



Comparative anatomy and palynology of two *Herniaria* L. species (*Caryophyllaceae*)

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İki *Herniaria* L. (*Caryophyllaceae*) türünün karşılaştırmalı anatomisi ve palinolojisi

Abstract: In this study, the anatomical and palynological features of *Herniaria incana* Lam. and *H. pisidica* Brummitt. species were investigated. Similarities and differences were discussed for species. Anatomy and pollen studies of these taxa are presented for the first time in this work. Scanning Electron Microscopy (SEM) and Light Microscopy (LM) studies on the pollen grains have revealed that they are prolate – spheroidal and their exine ornamentation is microechinate – punctate. The stem has a collateral vascular bundle and the mesophyll type is bifacial for both *H. incana* and *H. pisidica*.

Key words: Anatomy, *Caryophyllaceae*, *Herniaria*, pollen, Türkiye

Özet: Bu çalışmada *Herniaria incana* Lam. ve *H. pisidica* Brummitt. türlerinin anatomik ve palinolojik özellikleri incelenmiştir. İki tür için benzerlikler ve farklılıklar tartışılmıştır. Bu taksonlara ait anatomi ve polen çalışmaları ilk kez bu çalışmada sunulmaktadır. Taramalı Elektron Mikroskobu (SEM) ve Işık Mikroskobu (LM) çalışmaları sonucunda; polenlerin prolat – sferoidal olduğu, polen ornamentasyonlarının ise mikroekinat – punktata olduğu tespit edilmiştir. Her iki tür için; gövde, kollateral iletim demetlerine sahiptir ve yaprak mezofil tipi bifasiyaldir.

Anahtar Kelimeler: Anatomi, *Caryophyllaceae*, *Herniaria*, polen, Türkiye

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1. Introduction

Caryophyllaceae Juss. family includes approximately 105 genera and approximately 3000 species (POWO, 2024), mainly distributed in Holarctic regions (Bittrich, 1993; Heywood, 1998) with a center of diversity in the Mediterranean and Irano-Turanean areas (Hernández-Ledesma et al. 2015).

Caryophyllaceae family has 3 subfamilies (Alsinoideae Burnett, Caryophylloideae Arn., and Paronychioideae A.St). The genus *Herniaria* L. belongs to the subfamily Paronychioideae. *Herniaria* Linnaeus (1753: 218) includes 52 species according to POWO (2024) and have distributed in Asia, Africa and Europe (Lawrence, 2017; Bittrich, 1993).

The genus *Herniaria* was revised as eight species by Brummitt in the Flora of Turkey in 1967. In the same work, *Herniaria orientalis* F. Herm. was given as a questionable record, and *H. amoena* Çelebioğlu & Favarger was added to the 11th volume of the Flora of Turkey. Also, the subspecies *H. cinerea* D.C. subsp. *euphratica* was overlooked despite being published in 1967 and was not added to the additional volumes. However, the situation was noticed in the List of Turkish Plants and a total of 12 species of the genus *Herniaria* were included in the work. Six of these species are endemic and the endemism rate is 50% (Güner et al. 2012; Davis, 1967). According to recent studies, *Herniaria* includes 11 taxa, with *H. orientalis* F. Herm. being a questionable record.

Herniaria genus and anatomy of *Herniaria* was studied by Williams (1896) and Hermann (1937) for the first time. Later, it was presented by Van et al. (1968). Gangulee et al. (1972) and Mousaand Al-Jibouri (2019) were studied on anatomy of *H. hirsuta* L. Also, Zarinkamar (2001) surveyed on *H. hirsuta* and *H. incana*. Schweingruber (2007) has investigated of 4 *Herniaria* taxa (*H. glabra* L., *H. incana*, *H. hirsuta*, *H. alpina* Chaix.). In pollen studies, by Nowicke and Skvarla (1976), and Hutchinson (1959) on *H. hirsuta*, Punt and Hoen (1999) on *H. glabra*, Perveen and Qaiser (2003) on *H. cinerea* D.C. were studied. As a result, anatomical and palynological surveys on *Herniaria* are rare.

