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Investigations on anatomical and morphological characteristics of some *Crocus* L. taxa around Abant Lake

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Abant gölü çevresindeki bazı *Crocus* L. taksonlarının anatomik ve morfolojik özellikleri üzerine araştırmalar

Abstract: In this study; the two *Crocus* L. taxa endemic to Bolu province, *Crocus abantensis* T. Baytop et Mathew and *Crocus*× *paulineae* Pasche & Kerndorff (hybrid) together with *C. ancyrensis* (Herbert) Maw subsp. *ancyrensis*, and *C. olivieri* J.Gay, were used to reveal their detailed leaf anatomical features. In view of these characteristics, it was aimed to determine the true parents of the hybrid and possible other hybrid taxa distributed in the south-southeast coasts Abant Lake. These two *Crocus* taxa, which are endemic to this region, have no previous anatomical studies. In this sense, deficiencies related to *Crocus* taxonomy have been completed. The main differences of *C.* × *paulineae* from the other taxa; having the thickest cuticle (3.80 µm), the longest parenchyma (20.93 µm) cell in the mesophyll, and having papillae like structure on the keel corners of cuticle. This structure was also observed in *C. olivieri* over the cuticle at the corners of the keel. The other important differences was the number of small vascular bundles among the studied taxa. The chromosomal number of hybrid was also given for the first time.

Key words: Abant Lake, *Crocus*, anatomy, endemic, Bolu

Özet: Bu çalışmada; Bolu ili için endemik iki *Crocus* L. taksonu, *Crocus abantensis* T. Baytop et Mathew ve *Crocus*× *paulineae* Pasche & Kerndorff (melez), ile birlikte *C. ancyrensis* (Herbert) Maw subsp. *ancyrensis* ve *C. olivieri* J.Gay, Bull. ayrıntılı yaprak anatomik özelliklerini ortaya çıkarmak için kullanıldı. Bu özellikler ışığında Abant Gölü'nün Güney-Güneydoğu kıyısında yayılış gösteren hibrit ve olası diğer hibrit taksonların gerçek ebeveynlerinin belirlenmesi amaçlanmıştır. Bu bölgeye endemik olan bu iki *Crocus* taksonun daha önce anatomik çalışması yoktur. Bu anlamda *Crocus* taksonomisi ile ilgili eksiklikleri tamamlanmıştır. *C.* × *paulineae*'nın diğer taksonlardan temel farklılıkları; mezofildeki en kalın kütikül (3.80 µm), en uzun parankima (20.93 µm) hücresine ve kütikülün karina köşelerinde papilla benzeri bir yapıya sahip olmasıdır. Bu yapı *C. olivieri* de de gözlenmiştir. Diğer önemli farklılıklar, incelenen taksonlar arasındaki küçük damar demetlerinin sayısıdır. Hibritin kromozom sayısı da ilk kez verilmiştir.

Anahtar Kelimeler: Abant Gölü, çiğdem, anatomi, endemik, Bolu

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1. Introduction

Turkey is rich in floristic terms with the increasing number of new species and even compared to Eastern Europe and West Asia and North Africa. A total of 9753 natural species have been recorded and 3035 of them are endemic for Turkey (Güner et al., 2012). Therefore, endemic species percentage is 31.12% (Mathew, 1984; Güner et al., 2012). It is much more important to increase the number of taxonomically important studies that will add value to the wealth beside the number of wealth of Turkish Flora. Bulbous plants of Turkey constitute an important part of this richness, such as about 800 taxa (Mathew, 1984; Ekim et al., 2000; Çolak, 2005). *Crocus* genus has been reported to be around 200 species in recent years, increasing significantly in the world (Harpke et al., 2014; Ruksans, 2017).

Turkey's Flora comprises a total of 103 *Crocus* L. species, including a natural hybrid among 235 *Crocus* species in the world (Mathew, 1984; Özhatay, 2002; Alavi-Kia et al., 2008; Kerndorff et al., 2012; Yüzbaşıoğlu et al., 2015; Erol and Çiftçi, 2022). Tuberous plants are grown in temperate climate regions of the world. *Crocus* species have been used as ornamental plants in European countries such as England and Germany for quite long time (Bowles, 1954; Mathew, 2000; Goode, 2005). New hybrids are grown from natural species. These beautiful flowers are also economically valuable in the ornamental plants market.

There are many systematic studies on this genus in literature (Pasche, 1994; Kerndorff and Pasche, 1994; 1996a,b; 1997; 2004; Mathew, 1982; Coşkun et al., 2010; Uslu et al., 2012). Coşkun et al. (2010) studied the phylogenetic relationship between 15 *Crocus* taxa using morphological and anatomical characters. In recent years, more studies have been conducted in the form of new taxa definitions (Candan and Özhatay, 2013; Harpke et al., 2014; Erol et al., 2014 and 2015; Yüzbaşıoğlu and Celep, 2016). One of these new taxa is *C. ancyrensis* Maw. subsp. *Güneri* Yüzb., was identified from Amasya region (Yüzbaşıoğlu and Celep, 2016), therefore; *C. ancyrensis* in the Abant region, according to taxonomic rules it will be accepted as subsp. *ancyrensis* in this study.

