

**Anatomical and Palynological Characters of Endemic *Fritillaria gencensis* Yıld., Kılıç & A.Demirpolat**

Azize DEMİRPOLAT\*

\*Bingöl University/ Food, Agriculture, Animal, Vocational School, Plant and Animal Production Department, Bingöl, Turkey

[ademirpolat@bingol.edu.tr](mailto:ademirpolat@bingol.edu.tr)

Received: 26.05.2022 Received in revised: 03.06.2022 Accepted: 03.06.2022

**Abstract**

This study was carried out to determine the anatomical and palynological features of *Fritillaria gencensis*, a new species found in Turkey. *F. gencensis* is a new species and no previous systematic research has been conducted on this species. In this study, the anatomical and palynological features of *F. gencensis* were evaluated to define the species in more detail. Anatomical examinations were made on the surface section of leaf and stem. In the trunk cross-section, the cortex cells are 3-7 layers. These cells are thin, parenchymal and have little or no intercellular spaces. 1-2 rows of collenchyma cells are seen below the cortex. In the upper and lower superficial sections taken from the leaf, the stomata index was 49.88% in the upper epidermis and 48.66% in the lower epidermis. Stomata are of the anomocytic type because there are no specific types of adjacent cells surrounding the stomata. As a result of palynological studies, the average of the polar axis, which is the long axis of the pollen, was 34.38  $\mu\text{m}$ , the equatorial axis was 24.23  $\mu\text{m}$  and the average thickness of the exine was 1.25  $\mu\text{m}$ . Pollen shape was prolate. Pollen ornamentation was reticulated. With this study, the anatomical and palynological features of the *F. gencensis* species was determined and with the results contributed to the taxonomic studies on the subject.

**Key words:** *Fritillaria*, Anatomy, Palynology, Liliaceae

**Endemik *Fritillaria gencensis*'in Yıld., Kılıç & A.Demirpolat Anatomik ve Palinolojik Özellikleri**

**Öz**

Bu çalışma Türkiye'de bulunan ve yeni bir tür olarak belirlenen endemik *Fritillaria gencensis* Yıld., Kılıç & A.Demirpolat türünün, anatomik ve palinolojik özelliklerini tespit etmek amacıyla yapılmıştır. *F. gencensis* türü yeni bir tür olduğu için bu tür ile ilgili daha önce herhangi bir sistematik araştırma yapılmamıştır. Bu çalışmada, türün daha detaylı tanımlanabilmesi için *F. gencensis*'in anatomik ve palinolojik özellikleri incelenmiştir. Yaprakların ve gövde yüzey kesitlerinde anatomik incelemeler yapılmıştır. Gövdeden alınan enine kesitte, korteks hücreleri 3-7 katmanlıdır. Bu hücreler ince, parankimatiktir ve hücreler arası boşlukları yok denecek kadar azdır. Korteksin altında 1-2 sıralı kollenkima hücreleri görülür. Yapraktan alınan alt ve üst yüzeysel kesitlerde stoma indexi üst epidermada %49.88, alt epidermada %48.66 olarak belirlenmiştir. Stomaları çevreleyen belirli tipte komşu hücreler olmadığından dolayı anomositik tiptedir. Palinolojik çalışmalar sonucunda, polenlerin uzun eksenini olan polar axis ortalaması 34.38  $\mu\text{m}$ , ekvatorial axis 24.23  $\mu\text{m}$ , ekzin kalınlığı ortalama 1.25  $\mu\text{m}$  olarak ölçülmüştür. Polen şekli prolatdır. Polen süslemesi ağsı deliklidir. Bu çalışma ile *F. gencensis* türünün anatomik ve palinolojik özellikleri belirlenerek konuyla ilgili taksonomik çalışmalara katkı sağlanmıştır.

