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A phylogenetic analysis and biogeographical distribution of *Teucrium* Sect. *Teucrium* (Lamiaceae) and taxonomic notes for a new species from southwest Turkey

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Abstract: *Teucrium* L. is a well-known and cosmopolitan genus of the Lamiaceae family. In Turkey, the genus is represented by 36 species (48 taxa). *Teucrium* Sect. *Teucrium* that this study focuses on comprises around 30 species worldwide and is mainly distributed in countries of the Mediterranean Basin. With 11 species (14 taxa), it is the second-largest section of the genus *Teucrium* in Turkey. In this study, morphological and molecular findings were evaluated and the phylogeny and biogeographical distribution of Sect. *Teucrium* were analyzed. Also, five endemic taxa belonging to Sect. *Teucrium* and which play an informative role in the subsectional classification of the section were analyzed for the first time. A new species of the genus *Teucrium* from southwest Turkey was described as *T. semrae* Aksoy, Dirmenci & Özcan and analyzed using different approaches. The infrageneric position of *T. semrae* in sect. *Teucrium* and the phylogenetic structure of the section were determined according to morphological and micromorphological features and molecular nrITS and cpDNA data. The phylogenetic position of the new species was argued by comparing the closest members. Comparative figures and illustrations, distribution maps and an updated identification key for the Sect. *Teucrium* in Turkey are presented, and a new detailed classification is proposed in this study.

Keywords: *Teucrium*, identification key, nrITS, *trn*L-F, new species, Turkey

1. Introduction

Teucrium L. (Lamiaceae:Ajugoideae) is a well-known and cosmopolitan genus mainly distributed in the temperate regions of Europe, North Africa, and Asia (Figures 1 and 2) and is the second-largest genus of the subfamily Ajugoideae (Harley et al., 2004; Salmaki et al., 2016). It is noteworthy that this large and polymorphic genus is mostly distributed in the Mediterranean basin. If considered in terms of species, it is known that approximately 96% of around 250–300 species are distributed in this region (Cantino, 1992; Navarro and El Oualidi, 2000; Harley et al., 2004). In Turkey, the genus is represented by 36 species (48 taxa) while 17 of the 48 taxa are endemics. The endemism ratio is about 35 % (Dirmenci, 2012; Özcan et al., 2015a; Vural et al., 2015; Dinç and Doğu, 2016).

Taxonomic studies on the genus *Teucrium* were firstly mentioned in the second volume of Linnaeus' Species Plantarum in 1753. Linnaeus named 27 *Teucrium* species without dividing them into sections. In an edition from 1763, 31 species again without sections are mentioned. The classification of Linnaeus pioneered many different scientific studies. However, since forming small groups using certain similar characters would make the classification easier, researchers after Linnaeus thought that division of the *Teucrium* genus would result in more accurate and appropriate classification. The main characteristics in the separation of sections for the genus *Teucrium* were the calyx shape and structure, and verticillasters (Bentham, 1834, 1876; Boissier, 1879; Briquet, 1895; Ekim, 1982).Also, indumentum of calyx, corolla, nutlet, leaf, stem and leaf fragmentation have proofed to be very useful and to have significant importance for dividing the species into sections (Dinç et al., 2009, Dinç et al., 2011a, 2011b; Ecevit Genç et al., 2015, 2017; Ecevit Genç et al., 2018a, 2018b). Bentham (1834) classified 63 species of *Teucrium* under 9 sections (Labiatarum genera et species). Don (1838) classified *Teucrium* taxa under 9 sections similar to Bentham's classifications (General History of the Dichlamydeous Plants).

Boissier (1879) collected 42 species of *Teucrium* under 8 sections. In his study, Boissier classified the *Teucris* section (synonym of Sect. *Teucrium*) members according to their leaf shape and whether or not the filament exserted from the corolla. In its classification, *Teucrium brevifolium*

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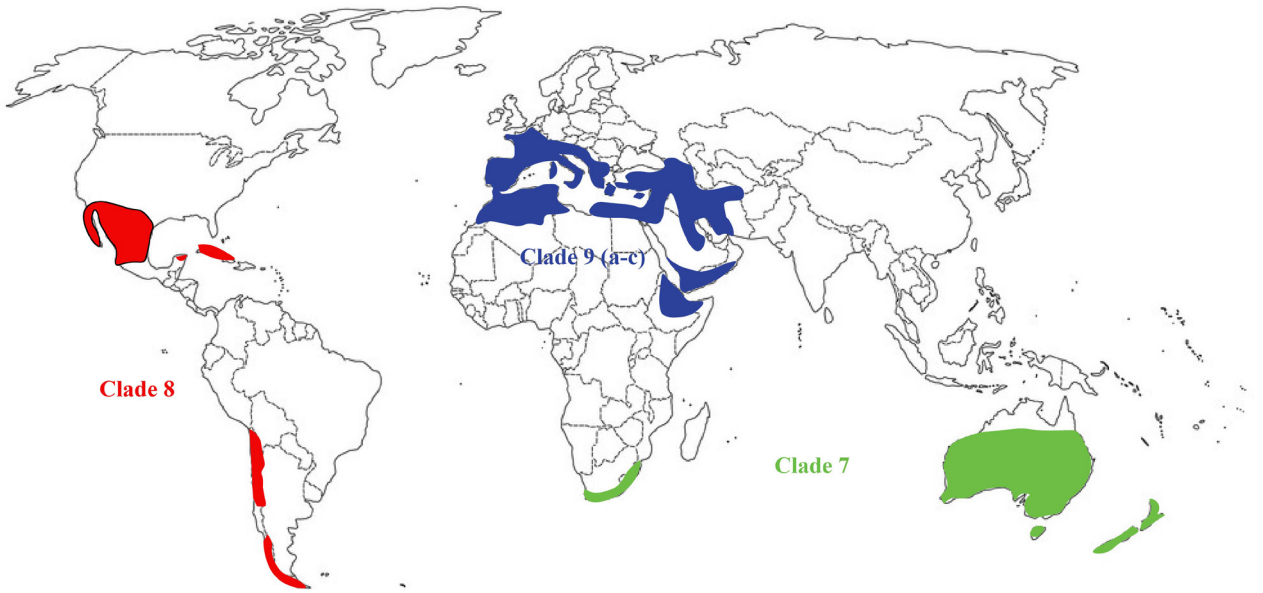

Figure 1. Distribution map of the members of section *Teucrium*. Clade 7 (in green): Australia, New Zealand and Capetown; Clade 8 (in red): North and South America; Clade 9 (in blue): Southern Europe, Northern and Northeastern Africa, and Western Asia.

Schreb. and *T. pestalozzae* Boiss., grown in Turkey, were classified in simple leaf ones. *Teucrium multicaule* Montb. & Aucher ex Benth., *T. orientale* L., *T. pruinosum* Boiss. were classified under exserted filaments from corolla, and *T. parviflorum* Schreb. were classified under not exserted. Briquet (1895) classified about 100 species under 10 sections, with *Teucris* being one of these sections. Today, the *Teucrium* genus is divided into 10 sections worldwide (Navarro and El Oualidi, 2000).

The Sect. *Teucrium* is distinguished by its calyces, which are not gibbous at base, and more or almost equal calyx teeth. The other feature that separates them from the other section members is their leaves forming entire to deeply dissected and revolute margins at the base.

Teucrium sect. *Teucrium* comprises 30 species worldwide (Navarro and El Oualidì, 2000; Parolly and Eren, 2007). In Turkey, the section includes 11 species (14 taxa and 5 endemics) and represents one of the important diversification centers of this section (Ekim, 1982; Duman, 2000). The species are distributed in the Mediterranean, in Central Anatolia, and east and southeast of Turkey (Figure 3). All endemic species belonging to Sect. *Teucrium* are found in the western Mediterranean part of Turkey [Figure 3-blue (b1) and green (c1) areas]. In addition to these endemic species, specimens belonging to prospective new species were collected from around the same area, too (Figure 3, star-shaped). The specimens were collected from Pinara Ancient City (Seydikemer, Muğla) by the authors in 2019 (Figure 3, star-shaped). After detailed investigations, these specimens were thought to belong to a new species of the Sect. *Teucrium* by their not gibbous calyces at the base and equal calyx teeth, and 2–3-partite (lobed) leaves. The new species is more related to *Teucrium ekimii* H.Duman and *T. pestalozzae* based on some morphological and molecular characters.

