



Article

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AN ANATOMICAL STUDY OF *Scilla* (SCILLOIDEAE) SECTION *Chionodoxa* AND *Scilla bifolia* IN TURKEY¹

Um Estudo Anatômico de Chionodoxa Incluída na Scilla (Scilloideae) e Scilla bifolia na Turquia

ABSTRACT - In the present study *Scilla luciliae*, *S. forbesii*, *S. sardensis*, *S. siehei*, *Scilla x allenii* and *S. bifolia* are compared anatomically. Some differences have been found in root, scape, and leaf anatomy of the taxa, and commented. *S. luciliae*, *S. forbesii*, *S. sardensis* and *S. siehei* have a metaxylem at the center of the root, others have 3-4 number metaxylem. Vascular bundles in two row in *S. luciliae* and *S. forbesii* in a single row in *S. sardensis*, *Scilla x allenii* and *S. bifolia* though in three rows in *S. siehei*. Aerenchyma tissue is present in mesophyll of five taxa leaf except *S. sardensis*. The anatomical variations in the taxa have been investigated by means of numerical methods (Analysis of variance and Pearson correlation). By the analysis of the investigated taxa from 12 anatomy related characters, it has been also found that the results from numerical analysis of anatomy characters can provide additional evidences, which correspond to the anatomy for the recognition of the taxa.

Keywords: anatomy, *Scilla* section *Chionodoxa*, statistics.

RESUMO - No presente estudo, *Scilla luciliae*, *S. forbesii*, *S. sardensis*, *S. siehei*, *Scilla x allenii* e *S. bifolia* foram comparadas anatomicamente. Algumas diferenças foram encontradas na raiz, no escapo e na anatomia foliar de táxons e, então, comentadas. *S. luciliae*, *S. forbesii*, *S. sardensis* e *S. siehei* possuem um metaxilema no centro da raiz, e as outras têm 3-4 metaxilemas. Há feixes vasculares de duas linhas em *S. luciliae* e *S. forbesii* e de linha única em *S. sardensis*, *Scilla x allenii* e *S. bifolia*, embora em *S. siehei* eles sejam de três linhas. Há presença de tecido aerênquima no mesofilo de folhas de cinco táxons, exceto de *S. sardensis*. As variações anatômicas nos táxons foram analisadas por meio de métodos numéricos (análise de variância e de correlação de Pearson). Pela análise dos táxons investigados, a partir de 12 caracteres relacionados anatomicamente, foi também descoberto que os resultados de análise numérica de caracteres anatômicos podem fornecer evidências adicionais correspondentes à anatomia para o reconhecimento dos táxons.

Palavras-chave: anatomia, *Chionodoxa* incluída na *Scilla*, estatísticas.

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INTRODUCTION

Chionodoxa and *Scilla* are close related genera of Liliaceae. *Chionodoxa* and *Scilla* transferred into subfamily Scilloideae of Asparagaceae family within the scope of APG III (Angiosperm Phylogeny Group) system (APG III, 2009; Chase et al., 2009; Reveal and Chase, 2011). In Flora of Turkey and the East Aegean Islands Volume 8, *Scilla* and *Chionodoxa* were evaluated and revised as two separate genera (Davis, 1984). According to the last world monocot checklist *Scilla* sect. *Chionodoxa* is represented with six species worldwide, 3 species of those are native to Turkey (*S. luciliae*, *S. sardensis*, *S. forbesii*, one species to Cyprus (*S. lochiae*) and 2 species to Crete (*S. nana* and *S. albescens*) (Govaerts, 2014). Davis (1984) treats *S. siehei* as a synonym of *S. forbesii*. In the scope of a Ph.D. thesis study by the first author the taxa of *S. sect. Chionodoxa* in Turkey have been revised between 2004–2010 based on morphological, molecular, and ecological data and the reproductive system (Yildirim, 2010). According to these results, *S. sect. Chionodoxa* is represented in Turkey by *S. luciliae*, *S. forbesii*, *S. sardensis* and *S. siehei*. Moreover, a natural hybrid (*Scilla* x *allenii*) between *S. siehei* and *S. bifolia* was found in this study. Except for *S. bifolia*, all other species are local endemic plants. Although, *S. siehei* (only growing in Nif Mountain), *Scilla* x *allenii* (only growing in Nif Mountain), *S. sardensis* (only growing in Mahmut Mountain) and *S. luciliae* (only grows in Bozdag Mountain) are distributed along the Bozdag Mountain series in Izmir Province, *S. forbesii* (only grows in Babadag Mountain) is distributed in Mugla. However, *Scilla bifolia* is a very common species distributed in several countries, Turkey, the Caucasus, Iraq, Iran, Syria along the danube river up to central Europe.

In this paper, the species of *S. sect. Chionodoxa* (*S. luciliae*, *S. forbesii*, *S. sardensis* and *S. siehei*), *S. bifolia* and the natural hybrid *S. allenii* are compared on the base of some anatomical features.

MATERIALS AND METHODS

Taxa have been collected from the following natural habitates by Hasan Yildirim during field studies for his thesis (Table 1).

