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The taxonomic value of leaf anatomy and trichome morphology of the genus *Cyclotrichium* (Lamiaceae) in Turkey

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The genus *Cyclotrichium* (Boiss.) Manden. & Scheng. is represented by six species in Turkey: *C. glabrescens, C. leucotrichum*, *C. longiflorum, C. niveum*, *C. origanifolium* and *C. stamineum*. They are aromatic perennial subshrubs used as spices or herbal teas in traditional Turkish medicine. The leaf anatomy and tomentum morphology of leaves and calyces of *Cyclotrichium* species in Turkey was investigated by scanning electron microscopy (SEM) and light microscopy (LM). The investigated species have equifacial (*C. niveum*, *C. origanifolium*) or bifacial leaves (*C. glabressens, C. leucotrichum*, *C. longiflorum, C. stamineum*). All species have peltate and capitate glandular, and simple (all species) or branched (*C. niveum*) eglandular trichomes and diacytic stomata. Peltate trichomes consist of a basal cell embedded in the epidermis, a stalk cell, and a broad 12 (–13)-celled secretory head arranged in two concentric circles. The capitate trichomes observed in *Cyclotrichium* can be grouped into five types, differing in structure and size. They consist of either a pear-shaped or globose unicellular head and uni-or bicellular stalk, or a bicellular head and bicellular stalk. The density of peltate, capitate and eglandular trichomes differs between species. Peltate trichomes are densely spaced only on the calyx and on the leaf surface of *C. niveum* and *C. origanifolium* and on the abaxial leaf surface of *C. longiflorum* and *C. stamineum*. The significance of trichome architecture for taxonomy in *Cyclotrichium* and Lamiaceae in general is discussed.

Turkey is regarded as an important centre of biodiversity for the Lamiaceae. In Turkey, the family is represented by 45 genera, 546 species and a total of 731 taxa. The genus *Cyclotrichium* Manden. & Scheng. belongs to the tribe Mentheae subtribe Menthinae (Lamiaceae, Nepetoideae; Harley et al. 2004). In earlier classifications, some species of *Cyclotrichium* were included in the genera *Micromeria* Benth. (sect. *Piperella* C. Presl), *Melissa* L. (sect. *Clinopodium* L.), and *Clinopodium* L. (Bentham 1834, 1848). Firstly, Boissier (1879) described *Cyclotrichium* as sect. *Cyclotrichium* Boiss. in the genus *Calamintha* Mill. Later, Briquet transfered it to the genus *Satureja* (Briquet 1897) and, finally, Mandenova and Schengelia (1953) introduced *Cyclotrichium* as a separate genus originally comprising six species.

Nine *Cyclotrichium* species have now been found in the world and they are distributed in Turkey, Lebanon (one species), Iraq and Iran (Leblebici 1982, Rechinger 1982). In Turkey, the genus is represented by six species: *C. glabrescens* (Boiss. & Kotsch ex Rech. f.) Leblebici, *C. leucotrichum* (Stapf) Leblebici, *C. longiflorum* Leblebici, *C. niveum* (Boiss.) Manden. & Scheng., *C. origanifolium* (Labill.) Manden. & Scheng. and *C. stamineum* (Boiss. & Hohen.) Manden. & Scheng. *Cyclotrichium glabrescens* and *C. niveum* are endemic to Turkey. All *Cyclotrichium* species in Turkey are suffrutescent perennial herbs and they generally grow on rocky, stony limestone slopes. The leaves are almost ovate in shape. The calyces are nearly regular, sub-bilabiate or bilabiate, straight or slightly curved. The corollas are violet, mauve, lilac, rose, pink or rarely white coloured. The nutlets are ovoid (Leblebici 1982, Davis et al. 1988, Dirmenci et al. 2010).

Several species of *Cyclotrichium* are locally known as 'dağ nanesi-mountaint mint', 'kız otu-girl grass', 'köpek nanesi-dog mint', 'karabaş otu-lavander' and 'nane ruhu-mint spirit' in the regions where they grow and they are used as culinary or medicinal herbs throughout Turkey (Başer et al. 1996). *Cyclotrichium niveum* has been used as tea and *C. origanifolium* is widely used as a flavoring agent in soups and salads. *Cyclotrichium niveum* is also used in the traditional Turkish medicine, for treating influenza, nausea and muscle pain disorders (Akkus-Cetinus et al. 2007, Alim et al. 2009). *Cyclotrichium* species have economic and medicinal importance because of their high essential oil content: *Cyclotrichium niveum*, *C. origanifolium*, *C. leucotrichum*, *C. stamineum* and *C. origanifolium* contain pulegone and menthone as major components (Başer et al. 1994, 1996, Tepe et al. 2005). The yields of leaf essential oils of the species *Cyclotrichium glabrescens*, *C. longiflorum, C. stamineum, C. leucotrichum, C. origanifolium*

and *C. niveum* were 0.5, 0.8, 1.2, 1.8, 2.3, and 3.4%, respectively (Kilic et al. 2007).

In the Lamiaceae, the morphology, distribution and frequency of glandular trichomes are used as discriminative characters at the subfamiliar level (Ascensão et al. 1995). Glandular trichomes are commonly found on reproductive and vegetative organs. They are the primary secretory organs of these plants and their structures can vary markedly among species (Serrato-Valenti et al. 1997). Gland structure of the family has been investigated by many researchers (Werker et al. 1985, Bosabalidis 1990, Serrato-Valenti 1997, Ascensão and Pais 1998, Ascensão et al. 1999, Bisio et al. 1999, Corsi and Bottega 1999, Kaya et al. 2003, 2007a, 2007b). In almost all studied species, two main types of glandular trichomes exist: peltate and capitate (Werker et al. 1985). Peltate trichomes in Lamiaceae are generally short with a uni- or bicellular stalk and a large secretory head with four to 18 cells arranged in one or two concentric circles (Ascensão and Pais 1998). Capitate trichomes are also widespread in Lamiaceae, but they are more variable in stalk length and head shape. They generally consist of one to two stalk cell(s) and one to two cell(s) forming a rounded to pear-shaped secretory head (Werker et al. 1985).

In a previous studies, the morphology and anatomy of *C. niveum* and *C. origanifolium* were described (Kaya and Başer 1996, Kaya et al. 2000). Trichome types in *Cyclotrichium* species are more variable, with sessile or subsessile glands, usually eglandular or pubescent trichomes. According to 'Flora of Turkey', some *Cyclotrichium* species are morphologically quite similar. For instance, *C. stamineum* is very closely related to both *C. glabrescens* and *C. longiflorum* while *C. leucotrichum* is related to *C. stamineum* (Leblebici 1974, 1982, Davis et al. 1988). Here, we focus on the trichome structure in Turkish species of *Cyclotrichium*. The results are compared with leaf anatomical evidence of the species in order to improve our knowledge of their anatomy, get a better understanding of their systematics and help separate similar species.

