

FOLIAR ANATOMY OF *CYNOGLOSSUM* L. (BORAGINACEAE) FROM NORTH ANATOLIA, TURKEY

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Abstract

Bracts, basal and cauline leaves of North Anatolian representatives of *Cynoglossum*, viz., *C. creticum* Miller, *C. officinale* L., *C. montanum* L. and *C. glochidiatum* Wall. were investigated anatomically. Foliar thickness, trichome length and types, stomata types, stomata index, the presence of collenchyma and sclerenchyma were found to be important for delimitation of *Cynoglossum* species. All examined species have isobilateral leaf. Stomata were anisocytic and anomocytic. All the studied species had trichomes. Simple long and short trichomes were seen in leaves of *C. creticum*. Long and short trichomes with cystoliths at the base were seen in all leaves of *C. montanum*. Unicellular and multicellular unbranched trichomes and glandular trichomes were found in leaves of *C. officinale*. Short and adpressed trichomes were seen in leaves of *C. glochidiatum*.

Introduction

Cynoglossum L. (Boraginaceae) is represented by eight species in Turkey (Riedl, 1978). It is taxonomically difficult genus because of the fairly uniformity in external morphology. They generally grow along roadsides, sand dunes or open woodlands (Riedl, 1978; Sutory, 2005). Some species of the *Cynoglossum* are used as remedies in Anatolian folk medicines and as ornamental plants in gardens and parks (Baytop, 1999).

Over the last couple of decades, several studies were carried out on *Cynoglossum* based on ecology, seed germination and chemical structure (Boorman and Fuller, 1984; Fisher *et al.*, 1989; Stabell *et al.*, 1998), however, very little is known about anatomical properties of this genus. Metcalfe and Chalk (1979) and Watson and Dallwitz (1991) highlighted characteristic properties of petiole and leaf anatomy of Boraginaceae. Dasti *et al.* (2003) stressed that the epidermal characteristics, such as shape and size of the epidermal cells, types of trichomes and of stomata provide extensive taxonomic data in Boraginaceae. Riedl (1978) states that most of the Turkish *Cynoglossum* representatives are poorly defined and some additional characters are needed for proper identification of *Cynoglossum*. Akçin and Bilgener (2000) carried out a chemotaxonomic study on some Turkish *Cynoglossum* representatives in order to explore their taxonomic value. Dasti *et al.* (2003) examined the epidermal morphology of *C. glochidiatum* Wall. and *C. tomentosa* (Wall.) Kazmi. Akçin (2008) studied the micromorphology of nutlet and seeds of some *Cynoglossum* species. However, no detailed anatomical studies were carried out so far on Turkish *Cynoglossum* species which are often difficult to distinguish from one another morphologically. The present study aims to examine the foliar anatomical properties of four North Anatolian species of *Cynoglossum*, namely *C. creticum* Miller, *C. officinale* L., *C. montanum* L. and *C. glochidiatum* Wall. and to evaluate their discriminative potential in taxonomy.

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Materials and Methods

All the four *Cynoglossum* species were collected from North Anatolia in Turkey during 2001-2009. Voucher specimens were kept at the Ondokuz Mayıs University Herbarium.

Samples for anatomical studies were fixed in 70% alcohol. Cross and surface sections were prepared from the stored leaf materials. Cross and surface sections of leaves were excised by hand and they were covered with glycerin-gelatin (Vardar, 1987). The photographs were taken with Nikon FDX-35 microscope. All measurements were calculated with an ocular-micrometer under light microscope. Stomata index was calculated according to Meidner and Mansfield (1968).

Table 1. The anatomical properties in cross-section of basal leaves of *Cynoglossum* species.

