

A Morphological and Anatomical Study on *Anchusa leptophylla* Roemer & Schultes (Boraginaceae) Distributed in the Black Sea Region of Turkey

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Received: 30.11.2006

Accepted: 08.06.2007

Abstract: The morphological and anatomical characteristics of *Anchusa leptophylla* Roemer & Schultes subsp. *leptophylla* and *A. leptophylla* subsp. *incana* (Ledep) Chamb. (Boraginaceae), which are distributed in the Black Sea region, were investigated. The morphological features of various organs of the plant such as the stem, flower, and fruit are given in detail. Features related to characteristics of the leaf and calyx were found to be important in separating the subtaxa morphologically. The shape of leaves is usually linear-lanceolate in *A. leptophylla* subsp. *incana*, while it is linear in subsp. *leptophylla*. In anatomical studies, cross-sections of the root, stem, and leaf parts, and the surface sections of the leaves of both subspecies were examined. The root is perennial. However, it was noted in *A. leptophylla* subsp. *leptophylla* that the periderm layer was thicker than in subsp. *incana*. The leaves are equifacial and have stomata cells that are anomocytic. The numbers of lower and upper parenchyma layers vary in the examined taxa. The mean number of stomata on the lower surfaces was higher than that on the upper one.

Key Words: *Anchusa leptophylla*, morphology, anatomy, Turkey

Türkiye' nin Karadeniz Bölgesi'nde Yayılış Gösteren *Anchusa leptophylla* Roemer&Schultes (Boraginaceae) Üzerinde Morfolojik ve Anatomik Bir Çalışma

Özet: Bu çalışmada, Karadeniz Bölgesi'nde yayılış gösteren *Anchusa leptophylla* Roemer & Schultes subsp. *leptophylla* ve *A. leptophylla* subsp. *incana* (Ledep) Chamb. (Boraginaceae)'nin morfolojik ve anatomik özellikleri incelenmiştir. Bitkilerin gövde, çiçek ve meyve gibi çeşitli organlarının morfolojik özellikleri ayrıntılı olarak verilmiştir. Yaprak ve kaliks özelliklerinin tür altı taksonların birbirinden ayrılmasında önemli olduğu tespit edilmiştir. *A. leptophylla* subsp. *incana*' da yaprak şeklinin genellikle linear-lanseolat iken, subsp. *incana*'da linear olduğu belirlenmiştir. Anatomik çalışmalarda, her iki alt türe ait örneklerin kök, gövde ve yaprak kısımlarından alınan enine kesitler ile yaprak yüzey kesitleri incelenmiştir. Kök çok yıllıktır. *A. leptophylla* subsp. *leptophylla* örneklerinde kökteki periderm tabakasının daha kalın olduğu tespit edilmiştir. Yapraklar ekvifasiyel ve stoma hücreleri anomositiktir. Üst ve alt parenkima tabaka sayısı, incelenen örneklerle bağlı olarak varyasyon göstermektedir. Yaprak alt yüzeyi üzerinde bulunan stoma sayısı, üst yüzey üzerindeki stoma sayısından daha fazladır.

Anahtar Sözcükler: *Anchusa leptophylla*, morfoloji, anatomi, Türkiye

Introduction

The genus *Anchusa* L. (Boraginaceae) is one of the largest genera centering in the Mediterranean and extending through Europe, Western Asia and Tropical Africa (Selvi & Bigazzi, 1998). The southern part of the Balkan Peninsula is a major centre of diversity of *Anchusa*, because here the Mediterranean, Central European, and endemic species come into contact and

overlap to a considerable extent (Selvi & Bigazzi, 2003). *Anchusa* is represented by 15 species, 2 of which are endemic to Turkey (Ekim et al., 2000). *A. leptophylla* subsp. *incana* (Ledep) Chamb. is a taxon endemic to Turkey (Chamberlain, 1977).

The name "Sığirdili" is used for *Anchusa* species in Turkey (Baytop, 1984, 1994). Some *Anchusa* species are known to cause diuresis and perspiration (Baytop, 1991).