In this study, *H. incana* and *H. pisidica* species were studied and compared in view of anatomical and palynological characters. For these species, similarities and differences were identified. In this study, anatomy and pollen examinations of these *Herniaria* taxa are presented for the first.

2. Materials and Method

The specimens were collected from Kastamonu province (*H. incana*) and Denizli province (*H. pisidica*) in Türkiye (Table 1). The collected specimens are deposited in the herbarium of Ankara Yıldırım Beyazıt University.

For anatomical studies; living material was kept in 70% ethanol. The paraffin method was used to obtain cross sections of stem and leaves. The specimens were embedded in paraffin wax. These materials were sectioned between 5

and 10 µm thickness with a Leica RM2245 rotary microtome. All species were stained with safranin–fast green. These stained sections were glued with entellan and permanent preparations were obtained according to Johansen (1940). Measurements and photos were taken using a Leica DM1000 binocular light microscope equipped with a Leica DFC280 camera.

For palynological investigations; pollen material were acquired from herbarium specimens. The pollen slides were prepared according to Wodehouse (1935) technique. For scanning electron microscopy (SEM), dry pollen samples were transferred on stubs, cover with gold, examined and photographed with a HITACHI SU5000 FE-SEM at Ankara Yıldırım Beyazıt University Central Research Laboratory Application and Research Center. Pollen morphological characters were calculated as minimum, maximum, standard deviation, average values and it presented as a table. Measurements were made on 20-30 pollen grains and based on the ratio of polar axis to equatorial axis (P/E), the pollen shape class was determined with Erdtman’s system (1969). Pollen morphologies were identified by using the glossary of pollen and spore terminology of Punt et al. (2007).

3. Results

3.1. Pollen morphology

It is found that pollen grains of *H. incana* and *H. pisidica* are polyporate, isopolar, radial symmetric and pollen shape

are prolate-spheroidal. Pollen ornamentation are microechinate – punctate for both taxon. Some differences were detected in terms of pollen characters such as exine and intine thickness, intense of spinule, number of punctum and these differences were presented figures and tables (Table 2, Fig. 1 and Fig. 2).

Table 1. Locality of *H. incana* and *H. pisidica*.

| Species of <i>Herniaria</i> | Locality | Herbarium number |
|------------------------------|---|------------------|
| <i>H. incana</i> | Amasya: Lokman Mountain, 1100 m. 19.06.2024. | M. Koç - 4350 |
| <i>H. pisidica</i> (Endemic) | Denizli: Babadağ, 37° 39' 52" N, 28° 57' 23.4" E, 1190, 28.06.2024. | M. Koç - 3611 |

3.2. Anatomy

3.2.1. Stem anatomy

— *H. incana* has epidermis 1-layered, consisting of rectangular and oval cells and is surrounded by a cuticle layer. The cortex tissue is consist of 6–7 layered, oval or cylindrical parenchymatic cells with chloroplasts and cortex has druse crystals. The sclerenchyma tissue is composed from 2 layered. The pith is consist of circular shaped parenchymatic cells. The stem has a collateral vascular bundle (Table 3, Fig. 3).

