

Anatomical investigations of the Turkish critically endangered species: *Achillea sivasica* Çelik et Akpulat (Asteraceae)

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Abstract – In this study, the root, stem, leaf midrib and leaf lamina anatomy and achene micromorphology of the Turkish critically endangered endemic *Achillea sivasica* were investigated for the first time. In this study, the root was found in late primary growth and in early secondary growth stage. It has a large cortex layer consisting of 12-16 cell rows beneath the periderm. Secretory ducts formed by 5-12 secretory cells embedded in the cortex and located near the vascular bundle were found at the root, which was in the early stage of secondary development. The stem was circular-pentagonal in cross-section. There was lamellar collenchyma beneath epidermis of pentagon corners, and cortex parenchyma between corners. Secretory ducts located near the phloem, between the cortex and endodermis on the interfascicular region, were also observed. An endodermis layer was evident and its cells have indentations and protrusions where they touch adjacent endodermis cells, which strengthens the connection between them. In addition, casparian strips were conspicuous in many endodermis cells. The leaf midrib area had a triangular cross section. There were secretory ducts, consisting of 4-5 secretory cells observed on both sides of the sclerenchymatous fibers that accompany the xylem. The leaf lamina was amphistomatic and stomata type was anomocytic. Mesophyll layer was equifacial. There was a large secretory duct and its diameter is bigger than the nearest main lamina vascular bundle. Achene shape of *A. sivasica* was lanceolate-oblong and its surface was ribbed and glabrous.

Keywords: *Achillea sivasica*, anatomy, Asteraceae, endemic, Turkey

Introduction

Asteraceae is the largest flowering plant family, comprising 23,000 species distributed in 13 subfamilies, 44 tribes and over 1600 genera worldwide (Funk et al. 2009, Panero et al. 2014). This family is distributed naturally on all continents outside of Antarctica, and its phylogenetic origin is thought to be in South America (Heywood 1978, Bremer 1994). In the flora of Turkey, the Asteraceae family is represented by 1209 species of which 447 species are endemic with an endemism ratio of 37% (Dogan et al. 2009). According to these numbers, Asteraceae is the largest plant family in Turkey, with the most endemic species.

Achillea L. is one of the youngest evolutionary genera of the Asteraceae family, with more than 100 species widely distributed throughout the world (Arabacı and Yıldız 2006, Goli et al. 2008). Turkey is one of the main diversity centers of the genus *Achillea*. In all, 47 species of *Achillea* are dis-

tributed and grow naturally in Turkey, and 24 of them are endemic for Turkey with a rate of endemism of 51% (Agar et al. 2015).

Anatomical studies on Asteraceae family have been carried out in the past (Metcalfe and Chalk 1950, Milan et al. 2006, Tekin and Meriç 2015). According to a literature survey, it was revealed that, although there are many pharmacognostical and phytochemical studies of taxa of the genus *Achillea*, there are very few anatomical studies. Grytsyk et al. (2016) studied the morpho-anatomy of four *Achillea* species (*A. millefolium* L., *A. stricta* Schleich., *A. carpatica* Bloski ex Dubovik and *A. distans* Wald. et Kit.) in the western region of Ukraine. Sulborska and Weryszko-Chmielewska (2006) studied the morphology, anatomy and ultrastructure of floral nectaries of *A. millefolium*. Akcin and Akcin (2010) studied the morphological and anatomical characteristics

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of two Turkish endemic *Achillea*; *A. phrygia* Boiss. et Bal. and *A. gypsicola* Hub.-Mor. Also, Akcin and Akcin (2014) studied the achene micromorphology of seven *Achillea* taxa including *A. biebersteinii* Afan., *A. coarctata* Poir., *A. grandifolia* Friv., *A. millefolium* ssp. *millefolium*, *A. millefolium* ssp. *pannonica* (Scheele) Hayek, *A. teretifolia* Willd. and *A. biserrata* Bieb. from Turkey.

Achillea sivasica Çelik et Akpulat was found as a new species in very limited area in Sivas province, Turkey (Çelik and Akpulat 2008). It is a local endemic, so Çelik and Akpulat (2008) suggested a threat category of “Critically Endangered (CR)” according to IUCN (2001) red list criteria for this species. No anatomical investigations have previously been carried out on *A. sivasica*, however, considering the medicinal importance of many *Achillea* species in Turkey; the chemical composition and phytochemicals, antioxidant, and antityrosinase activities of *A. sivasica* are recently being studied (Haliloglu et al. 2017, Özek et al. 2018). According to these studies *A. sivasica* is characterized by an extremely high percentage of the C18:3 fatty acid, and is a rich source of valuable volatile constituents such as β -pinene, 1,8-cineole camphor, and β -bisabolol. Haliloglu et al. (2017) reported that *A. sivasica* could be considered a natural source of active constituents for food supplements and therapeutic applications.

The aim of this study is to reveal the previously unknown anatomical features and fruit micromorphological features of *A. sivasica*, pharmacognosy studies of which have been carried out, as mentioned above. For this purpose, root, stem, leaf midrib area and leaf lamina cross sections of plant samples were taken. Photomicroscopic examinations were carried out and achene surface micrographs were examined using a scanning electron microscope.

