

Phylogenetic relationships of some Turkish *Crocus* (Iridaceae) taxa based on morphological and anatomical characters

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Abstract: In this study, relationships among the 15 taxa of the genus *Crocus* L. distributed in Turkey were analysed using 29 morphological and 4 anatomical characters. Analysis of the data set utilising maximum parsimony criterion with Branch-and-Bound search algorithm yielded 32 most parsimonious trees. Bootstrap analysis with the majority rule consensus algorithm generated a consensus tree supporting some branches. Our data mostly did not agree with the previous sectional and serial treatments. The most interesting result was the condition of *Crocus pallasii*, which was previously included in the section *Crocus*. *C. pallasii* showed a sister group relationship with *C. cancellatus* from the section *Nudiscapus* series *Reticulati* in this work. Previously described sections *Flavi* and *Reticulati* did not show monophyly for their taxa based on our analysis (e.g., *C. gargaricus* and *C. cancellatus* of the section *Reticulati* were not monophyletic based on our data set). Interestingly, *C. gargaricus* of the section *Reticulati* was sister to the section *Nudiscapus* series *Biflori* members, specifically with *C. leichtlinii*. Sectional and serial treatment of *C. fleischeri* was consistent with our results. Our data suggest that more morphological data along with molecular data are needed for reliable conclusions on the studied taxa.

Key words: Anatomy, *Crocus*, morphology, parsimony, phylogenetic

Türkiye’de yayılış gösteren bazı *Crocus* (Iridaceae) taksonları arasında morfolojik ve anatomik karakterlere dayalı filogenetik akrabalık ilişkileri

Özet: Bu çalışmada, 29 morfolojik ve 4 anatomik karakter kullanılmak suretiyle Türkiye’de yayılış gösteren 15 *Crocus* L. taksonu arasındaki akrabalık ilişkileri analiz edilmiştir. Maximum parsimony kriteri ile Dallandır-ve-Bağla algoritması kullanılarak yapılan veri setinin analizi 32 eşit şekilde parsimoni gösteren en tutumlu (most parsimonious) ağacı vermiştir. Bazı dallar Çoğunluk Uyumluluk metodu algoritması kullanılarak yapılan Bootstrap analiziyle desteklenmiştir. Verilerimiz çoğunlukla, önceden yapılmış olan seksiyonel ve serilere ait sonuçlarla uyuşmamıştır. Bu çalışmadaki en ilgi çekici sonuç, önceki çalışmalarda, *Crocus* seksiyonunda gösterilen *Crocus pallasii*’nin durumudur. *C. pallasii* bu çalışmada *Reticulati* serisinin *Nudiscapus* seksiyonundan *C. cancellatus* ile kardeş gurup özelliği göstermiştir. Önceden tanımlanmış *Flavi* ve *Reticulati* seksiyonları bizim analizimize göre monofili göstermemiştir (örneğin; *Reticulati* seksiyonundan *C. gargaricus* ve *C. cancellatus* bizim verilerimize göre monofiletik değildir). İlginç bir şekilde, *Reticulati* seksiyonundan *C. gargaricus*, *Biflori* serisinin üyelerinden *Nudiscapus* seksiyonu üyeleri ile kardeş gurup özelliği göstermiştir (özellikle *C. leichtlinii* ile). *C. fleischeri*’nin seksiyonel ve seriye ait işlemleri sonuçlarımızla tutarlıdır. Bizim verilerimiz, çalıştığımız taksonların üzerinde güvenilir sonuçlara ulaşılabilmesi için, moleküler verilerle birlikte daha fazla morfolojik veriye gereksinim olduğunu göstermektedir.

Anahtar sözcükler: Anatomi, *Crocus*, filogenetik, morfoloji, parsimoni

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Introduction

Iridaceae is a large and diverse family of about 92 genera and about 1800 species mainly distributed in the southern hemisphere (Ali & Mathew, 2000). *Crocus* L., which has elegant and stylish flowers, is a genus of the Iridaceae family.

The genus *Crocus* includes 88 species distributed from south-western Europe, through central Europe to Turkey and south-western parts of Asia, as far east as western China (Alavi-Kia et al., 2008; Petersen et al., 2008). *Crocus* is represented by 36 species in 71 taxa in Turkey. Thirty-five of these are endemic to Turkey (Mathew, 1984, 1988, 2000; Kerndorff & Pasche 2004; Özhatay et al., 2009). Due to taxon diversity, Turkey might be considered as the homeland of *Crocus* (Candan et al., 2009).

