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The Importance in Phylogenetic Relationships of The Regions Belonging to Nuclear and Plastid DNA among *Crocus biflorus* Subspecies

Aykut YILMAZ*[1]

Abstract

The genus *Crocus* L. (Iridaceae) composed of about 200 species is taxonomically very problematic, because of introgression and backcrossing observed among closely related species. Furthermore, determination of new taxa and variable characters observed in these new taxa are other important reasons of the taxonomic problems. Recently, many molecular based studies to understand the phylogenetic relationships of the *Crocus* taxa show the presence of the problems among the taxa, especially in *C. biflorus* subspecies. As a result of this, some researchers state that the term of subspecies must be changed in the genus and the most of subspecies must be categorized as species. For these reasons, in this study, the four regions belonging to nuclear and plastid DNA (*ITS1-5.8S rRNA-ITS2*, *psbA-trnH IGS*, *rpoC1* and *trnL-trnF IGS*) were used to understand the identification and separation abilities of taxa studied, in addition to understanding the taxonomy of *C. biflorus* subspecies.

Keywords: *Crocus*, introgression, backcrossing, *C. biflorus*

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1. INTRODUCTION

The genus *Crocus* L. belonging to the family Iridaceae consists of about 200 recognized species distributed from western Europe and north west Africa to western China. Balkan Peninsula and Turkey are considered as the center of species diversity for the genus [1, 2, 3, 4].

The genus *Crocus* is famous with their flowers in different colours and popular ornamentals. However, *C. sativus* is the best popular species of the genus because of the Saffron known as the world's most expensive spices [5].

The genus is very complicated and hard to understand as taxonomic and phylogenetic. In addition to intermediate or variable characters observed in closely related species caused by introgression and backcrossing [6, 7], especially the continuously determination of new taxa and not assigned to any series [8] are the main reasons of taxonomic problems and doubtful species identification within the genus. Furthermore, recent phylogenetic analyses clearly show that subspecies of *C. biflorus* occur in distinct clades even in different series on the dendrogram prepared for phylogenetic relationships [9, 10]. This situation reveal that subspecies status of the genus for *C. biflorus* is incorrect. In other words, the term "subspecies" which was brought into the genus taxonomy by Mathew (1982) [1] can not be maintained any more [2].

Molecular studies such as PCR-based and especially in last years the studies on DNA sequence informations accelerated understanding of the genus taxonomy. As a result of these studies, taxonomical situation of the genus *Crocus* changed [9, 10, 11, 12] and many taxonomic classifications belonging to the genus were revised because of incorrect phylogenetic relationships.

Furthermore, two new series named as *Isauri* and *Lyciotauri* for section *Nudiscapus* were described by Kerndorff et al. (2014; 2015) [13, 14] and some subspecies of *C. biflorus* were grouped into these series [15].

For these reasons stated, determination of phylogenetic relationships among *Crocus biflorus* subspecies and grouped according to their genetic similarity of subspecies is necessary.

In this study, different regions containing *ITS1-5.8S rRNA-ITS2*, *psbA-trnH IGS*, *rpoC1* and *trnL-trnF IGS* belonging to nuclear and plastid DNA were used to understand the taxonomy of *C. biflorus* subspecies and to contribute the solution of the still existing problems.

2. MATERIAL AND METHODS

Sequence informations of four different regions containing *ITS1-5.8S rRNA-ITS2*, *psbA-trnH IGS*, *rpoC1* and *trnL-trnF IGS* belonging to nuclear and plastid DNA were provided from National Centre of Biotechnology Information [16]. Sequence informations belonging to *Crocus biflorus* subspecies for each regions were separately examined to evaluate discrimination ability of each regions studied and to understand the taxonomy and phylogenetic relationships of *C. biflorus* subspecies. For this aim; 16 subspecies of *C. biflorus* for *ITS1-5.8S rRNA-ITS2*, 9 subspecies of *C. biflorus* for *psbA-trnH IGS*, 16 subspecies of *C. biflorus* for *rpoC1*, 17 subspecies of *C. biflorus* for *trnL-trnF IGS* and their sequence informations were performed by using Molecular Evolutionary Genetics Analysis (MEGA).

After the sequence informations for *C. biflorus* subspecies were obtained, multiple sequence alignments for each regions were seperately performed by using MEGA X [17].

After that, the alignment sequence informations for each barcoding regions studied were used to assign the variable sites, probabilities of substitution from one base to another base, transitional substitutions (%), transversional substitutions (%), transition/transversion rates for purines-pyrimidines and nucleotide frequencies (Table 5).

Moreover, the tables showing the variable sites were prepared for each regions separately (Table 1, 2, 3, 4).

