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LEAF AND STEM COMPARATIVE ANATOMICAL ANALYSIS OF THREE GENERA OF ALSINOIDEAE (CARYOPHYLLACEAE)

M. Keshavarzi & **S. Esfandani Bozchaloyi**

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Leaf and stem anatomical structures of three genera (8 taxa) of subfamily Alsinoideae (Caryophyllaceae) is provided for the first time from Iran. There are great morphological similarities among some species so there are difficulties in species identification. Leaf and stem anatomical studies of 42 populations from different habitats are used to distinguish different taxa. A total of 42 qualitative and quantitative anatomical characters are studied. Inter and intra-specific relationships among species are evaluated and diagnostic value of the anatomical features are considered. Statistical analysis confirms the diagnostic value of leaf and stem anatomical features in studied taxa.

Maryam Keshavarzi (correspondence<Neshat112000@yahoo.com>), Somayeh Esfandani Bozchaloyi, Biology Dept., Faculty of Science, Alzahra University, Tehran, Iran.

Key words: Anatomy; stem; Leaf; Caryophyllaceae; *Stellaria*; Iran

تشریح مقایسه‌ای ساقه و برگ در سه جنس از زیر تیره Alsinoideae تیره Caryophyllaceae

مریم کشاورزی، دانشیار گروه زیست شناسی دانشگاه الزهرا، تهران.

سمیه اسفندانی بزچلویی، دانشجوی کارشناسی ارشد سیستماتیک اکولوژی دانشگاه الزهرا، تهران.

ساختمان تشریحی ۳ جنس و ۸ تاکسون از زیرتیره Alsinoideae تیره Caryophyllaceae برای اولین بار از ایران گزارش می شود. نظر به وجود شباهت های ریختی بین گونه ها، مشکلات زیادی در جداسازی آنها وجود دارد. تنوع ساختمان تشریحی ساقه و برگ در ٤٢ جمعیت از زیستگاههای مختلف برای تشخیص بهتر تاکسون ها مورد ارزیابی قرار گرفت. در این پژوهش ٤٢ صفت کمّی و کیفی تشریحی مورد بررسی واقع شد. تنوع درون و بین گونه ای مورد ارزیابی واقع شده و ارزش افتراقی خصوصیات تشریحی مورد توجه قرار گرفتند. تجزیه و تحلیل های آماری ارزش افتراقی خصوصیات تشریحی برگ و ساقه را مشخص می کند.

INTRODUCTION

Stellaria L. (Caryophyllaceae Juss., Alsinoideae Beilschm.) consists of about 120 species (Morton 2005) throughout temperate areas worldwide and at higher altitudes in tropical areas. The genus is represented by 9 species in Iran (Rechinger 1988).There are a few worldwide weedy species such as *S. media* (L.) Vill. and *S. graminea* L. Members of this subfamily are distinguished from subfamily Caryophylloideae Arn. by their free sepals and from the subfamily Paronychoideae Meisn. by their non stipulate leaves (Conn 1983, Chater & Heywood 1993, McNeill 1962). The genus is characterized by the presence of five sepals and petals which are usually bifid; however in some species the petals are markedly reduced or absent (Fior et al. 2006, Harbaugh et al. 2010). Jankovic et al. (1982) have studied some leaf morpho – anatomical features of *Stellaria holostea* L., from the sterile shoot. Anatomy of Caryophyllaceae had been considered by Metcalfe and Chalk (1983). Anatomical characters are less influenced by environmental condition than morphological ones (Bokhari 1987). Generally, variation of morphological characters within family Caryophyllaceae makes taxa complicated to be delineated and identified (Fior et al. 2006). Schweingruber (2007) studied the xylem and phloem of 88 Caryophyllaceae from subtropical and temperate regions mainly in Western Europe and the Canary

Islands. He compared his findings with previous taxonomic classification, and assigned them to their ecological range.

Gyorge (2009) studied the adaptive structural and functional strategies which enable plants to survive in variable biotopes. The intensity of some parameters of abiotic factors can be estimated from the environment on the basis of anatomical studies. He considered populations growing in both open and shaded places from mountainous biotopes. The structure of the leaf blade in *Stellaria holostea* grown in open places and in shaded ones was similar. Kilic (2009) investigated anatomical characters in *Silene* from Turkey. Georgescu & Dinu (2010) observed stem anatomical structure of ephemeral plants as *Stellaria media*. Rani et al. (2012) have studied some stem and leaf anatomical features through the pharmacognostical study for quality control of *Stellaria media*. Arora & Sharma (2012) did pharmacognostic and phytochemical studies of *Stellaria media* and showed the presence of epidermis, palisade cells, trichomes and vascular bundles in leaf. Stem anatomical observations revealed the presence of epidermis, hypodermis, cortex, vascular bundles and pith. In present study leaf and stem anatomical observations for 6 *Stellaria* species and two related genera (*Mesostemma kotschyanum* (Fenzl in Boiss.) Vved and *Myosoton aquaticum* (L.) Moench.) has been done to find efficient diagnostic values for better separation of the taxa. *Mesostemma kotschyanum* is considered as the basionym of *Stellaria kotschyanum* Fenzl in Boiss. *Myosoton aquaticum* is sometimes treated as *Stellaria aquaticum* (L.) Scop. Recent molecular studies show that *M. aquaticum* nested within *Stellaria* clades (Mahdavi 2012).

