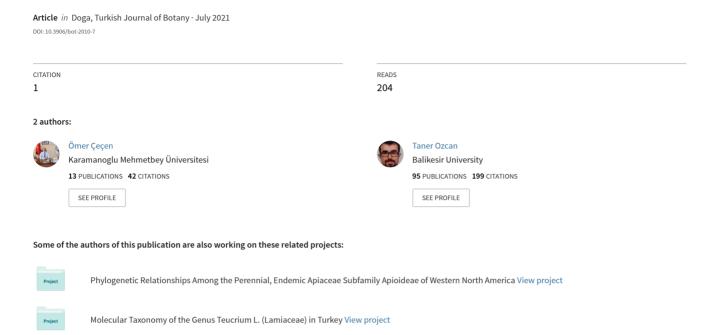
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A new *Teucrium* L. (Lamiaceae) Species from South Anatolia (Turkey)

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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. Peronematoideae, 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 disributed. 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

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 verticallasters 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

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).

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especially important in the infrageneric classification of the genus (Navarro and El Oualidi, 2000; Grubesic 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 (Orientale 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 pseudaroanium* 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 newone 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, micromophological 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 acronmys), 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. pseudaroanium* and *T. alyssifolium* was

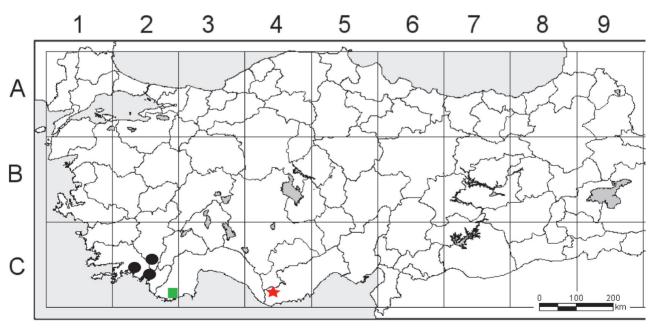


Figure 1. Distribution map of the new species *Teucrium turcicum*(\bigstar) and its allied species, *T. pseudaroanium* (\blacksquare) and *T. alyssifolium* (\bullet), in Turkey.



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 protographedusing 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. pseudaroanium*, 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 *trn*L-F and *rpl32-trn*L 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, *trn*L-c (5'-CGAAATCGGTAGACGCTACG-3') (Taberlet et al., 1991) and *trn*L-f (5'-ATTTGAACTGGTGACACGAG-3')

(Taberlet et al., 1991) primers for *trn*L-F region, and rpl32-F (5'-CAGTTCCAAAAAAACGTACTTC-3') (Shaw et al., 2007)and trnL^(UAG) (5'-CTGCTTCCTAAGAGCAGCGT-3') (Shaw et al., 2007) primers for *rpl*32-*trn*L 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 lowquality 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/Jacknife search. Boostrap anaylsis 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 squences edited in this study were deposited in the GenBank with their accesion 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. pseudaroanium*) 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. pseudaroanium* 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 $10-30\times4-14$ mm, obovate, cuneate at base, crenate and slightly revolute at margines, acute to obtuse at apex,villous green above and wooly grey beneath; veins are visible in both sides. Inflorescence 5-8 cm long, 6-10-flowered,verticillasters 2-flowered, stalked

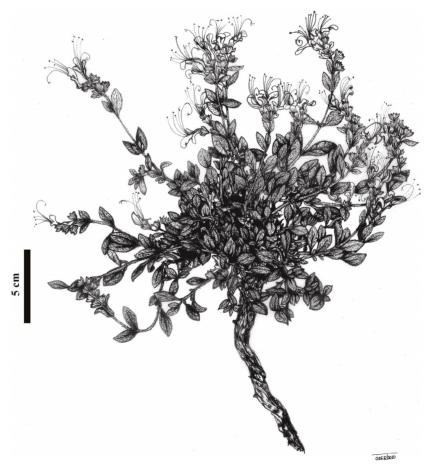


Figure 3. General appearance of *Teucrium turcicum* (collected by Ömer ÇEÇEN (ÖÇ 6012), drawn by Özer TÜRKOĞLU).

