



## Anatomical properties of four *Pilosella* Vaill. (Asteraceae) taxa from Eastern Black Sea Region of Turkey

### Türkiye'nin Doğu Karadeniz Bölgesi'nden dört *Pilosella* Vaill. (Asteraceae) taksonun anatomik özellikleri

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#### Abstract

Anatomical features of four *Pilosella* Vaill. taxa (*P. officinarum* Vaill., *P. piloselloides* (Vill.) Sojak subsp. *bauhini* (Schult.) S. Bräut. & Greuter, *P. × hypeurya* (Peter) Sojak, *P. densiflora* (Tausch) Sojak) collected from Black Sea Region of Turkey were investigated. Anatomical studies include transverse sections of root, stem and leaf with their illustrations. Although the anatomical properties are similar to the typical anatomy of dicotyledons, some differences were observed among them. While root anatomy of *P. officinarum*, *P. piloselloides* subsp. *bauhini* and *P. densiflora* consist of a single layer exodermis, there is 2-3 layer exodermis in the natural hybrid *P. × hypeurya*. Additionally the root pith of this hybrid taxa completely composed of schlerenchyma cells in contrast to other investigated taxa. The stem anatomy of the investigated taxa is similar to each other except the number of the tracheidal elements. The numbers of the stomata per  $\mu\text{m}^2$  distinctly differ in *P. piloselloides* subsp. *bauhini* from the rest examined taxa.

#### Özet

Bu çalışmada Türkiye'nin Doğu Karadeniz bölgesinden toplanan 4 *Pilosella* Vaill. taksonunun (*P. officinarum* Vaill., *P. piloselloides* (Vill.) Sojak subsp. *bauhini* (Schult.) S. Bräut. & Greuter, *P. × hypeurya* (Peter) Sojak, *P. densiflora* (Tausch) Sojak) anatomik yapıları incelenmiştir. Anatomik veriler kök, gövde, yaprak enine kesit ve yaprak yüzeysel kesitleri çizimleri ile birlikte verilmiştir. İncelenen türlerin anatomik yapıları tipik dikotiledon yapısına benzerlik göstermekle birlikte aralarında bazı farklılıklar tespit edilmiştir. *P. officinarum*, *P. piloselloides* subsp. *bauhini* ve *P. densiflora*'nın kök anatomilerinde tek sıra ekzodermis bulunurken, melez bir takson olan *P. × hypeurya* kök anatomisinde 2-3 sıra ekzodermise rastlanmıştır. Melez taksonun kök öz bölgesi diğer incelenen taksonlardan farklı olarak sadece sklerankima hücrelerinden oluşmaktadır. Trake sayıları dışında gövde anatomilerinin benzer olduğu görülmüştür. *P. piloselloides* subsp. *bauhini* 'de stoma sayıları belirgin şekilde diğer türlerden fazladır.

## INTRODUCTION

According to the monograph of Zahn (1921-1923), the genus *Hieracium* L. is divided into four subgenera and two of these, *Hieracium* L., *Pilosella* Vaill. are present in Turkey but Sell and West (1974) who prepared the account of *Pilosella* for the Flora of Turkey recognised these subgenera as a distinct genera. Phytochemical (Petrovic et al. 1999) and molecular (Shi et al. 1996) studies on the genus *Hieracium* s.l. strongly support the morphologically based classifications, however now *Pilosella* is generally treated as a distinct genus, based on a whole range of morphological, biochemical, cytological and genetical characteristics (Braütigam and Greuter 2007). *Pilosella* is well known for its taxonomic complexity and sufficiently different from the genus

*Hieracium* in its achene structure, stoloniferous habit and much higher capacity for sexual reproduction than *Hieracium* to merit elevation to generic status (Sell and West 1974; Sell 1987). The genus *Pilosella* was represented with 35 species including 13 hybrids between most species that grow together in Turkey (Coşkunçelebi 2012). *Pilosella* species are important for their medical value. It is reported that it is used for the treatments of bronchitis, bronchitic asthma and whooping cough (Grieve 1995).

In recent years, the genus *Pilosella* has been the subject of caryological (Bishop and Davy 1994; Gadella 1987), taxonomical (Sell 1987) and ecological (William et al. 1987) studies which improved our understanding of the systematics of this genus. There are also studies

undertaken in order to understand the ecology and evolution of this species, and to implement effective biological control (e.g. Jenkins and Jong 1997, Chapman et al. 2000).

