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Research article/Araştırma makalesi

Some morpho-anatomical studies on rare endemic *Muscari sivrihisardaghlarensis*

Okan SEZER [1], Kurtuluş ÖZGİŞİ [1], Ö. Koray YAYLACI [1], Onur KOYUNCU [*1]

[1] Eskişehir Osmangazi University, Faculty of Science and Art, Department of Biology, 26480 Eskişehir, Turkey

Abstract

In this study, some morpho-anatomical features of rare endemic *Muscari sivrihisardaghlarensis* Yıldırımlı & B. Selvi which is distributed in Central Anatolia has been investigated. Anatomical features, a detailed morphological description and a distribution map of *M. sivrihisardaghlarensis* are given. Also conservation status first time determinated. For identification of anatomical features, tranverse sections of the roots, scapes, leaves and surface sections of the leaves were investigated. Obtained morpho-anatomical characteristics of *M. sivrihisardaghlarensis* were compared with related species and its type description.

Key words: Asparagaceae, *Muscari sivrihisardaghlarensis*, anatomi, morfoloji, endemik

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Nadir endemic olan Sivrihisar Misk Sümbülü (*Muscari sivrihisardaghlarensis*) nün bazı morfo-anotomik özellikleri

Özet

Bu çalışmada, Orta Anadolu'da yayılış gösteren nadir endemik *Muscari sivrihisardaghlarensis* Yıldırımlı & B. Selvi (Sivrihisar Misk Sümbülü) türü üzerinde bazı morfo-anatomik incelemeler yapılmıştır. *M. sivrihisardaghlarensis*'in anatomik özellikleri, detaylı morfolojik deskripsiyonu ve bir yayılış haritası verilmiştir. Ayrıca taksonun koruma statüsü ilk kez belirlenmiştir. Anatomik özellikler için kök ve skap enine kesitleri ile yaprak enine ve yüzelsel kesitleri incelenmiştir. *M. sivrihisardaghlarensis*' in belirlenen morfo-anatomik özellikleri tip deskripsiyonu ve yakın ilişkili olduğu türler ile karşılaştırılmıştır.

Anahtar kelimeler: Asparagaceae, *Muscari sivrihisardaghlarensis*, anatomi, morfoloji, endemik

1. Introduction

According to the recent phylogenetic studies, Asparagaceae which is systematically situated between the families of the Iridaceae and Hypoxidaceae has approximately 143 genera and 3700 species all over the world (the plantlist.org, 2010; APG III, 2009). Furthermore, this family has been represented about 120 species in Turkey. Some of these species are native and also endemic for Turkey. Asparagaceae species have wide distribution in Turkey, more especially in the Mediterranean and Irano-Turanian floristic regions (Dane, 2006; Davis et al., 1984; Davis et al., 1988; Güner et al., 2000).

Muscari (L.) Miller (Grape hyacinth) which spring-flowering bulbous plants in the family of Asparagaceae has about 50 species in the World (the plantlist.org, 2010). This genus naturally grows in Caucasus to the Mediterranean basin, temperate regions of Europe, North Europe and Northwest parts of Asia (Johnson et al., 1996; Jafari et al., 2008).

Muscari was revised for the Flora of Turkey and the East Aegean Islands and 20 species recognized from Turkey (Davis & Stuart, 1984). In addition to these, 11 new species have been described from Turkey between the dates of 1984-2012 (Karlen, 1987; Tan, 1988; Speta, 1989; Cowley, Özhatay & Mathew, 1994; Güner & Duman, 1999; Güner et al., 2000; Yıldırımlı & Selvi, 2002; Uysal et al., 2007; Eker & Koyuncu, 2008; Doğu & Bağcı, 2009;

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +902222393750-2428; Fax.: +902222393750-2428; E-mail: okoyuncu@ogu.edu.tr
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Yıldırımlı, 2010). With the addition of these species, total number of *Muscari* species which determined from Turkey is reached to 31. 19 of 31 species are endemic for Turkey and endemism ratio is 61.2 %.