The main objectives of this study are; (i) to describe and classify the collected specimens which are thought to be a potentially new *Teucrium* species by comparing them with members of sect. *Teucrium* using different approaches, (ii) to investigate the morphological, micromorphological and molecular structure of this new species, (iii) to define the phylogenetic position of the new species in sect. *Teucrium*, (iv) to indicate the phylogenetic relationship and biogeographic diversification of the species belonging to sect. *Teucrium*, 5- to present a new, expanded and revised classification key of this section in Turkey.

2. Materials and methods

2.1. Plant materials

Specimens were collected from the Seydikemer district (Pinara Ancient City), Muğla province in southwestern Turkey (Figure 2, Appendix). The collected specimens were identified using the relevant literature (Ekim, 1982; Duman, 2000; Dirmenci, 2012; Seregin et al., 2018; Özhatay et al., 2019) and compared with materials stored in herbaria which the acronyms are indicated as ANK, B, BM, E, EGE, GAZI, HUB, ISTE, ISTO, K, LE, MA, W, and WU, as well as with our own herbarium specimens in the Balıkesir University Herbarium (see Appendix).

The research on trichome morphology of the new species and the related species *T. ekimii* and *T. pestalozzae* was carried out using tabletop scanning electron

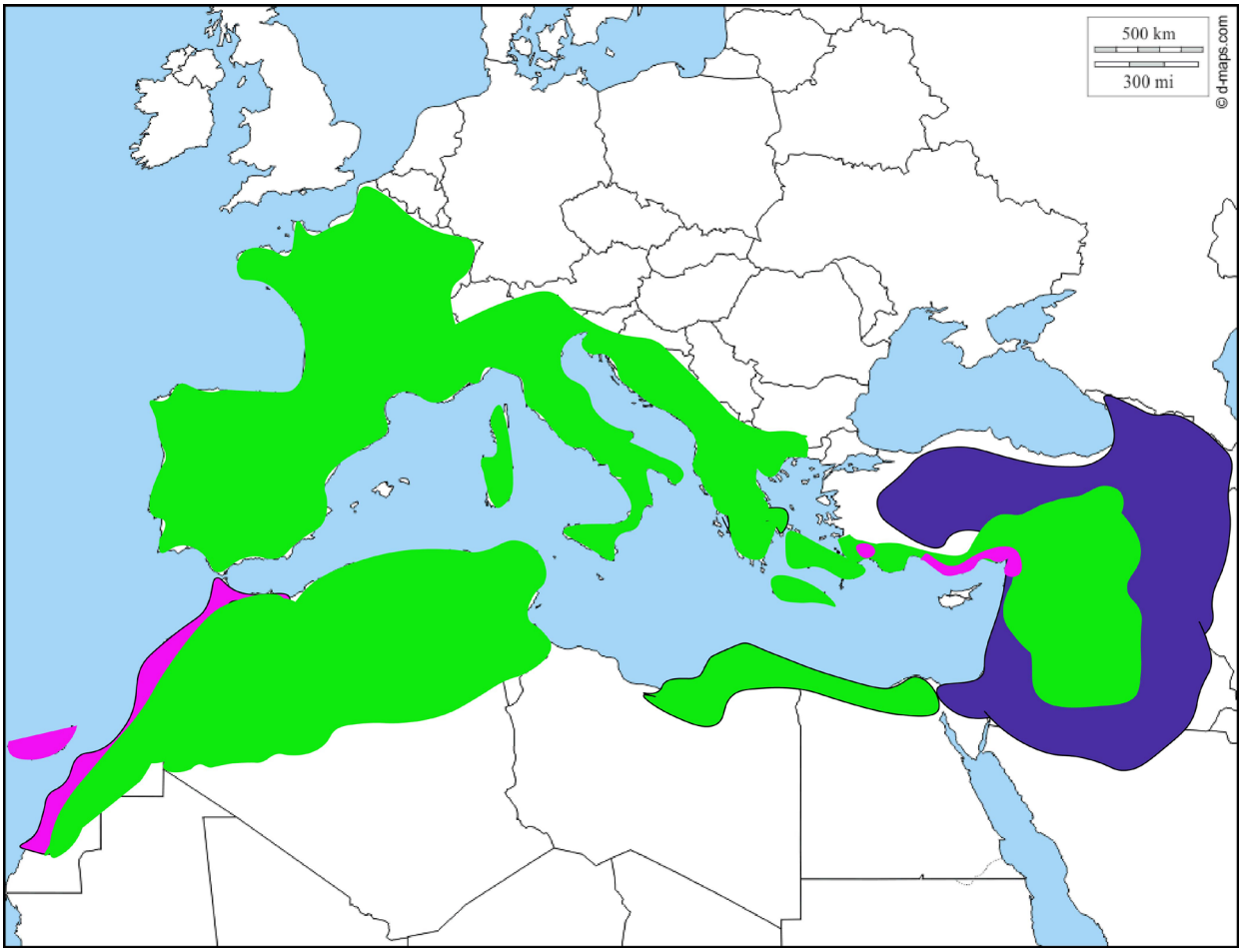

Figure 2. Distribution map of the members belonging to Clade 9 a-c (based on nrITS data). 9-a: Blue area, 9-b: Pink area, 9-c: Green area.

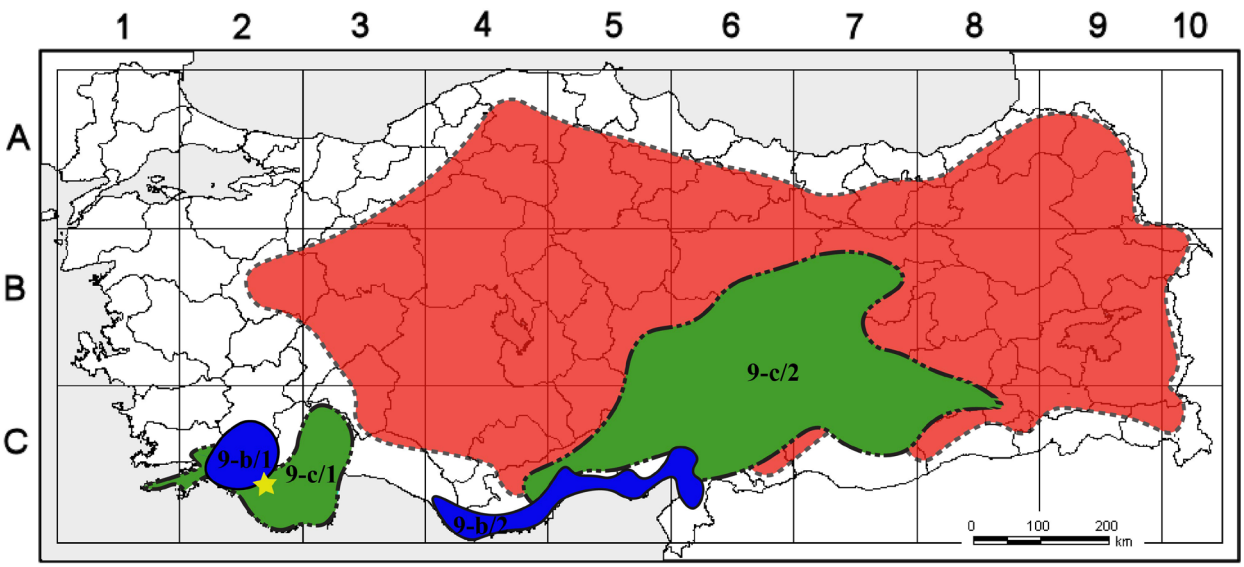

Figure 3. Distribution map of the section *Teucrium* growing in Turkey (based on nrITS data).Yellow star: *T. semrae*; red area: Clade 9-a; blue area: Clade 9-b1-2; green area: Clade 9-c1-2.

microscopy (SEM). Stems and leaves were investigated and photographed using a NeoScope JCM.