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Studies on the morphology of this species are limited (Selvi et al., 1996; Selvi & Bigazzi, 2000a, 2000b). A taxonomic revision of the genus *Anchusa* in Greece was performed by Selvi & Bigazzi (2003). The great diversity of forms exhibited by this heterogeneous genus has generated rather variable interpretations (Greuter et al., 1984; Brummit, 1992). However, due to the lack of revisions, the identity and taxonomic status of several species were still uncertain (Selvi & Bigazzi, 2003). Chamberlain (1977) reported that *A. leptophylla* is a widespread and variable species in Turkey. There are some characteristics, such as indumentum and branching pattern, that are shared by subsp. *leptophylla* and subsp. *incana*, which is the main reason for the identification of the members of the 2 subspecies (Chamberlain, 1977). Delimitation of some genera has been widely debated mainly due to a remarkable diversity of the forms' morphological, palynological, and karyological characters (Selvi & Bigazzi, 2001). Recent studies focused on the structure and micromorphology of reproductive organs (Bigazzi et al., 1997, 1999; Bigazzi & Selvi, 1998; Selvi & Bigazzi, 2000a). Some anatomical properties of Boraginaceae were reported by Metcalfe & Chalk (1979). However, so far there have been no detailed anatomical studies on *Anchusa* species naturally distributed in Turkey. The chromosome numbers of *A. leptophylla* are $2n = 16$ (Selvi & Bigazzi, 2000a). In addition, the pollen morphologies of some *Anchusa* taxa were determined by Diez (1994) and Bigazzi & Selvi (1998).

In the present research, the morphological and anatomical characteristics of *A. leptophylla* subsp. *leptophylla* and *A. leptophylla* subsp. *incana* were investigated.

Materials and Methods

Plant samples were collected from different localities in Samsun, Amasya, and its environs. These localities are listed below. Specimens for morphological studies were dried according to standard herbarium techniques and stored in Ondokuz Mayıs University Herbarium (OMUB). The vouchers were identified according to Chamberlain (1977). Fresh samples were used for morphological measurements. The morphological illustrations were drawn using a stereomicroscope.

Anatomical observations were performed using an average of 30 specimens belonging to each taxon. The

materials necessary for anatomical studies were fixed in 70% alcohol. Anatomical investigations were carried out on the cross-sections of the root, stem, and leaves as well as the surface sections of leaves taken free-hand. The cross and surface sections were covered with glycerin-gelatin (Vardar, 1987). Anatomic structures were measured using an ocular micrometer determining maximum and minimum boundaries. The photographs were taken with a Nikon Coolpix 5200 digital camera.

Specimens:

A. leptophylla Roemer & Schultes subsp. *leptophylla*

A5 Amasya: Köle mazar, nr. Amasya, roadside, 350 m, 04.vii.2005, Ulu 147.

A5 Amasya: Boğazköy, roadside, 300 m, 28.v.2005, Ulu 277.

A5 Amasya: Fındıklı village, roadside, 390 m, 03.vii.2004, Ulu 124.

A5 Amasya: nr. Kurnaz-Akören bridge, roadside, 385 m, 04.vii.2004, Ulu 150.

A5 Amasya: 10 km from Amasya to Merzifon, roadside, 400 m, 06.vii.2004, Ulu 305.

A5 Amasya: Suluova centre, roadside and rocky slopes, 380 m, 05.vii.2004, Ulu 159.

A6 Samsun: 15 km from Ladik to Taşova, roadside, 400 m, 11.vi.2005, Ulu 287.

A. leptophylla Roemer & Schultes subsp. *incana* (Ledeb) Chamb.

A5 Samsun: Ladik İstasyon, nr. Karadağ, Doruk village, fields, 900 m, 28.vi.2005, Ulu 304.

A5 Samsun: Havza, Taşkaracan village, fields and roadside, 750 m, 29.v.2004, Ulu 078.

A5 Samsun: Kavak, nr. Aksu, nr. Üçanlar bridge, fields, 500 m, 11.vi.2005, Ulu 296.

A5 Samsun: Ladik, Ahmetsaray village, fields, 900 m, 22.v.2004, Ulu 172.

A5 Samsun: Havza, Bekdiğın, roadside, 800 m, 11.vi.2005, Ulu 289.

A5 Amasya: Suluova, centre, roadside, 380 m, 29.vi.2005, Ulu 299.

A5 Amasya: 10 km from Amasya to Merzifon, fields, 380 m, 11.vi.2005, Ulu 297.

A6 Samsun: Kunduz Mountain, Zeytin village, 1500 m, 29.vi.2005, Ulu 306.

A6 Amasya: 15 km from Suluova to Ladik, roadside, 910 m, 29.v.2005, Ulu 309.

Results

In our research area, there are 2 subspecies of *Anchusa*: *A. leptophylla* subsp. *leptophylla* and *A. leptophylla* subsp. *incana*.