The anatomical properties are generally useful for the classification and delimitation of taxa. However there are few anatomical studies reports on the genus *Hieracium* sensu lato (Qureshi et al. 2009; Talukdar 2015; Tavakkoli et al. 2017) and *Pilosella* sensu stricto (Sepet 2017; Umdu (2000), but only of them including *Hieracium lasiochaetum* (Bornm. & Zahn) Sell & West and *Pilosella hoppeana* subsp. *lydia* (Bornm.& Zahn) Sell & West (= *Pilosella hoppeana* subsp. *testimonialis* (Naegli ex Peter) P.D.Sell & C.West) distributed in Turkey. Thus the main objective of this study is to determine the anatomical properties of *Pilosella* species distributed in Black Sea Region of Turkey. Also we can discuss all findings in a systematic aspects within the the genus.

## MATERIALS AND METHODS

Plant materials used in this study were collected from Black Sea Region of Turkey (Table 1). Some of the plant materials were dried according to the herbarium techniques and kept in the Herbarium of Biology, Karadeniz Technical University (KTUB). Some of the plant materials were preserved in FAA (Formalin-Glacial Acetic Acid-Ethyl Alcohol) at 24h and stored in ethyl alcohol (70%) for anatomical studies. Anatomical studies consist of several sections (cross, superficial) from root, stem, and leaf of *Pilosella* species identified according to the Flora of Turkey (Davis 1972). Sections were waited in sodium hypochloride at 5-10 minutes so as to loose their colour. Afterwards, these sections were stained with safranin-fastgreen (Algan 1981) and permanent slides were made by using entellene. All drawings (both morphological and anatomical) were made by using a lucida camera attached to a Olympus light microscope.

**Table 1.** Locality information of the examined taxa in the present study

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<i>Pilosella densiflora</i> (Tausch) Sojak
A7: Trabzon, Hıdırnebi Yaylası, 1400-1500m, Coşkunçelebi 164, 01 vii 1999, KTUB
<i>Pilosella officinarum</i> Vaill.
A7: Trabzon, Haçka Yaylası, 1400m, Coşkunçelebi 191, 22 vii 1999, KTUB
<i>Pilosella</i> × <i>hypeurya</i> (Peter) Sojak
A7: Trabzon, Hıdırnebi Yaylası, 1400-1500m, Coşkunçelebi 163, 01 vii 1999, KTUB
<i>Pilosella piloselloides</i> (Vill.) Sojak subsp. <i>bauhini</i> (Schult.) S. Bräut. & Greuter
A8: Gümüşhane, Vavuk Dağı, 1800m, Coşkunçelebi 168, 09 vii 1999, KTUB

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## RESULTS

The anatomical properties determined by the microscopic examinations of root, stem and leaf sections in alphabetical order are in the following.

### *Pilosella densiflora* (Tausch) Sojak

**Root:** Epidermis, exodermis, cortex and vascular cylinder take place from out side to the inner, respectively (Figure 1- A). Epidermis cells are quadrilateral or polygonal with smooth walls and have no intercellular space. The exodermis cells are polygonal and have smooth walls and no intercellular space. The cortex consists of parenchymatic cells. Diameters of parenchymatic cells are almost same and they have numerous intercellular spaces. The cells which are close to vascular cylinder are arranged concentric. Endodermis cells are visible and have no intercellular space. Pericycle cells have variable

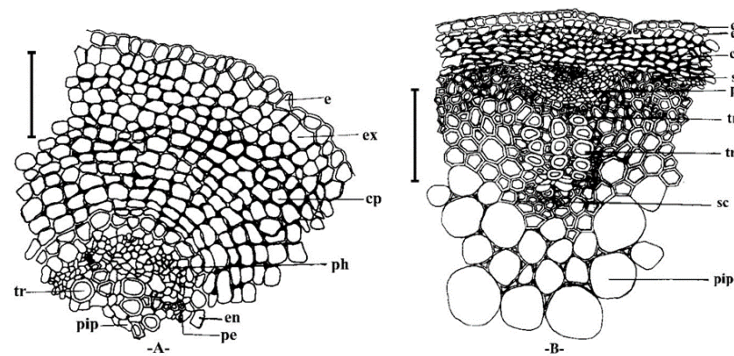
diameters with thin walls. The root is tetraarch. The phloem arranged among the xylem poles and have thin walls. Cambium, located between xylem and phloem, are not visible. Xylem poles are closely in pith but not connected. The pith consists of parenchymatic cells.

