

## Morphological, karyological and phylogenetic evaluation of *Cyclotrichium*: a piece in the tribe *Mentheae* puzzle

Tuncay DİRMENÇİ<sup>1</sup>, Ekrem DÜNDAR<sup>2,\*</sup>, Görkem DENİZ<sup>2</sup>, Turan ARABACI<sup>3</sup>, Esra MARTIN<sup>4</sup>,  
Ziba JAMZAD<sup>5</sup>

<sup>1</sup>Balıkesir University, Necatibey Faculty of Education, Department of Biology Education, 10100, Balıkesir - TURKEY

<sup>2</sup>Balıkesir University, Faculty of Arts and Sciences, Department of Biology, 10145, Balıkesir - TURKEY

<sup>3</sup>İnönü University, Faculty of Sciences and Arts, Malatya - TURKEY

<sup>4</sup>Niğde University, Faculty of Sciences and Arts, Niğde - TURKEY

<sup>5</sup>Research Institute of Forests & Rangelands, 13185-116, Tehran, IRAN

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**Abstract:** The genus *Cyclotrichium*, a member of the tribe *Mentheae* subtribe *Menthinae* (Lamiaceae, Nepetoideae), was analysed with respect to morphological revision, phylogenetic analysis, and cytogenetic properties. All species of the genus were investigated for morphological characters and ITS (internal transcribed spacers) of nrDNA sequence comparison (except *C. hausknechtii* for ITS). Six members of the genus were also analysed for chromosome numbers. The combined results strongly suggested that *Cyclotrichium* is a separate genus in *Nepetoideae* with distinct morphological, phylogenetic, and cytogenetic characteristics. For intrageneric phylogeny of *Cyclotrichium*, 3 groups were recognised: 1. *C. niveum*; 2. *C. origanifolium*; and 3. the remaining 6 species. *Clinopodium s.l.* and *Mentha* appear to be most closely related to *Cyclotrichium*. The phylogenetic relationship of *Cyclotrichium* with *Clinopodium s.l.*, *Mentha*, *Micromeria*, *Melissa*, and *Satureja* is discussed. This is the first report on the somatic chromosome numbers of 6 *Cyclotrichium* species and phylogenetic analysis of *Cyclotrichium* based on (nrDNA) ITS sequences.

**Key words:** *Cyclotrichium*, *Clinopodium*, *Satureja*, ITS, phylogeny

### *Cyclotrichium* cinsinin morfolojik, karyolojik ve filogenetik analizi: tribus *Mentheae* bulmacasında küçük bir çözüm

**Özet:** Bu çalışmada *Mentheae* tribusunun *Menthinae* alt tribusuna ait *Cyclotrichium* cinsi (Lamiaceae, Nepetoideae), morfolojik, filogenetik ve sitogenetik yönlerden analiz edilmiştir. Cinsin bütün türleri morfolojik karakterleri ve çekirdek ribozomal ITS (internal transcribed spacers) DNA dizileri açısından incelenmiş ancak *C. hausknechtii* tanımlandığından beri arazide hiç bulunamadığı için ITS dizi analizine katılamamıştır (sadece tip örneğinden morfolojik inceleme yapılmıştır). Cinsin altı türü karyotip analizine tabi tutulmuş ve kromozom sayıları tespit edilmiştir. Sonuçlar *Cyclotrichium*'un belirgin morfolojik, filogenetik ve sitogenetik özellikleriyle *Nepetoideae* içerisinde farklı bir cins

\* E-mail: dundar@balikesir.edu.tr

olduğunu göstermiştir. Cins içi filogenetiğe bakıldığında *Cyclotrichium*'un üç gruba ayrıldığı görülmüştür: 1. *C. niveum*, 2. *C. origanifolium* ve 3. geriye kalan altı tür. *Clinopodium s.l.* ve *Mentha* cinsi *Cyclotrichium*'a en yakın olarak gözlenmiştir. *Cyclotrichium*'un *Clinopodium s.l.*, *Mentha*, *Micromeria*, *Melissa* ve *Satureja* ile ilişkileri tartışılmıştır. Altı *Cyclotrichium* türünün somatik kromozom sayısı ve *Cyclotrichium* cinsinin filogenetik analizi bu çalışma ile ilk defa rapor edilmektedir.

**Anahtar sözcükler:** *Cyclotrichium*, *Clinopodium*, *Satureja*, ITS, filogenetik

## Introduction

A group of Lamiaceae that has caused much confusion over its generic boundaries is the *Satureja* L. complex. Briquet (1897) included a number of genera (e.g., *Satureja*, *Micromeria* Benth., *Clinopodium* L., *Acinos* Mill., and *Calamintha* Mill.) in *Satureja s.l.*, while other taxonomists (Bentham, 1848; Ball, 1972; Davis, 1982; Doroszenko, 1986) preferred a narrower circumscription of taxa and kept these genera separate.

The genus *Cyclotrichium* Manden. & Scheng. is related to these genera in the tribe *Mentheae* subtribe *Menthinae* (Lamiaceae, Nepetoideae) (Harley et al., 2004). In earlier classification, some species of *Cyclotrichium* were included in the genera *Micromeria* (sect. *Piperella* Benth.), *Melissa* L. (sect. *Clinopodium*), and *Clinopodium* (Bentham, 1834, 1848). The *Cyclotrichium* was first described as sect. *Cyclotrichium* Boiss. with 6 species in the genus *Calamintha* by Boissier in *Flora Orientalis* (1879). Later, Briquet classified it in *Satureia* as sect. *Cyclotrichium* Briq. (Briquet, 1897). Finally, Mandenova and Schengelia (1953) introduced *Cyclotrichium* as a separate genus with 6 species.

The small genus *Cyclotrichium* contains 9 species distributed in Turkey, Lebanon, Iraq, and Iran. Only one species of the genus is a Mediterranean element (*C. origanifolium* (Labill.) Manden. & Scheng.) and it

is distributed from Turkey to Lebanon, while all others are Irano-Turanian elements and are distributed nearby at the intersection of the Turkish, Iranian, and Iraqi boundaries (Figure 1). South-west Asia is a speciation centre for *Cyclotrichium* (Leblebici, 1974, 1982; Rechinger 1952, 1982; Davis, 1988; Güner et al., 2000).

