

## COMPARISON OF FOLIAR ANATOMY OF *SCORZONERA* L. (ASTERACEAE) TAXA FROM NORTH EAST ANATOLIA

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### Abstract

In the present study, the leaves of 18 *Scorzonera* L. (Asteraceae) taxa were studied by LM in order to assess anatomical variations that may serve as distinguishing characters and to evaluate their significance for the genus by numerical analysis. All the investigated species can be divided into distinct groups according to mesophyll and midrib structure. Firstly they can be divided in two groups based on presence/absence of cavity on midrib. Secondly two main vascular bundle types can be identified in transverse sections according to the presence or absence of secretory cells in phloem. Principal component analysis (PCA) showed that width of the palisade tissue beneath the upper epidermis, rate of spongy to the mesophyll (w/w), average number of epidermal cells for both sides and average number of stomata on upper side are the most important characters in explaining the total variations.

### Introduction

*Scorzonera* L. s.l. (Asteraceae) is the largest genera with 175 taxa in the tribe Lactuceae (Bremer, 1994). It is widely spread in arid regions of Eurasia and Africa (Nazarova, 1997). This large genus consists of several closely related species (Chamberlain, 1975). One of the major taxonomic difficulties of the genus is the enormous morphological variation (Bremer 1994) and this is not investigated well enough by taxonomists (Nazarova, 1997). In recent years, karyological (Nazarova, 1997; Guardia & Blanca, 1987), ethno botanical (Rivera *et al.*, 2006; Ertuğ, 2000), chemical (Zidorn *et al.*, 2003; Magiatis *et al.*, 2001), genetic (D'amato, 2000), phylogenetic studies (Mavrodiev *et al.*, 2004; Bremer, 1994) have been carried out in *Scorzonera*. Many species belonging to this genus have been used as folk remedies and vegetables (Rangahau, 2001).

There is no comprehensive anatomical study up to now on Turkish *Scorzonera* species but there are some studies related to phenetic systematic (Duran, 2002; Makbul, 2006a). Lersten & Curtis (1997) reported that leaf characters are more indicative for systematic classification than any other anatomical characters. Besides, Lersten & Curtis (2001) indicated that anatomical studies support to solve the systematic problems. Metcalfe & Chalk (1950) reported the general anatomical properties of Asteraceae including a few details on the genus *Scorzonera*. All these cited studies supply additional information for solve the systematic problems on the genus, but they are not enough.

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*Scorzonera* is represented by 49 species in Turkey (Hamzaoglu *et al.*, 2010) but most of them are not distributed in NE Anatolia. Although anatomical properties are very important characters in the *Scorzonera*, there is no sufficient data related to the Turkish species. The purpose of the present study which is a part of PhD Thesis of the first author, is to investigate the leaf anatomical properties of *Scorzonera* taxa distributed in NE Anatolia and determine their systematic importance by means of numerical methods.

## Materials and Methods

Plants were collected from North East Anatolia in the year of 2003 and 2006. The collection data for the examined specimens are given in Table 1. Specimens were dried according to standard herbarium techniques and stored in the Herbarium of Karadeniz Technical University, Department of Biology (KTUB).

The materials for anatomical study were fixed in FAA (Formaldehyde: Acetic Acid: Alcohol) for 24 hours and then preserved in 70 alcohols %. All observations were performed on transverse and surface sections of well developed leaves taken by hand. All sections were stained with hematoxylen for 30 minutes and mounted with glycerin-gelatin in order to get permanent slides (Vardar, 1987). Well-staining sections were photographed with Olympus BX51 from permanent slides. All measurements and observations were made three or four times.

Twenty six characters related to leaf anatomy seen in Table 2 were assessed by numerical analysis. Twenty two were quantitative including linear measurements, ratios of linear measurements and numbers. The remaining 4 characters were qualitative, each divided into two discrete categories. Two multivariate analyses were performed by using SYN-TAX PC 5.0 (Podani, 1993): cluster analysis (CA) and principal components analysis (PCA). For the CA, a pair-wise matrix of resemblance values was calculated from raw standardized data matrix, using Gower's as the coefficient of resemblance that is designed for mixed data sets (Sneath & Sokal, 1973). For PCA, the raw data were used to create a correlation matrix and two eigenvectors were extracted, providing two axes onto which the raw data were projected to give a two-dimensional plot of the taxa and characters. During PCA analysis, characters explained very low variances accounted by each component were removed from the original data sets and analysis was performed without them once again. So only the results of the characters; X<sub>9</sub>, X<sub>13</sub>, X<sub>15</sub>, X<sub>19</sub> and X<sub>20</sub> (Table 3) explained most of the variance has been given in the present paper.

**Anatomical results:** Foliar anatomic features of the examined taxa based on transverse sections of midrib and lamina and surface preparation of the lamina are in the following order. All the detailed measurements related to leaves are given in the appendix.

### *Scorzonera laciniata* L. subsp. *laciniata* (Figs. 1a-e)

Midrib having a large cavity is pentagonal in shape and consists of 5 main collateral bundles at corners and numerous small bundles among the big ones. Collenchyma is 6 or 8 layered and only located at the sides between the lower epidermis and big bundles. At the same time, midrib contains two layers of palisade parenchyma arranged among the bundles. Xylem (200-230 µm) is larger than the phloem (100-130 µm) having a monolayer of secretory cell (Figs. 1a-b). Mesophyll (280-300 µm) consists of 2 layers of palisade adjacent to both epidermises and 2-3 layers of spongy (Fig. 1c). Ecvifacial leaves have anomocytic stomata on both sides (Figs. 1d-e).

**Table 1. Localities information of the examined taxa.**

No	Species	Locality
1.	<i>S. laciniata</i> L. subsp. <i>laciniata</i>	Artvin: Yusufeli-Yokuşlu köyü, 815m, Makbul 074, KTUB
2.	<i>S. cana</i> (C.A. Mey.) Hoffm. var. <i>jacquiniana</i> (W. Koch) Chamb.	Trabzon: Araklı-Dağbaşı, 1850m, Makbul 054, KTUB
3.	<i>S. cana</i> (C.A. Meyer) Hoffm. var. <i>cana</i>	Rize: Cimil yaylası, 2300m, Makbul 057, KTUB
4.	<i>S. cana</i> (C.A. Meyer) Hoffm. var. <i>alpina</i> (Boiss.) Chamb.	Rize: Ovit yaylası, 2400 m, Makbul 029, KTUB
5.	<i>S. armeniaca</i> (Boiss. & Huet.) Boiss.	Bayburt: Bayburt kalesi, 1650m, Makbul 059, KTUB
6.	<i>S. suberosa</i> C. Koch	Bayburt: Çerçi köyü, 1700 m, Makbul 069, KTUB
7.	<i>S. mollis</i> subsp. <i>mollis</i> Bieb.	Giresun: Fındıkbeli geçidi, 1730m, Makbul 080, KTUB
8.	<i>S. mollis</i> subsp. <i>szowitzii</i> (DC) Chamb.	Gümüşhane: Tersun dağı, 2000m, Makbul 064, KTUB
9.	<i>S. inaequiscapa</i> Boiss.	Giresun: Alucra-Şiran, 15. km, 1670m, Makbul 079, KTUB
10.	<i>S. incisa</i> DC.	Bayburt: Kop dağı, 2150m, Makbul 085, KTUB
11.	<i>S. eriophora</i> DC.	Gümüşhane: Moğoldas dağı, 1650m, Makbul 044, KTUB
12.	<i>S. cinerea</i> Boiss.	Bayburt: Kop dağı, 2150m, Makbul 087, KTUB
13.	<i>S. seidlitzii</i> Boiss.	Artvin: Şavşat, Sahara Mezrası, 2150m, Makbul 022, KTUB
14.	<i>S. sericea</i> DC.	Bayburt: Kop dağı, 2450m, Makbul 089, KTUB
15.	<i>S. pseudolanata</i> Grossh.	Bayburt: Köse, 1650m, Makbul 040, KTUB
16.	<i>S. latifolia</i> (Fish. & Mey.) DC.	Bayburt: Kop dağı, 2160m, Makbul 094, KTUB
17.	<i>S. sosnowskyi</i> Lipschitz.	Bayburt: Kop dağı, 2150m, Makbul 086, KTUB
18.	<i>S. tomentosa</i> L.	Giresun: Alucra, 1400m, Makbul 012, KTUB

**Table 2. List of characters used in numerical analysis.**

Symbol	Characters
X <sub>1</sub>	Average row number of collenchyma on midrib (number)
X <sub>2</sub>	Width of phloem / width of xylem (µm/µm)
X <sub>3</sub>	Average row number of trachea (number)
X <sub>4</sub>	Diameter of tracheas (µm)
X <sub>5</sub>	Cavity on midrib, absent:0; present:1
X <sub>6</sub>	Palisade parenchyma along midrib: uncontinuous:0; continuous: 1
X <sub>7</sub>	Average row number of palisade cells beneath the upper epidermis (number)
X <sub>8</sub>	Average row number of palisade cells beneath the lower epidermis (number)
X <sub>9</sub>	Width of the palisade tissue beneath the upper epidermis (µm)
X <sub>10</sub>	Width of the palisade tissue beneath the lower epidermis (µm)
X <sub>11</sub>	Length of the palisade cells (µm)
X <sub>12</sub>	Average row number of spongy cells (number)
X <sub>13</sub>	Width of spongy tissue / Width of mesophyll tissue (µm/ µm)
X <sub>14</sub>	Average number of stomata on lower surface (mm <sup>2</sup> )
X <sub>15</sub>	Average number of epidermal cells on lower surface (mm <sup>2</sup> )
X <sub>16</sub>	Width/length of lower epidermal stomata (µm/µm)
X <sub>17</sub>	Width/length of lower epidermal cells (µm /µm)
X <sub>18</sub>	Stomata index of lower surface
X <sub>19</sub>	Average number of stomata on upper surface (mm <sup>2</sup> )
X <sub>20</sub>	Average number of epidermal cells on upper surface (mm <sup>2</sup> )
X <sub>21</sub>	Width/length of upper epidermal stomata (µm/µm)
X <sub>22</sub>	Width/length of upper epidermal cells (µm /µm)
X <sub>23</sub>	Stomata index of upper surface
X <sub>24</sub>	Average number of main bundles on midrib (number)
X <sub>25</sub>	Secretory cells into phloem; absent:0; present:1
X <sub>26</sub>	Lower and upper epidermis; glabrous:0; hairy:1

**Table 3. Percentage of variance as percentages of variables accounted for by first three components.**

Components	Percentage of variance explained by 26 characters	Percentage of variance explained by 5 characters
PC1	88.80	89.72
PC2	6.30	6.37
PC3	3.26	3.24
Total	98,36	99,33

***S. cana* (C.A. Mey.) Hoffm. var. *jacquiniana* (W. Koch) Chamb. (Figs. 2a-d)**

Triangular midrib has a large cavity surrounded with paranchymatous cells. There are 3 layers of collenchyma only at the sides between the main bundles and lower epidermis. Bundle sheath is not seen clearly at the every side of bundles. Xylem (150-170  $\mu\text{m}$ ) consisting a monolayer of secretory cells is larger than the phloem (90-100  $\mu\text{m}$ ). Small bundles distribute in the mesophyll (Fig. 2a). Palisade cells are clear in the midrib area. Mesophyll (300-330  $\mu\text{m}$ ) consists of 2 layers of palisade adjacent to both epidermises and 3-4 layers of spongy (Fig. 2b). Small bundles occur between the spongy cells. Ecvifacial leaves have anomocytic stomata on both sides (Figs. 2c-d).