**Stem:** Vascular bundles are placed in one line (Figure 1- B). The epidermis consists of one row cells. They are usually quadrilateral and the diameters are variable. A thin cuticle covers the epidermis. The paranchymatic cortex cells are located under the epidermis and have prominent intercellular spaces. The cortex consists of 6-7 rows cells and some of them which are close to epidermis have chloroplasts. Vascular bundles are located in one ring and they are very closely to each other. There are some schlerencymatic cells outside the phloem. Xylem takes 4 times larger place than phloem. Cambium, between xylem and phloem, is not visible. Trachea are in

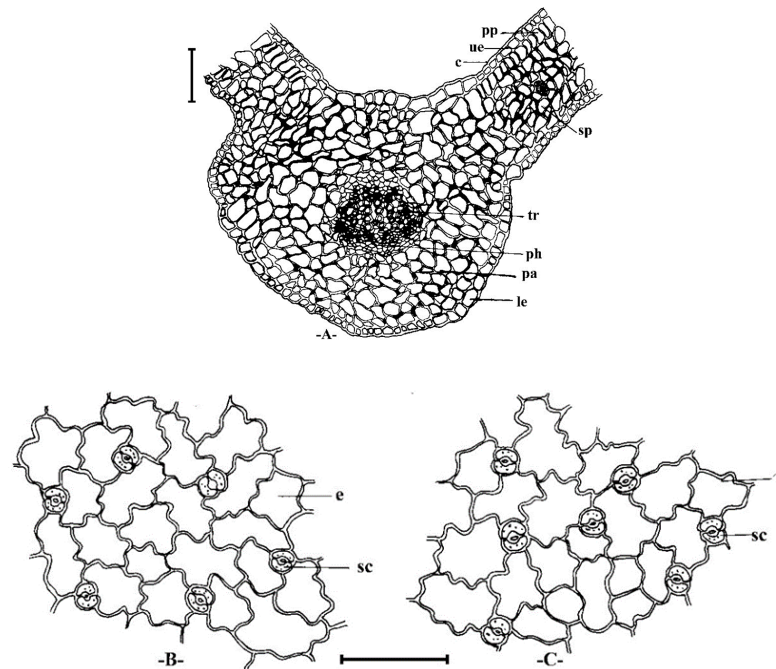
radial rows and there are 5 tracheas in each row. There are some schlerenchymatic cells outside the xylem which faces to the pith. The pith of the stem consists of parenchymatic cells which are usually oval and have numerous intercellular spaces. Diameters of the parenchyma cells increase in the pith.

**Leaf:** The epidermis cells are quadrilateral and have no intercellular space (Figure 2- A). In mesophyll tissue, palisade parenchyma consist of one row cells (usually quadrilateral) and have numerous chloroplasts. Spongy parenchyma cells cover three fourth of mesophyll. Among them intercellular spaces are widespread and they are in

variable shapes and diameters. The midrib is developed, 3 times wider than the leaf lamina. There is only one vascular bundle. Xylem takes more place and there are 5-6 tracheas in each radial row. Phloem covers one third of vascular bundles. Around the vascular bundle there is thin starch sheath consist of 2-3 rows cells. The vascular bundle is in the parenchyma tissue. Both sides of the leaf have anomocytic stomata. The size of the stomata placed on upper epidermis are about 22×30 µm, the others are 24×34 µm. There are about 100 stomata on upper epidermis and 80 stomata on lower epidermis per/mm<sup>2</sup> (Figure 2- B, C).



**Figure 1.** *Pilosella densiflora*. A- Cross section of root. B- Cross section of stem (c- cuticle, e- epidermis, ex- exodermis, cp- cortex parenchyma, is- intercellular space, en- endodermis, pe- pericycle, tr- trachea, trc- tracheid, ph- phloem, sc- sclerenchyma, pip- pith parenchyma). Scale bar: 100 µm)

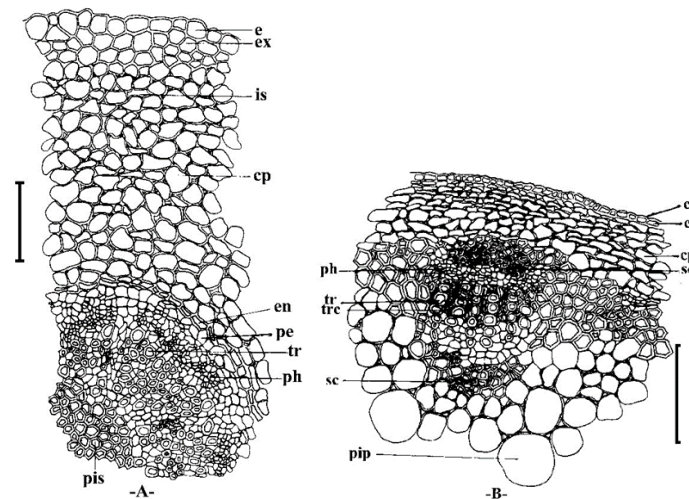


**Figure 2.** *Pilosella densiflora*. A- Cross section of leaf, B-The stomata from upper epidermal leaf, C- The stomata from lower epidermal leaf. (c- cuticle, e- epidermis, ue- upper epidermis, le- lower epidermis, pa- parenchyma, pp- palisade parenchyma, sp- spongy parenchyma, ph- phloem, tr- trachea, sc- stoma cell). Scale bar: 100 µm)

