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Trichome micromorphology of the genus *Stachys* sect. *Fragilicaulis* subsect. *Fragilis* and its taxonomic implications

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ABSTRACT
The leaf, stem, calyx, and corolla indumentum of seven endemic taxa belonging to *Stachys* subsect. *Fragiles*, growing in Turkey, were micromorphologically and anatomically analyzed under scanning electron and light microscopes with the use of cluster analysis and Principal Component Analysis. We detected 10 trichome types on the leaf, seven trichome types on the stem and calyx, and eight trichome types on the corolla. Especially, the type and distribution of the trichomes on the calyx and corolla surfaces were quite different. The calyx and corolla had seven and eight trichome types, including glandular or non-glandular, in the examined taxa, respectively. The most common type observed was a short clavate glandular trichome; however, conical thin-walled unicellular hairs were specific for the studied taxa. No branched trichomes were observed. In the examined taxa, the indumentum indicated variations and some important similarities. The indumentum types have a high taxonomical value for the species of the genus. A key has been offered for the identification of the studied taxa based on the trichome characters, by comparing the key of classic taxonomic characters.

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Introduction

Stachys L. is one of the largest genera of the family Labiatae (Lamiaceae) and comprises about 435 taxa belonging to 370 species (Harley et al. 2004; Güner 2016). It comprises annual and perennial herbs and shrubs. It has a wide distribution in the Mediterranean, southwestern Asia, North and South America, and Southern Africa (Akçiçek et al. 2012). Taxa of the genus are especially numerous in the warm temperate areas of the Mediterranean and southwestern Asia; they are not found naturally in Australia and New Zealand (Bhattacharjee 1980; Akçiçek et al. 2012). The genus is represented in Turkey by 118 taxa belonging to 90 species (Bhattacharjee 1980; Davis et al. 1988; Scheen et al. 2010; Güner and Akçiçek 2015; Celep and Dirmenci 2017).

Sect. *Fragilicaulis* R. Bhattacharjee comprises 30 taxa belonging to two subsects: *Fragiles* Rech. and *Multibracteolatae* R. Bhattacharjee. These taxa are rupicolous suffrutescent perennials and their stems are fragile at the base (Bhattacharjee 1982; Rechinger 1982; Davis et al. 1988; Güner and Akçiçek 2015; Karaismailoğlu and Güner 2019). Subsect. *Fragiles* comprises seven species, mainly distributed in the warm temperate regions of the Mediterranean, and is endemic to Turkey (Güner 2016). *Fragiles* species dwell on damp rocks by waterfalls and limestone crevices. The subsect. differs from other subsects. by its geographic distribution, inconspicuous bracteoles, and triangular to triangular-lanceolate calyx teeth (Bhattacharjee 1980, 1982).

Many taxonomical studies on *Stachys* have been conducted in various phytogeographical regions (Ball 1972; Nelson 1981; Bhattacharjee 1982; Rechinger 1982; Demissew and Harley 1992; Lindqvist and Albert 2002; Salmaki et al. 2012). In these studies, the diagnostic characteristics were frequently related to the number of flowers per verticillasters, structure of calyx tube, corolla colour, bracteole size, and stem structure (Salmaki et al. 2012). Numerous morphological features in *Stachys* are labile (Falciani 1997). Hence, finding additional diagnostic characters is crucial.

The taxonomic and systematic relevance of trichomes in Lamiaceae is well known, as well as in other families of Lamiales, such as Acanthaceae, Bignoniaceae, Plantaginaceae, Scrophulariaceae, and Verbenaceae (Metcalfe and Chalk 1950; El-Gazzar and Watson 1968, 1970; Ahmad 1974, 1978; Elias and Newcombe 1979; Mathew and Shah 1983; Abu-Asab and Cantino 1987; Cantino 1990; Rahn 1992; Navarro and El Oualidi 1999; Gairola et al. 2009; Ecevit-Genç et al. 2015). Trichomes and their presence–absence, typology, and distribution in the different plant structures are generally useful characters in the systematics of the Lamiaceae family (Davis and Heywood 1964; Rechinger 1982; Bhattacharjee 1982; Davis et al. 1988; Cantino 1990; Sebebe and Harley 1992; Servettaz et al. 1992; Marin et al. 1994; Navarro and El Oualidi 1999; Giuliani et al. 2008; El Beyrouthy et al. 2009; Salmaki et al. 2009; Kahraman et al. 2010; Baran et al. 2010; Rezakhanlo and Talebi 2010; Grujıć et al. 2014; Celep et al. 2014; Eiji and Salmaki 2016; Atalay et al. 2016; Sajna and

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Table 1. The studied *Stachys* taxa and their locations.

Subsection	Taxa	Location	Voucher
Fragiles	*Stachys longiflora* Boiss. & Balansa	Mersin: Işıktepe, 250 m, 10.07.2013	Ö.Güner 2364
	S. euadenia Davis	Mersin: Anamur-Ermenek, 1410 m, 10.07.2013	Ö.Güner 2369
	S. pinardii Boiss.	Antalya: Feslikan road, 300–350 m, 20.04.2013	Ö.Güner 2301
	S. antalyensis Ayaşligil & Davis	Antalya: Manavgat, 1275 m, 25.05.2013	Ö.Güner 2355
	S. buttleri Mill.	Antalya: Düden, 88 m, 12.07.2013	Ö.Güner 2372
	S. pseudopinardii R. Bhattacharjee & Hub.-Mor.	Mersin: Silifke, 30 m, 23.04.2013	Ö.Güner 2303
	S. chasmosericea Ayaşligil & Davis	Antalya: Manavgat, 825 m, 11.07.2013	Ö.Güner 2371

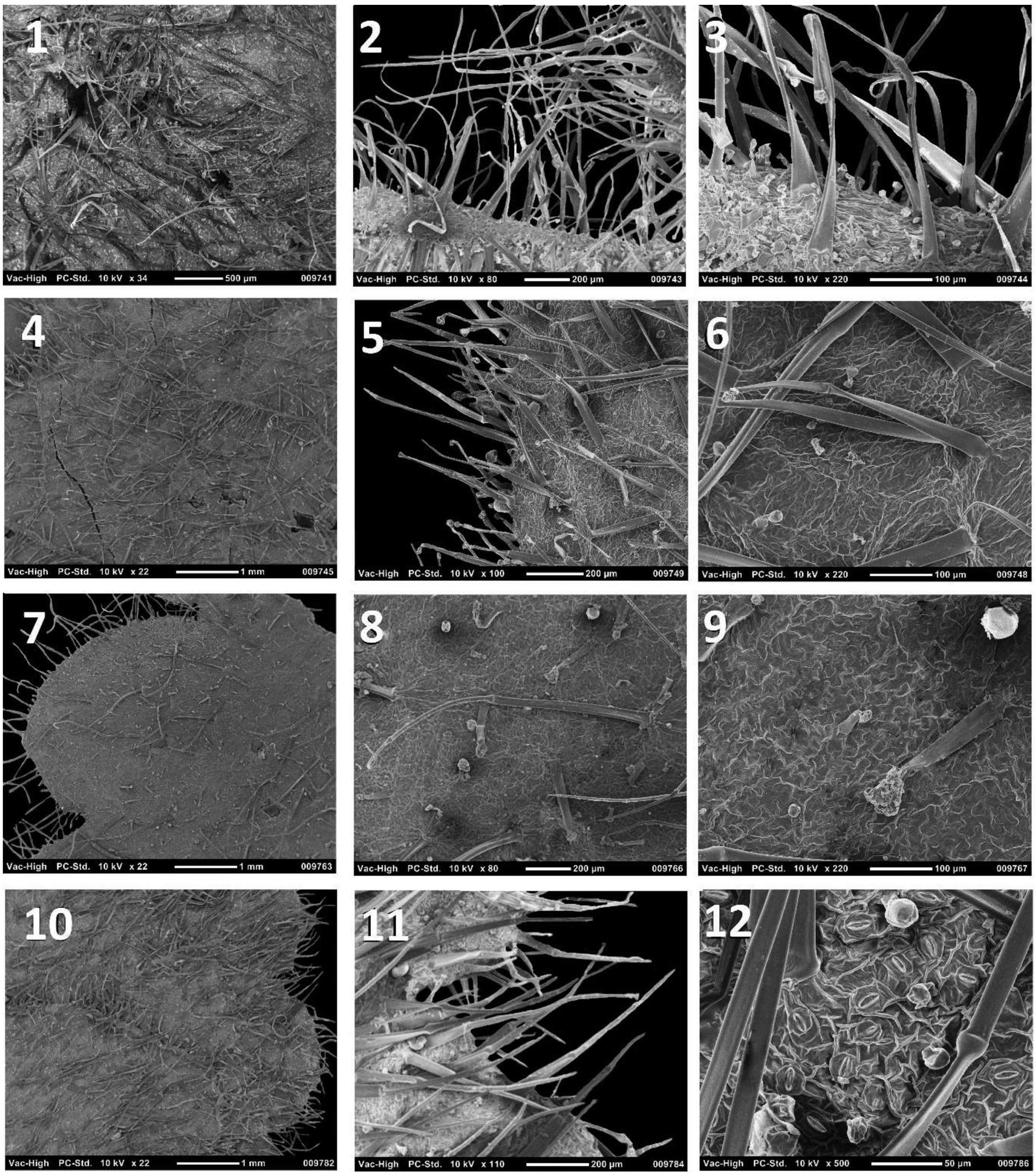

Figure 1. The upper surface of leaves of the examined *Stachys* taxa: **1–3:** *Stachys longiflora* (A1, A2, D1, D2, D3, and E2), **4–6:** *S. euadenia* (A1, A2, and D2), **7–9:** *S. pinardii* (A1, B, and E2), **10–12:** *S. antalyensis* (A1, A2, B, D2, and E2) (See Table 2 for character codes).

