Taxonomic and GIS-Based Ecologic Studies on Critically Endangered *Crocus wattiorum* B.Mathew (Olympos Saffron)

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> Abstract: Crocus wattiorum, endemic to the Olympos-Bey Mountains National Park of Antalva province. Turkey, is mainly distributed on rocky and stony areas under sparse Pinus brutia and maguis. As well as the leaf and scape anatomy, the palynological and seed micromorphological features of this species were investigated in this study. The leaves of C. wattiorum consist of two lateral arms with rectangular keel in the median region. The characteristic pale stripe running axially along the center of the leaf is caused by the parenchymatous cells in the keel which lack chloroplast and break down to form an air space. The seeds of the species are globose to ovate in shape, the seed surface is papillose. The pollen grains are prolate spheroidal, with a thin exine and a relatively thick intine. During the field study, it was endeavored to record all individuals found in their habitat, and the individuals' locations were recorded with GPS. Data concerning the habitat types, elevation, aspect, slope, vegetation and distance from the sea of the individuals was noted and evaluated using Geographic information system, ArcGIS 9.3. Additionally, potential

distribution areas of the species, which is categorized CR under IUCN threat category, were determined.

Keywords: anatomy, *Crocus*, morphology, palynology, Turkey.

Introduction

Turkey is the only country almost entirely covered by three of the world's 34 biodiversity hotspots: the Caucasus, Irano-Anatolian and Mediterranean. Turkey has a diverse ecology and is estimated to host around 11707 plant taxa, of which 3649 (32 %) are endemic to Turkey (GÜNER et al. 2012). In terms of plant diversity, Antalya province is the richest in Turkey, home to approximately 750 of the country's endemic species. Of these 750, about 250 are best described as locally endemic, as they are found only in the Antalya region. Totally 41 species and 79 taxa (1 hybrid and 1 culture form), - of which 55 are endemics of the genus *Crocus* L. - are distributed in Turkey (YÜZBAŞIOĞLU 2012). According to KERNDORFF & PASCHE (2004), the genus' centre of diversity is southwestern Turkey.

In autumn, flowering *Crocus wattiorum* B.Mathew, distributes mainly in rocky and stony spots, under sparse Red Pine and maquis in a certain area within the boundaries of Olympos-Bey Mountains National Park (Kemer, Antalya). Detailed field studies were carried out in 2012 and 2013. According to the field studies' results, 253 specimens of Olympos Saffron were identified both in forest habitats, and in clearings. The distribution of the species was modelled in three dimensions, based on the GPS location of each individual. As a result of statistical analysis, the general characteristics of *C. wattiorum*'s habitat were determined. In addition to the features of habitat and soil, topographical features such as exposure, gradient and altitude of the species were also noted.

Particularly in recent years, numerous plant species are facing risk of extinction due to the establishment of new stone quarries, the construction of Hydro-electric installations, improper land use, uncontrolled tourism activities and general misadministration. For these reasons, training seminars have been given to the relevant members of the forest administration. During these seminars, information about *C. wattiorum* was given to the participants about the habitat and distribution areas of the species. Additionally, information was provided to participants concerning further planned studies, aimed to ensure the survival of the species.

Anatomical, morphological and palynological features of *C. wattiorum* were also examined in the scope of this study. The pollen grains of *C. wattiorum* were examined by light microscope (LM) and scanning electron microscope (SEM) and the palynological features of the species determined. The micromorphological features of the seeds were also evaluated by SEM, and anatomical features of the leaf and scape were investigated by LM.

Material and Methods

Morphological and anatomical studies

Firstly, field excursions were planned in order to determine the populations of the species in nature. During the field studies, in order to ensure the generation of the species a certain number of individuals were transferred to the Botanic Garden of Akdeniz University without harming the natural populations. A number of plant samples were collected and dried according to herbarium techniques, and archived in the Akdeniz University Herbarium (AKDU). Morphological measurements were done on numerous seeds (about 100) under stereomicroscope. In this study, the seed coat morphology of the species was examined using SEM (Scanning electron microscopy) techniques. For the SEM study, the seeds were covered with gold on stubs. The microphotographs were taken with a Zeiss LEO-1430 Scanning Electron Microscope.

