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Some anatomical studies on rare endemic *Fritillaria serpenticola* from Turkey and reassessment the conservation status of it

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Abstract

Endemic *Fritillaria serpenticola* (Rix) Tekşen & Aytaç (*Liliaceae*) was described in "Flora of Turkey" by Rix. Plant samples have been collected from Eskişehir, Alpu, Alapınar Village-Turkey. The morphological and anatomical features of *F. serpenticola* are given in below. Some of the properties of various organs as stem, leaf, flower and bulb were determined in the morphological part of the study. Conservation status of *F. serpenticola* was reevaluated. The aim of this study is determine anatomical features of *F. serpenticola* to investigate conservation and distribution status thus assessing the utility of these obtained data for systematic purposes and biological diversity researches.

Keywords: Anatomy, morphology, ecology, *Liliaceae, Fritillaria*

Introduction

According to the Angiosperm Phylogeny Group (1); Genus *Fritillaria* L. is a large genus in *Liliaceae* family of *Monocotyledones* (1). The *Liliaceae* contains approximately 280 genera and 4000 species in world wide. *Fritillaria* is represented with about 170 taxa around the World (2). In these taxa, there are 25 in Greece, 24 in China, 22 in Russia and 18 in Iran (2). Turkish *Liliaceae* has 29 native genera, 15 non native genera, 556 native species, 32 non native species, 2 nothospecies, 36 subspecies, 594 native taxa, 276 endemic species (49.6 %), 9 endemic subspecies, 285 endemic taxa (47.9 %) (3). And Turkish *Fritillaria* has 40 native species, 4 subspecies, 26 endemic species (65.0 %) (3).

Taxonomy of *Fritillaria* was revised several times (4-6) and the classification proposed by Rix et al. (6) is supported, in its general lines, by the most recent phylogenetic studies (7, 8). According to these authors, the genus *Fritillaria* is monophyletic, but infra-generic phylogenetic relationships are unclear (9). *Fritillaria* genus is interpret by Rix in flora of Turkey.

Members of the family *Liliaceae* can be used as ornamental plants and vegetables as well as being used as medicinal plants due to the chemical substances they contain (10). The bulbs of *Fritillaria* species from cough and sore throat relieving, expectorant, fever reducer, lung moisturizer, high blood pressure lowering, asthma, secretory gland tumors and is reported that are being used to cure bronchitis (7, 10-14).

Fritillaria species have so far been investigated by different researchers according to their taxonomic, systematic, morphological, anatomical, palynological, pharmacological and ethnobotanical properties (2, 9, 15-35).

F. serpenticola was evaluated as a subspecies of *Fritillaria carica* in flora of Turkey by Rix. *Fritillaria carica* subsp. *serpenticola* is distinguished from subsp. *carica* by its leaves and flower characters. *F. serpenticola* is morphologically separated from *F. carica* by leaf number, shape, perigon shape and stylus characteristics. Mentioned characters are used for separate the *Fritillaria* species (not subspecies). Tekşen and Aytaç upgrade *Fritillaria carica* subsp. *serpenticola* to species level (19).

This study identifies some morphological anatomical features, distribution and conservation status of endemic *F. serpenticola* and evaluates the utility of these obtained data for taxonomic and biological diversity studies. The leaf, root and scape transversal and superficial cross sections of *F. serpenticola* have been studied for the first time in anatomical studies. The resulting *F. serpenticola* the morpho-anatomical features are compared with closely related species and type designation. Distribution and population conditions of *F. serpenticola* have been investigated and so IUCN category of taxon was reevaluated (6, 19, 36).

Materials and methods

Plant material

Fritillaria serpenticola samples were collected from Eskişehir province Alpu district, Alapınar village, N 39°59'18.1"-E 30°51'53.0" coordinates and 1279 metres in 14.04.2016 (Fig. 1). In addition, the distribution map of plant samples is given in Figure 7. Morpho-anatomical investigations were carried out using plant samples held in alcohol. Herbarium samples were prepared to provide a systematic review of the material obtained and these samples were preserved in the Herbarium of Eskişehir Osmangazi University as herbarium material (OUFE 20411).