Materials and methods

Study area

Achillea sivasica samples were collected from two districts of Sivas (Turkey) province. Sivas is located in Inner

Anatolia and is in the Iranian-Touranian phytogeographical area and has a continental climate. Localities where the plant samples were collected: Locality 1: B6 Sivas: Sivas to Ulaş district, Ziyarettepe, 39°33'08.7" N, 37°01'12.2" E, altitude 1441 m a.s.l., M. Tekin 1430, 11.05.2013; ibid. M. Tekin 1812, 06.06.2018 (This locality is also the *locus classicus*). Locality 2: B6 Sivas: Kangal to Gürün district, 8th km, calcareous area, 39°07'56.3" N, 37°13'48.0" E, altitude 1570 m a.s.l., M. Tekin 1252, 21.06.2012; ibid. M. Tekin 1484, 19.07.2013; ibid. M. Tekin 1836, 04.07.2019.

Sampling

Species identification was carried out by Dr. M. Tekin according to study of Çelik et Akpulat (2008) and dried plant samples were kept with collector name and number in Cumhuriyet University Faculty of Science Herbarium (CUFH) (Figs. 1, 2).

Ten plant samples which best represent the population were collected from each locality for anatomical studies. These samples were stored at +4 °C in 70% ethyl alcohol. Transverse sections were taken manually from the root, stem and leaf of *A. sivasica* using a razor. Root sections were taken from secondary roots. Stem sections were taken 15-20 cm above the ground and leaf sections were taken from the middle part. Also, superficial hand-made sections of the lower surface of the leaf lamina were taken with a razor. In order to determine pectin- or lignin-containing tissues, and thus to separate the tissues from each other, all sections were double stained with 1% Safranin (Sigma) and 1% Alcian Blue (Sigma) dyes in the ratio of 3/2 (Davis and Barnett 1997), and sections were kept about 5 min in the dye. Washing was performed in distilled water/glycerin 1:1 mixture after dyeing to remove excess paint from the sections. The stained sections were prepared with a glycerin-gelatin mixture (Brown 1960). Anatomical investigations on these sections were made using an Olympus BX22 light microscope. Micrographs were taken using an Olympus BX51 light microscope coupled with an Olympus DP70 digital camera. Anatomical measurements are based on at least 30 measurement

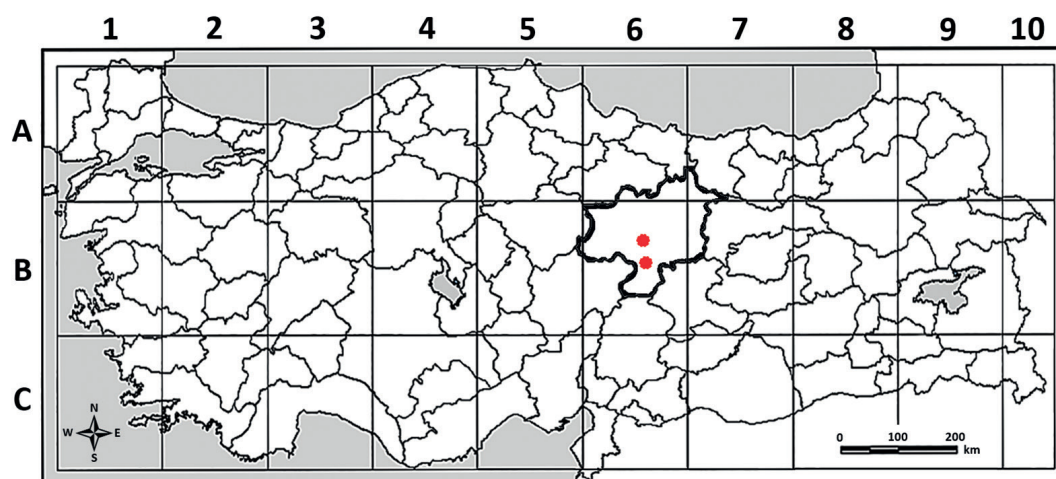


Fig. 1. Geographic distribution area of *Achillea sivasica* (●) in Sivas province in Turkey.

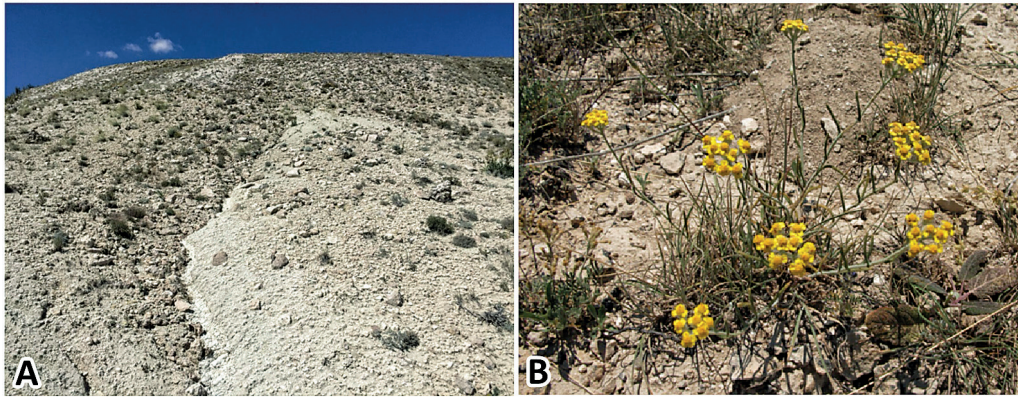


Fig. 2. Calcareous rocks are the natural habitat of *Achillea sivasica* (A) and the general appearance of the species in its habitat (B) Photos by M. Tekin.