Table 1 - Collection Sites of the *Scilla* taxa

Taxon	Collection Site	Date	Coordinates					Collector
<i>S. sardensis</i>	İzmir, Mahmut Mountain	18.03.2009	Locality 1, N: 38° 17' 41.0",					Hasan Yıldırım
<i>S. luciliae</i>	İzmir, Ödemiş Bozdag	16.05.2009	Locality 1, N: 38° 19' 25.32", E: 28° 06' 06.93", h: 2133 m	Locality 2, N: 38° 19' 57.28", E: 28° 06' 40.79", h: 1522 m	Locality 3, N: 38° 20' 02.36", E: 28° 06' 02.36", h: 1774 m	Locality 4, N: 38° 19' 01.66", E: 28° 06' 09.01", h: 1885 m		Hasan Yıldırım
<i>S. forbesii</i>	Muğla, Fethiye Babadağ	23.03.2009	Locality 1, N: 36° 31' 46.3", E: 029° 11' 04.9", h: 1908 m	Locality 2, N: 38° 21' 47.1", E: 027° 32' 33.3", h: 350 m	Locality 3, N: 38° 21' 00.4", E: 027° 30' 20.0", h: 1246 m	Locality 4, N: 38° 20' 15.3", E: 027° 30' 29.2", h: 1120 m	Locality 5, N: 36° 32' 20.5", E: 029° 10' 14.1", h: 1711 m	Hasan Yıldırım
<i>S. siehei</i>	İzmir, Kemalpaşa Nif Mountain	17.03.2009	Locality 1, N: 38° 23' 28.8", E: 027° 21' 33.0", h: 1434 m	Locality 2, N: 38° 22' 59.0", E: 027° 21' 31.1", h: 1372 m	Locality 3, N: 38° 23' 08.2", E: 027° 22' 07.9", h: 1174 m	Locality 4, N: 38° 22' 54.7", E: 027° 22' 19.2", h: 1097 m	Locality 5, N: 38° 23' 17.8", E: 027° 22' 20.1", h: 1013m	Hasan Yıldırım
<i>Scilla</i> x <i>allenii</i> (Hybrid)	İzmir, Kemalpaşa Nif Mountain	17.03.2009	Locality 1, N: 38° 23' 08.2", E: 027° 22' 07.9", h: 1174 m	Locality 2, N: 38° 22' 54.7", E: 027° 22' 19.2", h: 1097 m	Locality 3, N: 38° 23' 17.8", E: 027° 22' 20.1", h: 1013 m	Locality 4, N: 38° 22' 15.1", E: 027° 19' 30.3", h: 878 m		Hasan Yıldırım
<i>S. bifolia</i>	İzmir, Kemalpaşa Nif Mountain	17.03.2009	Locality 1, N: 38° 23' 28.8", E: 027° 21' 33.0", h: 1434 m	Locality 2, N: 38° 23' 08.2", E: 027° 22' 07.9", h: 1174 m	Locality 3, N: 38° 22' 54.7", E: 027° 22' 19.2", h: 1097 m			Hasan Yıldırım

Scilla siehei, *S. forbesii*, *S. sardensis*, *S. luciliae* and *Scilla x allenii* are local endemic plants that are only known from type localities. Each of these taxa have just one single population. For this reason, we used material obtained from subpopulations at different altitudes from each taxon population. Although, *S. bifolia* is a very common species, we used its Nif Mountain population, because of the hybrid taxon *Scilla x allenii* occurring nearby.

A total of 30 individuals collected from subpopulations at different altitudes were used. For anatomical studies plant specimens were fixed in 70% ethanol. The paraffin method (Algan, 1981) was used for preparing the cross-sections of root, scape and leaves for each sample. Roots of the same diameter have been used for cross-sections. Transverse sections 15-20 μm were made using a sliding microtome. Slides stained with safranin-fast green. Slides were photographed with motorized Leica DM 300 microscope. Species root, stem, and leaf cell size were measured using an ocular-micrometer. Minimum, maximum, mean, and standard deviation were determined.

RESULTS AND DISCUSSION

Root

All taxa show a one layered rhizodermis. *Scilla bifolia* has a 11-13 layered cortex, whereas the others have only a 6-8 layered root cortex. *Scilla luciliae*, *S. forbesii*, *S. sardensis* and *S. siehei* have a metaxylem vessel in the center of the root, while others have 3-4 metaxylem vessels. The endodermis cell wall of all taxa is thickened on all 4-sides o-type endodermis. *Scilla luciliae*, *S. forbesii*, and *S. siehei* have five xylem strands, while *S. sardensis*, *Scilla x allenii* and *S. bifolia* have six xylem strands (Figure 1 and 2). Also, root cross-sections of *S. siehei*, *Scilla x allenii* and *S. bifolia* have raphide crystals in cortex.

