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MORPHOLOGICAL, ANATOMICAL AND NUMERICAL STUDIES ON SOME *ANCHUSA* L. (BORAGİNACEAE) TAXA FROM TURKEY

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Abstract

This study used numerical methods to illustrate, describe and assess the taxonomic significance of morphological and anatomical features of three *Anchusa* species,, *Anchusa undulata* subsp. *hybrida* (Ten.) Coutinho, *A. azurea* Miller var. *azurea* and *A. pusilla* Guşul., collected from Northern Turkey. In this morphological study, it was determined that the ratio of calyx lobe to the calyx length and the arrangement of the anthers in the corolla tube were important characters in separating the taxa morphologically. Anatomical studies supported these morphological observations. Further, statistical analysis showed that corolla tube length was not important as a taxonomic character. However, the ratio of calyx lobe length to calyx length was the most significant character in distinguishing the taxa. The first two principal components explained 45.69 % of the total variance. Principal component analysis showed that no separation could be obtained among the species, although *A. azurea* specimens tended to compose a different group.

Introduction

Anchusa L., (Boraginaceae) is one of the major genera of flowering plants, consisting of about 170 taxa native to temperate and subtropical areas of the Old World. The major diversity centre of *Anchusa* is the southern part of the Balkan Peninsula (Selvi & Bigazzi, 2003). The present great form diversity in this heterogeneous genus has generated variable interpretations at both species and generic level (Guşuleac, 1927, 1928, 1929; Chater, 1972; Greuter *et al*., 1984; Brummit, 1992; Selvi & Bigazzi, 1998). Additionally, some species in this genus have been used as folk medicine (Baytop, 1991).

Morphological and anatomical studies are limited in the *Anchusa* taxa (Metchalfe & Chalk, 1989; Selvi *et al.,* 1996; Selvi & Bigazzi, 2000 a, b). Recent studies are mainly focused on leaf features (Nyauwame & Gill, 1990; Selvi & Bigazzi, 2001). Characters concerning the vascular system appear to be essentially uniform, while stomata size and density, structure of individual trichomes, type of indumentum, leaf and epidermis thickness and palisade arrangement were variable. Selvi & Bigazzi (2001) determined that most species had typical dorsiventral leaves, but some of them showed an isobilateral symmetry containing a layer of abaxial palisade tissue. Although the thickness of the lamina and the outer wall of the adaxial epidermis cells were thicker in xerophytic taxa of *Anchusa* species.

Fifteen *Anchusa* species are common in Turkey; some of them are endemic (Chamberlain, 1979; Ekim *et al*., 2000). Anatomical features are important characters in *Anchusa*, but there are no detailed data concerning Turkish originated species.

Different studies between and within populations have also revealed quantitative–genetic variation in several plant species. In these studies, phenotypic and genotypic correlations between morphological and developmental traits were frequently determined in plant populations (Schoen, 1982; Roach, 1986; Venable & Burquez, 1989). However, investigated populations displayed a continuous variation in most of the obtained morphological traits and the genetic basis of the present variation is unknown.

Due to lack of thorough revisions, the delimination and taxonomic status of the *Anchusa* taxa belonging to the Flora of Turkey is uncertain. A recent biometric investigation on *A. undulata* subsp. *hybrida* showed that these species represents a weakly differentiated geopraphical race in Italy (Selvi & Bigazzi, 1998). Additionally, *A. azurea* and *A. pusilla* are widespread and variable species in Turkey.

However, so far there are insufficient data concerning Turkish *Anchusa* species. Hence, the aim of this study was to explore patterns and ranges of variation regarding floral characters with potential taxonomic value within some *Anchusa* taxa populations in Turkey. In addition, we focused on anatomical and morphological features of *Anchusa* taxa distributed in Northern Turkey to explore their systematic importance.

Materials and Methods

Plant samples were collected from different localities in Northern Turkey during 2004-2005. These localities are shown in Table 1. A total of 280 specimens were studied. For morphological studies the specimens were dried according to standard herbarium techniques and stored at the Ondokuz Mayıs University Herbarium (OMUB). Materials prepared and used for anatomical studies were preserved in 70% alcohol.

Anatomical observations were performed on root, stem and leaf transverse sections and on leaf surface sections. The photographs were taken with a NICON COOLPIX 5200 digital camera. All measurements and observations were replicated three times.

The 14 characters presented in Table 2 were assessed by numerical analysis. To reduce the effect of individual variability, characters were measured three times and the corresponding average values were used for further calculation. Means and standard deviations were calculated to document the variation among each investigated character.

To demonstrate the present variation on diagramme level, obtained morphological data was used to carry out a so called principal component analysis (Backhaus *et al*., 1989) to create a scatter plot diagramm using the statistical software package SPSS (version 12, SPSS Inc., Chicago, USA).

The principal component analysis (PCA) involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables called principal components. The statistical procedure covers a factor analysis, where over every character and variable a correlation matrix between variable and character were calculated. Over this matrix the so called principal components were created and this components can be used to show the present variation on a scatter plot diagramme. Further statistical tests were also done using SPSS.

Results

Morphological characters

Anchusa undulata* subsp. *hybrida (Fig. 1)

Biennial or perennial, stem 22-73 cm, generally with strigose dense hairs. Basal leaves oblanceolate, 5.5-13.0x0.8-1.8 cm, margins undulate, surfaces covered with tuberculate or strigose hairs. Cauline leaves 2.5-18.0x0.6-2.5 cm, linear to lanceolate, deeply undulate-dentate. Indumentum dimorphic, with strigose and longer tuberculate hairs. Inflorescence rich with several branched cymes elongating considerably in fruit. Bracts lanceolate or ovate-lanceolate, 2.0-5.0x0.5-0.8 mm, with hirsute hairs. Calyx 5.5-8.5 mm, divided to ½ into subacute to acute lobes. Corolla usually dark blue and violet or purple with white throat, lobes 2.0-4.0 mm. Stamens 2.0-3.0 mm, usually not overlapping scales. Style 3.5-8.5 mm. Nutlets 1.5-3.0x2.0-3.5 mm, obliquely ovoid with a lateral beak and a thickened basal ring, brown, coat surface reticulate-rugose.

Table 1. Locality information of the examined *Anchusa* taxa.

Taxa	Localities
A. azurea var. *azurea* **(A)**	**1**: A5 Samsun: Kurupelit Campus of Ondokuz Mayıs University, 6 m, 27.04.2004, Ulu 096,
	2: A5 Samsun: Yeşilkent, 30 m, 09.05.2004, Ulu 048
	3: A5 Samsun: Taflan, 6 m, 27.04.2004, Ulu 014
	4: A5 Samsun: Ladik, 930 m, 11.06.2005, Ulu 269
	5: A5 Samsun: Alaçam, 25 m,06.06.2004, Ulu 082
	6: A5 Samsun: Karadağ, 900 m, 02.07.2004, Ulu 117
	7: A5 Samsun: Tekkeköy, 25 m, 12.06.2005, Ulu 261
	8: A6 Amasya: Taşova, 235 m, 11.06.2005, Ulu 249
	9: A6 Amasya: Suluova, 400m, 07.07.2004, Ulu 156
A. undulata subsp. *hybrida***(B)**	**1**: A5 Samsun: Çarşamba, 10 m, 17.04.2004,.Ulu 010
	2: A5 Samsun: Gelemen, 8 m, 13.06.2004, Ulu 201
	3: A5 Samsun: Çarşamba, Costal Village, 7 m, 27.05.2005, Ulu 179
	4: A5 Samsun: Çarşamba, Çınarlık, 5 m, 09.05.2005, Ulu 211
A. pusilla **(C)**	**1**: A5 Samsun: Havza, 710 m, 09.06.2005, Ulu 231
	2: A6 Amasya: Suluova, 390 m, 09.06.2005, Ulu 223
	3: A5 Samsun: Kavak, 900 m, 02.07.2004, Ulu 123

