

A taxonomic study for *Fumaria asepala* Boiss. and *Fumaria schleicheri* Soy.-Will. subsp. *microcarpa* Boiss. ex Hausskn. growing in Turkey: evidence from morphological, anatomical and palynological studies

Türkiye'de yetişen *Fumaria asepala* Boiss. ve *Fumaria schleicheri* Soy.-Will. *microcarpa* Boiss. ex Hausskn. için taksonomik bir çalışma: morfolojik, anatomik ve palinolojik çalışmalardan elde edilen kanıtlarla

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Abstract

The genus *Fumaria* L. belongs to the Papaveraceae family, and consists of plants that are difficult to distinguish morphologically. In this study, it is aimed to reveal the structural features of two species belonging to the genus *Fumaria*. To this end, leaf, sepal, petal dimensions and shapes, filament and anther lengths, and various morphological characteristics were examined and necessary measurements were made. Within the scope of anatomic studies, cross-sections were made manually from roots and stems of species, and preparations were prepared by reverse staining technique and photographed under a light microscope to determine anatomic characteristics. Additionally, light microscope and SEM studies were made to expose pollen characteristics. According to the results of the studies, *Fumaria asepala* Boiss. and *Fumaria schleicheri* Soy.-Will. subsp. *microcarpa* Boiss. ex Hausskn. species live together in the area and display an interesting characteristic. Following microscopic studies, although these two species are morphologically very similar to each other, *F. asepala* is different from *F. schleicheri* subsp. *microcarpa* by the absence of sepal. Again, fruit micromorphologies also provide valuable data to distinguish the species. Results of anatomic studies were also similar in both species. In palynological studies that examined pollens, it was observed that pollen types were suboblate for both species, and apertures of pollens were pantoporate/porate. The number of apertures was 6-7 in *F. asepala*, 4-8 in *F. schleicheri* subsp. *microcarpa*, and larger in *F. asepala* species. Additionally, the pollens of *F. schleicheri* subsp. *microcarpa* were larger compared to *F. asepala*. Original data were obtained about species after combining these results, and basic systematic data about genus was brought to the literature. This study is important as it is the detailed systematic study of *Fumaria* species grown in Elazığ province.

Özet

Fumaria L. cinsi Papaveraceae familyasına ait bir cins olup, morfolojik olarak ayırt edilmeleri oldukça güç bitkilerden oluşur. Bu çalışmada, *Fumaria* cinsine ait iki türün yapısal özelliklerini ortaya çıkarılması amaçlanmaktadır. Bu amaçla yaprak, sepal, petal boyutları ve şekilleri, filament ve anter uzunlukları ile çeşitli morfolojik özellikleri incelenmiş ve gerekli ölçümler alınmıştır. Anatomik çalışmalar kapsamında, türlerin kök ve gövdelerinden elle enine kesitler alınmış ve zıt boyama tekniği kullanılarak hazırlanan preparatlar, ışık mikroskobu altında fotoğraflanarak anatomik özellikleri belirlenmiştir. Ek olarak polen özelliklerini ortaya çıkarmak için ışık mikroskobu ve SEM çalışmaları yapılmıştır. Yapılan çalışmalardan elde edilen sonuçlara göre *Fumaria asepala* Boiss. ve *Fumaria schleicheri* Soy.-Will. subsp. *microcarpa* Boiss. ex Hausskn. türleri bulunduğu bölgede yan yana yaşayarak ilginç bir özellik sergilemektedir. Morfolojik olarak birbirine çok benzeyen bu iki tür yapılan mikroskobik çalışmalar sonucu *F. asepala* da sepallerin bulunmayışı özelliği ile *F. schleicheri* subsp. *microcarpa* 'dan ayrılmaktadır. Yine meyve mikromorfolojileri de türlerin ayırımında değerli veriler sunmaktadır. Anatomik çalışmalardan elde edilen sonuçlar ise her iki türde de benzer bulunmuştur. Palinolojik çalışmalarda incelen polenlerin, polen tipi her iki tür için de suboblate olduğu, polenlerin apertürleri Pantoporate/Porat olduğu gözlenmiştir. Apertür sayısı *F. asepala*'da 6-7, *F. schleicheri* subsp. *microcarpa* 'da 4-8 iken, apertür çapı *F. asepala*'da daha büyük ölçülmüştür. Ayrıca *F. schleicheri* subsp. *microcarpa* 'nın polenleri *F. asepala*'ya göre daha büyük olarak ölçülmüştür. Elde edilen bu sonuçlar kombine edilerek türlerle ilgili orijinal veriler elde edilmiş, cinse ait temel sistematik veriler literatüre kazandırılmıştır. Bu çalışma Elazığ ilinde yetişen bazı *Fumaria* türleri ile ilgili yapılan detaylı bir sistematik çalışma olması açısından önem taşımaktadır.

