

Notes on the stem anatomy of *Scorzonera* (Asteraceae) taxa from Northeast Turkey

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Abstract. Eighteen *Scorzonera* L. (Asteraceae) taxa collected from NE Turkey were examined by light microscopy (LM) in this study in terms of stem anatomy. The anatomical characteristics were numerically analyzed by cluster (CA) and principal component analysis (PCA) based on thirteen traits. Although it has been found that the general stem anatomical traits are very similar, some anatomical characters, such as presence and distribution of secretory cells and channels, arrangement and composition of vascular bundles, cavities in pith and hairs on epidermis were found to be important in delimiting the examined taxa. Additionally, it was also found that binary variables are more important than metric variables in delimiting the *Scorzonera* taxa examined in this study.

Key words: Anatomy, CA, PCA, *Scorzonera*, stem, Turkey

Introduction

Scorzonera L. s.l. (Asteraceae) is the largest genus with 175 taxa in the tribus *Lactuceae* (Bremer 1994). Taxonomically, *Scorzonera* is a very difficult genus consisting of several closely related taxa (Chamberlain 1975). The taxonomic difficulties are mainly due to phenetic plasticity (Bremer 1994), which is not sufficiently investigated by taxonomists (Nazarova 1997). The treatment of the genus has so far undergone considerable changes, while the concept of the genus introduced by Lipschiz (1964) was adapted in many regional Floras (Chamberlain 1975; Chatter 1976; Rechinger 1977). Since the genus *Scorzonera* was revised by Chamberlain (1975) for the *Flora of Turkey*, some new taxa such as *S. ekimi* A. Duran (Duran 2002a), *S. adilii* A. Duran (Duran 2002b), *S. ulrichii* G. Parolly & N. Kilian (Kilian & Parolly 2002), *S. karabelensis* G. Parolly & N. Kilian (Parolly & Kilian 2003), *S. gokcheoglui* O. Ünal (Ünal &

Göktürk 2003), *S. yıldırımlii* A. Duran & Hamzaoğlu (Duran & Hamzaoğlu 2004), and *S. aytachii* A. Duran & M. Sağıroğlu (Duran & Sağıroğlu 2002) have been recorded from Turkey. The genus includes about 49 species in Turkey, part of which are endemic (Hamzaoğlu & al. 2010).

In recent years, karyological (Guardia & Blanca 1987; Nazarova 1997), ethnobotanical (Ertuğ 2000; Rivera & al. 2006), chemical (Magiatis & al. 2001; Zidorn & al. 2003), genetic (D'Amato 2000), phenetic, and taxonomic studies (Bremer 1994; Mavrodiev & al. 2004) have been carried out into *Scorzonera*. Metcalfe & Chalk (1950) reported the general anatomical characteristics of Asteraceae, including some details on the genus *Scorzonera*. The first comprehensive anatomical study of some Turkish *Scorzonera* species was carried out by the first author in his PhD study (Makbul 2006).

Anatomical characteristics are very important in many plant genera, such as *Epilobium* (Makbul & al. 2008) and *Scrophularia* (Makbul & al. 2006) in terms

of their systematics, but there is no detailed anatomical data on the Turkish *Scorzonera* taxa. Thus the aim of the present study is to investigate the stem anatomy of the *Scorzonera* taxa distributed in NE Anatolia and to explore the systematic importance by means of numerical methods.

Material and methods

The plants were collected from Northeast Anatolia between 2003 and 2006 (Fig. 1). 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 material for anatomical study was fixed in FAA (Formaldehyde: Acetic Acid: Alcohol) for 24 hours and then preserved in ethanol (70 %). All observations were made on transverse sections of stem manually cut. All sections were stained with hematoxylen for 30 minutes and mounted with glycerine-gelatine, in order to obtain permanent slides (Vardar 1987). The well-stained sections were photographed with a digital photo-camera attached to