Table 2. Morphometric variables selected for numerical analyses

SL (cm)	Mean length of stem
IL (cm)	Mean length of inflorescence
SLW (cm)	Mean width of cauline leaves
SLL (cm)	Mean length of cauline leaves
BLL (cm)	Mean length of basal leaves
BL (mm)	Mean length of bracts
PL (cm)	Mean length of pedicel
CL (mm)	Mean length of calyx
CLL (mm)	Mean length of calyx lobe
CRTW (mm)	Mean width of corolla tube
CRTL (mm)	Mean length of corolla tube
CRLW (mm)	Mean width of corolla lobe
NW (mm)	Mean width of nutlet
NL (mm)	Mean length of nutlet

Anchusa azurea* var. *azurea (Fig. 2)

Perennial, stem erect, 30-115 cm, covered with hispid strigose with dense, tubercle-based bristles and shorter hairs. Basal leaves linear-lanceolate, 7-30x1.2-3.5 cm, hirsute on surfaces. Cauline leaves linear-lanceolate, 4-19x0.2-3.6 cm, indumentum dense, with soft or stiff setae. Inflorescence paniculate, elongating considerably in fruit. Bracts linear to ovate-lanceolate with hirsute hairs, 9-20x1.0-1.5 mm. Calyx 6-12 mm in fruit, divided almost to the base into 5 linear, acute lobes. Corolla violet or deep blue, lobes 4-6 mm. Stamens inserted at top of tube. Stylus 7-12 mm. Nutlets oblong-ovoid, erect, 5.0-9.0x2.0-4.5 mm, greyish-brown. Coat surface verrucose with prominent ridges and simple papillae.

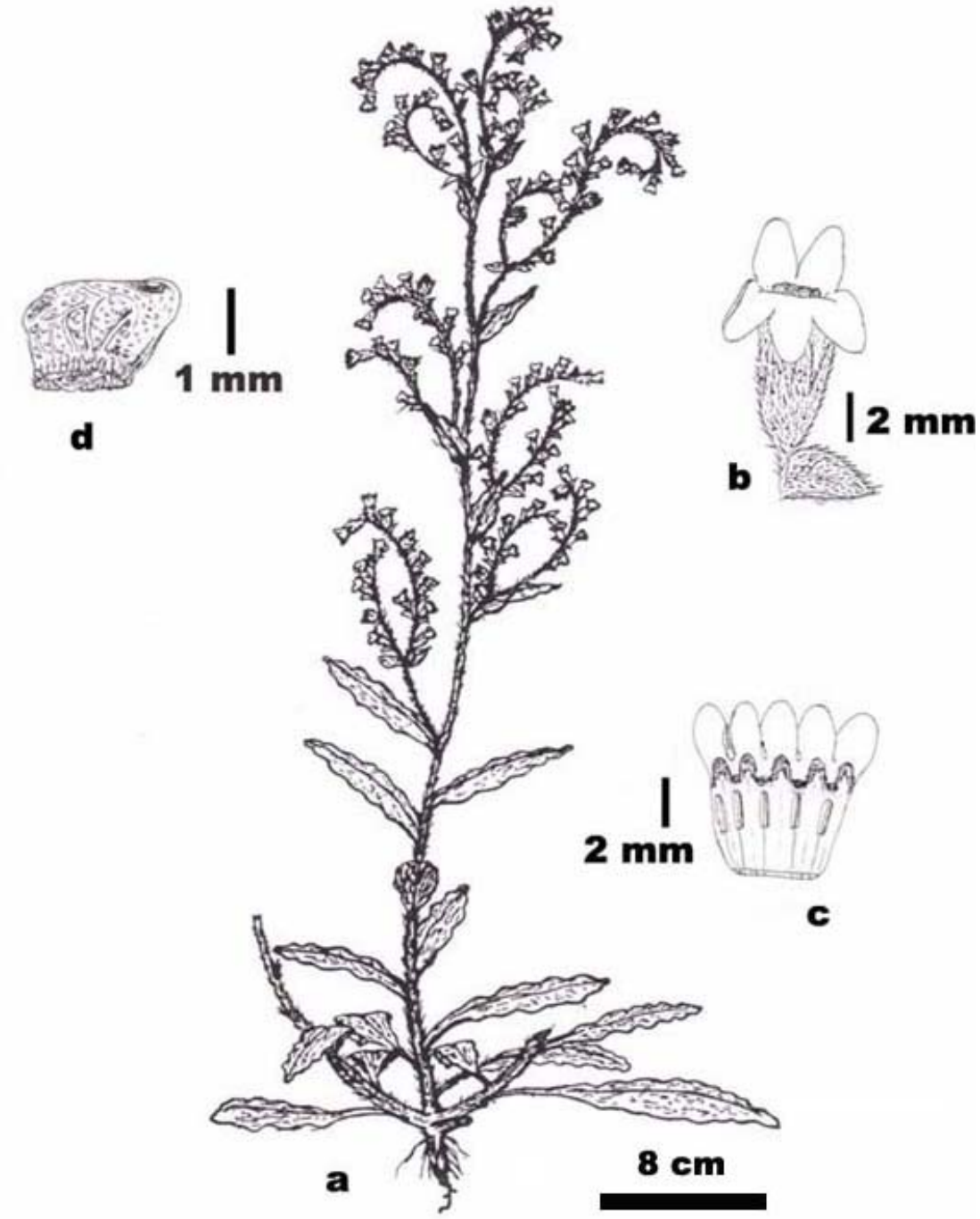


Fig. 1. *Anchusa undulata* subsp. *hybrida* (Ten) Coutinho (Ulu 010). a. General appearance, b. Flower with bract, c. Dissected corolla d. Nutlet.

Anchusa pusilla (Fig. 3)

Annual or biennial. Stem erect, 30-78 cm, with sparsely strigose hairs. Basal leaves oblanceolate, 2.5-12x0.8-1.5 cm. Cauline leaves linear-lanceolate to oblong-lanceolate with dentate margins, 4.0-9.0x0.3-2.0 cm. Inflorescence paniculate, consisting of several branched cymes elongating considerably after anthesis. Bracts linear to lanceolate with strigose hairs, 2.5-6.0x1.0-1.5 mm. Calyx 5.0-9.0 mm in fruit, divided almost to the base into linear, obtuse lobes. Corolla pale violet, lobes 2.5-5.5 mm, scales with long hairs and obliquely-ovoid. Stamens inserted near top of tube. Stylus 4.0-8.0 mm. Nutlets 3.0-4.0x2.5-4.0 mm., obliquely-ovoid, with a prominent basal annulus, greyish-brown, with a reticulation of prominent ridges and papillae lobed, rosette-like.

