

MORPHO-ANATOMICAL INVESTIGATIONS OF THREE CRITICALLY ENDANGERED ENDEMIC SPECIES

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Keywords: *Crocus*, Endemic species, Morpho-anatomical investigation, Critically endangered

Abstract

Morphological and anatomical characteristics of *Crocus ancyrensis* Herb. (Maw) (endemic), *C. antalyensis* Mathew (endemic), *C. chrysanthus* (Herb.) Herb. and *C. sativus* L. which are distributed in Central Anatolia, Turkey have been studied. The morphological characteristics of species have been compared with the Flora of Turkey. In anatomical studies, transverse sections of root, stem and leaf have been examined and supported by illustration. In addition, the determination of the anatomical characteristics of the species are given in this study for the first time. The leaf anatomy of *Crocus ancyrensis* has been illustrated elsewhere.

Introduction

Turkey is a rich country regarding the *Crocus* L. (Iridaceae) species. *Crocus* is distributed mainly in the Mediterranean region and includes 80 species worldwide. There are 70 taxa (including sub species and varieties) of *Crocus* in Turkey. Thirty-one of these are endemics for Turkey (Mathew 1984, Guner *et al.* 2000, Erol *et al.* 2011). Many species of Iridaceae are grown in parks and gardens as ornamental plants due to their beautiful flowers (Baytop 1999). Some *Crocus* species were used for making dye, perfume and medicaments as long ago as 1600 B.C. The saffron (*Crocus sativus* L.) was the first to be cultivated and had been grown for economic purposes since ancient times. Abdullaev (2003) pointed out that the saffron could be useful in cancer chemoprevention in the immediate future. Any other *Crocus* species except *C. sativus* were defined until 16th century (Ozdemir *et al.* 2006).

The natural habitats of the *Crocus* flowering become yellow, white, red, blue, lilac and orange which bring the good news of the arrival of the autumn and the spring season are in danger. The *Crocus* species are regarded as ornamental plants as they grow like tulipa and flower in different colours. Because of these properties the *Crocus* species can sustain their life forms when they are cultivated in parks and gardens. It is estimated that the *Crocus* which attracts people positively with their lovely flowers is going to be of an important economical value in the near future. Thus, the wide spread propagation of these important ornamental plants may be protected them more or less from extinction (Kandemir 2010). There are some reports that the *Crocus* species have antitumor, antimutagenic, cytotoxic activities and inhibits nucleic acid synthesis in human (Fatehi *et al.* 2003, Kravkaz *et al.* 2006).

The species known as endemic *C. ancyrensis* (Herb.) Maw “Ankara Çiğdemi”, *Crocus chrysanthus* (Herb.) Herb., “Sarı Çiğdem”, endemic *C. antalyensis* Mathew “Antalya Çiğdemi” and *C. sativus* L “Safran” in Anatolia (Guner *et al.* 2012).

The aim of this study was to investigate morphological and anatomical properties of *C. ancyrensis*, *C. chrysanthus* and *C. antalyensis* which are found to be under the danger of extinction. In addition, morphological and anatomical characteristics of *C. ancyrensis*, *C. chrysanthus* and *C. antalyensis* are described here for the first time. The leaf anatomy of *C. antalyensis* leaf has been illustrated elsewhere.

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Materials and Methods

Plant materials were collected from different localities in Eskişehir and were identified with the help of herbarium materials (Table 1, Map 1). Voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University (ESSE) in Eskişehir, Turkey.

The plant materials were identified as *Crocus* spp. using the Flora of Turkey and the East Aegean Islands (Mathew 1984). Herbarium specimens were used for description and detailed morphological drawings. A wild M5 A stereo microscope with drawing tube was utilized for morphological drawing.

For anatomical studies, samples were collected from plantation areas and preserved in 70% alcohol. The root, stem and leaves of the mature and flowered plants were used. Investigations were performed on the cross-section and surface section of the leaves and cross-sections of the flowering stem from the middle area. These sections were pointed by Sartur reagent and then covered with glycerin-gelatin. In anatomical studies, an Olympus BX 51 trinocular microscope and a digital camera were used. Cross sections of stems were drawn as schematically and corner of the stems were drawn from anatomical structure. Cross sections of leaves were drawn schematically and midrib were anatomically. In addition, root, stem (scape) and leaves were added to the anatomical drawings. A Leitz SM-LUX binocular light microscope with a drawing tube were used in the anatomical studies.