**Anahtar kelimeler:** *Fritillaria*, Anatomi, Palinoloji, Liliaceae

## Introduction

The genus *Fritillaria* L. belongs to the Liliaceae family and has about 165 taxa worldwide. The Liliaceae family has recently split into many different families. Although this situation causes some disagreements considering the morphological and phylogenetic studies, it was determined that the Liliaceae family consists of 6 tribes, 15 genera and approximately 900 species (Yıldız and Aktoklu, 2010). The genus *Fritillaria* is represented by about 43 species, 21 of which are endemic to Turkey (Özhatay et al., 2000). There are 25 taxa of this genus in Greece (Rix, 2001), 22 taxa in Russia (Lozina-Lozinskaya, 1968) and 18 taxa in Iran (Rechinger, 1990).

Also called inverted tulips, *Fritillaria* is known for its flower or inflorescence tilting towards the ground. The name *Fritillaria* is derived from the genus name fritillus, which means membrane in Latin (Öztaş and Öztaş, 2017). *Fritillaria* taxa generally prefer sandy, rich soils with good content (Beresford-Kroeger, 2004). Species of this genus bloom in early or late spring. The genus *Fritillaria* has been classified within itself by different researchers. *Fritillaria* species were divided into two different groups by Fay & Chase (2000), Eurasian *Fritillaria* species and North American *Fritillaria* species. However, *Fritillaria* species were divided into 7 subgenus and 2 sections by Rix (Tekşen & Aytaç, 2011 as cited in Rix, 2001). There are also studies showing that some *Fritillaria* species are used in folk medicine (Huang et al., 2004; Dhyanı et al., 2010; Mosaddegh et al., 2012; Khan et al., 2013; Mükemre et al., 2015). In some ethnobotanical studies, it is known that *Fritillaria* species are used in digestive problems, abdominal, kidney, rheumatism and joints pains, to relieve cough and wound healing (Mosaddegh et al, 2012; Khan et al., 2013; Mükemre et al., 2015). When the ethnobotanical studies on *Fritillaria* species are examined, it is seen that the part used in this genus is especially the bulbs (Dhyanı et al., 2010; Huang et al., 2004; Khan et al., 2013; Mosaddegh et al., 2012; Mükemre et al., 2015).

Endemic and new species *F. gencensis* is morphologically close to *F. alburyana* Rix, with lower leaves 2.5-4.5 cm wide (not 1-2.5 cm), ovate (not lanceolate); crests 4-6 mm wide (not 10-15 mm), oblong (not ovate); filaments 8-9 mm (not 10-15 mm), glabrous (not papillose); anthers yellow (not brown); styles 8-10 mm (not 9-15 mm). On the other hand, *F. pinardii* Boiss. morphologically close to the genus Boiss. with lower leaves 2.5-4.5 cm wide (not 0.5-2.4 cm), ovate (not lanceolate); the flowers are cylindrical to circular (not narrow bell-shaped), pale pink-gray, white-yellowish, filaments glabrous (not papillose) (Yıldırım et al., 2019).

There are studies on the anatomical, morphological, palynological features and phylogenetic studies of some species belonging to the genus *Fritillaria* in Turkey (Pehlivan and Özler 2002; Özler ve Pehlivan 2007; Alan, 2008, Sancar et al., 2021). However, since *F. gencensis* is a new species, no research has been done on this species. In this study, the anatomical and palynological features of *F. gencensis* were examined for the first time to define the species in more detail. Anatomical examinations were made on the surface sections of the leaf and stem, and the results were given.

## Material and Method

### Plant Material

The plant sample was collected from Bingöl-Genç district (AD 2221) and turned into a herbarium sample (Fig. 1). Fresh specimens of this new species, defined according to the flora of Turkey, were stored in 70% alcohol for anatomy studies. For palynology studies, with flowering specimens of *F. gencensis* were kept and used. Type, Turkey. B8 BİNGÖL: Genç, Çevirme village, 1650 m, 22.04.2019, Ş. Yıldırım et al. 45138, Ö. Kılıç & A. Demirpolat (holo. Yıldırım et al. Otluk (YO); iso. EGE, GAZİ, HUB, YO).