Systematic studies relating to *Crocus* have been presented by Pasche (1994a, 1994b), Kerndorff and Pasche (1994, 1996a, 1996b, 1997), but there have been a few studies on the morphology and anatomy of *Crocus* species in Turkey (Erol & Küçüker, 2005, 2007). Shorina (1975) investigated leaf structure in some saffrons in association with the evolution of the genus *Crocus*. Rudall and Mathew (1990) worked on leaf anatomy of *Crocus* in the context of the systematics of the subfamily. This particular study of Rudall and Mathew (1990) has an important place among these studies as it presents anatomical data belonging to the leaf for consideration as follows: general outline of cross sections, the existence of papillae, the presence of anticlinal walls of the epidermis cells as situate or smooth, the thickness of the sclerenchyma layer on vascular bundles, and the extension of sclerenchyma out of lamina. Pulido et al. (2004) carried out a comparative morpho-anatomical study of 3 species pertaining to the genus *Crocus*.

Pollen morphology data (Pınar et al., 2009a, 2009b) and karyomorphological data (Martin et al., 2009) can be employed in discussing taxonomic problems. On the other hand, anatomical and palynological data can also be used to infer phylogenetic relationships among the taxa of interest (Almeida et al., 2009).

Rudall (1993) investigated the anatomy and systematics of Iridaceae and assessed its phylogeny by means of a cladistic analysis utilising characters from morphology, anatomy, and biochemistry.

There are some very recent phylogenetic studies on the genus *Crocus* using molecular data (Petersen et al., 2008, Sık et al., 2008; Seberg & Petersen, 2009). Frello & Harrison (2000) investigated the chromosomal and species distributions of cloned non-homologous repetitive DNA elements from *C. vernus*. Alavi-Kia et al. (2008) examined analysis of genetic diversity and phylogenetic relationships in *Crocus* genus of Iran using inter-retrotransposon amplified polymorphism.

In this study, 15 *Crocus* taxa were analysed using 29 morphological and 4 anatomical characters (Table 1). This study provides information on phylogenetic relationships of 15 *Crocus* taxa from Turkey based on morphological and anatomical characters.

Materials and methods

Crocus taxa examined in this study were collected in and around the city of Balıkesir, Turkey during 2004-2006. Materials of 9 taxa are kept in the herbarium of Balıkesir University, Turkey. *Crocus* taxa used for the anatomical and morphological studies were collected from their natural habitat.

Collection data including localities and collector information for the examined specimens are given in Table 2.

Morphological studies

Biometric measurements of vegetative and generative organs of 9 *Crocus* taxa were performed over herbarium samples while biometric measurements of 6 *Crocus* taxa were adapted with the data obtained from variety of sources including Mathew (1984, 1988, 2000), Akan and Eker (2004), Özdemir et al. (2004, 2005), Işık and Dönmez (2006), Akan et al. (2007), Satıl and Selvi (2007). For morphological studies, 5 plant materials were sampled for each taxon. Morphological characters obtained from biometric measurements are provided in Table 3.

Anatomical studies

Fresh plants were fixed in 70% alcohol. Scape and leaves of flowered plants were used in the anatomical studies. For anatomical studies, 5 plant materials were also sampled for each taxon. Transverse sections of

Table 1. Classification of studied *Crocus* taxa.

Subgenus <i>Crocus</i>
Section <i>Crocus</i>
Series <i>Crocus</i>
<i>C. pallasii</i> Goldb. subsp. <i>pallasii</i>
Section <i>Nudiscapus</i>
Series <i>Reticulati</i>
<i>C. cancellatus</i> Herbert subsp. <i>damascenus</i>
<i>C. cancellatus</i> Herbert subsp. <i>mazziaricus</i> (Herbert) Mathew
<i>C. gargaricus</i> Herbert subsp. <i>gargaricus</i>
Series <i>Biflori</i>
<i>C. bifloris</i> Miller subsp. <i>nubigena</i> (Herbert) Mathew
<i>C. chrysanthus</i> (Herbert) Herbert
<i>C. danfordiae</i> Maw
<i>C. leichtlinii</i> (Dewar) Bowles
Series <i>Flavi</i>
<i>C. flavus</i> Weston subsp. <i>flavus</i>
<i>C. flavus</i> subsp. <i>dissectus</i> T.Baytop & Mathew
<i>C. antalyensis</i> Mathew
<i>C. olivieri</i> J.Gay subsp. <i>istanbulensis</i> Mathew
<i>C. candidus</i> E.D.Clarke
Series <i>Intertexti</i>
<i>C. fleischeri</i> J.Gay
Series <i>Speciosi</i>
<i>C. pulchellus</i> Herbert