Results and Discussion

Morphological: Crocus ancyrensis Herb. (Maw): The plant size of the taxon (9-) 13 - 25 cm. Corm tunic coarsely reticulate, (0.8-) 1.3 - 2.3 cm. Ring at base absent. Number of cataphyl 2 - 3. Leaves 3 - 8, synanthous, 0.5 - 1 mm broad. Prophyll absent. Bracts absent. Bracteole present. Number of flowers 1 - 3. Tube of perigon 3 - 7 cm. Throat of perianth orange, glabrous; segments 1.7 - 3 × 0.5 - 1 cm, obtuse to rounded, apex of segments obtuse-acute, base of segments cuneate, colour of segments orange, purplish at base and veins are brown striped. Filaments 4 - 10 mm, orange, glabrous; anthers 6 - 13 mm, yellow. Style 8 - 11 mm, dividing into 6 - 12 slender orange or reddish-orange branches. Indumentum of style glabrous (Figs 1, 4 and Table 2).

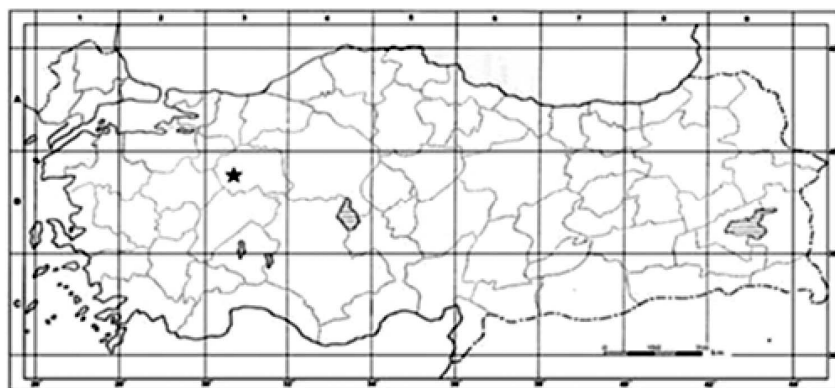
The flowering period is 2 - 4(-6). The plant are distributed from sea level to 1200 m altitude, in open rocky places, in scrub end in *Pinus* woods. Endemic, lr.-Tur. element. (Mathew 1984). New record.

Crocus antalyensis Mathew: The plant size of the taxon 9 - 23 cm. Corm tunic membranous, splitting into parallel fibres and with a long persistent neck at apex, (0.5-) 1 - 2 cm. Number of cataphyl 2 - 4. Leaves 3 - 8, synanthous, 1 - 2 mm broad. Prophyll absent. Bracts present. Bracteole present, much reduced and enclosed within bract. Number of flowers 1 - 4. Tube of perigon 3 - 8 cm. Throat of perianth yellow, pubescent; segments 2 - 4 × 0.5 - 1.5 cm, obtuse to subacute, apex of segments acute, base of segments cuneate. Colour of segments lilac-blue, outer ones sometimes buff or flecked with purple on outside or rarely white stained externally with blue near base. Filaments 3 - 7 mm, deep yellow, pubescent; anthers 8 - 13 mm, pale yellow. Style 9 - 14 mm, dividing into c. 6 - 12 slender orange or yellow branches. Indumentum of style pubescent. (Figs 2, 5 and Table 2). Endemic. East Mediterranean element (Mathew 1984). New record.

Crocus chrysanthus (Herb.) Herb: The plant size of the taxon 7 - 19 cm. Corm tunic membranous or coriaceous splitting into rings at base, rings entire or toothed, 0.9 - 2.2 cm. Number of cataphyl 1 2 - 4. Leaves (2-) 3 - 6, synanthous, 1 - 2 mm broad. Prophyll absent. Bracts present. Bracteole present, usually much narrower than bract. Number of flowers 1 - 3 (-4). Tube of perigon 3 - 10 cm. Throat of perianth orange, glabrous; segments 1.4 - 3 × 0.5 - 1.2 cm, subacute, apex of segments acute, base of segments cuneate. Colour of segments yellow to orange-

yellow, sometimes striped or suffused with bronze or purple outside, rarely creamywhite. Filaments 2 - 7 mm, yellow-orange, glabrous; anthers (2-) 4 - 14 mm mm, yellow, some times with black basal lobes, rarely wholly black-edged before dehiscence. Style 6 - 15 mm, dividing into 3 slender or expanded yellow to orange branches. Indumentum of style glabrous (Figs 3, 6 and Table 2).

Ir.-Tur. Element, New record (Mathew 1984).



Map 1. The study area of *Crocus* sp.