MATERIALS AND METHODS

Forty two populations of 6 *Stellaria* L. and two of its closest relatives have been gathered from different habitats in Iran (Table 1). Some species as *S. graminea, S. persica* and *S. holostea* comprises two stem types as sterile and fertile. Sterile stems are not bearing flowers and here we consider both of these two types of stems. Three individuals of each population were studied anatomically. The species were determined according to various floras (Schischkin 1936; Zohary 1966; Rechinger 1988). Vouchers are deposited at Herbarium of Alzahra University (AUH). Cross sections were taken from the middle part of the leaf blades and stems which were fixed in FAA for 48 hours. Samples were taken from the middle part of the leaf blade and lower part of 2nd internode of stems to have the identical parts to be compared. Cross sections were hand-made and double colored by methyl green 0.1% and carmine 1% for 30 seconds and 15 minutes respectively. We used light microscope with an Olympus DP12 digital camera attached. The quantitative characteristics were measured by UTHSCSA Image tool. The terminology of Cullen (1978) and Stearn (1983) was followed for the general outline of leaf and stem cross sections. For numerical analysis 42 characters were evaluated (Tables 2 and 3). Qualitative characters were coded as multistate characters and means of quantitative characters were used. Analysis of Variance (ANOVA) was applied to detect the significant differences in the studied characters of various taxa. To reveal the species relationships, we used cluster analysis and principal component analysis (PCA) (Ingrouille 1986). In order to determine the most variable anatomical characters among the studied species, a factor analysis based on the principal components analysis was performed. We have used SPSS, ver. 16 software for statistical analysis.

RESULTS AND DISCUSSION

Leaf anatomy

In this research most adopted characters show a significant variation in studied taxa. Sclerenchymatous cells in midrib are evident in *Mesostemma kotschyanum* (Fig. 15) and *S. alsinoides* (Figs. 13 and 14). Mesophyll is isobilateral in *Mesostemma kotschyanum*, in which there are palisade in adaxial and abaxial surfaces and a row of spongy cells between these two layers (Fig. 27). Isobilateral mesophyll is observed in *S. alsinoides* but with spongy layers (Figs. 25 and 26). Dorsiventral type of mesophyll is present in other studied taxa (Figs. 28-36).We observed two types of multicellular trichomes:

Type 1. Acerate hairs with 2-3 cells (present in *S. holostea*) (Figs. 20, 21, 32 and 33).

Type 2. Simple hairs With more than 3 cells, (present in *Mesostemma kotschyanum* subsp. *kotschyanum, S. alsinoides* and *Myosoton aquaticum*) (Figs. 15, 24, 25, 27 and 36). Other taxa don't show hairs but in *S. media* and *S. pallida* there are a lot of papillae on lower leaf surface. Midrib shape is dome shape in *S. graminea*, *S. persica* and *S. holostea* (Figs. 16-21) and arc shaped in *S. media, S. pallida, Myosoton aquaticum, Mesostemma kotschyanum* (Figs. 15, 22, 23 and 24) and *S. alsinoides* (Figs 13 and 14).

Collenchyma at midrib is present in *Mesostemma kotschyanum, S. graminea, S. persica* and *S. holostea* (Figs. 15-21) but is absent in other studied species.

Stem anatomy

There is a single layer epidermis with rectangular cells located close to several parenchymatous layers. Multi-layer sclerenchyma thick and thin walled cells that surround vascular bundle. The pith is hollow or filled

Table 1. Voucher details of *Stellaria* species and relative genera examined in this study.