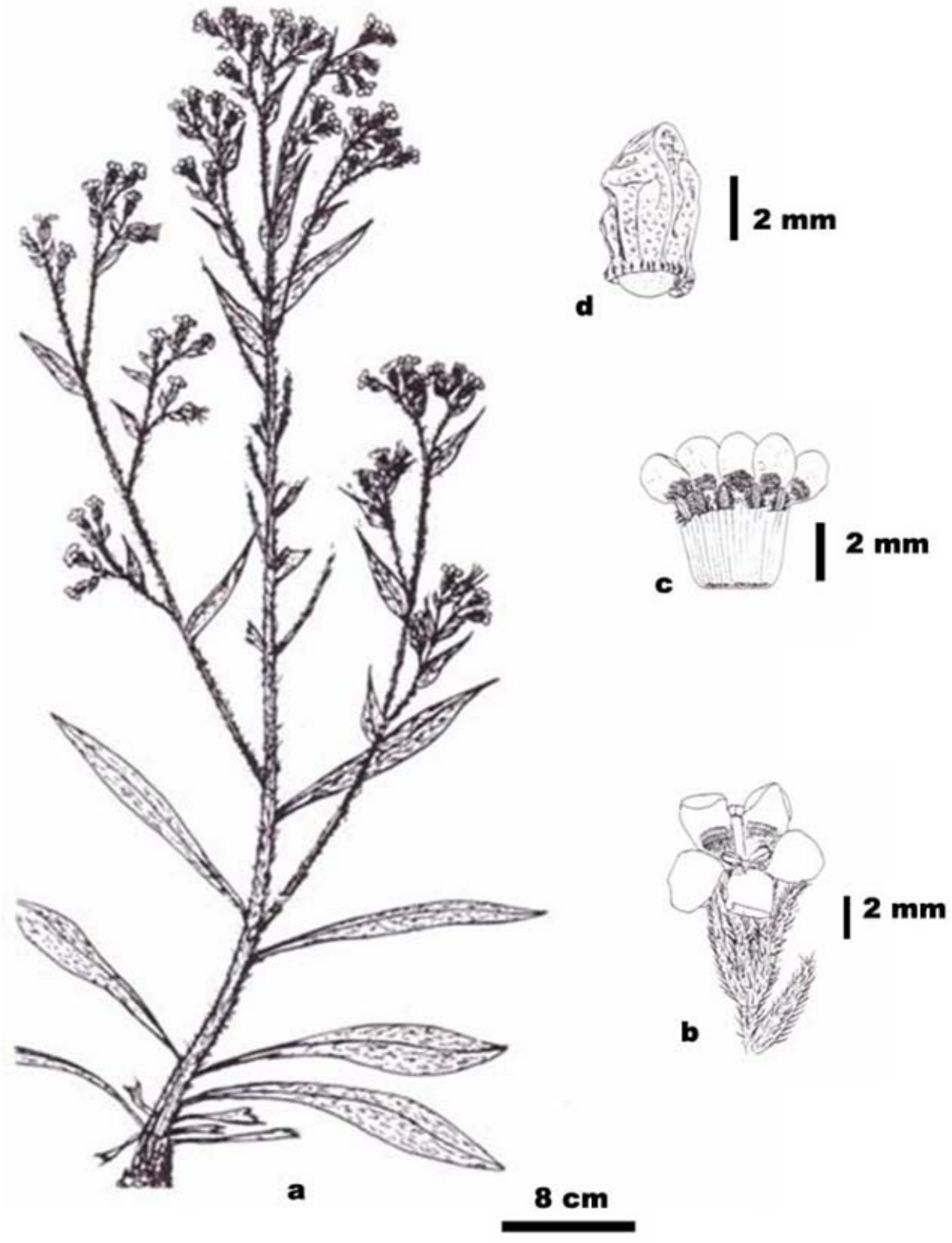


Fig. 2. *Anchusa azurea* Miller var. *azurea* (Ulu 023). a. General appearance, b. Flower with bract, c. Dissected corolla, d. Nutlet.

Comparison of morphological characteristics between *Anchusa undulata* subsp. *hybrida*, *Anchusa azurea* var. *azurea* and *Anchusa pusilla* are given in Table 3.

Table 3. Comparison of morphological characteristics between *A.undulata* subsp. *hybrida*, *A. azurea* var. *azurea* and *A. pusilla*.

	A.undulata subsp. *hybrida*	*A. azurea* var. *azurea*	*A. pusilla*
Life timing	Biennial or perennial	Perennial	Annual or biennial
Stem	Erect, 22-73 cm, generally with strigose dense hairs.	Erect, 30-115 cm, covered with hispid strigose or dense, tubercle- based bristles and shorter hairs.	Erect,30-78 cm, with sparsely strigose hairs.
Basal leaves	Oblanceolate, 5.5-13.0x0.8-1.8 cm, margins undulate with tuberculate or strigose hairs.	Linear-lanceolate, 7-30x1.2-3.5 cm, hirsute on surfaces.	Oblanceolate, 2.5-12x0.8-1.5 cm, with strigose hairs.
Cauline leaves	2.5-1.8x0.6-2.5 cm, linear to lanceolate, deeply undulate-dentate.	4-19x3.2-3.6 cm, linear-lanceolate with dentate margines.	4.0-9.0x0.3-2.0 cm linear-lanceolate to oblong-lanceolate with dentate margines.
Indumentum	Dimorphic, with strigose and longer tuberculate hairs.	Monomorphic, with soft of stiff setae or occasionally dimorphic.	Monomorphic with tuberculate-strigose hairs or occasionally dimorphic.
Inflorescence	Branched cymes.	Paniculate.	Paniculate, consisting of several branched cymes.
Bracts	2.0-5.0x0.5-0.8mm, lanceolate or ovate-lanceolate with hirsute hairs.	9-20x1.0 -1.5 mm, linear to ovate-lanceolate with hirsute hairs.	2.5-6.0x1.0-1.5 mm,linear to lanceolate with strigose hairs.
Calyx	5.5-8.5mm,divided to ½ into subacute to acute lobes.	6-12 mm, divided almost to the base into linear, acute lobes.	5-9 mm, divided almost to the base into linear, obtuse lobes.
Corolla	Usually dark blue and violet or purple, lobes 2-4 mm.	Violet or deep blue, lobes 4-6 mm.	Pale violet, lobes 2.5-5.5 mm.
Stamens	2.0-3.0 mm, usually not overlapping scales.	1.5-2.0 mm, inserted at top of tube.	1.5-2.5 mm, inserted near top of tube.
Stylus	3.5-8.5 mm.	7-12 mm.	4.0-8.0 mm.
Nutlets	1.5-3.0x2.0-3.5 mm, obliquely-ovoid with a lateral beak and a basal ring, coat surface reticulate- rugose	5.0-9.0x2.0-4.5 mm, oblong- ovoid, erect, coat surface with simple papillae.	3.0-4.0x2.5-4.0 mm, obliquely- ovoid with a prominent basal annulus and lobed rosette- like papillae