I- Morphological Characteristics

A. leptophylla Roemer & Schultes

Biennial or perennial herbs woody at the base. Stem prostrate-ascending, 35-145 cm, branched from the base, puberulous. Basal leaves 6.5-20.0 × 0.8-1.9 cm, linear. Cauline leaves 3.5-24.5 × 0.3-2.0 cm, linear,

linear-lanceolate or oblanceolate and puberulous. Cymes dense, elongating in fruit. Bracts 3.0-4.5 × 1.0-1.8 mm, linear, linear-lanceolate or ovate-lanceolate. Calyx 4.5-8.0 × 5.0-8.0 mm in flower, divided to $\frac{1}{2}$ - $\frac{2}{3}$ into obtuse lobes, elongating in fruit, lobes acute, subacute or obtuse. Corolla usually bright blue with throat, 5.0-9.3 × 5.0-11.0 mm, limb 2.0-4.0 mm, stamens inserted below the scales. Style 3.5-9.0 mm, long. Nutlets 2.0-3.5 × 2.5-5.5 mm, obliquely-ovoid with a lateral beak, greyish-brown, coat surfaces papillae with lobed, rosette-like shape (Figures 1 & 2).

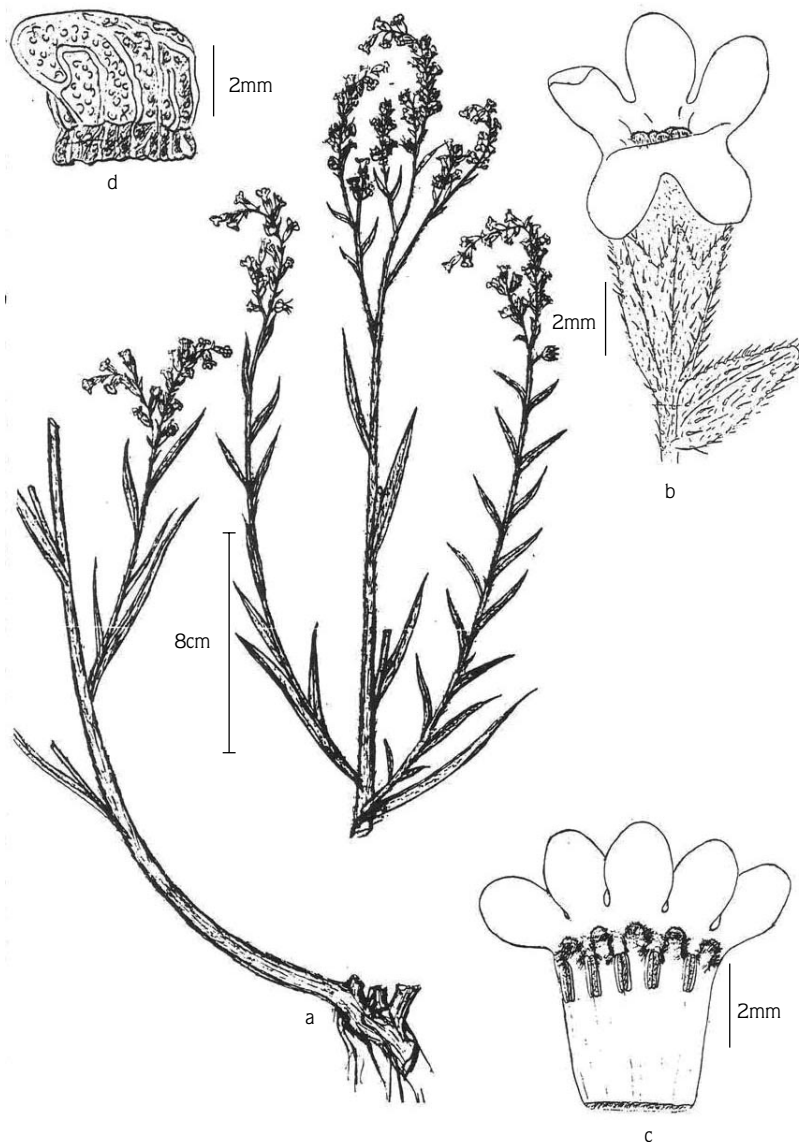


Figure 1. *A. leptophylla* subsp. *leptophylla*.
a) General appearance, b) Flower with bract, c) Dissected corolla, d) Nutlet.