It is observed that most of the studies in the world have been done on *C. sativus* species (Negbi et al., 1989; Rios et al.,

1996; Bhargava, 2011). Rudall and Mathew (1990) have studied *Crocus* in terms of leaf anatomy, and many researchers now refer to this work in leaf studies. Mathew (2002) reviewed the morphological characteristics of the *Crocus* genus. Akan and Eker (2004) studied, the morphological and anatomical features of *C. pallasii* subsp. *turcicus* B.Mathew and *C. cancellatus* subsp. *damascenus* (Herb.) B.Mathew. Özdemir et al. (2004) have made anatomical and morphological studies on two endemic species in Turkey, which *C. danfordia* Maw. and *C. fleischeri* Baker Kandemir (2011) also compared 14 *Crocus* taxa in terms of leaf anatomy. Anatomical studies on *Crocus olivieri* J.Gay, Bull. were made by Özdemir et al. (2011). In another study, morphological and leaf anatomy of two yellow-flowered endemic taxa of *Crocus* (*Crocus ancyrensis*, *Crocus siehenaus* Hort. ex B.L.Burtt) were studied (Candan, 2015). Raca et al. (2017) studied three *Crocus* taxa from Verni series in Serbia.

When the anatomical and morphological studies of *Crocus* genus are examined in the literature; it is observed that the two endemic *Crocus* for Bolu, *Crocus abantensis* T. Baytop et Mathew, and *Crocus* × *paulineae* Pasche & Kerndorff, taxa are not included. The other two taxa *C. olivieri* J.Gay, (Özdemir et al., 2011) and *C. ancyrensis* (Herbert) Maw subsp. *ancyrensis* (Candan, 2015) have been studied by various researchers. The study also includes chromosome numbers and karyotype of the hybrid, were presented for the first time. In addition, two other taxa spread around Abant Lake; *C. ancyrensis* subsp. *ancyrensis*, and *C. olivieri* were also included in the study. Because it was considered that it is necessary in order to reveal the parents of the endemic *C.* x *paulineae* and if any other hybrids.

2. Material and method

Four *Crocus* taxa, *Crocus abantensis*, *Crocus* × *paulineae*, *C. ancyrensis* subsp. *ancyrensis* and *C. olivieri*, were collected from the Abant Lake in February-March 2018 during the flowering of *Crocus*. The abbreviations (ABA, C×PAU, ANC, and OLV respectively) of taxa were used in the Tables and Figures. Collected samples were diagnosed according to the Flora of Turkey and East Aegen Islands (Mathew, 1984; Güner et al., 2012). Voucher specimens of the taxa were deposited in the Abant İzzet Baysal University, Department of Biology. For morphological studies, 15 plant specimens were collected from each taxa. For anatomical studies, 10 individuals were stored in 70% alcohol.

Anatomical sections were obtained from fully developed leaves of specimens stored in 70% alcohol. Transverse and superficial (top and bottom) parts of the leaves were sectioned manually. Due to the fact that there was not much difference in the anatomical features of the parts such as root and stem (scape) in the *Crocus*, the leaf structure was emphasized in the study. The sections were prepared by the Glycerine-Gelatine method (Jensen, 1962) and examined. The photos were taken with the DP71 digital camera, which is compatible with the Olympus BX51light microscope. In order to illuminate the tissues in the best way, different dye solutions (Bozdağ et al., 2016) have been applied. Safranin O was used alone or together with fast green.

For anatomical studies, nineteen characters previously used in various studies (Mathew, 1984; Erol and Küçüker, 2007; Kandemir et al., 2012), which gave good results, were selected and 10 repeats were made (Table 1). However; the characters that given in the table with "†" sign did not included in the analysis; as there was no difference in the studied taxa. In the superficial sections of the epithelial cells forming the abaxial and adaxial surfaces of the leaf, the length and width measurements of the cells were made using micrometric slide and ocular. Stoma index of leaf superficial sections was also calculated as 10 repeats for each taxa. Since there was no stoma in the adaxial surface epidermis of the leaf, this procedure was performed for the abaxial surfaces. The number of stomata in per mm^2 was calculated (Meidner and Mansifield, 1968). The counts on the microscope were estimated over the area calculation.

For karyotype studies, roots of *C.* × *paulineae* were fixed in ethanol-glacial acetic acid (3:1) at 4°C for 24 hours and stored in 70% ethanol at 4°C. Prior to staining, hydrolysis was done using 1 N HCL at 60°C for 15 minutes. Finally, they were stained with 2% aceto-orcein for 2 hours and squashes were made with 45% acetic acid.

The photographs were taken using an Olympus BX51 light microscope with camera DP71 attachment. Karyotype parameters were prepared from well-spread metaphase plates. The somatic chromosome number and karyotypic details were studied in at least five well-prepared metaphase plates, and the mean values were used in the analysis. Chromosome pairs were identified according to the nomenclature of Levan et al. (1964).

In Cluster Analysis, the distance matrix was calculated by measuring different micro-morphological and anatomical characters (Table 2, 3) from 15-10 individuals respectively and the proximity of taxa was examined. Ward's distance matrix was calculated by taking average values. Clustering analysis (UPGMA) was performed by using Past (Paleontological Statistics Software Package for Education and Data Analysis, Hammer et al., 2001) program in order to investigate the proximity of studied taxa (Figure 2). In order to explore the groupings of the studied taxa, Principal Component Analysis (PCA) was carried by individual taxa data (Figure 3).