Anatomical studies

In anatomical studies; root, scape, leaf transversal and superficial cross sections were taken from the samples kept in alcohol. The photographs of these sections were taken with a Cameram ™ digital camera and a Nikon 80i type microscope. Various basic anatomical books and studies have been used as plant identification resources (37-44).

Morphological studies

Fresh herbs and herbarium samples were used to determine the detailed morphological features. Morphological characteristics of the examined samples were compared with “Flora of Turkey” and, specified morphological features (19, 45).

Results and discussion

Morphological properties

Description Obtained from This Study: Bulb 0.7-1 x 0.8-1.3 cm, globose or obovate, tunica thin. Stem erect, 5-16 cm, glabrous. Leaves 4-5, sessile, alternate. Lowest leaves 2.3-5.2 x 0.7-1.3 cm, widely acute, lanceolate, leaves margin glabrous. Upper leaves linear, acuminate, 1.5-6. 4x 0.1-0.6 cm, bended at apex. Bract 1; linear, acuminate, 1.5-3.0 x 0.1 cm, bended at apex. Flowers 1(-3). Perigon conical, yellow, when the color fades orange. Outer tepals 10-20 x 3-6 mm, lanceolate, obtuse, short-haired at apex; inner tepals 9-18 x 4-6 mm, oblanceolate, obtuse, short-haired at apex. Nectaries brown, linear-lanceolate, 2-4 x 1 mm at base of tepal. Filaments flat, rare papillose, yellow 6-8 mm; anthers yellow, elliptical, basifixed 2.4-2.9 mm. Style surface is thinly papilled 9-11 mm; stigma flat. Capsule obovate, base cuneate 11-22 x 12-18 mm. Seeds flat, obovate 4-7 x 3-6 mm; testa shiny, brown, faveolate.



Figure 1. General view of *Fritillari serpenticola* in locality

Table 1. Analogy of some morphological characters of *F. serpenticola* and type description

Characters	***F. serpenticola* (This study)**	**Type description (Tekşen and Aytaç 2004)**	**Flora of Turkey (Rix 1984)**
Stem	Smooth	smooth	smooth
Leaves	4-5	3-5	3-5
Lowest leaves	2.3–5.2×0.7-1.3 cm, broadly lanceolate	2.5-7.0x0.4-1.8 cm, broadly lanceolate (4-6x as long as broad)	2.7-7.5x0.5-1.8 cm, lanceolate, acute
Lowest leaves margin	not papillose	not papillose	not papillose
Upper leaves	twisted	twisted	twisted
Perigon	conical, outside and inside yellow, fading to orange	conical, outside and inside yellow, fading to orange	conical, outside and inside yellow, fading to orange
Inner tepal	4-6 mm width	3.5-5 mm width	-
Filaments	6-8 mm, sparsely papillose	smooth	5-9 mm, smooth
Anthers	3-4 mm	2.5-3 mm	2.5-3 mm, elliptic
Style	9-11 mm, sparsely papillose	0.5 mm diameter, sparsely papillose or only at apex papillose	6-10 mm, scarcely papillose

Anatomical properties

Root anatomy

There is a thin cuticle covering the epidermis cell which is composed of squared or orbicular cells. Exodermis is 2-3 layered and composed of isodiametric cells. The cortex has 3-4 layered and consist of parenchyma. Parenchyma cells are square or irregular and thin walled. Endodermis is a single layer. A single layer pericycle is found under the endodermis layer. The center of vascular cylinder is composed of 1 metaxylem (Fig. 2, Table 2).

