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# Anatomical and Pollen Characters in the *Genus silene* L. (Caryophyllaceae) from Turkey

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**Abstract:** Anatomical and palynological features of *Silene caramanica* Boiss. and Heldr., *S. sipylea* O. Schwaz, *S. montbretiana* Boiss., *S. dianthoides* Pers., *S. pharnaceifolia* Fenzl., *S. odontopetala* Fenzl., *S. urvillei* Schott, *S. nuncupanda* Coode and Cullen collected from Turkey were examined and evaluated by numerical analysis in order to determine the taxonomic value of the observed internal peculiarities. Features related to pollen morphology (pollen shape and number of pore) and anatomical characters (idioblasts distribution, stomata index, stomata width and length, diameter of vascular bundle) were found to be important in separating the examined taxa. Principal compenent analysis of all characters showed that the anatomical and palynolological characters to be important in explanning the total variation among the examined taxa.

**Key words:** Anatomy, caryophyllaceae, numerical analysis, pollen morphology, *Silene*

## INTRODUCTION

The *Genus silene* L. belongs to the family Caryophyllaceae, having the greatest number of species in the Turkey. *Silene* is one of the larger genera of flowering plants in the world c. Seven hundred and fifty species, almost half of which occur in Mediterrenean area. South-west Asia and the South Balkan Peninsula are two main centres for the genus (Greuter, 1995). *Silene* is represented by c. One hundred and seventy species belongings to 31 sections in Turkey, 42% of which are endemic (Davis, 1967; Ozcelik, 2002; Duran and Menemen, 2003; Aytac and Duman, 2004; Ozhatay and Kultur, 2006). There is a very diffucult group taxonomically because of its fairly uniform external appearance (Coode and Cullen, 1967). In recent years, the relationships among *Silene* sp. have been realized in various studies, most of them focused on morphological features (Greuter, 1995; Yildiz, 2002; Kilic and Ozcelik, 2008), there have been very little study of anatomical and palynological properties. Metcalfe and Chalk (1950) reported the general anatomical properties of Caryophyllaceae, including a few details on the *Genus silene*. However, there have been no detailed anatomical studies on *Silene* sp., which are naturally in Turkey so far. Pollen grains of Caryophyllaceae received much attention in early research (Erdman, 1952).

In the flora of Turkey, the reports of species were based on a few specimens. This account does not exactly reflect the variability of the populations. In a taxonomic revision of the genus, used morphological characters, the relationships between the taxa appeared unresolved (Coode and Cullen, 1967). This research showed that morphology is insufficient for a deep understanding of the relationships in the *Silene*. Based on the results, there is a strong need for other data sources, such as anatomy, palynology, molecular, to better resolve *Silene* phylogeny. The presents research goal is to characterize the anatomy and pollen of the *Silene* in order to complement the anatomical and palynological studies in the genus, identify diagnostic characters for the genus and species and provide additional information in a poorly studied group as regards anatomy and pollen. It is hoped that the overall results of the study will help in filling the gap in their taxonomy and stimulate interest in further studies leading to their conservation.

## MATERIALS AND METHODS

Plants were collected from different localities in Turkey. The list of voucher specimens is given in Table 1. Plant samples were identified according to Coode and Cullen (1967). Specimens were dried according to standard herbarium techniques and stored in the herbarium of Suleyman Demirel University, Department of Biology.

The materials for anatomical study were fixed in FAA (Formaldehyde: Acetic acid: Alcohol) for 24 h and then preserved in 70% alcohol in the field. Anatomical observations were performed on transverse sections of stem and leaves cut by microtome (8-10 μm), surface sections of leaves by hand. All sections were stained with safranin-fast green for 24 h and mounted with glycerine-gelatine to make permanent slides (Vardar, 1987). Well

Table 1: Localities of the examined *Silene* taxa

| Taxa | Locality |
|---|---|
| *S. caramanica* Boiss. and Heldr* | C4 Konya: Alacabel mevkii, 1850 m, Kilic, 312 |
| *S. sipylea* O. Schwaz* | C3 Isparta: Kizildag, 1200 m, Kilic, 266 |
| *S. montbretiana* Boiss | B5 Kayseri: Pinarbasi, 1700 m, Kilic 161 |
| *S. dianthoides* Pers | B5 Nigde: Caykavak gecidi, 1500 m, Kilic 193 |
| *S. pharnaceifolia* Fenzl | B6 Sivas: Kangal 1750 m, Kilic 174 |
| *S. odontopetala* Fenzl | B5 Kayseri: Sariz, 1600 m, Kilic 153 |
| *S. urvillei* Schott* | C2 Mugla: Yilanli, 650 m, Kilic 169 |
| *S. nuncupanda* Coode and Cullen* | B6 Sivas: Gurun, 1700 m, Kilic 111 |

*Endemic

staining sections were photographed with an Olympus BX51 from permanent slides. All measurements and observations were made 3 or 4 times from several sections taken from at least two selected specimens. For the description of anatomical features, the terminology of Metcalfe and Chalk (1950) was used.

For a ligth microscopy, the pollen was first treated with 70% ethanol to remove oily substances, then embedded in glycerine-gelatin with basic fuchsin, following the method of Wodehouse (1935). The pollen was measured with a Olympus 220 microscope. Pollen micrographs were taken under an Olympus trinocular research microscope using a camera and then added to the study. Measurements were based on 50 pollen grains and the following parameters were measured. Pollen diameter (P: Polar axis; E: Equatorial axis), pore diameter (Plg, Plt), distance between pores (Pl-Pl), number of pore, exine and intine thickness were taken using an immersion object-lens (X100) and a scale ocular (10 X). The pollen terminology used in the present study follows Faegri and Iversen (1989) and Punt *et al*. (1994).

**Numerical analysis:** The 42 characters presented in Table 2 were assessed by numerical analysis: 30 related to anatomical ($X_1$-$X_{30}$) and 12 related to palynological ($X_{31}$-$X_{42}$) features. Arithmetic means of each quantitive variable, related to anatomical properties and pollen grains were calculated for each taxon separately in order to determine the values of particular characters for each taxon.

