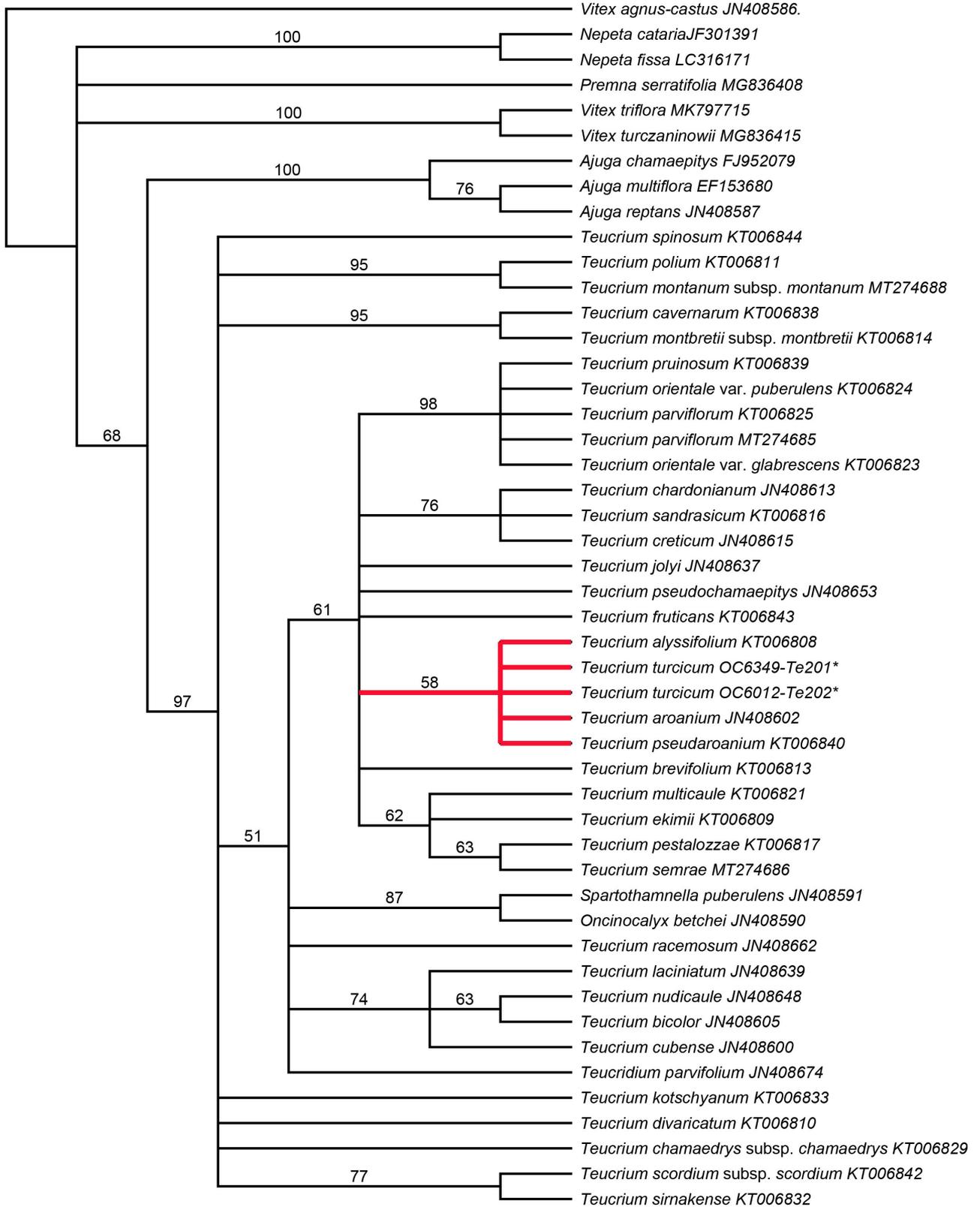
