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Crocus adamioides (Iridaceae) in the Bulgarian flora

Tsvetanka Raycheva[1], Kiril Stoyanov[1]*, Samir Naimov[2], Elena Apostolova-Kuzova[2]

[1] Agricultural University – Plovdiv, Department of Botany and Agrometeorology, Plovdiv, Bulgaria

[2] University of Plovdiv, Department of Plant Physiology and Molecular Biology, Plovdiv, Bulgaria

Abstract – *Crocus adamioides* Kernd. et Pasche, as it is currently known, was originally treated as *C. biflorus* Mill. subsp. *adamii* B. Mathew in the flora of Bulgaria by Mathew (1982) and verified by Rukšāns (2017). The taxon was afterwards described as a separate species (Kerndorff et al. 2012), the holotype being collected in Kırklareli Province, European Turkey. The species was for the first time mapped in two floristic regions of Bulgaria. The diagnostic characters based on the general morphology and leaf anatomy were defined from the natural populations of the species and compared to the type specimen and relevant data from the literature. The phylogenetic position of the species was clarified by sequencing of the internal transcribed spacer region (ITS: ITS1 + 5.SsrDNA + ITS2) and comparison of the obtained sequence with those annotated in NCBI. A phylogenetic tree was built using Bayesian phylogeny. Results have shown the highest degree of phylogenetic similarity with *C. adamioides* from Turkey. The closest relative *C. ranjeloviciorum* Kernd., Pasche, Harpke et Raca remains in the proximity. Our morphological, anatomical and molecular analyses have revealed that the Bulgarian population shows a peculiar combination of characters specific to *C. adamioides*.

Keywords: anatomy, Bulgaria, chorology, *Crocus*, ITS region, morphology

Introduction

The genus *Crocus* currently consists of about 230 taxa according to Rukšāns, (2017). It is distributed from Western Europe and Northwestern Africa to Western China, with the centre of the species diversity being on the Balkan Peninsula and west Turkey (Mathew 1982, Ranđelović et al. 2012, Harpke et al. 2014, 2016, Rukšāns 2017). As a result of this pattern Turkey represents an especially rich territory in terms of *Crocus* species, currently comprising approximately 140 taxa (Yüzbaşıoğlu 2019, Çiftçi et al. 2020). The number of endemic species recorded in these areas is increasing continuously, and to date there are approximately 40 Balkan endemic species (Miljković et al. 2016, Spirios et al. 2019). Most of the newly described species do not have a clear infrageneric position in the existing system of classification. Moreover, the conception about the volume of the polymorphic groups in the genus is complicated and debatable. One of the rather heterogeneous groups is *Crocus biflorus* s.l., belonging to *C.* sect. *Nudiscapus* B. Mathew, ser. *Biflori* B. Mathew. Recent phylogenetic analyses have proved several units of this series to be para- or polyphyletic, causing subsequent taxonomic problems (Petersen et al. 2008, Harpke et al. 2013). A DNA-based investigation showed that several subspecies of *C. biflorus* s.l. according to Mathew (1982) have been ranked as species instead of subspecies (Harpke et al. 2016).

According to recent Bulgarian floristic literature, the genus *Crocus* is represented by 9 species, growing wild (Assyov and Petrova 2012). The genus *Crocus* was studied taxonomically more than 50 years ago (Velchev 1964). Traditionally, all Bulgarian taxa with white or lilac flowers, with three to five striking purple longitudinal stripes on the outer tepals have been treated under the name *C. biflorus* (Velenovský 1898). In the 3rd edition of Flora of Bulgaria (Stojanov and Stefanov 1948) and Flora of the P.R. of Bulgaria (Velchev 1964), the variability of *C. biflorus* is represented as a single taxon – var. *violaceus* Boiss. This taxon is is considered synonymous with *C. biflorus* subsp. *adamii* (J.Gay) Mathew, with distributional area covering the territory between Bulgaria and Iran (Mathew 1982). In the 4th edition

* Corresponding author e-mail: nomtax@gmail.com

of Flora of Bulgaria (Stojanov et al. 1966), *C. biflorus* comprises three varieties: var. *biflorus*, var. *adamii* (Gay.) Bak, and var. *albus* Herb. Subsequently, Kerndorff et al. (2012), on the basis of phylogenetic studies in the ITS region, described *C. adamioides* as a new species, which is neither closely allied to *C. biflorus* nor to its subspecies *adamii*.