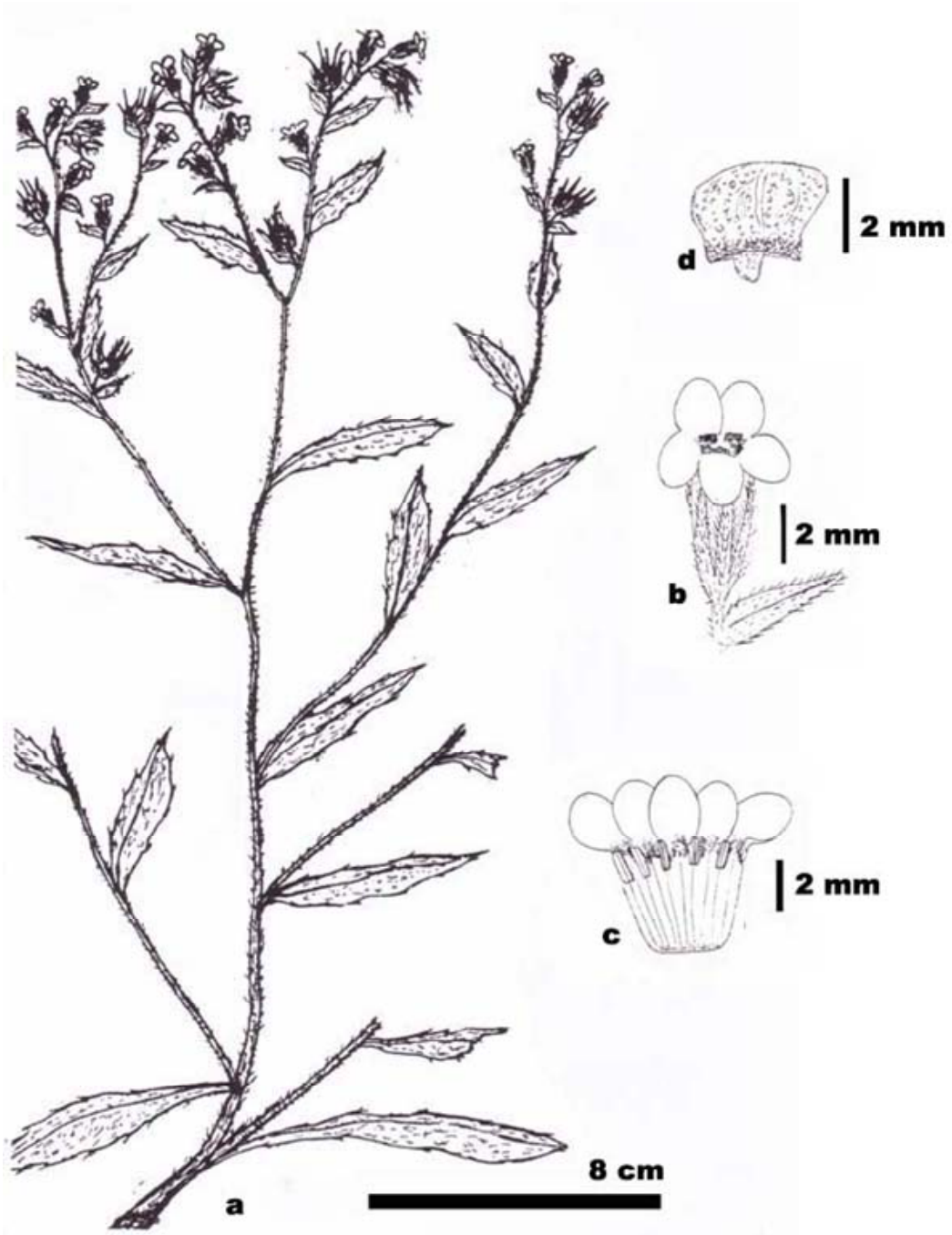


Fig. 3. *Anchusa pusilla* Guşul (Ulu 123). a. General appearance, b. Flower with bract, c. Dissected corolla, d. Nutlet.

Anatomical characters

Anchusa undulata subsp. *hybrida*

Root (Fig. 4)

A transverse section taken from the middle part of the root was investigated . Periderm is multilayered on the outer root surface and the cell size 50-500 µm. Cortex layer is 1000-2250 µm thick and consists of parenchymatic cells (Table 4). Further, the cambium cells are distinguishable and 2-3 layered. The neighboured xylem tissue is extensive (750-3875 µm). Primary pith rays consists of 4-17 layers. In the centre, there is a narrow pith which consists of parenchymatic cells.

Stem (Fig. 5)

The epidermis is composed of almost square or rectangular cells has compactly arranged cells. It's upper surface is covered with a relatively thick cuticle and contains eglandular and glandular hairs. The collenchyma tissues consisting of 2-5 layered rounded cells are located under the epidermis and the 3-10 layered parenchyma tissue consists of usually oval cells. Cambium cells and xylem members are more visible. Thickness of xylem tissue is 200-340 μm. The diameter of trachea cells are 20-50 μm, pith cells are large and cylindrical (Table 4).

Leaf (Figs. 6-7)

A transverse and surface section of the lamina and both epidermises was studied. Epidermal cells of both surfaces are arranged in a single layer. Glandular and eglandular hairs are dense on the lower surface. Both epidermises have thick cuticle and undulate cell walls. The stomata type is anomocytic and they occur on the surfaces of both sides, being more abundant on the lower surface (Fig. 6 and Table 4). Leaf has isobilateral symmetry with distinct upper and lower surfaces. Mesophyll consists of 2 or 3 layers of upper and lower palisade cells and isodiametric spongy parenchymatic cells with large intercellular cavities. Vascular bundles are surrounded by a parenchymatic bundle sheath (Fig. 7).

Anchusa azurea* var. *azurea

Root (Fig. 8)

A transverse section taken from the middle part of the root showed that the upper surface is covered with a periderm layer which consists of cells 40-300 μm in size (Table 4). Under the periderm there is a cortex consists of parenchymatic, rectangular cells. Cambium is not distinguishable. Thickness of xylem tissue including solitary vessels or clustered vessels is 660-3400 μm. Primary pith rays are 5-7 layered, sometimes 10-13 layered (Table 4). In the pith, primary xylem tissue is present.

Stem (Fig. 9)

The epidermis is composed of a single layer of rectangular or oval cells. They are covered with a thin cuticle. The epidermis had glandular and eglandular hairs. Under the epidermis there is collenchyma with 3-9 layered cells. Cortex parenchyma consists of 3-7 layers of usually large and oval cells. Cambium is not distinguishable. Cells of xylem tissue covers a large area 250-850 μm. Diameter of trachea cells are 50-60 μm (Table 4). The pith consists of large parenchymatic cells.

Leaf (Figs. 10-11)

A transverse and surface section of the lamina and both epidermises was studied. There is a single layered epidermis on the upper and lower surface of the leaf. Both epidermises are covered with a thick cuticle. In surface preparations, cell walls of both epidermises are undulate (Fig. 10). There are many prominent trichomes on the lower and upper epidermis. Leaf is ecvifacial and has anomocytic stomata cells. In terms of size, the adaxial stomata cells are usually slightly larger than those of the abaxial stomata. The abaxial stomata density is lower than the adaxial one (Table 4). Palisade parenchyma cells are 1 or rarely 2 layered and elongated on both lower and upper epidermis. Isodiametric spongy parenchyma cells have large intercellular cavities. Vascular bundles are surrounded by parenchymatous and orbicular cells (Fig. 11).