Species	Locality
Stellaria media (L.)Vill.	Tehran: Vanak, 1461m, Esfandani 993; Tehranpars, 1400m ,Esfandani 994; Evin, 1649m, Esfandani 995; Lavizan, 1500m, Esfandani 996; Milad Tower, 1300m, Esfandani 997; Enghelab sq., 1100m, Esfandani 998; Darband, 1600m ,Esfandani 1012; Shahriyar, 1600m, Esfandani 1016; Pasdaran street, 1500m, Esfandani 1018; Resalat street, 1400m , Esfandani 990; Delavaran street, 1500m, Esfandani 989; Narmak, Hafthouz, 1380m, Esfandani 988; Kohsar, 1580m, Esfandani 986. Alborz: Karaj, 1300m, Esfandani 1000; Karaj- Mardabad, 1280m, Esfandani 1020; Karaj, Chalus road, 1400m, Esfandani 1021; Karaj, Azimiyeh, 1320m, Taghipour 992 Isfahan: City center, 1500m, Esfandani 1010; Hezarjarib, 1600m, Habibi Tirtash 991 Markazi: Arak, Senejan, 1800m, Esfandani 1011 Azerbaijan: Tabriz, City center, 1450m, Habibi Tirtash 1013; University tabriz, 1400m, Habibi Tirtash 1014 Golestan: Gorgan, Gonbadkavous, 134m, Esfandani 1015; Gorgan, Ramian, 200m, Esfandani 1017; Gorgan, Gonbadkavous, 24m, Habibi Tirtash 1022 Mazandaran: Sari, 40m, Esfandani 1019; Ramsar, 65m , Esfandani 1025; Tonekabon, 23m, Esfandani 1029 Gilan: Rezvanshahr, 12m, Esfandani 1023 Qom: Qom, Masoume Shrine, 950m, Esfandani 1024 Qazvin: Avaj, 1900m, Esfandani 1027 Khorassan: Sabzevar, 980m, Esfandani 985
S. pallida (Dumort.) Pire	Golestan: Gorgan, Ramian Road, 200m, Esfandani 1030; Gorgan, Gonbadkavous, 24m, HabibiTirtash 1031
S. holostea L.	Alborz: Karaj, Chalus road, Siahbisheh, 2200m, Esfandani 1032 Gilan: Jirandeh, 1300m , Dadmehr1043
S. persica Boiss.	Alborz: Karaj, Chalus road, Kandovan Tunnel north slopes, 2750-2800m, Esfandani 1035
S. graminea L.	Alborz: Karaj, Chalus road, Kandovan north slopes, 2750-2800m, Esfandani 1036 Ardebil: Neour, 2400m, Esfandani1039
S. alsinoides Boiss. ex Buhse.	Mazandaran: Haraz road, EmamZad-e-Hashem, 2700-2800m, Esfandani 1038
Myosoton aquaticum (L.) Moench.	Gilan: Rezvanshahr, Khoshabireh, 50m, Esfandani 1040
Mesostemma kotschyanum (Fenzl in Boiss.) Vved. Subsp. *kotschyanum*	Tehran: Mountain of Tuchal, near fifth station of Tuchal Telecabin, 2900m, Esfandani 1041

with large thin walled cells. Studied taxa show some variations in their stem cross sections:

Stem cross section shape and size: In *S. persica, S. graminea, S. holostea* (Sterile) cross sections are rectangular (Figs. 3-7), in *Mesostemma kotschyanum* stem cross section is angular-circular (Fig. 2), in *S. alsinoides* rounded and has the smallest stem cross section (340 µm length and 242 µm width) (Fig. 1) and in other species is elliptical including fertile stems of *S. holostea* (Figs. 8-12). *Stellaria media* has the greatest size (2200 µm length and 1300 µm width).

The number of sclerenchymatous and collenchymatous layers in stem: Sclerenchymatous thick-walled cell layers are well developed in *Mesostemma kotschyanum* (5-6 layers) and occupy about 70% of stem radius (Fig. 2) whereas sclerenchymatous thin-walled cell layers are developed in *S. alsinoides* and *S. holostea* and *S. pallida* (2-4 layers) (Figs. 1, 8, 9 and 12).

Four different collenchymas type are observed in studied taxa: In *S. alsinoides, Mesostemma kotschyanum, S. pallida and Myosoton aquaticum* (Figs. 1, 2, 9 and 12) collenchymas are absent. In *S. graminea, S. persica,* (Figs. 3-6) multi collenchymas layers are present in corners without adherence. In *S. holostea* (Fertile) and *S. media* (Figs. 8, 10 and 11) there is a continuum of collenchymas under stem epidermis. In *S. holostea* (Sterile) (Fig. 7) there are collenchymas both under epidermis and at stem corners.

Pith status: Pith in *S. persica*, *S. graminea*, *S. holostea* and *S. alsinoides* is complete (Figs. 1 and 3-7) but in other studied taxa is hollow inside.

Hair type and structure: In *S. holostea* acerated 2-3

Table 2: Qualitative studied anatomical characters (leaf and stem).

No.	Characters	State of characters and their codes
1	Shape of the stem cross-section	1-round 2- elliptical 3- Rounded- elliptical 4-rectangular 5-angularcircular
2	Stem sclerenchyma status	0-absence 1- presence
3	Stem hairs	1-unilateral hair 2- multilateral hair
4	Stem hairs status	0-absence 1- presence
5	Collenchyma under the stem epidermis	0-absence 1- presence
6	Midrib shape of dorsal surface	1-dome- shaped 2-arc-shaped
7	Sclerenchyma nerve status	0-absence 1- presence
8	Midrib collenchyma	0-absence 1- presence
9	Hairs of midrib	0-absence 1- presence
10	Status of palisade cells	1-single-layered 2- multi-layered
11	Hair of dorsal leaf surface	0-absence 1- presence
12	Hair of ventral leaf surface	0-absence 1- presence

Table 3: Quantitative studied anatomical characters (leaf and stem).