INTRODUCTION

The genus *Fumaria* belongs to the "Fumarioideae" subfamily of the Papaveraceae family and there are 70 species worldwide (URL-1). Species of the genus grow in temperate and subtropical climate zones in the northern hemisphere except in the USA. *Fumaria* genus, found in almost every region of our country, from sea level to 2000 m altitude, in fields, roadsides, hill slopes, and between fences, has species that could grow in arid and moist soils. Among these species, some have climber characteristics. Genus is represented by 15 species in Flora of Turkey Vol.1 (Davis 1965), but represented by 17 taxa with recent update (Güner et al. 2012)

Fumaria species known as "Şahtere" in our country are plants 15-150 cm high, vertical, spreading or climber. The leaf is 5-7 cm, the petiole is thin and long, the inflorescence is tight or loose, raceme is opposite the leaf. The inflorescence is short, bracteoles are equal in length to pedicel and flowers are generally zygomorphic (Şener 1982). *Fumaria* is a genus of plants with medical characteristics. It contains intense alkaloids and sterols. Treatments of antihypertensive, diuretic, liver protective, purgative, gastrointestinal disorders, and abdominal cramps are some of the traditional characteristics of this genus (Reynier 1977, Topçam 2017)

Fumaria species are very similar to each other in appearance and their differentiation is very difficult for

native people. Descriptions of *Fumaria* species are difficult because of variability in vegetative and reproductive characteristics most probably due to interspecies hybridization (Murphy 2009). This study aimed to determine the taxonomic characteristics of *F. asepala* Boiss. and *F. schleicheri* Soy.-Will. subsp. *microcarpa* Boiss. ex Hausskn. that belong to the *Fumaria* genus. Determining morphological, anatomical and pollen characteristics of a plant will guarantee a true description of the plant and help standardization by demonstrating similarities and differences between species. This comprehensive study conducted in our country is important as it is the study performed on this species.

MATERIAL AND METHODS

Materials

Plant materials were collected from their natural environment in April 2021 and were identified in Firat University Herbarium (FUH). Some of the collected plants were reserved as herbarium material in terms of suitability for morphological studies, and the another parts in 70% ethanol were preserved as soon as they were collected for anatomic studies. Parts with matured flowers were enveloped for palynological studies. General appearances of plants are shown in Figure 1, and detailed locality data in Table 1.

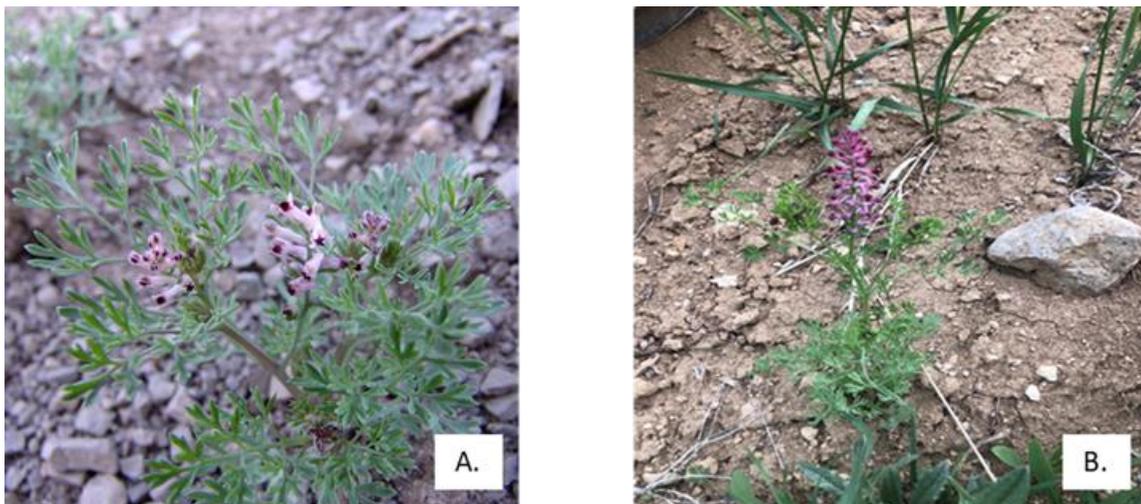


Figure 1. General appearance of *Fumaria* species (A. *F. asepala*, B. *F. schleicheri* subsp. *microcarpa*)

Table 1. Detailed location information in studied taxa

| Taxa | Collection date | Locality |
|---|-----------------|---|
| <i>Fumaria asepala</i> | 13.05.2021 | B7 Elazig: Baskil- Sancaklı village inner road, 1410 m. |
| <i>Fumaria schleicheri</i> subsp. <i>microcarpa</i> | | Lat: 38°35'13.061" Lon: 38°55'23.055" |

Methods

Morphological examinations include instant observations on samples in the field and macroscopic and microscopic examinations performed on the samples converted to herbarium material in the laboratory. Small structures were measured under a stereomicroscope and macroscopic structures with the naked eye using a ruler.

Within the scope of anatomic studies, anatomic structures of root and stem were examined in detail. Sections were made from morphological organs of interest and preparations were stained with the reverse staining (Safranin-Alcian Blue) method and images were taken under the microscope (Tekin and Civelek 2013).

The Wodehouse method was used for light microscope studies. Pollens treated with 96% alcohol for 1 minute were kept on the heating plate until alcohol evaporated, then basic fuchsine was added to pollens, and a piece of glycerine gelatine was melted and examined under a microscope by covering with lamella (Wodehouse, 1935; Demirpolat 2022a-b). Also, the pollens were imaged with SEM (Scanning Electron Microscopy) by procurement service from Firat University Central Laboratory and their surface micromorphological properties were determined.

Zeiss Primostar 3 light microscope, Leica EC3 stereomicroscope and Zeiss EVO MA10 electron microscope were used for all these studies.