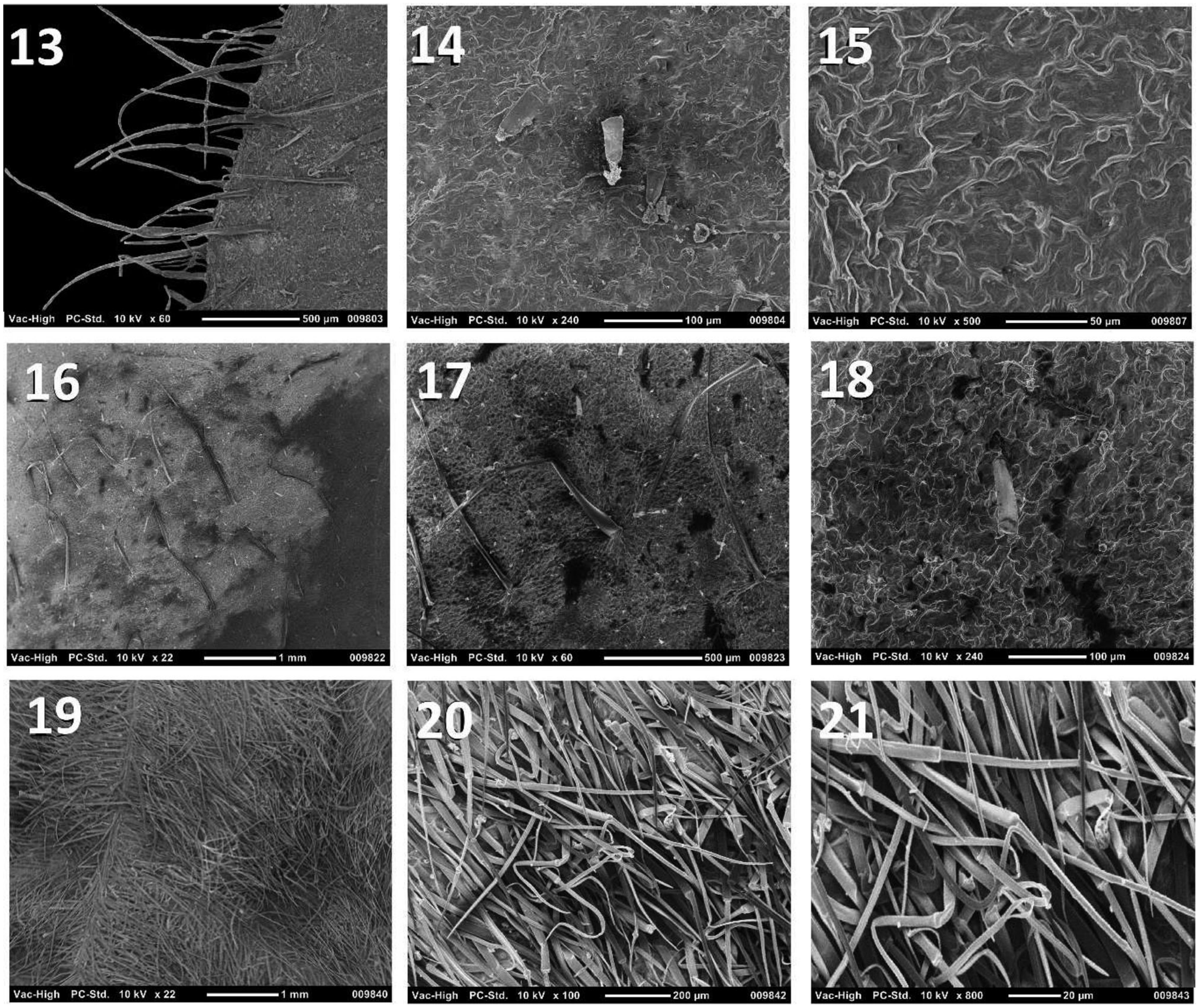

Figure 1 **(Cont.).** The upper surface of leaves of the examined *Stachys* taxa: **13–15:** *Stachys buttleri* (A1, A2, C, and E2), **16–18:** *S. pseudopinardii* (A1, B, and E2), **19–21:** *S. chasmosericea* (A2, B, E1, and E2) (See Table 2 for character codes).

Sunojkumar 2018); however, there is no comprehensive studies on trichome characters in the recognition and classification of taxa belonging genus *Stachys* from Turkey.

The aim of the present study was to examine the trichome morphology of the species of *Stachys* subsect. *Fragiles* to evaluate the utility of these characters.

Materials and methods

Studied taxa were collected from Mediterranean geographical region of Turkey by Ö. Güner. Herbarium vouchers were deposit in GAZI herbarium (Table 1).

The trichomes on the stems, leaves, calyces, and corollas of the examined taxa were analyzed. Samples for micromorphological observations were placed on a stub with silver epoxy, and surrounded with platinum-gold. Later, they were examined using a JEOL Neoscope-5000 scanning electron microscope (SEM) (Figures 1–5).

The trichome micromorphology and structure of the leaves, stems, calyx, and corolla were described and classified. The classification was generally based on that of Roe (1971) and Navarro and El Oualidi (1999). The characters considered in the classification and their character codes are presented in Table 2.

Anatomically, the trichome observations were made in samples of the stems, leaves, calyx, and corolla obtained from the herbarium materials. Cross-sections were taken using a fully automatic microtome (Thermo Shonda Met Finesse). Later, they were treated with various series of alcohol and xylene, stained with hematoxylin in a staining device (ASC 720 Medite), and enclosed in Entellan (Figure 6) (Gerlach 1977; Karaismailoğlu 2015, 2016). An Olympus CX21FS1 microscope was used to examine the samples.

The data obtained from the studied parameters were assessed using the Multi Variate Statistical Package (MVSP) software program (Kovach 2007). Firstly, a matrix was created according to data derived from the presence/absence (1/0) of the different types of trichomes distributed in the plant structures analyzed. Later, grouping of the taxa was done using the cluster analysis process (Unweighted Pair Group Method with Arithmetic mean = UPGMA) in accordance with the 10 trichome types in Table 3 (Figure 7). In addition, the similarity matrix, and Principal Component Analysis (PCA)