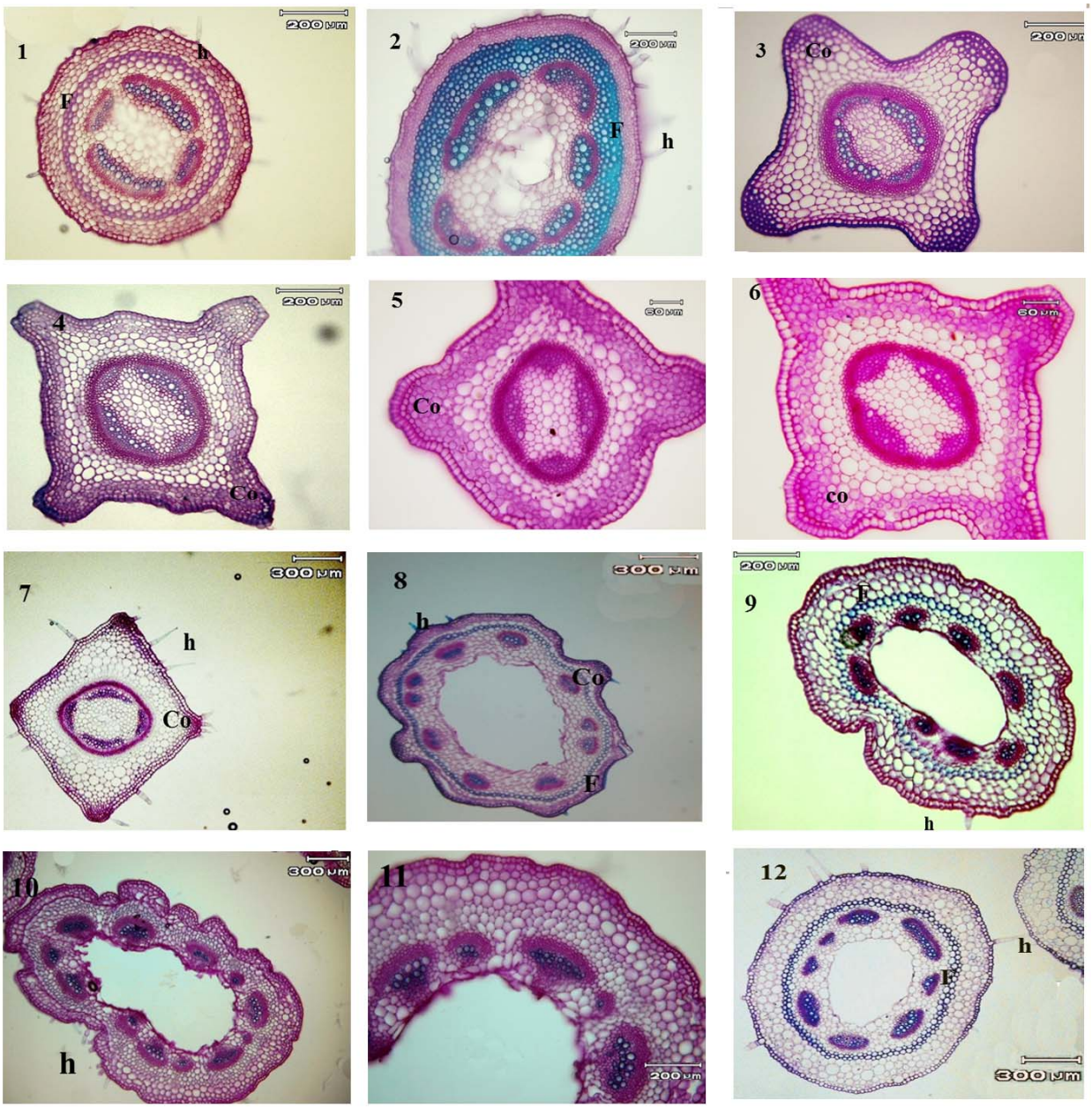
1	Thickness of stem vascular bundle	16	Average width of vascular bundles at midrib
2	Thickness of stem cortex	17	Lamina thickness of upper epidermis
3	Length of stem vascular bundles	18	Lamina lower cuticle diameter
4	Average width of stem vascular bundles	19	Lamina upper cuticle diameter
5	1st diameter of stem cross-section	20	Lamina lower epidermis diameter
6	2nd Diameter of stem cross-section	21	Lamina length of vascular bundles
7	Stem sclerenchyma diameter	22	Lamina average width of vascular bundles
8	Stem epidermis diameter	23	Lamina thickness of spongy parenchyma
9	Stem cuticle thickness	24	Lamina thickness of palisade parenchyma
10	Number of stem vascular bundles	25	Total thickness of lamina
11	Stem collenchyma diameter	26	Total thickness of the lamina excluding epidermis
12	Midrib diameter	27	Upper cuticle thickness of midrib
13	Midrib diameter excluding epidermis	28	Thickness of cortex parenchyma of midrib
14	Lower epidermis diameter of midrib	29	Length of vascular bundles of midrib
15	Upper epidermis diameter of midrib	30	thickness of lower cuticle of midrib