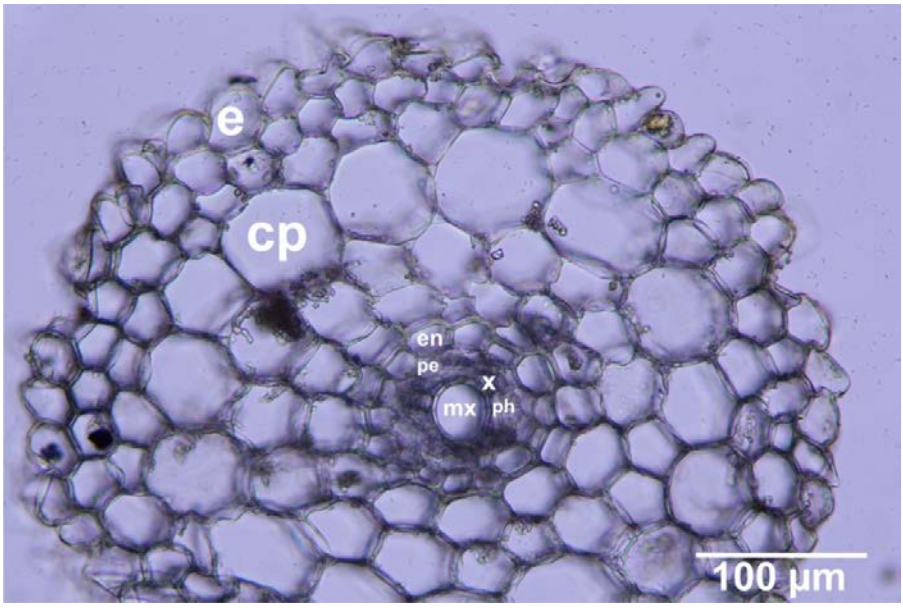

Figure 2. Cross section of the root of *Fritillari serpenticola*. e: epidermis, en: endodermis, cp: cortex parenchyma,pe: pericycle, x:xylem, mx: metaxylem, ph: phloem (20x10)

Scape anatomy

There is a thin cuticle covering the single-layered epidermis cell. Epidermal cells small orbicular and upper side of the cell wall is thickened. Beneath the epidermis is a 4-5 layered cortex of globular parenchymal cells. Cortex has a broad space between cells. Parenchyma cells are located under sclerenchyimatic 4-5 layered cortex cells. Under the sclerenchyma layer, there are bundles of vascular. At the center of the vascular bundle there are 6-7 protoxylem and 6-7 metaxylem (Fig. 3, Table 2).

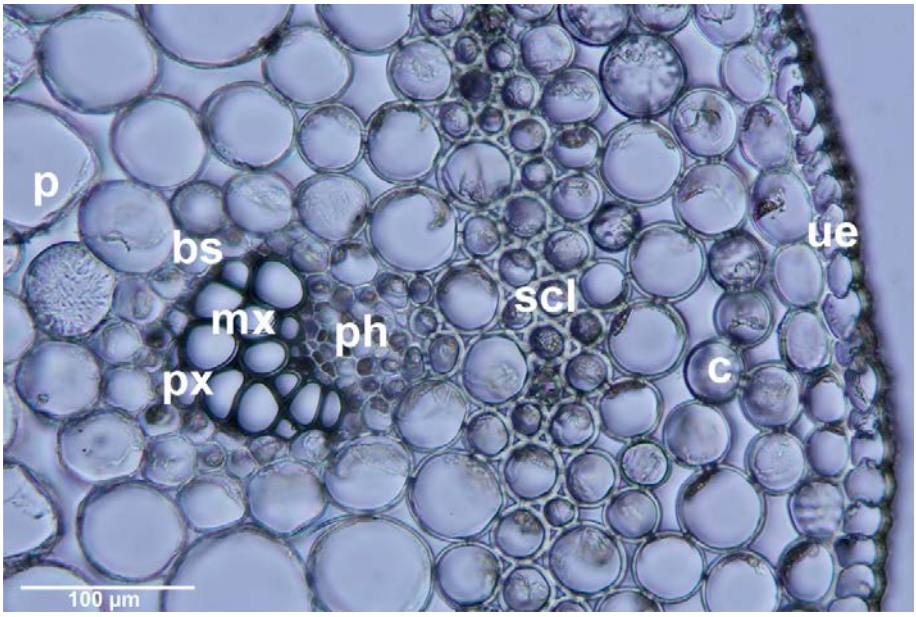

Figure 3. Cross section of the scape of *Fritillari serpenticola*.ue: upper epidermis, c: cortex, scl: sclerenchyma, ph: phloem, mx: metaxylem, px: protoxylem, p: pith (20 x10)