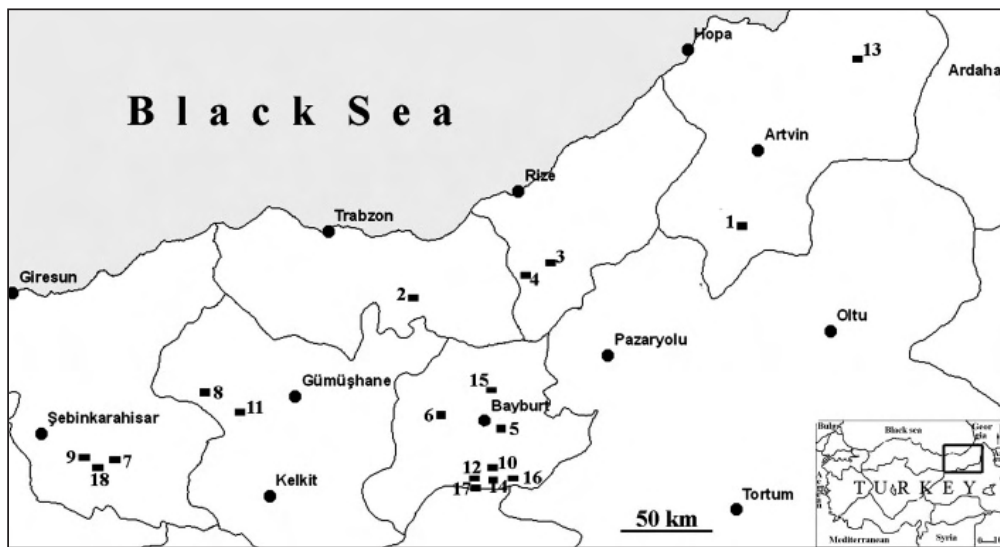


Fig. 1. Distribution map of the examined taxa (for the taxa numbers see Table 1).

Table 1. Locality information about the examined taxa.

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

an Olympus BX51 light microscope. A raw data matrix for the numerical analysis was created by using the average measurements and observations (Table 2). These measurements and observations were repeated at least three or four times for all the examined taxa.

Thirteen characters related to stem anatomy shown in Table 2 were assessed by numerical analysis. Three characters (X7, X8, and X13) were quantitatively scored as 0 or 1, and the remaining ten characters were quantified including linear measurements and numbers (Table 2). Two multivariate analyses were performed by Syn-Tax PC 5.0 (Podani 1993): cluster analysis (CA) and principal components analysis (PCA). For CA (UPGMA), a pair-wise matrix of resemblance values was calculated from raw standardized data matrix, using Gower's coefficient of resemblance designed for mixed data sets (Sneath & Sokal 1973). A dendrogram was generated by the unweighted pair-group method by using arithmetic averages (UPGMA). In order to determine how well this dendrogram represented the underlying matrix of resemblances, a cophenetic correlation coefficient (rcs) was also calculated. The simplicity of rcs has led to its extensive application (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.

Anatomical results

Stem anatomic features of the examined taxa based on transverse sections of the stem are given in Figs 2-3. All detailed measurements related to stem anatomy are given in the Appendix.

The epidermal tissue consists of a single row of rectangular or orbicular cells in all examined taxa. Although some of the epidermal cells are covered with dense simple (tomentose) or branched hairs, the others are generally smooth. The collenchyma is generally located very close to the epidermis, with one or two to three rows, but the row numbers vary, especially close to the vascular bundles. The cortex consists of usually parenchymatic oval cells, with thin walls, but their size and average row number differ among the examined taxa. The vascular bundles, interconnected with an intravascular sclerenchyma, formed one or two to three continuous rings in the stem of the examined taxa. Secretory cells are located very close to the phloem tissue, but their presence and distribution varies among the examined taxa. Similarly, scleranchymatous cells are present in the phloem and xylem. There is also large pith in the stem centre, but the presence and distribution of cavity and latex channels differ among the examined taxa.

Table 2. List of characters used in numerical analysis.