For anatomical studies, material of *C. wattiorum* was collected from the natural population, in November 2013, during the flowering period. Live samples were fixed in 70 % ethanol. Hand cuts were stained with sartur reagent. Prepared samples were photographed with the Olympus BH-2 microscope.

Palynological studies

For light microscopy (LM), pollens were first treated with 70% alcohol to remove oily substances, then embedded in glycerine jelly stained with basic fuchsin, following WODEHOUSE's method (1935). LM studies measured the equatorial axis (E), polar axis (P), exine, exintine and endintine thicknesses. The measured pollen diameters were based on 50 samples. To examine the exine sculptures and seed coat in detail, scanning electron microscopy (SEM) was also used. For SEM study, pollens were treated first with 70% alcohol, and then dried, before mounting on stubs and covered with gold. The microphotographs were taken with Zeiss LEO-1430 Scanning Electron Microscope. Pollen shapes and ornamentation were identified according to HOEN (2014).

GIS studies

The material of this study consisted of 253 specimens, which were observed and their GPS locations established during the field trips. Data concerning habitat types, elevation, aspect, slope, vegetation and the specimens' distance from the sea were recorded. Geographic Information System (GIS) ArcGIS 9.3 software, 8 digital elevation maps and 1/25000 raster maps were referenced during the study. (map ref. O24b3, O25a4, O24c2, O25d1, O24c3, O25d4, P24b2 and P25a1). The 3D Analysis module of the program was used for the creation of slope and elevation maps. Using the relevant modules of the program, the individuals' co-ordinates of were transferred into the maps.

Results

Anatomical and morphological studies

Leaf anatomy: The leaves of *C. wattiorum* consist of two lateral arms, the rectangular keel in the median region. The arms are ribbed and recurved towards the keel with a wide angle. The characteristic pale stripe running axially along the center of the leaf is caused by the parenchymatous cells in the keel, which lack chloroplast and break down to form an air space.

Both adaxial and abaxial surfaces have a thick cuticle. The epidermis is single layered on the abaxial and adaxial surfaces. Micropapillae are conspicuous on the cuticle. The hair like papillae is generally present on prominent ridges over the largest vascular bundles.

Stomata are anomocytic and occur only on the lower surfaces. The epidermis is single layered on abaxial and adaxial surface. The stomata are mainly confined to the groove parts. Mesophyll consists of palisade and spongy parenchyma. The palisade parenchyma is 1 layer located on the adaxial side of the arms. The spongy parenchyma is 3-4 layered, present on the abaxial side. There are four large vascular bundles and a different number of small vascular bundles in the leaves. Two of the large bundles are at the corners of the keel, and the others are at the end of the arms. The small bundles were between the arms and the keel. Crytals (styloids) occasionally are present in cells of the parenchymatous outer bundle sheath.

Scape anatomy: The cross sections of the scape show that the epidermis is covered by a thick cuticle. The epidermis is single layered and isomorphic. Upper and lower walls of epidermis are thickened. There is 4-5 cell layered cortex parenchyma under the epidermis. The cortex cells are thin walled and have intercellular spaces. A pith region is present under the cortex at the center of scape. The pith cells are thin and have intercellular spaces. Different sized vascular bundles are located in three circles. The vascular bundles at the center are large and 4-5 in number. At the edges, Vascular bundles are 9-10 in number and are smaller than the others. LM photographs of the leaf and scape anatomy are given in Fig. 1.

Seed morphology: The seeds of this autumn-flowering species are globose to ovate in shape, brownish coloured, $3-3.5 \times 2-2.5$ mm, covered by numerous, short conic papillae-like protrusions. Raphe and pronounced caruncle are present (Fig. 2. d-f).

Palynological studies

According to LM and SEM (Fig. 2: a-c) investigations, the pollen grains are large in size (51-100 μ m). The pollen shapes (based on P/E ratio) are prolate spheroidal, with a thin exine (exine sculpture with spinules and microperforate) and a thick intine. The main palynological features of *C. wattiorum* are given in the Tab. 1.