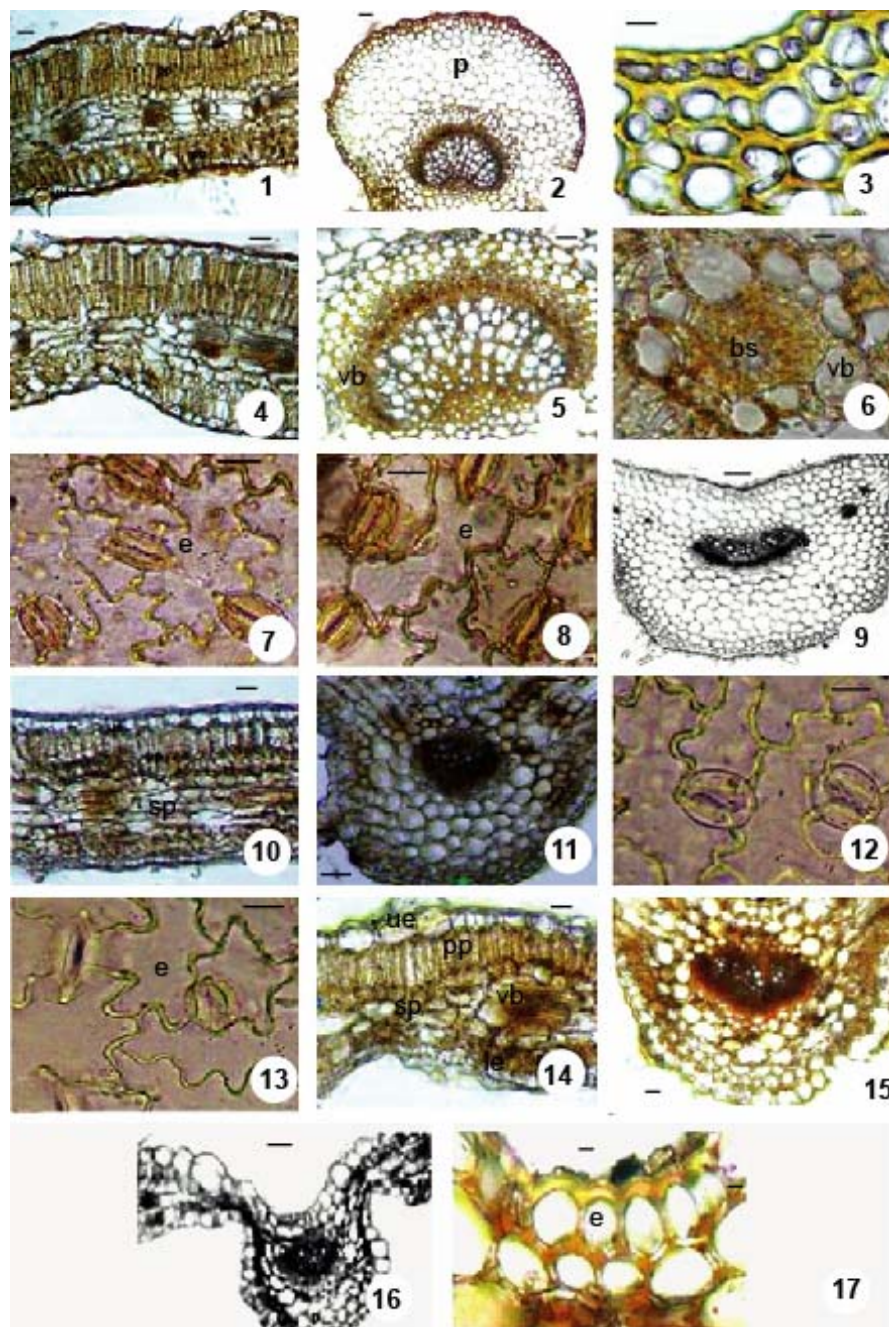
| Characters | <i>C. creticum</i> Min.-Max. | <i>C. montanum</i> Min.-Max. | <i>C. officinale</i> Min.-Max. | <i>C. glochidiatum</i> Min.-Max. |
|--|---------------------------------|---------------------------------|-----------------------------------|-------------------------------------|
| Leaf thickness (µm) | 250-460 | 190-220 | 180-230 | 340-400 |
| Cuticle thickness (µm) | 2.5-5.0 | 5.0-7.5 | 5.0-7.5 | 2.5-5.0 |
| Trichome length (µm) | 100-600 | 120-1500 | 120-600 | 100-270 |
| Palisade parenchyma (adaxial /abaxial surface) | 2 / 1-2 layered | 2 / 1 layered | 1/1 layered | 2/1 layered |
| Spongy parenchyma | 4-6 layered | 3-4 layered | 3-4 layered | 3-4 layered |
| Bundle sheath | parenchymatic | parenchymatic | parenchymatic | parenchymatic |
| Epidermis cell length (µm) | 25-70 | 35-40 | 35.0-37.5 | 30-50 |
| Epidermis cell width (µm) | 12.5-37.5 | 22.5-27.5 | 17.5-30.0 | 20-40 |
| Type of vascular bundle | collateral | collateral | collateral | collateral |