Material and methods

Plant material

Individuals of six *Cyclotrichium* species were collected during the flowering period (Jun/Aug 2002–2008) from different localities in Turkey (Table 1). Voucher specimens were deposited in herbarium ESSE. Voucher information can be found in Appendix 1.

Scanning electron microscopy (SEM)

Leaves and calyces were fixed with 3% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2) for 4 h at 4°C. After washing, the material was dehydrated in acetone and then critical-point dried. The specimens were mounted on stubs with double-sided adhesive tape and coated with gold. Photographs were taken with a scanning electron microscope (Zeiss EVO 50).

Light microscopy (LM)

Living material was kept in 70% alcohol for anatomical studies. Transverse and paradermal hand sections were made from the mid-part of mature leaves and calyces. Embedded material was prepared as follows: adult leaves and calyces were fixed with FAA for 24 h, then dehydrated in a graded ethanol series and embedded in glycerine-gelatine.

Trichome features were studied using light microscopy in addition to SEM. The number of cells in uniseriate stalks of trichomes of individual taxa was determined, as well as the number of cells in the secretory structure of trichome glands. Olympus BX51 and Nikon Eclipse E600 research microscopes with drawing tubes were used in drawing and observations of leaf and trichomes. The classification of glandular and eglandular trichomes follow Werker et al. (1985) and Fahn (2000).

Results

The leaf epidermal cells in all *Cyclotrichium* species were irregular or isodiametric. The epidermis consist of uniseriate oval, square and rectangular cells in transverse section (Fig. 1–2). The upper walls of both epidermises are thicker than the lower and lateral walls. In all *Cyclotrichium* leaves, cells of the adaxial epidermis are larger than those of the abaxial epidermis. Both the adaxial and the abaxial epidermises are covered by a thick cuticle. The stomata are diacytic and occur on the adaxial and abaxial side of leaves, being more numerous on the lower surface. The leaf is amphistomatic and the stomata in all *Cyclotrichium* species are of mesomorphic or slightly hygromorphic type. The investigated *Cyclotrichium* leaves

Table 1. Collection data of *Cyclotrichium* species in Turkey, collected during Jun/Aug 2002–2008.

Species	Collection data	Herb. no	Herb. acronym
C. niveum	B6 Malatya: Malatya to Darende	T. Dirmenci 3504	ESSE 14472
C. glabrescens	B9 Bitlis: Hizan, Karbastı village	T. Dirmenci 1440	ESSE 14518
C. longiflorum	C9 Hakkari: Hakkari to Çukurca	T. Dirmenci 2476	ESSE 14471
C. stamineum	C9 Şırnak: Şırnak to Hakkari	B. Yıldız 15158	ESSE 14470
C. leucotrichum	C8 Mardin to Kızıltepe	T. Dirmenci 3593	ESSE 14469
C. origanifolium	C4 İçel: Arslanköy, Bolkar Mountain	T. Dirmenci 2178	ESSE 14466

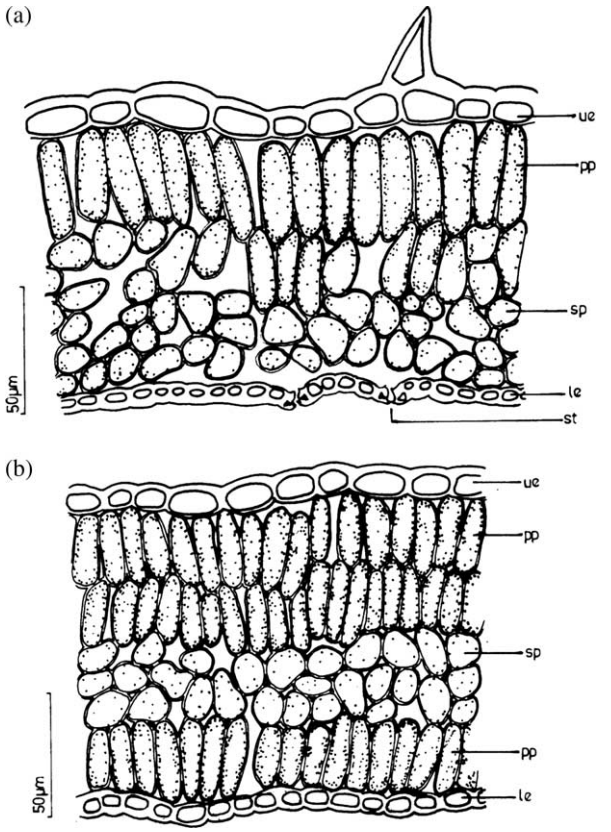

Figure 1. Mesophyll types in *Cyclotrichium* species. Bifacial type (a): *C. stamineum, C. leucotrichum*; equifacial type (b): *C. origanifolium, C. niveum.* Abbreviations: le = lower epidermis, pp = palisade parenchyma, sp = spongy parenchyma, st = stomata, up = upper epidermis.

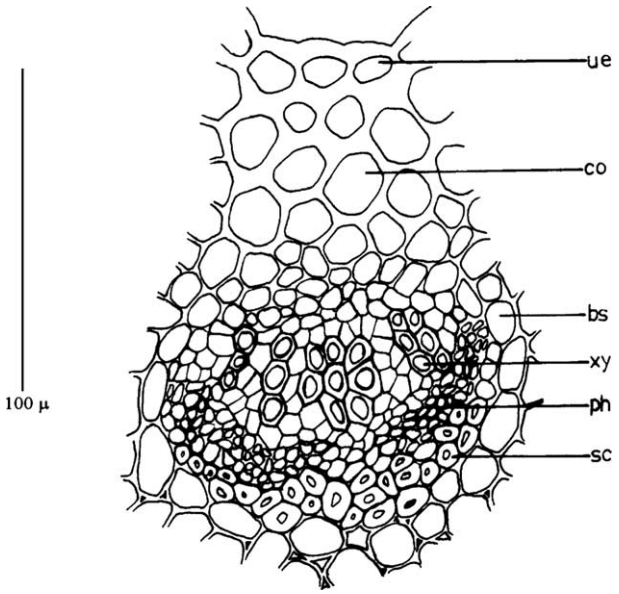

Figure 2. The midrib region in *Cyclotrichium* species. Abbreviations: le = lower epidermis, co = collenchyma, ph = phloem, pp = palisade parenchyma, sc = sclerenchyma, sp = spongy parenchyma, up = upper epidermis, xy = xylem.

are bifacial in *C. glabressens, C. leucotrichum*, *C. longiflorum* and *C. stamineum* and equifacial in *C. niveum* and *C. origanifolium* (Table 2). The mesophyll of bifacial leaves consists of one to two layers of palisade parenchyma and two to three (rarely four) layers of spongy parenchyma (Fig. 1a). In equifacial leaves, mesophyll tissue consists of one or two layers of palisade parenchyma and one or two layers of spongy parenchyma which is located in between the palisade parenchyma (Fig. 1b). The midrib region is well developed and forms a projecting part towards the outer surface in all species. The leaves have collateral vascular bundles with adaxial xylem and abaxial phloem (Fig. 2). Bi- to triseriate sclerenchymatous cell layers are located below the phloem, and several seriate collenchymatous cell layers are located below the upper and lower epidermises in the mid-rib region.