RESULTS

Morphological Observations

Morphological characteristics of two species that belong to the *Fumaria* genus that grow in every region in our country are reported here.

F. asepala's life form is annual, 20-30 cm in length with hairless stem. Leaf type is linear lanseolat, petiol is slender, bracteole pedicel is equal, and lanseolat. The flower colour is white, purple at the tips, a flower arrangement is rasemus. The most characteristic feature of the flower with zygomorphic symmetry is the absence of sepal. Corolla is 4 petal and coripetal. The number of stamens is 6 and is characteristically diadelf and united in groups of 3 at the base with expanded filaments. The ovary is at the top, stilus is long, stigma is segmented. The tip of the single-seeded fruit which is in nutlike shape is apiculate. The general colour of the plant is pale green (in this study).

F. schleicheri subsp. *microcarpa*'s life form is annual and has a 30-50 cm long hairless stem. Leaf type is linear, petiol slender, bracteole shorter than pedisel, and linear-lanseolat. The flower color is pink, purple at the tips, a flower arrangement is rasemus. In a flower with zygomorphic symmetry, sepals are ovate. Corolla is 4 petal and coripetal. The number of stamens is 6 and is characteristically diadelf and united in groups of 3 at the base with expanded filaments. The ovary is at the top, stilus is long, and stigma is segmented. The tip of the single-seeded fruit which is in nutlike shape is apiculate. The general colour of plant is vibrant green (in this study). Detailed measurements and morphological features of both species in comparison with the Flora of Turkey are given in Table 2.

When we look at fruit micromorphology, the tip of the fruit in both species is apiculate and nutlike shaped. In *F. asepala*, epidermal cells are randomly distributed, show a wrinkled surface, and reticulate ornamentations are observed (Figure 2A). In *F. schleicheri* subsp. *microcarpa*, epidermal cells are square, rectangle, or polygonal shaped. There are reticulate-granulate (weblike-point) ornamentations on the fruit surface (Figure 2B). Fruit

micromorphologies of both species are different from each other and provide valuable data about taxonomy.

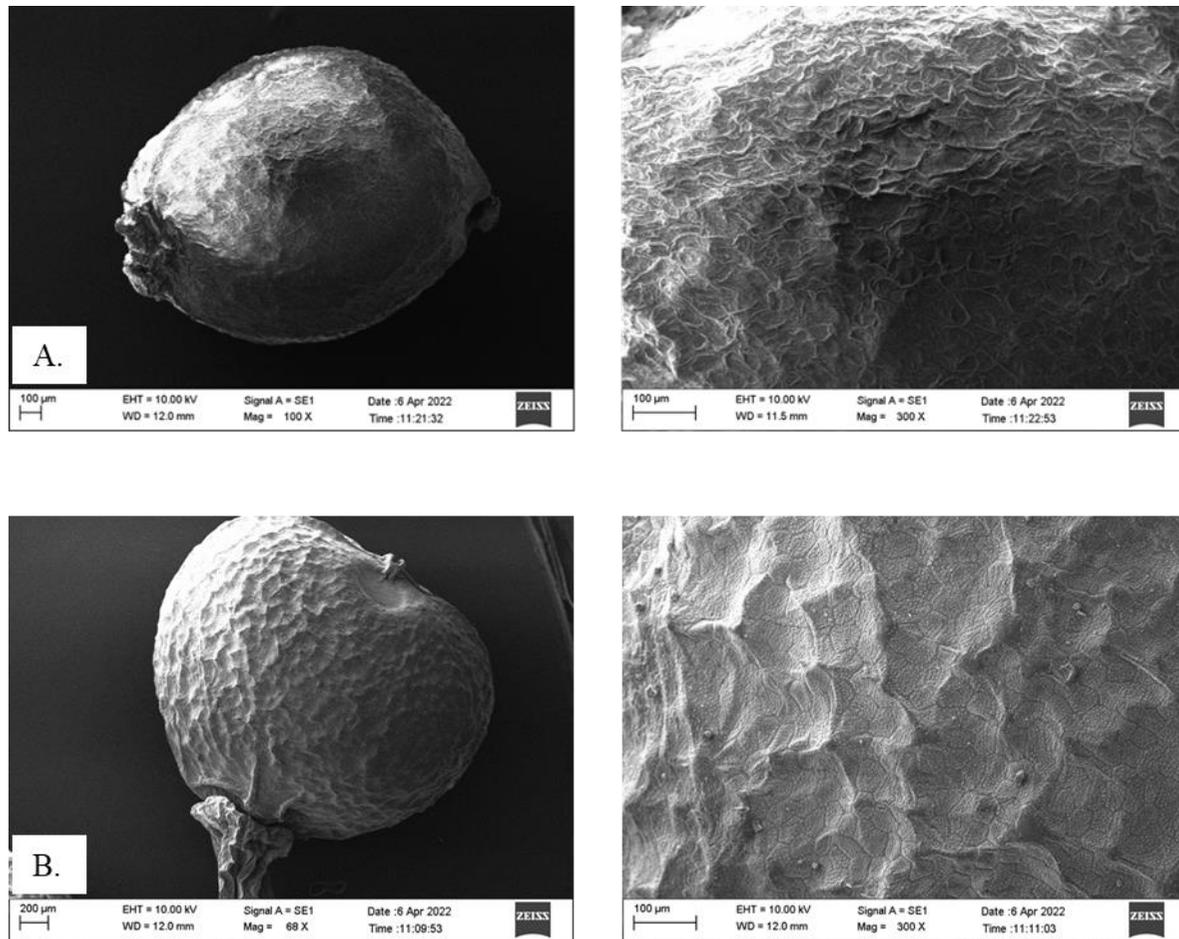


Figure 2. SEM images of fruit (A) *F. asepala* (B) *F. schleicheri* subsp. *microcarpa*