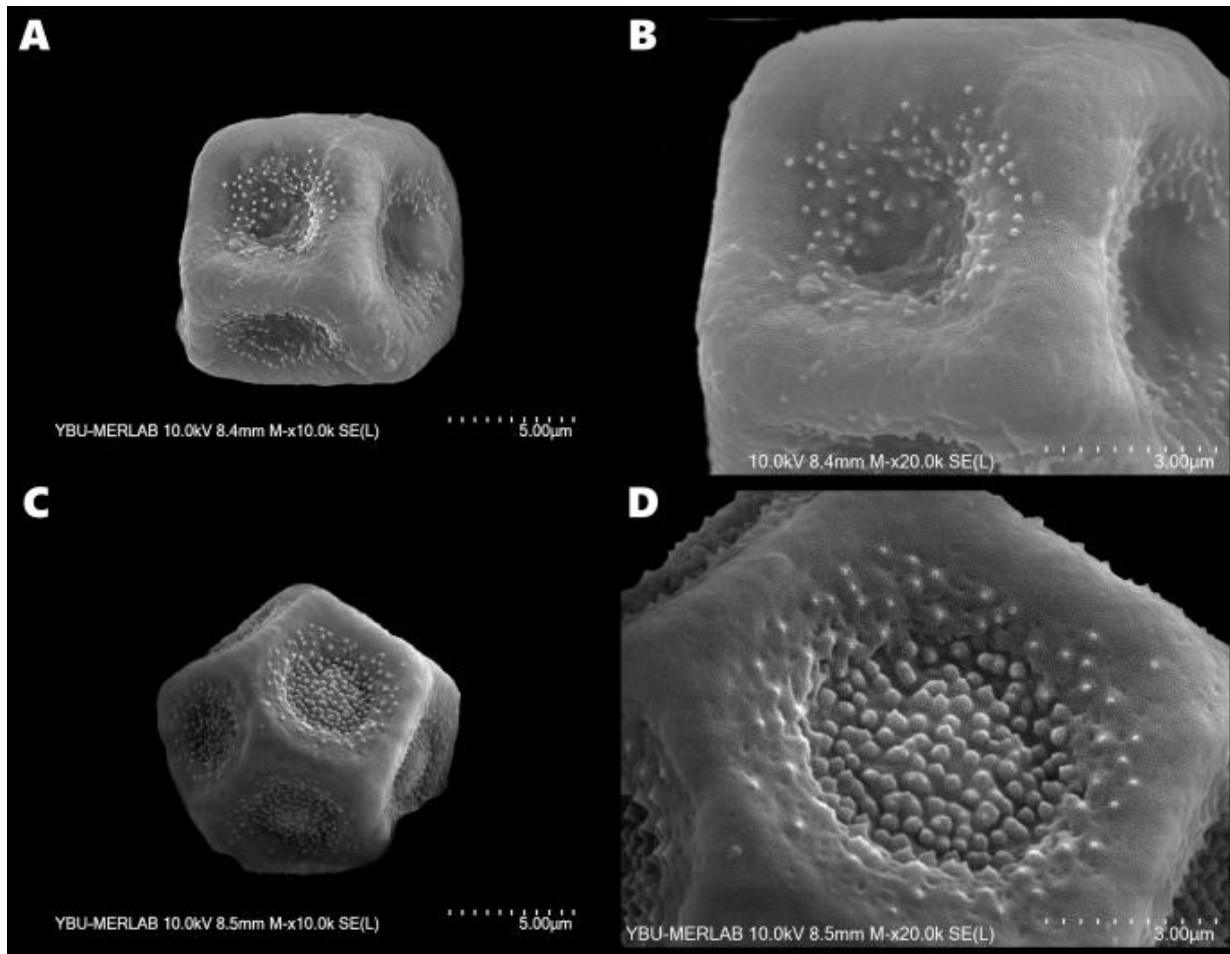


Figure 1. SEM micrographs of pollen grains. A–B: Equatorial view and exine sculpturing of *H. incana* C–D: Equatorial view and exine sculpturing of *H. pisidica*.