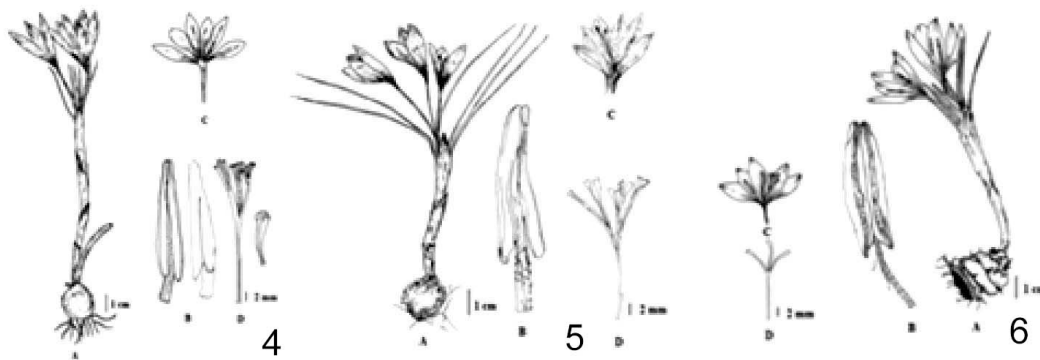
Figs 1-3. General appearance: 1. *Crocus ancyrensis*, 2. *C. antalyensis* and 3. *C. chrysanthus*.

Table 1. Information on the plant specimens.

Code	Collection date	Collection site	Voucher specimens no. (ESSE No.)
<i>C. ancyrensis</i> (A)	10.03.2013	B3: Eskisehir: Bozdag, 39° 56' 32'' K- 030° 30' 54'' D, 1079 m	14623
<i>C. chrysanthus</i> (B)	10.03.2013	B3: Eskisehir:Hekimdag,39°54'34''K-030° 33'13''D, 1289 m	14625
<i>C. antalyensis</i> (C)	10.03.2013	B3:Eskisehir:Bozdag,39°56'32''K-030° 30' 54''D, 1079 m	14624
<i>C. sativus</i> (D)	20.04.2013	B3:Eskisehir Passage Zone Agricultural Research Institute (TAGEM)	15406

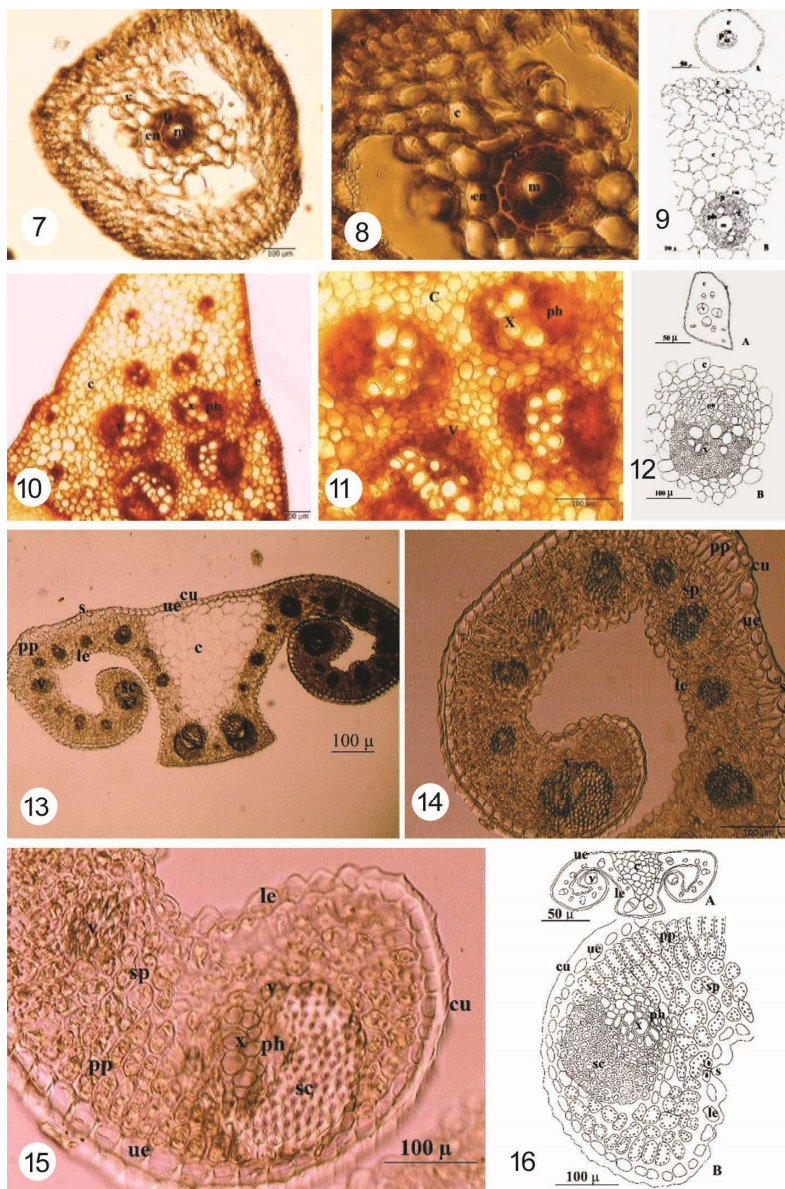
Table 2. The comparison of morphological characteristic between the present study and Flora of Turkey, with respect to *Crocus* species.