Table 2. Detailed measurements and the features of the morphological structures of *F. asepala* and *F. schleicheri* subsp. *microcarpa*

| Characters | <i>F. asepala</i> | | <i>F. schleicheri</i> subsp. <i>microcarpa</i> | |
|--|---|---|--|---|
| | Flora of Turkey | In this study | Flora of Turkey (<i>F. microcarpa</i>) | In this study |
| Life cycle | Annual | Annual | Annual | Annual |
| Stem | Dwarf herbs, with suberect or climbing stems | 20-30 cm, Ascending erect | Dwarf, stems ascending erect | 30-50 cm, Ascending erect |
| Hairiness condition of the stem | - | Hairless | - | Hairless |
| Leaf size and type | Leaves 2(-3) pinnatisect, the lobes flat, linear oblong | Leaves 5-7 cm long, Lamina bi-tripinnatisekt, lobes linear lanceolate | Leaves 2-3 pinnate with linear segments | Leaves 5-7 cm long, Lamina bi-tripinnatisekt, lobes linear |
| Petiole | - | Thin and long | - | Thin and long |
| Bract | - | Linear of equal length to the pedicel | - | Linear-lanceolate shorter than pedicel |
| Corolla colour | White | White, purple at the tips | Deep pink, maroon at the tip | Pink, purple at the tips |
| Inflorescens | Racemes very short, 6-12 flowered | It is a dense or loose racemus, located opposite the leaves. Inflorescence is short (1-1.5 cm), 10-16 flowered and frequent | Racemes dense, 30-flowered | It is a dense or loose racemus, located opposite the leaves. Inflorescence is short (1-1.5 cm), 10-16 flowered and frequent |

Tablo 2. (Continued)

| | | | | |
|------------------------------------|---|--|---|---|
| Flower symmetry | - | Zigomorf | - | Zigomorf |
| Calix | Sepals usually absent, occasionally present | No sepals | Sepals ovate, somewhat dentate, 1/10 of the length of the corolla | Sepals ovate, 0,5-0,7 mm long |
| Corolla | Corolla 4-5 mm, outer petals emarginate | Corolla 4-petal, coripetal | Corolla 4-5 mm long | Corolla 4-petal, coripetal, 5-6 mm long |
| Petal | - | The top of the upper petal is helmet, the sides of the helmet are straight, two of the petals are located on the sides | - | The apex of the upper petal is helmet, the edges of the helmet are backwards, the lower petal is pandurat, carinal at the apex. |
| Stamen number and condition | - | 6, Diadelf and united in groups of 3 | - | 6, Diadelf and united in groups of 3 |
| Filament | - | Enlarged at the base | - | Enlarged at the base |
| Pistil length | - | 3-5 mm | - | 3-5 mm |
| Ovary | - | Hypogin | - | Hypogin |
| Stylus | - | Tall | - | Tall |
| Stigma | - | Pieced | - | Pieced |
| Fruit | Apiculare, rugulose | Single seeded Nux | Apiculate, rugose | Single seeded Nux |

Anatomical Studies

Within the scope of this study, detailed anatomical structures of sections taken from root and stem were stained with reverse staining technique and

photographed after the examination. Table prepared based on the data are shown below (Table 3).

Table 3. Anatomical features and measurement results of *F. asepala* and *F. schleicheri* subsp. *microcarpa* (Root and Stem)

| Root | | |
|---------------------|---|--|
| Characters | <i>F. asepala</i> | <i>F. schleicheri</i> subsp. <i>microcarpa</i> |
| Epidermis | Single layer | Single layer |
| Sclerenchyma | None | None |
| Parenchyma | 8-9 layers | 12 layers |
| Phloem | 5-9 layers | 8-10 layers |
| Xylem | 21-25 layers | 9-17 layers |
| Pith | Xylem covers almost the entire pith region. | Xylem covers almost the entire pith region. |
| Stem | | |
| Characters | <i>F. asepala</i> | <i>F. schleicheri</i> subsp. <i>microcarpa</i> |
| Epidermis | Single layer | Single layer |
| Sclerenchyma | 5-8 layers (It contains sclereids) | 2-12 layers |
| Parenchyma | 5-9 layers, There is plate collenchyma in the corner parts of the stem section. | 5-7 layers |
| Phloem | 3-6 layers | 6-14 layers |
| Xylem | 6-9 layers | 5-9 layers |
| Pith | Empty | Empty |
| Stem shape | Pentagon, with pointed corners | Pentagon, with pointed corners |

Root Anatomy

In both species, the root is surrounded by a single layer of the epidermis and the epidermis tends to thicken outward. In root without sclerenchyma layer, cortex layer consists of 8-9 layers in *F. asepala* and reaches 12 layers in *F. schleicheri* subsp. *microcarpa*. While the phloem of

F. asepala (5-9 layers) is thinner than *F. schleicheri* subsp. *microcarpa* (8-10 layers), its xylem (21-25 layers) is thicker than *F. schleicheri* subsp. *microcarpa* (9-17 layers). Xylem completely covers pith zones in both species. In the center, xylem elements seem to be arranged in a fan-like fashion and divided into two by the secondary pith lines. Images of root sections are seen in Figure 3.