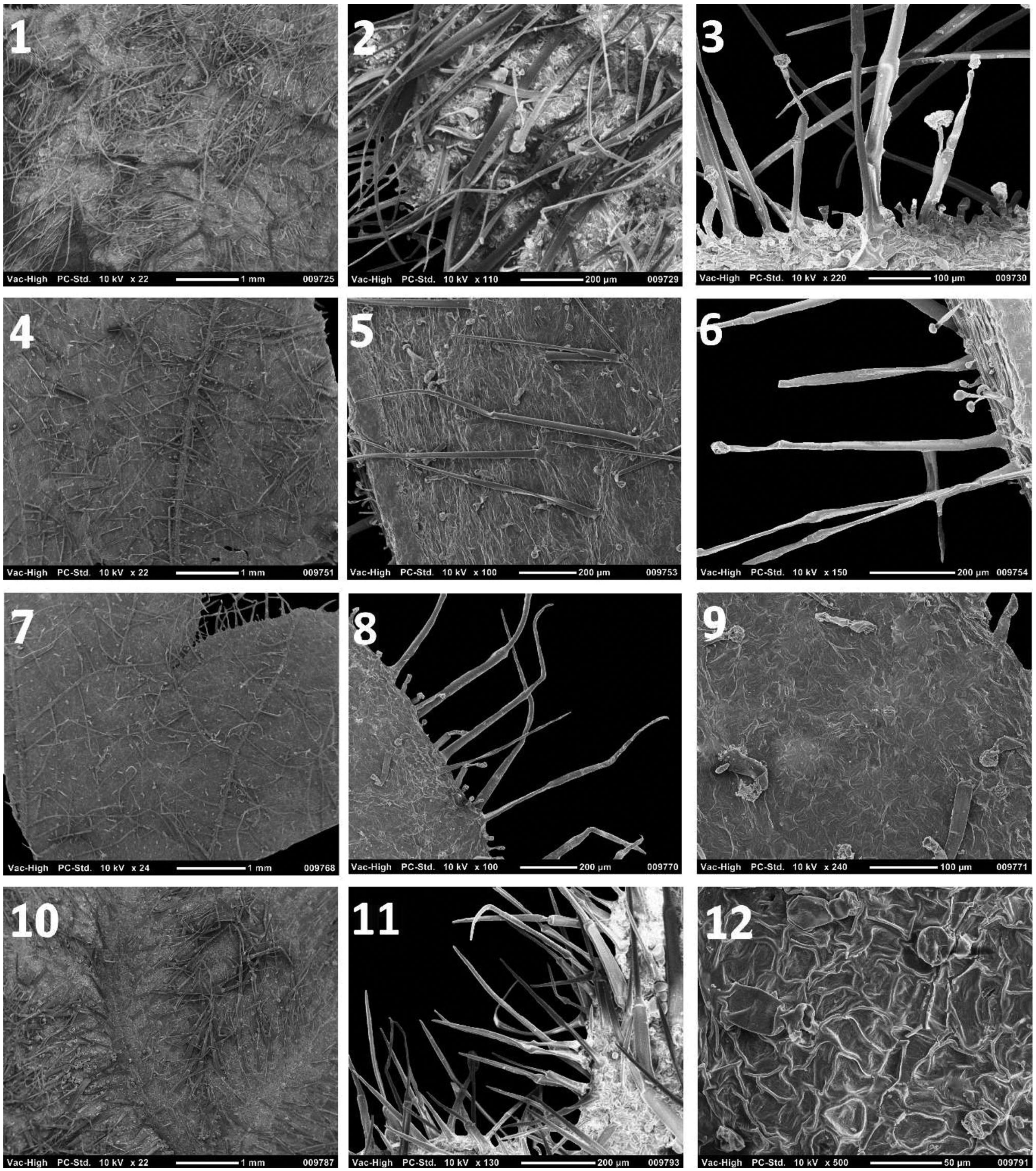

Figure 2. The lower surface of leaves of the examined *Stachys* taxa: **1–3:** *Stachys longiflora* (A1, A2, and D3), **4–6:** *S. euadenia* (A1, A2, B, and D2), **7–9:** *S. pinardii* (A1, A2, and D2), **10–12:** *S. antalyensis* (A1 and D2) (See Table 2 for character codes).

charts of the examined taxa were designed to determine the distance-proximity relationship among taxa (Table 4, Figure 8).

Results

The structure and distribution of the trichome types of taxa belonging to *Stachys* subsect. *Fragiles* from Turkey are presented in Table 3. SEM micrographs of the trichomes have demonstrated in Figures 1–5. The anatomical appearances of the trichomes have given in Figure 6.

In this study, two main trichome types have observed on the leaf, stem, calyx, and corolla: glandular and non-glandular. According to the cell number and shape, non-glandular trichomes (NGTs) can be separated into three subtypes: conical unicellular trichomes (CUTs), thin-walled trichomes (TNWTs) [triangular unicellular cells (1), 2–5 cells with acute apical cells (2), 3–5 cells, elongated and flexuous with distinct internodes (3)], and thick-walled trichomes (THWTs) [short

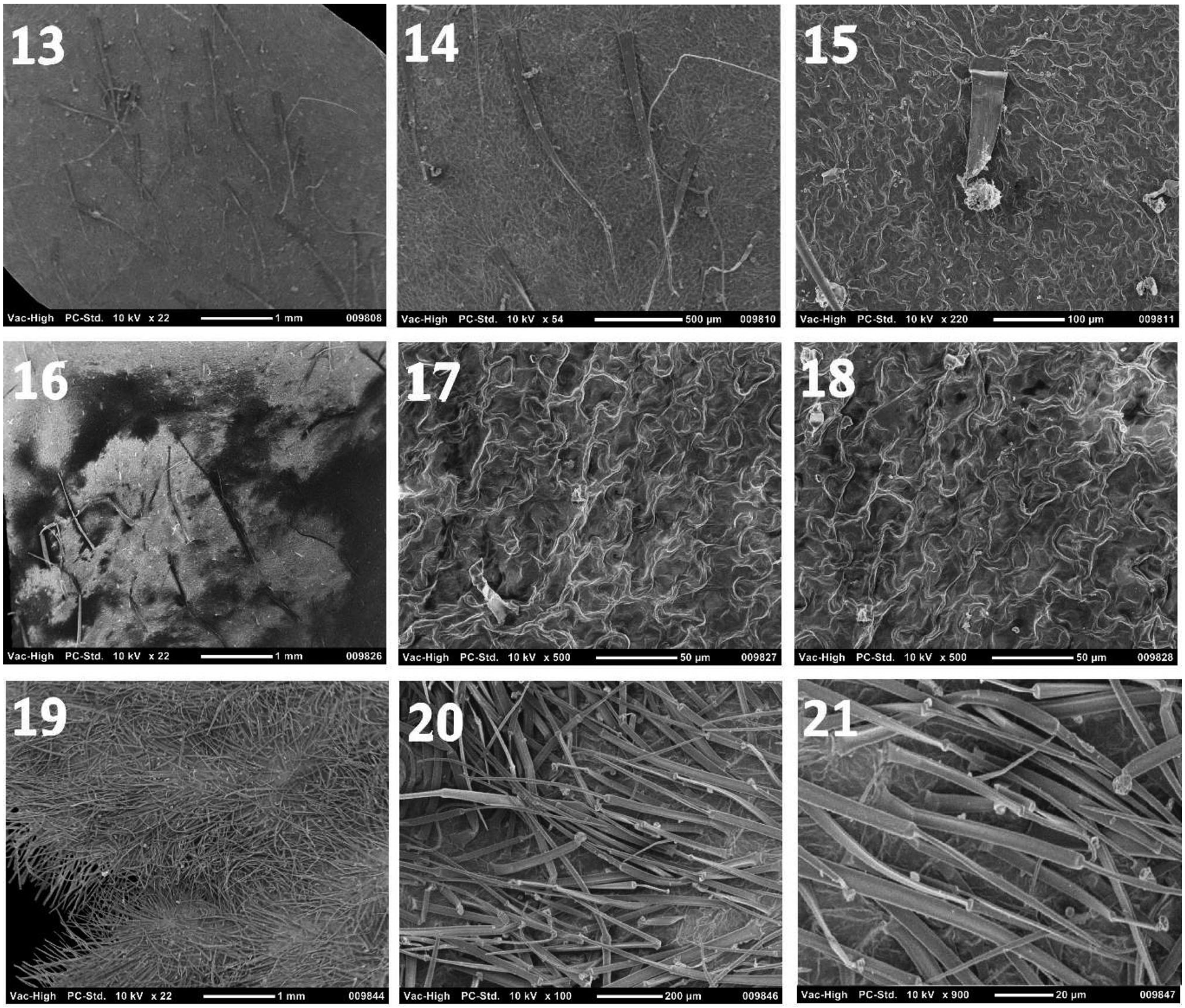

Figure 2 (Cont.). The lower surface of leaves of the examined *Stachys* taxa: **13–15:** *Stachys buttleri* (A2 and D2), **16–18:** *S. pseudopinardii* (A1 and D2), **19–21:** *S. chasmosericea* (A2, D2, E2, and E3) (See Table 2 for character codes).

and slightly conical 2-celled trichomes (1), elongated, adpressed, 3–5 cells, either hooked or curved in shape (2), elongated, erect, 3–7-celled trichomes (3)]. Based on the head dimension and stalk length, glandular trichomes (GTs) can be divided into clavate (CLA) and subsessile (SUB) trichomes. Depending on the dimensions of the stalk and morphology of the glandular head, CLA trichomes can be further divided into short: 1–2-celled (1) and long: 3–5-celled (2) trichomes.

The micromorphological characters and distribution of the trichomes in the examined taxa have exhibited a substantial difference in the trichome structure on the surfaces of the leaves. The indumentum on the leaves is clearly dense on the upper surfaces compared to the lower surfaces. While the indumentum in *S. chasmosericea* Ayaşligil & Davis is the most dense, the sparsest trichome distribution is observed in *S. buttleri* Mill. and *S. pseudopinardii* R. Bhattacharjee & Hub.-Mor. Ten different unbranched trichome types are detected on the leaves of subsect. *Fragiles*, however, branched trichomes are not seen (Table 2). The upper and lower surfaces of the leaves have partially or completely comprised the different types of trichomes. However, the upper and lower surface trichome types of *S. euadenia* Davis are almost the same (A1, A2 and D2) (Figures 1 and 2). On the upper surface, the most common types are A1, A2, and E2; however, type C is taxon-specific for *S. buttleri*. While type A1, A2, and D2 trichomes are mostly observed on the lower surfaces, type B in *S. euadenia*, type D3 in *S. longiflora* Boiss. & Balansa, and type E3 in *S. chasmosericea* are recorded as taxon-specific, as a feature that separates them from other taxa (Figure 2).

In this work, the observed trichome structures and distribution on the stem surfaces are significantly different. While trichome distribution is the most dense in *S. chasmosericea*, the trichome indumentum is observed in *S. buttleri*. Seven trichome types, including glandular or non-glandular trichomes, are observed on the stems in the examined taxa; however, no branched trichomes are detected (Table 3 and Figure 3). The glandular trichome type is more common than non-glandular ones. A1- and B-type trichomes are found on the stems of the examined taxa, except in *S. pinardii* Boiss.