In this study, Navarro and El Oualidi's (2000) indumentum classification for *Teucrium* was used. Descriptions and abbreviations of indumentum based on Navarro and El Oualidi (2000) are as follows (Table 1):

Glandular trichomes

A: Clavate glandular trichomes

A1-Short clavate glandular trichomes. Generally, with two, large and thin stalk cells.

A2-Long clavate glandular trichomes. Generally, with long 3–5 stalk cells.

B: Subsessile glandular trichomes, peltate trichomes

Nonglandular trichomes

F: Thin-walled trichomes.

F4. Short, thin-walled, 2–3-celled trichomes densely covered by micropapillae, with the apical cell elongated, slightly crumpled or falcate.

G: Thick-walled, 3(–5)-celled, falcate, erect or sometimes slightly curved, the basal cell with smooth trichomes.

I: Vermiform hairs, thick-walled with elongated cells.

2.2. DNA isolation

DNA isolation was performed using the DNA Plant Mini Kit (Qiagen GmbH, Düsseldorf, Germany). First, recently collected herbarium materials or dried leaf samples on silica were powdered using liquid nitrogen. All the procedures of the manufacturer were conducted with some modifications [more RNase (10 µl not 4 µl), more incubation time (more than 30 minutes instead of 30 minutes)]. Second elutions were obtained in the final stage. The DNA solution was stored at –20 °C until using, and the second elutions were used for PCR steps.

2.3. PCR amplifications

In this study, the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA (nrDNA) and chloroplast *trn*L-F sequences were used to identify the phylogenetic position of the new species and its allies. ITS nrDNA were performed using ITS5A (5'-CCTTATCATTTAGAGGAAGGAG-3') (Stanford et al., 2000) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990) primers, and *trn*L-F amplifications were performed with *trn*L-c (5'-CGA AAT CGG TAG ACG CTA CG-3') (Taberlet et al., 1991) and *trn*L-f (5'-

Table 1. Morphological and micromorphological comparison of *Teucrium semrae* and the related species *T. ekimii* and *T. pestalozzae*.

Characters	*T. semrae*	*T. ekimii*	*T. pestalozzae*
Flowering stems	fragile	fragile	robust
Stem indumentum	densely villose and glandular	glabrous to sparsely pubescent below, long villose and glandular above	retrorsely pubescent or long hairy above, sometimes with sessile glands
Leaves	tripartite, sligthly discolorous, densely villose and glandular	obovate to linear, sligthly discolorous, subglabrous, sparsely villose, sometimes sparsely glandular above, white tomentose to canescent at beneath	linear-oblanceolate to linear, discolorous, adpressced-pubescent or sometimes long villose above, white tomentose beneath
Bracts	tripartite, densely villose and glandular	elliptic to linear, rarely tripartite, densely villose with glandular	linear to linear-elliptic, adpressced-pubescent
Calyx teeth	broadly triangular, as long as or rarely longer than tube	triangular to slightly elliptic, as long as or longer than tube	lanceolate, longer than tube
*Stem indumentum	A1, A2, B, G	B, I	B, G
*Adaxial surface indumentum of leaves	B, G	B, G	G
*Abaxial surface indumentum of leaves	A1, B, G	B, G	B, G
*Nutlet surface micromorphology	B, F4	B, F4	B, F4

*according to Navarro & Oualidi J (2000).

ATT TGA ACT GGT GAC ACG AG-3') (Taberlet et al., 1991) primers. All PCR amplifications were conducted using the PCR procedure by Shaw et al. (2007).

2.4. DNA data analysis

The appropriate PCR products were sent to Genoks (Gene Research and Biotechnology Company, Ankara, Turkey) for sequencing. The sequenced DNAs were edited using Sequencer version 5.4 (Gene Codes Corporation, Ann Arbor, MI, USA). Some ambiguous nucleotides from the 5′ end and the 3′ end were cut, after that, the rest of the sequences were aligned using Bioedit 7.2.5 (Hall, 1999) and CLUSTAL W2 online software (Larkin et al., 2007). Phylogenetic cladograms were constructed using PAUP* 4.0a165 (Swofford, 2003).

3. Results

3.1. *Taxonomic treatments*

Teucrium semrae **Aksoy, Dirmenci & Özcan sp. nov. (Figures 4–7)**

Type: Turkey. Muğla: Seydikemer district, Minare village, Pınara ancient city, Aşı stream, 450-650 m, 28.04.2019, Aksoy 2993 (Holotype: GAZI, isotypes ANK, EGE, ISTE , Akdeniz University Herbarium)**.**

Diagnosis: *Teucrium semrae* is closely similar to *T. ekimii* and *T. pestalozzae* but it differs from *T. ekimii* by its stems densely villose and glandular throughout (not glabrous to sparsely pubescent below, long villose and glandular above), leaves tripartite, and densely villose and glandular throughout (not obovate to linear, subglabrous, sparsely villose, sometimes sparsely glandular), bracts tripartite (not elliptic to linear, rarely tripartite) (Figures 4– and 5). It differs from *T. pestalozzae* by its stems fragile (not robust), densely villose and glandular (not retrorsely pubescent or long hairy above, sometimes with sessile glands), leaves tripartite, densely villose and glandular, slightly discolorous (not linear-oblanceolate to linear, discolorous, adpressed pubescent or sometimes long villose above, white tomentose beneath), bracts tripartite (not linear) (Figures 4 and 5).

Description: Saxatile shrubs, stems prostrate to the substrate, up to 15 cm long, flowering stems fragile, whitish-grey at base, leafy and flowering stems yellowish-green or green, throughout densely villose and glandular. Leaves 7–2 × 4–6 mm, tripartite to 1/3–1/2, rarely 2-partite, very rarely entire or young leaves entire, slightly discolorous, upper surface bright green, lower surface greyish-green or greyish; densely villose and glandular on both surfaces, attenuate at the base; lobes lanceolate to obovate and obtuse to acute at apex, middle lobes usually longer and broader than laterals; middle lobes 4–6 mm long; lateral lobes 2–3 mm long. Bracts similar to leaves, deeply tripartite, 5–10 × 3–6 mm, longer than pedicel and shorter than calyx. Verticillasters 2-flowered. Pedicels 2–5 mm long, densely villose and glandular papillate. Calyx 4.5–6.5 mm long, broadly campanulate, densely villose and glandular papillate, distinctly 10-nerved, teeth triangular, mucronate/muticous, 2.5–3(–3.5) mm long, as long as tube or rarely longer. Corolla 6–8.5 mm long, blue, sparsely villose and glandular at upper lip and lower lip outside; upper lip 2-paired, lobes lanceolate; lower lip 3-partite, middle lobe broader than lateral lobes. Style unequally bifid and exceeding the calyx. Stamen 4, exceeding the upper lip; posteriors longer than inferiors. Nutlet pale brown, 2.2–2.7 mm long, densely hairy and glandular from 1/3 of base, hilum half of the inner surface.

Flowering and fruiting time: April–May.

Paratypes: Turkey. Muğla: Seydikemer district, Minare village, Pinara ancient city, Aşı stream, 400–750 m, 23.05.2019, Dirmenci (5250) (Hb. Dirmenci); Ibid. Aksoy 3004 (Hb. Dirmenci).

Etymology: The name *Teucrium semrae* was given in dedication to the first author's wife.

Proposed Turkish name: "Kaya Güzeli"

Habitat and ecology: The new species grows in calcareous steep rock crevices with or without maquis species (Figure 4). Species growing with the new species include *Inula heterolepis* Boiss., *Micromeria myrtifolia* Benth., *Origanum onites* L., *Phillyrea latifolia* L., *Pistacia terebinthus* L., *Salvia fruticosa* L., *Satureja thymbra* L., *Amygdalus orientalis* Mill., *Daphne sericea* Vahl. subsp. *sericea*, *Quercus coccifera* L.