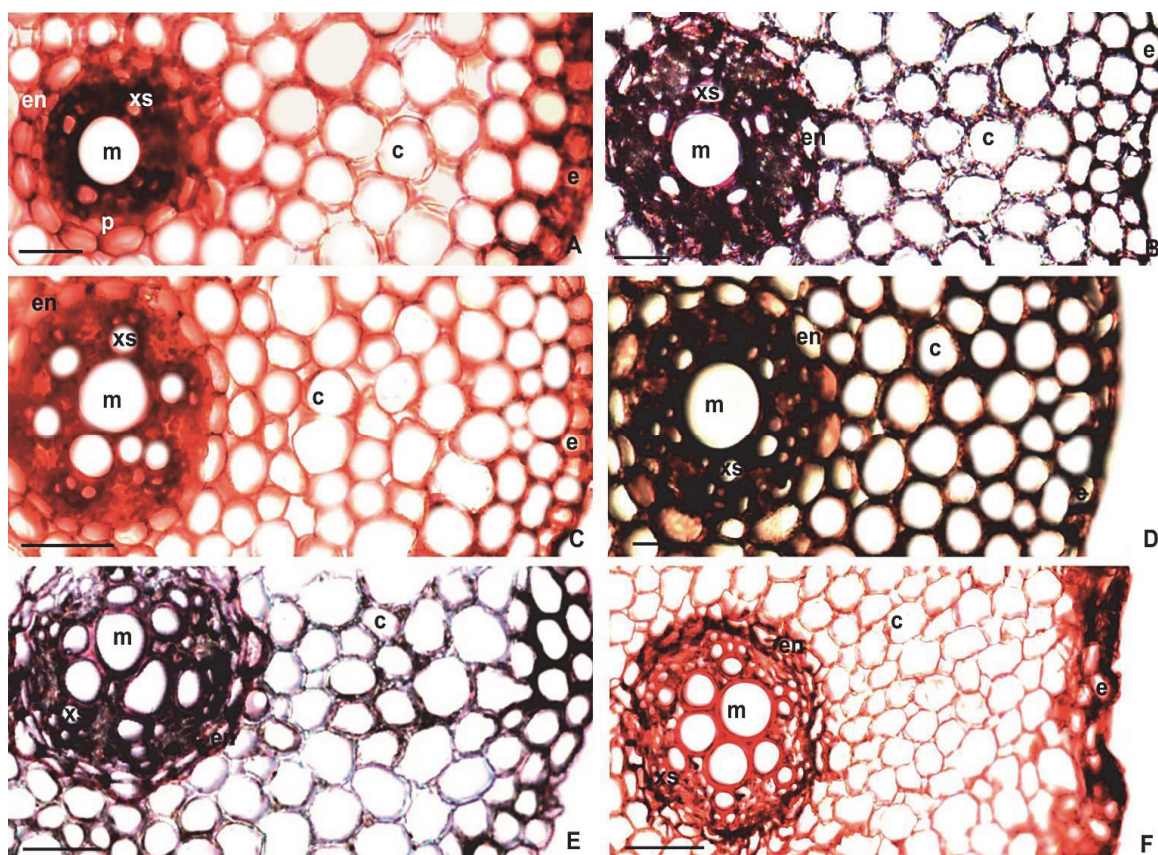


Figure 1 - Root cross-section of taxa, A: *S. luciliae*, B: *S. forbesii*, C: *S. sardensis*, D: *S. siehei*, E: *Scilla x allenii*, F: *S. bifolia*; c: cortex, e: epidermis, en: endodermis, m: metaxylem, p: pericycle, xs: xylem strands; Scale Bar: 50 μm .

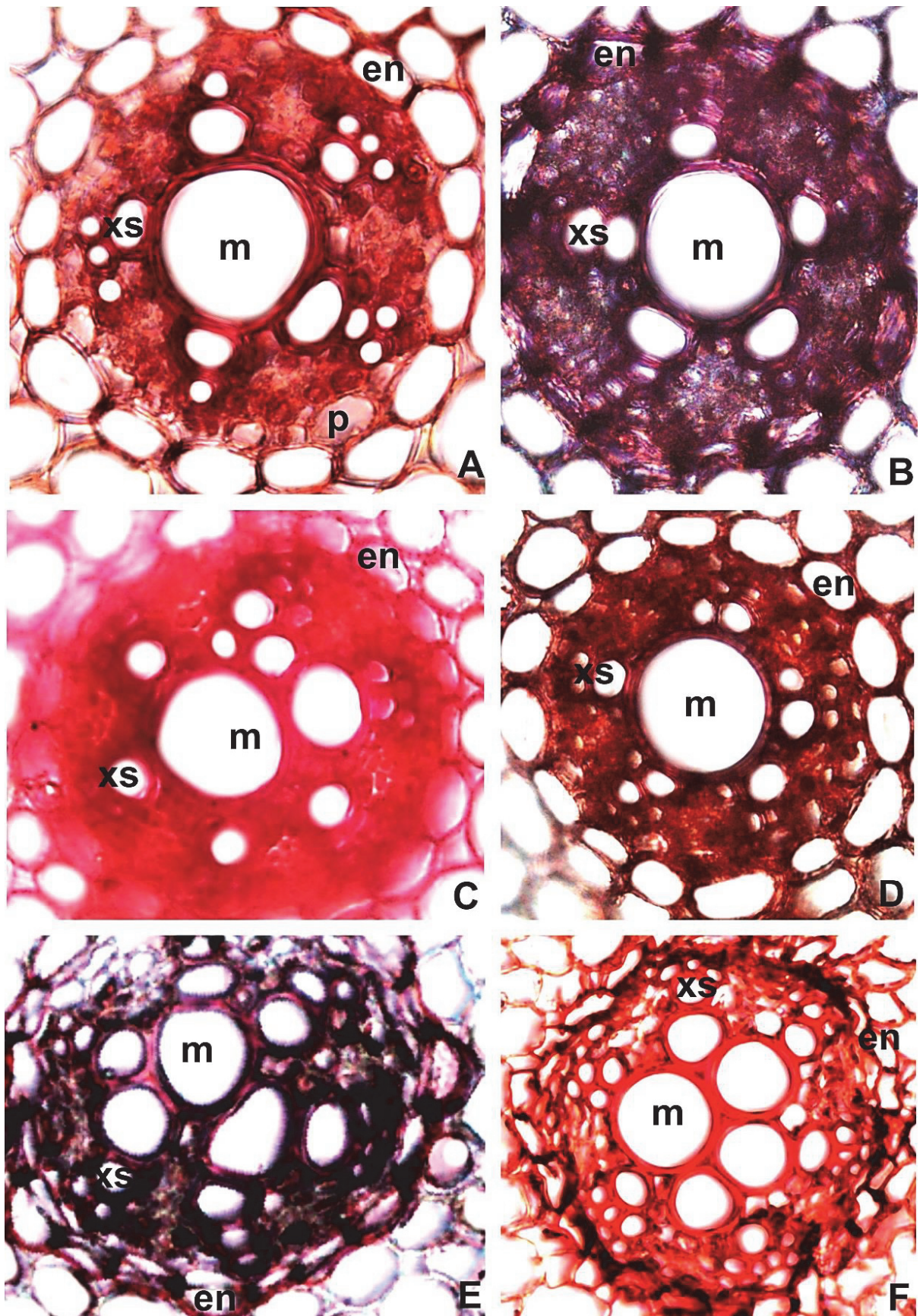


Figure 2 - Root cross-section of taxa, A: *S. luciliae*, B: *S. forbesii*, C: *S. sardensis*, D: *S. siehei*, E: *Scilla x allenii*, F: *S. bifolia*; en: endodermis, m: metaxylem, p: pericycle, xs: xylem strands.