Different taxa of Asparagaceae has been investigated according to their morphological, anatomical, cytological, palynological and ecological features by different researchers until today (Shoub & Halevy, 1971; Bentzer et al., 1974; Özyurt, 1978; Küçüker, 1985; Küçüker, 1990; Özhatay & Dalgıç, 1990; Uysal, 1992; Uysal, 1999; Kandemir et al., 2000; Pehlivan & Özler, 2003; Ocak et al., 2004; Hermann et al. 2006; Lynch et al., 2006; Satıl & Akan, 2006; Gürsoy & Şık, 2010; Kahraman et al., 2010; Doğu et al. 2011). In addition to these, like some Asparagaceae taxa, *Muscari sivrihisardaghlarensis* (Sivrihisar Misk Sümbülü) has not been studied previously and also such investigations on *Muscari* species are rather limited (Özhatay & Dalgıç, 1990; Uysal, 1992; Pehlivan & Özler, 2003; Gürsoy & Şık, 2010).

In this study, some morpho-anatomical characteristics of endemic *M. sivrihisardaghlarensis* which is distributed in Central Anatolia have been investigated. Furthermore, distribution of *M. sivrihisardaghlarensis* was revised. Also conservation status was the first time determinated.

This study aims to investigate morpho-anatomical features of *M. sivrihisardaghlarensis*, to determinate distribution and conservation status and so evaluate the usefulness of these obtained data for systematic purposes. In anatomical studies, tranverse sections of the roots, scapes, leaves and surface sections of the leaves were the first time investigated. Obtained morpho-anatomical characteristics of *M. sivrihisardaghlarensis* were compared with closely related species and its type description (Davis & Stuart, 1984).

2. Materials and methods

2.1. Plant material

The samples of *M. sivrihisardaghlarensis* were collected from three localities in B3: Eskişehir, Sivrihisar for this study in 2010-2012. Also a distribution map of plant samples is given in Figure 1. Fresh, fixed and dried plant samples were used for morpho-anatomical investigations. In order to ensure a systematic study of the material obtained, herbarium samples were prepared and these samples were protected as herbarium samples at the Eskişehir Osmangazi University Herbarium (OUFE).

Localities were collected plant samples:
1- B3 Eskişehir; Sivrihisar, from Günyüzü bridge and Bademlik petrol office, Sivrihisar mountains, towards peak, slopes, hilly, screes, gypseus places, steppe, 1000-1500 m (c. 1300 m), 17.04.2012, OUFE: 16475, (Type locality).
2- B3 Eskişehir; Sivrihisar, Kuzören-Karacaören road, slopes, hilly, screes, steppe, 39°18′58″N, 31°42′33″E, 1361 m, OUFE: 16473, 09.04.2012.
3- B3 Eskişehir; Sivrihisar, Arayit Mountain, Backside of radar station, slopes, hilly, screes, steppe, 39°18′12″N, 31°45′01″E, 1800 m, OUFE: 16474, 16.04.2012.

2.2. Anatomical studies

In anatomical studies; the root, scape and leaves were fixed in 70% alcohol and then kept in the same solution until the acquisition of cross and superficial sections. For sectioning, samples were taken from the alcohol by hand and scalpel. The sections were photographed by the Kameram™ digital camera and an Nikon 80i type microscope in our Department. A variety of foundation anatomical books and conducted studies were used as sources for identification of the plants (Esau, 1967; Fahn, 1967; Vardar, 1987; Özörgücü ve ark., 1991; Özörgücü, 1993; Yentür, 1995).

2.2. Morphological studies

Herbarium and fresh plant samples were used for identification of detailed morphological characteristics. Obtained morphological characteristics of samples compared with morphological characteristics in type description of *M. sivrihisardaghlarensis* and closely related species (*i.e. M. neglectum* and *M. armeniacum*), (Davis & Stuart, 1984; Gürsoy and Şık, 2010).

3. Results and discussion

3.1. Morphological properties

Description: Bulb 13-20 x 12-18 mm, ovoid, without offsets. Leaves (2-) 3 (-6) cm x 0.8-1 mm, linear, (3-) 4 (-5), canaliculated, green. Scape c. 3 cm, canaliculated. Raceme 1-1.5 x 0.8-1 cm, cylindrical, very dense, 9-14 fertile flowers and 6-20 sterile flowers, imbricate. Pedicels of fertile flowers 0.1-0.5 mm long, spreading or deflexed, shorter than flowers. Fertile flowers c. 3-4 x 2 mm, oblong-urceolate, dark violet to blackish, constricted distally; lobes c. 1 mm