The abbreviations of the series names; *Crocus*: CROC; *Reticulati*: RETI; *Biflori*: BIFL; *Flavi*: FLAV; *Intertexti*: INTE; *Speciosi*: SPEC. The abbreviations for supraspecific taxa are also used in Figures 1 and 2.

Table 2. Collection data of studied *Crocus* taxa.

Taxa	Collection data and collector's number
* <i>C. fleischeri</i>	B1 Manisa; Maldan district 450 m, 06.02.2003, Özdemir, Akyol, Alçıtepe; C2 Muğla: Yılanlı Mountain, 1350 m, 14.03.2001, O.O. 19.
* <i>C. gargaricus</i> subsp. <i>gargaricus</i>	B1 Balıkesir: Edremit, Kazdağı, Kartalçimen, 1700 m, 07.04.2005, F.S. 1382.
* <i>C. danfordiae</i>	A4 Ankara: S.İşık (1003), E.O.Dönmez, A.A.Dönmez (HUB); B1 Manisa: Spil Mountain, 1200 m, 02.02.2005, E.E. 124.
<i>C. chrysanthus</i>	B1 Balıkesir: Dinkçiler, Taşocağı, 140 m, 15.02.2005, S.V. 1011.
* <i>C. leichtlinii</i>	C7 Şanlıurfa: Siverek, Karacadağ, Rame Stream, 28.03.2003, 1390 m, İ.Eker 369.
* <i>C. bifloris</i> subsp. <i>nubigena</i>	B1 Balıkesir: Edremit, Kazdağı, 1400 m, 15.02.2005, F.S. 1384.
<i>C. flavus</i> subsp. <i>flavus</i>	B1 Manisa: Salihli-Bahçecik district, forest, 600 m, 31.01.2003, Baran 014.
* <i>C. flavus</i> subsp. <i>dissectus</i>	B1 Balıkesir: Çağış, 240 m, 16.03.2006, S.V. 1119.
* <i>C. antalyensis</i>	B2 Bilecik: Bozüyük, Erikli, 700m, 17.02.2004, R.P. 12.
* <i>C. olivieri</i> subsp. <i>istanbulensis</i>	B1 Balıkesir: Değirmen boğazı, 180 m, 21.03.2006, S.V. 1123.
* <i>C. candidus</i>	B1 Balıkesir: Edremit, Ortaoba village, 350 m, 28.02.2005, S.V. 1021.
<i>C. pallasii</i> subsp. <i>pallasii</i>	B1 Balıkesir: Savaştepe, Kozören village, 500 m, 14.12.2004, S.V. 1006.
<i>C. cancellatus</i> subsp. <i>damascenus</i>	C7 Şanlıurfa: NW of Şanlıurfa, Direkli Hills, E & N slope, around Huzurevi, rocky steppe, 600-800 m, 20.10.2001, İ.Eker 5.
<i>C. cancellatus</i> subsp. <i>mazziaricus</i>	B1 Balıkesir: Savaştepe, Kozören village, 500 m, 14.12.2004, S.V. 1007.
<i>C. pulchellus</i>	B1 Balıkesir: Savaştepe, Çukurhüseyin village, 200 m, 12.11.2006, S.V. 1196.

*Endemic taxon

Table 3. Morphological and anatomical characters used for phylogenetic analysis and their character states.