The data set include binary variables and quantitative. In another words, $X_6$, $X_7$, $X_{12}$, $X_{13}$, $X_{15}$, $X_{19}$, $X_{20}$, $X_{21}$, $X_{22}$, $X_{25}$, $X_{28}$, $X_{29}$, $X_{31}$, $X_{33}$, $X_{34}$, $X_{35}$, $X_{40}$, $X_{42}$ are binary variables, each diveded into two discrete catagories while the remaning variables are quantitative. The species were clustered using a Sorensen distance measurement and flexible beta linkage ($\beta$ = -0.25). Cluster analyses was performed by using PC-ORD. PCA was performed by using CAP (community analysis packet) (Legendre and Legendre, 1983). Correlation analysis between axes values of species obtained from PCA and characters were performed by using SPSS (Ozdamar, 1997) in Turkish (Ozkan and Suel, 2008).

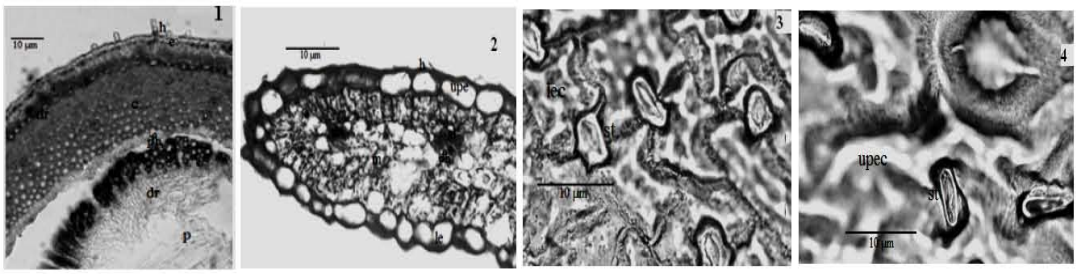
Table 2: Characters used in this study

| Symbols | Characters |
|---|---|
| $X_1$ | Cuticle thickness of stem (μm) |
| $X_2$ | Hair of stem; present: 1, absent: 0 |
| $X_3$ | Width/length of epidermal cells of stem (μm/μm) |
| $X_4$ | Width of cortex/Width of cylinder (μm/μm) |
| $X_5$ | Width of cortex (μm) |
| $X_6$ | Average number of cortex cells ($mm^2$) |
| $X_7$ | Idioblast in cortex; present: 1, absent: 0 |
| $X_8$ | Width of pith (μm) |
| $X_9$ | Idioblast in pith; present: 1, absent: 0 |
| $X_{10}$ | Width of trachea in stem (μm) |
| $X_{11}$ | Width of xylem in stem (μm) |
| $X_{12}$ | Width of phloem in stem (μm) |
| $X_{13}$ | Width/length of endodermal cells of stem (μm/μm) |
| $X_{14}$ | Hollow of pith; present: 1, absent: 0 |
| $X_{15}$ | Width of leaf (μm) |
| $X_{16}$ | Hair of leaf; present: 1, absent: 0 |
| $X_{17}$ | Width/length of upper epidermal cells (μm/μm) |
| $X_{18}$ | Average number of upper epidermal cells ($mm^2$) |
| $X_{19}$ | Average number of upper epidermal stomata ($mm^2$) |
| $X_{20}$ | Width/length of upper epidermal stomata (μm/μm) |
| $X_{21}$ | Stomata index of upper epidermis |
| $X_{22}$ | Width of mesophyll (μm) |
| $X_{23}$ | Idioblast in mesophyll; densely: 1, scarcely: 0 |
| $X_{24}$ | Width/length of lower epidermal cells (μm/μm) |
| $X_{25}$ | Average number of lower epidermal cells ($mm^2$) |
| $X_{26}$ | Average number of lower epidermal stomata ($mm^2$) |
| $X_{27}$ | Width/length of lower epidermal stomata (μm/μm) |
| $X_{28}$ | Stomata index of lower epidermis |
| $X_{29}$ | Width/length of leaf vascular bundle (μm/μm) |
| $X_{30}$ | Width of leaf trachea |
| $X_{31}$ | E: Equatorial axis (μm) |
| $X_{32}$ | P: Polar axis (μm) |
| $X_{33}$ | P/E rate |
| $X_{34}$ | plg: Length of pore (μm) |
| $X_{35}$ | plt: Width of pore (μm) |
| $X_{36}$ | pl-pl (μm) |
| $X_{37}$ | Average number of pore ($mm^2$) |
| $X_{38}$ | in: Intine (μm) |
| $X_{39}$ | ex: Exine (μm) |
| $X_{40}$ | Width of granules in exine (μm) |
| $X_{41}$ | Granules in exine; densely: 1, scarcely: 0 |
| $X_{42}$ | Pollen shape: oblate-spheroidae:1, spheroidae: 0 |

## RESULTS AND DISCUSSION

### Anatomical characters

***Silene caramanica*:** A transverse section taken from the middle part of the stem was observed (Fig. 1a). Cuticle layer 2-2.5 μm thick. Epidermis consist of a single layer of plane, rectangular or orbicular cells (7.5×10 μm). There are long and thin-walked multicellular hairs on the epidermis. The sclerenchyma is 14-layered, located close to the




(a) (b) (c) (d)

Fig. 1: *S. caramanica*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem

epidermis. The cortex (230-280 µm) consist of 15 or 17 layers of usually oval cells with thin walls and occupies 15-20% of the stem radius. The phloem measures 25-25 µm; xylem 60-75 µm, trachea width 15-20 µm, including solitary or clustered vessels, makes up 15% of the stem radius. The pith, with an evident empty central part, occupies 20% of the stem radius. Idioblasts, including druses, are scarcely distrubuted in pith.