Trichome morphology was investigated using light (Fig. 3) and scanning electron microscopy (Plate 1–30). *Cyclotrichium* species have simple or branched eglandular trichomes and capitate and/or peltate glandular trichomes.

The eglandular trichomes are covered by micro-papillae. Simple eglandular trichomes are composed of a basal epidermal cell and one to seven (rarely eight) cells arranged linearly to form trichome-like structures that are straight or curved and tapered towards the apex. Unicellular trichomes are conic (Fig. 3a). Branched trichomes are thick-walled and short or elongated with acute arms (Fig. 3b). They are observed only in *C. niveum,* which is thus distinct from the other species.

The peltate glandular trichomes consist of a basal cell, a short unicellular stalk and a broad head of 12 (–13) secretory cells. Of these cells, eight (–nine) are large and peripheral, four are small and occupy the central area of the head (Fig. 3c). Five general types of capitate trichomes were observed:

type I trichomes are short and consist of a stalk cell and a globose unicellular head (Fig. $3d_1$); type II trichomes are short or long and consist of a stalk cell and a pear-shaped unicellular head (Fig. $3d_2$); type III trichomes are short or long and consist of two stalk cells

Table 2. Anatomical characteristics of *Cyclotrichium* species in Tureky.

	C. niveum	*C. glabressens*	*C. longiflorum*	*C. stamineum*	*C. leocotrichum*	*C. origanifolium*
Mesophyll type	equifacial	bifacial	bifacial	bifacial	bifacial	equifacial
Layers of palisade parenchyma	l–2	2	1–2	2	2	2
Layers of spongy parenchyma	1–2	2–3 (–4)	2–3 (–4)	2–3 (–4)	2–3 (–4)	1–2

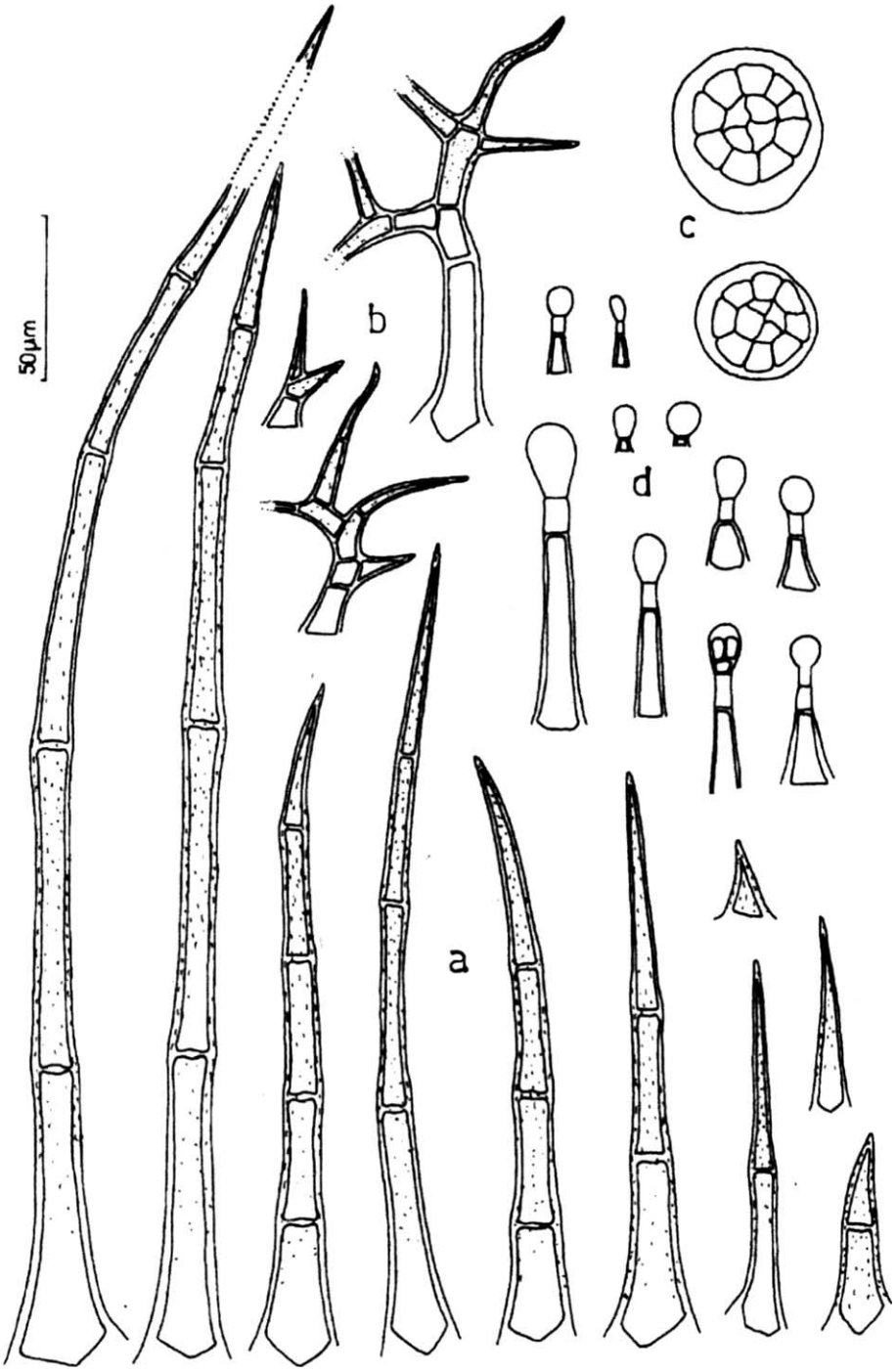

Figure 3. Trichomes of *Cyclotrichium* species: (a) eglandular simple, (b) eglandular branched, (c) glandular peltate, and (d) glandular capitate.

and a globose unicellular head (Fig. $3d_3$); type IV trichomes are short or long and consist of two stalk cells and a pear-shaped unicellular head (Fig. $3d_4$); type V trichomes are long and consist of two stalk cells and a bicellular head (Fig. $3d_5$).