MORPHOLOGICAL CHARACTERS	Character Number	Character States		
Flowering period	1	Autumn (0)	Winter (1)	Spring (2)
Flowering condition	2	synanthous (0)	hysteranthous (1)	
Flower colour	3	white (0)	bright yellow-orange (1)	blue-purple (2)
Anther orientation	4	inward-oriented (0)	outward-oriented (1)	
Anther colour	5	cream-whitish cream (0)	yellow-orange (1)	dark brown (2)
Anther base	6	spotless (0)	with spot (1)	
Perianth tip	7	subacute (0)	acute-acuminate (1)	obtuse (2)
Perianth segments	8	unequal (0)	equal (1)	
Tunica structure of corm	9	membranaceous (0)	membranaceous-leathery fibrous (1)	leathery-straight fibrous (2)
Tepal veins	10	non-distinct (0)	distinct (1)	
Tunica base of corm	11	without ring (0)	with ring (1)	
Tooth & ring condition of tunica base	12	without tooth (0)	toothed (1)	
Tunica fibrous neck formation	13	not prolonged (0)	prolonged (1)	
Perianth tube colour	14	white (0)	blue-purple (1)	yellow-orange (2)
Style colour	15	white (0)	red (1)	yellow-orange (2)
Number of styles	16	3-parted (0)	6-parted (1)	7 and over (2)
Number of cataphyll	17	3 (0)	5 (1)	
Profile condition	18	absent (0)	present (1)	
Bracteole	19	absent (0)	present (1)	
Anther length	20	13 mm and shorter (0)	longer than 13 mm (1)	
Filament length	21	8 mm and shorter (0)	longer than 8 mm (1)	
Filament colour	22	white-grayish (0)	yellow-orange (1)	
Length of style comparing to anthers	23	equal or not longer (0)	longer than anthers (1)	
Leaf width	24	3 mm and shorter (0)	longer than 3 mm (1)	
Style length	25	15 mm and shorter (0)	longer than 15 mm (1)	
Pollen aperture type	26	short furrow (0)	spiral furrow (1)	
Number of flowers	27	2-4 flowers (0)	single flower (1)	
Corm diameter	28	5-20 mm (0)	21-36 mm (1)	
Leaf width	29	5-39 mm (0)	40-79 mm (1)	
ANATOMICAL CHARACTERS				
Projecting bundle parts towards Epidermis (swollen)	30	absent (0)	present (1)	
Micropapil projections of cuticle	31	absent (0)	present (1)	
Number of vascular bundles	32	8 and lesser (0)	more than 8 (1)	
Trichome-like epidermal outgrowth	33	absent (0)	present (1)	

leaves and scape were cut manually. Tissues were stained with Floroglucin+HCl and embedded in glycerin-gelatine (Baytop, 1972). An Olympus BX50 phase contrast microscope with a drawing tube was used in anatomical studies. Anatomical characters and their character states are listed in Table 3.

Phylogenetic analysis

Table 4 shows characters and their states used to form data matrix during phylogenetic analysis using PAUP* (Swofford, 2001). In PAUP*, the following settings were used in the Branch-and-Bound search: Optimality criterion = Parsimony (MP), addition sequence = furthest, multiple trees ('Multrees') option in effect, initial 'MaxTrees' setting = 100, branches collapsed (creating polytomies) if maximum branch length was zero, topological constraints not enforced, trees were unrooted. The Bootstrap analysis (Felsenstein, 1985) with 1000 replicates was also performed to see how some branches were statistically supported during the phylogenetic analysis.

Results and discussion

We have investigated phylogenetic relationships among 15 *Crocus* taxa using 29 morphological and 4 anatomical characters. The characters and their character states for each taxon used during the analysis are shown in Table 3.

Analysis of data set utilising maximum parsimony criterion with Branch-and-Bound search algorithm yielded 32 most parsimonious trees. Only 4 branches of the tree received bootstrap values above 50%. The score of those MP trees were found as 72 steps. Tree number 1 of those 32 most parsimonious trees is shown in Figure 1. Indices and values obtained after the Branch-and-Bound search are as follows: Consistency Index (CI) = 0.5139, Homoplasy Index (HI) = 0.4861, Retention Index (RI) = 0.6277. Data set contained a total of 29 ordered type characters (Wagner). All characters had equal weight and 2 characters were constant. Seven variable characters were parsimony-uninformative whereas 24 characters were parsimony-informative. In Figure 1, 4-letter abbreviations in capitals on the right side of the taxon