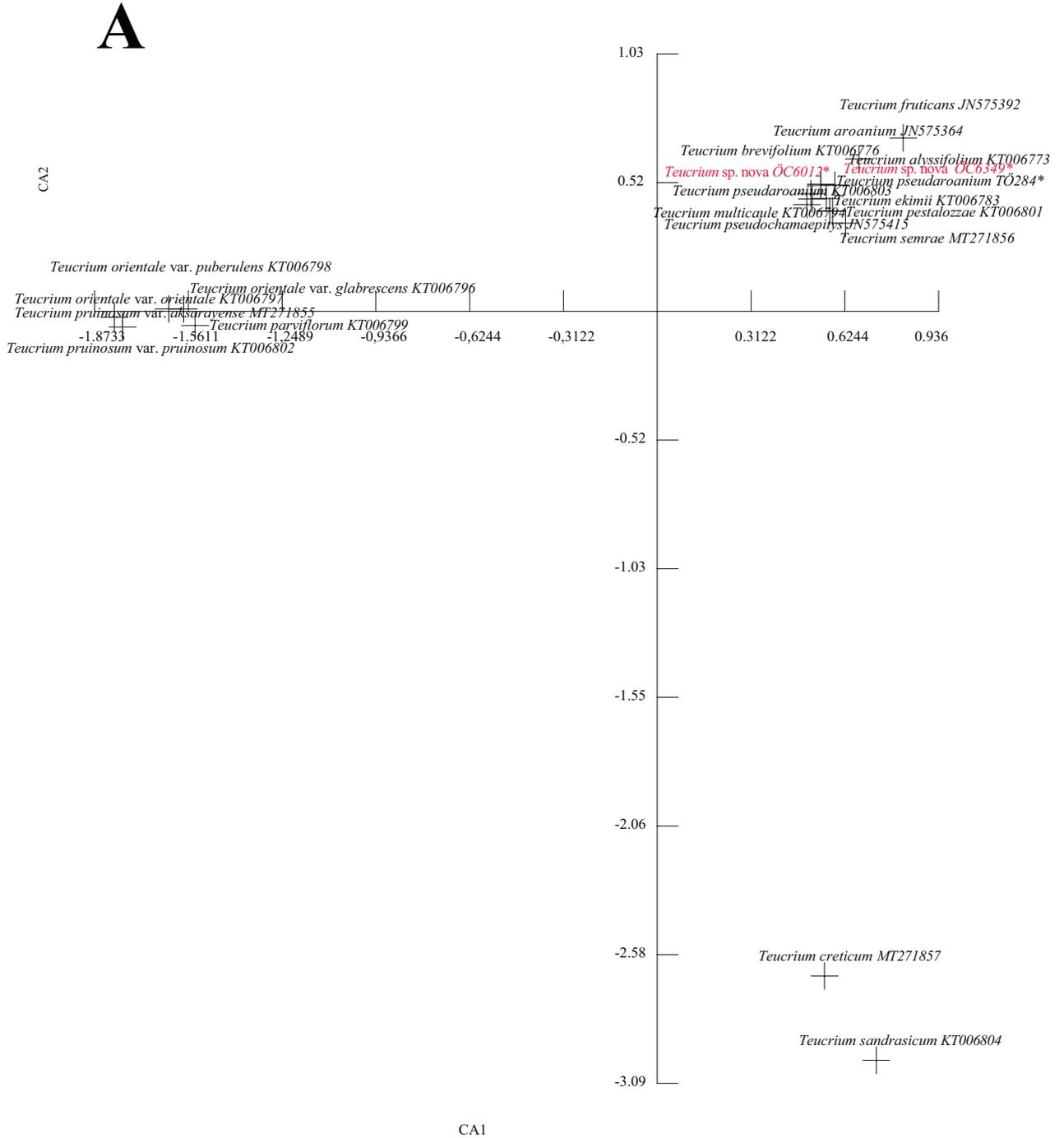