***S. cana* (C.A. Meyer) Hoffm. var. *cana* (Figs. 3a-d)**

Triangular midrib has a large cavity bordered with paranchymatous cells. There are 3 layers of collenchyma only at the sides between the main bundles and lower epidermis. Xylem (150-170  $\mu\text{m}$ ) is larger than the phloem (70-90  $\mu\text{m}$ ). Midrib has one main and several small bundles (Fig. 3a). Palisade cells are present in the midrib. Mesophyll (200-220  $\mu\text{m}$ ) consists of 2 layers of palisade adjacent to upper epidermis and a mono layered palisade beneath the lower epidermis and 3-4 layers of spongy (Fig. 3b). Leaf is ecvifacial with anomocytic stomata at both sides. (Figs. 3c-d).

***S. cana* (C.A. Meyer) Hoffm. var. *alpina* (Boiss.) Chamb. (Figs. 4a-d)**

Arc-shaped midrib has a small cavity surrounded with parenchymatous cells. There are 3 layers of collenchyma only at the sides between the main bundles and lower epidermis. Xylem (190-200  $\mu\text{m}$ ) occupies 65% of the bundle. Palisade tissue is clear in the midrib (Fig. 4a). Mesophyll (400-450  $\mu\text{m}$ ) consists of 2 layers of palisade adjacent to upper epidermis and 1-2 layers of palisade beneath the lower epidermis and 3-4 layers of spongy (Fig. 4b). Ecvifacial leaves have anomocytic stomata on both sides (Figs. 4c-d).

***Scorzonera armeniaca* (Boiss. & Huet) Boiss. (Figs. 5a-e)**

There is a distinct large cavity in the central part of the midrib. 3-4 layered collenchyma occurs only at sides between the lower epidermis and main vascular bundles surrounded by bundle sheath. Xylem (230-250  $\mu\text{m}$ ) occupies 45% of main bundles. Phloem in the main bundles (160-200  $\mu\text{m}$ ) has a mono layer of secretory cells (Figs. 5a-b). Palisade tissue is obviously in the midrib. Mesophyll (380-400  $\mu\text{m}$ ) consists of 3 layers of palisade adjacent to upper epidermis and 2 layers of palisade beneath the lower epidermis and 2-3 layers of spongy (Fig. 5c). Leaf is ecvifacial with anomocytic stomata at both sides (Figs. 5d-e).

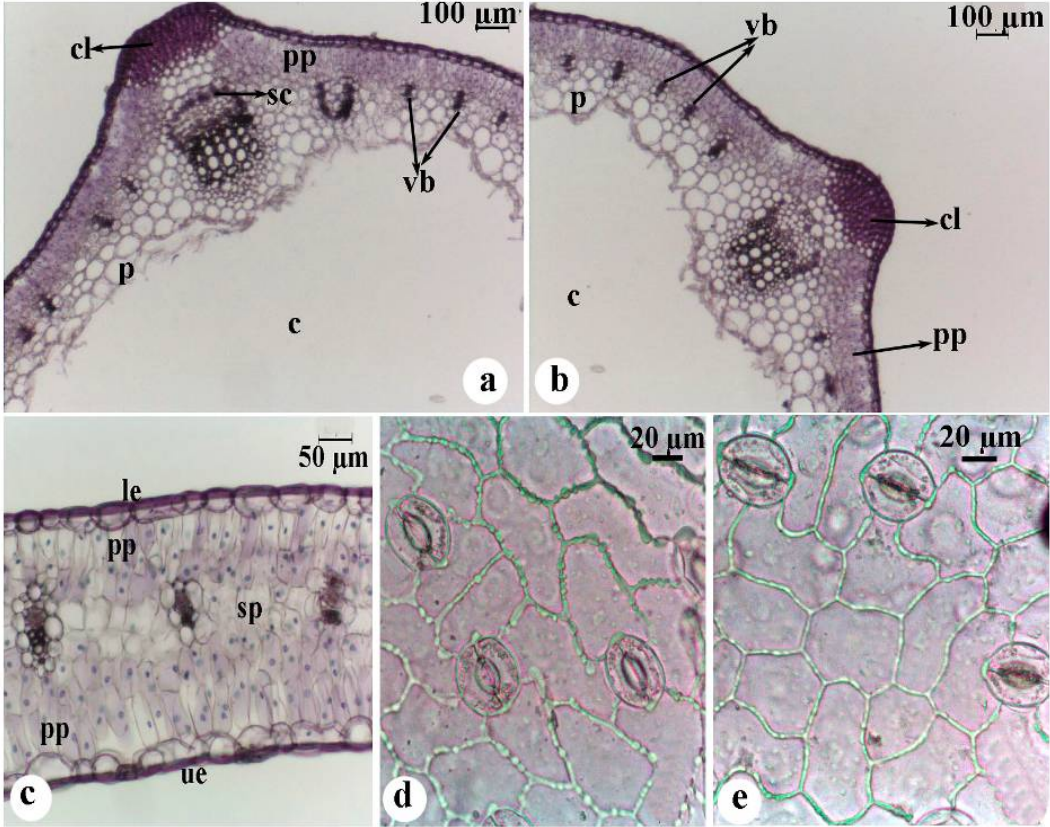


Fig. 1. *Scorzonera laciniata* subsp. *laciniata*, a-c. Cross sections of leaf, d. Superficial section of upper epiderma, e. Superficial section of lower epiderma.

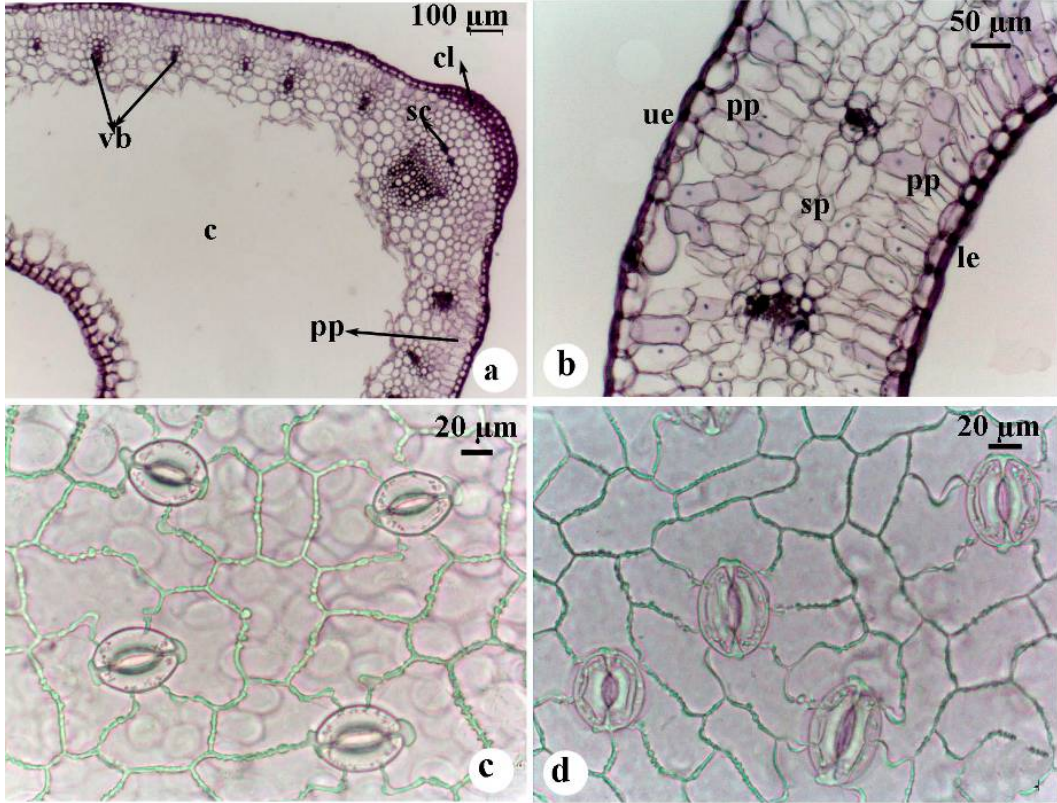


Fig. 2. *Scorzonera cana* var. *jacquiniana*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

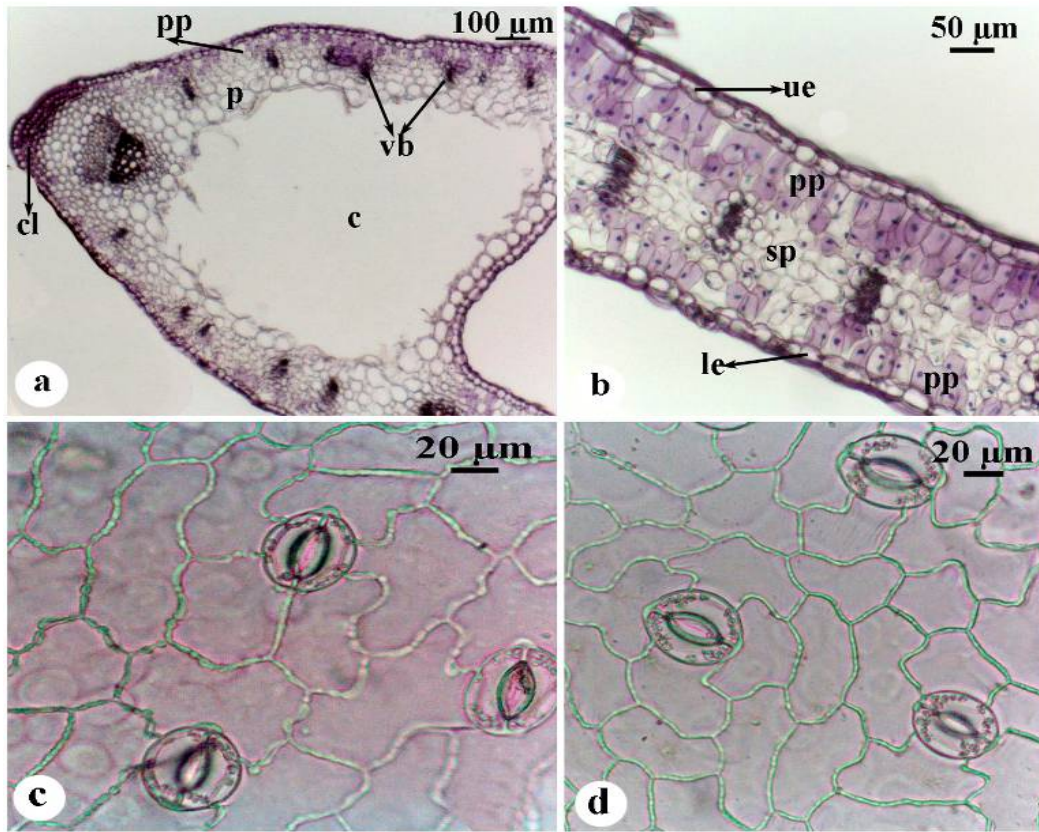


Fig. 3. *S. cana* var. *cana*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