The conventional morphological approach is not reliable enough for sufficient differentiation of taxa in polymorphic groups and needs to be combined with the molecular one. The internal transcribed spacer region (ITS: ITS1 + 5.8SrDNA + ITS2) and 5' external transcribed spacer (ETS) of the nuclear ribosomal DNA (rDNA) have proven to be useful phylogenetic markers in *Crocus* (Harpke et al. 2014). However, as a multigene family consisting of hundreds of tandemly repeated units in single or multiple clusters in the genome (NOR), the rDNA is subjected to concerted evolution. Processes like unequal crossing-over (Eickbush and Eickbush 2007) and gene conversion (Li et al. 2011) can lead to the homogenization of the different units. Moreover, frequent intra-individual polymorphic rDNA regions are reported for a few cases in *Crocus* (Harpke et al. 2013, 2014) and are often caused by e.g. hybridization or polyploidy (Petersen et al. 2008).

In January 2018, we found a population of plants that was identified as *Crocus* cf. *adamii* B. Mathew. The morphological analysis revealed that this population has a combination of specific characters of *C. adamioides*, instead of *C. adamii* (Kerndorff et al. 2012). Consequently, we decided to study the Bulgarian population of *C. adamioides* in detail (including its morphology, anatomy, and molecular data).

This paper provides full insight into the chorology and taxonomy of *C. adamioides* for the flora of Bulgaria.

Materials and methods

Plant material

After extensive field studies, the presence of the species was observed in two floristic regions – the Thracian Lowland and Toundja hilly plain (Mt Sakar). The plant material was collected in January-February 2019. Data about the habitat and populations of the species were derived from personal observations. On the other hand, the information about the distribution was based on relevant literature sources as well as on the authors' field observations. The sampling localities are represented on a map (BGMountains Project 2019). The voucher specimens are deposited in the herbarium of the Agricultural University – Plovdiv (SOA). A list of them is given below by floristic regions in Bulgaria, then by quadrant code of MGRS coordinates (10 × 10 km). Each specimen contains information about the description of the habitat and toponym, followed by coordinates (WGS84), altitude, dates of collections, collectors' names, herbarium code, and voucher numbers. The specimen used for molecular analysis is marked with an asterisk (*).

Thracian Lowland

35TLG86, Protected locality "Propadnaloto blato", near the village of Golyamo Asenovo, N42.12545 E25.64678, 145 m. 2019-01-29, 2019-02-02 (*coll*. V. Trifonov and K. Stoyanov) SOA **062524**, **062525**.

Toundja hilly plain (Sakar Mountain, a new record for the country)

35TMG43, Oak forest near Dervishka Mogila, N41.9073056 E26.3345, 404 m, 2019-02-02 (*coll*. Ts. Raycheva and K. Stoyanov) SOA **062626**. Oak forest, near the village of Moustrak, N42.0811944 E26.356, 322 m, (*coll*. Ts. Raycheva and K. Stoyanov) SOA **062627**.

35TMG44, Oak forest between the villages of Planinets and Oustrem, N41.9756111 E26.3885, 303 m, 2019-02-02 (*coll*. Ts. Raycheva and K. Stoyanov) SOA **062625* (**sequence MN955426**).**

35TMG45, Dry shrubs with *Quercus sp. div.* and *Paliurus* near the town of Topolovgrad, N42.0811944 E26.356, 322 m, 2019-02-02 (*coll*. Ts. Raycheva and K. Stoyanov) SOA **062624**.

35TMG54. Village of Stoudena, N41.9166667 E26.4013889, 295 m, 2019-02-21 (*coll*. V. Trifonov) SOA **062628**.

Morphological investigations

The collected fresh plants of *C. adamioides* were photographed in detail. The morphological measurements (Tab. 1) were made on both fresh and dried samples from three populations (35TMG43, 35TMG44, 35TMG54), 30 individuals from each population.

The morphological measurements, after statistical analysis (mean, standard deviation, minimum and maximum), were compared with those of *C. randjeloviciorum* Kernd., Pasche, Harpke et Raca as a rather similar and closely related species (Harpke et al. 2017).

Anatomical investigations

Developed leaves from flowering plants were used in the anatomical study. In order to examine the leaf anatomy, five individuals per population, from 3 populations in total (35TLG86, 35TLG86, 35TMG44) were collected and conserved in 75% ethanol. Five transverse cross sections and epidermal areas of leaves from each individual were made manually from the middle part. The microscope slides were prepared in glycerine jelly. Photographs of the microscope slides were taken using a Carl Zeiss Amplival microscope with an external attached ocular camera, calibrated using a micrometric slide.

The measurements of the characters were done using Micam 2.4 software (Van Westen 2018). Nineteen anatomical features, in the 30 leaf transverse sections from each population were measured: section width and height, arm length, white stripe, vascular bundle count (magnification 3.2×), palisade tissue height, height and width of pali-

Tab. 1. Comparison of morphological characters of *Crocus adamioides* Kernd. et Pasche (both Bulgarian – this study, and the Turkish populations – Kerndorff et al. 2012) and *C. ranjeloviciorum* Kernd., Pasche, Harpke et Raca (Serbia, Harpke et al. 2017).