results of each tissue or cell group from many sections obtained from plant samples from different locations. As a result of analysis of these measurements in SPSS package program, minimum, maximum, average values and standard deviation were determined. For scanning electron microscopy (SEM) analysis, fruit samples were transferred onto lead staples using double layer tape and then coated with gold. Micromorphological observations were made using the LEO 440 model SEM on the surface of the gold-coated staples and micrographs were taken at different magnifications. The nomenclature of all taxa follows IPNI (2020).

Results

Root anatomy

The root was of late primary and early secondary growth stage and had a circular cross-section (Fig. 3). The outermost layer, the periderm, consisted of phellem, phellogen and phelloderma, and all these layers were conspicuous. Phellem was only 1-3 layered on the surface of roots in the late stage

of primary development (younger root), and was multilayered on surface of roots in the early stage of secondary development (older root). Phellem and phellogen were single layered in both developmental types of root (Fig. 3). Under the periderma there was a very large layered cortex consisting of 12-16 parenchymatous cell rows. Cortex cells were oval and circular in the younger roots, whereas they were generally rectangular-oval and dorsiventrally pressed in the older roots. With the development, while young roots only have periclinal and anticlinal cell divisions along 6-7 cells rows close to the vascular bundle, in older roots, these divisions were seen in all cortex layer cell rows. In older roots, the presence of 5-7 secretory ducts formed by 5-12 unilayered secretory cells embedded in the cortex near the vascular bundle was found as an important difference compared to the younger roots (Fig. 3C, D). Most of the root volume was occupied by the cortex. The endodermis and pericycle consist of single cell row and they were prominent in older root but inconspicuous in younger root (Fig. 3B, D). In the center of the root, there was a tetrarch xylem and between its arms there was phloem which covering a small area at the corresponding locations. There was distinguishable cambium layer consist of 1-3 cell rows, between the phloem and the xylem in both younger and older root cross sections (Fig. 3, Tab. 1)

Stem anatomy

The stem had a circular-pentagonal shape in cross-section and was covered by a single-layered epidermis. Its cells generally were oval, circular, rarely squarish or rectangular with abundant non-glandular, elongated hairs (Fig. 4A, B, F). Beneath the epidermis, there were 7-10 cell rows of lamellar collenchyma at locations corresponding to the corners of the pentagonal cross-section. The cortex parenchyma was located under the epidermis between the corners of the pentagon (Fig. 4A, B). The cortex parenchyma consisted of 4-8 cells rows of oval and circular cells and occasionally contained intercellular spaces. Cortex parenchyma cells located close to the epidermis were usually small and had abundant chloroplasts, while those adjacent to the endodermis were larger and had fewer chloroplasts. Near the phloem, between the cortex and endodermis on the interfascicular region, se-

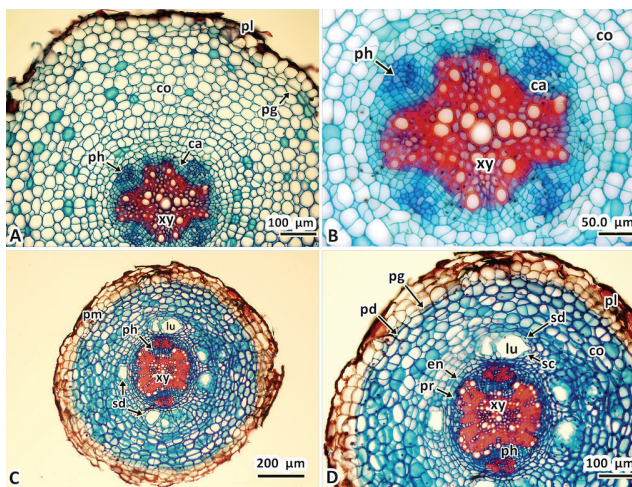
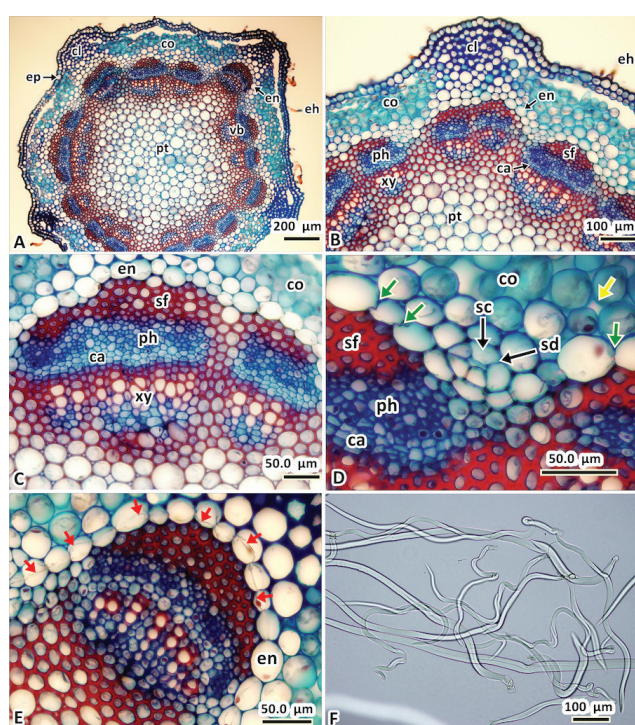