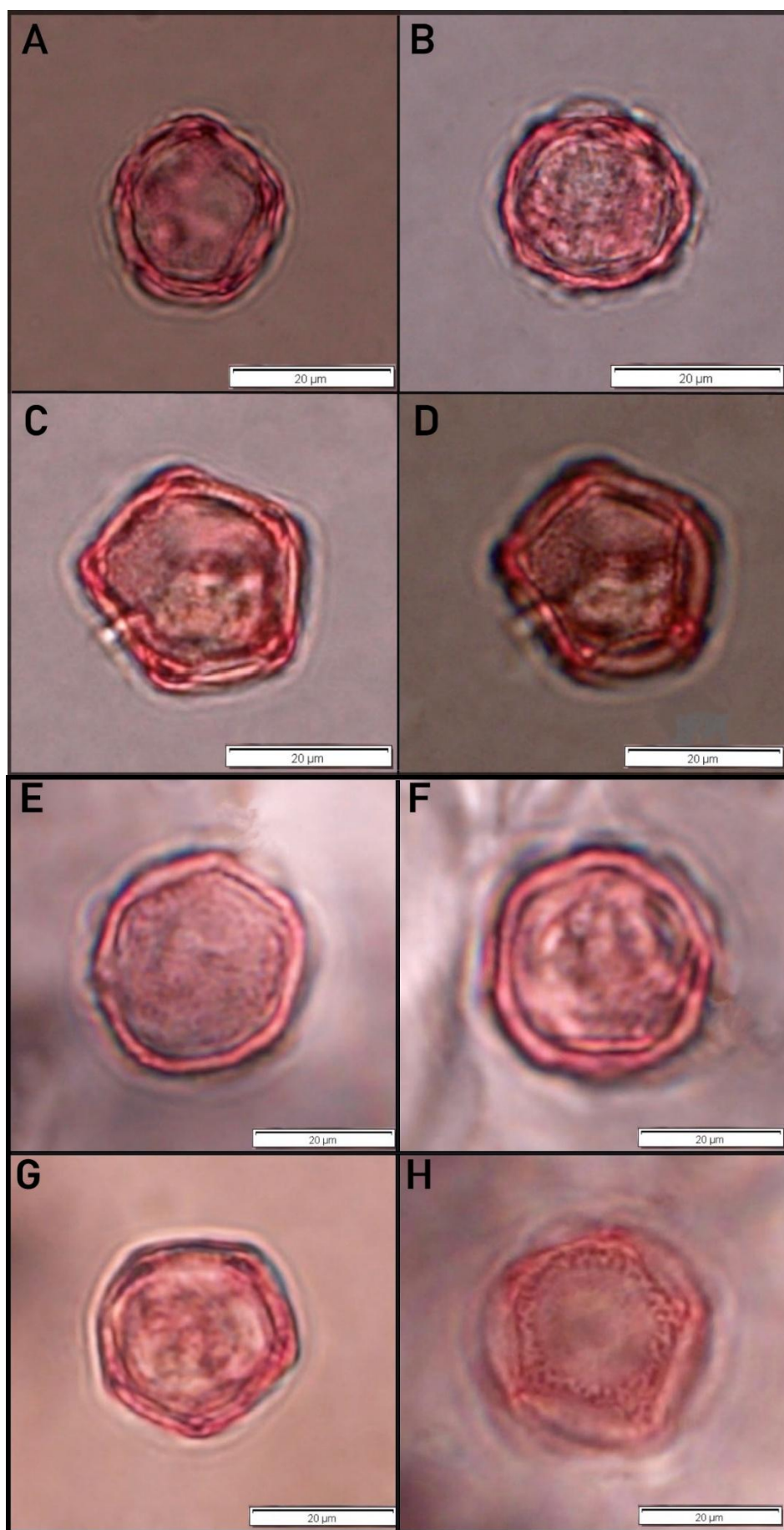


Figure 2. Light micrographs (LM) of the pollen grains. A–B: Equatorial view and ornamentation of *H. incana* C–D: Polar view and ornamentation of *H. incana*. E–F: Equatorial view and ornamentation of *H. pisidica* G–H: Polar view and ornamentation of *H. pisidica*.

Table 2. Comparative of the pollen characters *H. incana* and *H. pisidica*.

| Pollen Characters Min - (M±SD) – Max [µm] | <i>H. incana</i> | <i>H. pisidica</i> |
|--|--------------------------------|--------------------------------|
| Pollen diameter (µm) | 11.44 - (13.41 ± 1.68) - 16.22 | 13.34 - (14.21 ± 0.58) - 15.02 |
| Polar axes (µm) | 11.27 - (14.50 ± 1.45) - 16.51 | 11.16 - (14.14 ± 1.54) - 17.72 |
| Equatorial axes (µm) | 10.37 - (13.93 ± 1.34) - 16.96 | 11.49 - (13.75 ± 1.53) - 16.96 |
| Pollen shape (µm) | Prolate spheroidal | Prolate spheroidal |
| Pollen ornamentation (µm) | Microechinate - Punctate | Microechinate - Punctate |
| Exine thickness (µm) | 0.21 - (0.36 ± 0.12) - 0.63 | 0.74 - (0.86 ± 0.17) - 1.19 |
| Intine thickness (µm) | 0.26 - (0.36 ± 0.07) - 0.47 | 0.59 - (0.64 ± 0.09) - 0.89 |
| Pore diameter (µm) | 3.93 - (5.11 ± 1.78) - 9.25 | 4 - (6.44 ± 1.20) - 8.54 |
| Number of pore (µm) | 10 ± 3 | 12 ± 3 |
| Distance between two pores (µm) | 1.10 - (1.38 ± 0.23) - 2.03 | 0.76 - (1.36 ± 0.42) - 2.35 |
| Operculum diameter (µm) | 1.71 - (2.81 ± 1.22) - 5.68 | 2.38 - (3.78 ± 1.02) - 6.48 |
| Number of spinule (10µm ²) | 20 - 25 | >70 (intense) |
| Number of spinules on operculum (µm) | 5 - 7 | 8 - 10 |
| Number of punctum (10µm ²) | 20 - 25 | 40 - 45 |