Symbol	Characters	Unit/Score
X ₁	Width/length of epidermal cells	µm/µm
X ₂	Average row number of collenchyma across the bundles	number
X ₃	Width/length of collenchyma cells	µm/µm
X ₄	Average row number of cortex parenchyma	number
X ₅	Width/length of cortex cells	µm/µm
X ₆	Width of cortex	µm
X ₇	Arrangements of the bundles	One ring: 0; more than one or disordered: 1
X ₈	Secretory cells on phloem	presence:1; absence:0
X ₉	Average row number of trachea	number
X ₁₀	Average trachea number for every row	number
X ₁₁	Diameter of trachea	µm
X ₁₂	Diameter of pith cells	µm
X ₁₃	Latex channels	presence:1; absence:0

Numerical results

A dendrogram resulting from UPGMA based on 13 stem anatomical traits is shown in Fig. 4. As seen in Fig. 4, all investigated taxa fall into two main clusters. The first group of species, labeled "a" and linked to each other at 74.7% dissimilarity level, consists of *S. latifolia*, *S. cinerea* and *S. tomentosa*. The second cluster, labeled "b" and divided into smaller clusters, includes all the remaining taxa. PCA results based on 13 selected stem traits are given in Fig. 5. This figure shows the distribution of taxa and the variables of the first two components. Only the first three components were taken into account because of their eigenvalues. The eigenvalues of the first three components in percentages, based on ten variables, are as follows: the first, second and third component account for 38.54%, 16.31%, 11.87%, respectively (Table 3).