Characters	*C. adamioides*		*C. ranjeloviciorum*
	Bulgaria	Turkey	Serbia
Corm height/diameter, mm	9-18.5	10-15	9-11
Edge of the basal rings	Clearly toothed, with distance between the teeth 0.5-2.5 mm	Clearly toothed	Smooth, rarely with tiny teeth
Teeth length, mm	0.5-2	up to 1	0-0.5
Leaf indumentum	glabrous	glabrous, sometimes slightly ciliated	papillose
Leaf width, mm	0.7-1.6	1-1.5	1.2-2.5
Main color of flower segments	Whitish, rarely pale liliac	White or liliac	Violet-blue, rarely whitish
Outer perygone segments (length × width, mm)	13.5-23-31.9 × 3.2-7.1-13.7	15-19-25 × 6-8.7-13	21-25-30 × 6-9.5-14
Inner perygone segments (length × width, mm)	13.7-21.7-33.9 × 2.4-7.5-12.5	14-18-24	18-23-28 × 6-10.2-14
Anthers – length, mm	5.6-9-14	6-8.8-12	7-11.5-15.2
Filaments	glabrous	glabrous	hairy

sade and spongy cells (magnification 10×), and the height and width of the adaxial and abaxial epidermal cells (magnification 40×). The length and width of adaxial epidermal cells were measured (magnification 3.2×). Abaxial epidermal cells were measured in the area of the stomatal rows (between the ribs). The size of stomata (both stomatal cells) was also measured (magnification 10×). The values were processed using basic statistical analysis – mean, standard deviation, maximum and minimum, and compared with the data for *C. randjeloviciorum* (Harpke et al. 2017).

Molecular methods

The genomic DNA was isolated using DNeasy Plant Mini Kit (QIAGEN). Briefly, 50 μg of plant material was ground in liquid nitrogen and processed according to the manufacturer's requirements. DNA concentration and quality were determined spectrophotometrically at a wavelength of 260 nm using Epoch microtiter plate reader and T3 plate protocol. The ITS region (ITS1, 5.8S rDNA, ITS2) was amplified using the following primers: ITS-A (5'-GGAAGGAGAAGTCGTAACAAGG-3') and ITS-B (5'-CTTTTCCTCCGCTTATTGATATG-3'), as described in Tirel et al. (1996). The reaction was carried out in a 50 μL reaction mixture containing: 1x reaction buffer, 200 μM dNTPs, 0.2 μM of each primer, 100 ng plant genomic DNA, and 1 unit of Q5 High Fidelity DNA polymerase (New England Biolabs). The PCR thermal cycler steps were as follows: initial melting of the reaction mixtures at 94 °C for 45 sec, followed by 30 cycles at 94 °C for 10 sec for denaturation, 10 sec at 62 °C for primer annealing, 30 sec. at 72 °C for primer extension, and a final elongation step of 2 min at 72 °C. Amplified PCR products were separated by 0.8% agarose gel electrophoresis, excised from the gel, and purified using a QIAquick Gel Extraction Kit (QIAGEN). The purified DNA fragments were subsequently sequenced with Microsynth Company (Switzerland) technology. Chromatograms were corrected manually with DNAStar software (Lasergene, USA). The sequence was submitted in NCBI Gene database under accession number MN955426 (voucher SOA 062625).

Phylogenic analyses

The obtained nucleotide sequence was blasted against nucleotide sequences from the NCBI Nucleotide database (BLAST 2019). The best hits, all deposited by Harpke, were downloaded and used for the phylogenetic analysis. The alignment of the sequences was done using the ClustalW Multiple alignment (Thompson et al. 1994). The phylogenetic analysis was done using Bayesian phylogenetic inference with MrBayes 3.2 (Ronquist et al. 2012). The parameters of the analysis were the same as described by Harpke et al. (2017) – 2 times 4 chains for 2 million generations, nuclear data set ГТР+G+I, sampling tree per 1000 generations, 2 independent runs. The result was visualized as a tree using TreeGraph 2 (Stöver and Müller 2010). The analysis included 31 nucleotide sequences, cited as numbers of entries in the phylogenetic tree.

Results

Crocus adamioides Kernd. et Pasche, Stapfia 97: 11 (2012).

Holotypus: Turkey, Kırklareli Province, Yildiz Dağları, 900-1100 m, 26.01.2009, HKEP 0904 (Gatersleben, GAT 7136!)