***Pilosella* × *hypeurya*** (Peter) Sojak (*P. hoppeana* × *P. officinarum*)

**Root:** Exodermis, located under the endodermis, consists of 2-3 rows polygonal cells (Figure 3- A). There are parenchymatic cortex consists of 14-16 rows cells under the exodermis. The parenchyma cells have variable shapes, diameters and numerous intercellular spaces. Endodermis cells are almost similar to parenchyma cells but the endodermis cells have little diameters and no intercellular space. Pericycle, outer layer of the stele, is unistratose. It has variable diameters and thin walls. In vascular bundles xylem poles are close to each other in the pith but not connected. The pith consists of schlerenchymatic cells. Phloem is located as a narrow band.

**Stem:** Epidermis consists of quadrilateral cells and cortex consists of 7-8 rows parenchymatic cells (Figure 3- B). The shape and the diameters of the parenchymatic cells are variable. They are oval, polygonal or quadrilateral and some are long with numerous intercellular spaces. Diameters of the cells increase from the epidermis to vascular cylinder. Vascular bundles are located in one ring and there is a schlerenchyma ring around them. The schlerenchyma ring consists of 4-5 rows cells. In vascular bundles, xylem takes more place than phloem and consists of 5-6 radial rows cells and there are 5-7 tracheas in each row. Among the vascular bundles, there are parenchymatic cells. The pith parenchymatic cells are usually oval and diameters of them increase to the pith. Among them intercellular spaces are widespread.

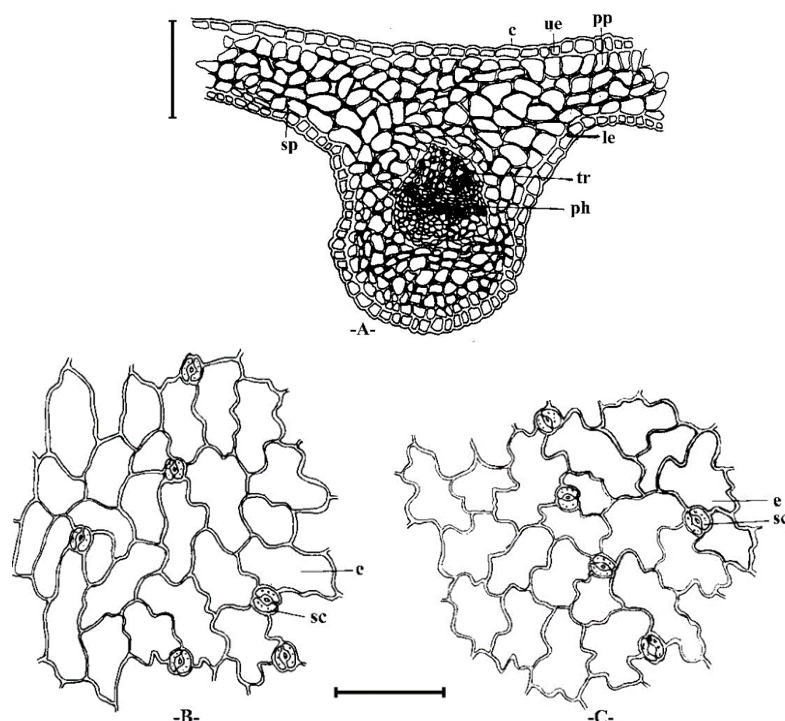


**Figure 3.** *Pilosella* × *hypeurya*. A- Cross section of root, B- Cross section of stem. (c- cuticle, e- epidermis, ex- exodermis, cp- cortex parenchyma, is- intercellular space, en- endodermis, pe- pericycle, tr- trachea, trc- tracheid, ph- phloem, sc- sclerenchyma, pip- pith parenchyma, pis- pith sclerenchyma. Scale bar: 100 µm