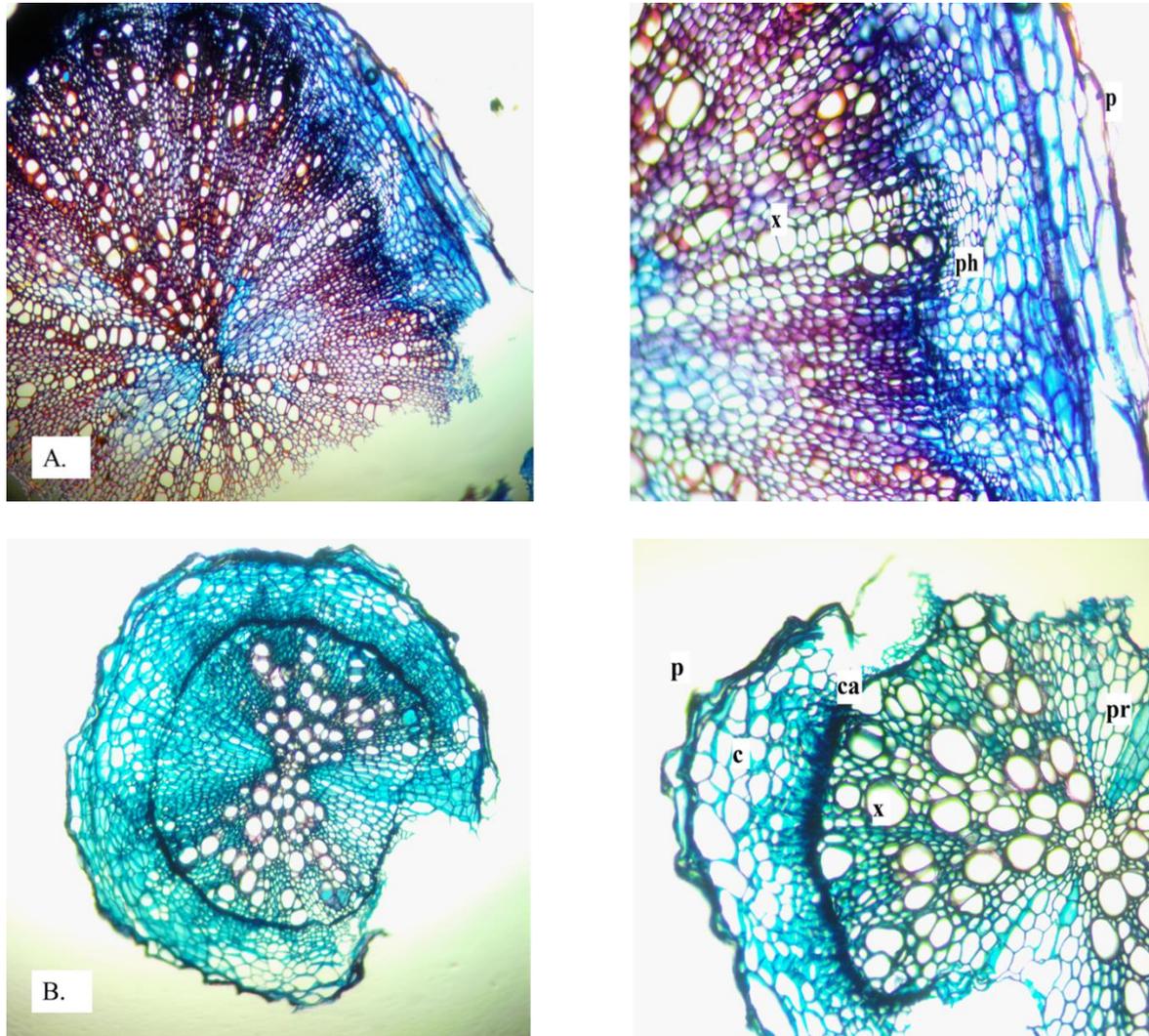


Figure 3. Photomicrographs of root (A) *F. asepala* (B) *F. schleicheri* subsp. *microcarpa* (p-peridermis, c-cortex, ca-cambium, x-xylem, pr-pith ray, ph-phloem)

Stem Anatomy

Cross-sections of the stem are grossly pentagonal in shape and have five prominent angles with raised corners. It is surrounded by a single row of the epidermis in both species, and just below it, the sclerenchyma layer has 5-8 layers in *F. asepala* and 2-12 layers in *F. schleicheri* subsp. *microcarpa*. In the corners of stem sections of *F.*

asepala species where the cortex layer is thicker, there is lamellar collenchyma (5-9 layers) and it is 5-7 layers in *F. schleicheri* subsp. *microcarpa*. There is one large vascular bundle at the corners and a small in between. Phloem is thin in *F. asepala* (3-12 layers), and thicker in *F. schleicheri* subsp. *microcarpa* (6-14 layers). Xylem is equal in thickness in both species. The pith region is empty in both species. Images of stem cross sections are seen in Figure 4.