The morphology and distribution of trichomes on calyx and leaves is shown in Table 3.

Cyclotrichium niveum: eglandular trichomes are common on leaves and calyces, and they are mostly branched (Fig. 3b), rarely simple with 1 or 2 cells (Fig. $3a_1$, $3a_2$); peltate trichomes (Fig. 3c) were observed in calyx grooves and on leaves under the branched trichomes; capitate trichomes are short, with 1 or 2 stalk cells (Fig. $3d_{1–4}$) and are rare (Plate 1–4).

Cyclotrichium glabrescens: the adaxial leaf surface has a papillate epidermis that is a diagnostic character (Plate 7). Eglandular trichomes are short and 1- or 2-celled (Fig. $3a_{1–2}$). They are rare or absent on the calyx. Short

Table 3. Distribution of trichomes on calyces and leaves of *Cyclotrichium* species. Symbols: (1) surface of calyx, (2) adaxial surface of leaves, (3) abaxial surface of leaves, (++) dense or too dense, (+) rare, (−) absent.

	C. niveum			*C. glabrescens*			*C. longiflorum*			C. stamineum			*C. leocotrichum*			*C. origanifolium*		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Eglandular trichomes																		
1–2-celled simple trichomes	+	+	+	−/+	−	+	+	++	++	+	++	++	+	−	−	++	++	++
3–7 (–8)-celled simple trichomes	−	−	−	−	−	−	++	−	−	+	−	−	+	+	+	+	+	+
branched trichomes	++	++	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glandular trichomes																		
Peltate trichomes	++	++	++	+	−	+	+	−	++	+	−	++	+	−	+	++	++	++
Capitate trichomes																		
Type I–II	+	+	+	++	+	++	++	+	+	++	+	+	++	++	++	++	++	++
Type III–IV	+	+	+	++	+	++	++	−	−	++	+	+	++	++	++	++	++	++
Type V	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−

eglandular trichomes are absent on the adaxial surface of the leaf, while they are rare on the abaxial surface. Peltate trichomes (Fig. 3c) are rare on the calyx, and are not present on the adaxial leaf surface. Capitate trichomes are like in *C. niveum*, but densely spaced. They can be observed in large numbers on the calyx ribs. Four types (I–IV) of capitate trichomes on the abaxial leaf surface are more common than on the adaxial surface (Fig. $3d_{1–4}$, Plate 5–9).

Cyclotrichium longiflorum: eglandular trichomes on the calyx are 1- or 7 (–8)-celled (Fig. $3a_{1–6}$). Short eglandular trichomes are sparsely present while long eglandular trichomes are more common. They are usually located on the calyx ribs. Both leaf surfaces have eglandular trichomes that are short, 1- or 2-celled. Peltate trichomes (Fig. 3c) are few and scattered on the calyx. They are observed in calyx grooves. Peltate trichomes are absent on the adaxial leaf surface, while they are abundant on the abaxial surface of leaves. Capitate trichomes are more common than peltate trichomes on the calyx. Capitate trichomes are mainly located on the sides of ribs and on calyx ribs. Types I–II of capitate trichomes are rarely found on both leaf surfaces, while types III–IV are absent (Fig. $3d_{1–4}$, Plate 10–15).

Cyclotrichium stamineum: eglandular trichomes are 1- to 2-celled and sparsely present on the calyx. Long, 3- or 6 (–7)-celled, eglandular trichomes are mainly observed on the teeth of the calyx. (Fig. $3a_{1-6}$). Eglandular trichomes on the adaxial and abaxial leaf surfaces are short, 1- or 2-cellular. Peltate trichomes (Fig. 3c) are rare on the calyx. Peltate trichomes, on the other hand, are dense on the abaxial leaf surface, but absent on the adaxial surface. Four types of capitate trichomes (Fig. $3d_{1–4}$) were observed in large numbers. They are predominantly located on the sides of ribs and on calyx ribs. Types I–IV of capitate trichomes are rarely found on the adaxial and abaxial leaf surfaces (Plate 16–19).

Cyclotrichium leucotrichum: eglandular trichomes are rare and 1- or 2 (–5)-celled on the calyx. They are long, 3- or 5 (–6)-celled and rare on both leaf surfaces (Fig. $3a_{1–6}$). Peltate trichomes (Fig. 3c) are rarely observed in calyx grooves. Peltate trichomes are absent on the adaxial leaf surface, and are very rarely found on the abaxial leaf surface. Types I–V of capitate trichomes (Fig. $3d_{1–5}$) are observed on the calyx. The long subtypes of capitate trichomes are more common than the short types. They are predominantly located on the sides of ribs and on calyx ribs. Type V capitate trichomes and peltate trichomes are rare. Capitate trichomes on leaf surfaces are as on the calyx (Plate 20–24).

Cyclotrichium origanifolium: eglandular trichomes are common, short and 1- or 2 (–4)-celled on the calyx ribs and on both leaf surfaces (Fig. $3a_{1–4}$). Both types of glandular trichomes are dense. Peltate trichomes (Fig. 3c) are mainly observed in calyx grooves. Type I and long subtypes (types II–IV) of capitate trichomes are predominantly located on calyx ribs (Fig. $3d_{1–4}$). Peltate trichomes and capitate trichomes (short subtypes), except type V, are found in large numbers on both leaf surfaces (Plate 25–30).

Discussion

Four of the six investigated species of *Cyclotrichium* have bifacial leaves (*C. glabrescens, C. leucotrichum*, *C. longiflorum*

