

Full Length Research Paper

# Comparative anatomical studies on the two *Stachys* species (sect. *Eriostomum*, subsect. *Germanicae*) growing in Turkey

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In this study, comparative anatomical studies on the two *Stachys* species (sect. *Eriostomum*, subsect. *Germanicae*) in Turkey were carried out on the plants collected from their type localities. These species are *Stachys balansae* Boiss. & Kotschy and *Stachys carduchorum* (R. Bhattacharjee) Rech.f. which are morphologically very similar to each other. Anatomical properties of *Germanicae* subsection was reported for the first time. In anatomical studies, the root, stem, leaf and petioles have been examined under light microscope in details; microphotographs was taken and micro-anatomical measurements of cells and tissues was done and presented in table. In anatomical results, there is almost no marked difference in anatomical structures of the roots and stems cross sections belonging to the taxa, however, anatomical differences are clear in vascular bundles present in median veins of the cross sections taken from the leaves and petioles. Anatomic properties of the two species was determined to be similar to the anatomic properties of other species of the genus *Stachys*.

**Key words:** Anatomy, *Eriostomum*, Lamiaceae, *Stachys*, Turkey.

## INTRODUCTION

*Stachys*, with around 300 species worldwide, is one of the largest genera of Lamiaceae. The genus is concentrated in the warm temperate regions of the Mediterranean and South West Asia, with secondary centres in North and South America and Southern Africa (Bhattacharjee, 1980).

Seventy two species were reported in a revision that R. Bhattacharjee conducted for the Flora of Turkey. Later studies have increased the species number to 90 (115 taxa), 54 of which 47% are endemic to Turkey (Bhattacharjee, 1982; Davis et al., 1982; Sümbül, 1990; Gemici and Leblebici, 1998; Duman, 2000; Dinç and Doğan, 2006; İlçim et al., 2008; Daşkın et al., 2009; Akçiçek, 2010; Yıldırım, 2010; Yılmaz et al., 2010;

Dirmenci et al., 2011; Erdoğan et al., 2011; Akcicek et al., 2012).

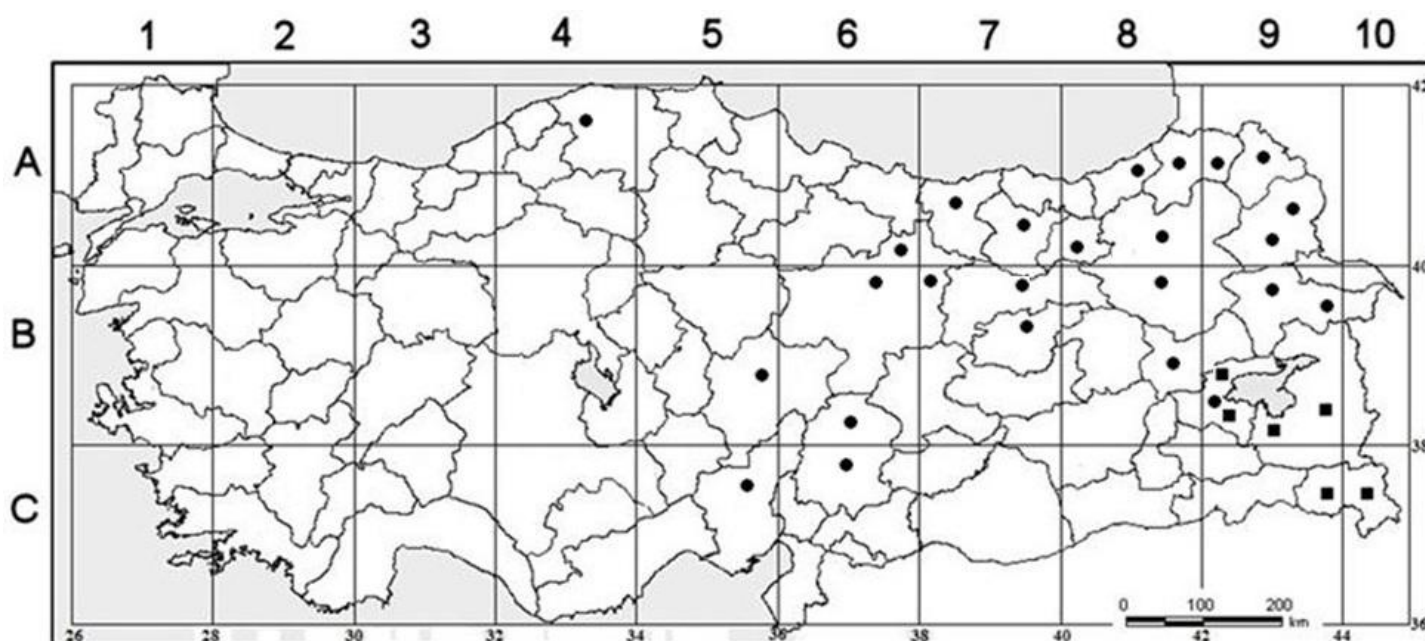
The section *Eriostomum* (Hoffmanns. & Link) Dumort. has 23 species (34 taxa) in Turkey. It is divided into three sub-sections, one of which is subsect. *Spectabiles* Bhattacharjee that is mainly distributed in oriental and Irano-Turanian regions. On the other hand, subsect. *Creticae* Bhattacharjee and subsect. *Germanicae* Bhattacharjee grow widely throughout Europe and Asia (Bhattacharjee, 1974, 1980; Falciani, 1997).