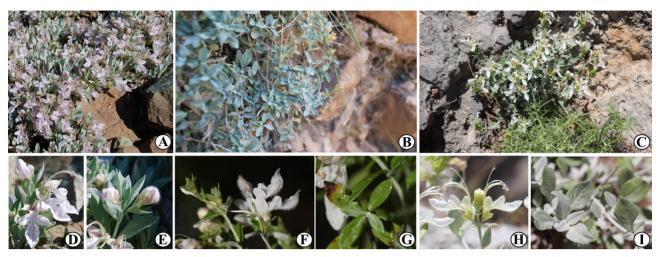


Figure 4. General morphological characters of stem, flowers, and leaves of *Teucrium alyssifolium* (A,D,E), *T. pseudaroanium* (B, F, G), and *T.turcicum* (C, H, I).

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, acuteat 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 exserted 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 carnosa* Willd., *Teucrium polium* L. subsp. polium, Onosma frutescens Lam., Lactuca tuberose Jacq., Scutellaria diffusa Benth., Phlomis leucophracta P.H.Davis & Hub.-Mor., Convolvulus cantabrica L., Micromeria myrtifolia Boiss. & Hohen., Gladiolus atroviolaceus Boiss., Mixhauxia thyrsoidea Boiss. & Heldr., Sideritis congesta P.H.Davis & Hub.-Mor., Centaurium 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 coggygria 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 km², 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 **pruinosum**
- 2.Calyx teeth straight
- 3.Corolla lavender-blue, 7.5–10 mm, clearly longer than calyx, to $2 \times \text{calyx}$ orientale
- - 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

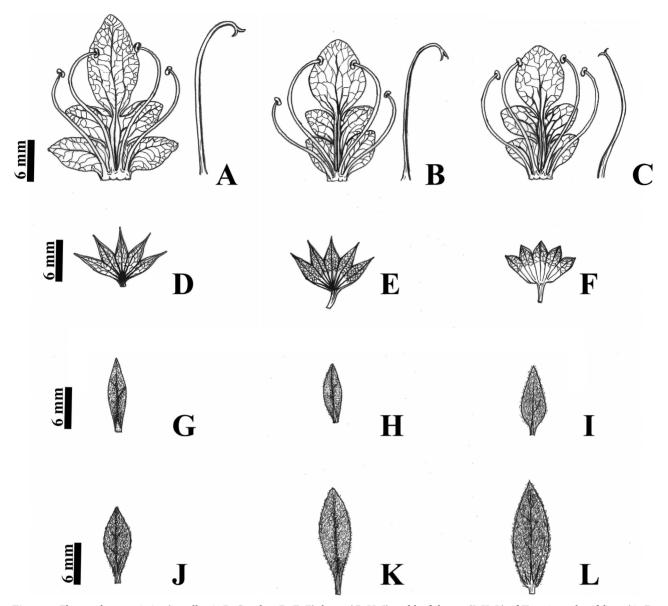


Figure 5. Flower characteristics (corolla: A, B, C; calyx: D, E, F), bract (G, H, I) and leaf shapes (J, K, L) of *Teucrium alyssifolium* (A, D, G, J), *T. pseudaroanium* (B, E, H, K), and *T.turcicum* (C, F, I, L).

- 8.Leaves discolorous, flat, strongly or slightly revolute at margins, obviously petiolate, calyx teeth triangular tobroadly triangular, corolla whitish to pale pinkish
- 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 oblong to obovate semrae

- 11. Plants eglandular; leaves lobes linear to linearlanceolate multicaule
 - 10. Leaves and bracts rarely 2-3 lobed