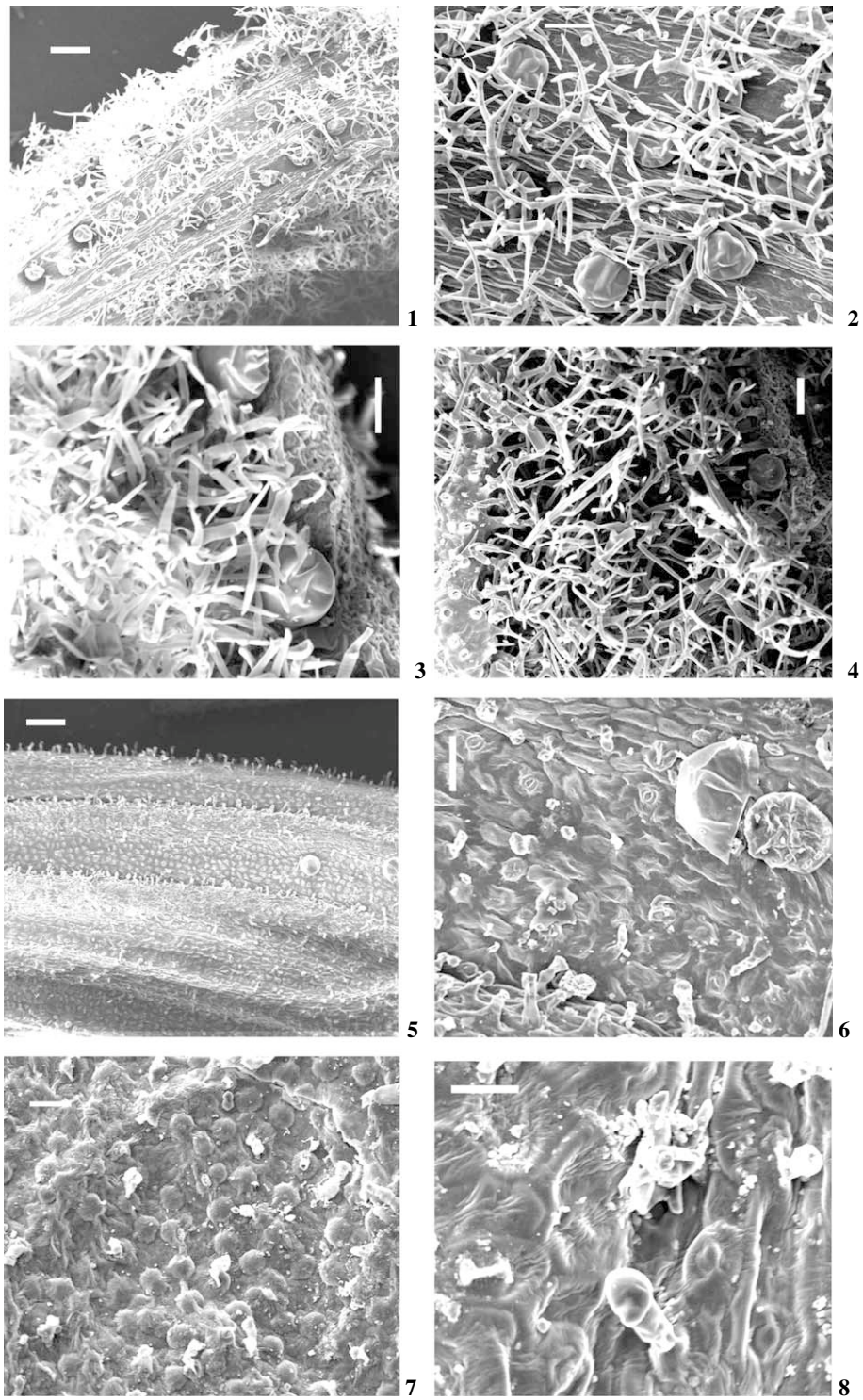

Plate 1–30. Scanning electron micrographs of *Cyclotrichium* trichomes. (1)–(4) *C. niveum*: (1)–(2) calyx, (3) adaxial surface of leaves, (4) abaxial surface of leaves); (5)–(9) *C. glabrescens*: (5)–(6) calyx, (7)–(8) adaxial leaf surface, (9) abaxial leaf surface; (10)–(15) *C. longiflorum*: (10)–(11) calyx, (12)–(13) adaxial leaf surface, (14)–(15) abaxial leaf surface; (16)–(19) *C. stamineum*: (16)–(17) calyx, (18) adaxial leaf surface, (19) abaxial leaf surface; (20)–(24) *C. leucotrichum*: (20) calyx, (21)–(22) adaxial leaf surface, (23)–(24) abaxial leaf surface; (25)–(30) *C. origanifolium*: (25)–(26) calyx, (27)–(28) adaxial leaf surface, (29)–(30) abaxial leaf surface. Scale bars: 1, 5, 10, 12, 14, 16, 21, 23, 25, 27, 29 = 200 μm; 20 = 150 μm; 4 = 100 μm; 2 = 90 μm; 3 = 80 μm; 6, 7, 9, 11, 13, 17, 18, 19, 22, 26, 28, 30 = 60 μm; 8, 15, 24 = 40 μm.

and *C. stamineum*) wheraes *C. niveum* and *C. origanifolium* have equifacial leaves. Both bifacial and equifacial leaves are characteristic for the Lamiaceae family (Metcalfe and Chalk 1950), and they have also been reported in Turkish *Satureja* L. species by Satıl and Kaya (2007). All studied species were found to have amphistomatic leaves with diacytic stomata,