Scape

The cuticle layer is very thick in *Scilla luciliae*, *S. sardensis*, *S. siehei*, *S. bifolia* and *Scilla x allenii*. However *S. forbesii* has a thinner cuticle than the other species. A single layered epidermis forms projections in the transversal section that form longitudinal ribs in all taxa except *Scilla x allenii*. The cortex is 4-6 layered in all taxa. Vascular bundles are arranged in two circles in *S. luciliae* and *S. forbesii* but in a single circle in *S. sardensis*, *Scilla x allenii* and *S. bifolia*, and in three circles in *S. siehei*. The pith area has a disjunction in *S. luciliae*, *S. forbesii*, *S. sardensis*, *Scilla x allenii* and *S. bifolia* (Figure 3 and 4). *S. forbesii*, *S. sardensis*, *Scilla x allenii* and *S. bifolia* taxa have needle-shaped raphide crystals in scape cross-sections (Figure 6).

Leaf

A cuticle layer is present on both surfaces in all taxa. The abaxial cuticle is thicker than the adaxial one. A prominent stomatal chamber is present in five taxa, except for *Scilla sardensis*. *S. luciliae* and *S. siehei*, which have palisade and spongy parenchyma, others have one type mesophyll cell. Aerenchyma tissue is present in the mesophyll in all taxa except for *S. sardensis*. A bundle sheath is present in leaf of all taxa (Figure 5). *Scilla forbesii*, *S. sardensis* and *Scilla x allenii* taxa have raphide crystals (Figure 6, Table 2).

Statistical analysis

The anatomical measurements of the investigated taxa were shown in Table 2. Difference relevance among the investigated taxa was evaluated by analysis of variance (Regression Analysis) and Pearson correlation (Correlation). The statistical analysis of the results is provided in Tables 3 and 4.

According to both methods of analysis, the differences among the investigated taxa are shown as B-D, B-E, C-F and D-F in Table 2 and Table 3, they are significant at level of 0.01P and 0.05P. Furthermore, a significant difference has been found at levels of 0.05 between A and B according to Table 3 based of Pearson correlation method (Correlation).

In this study, the species of *Scilla* sect. *Chionodoxa*, the closely related species *S. bifolia* and the natural hybrid association between *S. bifolia* and *S. siehei* (*Scilla x allenii*) were compared anatomically.

Considering root cross-sections, some similarities and differences were observed in all taxa. Satil and Akan (2006) have observed metaxylem and 4-5 xylem strands in *S. mesopotamica*, similar to those in *S. luciliae*, *S. forbesii*, and *S. siehei*. Endodermal thickenings are present in *S. mesopotamica* too. Crystals are a constant characteristic of the group. The shape and location of the crystals in plants are very important for taxonomic studies (Metcalfe and Chalk, 1983; Yentür, 1995; Fahn, 1990). Needle-shaped raphide crystals were found in roots of *S. siehei*, *Scilla x allenii* and *S. bifolia*, but they were absent in other studied taxa. *S. mesopotamica* has raphide crystals in root cross-section (Satil and Akan, 2006).

According to Satil and Akan (2006), 7-10 number of vascular bundles in a row in *S. mesopotamica* is similar to *S. sardensis*, *Scilla x allenii* and *S. bifolia* taxa. Vascular bundles in three rows of *S. peruwiana* are similar to *S. siehei*, and those arranged in two of *S. verna*, *S. luciliae* and *S. forbesii* (Rubim et al., 2013). *Scilla x allenii*'s scape shape is less protruding from others. Needle shaped raphide crystals present in *S. forbesii*, *S. sardensis*, *Scilla x allenii* and *S. bifolia*. *S. mesopotamica*'s scape have raphide crystals too (Satil and Akan, 2006).

The presence of cavities and a homogeneous mesophyll is the rule in the taxa of *Hyacinthoides* and *Scilla* (Rubim et al., 2013). In leaf anatomy, stomatal cavity present under the stomata in five taxa, except for *S. sardensis*. Rubim et al. (2013) has reported that *S. monophyllos*, *S. odorata*, *S. ramburei*, *S. verna*, *S. merinoi*. In our study, *S. forbesii*, *S. sardensis*, *Scilla x allenii* and *S. bifolia* taxa have homogeneous mesophyll, while *S. luciliae* and *S. siehei* species have heterogeneous mesophyll. Aerenchyma cavities present in mesophyll in leaves of five taxa except *S. sardensis*. Cavities have been observed in leaves of *S. monophyllos*, *S. odorata*, *S. ramburei*, *S. verna*, *S. merinoi*,