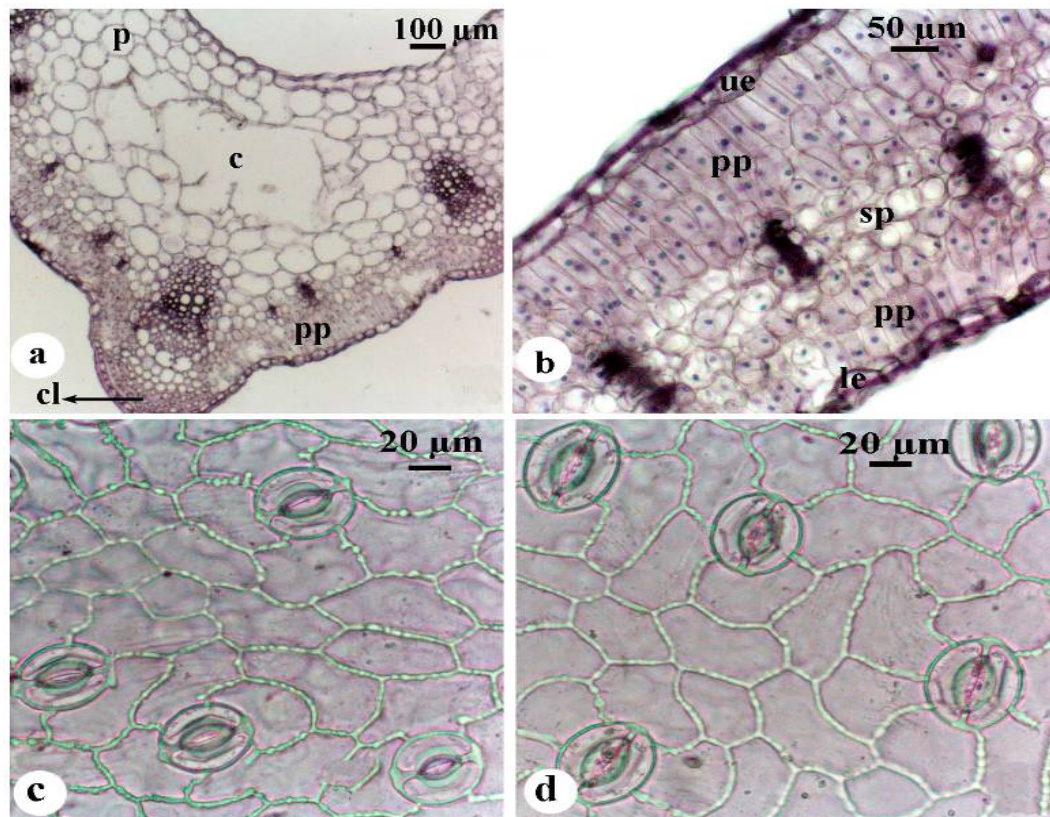


Fig. 4. *S. cana* var. *alpina*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

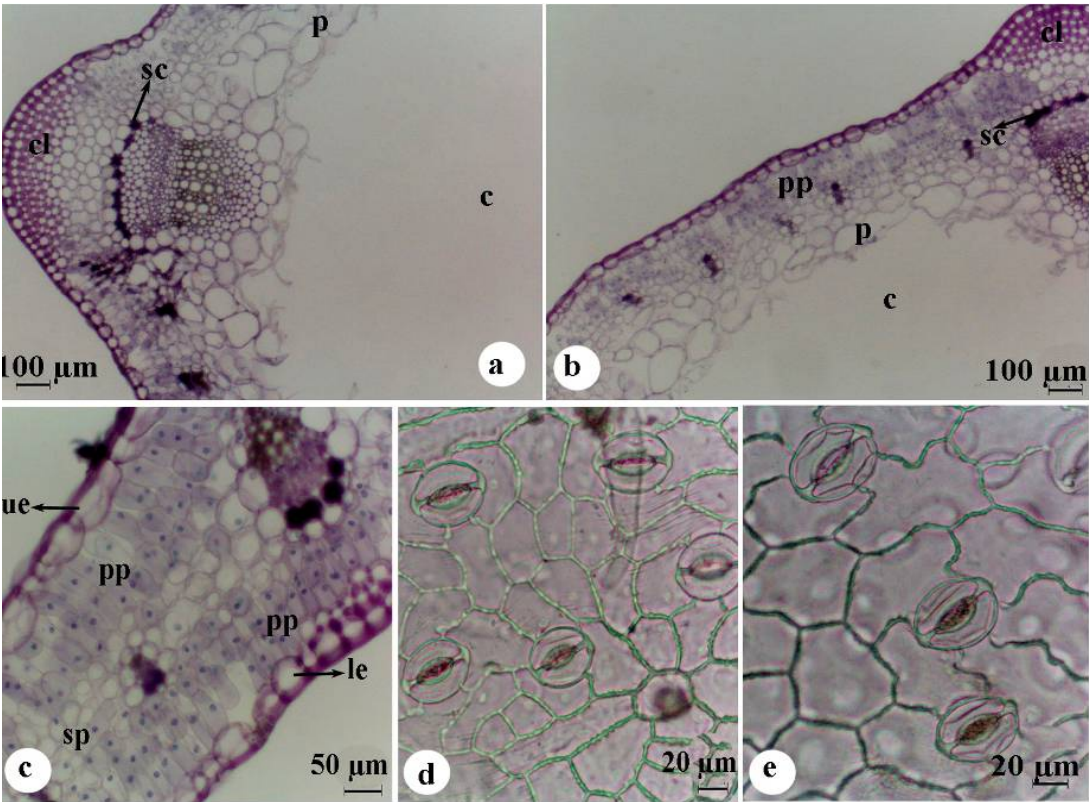


Fig. 5. *S. armeniaca*, a-c. Cross sections of leaf, d. Superficial section of lower epiderma, e. Superficial section of upper epiderma.

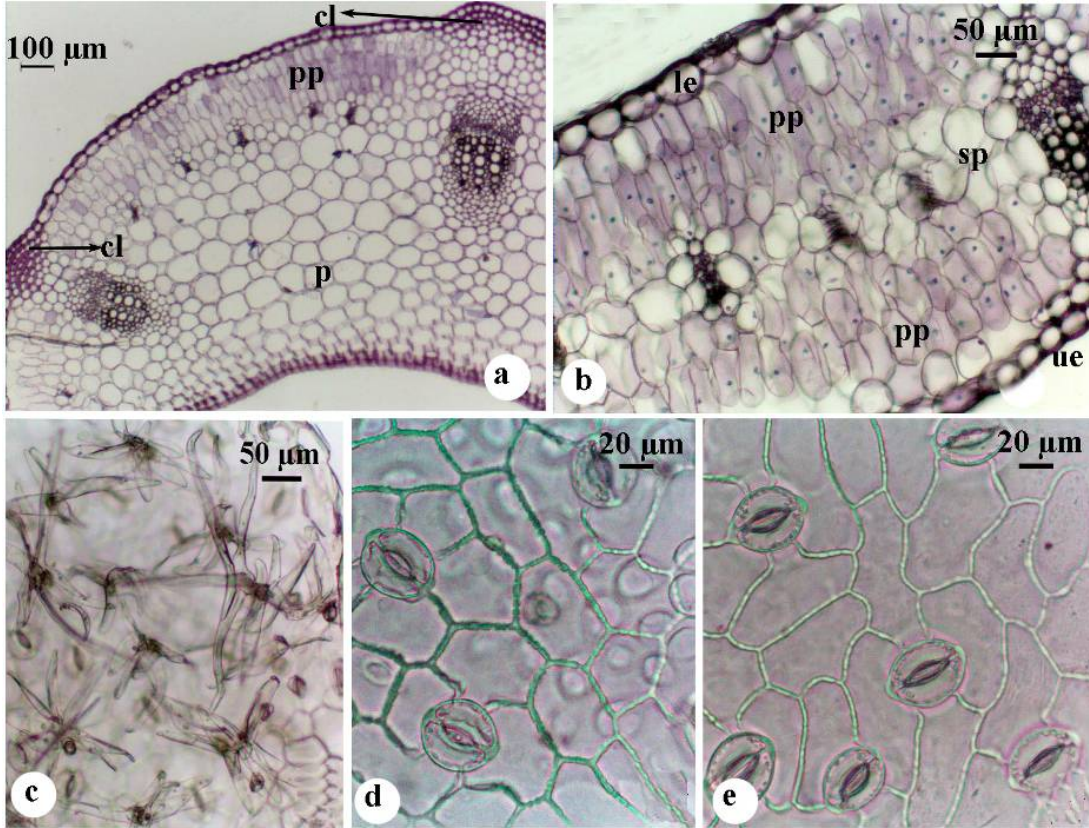


Fig. 6. *S. suberosa*, a-b. Cross sections of leaf, c-d. Superficial section of lower epiderma, e. Superficial section of upper epiderma.

***Scorzonera suberosa* C. Koch (Figs. 6a-e)**

Arc-shaped midrib has three main vascular bundles. Collenchyma is as 2-3 layers at sides between the bundles and lower epidermis. Xylem (250-300  $\mu\text{m}$ ) occupies 60-70% of the bundles. Phloem is 100-150  $\mu\text{m}$  and does not consist of secretory cell. Palisade tissue extends into the midrib region (Fig. 6a). Mesophyll (400-450  $\mu\text{m}$ ) consists of 2-3 layers of palisade cells adjacent to both epidermises and monolayer of spongy (Fig. 6b). All lower surface of the leaf is covered with densely star-shaped hairs (Fig. 6c). Ecvifacial leaves have anomocytic stomata on both sides. (Figs. 6d-e).

***Scorzonera mollis* subsp. *mollis* Bieb. (Figs. 7a-e)**

There is a distinct large cavity in the central part of the midrib composed of several bundles that vary in size. There are 6-8 layers of collenchyma between the lower epidermis and main bundles and a monolayer of collenchyma adjacent to upper epidermis. Xylem (450-470  $\mu\text{m}$ ) occupies 70% of the bundles. Phloem consisting a monolayer of secretory cells is 150-170  $\mu\text{m}$ . Palisade tissue extends into the midrib (Figs. 7a-b). Mesophyll is 250-300  $\mu\text{m}$  and consists of 5 layers of palisade cells (3 layers beneath upper epidermis and 2 layers beneath the lower epidermis) and a mono layer of spongy (Fig. 7c). Leaf is ecvifacial with anomocytic stomata on both sides (Figs. 7d-e).

***Scorzonera mollis* subsp. *szowitzii* (DC) Chamb. (Figs. 8a-d)**

Semi circular midrib contains several numbers of bundles. Collenchyma is 5-7 layers between the lower epidermis and main bundles and it is a mono layer inside of the upper epidermis. Xylem is 200-220  $\mu\text{m}$  and occupies 55-60% of bundle. Phloem surrounding by monolayer of scleranchyma is 130-150  $\mu\text{m}$ . Palisade cells are present in the midrib (Fig. 8a). Mesophyll is 350-400  $\mu\text{m}$  and consists of 2-3 layers of palisade inside of upper epidermis and 2 layers of palisade inside of lower epidermis and 1-2 layers of spongy (Fig. 8b). Ecvifacial leaves have anomocytic stomata on both sides (Figs. 8c-d).

***Scorzonera inaequiscapa* Boiss. (Figs. 9a-e)**

Midrib having a cavity surrounded by parenchymatous cells is arc-shaped and has several different size bundles. There are 4 layers of collenchyma located between the lower epidermis and main bundles. Xylem is 230-250  $\mu\text{m}$  and occupies 65-70% of the vascular bundle. Phloem is 100-130  $\mu\text{m}$  without any secretory cell or sclerenchyma fibers. Palisade cells are obviously in the midrib (Fig. 9a). Mesophyll is 350-370  $\mu\text{m}$  and consists of 2-3 layers of palisade inside of the upper epidermis, 3 layers of palisade beneath the lower epidermis and 2 layers of spongy (Fig. 9b). Leaf is ecvifacial and has anomocytic stomata cells at both sides. All lower surface of leaf is covered with densely star-shaped hairs (Figs. 9c-e).

***Scorzonera incisa* DC. (Figs. 10a-d)**

Midrib is circular and has several equal main vascular bundles spreading parallel to the lower epidermis. There are 4-5 layers of collenchyma between the lower epidermis and main bundles and 2 layers of collenchyma inside of upper epidermis. Xylem is 400-450  $\mu\text{m}$  and occupies 75% of the bundles. Phloem consisting of orbicular parenchyma cells is 100-150  $\mu\text{m}$ . Midrib doesn't consist of palisade cells (Fig. 10a). Mesophyll is 280-300  $\mu\text{m}$  and consists of 2-3 layers of palisade in the upper side, 2 layers of palisade in the lower side and 1-2 layers of spongy (Fig. 10b). Leaf is ecvifacial with anomocytic stomata cells at both sides (Figs. 10c-d).