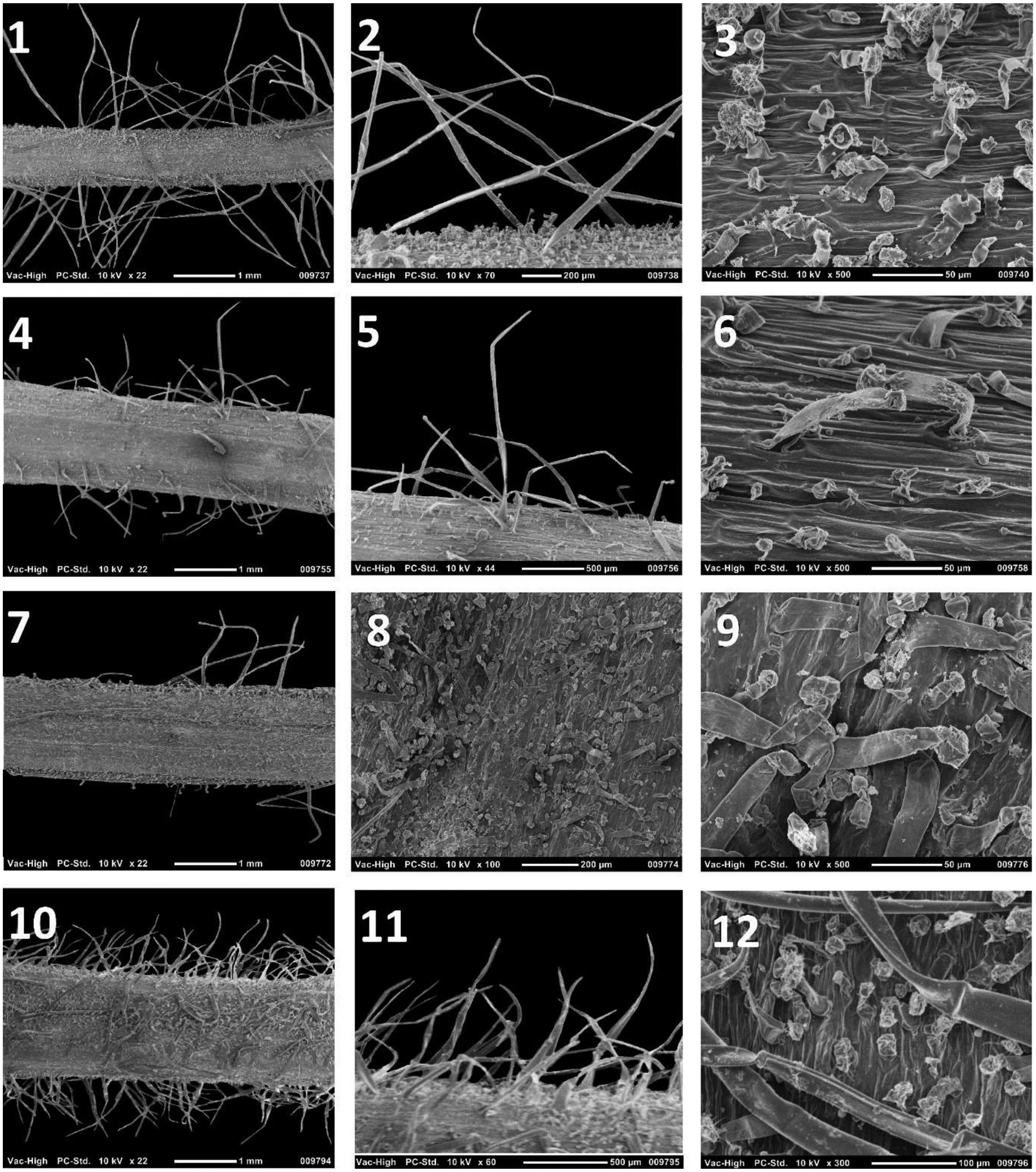

Figure 3. The surface of stem of the examined *Stachys* taxa: **1–3:** *Stachys longiflora* (A1, B, and E3), **4–6:** *S. euadenia* (A2, B, D3, and E2), **7–9:** *S. pinardii* (A2 and D2), **10–12:** *S. antalyensis* (A1, B, and E2) (See Table 2 for character codes).

Moreover, D2- and D3-type trichomes are only found on *S. pinardii* and *S. euadenia*, respectively.

The type and distribution of the trichomes on the calyx and corolla surfaces are quite different. The calyx and corolla have seven and eight trichome types, including glandular or non-glandular, in the examined taxa, respectively. While type A1, A2, D2, and E2 trichomes are commonly seen on the calyx, type E3 is found only in *S. antalyensis* Ayaşligil & Davis. The indumentum in the calyx of *S. chasmosericea* is quite dense when compared to the other taxa, while *S. buttleri* has a rather sparse indumentum (Figure 4). Moreover, the calyxes of *S. longiflora* and *S. euadenia* have the same trichome types (Table 2). The corolla is much sparser in terms of the indumentum, unlike the other studied parts. *S. chasmosericea* have four different corolla trichome types (A1, B, D1, and D2), whereas S. *euadenia* and *S. pinardii* contain a uniform

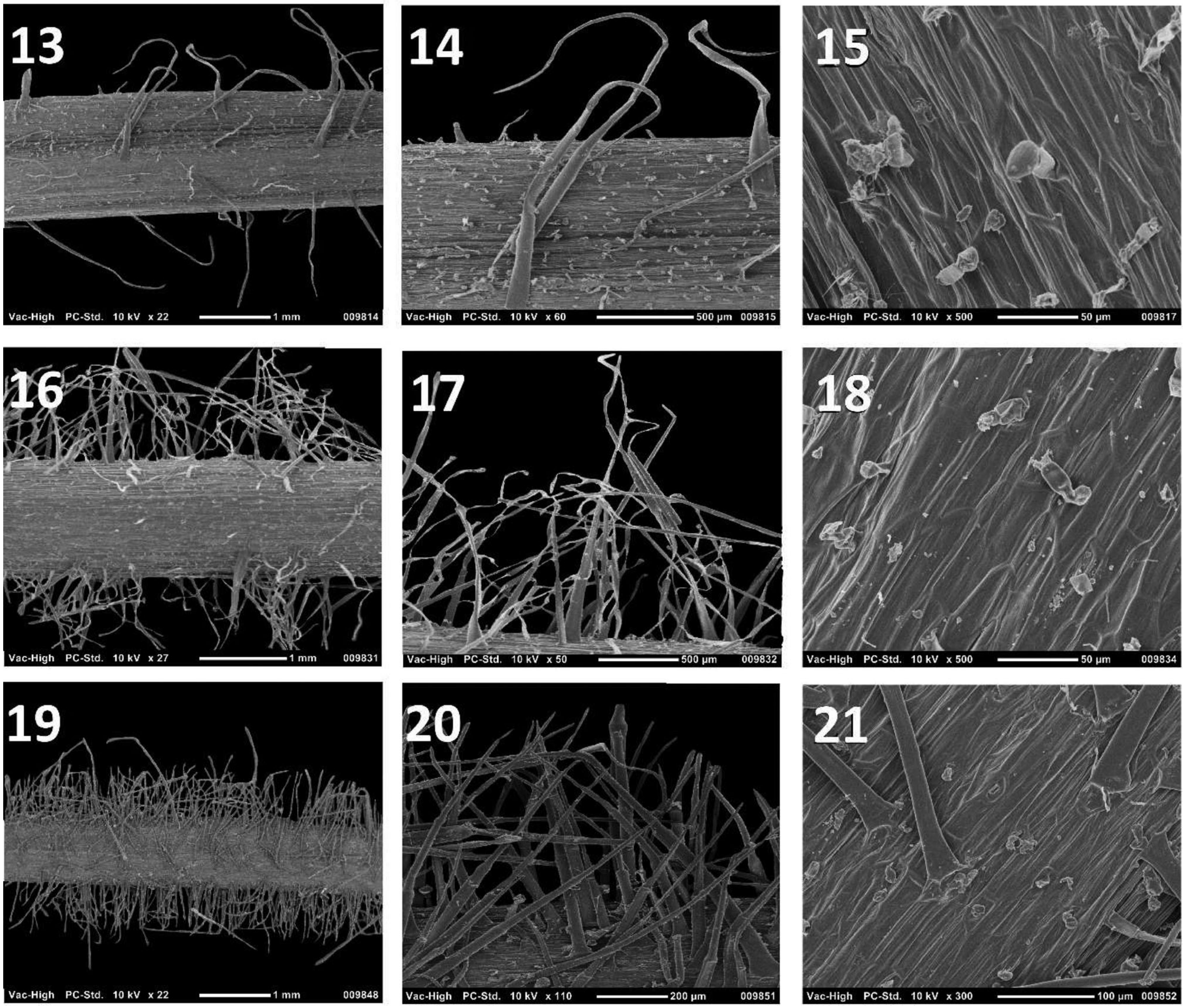

Figure 3 (Cont.). The surface of stem of the examined *Stachys* taxa: **13–15:** *Stachys buttleri* (A1, B, and E2), **16–18:** *S. pseudopinardii* (A1, B, E2, and E3), **19–21:** *S. chasmosericea* (A1, B, and E3) (See Table 2 for character codes).

indumentum, with trichome types A2 and A1, respectively (Figure 5 and Table 3). E1 type is specific to *S. longiflora*, while trichome types E2 and C are only observed in *S. pseudopinardii* (Table 3).

A dendrogram showing the variations or similarities among the examined taxa has formed using the numerical analysis of the trichome characteristics, based on the presence or absence of 10 different trichomes in each part of the five different parts studied. The cophenetic correlation coefficient has calculated using the relation between the dendrogram and the similarity matrix (Figure 7 and Table 4). The cophenetic relation between the similarity matrix and the dendrogram was determined as 0.61. This coefficient varies between 0 and 1, and the higher value indicates that characters used are useful for separating taxa.