Description (based on Bulgarian materials): Perennial synanthous geophyte, 6-18 mm in height (Fig. 1). Corm subglobose 9-18.5 mm in width and 7-19 mm in height; tunics coriaceous, inner softer; neck bristly, up to 5 mm long,

Fig. 1. *Crocus adamioides* Kernd. et Pasche – specimen SOA 062626 near the village of Moustrak, Toundja hilly plain (Sakar Mountain), southeastern Bulgaria.

split into broad segments; ring present, basal detachable, with small teeth 0.5-1.5(-2) mm long, evenly spaced (Fig. 2C). The tunic teeth details depend on age. In young corms (3-4 years old), the teeth of the basal rings are sparsely situated, 1-1.3 mm long and the distance between them is about 2.5 mm. In mature corms (9-10 years old), the teeth are 1.5-1.7(-2) mm long, with the distance between them about 1.5 mm. Cataphylls silvery-whitish to yellowish. Leaves (2-)3-4 during flowering time, dark green. Bract 1.5-2 mm wide, skinny, yellowish. Bracteole 3-4 mm wide, membranous, transparent. Flowers 1-2(-3). Perigone segments white to pale blue or lilac, usually with acute apical regions. Outer perigone segments 13-32 mm long and 3-14 mm wide, with 3 violet-lilac stripes on the outside. Inner perigone segments 13.5-34 mm long and 4.5-12.5 mm wide, without stripes, but most of them with a distinct blotch on the outside. Perigone tube yellow, smooth, 20-50 mm long. Anthers 5-14 mm long, yellow, divided by a distinct white connective. Stigma trilobate, stylodia 2-6 mm, equal, or longer compared to the stamens (Fig. 2A, B).

Habitat and distribution

In Bulgaria, the species occurs in the Thracian Lowland and on Sakar Mountain (the floristic region of Toundja hilly plain), on hilly terrain at an altitude between 140 and 410 m a.s.l. (Fig. 3, see also the description in Materials and methods) The flowering period of the collected samples starts at the end of January and lasts until the end of February. The accompanying species are: *Quercus cerris* L., *Q. robur* subsp.

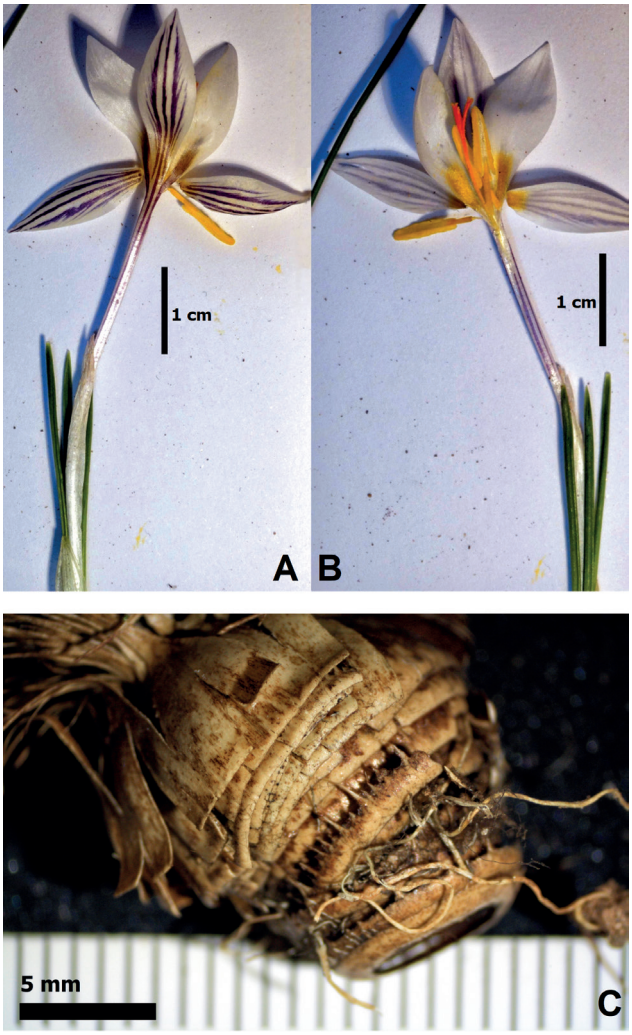

Fig. 2. *Crocus adamioides*, specimen SOA 062626 near village of Moustrak, Toundja hilly plain (Sakar Mountain), southeastern Bulgaria. A, B – flower; C – corm.

pedunculiflora (K.Koch) Menitsky, *Paliurus spina-christi* Mill., *Fagus sylvatica* L., *Viola odorata* L., *Dipsacus fullonum* L., *Centaurea salonitana* Vis., *Teucrium capitatum* L., *Sanguisorba minor* Scop., *Euphorbia amygdaloides* L., *Iris sin-*