Results

Cynoglossum creticum

Epidermis consists of a single layer both in abaxial and adaxial surface of basal leaf (Table 1, Figs 1-3). Leaf is isolateral. Vascular bundles are surrounded by bundle sheath with big starch grains. Collenchymatic cells are located under the adaxial epidermis in the midrib. Some parenchymatic cells contain crystal near the median vein. On the abaxial side of the leaves trichome frequency is higher than adaxial side. The stomata are present on both adaxial and abaxial epidermis. Stomatal types are of anomocytic and anisocytic, but mainly anomocytic. The stomata index is 20.98 for adaxial surface and 30.66 for abaxial surface. Cauline leaf is isolateral. Mesophyll consists of 2 layers palisade cells in adaxial surface, 1-2 layers in abaxial surface and 2-3 layers spongy parenchyma cells. Vascular bundles are surrounded by a parenchymatic bundle sheath and collateral type. Both anomocytic and anisocytic stomata were found. The stomata index is 19.79 for adaxial surface and 23.59 for abaxial surface. There are trichomes on both sides of the leaves and trichomes frequency is almost same (Table 2, Figs 4-8).

In bract, palisade parenchyma cells are 2 layered on adaxial surface and 1-2 layered on abaxial surface. Vascular bundles more frequent than in cauline leaves. The number of epidermal cells on adaxial surface is 290- 352 and these values are nearly same on abaxial surface (Table 3, Fig. 9). The stomata index is 23.02 for adaxial surface and 28.50 for abaxial surface.



Figs 1-17. Transverse and surface section of *Cynoglossum creticum* and *C. montanum* leaves. 1-9. *C. creticum*. 1-3. basal leaf. 4-8. cauline leaf. 9. bract; 10-17. *C. montanum*. 10-13. basal leaf. 14-15. cauline leaf. 16-17. bract. e- epidermis; ue- upper epidermis; le- lower epidermis; cl- collenchyma; p- parenchyma; pp- palisade parenchyma; sp- spongy parenchyma; vb- vascular bundle; bs- bundle sheath; st- stomata; h- trichomes. Bar: 15 μ m (Figs 7-8); 20 μ m (Figs 12-13, 17); 40 μ m (Figs 10, 14-16); 50 μ m (Fig. 4); 70 μ m (Figs 1,3,5-6); 100 μ m (Figs 2, 9, 11).

Cynoglossum montanum

In basal leaf epidermis consists of irregular shaped cells. Leaf is isolateral. Mesophyll composed of 2 layered palisade parenchyma cells in adaxial surface, 1 layer in abaxial surface and 3-4 layers spongy parenchyma cells. The stomata are more frequent on the abaxial side when compared to the on adaxial side. Stomatal types are anomocytic and anisocytic (Table 1, Figs 10-13). The stomata index is 20.58 for adaxial surfaces and 23.25 for abaxial surfaces.