Neighbour-joining dendrograms showing bootstrap values on branches were provided for each regions examined to determine the species identification abilities and phylogenetic relations among *C. biflorus* subspecies.

All positions containing gaps and missing data for each regions were eliminated with the complete deletion option of the program for effective analyses.

3. RESULTS AND DISCUSSIONS

3.1. Analysis results for ITS1-5.8S rRNA gene-ITS2

Sequence informations belonging to 16 subspecies of *C. biflorus* were provided from NCBI [8, 10]. These DNA regions containing sequence informations of *ITS1-5.8S rRNA gene-ITS2* were aligned by using Molecular Evolutionary Genetics Analysis (MEGA X). The sites with missing/ambiguous data and gaps were excluded for effective analyses in the determination of alignment length and variable sites. After the exclution of these regions, alignment length for taxa studied was established as 604 bp. Totally 62 variable sites for taxa examined were determined (Table 1).

Furthermore, the probabilities of substitutions from one base to another base were computed as transitional and transversional substitutions (Table 5). It was observed that rate of transitional substitutions with 75.72 % is higher than the transversional substitutions. In other words, it can be said that the variable sites among the *C. biflorus* taxa are highly caused by the substitutions between same base groups (purines; A ↔ G or pyrimidines; C ↔ T).

In addition to the rate of base substitutions, transition/transversion rate for purines (k_1), pyrimidines (k_2) and overall transition/transversion rate (R) were assigned as 3.73, 8.84 and 2.89, respectively.

The nucleotide frequencies for *ITS1-5.8S rRNA gene-ITS2* of *C. biflorus subspecies* were analysed as 37.42 % (A+T/U) and 62.58 % (C+G) (Table 5). In other words, it can be said that the DNA region examined consists of highly G and C bases.

Finally, Neighbor-Joining (NJ) dendrogram based on the sequence informations of *ITS1-5.8S rRNA gene-ITS2* for *C. biflorus* subspecies was drawn to evaluate the phylogenetic relationships of taxa studied (Figure 1). Furhermore, the separation ability of *ITS1-5.8S rRNA gene-ITS2* was evaluated for the taxa studied. Branch lengths in dendrogram infer the evolutionary distances. The evolutionary distances in dendrogram were computed using the Maximum Composite Likelihood method [18].

Table 1

Subspecies of *C.biflorus* and variable sites belonging to *ITS1-5.8S rRNA-ITS2* (The numbers show variable nucleotides)