long, white, obtuse, erect. Stamens biseriate at middle of tube; anters 0.5 mm long, blackish. Pedicels of sterile flowers 0.1-0.2 mm, ascending. Sterile flowers c. 3 x 2 mm, c. 6, white to light blue. Capsule obovate-orbicular, 4.0x4.5mm, shortly emarginate, 3 locules 2 seeds per locules. Seeds black, ovate to globose, 1.2x1.5mm. Fl. 3-4, Fr. 4-5. Stony slopes, hilly, screes, gypseus places, steppe, s.l.1000-1900 m. (Figure 1, Table 1).
In the end of morphological investigations some characters which has not mentioned in type description are indicated (Capsule and Seed features, Flowering and Fruiting time). In addition to some characters in type description are identified again (Fertile flowers and Sterile flowers number) (Table 1). As a result of a wide description has been given about *M. sivrihisardaghlarensis*.



Figure 1. General appearance of *Muscari sivrihisardaghlarensis* in nature

Table 1. Comparison of some morphological characters of *Muscari sivrihisardaghlarensis* and type description (Yıldırımlı and Selvi, 2002)

Characters	***M. sivrihisardaghlarensis* (this study)**	**Type description (Yıldırımlı and Selvi, 2002).**
Bulb	13-20 x 12-18 mm, ovoid, without offsets	13-20 x 12-18 mm, ovoid, without offsets
Leaves	(2-) 3 (-6) cm x 0.8-1 mm, linear, (3-) 4 (-5), canaliculated, green.	(2-) 3 (-6) cm x 0.8-1 mm, linear, (3-) 4 (-5), canaliculated, green.
Scape	c. 3 cm, canaliculated.	c. 3 cm, canaliculated.
Raceme	1-1.5 x 0.8-1 cm, cylindrical, very dense, c. 9 fertile flowers and c. 6 sterile flowers, imbricate.	1-1.5 x 0.8-1 cm, cylindrical, very dense, c. 9 fertile flowers and c. 6 sterile flowers, imbricate.
Fertile flowers	9-14, c. 3-4 x 2 mm, oblong-urceolate, dark violet to blackish, constricted distally; lobes c. 1 mm long, white, obtuse, erect.	c. 9, c. 3-4 x 2 mm, oblong-urceolate, dark violet to blackish, constricted distally; lobes c. 1 mm long, white, obtuse, erect.
Sterile flowers	6-20, c. 3 x 2 mm, c. 6, white to light blue.	c. 6, c. 3 x 2 mm, c. 6, white to light blue.
Pedicels of fertile flowers	0.1-0.5 mm long, spreading or deflexed, shorter than flowers.	0.1-0.5 mm long, spreading or deflexed, shorter than flowers.
Pedicels of sterile flowers	0.1-0.2 mm, ascending.	0.1-0.2 mm, ascending.
Stamens	biseriate at middle of tube; anters 0.5 mm long, blackish.	biseriate at middle of tube; anters 0.5 mm long, blackish.
Capsule	obovate-orbicular, 4.0x4.5mm, shortly emarginate, 3 locules, 2 seeds per locules	unknown
Seeds	black, ovate to globose, 1.2x1.5mm	unknown
Flowering	3-4	-
Fruiting	4-5	-

3.2. Anatomical properties

3.2.1. Root anatomy

The epidermis is made up of a single layer of nearly globoid cells on the outermost surface. Epidermis is fallowed by single layered exodermis. Exodermis is composed of relatively bigger and thin walled cells than epidermal cells. After that, the cortex covers a very large area, and underneath is the endodermis, which is consists of 7-9 layers of mainly polygonal parenchymatous cells. Following this, single layered endodermis is composed of thick walled cells. The direction of these thickneses is towards the pericycle, which consists of single layered and thin walled cells. In pith, radially arranged vascular bundle has 6 protoxylem ridges between the phloem strands. Center of vascular bundle is full of 7-9 metaxylems (Figure 2).