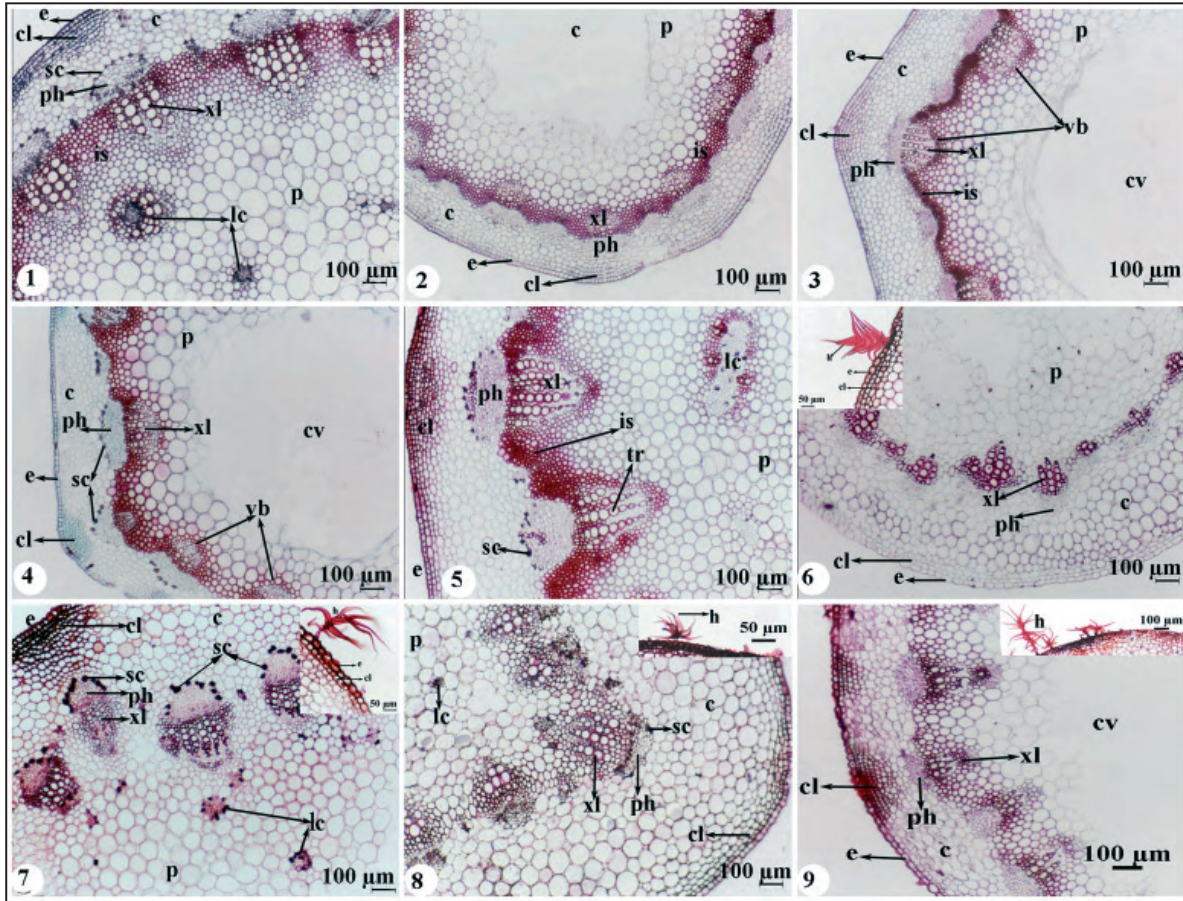


Fig. 2. Cross sections of stem (for the taxa numbers see Table 1). Abbreviations: c – cortex, cl – collenchyma, cv – cavity, e – epidermis, h – hair, lc – latex channel, is – intravascular sclerenchyma, p – parenchyma, ph – phloem, phs – phloem sclerenchyma, sc – secretory cell, xl – xylem, xls – xylem sclerenchyma, tr – trachea, vb – vascular bundle.

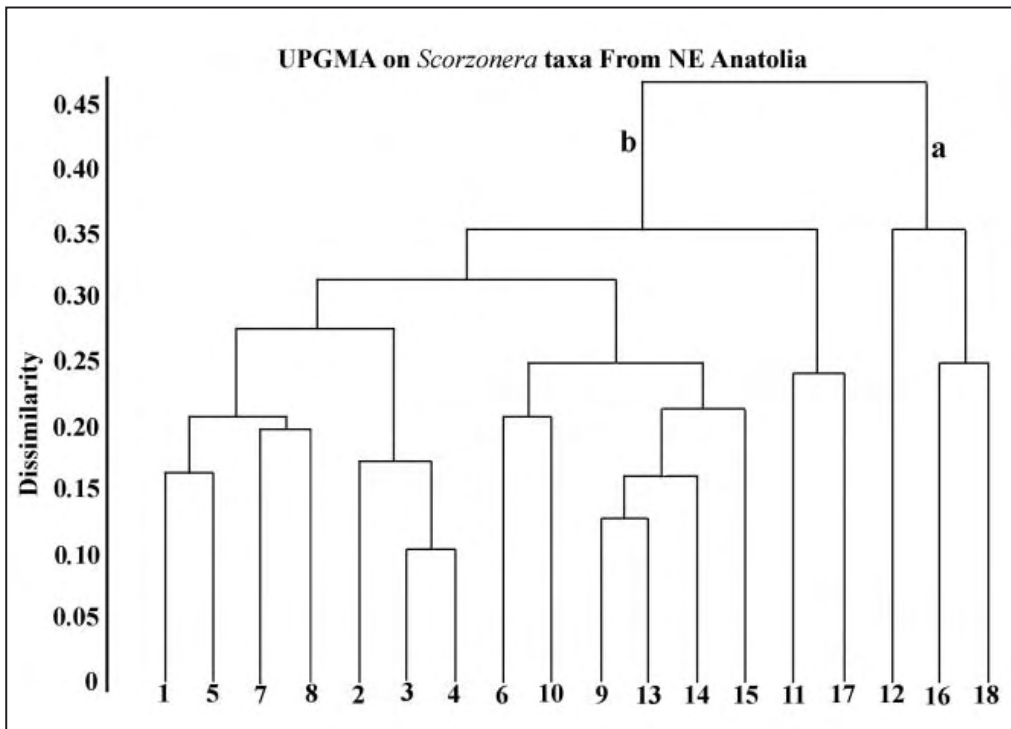


Fig. 4. Cluster analysis – UPGMA (for the taxa numbers see Table 1).