Fig. 3. Photomicrographs of root cross sections in the late stage of primary development (A-B) and early stage of secondary development (C-D) of *Achillea sivasica*. Abbreviations: ca – cambium, co – cortex, en – endodermis, lu – lumen, pd – phelloderma, pg – phellogen, ph – phloem, pl – phellem, pm – periderm, pr – pericycle, sc – secretory cell, sd – secretory duct, xy – xylem.

Tab. 1. Anatomical measurement results of the root, stem, leaf midrib area and leaf lamina of *Achillea sivasica* (n = 100, max – maximum, min – minimum, SD – standard deviation).

	Width/Diameter (μm)		Length (μm)	
	Min–Max	Mean \pm SD	Min–Max	Mean \pm SD
Root				
Cortex cells	9.45 – 38.65	25.6 \pm 8.26	11.59 – 52.50	33.05 \pm 11.70
Cambium cells	2.04 – 8.05	4.36 \pm 1.29	3.73 – 11.31	8.35 \pm 1.62
Trachea diameter	12.13 – 35.27	21.89 \pm 5.14	–	–
Stem				
Cuticle thickness	0.61 – 1.23	0.85 \pm 0.19	–	–
Cortex cells	13.50 – 40.45	24.04 \pm 7.81	14.02 – 44.09	26.91 \pm 8.62
Epidermis cells	8.51 – 32.81	20.31 \pm 5.82	16.52 – 39.75	25.66 \pm 5.46
Endodermis cell	14.23 – 36.18	24.89 \pm 5.48	19.44 – 43.84	32.28 \pm 6.96
Cambium cells	4.48 – 8.08	5.93 \pm 0.83	9.80 – 16.36	12.19 \pm 1.69
Pith cells	20.54 – 83.65	46.34 \pm 20.58	21.76 – 91.41	51.03 \pm 22.11
Trachea diameter	11.64 – 23.96	19.39 \pm 2.89	–	–
Leaf midrib area				
Cuticle thickness	0.65 – 1.07	0.86 \pm 0.09	–	–
Epidermis cells	9.27 – 41.36	21.69 \pm 8.49	19.96 – 51.66	31.43 \pm 7.54
Chlorenchyma cells	15.09 – 42.52	21.70 \pm 5.72	18.10 – 51.77	31.98 \pm 9.03
Non-photosynthetic parenchymatous cells	7.62 – 59.52	30.99 \pm 15.35	15.77 – 69.13	39.42 \pm 16.79
Trachea diameter	6.10 – 18.08	12.23 \pm 3.79	–	–
Leaf lamina				
Cuticle thickness of upper epidermis	0.52 – 1.23	0.76 \pm 0.20	–	–
Cuticle thickness of lower epidermis	0.60 – 1.33	0.97 \pm 0.19	–	–
Upper epidermis cell	12.16 – 37.72	25.59 \pm 6.83	21.76 – 41.04	32.87 \pm 4.77
Lower epidermis cell	19.28 – 47.33	30.14 \pm 7.74	25.52 – 55.98	37.22 \pm 6.54
Palisade parenchyma cell	13.12 – 24.06	19.73 \pm 3.29	32.21 – 80.33	54.73 \pm 12.52
Spongy parenchyma cell	11.66 – 28.97	20.51 \pm 3.73	16.43 – 39.84	27.34 \pm 5.29
Mesophyll thickness	170.26 – 302.60	248.12 \pm 38.81	–	–
Leaf lamina thickness	222.66 – 378.82	314.68 \pm 37.83	–	–

cretory ducts were observed (Fig. 4D). Beneath the cortex, there was a single cell row of the endodermis layer, consisting of oval cells that separate the cortex layer and vascular



bundles from each other. Endodermis cells have indentations and protrusions in the places where they touch adjacent endodermis cells, which strengthens the connection between them (Fig. 4D). In addition, a Casparian strip was clearly obvious in many endodermis cells (Fig. 4E). Many open collateral vascular bundles located below the endodermis were arranged more or less in a regular ring, which is typical for dicotyledons. Sclerenchymatous fibers are found in vascular bundles densely on the phloem. There were 3-7 cell rows of sclerenchymatous fibers layer on each vascular bundle and also these fibers occupied the area around the xylem, so that it fills the interfascicular area and forms a continuous internal ring throughout the stem. Underneath it, there were