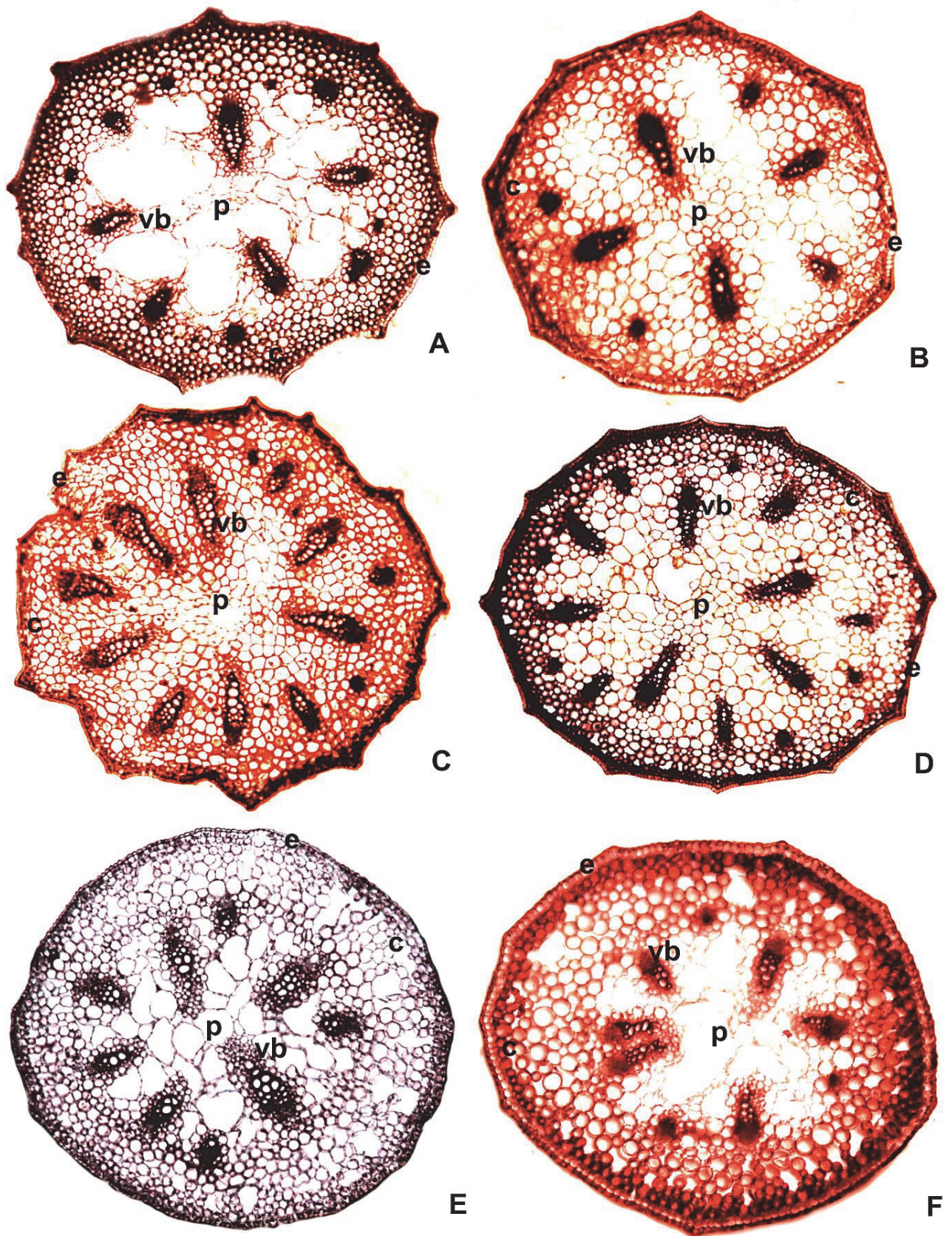


Figure 3 - Scape cross-section of taxa, A: *S. luciliae*, B: *S. forbesii*, C: *S. sardensis*, D: *S. siehei*, E: *Scilla x allenii*, F: *S. bifolia*; c: cortex, e: epidermis, p: pith, vb: vascular bundle.