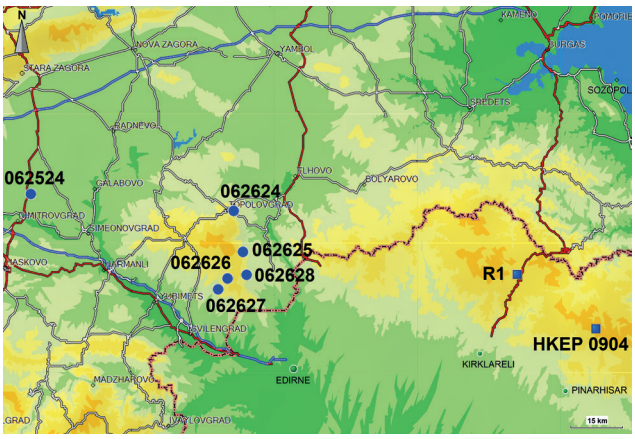

Fig. 3. Distribution of *Crocus adamioides*: numbered – new records in Bulgaria. HKEP 0904 – type locality in Turkey, R1 – data provided by J. Rukšāns (personal communication).

tenisii Janka, *Fragaria viridis* Weston, *Verbascum phoeniceum* L., *Clematis viticella* L., *Asparagus officinalis* L., *A. verticillatus* L., *Teucrium chamaedrys* L., etc.

Leaf anatomy

The cross-section of the leaf is typical of the genus *Crocus* with a central square or rectangular "keel" and two lateral "arms" (Fig. 4A). Each arm has 2 well-defined ribs. The cross-section width is 1053-1931 μm and height 216-610 μm. Large, thin-walled parenchymal cells are oriented in the central part of the keel, forming a lacuna area, visible as a longitudinal white strip on the top of the leaf.

The total of 9 – 12 – 15 collateral vascular bundles are positioned in one row on the abaxial leaf side along the leaf mesophyll; the 6-10 located on the lateral arms and the 4-5 in the keel. Four of these bundles are larger than the other, with well-developed sclerenchyma tissue like a "cap". The biggest pair (width 92-155 μm and height 68-127 μm) of the vascular bundles is located at the ends of the arms (Fig 4B). Another pair of big vascular bundles (width 78-158 μm and height 66-135 μm) is positioned in the corners of the keel. The xylem is oriented towards the adaxial side with the phloem beneath. The epidermal cells of both surfaces have thickened external cell walls, a thick cuticular layer and uniform micropapillary relief (Fig. 4C). The assimilating parenchyma has a distinct palisade layer of 1-2 rows of cells. The spongy parenchyma is located in the zones around the stomata (Fig. 4D) between the ribs of the vascular bundles. The measured anatomical characters are listed in Tab. 2 and compared with those of *C. randjeloviciorum* (Harpke et al. 2017).

The epidermal cells are elongated, rectangular to elliptical, with straight anticlinal walls (Fig. 5). The cells of the adaxial epidermis (Fig. 5A) are 264-785 μm long and 10-39 μm wide, distinctly longer than those of the abaxial epidermis. The abaxial epidermis (Fig. 5B) is represented by basic cells (length 44-111 μm and width 15-29 μm) and stomata. The stomata are anomocytic, *Amaryllis* – type (length 23-34 μm and width 15-26 μm), situated only on the lower surface of the leaf, in the zone of the leaf arms and in the lateral zone of the keel, between the nerves.

ITS sequences

The BLAST analysis of the obtained ITS sequence compared with the Nucleotide data from NCBI displayed similarity (99.34%) with the sequences LT222361 (*Crocus adamioides*, Harpke et al. 2016, GAT7136, holotypus) and