Distribution and conservation status: *Teucrium semrae* is an East Mediterranean element known only from around the type location in southwest Turkey where its distribution area is less than 10 km^2. The total number of individuals was estimated to be approximately 500 (B2abi-v). Therefore, it should be regarded, according to the World Conservation Union (IUCN), as a critically endangered (CR) species (IUCN Species Survival Commission, 2014).

Species identification key of Sect. *Teucrium* in Turkey.

1.Leaves 2–3 pinnatisect; flowers born in spreading panicles

2.Plants densely pruinose; calyx teeth recurved to hooked at apex ..... **pruinosum**

2.Plants green or purple above; 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**

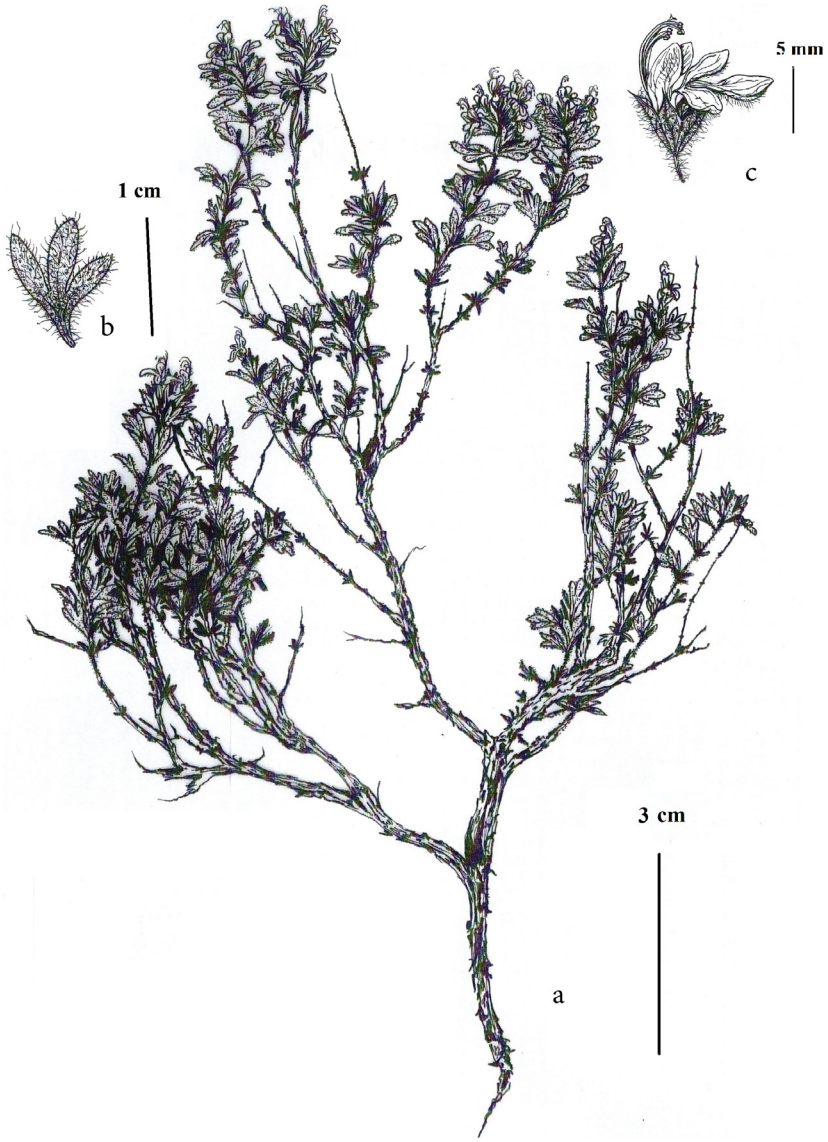

Figure 4. Habitus (a), leaf (b), and flower (c) of *Teucrium semrae* (illustrated by Özer Türkoğlu).

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 to lanceolate, corolla pink or lavender-blue

8.Leaves concolorous, flat, adpressed-tomentose on both surfaces, corolla pink to pinkish-white **... alyssifolium**

8.Leaves discolorous, flat, strongly or slightly revolute at margins, corolla whitish to lavender-blue

9.Leaves flat or slightly enrolled; calyx teeth triangular, acute, corolla whitish to whitish-blue **..... pseudaroanium**

9.Leaves strongly revolute at margins, calyx teeth lanceolate to acuminate, corolla lavender-blue

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

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

4.Leaves and bracts entire to 3-lobed

11. Leaves and bracts mostly 3-lobed,

12.Plants densely glandular; leaves lobes oblong to obovate ..... **semrae**

12.Plants e-glandular; leaves lobes linear to linear-lanceolate ..... **multicaule**

11.Leaves and bracts rarely 2-3 lobed

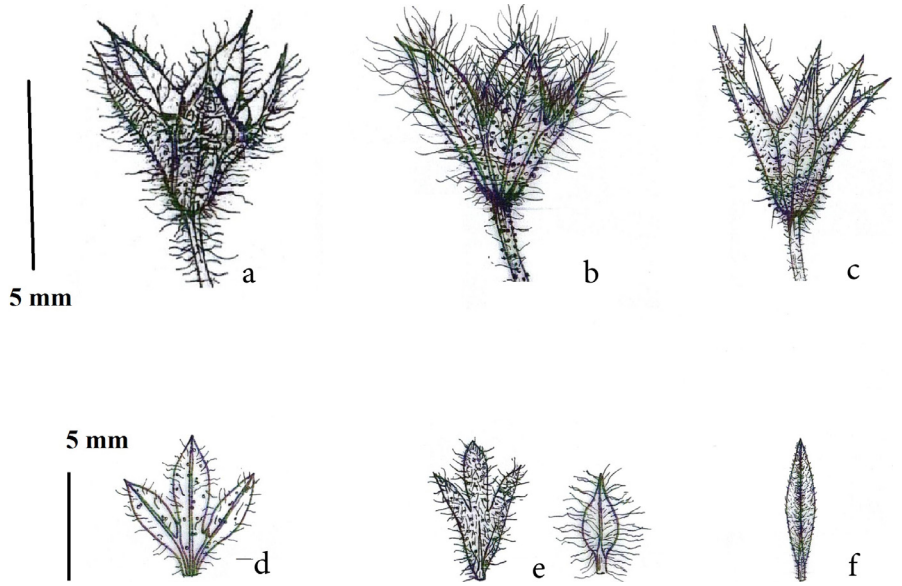

Figure 5. Calyx and bracts of *Teucrium semrae* (a, d), *T. ekimli* (b, e), and *T. pestalozzae* (c, f) (illustrated by Özer Türkoğlu).

Figure 6. Habitat and general appearance of *Teucrium semrae* (photo by Ahmet Aksoy).

Figure 7. Leaves, bracts and flowers of *Teucrium semrae* (a, d), *T. ekimii* (b, e, f), and *T. pestalozzae* (c, g) (photos by Ahmet Aksoy and Tuncay Dirmenci).

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

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

3.2. Micromorphological results

The findings regarding the indumentum on the stem, leaf, and nutlet of *Teucrium semrae*, *T. ekimii* and *T. pestalozzae* were evaluated according to Navarro and El Oualidi (2000) (Figures 8a–8l and 9a–9i). As can be seen in the Table 1 and Figures 8 and 9, *Teucrium semrae*, *T. ekimii* and *T. pestalozzae* have indumentum types as following, respectively: on stems type A1, A2, B and G (Figures 8a and 8d), adaxial of leaf type B and G (Figure 8g), abaxial of leaves type A1, B, G (Figure 8j), and nutlet surface type B and F4 (Figures 9a, 9d and 9g); on stems type B and I (Figures 8b and 8e), adaxial of leaf type B and G (Figure 8h), abaxial of leaves type B and G (Figure 8k), and nutlet surface type B and F4 (Figures 9b, 9d and 9h); on stems type B and G (Figures 8c and 8f), adaxial of leaf type G (Figure 8i), abaxial of leaves type B and G (Figure 8l), and nutlet B and F4 (Figures 9c, 9f and 9i).