*Stachys* species are known in Anatolia as Ada çayı and Dağ çayı and used as sage and in popular medicines to treat genital tumours, sclerosis of the spleen, inflammatory tumours, coughs and ulcers (Potoğlu-Erkaya and Koyuncu, 2007). Teas prepared from the whole plant or leaves are used in phytotherapy, possessing sedative, antispasmodic, diuretic and emmenagogue activities (Jovanovic et al., 2008). In addition, its aerial parts are

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**Table 1.** Collection data of *S. balansae* and *S. carduchorum* from Turkey.

Species	Collection data and collector number
<i>S. balansae</i>	Turkey, <b>A8 Erzurum</b> : Aşkale-Kop Mountain, gravel field, 40° 00' 600"N, 040° 28' 800" E, 2450 m, 03.08.2008, B. Yıldız (BY 16851)
	Turkey, <b>A9 Artvin</b> : Ardanuç, Güleş village-Bilan plateau, 2150 m, 06.08.2008, B.Yıldız (BY 16870).
<i>S. carduchorum</i>	Turkey, <b>B9 Bitlis</b> : KarzDağ, above Kotum, 6500 ft., masy ground, 28.06.1954, P.H.Davis 22230 & Q.Polunin, (K)
	Turkey, <b>B9 Van</b> : Çatak, Kavuşşahap mountain, Karapet pass, between Çatak and Bahçesaray, limestone ravines, 38° 09' 154"N, 042° 52' 646" E, 2750 m, 24.07.2009, Akçiçek 5335, Dirmenci (ISTE)

**Figure 1.** Distribution map of *S. balansae* (●) and *S. carduchorum* (■) in Turkey.

(orally used as herbal tea in the treatment of various infections, asthmatic, antibacterial, antioxidant rheumatic and other inflammatory disorders (Couladis et al., 2003; Grujic-Jovanovic et al., 2004; Matkowski and Piotrowska 2006; Jovanovic et al., 2008; Ebrahimabadi et al., 2010). Anatomical studies on *Stachys* taxa are limited (Uysal, 2002, 2003; Potoğlu-Erkaya, 2007; Dinç and Öztürk, 2008). However, there has been no anatomical investigation species from section *Eriostomum* species as yet.

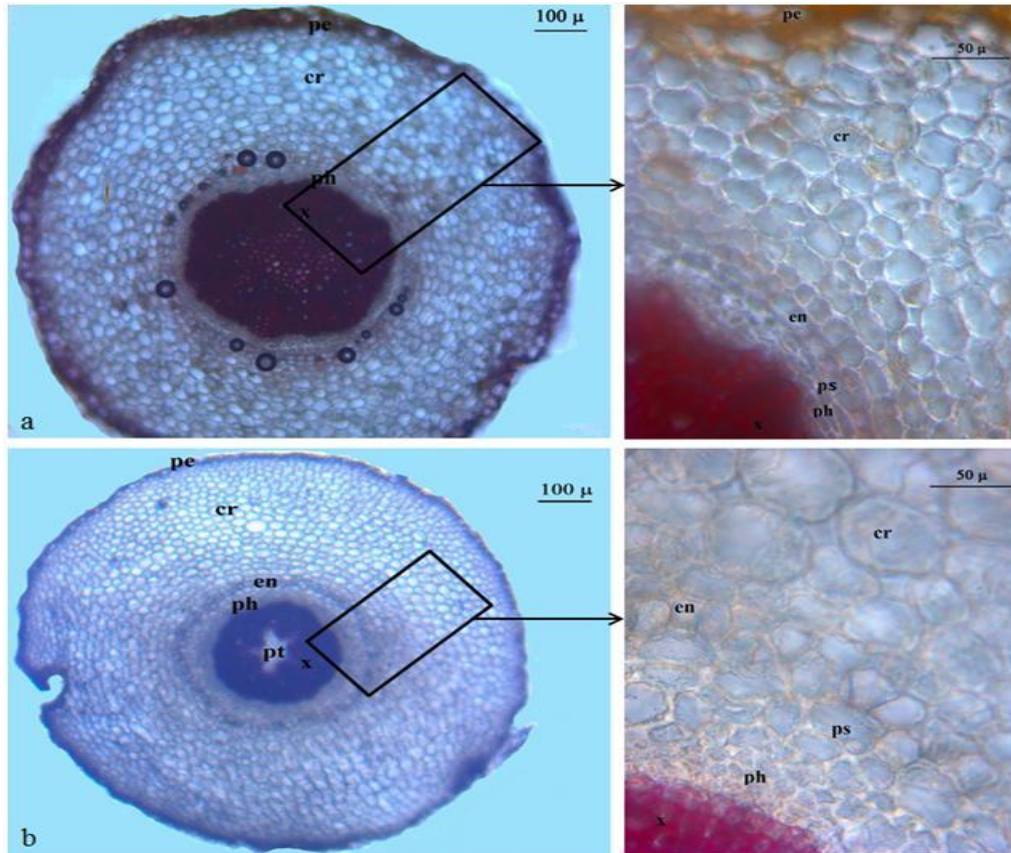
In a previous study, we investigated the morphological and ecological features of *S. balansae* and *S. carduchorum* (Erdoğan et al., 2011). Here, we report on the anatomy of these two species. In this study, the anatomical structure of root, stem, leaf and petiole in the

two species of *Eriostomum* section grown in Turkey is studied for the first time.