The outcomes of the cluster analysis have showed that there are two main clusters: A and B: Cluster A is consisted of *S. chasmosericea*, *S. pinardii*, and *S. pseudopinardii*. Cluster B1 is consisted of *S. buttleri* and *S. antalyensis*. Cluster B2 is comprised *S. longiflora* and *S. euadenia* (Figure 7). *S. buttleri* and *S. antalyensis* are the most closely correlated taxa (similarity coefficient of 0.923). Moreover, *S. antalyensis* and *S. chasmosericea* are the most indistinctly correlated taxa (similarity coefficient of 0.123) (Figure 8 and Table 4).

Discussion

This study has presented the first comprehensive study of the trichome micromorphology of *Stachys* subsect. *Fragiles*. The diverse trichome types offered here noticeably increase the importance of these characters in the classification of the examined subsect. As in former studies, including trichome features in the family Lamiaceae (Metcalfe and Chalk 1950; Cantino 1990; Kaya et al. 2007; Grubesic et al. 2007; Dinç and Öztürk 2008; Gonzáles et al. 2008; Gairola et al. 2009; Kahraman et al. 2010; Celep et al. 2014; Ecevit-Genç et al. 2015; Atalay et al. 2016; Talebi et al. 2018; Sajna and Sunojkumar 2018), the outcomes obtained from this study have confirmed the usefulness of such characters in the

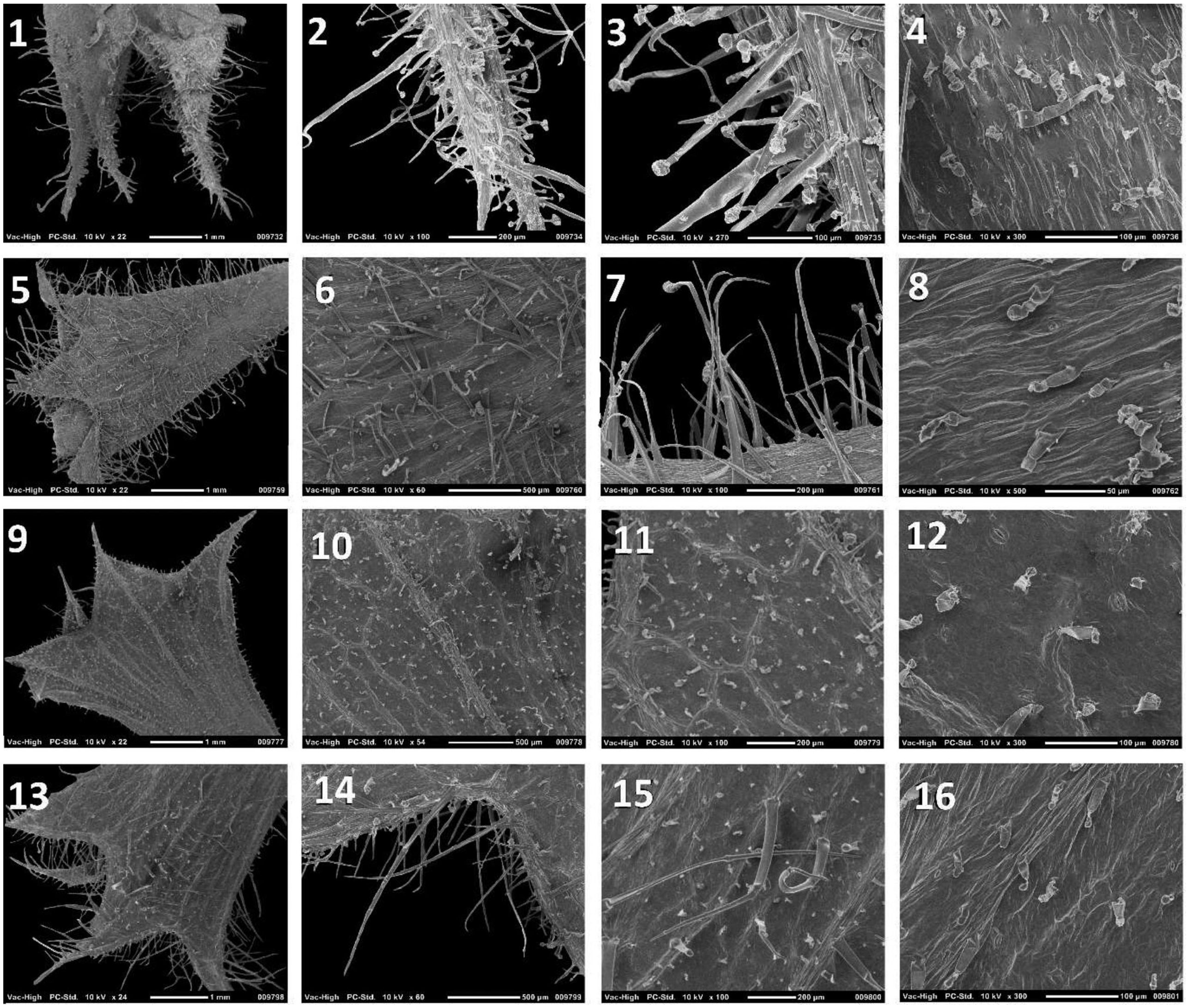

Figure 4. The surface of calyx of the examined *Stachys* taxa: **1–4:** *Stachys longiflora* (A1, A2, D2, and E2), **5–8:** *S. euadenia* (A1, A2, D2, and E2), **9–12:** *S. pinardii* (A1, B, and D1), **13–16:** *S. antalyensis* (A1, A2, D1, E2, and E3) (A2, B, D1, and D2) (See Table 2 for character codes).

separation of taxa at various taxonomic levels and makes it possible to display systematic affiliations among taxa.

GTs are of great taxonomical significance at the generic and subgeneric level, and their absence or presence in plant parts may be utilized as a phylogenetic character (Mraz 1998; Navarro and El Oualidi 1999). GTs are denser than non-glandular hairs on the corolla when compared to the other examined parts. GT presence or absence has separated taxa in subsect. *Fragiles*. Type A1 trichomes are widely available, except in *S. chasmosericea* in the leaves and calyces, and in *S. euadenia* and *S. pinardii* in the stems and corolla. It is most common among trichome types. *S. euadenia* has only type A2 GTs on the corolla surface, which distinguishes it from the other taxa. Type B trichomes have a distinctive character among the taxa according to their absence or presence on the examined parts, e.g. the presence only in *S. euadenia* on the upper surfaces of the leaf or the absence only in *S. pinardii* on the stem surface (Figures 1–6, Table 3). This GT is one of the most common types in the family and it presents an ancestral state (Navarro and El Oualidi 1999; Salmaki et al. 2009). This similar systematic importance of GTs has highlighted in various genera in Lamiaceae (Mraz 1998; Navarro and El Oualidi 1999; Ecevit-Genç et al. 2015; Sajna and Sunojkumar 2018). This situation has been reported for *Stachys scardica* by Grujić et al. (2014). Moreover, the presence of GTs is obviously connected with their role in pollination. It has been assumed that GTs play a key role in visual attraction for visiting insects (Fahn 1979; Navarro and El Oualidi 1999).

NGTs generally have a high systematic value in the family Lamiaceae (Navarro and El Oualidi 1999). CUTs, TNWTs, and THWTs are observed in all of the examined parts and they are widespread, except for in the corolla. CUTs are the rarest trichome type in the examined taxa, and are only present on the upper surface of the leaves of *S. buttleri* and the corolla surface of *S. pseudopinardii* (Figures 1–6, Table 3). This trichome type distinguishes both of the mentioned taxa from the other studied taxa. Simple thin-walled trichomes are frequently observed in the examined subsect. and they are systematically valuable in separating closely-related taxa and