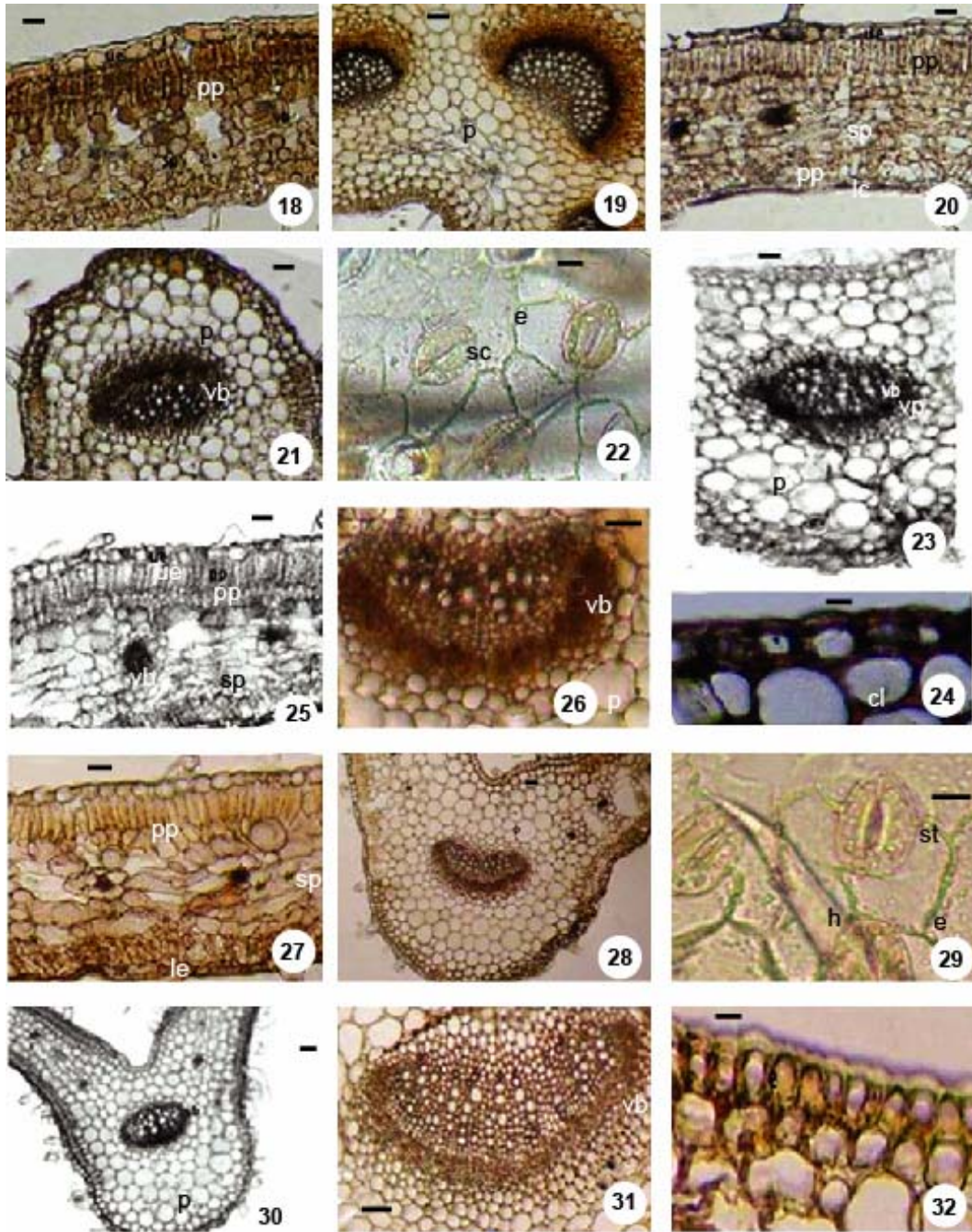
Table 2. The anatomical properties in cross-section of cauline leaves of *Cynoglossum* species.