According to the latest generic description by Harley et al. (2004), all *Cyclotrichium* species are perennial subshrubs, hairs simple or dendroid; leaves entire or toothed; inflorescence of auxiliary cymes, sometimes very shortly pedunculate, forming terminal thyrse of 6 to many flowered, usually remote verticillasters; bracts, at least the lowest leaf-like, upper small, inconspicuous, bracteoles  $\pm$  inconspicuous, smaller than to equalling calyx; calyx weakly 2-lipped, 5-lobed (3/2), lobes triangular-subulate, posterior lobes usually shorter than anterior; calyx-tube cylindrical, straight or shortly curved, 13-nerved, throat hairy; corolla resupinate (Figure 2), 2-lipped, 4-lobed (3/1), white or lilac, posterior (lower) lip entire or emarginate, anterior (upper) lip 3-lobed, slightly deflexed, median lobe larger, corolla tube twisted, annulate, near middle; stamens 4,  $\pm$  equal, long exserted, anthers ellipsoid, thecae 2, parallel, distinct with short connective; stigma-lobes unequal,  $\pm$  subulate, posterior lobe longer; disc weakly lobed; nutlets ovoid, smooth, glabrous.

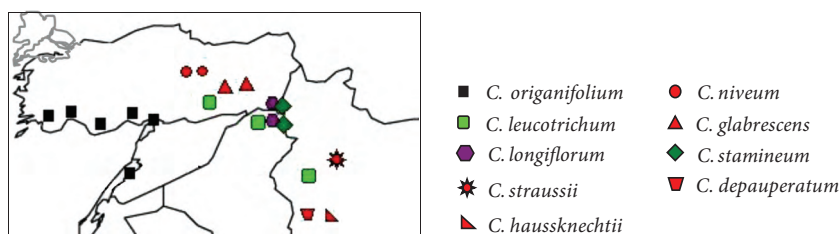


Figure 1. Distribution map of *Cyclotrichium* species.

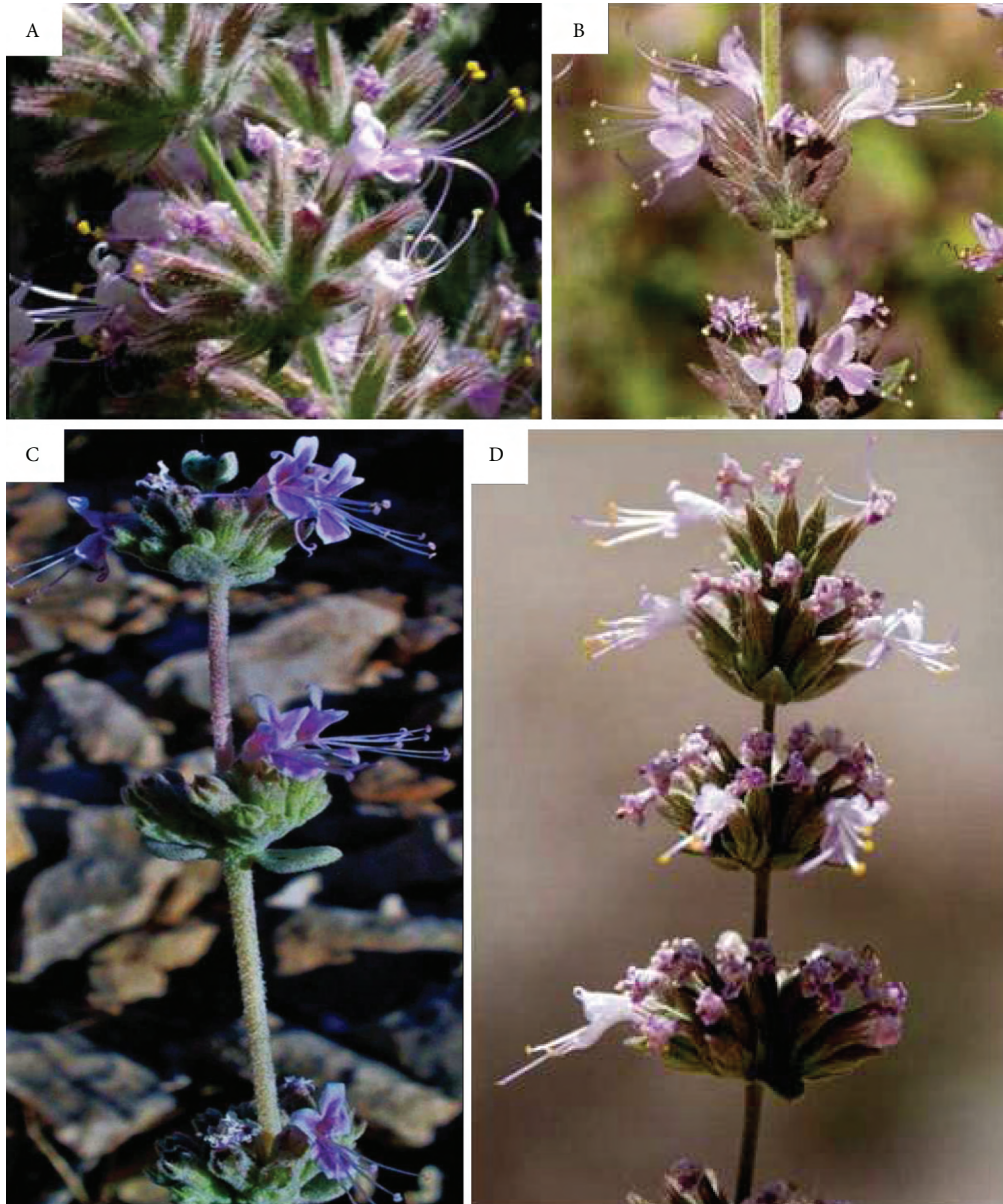


Figure 2. Verticillasters of *C. longiflorum* (A); *C. stamineum* (B); *C. niveum* (C) and *C. glabrescens* (D).

Species of *Cyclotrichium* are as follows (pictures are provided if available): *C. depauperatum* (Bunge) Manden. & Scheng., *C. glabrescens* (Boiss. & Kotschy ex Rech.f.) Leblebici, *C. leucotrichum* (Stapf. ex Rech.f.) Leblebici, *C. longiflorum* Leblebici (Figure 2A), *C. hausknechtii* (Bunge) Manden. & Scheng., *C. niveum* (Boiss.) Manden. & Scheng., *C. organifolium*, *C. stamineum* (Boiss. & Hohen) Manden. & Scheng. (Figure 2B), and *C. straussii* (Bornm.) Rech.f.