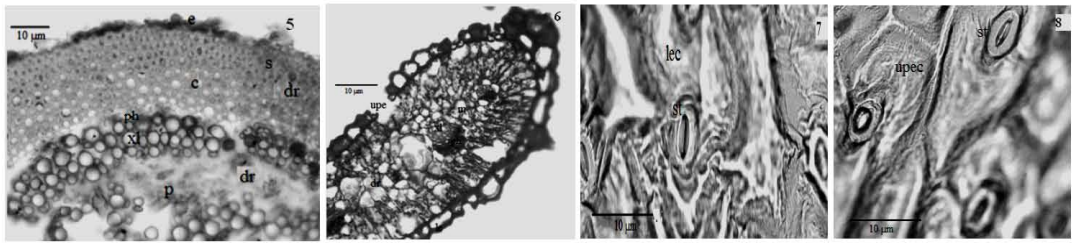
The anatomical features of lamina and leaf surfaces were analyzed (Fig. 1b). The upper and lower epidermises comprise uniseriate, oval and rectangular cells. Both epidermises are covered with cuticle. The upper epidermal cells are larger than lower ones. There are trichomes on both epidermises. Mesophyll consist of 6 or 7 layers of elongated palisade cells (200-230 µm). Vascular bundles are surrounded by a parenchymatic bundle sheath. Leaves unifacial and anamocytic stomata cells. Stomata occur on the both surfaces, level with neighboring cells. The stoma index is 42,85 for the lower and 27, 53 for the upper surface (Fig. 1c and d).

***S. sipylea*:** The transverse section taken from the middle part of the stem revealed the following: In general, the stem is circular. The epidermis consist of rectangular and ovoidal cells forming a single layer (10×12.5 µm) and is surrounded by a cuticle layer (2.5-3.75 µm). Underneath the epidermis, there is cortex with 13-layered make up ovoidal parenchymatous cells (200-250 µm) and occupies 15% of the stem radius. Druses are densely distrubuted in cortex. Continuous sclerenchyma under epidermis is 10-11 layered, its cells are ovoid shaped. The endodermis is distinct (10×12.5 µm) and composed of a single layer of rectangular cells. Cambium was distinguishbale. The phloem (27.5 µm) and the xylem members (112.5 µm) are clear. Xylem elements were arranged in parallel rows and occupies of 6% of stem radius. The pith is hollow in the centre and the outher part consist of deformed cells and occupies 50% of the stem radius (Fig. 2a).

A transverse section of the lamina and both epidermis was studied (Fig. 2b). Vascular bundles are surrounded by parenchymatous and orbicular cells. Mesophyll, not including spongy, consist of 4 or 5 layers of elongated palisade cells (100-140 µm). Idioblasts, including druses, were seen between the palisade cells. Both epidermises are covered by cuticle. Upper epidermal cells are larger than the lower ones; they have undulate cell and no trichomes. Leaves unifacial and are amphistomatic with caryphyllaceous stomata. The stoma index is 20 for the lower and 26, 98 for the upper surface (Fig. 2c and d).

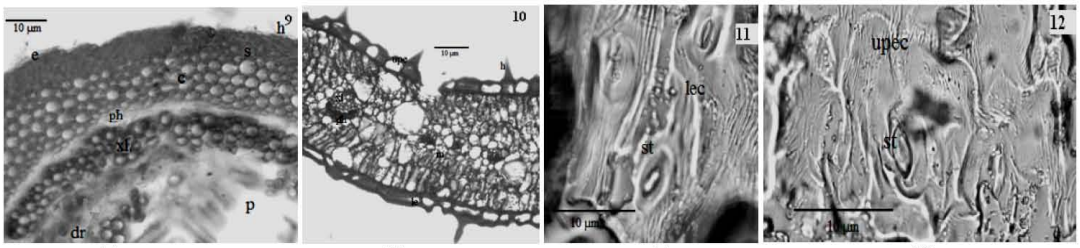
***S. montbretiana*:** A transverse section taken from the middle part of the stem was observed (Fig. 3a). Cuticle layer 2.5-3.5 µm. Epidermis consist of a single layer of rectangular or orbicular cells (7.5×20 µm) and there are hairs on it. Under the epidermis, there is plate sclerenchyma with 4-5 layered. Stem cortex (150-180 µm) consist of usually spheroidal aerenchyma cells and makes up 10% of the stem. Cambium is not distinguishable. The phloem (25 µm) and the xylem members (62 µm) are clear. Xylem tissue, including solitary or clustered vessels, is not extensive and occupied 5% of the stem radius. The pith, with an evident empty central part, occupies 50% of the stem radius.

The transverse section of lamina and surface preparations of both epidermises revealed that the upper and lower epidermises comprise uniseriate, oval and rectangular cells. The upper epidermis cells are larger than the lower ones. Both epidermises are covered with a cuticle. There are long and thin-walked hairs on both epidermises, but the trichome density of the upper epidermis is not as high as that of the lower epidermis. Palisade parenchyma cells are 2-layered below both epidermises (400-425 µm). Vascular bundles are surrounded by a parenchymatic bundle sheath. Leaves are unifacial and amphistomatic. The stomata




(a) (b) (c) (d)

Fig. 2: *S. sipylea*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem




(a) (b) (c) (d)

Fig. 3: *S. montbretiana*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem

caryphyllaceous and the guard cells form level with neighboring cells. The stoma index is 25 for the lower and 31, 03 for the upper surface (Fig. 3b-d).

***S. dianthoides*:** The transverse section taken from the middle part of the stem revealed the following: the stem is circular. Cuticle layer is 2-3 µm. Epidermal cells consist of a single layer and are plane and are rectangular or orbicular (12×14 µm). There are short hairs on the epidermis. Under the epidermis are 4-5 layers plate sclerenchyma. Stem cortex (300-350 µm) consist of 16-18 layers of usually oval cells and occupies 20% of the stem radius. There are druses densely distrubuted in cortex. Vascular bundles are scattered in a circular manner below the parenchymatous tissue. The phloem (20-25 µm) occupies a small area and xylem (45-50 µm) makes up most of bundles (trachea 5-7.5 µm). Pith is hollow and occupy 32% of stem radius (Fig. 4a).