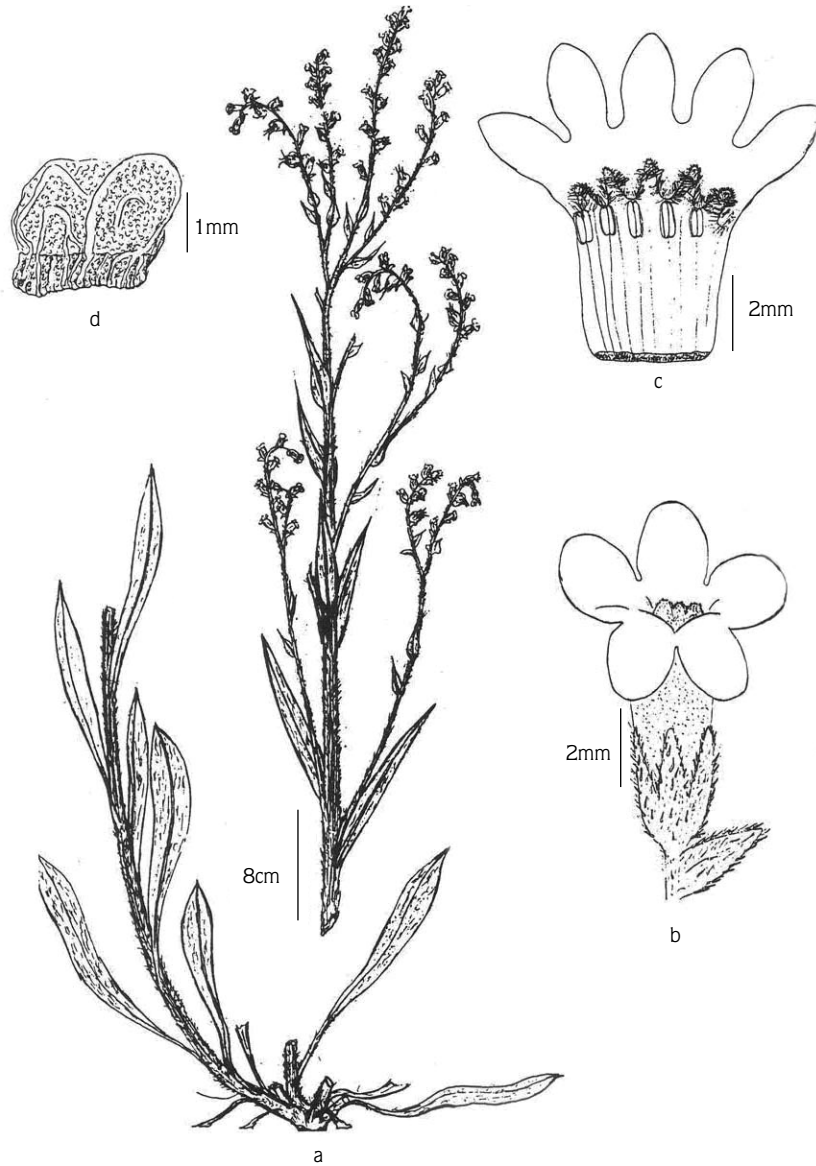


Figure 2. *A. leptophylla* subsp. *incana*.
 a) General appearance, b) Flower with bract, c) Dissected corolla, d) Nutlet.

Some morphological differences were observed between the subspecies *leptophylla* (Figure 1) and *incana* (Figure 2). Leaf shape and size and the ratio of calyx lobe length to calyx length differ between the 2 subspecies. These differences are shown comparatively in Table 1.

II- Anatomical Characteristics

Root (Figures 3 & 4)

Root is biennial or perennial and shows secondary growth. Periderm is multilayered in the root. Cortex is

parenchymatic. Parenchymatic cells are 500-2125 µm in subsp. *leptophylla* (Figure 3) and 750-3500 µm in subsp. *incana* (Figure 4). Cambium cells are distinguishable. Primary pith rays are usually 20 layered in *A. leptophylla* subsp. *leptophylla* while these rays are 5-30 layered in *A. leptophylla* subsp. *incana*. Pith is completely filled with secondary xylem cells. The comparative differences in the root anatomy of the investigated subspecies are given in Table 2.

Table 1. Comparison of morphological characteristics between *Anchusa leptophylla* subsp. *leptophylla* and *Anchusa leptophylla* subsp. *incana*.