Fig. 4. Photomicrographs of stem cross sections of *Achillea sivasica* (A-E) and detail of non-glandular hairs (F). General view of stem (A); detailed view of stem (B); detailed view of open collateral vascular bundle (C); detailed view of endodermis and secretory duct (green arrows indicated indentations and protrusions between adjacent endodermis cells and yellow arrows indicated intercellular area between cortex parenchyma cells) (D); detailed view of Casparian strips in endodermis (red arrows indicate Casparian strips in endodermis cells). Abbreviations: ca – cambium, cl – collenchyma, co – cortex, eh – epidermal non-glandular hair, en – endodermis, ep – epidermis, ph – phloem, pt – parenchymatous pith, sc – secretory cell, sd – secretory duct, sf – sclerenchymatous fibers, vb – vascular bundle, xy – xylem.

many cell rows of phloem. Between phloem and xylem there was cambium consisting of 2-3 cell rows that were dorsiventrally flattened (Fig. 4C). We observed only a fascicular cambium; no interfascicular cambium had been yet formed. The center of the stem is filled by pith parenchyma. Pith cells were mostly circular, hexagonal, sometimes oval, and there were wide intercellular spaces between them. There was no pith cavity in the center of the stem (Fig. 4, Tab. 1).

Leaf midrib area anatomy

The midrib area had a triangular cross section (Fig. 5A). It was covered by a single layered upper and lower epidermis composed of cells of a similar size, oval and squarish in shape. On the surface of both epidermises were found non-glandular trichoms. Beneath the epidermis, there were 2-6 cell layered chlorenchyma which are loosely aligned and have wide intercellular spaces. Below it, and especially around the midrib, there was a tightly aligned, non-photosynthetic ground parenchyma. Between the lower epidermis and the ground parenchyma, there were 2-3 cell layered collenchyma tissue (Fig. 5A, B). There were 5 vascular bundles in the cross section of each leaf midrib area. The main vascular bundle (midrib) was almost circular and was supported by sclerenchymatous fibers from upper and lower side. There were two secretory ducts, one on each side of the sclerenchymatous fibers accompanying xylem. The epithel consisted of 4-5 cells which are unilayered (Fig. 5A, C, Tab. 1).

Leaf lamina anatomy

The leaf lamina was surrounded by a single layered epidermis on the upper and lower sides in cross-section. Both epidermis cells were oval and rectangular, occasionally circular or squarish. There was a thick cuticle layer on the surface of both epidermises (Tab. 1). Stomata were observed on both sides of the lamina so the leaves belong to the amphistomatic type. The stomata were anomocytic and hydrophytic (Fig. 5D, E). Beneath upper and lower epidermis, there were 1-3 cell rows of palisade parenchyma, consisting of cylindrical shaped cells and large intercellular spaces. Between palisade parenchymas, there were 2-4 cell rows of spongy parenchyma the cells of which are generally oval and sometimes circular. According to the mesophyll layer arrangement, lamina was equifacial. The vascular bundles were embedded in the spongy parenchyma and surrounded by the

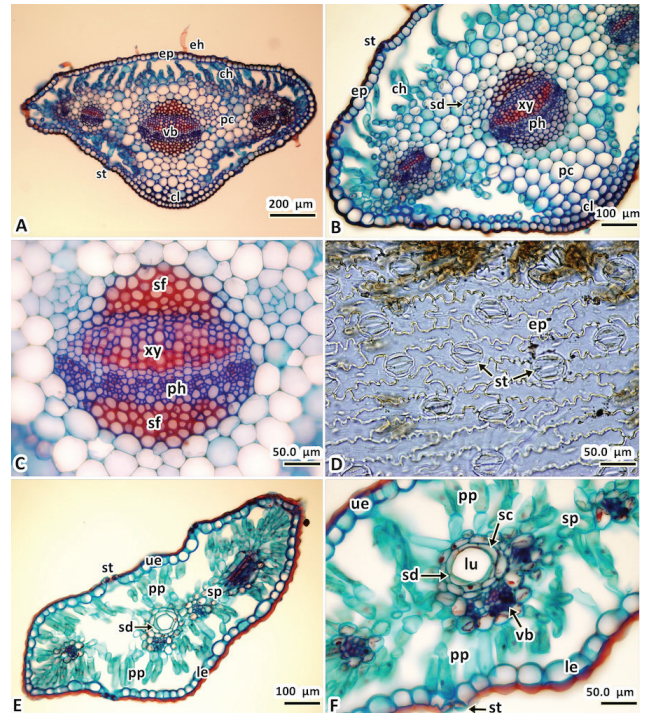


Fig. 5. Photomicrographs of *Achillea sivasica* leaf (A-F). Leaf midrib area in cross sections, general view (A), detailed view (B) and detailed view of midrib (C). Leaf lamina superficial section of lower epidermis (D). Cross sections of leaf lamina, general view (E) and detailed view (F). Abbreviations: cl – collenchyma, ch – chlorenchyma, eh – non-glandular hair, ep – epidermis, le – lower epidermis, lu – duct lumen, pc – parenchymatous cells, ph – phloem, pp – palisade parenchyma, sc – secretory cell, sd – secretory duct, sf – sclerenchymatous fibers, sp – spongy parenchyma, st – stoma, ue – upper epidermis, vb – vascular bundle, xy – xylem.

single cell row parenchymatous bundle sheath. Above the main vascular bundle of lamina, there was a secretory duct consisting of 4-5 secretory cells. Secretory duct was surrounded by the ground parenchyma cells and its diameter was bigger than the nearest main vascular bundle of lamina (Fig. 5D, F, Tab. 1).