3.2. Micromorphological results

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

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

Characters		T. alyssifolium	T. pseudaroanium	T. turcicum							
structure		quadrangular, mostly ascending, 3.5-9 cm	slender, ascending to erect, 2–18 cm	slender, ascending to erect, 10–30 cm							
Stem	indumentum	densely tomentose with sessile glands	puberulent to white tomentose	densely white tomentose							
	trichome types*	H, B (very rare)	I, B	Н							
	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	$(4-)10-20(-25) \times (2-) 5-10(-12) \text{ mm}$	5-30 mm × 2-14 mm							
Leaf	adaxial indumentum	12 1 1	arachnoid and green								
	abaxial indumentum	white adpressed-tomentose	densely white-tomentose to canescent	densely white-tomentosa							
	trichome types*	H, B, A1(rare) (adaxial-abaxial)	I, B (adaxial)	H, B (abaxial) distinctly petiolate, 2–7 mm							
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							
	colour	light pinkish-lavender	white to pale pinkish mauve	white to yellow-creamy rarely purple striped							
0 11	size	20–25 mm	12–17 mm	18–22 mm							
Corolla	tube	2 mm	1.5–2.5 mm	5–6 mm							
	Lower lips	12(-17) mm	10-15(-18) mm	11–14 mm							
	length	18–22 mm	filaments arcuate, c. 14–18 mm	20-24 mm							
	color	whitish-pink	whitish-pink, somewhat darker than corolla	whitish							
Filament	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							
	shape	light brown, broadly elliptic-obovate, $2-2.7 \times 1.2-3.1$ mm, alveolate	light brown, obovate-oblong, 2.5–3.0 \times 1.8–2, alveolate, alveolishallow	light brown, obovoid, 2.5×1.3 mm, alveolate							
Nutlet	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 typeson 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 discolorous 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 disribute on the nutlets in the taxa studied (Figures 7A, 7B, 7C, 7D, 7E, 7F, 8F). Also, B type is the most general typepresent on the nutlets and leaves of three species. While B type trichomes can be observed more easily on the nutlets, these trichomes

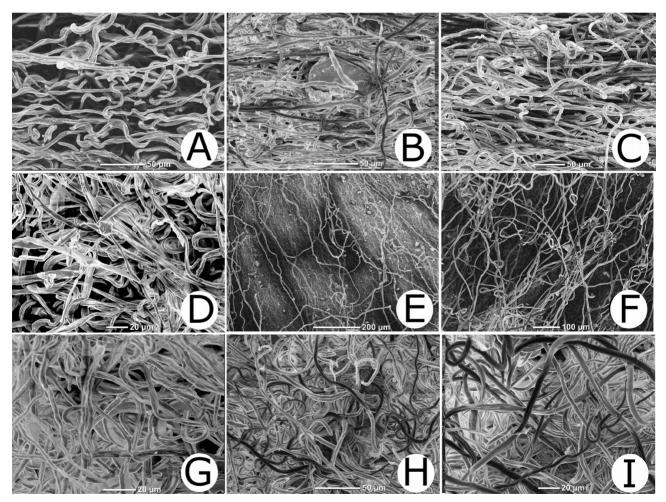


Figure 6. Micromorphological characters of stem (A-C), and adaxial(D-F) and abaxial (G-I) sides of leaves of *T. alyssifolium* (A, D, G), *T. pseudaroanium* (B, E, H), and *T.turcicum* (C, F, I).

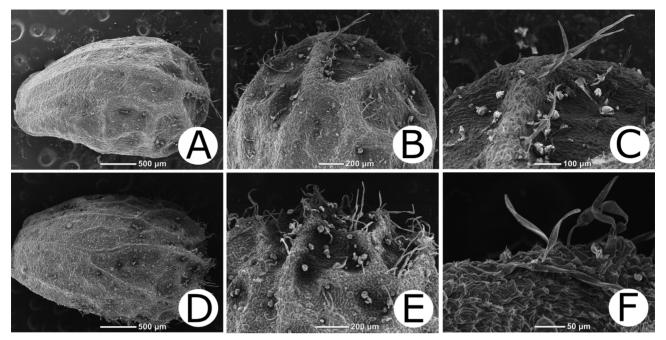


Figure 7. Micromorphological characters of nutlets of Teucrium pseudaroanium (A, B, C) and T.turcicum (D, E, F).

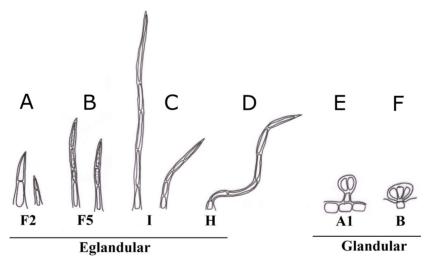


Figure 8. Trichome types of eglandular and glandular that observed in the studied taxa. Non glandular trichomes; A) F2. Large, very thin-walled, 2(-5)-celled trichomes with an acute apical cell, with ridges and marked internodes. B) F5. Elongated and flexuose, with thin-walled 3–7(-11)-celled trichomes, 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. pseudaroanium* on their nutlets (Figure 8B, 8F).