celled hairs are present all aound the stem cross sections (Figs. 7 and 8) but in *S. media* and *S. pallida* there are simple cellular hairs in olnly one side of stem cross sections (Figs. 9 and 10). In *Myosoton aquaticum* and *Mesostema kotschyanum* subsp. *kotschyanum* these simple multicellular hairs are present all around stem (Figs. 12 and 2). Other studied species have not hairs in their stems.

Number of vascular bundles: Stem vascular bundles are 8 in *S. pallida* and *S. holostea* (Fertile) (Figs. 9 and 8), in *S. media* is 11 (Figs.10 and 11), but in other studied taxa are 4 (as in Sterile stem of *S. holostea*, Fig. 7).

Statistical analysis

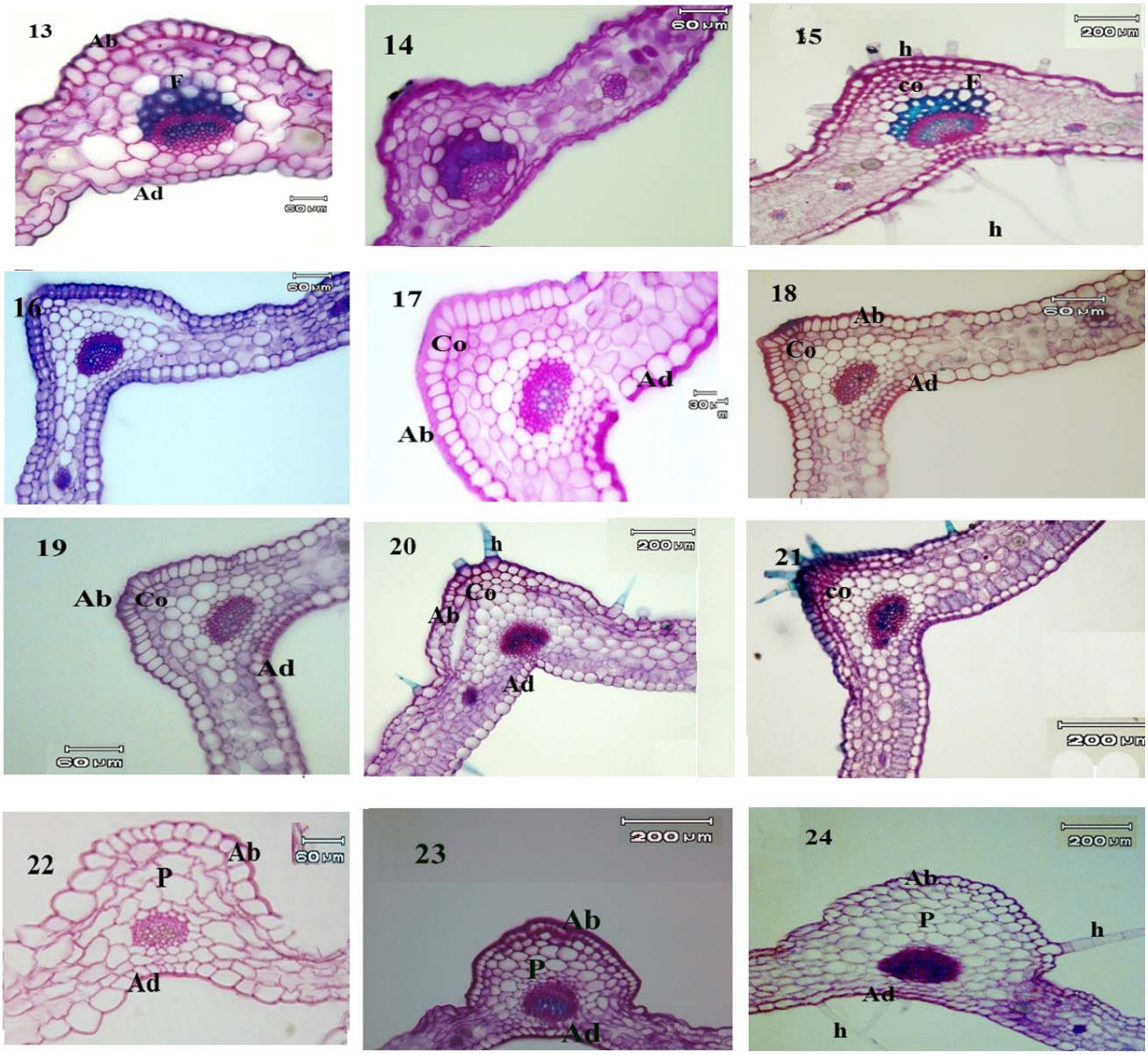
The studied species differ significantly in most quantitative characters, as revealed by the ANOVA test. Cluster analysis and PCA ordination of *Stellaria* species and relative genera examined in this study based on both quantitative and qualitative anatomical characters have produced similar results (Figs. 37 & 38). In cluster analysis, two major clusters were formed. The first cluster comprised two sub-clusters: In the first sub-cluster, the *S. graminea* (Fertile stems) and *S. graminea* (Sterile stems) of section *Stellaria*, are located close to each other (sub-cluster1). Furthermore, species of *S. persica* (Fertile stems) and *S. persica* (Sterile stems) *(Stellaria section)* are also located beside each other (sub-cluster2). In *S. persica* and *S. graminea* despite *S. holostea* there are identical fertile and sterile stem and there were no differences between fertile and sterile leaf and stem anatomy. Other species has not two stem types. *Stellaria graminea* and *S. persica* are grouped as a sub-cluster due to anatomical similarities, including shape of stem cross-section, which is rectangular, absence of hair, absence of sclerenchyma in stem, quaternary vascular bundles, collenchymas in stem corners, midrib shape and