| Characters | <i>C. creticum</i> | <i>C. montanum</i> | <i>C. officinale</i> | <i>C. glochidiatum</i> |
|---|--------------------|--------------------|----------------------|------------------------|
| | Min.-Max. | Min.-Max. | Min.-Max. | Min.-Max. |
| Leaf thickness (µm) | 200-300 | 130-180 | 200-250 | 170-200 |
| Cuticle thickness (µm) | 5.0-7.5 | 5.0-7.5 | 2.5-5.0 | 5.0-7.5 |
| Trichome length (µm) | 80-440 | 180-500 | 100-300 | 180-200 |
| Palisade parenchyma (adaxial/abaxial surface) | 2/1-2 layered | 2/1 layered | 1/1 layered | 2/1 layered |
| Spongy parenchyma | 2-3 layered | 2-3 layered | 3-4 layered | 3-4 layered |
| Bundle sheath | parenchymatic | parenchymatic | parenchymatic | parenchymatic |
| Epidermis cell length (µm) | 30-50 | 30-50 | 15.0-37.5 | 20.0-37.5 |
| Epidermis cell width (µm) | 20-30 | 20.0-22.5 | 12.5-20.0 | 22.5-27.5 |
| Type of vascular bundle | collateral | collateral | collateral | collateral |

Table 3. The anatomical properties in cross-section of bracts of *Cynoglossum* species.

| Characters | <i>C. creticum</i> | <i>C. montanum</i> | <i>C. officinale</i> | <i>C. glochidiatum</i> |
|--|--------------------|--------------------|----------------------|------------------------|
| | Min.-Max. | Min.-Max. | Min.-Max. | Min.-Max. |
| Leaf thickness (µm) | 190-270 | 200-220 | 200-300 | 272-300 |
| Cuticle thickness (µm) | 5.0-7.5 | 2.5-5.0 | 2.5-5.0 | 2.5-5.0 |
| Trichome length (µm) | 100-320 | 40-100 | 40-250 | 150-300 |
| Palisade parenchyma (adaxial /abaxial surface) | 2/1-2 layered | 2/1 layered | 1/1 layered | 2/1 layered |
| Spongy parenchyma | 3 layered | 3-4 layered | 3-4 layered | 3-4 layered |
| Bundle sheath | parenchymatic | parenchymatic | parenchymatic | parenchymatic |
| Epidermis cell length (µm) | 15-30 | 40.0-42.5 | 25.0-32.5 | 25-30 |
| Epidermis cell width (µm) | 10-20 | 20-40 | 20.0-12.5 | 12.5-22.5 |
| Type of vascular bundle | collateral | collateral | collateral | collateral |