Characteristic	<i>C. ancyrensis</i>		<i>C. chrysanthus</i>		<i>C. antalyensis</i>	
	This study	Flora of Turkey	This study	Flora of Turkey	This study	Flora of Turkey
Morphological and morphometrical characteristic						
Plant size (cm)	(9-)13 - 25	"	7 - 19	"	9 - 23	"
Corm size (cm)	(0.8-)1.3 - 2.3	"	0.9 - 2.2	"	(0.5-)1 - 2	"
Number of cataphyl	2-3	"	2 - 4	"	2 - 4	"
Number of leaves	3-8	2 - 6	(2-)3 - 6	3-5(-6)	3 - 8	3 - 8
Broad size of leaves (mm)	0.5-1	0.5 - 1 (-2)	1 - 2	0.5-2.5	1 - 2	1 - 2.5
Bracts	Absent	"	Same	Present	Same	Present
Number of flowers	1-3	"	1 - 3(-4)	"	1 - 4	"
Tube of perigon (cm)	3-7	"	3 - 10	"	3 - 8	"
Throat of perigon	Orange	Yellow	Orange	Yellow	Same	Yellow
Segments (cm)	1.7-3 × 0.5-1	(1.3-)1.5 - 3x (0.7-) 0.9 - 1.3	1.4 - 3 × 0.5 - 1.2	1.5 - 3.5 × 0.5 - 1.1	2 - 4 × 0.5 - 1.5	2 - 3.5 × 0.6 - 1.1
Shape of segments	Same	Obtuse to rounded	Subacute	"	Same	Obtuse to subacute
Apex of segments	Obtuse-acute	"	Acute	"	Acute	"
Base of segments	Cuneate	"	Cuneate	"	Cuneate	"
Colour of filaments	Orange	"	Yellow-orange	Yellow	Same	Deep yellow
Size of filaments (mm)	4 - 10	2 - 4	2-7	3 - 6	3 - 7	3 - 5
Anther size (mm)	6 - 13	(6-)8 - 13	(2-)4-14	6 - 12	8 - 13	10 - 12
Size of style (mm)	8 - 11	"	6-15	"	9 - 14	"
Indumentum of style	Glabrous	"	Glabrous	"	Pubescent	"

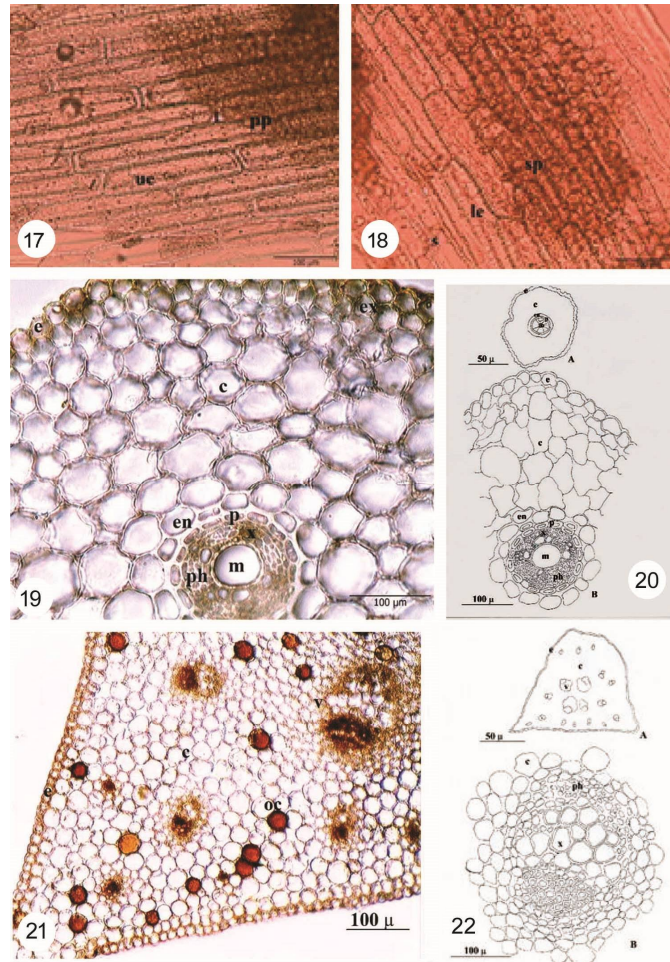


Figs 4-6. General appearance: 4. *C. ancyrensis* (ESSE: 14623), 5. *C. antalyensis* (ESSE: 14624), 6. *C. chrysanthus* (ESSE: 14625). A - Habit, B - Stamen - perianth segments and D - Style and stigma.