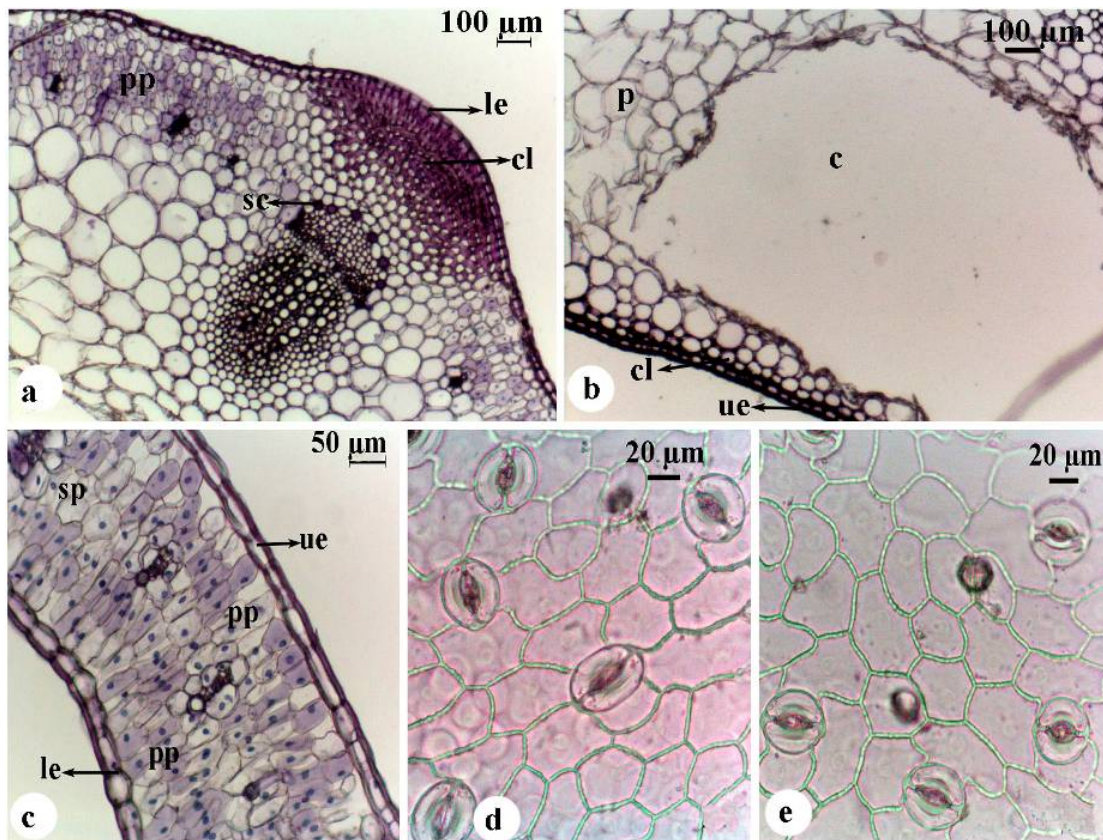


Fig. 7. *S. mollis* subsp. *mollis*, a-c. Cross sections of leaf, d. Superficial section of lower epiderma, e. Superficial section of upper epiderma.

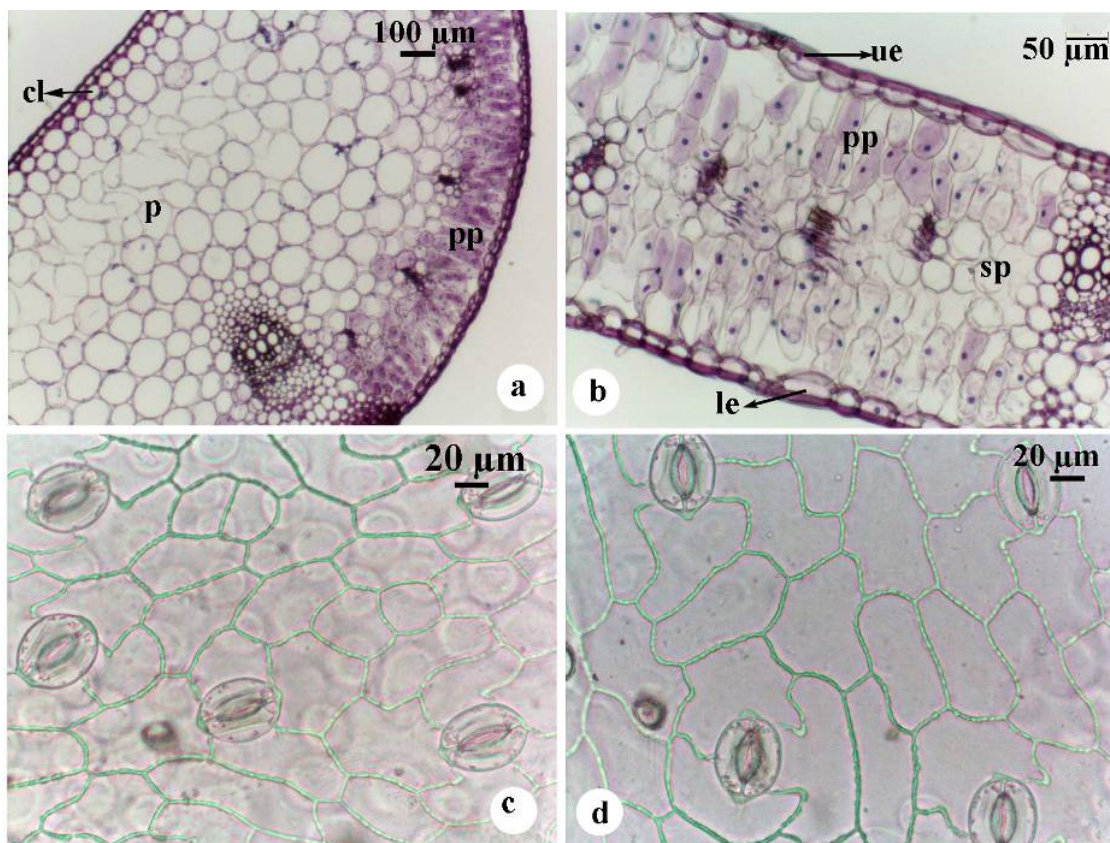


Fig. 8. *S. mollis* subsp. *szowitzii*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

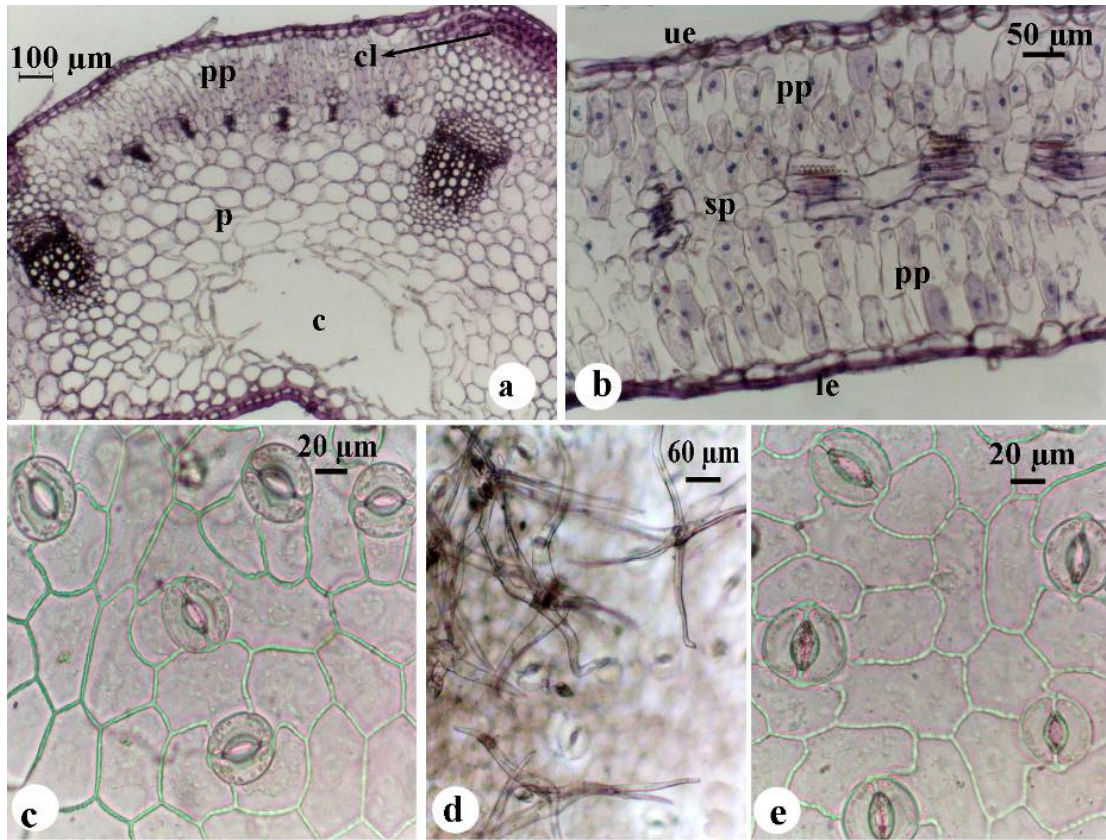


Fig. 9. *S. inaequiscapa*, a-b. Cross sections of leaf, c-d. Superficial section of lower epiderma. e. Superficial section of upper epiderma

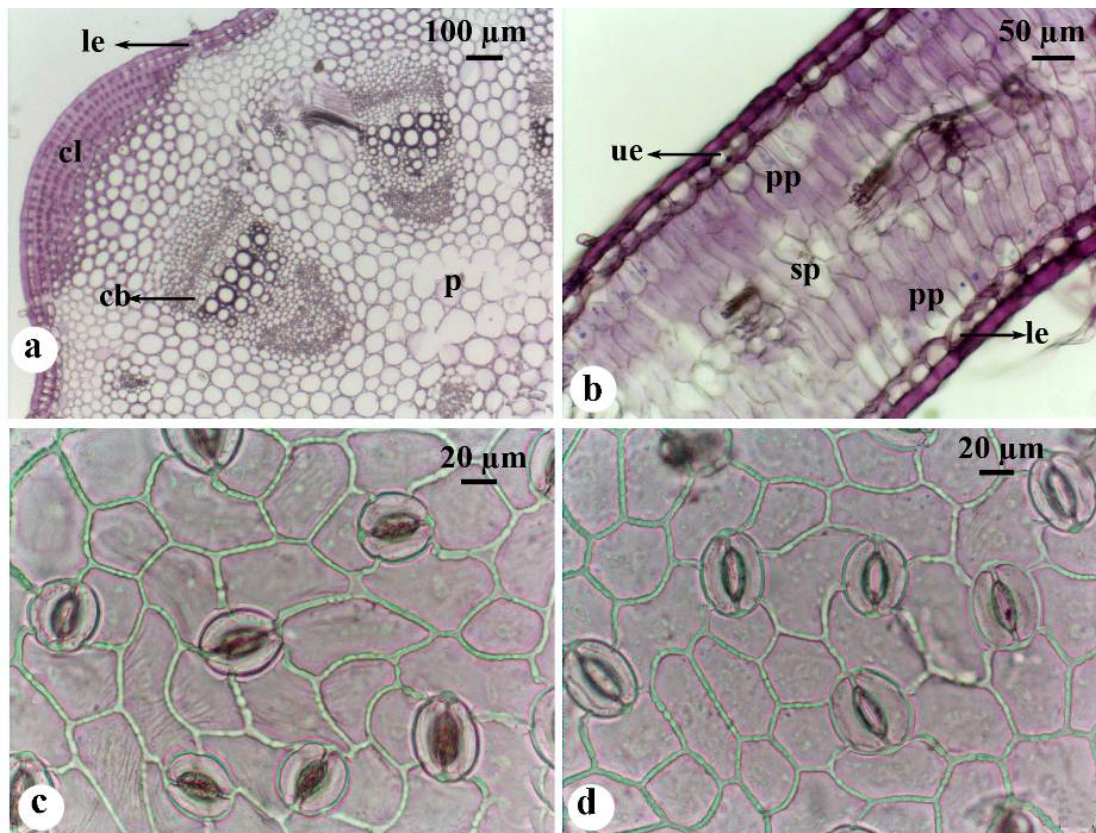


Fig. 10. *S. incisa*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

***Scorzonera eriophora* DC. (Figs. 11a-d)**

Midrib having a cavity at centre is V-shaped and has several main vascular bundles arranged as parallel to the lower epidermis. There are 4-5 layers of collenchyma between the lower epidermis and main bundles and a monolayer of collenchyma adjacent to upper epidermis. Xylem is 350-400  $\mu\text{m}$  and occupies 65-70% of the bundles. Phloem is 150-200  $\mu\text{m}$  and consists of parenchymatic cells. Palisade cells are clear in the midrib tissue (Fig. 11a). Mesophyll is 500-550  $\mu\text{m}$  and consists of 2 layers of palisade in the upper sides, 2-3 layers of palisade in the lower sides and 4-5 layers of spongy. There are sparsely simple hairs throughout the lower surface (Fig. 11b). Ecvifacial leaves have anomocytic stomata at both sides (Figs. 11c-d).