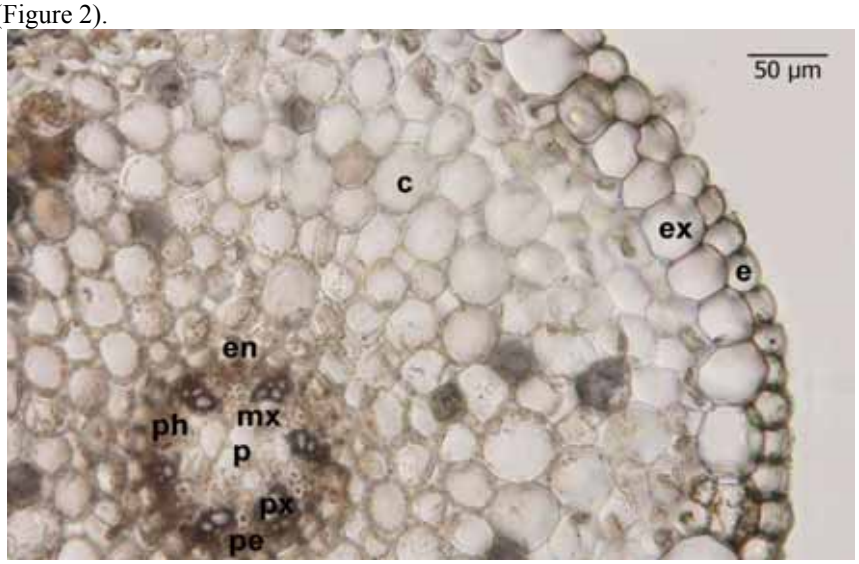


Figure 2. Cross section of the root of *Muscari sivrihisardaghlarensis*. **e:** epidermis, **ex:** exodermis, **c:** cortex, **en:** endodermis, **pe:** pericycle, **px:** protoxylem, **mx:** metaxylem, **ph:** phloem, **p:** pith (20x10)

3.2.2. Scape anatomy

Epidermis is composed of one cell layer. Epidermal cells square-rectangular shaped and cell wall thickened in the upper parts. There are no hairs on epidermis and epidermal cells covered by thick cuticle. Under the epidermis, 5-7 layered cortex which made up of globular parenchymatic cells is situated. Cortex has wide intercellular spaces. 4-5 layered sclerenchymatic cells are located under the parenchymatic cortex cells. Following this, closed collateral vascular bundles are numerous and scattered throughout the pith. Every vascular bundle is surrounded by a sclerenchymatic bundle sheath. Also orbicular or hexagonal parenchymatic cells are located at pith (Fig. 3).

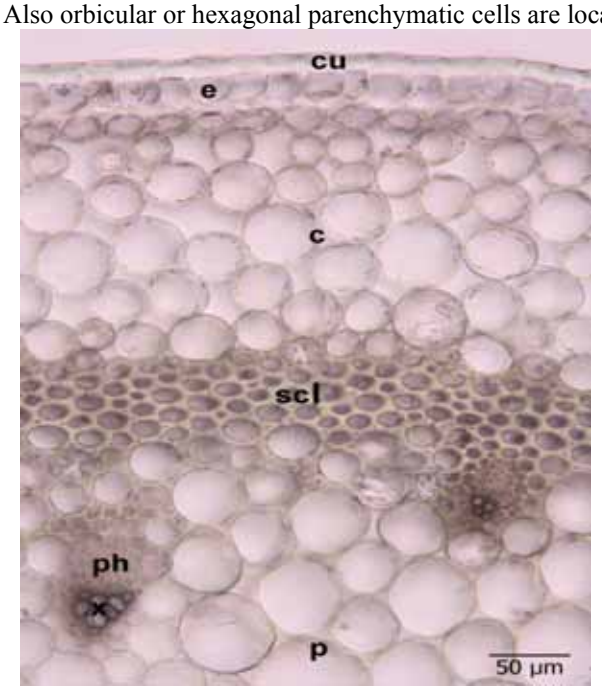


Figure 3. Cross section of the scape of *Muscari sivrihisardaghlarensis*. **cu:** cuticle, **e:** epidermis, **c:** cortex, **scl:** sclerenchyma, **ph:** phloem, **x:** xylem, **p:** pith (20x10)

3.2.3. Leaf anatomy

Epidermis is composed of a single layer of somewhat isodiametric epidermal cells in cross section. Upper and lower walls of epidermal cells are thicker than lateral. Typically length of epidermal cells is longer than width (Figure5). The outer surface of epidermis is covered with a thick cuticle. The leaf is amphistomatic and equifacial. Beneath both the surfaces of the mesophyll is present 1-2 layered palisade parenchyma. Spongy parenchyma located between the palisade. Some Spongy parenchyma cells are include raphide crystals. Same sized vascular bundles are arranged in a single row. Lacunae are present between vascular bundles especially interior ones. Leaves haven't got a distinct midrib. Stomata are anomocytic and also lie at the same level with epidermal cells (Figure 4,5).