Anatomical: The sections taken from root, stem and leaves of *C. ancyrensis*, *C. chrysanthus* and *C. antalyensis* were examined and the anatomic features were determined, compared and presented in Figs 7 - 37 and Tables 3 - 5.

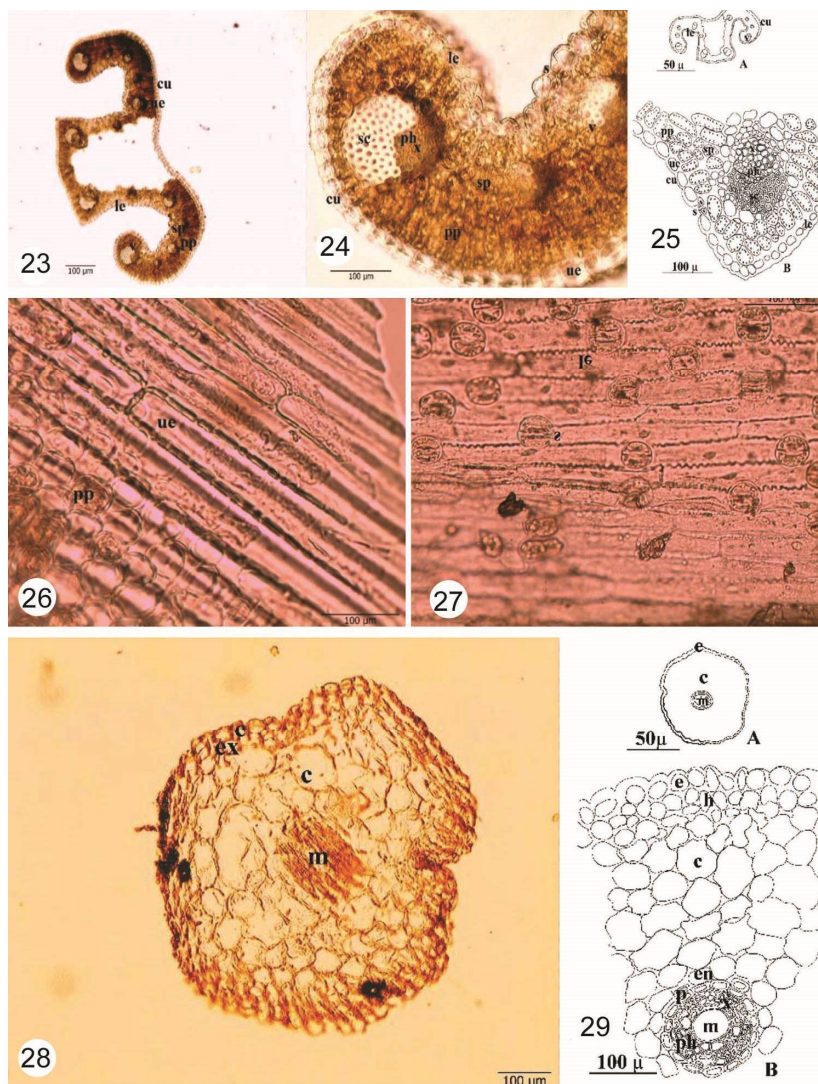


Figs 7-16. *Crocus ancyrensis*: Cross-section of root; c - cortex; e - epidermis; h - hypodermis; en - endodermis; m - metaxylem.; ph - phloem and x - xylem. 10-12. *C. ancyrensis*: Cross-section of stem; c - cortex; e - epidermis; ph - phloem; x - xylem and v - vascular bundle. 13-16. *C. ancyrensis*: Cross section of leaf,



Figs 17-22: 17. Adaxial surface-section of leaf, 18. Abaxial surface-section; cu - cuticula, ue - upper epidermis, sc - scleranchyma, s - stomata, pp - palisade parenchyma, sp - spongy parenchyma, v - vascular bundle and le - lower epidermis. 19-20. *C. antalyensis*: Cross - section of root; c - cortex; e - epidermis; h - hypodermis; en - endodermis; ex - exodermis; m - metaxylem; ph - phloem and x - xylem. 21-22. *C. antalyensis*: Cross - section of stem; c - cortex; e - epidermis; ph - phloem; x - xylem and v - vascular bundle.

