

Micromorphological studies on nutlet and leaf indumentum of genus *Teucrium* L. (Lamiaceae) in Iran

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Abstract: The micromorphology of leaf indumentum and nutlet surface of 12 species belonging to the 6 sections of the genus *Teucrium* L. was studied by scanning electron microscope (SEM). Eight types of trichomes are identified including unbranched unicellular trichomes, unbranched multicellular trichomes, sessile glands, subsessile glands, stalked-multicellular glands long clavate glandular trichomes (with 3-5 cells), trichomes with large thin-wall, trichomes with non-micropapillate basal cell, which are the most general types on the adaxial side of the leaves, and branched trichome with long axis is the exclusive type and present in *Teucrium polium* L. Thus, trichomes can be used as a distinctive taxonomic character in species classification. Nutlet shapes in most species are elliptic-ovate, the surface of which is polygonal with different ornamentations and wall thickness, and the indumentum varies in different species, but it has some significant similarities within the sections. The absence or presence of the indumentum and its different types has high taxonomical values among the species.

Key words: *Teucrium*, Lamiaceae, micromorphology, SEM, leaf indumentum, nutlet surface, Iran

Introduction

The genus *Teucrium* L., with about 100 species, is a large genus distributed in Europe, North Africa, and temperate parts of Asia, but mainly in the Mediterranean region (Mabberley, 1997) along with the genera *Stachys* L. (Akçiçek, 2010) and *Sideritis* L. (Güvenç & Duman, 2010). *Teucrium* has 19 taxa in Iran, which mainly grow in the Irano-Turanian region between 700 and 2000 m above sea level (Rechinger, 1982). Among them *Teucrium persicum* Boiss. is the only one endemic to Iran (Rechinger, 1982). *Teucrium*

species are distributed in most regions of Iran. i.e. *T. persicum* is present only at elevations of southern regions as a Saharo-Sinidian element, while *T. hyrcanicum* L., which is a hyrcanian element, grows in the north of Iran. Some species, namely *Teucrium polium* L. and *T. orientale* L., are widely distributed in steppe, arid, and semiarid regions.

Micromorphological characters, especially trichomes, are one of the most useful taxonomic features in *Teucrium*. Their absence or presence and also their typology have a significant role in

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classification of the genus. Several studies are present on *Teucrium* morphology and micromorphology. Research on *Teucrium* species based on pollen micromorphology was presented by Dönmez et al. (1999). There is a comparative anatomical and micromorphological study between *Teucrium creticum* L. and *Teucrium orientale* var. *orientale* by Dinç et al. (2009); also a survey by Grubešić et al. (2007) related to the micromorphology in *Teucrium* species growing in Croatia. A paper by Navarro and El Oualidi (2000) focusing on trichome morphology in *Teucrium* has useful information about trichome distribution on the calyx, corolla, leaves, and nutlets of *Teucrium* L. species of the Mediterranean area.

In this survey the studied species include 6 sections as follows: section *Polium*: *T. polium* and *T. stocksianum* Boiss., section *Chamaedrys*: *T. chamaedrys* L., section *Isotridon*: *T. persicum*, section *Scordium*: *T. scordium* L. subsp. *scordioides*. Schreb., *T. scordium* L. subsp. *serratum*. (Benth.), and *T.*

melissoides Boiss. & Hausskn. ex Boiss.; section *Stachyobotrys*: *T. hyrcanicum* L.; section *Teucris*: *T. parviflorum* Schreb., *T. oliverianum* Gingins., and *T. orientale* subsp. *orientale*, subsp. *taylori* (Boiss.) Rech.f., subsp. *gloeotrichum* Rech.f., subsp. *glabrescens* (Hausskn. & Bornm.) Rech. f.

Materials and methods

This present study is based on herbarium specimens of Central Herbarium of Tehran University (TUH) and IRAN herbarium. The list of studied specimens is given in Table 1.

For SEM studies, leaves and mature nutlets were prepared, and then were mounted directly on 12.5 nm diameter stubs. The specimens were coated in a sputter coater with approximately 25 nm of gold-palladium. They were observed and photographed by Leo-440i Scanning Electron Microscope.

Table 1. List of species, with location of voucher specimens of leaf indumentum and seed.