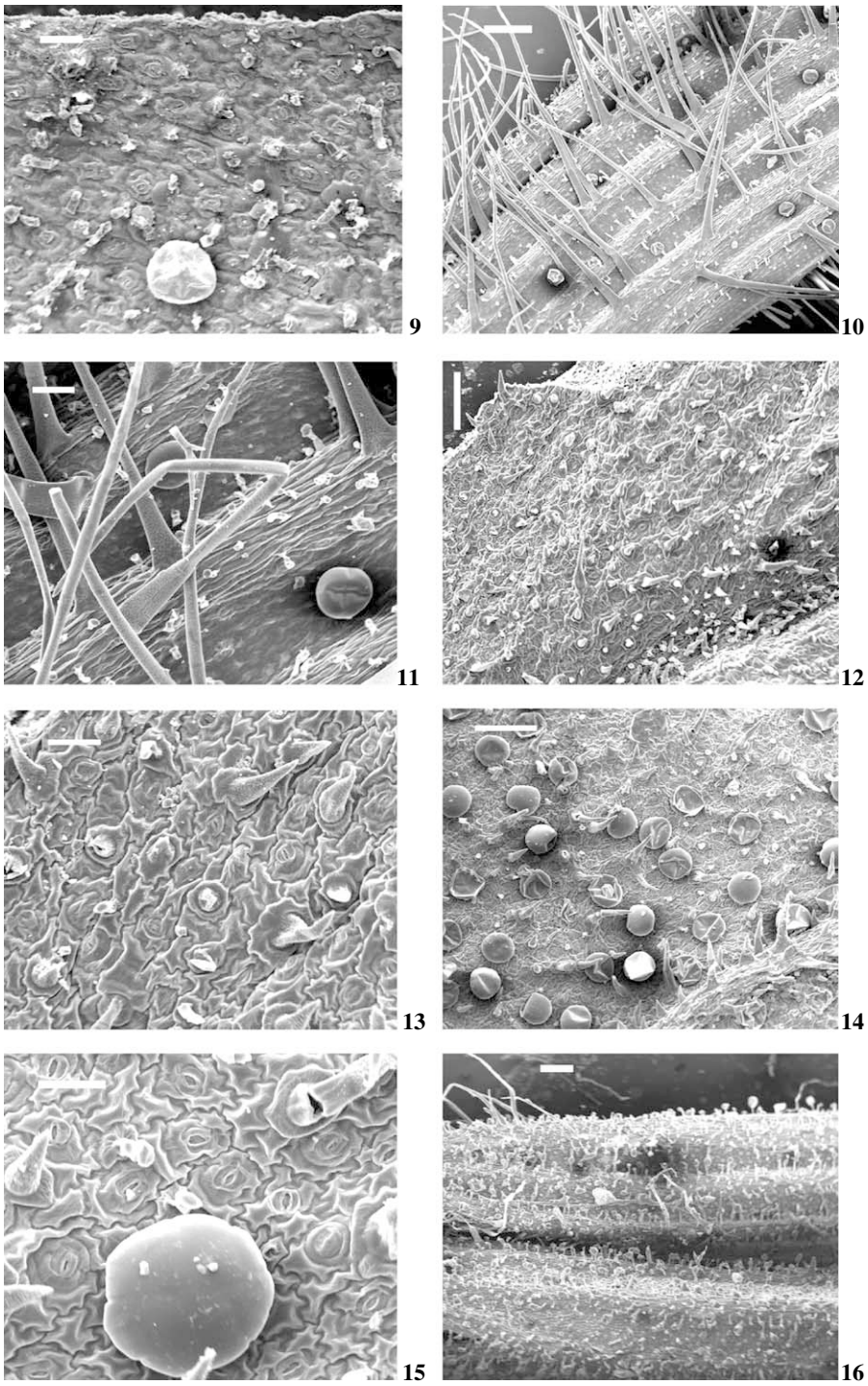

Plate 1–30 (*Continued*)

which are common in the Lamiaceae. In an extensive survey of 127 genera of Lamiaceae, Cantino (1990) found both hypostomatic and amphistomatic leaves in the members of the family, the latter type being slightly more frequent.

Plants may develop structural characteristics that are adaptations to arid habitats. Plants such as *Cyclotrichium* species are termed xeromorphic plants. Xeromorphism, however, is not confined to xerophytes, and not all xerophytes exhibit xeromorphic characters. One of the most obvious features of xeromorphic leaves is the small ratio of the external leaf surface to its volume. The reduction of the external surface is accompanied by certain changes in the internal structure of the leaf as, for instance, reduction in cell size, increase in cell wall thickness, greater density of both the vascular system and of the stomata, and an increased development of palisade tisssue at the expense of spongy tissue (Fahn 2000). Xeromorphic characters, like

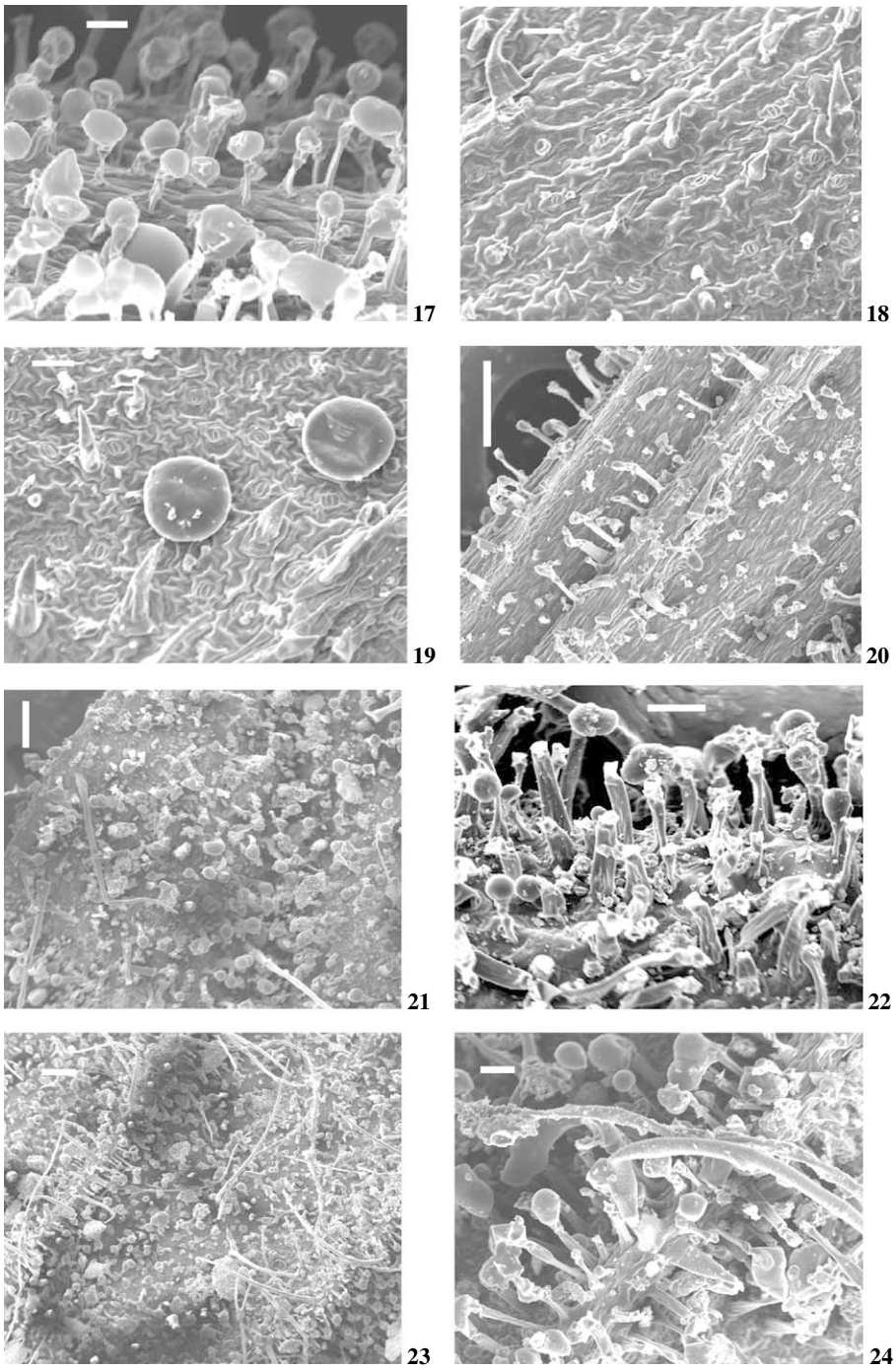

Plate 1–30 (*Continued*)

thick walls and cuticle and the additional development of sclerenchyma, have been observed in the internal structure of *Cyclotrichium* leaves. Xeromorphic leaves are also often covered with trichomes which are generally very common on calyces and leaves of *Cyclotrichium* species. Branched eglandular trichomes are restricted to *C. niveum*, and allows distinguishing this species from the remainder of the genus. Branched trichomes, with well developed axes, covered by micro-papillae, have also been reported in some *Teucrium* L. (Navarro and Oualidi 2000). The eglandular trichomes in other species are always simple and 1- or 7 (–8)-celled. According to our results, multicellular simple eglandular trichomes are not present on the calyx or leaf surfaces of *C. glabrescens* and *C. niveum*.