	42	43	47	52	53	57	59	61	64	65	67	71	74	78	84	90
fibroannulatus	T	C	T	C	T	G	C	C	G	T	C	C	C	T	C	G
pulchricolor	.	T	C	A	A	.	.	T	.	.	.	.	.	C	.	.
albocoronatus	.	.	.	.	.	.	A	.	.	.	.	.	T	.	T	A
wattiorum	C	T	.	A	A	A	.	T	.	.	.	.	.	C	.	.
atrospermus	.	T	.	A	A	A	.	T	.	.	.	.	T	C	.	.
artvinensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
isauricus	.	T	.	A	A	A	.	T	.	C	.	.	.	C	.	.
tauri	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
punctatus	.	T	C	A	A	.	.	T	.	C	.	.	.	C	.	.
nubigena	.	T	C	A	A	A	.	T	.	.	.	.	T	C	.	.
yataganensis	.	T	.	A	A	.	.	T	T	.	.	.	.	C	.	.
caricus	.	T	.	A	A	A	.	T	.	.	.	.	.	C	.	.
ionopharynx	.	T	.	A	A	A	.	T	.	.	.	.	T	C	.	.
leucostylosus	.	T	C	A	A	.	.	T	.	C	.	.	.	C	.	.
crewei	.	T	.	A	A	A	.	T	.	.	T	T	.	C	.	.
pseudonubigena	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	99	100	101	112	125	136	137	138	143	144	145	149	152	166
fibroannulatus	T	A	A	A	A	G	C	G	C	C	A	T	C	C	G
pulchricolor	.	G	.	.	.	.	.	.	.	G	.	.	T	T	C
albocoronatus	.	.	.	.	.	A	.	A	.	.	.	.	.	.	.
wattiorum	.	G	.	.	.	.	.	.	.	G	.	C	T	.	C
atrospermus	.	G	.	.	.	.	T	.	.	G	.	.	T	.	C
artvinensis	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
isauricus	.	G	G	.	C	.	.	.	T	G	.	.	T	T	C
tauri	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	4 7 6	4 7 7	4 8 3	5 0 9	5 4 3	5 5 3	5 7 8	5 8 9	5 9 1	5 9 4	6 0 8	6 1 4	6 2 6	6 2 7	6 2 8	6 2 9
punctatus	.	G	.	.	.	.	.	.	.	G	.	.	T	T	C	
nubigena	.	G	.	.	.	.	.	.	.	G	.	.	T	T	C	
yataganensis	.	G	.	.	C	.	.	.	.	G	.	.	T	.	C	
caricus	.	G	.	.	C	.	.	.	T	G	.	.	T	T	C	
ionopharynx	C	G	.	.	.	.	.	.	.	G	G	.	T	T	C	
leucostylosus	.	G	.	.	.	.	.	.	.	G	.	C	T	T	C	
crewei	.	G	.	.	.	.	.	.	.	G	.	.	T	.	C	
pseudonubigena	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	
fibroannulatus	C	T	G	T	A	T	C	C	C	C	T	G	C	T	C	C
pulchricolor	.	.	.	.	T	.	.	.	T	.	.	A	T	C	.	G
albocoronatus	.	.	A	.	.	.	.	T	.	.	.	.	.	C	T	.
wattiorum	T	.	.	C	T	.	T	.	T	.	.	A	T	.	.	G
atrospermus	T	.	.	.	T	.	.	.	T	T	.	A	T	.	.	G
artvinensis	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.
isauricus	.	C	.	.	T	.	.	.	T	.	.	A	T	C	.	G
tauri	.	.	.	.	.	.	.	.	.	.	.	.	T	C	.	.
punctatus	.	C	.	.	T	.	.	.	T	.	.	A	T	C	.	G
nubigena	.	.	A	.	T	C	.	.	T	.	.	A	T	.	.	G
yataganensis	T	.	.	.	T	.	.	.	T	.	.	A	T	.	.	G
caricus	.	C	.	.	T	.	.	.	T	.	.	A	T	.	.	G
ionopharynx	.	C	.	.	T	C	.	.	T	.	.	A	T	.	.	G
leucostylosus	.	C	.	.	T	.	.	.	T	.	.	A	T	C	.	G
crewei	T	.	.	.	T	.	.	.	T	.	C	A	T	.	.	G
pseudonubigena	.	.	A	.	.	.	.	.	.	.	.	.	T	C	.	.

	6 3 2	6 3 4	6 3 7	6 3 8	6 3 9	6 4 0	6 4 2	6 4 3	6 4 8	6 4 8	6 4 8	6 4 8	6 4 9	6 5 1	6 5 2	6 5 4
fibroannulatus	T	G	T	C	T	T	G	G	G	A	G	T	G	T	G	
pulchricolor	.	.	.	T	C	G	C	A	.	G	A	G	C	A	.	
albocoronatus	.	.	.	.	.	.	.	.	A	.	.	G	A	.	.	
wattiorum	.	.	.	T	C	G	C	A	.	G	A	G	.	C	.	
atrospermus	.	.	.	T	C	G	T	A	.	G	A	G	.	C	.	
artvinensis	.	.	.	.	.	C	.	.	.	.	.	.	A	.	.	
isauricus	.	.	.	.	C	G	C	A	.	G	A	G	.	C	.	
tauri	.	T	C	T	.	G	.	.	A	.	.	G	A	.	.	
punctatus	.	.	.	T	C	G	C	A	.	G	A	G	C	C	.	
nubigena	.	.	.	T	C	G	C	A	.	G	A	G	C	C	.	
yataganensis	.	.	.	T	C	G	T	A	.	G	A	G	.	C	.	
caricus	.	.	.	T	C	G	C	A	.	G	A	G	.	C	.	
ionopharynx	.	.	.	T	C	G	C	A	.	G	A	G	C	C	.	
leucostylosus	.	.	.	T	C	G	C	A	.	G	A	G	C	C	.	
crewei	.	.	.	T	C	G	C	A	.	G	A	G	C	C	A	
pseudonubigena	C	T	C	T	.	G	.	.	A	.	.	G	A	.	.	