Abbreviations: M: Average, SD: Standart deviation, Min: Minimum, Max: Maximum, µm: Micrometer

Table 3. Comparative anatomy of the stem *H. incana* and *H. pisidica*.

| Stem Characters Min - (M±SD) – Max [µm] | <i>H. incana</i> | <i>H. pisidica</i> |
|--|---------------------------------|---------------------------------|
| Epidermis Length | 7.83 - (15.62 ± 5.60) - 22.95 | 9.97 - (17.71 ± 3.20) - 24.52 |
| Epidermis Width | 9.33 - (19.58 ± 6.79) - 32.47 | 9.94 - (23.40 ± 6.3) - 41.95 |
| Cortex Length | 8.21 - (22.02 ± 7.91) - 35.83 | 9.73 - (19.12 ± 4.01) - 32.77 |
| Cortex Width | 14.18 - (25.59 ± 10.26) - 48.71 | 14.84 - (27.68 ± 6.14) - 49.55 |
| Sclerenchyma Length | 11.19 - (14.48 ± 1.76) - 17.91 | 17.48 - (25.02 ± 3.33) - 34.96 |
| Sclerenchyma Width | 5.59 - (8.13 ± 1.55) - 10.07 | 5.57 - (9.93 ± 2.42) - 15.84 |
| Xylem Length | 7.27 - (12.74 ± 2.80) - 17.54 | 9.51 - (18.44 ± 4.85) - 30.7 |
| Xylem Width | 5.59 - (10.69 ± 2.80) - 16.79 | 7.88 - (13 ± 2.19) - 18.28 |
| Core Length | 17.35 - (42.57 ± 20.01) - 81.18 | 9.01 - (20.22 ± 10.01) - 68.92 |
| Core Width | 6.71 - (21.31 ± 11.32) - 51.51 | 11.51 - (28.71 ± 16.06) - 103.9 |

Abbreviations: M: Average, SD: Standart deviation, Min: Minimum, Max: Maximum, µm: Micrometer

— *H. pisidica* has epidermis 1-layered, consisting of rectangular and oval cells and is surrounded by a cuticle layer. The cortex tissue, which is located under the epidermis, is composed 7–8 layered, oval or cylindrical parenchymatic cells with chloroplasts and cortex has druse crystals. The sclerenchyma tissue is composed from 3 layered. The pith is consist of circular shaped parenchymatic cells and has druse crystals. The stem has a collateral vascular bundle (Table 3, Fig. 3).

3.2.2. Leaf Anatomy

— *Herniaria incana* shows the lamina with upper and lower epidermis covered by a cuticle layer. Both epidermis are consist of uniseriate oval and rectangular cells. The mesophyll is consist of 2-3 layer of elongated palisade parenchyma cells. Spongy parenchyma cells are 7-8 layers. Mesophyll has druse crystals. The mesophyll type is bifacial (Table 4, Fig. 4).

— *Herniaria pisidica* shows the lamina with upper and lower epidermis covered by a cuticle layer. Both epidermis consist of uniseriate oval and rectangular cells. The mesophyll is consist of 2-3 layer of elongated palisade

parenchyma cells. Spongy cells are 7-8 layers. Mesophyll has druse crystals. The mesophyll type is bifacial. (Table 4, Fig. 4).