Leaf anatomy

Epidermis consists of a single layer of square and orbicular epidermal cells. The upper epidermis cells are smaller than the lower epidermis cells. There is a thin cuticle in the upper and lower epidermis. Palisade and sponge parenchyma of the mesophyll does not vary. Mesophyll 8-10 layered cells oval or slightly elongated. Some spongy parenchyma cells are includes raphide crystals. Vascular bundles in one direction and in different dimensions are found in the mesophyll cells (Fig. 4). The stomata are located at the same level as the epidermal cells and they are anomocytic (Fig. 5, 6, Table 2).

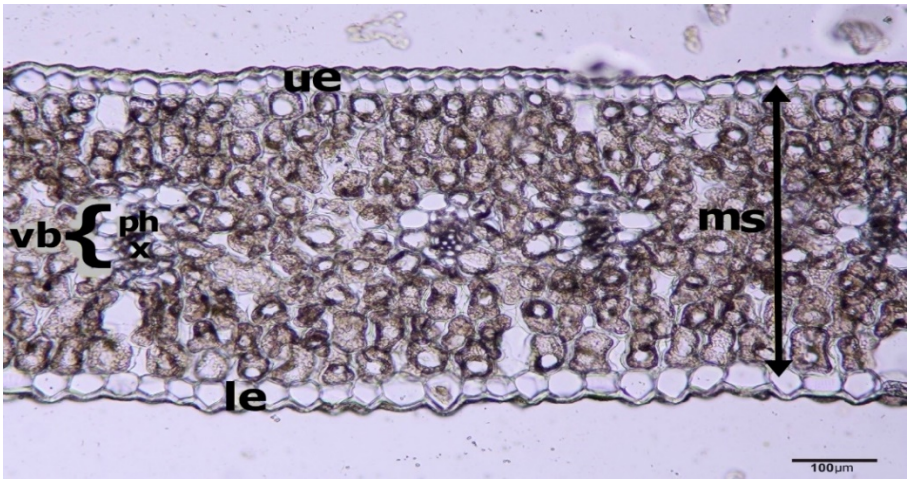

Figure 4. Transverse section of the leaf of *Fritillaria serpenticola.* ue: upper epidermis, ms: mesophyll, ph: phloem, x: xylem, le: lower epidermis, vb: vascular bundle (10 x 10)

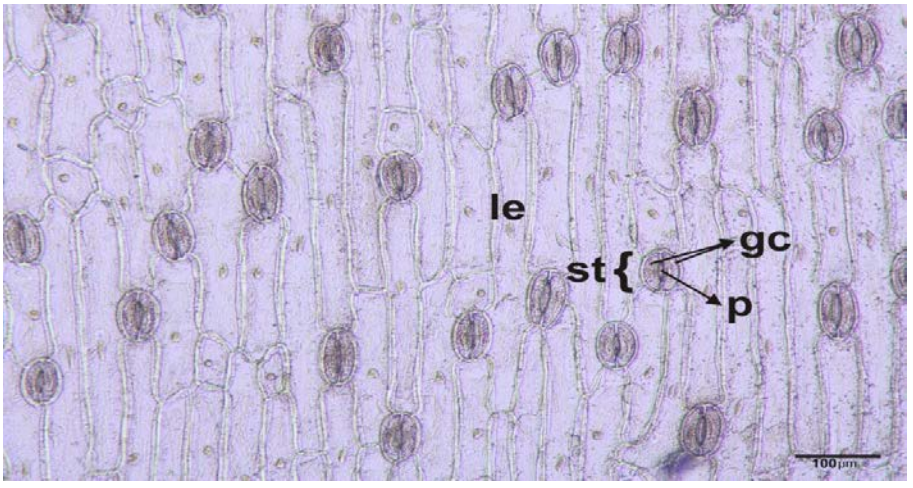

Figure 5. Upper surface section of the leaf of *Fritillaria serpenticola* le: lower epidermis, st: stoma (10 x 10)