Root in all the species is formed by epidermis and hypodermis single layered on the outer surface. In transverse section these cells are 4 sided and thin walled. Hypodermis is absent from the *C. chrysanthus*. Exodermis is 4 - 7 layered in *C. ancyrensis*, 1 - 2 layered in *C. antalyensis*, 4 - 6 layered in *C. chrysanthus*. Cortex is 6-9 layered in *C. ancyrensis*, 5 - 7 layered in *C. antalyensis*, 4 - 5 layered in *C. chrysanthus*, ovoidal parenchymatic with intercellular spaces. Endodermis cells are thin walled. Pericycle is located inside endodermis. Single metaxylem is present on the median part of vascular cylinder. Xylem strands are present on the periphery of the vascular cylinder and these strands reach the pericycle. Xylem is triarch in *C. ancyrensis*, pentaarch in *C. antalyensis* and tetraarch in *C. chrysanthus*. Raphide crystal is absent from the *C. chrysanthus* (Figs 7 - 9, 19 - 20, 28 - 29; Table 3).



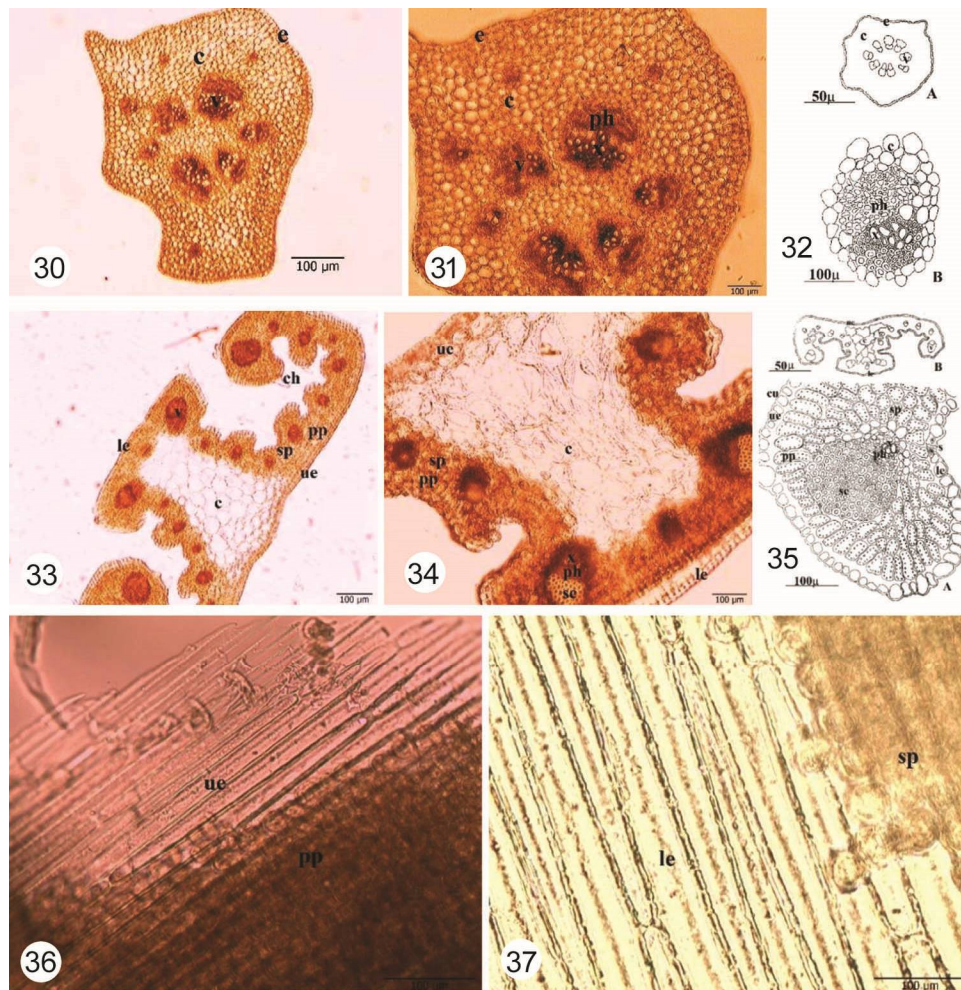
Figs 23-29. *C. antalyensis*: Cross section of leaf, 26: Adaxial surface-section of leaf, 27: Abaxial surface-section; cu - cuticula, ue - upper epidermis, sc - sclerenchyma, s - stomata, pp - palisade parenchyma, sp - spongy parenchyma, v - vascular bundle and le - lower epidermis. 28-29. *C. chrysanthus*; Cross - section of root; c - cortex; e - epidermis; h - hypodermis; en - endodermis; m - metaxylem.; ph - phloem and x - xylem.

Table 3. The comparison of anatomical characteristics of root in *Crocus* species.

	Hypodermis	Exodermis	Cortex	Raphide crystal	Xylem	Metaxylem
<i>C. ancyrensis</i>	Present	4-7 layered	6-9 layered	Present	Triarch	1
<i>C. antalyensis</i>	"	1-2 "	5-7 "	"	Pentaarch	1
<i>C. chrysanthus</i>	Absent	4-6 "	4-5 "	Absent	Tetraarch	1

Table 4. The comparison of anatomical characteristics of stem (scape) in *Crocus* species.