Although *T. turcicum* is closer to *T. pseudaroanium* 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. pseudaroanium* and *T. alyssifolium* 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 *trn*L-F, likelihood settings from best-fit model (TVM+I) were selected by BIC with jModeltest. After alignment of *trn*L-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. turcium, 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 toquite 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					
Exan	nined taxa	47	43					
Total	characters	675	942					
	Constant	351	735					
	Parsimony-uninformative	85	86					
	Parsimony-informative	239	121					
Rete	ntion index	0.696	0.864					
Cons	sistency index	0.541	0.805					
Hom	oplasy index	0.475	0.195					
	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					
BIC	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 islocated 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

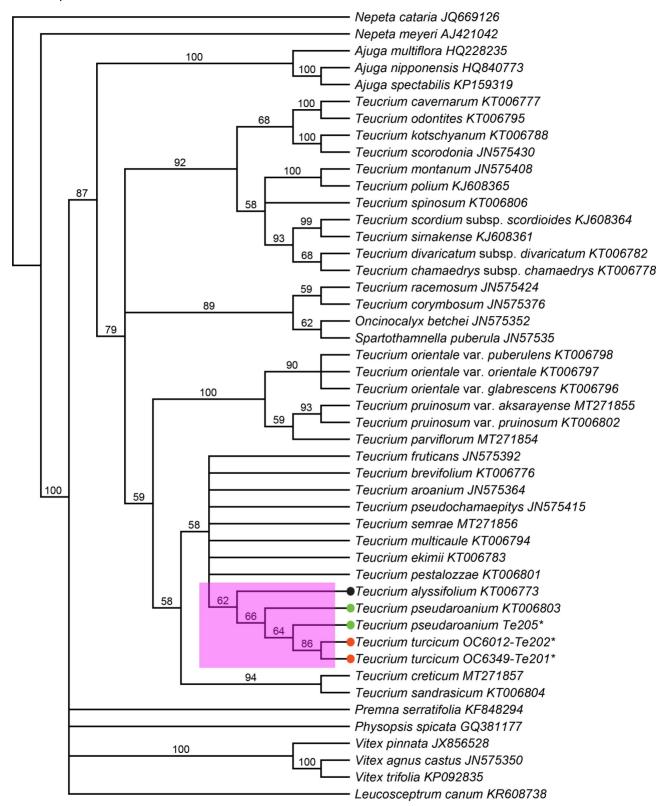


Figure 9. Phylogenetic cladogram of nrITS sequences based on MP analysis.

Bootstrap consensus tree

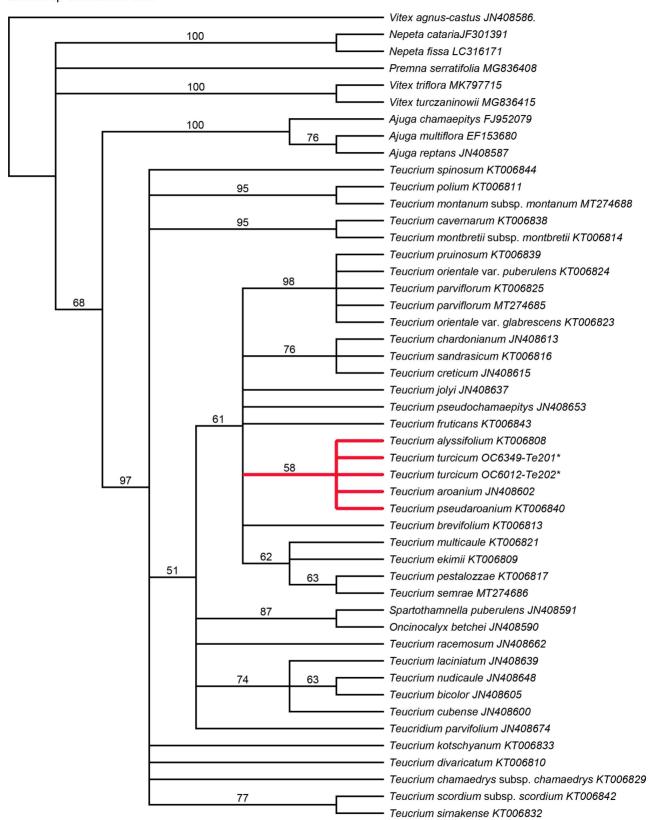


Figure 10. Phylogenetic cladogram of trnL-F sequences based on ML analysis.