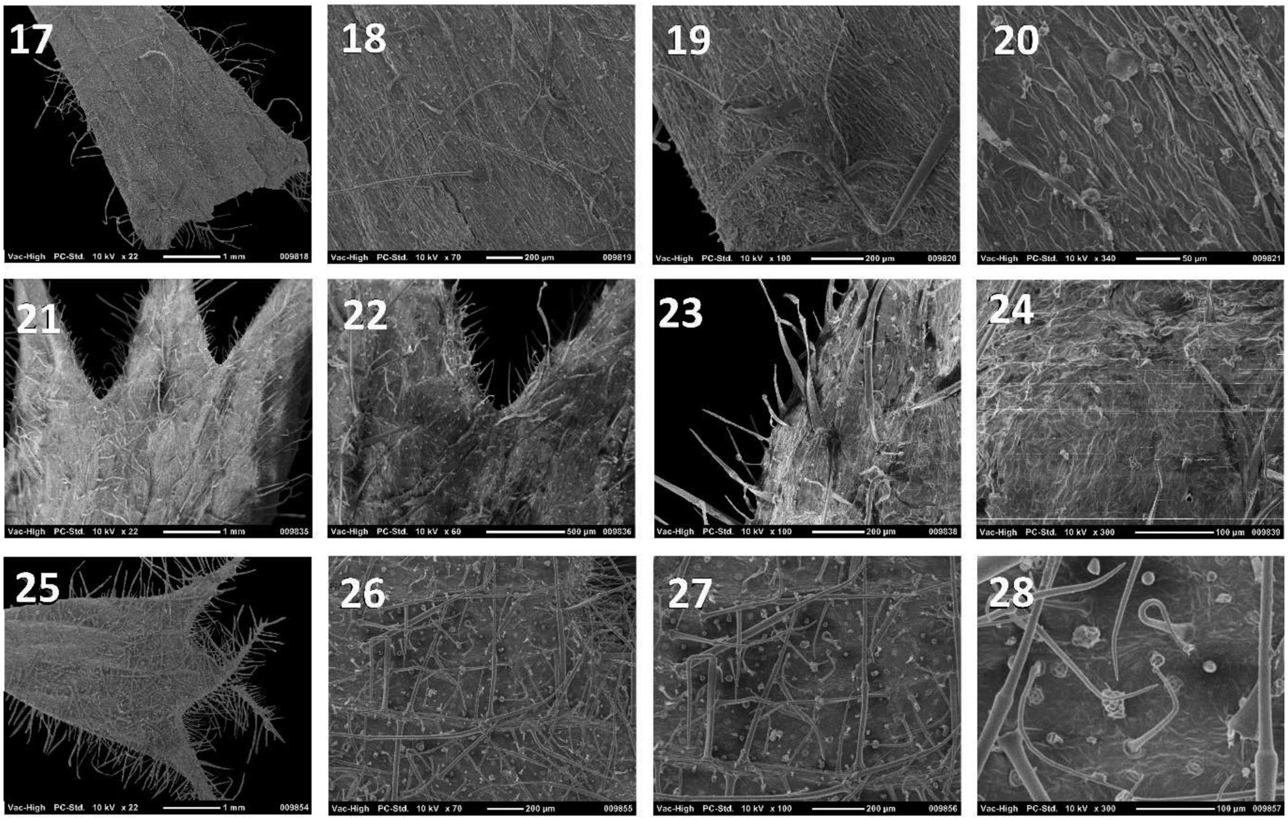

Figure 4 (Cont.). The surface of calyx of the examined *Stachys* taxa: **17–20:** *Stachys buttleri* (A1, B, and E2), **21–24:** *S. pseudopinardii* (A1, A2, D1, and D2), **25–28:** *S. chasmosericea* (A2, B, D1, and D2) (See Table 2 for character codes).

identifying some of the floral properties for pollination, as in some previous studies, such as that by Marin et al. (1994), Navarro and El Oualidi (1999), and Ecevit-Genç et al. (2015). TNWTs are significantly greater on the stem, calyx, and upper surface of the leaf of the examined taxa. *S. chasmosericea* clearly has more thin-walled trichomes than the other taxa. THWTs are the most common type of trichome in the examined taxa for types D1 and D2. However, it is rarely observed on the corolla surface in *S. longiflora* and *S. chasmosericea*. Type D3 is less commonly observed than the other THWTs; it is only observed on the upper and lower surfaces of the leaves in *S. longiflora* and on the stem surface in *S. euadenia*. THWTs (E1, E2, and E3) are the unbranched and thick-walled trichomes that are frequently observed in the taxa. These trichome types are present in *S. euadenia* only on the upper leaf surface, in *S. pinardii* only on the stem surface, in *S. chasmosericea* only on the lower leaf surface, and in *S. longiflora* only on the corolla surface. The basic common type of THWT is E2; however, E1 is the rarest type, which is only observed on two parts. Generally, NGTs are a good taxonomic indicator for the delimitation of taxa in the subsect. *Fragiles*, which is a heterogeneous subsect. with regards to its trichome types. At the same time, Dinç and Öztürk (2008) reported that NGTs are an important taxonomic character for the delimitation of *Stachys* sects. *Ambleia* Benth. and *Zietenia* (Gled.) Benth. The sect. *Ambleia* is characterized by dendroid branched trichomes, unseen in our study, and this feature has separated it from the other sects. of the genus *Stachys* (Bhattacharjee 1980, 1982). Moreover, Salmaki et al. (2009) has reported many types of NGTs, which showed significant variation among the species and presented valuable characters in the delimitation of the sects. and species, in their inclusive study of Iranian *Stachys* taxa. Similar results were also obtained in this study. Distinctive types of NGTs are found at the species level; however, there are common types at the subsect. level as well. Aside from in *S. chasmosericea*, NGTs are clearly dense in the other studied species. This may provide it with some advantages, such as adaptation to environmental situations and defense from various external threats, such as UV radiation, extreme temperatures, and herbivores.

Systematic correlations among the some *Stachys* taxa have been established (Giuliani et al. 2008; Dinç and Öztürk 2008; Salmaki et al. 2009; Rezakhanlo and Talebi 2010; Erdogan et al. 2011; Giuliani and Maleci Bini 2012; Grujić et al. 2014) on the basis of the presence or absence of trichomes and their distribution in vegetative or reproductive organs. These studies have confirmed that the obtained outcomes are systematically significant for providing distinction among closely related taxa with the trichome type and its distribution. Our micromorphological results lead us to agree with the conclusions from previous studies.

A dendrogram was created for an evaluation of the trichome characters of the leaf, calyx, and corolla of taxa in *Stachys* subsect. *Fragiles* using UPGMA cluster analysis. The dendrogram exhibiting the two main groups is compatible with the classification of Bhattacharjee (1982). The trichome

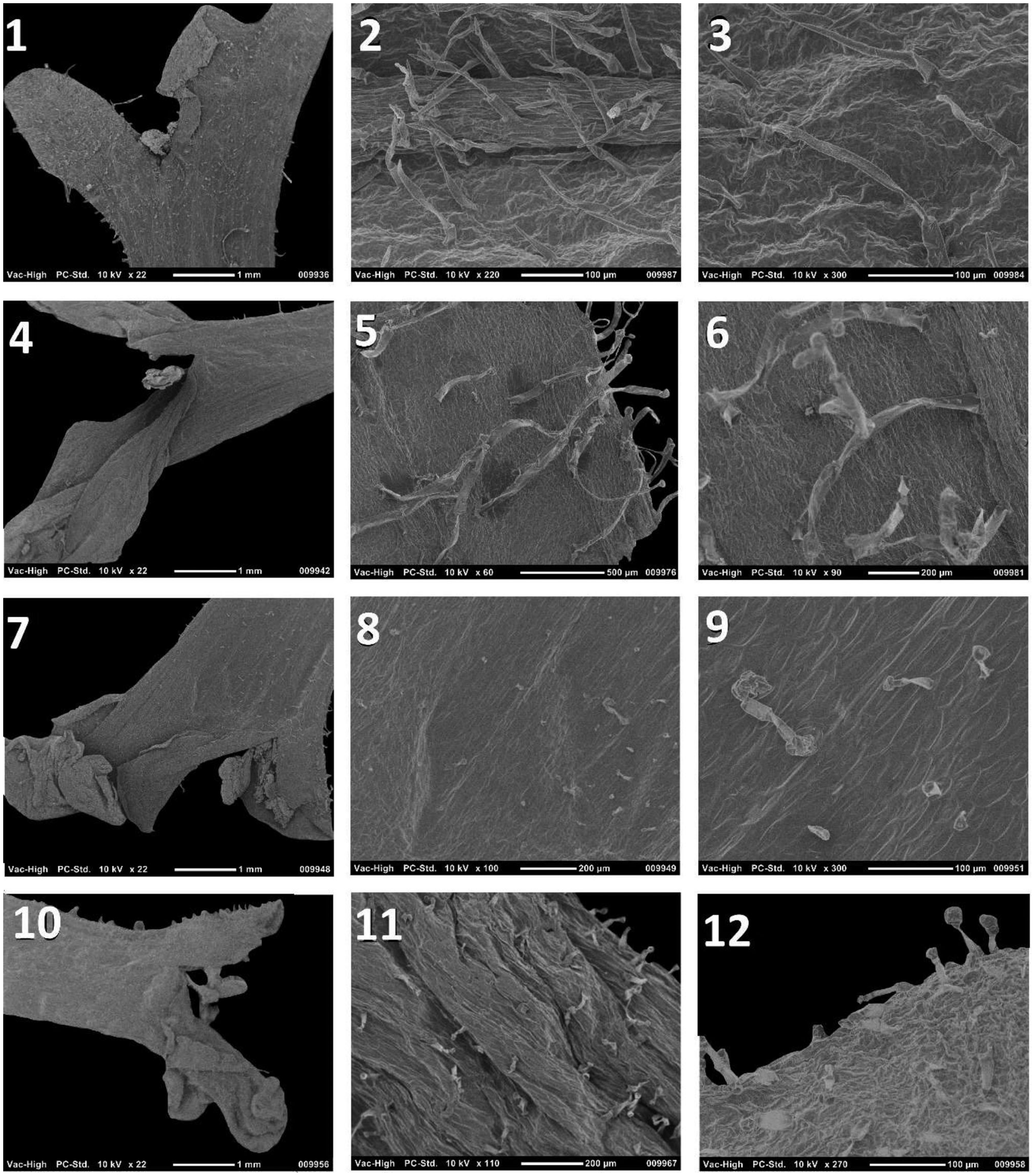

Figure 5. The surface of corolla of the examined *Stachys* taxa: **1–3:** *Stachys longiflora* (A2, D2, and E1), **4–6:** *S. euadenia* (A2), **7–9:** *S. pinardii* (A1), **10–12:** *S. antalyensis* (A1 and A2) (See Table 2 for character codes).

variations are observed at the species level (Figures 7 and 8, Table 4). It appears that the trichome characters are partially compatible with the current classification. Besides, the key prepared according to traditional characters and the key generated from this study are provided and the relationship between the two has been discussed.