3. Results

Leaf cross-section of studied taxa was made manually and examples were given in Figure 1. Among them *C. olivieri* was the largest one, in Figure 1D. Epidermis cells (abaxial) were observed as a single row and square shape in all the studied taxa. *Crocus olivieri* and *C.x paulinea* had single-row epidermis and micro-papilla protrusions over the cuticle at the corners of the keel (Figure 1-B,D).

In the leaves of *Crocus* genus; the parenchymatic cells in the middle of the leaf arms, melted and formed the air space called lacuna, rectangular in shape, triangular or in the form of a central space. Among the taxa studied, *C. abantensis* and *C. olivieri* this space was very clearly rectangular (Figure 1-A, D). In *C. ancyrensis* subsp. *ancyrensis*, it was; closer to the triangular structure, while *C.*×*paulineae* could not be detected in a very obvious shape (Figure 1-C, B). The maximum lacuna space length and width was found in *C. ancyrensis* subsp. *ancyrensis*, while the least lacuna space length and width was in *C.*×*paulineae* (Table 2). The longest arm length (155.87 µm) was in *C. olivieri* the shortest one was in *C. abantensis*, the other taxa were ranged in between them (Table 2).

Table 1. The list of character names and their codes that used in the study (†: The characters were not included analysis, due to have no differences among the taxa).

Morphological characters	Anatomical characters
Corm Length (CL)	Thickness of Cuticle (TUC)
Corm Width (CW)	Upper Epidermis Length (UEL)
Corm Diameter (CD)	Upper Epidermis Width (UEW)
Leaf Length (LL)	Lower Epidermis Length (LEL)
Cataphyll Length 1 (CTL1)	Lower Epidermis Width (LEW)
Cataphyll Length 2 (CTL2)	Number of Large Vascular Bundle (NLV)†
Cataphyll Length 3 (CTL3)	Number of Medium Vascular Bundle (NMV)
Cataphyll Width 1 (CTW1)	Number of Small Vascular Bundle (NSV)
Cataphyll Width 2 (CTW2)	Lacuna Space Length (LSL)
Cataphyll Width 3 (CTW3)	Lacuna Space Width (LSW)
Bracte Length (BRTL)	Number of Palisade Cell (NPC)†
Bracteol Length (BRLL)	Palisade Cell Length (PCL)
Perianth Tube Length (PTL)	Palisade Cell Width (PCW)
Perianth Inner Segment Length (PISL) †	Number of Sponge Cell (NSC) †
Perianth Inner Segment Width (PISW)	Narrowest Carina Base Length (NCL)
Perianth Outer Segment Length (POSL)	Carina Arm's Length (CAL)
Perianth Outer Segment Width (POSW)	Stoma Number of Lower Epidermis (STN) †
Anther Length (AL)	Numbers of epidermal Cell (NEC)
Filament Length (FL) †	Stoma Index: (STI=STN/STN+NEC) * 100
Style Length (STYL)	
Stigma Length (STGL)	
Scape Length (SCL)	
Ovarium Length (OVL)	
Ovarium Length/Scape Length (P3) †	
Perianth Inner S. Length/Width (P4)	
Style Length (STYL)	
Perianth Outer S. Length/Width (P5)	

Table 2. Mean values of the anatomical characters (long names are given in Table 1) and standard deviations of the studied taxa.

Characters	ABA	ANC	PAU	OLV
TUC (µm)	3.27 ± 0.80	3.33 ± 0.49	3.80 ± 0.41	2.60 ± 0.51
UEL (µm**)**	6.03 ± 0.85	5.33 ± 0.49	6.00 ± 0.65	5.77 ± 0.68
UEW (µm)	5.67 ± 0.45	5.87 ± 0.83	5.67 ± 0.72	5.00 ± 0.76
LEL (µm)	6.73 ± 1.10	5.73 ± 0.70	5.27 ± 0.46	5.73 ± 0.80
LEW (µm)	6.90 ± 1.11	5.93 ± 0.70	5.53 ± 0.52	6.60 ± 0.99
NMV	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	6.20 ± 2.08
NSV	1.20 ± 0.41	4.53 ± 1.41	5.60 ± 0.51	7.13 ± 2.00
LSL (µm)	35.33 ± 2.44	40.93 ± 8.30	32.00 ± 3.96	34.40 ± 4.67
LSW (µm)	13.00 ± 2.17	25.60 ± 4.47	15.93 ± 1.87	17.73 ± 3.22
PCL (µm)	16.33 ± 3.98	17.27 ± 1.71	20.93 ± 2.46	18.67 ± 2.82
PCW (µm)	4.67 ± 0.49	3.97 ± 0.58	3.83 ± 0.24	4.97 ± 0.64
NCL (µm)	61.53 ± 1.92	58.47 ± 3.85	48.33 ± 4.37	43.73 ± 7.42
NSC	2.73 ± 0.46	3.07 ± 0.59	3.00 ± 0.00	2.73 ± 0.46
CAL (µm)	113.53 ± 4.78	127.40 ± 18.04	119.00 ± 6.60	155.87 ± 17.71
STN	63.80 ± 12.17	69.60 ± 11.59	68.87 ± 7.93	70.80 ± 13.23
NEC	128.47 ± 22.24	135.87 ± 17.15	118.93 ± 8.48	135.73 ± 19.15
SI (%)	33.25 ± 4.18	33.83 ± 3.10	36.61 ± 2.49	34.22 ± 3.71