3.3. Molecular results

Parsimony analysis with neighbour joining search was performed onto analyzed nrITS and cpDNA sequences. nrITS DNA had 682 characters for 67 taxa. 295 characters were constant and the proportion was 0.432. The variable parsimony-uninformative characters were 115, and the number of parsimony-informative characters was 272. Parsimony tree scores are as follows: consistency index (CI): 0.459, retention index (RI): 0.679 and homoplasy index (HI): 0.541.

70 taxa were analyzed with *trn*L-F sequences and 946 characters were obtained after being edited. 734 characters were constant and the proportion was 0.775, the variable parsimony-uninformative characters were 87, and the number of parsimony-informative characters was 125. Parsimony tree scores are as follows: consistency index (CI): 0.782, retention index (RI): 0.878 and homoplasy index (HI): 0.218.

According to the DNA results, nrITS data had more single nucleotide polymorphism in comparison to *trn*L-F data. So, constant character proportion was smaller and the number of parsimony-informative characters was higherin ITS than in *trn*L-F sequences. But on the other hand, there are a few insertions-deletions nucleotide sites in *trn*L-F data and they are very important to separate the *T. multicaule*/*T. ekimii*/*T. pestalozzae*/*T. semrae* group from the other members of sect. *Teucrium*.

4. Discussion

With 11 species (14 taxa), Sect. *Teucrium* is the second largest out of the eight sections in Turkey (Dirmenci,

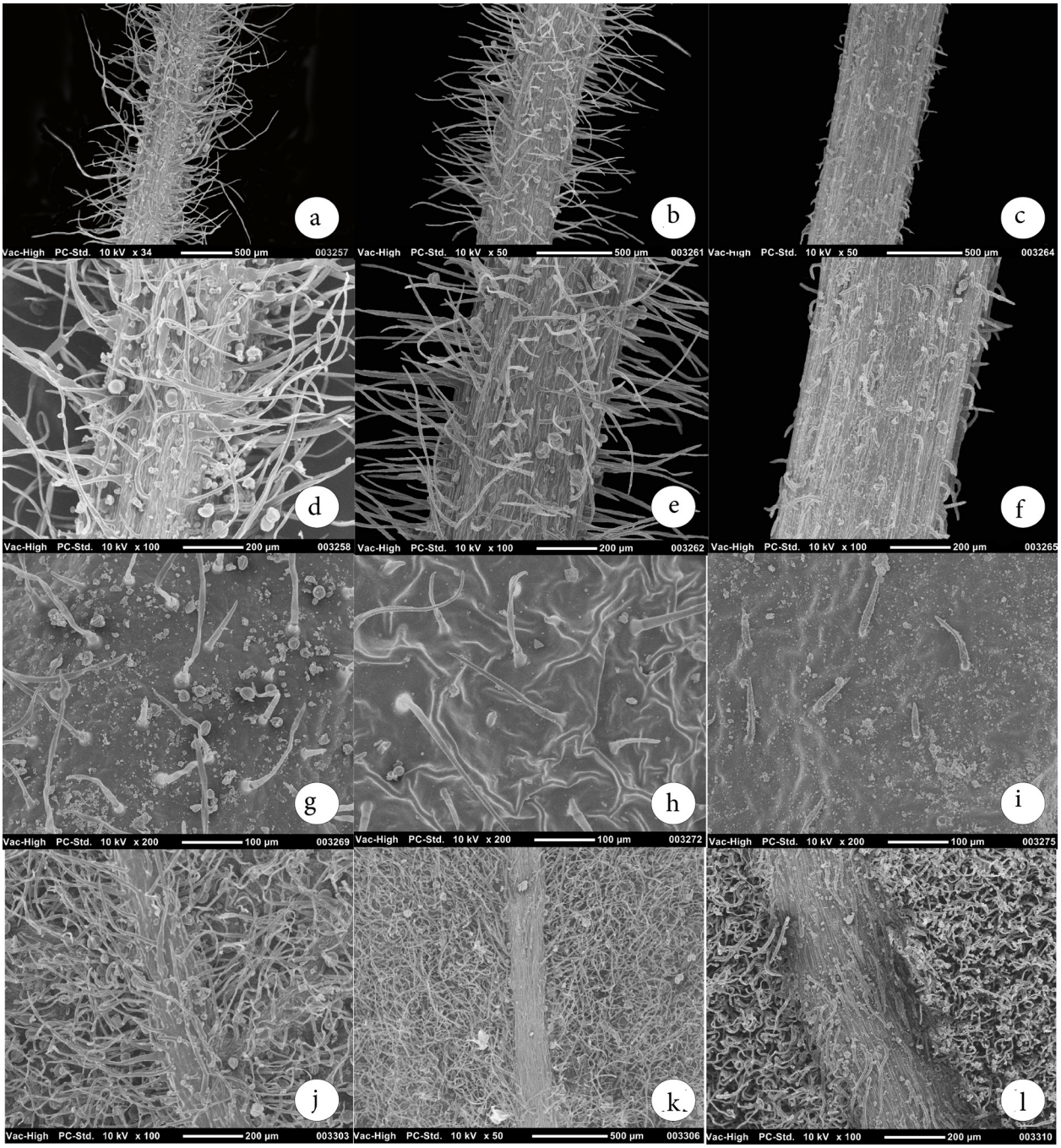

Figure 8. SEM micrographs of stems (*Teucrium semrae* a, d; *T. ekimli* b, e; *T. pestalozzae* c, f), adaxial and abaxial surface of leaves (*Teucrium semrae*. g, j; *T. ekimi* h, k; *T. pestalozzae* i, l).

2012; Ecevit Genç et al., 2015, 2017, 2018a, 2018b). In the Flora of Turkey (Ekim, 1982), the section *Teucrium* was divided into 2 groups based on leaf fragmentation and some calyx/corolla characters. In the first group, most of the leaves are 2–3 pinnatisect (*T. orientale*, *T. parviflorum*, and *T. pruinosum*). In the second group, the leaves are entire (*T. alyssifolium* Stapf, *T. brevifolium*, *T. creticum* L., *T. pseudaroanium* Parolly, Erdağ & Nordt, *T. sandrasicum* O.Schwarz), or entire to partly 3-partite (*T. multicaule*, *T. ekimii*, *T. pestalozzae*). (Ekim, 1982; Duman, 2000; Dirmenci, 2012; Ecevit Genç et al., 2015, 2017, 2018a, 2018b; Dinç and Doğu, 2016). According to our morphological observations and based on the Flora of Turkey, the members of Sect. *Teucrium* distributed