Sect. <i>Polium</i>	
<i>Teucrium polium</i>	Kurdistan: Sanandaj, 1360 m, 15.6.1992, Attar et al. 14276-TUH.
<i>T. stocksianum</i>	Hormozgan: Bandar-Abbas, Tange zagh, 10 m, Ghahreman 8324-TUH.
Sect. <i>Chamaedrys</i>	
<i>T. chamaedrys</i>	Ardebil: Ahar, Kaleybar, 1580 m, 17.7.1993, Attar 17255-TUH.
Sect. <i>Isotridon</i>	
<i>T. persicum</i>	Bushehr: Bandar-e Taheri, Assalouyeh, 450 m, 21.3.2005, Ghahreman & Attar 32895-TUH.
Sect. <i>Scordium</i>	
<i>T. scordium</i> subsp. <i>scordioides</i>	W. Azerbaidjan: Between Urumieh and Salmas, 1380 m, 16.7.1997, Mozaffarian 70100-TUH.
<i>T. scordium</i> subsp. <i>serratum</i>	Khorassan: south of Birjand, Razg, 1800 m, 24.10.1997, Aliabadi 22181-TUH.
<i>T. melissoides</i>	Lorestan: road of Andimeshk, Pol-e Dokhtar, 750 m, 28.10.1993, Ghahreman et al. 17611-TUH.
Sect. <i>Stachyobotrys</i>	
<i>T. hyrcanicum</i>	Gilan: Lahijan, Hassan be Kandeh, 25 m, 20.6.2001, Naghinezhad 27837-TUH.
Sect. <i>Teucris</i>	
<i>T. parviflorum</i>	Lorestan: Khorramabad, road of Sefid-dasht, 50 km after bifurcation of Khorramabad-Sefid dasht, 1950 m, 18.6.1998, Ghahreman et al. 21841-TUH.
<i>T. oliverianum</i>	Khouzestan: Bagh-Malek, Haparou to Sidan, 800 m, 30.5.1979, Iranshahr & Moussavi 25709.1-IRAN.
<i>T. orientale</i> subsp. <i>glabrescens</i>	Hamadan: Razan to Avaj, 10 km to Avaj, 2200-2300 m, 10.7.1994, Chehregani & Zarre 17891-TUH.
<i>T. orientale</i> subsp. <i>gloeotrichum</i>	Lorestan: Khoramabad, Cham-Divan, Chal-e Ahmad, 1400 m, 20.5.1999, Veiskarami 23934-TUH;
<i>T. orientale</i> subsp. <i>orientale</i>	Tehran: Jajrud, 1968, Farideh Zagh 8312-TUH.
<i>T. orientale</i> subsp. <i>taylori</i>	East Azerbaidjan: N. slopes of Mishou-Dagh, south of the main road, near Yam (S. of Marand), 1950 m, 19.6.2001, D.Podlech & Sh.Zarre 55248-TUH.
<i>T. orientale</i> subsp. <i>taylori</i>	Kordestan: Bidjar to Sanandaj, 72 km to Sanandaj, 1950 m, 1.7.1971, Terme 25748.2-IRAN.

Results

Micromorphological characters of upper surface indumentum of leaf

Generally, trichomes are branched, long and knotted, simple, glandular and eglandular, one-cellular to multicellular. The shape, length and surface of trichomes in different species varied. Basal cell can be conic and the terminal cell lance-shaped, also surface of trichomes can be smooth, micro-papillate or macropapillate. Some trichomes are short clavate or long clavate. Branched trichome is exclusive to *T. polium*. In most species single secretory cells are observed.

Descriptions and abbreviations of indumentums present based on Navarro and El Oualidi (2000) are as follows (Table 2):

1. Glandular trichomes

1.1. Simple glandular trichomes

A: Clavate glandular trichomes

A1. Short clavate glandular trichome, with 2 large and thin stalk cells or 3 stalk cells, with reduced neck and usually without micro-papillae (Figure 1A-B). A2. Long clavate glandular trichomes with 3(5) cells with thick-walled cells, with long thin-walled basal cell without micropapillae (Figure 1C-F).

B: Subsessile glandular trichomes

B1. One-celled (Figure 1F-H).

B2. Two-celled.

B4. Four-celled.

2. Non-glandular trichomes

2.1. Simple unbranched trichomes

F: Thin-walled trichomes

Table 2. Indumentum types and their diversity in different species of *Teucrium*.

Taxa	A1	A2	B1	B2	B4	F2	F4	F5	G2	G3	I	N
Sect. <i>Polium</i>												
<i>T. polium</i>					•							•
<i>T. stocksianum</i>		•	•									•
Sect. <i>Chamaedrys</i>												
<i>T. chamaedrys</i>			•						•			
Sect. <i>Isotrion</i>												
<i>T. persicum*</i>		•	•								•	
Sect. <i>Scordium</i>												
<i>T. scordium</i> subsp. <i>scordioides</i>		•	•			•						
<i>T. scordium</i> subsp. <i>serratum</i>	•		•						•			
<i>T. melissoides</i>	•	•		•					•			
Sect. <i>Stachyobotrys</i>												
<i>T. hyrcanicum</i>		•	•			•	•					
Sect. <i>Teucriis</i>												
<i>T. parviflorum</i>			•			•						
<i>T. oliverianum</i>			•			•						
<i>T. orientale</i> subsp. <i>orientale</i>			•			•						
<i>T. orientale</i> subsp. <i>glabrescens</i>		•	•									
<i>T. orientale</i> subsp. <i>gloeotrichum</i>			•				•		•			
<i>T. orientale</i> subsp. <i>taylori</i>			•						•			