Figs 1-12. Stem cross section in: **1**, *S. alsinoides*; **2**, *Mesostemma kotschyanum*. subsp. *kotschyanum* ;**3**, *S. graminea* (fertile stems); **4**, *S. graminea* (sterile stems); **5**, *S. persica* (sterile stems); **6**, *S. persica* (fertile stems); **7**, *S. holostea* (sterile stems); **8**, *S. holostea* (fertile stems); **9**, *S. pallida*; 10-**11**, *S. media*; **12**, *Myosoton aquaticum*; Abbreviations: h= Hair; Co= Collenchyma; F= fiber sclerenchyma.

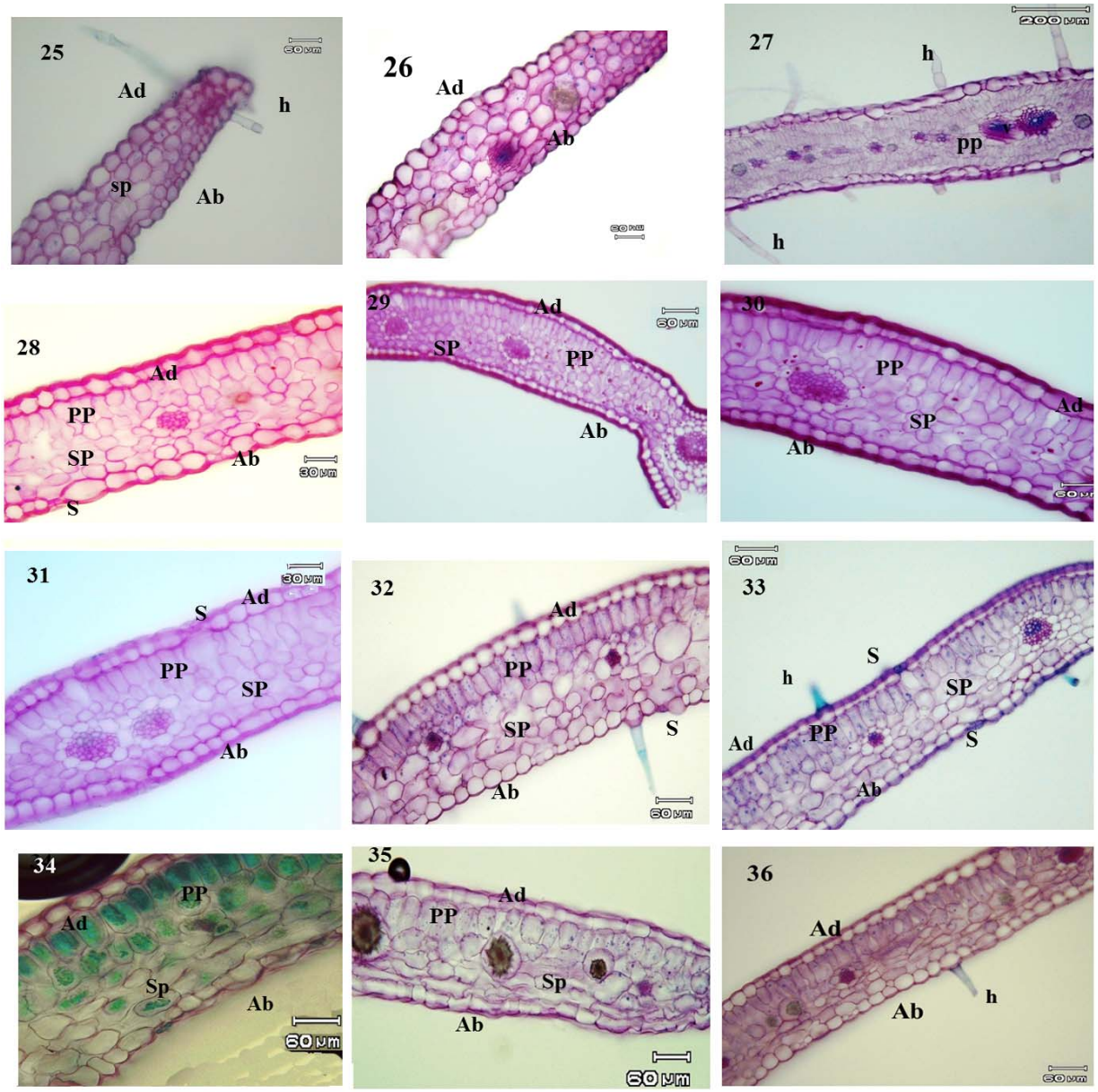
dorsiventral leaf blade. These two species are separated from the other *Stellaria* species. The second major cluster comprised two sub-clusters. *M. kotschyanum* form a separate clade. Due to anatomical differences the genus *Mesostemma* is separated from the genera *Myosoton* and *Stellaria*. These differences are because of having only palisade parenchyma, absence of spongy parenchyma in cross section of lamina, having a circular -angular shape and having several layers of thick sclerenchyma in stem. The phenogram resulted from the analysis does not clarify the relationships among taxa (Fig. 37).