The aim of this paper was carried out in order to provide insight into anatomy two *Stachys* species. Moreover, a better understanding of systematics helps separate morphologically similar species from each other.

#### MATERIALS AND METHODS

Plant specimens of the 2 species were collected from their type localities (Table 1, Figure 1). Species collected was examined in the herbaria and were determined using the relevant literature (Ball, 1972; Bhattacharjee, 1982; Davis, 1982; Duman, 2000). The specimens are dried according to standard herbarium techniques and stored in the Herbarium of Necatibey Education Faculty,



**Figure 2.** Cross-section of the root of *S. balansae* (a) and *S. carduchorum* (b). pe, Periderm; cr, cortex; en, endodermis; ps, pericycle; ph, phloem; x, xylem; t, trache; pt, pith.

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Anatomical studies were carried on specimens kept in 70% alcohol. Cross-sections of roots, stems, petioles, leaves and surface sections of leaves were taken by hand. Then, sections were stained with Floroglucin and then mounted with Glycerin-Jelatin (Yakar-Tan, 1982). Measurements and photographs of anatomic sections were taken using Olympus CX21 binocular light microscope with an Olympus Camedia camera.

For images of stomata at Scanning Electron Microscope (SEM), leaves of the dried plant samples were mounted on standard SEM pin mount stubs using a double-sided conductive carbon tape. The samples were then coated with a thin layer of gold-palladium using a Cressington 108 Auto sputter coater to reduce charging. The coated samples were imaged using a Hitachi S-4800 Scanning Electron Microscope (SEM) at an accelerating voltage of 5 to 15 kV and working distances ranging from 20 to 22 mm at University of Toledo, Ohio, USA. The SEM micrographs were then analyzed.

## RESULTS

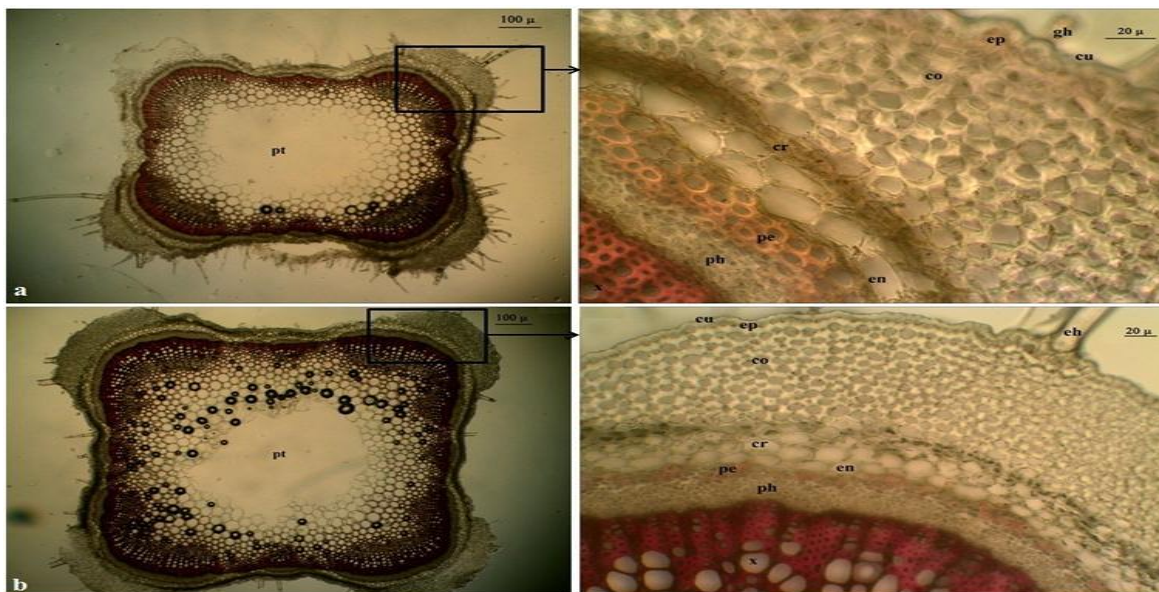
### Root

Cross-sections taken from the root of *S. balansae* and *S. carduchorum* have revealed that the periderm layer on the outermost surface of the root is consisting of phelloderm by 3 to 5 layers and phelloderm and phellogen are not

distinguishable. There is a multi-layered parenchymatic cortex (8 to 13 layers) under the periderm. Endodermis is one rowed and under of the endodermis pericycle is indistinguishable. Phloem is located under endodermis and 4 to 12 layered. Cambium cells are indistinguishable. The xylem is composed of vessels and tracheids. Pith rays comprise 1 to 3-rowed rectangular cells. Pith region of *S. balansae* consist of xylem elements whereas *S. carduchorum* consist of polygonal or orbicular parenchymatous cells (Figure 2).

### Stem

Cross-sections taken from the stem have exhibited a monolayer epidermis covered by a thin cuticle. The epidermis is composed of oval, cubic or rectangular cells. There are glandular and eglandular hairs (simple and unbranched, 1 to 4 cells) on epidermis. On young stems are seen diacytic type stomatas. Underneath the epidermis, multilayered collenchyma cells are located at the corners (7 to 13 layers) and there are 1 to 2 rows of chlorenchyma cells between them. Beneath this is situated the cortex which consists of 2 to 6 layers of oval, ovate or orbicular parenchymatous cells. Endodermis



**Figure 3.** Cross-section of the stem of *S. balansae* (a) and *S. carduchorum* (b). cu, cuticle; gh, glandular hair; eh, eglandular hair; ep, epidermis; co, collenchyma; cr, cortex parenchyma; en, endodermis; pe, pericycle; ph, phloem; x, xylem; pt, parenchymatic pith.

under the cortex is one rowed. Pericycle is indistinguishable. Sclerenchymatic cell clusters (1 to 4 layers) are situated in beneath endodermis. Phloem is 3 to 6-layered and consists of oval and rectangular cells. Cambium is indistinguishable. The xylem is composed of trachea and tracheids. Trachea is orbicular or ovoid while tracheids are polyhedral. Rays are 1 to 2 rowed. The pith is large and comprised of hexagonal or orbicular parenchymatic cells with intercellular spaces in the centre of stem (Figure 3).