### Anatomical Study

For the anatomical study, transverse sections from the stem and leaf of the samples prepared with 70% alcohol and superficial sections from the upper and lower surfaces of the leaf were made by hand. Safranin-fast green (Bozdağ et al., 2016) was used for painting anatomical sections (Table 1 and Figure 2). Anatomical photography and measurement of the specimens were performed with the help of a digital camera with a Euromex CMEX-10PRO trinocular microscope.

### Palynology Study

The pollen morphology of the taxon in the study was examined with light microscopy and Scanning Electron Microscopy. The pollens obtained from the samples in light microscopy examinations were prepared according to the method of Wodehouse (1935). Measurements were performed and the photographs were taken with Euromex CMEX-10PRO trinocular microscope (100x). Polar axis (P), equatorial axis (E), and exine thickness (Ex). Sulcus width-length was measured from thirty pollen. The terminology used is mainly from Faegri & Iversen (1935) and the study of Kılıç et al. (2020). These measurements are reported in Table 2 and micrographs in Figure 3.

Pollens belonging to the genus *F. gencensis* were placed on metal pollen carriers (stap) with double-sided adhesive tape under a binocular

microscope to be examined by SEM and covered with gold to provide the appearance of the pollen in the electron microscope. The pollen appearances and detailed surface ornamentations of the studied pollen were obtained from Bingöl University Central Laboratory. Microphotographs at 1500x- 10000x magnification were taken for each taxon.

**Result and Discussion**

**Stem anatomy of *Fritillaria gencensis***

In the cross-section of the stem, there are epidermal cells in a row and oblong-oval shape. The epidermal layer is covered with a cuticle in some sections. The cortex contains 4-11 layers sclerenchyma cells. Vascular bundles are denser in the vascular cylinder. Cortex cells are 3-7 layers (Table 1). They are thin, parenchymal cells with little or no intercellular spaces. 1-2 layers of collenchyma cells are below the cortex. Xylem occupies a larger area than phloem. Xylem cells are stained with safranin. There is a scape extract center with thin walls and intercellular space of normal parenchymal cells. The cross-section taken from the middle part of the stem was observed as follows (Figure 2).

**Leaf anatomy of *Fritillaria gencensis***

In the cross-section taken from the middle part of the leaf, the epidermal cells differ in shape and size. The epidermal cells on both the lower and upper surfaces are rectangular and the larger epidermal cells are oval in shape. Spongy and palisade parenchymal cells are undifferentiated in the mesophyll layer. It has intercellular spaces.

Parenchymal cells are mainly flat and transversely elongated. Vascular bundles are regular and less numerous at the periphery of the trunk. The bundle sheath around the xylem or phloem element consists of a single layer of large parenchymal cells. The pith has large, oval, or round-shaped, thin-walled parenchymal cells. The following Fig. 2 cells are observed in the superficial section taken from the upper and lower regions of the leaf. There are stomata on both surfaces of the superficial sections taken from the upper and lower regions of the leaf. Stomata are of the anomocytic type because there are no specific types of adjacent cells surrounding the stomata. In the upper and lower superficial sections taken from the leaf, the stomata index is 49.88% in the upper epidermis and 48.66% in the lower epidermis (Table 1).

**Pollen anatomy of *Fritillaria gencensis***

The mean polar axis, which is the long axis of the pollen is 34.38 µm, equatorial axis is 24.33 µm and exine thickness is 1.25 µm. Pollen shape is prolate. Pollen ornamentation is reticulated. The reticles are not thin and taper slightly towards the edge of the sulcus. Sulcus is a long longitudinal slit, running parallel to the equatorial axis. The mean sulcus length is 24.99 µm, and the sulcus width is 12.23 µm (Table 2). Sulcus membrane is granulate. The sulcus tip is rounded. The ornamentation of the pollen exine is a valuable character in palynology. *F. gencensis* pollen grains are operculate and monosulcate.