Fig. 1. Cross section of *C. wattiorum*. A-B: Leaf, C: Scape; ab: abaxial epidermis, ad: adaxial epidermis, c: cortex, e: epidermis, lc: lacuna, m: mesophile, ph: phloem, pi: pith, sc: sclerenchyma, t: trachea, vb: vascularbundle.

Tab. 1. Pollen characters of C. wattiorum.

Taxon	Aperture type	Р		E		Evino	Evipting	Endintino		
		M (µm)	SD	V (µm)	M (µm)	SD	V (µm)	(µm)	(µm) (µm)	(µm)
C. wattiorum	Spiral furrow	73.39	±2.8	68-79	72.16	±3.12	65-80	(1)-1.7-(2)	(3)-4.13-(7)	(0.7)-1-(1.5)



Fig. 2. SEM images of pollen and seeds of *C. wattiorum*. a-c: Pollen (a: Pollen with spiral furrow; b-c: Exine sculpture with spinules and microperforations); d-f: Seed (d: Seed with raphe and caruncle; e-f: densely papillose coat surface).

GIS studies

The GPS coordinates of every individual were recorded during the field studies which established 253 individuals of *C. wattiorum*. According to this data, it was determined that the individuals of the species are distributed in an area of 10428 meters in an east-west direction and 24314 meters in a north-south direction.

C. wattiorum is distributed in an area of total 253.5 km^2 according to these values. Additionally, it was determined that the populations are concentrated in 15 different locations. The distribution area of the species can be shown in the Fig. 3.



Fig. 3. Distribution areas of *C. wattiorum* (a); Potential distribution areas (b); Vegetation types in distribution areas of *C. wattiorum* (c).

Vegetation type: First of all, the vegetation in the distribution areas of *C. wattiorum* was examined. The vegetation types in the area are: Open areas (rocks and stony areas), sparse to dense Red Pine, sparse to dense Cedar and Black Pine (Fig. 3). According to data obtained, it was determined that 47% of the individuals prefered sparse Red Pine to bare areas or being under maquis.

Elevation (m): The individuals of this species are distributed in altitudes between 113 m and 1351 m. It was determined that the mean value of the elevation for this species is 489 m. The high standard deviation value of 429 m demonstrates the hetrogeneous altitude distribution of this species.

Aspect: While most of the individuals investigated preferred a southerly aspect, examples were found in all aspects, ranging from North to 349°.

Slope (%): As results of this analysis, the individuals of the species show preference for sloping land between 6-80 %, the average slope where individuals



were found was 38%. The high standard deviation value of 22.6 tells us that his species has a hetrogeneneous distribution in regard to slope.

Rock Density (%): In order to investigate the preferred habitat of the species, the rock density of the sites were evaluated on scale of 0 to 100, with 100 being the highest value. Results show that the species prefer rocky areas with a mean rock density of 78, and that this species does not exist in rock-free areas.

Sun Exposure (%): Levels of sun exposure were also examined in respect to the situation of the specimens. Full shade is represented as 0, full sun as 100. It was established that the species prefers partial shade, with a mean cover index of 53%.

Proximity to coast (m): The distance to the sea of every individual of *C. wattiorum* was determined. As the results of these measurements the individuals proximity to the sea varies between 455m and 10280 m. The mean from sea is 4581 and the standart deviation is 1784 m.

The geographical features of Olympos Saffron are shown on Tab. 2, Tab. 3 shows the results of GIS analysis.

Managurad variables	Statistical Values	Species		
Measureu variables	Statistical values	Olympos Saffron		
Elevation (m)	Mean	489.6		
	Minimum	113.0		
	Maximum	1351.0		
	Standard deviation	429.3		
Aspect	Mean	178.7		
	Minimum	0.0		
	Maximum	349.0		
	Standard deviation	107.6		
Slope (%)	Mean	38.0		
	Minimum	6.0		
	Maximum	80.0		
	Standard deviation	22.6		
Rock Density (%)	Mean	78.5		
	Minimum	50.0		
	Maximum	100.0		
	Standard deviation	12.8		
Sun Exposure (%)	Mean	53.5		
	Minimum	5.0		
	Maximum	95.0		
	Standard deviation	22.0		
Proximity to coast (m)	Mean	4581.5		
	Minimum	455.0		
	Maximum	10280.0		
	Standard deviation	1784.5		

Tab. 2. The geographical features of Olympos Saffron.