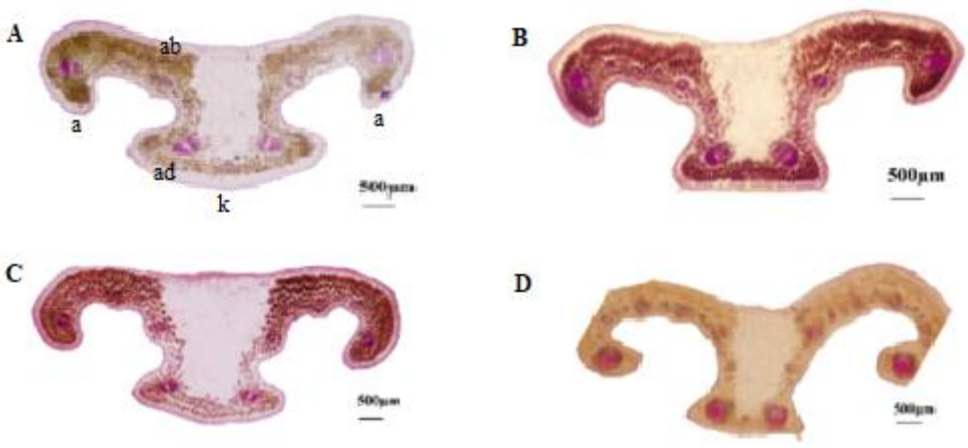

Figure 1. A- *Crocus abantensis* **B-** *Crocus* × *paulineae*,**C-** *C. ancyrensis* subsp. *ancyrensis*, **D-** *C. olivieri*. (a: arms; ab: abaxial epidermis; ad: adaxial epidermis; p: parenchyma also referred lacuna area; k: keel; m: mesophyle; v: vascular bundle).

Cuticle thickness was high (3.80 µm) in hybrid taxon, the least thickness (2.60 µm) was in *Crocus olivieri*. The number of palisade parenchyma cell line was 2, and the number of large vascular bundles was 4 in all the taxa; for this reason, they were not included in the Table 2. Large vascular bundles are located at the ends of the arms and keel corners (Figure 1 A-D). Small and medium vascular bundles are scattered between large ones. There was a difference in terms of number of small bundles, the least number (average 1.2) was in *C. abantensis.* On the other hand the average number of small bundles was; 5.60 in *C.*×*paulineae*, 7.13 in *C. olivieri* and, 4.53 in *C. ancyrensis* subsp. *ancyrensis* (Table 2).

Table 3. Mean values of the morphological characters (long names are given in Table 1) and standard deviations of the studied taxa.

	ABA	ANC	PAU	OLV
CL	0.68 ± 0.15	0.83 ± 0.16	0.61 ± 0.15	1.17 ± 0.41
CW	0.81 ± 0.22	0.86 ± 0.17	1.29 ± 0.37	1.13 ± 0.19
CD	2.67 ± 0.51	2.57 ± 0.40	3.59 ± 0.65	3.45 ± 0.69
LL	8.51 ± 1.39	11.21 ± 2.03	7.65 ± 1.26	14.47 ± 3.67
CTL1	1.93 ± 0.63	1.83 ± 0.46	2.75 ± 1.08	2.35 ± 0.64
CTL2	4.26 ± 1.42	3.67 ± 0.91	4.03 ± 1.06	5.85 ± 2.07
CTL3	5.63 ± 1.05	5.24 ± 1.09	6.49 ± 0.78	7.44 ± 2.53
CTW1	0.78 ± 0.19	0.71 ± 0.15	1.00 ± 0.21	1.09 ± 0.31
CTW2	0.79 ± 0.19	0.85 ± 0.23	2.55 ± 0.74	2.13 ± 0.70
CTW3	0.94 ± 0.20	0.85 ± 0.30	3.65 ± 1.66	4.55 ± 0.56
BRTL	5.61 ± 1.11	5.85 ± 1.14	3.52 ± 2.60	8.39 ± 2.19
BRLL	5.27 ± 0.98	4.01 ± 1.10	4.88 ± 0.68	8.89 ± 1.85
PTL	5.79 ± 1.18	5.71 ± 0.94	5.43 ± 0.77	7.53 ± 1.24
PISW	1.17 ± 0.26	0.71 ± 0.20	0.95 ± 0.12	0.96 ± 0.19
PISL	24.80 ± 0.27	23.13 ± 0.38	24.47 ± 0.38	24.40 ± 0.27
POSL	2.68 ± 0.18	2.59 ± 0.54	2.33 ± 0.29	2.42 ± 0.19
POSW	1.18 ± 0.18	0.72 ± 0.28	1.10 ± 0.22	0.82 ± 0.05
AL	1.19 ± 0.12	1.33 ± 0.37	0.97 ± 0.12	0.98 ± 0.14
STYL	6.79 ± 0.49	6.27 ± 0.95	6.09 ± 0.94	7.10 ± 0.93
FL	0.83 ± 0.20	0.75 ± 0.21	0.83 ± 0.18	0.75 ± 0.17
STGL	0.58 ± 0.14	0.51 ± 0.11	0.70 ± 0.23	0.61 ± 0.17
SCL	2.82 ± 0.48	3.71 ± 0.69	3.33 ± 0.92	4.17 ± 1.44
OVL	0.43 ± 0.12	0.58 ± 0.10	0.45 ± 0.07	0.55 ± 0.11
P1	5.77 ± 0.60	5.01 ± 1.38	6.35 ± 1.22	6.72 ± 1.46
P2	2.47 ± 0.41	1.73 ± 0.38	1.97 ± 0.66	1.85 ± 0.89
P3	0.19 ± 0.05	0.16 ± 0.05	0.15 ± 0.05	0.15 ± 0.06
P4	2.20 ± 0.41	3.46 ± 0.95	2.63 ± 0.41	2.69 ± 0.65
P5	2.34 ± 0.37	4.24 ± 2.03	2.39 ± 0.61	2.79 ± 0.26
P5	2.34 ± 0.37	4.24 ± 2.03	2.39 ± 0.61	2.79 ± 0.26