**Table 1.** Anatomical characters of *F. gencensis*

<i>Stem</i>				
Epiderma	Cortex	Sclerenchyma	Collenchyma	Pith region
Row and oblong-oval shape	3-7 layers	4-11 layers	1-2 layers	Oval shaped
<i>Leaf anatomical characters</i>				
Upper and lower epiderma	Mesophyll	Stoma type	Stomata index	
			upper epidermis	lower epidermis
Rectangular and oval shape	7-19 layers oval shape	Anomocytic type	49.88%	48.66%

**Table 2.** The pollen morphological characters of *F. gencensis* (values in µ m)

<i>F. gencensis</i>	P		E			Exine	Shape	Sulcus		Ornamentation
	Min.	Max.	Min.	Max.	Mean			Sulcus length	Sulcus width	
	22.80	41.38	34.38	20.01	34.60			24.23	1.25	



**Figure 1.** General appearance of *F. gencensis*

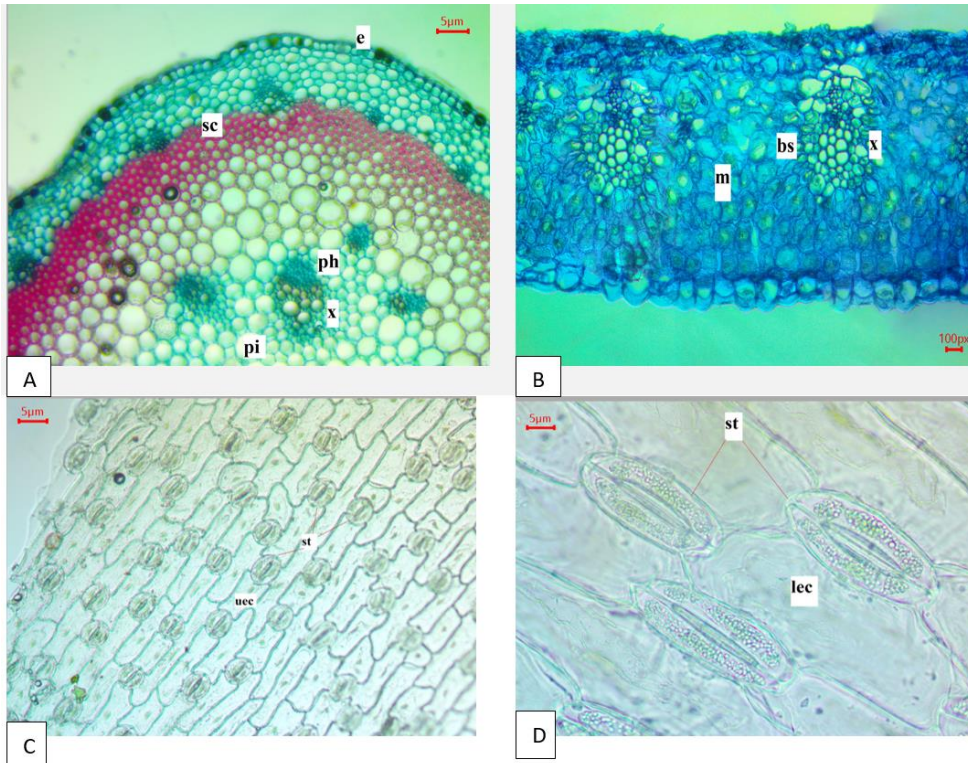
Although the previously studied *Fritillaria* species had common anatomical characteristics, *F. gencensis* species has somewhat distinct anatomy (the presence of sclerenchyma and the number of layer, size, and shape of epidermal cells, the number of subepidermal parenchymal layers, the presence of vascular bundle, the stoma index). To distinguish taxa and to reduce systematic problems, similarities in morphological features of taxa can be distinguished by anatomical characters. In addition, Charlton (1988) reported that anatomical data can be used to solve problems.