Figure 10. Phylogenetic cladogram of *trnL-F* sequences based on ML analysis.



**Figure 11.** MCA Scatter Plot showing the position of *Teucrium turcicum* in sect. *Teucrium* according to nrITS sequences (A) and *trnL-F* sequences (B).

distribution area around Denizli-Muğla provinces than other two species. On the other hand, *T. pseudaroanium* is distributed around Demre stream valley (Antalya province) and the distribution area of *T. turcicum* is restricted in Anamur (Mersin). This difference in distribution allows these three species to stay away and

to be isolated from each other. *T. alyssifolium* grows in serpentine rocks, but *T. pseudaroanium* and *T. turcicum* grow in marble-calcerous cliffs and crevices. Although the distribution areas of the three species do not coincide, the soil types on which they grow also differ. In addition, while *T. alyssifolium* individuals are obviously fragrant in

**B**

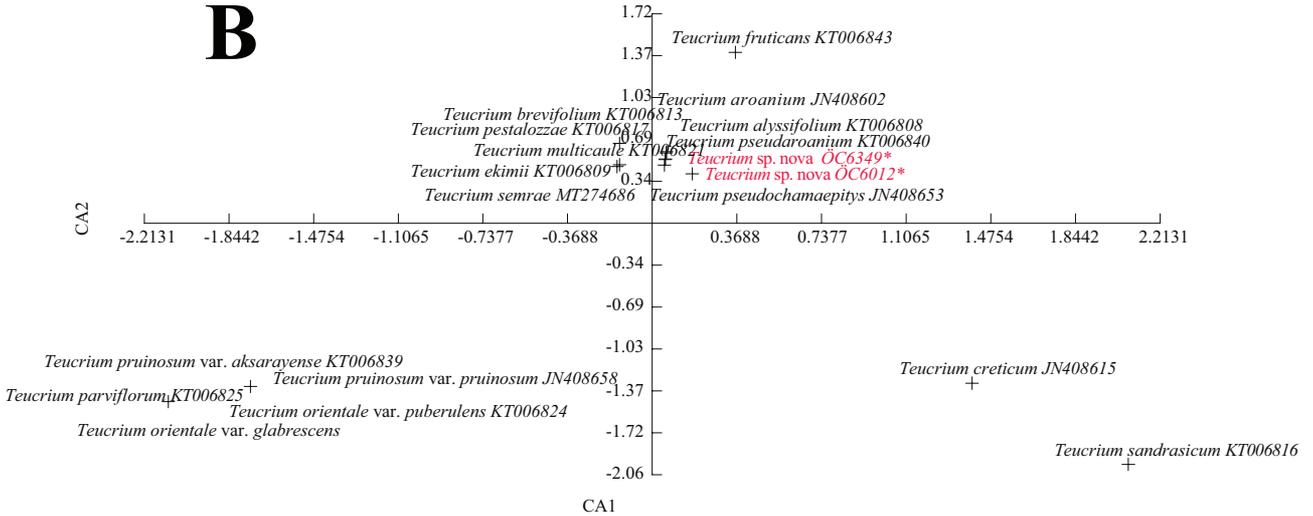


Figure 11. (Continued).

their natural environment and while herbarium materials, no obvious odor is detected in other two species. On the other hand, number of flowers in the inflorescence of *T. alyssifolium* can be six flowers in maximum, while minimum number of flowers in *T. pseudaroanium* and *T. turcicum* has six and usually up to 10–12 flowers. While *T. turcicum* has whitish to creamy-yellow corolla and purple anthers in flowering period and brown when anthers dry, *T. pseudaroanium* has white-light pinkish mauve corolla and yellowish anthers (Figures 4D, 4F, 4H). The longest filament size is observed in *T. turcicum* with up to 24 mm, while up to 22 mm and 18 mm in *T. alyssifolium* and *T. pseudaroanium*, respectively (Table 1).

According to Ecevit-Genç et al. (2015), the abaxial and adaxial surfaces of leaves of *T. brevifolium*, *T. sandrasicum*, *T. alyssifolium*, *T. parviflorum*, and *T. orientale* var. *orientale* have the same type of trichomes while the rest of the taxa in sect. *Teucrium* have different. In this study, lower and upper surfaces of *T. alyssifolium* have same density and color with H type eglandular trichomes, while in *T. pseudaroanium* and *T. turcicum* lower surfaces of leaves are whitish and upper sides greenish. On the other hand, while *T. pseudaroanium* has I type eglandular trichomes on both sides, *T. turcicum* has H type eglandular trichomes like *T. alyssifolium* (Table 1, Figures 6A, 6B, 6C, 6D, 6E, 6F, 8C, 8D). H type trichomes in *T. alyssifolium* and I type trichomes in *T. pseudaroanium* were presented by Ecevit-Genç et al. (2015) and Parolly and Eren (2007), respectively, and also approved in this study. Consequently, leaves of *T. turcicum* are similar to *T. pseudaroanium* in general morphology, but its trichome types are similar to *T. alyssifolium*. In addition, *T. alyssifolium* had sparsely A1 type glandular trichomes; this type trichome was not observed on *T. turcicum*.