*C. niveum* (Figure 2C) and *C. glabrescens* (Figure 2D) are endemic to Turkey, while *C. hausknechtii*, *C. straussii*, and *C. depauperatum* are endemics of Iran.

*Cyclotrichium* species contain aromatic essential oils and hence they are often utilised as spices. They are also consumed as herbal tea in Turkey. Local names of these plants are “dağ nanesi”, “kız otu”, “köpek nanesi”, “karabaş otu”, and “naneruhu” (Baser et al., 1996).

Major secondary metabolites of *Cyclotrichium* species are isopinocampone, terpinen-4-ol, spathulenol, menthone, thymol, carvacrol, and pulegone (Baser et al., 2001; Kilic et al., 2007). Secondary compounds based on species are as follows: *C. niveum* contains pulegone; *C. glabrescens* contains thymol and carvacrol; *C. longiflorum* and *C. stamineum* contains isopinocampone; *C. leucotrichum* contains pulegone, p-mentha-3.8-diene, and t-cadinol; *C. origanifolium* contains isopinocampone and menthone (Baser et al., 1994; Baser et al., 1996; Baser et al., 2001; Tepe et al., 2005; Aslan et al., 2007, Kilic et al., 2007); *C. straussii* contains  $\beta$ -caryophyllene and germacrene D (Nori-Sharg & Baharvand, 2006); and *C. depauperatum* contains *cis*-pinocampone and pulegone (Sajjadi & Mehregan, 2006).

Although *Cyclotrichium* has been established as a separate genus since 1953 (Mandenova & Schengelia, 1953), it had long been confused with *Micromeria*, *Melissa*, *Clinopodium*, *Calamintha*, *Acinos*, and *Satureja* (see above). Moreover, *Calamintha* and *Acinos*, which are closely related to *Cyclotrichium*, have recently been transferred into *Clinopodium* (Govaerts, 1999; Harley & Granda, 2000; Ryding, 2005; Bräuchler et al., 2006) although the word *Calamintha* has not been completely abandoned (Özhatay et al., 2009). Hence a molecular phylogenetic analysis will greatly help in the establishment of the correct classification of this complex in addition to intrageneric revision of *Cyclotrichium*. For this purpose, an ITS (Internal Transcribed Spacers) sequence based phylogenetic analysis covering all of the *Cyclotrichium* species (except *C. hausknechtii*, which was never recollected) along with the related genera mentioned above were conducted. Utilisation of ITS phylogeny in plants has been effective (Baldwin et al., 1995; Álvarez & Wendel, 2003) and it has been validated by numerous reports (e.g., Liston et al., 1999; Steane et al., 1999; Bellarosa et al., 2005). Cytogenetic analysis comparing the chromosome number of *Cyclotrichium* species and morphological revision (including *C. hausknechtii* from the type specimen and isotype specimens) of the genus was also conducted.

## Materials and methods

### Morphological Evaluation

All *Cyclotrichium* species were morphologically evaluated from collected specimens and/or herbarium specimens (including type specimens and isotype specimens) in ANK (Ankara University), BM (Natural History Museum, London), E (Edinburgh), G-Boiss (Geneva), GAZI (Gazi University), HUB (Hacettepe University), ISTE (İstanbul University, School of Pharmacy), ISTO (İstanbul University, Faculty of Forestry), K (Kew), W (Natural History Museum), and in WU (Wien University). Voucher information can be found in the Appendix.

### Genomic DNA isolation, PCR, and Sequencing

Genomic DNA isolation was performed both manually as described by Dellaporta et al. (1983) and using a Plant DNeasy kit (Qiagen GmbH, Hilden, Germany). PCR was run using the published ITS primers (White et al., 1990; Sang et al., 1995) with the following protocol on a Techne Thermal Cycler (Techne, Cambridge, UK): 5 min 95 °C initial denaturation, 35 cycles of 30 s 94 °C denaturation, 30 s 50 °C annealing and 1 min 72 °C extension, followed by a 10 min final extension at 72 °C. The primers used to amplify ITS regions were also used for sequencing, which was accomplished commercially by a biotechnology company (RefGen, Ankara, Turkey).

### Phylogenetic Analysis

Phylogenetic analysis was conducted using the programs BioEdit (Hall, 1999) and PAUP 4.0b10 (Swofford, 2001). Multiple ITS sequences for the same species collected from conditions with potential phylogenetic importance were included in the tree although no sequence difference was detected. ITS sequences of 3 species to represent each of the related genera were obtained from GenBank and their accession numbers were indicated in parentheses on the tree. Since *Clinopodium s.l.* is the most complex genus related to *Cyclotrichium*, members of this genus from the Old World (former *Calamintha* taxa *Clinopodium betulifolium* (Boiss. & Balansa) Kuntze, *C. tauricolum* (P.H.Davis) Govaert, and *C. grandiflorum* (L.) Kuntze, and former *Acinos* species (*Clinopodium alpinum*) and from the New World (*Clinopodium georgianum* R.M.Harper, *C. dentatum* (Chapm.) Kuntze, and *C. coccineum* (Nutt. ex Hook.) Kuntze) were also included in the tree in addition to

the fact that the Old World species are also new transfers from former genera (*Calamintha* and *Acinos*). For both *Melissa* and *Acinos* there was only one ITS sequence for each in the databases available. ITS sequences of *Clinopodium betulifolium*, *Clinopodium tauricum*, and *Clinopodium grandiflorum* (former *Calamintha* taxa) were obtained through collecting specimens, PCR amplification, and sequencing as described above.

### Karyology

Plant materials from Turkey were collected between 2005 and 2008. Vouchers have been deposited at the Balıkesir Herbarium and Biology Department of Balıkesir University, Turkey. Root tips were obtained from surface-sterilised seeds germinated for 2 days on wet filter paper for the somatic chromosome numbers. Root tips were pre-treated with  $\alpha$ -monobromonaphthalene for 16 h at 4 °C, fixed in 3:1 ethanol:glacial acetic acid for 24 h, and stored at 4 °C until use. The root tips were washed in distilled water to remove the fixative, hydrolysed in 1 N HCl for 12 min at room temperature, and stained with 2% acetorcein for 2 h. Permanent slides were made with the standard liquid nitrogen method. The slides were dried for 24 h at room temperature and mounted in Depex.