In the palynology revision of the *Fritillaria* genus, Tekşen et al. (2010) determined that the *F. pinardii* species, which is morphologically close to the *F. gencensis* species (Yıldırım et al., 2019), has the ornamentation as reticulate perforate and enter the Type II pollen decoration section. *F. alburyana* is studied in the Type III pollen ornamentation section with its fine reticulate ornamentation. Again, according to that study, the pollen form of *F. alburyana* species was subprolate, sulcus membrane verrucate, and sulcus apex sharp

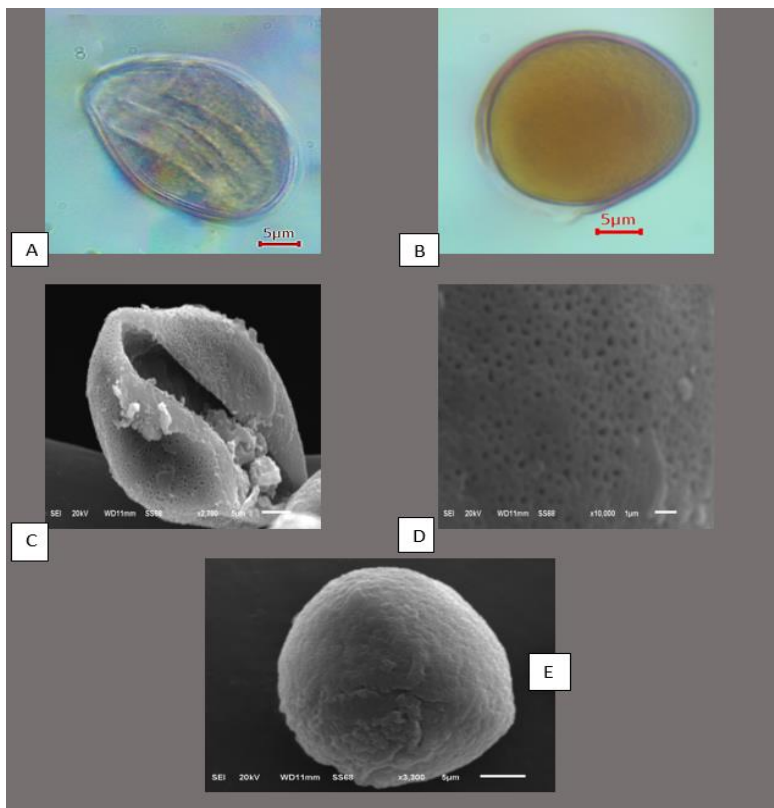
(Tekşen et al., 2010). The pollen shape of *F. pinardii* species was prolate, sulcus membrane gemmate, sulcus apex round. The aperture type of both species was monosulcate (Tekşen et al., 2010). In our study the results were similar and aperture type in *F. gencensis* is monosulcate. The apex of *F. gencensis* sulcus is round-shaped, as in *F. pinardii* of these two species to which it is morphologically close.

According to Özler and Pehlivan (2007), while the mean polar axis of *F. pinardii* species was 49.68  $\mu\text{m}$ , the mean equatorial diameter was 35.68  $\mu\text{m}$ . On the other hand, Tekşen et al. (2010) measured the mean polar axis of *F. pinardii* as 44.3  $\mu\text{m}$ , the mean equatorial diameter as 34.9  $\mu\text{m}$ . In our study, the mean polar axis was 34.38  $\mu\text{m}$  and the equatorial axis was 24.23  $\mu\text{m}$ . In this respect, it was determined that *F. gencensis* has smaller pollen grains.

Kandemir et al. (2022) found that the region in the stem cross-section of 9 *Fritillaria* species consists of large parenchymal cells in all taxa, but the shape differs. The shape of the parenchymal cell is oval in the pith of *F. asumaniae*, *F. byfieldii* and *F. mughlae*; oval and orbicular in *F. carica*, *F. elwesii*, *F. forbesii* and *F. milasense*; oval and hexagonal in *F. özdemir-elmassii* (Kandemir et al., 2022). In our study, the shape of the large parenchymal cell in the pith was oval and hexagonal in the cross-section of the trunk. Again, phloem and xylem elements, vascular bundles, and sheaths were observed in all taxa examined according to Kandemir et al. (2022). The bundle sheath consists of oval-shaped, large parenchymal cells without chloroplasts (Kandemir et al., 2022). In this respect, they are similar to the *F. gencensis* species in our study. The number of layers of sclerenchymatous tissue varied among taxa and thus it was used as an important distinguishing anatomical feature (Kandemir et al. 2022, Namazi et al. 2017; Corneanu & Popesco, 1981; Pinar & Behçet, 2012; Alan, 2008). It was reported by Namazi et al. (2017) that, the number of layers of the sclerenchymatous sheath also differs among *Fritillaria* species in Iran. In our study, it was observed that the *F. gencensis* stem cortex contains 4-11 layers sclerenchyma cells.