Fruit micromorphology

Achene of *A. sivasica* were yellowish-brown with lanceolate-oblong shapes. Their surface is ribbed and glabrous. Slime cells were present on achene surface but they are inconspicuous (Fig. 6).

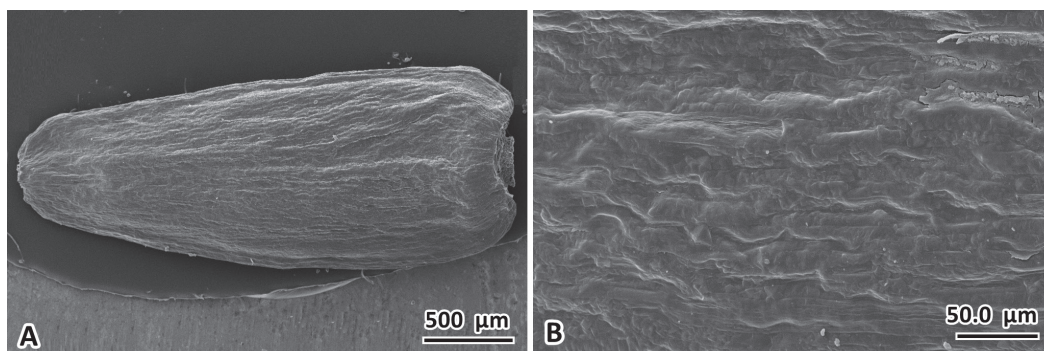


Fig. 6. Achene micrographs of *Achillea sivasica*. A – general appearance, B – detailed appearance of surface ornamentation.

Discussion

This study is the first report focused on investigations of anatomical and fruit micromorphological characters of the Turkish critically endangered *A. sivasica*. Metcalfe and Chalk (1950) determined the general anatomical characters of the family Asteraceae. Anatomical features of *A. sivasica* obtained as a result of the present study in general overlap with those given by the mentioned authors. These authors described the existence of secretory system elements in the roots of taxa belonging to the Asteraceae family. It was reported that presence of secretory elements is an important taxonomical character, and their limited distribution has a great diagnostic value (Metcalfe and Chalk 1950, Fahn 1979). We observed secretory ducts in the roots of *A. sivasica* in the early secondary growth phase, in stem and leaves whereas secretory ducts were not reported on any of the vegetative organs of *A. phrygia* and *A. gypsicola* (Akcin and Akcin 2010).

Achillea sivasica has both primary and secondary root structure according to growth stage, whereas the anatomical description of *A. phrygia* and *A. gypsicola* shows only a secondary root structure (Akcin and Akcin 2010). Tekin and Meriç (2015) studied the anatomical characters of six Turkish endemic *Tanacetum* L. (Asteraceae) taxa which are very similar taxonomically to *Achillea*. The roots of *Tanacetum* taxa studied by Tekin and Meriç (2015), were found only in the primary growth stage. However, in roots studied by us, primary xylem ridges were determined as tetrarch whereas they were found as triarch in *Tanacetum albipannosum* Hub. Mor. et Grierson, *T. densum* (Lab.) Schultz Bip. ssp. *sivasicum* Hub. Mor. et Grierson, *T. haussknechtii* (Bornm.) Grierson and *T. heterotomum* (Bornm.) Grierson, pentarch in *T. cappadocicum* (DC.) Schultz Bip. and hexarch

in *T. argenteum* (Lam.) Willd. ssp. *argenteum* according to mentioned study. Secretory ducts were observed in the early secondary growth roots of *A. sivasica* while no secretory ducts were found in the roots of any of the samples studied by Tekin and Meriç (2015) *Tanacetum* taxa. The existence of an endodermis on the vegetative organs was reported by some studies on Asteraceae taxa (Melo-De Pinna and Menezes 2002, Topsakal et al. 2019). Tekin and Meriç (2015) was found the presence of endodermis layer in root and stem of all studied *Tanacetum* taxa. According to study of Akcin and Akcin (2010) *A. phrygia* and *A. gypsicola* had endodermis in root and stem, which is the same result as in the present study. In *A. sivasica* there is typical endodermis in early secondary growth stage of root and primary growth stage of stem. In addition, the pith area was occupied by a primary xylem element in *A. sivasica*, which is the same as in *A. phrygia* and *A. gypsicola*. While most of the root volume in *A. sivasica* is filled by the cortex parenchyma, in *A. phrygia* and *A. gypsicola* it is filled by the secondary xylem (Akcin and Akcin 2010).