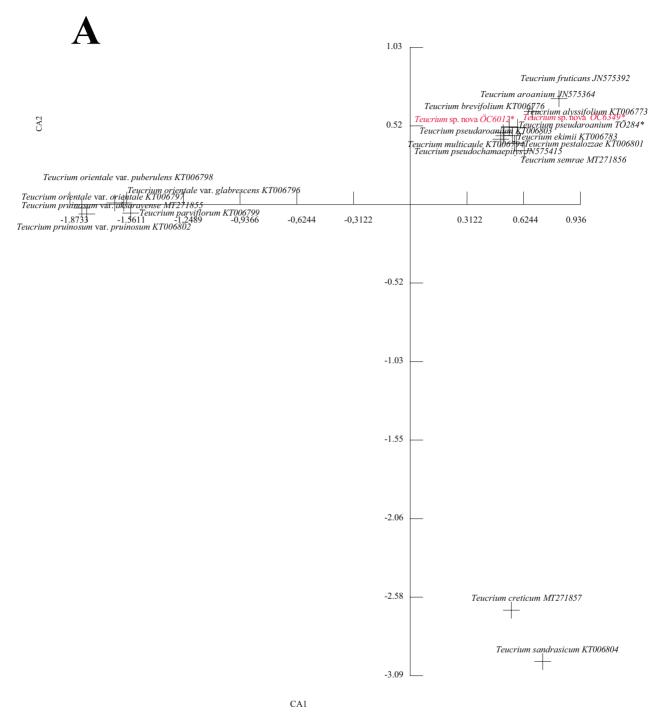


Figure 11. MCA Scatter Plotsshowing the position of *Teucrium turcicum*in sect. *Teucrium* according to nrITS sequences (A) and *trn*L-F sequences (B).

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

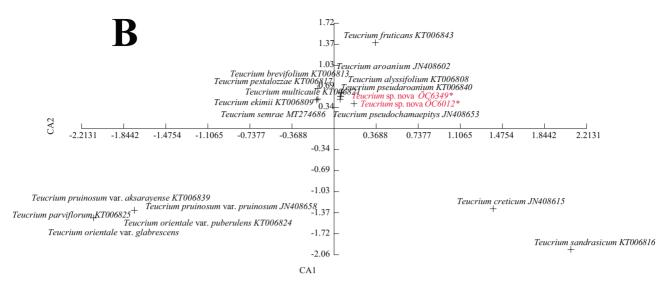


Figure 11. (Continued).

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. pseudaroainum* 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 mavue 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. pseudaroainum*, 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 micropapillae) 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; Grubesic et al.,

Table 3. Single polymorphic regions separated *Teucrium turcicum* and its allies from some examined taxa(based on chloroplast *rpl*32-trnL region).