	<i>A. leptophylla</i> subsp. <i>leptophylla</i>	<i>A. leptophylla</i> subsp. <i>incana</i>
Stem	Erect, 45-145 cm, stems usually branched near base	Erect, 35-117 cm, stems branched throughout
Cauline leaves	4.5-24.5x 0.4-1.0 mm, linear, entire	3.5-22.0 x 0.3-2.0 mm, linear-lanceolate, entire
Inflorescence	Spreading strigulose, elongating in fruit	Sericeous, elongating in fruit
Bracts	3.0-4.5 mm, ovate-lanceolate	3.0-4.0 mm, linear-lanceolate or ovate-lanceolate
Calyx	4.5-8.0 mm, divided to 1/3, densely sericeous	5.0-8.0 mm, divided to 1/2 or more, spreading hairs
Corolla	6.0-11.0 x 5.0-9.0 mm, lobes 2.0-4.0 mm	5.0-9.5 x 4.0-6.0 mm, lobes 2.0-4.0 mm
Stamens	Inserted below the scales	Inserted below the scales
Nutlet	2.0-3.5 x 2.5-5.5 mm, obliquely ovoid, greyish-brown	2.0-3.5 x 2.0-4.0 mm, obliquely ovoid, greyish-brown

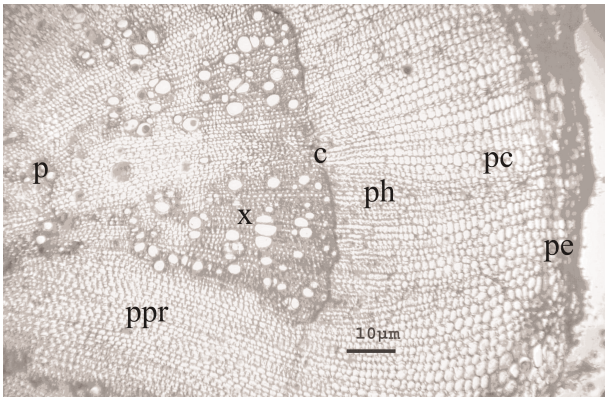


Figure 3. Cross-section of root of *Anchusa leptophylla* subsp. *leptophylla*.
pe) Periderm, pc) Primer cortex, ph) Phloem, c) Cambium, x) Xylem, p) Pith, ppr) Primary pith ray.

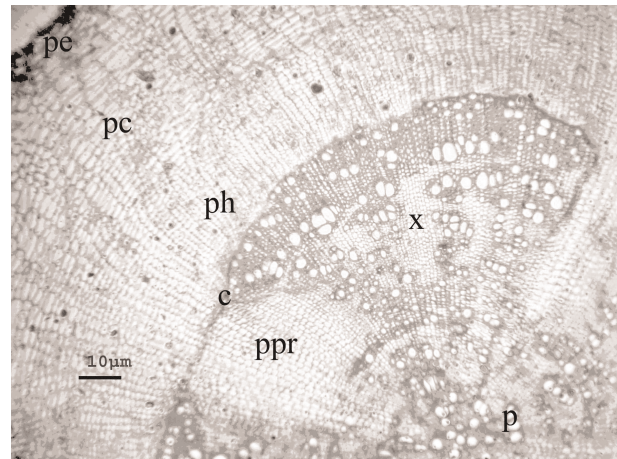


Figure 4. Cross-section of root of *Anchusa leptophylla* subsp. *incana*.
pe) Periderm, pc) Primer cortex, ph) Phloem, c) Cambium, x) Xylem, p) Pith, ppr) Primary pith ray.

Stem (Figures 5-7)

The epidermis is composed of arranged cells. The upper surface is covered with a relatively thick cuticle. There are glandular and eglandular hairs on the

epidermis. They are unicellular and multicellular (Figure 5a-c). The collenchyma tissues consisting of 3-7 layers of cells are located under the epidermis. Parenchyma tissue consists of usually round cells. Thickness of this tissue is