## Results

### Revised Dichotomous Key

Below is a revised dichotomous key generated in light of the characters listed in the Table, based on the *Flora of Turkey and the East Aegean Islands* (Davis, 1982) and the *Flora Iranica* (Leblebici, 1982; Rechinger, 1982), along with our morphological evaluation of both collected specimens and herbarium materials.

1. Plant densely white-tomentellous with dendroid hairs ..... ***C. niveum***
1. Plant glabrous to various hairy, glandular or not
  2. Calyx conspicuously bilabiate, upper lip divided to halfway into  $\pm$ recurved, triangular-acuminate teeth ..... ***C. organifolium***
  2. Calyx sub-bilabiate, (often inconspicuously); teeth erect, triangular-lanceolate to subulate
  3. Calyx glabrous or with minute glandular papillose and sessile glands, without long hairs
    4. Stems 10-15 cm, canescent, leaves ovate, acute, verticillasters many flowered, bracteoles as long as calyx, calyx grabrous or with minute glandular papillose (see Discussion) ..... ***C. haussknechtii***
    4. Stems to 35 cm, hirsute-villose, leaves suborbicular, obtuse to rounded, verticillasters lax, bracteoles as long as pedicel ..... ***C. depauperatum***
3. Calyx glandular puberulent or glandular pruinose, sometimes with pilose or villose hairs.
  5. Stems at least densely long pilose at verticillasters, eglandular or glandular, calyx densely long pilose
    6. Bracteoles 2.5-5 mm, calyx 5-7 mm ..... ***C. straussii***
    6. Bracteoles 4.5-6 mm, calyx 8-11 mm ..... ***C. longiflorum***
  5. Stems glandular-puberulent or glandular-pruinose with or without long hairs
    7. Stems glandular puberulent, with long spreading eglandular hairs at least at nodes; lower calyx teeth 1.5-2.5 mm, not ciliate ..... ***C. leucotrichum***
    7. Stems glandular-pruinose, lacking long eglandular hairs; lower calyx teeth 2.5-4 mm, often ciliate.
    8. Bracteoles 3-4 mm, linear-lanceolate, shorter than tube, calyx straight, tube with or without long hairs; teeth ciliate or not ..... ***C. stamineum***
    8. Bracteoles 5-6 mm, lanceolate, as long as or longer than tube, calyx slightly curved, without long hairs, teeth ciliate ..... ***C. glabrescens***

### Phylogenetic Tree

A cladogram displaying the phylogenetic position of *Cyclotrichium* species with respect to each other and with respect to related genera (*Clinopodium s.l.*, *Mentha*, *Micromeria*, *Satureja*, *Thymus*, and *Melissa*) was constructed (Figure 3). ITS sequences for *Clinopodium betulifolium*, *C. tauricum* and *C.*

Table. Morphological traits of *Cyclotrichium* species.

Species / Trait	Stem	Leaves	Bracteoles	Calyx	Corolla
<i>C. haussknechtii</i>	10-15 cm, glandular pubescent, canescent	ovate, 8-10 × 5-7 mm, glandular papillate, hairy	as long as calyx, 5.5-7 mm, lanceolate	6.5-8 mm, scarcely bilabiate, glabrous rarely glandular, teeth lanceolate, ciliate, upper teeth 1.7-2 mm lower teeth c. 2.5	6-7 mm
<i>C. straussii</i>	25-30 cm, hirsute-villose, glandular papillate	ovate-orbicular, 10-15 × 8-14 mm, hirsute-villose	2.5-5 mm, linear-lanceolate	subbilabiate, 5-7 mm, hirsute-villose with glandular papillate, teeth lanceolate, the uppers to 2 mm, the lowers c. 3 mm	10-12 mm
<i>C. depauperatum</i>	35 cm, pubescent with short crispy hairs, sessile glands	ovate-orbicular, c. 10 × 10 mm, short villose-tomentose	as long as pedicel, 2-3 mm, lanceolate	± actinomorphic, 6-7 mm, without long hairs (rarely villose), glandular papillate with sessile glands, teeth 1.5-2 mm	9-11 mm
<i>C. niveum</i>	20-50 cm, white-tomentellous, dendroid hairy, sessile glands	ovate-elliptic, 8-14 × 4-9 mm dendroid hairy	shorter than tube, 1.5-2 mm, linear to linear-subulate	4-6 mm, subbilabiate dendroid hairy upper teeth 0.5-1 mm, lower teeth 1-1.5 mm	7-9 mm
<i>C. origanifolium</i>	to 25 cm, hirsute or glabrous, with sessile or subsessile glands	ovate to ovate-orbicular, 6-15 × (4-5)-13 mm, hirsute, with sessile glands or subsessile glands	as long as calyx tube, 4-5 mm, lanceolate-acuminate to subulate	clearly bilabiate, 4.5-7.5 mm, slightly curved, glabrous to densely hirsute, upper teeth 0.8-1.5 mm, lower teeth, 1.2-2.5 mm	8-12 mm
<i>C. leucotrichum</i>	15-45 cm, yellowish-green glandular-puberulent	ovate, ovate-elliptic, 18-25 × 10-15 mm, glandular papillate, long hairy	shorter than tube, 3.5-5 mm, lanceolate-subulate	subbilabiate, 5.5-8 mm, straight, densely glandular papillate upper teeth 1-1.5 mm, lower teeth 1.5-2.5 mm	9-12 mm
<i>C. glabrescens</i>	10-25 cm, glaucescent-green, glandular pruinose, lacking long hairs	ovate to broadly ovate, 10-23 × 8-20 mm, densely glandular papillate with sessile glands below	5-6 mm, lanceolate	subbilabiate, 6.5-8 mm, slightly curved, glandular papillate, lower teeth 2.5-3 mm, upper teeth 2-2.5 mm	9-12 mm
<i>C. stamineum</i>	to 35 cm, glaucescent-green, minutely glandular-pruinose without or sparsely long hairs	ovate, 15-20 × 7-15 mm densely glandular papillate with or without sparsely long hairs	½-1 × tube, 3-4 mm, linear-lanceolate	subbilabiate, 6-8 mm, straight, glandular papillate, upper teeth 1.2-2 mm, lower teeth 2-3 (3.5) mm	8-12 mm
<i>C. longiflorum</i>	to 45 cm, puberulent, sessile glands, slightly glandular papillate	ovate to broadly ovate, 15-25 × 10-18 mm, without long hairs, with sessile glands	shorter than tube, 4.5-6 mm, linear-lanceolate	subbilabiate to bilabiate, 8-11 mm, slightly curved, densely pilose, upper teeth 2-3 mm, lower teeth 3-4 mm	10-13 mm