S. media, *S. pallida*, *S. alsinoides* and *Myosoton*



Figs 13-24. Midrib cross section in: **13-14**, *S. alsinoides*; **15**, *Mesostemma kotschyanum*. subsp. *kotschyanum* ; **16**, *S. graminea* (fertile stem); **17**, *S. graminea* (sterile stem); **18**, *S. persica* (sterilse stem); **19**, *S. persica* (fertile stem); **20**, *S. holostea* (sterile stem); **21**, *S. holostea* (fertile stem); **22**, *S. pallida*; **23**, *S. media*; **24**, *Myosoton aquaticum*; Abbreviations: h= Hair; S= Stomata; Ab; Abaxial surface; Ad= Adaxial surface; P= Parenchyma; Co= Collenchyma; F= Fiber sclerenchyma.

aquaticum are located close to each other. Although *S. media* and *S. pallida* show similar morphology, the present leaf anatomical observations is in concordant with these similarities but stem anatomical structure shows considerably differences. *S. media* has 11 vascular bundles, 3-7 layered cortex parenchyma and absence of sclerenchyma around the vascular bundle. *Stellaria pallida* has 8 vascular bundles, 2-3 layered cortex parenchyma and sclerenchyma around the vascular bundle. *Myosoton aquaticum* has hairs all around in stems and leaves cross-sections so is separated from *S. media* and *S. pallida*. *Myosoton* is anatomically similar to *S. pallida* and *S. media* in midrib shape and stem corss section shape, absence of sclerenchymas and collenchymas in midrib and having dorsiventral leaf blades. These species are morphologically similar (Mahdavi et al. 2012) but the *S. alsinoides* from a separate section of this genus with a different morphology and anatomy, is not clearly separated in phenogram (Fig. 37). *Stellaria holostea*



Figs 25-36. Lamina cross section in: **25-26**, *S. alsinoides*; **27**, *Mesostemma kotschyanum*. subsp. *kotschyanum* ; **28**, *S. graminea* (fertile stem); **29**, *S. graminea* (sterile stem); **30**, *S. persica* (sterile stem); **31**, *S. persica* (fertile stem); **32**, *S. holostea* (sterile stem); **33**, *S. holostea* (fertile stem); **34**, *S. pallida*; **35**, *S. media*; **36**, *Myosoton aquaticum*; Abbreviations: PP= Palisade parenchyma; Sp= Spongy parenchyma; h= Hair; S= Stomata; Ab; Abaxial surface; Ad= Adaxial surface.

appears in the same group. Fertile and sterile stems of *S. holostea* show many anatomical differences despite their total morphological similarities. Diagnostic characters in these two stem types are: shape of stem cross-section, having a ring sclerenchyma, collenchyma status and number of vascular bundles in cross section.