**Leaf:** Epidermis is uniseriate and have quadrilateral cells (Figure 4- A). Upper epidermis cells are similar to the lower epidermis cells in shapes but the upper epidermis cells are bigger than the others. Palisade parenchyma cells, under the upper epidermis, have numerous chloroplasts and under them there are spongy parenchyma cells consist of 3-4 rows variable shaped cells. They have less chloroplasts than palisade parenchyma cells and numerous intercellular spaces. The midrib are about 3 times wider than the lateral sides. In the middle there is a developed collateral bundle. Xylem faces towards upper epidermis and phloem faces towards

lower epidermis. In xylem, tracheas are in radial rows and there are 3-5 tracheas in each row. Phloem takes less place than xylem. Vascular bundles are among parenchymatic tissue. Parenchymatic cells that are close to vascular bundles have numerous chloroplasts and the others that are close to epidermis have less chloroplasts than the others. Among them intercellular spaces are widespread. Anomocytic stomata that placed on upper epidermis are of the size of 22×26 µm, the others placed on lower epidermis are of the size of 22×30 µm. The number of the stomata placed on upper epidermis are 30, the others are 80 per/ mm<sup>2</sup> (Figure 4- B, C).





**Figure 4.** *Pilosella x hypeurya*. A- Cross section of leaf, B-The stomata from upper epidermal leaf, C- The stomata from lower epidermal leaf. (c- cuticle, e- epidermis, ue- upper epidermis, le- lower epidermis, pp- palisade parenchyma, sp- spongy parenchyma, ph- phloem, tr- trachea, sc- stoma cell. Scale bar: 100  $\mu$ m)

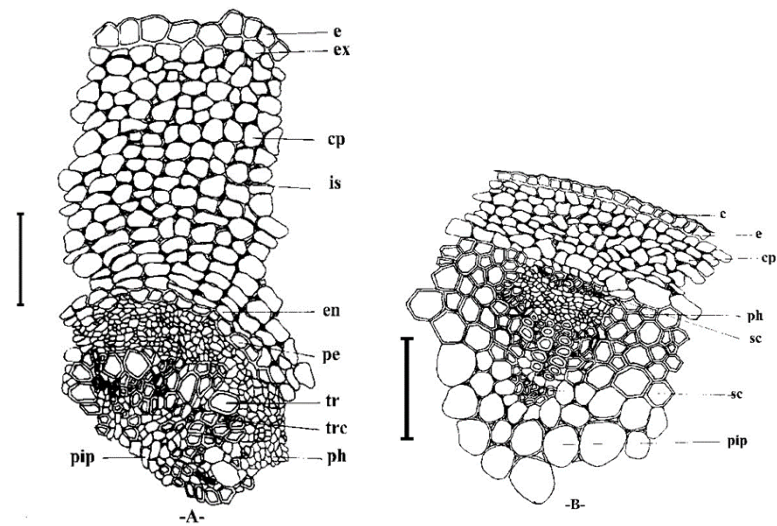
***Pilosella officinarum* Vaill.**

**Root:** One row epidermis, 1-2 rows exodermis and 14-15 rows parenchymatic cortex are present. Parenchymatic cortex cells varies in shape but mostly oval or polygonal (Figure 5- A) and consist of numerous intercellular spaces. The endodermis does not separate from the parenchymatic cortex simply but the endodermis cells have little diameters and no intercellular space. Pericycle, placed under the endodermis, consist of 1-2 rows cells. Tetraarch root and the xylem poles are attached in the pith. Phloem takes less place than xylem. The pith consists of parenchymatic cells.