***Scorzonera cinerea* Boiss. (Figs. 12a-e)**

Triangular midrib contains one main vascular bundle. 2-3 layers of collenchyma occur beneath the upper epidermis and 4-5 layers close to lower epidermis along the midrib. Xylem is 200-220  $\mu\text{m}$ . Phloem consisting of densely secretory cells is 100-120  $\mu\text{m}$ . Palisade cells extend into the midrib (Fig. 12a). Mesophyll is 430-450  $\mu\text{m}$  and consists of 2 layers of palisade inside of upper and lower epidermises and 4-5 layers of spongy. There are distinct stellate hairs sparsely throughout the lower and upper surface (Figs. 12b-c). Leaf is ecvifacial with anomocytic stomata at both sides (Figs. 12d-e).

***Scorzonera seidlitzii* Boiss. (Figs. 13a-d)**

Arc-shaped midrib has 3-4 layers of collenchyma located between the lower epidermis and a single vascular bundle located at the centre of midrib. Xylem is 150-170  $\mu\text{m}$  and occupies approximately 50% of bundles. Phloem is 80-90  $\mu\text{m}$  without secretory cells. Palisade cells are present only along inside of upper epidermis in the midrib region (Fig. 13a). Mesophyll is 310-360  $\mu\text{m}$  and consists of 3 layers of palisade inside of upper epidermis, 2 layers of palisade beneath the lower epidermis and 2-3 layers of spongy (Fig. 13b). Leaf is ecvifacial with anomocytic stomata on both sides (Figs. 13c-d).

***Scorzonera sericea* DC. (Figs. 14a-d)**

Arc-shaped midrib has 4-5 layers of collenchyma located between the lower epidermis and vascular bundle. Xylem is 120-150  $\mu\text{m}$  and occupies approximately 60% of bundles. Phloem is 80-90  $\mu\text{m}$  without secretory cells. Palisade cells are obvious only along inside of upper epidermis in the midrib region (Fig. 14a). Mesophyll is 300-320  $\mu\text{m}$  and consists of 3 layers of rectangular palisade cells inside of upper epidermis, 2 layers of palisade inside of the lower epidermis and 3-4 layers of spongy. Both epidermal surfaces covered with simple hairs (Fig. 14b). Ecvifacial leaf has stomata on both sides (Figs. 14c-d).

***S. pseudolanata* Grossh. (Figs. 15a-d)**

Lower and upper epidermis consists of monolayer of rectangular or orbicular cells. Triangular-shaped midrib has 2-3 layers of collenchyma located between the lower epidermis and vascular bundle. Xylem is 180-220  $\mu\text{m}$  and occupies 70% of the vascular bundle. Phloem is 65-75  $\mu\text{m}$ . Midrib doesn't consist of palisade cells (Fig. 15a). Mesophyll is 250-300  $\mu\text{m}$  and consists of 2 layers of palisade inside of both epidermises and 2-3 layers of spongy (Fig. 15b). Ecvifacial leaves have anomocytic stomata on both sides (Figs. 15c-d).

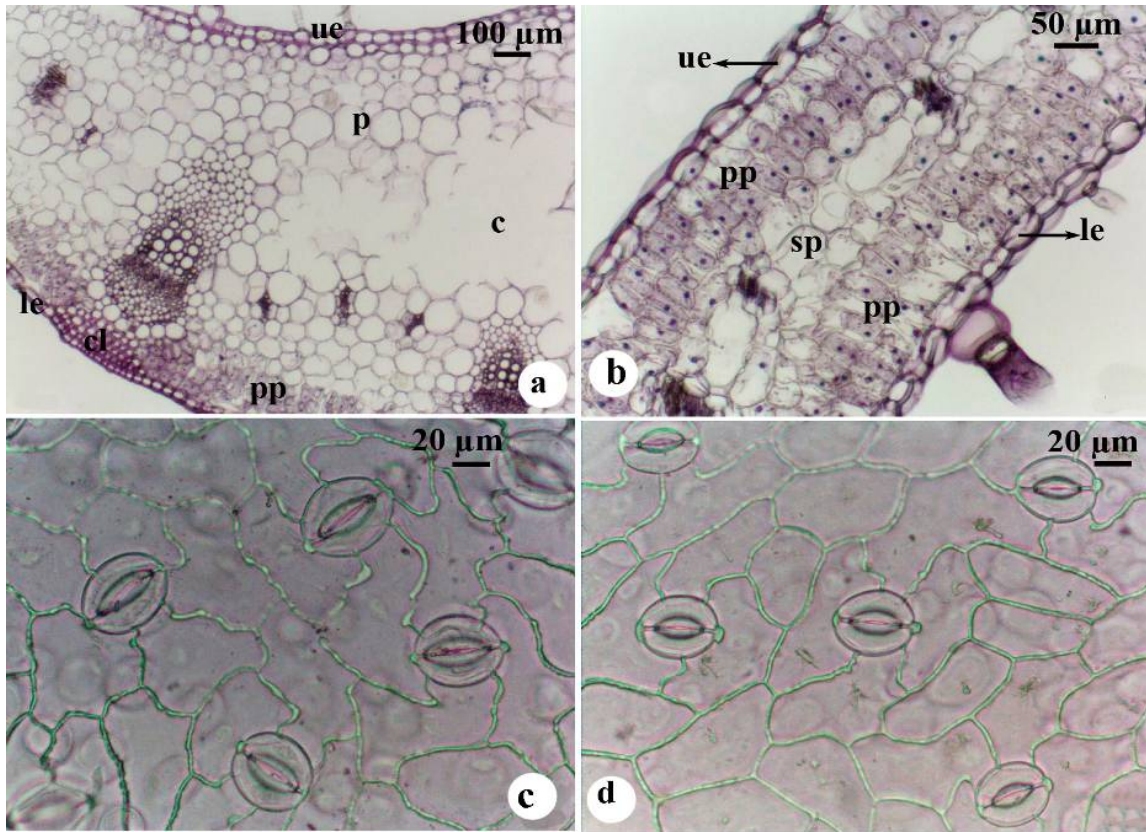


Fig. 11. *S. eriophora*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

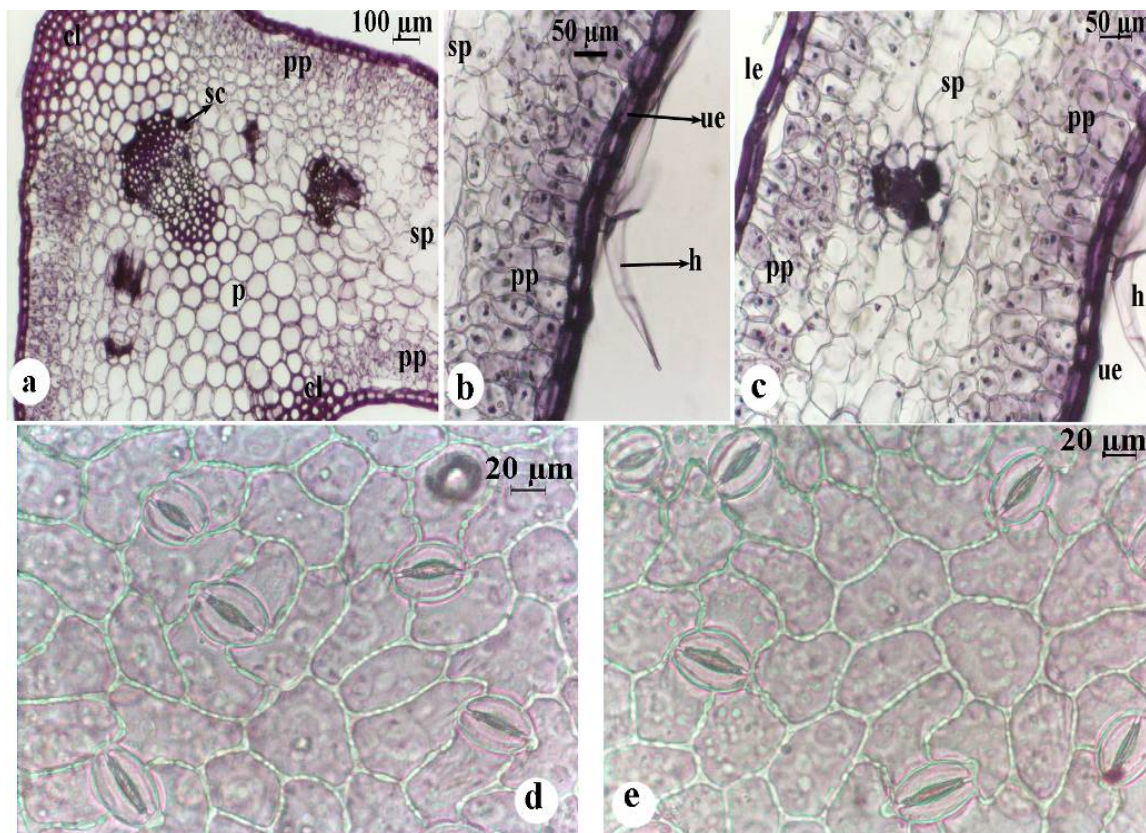


Fig. 12. *S. cinerea*, a-c. Cross sections of leaf, d. Superficial section of lower epiderma, e. Superficial section of upper epiderma.