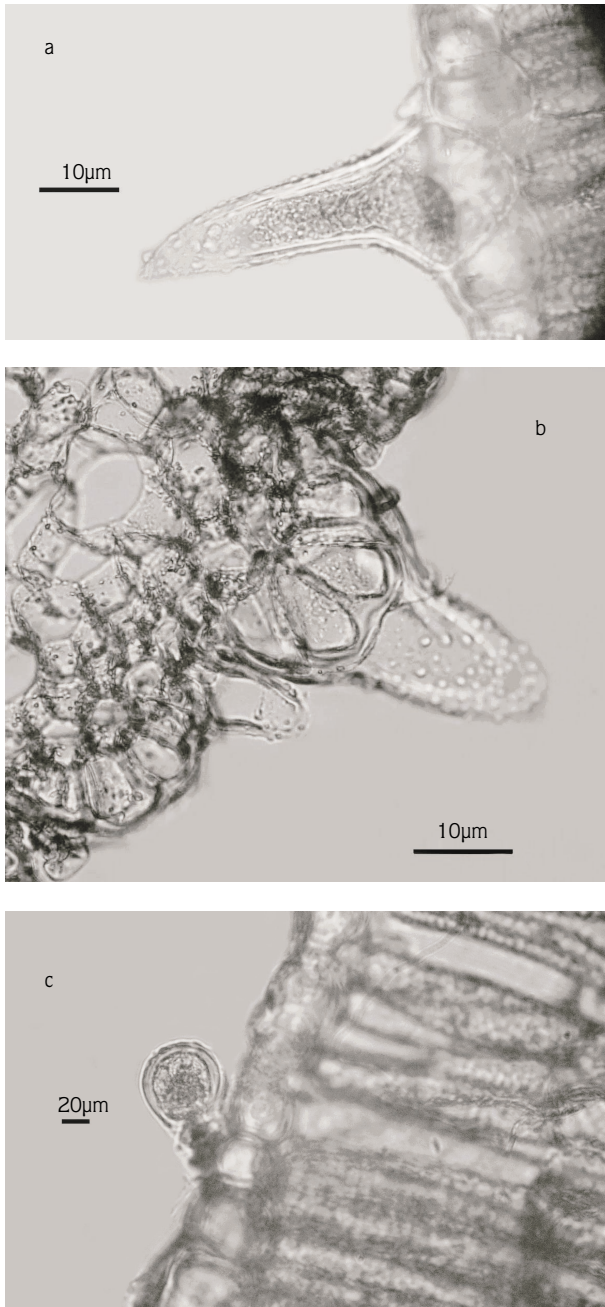


Figure 5. Hair types of *A. leptophylla* taxa.
 a,b) Egladular hairs of the stem and leaf
 c) Glandular hairs of the stem and leaf.

100-320 µm in *A. leptophylla* subsp. *leptophylla* (Figure 6) and 130-450 µm in subsp. *incana* (Figure 7). Cambium is distinguishable and located under the phloem. Diameters of trachea are 40 µm (Table 2). The pith consists of large parenchymatic cells.

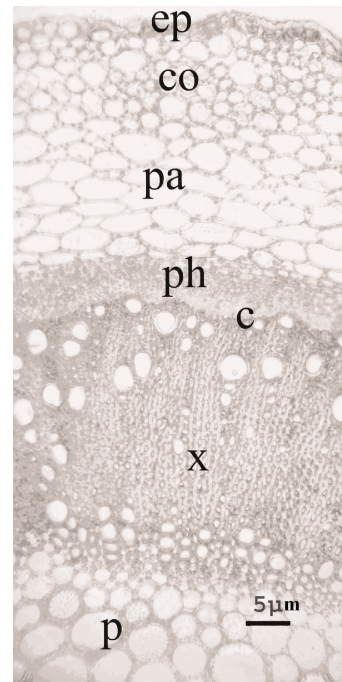


Figure 6. Cross-section of stem of *Anchusa leptophylla* subsp. *leptophylla*.
 ep) Epidermis, co) Collenchyma, pa) Parenchyma,
 ph) Phloem, c) Cambium, x) Xylem, p) Pith.

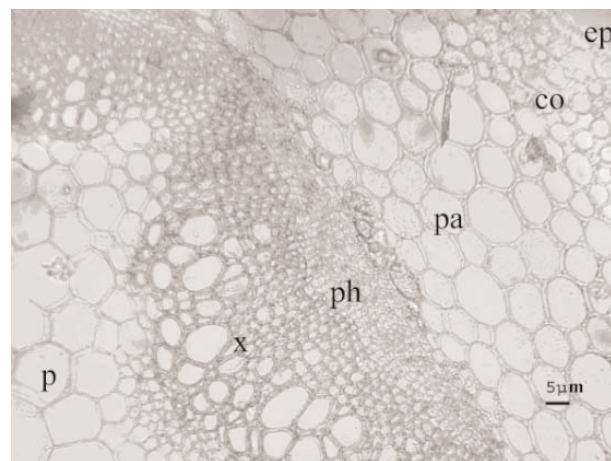


Figure 7. Cross-section of stem of *Anchusa leptophylla* subsp. *incana*.
 ep) Epidermis, co) Collenchyma, pa) Parenchyma,
 ph) Phloem, x) Xylem, p) Pith.

Leaf (Figures 5 & 8-10)

Leaves have obviously thick cuticle on both surfaces. The upper and lower epidermises consist of a single layer of cells. Unicellular and multicellular glandular and egladular hairs are dense on both surfaces (Figure 5a-c).

Table 2. Comparison of anatomical characteristics between *Anchusa leptophylla* subsp. *leptophylla* and *Anchusa leptophylla* subsp. *incana*.