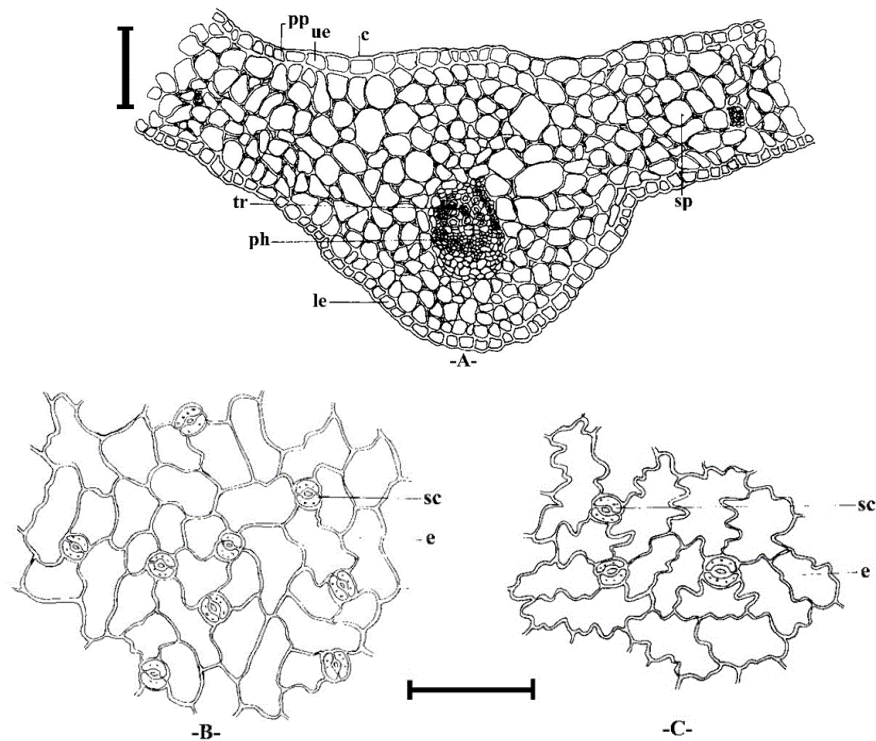
**Stem:** The cuticle covers all parts of the stem epidermal surface. The epidermis consists of one row cells with wavy periferal walls. Cortex consists of 5-7 rows of parenchymatic cells. The cells located inside of the cortex are bigger and variable in shapes. Cortex parenchymatic cells are commonly quadrilateral or oval with numerous intercellular spaces. The parenchymatic cells close to endodermis have prominent chloroplasts. There are discrete vascular bundles and among them there are schlerenchymatic cells in 2-3 rows. Schlerenchymatic

cells cover outside the phloem. Xylem is arranged in radial rows and tracheids placed among them. There are schlerenchymatic cells outside xylem towards central part. Among the vascular bundles, there are parenchymatic cells increasing from the vascular bundles to the pith. They are usually oval or polygonal in shape and consist of numerous intercellular spaces (Figure 5- B).

**Leaf:** Palisade tissue is not visible. Mesophyll, places near the leaf lamina, usually consist of 4-5 rows parenchymatic cells. These are oval, cylinder or sometimes long in shape with several intercellular spaces. Epidermal cells except the cells close to mesophyll are usually quadrilateral in shape. Leaf lamina is twice thicker than the lateral sides (Figure 6- A). In leaf lamina, there is only one vascular bundle. In xylem, tracheas are arranged in radial rows and among them there are tracheas. Phloem located as a thin band. The vascular bundle arranged in parenchymatic tissue. Anomocytic stomata are placed on upper epidermis and its size is 22x28  $\mu$ m, and the others on lower epidermis are 22x26  $\mu$ m. The number of them is about 160 per/mm<sup>2</sup> (Figure 6- B,C).



**Figure 5.** *Pilosella officinarum*. A- Cross section of root, B- Cross section of stem. (e- epidermis, ex- exodermis, cp- cortex parenchyma, is- intercellular space, en- endodermis, pe- pericycle, tr- trachea, trc- tracheid, ph- phloem, sc- sclerenchyma, pip- pith parenchyma. Scale bar: 100 μm)



**Figure 6.** *Pilosella officinarum*. A- Cross section of leaf, B- The stomata from upper epidermal leaf, C- The stomata from lower epidermal of leaf. (c- cuticle, e- epidermis, ue- upper epidermis, le- lower epidermis, pp- palisade parenchyma, sp- spongy parenchyma, ph- phloem, tr- trachea, sc- stoma cell. Scale bar: 100 μm)

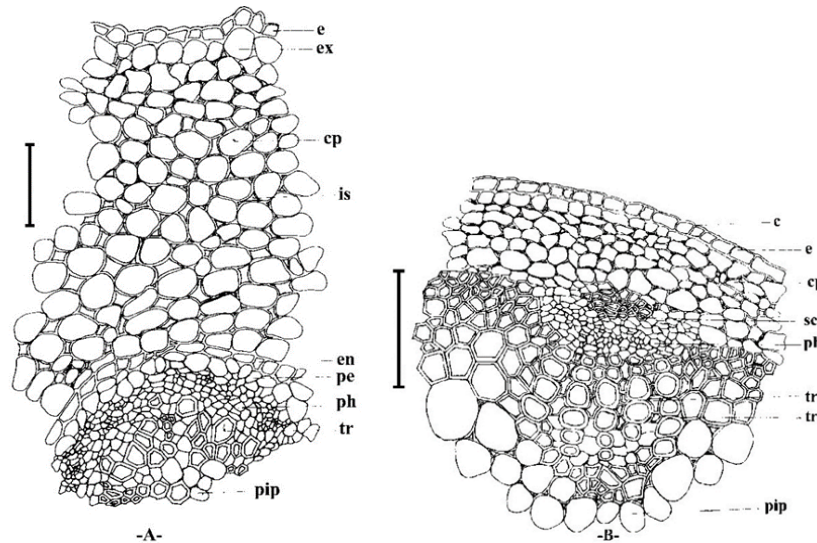
***Pilosella piloselloides* (Vill.) Sojak subsp. *bauhini* (Schult.) S. Bräut. & Greuter**