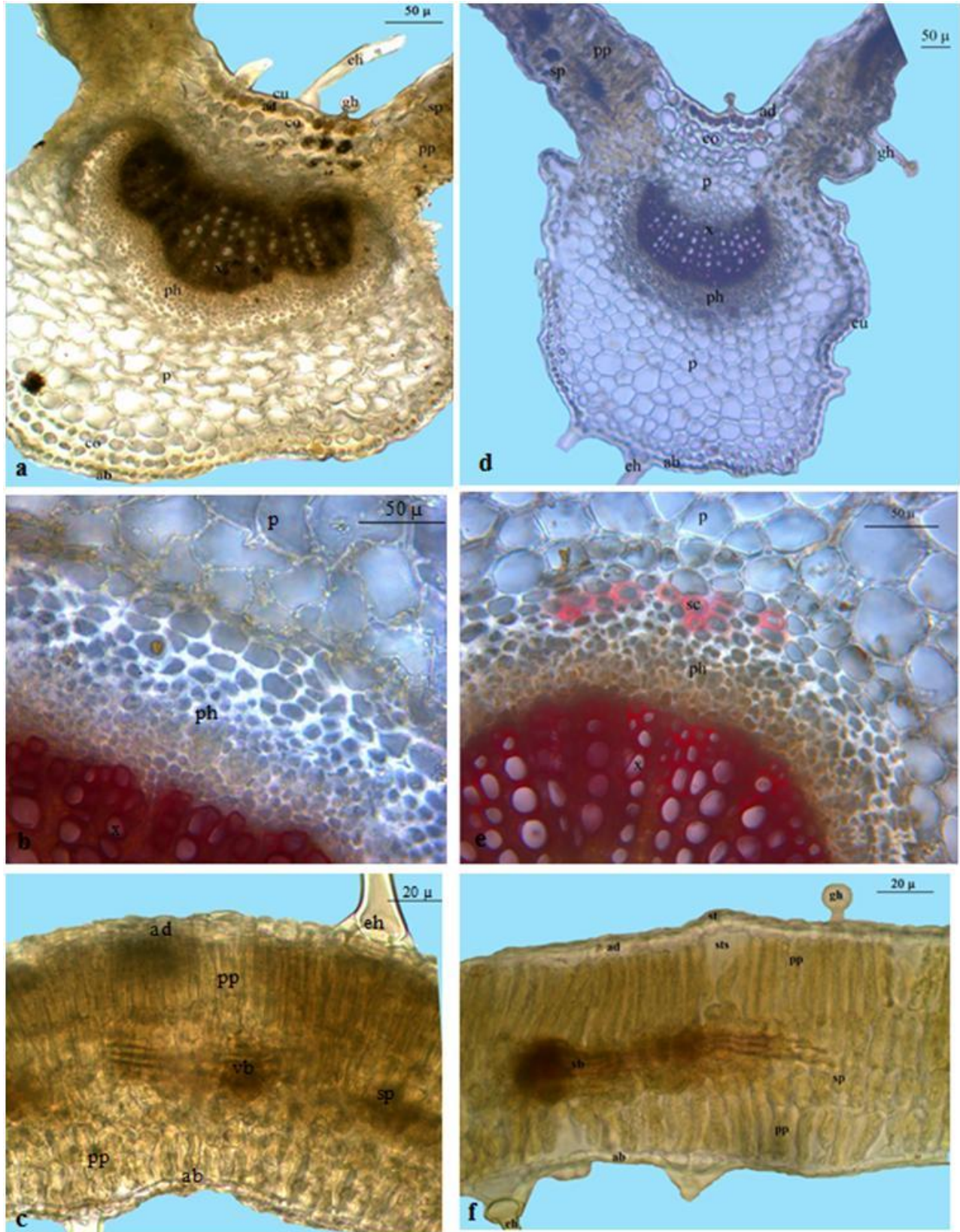
### Leaf

The epidermis is composed of a single layer cells and rectangular or oval cells. Adaxial epidermis is larger than lower and covered with a thick undulate cuticle. There are glandular and eglandular hairs (simple and unbranched, 1 to 2 cells) and on the surfaces of both epidermis. Stomata type is diacytic and occurs on the surfaces of both epidermis being more abundant on the lower surface. Stomata is located on the same level with epidermal cells. Leaf is isobilateral. Mesophyll occurs palisade 2 seriate beneath adaxial epidermis and 1 to 2 seriate above abaxial epidermis, at the median region of mesophyll is located spongy parenchyma that is 2 to 5-midrib that is located to beneath the epidermis is seen collenchyma. Parenchyma that is located beneath collenchymas is shaped polygonal and oval and 3 to 6 layered at adaxial surface and 3 to 9 layered at adaxial surface. Vascular bundle is surrounding by parenchyma cells. The xylem faces towards the adaxial surface while phloem faces the abaxial surface. The phloem tissue is 3