Diagnostic key based on the morphological characters used in the taxonomy of the genus (Bhattacharjee 1982; Güner 2016):

1. Verticillasters 2(–4) – flowered
  2. Calyx 7.5–14 mm, calyx teeth 1/1–1/2 x tube; corolla 22–32 mm **S. longiflora**
  2. Calyx 6–10 mm, calyx teeth 1/3 x tube; corolla (11-)14–20 mm **S. buttleri**
1. Verticillasters 4–8(–10) flowered
  3. Bracteoles usually absent, rarely present
    4. Flowering stems ascending-erect; verticillasters approximate above, 6–10–flowered **S. antalyensis**

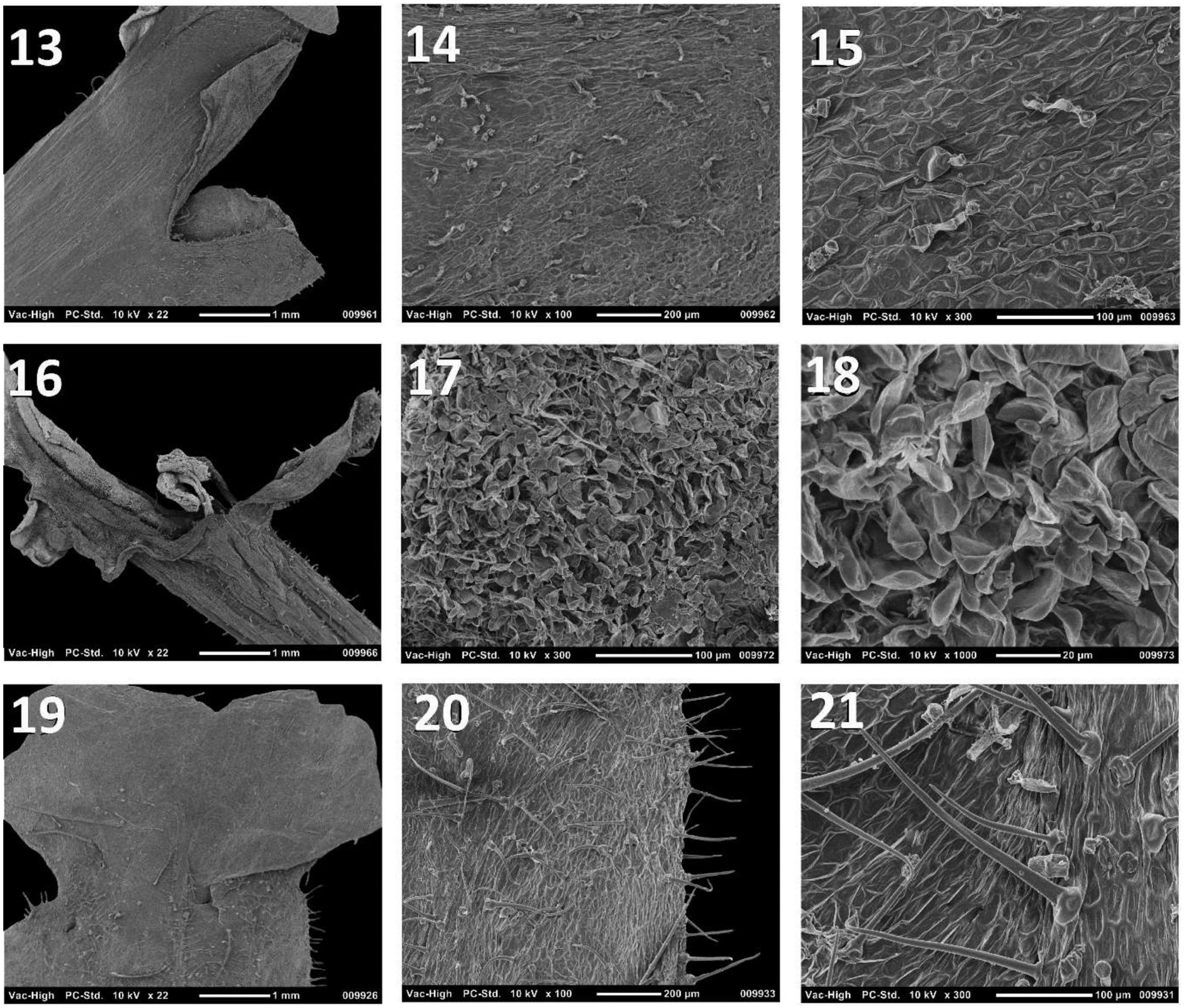

Figure 5 (Cont.). The surface of corolla of the examined *Stachys* taxa: **13–15:** *Stachys buttleri* (A1, A2, and B), **16–18:** *S. pseudopinardii* (C and E2), **19–21:** *S. chasmosericea* (A1, B, D1, and D2) (See Table 2 for character codes).

4. Flowering stems pendent; verticillasters remote, 4–6–flowered
   5. Calyx ± regular, infundibular, 6–8 mm; teeth ± equal; corolla purplish **_S. euadenia_**
   5. Calyx sub–bilabiate, 9.5–10.5 mm; teeth unequal; corolla white **_S. pseudopinardii_**
3. Bracteoles setaceous, few
   6. Calyx 7-12 mm; corolla white, 13–20 mm, tube exserted from calyx **_S. pinardii_**
   6. Calyx 5-7 mm; corolla rose-pink, 12 mm, shortly exserted from calyx **_S. chasmosericea_**

Diagnostic key based on according to trichome characters obtained from this study:

1. Glandular and non-glandular trichomes prominently dense **_S. chasmosericea_**
1. Glandular and non-glandular trichomes relatively much less
   2. Glandular trichomes in stem A1 type
      3. E2 type non-glandular trichomes on the calyx absent **_S. pseudopinardii_**
      3. E2 type non-glandular trichomes on the calyx present
         4. Non-glandular trichomes on the corolla present **_S. longiflora_**
         4. Non-glandular trichomes on the corolla absent
            5. B type glandular trichomes present **_S. buttleri_**
            5. B type glandular trichomes absent **_S. antalyensis_**
   2. Glandular trichomes in stem A2 type
      6. Glandular trichomes on the corolla A1 type **_S. pinardii_**
      6. Glandular trichomes on the corolla A2 type **_S. euadenia_**

Trichome characters have been found to be very good characters in separation of taxa in this study. However, the dichotomous key created with these characteristics is not generally compatible with the key created with traditional characters.

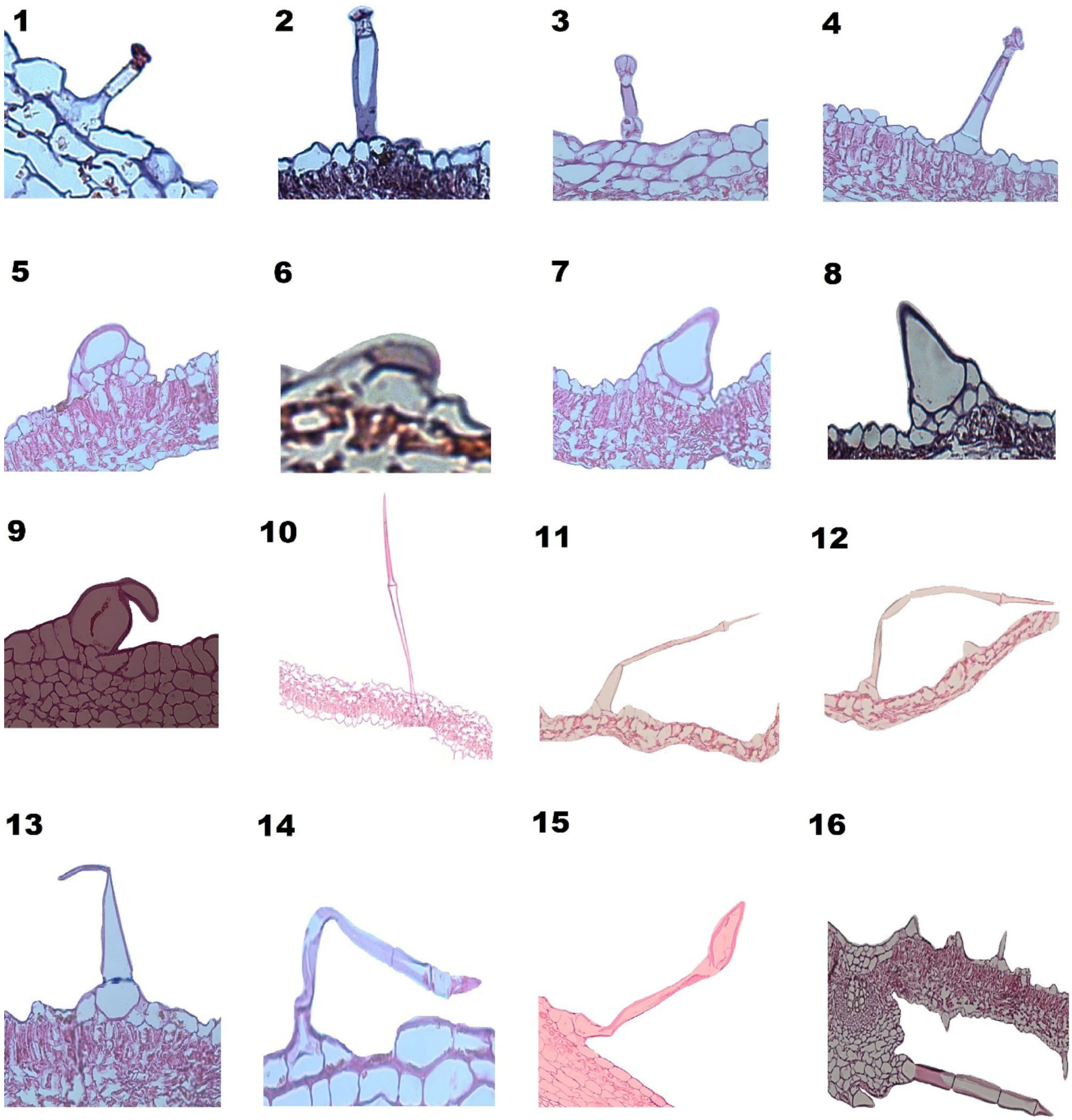

Figure 6. Trichome types of the examined *Stachys* taxa; **A1:** 1–2, **A2:** 3–4, **B:** 5–6, **C:** 7–8, **D1:** 9, **D2:** 10, **D3:** 11–12, **E1:** 13, **E2:** 14–15 and **E3:** 16.