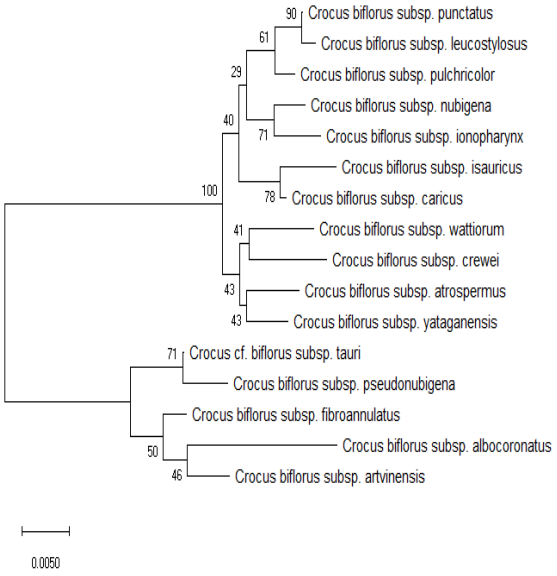

Figure 1 Neighbor-Joining dendrogram provided from *ITS1-5.8S rRNA-ITS2*

3.2. Analysis results for psbA-trnH IGS

Sequence informations belonging to 9 subspecies of *C. biflorus* were provided from NCBI [9]. These DNA regions containing sequence informations of *psbA-trnH IGS* were aligned by using Molecular Evolutionary Genetics Analysis (MEGA X). The sites with missing/ambiguous data and gaps were excluded for effective analyses in the determination of alignment length and variable sites.

Alignment length for taxa studied was established as 603 bp. Variable sites showing the phylogenetic relationships among the taxa examined were determined in 6 nucleotides (Table 2).

Transitional and transversional substitutions expressing the substitutions between same or different base groups were determined as 70.39 % and 29.61 %, respectively. Moreover, transition/transversion rate for purines (k_1), pyrimidines (k_2) and overall transition/transversion rate (R) were assigned as 2.30, 7.12 and 2.22, respectively (Table 5).

The nucleotide frequencies for psbA-trnH IGS of *C. biflorus subspecies* were analysed as 62.41 % (A+T/U) and 37.59 % (C+G) (Table 5).

Finally, Neighbor-Joining (NJ) dendrogram based on the sequence informations of *psbA-trnH IGS* for *C. biflorus* subspecies was drawn to evaluate the phylogenetic relationships of taxa studied and to understand the discrimination ability of *psbA-trnH IGS* for the taxa studied (Figure 2).

Table 2

Subspecies of *C.biflorus* and variable sites belonging to *psbA-trnH IGS* (The numbers show variable nucleotides)

	2 9	7 1	1 1	1 1	1 3	1 0
Crocus biflorus subsp. weldenii	C	A	T	C	T	C
Crocus biflorus subsp. leucostylosus	T	.	.	.	.	.
Crocus biflorus subsp. adamii	.	.	A	G	A	.
Crocus biflorus subsp. pseudonubigena	.	G	.	.	.	.
Crocus biflorus subsp. artvinensis	.	G	.	.	.	A
Crocus biflorus subsp. melantherus	.	.	.	.	.	.
Crocus biflorus subsp. pulchricolor	T	.	.	.	.	.
Crocus biflorus subsp. punctatus	T	.	.	.	.	.
Crocus biflorus subsp. stridii	T	.	A	G	A	.

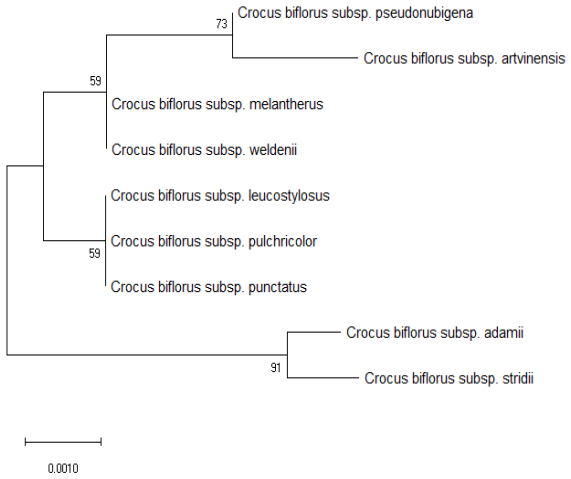

Figure 2 Neighbor-Joining dendrogram provided from *psbA-trnH IGS*

3.3. Analysis results for rpoC1

The DNA sequences belonging to 16 subspecies of *C. biflorus* for *rpoC1* were provided from NCBI [9] and than these sequence informations were aligned by MEGA X for further analysis.

Alignment length for 16 taxa belonging to *C.biflorus* subspecies was established as 575 bp, after the exclution of the positions containing gaps and missing data for effective analyses. Totally 8 variable sites were determined among the taxa studied (Table 3).

The rate of transitional substitutions for *rpoC1* sequence is higher with 91.83 % than transversional substitution. In addition to transitional and transversional substitutions, transition/transversion rates were assigned as 30.85 for purines (k_1) and 13.60 for pyrimidines (k_2). The overall transition/transversion rate (R) was 11.13. The nucleotide frequencies of A+T/U and G+C were determined as 58.03 % and 41.97, respectively (Table 5).