**Root:** Epidermis and exodermis consist of 1 row cells, but the exodermis cells are bigger than the endodermis cells (Figure 7- A). Parenchymatic cortex consists of 12-13 rows cells with numerous intercellular spaces. Endodermis, unistratose cylinder of cells line the inner boundary of

cortex, is clearly different with small cells. There are no intercellular space between endodermal cells and they have smooth walls. Pericycle is unistratose, pericycle cells are with thin walls and variable diameters. Phloem is located among the xylem poles. Xylem poles are placed closely to each other, but not connected. The pith is parenchymatic.

**Stem:** Epidermis cells are quadrilateral and the centrifugal walls are wavy (Figure 7- B). The cortex parenchyma, placed under the epidermis, consist of 5-6 rows oval or polygonal shaped cells. The cells close to epidermis have little diameters and numerous chloroplasts, the others close to vascular bundles have large diameters and have no chloroplast. Intercellular spaces are widespread among them. In this taxa vascular bundles are located in one ring. Outside the phloem there

are some schlerenchyma cells. Phloem has a thick cap of phloem fibers; the conducting phloem is quite abundant. Xylem arranged in radial rows (5) and each row consist of 4-5 tracheas. Among the vascular bundles there are schlerenchyma consisting of 2-3 rows cells. The pith is parenchymatic. The pith parenchymatic cells are usually oval or polygonal and have numerous intercellular spaces.

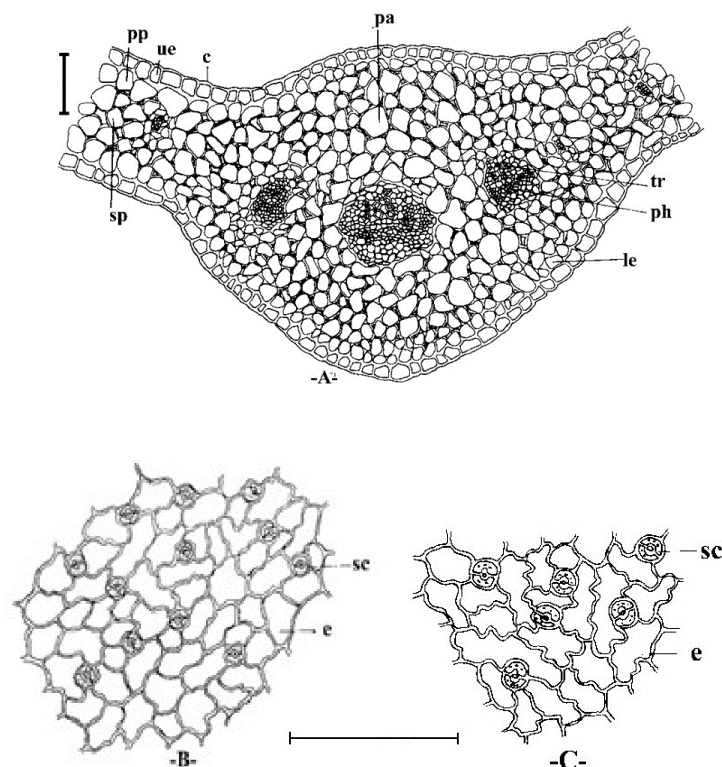


**Figure 7.** *Pilosella piloseloides*. A- Cross section of root, B- Cross section of stem. (c-cuticle, e- epidermis, ex- exodermis, cp- cortex parenchyma, is- intercellular space, en- endodermis, pe- pericycle, tr- trachea, trc- tracheid, ph- phloem, sc- sclerenchyma, pip- pith parenchyma. Scale bar: 100 µm)

**Leaf:** Epidermis consist of one row cells and the cells are usually quadrilateral (Figure 8- A). In the leaf mesophyll, palisade and spongy parenchyma cells can not be separate simply as *P. officinarum*. But it is seen that there is a palisade parenchyma consists of one row cells that have numerous chloroplasts and no intercellular space. Spongy parenchymatic cells have variable diameters and numerous intercellular spaces. The midrib is about twice thicker than the other sides of the leaf. There are 3 vascular bundles. The biggest one is in the middle. Above the vascular tissue is a mass of fibres, below that is xylem, in xylem tracheas arranged in radial rows and there are 3-

4 tracheas in each row. The vascular bundles are among the parenchyma tissue. Parenchyma cells that are close to vascular bundles have chloroplast while the others that are close to epidermis have not. Stomata are anomocytic as the other taxa and the stomata that placed on upper epidermis are of the size of 16×18 µm and the others placed on lower epidermis are 18×20 µm. The number of stomata placed on upper epidermis are about 280, the others are about 220 per/mm<sup>2</sup> (Figure 8- B, C).