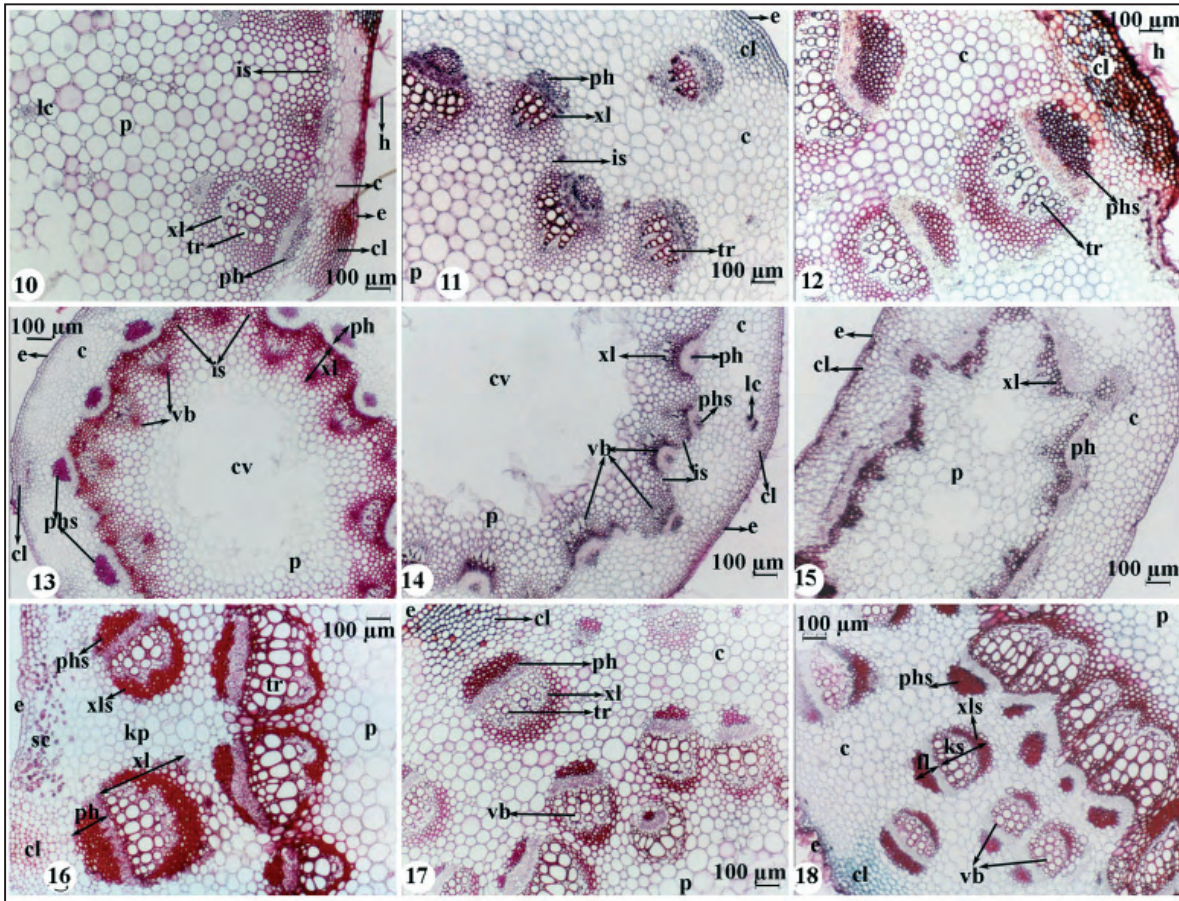


Fig. 3. Cross sections of stem (for the taxa numbers see Table 1; for the abbreviations see Fig. 2).