	Shape (transverse section)	Tricho- me	Ergastic substance	Vascular bundle	The number of vascular bundles in the peripheral/centre
<i>C. ancyrensis</i>	Triangular	Absent	---	Scattered	5 - 10/3-4
<i>C. antalyensis</i>	"	"	Oil cells present	"	10 - 15/3-4
<i>C. chrysanthus</i>	Roundish	"	--	Circular	5 - 8/5-9



Figs 30-37. *C. chrysanthus*: Cross-section of stem; c - cortex; e - epidermis; ph - phloem; x - xylem and v - vascular bundle. 33-37. *C. chrysanthus*: Cross section of leaf, 36 Adaxial surface-section of leaf, 37: Abaxial surface-section. cu - cuticula, ue - upper epidermis, sc - sclerenchyma st - stomata, pp - palisade parenchyma, sp - spongy parenchyma, v - vascular bundle and le - lower epidermis.

Cross-sections from the stem of in all the species show that the cuticle is the outer layer. The epidermis in all the species under the cuticle and has isodiametric cells. The epidermis is without hairs. Cortex cells are parenchymatic layered under epidermis. Parenchyma cells are oval or hexagonal and are under the epidermis. These cells have no intercellular spaces. Vascular bundles are located to the periphery and centre of stem. These vascular bundles vary in size. The number of vascular bundles in the peripheral part is usually 5 - 10 in *C. ancyrensis*, 10 - 15 in *C. antalyensis*, 5 - 8 in *C. chrysanthus*. The number of vascular bundles in the centre is usually 3 - 14 in *C. ancyrensis*, 3 - 4 in *C. antalyensis*, 5 - 9 in *C. chrysanthus*. Vascular bundles are numerous and scattered, circular in *C. chrysanthus*. The xylem members are more visible. In the centre, there is a pith which consists of parenchymatic cells (Figs 10 - 12, 21 - 22, 30 - 32 and Table 4).

Table 5. The comparison of anatomical characteristics of leaf in *Crocus* species.

	Cuticle	Stomata type	Leaf type	Cortex (collenchyma)	Palisade parenchyma	Spongy parenchyma	Vascular bundle type
<i>C. ancyrensis</i>	Thick	Anomocytic amaryllis type	Isolateral	Present	1 - 3 layered	3 - 4 layered	Collateral
<i>C. antalyensis</i>	Thin	Anomocytic amaryllis type	"	Absent	1 - 2 "	2 - 3 "	"
<i>C. chrysanthus</i>	Thin	Anomocytic amaryllis type	"	Present	1 - 3 "	3 - 4 "	"

In the cross-section of the leaves, the cuticle is on the outer layer and it was determined that the upper and lower epidermis layers are similar. Epidermis cells are isodiametric and oval. The outside walls are thicker than the internal and lateral walls. Both the epidermal surfaces are covered with thick in *C. ancyrensis*, thin in *C. antalyensis* and *C. chrysanthus* cuticle. The stomata is anomocytic type and located slightly upper than the epidermal cells level. The leaf is isolateral in all the taxa. The palisade parenchymatic cells are 1 - 3 layered in *C. ancyrensis* and *C. chrysanthus*, 1 - 2 layered in *C. antalyensis* with chloroplast in abundance. There are spongy parenchymatic cells, are 3 - 4 layers in *C. ancyrensis* and *C. chrysanthus*, 2 - 3 layered in *C. antalyensis* under the parenchymatic cells. Among the parenchymatic cells, there are large and rarely small vascular bundles; both on the upper and lower side of leaves, there are amaryllis type stomata. The vascular bundle is collateral type. The xylem is towards the upper surface and the phloem towards the lower surface. Thick lateral veins in both sides of middle vein are arranged till edge of palm and middle vein has made a deep outgrowth. Lateral veins in view of anatomic are the same structure as middle veins, but vascular are much more reduced. The walls of abaxial epidermis cells are sinuous in surface sections of the leaf. Abaxial epidermis has stomata while the adaxial epidermis has no stomata (Figs 13 - 18, 23 - 27, 33 - 37 and Table 5).

Three taxa of *Crocus*, namely *C. ancyrensis* (Endemic), *C. antalyensis* (Endemic) and *C. chrysanthus* which flower in spring, were observed. This study was aimed at comparing the morphological and anatomical features of the three species with a view to establishing further proof of their taxonomical identity. The illustrations of each species are shown in Figs 1 - 6. The morphological differences between the species are summarized in Table 2.

Descriptions of the taxa have been defined in more detail than those in Flora of Turkey and also lacking the knowledge on the properties habit and perianth have been completed. On the other hand, all detailed figures which help to define the taxa have been given first time in this study.