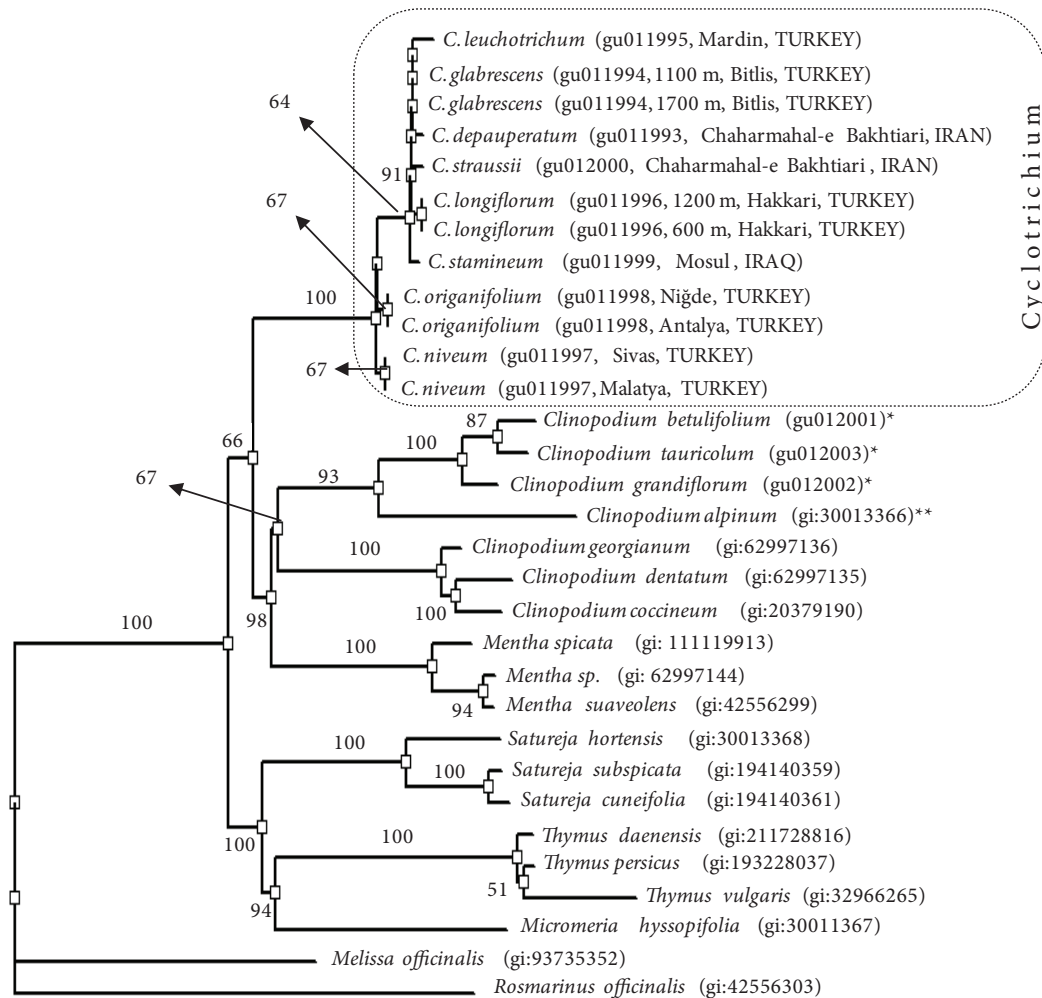


Figure 3. The cladogram (generated from ITS sequences) displaying the phylogenetic position of *Cyclotrichium*. *Rosmarinus officinalis* was used as an outgroup. The alignment of the sequences was generated using the Clustal W algorithm (Thompson et al., 1994) and DNADist function of BioEdit program (Hall, 1999) was used to generate the tree. Percentage bootstrap values (of 1000 replicates) shown on branches of the tree were calculated by PAUP program (Swofford, 2001). Branch lengths are proportional to distances. The accession numbers of ITS sequences obtained from GenBank are indicated in parentheses. *Cyclotrichium* species are highlighted in a rounded rectangle. Factors with potential phylogenetic importance such as geographical distance and altitude for the same species are indicated in parentheses for each relevant taxon of *Cyclotrichium* (also see the Discussion). \*Former *Calamintha* species. \*\*Former *Acinos* species. Accession numbers starting with "gu" belong to sequences that were obtained through this study.

*grandiflorum* were generated along with *Cyclotrichium* specimens as described in the Materials and Methods, while all other sequences were taken from GenBank. *Cyclotrichium* species clearly formed a distinct branch displaying a monophyletic genus (Figure 3). Specimens of the same species collected from different geographical regions and from different altitudes

displayed no ITS sequence difference. ITS sequences for *C. hausknechtii* were not included in the phylogenetic tree because this species has never been re-collected anywhere in the field since it was first collected. Morphological evaluation, however, has been conducted on the type specimen (in G-Boiss.) and on isotype specimens (in K and in BM).

### Karyological features

The somatic chromosome numbers of 6 *Cyclotrichium* species, collected from different localities in Turkey, were determined for the first time. *Cyclotrichium* species investigated were diploid with chromosome numbers counted as  $2n = 16$ . The basic chromosome number of the genus is  $x = 8$ . Analyses were performed in the following species: *Cyclotrichium leucotrichum* (T. Dirmenci 3593), *C. glabrescens* (T. Dirmenci 3592-b), *C. origanifolium* (T. Dirmenci 2178), *C. niveum* (T. Dirmenci 3566), *C. stamineum* (B. Yıldız 16935), and *C. longiflorum* (B. Yıldız 16922).