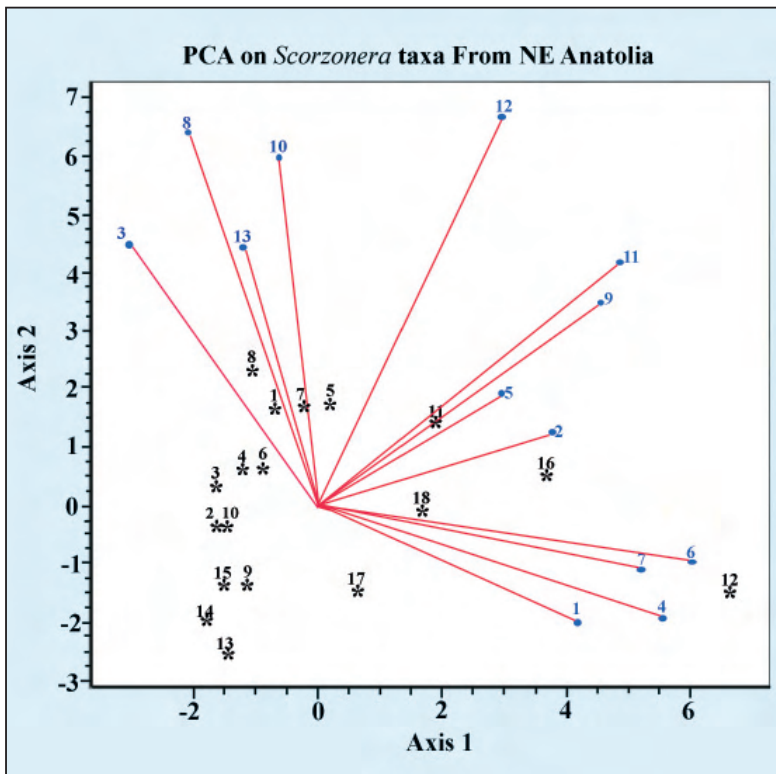


Table 3. Percentage of variance accounted for by the first three components.

	PC1	PC2	PC3
Percentage of variance explained	38.54	16.31	11.87
Cumulative of variance explained	38.54	54.85	66.73

Fig. 5. Principal component analyses of 18 taxa and 13 variables projected onto the axis 1 and axis 2 (for the variable number explanations see Table 2).

Discussion

Internal stem structure of 18 *Scorzonera* taxa distributed in NE Anatolia were explored in this study, in order to explain the systematic value of anatomical traits and provide useful information for the systematics of *Scorzonera* taxa.

Plant systematics is a broad discipline, which includes the study of evolutionary relationships between plant species. Species identification is a typical first step in analyzing botanical evidence for casework. Plant anatomy uses several features of stem, leaf, and fruit and seed internal morphology to help in species identification. Thus plant anatomy has been used in some cases to differentiate the species, although it has traditionally proven to be most useful at genus level and higher. The most fundamental work in plant anatomy is *Anatomy of Dicotyledons* written by Metcalfe & Chalk (1950). However, there are many sources in literature which stress the significance of anatomy in plant taxonomy (Carlquist 1961; Özörgücü & al. 1991; Dickison 2000; Lersten & Curtis 2001; Makbul & al. 2006, 2008). As indicated in the above-mentioned literature, we have found that the presence and distribution of latex channels and secretory cells, collenchyma and sclerenchyma features are particularly important in the examined taxa.

These taxa were treated under the subgenera of *Scorzonera* by Lipschiz (1964). While *S. seidlitzii* and *S. latifolia* were grouped in the same clade, according to ITS sequence data by Mavrodiev & al. (2004) and according to caryological data (Nazarova 1997), these two taxa were clustered in a different group based on stem anatomical characteristics (Fig. 4). This shows that anatomical traits can be useful in delimiting these two taxa. However, all subspecies of *S. cana*, *S. laciniata* subsp. *laciniata* and *S. armeniaca* were aggregated in the same cluster, as indicated by Mavrodiev & al (2004), on the basis of the ITS sequence data. However, the results based on phenetic features reported by Makbul (2006) were in accordance with the present findings.

It is well known that different types of anatomical traits provide numerous significant characters at different taxonomical level (Carlquist 1961). Yentür (2003) indicated that arrangement of bundles provides valuable information in comparative anatomy. In the present study, the examined taxa fall into two clusters, according to the number of the bundle rings. While in *S. cinerea*, *S. latifolia* the bundles are arranged in two

rings, or disordered in *S. tomentosa*, in the rest of taxa they are arranged in one ring.