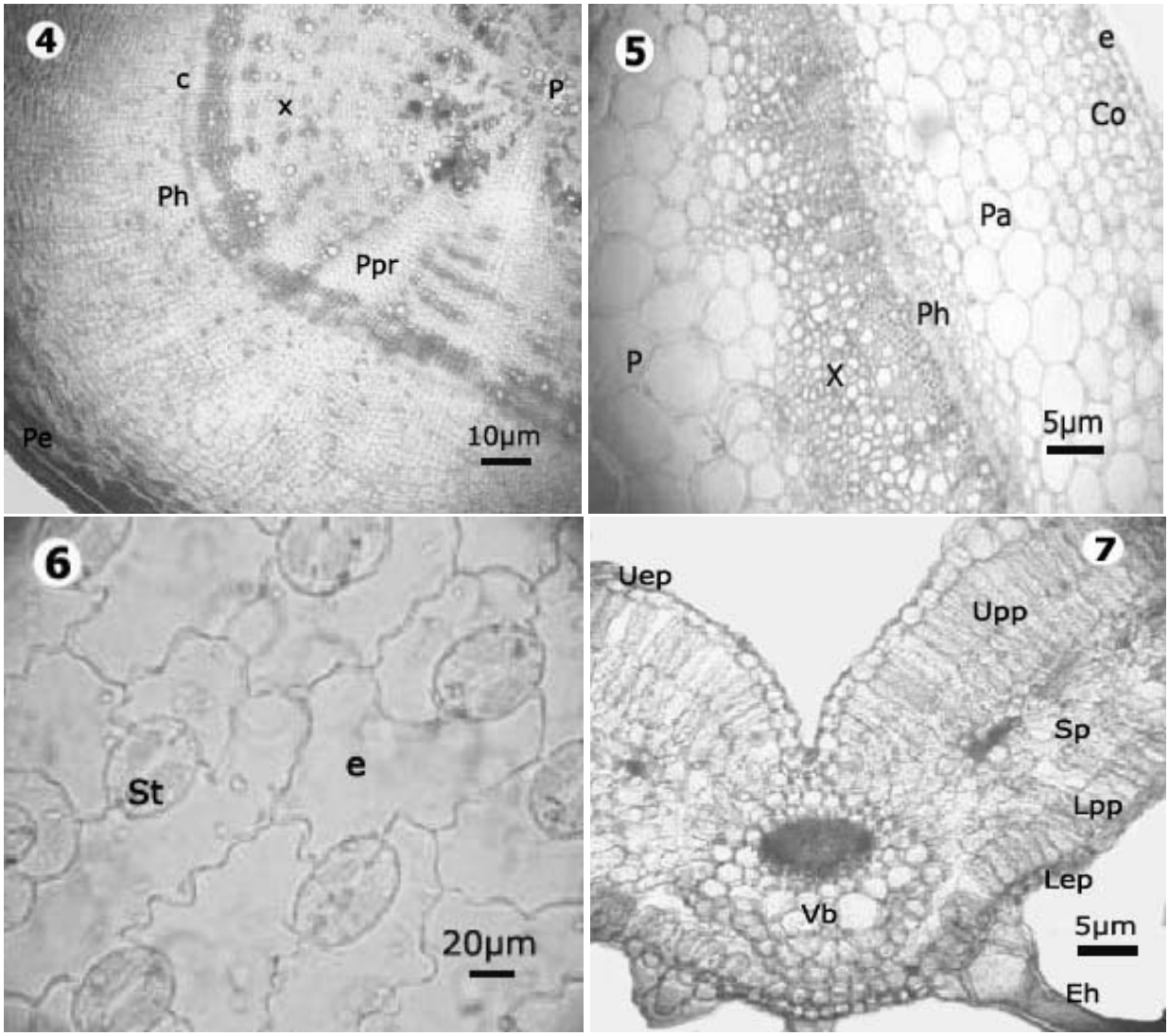


Fig. 4-7. *Anchusa undulata* subsp. *hybrida*. Fig. 4. Transverse section of root. Fig. 5. Transverse section of stem. Fig. 6. Surface section of leaf. Fig. 7. Transverse section of leaf. C: Cambium, Co: collenchyma, e: epidermis, Eh: eglandular hair, Lep: lower epidermis, Lpp: lower palisade parenchyma, p: pith, Pa: parenchyma, Ph: phloem, pr: periderma, Ppr: primer pith rays, Sp: spongy parenchyma, St: stoma, Uep: upper epidermis, Upp: upper palisade parenchyma, Vb: vascular bundle.

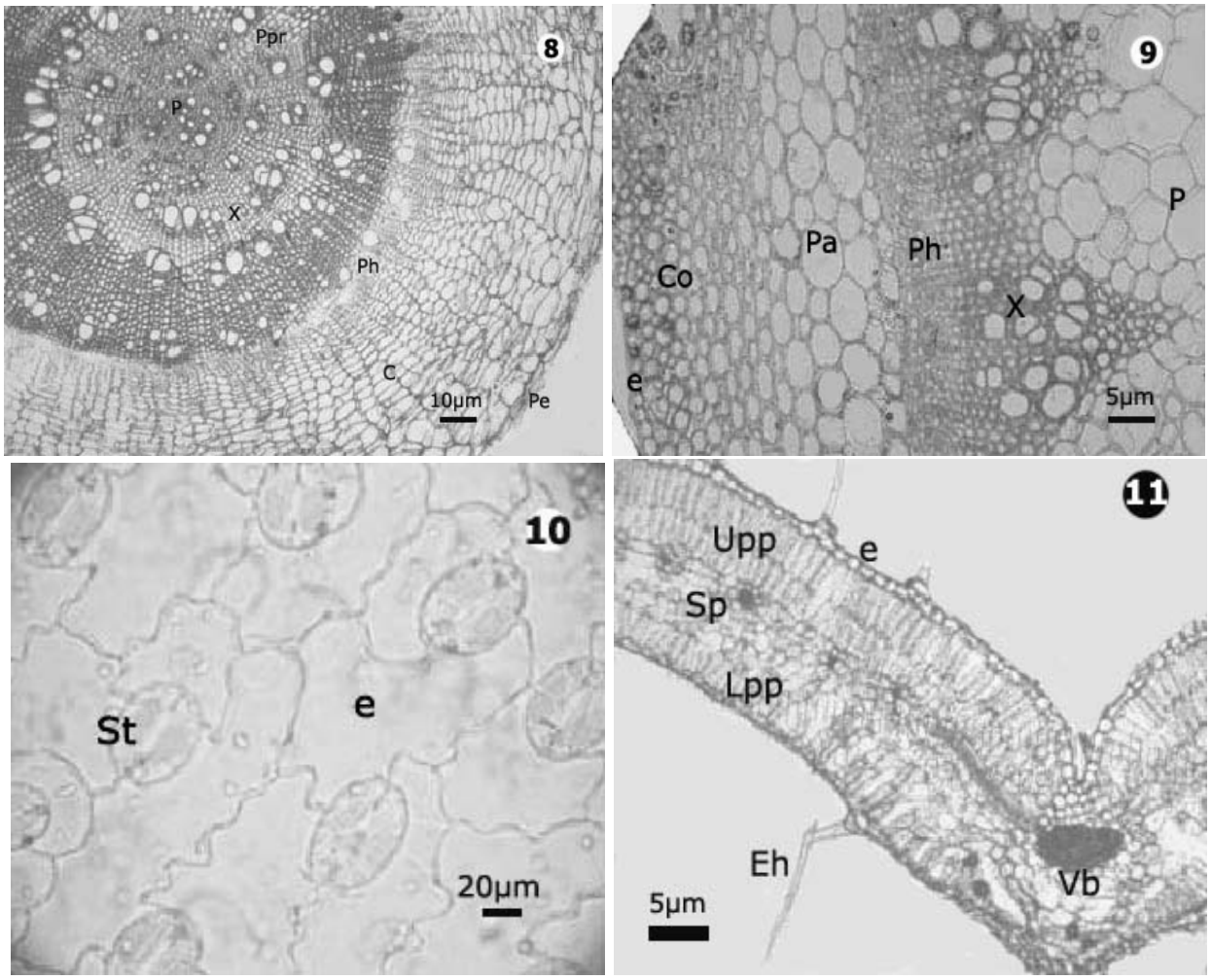
Anchusa pusilla

Root (Fig. 12)