In order to determine the most variable characters of the studied species, a Factor Analysis based on PCA has been performed, revealing that the first 3 factors comprised over 75.81 % of the total variation. In the first factor with about 40 % of total variation, such characters as total thickness of the lamina and midrib, total thickness of the midrib excluding epidermis, average width of vascular bundles in leaf midrib and

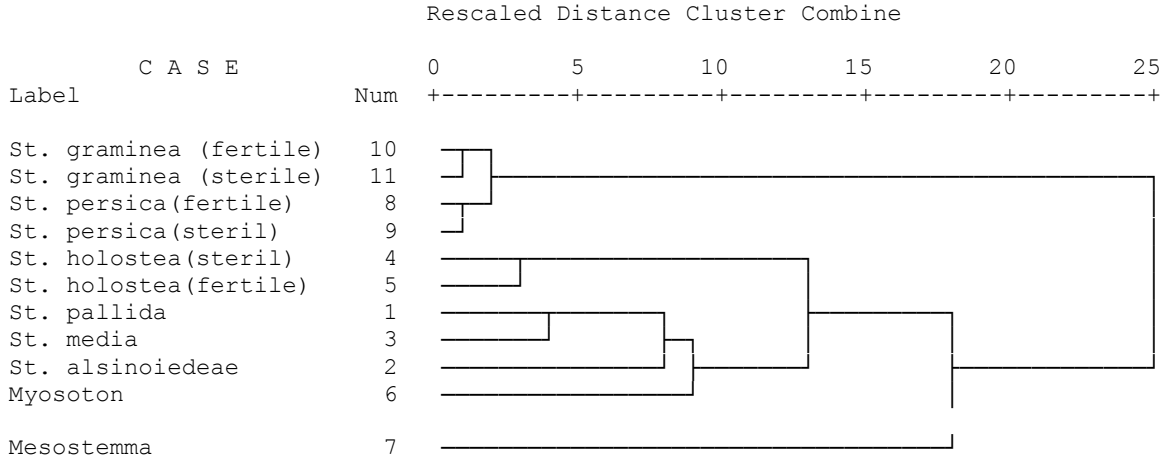


Fig. 37. WARD Phenogram based on leaf and stem anatomical characters in *Stellaria* species and relative genera.

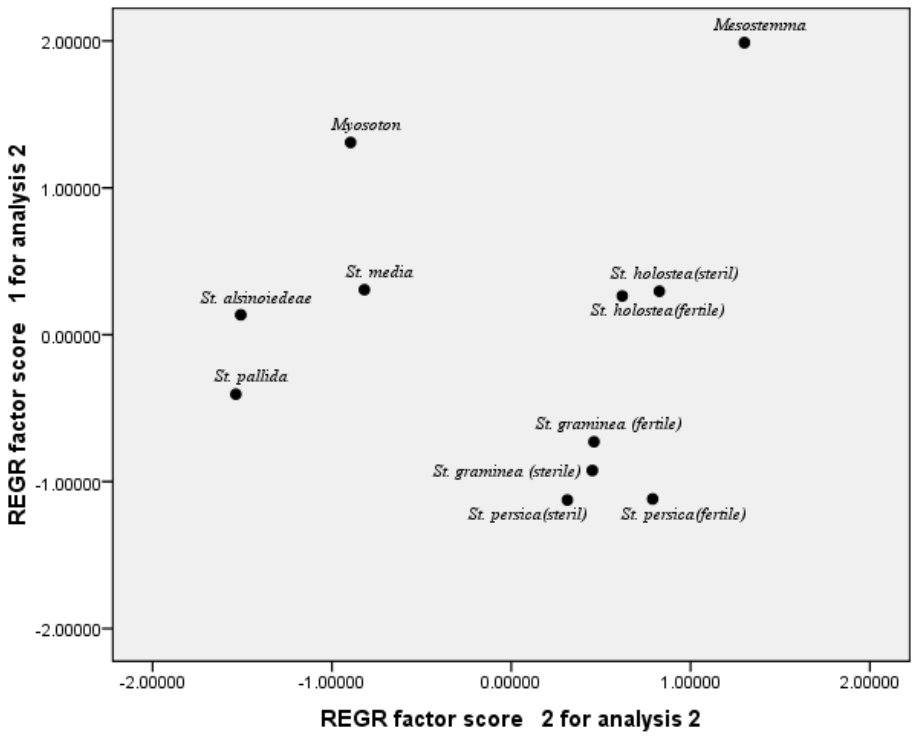


Fig. 38. PCA ordination of leaf and stem anatomical characters in *Stellaria* species and relative genera.

stem, total thickness of the lamina, thickness of the upper and lower epidermis (in leaf), length and width of vascular bundles (leaf), thickness of stem sclerenchyma, thickness of midrib upper epidermis, thickness of cortex parenchyma at midrib and stem, have shown the highest correlation (>0.7). In the second factor with over 21 % of total variation, sclerenchyma status at midrib, thickness of upper and lower cuticle at midrib, Shape of stem cross-section, thickness of leaf upper cuticle has shown the highest correlation. The third factor with 14 % of total variation and comprising sclerenchyma status at midrib, thickness of vascular bundle (stem) has featured the highest correlation. These are the most variable anatomical characters among the studied taxa.

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