* Endemic to Iran

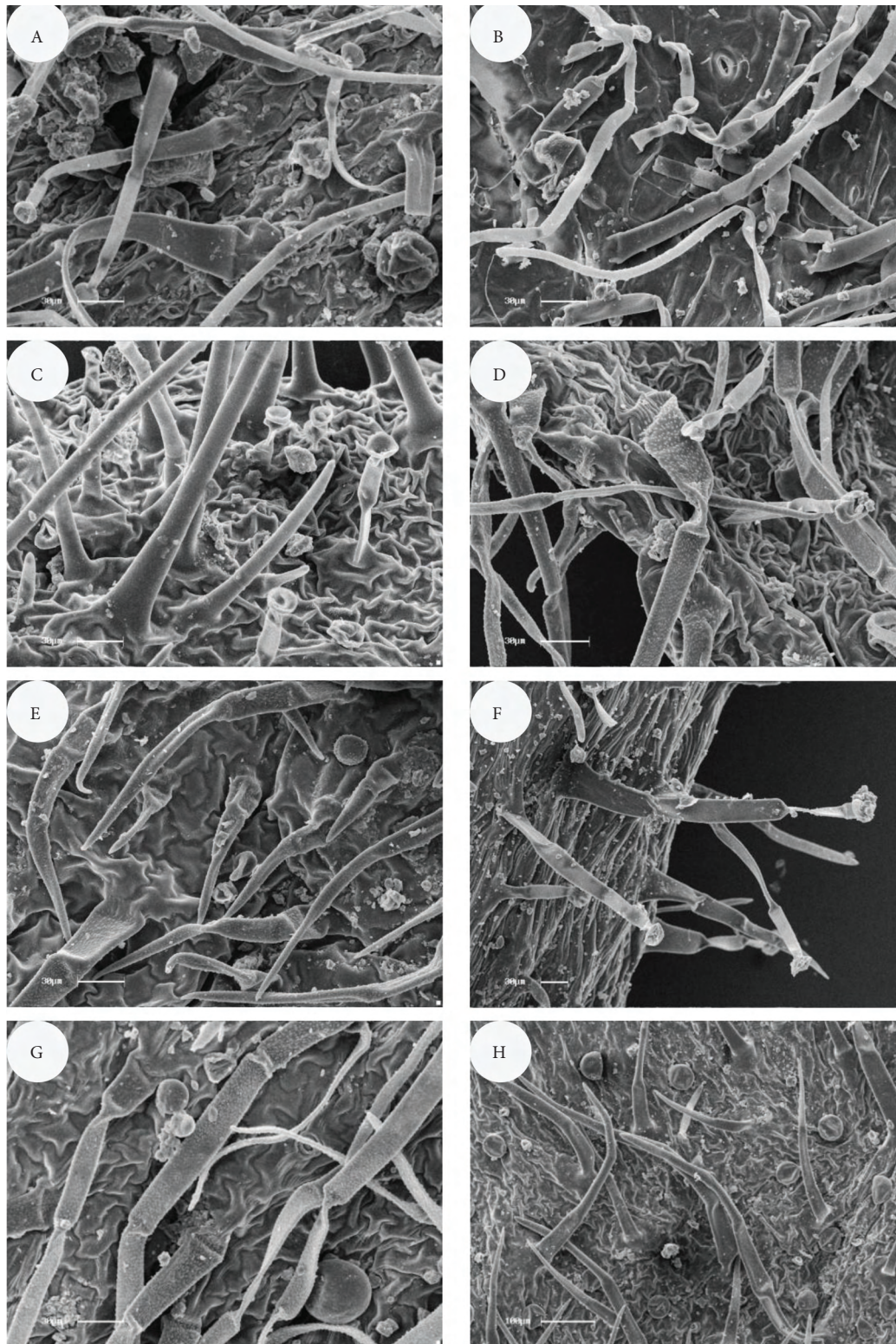


Figure 1. Scanning electron micrographs of indumentum in species of *Teucrium*. A- *T. scordium* subsp. *serratum*, B- *T. melissoides*, C- *T. persicum*, D- *T. scordium* subsp. *scordioides*, E- *T. hyrcanicum*, F- *T. orientale* subsp. *glabrescens*, G- *T. orientale* subsp. *taylori*, H- *T. chamaedrys*. A, B, C, D, E, F, G: scale bar = 30 μ m; H: scale bar = 100 μ m.

F2. Large, very thin-walled, 2(5)-celled trichomes, with an acute apical cell, with ridges and marked internodes (Figure 2A-C). F4. Short thin-walled, 2-celled trichomes densely covered by micro-papillae, with the apical cell elongated, in triangular shape (Figure 2D). F5. Elongated and flexuose, thin-walled, 3-7(11)-celled trichomes, with internodes distinct, the apical cell acute with micro-papillae, the basal cell smooth, each cell transverse to the preceding one, without micropapillae.

G: Thick-walled trichomes

G2. Elongated, generally adpressed, thick-walled 3(5)-celled trichomes, folded with rounded micro-papillae, hooked (Figure 2D). G3. Elongated thick-walled trichomes (3)5-7(8)-cells, erect with rounded epidermal cell and elongated stalk cells and slightly flexuose.