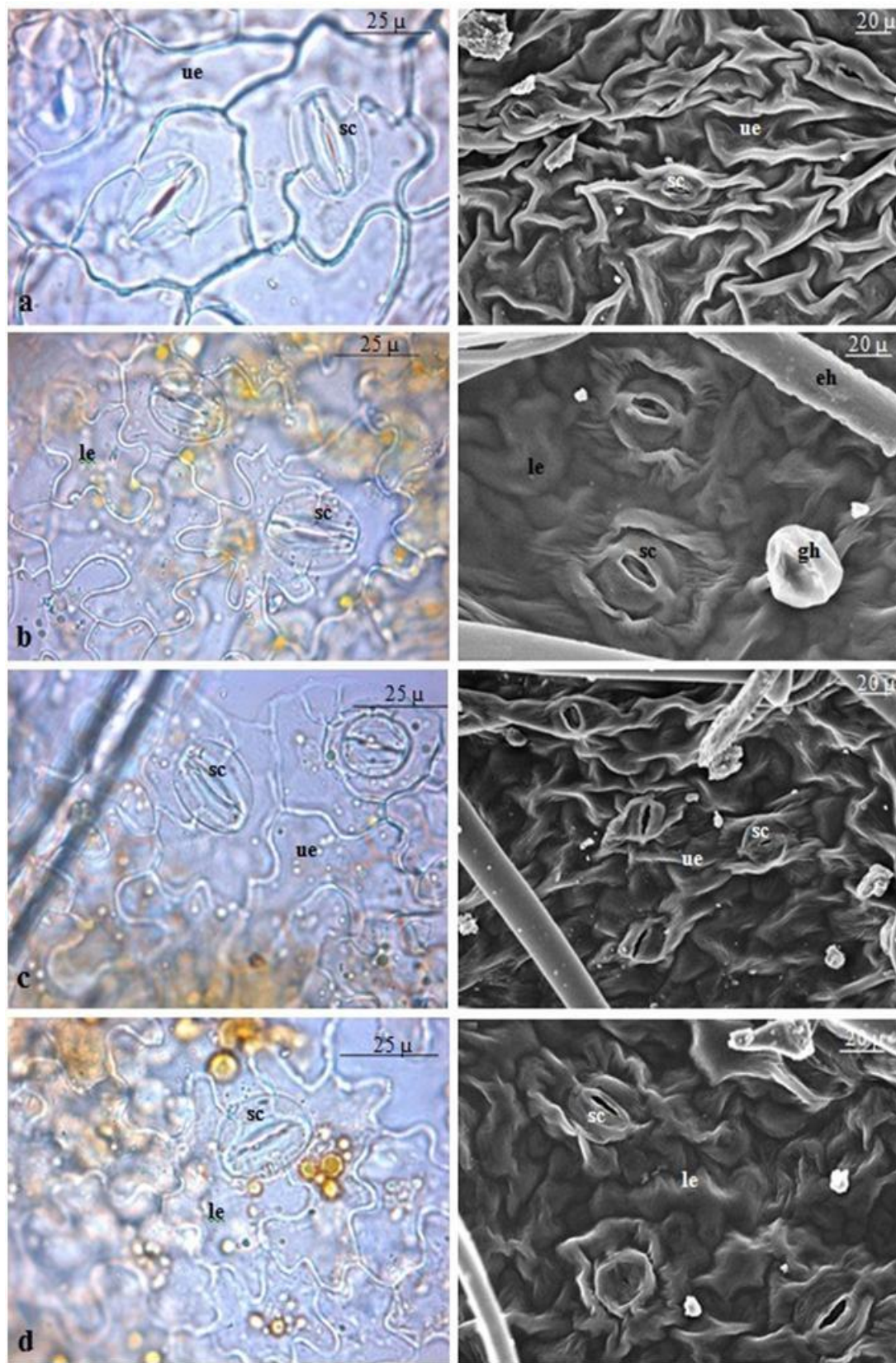
to 8 layered and surrounded by parenchymatic cells (Figure 5a and c). In the phloem cells of *S. balansae* are not seen sclerenchymatic cells while phloem cells of *S. carduchorum* are seen (1 to 2 rowed) (Figure 4).