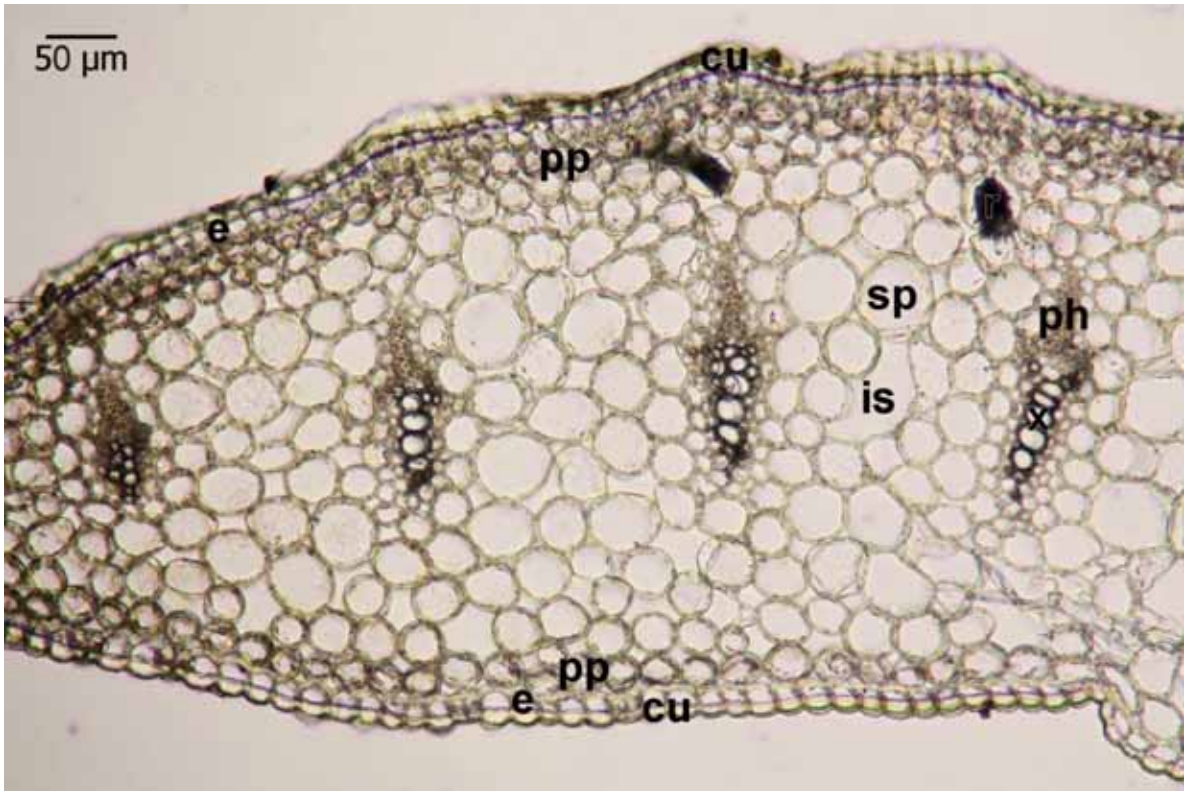


Figure 4. Transverse section of the leaf of *Muscari sivrihisardaghlarensis*. **cu:** cuticle, **e:** epidermis, **pp:** palisade parenchyma, **sp:** spongy parenchyma, **ph:** phloem, **x:** xylem, **is:** intercellular space, **r:** raphide crystals (10x10)