I. Vermiform hairs, thick-walled with elongated cells (Figure 2E).

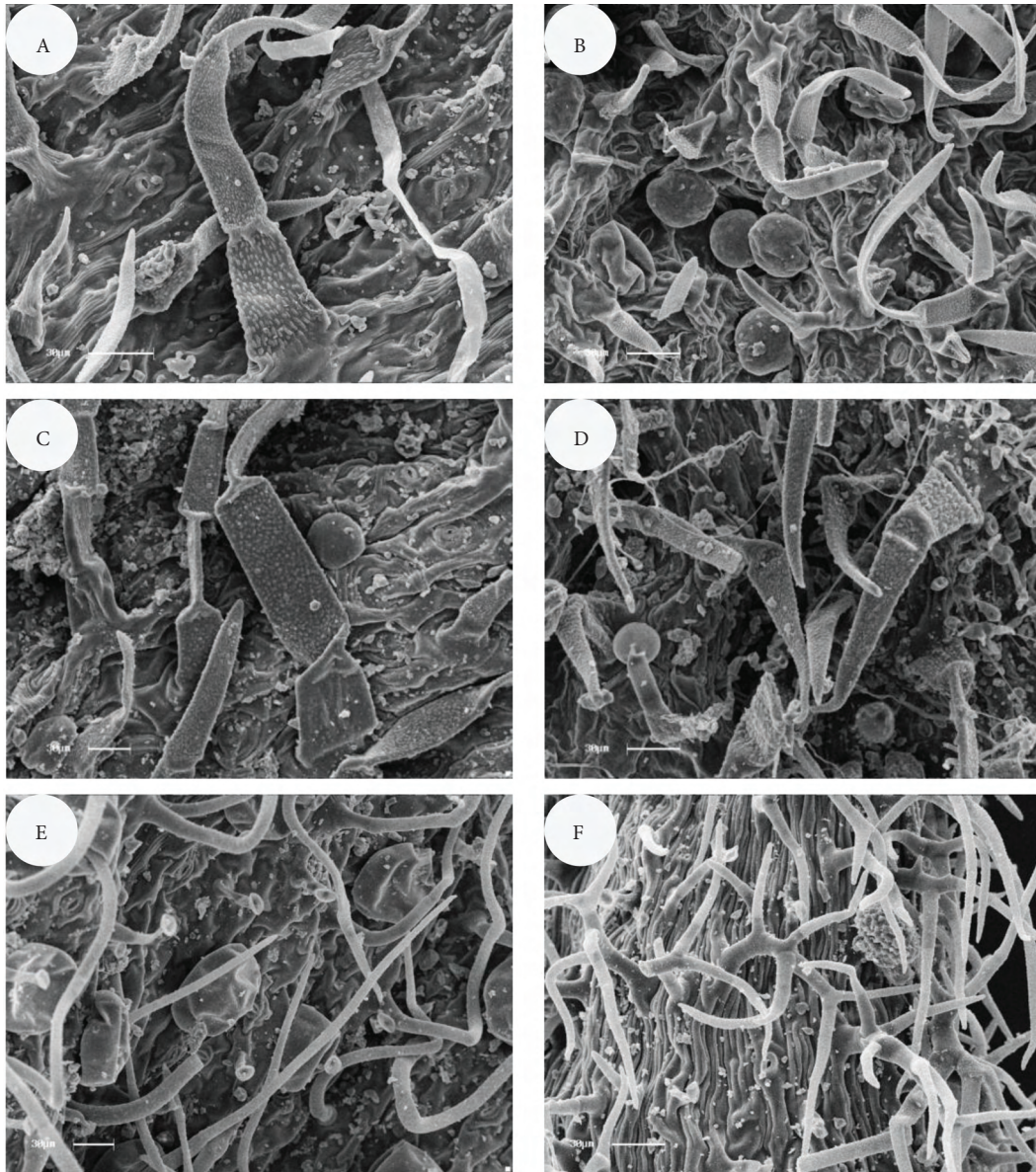


Figure 2. Scanning electron micrographs of indumentum in species of *Teucrium*. A- *T. parviflorum*, B- *T. oliverianum*, C- *T. orientale* subsp. *orientale*, D- *T. orientale* subsp. *gloeotrichum*, E- *T. stocksianum*, F- *T. polium*. Scale bar= 30 µm.

2.2. Branched trichomes

N. Long branched hairs, with short axis and very long thick-walled arms with long axis (Figure 2F).

Nutlet shape and surface

Three specific nutlet shapes, namely elliptic, elliptic-ovate, and ovate, were observed among the studied species. The size of nutlets ranges from 1.02 to 3.5 mm in length and 0.73 to 1.95 mm in width (Table

3). The nutlet surface is generally alveolate, but it has some specific differences in depth and shape between species. Among the examined species *T. parviflorum* and *T. orientale* subsp. *orientale* have the largest nutlets while species such as *T. hyrcanicum* (Figure 3) possess very small ones (Table 3). The alveoli of the nutlet surface differ in depth and shape. In some species such as *T. polium* and *T. stocksianum* the alveoli are deep while in some others like *T. scordium*

Table 3. Micromorphological characters of nutlet coat and their indumenta.