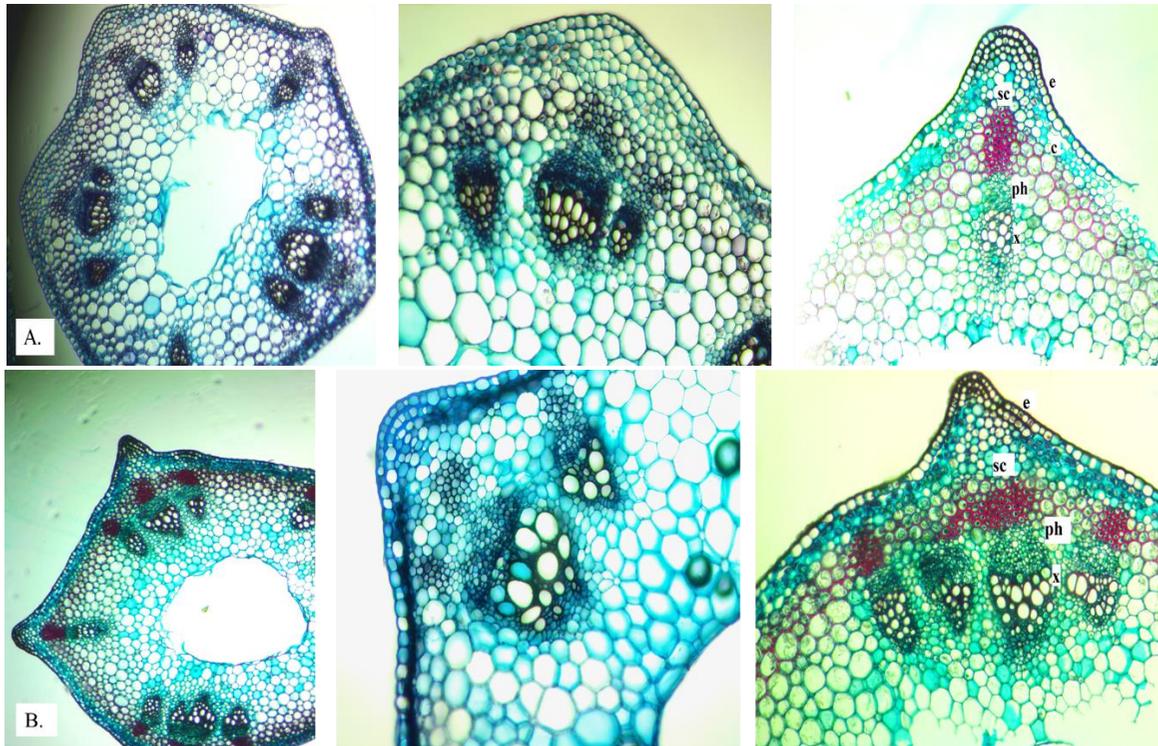


Figure 4. Photomicrographs of stem (A) *F. asepala* (B) *F. schleicheri* subsp. *microcarpa* (e-epidermis, ph-phloem, x-xylem, sc-sclerenchyma, c-cortex)

Palynological Studies

The measurements taken within the scope of palynological studies and the images of pollen belonging

to the species are given in the table and figure below (Table 4 and Figures 5-6).

Table 4. Pollen qualitative and quantitative morphological data of two *Fumaria* species

| Taxa | P | E | P\E | Pollen shape | Number of Aperture/ Type/Diameter | Exine | Ornamentation |
|---|-------|-------|------|--------------|--------------------------------------|-------|---------------|
| <i>F. asepala</i> | 29.89 | 29.90 | 0.99 | Suboblate | 6-7/Pantoporate-Porat/ 13.96 | 2.95 | Verrucate |
| <i>F. schleicheri</i> subsp. <i>microcarpa</i> | 36.15 | 36.19 | 0.99 | Suboblate | 4-8/Pantoporate-Porat/ 11.29 | 3.04 | Verrucate |

As a result of palynological studies, it was determined that the shape of examined pollen is suboblate, symmetry radial, aperture verrucate, and ornamentation

pantoporate. In terms of size, pollens are between 27 and 42 μm (Figure 5-6).