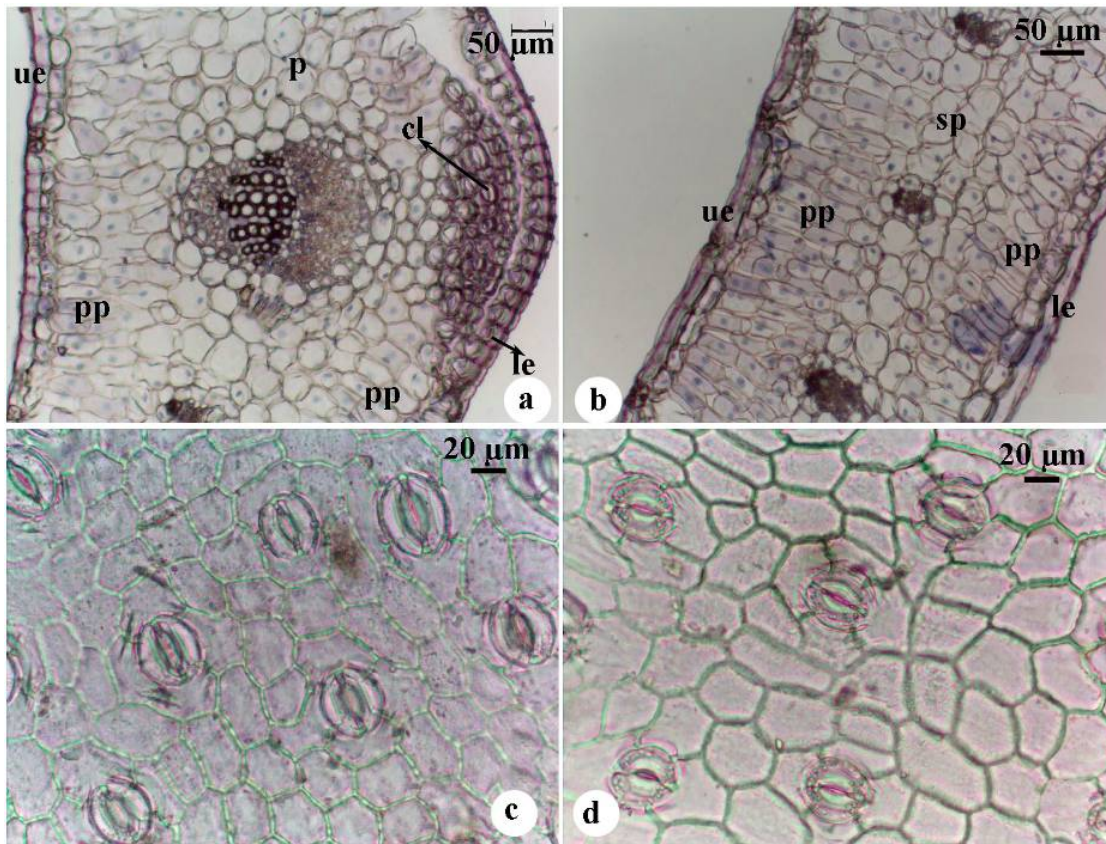


Fig. 13. *S. seidlitzii*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

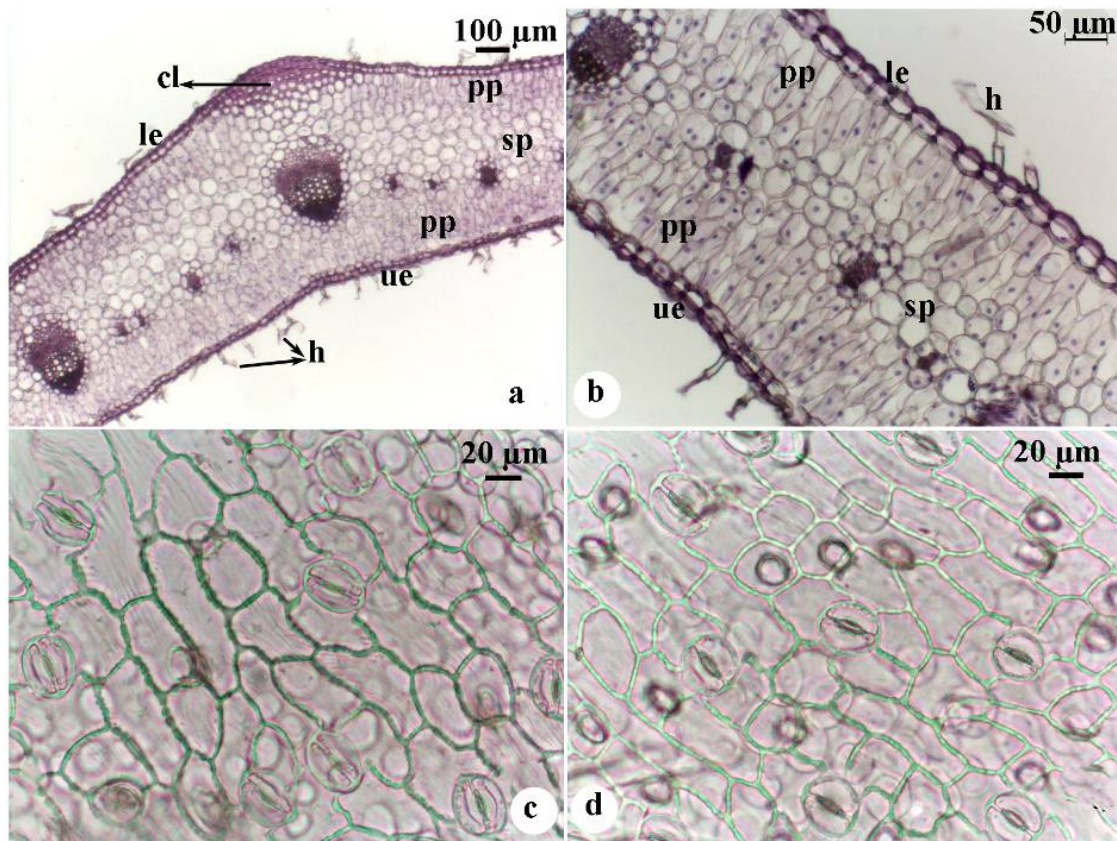


Fig. 14. *S. sericea*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

***Scorzonera latifolia* (Fish. & Mey.) DC. (Figs. 16a-d)**

Midrib is semi circular with a main bundle and consists of 10-12 rows collenchyma in lower part and 4-5 rows in upper part. Besides, 2 layers of collenchyma continue along the all upper epidermis. Both epidermises surface are covered with densely simple hairs. Xylem is 300-350  $\mu\text{m}$  and occupies 60% of the bundle. Phloem is 200-250  $\mu\text{m}$  and consists of densely scleranchymatic fibers and secretory cells as a monolayer. Palisade cells extend into the midrib (Fig. 16a). Mesophyll is 500-600  $\mu\text{m}$  and consists of 2-3 layers of rectangular palisade cells in upper sides, 2 layers of palisade in lower sides and 2-3 layers of spongy (Fig. 16b). Ecvifacial leaves have anomocytic stomata on both sides (Figs. 16c-d).

***Scorzonera sosnowskyi* Lipschitz. (Figs. 17a-d)**

Midrib is semi circular with a main and consists of 6-9 rows collenchyma inside of the both sides. Xylem is 250-300  $\mu\text{m}$  and phloem with densely sclerenchyma fibers is 180-220  $\mu\text{m}$ . Midrib doesn't consist of palisade cells (Fig. 17a). Mesophyll is 350-400  $\mu\text{m}$  and consists of 2-3 layers of rectangular palisade cells adjacent to upper epidermis, 2 layers of palisade beneath the lower epidermis and 3 layers of spongy (Fig. 17b). Ecvifacial leaf has anomocytic stomata on both sides (Figs. 17c-d).

***Scorzonera tomentosa* L. (Figs. 18a-e)**

Midrib consists of 5-7 layers of collenchyma beneath the upper and lower epidermis. It is semi circular with a main bundle. Xylem is 310-330  $\mu\text{m}$ . Phloem consisting densely sclerenchyma fibers is 220-240  $\mu\text{m}$ . There are densely simple hairs on both sides of epidermis (Fig. 18a). Mesophyll is 340-370  $\mu\text{m}$  and consists of 2-3 layers of rectangular palisade cells inside of upper epidermis, 2 layers of palisade beneath the lower epidermis and 2-4 layers of spongy (Fig. 18b). Ecvifacial leaf has anomocytic stomata on both sides (Figs. 18c-e).

**Numerical results:** Dendrogram resulting from UPGMA based on 26 leaf variables is represented in Fig. 19. As seen in the Fig. 19, all the investigated taxa fall into two main clusters. The first group labeled as "a" linked to each other at 90% dissimilarity level consists of *S. mollis* subsp. *mollis*, *S. mollis* subsp. *szowitzii*, *S. seidlitzii*, *S. latifolia*, *S. sosnowskyi*, *S. cinerea*, *S. sericea*, *S. tomentosa*, *S. pseudolanata* and *S. incisa*. The second cluster labeled as "b" divided into smaller clusters includes all remaining taxa.

PCA results based on both 18 and 5 selected foliar traits are given in Fig. 20 respectively. This figure also shows the taxa and the variables on the first two components. Most of the traits used in this study are not important in explaining the total variation, so only PCA results using five characters are taken into consideration but the eigenvalues as percentages of the explained variance based on both selected 5 traits and also 26 are given in Table 3. As seen in Table 3, the eigenvalues of the first, second and third components base on 5 and 26 variables vary between 89.72-88.80%, 6.37-6.30% and 3.24-3.26% respectively.