There are some important differences in stem indumentum of *T. pseudaroanium* and *T. turcicum*. *T. pseudaroanium* has a sparsely indumentum with I and B type trichomes. On the other hand, stem indumentum of *T. turcicum* has denser than *T. pseudaroanium* with H type eglandular trichomes, and it is sparser than *T. alyssifolium*.

Calyx characters are also very useful to distinguish of three species (Figures 4E, 4F, 4H, 5D, 5E, 5F). *Teucrium alyssifolium* has the longest calyx up to 13 mm in length and the longest calyx teeth up to 6 mm in length. Calyx teeth in *T. alyssifolium* are narrowly triangular and are almost equal to calyx tube (Figures 4E, 5D). The shortest calyx teeth are observed in *T. turcicum*, and they are almost 1/3 of calyx. The calyx teeth are obviously shorter than tube and not mucronulate at apex easily distinguishes *T. turcicum* from the closest species *T. pseudaroanium* (Figures 4E, 4F, 4H, 5D, 5E, 5F).

Ecevit-Genç et al. (2015) and Parolly and Eren (2007) data were used to compare the nutlet characters of the new species and its allies because of lackness of the mature seeds of *T. alyssifolium*. According to relevant literature, B type trichomes were observed on the nutlets of *T. alyssifolium* and *T. pseudaroanium*, and F2 type (without micro-papillae) and F5 type (with micro-papillae) eglandular trichomes were seen in *T. alyssifolium*. *T. pseudaroanium*, F5 type is denser in *T. alyssifolium*. In this study, B type and F2 type trichomes were observed on the nutlets of *T. pseudaroanium* and *T. turcicum*, additionally, F5 type trichomes in *T. turcicum* were rarer than *T. pseudaroanium* (Figures 7A, 7B, 7C, 7D, 7E, 7F, 8A, 8B, 8C).

Indumentum structure and trichome differentiation is very informative for classification and also useful for distinguishing different species of *Teucrium* genus (Marin et al., 1994; Navarro and El Oualidi, 2000; Grubestic et al.,



sect. *Teucrium*. The new species has a close relationship and formed a group with *T. pseudaroanum* and *T. alyssifolium* according to molecular data, and morphologically similar to *T. pseudaroanum* than *T. alyssifolium*. On the other hand, indumentum density and types can be useful to differ these three species from each other.

With the new current species from Turkey, the species number of the genus is raised to 38 (50 taxa), and the number of taxa belonging to sect. *Teucrium* has been raised to 16 (13 species). With the addition of this new species, there are eight taxa (seven species) endemics in sect. *Teucrium*, and 19 of 50 taxa are endemics in Turkey. The endemism ratio is raised to 38% in Turkey.

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**Appendix**

**GenBank accession numbers**

ITS Sequences: *Teucrium pseudaroanum* Te205 (MW750193);  
*T. turcicum* OC6012 (MW750194); *T. turcicum* OC6349  
(MW750195).

*trnL-F* Sequences: *Teucrium turcicum* OC6349 (MW788643); *T.*  
*turcicum* OC6012 (MW788644).

*rpl32* Sequences: *Teucrium orientale* OC5727 (MW788645);  
*T. pruinosum* OC4694 (MW788646); *T. orientale* OC6390  
(MW788647); *T. turcicum* OC6349 (MW788648); *T. turcicum*  
OC6012 (MW788649); *T. pseudaroanum* TO284 (MW788650);  
*T. semrae* TD5250 (MW788651); *T. alyssifolium* TD4549  
(MW788652).