### Discussion

Although *Cyclotrichium* has been established as a distinct genus since 1953 (Mandenova & Schengelia, 1953), it has a long confused taxonomical history involving *Clinopodium s.l.*, *Micromeria*, *Melissa*, and *Satureja* (see Introduction). The *Satureja* L. complex that *Cyclotrichium* belongs to is still undergoing taxonomical changes as in the examples of *Calamintha*, *Micromeria* sect. *Pseudomelissa*, and *Acinos*'s recent transfer into *Clinopodium* (Govaerts, 1999; Harley & Granda, 2000; Ryding, 2005; Bräuchler et al., 2006). With this respect, revised taxonomy of these genera backed with molecular analysis is still needed. For this purpose, phylogenetic analysis of *Cyclotrichium* has been conducted for the first time. The Old World members of *Clinopodium s.l.* (former *Calamintha* taxa *Clinopodium betulifolium*, *C. tauricum*, and *C. grandiflorum*) appeared closer to *Cyclotrichium* than other previously confused genera (such as *Satureja*, *Micromeria*, and *Melissa*) in the phylogenetic tree, suggesting its closer relation to *Clinopodium s.l.*

The phylogenetic tree (Figure 3) clearly displays *Cyclotrichium* as a monophyletic genus and it is in very good accordance with morphological taxonomy. The tree also displays all the genera included as separate groups as well as *Cyclotrichium*, which was previously quite confused with the other mentioned genera. Hence, our phylogenetic analysis strongly supports Mandenova and Shengalia's (1953) suggestion based on morphological taxonomy that *Cyclotrichium* is a distinct genus from all the others in question.

As for the intrageneric phylogeny, the cladogram is in very good accordance with the morphological classification. As can be seen from the dichotomous key and the detailed morphological traits table (Table), the genus splits into 3 groups: 1. *C. niveum* group, 2. *C. origanifolium* group, and 3. the remaining 6 species. *C. niveum* is full of white-tomentellous and dendroid hairs on all surfaces of the plant. In this respect, *C. niveum* is distinctly separated from all other species. *C. origanifolium* has a clearly bilabiate calyx with a recurved upper lip that is also distinct from all other species. The third group has a sub-bilabiate calyx and simple hairs in common. This is almost exactly the case in the phylogenetic tree, where the 3 distinct groups mentioned can be clearly seen (Figure 3).

Morphological analysis of *C. hausknechtii* revealed some observations that are important for the dichotomous key. In the original description of *C. hausknechtii* there is no mention of a hairy calyx. Likewise, in *Flora Orientalis* (Boissier, 1879) and in *Flora Iranica* (Rechinger, 1982), it differs from the other species with its calyx having no hair. In our detailed morphological evaluation of type specimen (in G-Boiss.) and isotype specimens (in K and in BM), however, dense minute glandular papillose hairs on the calyx were observed. These hairs cannot be clearly seen on the isotype in K because the specimen is highly deformed and very little material is left. The type specimen in G-Boiss., however, has been very well preserved and all the morphological details can be clearly seen. Based on these observations, we suggest *C. hausknechtii* is more closely related to *C. leucotrichum* rather than to *C. depauperatum*. Therefore, to locate and collect new specimens of *C. hausknechtii* is essential to assess its correct systematic position as well as to finalise the necessary modifications in the key.

One interesting observation based on both morphology and molecular data is that significant differences with potential phylogenetic importance (such as long geographical distance and high altitude differences) caused no difference in *Cyclotrichium*'s intrageneric taxonomy. Two different specimens of *C. origanifolium* collected from Antalya and Niğde for instance, displayed no taxonomical difference despite about 550 km distance and a different climate.



Likewise, no taxonomical difference was observed for the 2 specimens of *C. longiflorum* that were collected at 600 m and 1200 m respectively, in Hakkari (Figure 3).

Related to the genus *Cyclotrichium*, no karyological studies have been reported in the literature. This is the first record on the somatic chromosome numbers of 6 species of the genus that are natively distributed in Turkey. As in the historical confusion based on morphology, *Clinopodium s.l.*, *Mentha*, *Micromeria*, *Satureja*, and *Melissa* are somewhat intermingled in terms of chromosome numbers. Somatic chromosome number (2n) of *Clinopodium s.l.* is 2n = 18, 20, 22, 24, 48 (Nilsson & Lassen, 1971; Löve & Kjellqvist, 1974; Ubera, 1979; Fernandes & Leitao, 1985; Morales, 1990, 1994), *Mentha* has 2n = 18, 20, 24, 36, 40, 48, 50, 72, 96, 120 (Harley & Brighton, 1977; Chambers & Hummer, 1994) *Micromeria* has 2n = 20, 22, 26, 30, 48, 60 (Cardona, 1973; Cardona & Contandriopoulos, 1983; Fernandes & Leitao, 1985; Morales, 1990, 1991; Luque

& Lifante, 1991; Bräuchler, et al., 2008), *Satureja* has 2n = 30 (Löve & Kjellqvist, 1974; Lopez, 1982), and *Melissa* has 2n = 32 (Fernandes & Leitao, 1985). *Cyclotrichium*, however, has 16 somatic chromosomes (2n) and this number clearly separates this genus from all the others mentioned.

In summary, this is the first report that presents a morphological and phylogenetic revision including all species of *Cyclotrichium*, and also suggests a different position of the genus based on chromosome numbers.

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## Appendix

Voucher specimens of the genera *Cyclotrichium* Manden. & Scheng. and *Clinopodium s.l.* Mill., which are examined in the present study. \*specimens that were used for genomic DNA extraction.

***Cyclotrichium depauperatum*: Iran** Iter Syriaco-Armeniacum, Bors valley fl. Chyrsan (Khersan), 3000 ped. 08.1868, *Hauskn. s.n.* (**holotype G, isotype K, BM**). Chaharmahal-e Bakhtiari, Dareh Bazoft, Kuh-e Keynu, 2150-2400m, *Mozaffarian* 58000\* (TARI).

***Cyclotrichium glabrescens*: Turkey** B8 Muş: Muş in subalpinis, c. 2000 m, 09.09.1859, *Kotschy* 1859: Suppl. 1688 (**isotype G, K**). Diyarbakır: Hazro, Uzunargit, around Değirmen, stream banks and rocky slopes, 890-930 m, 03.08.1974, *H.Demir, S.Akkuş, H.Olgaç* (Dicle Ün. Herb.). B9 between Bitlis and Baykan 26 km, N 38° 14' 860'', E 041° 57' 329'', ca. 1100 m, 07.08.2007, *Dirmenci* 3592-b. Bitlis: Hizan, Karbastı village, Yamaç, 1500 m, 24.07.2002, *T.Dirmenci* 1440\*. Between Bitlis and Baykan 26 km, N 38° 14' 860'', E 041° 57' 329'', ca. 1100 m, 27.06.2007, *T.Dirmenci* 3441\*; *ibid.*, 07.08.2007, *T.Dirmenci* 3592-b & M. Fırat. Şeyh Habib Mt., 1700 m, 05.08.2002, *A.Altiok* 2759 (VANF). Kurtboğan hill, 1650 m, 08.08.2001, *A.Altiok* 1756 (VABF). Around Karınca Karakolu, 1300 m, *A.Altiok* 1539 (VANF). *ibid.*, 1300 m, 15.07.2001, *A.Altiok* 1536 (VANF). Bitlis: Hizan, Karbastı village, Gerzemel Mountain, rocky places, 1600-1700 m, 17.07.2001, *Dirmenci* 1440.