Leaf features in a transverse section taken from the lamina and surface preparations of both epidermises of *S. dianthoides* are shown in Fig. 4b. Upper epidermal cells are larger than the lower ones; they have undulate cell and no trichomes. Both epidermises are covered with a cuticle. Vascular bundles surrounded with parenchymatous and orbicular shaped are collateral thype with a visible cambium. Scleranchyma cells in bundles are dense. One layered sclerenchyma is seen inside of the upper and lower epidermis in the midribs. Palisade and spongy are not distinct, that is, mesophyll tissue (180 230 µm) is homogeny and idioblasts are scarcely seen in mesophyll.

Stomata cells ocur on the both surfaces at the same level with neighboring cells are caryphyllaceous. Number of stomata and their size are different from upper and lower surfaces. The stoma index is 37 for the lower and 39, 77 for the upper surface (Fig. 4c and d).

***S. pharnaceifolia*:** A transverse section taken from the middle part of the stem was observed (Fig. 5a). The stem possesses a thick layer of outher cuticle (1.5-2.5 µm) followed by a single layer of flat epidermal cells (2×9 µm). The sclerenchyma is 2-3 layered, located close to the epidermis. The cortex (130-160 µm) consist of 7-8 layered ovoidal parenchymatic cells and occupies 16% of stem radius. Idioblast, including druses, scarcely scattered in cortex. The phloem measures 20-25 µm; xylem 30-37 µm, trachea width 7-12 µm, including solitary or clustered vessels, makes up 5% of the stem radius. The pith, with an evident empty central part, occupies 45% of the stem radius.

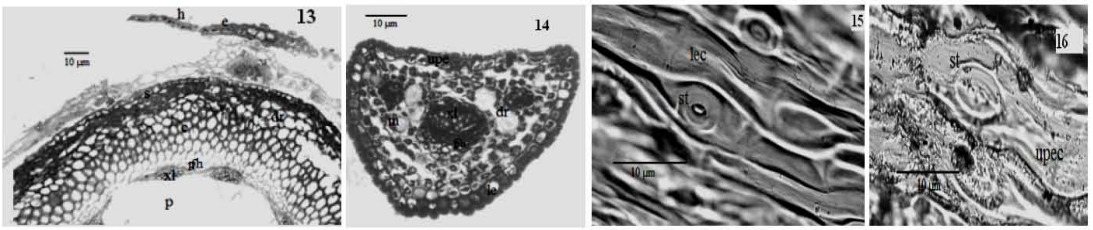



Fig. 4: *S. dianthoides*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem

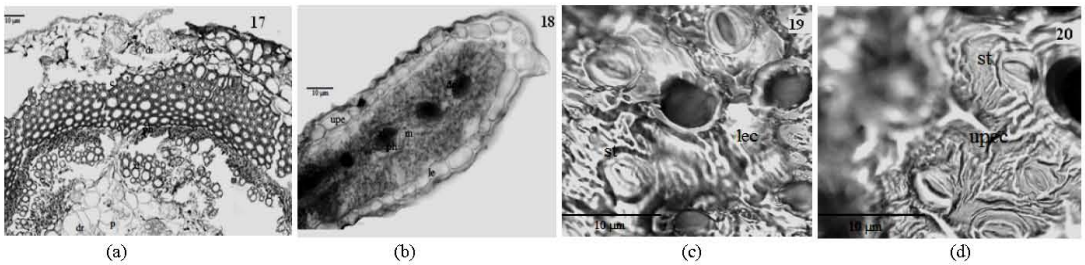



Fig. 5: *S. pharnaceifolia*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem

In transverse section, the upper and lower epidermises comprise uniseriate and oval cells. Mesophll is not well defferentiated consisting of 3-4 layers of isodiametric parenchymatic cells (250-320 μm). Druses are densely seen between parenchymatic cells. Vascular bundle is surrounded by elliptical or spheroidal parenchymatous cells. Upper epidermal cells are larger than the lower ones. There are no trichomes on the both of the epidermis. Both epidermises are covered with a cuticle. Leaf unifacial and has caryphyllaceous stomata cells. Stomata ocur on the both surfaces, upper than neigboring cells. Number of stomata and their size are different for upper and lower surfaces. The stoma index is 45, 26 for the lower and 38, 02 for the upper surface (Fig. 5b-d).

***S. odontopetala*:** The transverse section of the stem is circular shaped. The epidermis consist of single layer rectangular cells (12×25 μm) and is surrounded by a cuticle layer (1-1.5 μm). There are multi-cellular hairs on the epidermis. Underneath the epidermis, there is sclerenchyma with 5-6 layered, its shaped is ovoid. The cortex (200-280 μm) is 11-12 layered and parenchymatic, with cells rectangular and polygonal and occupies 15% of stem radius. Druses is densely distrubuted in cortex the vascular bundles are surrounded by sclerenchyma fiber. The phloem occupies a 20-25 μm area and xylem (50-60 μm) makes up most of bundles (trachea 12-15 μm). Pith is hollow and occupy 35% of stem radius (Fig. 6a).

The transverse section of lamina and surface preparations of both epidermises revealed the following elements. There are trichomes on the both of the epidermis. Vascular bundles are are-shaped and surrounded by parenchymatic bundle sheath. Mesopyhll consist of 4-5 layers of elongated palisade cells (330-350 μm) and druses is densely distrubuted in mesopyhll. Both epidermises are covered by cuticle. Leaf is unifacial and has caryphyllaceous stomata. The stoma index is 42, 68 for the lower and 40, 27 for the upper surface (Fig. 6b-d).