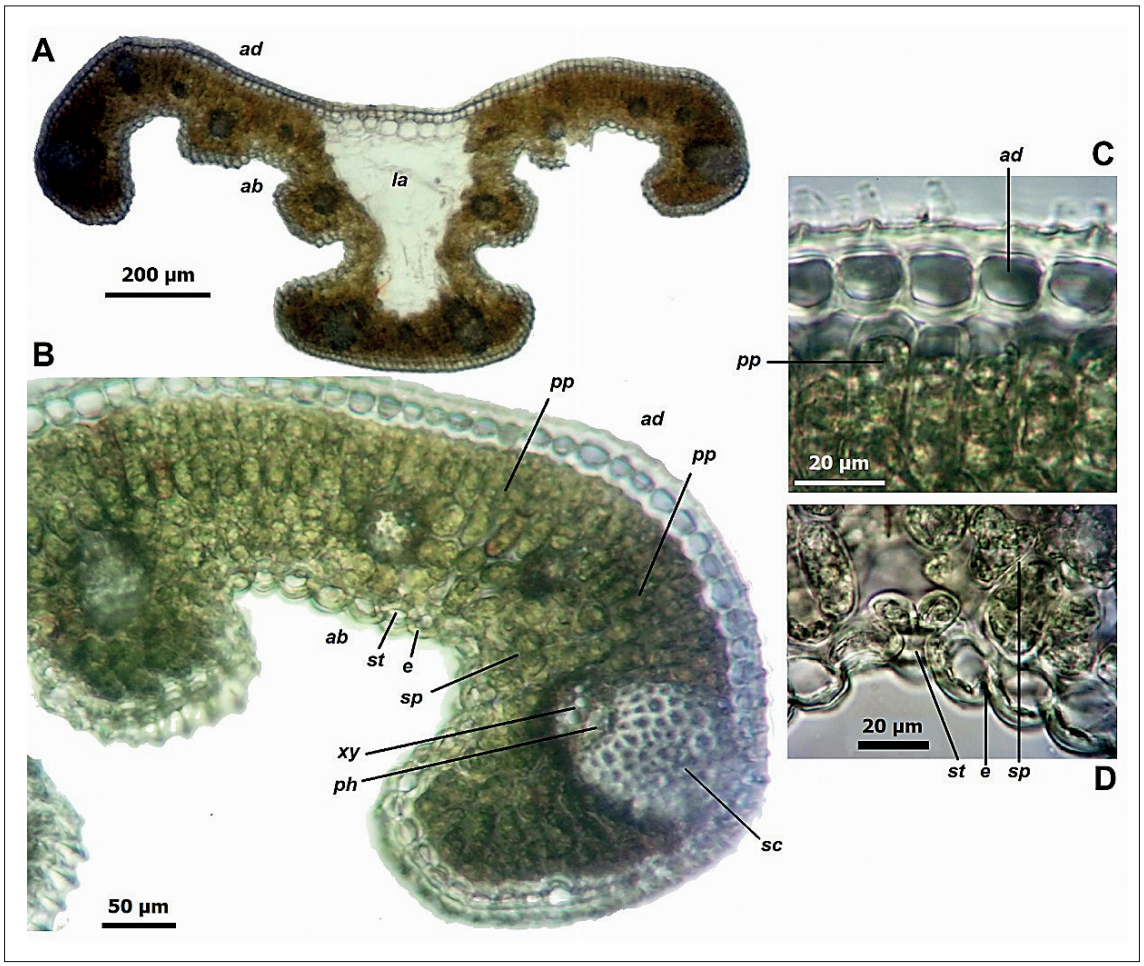

Fig. 4. *Crocus adamioides* from Toundja hilly plain (voucher SOA 062625): A – leaf cross sections (3.2×), B – arm detail (10×), C – adaxial epidermis (40×), D – abaxial epidermis (40×). Abbreviations: ad – adaxial side, ab – abaxial side, la – lacuna area, e – epidermis, pp – palisade parenchyma, sp – spongy parenchyma, sc – sclerenchyma cap, ph – phloem, xy – xylem).

Tab. 2. Leaf anatomy character measurements (range and mean ± standard deviation (SD)) of Bulgarian collections of *Crocus adamioides* Kernd. et Pasche, compared to the data of *C. randjeloviiciorum* Kernd., Pasche, Harpke et Raca (Harpke et al. 2017).

	Crocus adamioides		*Crocus randjeloviciorum*	
	Range	Mean ± SD	Range	Mean ± SD
Section width, µm	1053 – 1931	1555 ± 308	2644 – 3668	3154 ± 311
Section height, µm	216 – 610	399 ± 103	446 – 692	563 ± 75
Arm width, µm	358 – 681	530 ± 105	1164 – 1726	1393 ± 170
Vascular bundles, count	9 – 17	12 ± 3	11 – 21	18 ± 3
Palisade tissue – height, µm	25 – 84	51 ± 13	51 – 90	68 ± 11
Spongy tissue – height, µm	22 – 63	42 ± 9	37 – 68	50 ± 8
White stripe, µm	224 – 291	275 ± 24	323 – 599	434 ± 91
Adaxial epidermal cell – length, µm	264 – 785	531 ± 131	.	.
Adaxial epidermal cell – height, µm	16.5 – 30.5	22 ± 3.2	14 – 19	16 ± 2
Adaxial epidermal cell – width, µm	7.7 – 18.8	12.6 ± 2.3	13 – 18	16 ± 1
Palisade cell – height, µm	24.4 – 42.8	35 ± 4.7	34 – 61	46 ± 6
Palisade cell – width, µm	10.3 – 16.9	12.8 ± 1.6	13 – 19	17 ± 1
Spongy cell – height, µm	8 – 23.7	14.5 ± 3.7	14 – 22	18 ± 2
Spongy cell – width, µm	12 – 25.3	19.2 ± 4.2	21 – 33	26 ± 3
Abaxial epidermal cell – height, µm	7 – 18	12.1 ± 2.9	12 – 20	16 ± 2
Abaxial epidermal cell – width, µm	7 – 21	14.8 ± 3.5	13 – 22	18 ± 2
Abaxial epidermis cell – length, µm	44 – 111	76 ± 19	.	.
Stomata – length, µm	24 – 34	28 ± 3	.	.
Stomata – width, µm	15 – 26	21 ± 2	.	.