A transverse section taken from the middle part of the root was investigated. The periderm layer is 25-125 µm thick (Table 4). Cortex is multilayered and consists of cylindrical cells. The 6-20 layered compressed phloem ring have small cells. Cambium cells are not distinguishable. The xylem which extend all around the primary xylem is 550-2750 µm. Primary pith rays are 2-5 layered (Table 4). In the centre, there is a pith which consists of parenchymatic cells.

Table 4. Comparison of anatomical characteristics between *A. undulata* subsp. *hybrida*, *A. azurea* and *A. pusilla*.

	Anatomical characters	***A. undulata* subsp. *hybrida***	***A. azurea* var. *azurea***	***A pusilla***
Root	Thickness of periderm (μm)	50-500	40-300	25-125
	Thickness of cortex (μm)	1000-2250	700-4375	300-750
	The layer numbers of phloem	Not distinguishable	8-33	6-20
	Cambium	Distinguishable	Not distinguishable	Not distinguishable
	Thickness of xylem (μm)	750-3875	660-3400	550-2750
	Number of primary pith rays	4-17	5-7, sometimes 10-13	2-5
	Pith	Parenchymatic	Parenchymatic	Parenchymatic
Stem	Thickness of epidermis (μm)	20-40	20-30	20-40
	Thickness of collenchyma (μm)	70-220	100-350	70-280
	Thickness of parenchyma (μm)	100-400	120-750	70-370
	Thickness of phloem (μm)	50-120	60-140	40-90
	Cambium	Distinguishable	Distinguishable	Not distinguishable
	Thickness of xylem (μm)	200-340	250-850	160-400
	Diameter of trachea (μm)	20-50	50-60	20-40
	Pith	Parenchymatic	Parenchymatic	Parenchymatic
Leaf	Thickness of upper epidermis (μm)	30-50	20-40	20-40
	The layer numbers of upper palisade parenchyma	2-3	1-2	2
	Thickness of upper palisade parenchyma (μm)	10-26	10-25	10-28
	Thickness of spongy parenhcyma (μm)	120-300	60-250	110-350
	Thickness of lower palisade parenchyma (μm)	70-120	50-140	70-150
	Thickness of lower epidermis (μm)	20-40	10-40	20-60
	Stoma length (μm)	33-57	32-68	40-62
	Stoma width (μm)	25-33	25-41	24-41
	Number of stomata on upper epidermis	7±2	7±2	5±2
	Number of stomata on lower epidermis	10±2	8±2	5±1



Figs. 8-11. *Anchusa azurea* var. *azurea*. Fig.8. Transverse section of root. Fig.9. Transverse section of stem Fig. 10. Surface section of leaf. Fig.11. Transverse section of leaf. c: cortex, Co: collenchyma, e: epidermis, Eh: eglandular hair, Lpp: lower palisade parenchyma, p: pith, Pa: parenchyma, Pe: periderma, Ph: phloem, Pr: periderma, Ppr: primer pith rays, Sp: spongy parenchyma, St: stoma, Upp: upper palisade parenchyma, X: xylem, Vb: vascular bundle.

Table 5. Variation among the studied species (Means ± SD).

Character	*A. azurea* var. *azurea*	*A. undulata* subsp. *hybrida*	*A.pusilla*
SL	56.06 ± 20.34	43.44 ± 12.99	45.72 ± 17.31
IL	39.67 ± 15.13	29.46 ± 11.58	15.27 ± 7.05
SLW	3.61 ± 0.79	1.98 ±0.55	2.50 ± 0.76
SLL	9.73 ± 3.35	6.50 ± 2.43	7.66 ± 3.45
BLL	11.78 ± 4.69	9.69 ± 1.75	6.28 ± 2.09
BL	4.98 ± 2.12	3.84 ± 0.80	4.01 ± 0.42
PL	3.50 ± 1.61	1.81 ± 0.74	3.15 ± 0.40
CL	8.64 ± 0.76	6.97 ± 0.58	6.23 ± 0.53
CLL	7.52 ± 0.35	3.28 ± 0.62	5.27 ± 0.46
CRTW	7.18 ± 1.08	5.01 ± 0.69	4.67 ± 0.58
CRTL	7.51 ± 1.11	8.35 ± 1.08	6.24 ± 1.44
CRLW	4.81 ± 0.78	3.12 ± 0.60	2.81 ± 0.47
NW	1.38 ± 0.86	1.33 ± 0.28	1.42 ± 0.45
NL	2.01 ± 1.13	1.22 ± 1.16	3.01 ± 1.27

Table 6. PCA factor loadings on the two first principal components with relative percentage of explained variance.

	Taxa and localities	
	Component 1	**Component 2**
Eigenvalue (%)	27.46	18.18
Cumulative (%)	27.46	45.69
SL	0.216	0.553
IL	0.268	0.559
SLW	0.550	0.133
SLL	0.366	0.188
BLL	0.78	– 0.265
BL	0.585	– 0.110
PL	0.725	– 0.380
CL	0.672	– 0.272
CLL	0.907	– 0.247
CRTW	0.763	– 0.079
CRTL	0.087	– 0.094
NW	0.110	0.704
NL	0.236	0.831