Anatomical measurements	<i>A. leptophylla</i> subsp. <i>leptophylla</i>	<i>A. leptophylla</i> subsp. <i>incana</i>	
ROOT	Thickness of periderm (µm)	150-825	50-125
	Thickness of cortex (µm)	500-2125	750-3500
	The layer numbers of phloem	5-12	19-35
	Thickness of xylem (µm)	1000-5000	1125-4625
	Number of primary pith rays	18-22	5-30
STEM	Thickness of epidermis (µm)	10-30	20-30
	Thickness of collenchyma (µm)	80-200	70-180
	Thickness of parenchyma (µm)	100-320	130-450
	Thickness of phloem (µm)	70-180	40-110
	Thickness of xylem (µm)	300-580	220-620
	Diameter of trachea (µm)	40	40
LEAF	Thickness of upper epidermis (µm)	20-40	20-50
	The layer number and thickness of upper palisade parenchyma (µm)	2-3 layered 9-33	Usually 2, rarely 1 layered 15-33
	Thickness of spongy parenchyma (µm)	120-240	120-250
	The layer number and thickness of lower palisade parenchyma (µm)	1-2 layered 80-250	Usually 1, rarely 2 layered 70-250
	Thickness of lower epidermis (µm)	2-4	2-4
	Stoma length (µm)	41-60	27-55
	Stoma width (µm)	27-35	22-35
	Number of stomata on upper epidermis	8 ± 1	10 ± 1
	Number of stomata on lower epidermis	9 ± 2	13 ± 2

The leaves are amphistomatic. In both subspecies, the epidermal cells of the upper and lower surfaces are equal. Stomata are anomocytic (Figure 8). Stomata cells are present in both epidermises. The stomata are more abundant on the lower surface in both subspecies (Table 2). Leaf is equifacial. Palisade cells are usually 2 layered on lower surfaces of subsp. *leptophylla* (Figure 9), while it is usually 1 layered on the subsp. *incana* (Figure 10). The upper and lower palisade parenchyma cells contain chloroplast. The spongy parenchyma is situated between 2 palisade layers. Thickness of the spongy parenchyma is usually 120-250 µm in both subspecies (Table 2). It contains intercellular spaces. In the median region of the leaf, there is a large vascular bundle. The type of vascular bundle is similar to that in the stem.

Discussion

Two subspecies of *A. leptophylla* were investigated and compared anatomically and morphologically.

The results obtained from morphological studies were generally consistent with the description given in the *Flora of Turkey* (Chamberlain, 1977). Some morphological characters of these 2 subtaxa not given in the *Flora of Turkey*, such as the width of cauline leaves, are provided in this study. Our studies indicated that the shape and dimension of leaves are sufficient to distinguish the subtaxa. Detailed morphological studies have shown that the shape of leaves is usually linear-lanceolate in subsp. *incana*, while it is linear in subsp. *leptophylla*. Therefore, in this study the shape of leaves is used as a

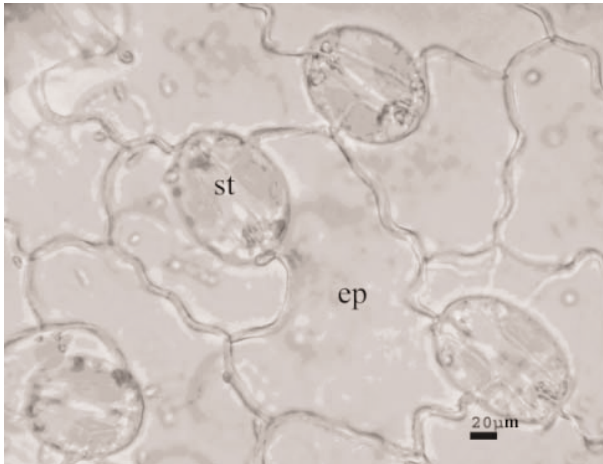


Figure 8. Surface-section of leaf of *Anchusa leptophylla* taxa. st) Stoma, ep) Epidermis.

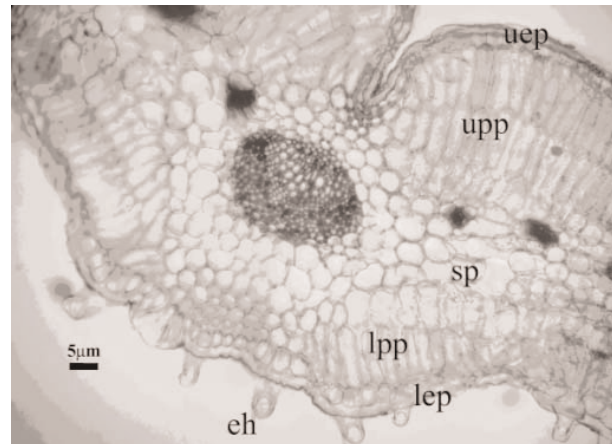


Figure 9. Cross-section of leaf of *Anchusa leptophylla* subsp. *leptophylla*.

uep) Upper epidermis, upp) Upper palisade parenchyma, sp) Spongy parenchyma, lpp) Lower palisade parenchyma, lep) Lower epidermis, eh) Eglandular hair.