Tab. 3.	The	results	of the	GIS	analy	yses.
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Measured variables			Oympos Saf	ron			
X Minimum			271587				
X Maximum		282015					
X Difference (East-West)			10428				
Y Minimum			4052513				
Y Maximum			4076827				
Y Difference (North-South)			24314				
Distribution Area (km ²)			253.5				
Number of Individuals			253				
Number of Group Location			15				
Vegetation type	Number of individuals	Aspect groups	Number of individuals				
Openings	-	0-22.5	8				
Sparse Red Pine	30	112.5-157.5	15				
Sparse Red Pine (Reforestation	-	157.5-202.5	27				
Sparse Red Pine and Macchie	9	202.5-247.5	22				
Sparse Red Pine, Stony area	11	22.5-67.5	25				
Sparse Red Pine and Macchie	5	247.5-292.5	28				
Sparse Red Pine, Stony area	119	292.5-337.5	43				
Sparse Cedar and Black Pine	43	337.5-360	15				
Dense Red Pine	28	67.5-112.5	63				
Dense Cedar and Black Pine	8	Flat	7				
Total		253	Total	253			
Slope groups (%)	Number of i	ndividuals	Altitude	Number of individuals			
0-12	45	i	0-250	78			
12-30 52			250-500	117			
30-60 12'		1	500-750	4			
60-100	i	1000-1250	19				
		1250-1500	35				
Total	253	3	Total	253			

Conservation status: Conservation status of the *C. wattiorum* was assessed according to IUCN (2014), described to the rules of citation below. Considering the IUCN criteria, the species resides in the CR (Critically Endangered) category; The Criteria For Critically Endangered, Endangered and Vulnerable.

This species is found in the area between Konyaaltı and Kemer, in Olympos-Bey Mountains Coastal National Park. Olympos Saffron is under threat due to the tourism activities in its distribution area, and uncontrolled picking (CR A1ac). The area of occupancy of the species was determined using GPS. The extent of occurrence (EOO) and area of occupancy (AOO) values were calculated. The EOO value of the species was determined as 150 km² (CR B1b(iii,iv)) taking both locations of occupancy and the area contained within the shortest continuous imaginary boundary. The AOO value in this area, where the species was identified was calculated as 15 km² (CR B2b(ii,iii)). The number of individuals identified in the populations of the species was determined as 253

(CR C2a(i)). The distribution of the species in the national park area is very scattered and the individual number of the populations is so few. Also, uncontrolled picking threats significantly the next generation of the species. When considering all these, the threat category of Olympos Saffron was evaluated as critically endangered (CR).

Potential distribution areas of C. wattiorum: The potential distribution areas of the species have been calculated using relevant modules of the GIS software considering optimal slope, altitude and vegetation type for *C. wattiorum*. The extrapolated distribution areas are rocky and stony areas under sparse Red Pine and these areas overlap into maquis (Fig. 3).

Discussion

To date, various studies have been made about the leaf and scape anatomy of the genus *Crocus*. RUDALL & MATHEW (1990) pointed out that the leaves of most species of *Crocus* have a distinctive cross sectional outline with a central square or rectangular "keel" and two lateral recurved arms, which may be taxonomically significant. Our result supported the result of EROL & KÜÇÜKER (2007) about *C. wattiorum*'s leaves. The leaves of the species have a pale stripe running axillary along the centre of the leaf. This is a common character in *Crocus* (RUDALL & MATHEW 1990, EROL & KÜÇÜKER 2007). The presence of micropapillae is an important taxonomical structure of the leaf anatomy for the genus *Crocus* and they are conspicuous on the cuticle in *C. wattiorum*.