**Figure 2.** Photomicrographs of stem (A) and leaf (B) cross sections of *F. gencensis*. Leaf superficial sections (C-D), e-epidermis, ph-phloem, x-xylem, pi-pith, sc-sclerenchyma, bs-bundle sheath, m-mesophyll, st-stoma, lec-lower epidermis cell, uec-upper epidermis cell.



**Figure 3.** Pollen grains photos of *F. gencensis*; A-C: an equatorial view, B-E: a polar view, D: detailed apertures and exine ornamentation in a equatorial view.

In our study, we found that *F. gencensis* leaf cross-section is unifacial in terms of mesophyll. Our study is parallel to the studies conducted in this regard (Kandemir et al., 2022; Behçet & Pınar, 2012; Corneanu & Popesco, 1981). There are anomocytic stomata in the lower and upper surfaces of *F. gencensis* leaf in our study. A similar situation has been reported in other studies of the *Fritillaria speciewa* (Kandemir et al. 2022; Kumar, 2006; Pınar & Behçet, 2012; Alan, 2008).

## Conclusion

In this study, with its distinctive anatomical and palynological features, *F. gencensis* was defined as a new species. The fact that it is a morphologically new endemic species within the genus was supported by anatomical and pollen examination. The stem and leaf sections are anatomically different from *F. alburyana* and *F. pinardii* species, both of which are morphologically close due to the absence of sclerenchyma. Palynologically, its pollen shape is close *F. pinardii* but different due to its small size.