***S. urvillei*:** A transverse section taken from the middle part of the stem show that the epidermis (8×20 μm) consist of a monolayer of thin-walled rectangular cells, its above is covered a thin cuticle (1.5-2 μm). The sclerenchyma beneath the epidermis is 6-7 layered. The stem cortex (300-350 μm) consist of 10-11 layers of usually oval cells and occupies 25% of the stem radius and it has a few

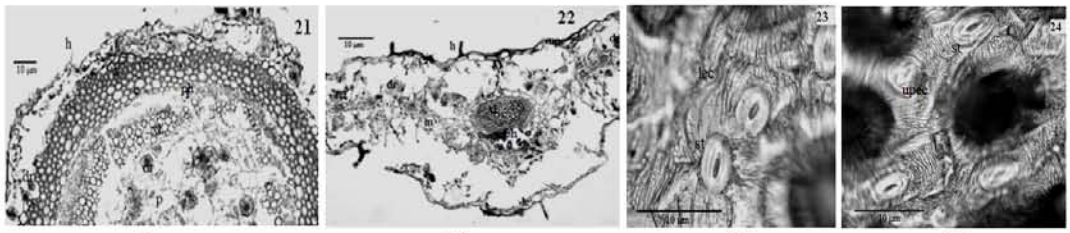



Fig. 6: *S. odontopetala*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem

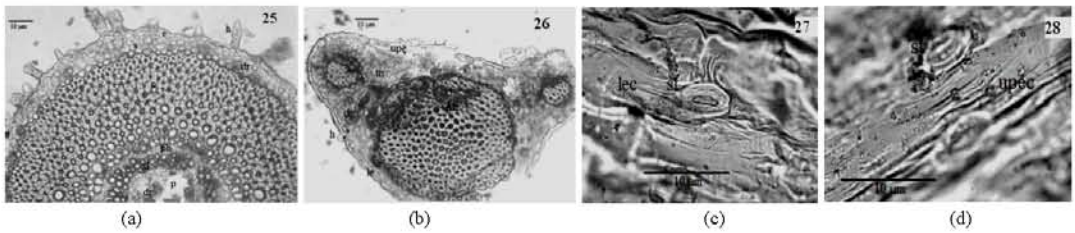



Fig. 7: *S. urvillei*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem

druses. Vascular bundles occupy 10% of the stem radius and are surrounded with a monolayer cell. The phloem is 60-65 μm, xylem is 65-70 μm. Pith without a free-central part accounts for 15% of the stem radius. The pith contain densely druses (Fig. 7a).

The transverse section of lamina and surface preparations of both epidermises revealed that the upper and lower epidermises comprise uniseriate, oval and rectangular cells. The upper epidermis cells are larger than the lower ones. There are long and thin-walked hairs on both epidermises, but the trichome density of the upper epidermis is as high as that of the lower epidermis. Both epidermises are covered with a cuticle.

Vascular bundles surrounded with parenchymatous and orbicular shaped cells. Scleranchyma tissue in bundles are dense. Palisade and spongy parenchyma are not distinct, that is, mesophyll is homogeny (400-450 μm). Druses is densely distrubuted in mesopyhll. Leaves are amphistomatic. The stomata caryphyllaceous and the guard cells form level with neighboring cells. The stoma index is 21, 79 for the lower and 21, 42 for the upper surface (Fig. 7b-d).

***S. nuncupanda*:** The transverse section taken from the middle part of the stem shows a uniserate epidermis (9×30 μm) consisting of large rectangular cells. Cuticle layer is 0.5-1 μm. The cortex (650-700 μm) is surrounded by a bilayer of sclerenchyma consist of 14-15 rows of usually oval cells, with several intercellular spaces and a few druses.

It occupies 5% of the stem radius. The phloem (15-18 μm) contains solitary or clustered sclerenchymatic fibers and the xylem (70-80 μm) occupies 10% of the stem radius. The stem center is filled with large, thin-walled parenchymatous cells and pith occupy 10% of the stem radius (Fig. 8a).

A transverse section of the lamina and both epidermis were studied. Mid-rib is semicircular shaped and consist of 1-2 layered sclerenchyma located bolow the both epidermal cells. Vascular bundles are surrounded by thin-walled, with parenchymatous cells. The mesophyll (180-260 μm) consists of 2 or 3 layers of elongated palisade cells. Druses is scarcely distrubuted in mesopyhll. The leaf is unifacial and both upper and lower epidermal cells have undulate walls and a few simple hairs. Both epidermises are covered by cuticle. Laminas are amphistomatic. The stomata caryphyllaceous and the guard cells form level with neighboring cells. The stoma index is 36, 76 for the lower and 36, 04 for the upper surface (Fig. 8b-d).

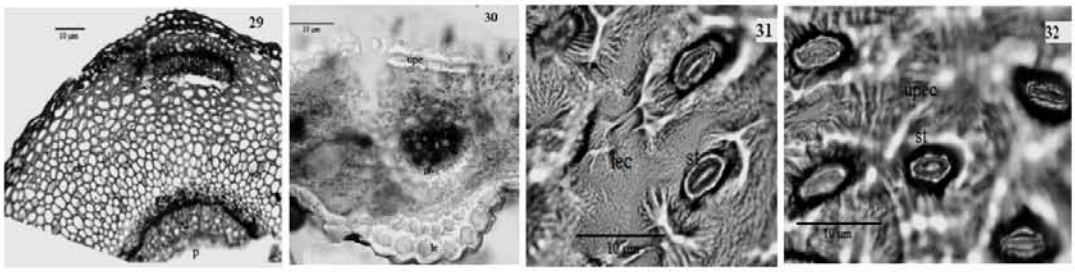



Fig. 8: *S. nuncupanda*, a) Transverse section of stem, b) Transverse section of leaf, c) Lover surface of leaf, d) Upper surface of leaf. c: cortex, dr: druses crystals, e: epidermis, h: hair, le: lower epidermis, lec: lower epidermis cell, m: mesophyll, p: pith, ph: phloem, s: sclerenchyma, st: stomata, upe: upper epidermis, upec: upper epidermis cell, xl: xylem

**Pollen characters**

***Silene caramanica***: The pollen grains are radially symmetrical and isopolar. Pollen shape is spheroidal, with the polar axis 26.8 μm and equatorial axis 26.4 μm. The grains are periporate (25-28 porate). Pores±circular (Plg: 2.8 μm, Plt: 2.6 μm), distance between of pores (Pl-Pl) 5.2 μm. The exine is tectate and 3.8 μm. Intine thichness is 0.5 μm on the equatorial axis. There are densely granules in exine (Fig. 9a and b).