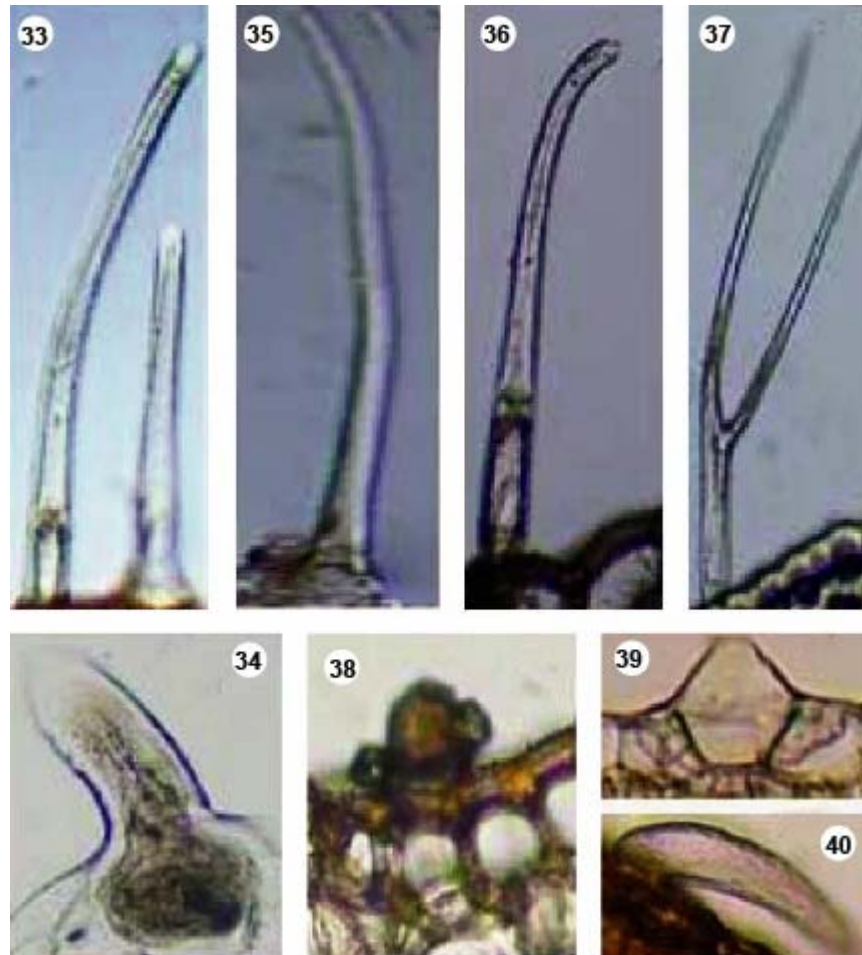
Cauline leaf is isolateral. Bundle sheath is not distinguishable. There are sclerenchymatic cells in the midrib. Collenchymatic cells are located under the adaxial epidermis. Stomatal types are of anomocytic and anisocytic (Table 2, Figs 14-15). The stomata index is 20 for adaxial surface and 17.79 for abaxial surface. In bract, mesophyll composed of 2 layered palisade parenchyma cells in adaxial surface, 1 layered in abaxial surface and 3-4 layered spongy parenchyma cells. Distinguishable angular collenchyma is present in the median region of leaf (Table 3, Figs 16-17). The stomata index is 20.45 for adaxial surface and 23.30 for abaxial surface.



Figs 18-32. Transverse and surface section of *Cynoglossum officinale* and *C. glochidiatum* leaves. 18-24. *C. officinale*. 18-19. basal leaf. 20-22. cauline leaf. 23-24. bract; 25-32. *C. glochidiatum*. 25-26. basal leaf. 27-29. cauline leaf. 30-32. bract. Bar: 15 μ m (Fig. 29); 20 μ (Fig. 22); 40 μ m (Figs 18, 20, 24); 70 μ m (Figs 25, 27, 28, 30, 32); 80 μ m (Figs 19, 21, 23); 100 μ m (Figs 26, 31).

Cynoglossum officinale

Basal leaf is isolateral. The stomata index on the adaxial side is 20.98, on the abaxial side values is 30.68 (Table 1, Figs 18-19). Cauline leaf is isolateral. Palisade parenchyma cells are 1 layered in both surface. Spongy parenchyma cells are 3-4 layered. Stomata type is anomocytic and anisocytic. The stomata index is 15.78 for adaxial surface and 27.27 for abaxial surface (Table 2 Figs 20-22). In bract, palisade parenchyma cells are 1 layered in both adaxial and abaxial surfaces. Spongy parenchyma cells are 3-4 layered. Both anomocytic and anisocytic types of stomata were found, but mainly anomocytic type was observed (Table 3, Figs 23-24). The stomata index is 16.21 for adaxial surface and 24.71 for abaxial surface.



Figs 33-40. Trichomes of *Cynoglossum* species. Fig. 33. Simple trichomes of *C. creticum*. Figs 34-35 Short trichomes with cystolith at the base and long simple trichomes of *C. montanum*. Figs 36-38. Unbranched, branched trichomes and glandular trichomes of *C. officinale*. Figs 39-40. Simple trichomes of *C. glochidiatum*.