Taxon	Nutlet length × width (mm)	Nutlet shape	Nutlet hairs
Sect. <i>Polium</i>			
<i>T. polium</i>	2.07 × 1.5	Elliptic-ovate, polygonal, alveoli deep, subregular, very thick wall	Glabrous
<i>T. stocksianum</i>	1.6 × 1.08	Elliptic-ovate, alveoli deep, regular, thin wall	Glabrous
Sect. <i>Chamaedrys</i>			
<i>T. chamaedrys</i>	2.18 × 1.25	Elliptic, indistinctly polygonal, alveoli irregular, thick wall	Glabrous
Sect. <i>Scordium</i>			
<i>T. scordium</i> subsp. <i>serratum</i>	1.07 × 0.77	Ovate, indistinctly polygonal, alveoli large shallow, thin wall	Unicellular hairs, sparse
<i>T. melissoides</i>	1.3 × 0.75	Elliptic-ovate, alveoli irregular, thin wall	Unicellular hairs, sparse
Sect. <i>Stachyobotrys</i>			
<i>T. hyrcanicum</i>	1.02 × 0.73	Elliptic-ovate, polygonal alveoli large, deep, regular, thick wall	Unicellular hairs, sparse
Sect. <i>Teucriis</i>			
<i>T. parviflorum</i>	3.5 × 1.95	Elliptic-ovate, polygonal, alveoli indistinct, irregular, shallow, thick wall	Unicellular hairs, dense; glands, 2-cellular; micropapillate; multicellular trichomes
<i>T. oliverianum</i>	2.7 × 1.6	Elliptic-ovate, polygonal, alveoli large, shallow, irregular, thick wall	Dense multicellular trichomes, immixed papilla
<i>T. orientale</i> subsp. <i>orientale</i>	2.92 × 1.72	Elliptic-ovate, polygonal alveoli large, regular, very thick wall	Unicellular hairs, very dense sparsely long multicellular trichomes
<i>T. orientale</i> subsp. <i>glabrescens</i>	2.58 × 1.38	Ovate, large distinct irregular polygonal, very thick wall	Subsessile glands, with sparse thin branched appressed trichomes
<i>T. orientale</i> subsp. <i>gloeotrichum</i>	2.07 × 1.38	Ovate, polygonal, alveoli very large, shallow, irregular, very thick wall	Unicellular hairs, dense; sessile glands
<i>T. orientale</i> subsp. <i>taylori</i>	2.6 × 1.6	Elliptic-ovate, polygonal, alveoli very large, shallow, irregular, very thick wall	Subsessile unicellular hairs, with dense thick branched appressed trichomes