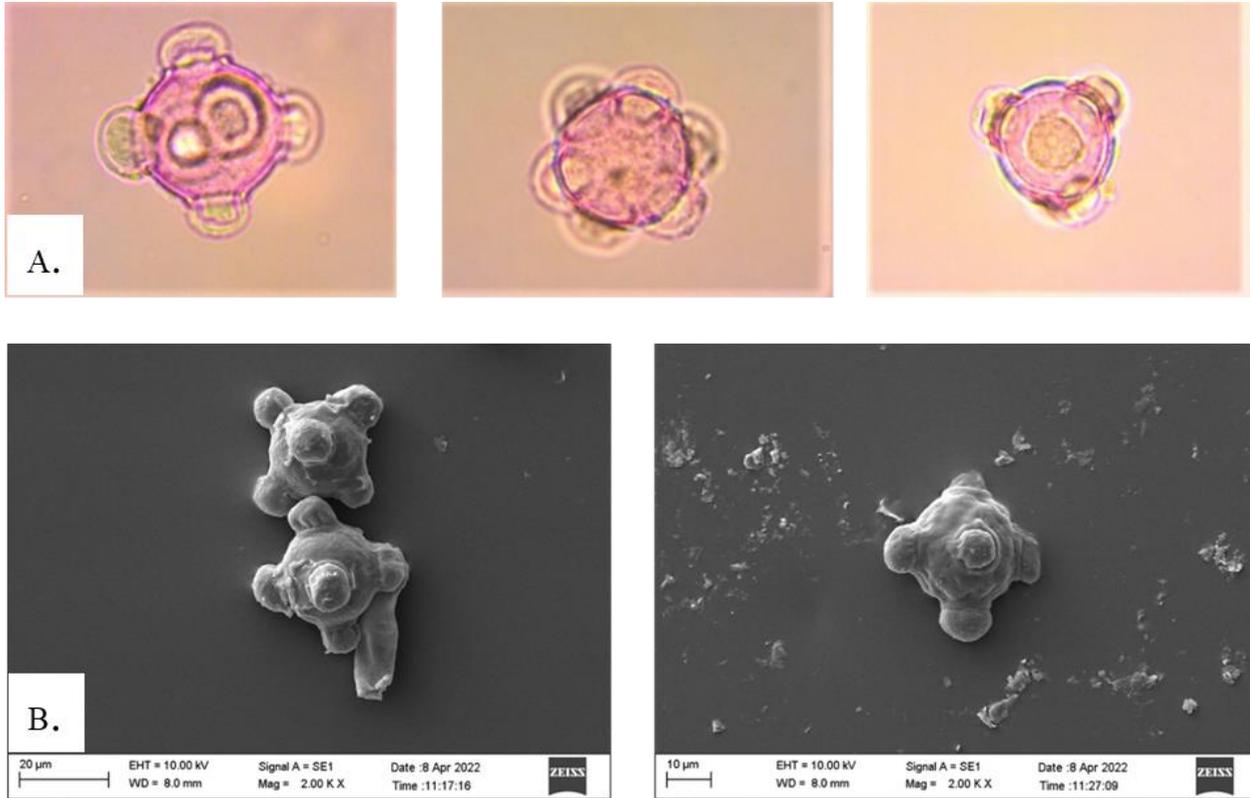


Figure 5. *F. asepala* pollen (A-Light microscope images B-SEM images)

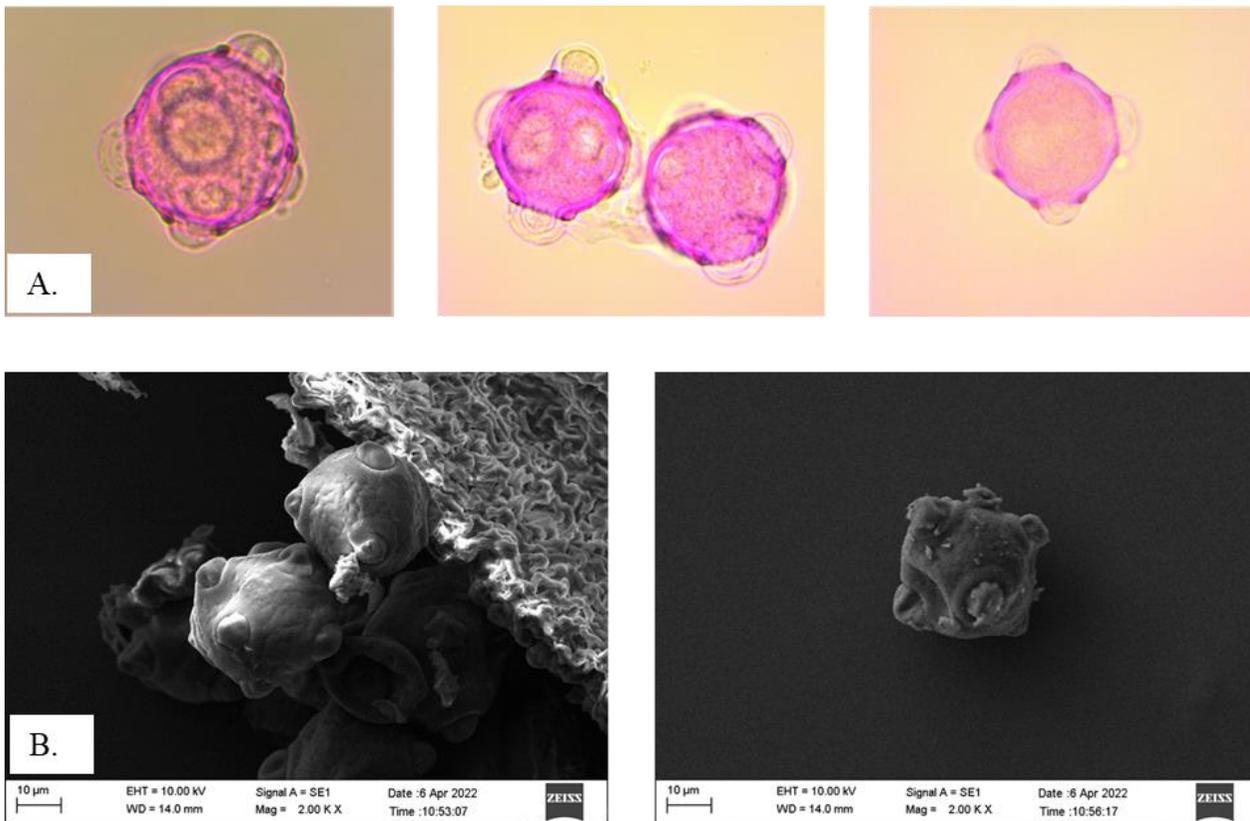


Figure 6. *F. schleicheri* subsp. *microcarpa* pollen (A-Light microscope images B-SEM images)