In the stem anatomy of *A. sivasica*, the presence of unilayered epidermis with abundant non-glandular hairs and lamellar collenchyma at the corners are features similar to those found in *A. phrygia* and *A. gypsicola*. In terms of anatomical features of the stem, a significant difference between *A. phrygia*, *A. gypsicola* and *A. sivasica* is epidermis cell size, the largest being found in *A. sivasica* (Tab. 2). *A. sivasica* has a single layered endodermis which is the boundary layer between cortex and stele as in *A. gypsicola*, but it is different from *A. phrygia* which has one- or two-layered endodermises (Akcin and Akcin 2010). Endodermis cells of *A. sivasica* have indentations and protrusions where they touch adjacent endodermis cells. We suggest it is important to increase the durability of the endodermis layer. In addition, the Caspar-

Tab. 2. Comparison of the anatomical measurement results of root, stem and leaf lamina obtained *A. sivasica* with present study and the results of *A. phrygia* and *A. gypsicola* studied by Akcin and Akcin (2010). Mean \pm standard deviation is presented.

	Width (μm)			Length (μm)		
	<i>A. sivasica</i> n = 100	<i>A. phrygia</i> –	<i>A. gypsicola</i> –	<i>A. sivasica</i> n=100	<i>A. phrygia</i> –	<i>A. gypsicola</i> –
Root						
Cortex cells	25.6 \pm 8.26	15.00 \pm 2.51	13.20 \pm 1.62	33.05 \pm 11.70	30.95 \pm 6.18	37.20 \pm 5.07
Trachea diameter	21.89 \pm 5.14	26.30 \pm 11.79	20.45 \pm 2.38	–	–	–
Stem						
Epidermis cells	20.31 \pm 5.82	9.00 \pm 1.24	14.05 \pm 2.36	25.66 \pm 5.46	11.90 \pm 1.66	17.90 \pm 2.84
Cortex cells	24.04 \pm 7.81	11.05 \pm 2.39	22.50 \pm 4.74	26.91 \pm 8.62	18.15 \pm 3.97	38.35 \pm 5.16
Endodermis cells	24.89 \pm 5.48	15.90 \pm 3.21	16.90 \pm 3.21	32.28 \pm 6.96	22.25 \pm 4.72	30.60 \pm 10.79
Pith cells	46.34 \pm 20.58	38.30 \pm 12.33	45.70 \pm 7.38	51.03 \pm 22.11	–	–
Trachea diameter	19.39 \pm 2.89	15.50 \pm 3.47	25.30 \pm 3.30	–	–	–
Leaf lamina						
Upper epidermis cells	25.59 \pm 6.83	9.35 \pm 1.18	10.25 \pm 2.20	32.87 \pm 4.77	15.05 \pm 2.56	22.30 \pm 5.58
Palisade parenchyma cells	19.73 \pm 3.29	11.20 \pm 3.06	11.45 \pm 2.27	54.73 \pm 12.52	31.50 \pm 6.14	25.50 \pm 4.53
Spongy parenchyma cells	20.51 \pm 3.73	10.20 \pm 2.25	12.75 \pm 3.60	27.34 \pm 5.29	11.45 \pm 2.91	17.45 \pm 2.06
Lower epidermis cells	30.14 \pm 7.74	21.15 \pm 6.84	12.90 \pm 3.73	37.22 \pm 6.54	22.80 \pm 7.08	22.45 \pm 5.82

ian strip which consists of hydrophobic substance to restrict apoplastic flow of water is clearly obvious in many of the endodermis cells of *A. sivasica*. There is no report on these endodermis features in *A. phrygia* and *A. gypsicola*. The stem of *A. sivasica* is in the primary growth stage and has no pith cavity in the center, whereas the stems of *A. phrygia* and *A. gypsicola* are in the secondary growth stage and contain a pith cavity in the center of the stem. This feature of the stem was reported by Tekin and Meriç (2015) only for *T. haussknechtii*, whereas the other five *Tanacetum* taxa studied (*T. albipannosum*, *T. densum* ssp. *sivasicum*, *T. heterotomum*, *T. cappadocicum* *T. argenteum* ssp. *argenteum*) had no pith cavity in the center of the stem. The fascicular cambium is very distinguishable in *A. sivasica* while there were no reports on the presence of cambium in *A. phrygia* and *A. gypsicola*. The general stem anatomy of *A. sivasica*, including the presence of unilayered epidermis, epidermal hairs, interrupted collenchyma beneath epidermis, single-layered endodermis is similar to that of the six *Tanacetum* taxa described by Tekin and Meriç (2015). The only difference is that some of these taxa have no distinguishable cambium, as was observed very clearly in *A. sivasica*.