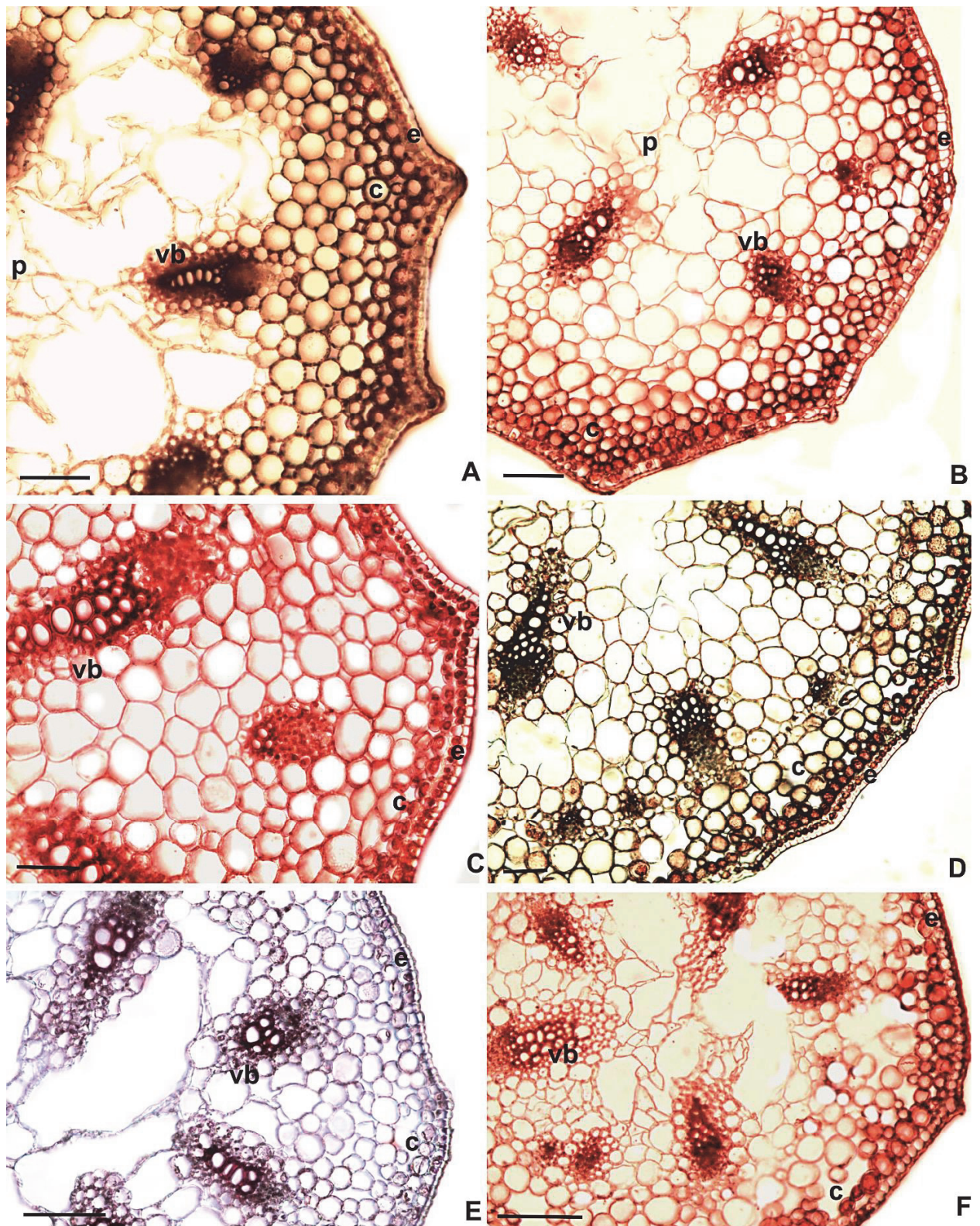


Figure 4 - Scape cross-section of taxa, A: *S. luciliae*, B: *S. forbesii*, C: *S. sardensis*, D: *S. siehei*, E: *Scilla x allenii*, F: *S. bifolia*; c: cortex, e: epidermis, p: pith, vb: vascular bundle; Scale Bar: 100 μ m.