Fig. 5. *Crocus adamioides* from Toundja hilly plain (voucher SOA 062625)**.** Leaf epidermis: A – adaxaial epidermis (10×), B – abaxial epidermis (10×).

HE664018 (*C. biflorus* subsp. *pulchricolor*, Harpke et al. 2013), followed byMF766260 (99.18%, *C. randjeloviciorum*, Harpke et al. 2017). The region 5.8s rRNA was identical in the whole genus and this fact allows use of the neutral evolutionary model in the phylogenetic analysis of the whole ITS1-5.8SrRNA-ITS2 sequence. The resulting tree looks similar to that published by Harpke et al. (2017). The Bulgarian specimen was placed in a clade together with the specimens designated as *C. adamioides* and *C. pulchricolor*, in proximity with the clade containing *C. randjeloviciorum*. The specimens of *C. biflorus* subsp. *adamii* s.str. remain as an outgroup (Fig. 6).

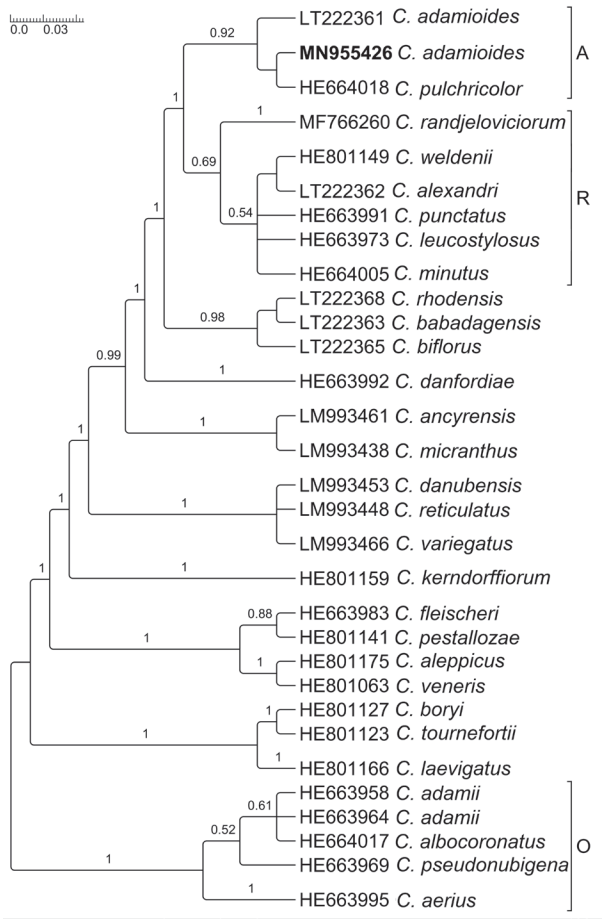

Fig. 6. Phylogenetic tree obtained by Bayesian phylogenetic inference of the nuclear rDNA ITS regions using the methodology of Harpke et al. (2017). A – clade of *Crocus adamioides*, R – clade of *C. randjeloviciorum*, O – clade of *C. biflorus* subsp. a*damii* s.str. Posterior probabilities designated by numbers.

Discussion

The genus of *Crocus* consists of critical taxa, and their discussion remains open. After the monograph of Mathew (1982), the status of the infraspecific taxa in the polymorphic groups was reassigned as species (Mathew et al. 2009).

The first report about *C. adamioides* was from Turkey, the mountain Yıldız Dağları in the province of Kırlkareli, at an altitude between 900 and 1100 m a.s.l. (Kerndorff et al. 2012). Later, Rukšāns confirmed the species with a locality near Kofçaz – a place very close to the border with Bulgaria. He believed that the mountain ridge where it was found stretches further into adjacent Bulgaria, and *C. adamioides* can be found there, too (Rukšāns 2017).