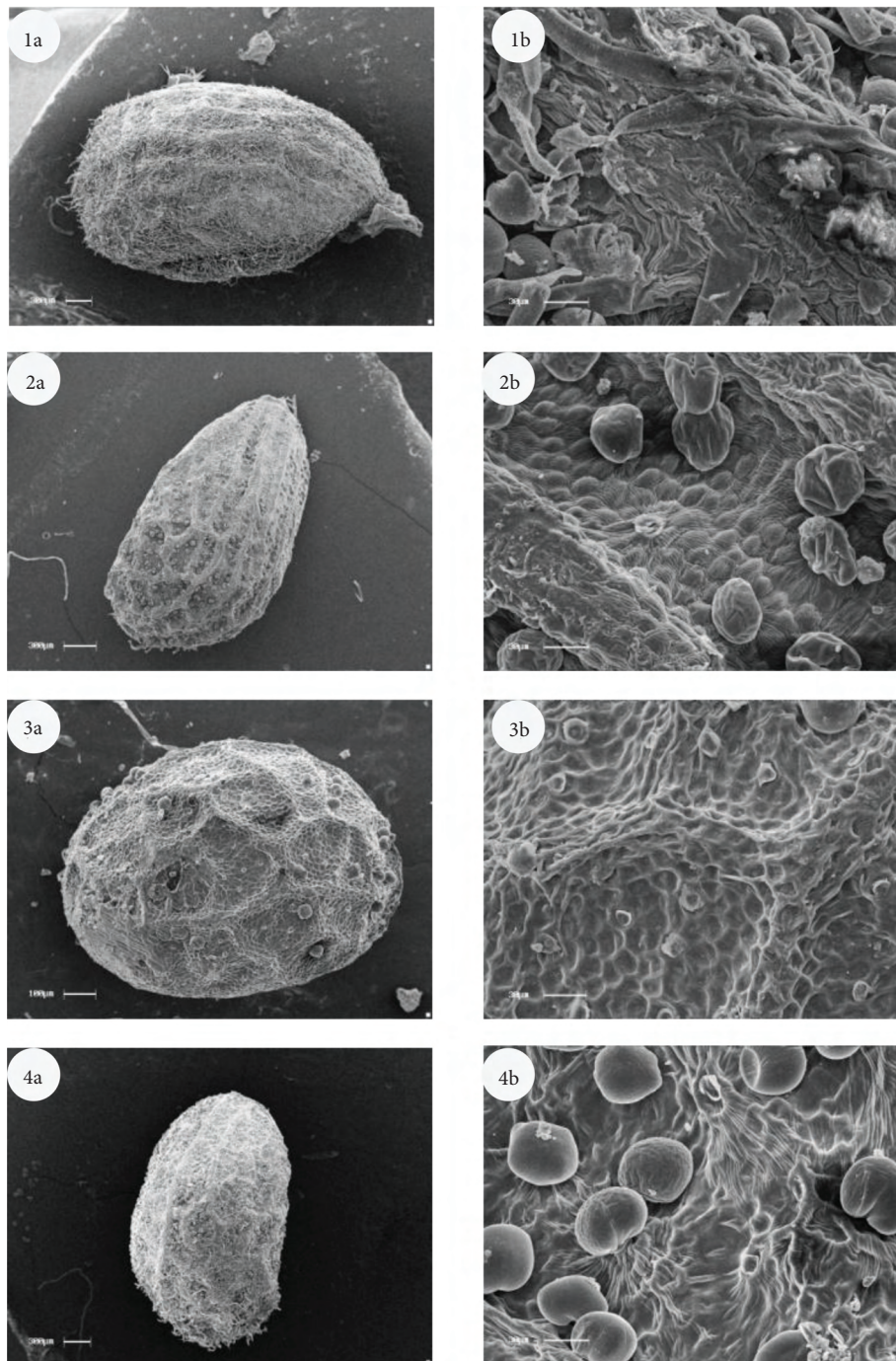


Figure 3. Scanning electron micrographs of nutlets in species of *Teucrium*. 1a-b. *T. parviflorum*, 2a-b. *T. orientale* subsp. *orientale*, 3a-b. *T. hyrcanicum*, 4a-b. *T. orientale* subsp. *gloeotrichum*. 1a, 2a, 4a: scale bar = 300 μm ; 3a: scale bar = 100 μm ; 1b, 2b, 3b, 4b: scale bar = 30 μm .