		3 2	1 2 0	1 4 1	1 9 3	2 2 7	2 4 2	2 8 1	2 8 2	2 8 3	2 8 4	2 8 5	2 8 6	2 8 7	3 0 3	3 3 0	3 5 5	3 5 6	3 5 7	3 5 8	3 5 9	3 6 0	3 6 1	3 6 2	3 6 3	3 9 3	3 9 4	4 1 3	4 4 0	4 5 5	4 6 2	4 9 0	4 9 7		5 6 0
Section Teucrium	T. orientale OC5727*	A	С	G	A	G	Т	-	-	-	-	-	-	-	Т	G	A	A	Т	Т	С	A	Т	A	G	G	Т	С	С	G	A	С	Т	G	G
	T. pruinosum OC4694*	A	С	G	A	G	Т	-	-	-	-	-	-	-	Т	G	A	A	Т	Т	С	A	Т	A	G	G	Т	С	С	G	A	С	Т	G	G
	T. orientale OC6390*	A	С	G	A	G	Т	-	-	-	-	-	-	-	Т	Т	A	A	Т	Т	С	A	Т	A	G	G	Т	С	С	G	A	С	Т	G	G
	T. semrae TD5250*	G	G	G	С	A	С	Т	Т	Т	A	С	С	Т	A	G	A	A	Т	Т	С	С	Т	-	Т	Т	С	Т	С	A	С	Т	С	A	С
	T. turcicum OC6349*	G	G	A	С	A	С	Т	Т	Т	A	С	С	Т	A	G	-	-	-	-	-	-	-	-	-	Т	С	Т	A	A	С	Т	С	A	С
	T. turcicum OC6012*	G	G	A	С	A	С	Т	Т	Т	A	С	С	Т	A	G	-	-	-	-	-	-	-	-	-	Т	С	Т	A	A	С	Т	С	A	С
	T. pseudaroanium TÖ284*	G	G	G	С	A	С	Т	Т	Т	A	С	С	Т	A	Т	-	-	-	-	-	-	-	-	-	Т	С	Т	С	A	С	Т	С	A	С
	T. alyssifolium TD4549*	G	G	G	С	A	С	Т	Т	Т	A	С	С	Т	A	G	-	-	-	-	-	-	-	-	-	Т	С	Т	С	A	С	Т	С	A	С
T. polium subsp capitatum JQ044770		A	С	G	С	G	С	-	-	-	-	-	-	-	Т	G	-	-	-	-	-	-	-	-	-	G	G	Т	С	G	С	С	Т	G	С
T. scorodonia subsp baeticumJF694912		A	G	G	С	G	С	-	-	-	-	-	-	-	Т	Т	-	-	-	-	-	-	-	-	-	G	G	Т	С	G	С	С	Т	G	G
T. flavum subsp flavumHQ646976		A	С	G	С	G	С	-	-	-	-	-	-	-	С	G	-	-	-	-	-	-	-	-	-	G	G	Т	С	G	С	С	Т	G	G

^{*:} Examined in this study

2007; Eshratifar et al., 2011; Dinç et al., 2011a, 2011b; Dinç & Doğu, 2016; Doğu et al., 2013; Ecevit-Genç et al., 2015, 2017; Marzouk et al., 2016; Aksoy et al. 2020; Moghadam and Kharazian, 2020).

Molecular data obtained in this study supports to define phylogenetic position of *T. turcicum* with its close allies. Obtained data based nrITS and *trnL*-F sequences supports the separation of sect. *Teucrium* presented by the previously above-mentioned studies (presented by Melnikov, 2014; Salmaki et al., 2016; Aksoy et al., 2020). *Teucrium turcicum* is a characteristic member of *Fruticantia* subgroup of sect. *Teucrium*. The general morphological and micromorphological characters, nrITS and *trnL*-F phylogenetic cladograms also show that *T. turcicum* forms a group with *T. alyssifolium* and *T. pseudaroanium*. Although these three species contain simple and entire leaves, they have the widest leaf shape compared with the members of the section distributed in Turkey. According to molecular data, these three species

are close to other species with simple and entire leaves. Moreover, Figures 9 and 10 (especially Figure 10) reveal that, although *T. aroanium* Boiss. is molecularly close to this group, they are easily distinguished by the following morphological features: Their natural distribution is limited to South Greece, their flowers are light purple to pink, and they have thin and silvery leaves. Althougth *T. aroanium* has purple anthers in flowering time, it has 4–6 flowers in its inflorescence. It is surprising that molecular data are consistent with morphological data. Calyx structure, longest filaments, leave shapes are the characteristic properties supporting that *T. turcicum* has a close relationship with *T. pseudaroanium* and *T. alvssifolium*.

5. Conclusion

According to morphological observations, indumentum characters, and nrITS and *trn*L-F sequences based on molecular data, the new species is a characteristic member of

sect. *Teucrium*. The new species has a close relationship and formed a group with *T. pseudaroanium* and *T. alyssifolium* according to molecular data, and morphologically similar to *T. pseudaroanium* 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 pseudaroanium* Te205 (MW750193); *T. turcicum* OC6012 (MW750194); *T. turcicum* OC6349 (MW750195).

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

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