Number of stomata in per/mm<sup>2</sup> from examined taxa are in Table 2 below.



**Figure 8.** *Pilosella piloselloides* subsp. *bauhini*. A- Cross section of leaf, B-The stomata from upper epidermal leaf, C- The stomata from lower epidermal leaf. (c-cuticle, e- epidermis, ue- upper epidermis, le- lower epidermis, pa- parenchyma, pp- palisade parenchyma, sp- spongy parenchyma, ph- phloem, tr- trachea, sc- stoma cell. Scale bar: 100  $\mu$ m

**Table 2.** Number of stomata in per/ $\text{mm}^2$  from examined taxa.

Taxa	Number of stomata (per/ $\text{mm}^2$ ) from upper epidermis	Number of stomata (per/ $\text{mm}^2$ ) from lower epidermis
<i>Pilosella densiflora</i> (Tausch) Sojak	100	80
<i>Pilosella</i> $\times$ <i>hypeurya</i> (Peter) Sojak	30	80
<i>Pilosella officinarum</i> Vaill.	160	160
<i>Pilosella piloselloides</i> (Vill.) S	280	220
Sojak subsp. <i>bauhini</i> (Schult.) S. Bräut. & Greuter		

## DISCUSSION

In this study, 4 *Pilosella* taxa (*P. officinarum*, *P. piloselloides* subsp. *bauhini*, *P.  $\times$  hypeurya*, *P. densiflora*) collected from different localities in Northeast Anatolia were investigated in term of anatomy.

It is well known that general root anatomy does not vary much, and the taxonomic value of roots is very limited in many plant genera (Fahn 1990). It was found some differences among the root anatomy of the investigated taxa. For example; in *P. officinarum*, *P. piloselloides* subsp. *bauhini* and *P. densiflora* consist of a single layer exodermis, while *P.  $\times$  hypeurya* is consist of 2-3 layer

exodermis. In addition to this, the root pith of *P.  $\times$  hypeuryum* is completely composed of sclerenchyma cells in contrast to other investigated *Pilosella* taxa. Our results are generally congruent with the findings of Sepet (2017) however root characters are not useful at the specific level within the examined taxa.

Metcalf and Chalk (1972) indicated that sclerenchyma patterns in stem can be used as a taxonomic feature in grouping different plant taxa. The stem anatomy of the investigated taxa is generally similar to each other, however distribution of phloem sclerenchyma vary among taxa.



The leaf anatomy are almost similar but there are some differences among them. In *P. piloseloides*, there are 3 vascular bundles while the others have just one. In *P. × hypeurya* and *P. densiflora*, the palisade parenchyma is composed of a single layer and they are almost quadrilateral, however in *P. piloseloides* and *P. officinarum* to separate palisade parenchyma cells and spongy parenchyma cells is very difficult.

Tavakkoli et al. (2017) indicated that presence or absence of lateral vascular bundles; main midrib shape; the thickness of epidermis, the number of lateral vascular bundles and stomata size are generally in accordance with the phenetic differences in the genus *Hieracium* s. l. Our observations based on leaf anatomical characteristics partly support the findings of Qureshi et al. (2009), Tavakkoli et al. (2017) and Sepet (2017).

The leaves are amphistomatic. The stomata are in type of anomocytic like the other members of *Compositae* as described by Metcalfe and Chalk (1972). In *P. piloseloides*, the numbers of the stomata are noticeable more than the other examined taxa (Table 1).

*P. × hypeurya* is a natural between *P. officinarum* and *P. hoppeana* (Coşkunçelebi, 2012). The anatomical properties of hybrid taxon is generally similar with the one of the ancestor taxa (*P. officinarum*), but differs from it by having 2-3 layer of exodermis (not 1-2 layer) and unilayer of pericycle not 1-2 layer) and number of stomata on the lower epidermis.

In conclusion, the anatomical properties of the four *Pilosella* taxa investigated here are similar to the description of typical anatomy of dicotyledons except of some differences described above.

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