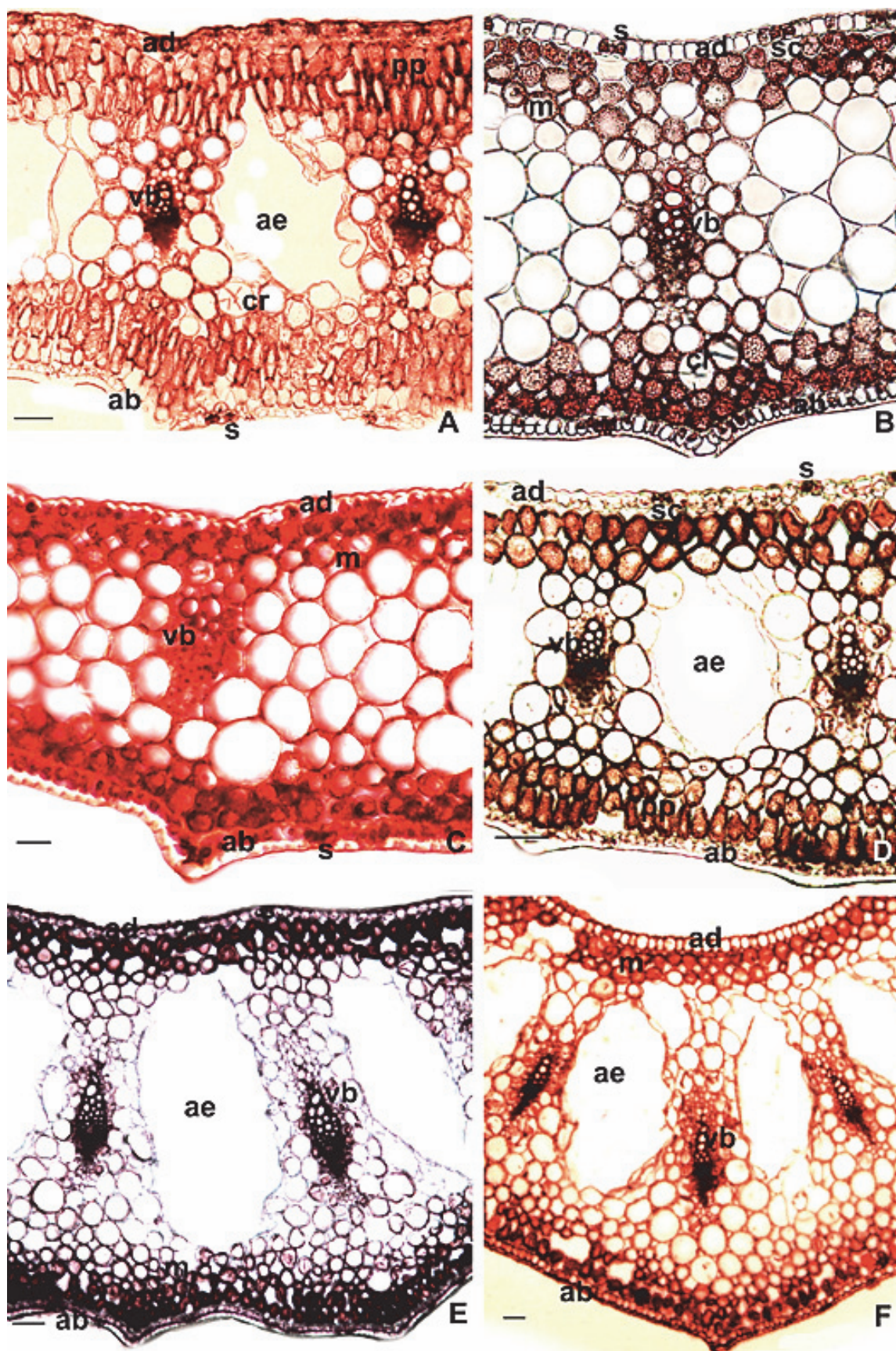


Figure 5 - Leaf cross-section of taxa, A: *S. luciliae*, B: *S. forbesii*, C: *S. sardensis*, D: *S. siehei*, E: *Scilla x allenii*, F: *S. bifolia*; ab: abaxial epidermis, ad: adaxial epidermis, ae: aerenchyma, cr: crystals, m: mesophyll, pp: palisade parenchyma, s: stoma, sc: stomatal cavity, vb: vascular bundle; Scale Bar: 50 μ m.

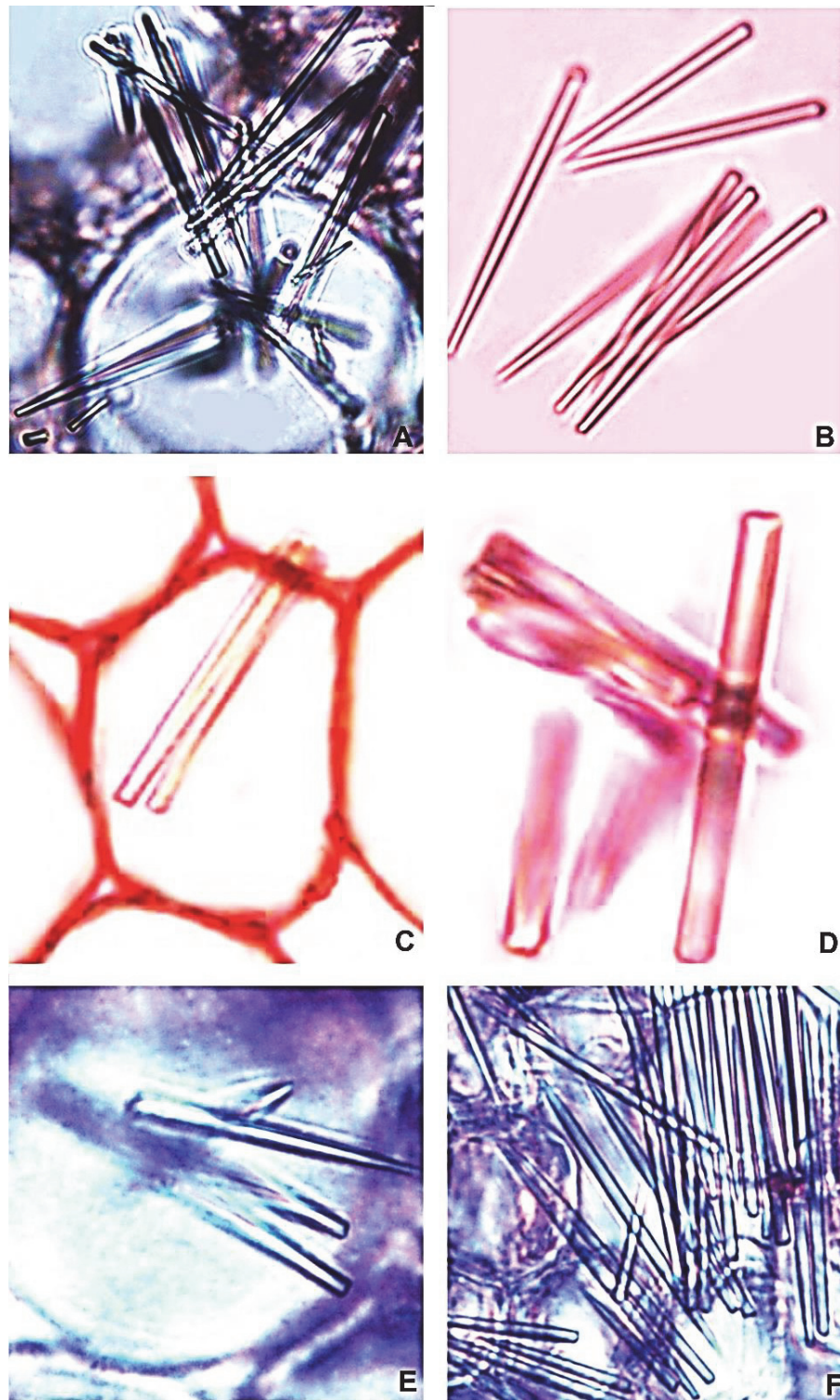


Figure 6 - Raphide crystals A: leaf of *S. forbesii*, B, C: scape of *S. sardensis*, D: leaf of *S. sardensis*, E: leaf of *Scilla x allenii*, F: scape of *Scilla x allenii*.

S. hyacinthoides, *S. lilio-hyacinthus* (Rubim et al., 2013) and *S. mesopotamica* (Satil and Akan, 2006). Bundle sheath is present in leaf of all studied taxa. Satil and Akan (2006) have observed bundle sheath in *S. mesopotamica*, Also they have raphide crystals in leaf cross section of the latter which are similar to *S. sardensis* and *Scilla x allenii* taxa.