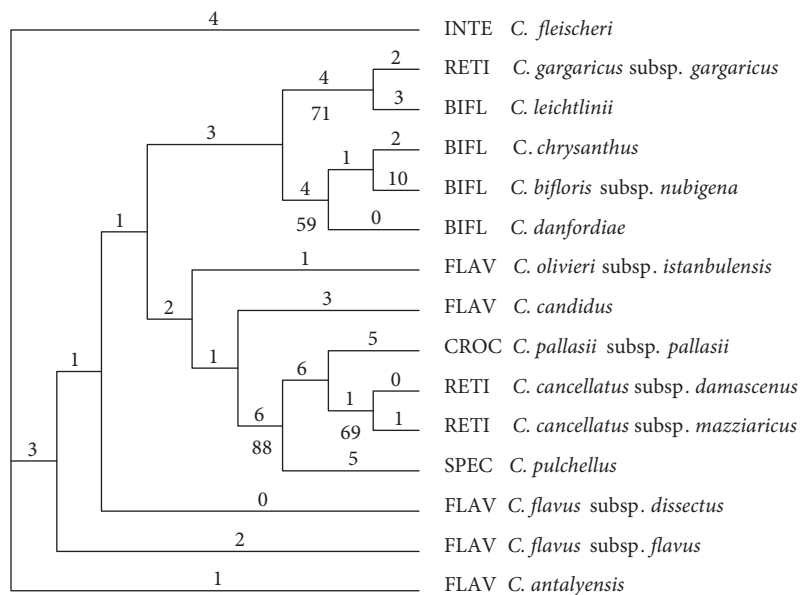


Figure 1. Tree number 1 of the 32 Most Parsimonious trees of *Crocus* based on morphological and anatomical characters (Numbers above and under branches indicate branch lengths and Bootstrap supports, respectively).

Table 4. Data matrix used for phylogenetic analysis (see Table 3 for the explanation of characters and their states).

↓ SPECIES/CHARACTERS →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
<i>C. fleischeri</i>	1	0	0	1	1	0	1	1	1	0	0	0	0	2	2	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	1	1	1		
<i>C. gargaricus</i> subsp. <i>gargaricus</i>	2	0	1	1	1	0	2	1	2	0	0	0	0	2	2	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	1	1		
<i>C. chrysanthus</i>	2	0	1	1	1	1	0	1	0	0	1	2	0	2	2	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0		
<i>C. danfordiae</i>	2	0	1	1	1	1	0	1	0	0	1	2	0	2	2	0	1	0	1	0	0	1	0	0	1	1	0	0	0	0	1	1	1		
<i>C. biflorus</i> subsp. <i>nubigena</i>	0	0	2	1	2	1	0	1	0	1	1	2	0	2	2	0	1	0	1	0	0	1	0	1	1	0	0	1	0	1	1	0	0		
<i>C. leichtlinii</i>	2	0	1	1	0	0	2	1	2	0	0	0	0	2	2	0	0	0	1	0	0	1	0	0	1	1	1	0	0	0	1	1	1		
<i>C. flavus</i> subsp. <i>flavus</i>	2	0	1	1	1	0	0	1	0	0	0	0	1	2	2	0	1	0	1	1	0	1	0	1	0	1	1	0	0	0	1	1	1		
<i>C. flavus</i> subsp. <i>dissectus</i>	2	0	1	1	1	0	0	1	0	0	0	0	2	2	1	1	0	1	1	0	1	0	1	0	1	0	1	0	0	0	1	1	1		
<i>C. antalyensis</i>	1	0	2	1	1	0	0	1	0	0	0	0	0	2	2	1	1	0	1	0	0	1	0	0	0	1	1	0	0	0	1	1	1		
<i>C. olivieri</i> subsp. <i>istanbulensis</i>	2	0	1	1	1	0	0	1	0	0	0	0	0	2	2	1	1	0	1	1	1	0	1	1	0	1	1	0	1	0	1	1	1		
<i>C. candidus</i>	2	0	0	1	1	0	2	1	0	0	0	0	0	2	2	1	1	0	1	0	0	1	0	1	1	1	0	1	1	0	1	1	1		
<i>C. pallasii</i> subsp. <i>pallasii</i>	0	1	2	1	1	0	1	1	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0	0	0	1	0	0	1	0	0	1	0	1	
<i>C. cancellatus</i> subsp. <i>damascenus</i>	0	1	2	1	1	0	1	1	1	1	0	0	1	1	2	0	1	0	1	1	0	1	0	0	1	1	1	1	0	0	1	0	1	0	1
<i>C. cancellatus</i> subsp. <i>mazziaricus</i>	0	1	2	1	1	0	1	1	1	1	0	0	1	1	2	0	1	0	1	1	0	1	0	1	1	1	1	1	0	0	1	0	1	0	1
<i>C. pulchellus</i>	0	1	2	1	0	0	0	1	0	1	1	0	1	1	2	1	1	0	1	1	0	1	0	1	0	1	0	1	0	1	1	0	1	1	1