In the Lamiaceae, glandular trichomes are generally classified as capitate (clavate) or peltate (subsessile; Fahn 2000). Glandular trichomes have a taxonomic value at the species level. The presence and absence of peltate and capitate glandular trichomes is a characteristic feature of Lamiaceae species (Ascensão et al. 1999). Their typology

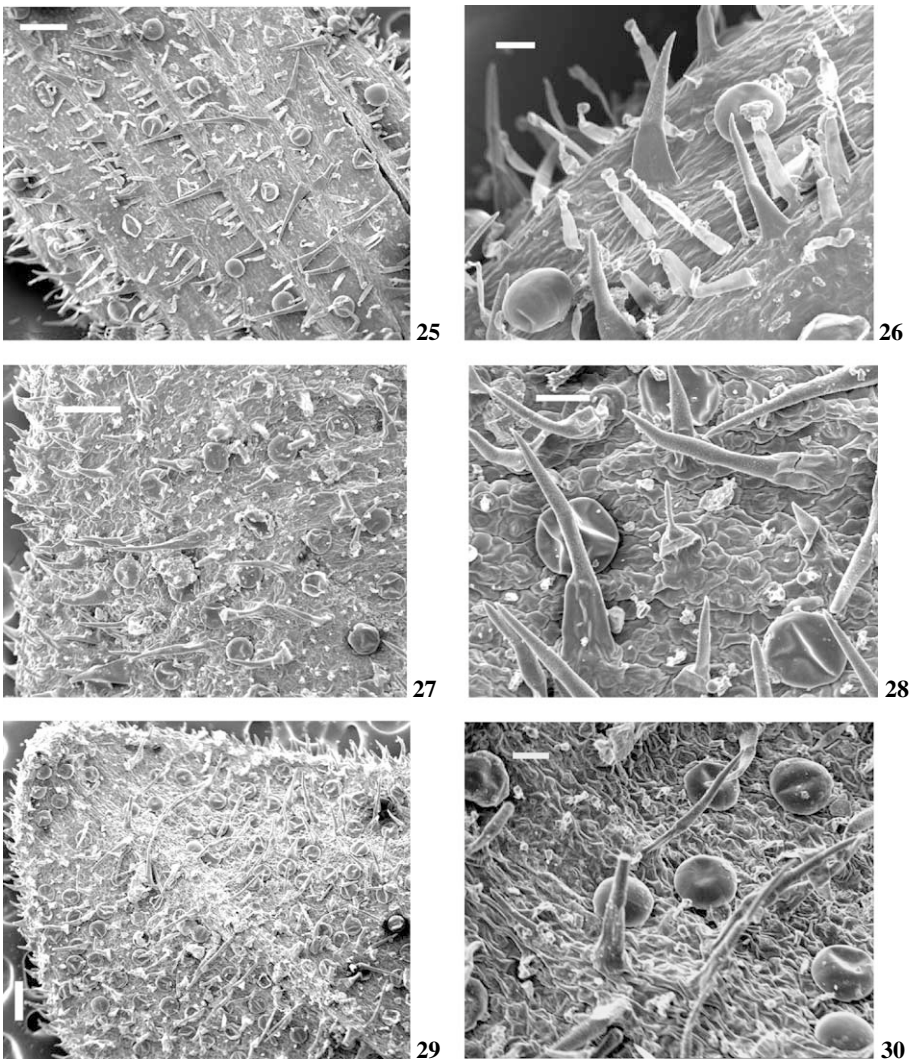

Plate 1–30 (*Continued*)

can be used as taxonomic markers in the infrageneric classification of the genus *Teucrium*, while the infrasectional classification of section *Polium* (Mill.) Schreb. is based almost exclusively on the typology of the trichomes (Navarro and El Oualidi 2000). Unlike peltate trichomes, that have a rather uniform morphology, capitate trichomes varies in terms of morphological characters which reflect different secretory processes and, probably, distinct functions (Ascensão et al. 1999).

The heads of peltate trichomes observed in the present study were made up of twelve (or rarely thirteen) secretory cells arranged in two concentric circles. Similar trichomes have been reported for species of *Salvia* L. (Corsi and Bottega 1999, Özdemir and Şenel 1999), *Satureja* L. (Bosabalidis 1990, Satıl and Kaya 2007) and species of *Salvia*, *Majorana* Mill. and *Rosmarinus* L. examined by Werker et al. (1985). In other members of the family, such as *Leonotis leonurus* (L.) R. Br. (Ascensão and Pais 1998), *Plectranthus ornatus* Codd (Ascensão et al. 1999), *Nepeta racemosa* Lam. (Bourett et al. 1994) and *Nepeta congesta* Fisc. & Mey. var. *congesta* (Kaya et al. 2007a), a lower number of head cells are arranged in a single circle. According to our results, the presence or absence, as well as the density, of peltate trichomes are diagnostic characters of individual species. In particular *C. niveum* and *C. origanifolium* have densely peltate trichomes on both the calyx and leaf surfaces while they are absent on the adaxial leaf surface in the remaining species.

Capitate trichomes are widespread in the Lamiaceae, but they vary greatly in structure and size (Werker et al. 1985). Five types of capitate trichomes were observed by us in *Cyclotrichium*. Type I of capitate trichomes is the most common in the Lamiaceae family, occuring in nearly all studied species (Ascensão et al. 1999, Bisio et al. 1999, Kaya et al. 2003, 2007a, 2007b). Also, type II of capitate trichomes is similar to those reported for *Salvia blepharophylla* by Bisio et al. (1999). Type V of the capitate trichomes is found only on calyces and leaves of *C. leucotrichum* and resemble trichomes reported for *Salvia glutinosa* L. (Kaya et al. 2003), *Salvia halophila* Hedge (Kaya et al. 2007b) and *Salvia huberi* Hedge (Özdemir and Altan 2005).