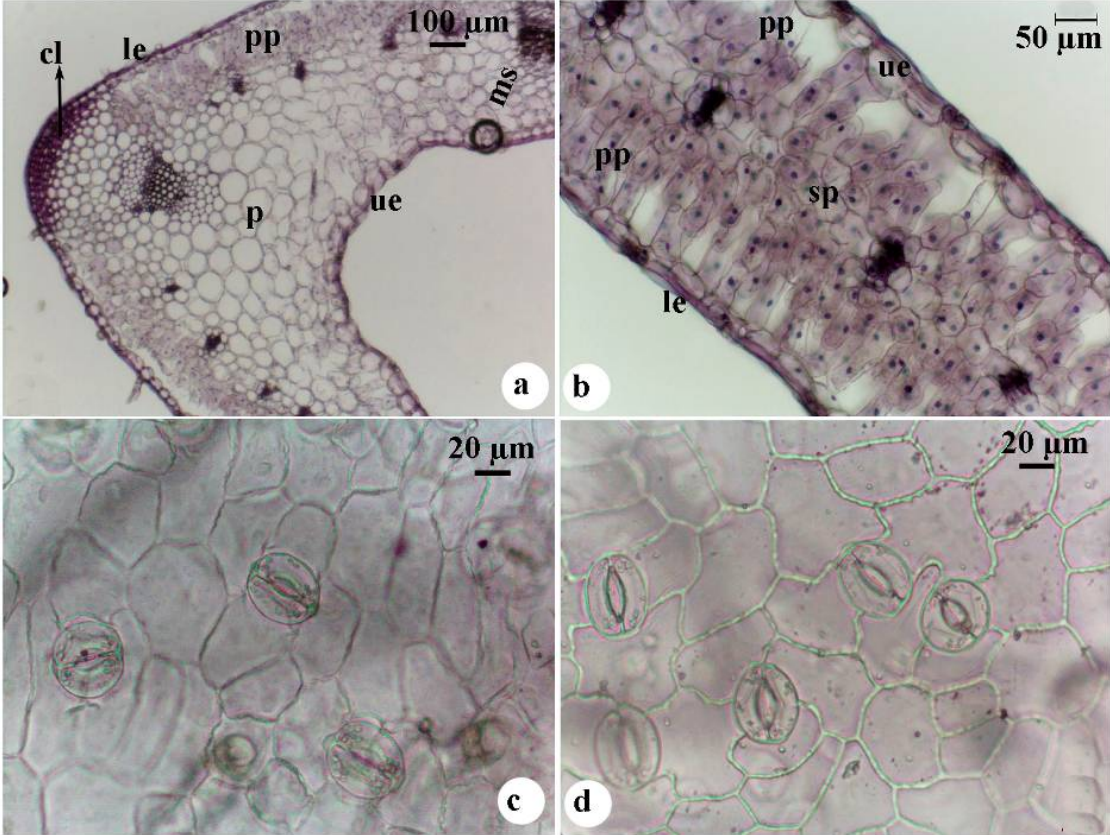


Fig. 15. *S. pseudolanata*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

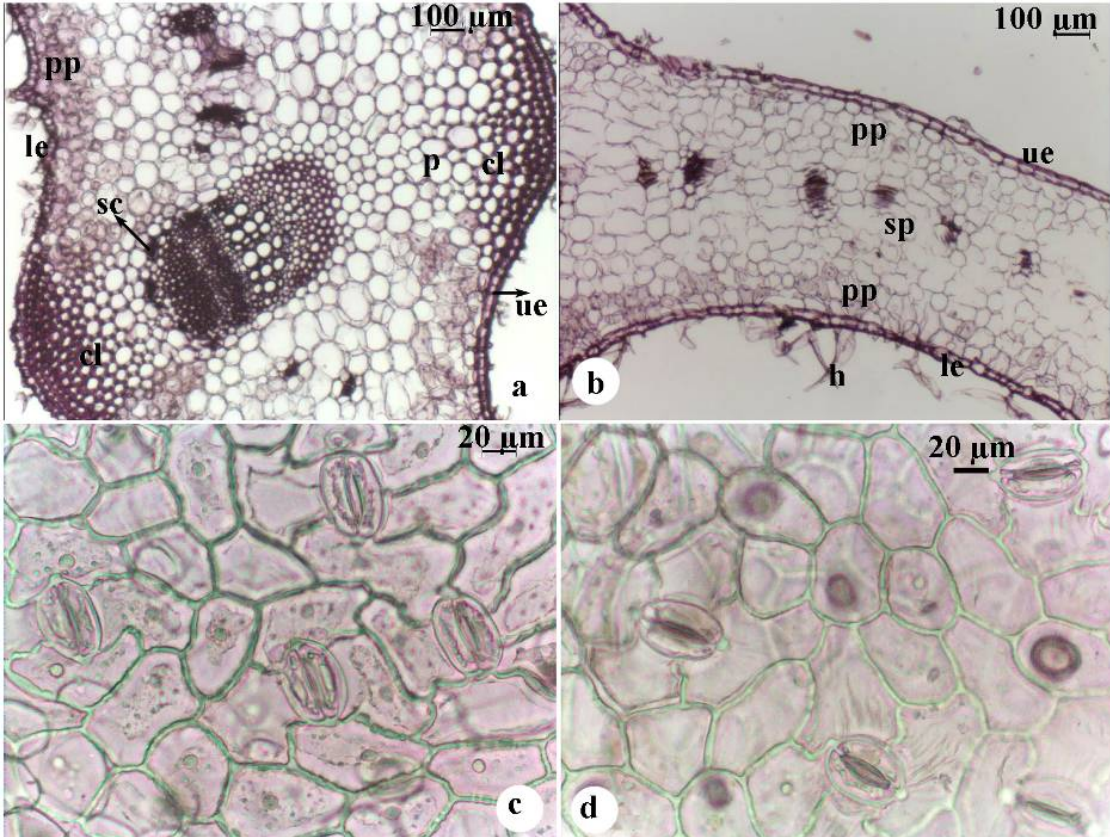


Fig. 16. *S. latifolia*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

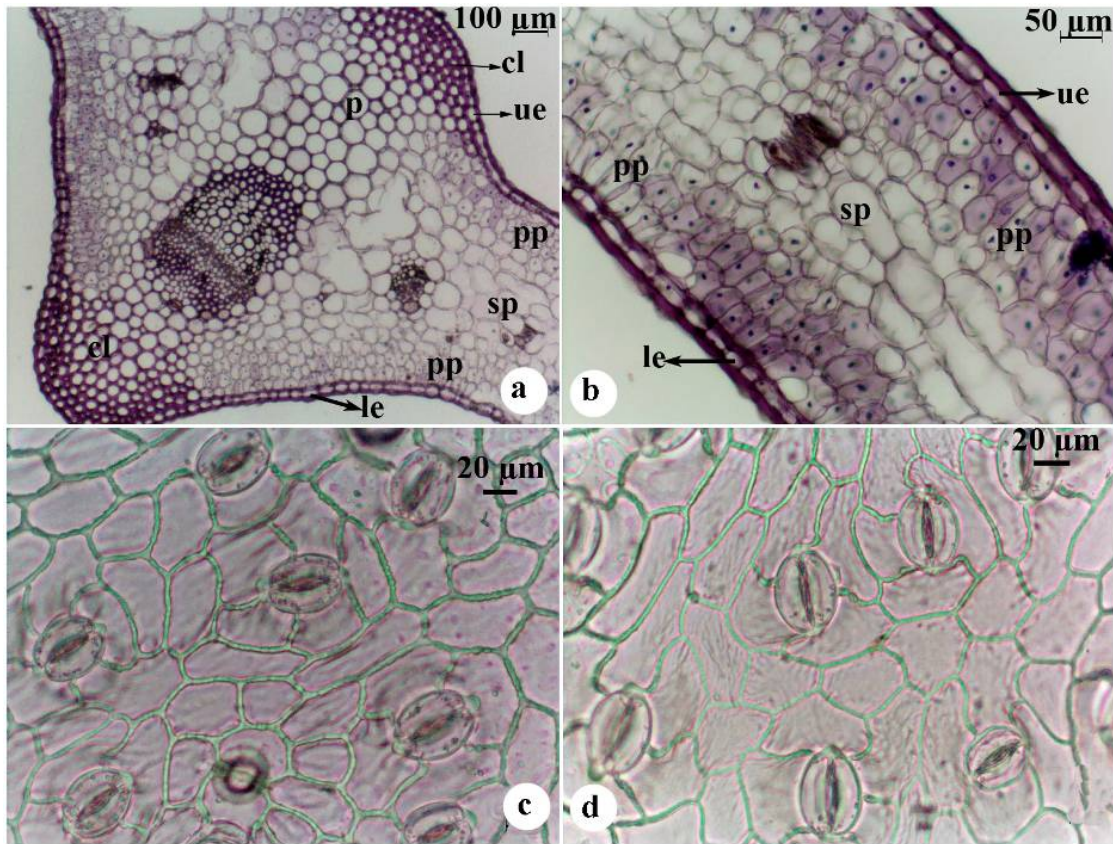


Fig. 17. *S. sosnowskyi*, a-b. Cross sections of leaf, c. Superficial section of lower epiderma, d. Superficial section of upper epiderma.

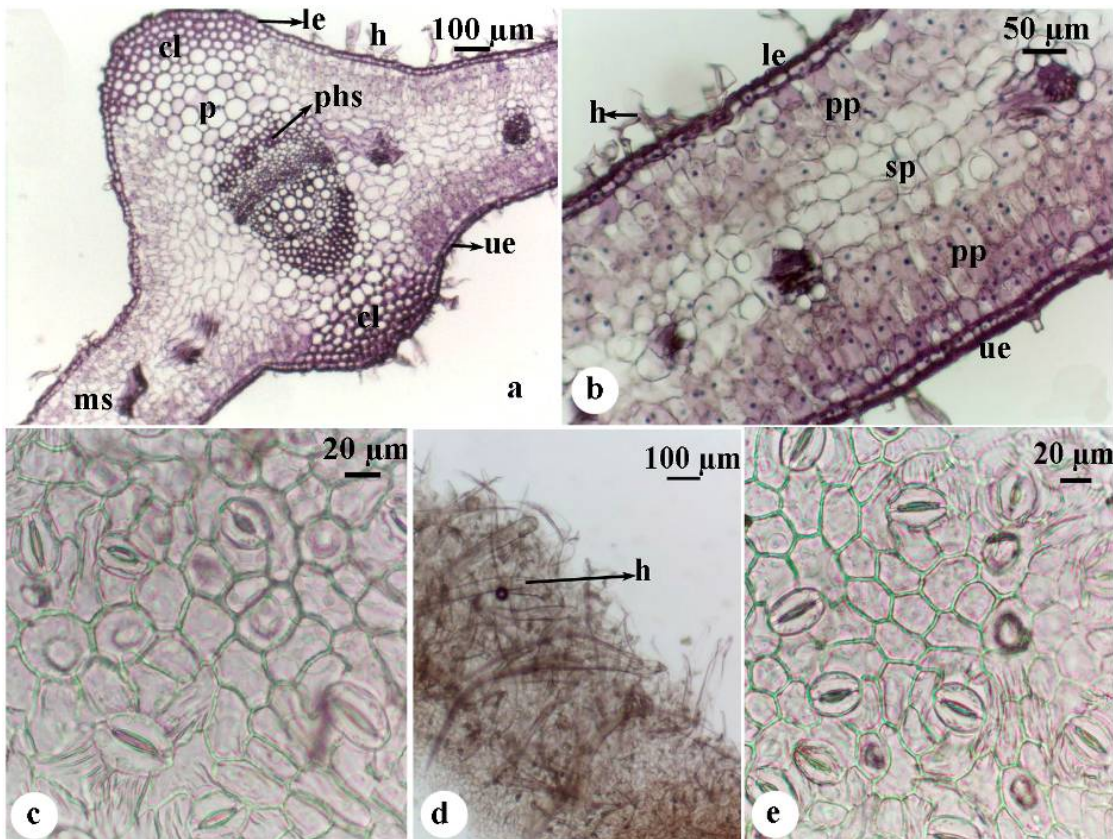


Fig. 18. *S. tomentosa*, a-b. Cross sections of leaf, c-d. Superficial sections of lower epiderma, e. Superficial section of upper epiderma.



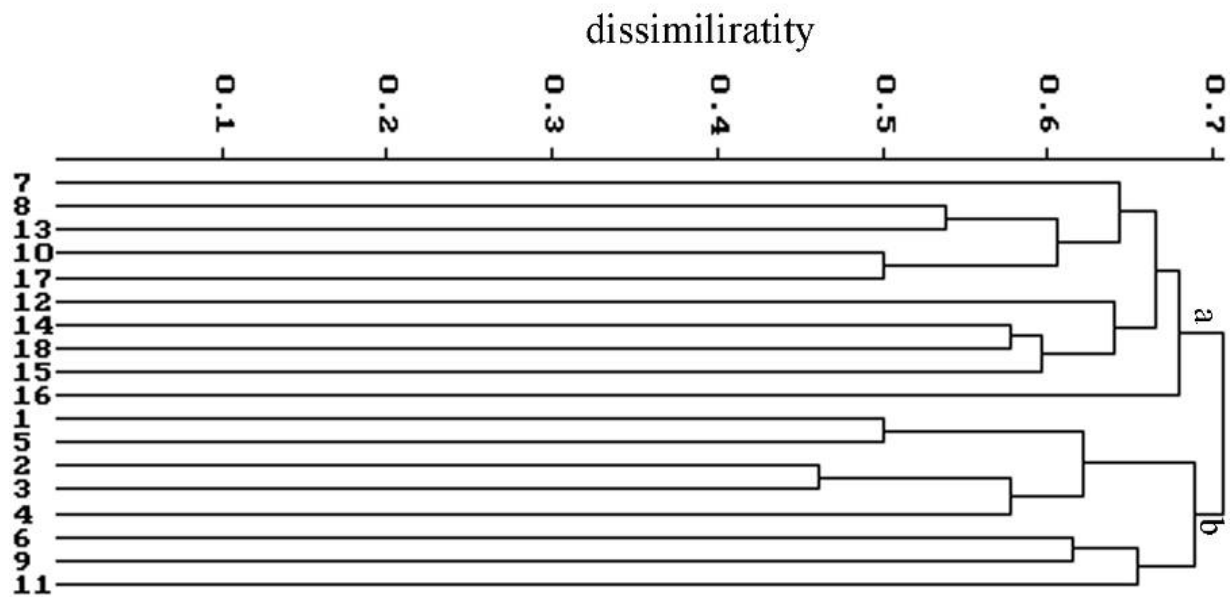


Fig. 19. Cluster analysis – UPGMA (For the taxa numbers see Table 1)