4. Discussions

In this study, anatomical and palynological data of *H. incana* and *H. pisidica* were obtained. The anatomical and palynological findings for *H. pisidica* and *H. incana* are reported first time in this work. Data on pollen and anatomy studies are compared with other similar *Herniaria* taxa.

Compared to palynological study; exine and intine thickness of *H. pisidica* are thicker than *H. incana*. The number of spinules is very intense in *H. pisidica*. Two taxa were compared the number of punctum; *H. pisidica* is approximately twice as much in *H. incana*. Compared to anatomical studies; *H. incana* leaf characters are smaller than compared to *H. pisidica*.

Metcalf (1946), the leaf mesophyll layer in *Herniaria* is chlorenchymatous and palisade tissue is developed. Calcium oxalate crystals (druse) generally form large and conspicuous masses in the mesophyll. These findings are similar to our study.

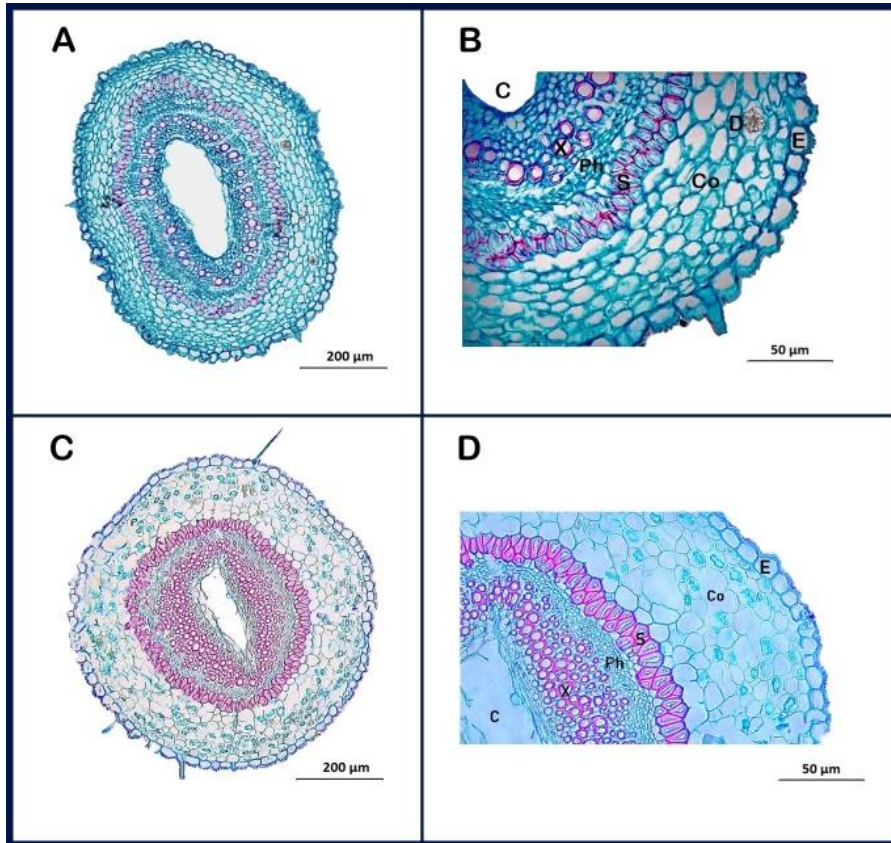


Figure 3. Cross-section of the stem. A–B: General and detailed view of *H. incana*, C–D: General and detailed view of *H. pisidica*. E: Epidermis, Co: Cortex, S: Sclerenchyma, Ph: Phloem, X: Xylem, C: Core, D: Druse crystal.