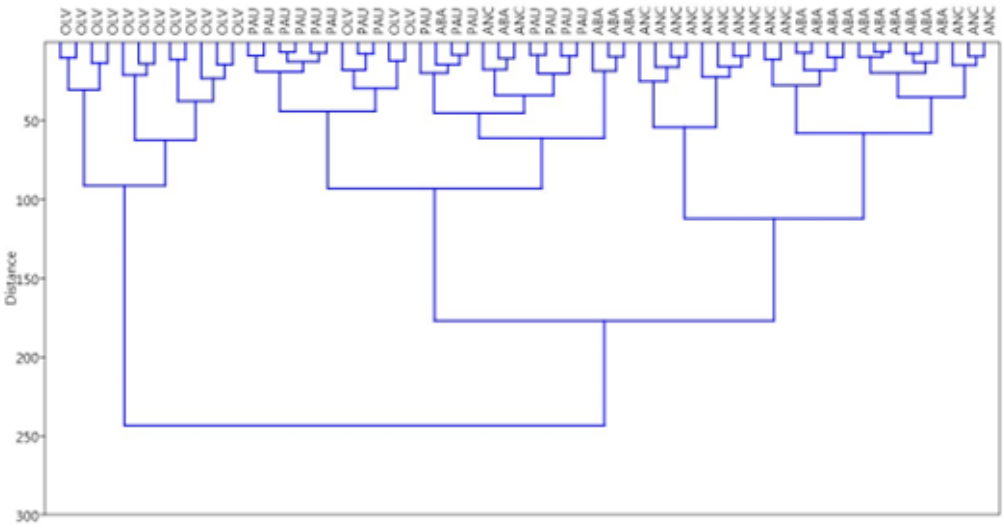

Figure 2. Graph of the taxa studied according to Ward's distance matrix by UPGMA method

In all the studied taxa; stomas were observed only in the lower surfaces of the leaves with the same plane as the epidermis cells. Stoma number (0.01 mm^2), and stoma indexes were calculated for each taxa (Table 2). Although there is no big difference between taxa; the lowest stoma average number was, 63.80, found in *C. abantensis*, and 69.60 in *C. ancyrensis* subsp. *ancyrensis*, 68.87 in hybrid taxon, and finally; 70.80 in *C. olivieri*. In terms of stoma index, all taxa were found very close to each other and the index was calculated between 33.25-36.61% (Table 2).

Among the morphological characters; corm diameter (CD: 3.59 cm), first cataphyll length (CTL1:2.75 cm), second cataphyll width (CTW2: 2.55 cm) and stigma length (STGL: 0.70 cm) values were, observed highest in hybrid taxon compared to other taxa (Table 3). Beside these, filament length, (0.83 cm) was also found high in both taxa *C. abantensis* and the hybrid. On the contrary to these characters, leaf length (LL: 7.65 cm), perianth tube length (PTL: 5.43 cm), anther length (AL: 0.97 cm), style length (STYL: 6.09 cm) and bracte length (BRTL: 3.52 cm); was the lowest value observed in the hybrid taxon (Table 3). Index type characters P1, P2, P3, P4, P5 were not found very useful for discriminating the taxa.

Crocus leaf has two arms and keel in the middle of these arms. The structure of arms; curved towards the keel, and differences are observed between the taxa such that the curl is at a narrow or wide angle (Table 4). The curved arms are usually extending parallel to the keel. Arm ends curved towards to keel with a narrow angle, and reach 2/3 of the keel; except *C. olivieri*. In this taxon, the arm ends usually reach the base of the keel (Figure 1-D; Table 4).

In the cluster analysis graph; *C.* × *paulineae*, *C. abantensis* and *C. ancyrensis* subsp. *ancyrensis* were creating a cluster together, *C. olivieri* formed the other cluster (Figure 2). Similarly in order to explore the groupings of the studied taxa, Principal Component Analysis (PCA) was carried by individual taxa data. The most important characters; their eigenvalues and percentages were given in Table 5. According to this graph, all the taxa were overlapped with each other (Figure 3).