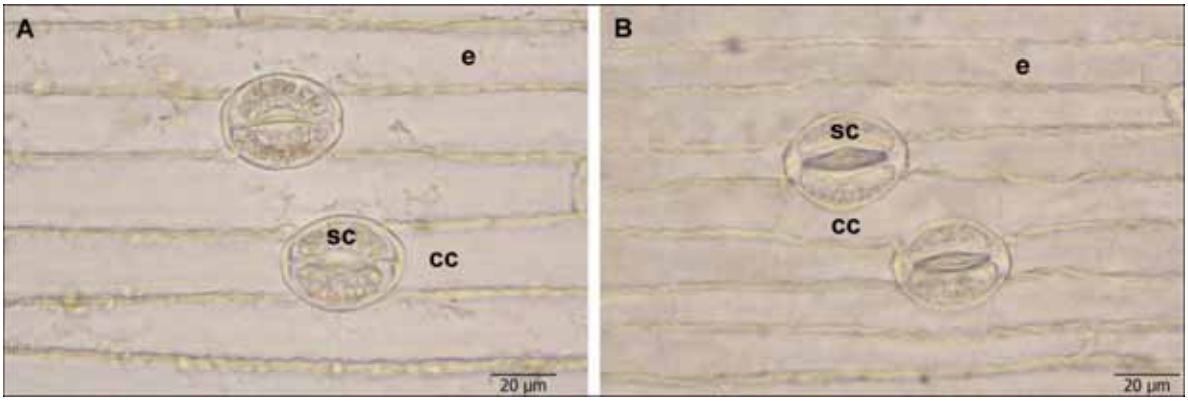


Figure 5. Surface section of the leaf of *Muscari sivrihisardaghlarensis*. **A:** stomata from upper epidermis; **B:** Stomata from lower epidermis; **sc:** stomatal cell, **cc:** companion cell, **e:** epidermis (60x10)

As a consequence of anatomical studies, some anatomical characters of *M. sivrihisardaghlarensis* are firstly identified (Table 2). Anatomical findings of *M. sivrihisardaghlarensis* are compared as systematically with closely related taxa *M. armeniacum* and *M. neglectum* (Table 2). According to these anatomical findings; *M. sivrihisardaghlarensis* shows general similarities with *M. armeniacum* and *M. neglectum*. However, some properties has differences; while the cuticle in the root of *M. sivrihisardaghlarensis* is "**±",** *M. armeniacum* and *M. neglectum* are "+". While, root cortex of the *M. sivrihisardaghlarensis* is 6-12 layered, *M. armeniacum* and *M. neglectum* are 7-10

layered. Whereas, Scape cortex of *M. sivrihisardaghlarensis* is 4-6 layered, *M. armeniacum* and *M. neglectum* are 3-5 layered. When sclerenchyma in the cortex of *M. sivrihisardaghlarensis* has 3-6 cell layered, *M. armeniacum* and *M. neglectum* has 3-5 cell layered. While sponge parenchyma in the leaf of *M. sivrihisardaghlarensis* is 6-9 layered, *M. armeniacum* and *M. neglectum* are 6-7 layered (Gürsoy & Şık, 2010). As a consequently, similarities and differences of investigated species are identified.

Table 2. Comparison of some anatomical characters of *Muscari sivrihisardaghlarensis*, *M. armeniacum* and *M. neglectum*

Parts	**Characters**	***M. sivrihisardaghlarensis***	***M. armeniacum* (Gürsoy&Şık 2010)**	***M. neglectum* (Gürsoy&Şık 2010)**
Root	Cuticle	±	+	+
	Epidermis	1-layered, isodiametric cells	1-layered, isodiametric cells	1-layered, isodiametric cells
	Exodermis	1-layered, isodisametric cells	1-layered, isodisametric cells	1-layered, isodiametric cells
	Endodermis	1-layer	1-layer	1-layer
	Cortex	6-12 layered, thin-walled, oval and consists of hexagonal parenchymatic cells	7-10 layered, thin-walled, oval and consists of hexagonal parenchymatic cells	7-10 layered, thin-walled, 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, thickened-walled isodiyametric cells	1-layered, thickened-walled isodiyametric cells	1-layered, thickened-walled isodiyametric cells
	Cortex	4-6 layered, ovoid and polygonal-shaped cells	3-5 layered, ovoid and polygonal-shaped cells	3-5 layered, ovoid and polygonal-shaped cells
	Sclerenchyma	3-6 layered	3-5 layered	3-6 layered
	Vascular Bundle	collateral	collateral	collateral
	Pith	parenchymatic cells	parenchymatic cells	parenchymatic cells
Leaf	Cuticle	+	+	+
	Epidermis	1- layer, ovoid and rectangular cells	1- layer, ovoid and rectangular cells	1- layer, ovoid and rectangular cells
	Palisade Parenchyma	1-2 layered, ovoid and cylindrical cells	1-2 layered, ovoid and cylindrical cells	1-2 layered, ovoid and cylindrical cells
	Spongy Parenchyma	6-9 layered, large cells	6-7 layered, large cells	6-7 layered, large cells
	Phloem	Upper side	Upper side	Upper side
	Xylem	Inner side	Inner side	Inner side
	Intercellular Space	+	+	+
	Raphide Crystals	+	+	+