The findings were compared with those in the Flora of Turkey and it was determined that there are some differences between this study and the characteristics given in the Flora of Turkey (Table 2).

Some morphological characters such as the height of the plant and corms, number of cataphyl, bracts, number of flowers and size and indumentum details of style of these three taxa are not given in the Flora of Turkey.

These characters are provided in this study. There are some differences between the Flora of Turkey and our findings but with these differences the descriptions of the taxa are widened; outside the filament size of *C. ancyrensis* in general, the findings of this study are in agreement with those in the Flora of Turkey.

According to present results, leaf length specially show remarkable differences among the species. Because of the morphological similarities the definition of diagnostic characters has difficulties. This situation is in general accordance with previous reports stating taxonomic problems of this genus (Mathew 1984). Our research shows that despite a limited area coverage, morphological results Mathew's (Mathew 1984) compatible with the findings of a variation limit.

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In morphological and taxonomic studies related to species, *C. antalyensis* is divided into 3 subspecies; *antalyensis*, *gemicii*, *striatus* (Erol *et al.* 2011). Endemic taxa distributed in Eskişehir province are categorized as *C. ancyrensis* (EN), *C. antalyensis* (EN) and *C. chrysanthus* (NT), respectively. *C. ancyrensis*, *C. antalyensis* and *C. chrysanthus* are new record for square B3: Eskişehir. *C. chrysanthus* is the most widespread species in this area.

Results of the anatomical study of root, stem and leaves of the taxa show that there are some variations in between species of *Crocus* growing in Turkey. However, many characters are same in all of them. Root in all the species is formed by epidermis and hypodermis with single layer on the outer surface.

In transverse section with cells are 4 sided and thin walled. Hypodermis is absent from the *C. chrysanthus*. Exodermis is 4 - 7 layered in *C. ancyrensis*, 1 - 2 layered in *C. antalyensis*, 4 - 6 layered in *C. chrysanthus*. Cortex is 6 - 9 layered in *C. ancyrensis*, 5 - 7 layered in *C. antalyensis*, 4 - 5 layered in *C. chrysanthus*, ovoidal parenchymatic with intercellular spaces. Xylem is triarch in *C. ancyrensis*, pentaarch in *C. antalyensis* and tetraarch in *C. chrysanthus*. Raphide crystal is absent from the *C. chrysanthus*.

Key to the taxa

1. Hypodermis present in root, triangular of stem shape, vascular bundle scattered of stem (scape), raphide crystal present
2. Xylem triarch, exodermis 4 - 7 layered in root, the number of vascular bundles in the peripheral/centre 5 - 10/3 - 4 of stem, cortex (collenchyma) of leaf present *C. ancyrensis*
2. Xylem pentaarch exodermis 1 - 2 layered in root, the number of vascular bundles in the peripheral/centre 10 - 15/3 - 4 of stem, cortex (collenchyma) of leaf absent *C. antalyensis*
1. Hypodermis absent in root, roundish of stem shape, vascular bundle circular of stem (scape), xylem tetraarch, exodermis 4 - 6 layered in root, the number of vascular bundles in the peripheral/centre 5 - 8/5 - 9 of stem, cortex (collenchyma) of leaf present *C. chrysanthus*

Cross-sections of the stem in all the species show that the cuticle is the outer layer. The number of vascular bundles in the peripheral part is usually 5 - 10 in *C. ancyrensis*, 10 - 15 in *C. antalyensis*, 5 - 8 in *C. chrysanthus*. The number of vascular bundles in the centre is usually 3 - 14 in *C. ancyrensis*, 3 - 4 in *C. antalyensis*, 5 - 9 in *C. chrysanthus*. Vascular bundles are numerous and scattered, circular in *C. chrysanthus*.

In the cross-section of the leaves, the cuticle is on the outer layer and it was determined that the upper and lower epidermis layers are similar. The leaf is isolateral in all the taxa. The palisade parenchymatic cells are 1 - 3 layered in *C. ancyrensis* and *C. chrysanthus*, 1 - 2 layered in *C. antalyensis* with chloroplast in abundance. There are spongy parenchymatic cells, are 3 - 4 layers in *C. ancyrensis* and *C. chrysanthus*, 2 - 3 layered in *C. antalyensis* under the parenchymatic cells.

In conclusion, the morphological and anatomical features of *C. ancyrensis*, *C. antalyensis* and *C. chrysanthus* were examined in this study. It may be concluded that three species have some characteristic morphological and anatomical features such as trilobed style and leaves with triangular keel in spite of the morphological and anatomical similarity with other species of *Crocus* and members of Iridaceae.

Acknowledgements

The authors are grateful to Mr. Ismail Kara for collecting the *Crocus sativus*. This study is a part of a Master's thesis.

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(Manuscript received on 7 November, 2018; revised on 24 April, 2019)