***S. sipylea***: The pollen grains are radially symmetrical and isopolar. Pollen shape is spheroidal, with the polar axis 29.4 μm and equatorial axis 28 μm. The grains are periporate (24-29 porate). Pores circular (Plg: 4.2 μm, Plt: 4.2 μm), distance between of pores (Pl-Pl) 5.1 μm. The exine is tectate and 2.7 μm. Intine thichness is 1 μm on the equatorial axis. There are densely granules in exine (Fig. 9c and d).

***S. montbretiana***: The pollen grains are radially symmetrical and isopolar. Pollen shape is oblate-spheroidal, with the polar axis 30 μm and equatorial axis 31.8 μm. The grains are periporate (32-36 porate). Pores±circular (Plg: 4.2 μm, Plt: 4 μm), distance between of pores (Pl-Pl) 5.8 μm. The exine is tectate and 1.7 μm. Intine thichness is 1 μm on the equatorial axis. There are densely granules in exine (Fig. 9e and f).

***S. dianthoides***: The pollen grains are radially symmetrical and isopolar. Pollen shape is spheroidal, with the polar axis 29.2 μm and equatorial axis 29 μm. The grains are periporate (22-28 porate). Pores±circular (Plg: 3.8 μm, Plt: 3.6 μm), distance between of pores (Pl-Pl) 7.6 μm. The exine is tectate and 1.8 μm. Intine thichness is 0.5 μm on the equatorial axis. There are densely granules in exine (Fig. 9g and h).

***S. pharnaceifolia***: The pollen grains are radially symmetrical and isopolar. Pollen shape is spheroidal, with the polar axis 31.2 μm and equatorial axis 31 μm. The grains are periporate (30-45 porate).

Pores circular (Plg: 5 μm, Plt: 5 μm), distance between of pores (Pl-Pl) 5.2 μm. The exine is tectate and 3 μm. Intine thichness is 1 μm on the equatorial axis. There are scarcely granules in exine (Fig. 9i and j).

***S. odontopetala***: The pollen grains are radially symmetrical and isopolar. Pollen shape is spheroidal, with the polar axis 31 μm and equatorial axis 30.6 μm. The grains are periporate (20-27 porate). Pores±circular (Plg: 4.8 μm, Plt: 4.6 μm), distance between of pores (Pl-Pl) 7.6 μm. The exine is tectate and 2.5 μm. Intine thichness is 1 μm on the equatorial axis. There are densely granules in exine (Fig. 9k and l).

***S. urvillei***: The pollen grains are radially symmetrical and isopolar. Pollen shape is spheroidal, with the polar axis 29.6 μm and equatorial axis 29.4 μm. The grains are periporate (27-33 porate). Pores±circular (Plg: 4.9 μm, Plt: 4.7 μm), distance between of pores (Pl-Pl) 5.1 μm. The exine is tectate and 2.8 μm. Intine thichness is 1 μm on the equatorial axis. There are densely granules in exine (Fig. 9m and n).

***S. nuncupanda:*** The pollen grains are radially symmetrical and isopolar. Pollen shape is spheroidal, with the polar axis 27.8 μm and equatorial axis 26.6 μm. The grains are periporate (27-29 porate). Pores±circular (Plg: 4.4 μm, Plt: 4.1 μm), distance between of pores (Pl-Pl) 5.2 μm. The exine is tectate and 2.9 μm. Intine thichness is 1 μm on the equatorial axis. There are densely granules in exine (Fig. 9o and p).