Cynoglossum glochidiatum

Epidermis is single layered on both adaxial and abaxial surfaces of basal leaf. Leaf is isolateral. Palisade parenchyma cells are 2 layered on adaxial surface and 1-layered on abaxial

surface. Spongy parenchyma cells are 3-4 layered. *C. glochidiatum* have trichomes and stomata both adaxial and abaxial sides. Stomata length is 20-25 μm and stomata width is 5-10 μm (Table 1, Figs 25-26). There is a thick cuticle on cauline leaf. Epidermal cells are isodiametric and oval. Leaf is isolateral. Stomata type is anomocytic and anisocytic (Table 2, Figs 27-29). The stomata index is 29.41 for adaxial epidermis, 20.45 for abaxial epidermis. In bract epidermis consists of uniseriate, rectangular cells. Palisade parenchyma cells are 2 layered on adaxial surface and 1 layered on abaxial surface. Spongy parenchyma cells are 3-4 layered. Collenchymatic cells are located under the epidermis in midrib region. The bundle sheath is parenchymatic (Table 3, Figs. 30-32). The stomata index is 22.04 for adaxial surfaces and 22.64 for abaxial surface.

All the studied species had trichomes. Simple long and short trichomes were seen in basal, cauline leaf and bract of the *C. creticum* (Fig. 33). Long and short trichomes with cystoliths at the base were seen on the both epidermises on basal and cauline leaves of *C. montanum* (Figs 34-35). Unicellular and multicellular unbranched trichomes and glandular trichomes were found in leaves of *C. officinale*. Also branched multicellular with nearly two equal arms trichomes were rarely presented in basal leaf of this species (Figs 36-38). Usually short and adpressed trichomes were seen in all leaves of *C. glochidiatum* (Figs 39-40).

Discussion

In the present study isobilateral (isolateral) types of leaf were observed in all examined species. Metcalfe and Chalk (1979) reported that there are centric or isobilateral mesophyll types in Boraginaceae. While the row number of palisade cells varies among species, it is very stable in the same species. Presence of collenchyma and sclerenchyma in leaves is an important feature (Mavi *et al.*, 2011). Sclerenchymatic and collenchymatic cells were located in the midrib of *C. montanum*. The other species had collenchymatic cells. Metcalfe and Chalk (1979) reported that there are both anomocytic and anisocytic stomata in Boraginaceae, but Özürgücü *et al.* (1991) reported only anomocytic stomata in this family. Our findings support Metcalfe and Chalk (1979). It was also found that all species have stomata on both upper and lower surfaces. Dasti *et al.* (2003) reported that anomocytic and helicocytic stomata were seen in leaves of *C. glochidiatum* and *C. tomentosa*. Stomata were anisocytic and anomocytic in *Onosma angustissimum* Hausskn. & Bornm. and *O. cassium* Boiss. (Boraginaceae) (Akçin and Binzet, 2010). The stomata numbers are more in abaxial surface than adaxial surface in the most of examined species. The stomata index differs for the adaxial and abaxial epidermis among four examined species. Stomata index is important as the number of stomata may be changed by the age of the leaf, but the stomata index remains constant for a species (Trease and Evans, 1982). Metcalfe and Chalk (1979) and Watson and Dallwitz (1991) reported that crystalloids, especially calcium oxalate crystals were present in the mesophyll of leaves of Boraginaceae family. Fisher *et al.* (1989) suggested that nuclear crystalloids were found in high percentage in Boraginaceae family and these inclusions could be valuable as a systematic character. In this study, it was found that cystoliths were present in hair base in all studied species.

The present study based on foliar anatomical characters supports the traditional taxonomic treatments of *Cynoglossum*. Leaves of *Cynoglossum* species contain diagnostic features such as foliar thickness, trichome length, stomata types, stomata index, the presence of collenchyma and sclerenchyma, and their arrangement around the vascular bundles.

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