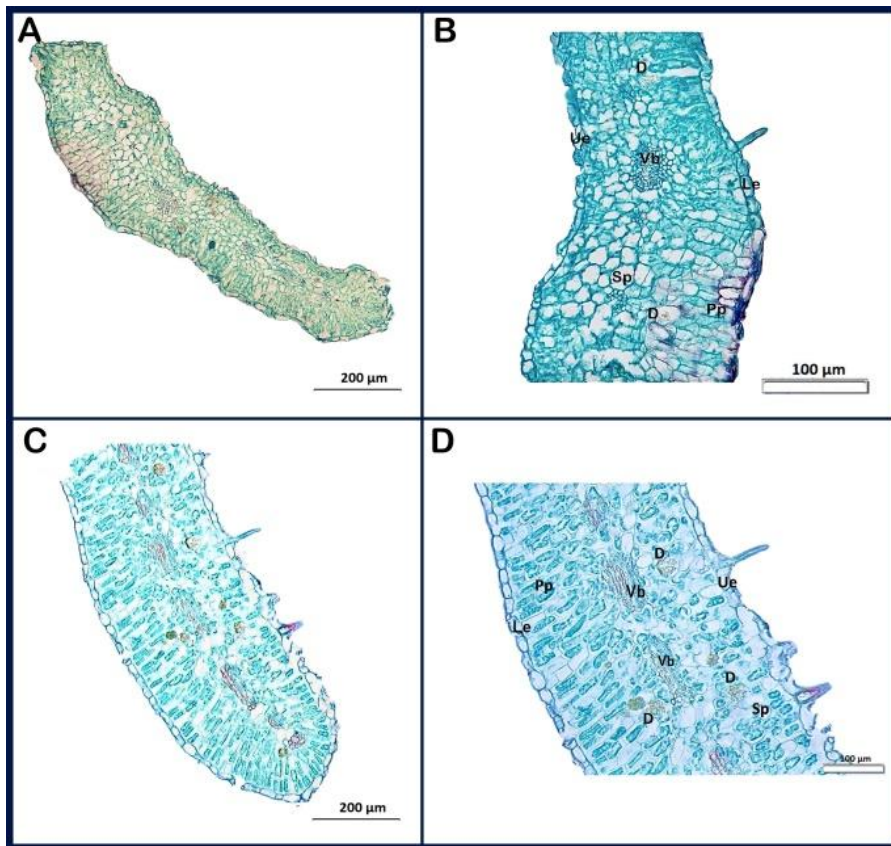


Figure 4. Cross-section of the lamina. A–B: General and detailed view of *H. incana*, C–D: General and detailed view of *H. pisidica*. Ue: Upper Epidermis, Pp: Palisade parenchyma Sp: Spongy parenchyma, Vb: Vascular bundle, D: Druse crystal, Le: Lower Epidermis.

Table 4. Comparative anatomy of the leaves *H. incana* and *H. pisidica*.

| Leaf Characters Min - (M±SD) – Max [µm] | <i>H. incana</i> | <i>H. pisidica</i> |
|--|--------------------------------|--------------------------------|
| Upper Epidermis Length | 4.79 - (6.60 ± 1.29) - 10.87 | 9.9 - (16.45 ± 4.81) - 34 |
| Upper Epidermis Width | 5.37 - (12.75 ± 4.62) - 23.67 | 18.03 - (27.99 ± 8.02) - 47.05 |
| Lower Epidermis Length | 4.47 - (6.35 ± 1.06) - 8.58 | 11.01 - (16 ± 3.41) - 25.9 |
| Lower Epidermis Width | 4.85 - (11.82 ± 3.43) - 22.76 | 14.02 - (26.04 ± 6.71) - 41.03 |
| Palisade Parenchyma Length | 11.19 - (17.95 ± 3.88) - 25.91 | 28.2 - (44.83 ± 8.34) - 66.19 |
| Palisade Parenchyma Width | 3.91 - (5.71 ± 1.48) - 9.70 | 9.04 - (13.7 ± 2.27) - 19.05 |
| Spongy Parenchyma Length | 6.34 - (12.11 ± 3.55) - 22.71 | 17.96 - (26.12 ± 5.53) - 40.9 |
| Upper Epidermis Length | 4.79 - (11.27 ± 2.60) - 16.79 | 20.51 - (26.19 ± 5.3) - 38 |

Abbreviations: M: Average, SD: Standart deviation, Min: Minimum, Max: Maximum, µm: Micrometer

Van et al. (1968), for *Herniaria* genus, the leaf is positioned in the center. Stomata is located on both surfaced of the leaf. The mesophyll is chlorenchymatous. Palisade texture is abundant and developed. Calcium oxalate crystals are usually located in the mesophyll and form large, conspicuous masses (or compound crystals). In our study, it was determined that the mesophyll layer contains intense chlorophyll and the palisade parenchyma appears in 2–3 layers, the spongy parenchyma has an average of 7-8 layers and it covers a lot of surface area, and the druse crystals are located in masses spread over the mesophyll. Our results are generally consistent this study results (apart from mesophyll layer).