Table 2. The characters used in the classification and their character codes.

Trichome types	Character codes	Features
1. Glandular trichomes (GTs)		
Clavate glandular trichomes (CLA)	A1	Short clavate glandular trichomes, with 2 large and thin stalk cells, with reduced neck and without micro-papillae
	A2	Long clavate glandular trichomes, with 3-5 thick walled cells
	B	Subsessile glandular trichomes (SUB)
2. Non-glandular trichomes (NGTs)		
2.1. Simple unbranched trichomes	C	Conical thin-walled unicellular hairs (CUTs)
	D	Thin-walled trichomes (TNWTs)
	D1	Triangular large, and very thin-walled unicellular hairs
	D2	Large very thin-walled, 2-5 celled trichomes, with an acute apical cell
	D3	Elongated and flexuous, thin-walled 3-5 cells, with distinct internode
	E	Thick-walled trichomes (THWTs)
	E1	Short slightly conical, 2-celled
	E2	Elongated, generally adpressed, 3–5 celled, hooked or curved
	E3	Elongated, thick walled 3–7 celled, erect with rounded epidermal cell and marked internode.

Table 3. Trichome types and their codes of the examined *Stachys* taxa.

Taxa	Upper leaf surface	Lower leaf surface	Stem	Calyx	Corolla
S. longiflora	A1, A2, D1, D2, D3, E2	A1, A2, D3	A1, B, E3	A1, A2, D2, E2	A2, D2, E1
S. euadenia	A1, A2, D2	A1, A2, B, D2	A2, B, D3, E2	A1, A2, D2, E2	A2
S. pinardii	A1, B, E2	A1, A2, D2	A2, D2	A1, B, D1	A1
S. antalyensis	A1, A2, B, D2, E2	A1, D2	A1, B, E2	A1, A2, D1, E2, E3	A1, A2
S. buttleri	A1, A2, C, E2	A2, D2	A1, B, E2	A1, B, E2	A1, A2, B
S. pseudopinardii	A1, B, E2	A1, D2	A1, B, E2, E3	A1, A2, D1, D2	C, E2
S. chasmosericea	A2, B, E1, E2	A2, D2, E2, E3	A1, B, E3	A2, B, D1, D2	A1, B, D1, D2

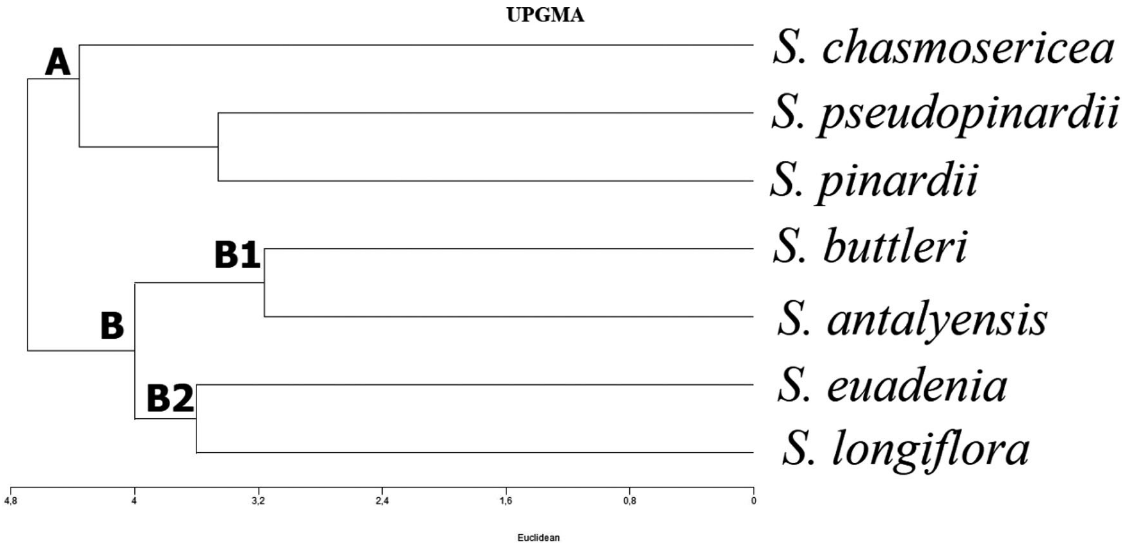

Figure 7. Cluster analysis of the studied *Stachys* taxa.

Table 4. The similarity matrix of the examined *Stachys* taxa.

Taxa	*S. longiflora*	*S. euadenia*	*S. pinardii*	*S. antalyensis*	*S. buttleri*	*S. pseudopinardii*	*S. chasmosericea*
S. longiflora	1	–	–	–	–	–	–
S. euadenia	0.893	1	–	–	–	–	–
S. pinardii	0.427	0.418	1	–	–	–	–
S. antalyensis	0.722	0.546	0.588	1	–	–	–
S. buttleri	0.714	0.653	0.559	0.923	1	–	–
S. pseudopinardii	0.721	0.698	0.905	0.614	0.567	1	–
S. chasmosericea	0.490	0.439	0.670	0.121	0.138	0.610	1

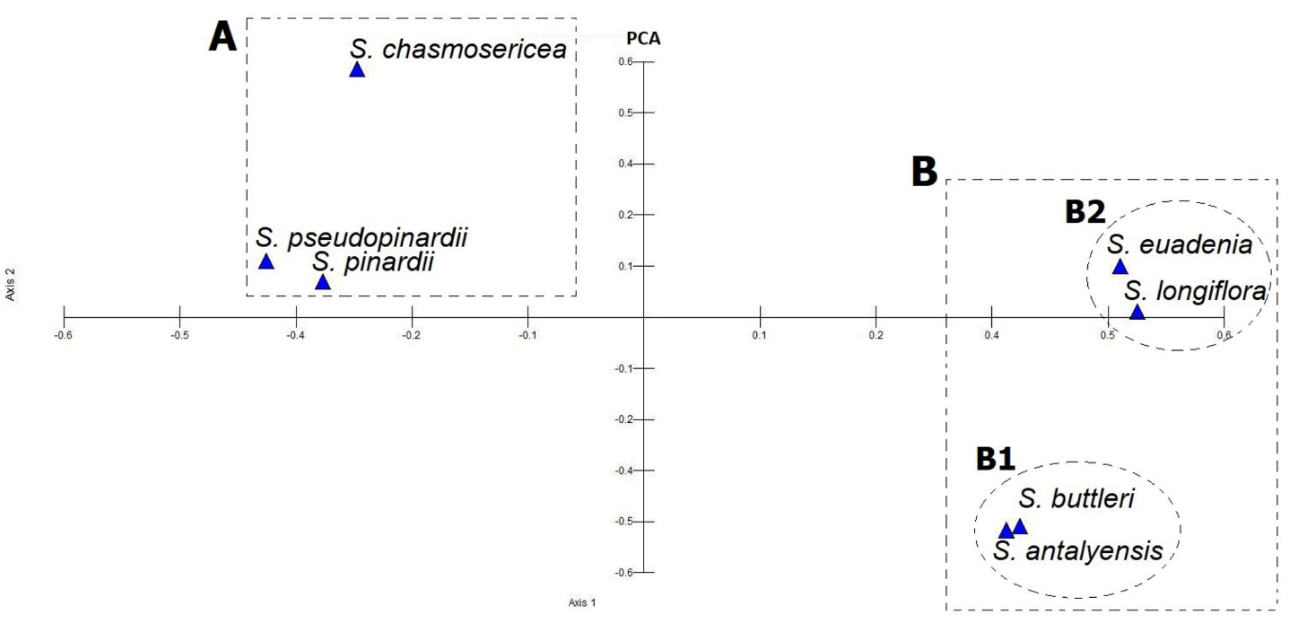

Figure 8. Principal component analysis of the examined *Stachys* taxa.

Conclusion

This investigation has shown that trichome micromorphology allows for a better understanding of the correlation among the examined taxa. Indumentum types displayed greater variability among taxa in comparison with other examined characters and, thus, they represent useful characters for the separation of the species. The trichome features that can be defined as systematic indicators are distribution, type and surface, density, and cell wall thickness.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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