The non-glandular and glandular hairs as a micro character of leaves could be occasionally used in the classification, especially at generic and specific level (Kallersjö 1986; Mukherjee & Sarkar 2001). Together with other characters, hairs can be important in taxonomic and phylogenetic studies of Asteraceae (Ciccarelli & al. 2007). In the present study, simple tomentosa hairs occur in the stem of *S. tomentosa*, *S. latifolia*, *S. seidlitzii*, *S. sericea*, *S. cinerea*, and *S. eriophora* (Fig. 3: 12, 14, 18); branched hairs occur in the stem of *S. incisa*, *S. inaequiscapa*, *S. mollis* subsp. *mollis*, and *S. mollis* subsp. *szowitzii* (Fig. 2: 7, 8, 9 and Fig. 3); and imbricate hairs in the stem of *S. suberosa* (Fig. 2: 6). The stem surface of the other taxa is without hairs.

It is well known that the position and the average row number of collenchyma tissue in plants are important for the comparative anatomical studies (Özörgücü & al. 1991). In the present study, it was also determined that the average row number of collenchyma across the main bundles differs among the examined taxa.

All investigated taxa fall into two groups based on the presence of secretory cells in the phloem. While *S. laciniata* subsp. *laciniata*, *S. cana* var. *jacquiniana*, *S. cana* var. *cana*, *S. cana* var. *alpina*, *S. armeniaca*, *S. mollis* subsp. *mollis*, and *S. mollis* subsp. *szowitzii* consist of secretory cells adjacent to the phloem, the others do not. Moreover, there are distinct sclerenchymatic cells in the phloem of *S. armeniaca*, *S. incisa*, *S. eriophora*, *S. cinerea*, *S. seidlitzii*, *S. sericea*, *S. latifolia*, *S. sosnowskyi*, and *S. tomentosa*. Baran and Özdemir (2006) determined that the secretory sclerenchymatous cells in the phloem supply additional taxonomic information in grouping the different plant taxa.

According to Metcalfe and Chalk (1950), laticiferous channels are commonly observed in the structure of leaves of species belonging to the Asteraceae. Latex channels are very important in the comparative anatomical studies and their contents and distribution differ among the Asteraceae members (Milan & al. 2006). In our study, there were secretory channels with varying size and density in the pith of *S. laciniata* subsp. *laciniata*, *S. armeniaca*, *S. suberosa*, *S. mollis* subsp. *mollis*, *S. mollis* subsp. *szowitzii*, *S. incisa*, *S. eriophora*, *S. sericea*, and *S. sosnowskyi*.

The cophenetic correlation coefficient (r_{cs}) of the dendrograms from UPGMA was calculated as 0.68,

which means that the dendrogram provides accurate representation of the resemblances. It generally varies from 0.6 to 0.95 (Sneath & Sokal 1973).

As it is seen in Fig. 4, all investigated taxa fall into two major clusters, at 0.45 dissimilarity level based on the raw anatomical data matrix. One, labelled “a” and consisting of three species, belongs to the caulescent group (*S. cinerea*, *S. latifolia* and *S. tomentosa*), while the other, labelled “b”, includes all remaining species representing the caulescent, scapigerous or subscapigerous taxa. As it is seen in Fig. 4, all representatives of the caulescent taxa are not grouped in the same cluster. While *S. sosnowskyi* occurs in group “b”, including only caulescent taxa, the other caulescent taxa (*S. cinerea*, *S. latifolia* and *S. tomentosa*) occur in cluster “a”, with the other scapigerous and subscapigerous taxa. When carefully examined, the dendrogram shows that stem anatomical results from UPGMA generally support the morphological delimitation reported by Chamberlain (1975).