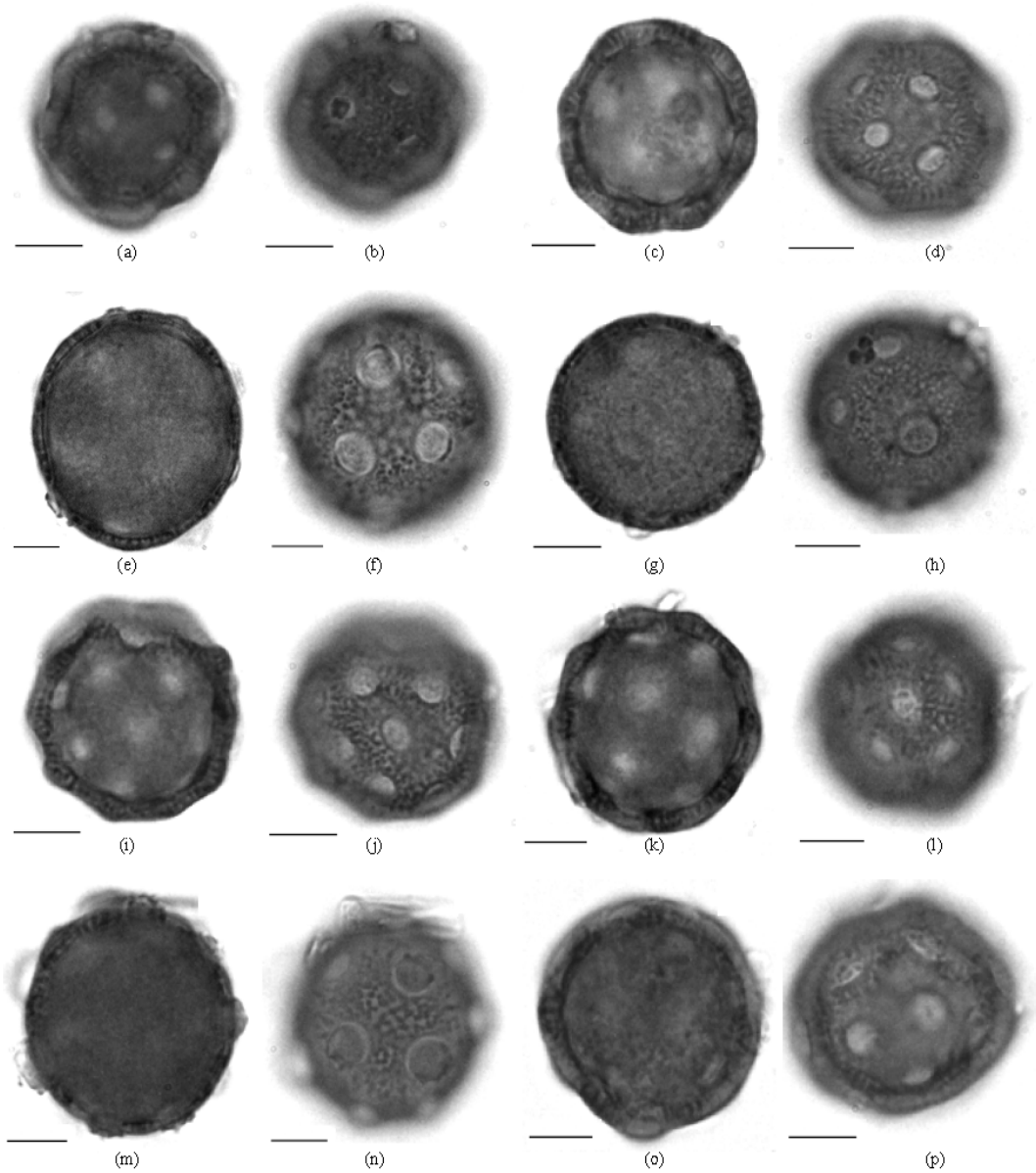



Fig. 9: LM photographs of examined *Silene* taxa. a-b) *S. caramanica*, c-d) *S. sipylea*, e-f) *S. montbretiana*, g-h) *S. dianthoides*, i-j) *S. odontopetala*, k-l) *S. pharnaceifolia*, m-n) *S. urvillei*, o-p) *S. nuncupanda*. Scale bar are 5 μm. Figure 9a-o a general viev of polen (Fig. 9b-p) detailed sculpture and aperture)

This is an anatomical and palynological report of Turkish representives of some *Silene* taxa. The present investigation sought to provide an additional perspective on the relations among the different taxa studied.

Stem anatomy is similiar in the examined taxa, but width of cortex, the presense and distrubition of crystals in the cortex and pith, width of trachea and the presense of hairs on epiderma, varies among the taxa. Metcalfe and Chalk (1950) gave information about the general anatomical characteristics of the family Caryophyllaceae.

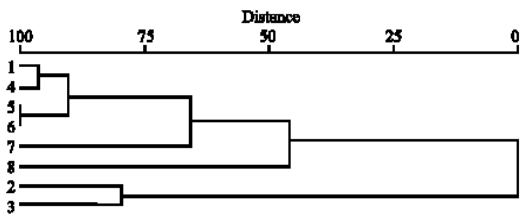



Fig. 10: Phenogram of the 8 studied taxa clustering with (UP-GMA) method. Taxa numbers given in Table 1

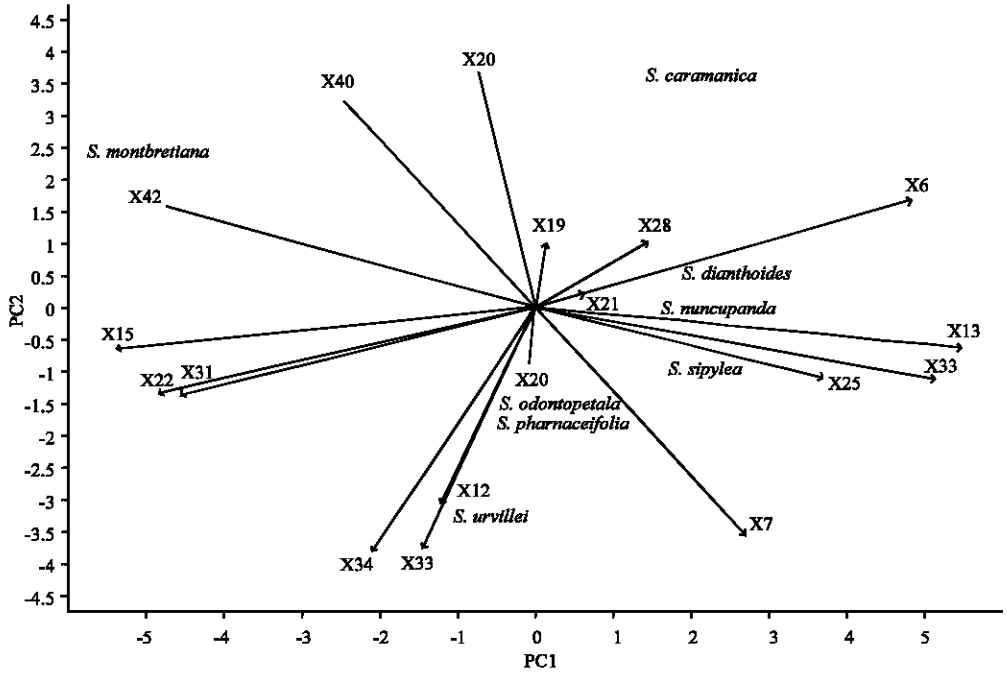



Fig. 11: Principal component analysis of 8 taxa and 18 variables projected onto PC1 and PC2. Variables numbers explained in Table 2

They state that stem anatomy of *Silene*; there are multicellular hairs on epidermis and calsium oxalate crystals in the endodermis of some species. But in this examination, although, it wasn't encountered to the crystals in endodermis, in the taxa examined, the crystals were identified both in the cortex and the pith. It was also defined that their availibity of frequency which is situated in stem cortex and pith are different from the taxa exemined before. While, the crystals weren't found in the stem cortex of the *S. caramanica* and *S. montbretiana*, they were found in very few amounts in *S. sipylea*, *S. pharnaceifolia*, *S. urvillei* and *S. nuncupanda* and were found in intensive amounts in the others. Moreover, in the pith, while the crystals weren't found in other taxa,they were found in intensive amounts in *S. sipylea*, *S. odontopetala* and *S. urvillei* and found in very few amounts in *S. caramanica* and *S. montbretiana.*