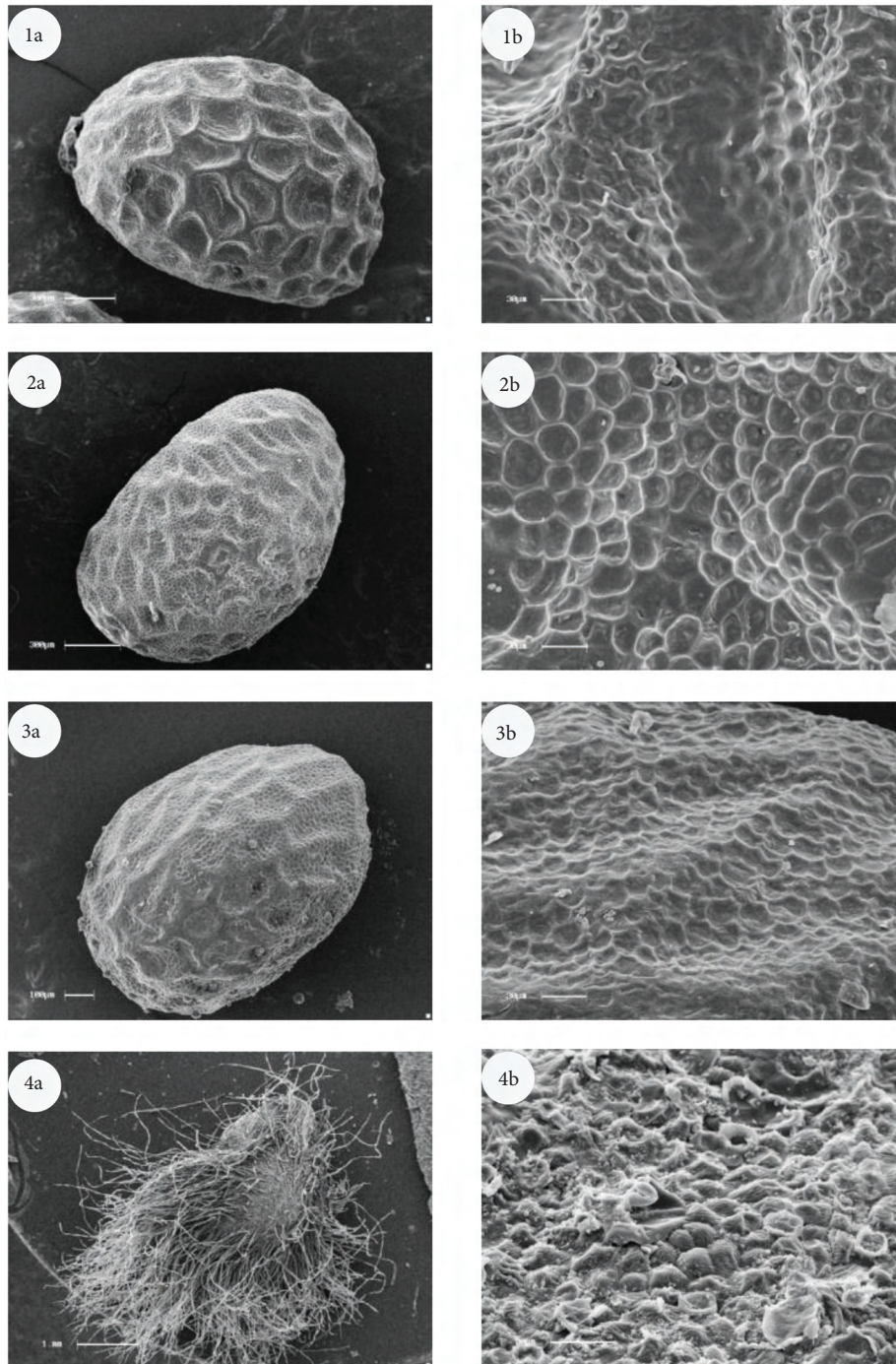


Figure 4. Scanning electron micrographs of nutlets in species of *Teucrium*. 1a-b. *T. polium*, 2a-b. *T. stocksianum*, 3a-b. *T. scordium* subsp. *serratum*, 4a-b. *T. oliverianum*. 1a, 2a: scale bar = 300 μ m; 3a: scale bar = 100 μ m; 4a: scale bar = 1 mm; 1b, 2b, 3b, 4b: scale bar = 30 μ m.

subsp. *serratum*, *T. parviflorum*, and *T. oliverianum* (Figure 4) they are shallow. The wall thickness of nutlets in all species of section *Teucrium* is thick and

very thick while in some other species like *T. scordium* subsp. *serratum* and *T. melissoides* (Figure 5) the nutlet wall is thin.

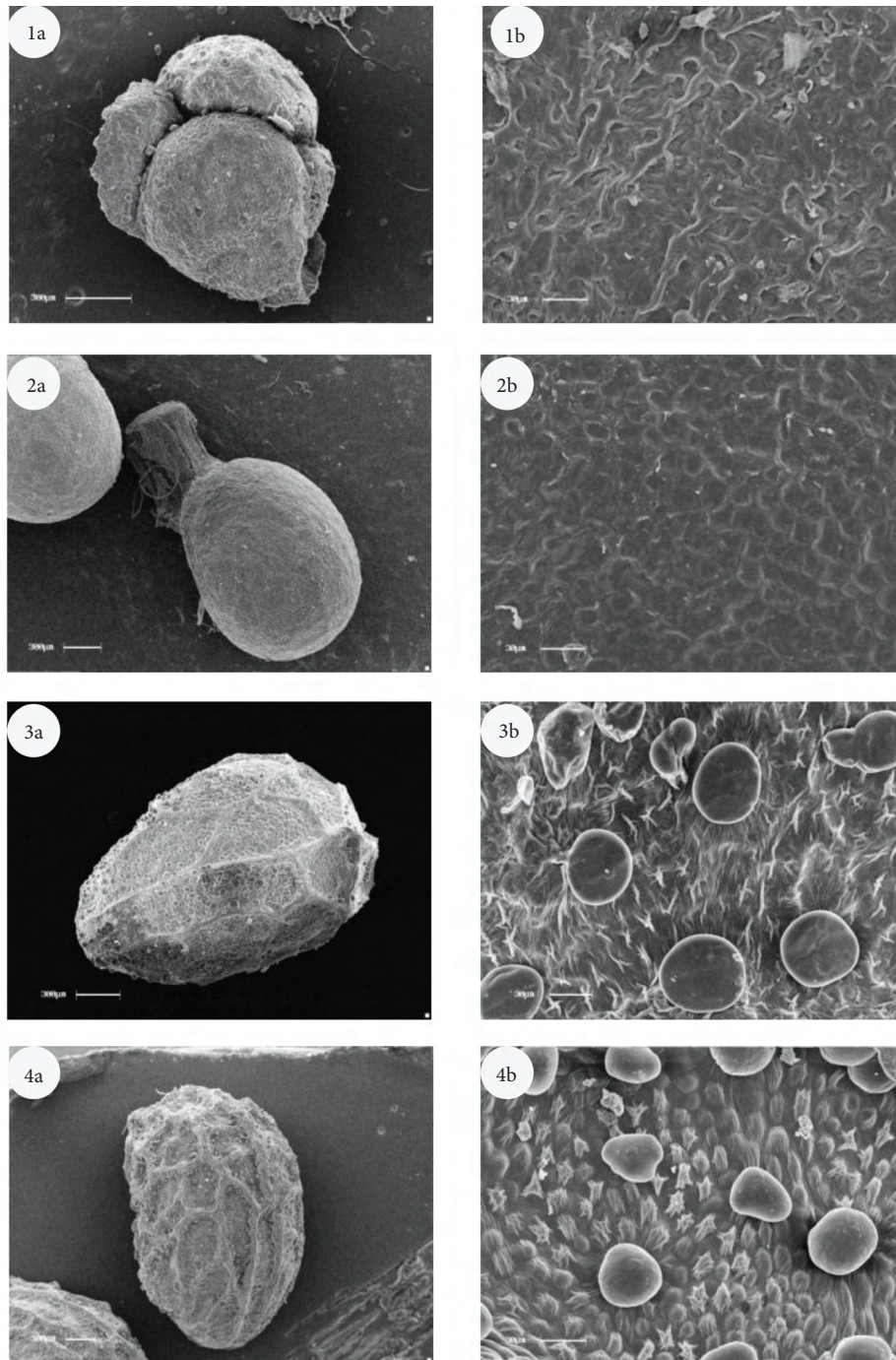


Figure 5. Scanning electron micrographs of nutlets in species of *Teucrium*. 1a-b. *T. melissoides*, 2a-b. *T. chamaedrys*, 3a-b. *T. orientale* subsp. *glabrescens*, 4a-b. *T. orientale* subsp. *taylori*. 1a, 2a, 3a, 4a: scale bar = 300 μm; 1a, 2b, 3b, 4b: scale bar = 30 μm.