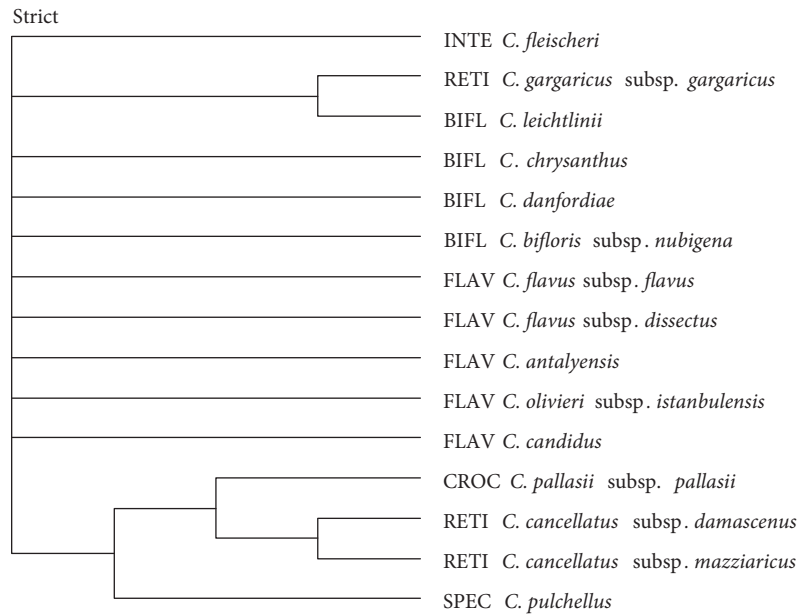


Figure 2. Strict consensus tree of the 32 most parsimonious trees after Branch-and-Bound search for 15 *Crocus* taxa.

names show the sectional and serial treatments suggested by previous workers (Mathew, 1982; Rudall & Mathew, 1990; Petersen et al., 2008) for the studied taxa. Figure 2 shows the strict consensus of 32 most parsimonious trees after Branch-and-Bound search.

The most interesting result was the sister group relationship of *C. pallasii*, which was previously included in the section *Crocus* (Figure 1). With a strong Bootstrap support (89%, Figure 1), *C. pallasii* demonstrated monophyly with *C. cancellatus*, which was placed in the section *Nudiscapus* series *Reticulati* by Rudall and Mathew (1990) and Petersen et al. (2008). Based on our analysis, previously described sections (see Rudall & Mathew, 1990; Petersen et al., 2008) *Flavi* and *Reticulati* did not show monophyly for the taxa included in them. For example, *C. gargaricus* and *C. cancellatus* of the section *Reticulati* were not monophyletic depending on our data set. Section *Nudiscapus* series *Reticulati* was polyphyletic. *C. gargaricus* of section *Reticulati* showed monophyly with section *Nudiscapus* series *Biflori* members. Sectional and serial treatment of *C. fleischeri* was consistent with our results. Our data mostly did not

agree with the sectional and serial treatments of Rudall and Mathew (1990) and Petersen et al. (2008). Sık et al. (2008) pointed out that Turkey could be one of the centres of origin for the genus *Crocus* based on the existing high level of genetic variation depending on RAPD and ISSR markers.

There are several detailed molecular studies covering nearly all of *Crocus* taxa (Petersen et al., 2008; Seberg & Petersen, 2009). However, they are not covering both morphological and molecular aspects of the genus for the same analysis, i.e. they are not using both data sets for the analysis. We should acknowledge that it is beyond the scope of this article to address all the issues of the genus *Crocus*; however, we should here express the need to perform a phylogenetic analysis using both morphological and molecular data sets to establish a reliable phylogeny of either the studied taxa or all of the taxa within this genus. The Iridaceae family is a taxonomically difficult to analyse and a phylogenetically poorly understood family. The generic boundaries, species affiliations, and phylogenetic relationships vary from one author to another (Rodríguez & Catedral, 2003).

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