Table 4. Comparison of leaf anatomical parts

	Leaf shape (general appearance)	**Mesophyll parenchyma**	**Epidermis (adaxial)**
Crocus×*paulineae*	The curved ends of the arms are curved at a narrow angle towards the keel and reach 2/3 of the keel. Wide keel base, slightly rounded corners, with a few papillae at corners	Spongy: elliptical Palisade: rectangular and two-rows	Single row and square shape, micro papillae at corners
Crocus abantensis	Curved; the arms extending parallel to the keel, reach 2/3 of the keel. Keel base is quite wide, and have rounded corners.	Spongy: oval-elliptical Palisade: rectangular and two-rows	Single row and square shape
Crocus ancyrensis subsp. *ancyrensis*	Curved arm tips are directed towards the keel at an acute angle and the arms reach 2/3 of the keel. The carina area is long and wide. Keel base is quite wide, with rounded corners.	Spongy: oval Palisade: rectangular and two-rows	Single row and square shape
Crocus olivieri	The curved arm ends are curled at a right or narrow angle and approach to the keel. The ends almost reach the base of the keel. Keel pointed corners, base wide. There are several protrusions on the cuticle.	Spongy: oval-elliptical Palisade: rectangular and two-rows	Single-row and square, micro papillae-like protrusions present in the cuticle

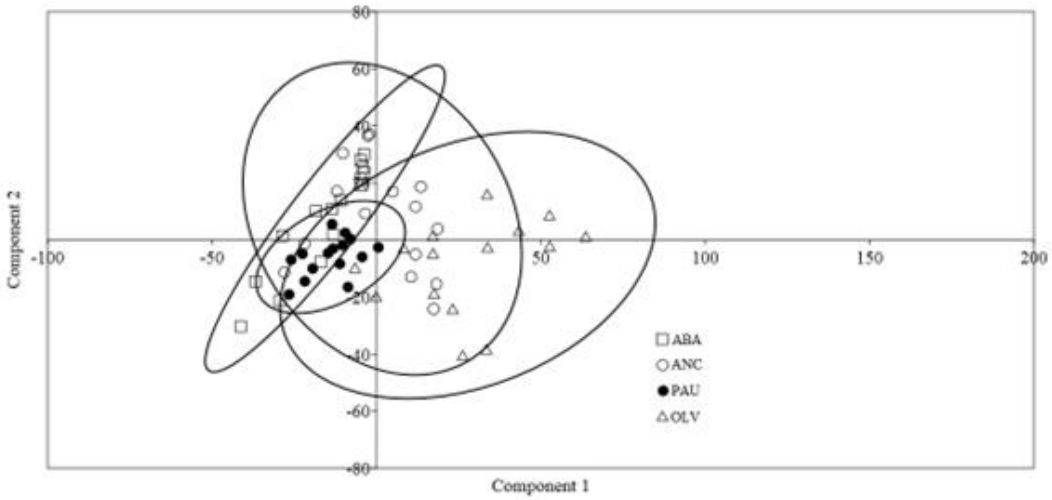

Figure 3. Groupings of the studied *Crocus* taxa by Principal Component Analysis (Cumulative percentage was about 62.1%).

Table 5. The most important characters; their eigenvalues and percentages for PCA graph.

PC	Eigenvalue	Variance (%)
1	20.1504	49.496
2	5.11277	12.559
3	3.06262	7.5228
4	2.6368	6.4769

The morphological characters of the studied taxa were compared in Table 6. Among these characters *C. x paulineae* and *C. olivieri* shared the similar structure for style, having 3 brached and bifurcate end. On the other hand *C. olivieri* has membranous tunica while the other taxa have reticulate fibrous structure (Table 6).

C. × *paulineae* chromosomal counts and its karyotype was prepared for the first time (Figure 4). There were 14 chromosomes; 2 pairs of them were sub-metacentric, and the rest were metacentric type chromosomes. Haploid component of its chromosomes was 24.79 µm and the length ranges were between 1.98-4.93 µm.

4. Discussions

Epidermis cells on leaf surface were observed as single row and square shape in all studied *Crocus* taxa. Only difference; *Crocus olivieri*, and *C. x paulineae* have a papilla-like structure on the cuticle above the corners of the keel (Figure 1-D, B). Similar structures were also observed in *C. caspius* Fisch. & Amp; C.A.Mey and *C. pallasii* Goldb. species (Rudall and Mathew, 1990). Likewise; these structures, were also found on their arms of *C. cansellatus* subspecies, Herb. ssp. *cancellatus*, ssp. *pamphylicus* B. Mathew and ssp. *damascenus* (Herb.) B. Mathew (Kandemir, 2011). Walls of the epidermis in stomatal regions (abaxial) for all the taxa are generally sinuous. The shape of transversal cross-section, in the widest part of the leaf, is highly relevant for taxonomy; as it is already assumed by Rudall and Mathew (1990). Structure of arms; keel, and this curl with narrow or wide angle, such differences between taxa were observed in this study as well (Table 4).