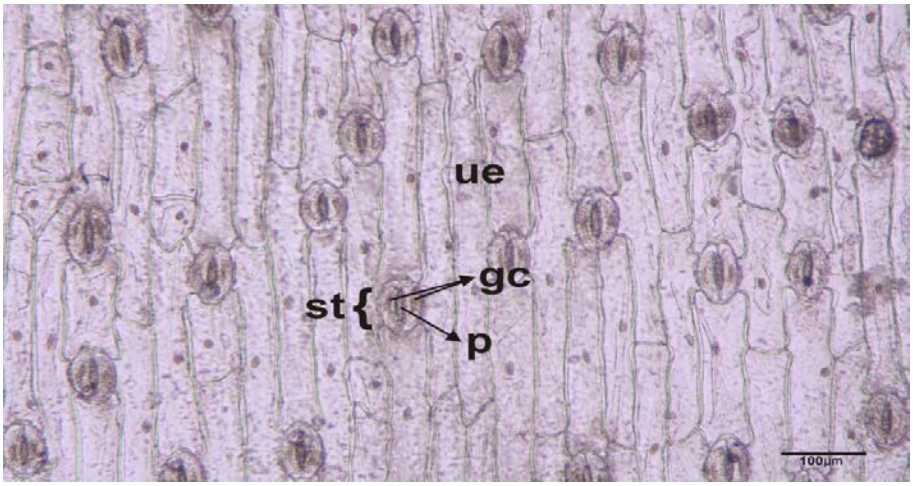

Figure 6. Lower surface section of the leaf of *Fritillaria serpenticola* ue: upper epidermis, st: stoma (10 x 10)

Obtained some anatomical data from these study is compared with other related taxa (Table 2).

Some anatomical similarities and differences were identified in some performed anatomical comparisons between taxonomically related taxa (Table 2).

Table 2. Comparison of some anatomical characters of *F. serpenticola*, *F. baskilensis* and *F. caucasica*

Parts	Characters	***F. serpenticola*** (This study)	***F. baskilensis*** (Pinar and Behçet 2012)	***F. caucasica*** (Akyol et al. 2014)
Root	Cuticle	+	+	+
	Epidermis	1-layered, square or orbicular cells	1-layered, squarel cells	1-layered, squarel cells
	Exodermis	2-3-layered, isodisametric	2-3-layered, isodisametric cells	1-2 layered, isodisametric
	Endodermis	1-layer	1-layer	1-layer
	Cortex	3-4 layered, square or irregular parenchymal cells	4-5 layered, irregular or oval parenchymatic cells	3-4 layered, oval and consists of hexagonal parenchymatic cells
	Pericycle	1-layer	1-layer	1-layer
	Vascular bundle	Collateral	collateral	collateral
	Pith	parenchymatic cells	parenchymatic cells	parenchymatic cells
Scape	Cuticle	+	+	+
	Epidermis	1-layered, small orbicular cells	1-layered, small orbicular cells	1-layered, orbicular cells
	Cortex	4-5 layered, globular parenchymal cells	3-5 layered, oval or orbicular cells	4-5 layered, oval or orbicular cells
	Sclerenchyma	4-5 layered	4-5 layered	3-5 layered
	Vascular bundle	Collateral	collateral	collateral
	Pith	parenchymatic cells	parenchymatic cells	parenchymatic cells
Leaf	Cuticle	+	+	+
	Epidermis	1-layered, square and orbicular cells	1-layered, square and orbicular cells	1-layered, square and orbicular cells
	Mesophyll	8-10 layered cells, oval or slightly elongated	8-10 layered cells, oval or slightly elongated	9-10 layered, oval or slightly elongated
	Phloem	upper side	inner side	upper side
	Xylem	inner side	upper side	inner side
	Intercellular space	+	+	+
	Raphide crystals	+	+	+

Plant Localities

Type. Turkey. C2 Antalya: Between Karaçulha and Altınyayla, in bare brown scree, 1700 m, 05.04.1980, O. Sonderhousen 680 (holo. E!). (Rix 1984).