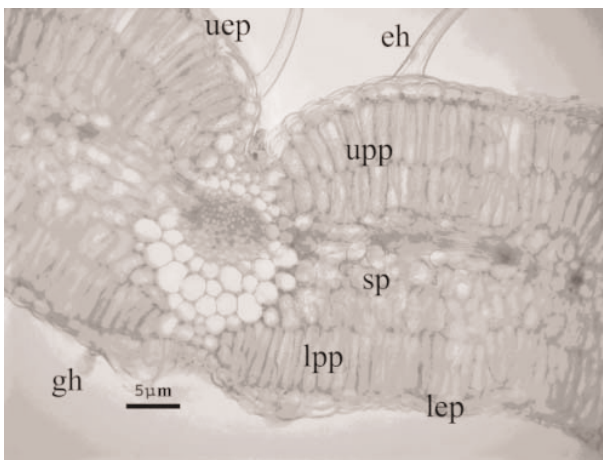


Figure 10. Cross-section of leaf of *Anchusa leptophylla* subsp. *incana*. uep) Upper epidermis, upp) Upper palisade parenchyma, sp) Spongy parenchyma, lpp) Lower palisade parenchyma, lep) Lower epidermis, eh) Eglandular hair, gh) Glandular hair.

diagnostic character to distinguish these subspecies in addition to the characters given in the *Flora of Turkey*. However, after a careful examination of the collected specimens, it was found that the indumentum is not useful in distinguishing subspecies, because this character was affected by different ecological factors (Selvi & Bigazzi, 2001). These observations are consistent with the fact that hairlessness exhibits a great deal of variation (Chamberlain, 1977).

The anatomical properties given in this paper provide the first detailed description of *A. leptophylla* subsp. *leptophylla* and *A. leptophylla* subsp. *incana*. Anatomical investigations show that there is no clear difference between the 2 subspecies. Analysis of the root cross-section showed that the root has secondary growth. Primary pith rays were considerably distinct. However, it was observed in *A. leptophylla* subsp. *leptophylla* that the periderm layer was thicker than it was in subsp. *incana* (Table 2). In the pith, primary xylem tissue was present. The stem was covered by a thin cuticle layer. Collenchyma was 3-7 layered at the corners. Cortex cells were parenchymatic. There was a pith composed of parenchymatic cells. There were glandular and eglandular hairs on the epidermis of the stem and leaf. Eglandular hairs were more common than glandular hairs. Metcalfe & Chalk (1979) reported that there are glandular and eglandular hairs in Boraginaceae. The leaves were isolateral (isobilateral). However, numbers of lower and upper parenchyma layers were variable in the examined taxa. This variation might be attributed to the morphological and ecological diversification. In *A. leptophylla* subsp. *leptophylla*, the palisade parenchyma cells were usually 2 layered and more regular than those of *A. leptophylla* subsp. *incana*. It was determined that most *Anchusa* species have typical dorsiventral leaves, but some have isobilateral leaves (Selvi & Bigazzi, 2001). Jodin (1903) and Selvi & Bigazzi (2001)

determined that the majority of *Anchusa* taxa have typical dorsiventral leaves; however, the abaxial palisade tissue in some *Anchusa* taxa provided noticeable exceptions to this rule. Our results are consistent with those given by Selvi & Bigazzi (2001). Metcalfe & Chalk (1979) pointed out that there were both anomocytic and anisocytic stomata in the Boraginaceae. In addition, it was reported that there were anomocytic stomata in this family (Özörgücü, 1991). In this study, the stomata were anomocytic and the mean number of

stomata on the lower surfaces was higher than that on the upper one. The numbers of stomata on the upper and lower epidermises in subsp. *leptophylla* and *incana* were 8 ± 1 and 10 ± 1 , and 9 ± 2 and 13 ± 2 , respectively (Table 2). Selvi & Bigazzi (2001) determined that in most of the *Anchusa* the adaxial density of stomata was lower than the abaxial one. Our results are in agreement with those reported by Selvi & Bigazzi (2001) and should be useful in future studies about this genus.

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