As shown in Table 3 and Table 4, there are important correlations between *S. siehei* and *S. bifolia*, which are the two closely related taxa. *Scilla x allenii* is hybrid taxa between *S. siehei*

Table 2 - Anatomical measurements of the studied taxa of *Scilla*

Anatomical characters	A	B	C	D	E	F
	<i>S. luciliae</i> (μm) Mean SD	<i>S. forbesii</i> (μm) Mean SD	<i>S. sardensis</i> (μm) Mean SD	<i>S. siehei</i> , (μm) Mean SD	<i>Scilla</i> \times <i>allenii</i> (μm) Mean SD	<i>Scilla bifolia</i> (μm) Mean SD
Root						
1 - Epidermis width	21.3 \pm 2.51	13.9 \pm 1.70	15.3 \pm 3.79	43.2 \pm 10.6	33.2 \pm 5.56	55.2 \pm 8.50
2 - Epidermis length	32.5 \pm 4.78	24.7 \pm 4.44	16.3 \pm 3.95	54.7 \pm 5.06	44.7 \pm 3.06	37.6 \pm 3.76
3 - Cortex	24.0 \pm 2.77	17.9 \pm 3.01	16.61 \pm 3.58	15.3 \pm 3.17	11.3 \pm 1.16	25.3 \pm 2.10
4 - Metaxylem	22.9 \pm 1.22	29.0 \pm 4.88	17.1 \pm 4.18	17.9 \pm 2.78	10.9 \pm 1.00	10.8 \pm 2.00
Scape						
5 - Epidermis width	30.6 \pm 4.09	24.5 \pm 4.14	20.5 \pm 5.21	60.0 \pm 12.2	50.0 \pm 2.25	50.7 \pm 10.2
6 - Epidermis length	26.2 \pm 6.51	7.50 \pm 1.86	13.1 \pm 3.34	35.0 \pm 10.9	25.0 \pm 6.50	25.0 \pm 9.10
7 - Cortex	12.3 \pm 1.98	10.2 \pm 3.03	9.00 \pm 3.97	24.4 \pm 4.96	14.4 \pm 2.66	14.4 \pm 2.00
8 - Tracheae	29.0 \pm 11.9	12.7 \pm 3.28	20.3 \pm 5.28	65.0 \pm 15.0	55.0 \pm 15.5	35.0 \pm 5.01
Leaf						
9 - Adaxial Epidermis width	15.0 \pm 2.35	11.5 \pm 1.76	12.9 \pm 2.79	40.3 \pm 12.9	30.3 \pm 10.0	35.3 \pm 10.9
10 - Abaxial Epidermis length	18.3 \pm 4.87	13.9 \pm 1.23	22.2 \pm 4.62	16.4 \pm 2.79	10.4 \pm 2.09	20.6 \pm 1.75
11 - Adaxial Epidermis width	33.5 \pm 6.30	28.0 \pm 3.94	26.4 \pm 4.07	71.6 \pm 12.4	61.56 \pm 8.40	60.6 \pm 10.0
12 - Adaxial Epidermis length	39.3 \pm 6.27	20.0 \pm 3.27	20.4 \pm 10.6	57.1 \pm 33.8	45.1 \pm 13.6	45.7 \pm 13.6
1-12: Anatomical characters			A-E: Taxon			

Table 3 - Pearson correlation (Correlation) based on anatomical characters of the investigated taxa

Taxa	A	B	C	D	E
B	0.055*				
	0.865				
C	0.216	0.060			
	0.500	0.853			
D	0.089	0.034*	0.290		
	0.784	0.918	0.360		
E	0.089	0.620	0.481	0.192	
	0.783	0.031*	0.113	0.550	
F	0.151	0.183	0.005**	0.727	0.282
	0.639	0.568	0.983	0.007**	0.375

* Significant at the level of 0.05. ** Significant at the level of 0.01.

and *S. bifolia*. It is mentioned that *Chionodoxa* and *Scilla*. are closely related genera of Liliaceae (Mathew, 1987). Also, according to the statistical results found, there is a considerable relation among the species of the two genera, at the level of $P < .01$ and $P < .05$. It has been also found that the results from numerical analysis of anatomy characters can provide distinct evidences, which correspond to the anatomy for recognition of the taxa. By the analysis of the investigated taxa from 12 anatomy characters, it has been determined that metaxylem number in roots and vascular bundle row number in scapes are the best character pairs that represent the variations in them.

Consequently *Scilla* section *Chionodoxa* and *S. bifolia* thought to have originated of the section are similar in many anatomical characters. Meanwhile, some characters such as metaxylem vessel number in roots, vascular bundle row number in scapes, and mesophyll structure in leaves, are useful to taxonomically distinguish these taxa.

Table 4 - The results of Analysis of Variance of 6 investigated *Scillae* taxa (Regression Analysis)

Taxon	MS	F- Value	Probability	Significance
A-B	2.770	0.030	0.860	NS
A-C	42.60	0.490	0.500	NS
A-D	7.190	0.080	0.780	NS
A-E	7.260	0.080	0.740	NS
A-F	20.85	0.230	0.600	NS
B-C	5.700	0.040	0.800	NS
B-D	193.7	8.010	0.030	*
B-E	610.5	6.250	0.030	**
B-F	53.40	0.350	0.560	NS
C-D	205.7	0.920	0.600	NS
C-E	563.7	3.010	0.110	NS
C-F	182.4	8.200	0.050	*
D-E	68.70	0.380	0.550	NS
D-F	983.0	11.20	0.007	**
E-F	102.3	102.3	0.375	NS

MS: Mean square. NS: Not significant. * $P < .05$. ** $P < .01$.

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