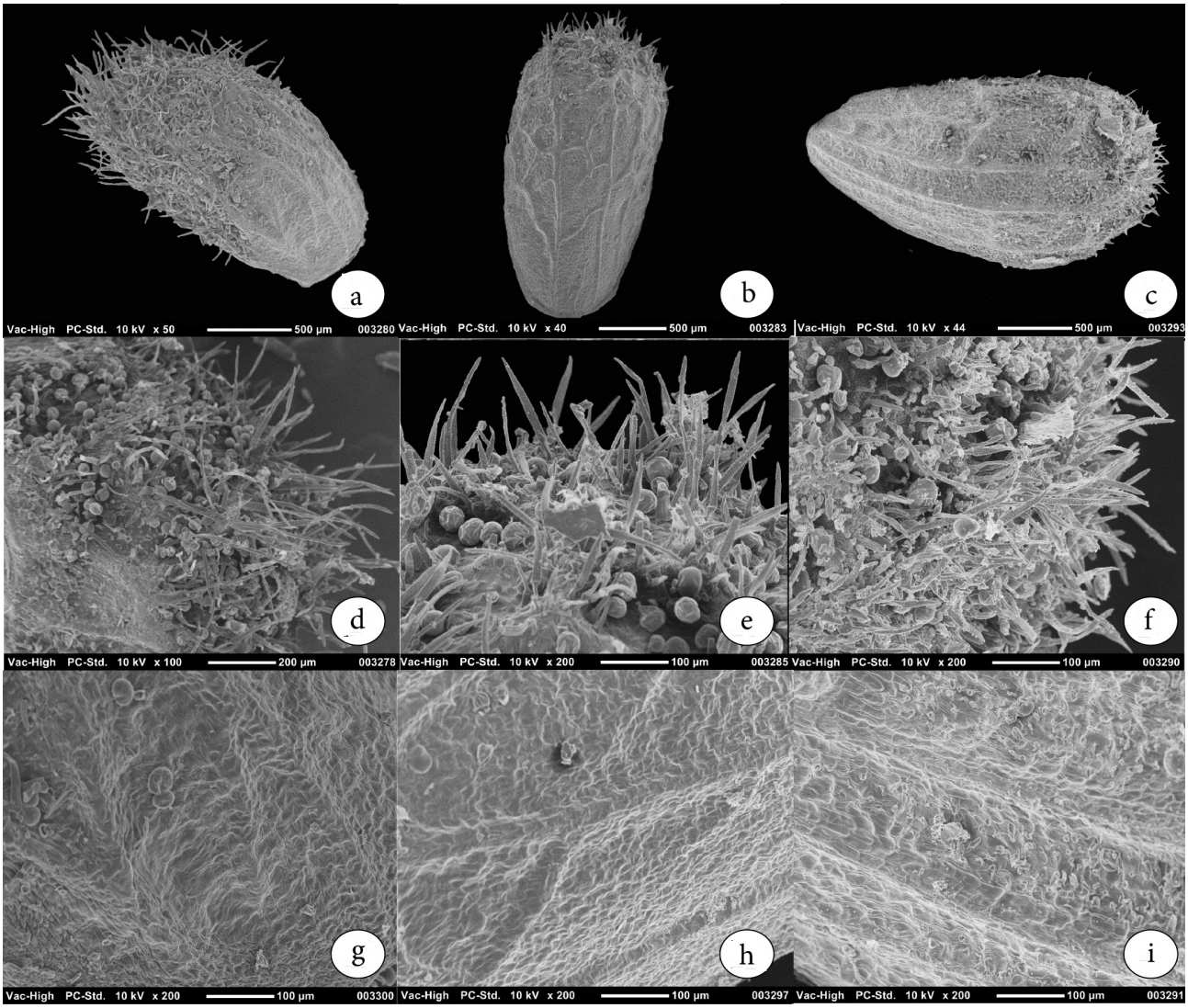

Figure 9. SEM micrographs of nutlets of *Teucrium semrae* (a, d, g), *T. ekimii* (b, e, h), and *T. pestalozzae* (c, f, i).

in Turkey should be classified under three groups as follows: The first group consists of *T. alyssifolium*, *T. pseudaroanium*, *T. brevifolium*, *T. creticum*, *T. sandrasicum* (taxa with simple and entire leaves); the second group consists of *T. ekimii, T. pestalozzae*, *T. multicaule* (with simple to 3-partite leaves); and the third group consists of *T. orientale s.l.* (var. *glabrescens* Hausskn. ex Bornm., var. *orientale*, var. *puberulens* Ekim), *T. parviflorum*, *T. pruinosum s.l.* (var. *pruinosum*, var. *aksarayense* M. Dinç & S. Doğu) (with 2–3 pinnatisect leaves).

nrITS and *trn*L-F data show that the distinction of the clades in Figures 10 and 11 coincides with the morphological structure of leaves. *T. creticum*/*T. sandrasicum* and *T. brevifolium*/*T. alyssifolium*/*T. pseudoaroanium* groups have simple and entire leaves, the *T. ekimii*/*T. pestalozzae*/*T. multicaule*/*T. semrae* group has simple and 3-partite leaves, while on the contrary, the *T. orientale* group members have obviously multipartite (2–3 pinnatisect) leaves and they are very different from the other members of this section. Although molecular data show great similarity with morphological classification, there are also important differences. First, three main clades of sect. *Teucrium* can be distinguished worldwide. Members of clade 7 (nrITS in Figure 10, clade 8 in Figure 11) growing in Australia and close countries, clade 8 (nrITS in Figure 10, clade 7 in Figure 11) comprises species distributed only in New World countries, and clade 9 (Figures 10 and 11) includes Turkish, European, North African, and West Asian species. Our results support Salmaki et al. (2016)'s molecular data. According to their results, species, which were previously placed in the sect. *Teucrium*, are monophyletic, called *Teucrium* core clade, and are divided into two major

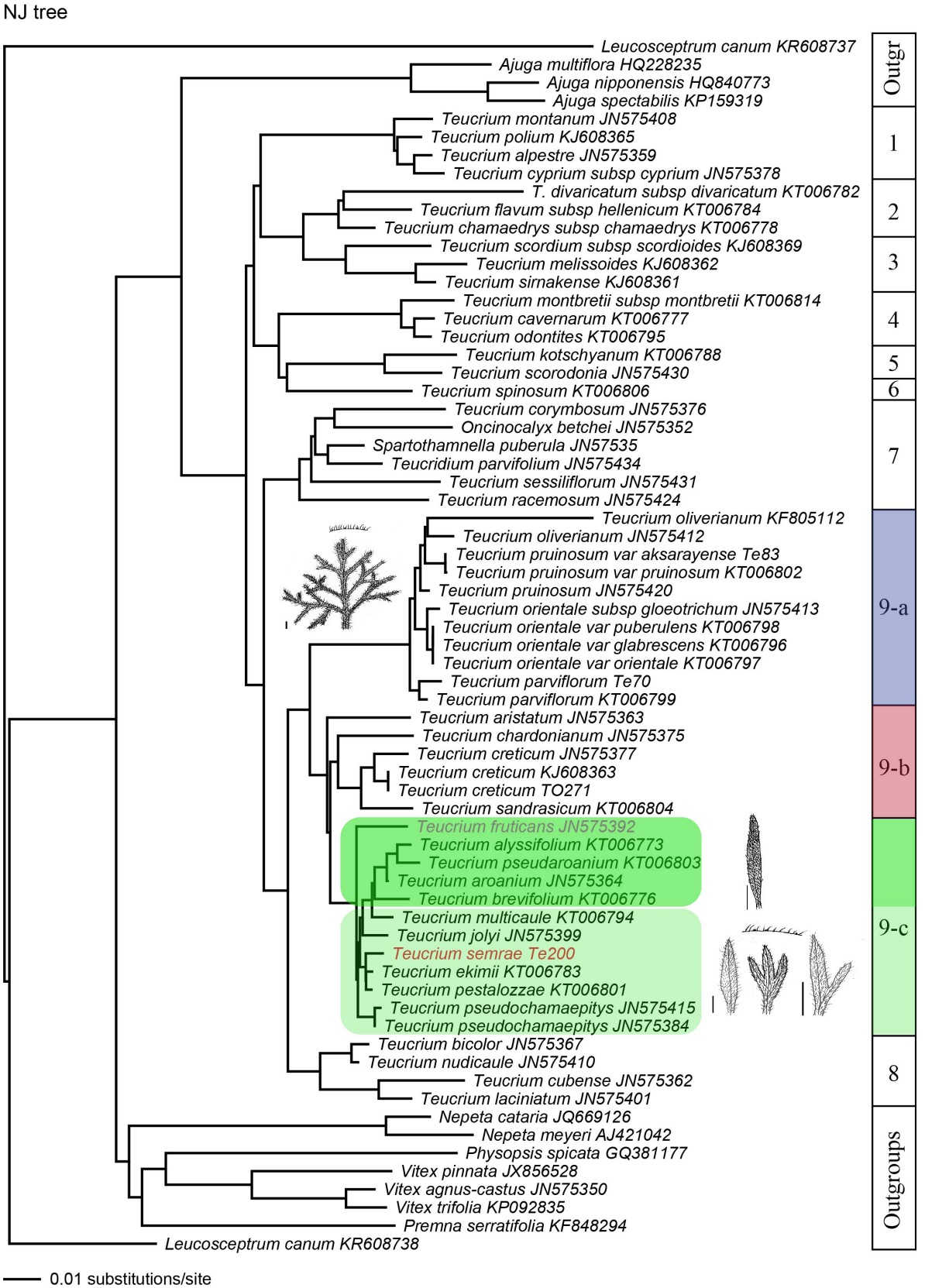

Figure 10. Neighbour-joining cladogram based on nrITS DNA data with demonstration of leaf distinction.