### Petiole

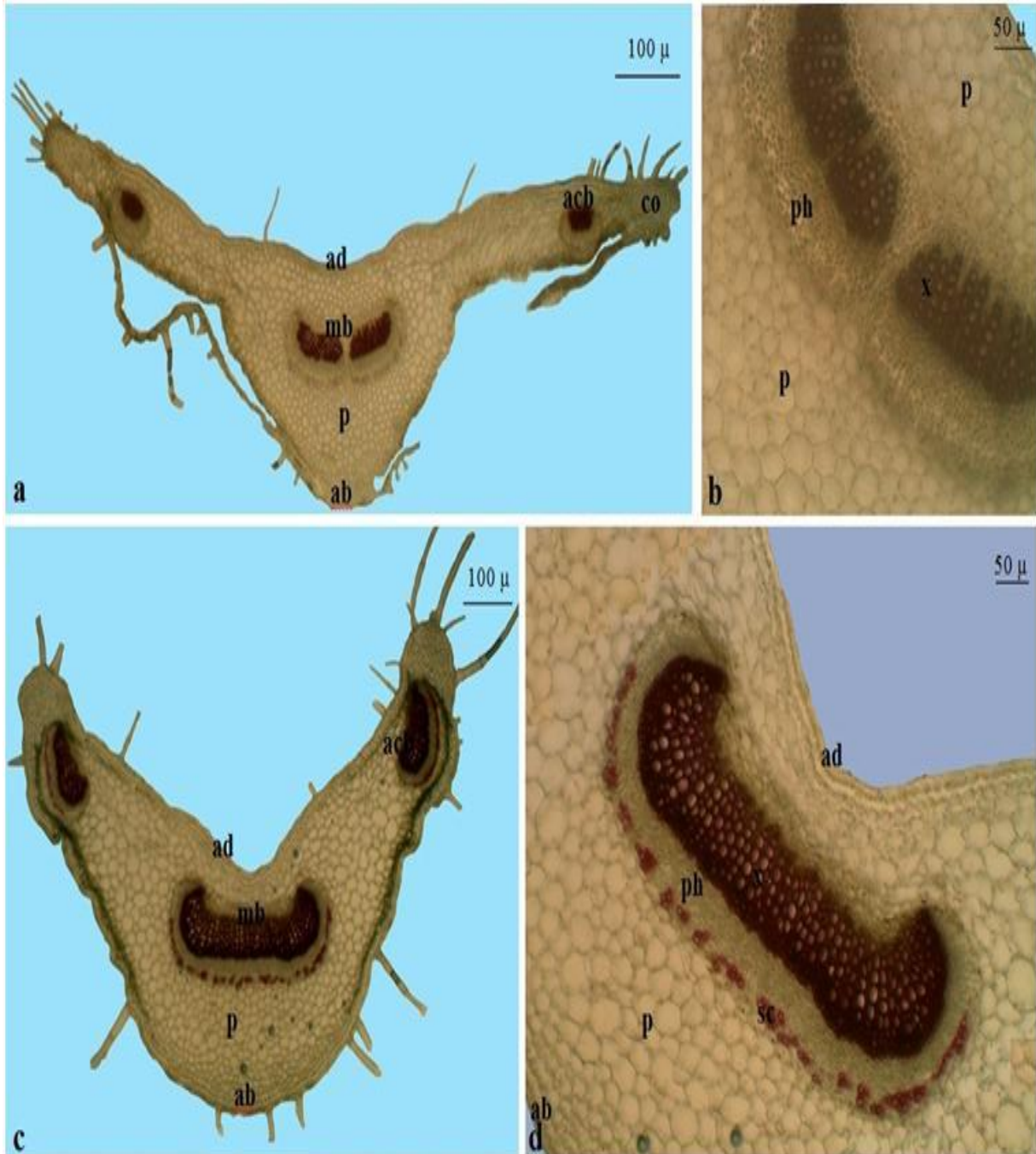
A cross section of the petioles shows that the adaxial surface is concave and slightly curved and the abaxial surface is convex and acute. There are eglandular (simple, 1 to 5 cells) and glandular hairs on both adaxial and abaxial surface. The epidermis is composed of oval and rectangular cells 1 layered. Collenchyma is situated under epidermis. It is 3 to 8 layered on abaxial surface, 1 to 2 layered underadaxial surface and 4 to 12 layered the ends of the petiolar wings. Parenchyma cells are hexagonal or oval shape. They are 2 to 5 layered on nearly flat and abaxial surface is broadly convex while adaxial surface of *S. carduchorum* deeply concave (V-shaped) and abaxial surface rounded-convex. 1 to 3 rowed in adaxial surface and 2 to 4 rowed in abaxial surface of collateral vascular bundle is located in the midrib region. seriate and composed of polygonal or rounded cells. The adaxial surface and 4 to 10 layered on abaxial surface. In the midrib, adaxial surface of concave-curved or deeply concave (V-shaped); abaxial surface is convex or rounded-convex. In the middle region of petiole, there are one (*S. carduchorum*) or two (*S. balansae*) collateral large vascular bundles crescent shape and one small subsidiary collateral bundle at the ends of petiolar wings. The phloem tissue is 3 to 8 layered and surrounded by



**Figure 4.** Cross-section of leaf lamina of *S. balansae* (a-c) and *S. carduchorum* (d-f). cu, cuticle; gh, glandular hair; eh, eglandular hair; ad, adaxial epidermis; ab, abaxial epidermis; pp, palisade parenchyma; sp, spongy parenchyma; vb, vascular bundle; st, stomata; sts, stomata space; co, collenchyma; p, parenchyma; sc, sclerenchyma; ph, phloem; x, xylem.



**Figure 5.** Surface sections of the leaves *S. balansae* (a, b) and *S. carduchorum* (c, d). Upper surface (a, c), lower surface (b, d). Ue, upper epidermis; le, lower epidermis; gh, glandular hair; eh, eglandular hair; sc, stoma cell.



**Figure 6.** Cross-section of the petiole of *S. balansae* (a, b) and *S. carduchorum* (c, d). ab, abaxial epidermis, ad, adaxial epidermis, co, collenchyma, p, parenchyma, mb, median bundle, acb, accessory bundle, sc, sclerenchyma, ph, phloem, x, xylem.

parenchymati cells. In the phloem cells of *S. balansae* are not seen sclerenchymatic layer while phloem cells of *S. carduchorum* are seen (1 to 2 layered). The xylem is composed trachea and tracheids. Xylem faces towards the adaxial surface while phloem faces the abaxial surface (Figure 6).