DISCUSSIONS

Our study aimed to determine the systematic characteristics of two *Fumaria* species that grow in Elazığ. To this aim, both external and internal morphological characteristics (Anatomic) were determined after taking anatomical sections. Additionally, pollen morphology was determined by both light microscope and electron microscope by conducting palynological studies. By combining all these studies, original data about species were obtained, and basic systematic data about genus were brought into the literature. Our study is important as it is the detailed systematic study on two *Fumaria* species that grow in Elazığ province.

When we review the studies on *Fumaria* species; Sener (1982) conducted a study to examine the morphological characteristics of some *Fumaria* taxa that grow in Turkey, and determine the morphological characteristics and alkaloid contents of about 38 species. With data in hand, he prepared a new identification key that belongs to the genus. In his study, he provided morphological data on *F. asepala* and *F. schleicheri* subsp. *microcarpa*. Our morphological data are in line with Sener's findings. Păltinean et al (2013) conducted morphological studies using a stereomicroscope on flower and fruit parts of 7 species that belong to *Fumaria* genus and found some differences and similarities. They suggested that the fruit types of each species have some distinctive characteristics. In this study, we also found that the fruit morphologies of *F. asepala* and *F. schleicheri* subsp. *microcarpa* are very different from each other. In this study, the researcher said that *F. asepala* showed a wrinkled surface with compact ornaments. In addition to this, epidermal cells are randomly distributed and reticulated ornaments are observed in *F. asepala* in our study.

Araii et al (2011) examined fruit and seed micromorphologies of 21 populations belonging to 6 *Fumaria* taxa that grow in Iran. In that study in which *F. parviflora*, *F. vaillantii*, *F. indica*, *F. asepala*, *F. officinalis* and *F. densiflora* species were examined, it was concluded that characteristics examined in seed and fruit micromorphology are quite valuable in terms of

taxonomy. While the *F. parviflora* and *F. vaillantii* were similar to each other, *F. indica*, a hybrid between *F. asepala* and *F. parviflora* showed intermediate micro-morphological characteristics. *F. officinalis* was very different from the others in terms of shape and structure.

Keshavarzi et al (2011a) made a comparative anatomic study on some *Fumaria* species that grow in Iran and stated that there are some differences in anatomical characteristics between species. They observed some differences in terms of the shape of stem sections, number of vascular bundles, the wall thickness of fruit, and wall structure. In conclusion, they stated that as *F. parviflora* and *F. vaillantii* are recorded as tetraploid ($2n=32$), high anatomical variation interval might be due to high ploidy levels in these taxa. Anatomical examination of stem and root sections showed the diagnostic significance of symmetry, presence of angles in the section, and the shape of xylem are of diagnostic importance. Just as in morphology, the structure of the fruit contains characteristics of diagnostic significance in anatomy. According to the data obtained from the study including *F. asepala* and *F. schleicheri* (It is not specified which subspecies it belongs to), root and stem characteristics were found similar to our study.

Păltinean et al (2015) examined histo-anatomical characteristics of 5 *Fumaria* species that grow in Romania and concluded that the results obtained together with the morphologic data could be meaningful in terms of taxonomy. They said that the *F. schleicheri* (It is not specified which subspecies it belongs to) species has distinct corners. In our study, we proved that both *F. asepala* and *F. schleicheri* subsp. *microcarpa* species have distinct corners. Again, in the palynological study of Keshavarzi et al (2011b) that was conducted on *Fumaria* species that grow in Iran, pollen morphology and exine structures of 7 *Fumaria* L. species were examined under a light microscope and scanning electron microscope. In this research, stated that both *F. asepala* and *F. schleicheri* pollen grains are oblate spheroidal, but in the our study, it was observed that the pollen type was suboblate for both species, and the apertures of the pollen were Pantoporate/Porat. Results showed that

pollen characteristics of *Fumaria* species might provide diagnostic features for taxonomic applications.

In a palynological study by Yılmaz Çıtak et al. (2016), pollen grains belonging to *F. officinalis* were examined and found that they have suboblate pollen type. In the data we obtained in our study is that the pollen type of both species studied is the same as *F. officinalis*. Perveen & Qaiser (2004) examined pollen morphology of 9 species belonging to Fumarioideae subfamily from Pakistan under a light and scanning electron microscope. They stated that pollen grains are generally radial symmetrical and although they seem to be similar, they are significant in terms of taxonomy. While the pollen of the *F. indica* species examined in this study is oblate spheroidal, without any resemblance it is suboblate with the taxa we studied.

Our results show similar features in parallel to literature. According to the data we obtained as a result of this study, the most distinctive feature of the two taxa studied is the absence of sepals in *F. asepala*. Leaf types and flower colors are another important feature that distinguishes the two species. Micromorphological features of fruits are an important taxonomic distinguishing feature. In addition, they have similar anatomical and palynological features. It is thought that the obtained data will be very useful in distinguishing these two species, which are very similar to each other.

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