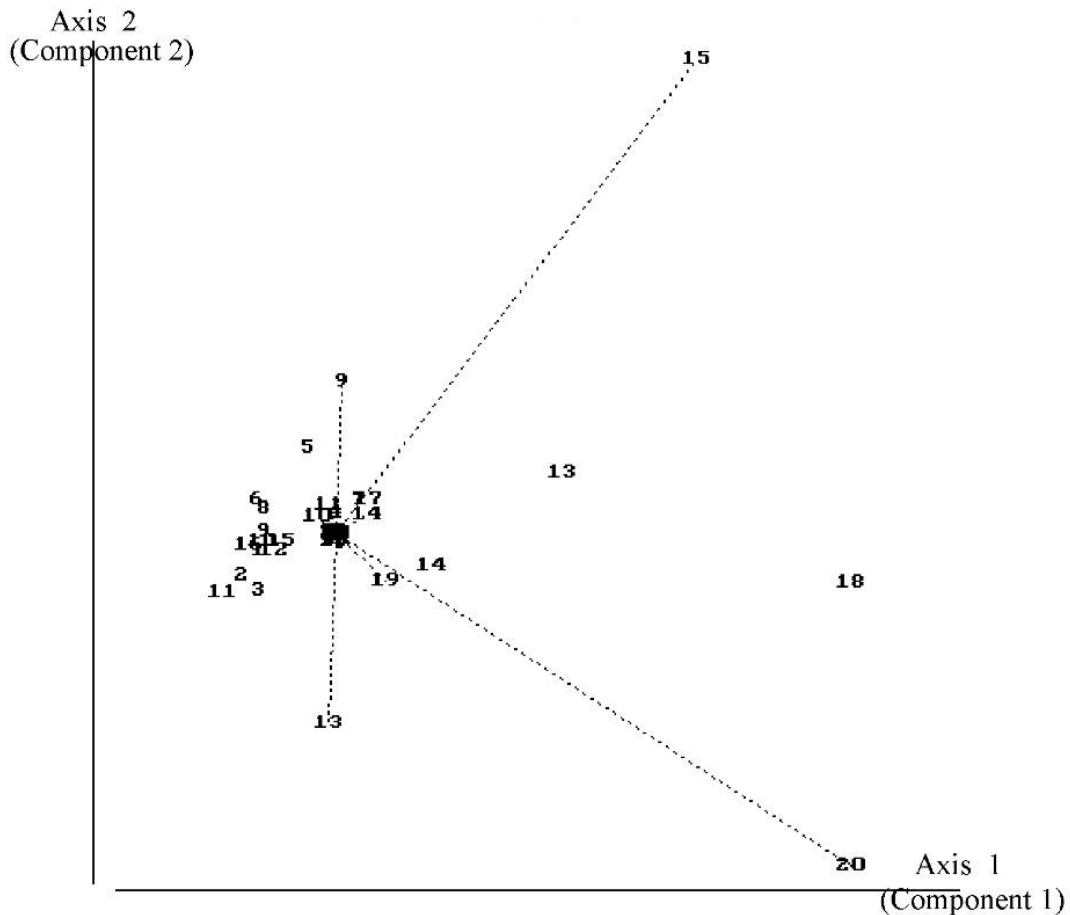


Fig. 20. Principal component analyses of 18 taxa and 5 variables projected onto the first two axes. For the variable number explanations see Table 1.

**Abbreviations**

(c) cavity, (cl) collenchyma, (p) parenchyma, (le) lower epidermis, (ue) upper epidermis, (sp) spongy parenchyma, (pp) palisade parenchyma, (ms) mesophyll, (sc) secretory cell, (vb) vascular bundle, (phs) phloem sclerenchyma, (cb) cambium, (h) hair, (µm) micrometer.

## Discussion

In this study, internal foliar traits of 18 *Scorzonera* species distributed in NE Anatolia were examined in order to provide useful and additional information to the systematics. This is the first anatomical report on the Turkish representatives of *Scorzonera*.

It is well known that the leaf anatomy varies greatly and has systematically significant value in many genera such as *Epilobium* (Makbul *et al.*, 2008) *Scrophularia* (Makbul *et al.*, 2006b; Lersten & Curtis, 2001). As indicated in the above literatures, we found that the presence and distribution of secretory cells, main bundles, hairs, mesophyll and surface features are particularly important in the examined taxa. Nevertheless, leaf phenetic characters among the morphological traits generally are the most important in the delimitation of *Scorzonera* species (Makbul, 2006a). According to Makbul (2006a) especially leaf margin shapes vary from taxon to taxon. Chamberlain (1975) also reported that the width of leaf, pubescence of leaf, margin shapes and basal and upper leaf characters are one of the most important phenetic characters in the genus *Scorzonera*. In the present study, it was found that foliar phenetic differences are generally accordance with foliar anatomic ones. Makbul (2006a) also indicated that anatomic properties of root, stem and achenes can be also used in conjunction with morphological ones in *Scorzonera*.

According to Mavrodiev *et al.*, (2004), based on ITS sequence data *S. latifolia* and *S. seidlitzii* are present in the same clade but these two taxa are easily separated by each other based on leaf anatomical traits as seen in Fig. 19. Besides the results based on caryological data (Nazarova, 1997) is supported by Mavrodiev *et al.*, (2004) study based on ITS data.

Yentür (2003) indicated that arrangement of bundles in midrib and mesophyll is valuable traits in comparative leaf anatomy, but the density of the bundles in mesophyll varies ecologically. In the present study, while *S. laciniata* subsp. *laciniata*, *S. eriophora*, *S. incisa*, *S. mollis* subsp. *mollis* have five main bundles, *S. cana* var. *jacquiniana*, *S. cana* var. *cana*, *S. cana* var. *alpina*, *S. latifolia*, *S. armeniaca*, *S. suberosa*, *S. mollis* subsp. *szowitzii*, *S. inaequiscapa* have three and the rest have only one main bundle. But this is not supported by numerical analysis. Numerical analysis show that width of the palisade tissue beneath the lower epidermis, and rates of spongy to the total mesophyll varies in among the examined taxa.

It is well known that the distribution site and the average number of collenchyma tissue in plants are important in comparative anatomical studies (Özörgücü *et al.*, 1991). In the present study, it was also for that the average row number of collenchyma across the main bundles in midrib differs from species to species, but it is not supported by numerical analysis.

All leaves of the investigated taxa are ecvifacial with anomocytic stomata. The average number of upper and lower epidermal cells varies from 359 to 1555 and 307 to 1117 respectively and average number of stomata on upper and lower surface varies from 51 to 192 and 51 to 128 in all examined taxa. These two traits are among the numerically important characters. It was also observed that there are distinct differences in terms of stomata index. While the stomata index varies from species to species, it is also well known that this trait is among the environmentally influenced anatomical character (Özörgücü *et al.*, 1991).

All investigated taxa fall in two groups based on the presence and distribution of secretory cell in phloem. Although this character was not among the numerical important characters, the secretory cells in the stem supply additional taxonomic information in grouping the *Scorzonera* taxa as indicated by Makbul (2006a) and with the present study. While *S. cana* var. *jacquiniana* have several secretory cells, there is not any secretory cell in the rest two *S. cana* varieties. Similarly, while one of the subspecies of *S. mollis* (*S. mollis* subsp. *mollis*) includes secretory cells, the other does not. *S. latifolia* morphologically similar to *S. sosnowskyi* is easily separated by the presence of secretory cell in its stem.

There were also some distinct differences among the investigated taxa by means of other foliar peculiarities such as width of the palisade tissue beneath the upper epidermis, rates (width/width) of spongy to the mesophyll, average number of epidermal cells for lower and upper surface and average number of stomata on upper surface. These findings are in accordance with the information described by Metcalfe & Chalk (1950) for Asteraceae, but numerical analysis did not support their value in explaining the total variations.

The cophenetic correlation coefficient ( $r_{cs}$ ) was also calculated in this study. The simplicity of  $r_{cs}$  has led to its extensive application (Sneath & Sokal, 1973). It has generally been found to vary from 0.6 to 0.95 (Sneath & Sokal, 1973). Our dendrogram had a cophenetic correlation of 0.76, suggesting that the dendrogram provides accurate representation of the resemblances.

As seen in Fig. 19, all investigated taxa fall into two major clusters at 97.4% dissimilarity levels. One labeled as “a”, consists of species belongs to all caulescent and some scapigerous taxa and the other labeled as “b” includes all remaining species representing scapigerous or subscapigerous taxa. When the dendrogram is carefully examined, it is seen that anatomical results of UPGMA generally supports morphological results reported by Chamberlain (1975) and Makbul (2006a).

Cluster “a” divided into two small groups (Fig. 19). While the first group consists of mostly scapigerous (*S. mollis* subsp. *mollis*, *S. mollis* subsp. *szowitzii*, *S. seidlitzii*), subscapigerous (*S. incisa*) and caulescent (*S. sosnowskyi*) taxa, the second group consists of generally caulescent taxa except for scabous species (*S. sericea* and *S. pseudolanata*). As seen in Fig. 7-8, these morphologically closely related taxa are easily separated by means of foliar anatomical traits such as presence of secretory cells and cavity in midrib, thickness of collenchyma and average number of spongy parenchyma. Similarly, the two subsp of *S. mollis* are not linked to each other very closely, this means that foliar anatomical traits supplies useful information for delimiting the two examined subspecies. *S. sosnowskyi* is the most different species in group “a” in terms of phenetic properties and it is closely related species of *S. latifolia* (Chamberlain, 1975), our results from UPGMA based on foliar traits are easily separating these two taxa at specific level (Fig. 19).

Cluster “b” also divided into two small groups as group “a”. First group consisted of only scapigerous and subscapigerous taxa include all the representatives of *S. cana* at subspecific level. This shows that all the representatives of *S. cana* are also very similar based on anatomical traits. Chamberlain (1975) reported that *S. cana* allied to *S. laciniata* subsp. *laciniata*, but these taxa are separated from each other in terms of some leaf anatomic characters such as general midrib shape, number of main bundles in midrib and mesophyll features. Our results from UPGMA are not in support of this view (Fig. 19). Chamberlain (1975) indicated that *S. armeniaca* and *S. laciniata* subsp. *laciniata* are closely related taxa because of the high phenetic resemblance. This view is confirmed with results from UPGMA based on general leaf anatomical traits.

Second group of cluster “b” consists of *S. suberosa* and *S. inaequiscapa* which are scapigerous and *S. eriophora* which is subscapigerous. *S. suberosa* and *S. inaequiscapa* are very similar species in terms of phenetic properties and so should be in cluster “a” according to morphological properties (Chamberlain, 1975; Makbul, 2006a), but they are in cluster “b” based on foliar anatomical features (Fig. 19). Additionally these two taxa are morphologically similar to the *S. mollis* which are in group “a”. This means that foliar trait can also be useful to distinguish these two species from *S. mollis* at specific level.

As it can be seen in Table 3, while the first three components account together for 99.33% total variation based on five selected variables, they explain 98.37% total variation based on 26 variables. This means that some characters among the 26 examined traits are more important in separating the examined *Scorzonera* taxa. These characters are as follows; width of the palisade tissue beneath the upper epidermis ( $X_9$ ), width of spongy tissue / width of mesophyll tissue ( $X_{13}$ ), average number of epidermal cells for lower ( $X_{15}$ ), average number of stomata on upper surface ( $X_{19}$ ) and average number of epidermal cell on lower surface ( $X_{20}$ ). As conclusion anatomical features supplies many valuable information in separating the *Scorzonera* taxa distributed in NE Anatolia.

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