3.3. Conservation status

The *M. sivrihisardaghlarensis* in endemic to Central Anatolia. It was found only near the three localities by us. First locality is between Kuzören and Karacaören (Sivrihisar-Eskişehir). It has very limited distribution in this locality. Population is composed of 50 individuals and covers less than 2 km^2. Abundance of the population is decrease in time because of grazing and other biotic factors in this locality. Second locality is peak of the Arayit mountain (Sivrihisar-Eskişehir). It has very limited distribution in this locality too. Population is composed of 80 individuals and covers less than 2 km^2. This locality is conserved area comparing to others and biotic effects on the population is less than other two localities. However, size of population is still very small because of abiotic factors like climatic factors. Third locality is type locality of species. This locality is situated around Günyüzü bridge and Bademlik petrol office (Sivrihisar-Eskişehir). Size of the populations is averagely 100 and they cover 3 km^2. Populations are under treat here because of some anthropogenic factors. Especially, agricultural works and grazing have major effect on size and abundance of populations (Figure 6).

According to these obtained data, *M. sivrihisardaghlarensis* is known from three locality and severely fragmented (criterion B2 a), with an estimated area of occupancy is less than 10 km^2 (criterion B2). The population is unhealthy with less than 250 individuals (criterion C). Therefore, it should be classified as "Critically Endangered (CR)" based on the criteria of the IUCN Red List Categories, and populations should be protected (IUCN 2001).

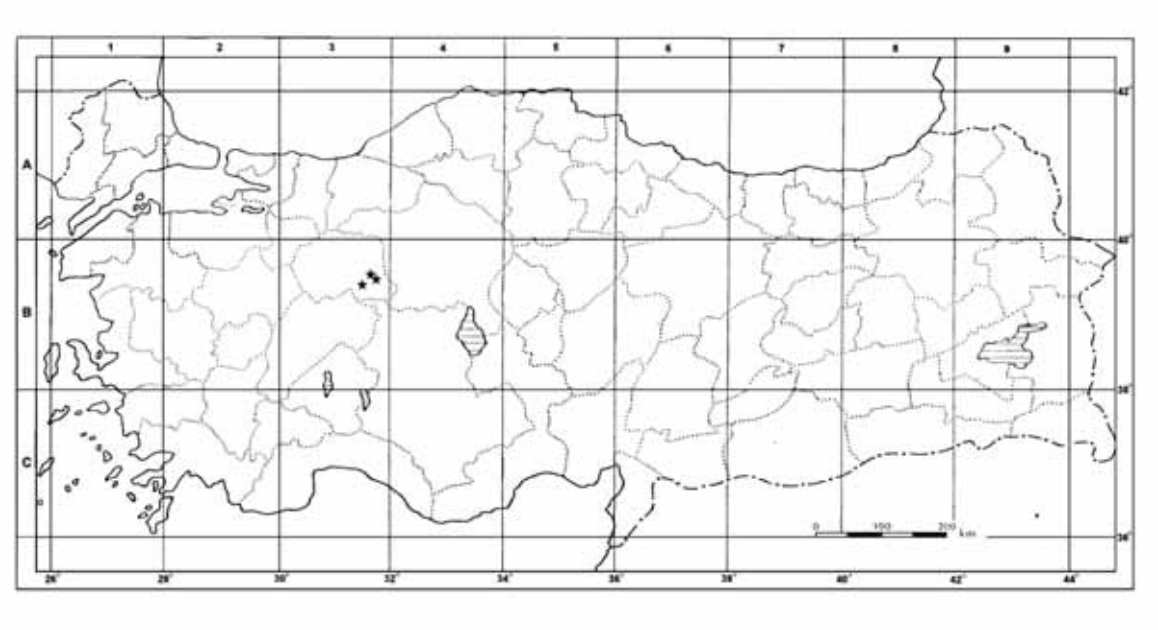


Figure 6. Distribution map of *Muscari sivrihisardaghlarensis* in Turkey

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Biological Diversity and Conservation

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