C2 Burdur: Altınyayla, Dirmil pass, 36°58561N, 29°34419E, 1500-1550 m, 18.04.2000, M.Tekşen 1975 (fl.) (GAZI); ibid., 13.07.2001, M.Tekşen

2110 (fr.) (GAZI) (24).

B3 Eskişehir: Gökçekaya road, 59 km, 1100 m, H.Malyer 1454 (ANK, AEF) (24) (Any population does not found in this locality where previously mentioned in the flora of Turkey).

B3 Eskişehir: Alpu district, Alapınar village, N 39°59'18.1"-E 30°51'53.0", 1279 m, 14.04.2016, OUFE 20411 (This study).

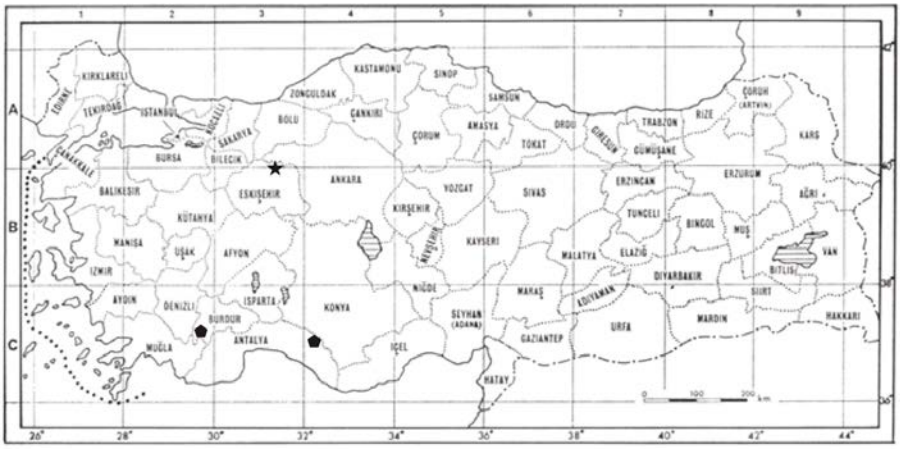

Figure 7. Distribution map of *Fritillaria serpenticola* in Turkey.

Conservation Status

In this study, population properties of endangered *Fritillaria serpenticola* which were described from serpantine rich habits in Eskişehir province have been observed between 2015-2017 years. *F. serpenticola* is high habitat selective taxon. Today, it founds in only two localities where have serpentine rich soil and base rock in Anatolia. Totally, 472 individuals were counted from two populations. Population size and individual numbers approximately decrease 10 % (50 individuals) in this period. According to the obtained data from observations, ecological factors which effect to the populations of *F. serpenticola* have been determined. In the light of obtained data, endemism categetory of *F. serpenticola* suggested as EN B2b (iii) (36).

Discussion

There have been some previous morphological, anatomical and palynological studies of the *F. serpenticola* (19, 26, 30). In this study, *F. serpenticola* from Eskisehir-Alpu were investigated in terms of anatomical, morphological properties and conservation status. In conclusion, the morphological properties of *F. serpenticola* have generally been matched with related literatures most of the anatomical properties of *F. serpenticola*

resemble the general characteristic of monocotyledons (19, 26, 30, 36, 38). Some anatomical characters of *F. serpenticola*, *F. baskilensis* and *F. caucasica* have been compared in this study. High anatomical similarities observed between related taxa. For example, epidermis, endodermis, pericycle, vascular bundle, pith and cuticle characteristics have high similarities. Some differences between exodermis, cortex, sclerenchyma, mesophyll characteristics are given Table 2.

According to the conservation studies, any noticeable change in population sizes and distributions are not observed. Especially antropogenic factors have high effects on *F. serpenticola* populations. But in 2 years period, these changes do not require any conservation precations urgently. In the light of the our observations, any changes not to be need on last suggested status by Tekşen and Aytaç (19).

This study intend to identify some morphological anatomical features, to investigate conservation and distribution status thus assessing the utility of these obtained data for systematic purposes and biological diversity researches.

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