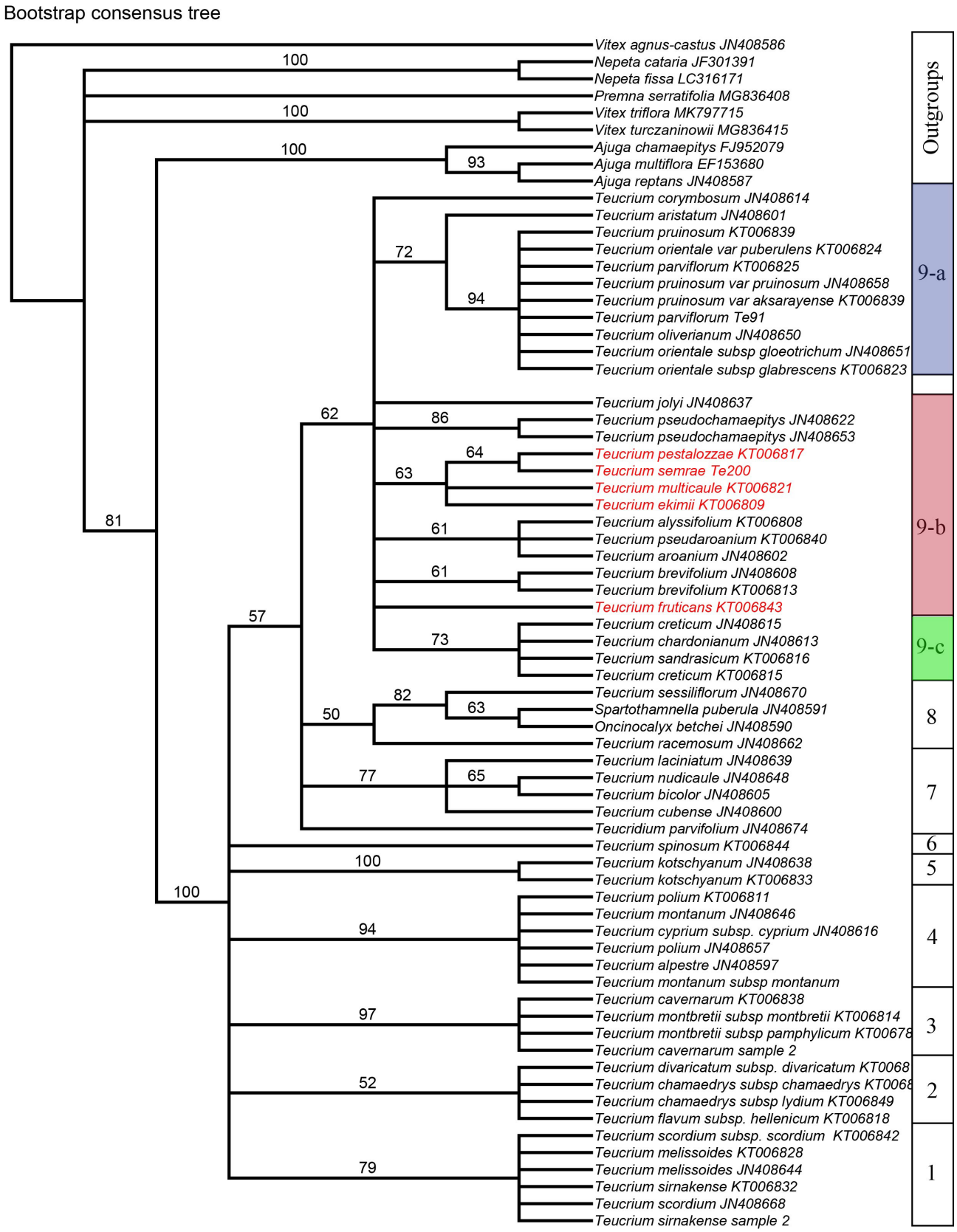

Figure 11. Maximum parsimony cladogram based on cp*trn*L-F DNA data.

lineages. The first one consists of subsect. *Cretica* Kästner and subsect. *Fruticantia* Kästner members, including *T. fruticans* L., type species of the genus. On the other side, *Teucridium* Hook.f., *Spartothamnella* Briq. And *Oncinocalyx* F.Muell. species and some Australian *Teucrium* species belong to the second one. In that study, *Teucridium*, *Spartothamnella* and *Oncinocalyx* species and some Australian Sect. *Teucrium* species differ from the New World Sect. *Teucrium* and Mediterranean Sect. *Teucrium* clades. In addition, when the clade consisting of Mediterranean Sect. *Teucrium* species (also mentioned as Clade 9-c in this study) is analyzed, three subclades can be distinguished: *T. brevifolium* clade, *T. creticum* clade and *T. orientale* clade. Their results coincide with the results obtained in this study. The first group comprises *T. orientale s.l.*, *T. parviflorum*, *T. pruinosum s.l.* and *T. oliverianum*. All the members of these four species have obviously 2–3 pinnatisect leaves and 3-partite bracts. In fact, these above-mentioned taxa (*Teucrium orientale* group) are different than the section *Teucrium* and the other sections' taxa with these leave characteristics. Despite these important distinctive features, not gibbous calyces at the base, almost equal calyx teeth, inflorescence structure, entire leaf margins and revolute leaves at the bottom cause these taxa to be included in the section *Teucrium*. The second group consists of *T. sandrasicum,T. creticum* and *T. chardonianum* Maire, Wilczek & Maire. Leaf structures of *T. sandrasicum* and *T. creticum* species are more similar than compared to *T. chardonianum* and other species. Although *T. chardonianum* spreads in Morocco and the shape of its leaves is quite different, it is similar to *T. sandrasicum* and *T. creticum* regarding the general morphology and woody stem. *Teucrium creticum* and *T. chardonianum* are sister species according to Salmaki et al. (2016) but their subsections are different: *Cretica* and *Fruticantia*, respectively. The third group is more complex. *Teucrium semrae* is a member of this group, (together with all the members of section *Fruticantia)*, which can be divided into three subgroups according to morphological and DNA data (clade 9-c in ITS tree, clade 9-b in *trn*L-F tree): shrub/shrublet and woody at base: *T. fruticans** and *T. brevifolium*; dwarf, suffruticose and large corolla: *T. alyssifolium, T. pseudaoranium* and *T. aroanium**, and the members of the last group have entire to 2–3-partite leaves and bracts: *T. ekimii*, *T. multicaule, T. pestalozzae*, *T. pseudochamaepitys** and *T. semrae* (*: these species are not distributed in Turkey).