## DISCUSSION

In this study, two taxa belonging to the same subsection (*Germanicae*) and morphologically difficulty in distinguishing from each other is investigated. Moreover, anatomical properties of *Germanicae* subsection have

**Table 2.** Stem anatomical characters of *S. balansae* and *S. Carduchorum*.

Species	Stem anatomical characters					
	Collenchyma layers		Parenchyma layer	Sclerenchyma cluster	Phloem layer	Pith ray row
	Corner	Between of corner				
<i>S. balansae</i>	8-12	1-2	2-6	1-4	4-6	1-2
<i>S. carduchorum</i>	7-13	1-2	2-5	1-3	3-5	1-2

\*ad: adaxial surface (upper epidermis) \*ab: abaxial surface (lower epidermis)

**Table 3.** Leaf anatomical characters of *S. balansae* and *S. carduchorum*.

Species	Leaf anatomical characters									
	Mesophyll type	Mesophyll region			Median vascular bundle					
		Palisade		Spongy	Collenchyma		Parenchyma		Sclerenchyma	Phloem
		ad*	ab*		ad*	ab*	ad	ab		
<i>S. balansae</i>	Isobilateral	2	1-2	2-5	1-3	1-4	3-8	3-6	-	3-6
<i>S. carduchorum</i>	Isobilateral	2	1-2	3-4	1-3	2-4	3-7	3-5	1-2	3-8

\*ad, Adaxial surface (upper epidermis); \*ab, abaxial surface (lower epidermis).

**Table 4.** Petiol anatomical characters of *S. balansae* and *S. carduchorum*.

Species	Petiol anatomical characters								
	Number of vascular bundle			Median Region					
	Median region	Wings of petiole	of	Collenchyma		Parenchyma		Sclerenchyma	Phloem
				ad*	ab*	ad	ab		
<i>S. balansae</i>	1	1		1-2	4-9	2-5	1-2	2-5	3-5
<i>S. carduchorum</i>	2	1		1-2	3-5	7-12	4-7	2-5	3-5

\*ad, Adaxial surface (upper epidermis); \*ab, abaxial surface (lower epidermis).

been reported for the first time. Our anatomical results are similar to Metcalfe and Chalk (1950) and Salmaki et al. (2011). The results obtained from anatomical investigation carried out on two species were generally similar. No important differences observed in root and stem anatomical features of *S. balansae* and *S. carduchorum*. However, Pithregion (root) of *S. balansae* consist of xylem elements while *S. carduchorum* consist of polygonal or orbicular parenchymatous cells.

Of the anatomy of leaf is appeared two significant differences. The first; in the midrib, adaxial surface of *S. balansae* is nearly flat and abaxial surface is broadly convex while adaxial surface of *S. carduchorum* deeply concave (V-shaped) and abaxial surface rounded-convex. The second; in the phloem cells of *S. balansae* are not seen sclerenchymatic cells while phloem cells of *S. carduchorum* are seen.

Of the anatomy of petiole is appeared significant difference. In the midrib, adaxial surface of *S. balansae* is slightly concave-curved and abaxial surface is convex

while adaxial surface of *S. carduchorum* deeply concave (V-shaped) and abaxial surface rounded-convex. Besides, large vascular bundles in the middle region of petiole are one bundle of *S. carduchorum* and two bundles of *S. balansae*. Moreover; in the phloem, cells of *S. balansae* are not seen sclerenchymatic layer while phloem cells of *S. carduchorum* are seen.

Comparative anatomical characters of stem, leaf and petiole are given (Table 2 to 4). In addition to comparative micro-anatomical measurements, various tissues of *S. balansae* and *S. carduchorum* are presented in Table 5.

Selected micrographs of common indumentum types of stem, leaf and petiole of *S. balansae* and *S. carduchorum*, are presented in Table 6 and Figure 7. Two basic types of trichomes can be distinguished: Glandular and non-glandular trichomes. Nonglandular trichomes are unbranched and unicellular (Figure 7g and j) or multicellular (Figure 7f). Glandular trichomes are two types: Capitulate and peltate. Capitulate trichomes consist of short stalked (single cell base, short single cell neck, and