While the first group consists only of caulescent taxa (*S. cinerea*, *S. latifolia*, *S. tomentosa*), the second group including the rest of the taxa is divided into three subgroups. As it is seen in Fig. 4, morphologically closely related taxa (Makbul 2006) are easily differentiated by means of some stem anatomical traits, such as secretory cells in the cortex, position of the vascular bundle and phloem and xylem sclerenchyma features. *S. sosnowskyi* is closely related to *S. latifolia*, according to Chamberlain (1975), but the present results from UPGMA differentiate these taxa at specific level. Cluster “b” also divides into two subgroups. While the first one consists of two caulescent taxa (*S. sosnowskyi* and *S. eriophora*), the second consists of caulescent and scabous taxa. All varieties of *S. cana* are aggregated in a single subgroup (Fig. 4). This shows that all representatives of *S. cana* are very similar on the basis of anatomical traits. According to Chamberlain (1975), *S. cana* is very close to *S. laciniata* subsp. *laciniata*, but they are differentiated from each other in terms of some stem anatomic characters, such as the row number of collenchyma and the presence of latex channels. Chamberlain (1975) also indicated that *S. armeniaca* and *S. laciniata* subsp. *laciniata* are closely related taxa, which was confirmed presently by UPGMA. Similarly, the subspecies of *S. mollis* have been aggregated closely into a small cluster, which means that the stem anatomical traits are not effective for delimiting the two examined subspecies.

Chamberlain (1975) and Makbul (2006) indicated that *S. suberosa* and *S. inaequiscapa* are two closely related taxa on the basis of morphological traits. But, these taxa have been located in different subgroups on the basis of stem anatomic features, as seen in Fig. 4. The main difference between these two taxa is the position of the collenchyma in the stem. The collenchyma is present only across the vascular bundles in *S. inaequiscapa*, but is homogeneously distributed in the cortex tissue of *S. suberosa*. This means that stem anatomical traits can also be useful to distinguish these two related species.

The results of PCA analysis of 18 taxa based on 13 variables is given in Fig. 5 and Table 3. The first, second and third components claim 38.54%, 16.3% and 11.87%, respectively. Together the three components account for 66.73% of the total variation. While the first component accounts for 38.54% of the variation, the second component accounts for 16.31%, and the third component for 11.87%. This shows that the first three components explain most of the total variations among the examined *Scorzonera* species on the basis of 13 stem anatomical characters. PCA analysis has also shown that some of the 13 examined traits are important in delimiting the examined *Scorzonera* taxa. These variables include the presence of latex channels, diameter of the trachea, presence of secretory cells on the phloem, and arrangement of bundles. In conclusion, the stem anatomical features supply additional information for differentiating the examined *Scorzonera* taxa in the present study. The positions of the 18 examined taxa in the first two components of PCA are given in Fig. 5. It could be seen that all taxa examined in this study split into three clusters. These clusters are related to the results from UPGMA, except for *S. cinerea*. As it is seen in Fig. 4, *S. cinerea* is nested in the sub-cluster of *S. latifolia* and *S. tomentosa*, but it is located in a different position in the biplot of PCA (Fig. 5). This situation could be explained by the presence of intense sclerenchymatic cells in the phloem tissue of *S. cinerea*. There is no other contradiction between PCA and UPGMA in the distribution of OTUs.

Conclusion

In conclusion, palynological (Blackmore 1982), cytological (Nazarova 1997; Owen & al. 2006) and molecular data (Mavrodiev & al. 2004; Owen & al. 2006) have proved themselves useful in investigating the in-

tra- and intertaxa relationships in *Scorzonera* s.l. Similarly, anatomical data can be also used in delimiting the investigated *Scorzonera* taxa at specific level. The present study, which is a preliminary step in the analysis of anatomical characters, has provided results that basically agree with the traditional taxonomic treatments of *Scorzonera*. Also, binary anatomical characters seem to be more important than quantitative ones in differentiating the *Scorzonera* species. Among these anatomical traits, the presence of latex channels and secretory cells is of greatest importance. The result of anatomical data will also contribute to filling in the gaps in the knowledge of Turkish *Scorzonera*.

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