Key to species of *Cyclotrichium* based on trichomes

1. Calyx and leaves with branched eglandular trichomes . . . . . *C. niveum*
   – Calyx and leaves with only simple eglandular trichomes 2
2. With capitate trichomes with bicellular head and bicellular stalk (type V) on calyx and leaves . . . . . *C. leucotrichum*
   – Type V trichomes generally absent. 3
3. With peltate trichomes on adaxial leaf surface . . . . . *C. origanifolium*
   – Without peltate trichomes on adaxial leaf surface 4
4. Without eglandular trichomes on adaxial leaf surface or multicellular eglandular trichomes on calyx . . . . . *C. glabrescens*
   – With eglandular trichomes on adaxial leaf surface and multicellular eglandular trichomes on calyx 5
5. Dense multicellular eglandular trichomes on calyx and without type III–IV capitate trichomes on both surfaces of leaves . . . . . *C. longiflorum*
   – Rare multicellular eglandular trichomes on calyx and with type III–IV capitate trichomes on both surfaces of leaves . . . . . *C. stamineum*

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Appendix 1. Voucher information:

Cyclotrichium glabrescens: Turkey B8 Muş: Muş in subalpinis, ca 2000 m a.s.l., 9 Sep 1859, Kotschy 1859: suppl. 1688 (isotype: G, K). Diyarbakır: Hazro, Uzunargıt, around Değirmen, stream banks and rocky slopes, 890–930 m a.s.l., 3 Aug 1974, H. Demir, S. Akkuş, H. Olgaç (Dicle Ün. Herb.). B9 Bitlis: Hizan, Karbastı village, Yamaç, 1500 m a.s.l., 24 Jul 2002, T. Dirmenci 1440. Bitlis: Şeyh Habib Mt, 1700 m a.s.l., 5 Aug 2002, A. Altıok 2759 (VANF).

Cyclotrichium leucotrichum: Turkey C8 Mardin: Richemil (Rişmil), 23 Jul 1888, Sintenis 1352 (isotype: E, K). Mardin: 20 km from Mardin to Diyarbakır, south of Sultan Şeyhmus village, 15 Sep 2007, T. Dirmenci 3592. Mardin to Kızıltepe T. Dirmenci 3593. Between Kızıltepe and Mardin, 1500 m a.s.l., 23 Jul 1974, M. Koyuncu 4466 (AEF, GAZI, E). Mardin: Karaman village, 24 Jul 1970, T. Baytop, ISTE 18228 (E). Batman: 2 km from Gerçüş to Mardin, rocky slopes, 15 Sep 2007, T. Dirmenci 3594.

Cyclotrichium longiflorum: Turkey C9 Hakkari: between Hakkari and Çukurca, 13 km, 1200 m a.s.l., 17 Jun 2004, Dirmenci 2476. Şırnak: 60 km from Şırnak to Hakkari, 600 m a.s.l., 8 Jun 2002, Yıldız 15158, Dirmenci and Arabacı. 63 km from Şenoba to Hakkari, rocky slopes, 15 Jul 2001, Z. Aytaç 8171 (ESSE, GAZI, ANK).

Cyclotrichium niveum: Turkey B6 Malatya: in Cappadocia centrali inter Ketche-Mesera et Guruno (Gürün), Tchihatcheff 1854:561 (holotype: G-Boiss.). B6 Sivas: Gürün, Barsakdere, 1500–1600 m a.s.l., 29 Jul 1992, Yıldız 9902. Malatya: between Malatya and Darende, 1 km west of Develi village, ca 1600 m a.s.l., 38°23′184″N, 037°54′571″E, 10 Aug 2007, T. Dirmenci 3504. Sivas: Deli Da., Bornm. 1893, p. 3482 (E, K). Malatya: between Gürün and Malatya, 65 km from Malatya, ca 1500 m a.s.l., 7 Aug 1956, McNeil 450 (E). Doğanşehir, between Erkenek and Alıçlı, 28 Jul 1987, Aktoklu 855 (HUB). B7 Erzincan: Kemaliye (Egin) Sarıkonaklar village, 1800 m a.s.l., 10 Jul 1982, M. Koyuncu et al., (AEF-23266). Adıyaman: Gölbaşı, Hamzaköy, 1060 m a.s.l., 15 Sep 2001, A. Dönmez 10127 (HUB).

Cyclotrichium origanifolium: Turkey C2 Denizli: d. Acıpayam, Bozdağ, 1670 m a.s.l., 16 Jul 1947, Davis 13404 (E). C3 Isparta: Selçuklu, Çimenova, west side of Sang Da., 18 Jul 1949, Davis 15591 (E). C3 Antalya: Akdağ, no. Karabuynus Y., 2000 m a.s.l., 31 Aug 1947, Davis 14533 (E). C3 Antalya: Tahtalı Da., 2100 m a.s.l., 16 Aug 1947, Davis 14144 (E). C4 Antalya: Gebiz, west side of Bozburun Mountain, 1800–1900 m a.s.l., 12 Jul 2002, Dirmenci 1949. C5 İçel: Arslanköy, Bolkar Moun., Gökkol Yayla, 2400 m a.s.l., calcareous, 7 Aug 2002, Dirmenci 2178 and Yıldız. C5 Niğde: Bulgar Maden, 1500 m a.s.l., Jul 1912, Shie 283 (E). C5 Mersin: Anamur, Olucak, between Ermenek and Anamur, 18 Aug 1949, Davis 16334B (E). C5 Adana: Karaisalı, Bulgar Da., between Pozantı and Meydan, 1500 m a.s.l., 1 Sep 1949, Davis 16585 (E). C6 Osmaniye: Amonos, Düldül, 1500–2000 m a.s.l., Jul 1911, Haradj. 3846 (E).

Cyclotrichium stamineum: Iraq in rupestribus umbrosis montis Gara, Kotschy 1841, p. 311 (holotype: G, isotype: K). Turkey: C9 Siirt: Eruh, Yassıdağ (Serikur Da.,) Meşindağ pass (Birini pass), 1640 m a.s.l., 18 Jul 1981, E.Tuzlacı (ISTO-47340). Şırnak: Şırnak to Hakkari B. Yıldız 15158. Hakkari: 2 km from Çukurca to Narlı village, 1200 m a.s.l., 9 Jun 2006, T. Dirmenci 3357a. Çukurca, 1200 m a.s.l., rocky slopes in open *Quercus* sp. forest, 2 Jun 1966, Davis 44745 (ISTO-11473, E). C10 Hakkari: Cilo Da. in Diz dere, 5700 ft, gravel terraces, 8 Aug 1954, Davis 23924 (ANK, E).Yüksekova: no. Oramar (S. of Cilo Da.) 1830 m a.s.l., Trelawny 1845 (E).