**Table 5.** Comparative micro-anatomical measurements various tissues of *S. balansae* and *S. carduchorum*.

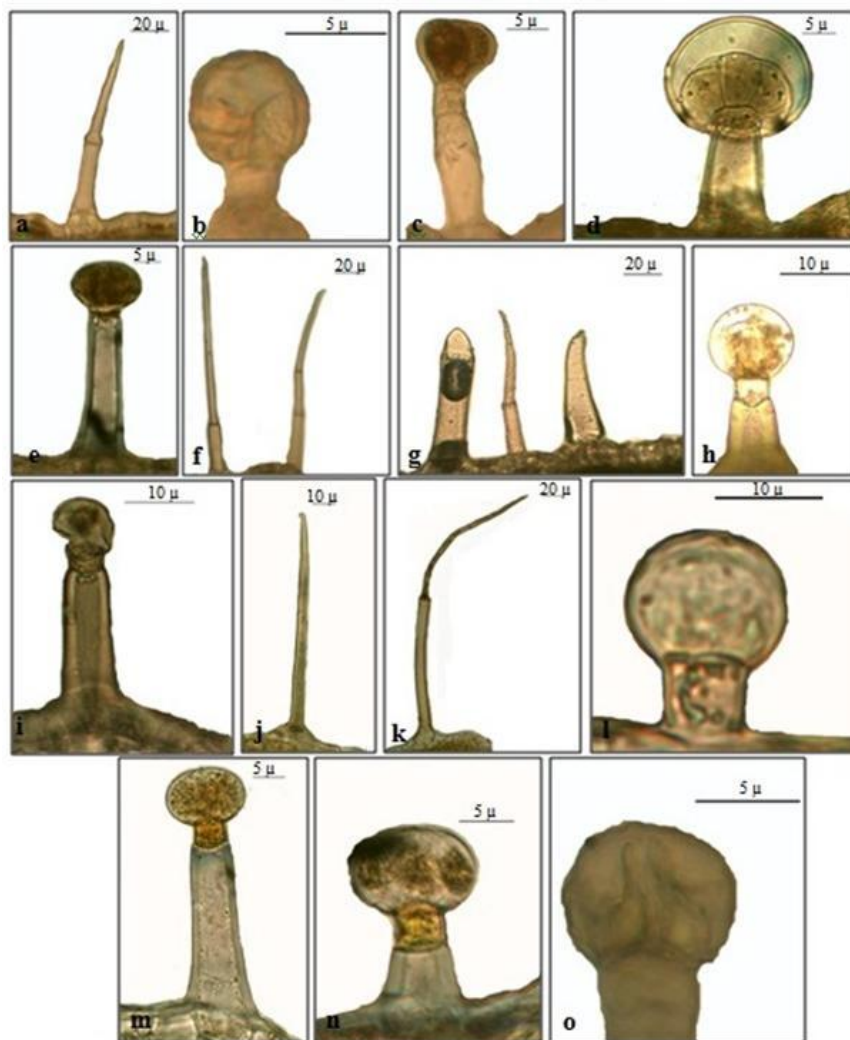
Tissues	<i>S. balansae</i>				<i>S. carduchorum</i>			
	Width ( $\mu\text{m}$ )		Length ( $\mu\text{m}$ )		Widths ( $\mu\text{m}$ )		Length ( $\mu\text{m}$ )	
	Min	Max	Min	Max	Min	Max	Min	Max
<b>Root</b>								
Peridermis	19	48	-	-	28	69	-	-
Parenchyma layers	184	261	-	-	160	250	-	-
Parenchyma cells	12	45	16	42	16	38	17.5	32.6
Endodermis layers	10	14	-	-	8	12	-	-
Phloem layers	13	45	-	-	21	42	-	-
Xylem layers	140	200	-	-	170	220	-	-
Trache cells	7	15	8	17	5	21	4	23
<b>Stem</b>								
Epidermis cell	5	12	5	14	7	16	6	14
Parenchyma layers	50	90	-	-	47	85	-	-
Endodermis layers	13	43	-	-	13	30	-	-
Phloem cells	13	18	10	17	11	19	10	18
Trache cells	4	47	6	24	5.7	22.4	7.3	16.8
Pith cells	21	97	18	102	16	89	15	95
<b>Leaf</b>								
Cuticle	0.8	2.5	-	-	0.5	2	-	-
Adaxial epidermis cells	7	13	10	22	9	20	8	23
Abaxial epidermis cells	4	11	9	18	8	18	9	21
Mesophyll layers	80	110	-	-	75	120	-	-
Palisade cells parenchyma	3	8	13	38	3.3	9.5	5	39
Spongy cells cellparenchyma	6.4	15.06	9.5	14.6	6	15	9	14
<b>Petiole</b>								
Adaxial cells	13.8	28.1	13.8	22.8	11	22	10	21
Abaxial cells	11.1	29.4	12.2	26.5	10	31	10	33
Parenchyma cells	14.2	30.6	10.2	24.9	14.2	30.6	10.2	24.9
Trache cells	3	12	5	13	8	19	6	17

**Table 6.** Characterisation of the trichome types of *S. balansae* and *S. carduchorum*.

Trichomes	Type	Description	<i>S. balansae</i>	<i>S. carduchorum</i>	Tissue
<b>Non glandular trichomes</b>	A	Acicular or curved, unbranched with one or more cells, mostly having one to five cells, arranged in a single row and having no cuticle with micropapillae	+	+	Stem
			+	+	Leaf
			+	+	Petiole
	B1	Peltate, 1 short stalk cell, 1 short neck cell and enlarged with 40 to 60 $\mu\text{m}$ ahead of 4 to 8 secretory cells	-	+	Stem
			+	+	Leaf
			-	-	Petiole
<b>Glandular trichomes</b>	B2	Peltate; Longer 1 stalk cell, 1 short neck cell and enlarged with 40 to 60 $\mu\text{m}$ ahead of 4 to 8 secretory cells	-	+	Stem
			+	+	Leaf
			-	-	Petiole
	C	Capitate; consisted of a short unicellular stalk and a globose head cell (20 to 30 $\mu\text{m}$ )	+	+	Stem
			+	+	Leaf

Table 6. Contd.

		+	+	Petiole
D	Capitate; 1 stalk of 2 to 5 cell, a short neck cell and a glandular head.	+	-	Stem
		-	-	Leaf
		-	+	Petiole



**Figure 7.** Trichome morphologies stem, leaf and petiole of *S. balansae* (a-e) and *S. carduchorum* (f-o). Stem (a-b; f-i), leaf (c-d; j-m), petiole (e; n-o).

oval one or two cell head) (Figure 7b, l and o) and long stalked trichom (extended two cell base, single cell neck, and oval two cell head (Figure 7e and i). Peltate trichomes consist of single cell base (short or long), single cell neck and six-ten cell head (Figure 7c, d, m and n)

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