In this way, the existence of sclerenchyma in the cortex, was defined primarily in this study. The ordinal cellular number and intensivity of this layer shows difference among the taxa exemined. The presence and distrubition of sclerenchymatic tissue in cortex of examined species are an additional taxonomic trait for identifying these taxa. While, this layer is defined in the most excessive amounts in the *S. caramanica* (14-), it is defined in the fewest amounts in *S. nuncupanda*. The xylem forms a continuous cylinder and a large proportion of the phloem often consist of parenchyma in all the examined taxa, as indicated by Metcalfe and Chalk (1950).

Leaves anatomy; both upper and lower epidermal cells have undulate walls and a few simple hairs, the whole of the mesophyll is composed of palisade tissue (unifacial) and cover with abundant chloplast in the parenchyma and their mesophyll has calsium oxalate crystals. The xylem is towards the upper surface and the phloem towards the lower surfaces. Metcalfe and Chalk (1950) pointed out that the leaves of the family Caryophyllaceae species are usually accompanied vascular bundles by sclerenchyma and stomata generally of the caryophyllaceous thype. The findings of this study correspond to the ideas of Metcalfe and Chalk (1950). The leaves are amphistomatic in all the exemined taxa. The stomata index which are located below and above surfaces of the leaves are different from each other. On the below surface of the leave the stomata index found in the fewest amounts in *S. sipylea* (20) and found in the most excessive amounts in *S. pharnaceifoli* (45.26). Additionally, on the above surface of the leave the stomata index found in the fewest amounts in *S. urvillei* (21.42) and found in the most excessive amounts in *S. odontopetala* (40.27).

While, the stomata index and the distrubution and existence of the crystals in the stem and leaves varies from species to species, it is well known that it is an environmentally influenced anatomical character, so it is not so useful in grouping the examined taxa. These findings supplement the information about the genus and family given by Metcalfe and Chalk (1950) and could be useful in future studies on this genus.

The palynological data presented here reinforce the close relantionship among examined species. According to these results, pollen grains of examined species are uniform in the fundamental characteristics of symmetry (radially symmetrical), polarity (isopolar), aperture type (periporate, 24-34 porate). The Polar/Equatoral (P/E) ratio is 0.94 and pollen shape is oblate-spheroidal in *S. montbretiana*; in the others the ratio ranges from 1.01- 1.05 and pollen shape is spheroidal. We found that diameter of pore, distance between two pori and number of pore vary among the examined taxa. But with the exception of the pollen size, the value of pollen characters as taxonomic tools within examined species of *Silene* studied here is restricted to a small number of cases and does not very remarkable. The differences existing among the pollen grains of the species investigated are not very important characters for taxonomy of this genus.

The cluster dendrogram based on 42 characters is portrayed in Table 2. Eight species fall into 2 major clusters, one with two of the six species (*S. sipylea* and *S. montbretiana*) and the other (*S. caramanica*, *S. dianthoides*, *S. pharnaceifolia*, *S. odontopetala*, *S. urvillei* and *S. nuncupanda*) with remaining ones. At the same time, as seen in (Fig. 10), *S. caramanica* and *S. dianthoides* are linked to each other at a low dissimilarity level, *S. urvillei* and *S. nuncupanda* are linked at a high dissimilarity level. Hence, we suggest that these clustering methods are more suitable than other methods for classifying the genus at the species level.

According to Coode and Cullen (1967), traditional relationships are based on their morphological characters (leaf, inflorescence), *S. caramanica* is close to *S. sipylea*. But in this dendogram and PCA ($X_{28}$-$X_{33}$), it was far from each other (Table 2). Therefore, this is in contrast with the considerable similiar in morphological characters found by Coode and Cullen (1967) (Fig. 11).

Yildiz and Minareci (2008), determined the pollen morphologies of *S. urvillei* in her studies, which reported that diameter of pollen is 32.07-36.18 μm, diameter of pore 5.15 -6.21 μm, distance between two pores 5.9-10.7 μm, number of granules on pore 15-19. But, the present investigation has revealed that contradict with the results of their ones.

PCA was applied on the data given in Table 2. The first four components were taken into account because of their high eigenvalues and persentage of variance (Fig. 11). The first, second, third and forth components accounted for 24.77, 21.43, 16.61 and 13.60% of the variance, respectively. These components explained 76.42% of total variance.

Pearson and Kendall Correlations with ordination axes were evaluated in order to determine the most important characters. The significant correlations with $AX_1$ are $X_6$, $X_{13}$, $X_{15}$, $X_{22}$, $X_{31}$, $X_{33}$, $X_{42}$. The characters related to $X_2$ are $X_7$, $X_{29}$, $X_{34}$, $X_{35}$, $X_{40}$. $X_{12}$, $X_{19}$, $X_{21}$, $X_{28}$ are significantly correlated with $X_3$. For $X_4$, the most important characters are $X_{20}$, $X_{25}$. PCA result dealing with first two axes was given in Fig. 10 because of the first axes highest eigenvalues and persentage.

## CONCLUSION

As a result, anatomy and palynology can be used to distinguish or combine as regards genus and taxa. But PCA analysis shows that some 42 examined traits are more important in delimiting the examined *Silene* sp. average number of cortex cells and pith, width of phloem in stem, width/length of endodermal cells of stem, average number of lower and upper epidermal cells. In this way, to define the relation of connection among the examined taxa, it is seen that the anatomic characters are in more forefront. Unfortunately, however, the criteria that used to assign taxonomic rank are sometimes unclear because they are not always explicitly mentioned. The evidence from anatomical and palynological studies shows that variations exist among the species of *Silene* studied.

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