Zarinkamar (2001), who examined anatomically two *Herniaria* taxa from Iran (*H. hirsuta* and *H. incana*), in terms of leaf anatomy. In these types reported: thick, granular waxy layer deposits on the epidermal cell walls and the cuticle rough on the outer surface, higher stomatal frequency, collenchyma and more mechanical sclerenchymatous tissue on the leaf edges, salt deposits in the form of large crystals around the vascular bundle in the middle of the palisade parenchyma have been reported in the literature. It has been emphasized that these characters originate from the resistance to xerophyte status in the Caryophyllaceae family. In addition, it has been reported in the literature that the variability of anatomical structures even among species of the same genus is related to the microclimatic conditions of the region where the species lives. In our study, it is observed that the epidermal cell walls are thick due to the xerophytic structure, the vascular bundle, including the sclerenchymatic tissue more mechanical in all taxa and the sclerenchymatous tissue covers a large area. Druse crystals appear as large and small structures spread throughout the mesophyll layer. The stomata has become smaller and located just under the upper and lower epidermis, and the average presence of one stoma each supports the xeromorphic structure. In general, the study results are consistent with our research.

Schweingruber (2007), were examined on four *Herniaria* taxa (*H. glabra*, *H. incana*, *H. hirsuta*, *H. alpina*). He found the presence of thick-walled vessels (2 µm), widespread parenchyma, absence of radial symmetry, sclereids and druse crystals in the cortex were noted. In our study, the presence of widespread parenchyma and the absence of radial symmetry were detected in our study. Calcium oxalate crystals are located in the cortex for all taxa. The results are generally consistent with our research.

When compared all these studies with our work; *H. pisidica* has druse crystals both in the cortex and the core were observed. For *H. incana* druse crystals were located in cortex only.

Mousa and Al-Jibouri (2019) studied the stem anatomy of *H. hirsuta* distributed in Iraq and reported that measurements (average) of stem anatomy are: epidermis thickness 16 µm, cortex parenchyma thickness 62 µm, xylem thickness 103 µm, thickness in pith cells 288 µm. According to these results, our measurements in *Herniaria* taxa are much smaller than the study by Mousa and Al-Jibouri (2019) except for epidermis.

H. hirsuta pollen grains are pantoporate (Nowicke and Skvarla 1976; Hutchinson, 1959). These studies support the idea that the genus *Herniaria* (previously included Illecebraceae family) differs in terms of pollen grains. The genus *Herniaria* is separated from Illecebraceae family both morphologically and palynologically.

Punt and Hoen (1999) reported that 4–6 porate (rarely more) pollen grains of *H. glabra*. For *H. cinerea*, pollen shape is spheroidal, and pollen grains are 6-12 porate, exine ornamentation is scabrate-spinulose. Pollen diameter is 20.11 ± 0.09 µm, and equatorial axis 19.69 ± 0.28 µm, exine thickness 1.72 ± 0.1 µm (Perveen and Qaiser, 2003). The results of these study are not consistent with our study consequence.

When our study results was compared with the literature, we found that inconsistent findings for anatomy and pollen on *Herniaria*. This situation leads to confusion on *Herniaria*. This information pollution can be eliminated. For this purpose, the next study aims to carry out micromorphology studies on all taxa of *Herniaria*. As mentioned above, anatomical and palynological differences are very important for taxonomic descriptions. Consequently, it is predicted that the information acquired with this work will contribute to the literature.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contribution

Y.C: Literature research, data collection, organize the execution of the study, writing the article and carrying out experimental studies, data entry and measurement. M.K: Provision of the plant material, provide a working environment and tools, create an idea.

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