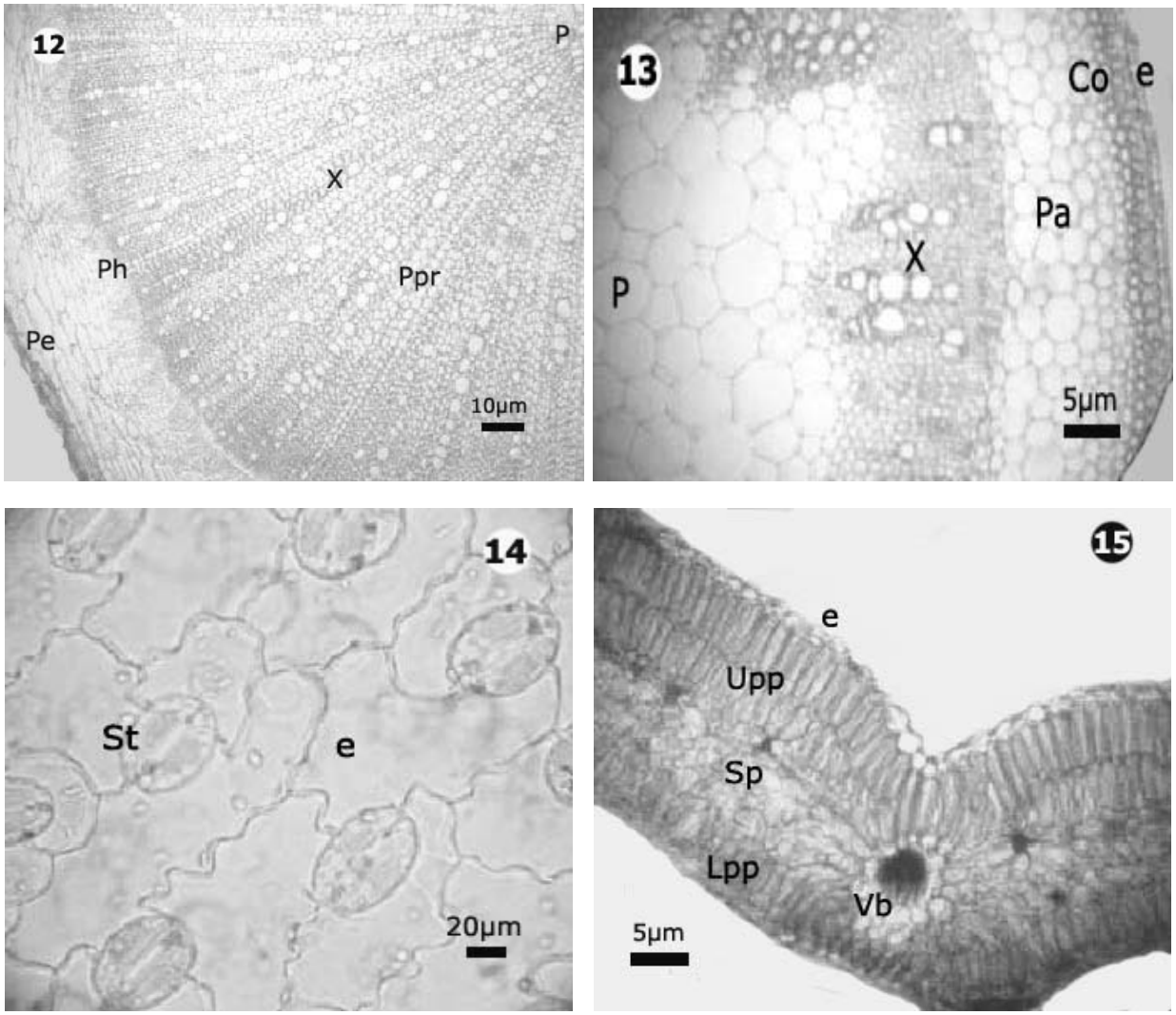
Stem (Fig. 13)

A transverse section of the stem showed that the cuticle is on the outer layer. The epidermis is positioned under the cuticle and isodiametric cells. Glandular and eglandular hairs were clearly seen on epidermis. Collenchyma is located under the epidermis. This tissue 2-8 layered at the corners. Parenchyma tissue is 2-6 layered and consists of large and oval cells. Cambium is not distinguishable. Secondary xylem covers a large area (160-400 μm thick). Diameter of trachea cells are 20-40 μm (Table 4). Pith cells are large and cylindrical.

Leaf (Figs. 14-15)

Investigation of the transverse and surface section of the lamina and epidermises showed that leaves are covered by a thick cuticular layer on both upper and lower surfaces. The upper and lower epidermis consists of a single row of cell in which the width and length are almost equal. Both epidermises have prominent undulate cell walls (Fig.14). There are many glandular and eglandular hairs on the epidermis. Leaf is ecvifacial. Anomocytic stomata cells oval in shape are present in both the upper and lower epidermis. Distribution of the stomata is fairly homogeneous on the both surfaces (Table 4). The mesophyll is differentiated into palisade and spongy parenchyma. The upper and lower palisade have long rectangular parenchyma cells and consists of usually 1 or 2 layers. The cells of upper palisade parenchyma are more regular than those of the lower palisade parenchyma cells. Spongy parenchymatic cells possessing large intercellular cavities are isodiametric. Vascular bundles are surrounded by parenchymatous cells (Fig. 15).

The comparative differences in the root, stem and leaf anatomy of the investigated taxa are given in Table 4.



Figs. 12-15. *Anchusa pusilla*. Fig. 12. Transverse section of root. Fig. 13. Transverse section of stem. Fig. 14. Surface section of leaf. Fig. 15. Transverse section of leaf. Co: collenchyma, e: epidermis, Lpp: lower palisade parenchyma, p: pith, Pa: parenchyma, Pe: periderma, Ph: phloem, Ppr: primer pith rays, Sp: spongy parenchyma, St: stoma, Upp: upper palisade parenchyma, X: xylem, Vb: vascular bundle.

Numerical analysis

All size related characters showed large ranges between investigated three species as can be seen in Table 5. Mean length of stem (SL) showed the highest variation. However, mean length of inflorescence (IL), mean length of basal leaves (BLL) and mean length of cauline leaves (SLL) were high variable characters. The most variable floral character was the mean length of corolla tube (CRTL). Calyx and calyx lobe measurements (CL and CLL) were less variable floral characters than the others (Table 5).

Principal component analysis

Morphological data were obtained to carry out a Principal Component Analysis (Table 6 and Fig. 16). The calculated principal components were used to differentiate localities and taxa (Fig. 16). In Table 6, the values for the principal components and correlation values for each character are given.

Table 5 and Table 6 must be added to this section.