Along a straight line, Bulgarian localities of *C. adamioides* are found 90 km northwest of the locus classicus. Not only the distance but also the lack of high mountains functioning as a dividing system are the reasons for the uninterrupted distribution range of the species on Bulgarian territory, outlining the presence of *C. adamioides* in the southern part of the Balkan Peninsula. This plant is not uncommon in the investigated Bulgarian localities, where most of the populations have numerous individuals. Nevertheless, we did not find specimens of this taxon deposited in the national herbaria in Bulgaria, or as a species mentioned in the Bulgarian floristic literature. However, in a study of *C. adamioides*, Rukšāns (2017) commented on his observations on the morphology of this species, based on material from Bulgaria. It is plausible that the species in Bulgaria had been previously unnoticed due to its inconspicuous habit. We suppose that it is due to the ephemeral flowering period, that the species has remained unnoticed until now. The flowering period of the collected samples starts at the end of January and lasts until the end of February, similarly to the samples described from Kırklareli province. Moreover, when the weather is cold or cloudy, the flowers remain closed and almost invisible. The described facts suggest the high probability that the area of distribution is wider than represented here, occurring in similar habitats in other localities in the country.

Crocus adamioides has morphological similarities with *C. randjeloviciorum*. The main morphological differences between the two species are listed in Tab. 1. One of the clearly defined morphological features that distinguish *C. adamioides* from the closely related species *C. randjeloviciorum* is the distinct separation of the teeth on the basal rings of the corm. The teeth of *C. adamioides* are much longer (0.5-1.5-2 mm) and more evenly spaced than those of *C. randjeloviciorum*, which are tiny (less than 0.5 mm) and rarely present. The flower segments of the specimens from Bulgaria have similar dimensions to those from Turkey, while the flower size of *C. randjeloviciorum* has greater values. Another clearly distinctive morphological character is the indumentum of the filaments – hairy in *C. randjeloviciorum*, and glabrous in *C. adamioides*. The investigated plants have glabrous filaments.

The anatomical cross-section of the leaf is a specific taxonomical marker in the genus *Crocus*. It is a useful character for the determination of the species, especially in the lack of discretion between the morphological criteria. The anatomical sections of the studied plants are similar to those of *C. randjeloviciorum*. The quantitative morphological characters of the leaves show higher values in *C. randjeloviciorum* than those from the Bulgarian populations (Tab. 2). The keel is not squared but slightly curved. The arms are at least twice as short as those of *C. randjeloviciorum*. The white stripe takes up 15-21% of the leaf cross section, while the white stripe of the leaves of *C. randjeloviciorum* takes 12-16% of the cross section. There are few vascular bundles than in *C. randjeloviciorum*. The adaxial epdermis is thicker than that of *C. randjeloviciorum*. The biggest vascular bundles in the sections of the collected material of *C. adamioides* from Bulgaria are terminal in the arms (Fig. 4 B). In comparison, the sections of *C. randjeloviciorum* have a terminal smaller bundle. Papillae are not presented but the cuticles all adaxial epidermal cells are slightly as elongated like micropapillae (Fig. 4 C), not longer than the height of the epidermal cells. Because of the limited distribution of the taxon, data on the anatomical quantitative and qualitative characteristics of the

leaf in *C. adamioides* are not reported in the literature. We believe that they will be useful in future studies of other closely related species in the *Nudiscapus* section.

Genetic distances in the genus *Crocus* frequently correlate with geographic distances (Kerndorff et al. 2017). This suggests that a combination of polyploidy and geographical speciation may have driven cladogenesis in the group. Despite the large number of species identified in the last decade, supported solely by ITS sequences, intra- and inter-population variability of these species have not been investigated, making it difficult to comment on our results in the general model of the *Nudiscapus* polymorphic group. The results show a relationship between the polymorphism and the genetic diversity of the *C. biflorus* group. The samples of *C. adamioides* remain in one node with *C. randjeloviciorum* but in distinct clade (Fig. 6). The phylogenetic analysis based on ITS sequences confirms that the investigated population belongs to *C. adamioides*.

The represented analyses confirm the presence of *C. adamioides* in Bulgaria. On the other hand, the presence of the taxa belonging to the putative *C. biflorus* group, which was considered widespread in the Balkans – from Greece in the south to Rhodes, Turkey (Rukšāns 2017) and Bulgaria (Velchev 1964) – remains questionable. Even though taxonomic revisions have led to the description of the new species in the neighbouring Balkan regions (Kerndorff et al. 2013, Harpke et al. 2017), the taxonomic structure of the taxa in Bulgaria is still unresolved, since no nomenclatural or taxonomic revisions of the species composition of the genus have been carried out. Future research should be focused on taxa from the *C. biflorus* s.l. group with unclear phylogenetic relationships and with no clearly distinguishing morpho-anatomical features. Revision of all specimens deposited as *C. biflorus* in many collections in Bulgaria is necessary. In order to disentangle the complex taxonomic status and relationships, the traditional morphological approach should be combined with molecular studies. Such an analysis would confirm the hypothesis that it is an aggregate of species with a severe morphological syndrome, possibly due to ongoing hybridization, species formation processes, and recent divergence.

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