***Cyclotrichium haussknechtii*: Iran** in fissuris rup. Teng Tokab, Nekbehan, 3000 ped., 06.1868, *Hauskn. s.n.* (**holotype G, isotype K, BM**).

***Cyclotrichium leucotrichum*: Turkey** C8 Mardin: Richemil (Rışmil), 23.07.1888, *Sintenis* 1352 (**isotype E**). Mardin: 20 km from Mardin to

Diyarbakır, S. of Sultan Şeyhmus village, 15.09.2007, *T.Dirmenci* 3592. between Kızıltepe and Mardin, 1500 m, 23.07.1974, *M.Koyuncu* 4466 (AEF, GAZI, E). between Mardin and Kızıltepe, 1 km, calcareous rocky place, 22.07.2005, *Arabacı* 2139\*; *ibid.*, rocky slopes, ca. 1000 m, 15.09.2007, *T.Dirmenci* 3593. Mardin: Karaman village, 24.07.1970, *T.Baytop*, ISTE 18228 (E). Batman: 2 km from Gerçüş to Mardin, rocky slopes, 15.09.2007, *T.Dirmenci* 3594. 3 km from Hasankeyf to Mardin, rocky slopes, 16.09.2007, *T.Dirmenci* (observation).

***Cyclotrichium longiflorum*: Iraq** Mosul: ad confinae Turciae prov. Hakkari, in ditone pagi Sharanish, in montibus calc. a Zakho, septentrionem versus. Jabal Khantur, in saxosis, 1200 m. 4-9.07.1957, *K.H.Rechinger* 10794 (**holotype E, isotype G, W**). Mosul: ad confinae Turciae prov. Hakkari, in ditone pagi Sharanish, in jugo montis Zawita, ca. 1800 m, 09.07.1957, *KH.Rechinger* 11992 (E, G, W). Mosul: ad confinae Turciae prov. Hakkari, in ditone pagi Sharanish, in jugo montis calc. a Zakho, septentrionem versus, in saxosis faucium supra marsis, ca. 1200 m, 09.07.1957, *KH.Rechinger* 10891 (E, G, W). Mosul: In fissures rupium calc. Septentr. Versus expositis supra pagum Basingera, ca 1200 m, 09.07.1957, *K.H.Rechinger* 11512 (W). **Turkey** C9 Hakkari: between Hakkari and Çukurca, 13 km, 1200 m, 17.06.2004, *Dirmenci* 2476\*; *ibid.*, 15 km, rocky places, c. 1200 m, 09.06.2006. *Dirmenci* 3356. Şırnak: 60 km from Şırnak to Hakkari, 600 m, 08.06.2002, *Yıldız* 15158\*, *Dirmenci* & *Arabacı*. 63 km from Şenoba to Hakkari, rocky slopes, 15.07.2001, *Z.Aytaç* 8171 (ESSE, GAZI, ANK).

***Cyclotrichium niveum*: Turkey** B6 Malatya: in Cappadocia centrali inter Kette-Mesera et Guruno (Gürün), *Tchihatcheff* 1854:561 (**holotype G-Boiss.**). B6 Sivas: Gürün, Barsakdere,

1500-1600 m, 29.07.1992, *Yıldız* 9902\*. Divriği, kale çevresi, hareketli yamaç, kalker, 1100 m, 08.09.1994, *A.A.Dönmez* (4270) & *Z.Yeşilyurt* (GAZI). 20 km from Gürün to Darande, 23.08.1991, *M.Kara* (ESSE 9228). Malatya: between Malatya and Darende, 1 km west of Develi village, c. 1600 m, N 38° 23' 184'', E 037° 54' 571'', 10.08.2007, *Dirmenci* 3506 & *Arabacı*. Between Gürün and Darende, 15 km, 1570 m, 11.07.2008, *Z.Aytaç* (9094) et al., (GAZI). Malatya/Sivas: between Gürün-Darende, rocky slopes, 1600 m, 28.06.1974, *K.Karamanoğlu* & *M.Koyuncu* (AEF-4532). Sivas: Deli Da., Bornm. 1893:3482 (E, K). Malatya: between Gürün and Malatya, 65 km from Malatya, c. 1500 m, 07.08.1956, *McNeil* 450 (E). Gürün, 40 km from Malatya, c. 1400 m, *McNeill* 466 (E, K). Malatya: between Malatya and Darende, South of Develi village, rocky place, 1400 m, 20.07.2005, *Arabacı* 2137\*. Doğanşehir, between Erkenek and Alıçlı, 28.07.1987, *Aktoklu* 855 (HUB). Darande, kayalıklar, 1200-1800 m, *A.Rıza Gürgen* (AEF-17918). Between Malatya and Darende, 1 km E of Develi village, N 38 23 184, E 037 54 571, 4795 ft., 10.08.2007, *T.Dirmenci* 3504 & T. Arabacı. **B7** Erzincan: Kemaliye (Egin) Sarıkonaklar village, 1800 m, 10.07.1982, *M.Koyuncu* et al., (AEF-23266). Kemaliye (Egin) around of Sarıkonaklar village, 1400 m, 09.07.1982, *M.Koyuncu* (5780) et al., (AEF-10532). Kemaliye (Egin), Ariki Da., 07.07.1977, *O.Soner* (AEF-6166). Kemaliye, Aşağı Umutlu, Gökseki Da., 1500-2400 m, *M.Tanker* & *M.Çoşkun*, 26.09.1984 (AEF-14675). Kemaliye (Egin), Kotachan, in dedivil. Lapidosis, 05.07.1890, *Sintenis* 2901 (K). Kemaliye, around of Sandıkbağı, 900 m, 17.11.1980, *Ş.Yıldırım* 4168 (HUB). Kemaliye, Başpınar, Buğdaypınar, 1000-1200 m, *Ş.Yıldırım* 4225 (HUB). Adıyaman: Gölbaşı, Hamzaköy, 1060 m, 15.09.2001, *A.Dönmez* 10127 (HUB).