## References

- Alan, S. 2008. An endemic species in Turkey: Morphological and anatomical investigation on *Fritillaria fleischeriana* Steudel & Hochst. ex Schultes & Schultes fil. (Liliaceae). *Ot Sistematic Botanic Dergisi*, 15(2): 115-124.
- Beresford-Kroeger, D. 2004. *A Garden for Life: The Natural Approach to Designing, Planting, and Maintaining a North Temperate Garden*. Ann Arbor: University of Michigan Press.
- Bozdağ, B., Kocabaş, O., Akyol, Y., Özdemir, C. 2016. Bitki Anatomisi Çalışmalarında El Kesitleri İçin Yeni Boyama Yöntemi. *Marmara Pharmaceutical Journal*, 20, 184-190.
- Charlton, W.A., 1988. Stomatal pattern in four species of monocotyledons. *Ann. Bot.* 61, 611-621.
- Corneanu, G.C., Popesco, G.G. 1981. Distributional and anatomical studies on *Fritillaria* (Liliaceae) in Romania. *Willdenowia*, 11, 307-315.
- Faegri, K., Iversen, J. 1975. *Textbook of Pollen Analysis*, Hafner Press, New York.
- Fay, M.F. and Chase, M.W. 2000. Modern concepts of Liliaceae with a focus on the relationships of *Fritillaria*. *Curtis's Bot Mag*, 17(3), 145.
- Kandemir, N., Çelik, A., Ullah F. 2022. Comparative micro-anatomical features of endemic *Fritillaria* taxa growing in the Mediterranean region (Turkey). *Flora*, 290.
- Khan, S. M., Page, S., Ahmad, H., Shaheen, H., Ullah, Z., Ahmad, M., ve Harper, D. M. 2013. Medicinal flora and ethnoecological knowledge in the Naran Valley, Western Himalaya, Pakistan. *Journal Ethnobiol and Ethnomedical*, 9 (1): 4.
- Kılıç, N., Yılmaz Dağdeviren, R., Caner, H., Akkemik, Ü. 2020. Türkiye’de Kullanılmakta Olan Palinoloji ve Polen Terimleri Üzerine Bir Değerlendirme ve Öneriler. *Avrasya Terim Dergisi*, 8 (2): 98-108.
- Kumar, F.Z. 2006. Density, size and distribution of stomatal in different monocotyledons. *Pakistan Journal Biology Science*, 9, 1650-1659.
- Lozina-Lozinskaya, A.S. 1968. *Fritillaria* L. – Pp. 232-246 In: Kamarov, V. L. (ed.), *Flora of the U.S.S.R.*, 4, Jerusalem.
- Mosaddegh, M, Naghibi, F, Moazzeni, H, Pirani, A., Esmaeili, S. 2012. Ethnobotanical survey of herbal remedies traditionally used in Kohghiluyeh va Boyer Ahmad province of Iran. *Journal Ethnopharmacology*, 141(1): 80-95.
- Mükemre, M., Behçet, L. ve Çakılcıoğlu, U. (2015). Ethnobotanical study on medicinal 51 plants in villages of Çatak (Van-Turkey). *Journal Ethnopharmacology*, 166, 361-374.
- Namazii, F., Tehranii, S.M., Shabani, L. 2017. Anatomical study on *Fritillaria* species in Iran. *Bangladesh Journal of Botanic*, 46, 93-101.
- Özhatay, N. 2000. *Fritillaria* L. – Pp. 243-246 In :Güner, A., Özhatay, N., Ekim, T. & Başer, K.H.C. (eds.) *Flora of Turkey and the East Aegean Islands*, 11. Edinburgh.
- Özler, H. and Pehlivan, S. 2007. Comparison of pollen morphological structures of some taxa belonging to *Asparagus* L. and *Fritillaria* L. (Liliaceae) from Turkey. *Bangladesh Journal of Botany*, 36(2): 111-120.
- Öztaş, H. and Öztaş, F. 2017. *Torosların Gizemli Bitkileri*. Konya: Ermenek Belediyesi.
- Pehlivan, S. and Özler, H. 2002. Pollen morphology of Turkish endemic *Fritillaria michailovskyi* Fomin and *Fritillaria armena* Boiss. (Liliaceae) species. 6st Plant Life of Southwest Asia Symposium, Van.
- Pınar, S.M., Behçet, L. 2012. Anatomical, morphological and palynological study on Turkish endemic *Fritillaria baskilensis* (Liliaceae). *Bocconeia*, 24, 305-310.
- Rechinger, K.H. 199. *Fritillaria* L. Pp. 61-76 in: *Flora Iranica*, 165, Graz.
- Rix, E.M. 2001. *Fritillaria*: A revised classification- *The Fritillaria Group of the Alpine Garden Society*, United Kingdom.
- Sancar, P.Y., Taskin, İ.İ., Kursat, M., Munzuroğlu, O. 2021. Phylogenetic analysis for endemic

- Fritillaria baskilensis* Behçet (Liliaceae): Evidence from cpDNA “trn” sequences. *Biyolojik Çeşitlilik ve Koruma*, 14(3): 431-441.
- Tekşen, M., Aytaç, Z., Pınar, N.M. 2010. Pollen morphology of the genus *Fritillaria* L. (Liliaceae) in Turkey. *Turkish Journal of Botany*, 4, 397-416.
- Tekşen, M. and Aytaç, Z. 2011. The revision of the genus *Fritillaria* L. (Liliaceae) in the Mediterranean region (Turkey). *Turkish Journal of Botany*, 35, 447-478.
- Wodehouse, R. P. 1935. *Pollen grains*. – New York.
- Yıldırım, Ş., Kılıç, Ö., Demirpolat A. 2019. A new unexpected species of *Fritillaria*, *F. gencensis* (Liliaceae) from Genç, Bingöl, Turkey. *OT Sistematiik Botanik Dergisi*, 26 (1): 1-11.
- Yıldız, B. and Aktoklu, E. 2010. *Bitki Sistematiği İlk Karasal Bitkilerden Bir Çenekliler*. Ankara: Palme Yayıncılık.