Among the taxa, *C. abantensis* and *C. olivieri* this space was clearly observed as a rectangle shape. *C. olivieri* was also reported to be rectangular by Özdemir et al. (2011); similarly this shape was rectangular some other *Crocus* taxa (Kandemir, 2009). In *C. ancyrensis* subsp. *ancyrensis*, this space was closer to the triangular shape, whereas in *C.x paulineae* there was not any obvious shape (Figure 1-B). Özdemir et al. (2006) in *C. flavus* subsp. *flavus* this shape was observed as triangular. The length of the arms and curl varies according to taxa; such as, *C. olivieri* has the longest arm (155.87µm), while *C. abantensis* has the least arm length (113.53 µm). Similarly, the arms of these two taxa approached to the keel at a narrower angle (Figure 1-A, D). Furthermore, in a study of Erol and Küçüker (2007), the

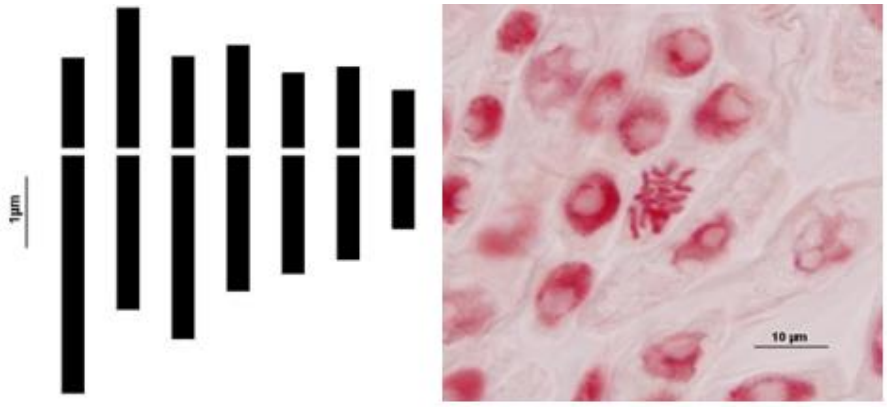

Figure 4. Karyotype and chromosome of *C. x paulineae*

arm length and curl variations were observed; among the taxa of *C. candidus* Clarke, *C. istanbulensis* (B.Mathew) Rukšāns. In another study, the length of arms and their curving degree differ even among the populations of *Crocus cf. heuffelianus* Herb. (Raca et al., 2019).

Rectangular shape palisade cells were found in the mesophyll, and almost round shape sponge parenchyma cells were present in all the taxa. The maximum length of palisade cell was, 20.93 µm which, observed in *C. x paulineae*. The second maximum length was, 18.67 µm, belong to the *C. olivieri* (Table 2). *Crocus ancyrensis* subsp. *ancyrensis* had 17.27 µm average length in this study, while maximum length was found 12.0 µm in the same taxon by Candan (2015). However, the length of palisade cell variations was between 37-72 µm in the *Crocus cf. heuffelianus* of different populations (Raca et al., 2019). The number of palisade cell was 2, sponge cell was 3-4 in the studied taxa; and in many studies (Özdemir et al., 2004; Satıl and Selvi, 2007; Kandemir, 2009; Raca et al., 2019). The vascular bundle was variable in terms of shape and number compared to the studied taxa. The number of medium and large vascular bundles was generally found to be 4 in all the taxa. Large vascular bundles were located at the ends of the arms with keel corners (Figure 1-A-D). Small and medium vascular bundles were scattered between them. Generally, there was a difference in terms of number of small vascular bundles among the taxa. The least average number was found in *C. abantensis* with the average number 1.20, while the highest number, 7.13 was seen in *C. olivieri* (Figure 1-A, D). The average number was 5.60 in hybrid taxa; 4.53 in *C. ancyrensis* (Figure 1-B,C). In similar studies, the number of large vascular bundles was four, and the number of small vascular bundles was varied at keel corners and arm ends (Kandemir, 2011; Erol and Küçüker, 2007; Satıl and Selvi, 2007; Raca et al., 2019).

Corm diameter was between 5-36 mm and leaf length between 5-79 mm among 15 *Crocus* taxa studied by Coşkun et al. (2010). In this study, corm diameter was found to be 2.57-3.59 cm, and leaf length 7.65-14.47 cm which was almost in the similar range as those *Crocus* data except leaf length was high in this study (Table 3). Leaf length can be related to the taxa. Similarly; anther length was 0.97-1.33 cm, filament length 0.75-0.83 cm, and style length 6.09-7.10 cm in this study. Anther length shorter or longer than 13 mm, filament length shorter or longer than 8.00 mm and style length shorter or longer than 15 mm was found in the study of Coşkun et al. (2010). In the study of Harpke et al. (2014) 9 different species was used and, filament length was found between 3.70-7.00 mm, anther length 7.00-14.00 mm and style length 5.00-11.50 mm. In this study periant outer segment length and width (POSL, POSW) was between 2.33-2.69 cm and 0.72-1.18 cm (Table 3) while, in Harpke et al. (2014) study, they were in the range of 21-30 mm and 5.00-10.50 mm respectively.