The leaves appear bifacial with two types of parenchyma, but it is homologous to unifacial leaves (RUDALL 1990, RUDALL & MATHEW 1990). The results of the study show that there is 1–layered palisade parenchyma and 3–4 layered spongy parenchyma in mesophyll. These features have been observed in *C. antalyensis* Mathew, *C. gargaricus* Herb. subsp. *gargaricus*, *C. gargaricus* Herb. subsp. *herbertii* Mathew, *C. biflorus* Mill. subsp. *nubigena* (Herbert) Mathew, *C. flavus* Weston subsp. *dissectus* Baytop & Mathew, *C. fleischeri* J. Gay, *C. candidus* E.D.Clarke, *C. pallasii* Goldb. subsp. *pallasii, C. pestalozzae* Boiss. and *C. pulchellus* Herb. (EROL & KÜÇÜKER 2007, SATIL & SELVI 2007, KANDEMIR 2009), whereas it has not been observed in the leaves of *C. leichtlinii* (Dewer) Bowles and *C. olivieri* J.Gay subsp. *istanbulensis* Mathew, *C. cancellatus* Herb. subsp. *mazziaricus* (Herbert) Mathew (AKAN et al. 2007, SATIL & SELVI 2007). Styloid crystals found in leaves of Iridaceae taxa (BRIGHTON et al. 1983, RUDALL et al. 1986, RUDALL 1994, PRYCHID & RUDALL 1999). We observed styloid crystals in the leaf in spite of EROL & KÜÇÜKER (2007), they had not observed styloids.

The intercellular spaces are present in the cortex of the scape. The different sized vascular bundles are located in the peripheral and central parts of the scape. These bundles are in ring position. Same features have been observed in *C. aerius* Herb., *C. biflorus* Miller subsp. *pulchricolor* (Herbert) Mathew, *C. danfordiae* Mawand, *C. fleischeri*, *C. olivieri* subsp. *olivieri*, *C. cancellatus* subsp. *damascenus* (Herbert) Mathew (ÖZYURT 1978, ÖZDEMIR et al., 2004, 2011, AKAN & EKER 2004; AKYOL 2014). However, the vascular bundles are found only in the

central part of the stem of *C. pallasii* subsp. *turcicus* Mathew (AKAN & EKER 2004).

There have been few studies of the seed micromorphology of the genus *Crocus*. That the seed surface of *C. wattiorum* is covered with densely papillae and papillae-like protrusions was determined on the seeds of *C. biflorus* subsp. *biflorus* and *C. imperati* Ten. by CAIOLA et al. (2010). The seed surface of this species is similar to *Crocus chrysanthus* (Herb.) Herb. subsp. *chrysanthus* var. *atroviolaceus* F.Candan and N.Özhatay which its seed surface was indicated as irregularly aculeate (CANDAN & ÖZHATAY 2013).

The pollen grains of the genus *Crocus* have various aperture types: a spiral furrow (spiraperturate), more or less extensive furrows, or short furrows. The most common type of aperture is the spiral furrow (IŞIK & DÖNMEZ 2006). The pollen type of *C. wattiorum* is also spiraperturate and similar the taxa [e.g. *C. aerius* Herb., *C. anycrensis* (Herb.) Maw, *C. biflorus* Miller subsp. *isauricus* (Siehe ex Bowles) Mathew, *C. cancellatus* Herb. subsp. *cancellatus*, *C. chrysanthus* (Herb.) Herb., *C. danfordiae* Maw, *Crocus flavus* Weston subsp. *dissectus* T.Baytop & B.Mathew, *C. pulchellus* Herb., *C. sieheanus* Barr ex B.L.Burtt] previously determined (IŞIK & DÖNMEZ 2006; KARACA et al. 2007, DÖNMEZ & IŞIK 2008; CANDAN et al. 2009). In this study pollen shape of *C. wattiorum* determined as prolate spheroidal and large in size (Tab. 1).

Besides the anatomical and morphological studies, IUCN threat category of this species was determined as CR in the present study. The potential distribution areas of Olympos Saffron were established in order to help conservation studies planned for the near future. In a short time, field studies will be organized to establish whether the species distributes in these areas or not.

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