***Cyclotrichium origanifolium*: Turkey**  
 C2 Denizli: d. Acıpayam, Bozdağ, 1670 m, 16.07.1947, *Davis* 13404 (E). C3 Isparta: Selçuklu, Çimenova, W side of Sang Da., 18.07.1949, *Davis* 15591 (E). Isparta: Sütçüler, Dedegöl Da., 2200 m, 02.08.1949, *Davis* 15993 (E). Antalya, Kemer, Akdağ, 1600 m, 10.07.1949, *Davis* 15111 (E). C3 Antalya: Akdağ, nr. Karabuynus Y., 2000 m, 31.08.1947, *Davis* 14533 (E). C3 Antalya: Tahtalı Da., 2100 m, 16.08.1947, *Davis* 14144 (E). C4 Antalya: Gebiz, west side of Bozburun Mountain, 1800-1900 m, 12.07.2002, *Dirmenci* 1949\*. C5 İçel: Arslanköy, Bolkar Da., Gökkol Yayla, 2400 m, calcareous, 07.08.2002, *Dirmenci* 2178 & *Yıldız*. C5 Niğde: Çamardı, Aladağ, road of Yedigöller, c. 2000-2200 m, 04.08.2006, *Yıldız* 16443\* & *Dirmenci*. C4 Antalya: Akdağ, S. of Geyik Da., above Gözübüyük yayla, 2200 m, 28.08.1947, *Davis* 14327 (E). C4 Antalya: distr. Elmalı, Üçkuyular, 1750 m, 28.07.1960, *Khan* et al. 274 (E). C5 Mersin: Anamur, Olucak, between Ermenek and Anamur, 18.08.1949, *Davis* 16334B (E). C5 Adana: Karaisalı, Bulgar Da., between Pozantı and Meydan, 1500 m, 01.09.1949, *Davis* 16585 (E). C5 Niğde: Regione alpine du Taurus, Pres de Gülek-Maden, 16.08.1855, *Balansa* 500 (E). C5 Niğde: Bulgar Maden, 1500 m, 07.1912, *Shie* 283 (E), Bulgar maden, 1400-2000 m, *Shie* 1896:586 (E). C5 Niğde: S valley of Maden, 30.07.1969, *Darrah* 348 (E). C6 Osmaniye: Amonos, Düldül, 1500-2000 m, 07.1911, *Haradj*. 3846 (E).

***Cyclotrichium stamineum*: Iraq** in rupestribus umbrosis montis Gara, *Kotschy* 1841:311 (**holotype G, isotype K**). in rupestribus m. Gara, regione alpinam, 24.07.1841, *Hohanacker s.n.* (K). Sarsang, Gara Dagh, 1050-1250 m, 12.06.1958, *E.Chapman* 26409 (K). Mosul: Zawita, 21 km a Dohuk orientem versus in pinetis (*P. brutia*) saxosis, 800 m, 10-12.07.1957, *Rechinger* 11546 (G, K, W). 25 km a Dohuk c. 800 m, *Rechinger* 11541 (G, W). Inter Dohuk et Amadiye, Amadiye 3 km occidentem versus, 1000 m, 12.07.1957, *Rechinger* 11618 (G, W). Galli Ali beg, 500 m, 20.08.1958, *Ali Rawi* 26795 (K). Bekhal, mountain slope, 07.07.1971, *S.Omar* et al., 38408 (K). Mosul, Zawita gorge, rocky crevices in *Pinus brutia* forest, 23.07.1961, *Agnew* 733 (E). Sirsang, 4000 ft, 06.07.1955, *R.H.Heines* 447 (E). Mosul: Sirsang, Gara mt. 10.07.1961, *Agnew* 648 (E). Mosul: ad confinae Turciae prov. Hakkari, inter Dohuk et Amadiya, Zawita, 21 km a Dohuk orientem versus, in fissuris dopium dolomit, c. 800 m, 12.07.1952, *K.H.Rechinger* 11541\* (E, K). Amadiya, Zawita gorge, 23.07.1961, *Agnew* 733 (E). **Turkey:** C9 Siirt: Erüh, Yassıdağ (Serikur Da.) Meşindağ pass (Birini pass), 1640 m, 18.07.1981, *E.Tuzlacı* (ISTO-47340). Hakkari: 2 km from Çukurca to Narlı village, 1200 m, 09.06.2006, *T.Dirmenci* 3357a. *ibid.*, 17.08.2008, *Yıldız* 16935, *Dirmenci* & *Fırat*. 5 km W of Çukurca, c. 1000 m, 09.06.2006. *T.Dirmenci* 3358.

Çukurca, 1200 m, rocky slopes in open *Quercus* sp. forest, 2.06.1966, *Davis* 44745 (ISTO-11473, E). C10 Hakkari: Cilo Da. in Diz dere, 5700 ft, gravel terraces, 06.08.1954, *Davis* 23924 (ANK, E). Yüksekova: nr. Oramar (S. of Cilo Da.) 1830 m, *Trelawny* 1845 (E).

***Cyclotrichium straussii*: Iran:** Persia occident., in ditone oppidi Sulatanabad, Strauss s.n. (**isotype K, BM**). Luristan, left bank of Kashgan Rud: above Pul-i-Khalor, 60 km W. of Khoramabad: dryish ledges NW of facing limestone cliffs, c. 1130 m, 11.07.1966, *J.C.Archibald* 2645\* (E). Lorestan, Shrab-Tak, 2300-2500 m, June-July, *M.S.Mossadegh* 44 (E).

Chaharmahal-e Bakhtiari, Lordegan, Kuh-e Karkunji, 1500-2300 m, *Mozaffarian* 54939 (TARI).

***Clinopodium betulifolium*: Turkey**  
 C5 İçel: above Gözne, calcareous, c. 1200 m, N 37° 00' 26'', E 34° 34' 70'', 07.08.2007, *Dirmenci* 3487\*, *Arabacı* & *Brauchler*.

***Clinopodium grandiflorum*: Turkey**  
 A8 Rize: c. 15 km from İnkizdere to Başköy, 1260 m, 01.09.2008, *Dirmenci* 3600\* & *Akçiçek*.

***Clinopodium tauricolum*: Turkey** C5 İçel: between Gülnar and Ermenek, around Güneşli village, *Cedrus libani* frost, 1200 m, 07.08.2007, *Dirmenci* 3483\*, *Arabacı* & *Brauchler*