The plot of Cluster and Principal Component Analysis, was based on two purposes. First of all; the proximity of the studied taxa was examined; and secondly, *C. x paulineae* as the natural hybrid and its parents of this hybrid was investigated. In the literature, this taxon has not been studied much since, the diagnosis of the hybrid (Pasche and Kerndorff, 1999). The parents of this endemic natural hybrid was given as *C. abantensis* and *C. ancyrensis* subsp. *ancyrensis* by Pasche and Kerndorff (1999). However, another yellow-flowered taxon, *C. olivieri* flowering in the same period was also present in the same area. In order to identify whether this taxon might also have been another parent of the hybrid; these graphs were drawn. The result of cluster analysis and PCA of the studied taxa was given in Figure 2 and 3 respectively. There were 2 main groups in CA; hybrid and its parent's *C. abantensis* and *C. ancyrensis* subsp. *ancyrensis*. In the second group *C. olivieri* was joined to the first group (Figure 2). However; in the first group hybrid was joined to the parental groupings from outside, it did not form between *C. abantensis* and *C.*

Table 6. Comparison of leaf morphological parts.

***Crocus* taxa**	**Corm, Tunica, Leaf length, Periant tube Length**	**Periant inner and outer segment**	**Style and Filamanet**
Crocus×paulineae	Corm diameter is the biggest, corm length is the smallest one. Tunica densely reticulate fibrous type. There is no ring condition at tunica base. Leaf length is short according to the other taxa. Periant tube length is also shorter than the others.	Inner segments shape are broadly elliptical with brownish yellow color. Outer segments are elliptical shape having acute tips. Color is yellow with brown or purple spotted.	Style is 3 branched with bifurcate end, yellow or orange color. Filaments are 8 mm and yellow.
Crocus abantensis	Corm diameter is the second small taxon. Tunica densely reticulate fibrous. No ring formation at tunica base. Leaf length is the second short taxon. Periant tube length is longer than *C x paulineae*.	Inner segments shape are inverted ovoid with blue-liliac color. Outer segments are almost same with inner segments having obtuse or pointed tips. Color is blue-liliac.	Style is 3 branched with orange color. Filaments are about 8 mm and light yellow.
Crocus ancyrensis subsp. *ancyrensis*	Corm diameter is the smallest taxon. Tunica coarsely reticulate fibrous. No ring formation at tunica base. Leaf length is the second long taxon. Periant tube length is the second short taxon.	Inner segments shape are ovoid elliptic with broadly acute tips. Color is bright yellow. Outer segments shape are same with inner segments having acute tip. Color is bright yellow.	Style yellow or orange-red to orange, 3 branched. Filaments are 7.5 mm yellow.
Crocus olivieri	Corm diameter is the smallest taxon. Tunica coarsely reticulate fibrous. No ring formation at tunica base. Leaf length is the second long taxon. Periant tube length is the second short taxon.	Inner segments shape are narrowly elliptical with broadly acute tips. Color is yellow to golden yellow. Outer segments shape are almost same with inner segments. Color is golden yellow.	Style is 3 branched with bifurcate end, yellow to orange color. Filaments are 7.5 mm and dark yellow color.

ancyrensis subsp. *ancyrensis* (Figure 2). Similar groupings were also observed in PCA; *C x paulineae, C. abantensis, C. ancyrensis* subsp. *ancyrensis,* and *C. olivieri* were also overlapped with those taxa. (Figure 3). Therefore, according to these graphs both taxa *C. olivieri* and *C. acncyrensis* subsp. *ancyrensis* still have the possibility to be one of the parents of *C. x pauline*.

The study was aimed to show anatomical structure of Abant Lake *Crocus* taxa. There has been no records about hybrid taxon, *C.* × *paulineae* research. Especially; it is the first study for the natural hybrid and one of its parent *C. abantensis*, which were the two important and endemic species for Bolu. The main differences of hybrid from the other taxa; having the thickest cuticle (3.90 µm), the longest parenchyma (20. 80 µm) cell in the mesophyll, having papillae like structure on the keel corners of cuticle, sponge cell shape was oval-elliptic (Table 2-4). The last two characters; were in common with *C. olivieri*.

The chromosomal counts of the hybrid was 14 and its karyotype was also presented in this study for the first time (Figure 4). In the earlier study *C. ancyrensis* subsp. *ancyrensis* and *C. olivieri* chromosome counts were both 2n = 6 and all the chromosomes were subtelosentric type, and *C. abantensis* 2n = 8, which 2 pairs were submetacentric, the rest was metacentric (Table 7) (Uslu et al., 2012). According to chromosomal comparison hybrid taxon shows more chromosomal similarity to one of the its parents, *C. abantensis*. On the other hand the other parent, *C. ancyrensis* subsp *ancyrensis* shows similarity with *C. olivieri* (Table 7). This information also correlates that one of the parents of natural hybrid *C. ancyrensis* subsp. *ancyrensis*, shares the same chromosomal counts and characteristics with *C. olivieri*.

Table 7. Comparison of chromosome numbers, karyotypic descriptions and morphometric parameters of studied *Crocus* taxa

Taxon name	Chromosome Number	Karyotypic Description	Haploid Complement (µm)	L/S	I^C	A_1	A_2
C. abantensis	2n = 8	6 m + 2 sm	17.41	1.49	0.40	0.33	0.15
C. olivieri	2n =6	6 st	27.24	4.82	0.32	0.79	0.12
C. ancyrensis subsp. ancyrensis	2n =6	6 st	31.06	4.28	0.19	0.77	0.13
C. x paulinea	2n = 14	10m + 2 sm	24.79	1.59	0.39	0.77	0.29

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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