There is another important study about the morphological classification of sect. *Teucrium*. Melnikov (2014) accepted the section *Teucrium* as a subgenus, and this subgenus was divided into three sections based on some morphological features, mainly leaf fragmentation and calyx structure. The first section *Teucrium* consists of *T. bicolor* Sm.*, T. brevifolium, T. fruticans*; the second section *Orientalia* Melnikov comprises of *T. aristatum* Pérez Lara, *T. campanulatum* L., *T. caucasigenum* Melnikov, *T. cravense* Maire, Molin. & Tallon, *T. cubense* Jacq., *T. fililobum* F. Muell. ex Benth., *T. laciniatum* Torr., *T. nudicaule* Hook., *T. orientale*, *T. pseudochamaepitys*, *T. sessiliflorum* Benth., *T. taylorii* Boiss, and the third section *Cretica* (Kästner) Melnikov includes *T. africanum* Thunb., *T. capense* Thunb., *T. corymbosum* R.Br., *T. creticum*, *T. parviflorum*, *T. racemosum* R.Br., *T. riparium* Hochst., *T. sandrasicum*. It is quite logical to separate Sect. *Teucrium* to different clades (subgroups). It can be seen from Figure 10 that Sect. *Teucrium* members are divided into three clades [Clade 7, 8, 9(a-c)]. Clade 8 (New World species) is the sister clade of Clade 9 members and Clade 7, containing South African and Australian Sect. *Teucrium* members, has close relationship with Clade 8 and 9. So, Sect. *Teucrium* has some major groups. On the other hand, Melnikov's sectional classification system (MGCS) is not fully consistent with results presented in Salmaki et al. (2016) and with the results of the morphological and molecular data presented in this study, especially regarding two major points: First, *T. brevifolium* and *T. fruticans* belong to the same group but *T. bicolor* is quite different morphologically and molecularly, as well as separated geographically. Further, *T. creticum* and *T. sandrasicum* can be classified into the same group, but *T. parviflorum* is more similar to *T. orientale*, not *T. creticum* and *T. sandrasicum*.

Molecular data obtained in this study supports the morphological and micromorphological differentiation of the new species and its allies. As seen from molecular results, the CI values are high, and the HI values are low for both of nrITS (0.541) and *trn*L-F (0.218) data. According to nrITS and cp*trn*L-F data, the new species belongs to the section *Teucrium*. Molecular data NJ and MP phylograms (Figures 10 and 11) indicate that *T. semrae* is a sister species to *T. ekimii, T. pestalozzae* and *T. multicaule.* These results are not surprising since they are associated with morphological data, especially leaf, bract, and calyx structure. *T. semrae*, *T. ekimii* and *T. pestalozzae* are Mediterranean elements and endemics that are distributed in restricted areas (Figure 3, green area 9b-1) and all these species are of chasmophytic habitus. On the other hand, *T. multicaule* is close to these species, but its distribution area is far away from them (Figure 3, green area 9b-2). *T. multicaule* is an Ir.-Tur. element, an erect and elongated species, and can grow in steppe or fallow fields. This subgroup includes also *T. pseudochamaepitys*. Although this species is not distributed in Turkey, it is very close to *T. multicaule* with

the structure of its leaves and bracts, general habitus and herbaceous stem.

Molecular data obtained in this study and the contributions ofthe above mentioned studies can be very useful to determine the position of *Teucrium semrae*. Also, morphological and indumentum characters were used to describe the new species. As used in many genera of Lamiaceae, the trichome morphology is very useful for the classification of all taxonomic levels (Marin et al., 1994; Navarro and El Oualidi, 2000; Grubesic et al., 2007; Moon et al., 2009; Salmaki et al., 2009; Eshratifar et al., 2011; Dinç et al., 2011a, 2011b; Doğu et al., 2013; Ecevit Genç et al., 2015, 2017; Marzouk et al., 2016; Karaismailoğlu and Güner, 2019). *T. semrae* was included in the Clade 9-c (Figure 10, 9-b in Figure 11) and its leaves and bracts are mostly 3-partite. It is closely related to the three Turkish endemic species *T. ekimii, T. pestalozzae,* and *T. multicaule* but can be distinguished from them especially by the fact that its leaves and bracts are mostly 3-partite, that there are clavate glandular trichomes on the stems, and that they have denser nonglandular and glandular trichomes on all aerial parts. As can be seen in the Table 1, adaxial and abaxial surfaces of the leaves of the three species generally have same trichome types, but the abaxial surfaces of leaves of *T. semrae* have different trichome type: A1. Also, *T. pestalozzae* had no B type on the adaxial surface of its leaves. Important differences were observed in the stem indumentum. Namely, *Teucrium pestalozzae* has G and B types, *T. ekimii* has I and B types, *T. semrae* has G, B, A1, and A2 types. Type A trichome was found on the nutlets of *Teucrium orientale* var. *glabrescens* and *T. pruinosum* with leaves 2–3 pinnatipartite in previous studies (Ecevit Genç et al., 2015). The data obtained in this study is consistent with previous studies and presents some important characters used to distinguish the new species and its allies.

The chromosome number counted in the genus *Teucrium* differs significantly in terms of taxonomy. In the literature it ranges from 2n = 10 to 104 in the genus *Teucrium* (http://www.tropicos.org/Project/IPCN). Chromosome changes in the sect. *Teucrium* can be useful and contribute to separate the section into subgroups. Reported chromosome numbers of *T. creticum, T. sandrasicum* (Özcan et al., 2015b), *T. aristatum* (Bayon, 1990), *T. fruticans* (Valdes-Bermejo and Crespo, 1978; Bayon, 1989; Gammar Ghrabi et al., 1989; Ruíz de Clavijo, 1990), *T. aroanium* (Bayon, 1990), *T. multicaule* (unpublished data), *T. pseudochamaepitys* (Valdes-Bermejo and Crespo, 1978) were 2n = 30. These species belong to Clade 9b and 9c in Figure 10. However, chromosomes of the Clade 9a members were not reported as 2n = 30, on the contrary, there is no evidence and record that they have more than 28 chromosomes. Chromosome numbers of *T. oliverianum* (Ghaffari, 2006), *T. orientale* (Kovdisheva and Akhmed-Zade, 1968; Aryavand, 1977) and *T. orientale* var *puberulens* (unpublished data) change from 2n = 18 to 2n = 28. Chromosome numbers of Clade 9a members distinguish them to Clade 9b and 9c. But a more detailed study about chromosome number is needed to support our results.

5. Conclusion

All the results obtained from different approaches demonstrated that the new species belongs to the sect. *Teucrium*. Its morphology is more similar to *T. ekimii*, *T. pestalozzae* and *T. multicaule* than to the other members of the section. With the new species from Turkey, the species number of the genus is raised to 37 (49 taxa).

Accordingly, sect *Teucrium* can be divided into three groups with the following species: *Fruticantia*: *T. fruticans, T. brevifolium, T. alyssifolium, T. pseudoaroanium, T. aroanium, T. jolyi, T. pseudochamaepitys, T. ekimii, T. pestalozzae, T. multicaule* and *T. semrae*; *Cretica*: *T. creticum, T. sandrasicum, T. chardonianum*; *Orientalia*: *T. oliverianum, T. orientale, T. parviflorum, T. pruinosum*.

We further conclude that the morphological system of Melnikov (2014) should be revised with more morphological and molecular data.

In addition, the data obtained in this study on the sect. *Teucrium* coincides with previous studies, provides some molecular data in addition to Salmaki et al. (2016), and presents some subsectional classification data in addition to the morphological classification system of Melnikov (2014). Sect. *Teucrium* should be divided into three groups as Melnikov mentioned, but it was concluded that Melnikov's system should be reviewed with additional morphological and molecular data. In addition, it was stated that it would be more correct to divide it into three subsections as *Orientalia*, *Fruticantia* and *Cretica*, not into two subsections as in Salmaki et al. (2016). As a result, this study has considered the previous studies and offers a more current and comprehensive classification using more specimens belonging to the Sect. *Teucrium*.

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Appendix

Examined specimens in this study and their GenBank codes: **ITS sequences:** *Teucrium parviflorum* Te70: MT271854; *Teucrium pruinosum* var. *aksarayense* Te83: MT271855; *Teucrium semrae* Te200: MT271856; *Teucrium creticum* TO271: MT271857.

trn*L-F Sequences:Teucrium parviflorum* Te91: MT274685; *Teucrium semrae* Te200: MT274686; *Teucrium sirnakense* Te48-2: MT274687; *Teucrium montanum* subsp. *montanum* Te95: MT274688; *Teucriumc avernarum* Te34: MT274689.