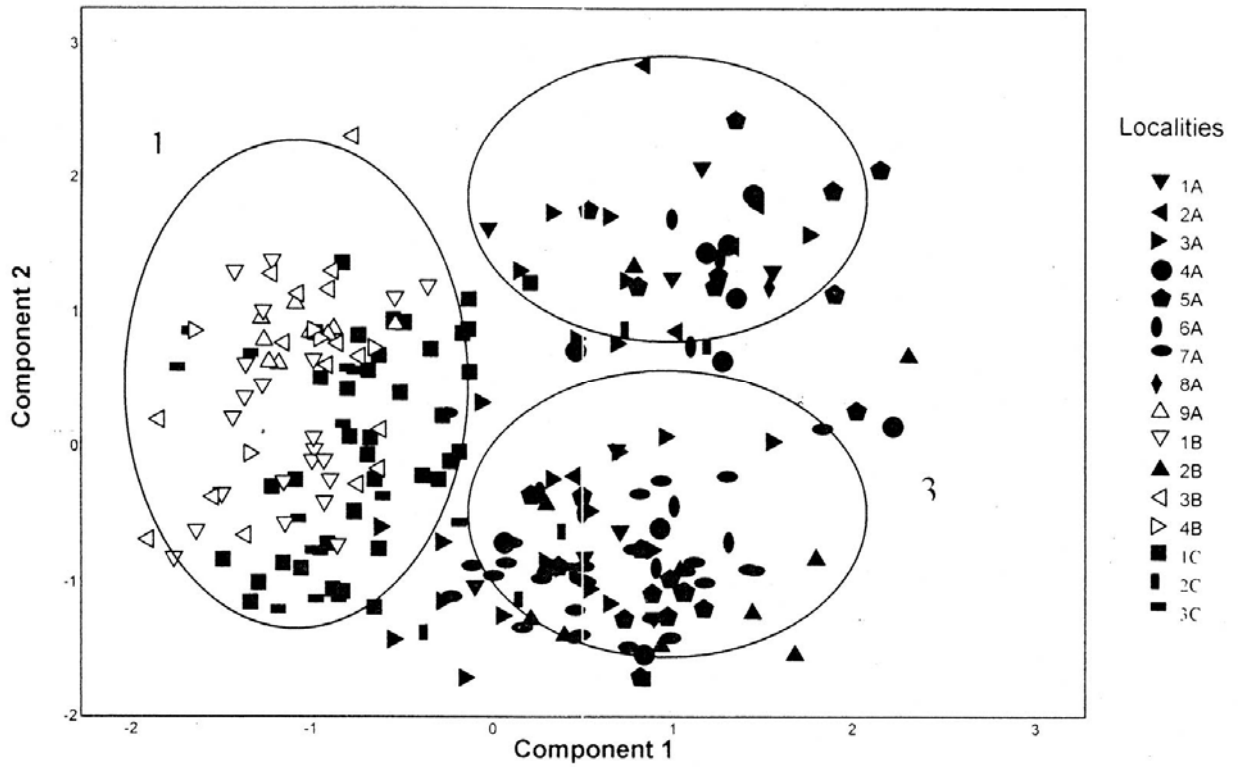


Fig. 16. Scatterplot of Principal Component Analysis of the geopraphic provenances of the examined *Anchusa* species. **A**: *A. azurea* var. *azurea*, **B**: *A.undulata* subsp. *hybrida,* **C**: *A. pusilla.* (Locality information of the *Anchusa* taxa are given in Table 1).

The 1st principal component accounts for 27.46% of the total variation and the 2nd principal component for 18.18 %. Both components accounts for 45.69% of the total variation. These components were partially effective in separating the investigated material based on obtained and evaluated characters.

The created Scatter Plot diagram is given in Fig. 16. Investigated taxa and localities are represented by different symbols and names (A: *A. azurea* var. *azurea*, B: *A. undulata* subsp. *hybrida* and C: *A.pusilla*). Based on investigated characters *A. azurea* var. *azurea* showed a considerable extensive distribution, whereas *A. undulata* subsp. *hybrida* and *A. pusilla* are distributed in the same group (shown within a circle) regarding collected localities. This means that these two taxa display similar attributes regarding investigated characters. This is underlined by the fact, that they are in the same group although they were collected from different localities.

Discussion

In this study, the characteristics of some *Anchusa* taxa collected from Northern Turkey were demonstrated by analyzing the results obtained from morphological, anatomical and numerical investigations. It was determined that morphological characters such as the relative length of teeth and tube of the calyx, the point of insertion and arrangement of the anthers in the corolla In *A. undulata* subsp. *hybrida* the calyx was divided to ½ -2/3 into lobes, while it was divided almost to the base in *A. azurea* and *A. pusilla*.

Faucal scales were also micromorphological characters with systematic relevance (Selvi & Bigazzi, 2003). In a typical *A. undulata* subsp. *undulata* species staminal

filaments are placed at the base of faucal scales with anthers completely overlapping, while *A. undulata* subsp. *hybrida* stamens were inserted below the scales. But it shows notable variation in habit, indumentum, plant size and leaf shape (Selvi & Bigazzi, 1998, 2001). Selvi & Bigazzi (1998) reported that in *A. undulata* subsp. *hybrida* intermediate specimens with partially overlapping anther and scales occur in Greece, Israel and Southern Italy. Similarly, we also determined stamens partially overlapping faucal scales in some *A. undulata* subsp. *hybrida* specimens collected from Turkey. All our observations were similar to those reported by Selvi & Bigazzi (1998).

Other characters reported in the literature such as type of indumentum are hardly reliable because they are very variable in the *Anchusa* taxa (Chamberlain, 1979). In the Flora of Turkey, *A. undulata* subsp. *hybrida* has dimorphic indumentum with short soft and longer stiff tuberculate hairs. However, in our study, it was determined that this type of indumentum can be present in other investigated *Anchusa* taxa. On the other hand, indumentum was considerably variable character in specimens from different localities. Leaf and stem surfaces of all taxa were more or less densely covered by different types of trichomes forming an indumentum of variable texture and density. Metchalfe & Chalk (1979) determined that the family Boraginaceae had glandular and eglandular hairs. Similarly, there are glandular and mostly eglandular hairs on the epidermis of stem and leaf were determined in this study.

The anatomical analysis given in this work provides the first detailed description of the three examined taxa of *Anchusa*. The most of anatomical root properties in *Anchusa* taxa resemble in the general characteristics. However, in *A. pusilla* the pith are more extensive than those in the other taxa.

The analysis of the stem cross-section showed that the cortex is narrow. The presence of collenchyma especially in stem ridges was prominent. The cambium is not prominent in *A. azurea* var. *azurea* and *A. pusilla*, unlike *A. undulata* subsp. *hybrida*.

Our results showed that all of the examined taxa showed an isobilateral structure with abaxial photosynthetic tissue. Jodin (1903) and Selvi & Bigazzi (2001) determined that the majority of the *Anchusa* taxa had typical dorsiventral leaves, however the abaxial palisade tissue in *A. azurea* and a few others provided notable exceptions to this general rule. Metchalfe & Chalk (1950) reported that efficient control of water loss may be the cause for the prevalence of amphistomatous species in the Boragineae. Anomocytic stomata in the family Boraginaceae was previously described by Özörgücü (1991). In addition, Metchalfe & Chalk (1979) pointed out that there were both anisocytic and anomocytic stomata in this family. In our study, it was determined that all of the examined taxa had anomocytic stomata and amphistomatous leaves and the distribution of the stomata was homogeneous on both surfaces. However, in *A. undulata* subsp. *hybrida* the adaxial density was lower than the abaxial one. It was determined that in some species of very xeric habitats such as *A. crispa*, *A. cespitosa* stomatal density is higher in the adaxial rather than in the abaxial surface (Selvi & Bigazzi, 2001). The much greater potential of stomatal aperture of the leaves of the xerophytic Boragineae in comparison to the mesophytic ones in terms of size and total density of both surfaces is a clear adaptive response to water stress and light intensity (Ticha, 1982; Willmer & Fricker, 1996; Weyers & Lawson, 1997). On the other hand, phyletic and ecological causes may account for the lack of adaxial stomata in tropical species of *Cordia* L., and *Ehretia* P. Br., which are considered as ancestral members of the Boraginaceae (Metchalfe & Chalk, 1950; Nyawuame & Gill, 1990; Al- Shehbaz, 1991).

Numeric analysis showed that variation of floral characters appears to show little correlation with geographical or ecological factors. This is connected to its continuous ditribution area. The ratio of calyx lobe length to the calyx length was the most significant morphological character distinguishing *Anchusa* species (Table 6). This character previously reported as taxonomically useful character (Chamberlain, 1979). However, corolla tube length was not important as a taxonomic character even in statistical terms (Table 6).

Despite the large variation in floral and other taxonomic characters, the plants seem to belong to three species. PCA analysis were unsuccessful in seperating individuals into clearly recognizable groups, but we could be able to show that, *A. azurea* var. *azurea* was presented in two groups shown in circles (Group 2 and 3). Also, the two *Anchusa* taxa, *A. undulata* subsp. *hybrida* and *A. pusilla*, were represented in one group (left circle, Group 1).

Based on our investigations and observations on *Anchusa* species collected from Northern Turkey we could say, that further ecological, karyological, palynological and micromorphological studies are necessary to shed more light on the taxonomic position of *Anchusa* species.

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