According to Metcalfe and Chalk (1950), anatomical diversity in taxa of the family Asteraceae is commonly observed in the leaf structure. They reported that there were both anomocytic and anisocytic stomata types for representatives of Asteraceae. Stomata of *A. sivasica* were found as anomocytic type which is the same as with mentioned study. In the cross section of the *A. sivasica* leaf we found single layered upper and lower epidermis, parenchymatous bundle sheath and anomocytic stomata, which is similar to the general anatomy of *A. phrygia* and *A. gypsicola* leaves described by Akcin and Akcin (2010). But there is important difference between the epidermis cell sizes of the mentioned species. Both epidermis cell sizes of *A. sivasica* are significantly larger than those in *A. phrygia* and *A. gypsicola* (Tab. 2). Grytsyk et al. (2016) were compared anatomically and morphologically, different *Achillea* species. They reported *A. stricta* Schleich. ex. W.D.J. Koch, *A. distans* Waldst. et Kit. ex. Willd. and *A. carpatica* Blocki ex Dubovik have anomocytic type stomata which is the same result as in the present study. The most important difference in terms of leaf lamina anatomy is the presence of equifacial mesophyll in *A. sivasica*, while mesophyll was found as dorsiventral in *A. phrygia* and *A. gypsicola*. However, there are no glandular and non-glandular hairs on *A. sivasica* leaf lamina, as was described for *A. phrygia* and *A. gypsicola* leaves. In *A. sivasica* leaves the mesophyll consists of 1-3 cell rows palisade parenchyma and 2-4 cell rows spongy parenchyma, while in the leaf of *A. phrygia* and *A. gypsicola* palisade parenchyma was composed of 1-2 and single cell rows and spongy parenchyma composed of 6-8 and 4-5 cell rows, respectively. According to these results palisade and spongy parenchyma cell sizes of *A. sivasica* are distinctly larger than those in *A. phrygia* and *A. gypsicola*. In our study we found big secretory ducts near main vascular bundle of leaf lamina, which is similar with the results obtained for *Achillea* species by Grytsyk et al. (2016). In contrast, Akcin and

Akcin (2010) did not report the presence of secretory ducts in the leaf lamina of *A. phrygia* and *A. gypsicola*. In leaves of *A. sivasica* stomata were observed on both sides of lamina in almost equal number, whereas in *A. phrygia* and *A. gypsicola*, stomata were reported as densely present on the lower epidermis as compared to the upper epidermis (Akcin and Akcin 2010). In *A. sivasica* we found hydroptic type of stomata that were located higher than adjacent epiderma cells. There were no reports of this character of *A. phrygia* and *A. gypsicola* by Akcin and Akcin (2010). Tekin and Meriç (2015) stated that all studied *Tanacetum* taxa had mesomorphic stomata, located at the same level as the adjacent epidermal cells.

The anatomical and micromorphological features of the achene surface and its sculpturing have been studied using SEM in some Asteraceae genera, and these characters have been reported to provide additional information for evaluation regarding family classification (Zhu et al. 2006). Also, in flowering plants, the presence of slime cells on the surface of the fruit and slime envelope formation are known as diagnostic characters in several families, including the Asteraceae (Kreitschitz and Valles 2007, Inceer 2011). However, there are very restricted studies on achene micromorphology of the genus *Achillea* (Akcin and Akcin 2010, 2014). In those mentioned two studies, the authors described achene shape as oblong (*A. phrygia*, *A. biebersteinii*, *A. biserrata*, *A. coarctata*, *A. millefolium* ssp. *millefolium*, *A. millefolium* ssp. *pannonica*) oblong lanceolate (*A. grandifolia*, *A. teretifolia*) and ovoid or obovoid (*A. gypsicola*). In the present study, achene shape of *A. sivasica* were found to be oblong-lanceolate which is the same shape as *A. grandiflora* and one of the other Turkish endemics, *A. teretifolia*. Akcin and Akcin (2014) reported that achene colour of *A. biserrata* and *A. coarctata* is yellowish-brown. This feature is the same for *A. sivasica* which has yellowish-brown achene. There are some studies on fruit surface and slime cell features on *Matricaria* L. (Inceer 2011), *Tripleurospermum* Sch. Bip. (Inceer et al. 2012), and *Artemisia* L. taxa (Yakovleva et al. 2002, Kreitschitz and Valles 2007) of the Asteraceae family. In addition, Akcin and Akcin (2010, 2014) studied slime cell features on achene of some taxa of the genus *Achillea* in Turkey. Akcin and Akcin (2010) found slime (myxogenic) cells on achene surface of *A. phrygia* and *A. gypsicola*, but they did not give these cells' shapes because they were not obvious enough. Accordingly, in present study, the slime cells found on achene surface are not conspicuous. Akcin and Akcin (2014) reported slime cells of ladder-like columns in parallel to the long axis of the fruit found in *A. teretifolia* and *A. coarctata*.

As a conclusion, the present study is the first and the only comprehensive report on the anatomy and achene micromorphology of the recently found critically endangered Turkish endemic *A. sivasica*. This study is important for the taxonomy of the genus, in terms of identifying the secretory system elements as well as determination of the significant anatomical differences between *A. sivasica* and related *Achillea* taxa; they were found to have equifacial mesophyll layer, hydrophylic type stomata although it is a plant living in arid environment and having significant Casparian strip

on stem endodermis. We believe that the results of the present study will contribute to the studies on anatomy and micromorphology that will be carried out in the future aimed at determining the structural features of other *Achillea* taxa and investigating their place in the systematics.

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