Nutlet hairs

Nutlets are glabrous in species such as *T. polium*, *T. stocksianum*, and *T. chamaedrys* (Figure 5). The type of hairs also shows variability among the species. Hairs are unicellular, sparse and dense, multicellular or branched appressed trichomes. Sparse unicellular hairs are present in species like *T. scordium* subsp. *serratum*, *T. melissoides*, and *T. hyrcanicum*, multicellular trichomes as in *T. parviflorum*, *T. oliverianum*, and *T. orientale* subsp. *orientale*, and finally branched appressed trichomes which are observed in *T. orientale* subsp. *glabrescens* and subsp. *taylori* (Figure 5), the former being sparse and the latter being dense.

Discussion

In this study, according to Table 2, 12 trichome types were observed in different species of *Teucrium*. Among them B1 is the most general type and is present in most species like *T. stocksianum*, *T. chamaedrys*, *T. persicum*, *T. scordium* subsp. *scordioides*, *T. scordium* subsp. *serratum*, *T. hyrcanicum*, *T. parviflorum*, *T. oliverianum*, and all 4 subspecies of *T. orientale*. It should be mentioned that this type of trichome is present in *T. chamaedrys* and *T. orientale*, which were studied by Navarro and El Oualidi (2000). The second common type is A2, which is present in the following 6 species: *T. stocksianum* (also reported by Navarro and El Oualidi (2000)), *T. persicum*, *T. scordium*, *T. melissoides*, *T. hyrcanicum*, and *T. orientale* subsp. *glabrescens*. F2 and F5 are frequent in some taxa as well. F2 is observed among *T. scordium* subsp. *scordioides*, *T. hyrcanicum*, *T. parviflorum*, *T. oliverianum* and *T. orientale* subsp. *orientale*, also F5 is present in *T. scordium* L. subsp. *serratum*, *T. melissoides*, and *T. orientale* subsp. *taylori*. Type F5 was observed in *T. melissoides* examined by Navarro and El Oualidi (2000) as well. Other trichome types are more specific to some certain species. A1 type can be seen in *T. scordium* subsp. *serratum* and *T. melissoides*. F4 is only observed in 2 species: *T. hyrcanicum* and *T. orientale* subsp. *gloeotrichum*. G2 type includes *T. chamaedrys* (also reported by Navarro and El Oualidi (2000) and *T. orientale* subsp. *gloeotrichum*. The types B2, G3, I, and N are rare in the genus. Each is present in only one

species: B2 in *T. melissoides*, G3 in *T. persicum*, I in *T. stocksianum*, and type N in *T. polium*.

Based on the micromorphological studies of nutlet the following results were obtained: Generally, nutlet shape is categorised in 3 types: ovate, elliptic-ovate, and elliptic.

Ovate in *T. scordium* subsp. *serratum*, *T. orientale* subsp. *glabrescens* and subsp. *gloeotrichum*, elliptic in *T. hyrcanicum* and *T. oliverianum*, and elliptic-ovate in the rest of the species.

Nutlet is glabrous in *T. polium*, *T. stocksianum*, and *T. chamaedrys*. In 3 species, namely *T. scordium* subsp. *serratum*, *T. hyrcanicum*, and *T. melissoides*, the sparse unicellular hairs are observed. Nutlet in *T. parviflorum* and *T. oliverianum* is covered with dense multicellular trichomes; *T. parviflorum* has the micropapillate trichomes also. Indumenta of 2 subspecies, namely *T. orientale* subsp. *gloeotrichum* and subsp. *taylori*, are similar to each other.

The nutlet surface is polygonal regular or irregular, of which the alveoli are deep or shallow. Wall thickness in 4 species, namely *T. polium*, *T. orientale* subsp. *gloeotrichum*, subsp. *glabrescens*, and subsp. *taylori*, is very thick, in *T. chamaedrys*, *T. hyrcanicum*, *T. parviflorum* and *T. oliverianum* is thick, and in the rest of the species is thin.

Based on Table 3 some micromorphological characters like nutlet shape, surface, and indumentum type are the significant characters for classifying the species. As shown in Table 3, the nutlet shape is generally similar among the species of each section. e.g., the 2 species belonging to the section *Polium* are the same in nutlet shape and hair type. Also in the section *Teucris* some meaningful nutlet similarities such as shape, surface, and hair type are observed among the species.

Combining all data, micromorphology of *Teucrium* has high systematic value and strongly supports the actual subdivision within this genus based on morphological data.

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