Finally, Neighbor-Joining (NJ) dendrogram based on the sequence informations of *rpoC1* for *C. biflorus* subspecies was drawn to evaluate the phylogenetic relationships of taxa studied and to understand the discrimination ability of rpoC1 for the taxa studied (Figure 3).

Table 3

Subspecies of *C.biflorus* and variable sites belonging to *rpoC1* (The numbers show variable nucleotides)

	2 9 2	2 7 0	3 8 7	4 1 6	4 0 7	5 3 7	5 0 6	5 6 6
Crocus biflorus subsp. wattiorum	A	A	A	T	T	G	G	C
Crocus biflorus subsp. weldenii	G	.	G	C	.	.	.	.
Crocus biflorus subsp. leucostylosus	G	.	G	.	.	.	.	.
Crocus biflorus subsp. adamii	G	.	.	.	.	.	.	.
Crocus biflorus subsp. pseudonubigena	G	.	.	.	.	.	.	.
Crocus biflorus subsp. artvinensis	G	.	.	.	.	.	A	.
Crocus biflorus subsp. melantherus	G	C	.	.	C	.	.	.
Crocus biflorus subsp. pulchricolor	G	.	G	.	.	.	.	.
Crocus biflorus subsp. punctatus	G	.	G	.	.	.	.	.
Crocus biflorus subsp. stridii	G	.	G	.	.	.	.	.

Crocus biflorus subsp. alexandri	G . G . . . . .
Crocus biflorus subsp. isauricus	G . . . .
Crocus biflorus subsp. crewei	G . . . . A . .
Crocus biflorus subsp. biflorus	G . . . .
Crocus biflorus subsp. nubigena	G . G . . . . .
Crocus biflorus subsp. tauri	G . . . . . A T

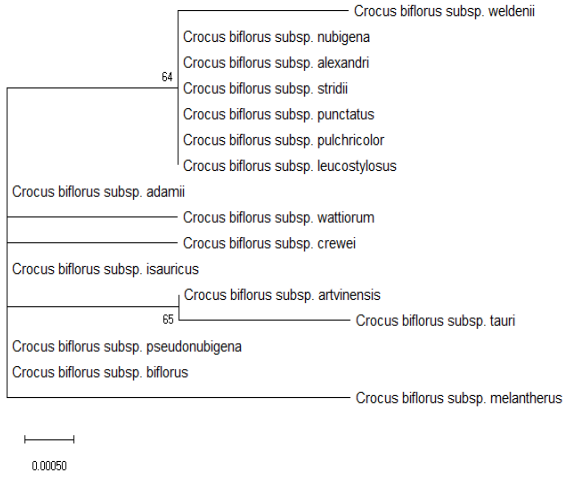

Figure 3 Neighbor-Joining dendrogram provided from *rpoC1*

3.4. Analysis results for trnL-trnF IGS

Sequence informations belonging to 17 subspecies of *C. biflorus* for *trnL-trnF IGS* were provided from NCBI [10]. After that, these DNA sequences for *C. biflorus* subspecies were aligned by using Molecular Evolutionary Genetics Analysis (MEGA X). The sites with missing/ambiguous data and gaps were excluded in the analysis of alignment length and variable sites.

Alignment length was established as 670 bp for taxa studied. Variable sites which were very important in species identifications and phylogenetic relationships among the taxa were determined in 9 nucleotides (Table 4).

The rates of transitional and transversional substitutions were determined as 79.77 % and 20.23 %, respectively. In other words, it can be said that the most of variable sites among the taxa were caused by the substitutions between same base groups.

Furthermore, transition/transversion rate for purines (k_1), pyrimidines (k_2) and overall transition/transversion rate (R) were assigned as 8.45, 7.21 and 3.65, respectively (Table 5).

The nucleotide frequencies belonging to trnL-trnF IGS sequences of *C. biflorus subspecies* were analysed as 65.08 % (A+T/U) and 34.92 % (C+G) (Table 5).

Finally, Neighbor-Joining (NJ) dendrogram was drawn to evaluate the phylogenetic relationships of *C. biflorus* taxa and to understand the discrimination ability of *trnL-trnF IGS* for the taxa studied (Figure 4).

Table 4

Subspecies of *C.biflorus* and variable sites belonging to *trnL-trnF IGS* (The numbers show variable nucleotides)

	1 2 9	2 4 8	2 8 6	3 6 2	5 6 6	5 8 0	6 0 3	6 5 2	6 5 8
Crocus biflorus subsp. stridii	G	C	G	G	A	G	T	T	G
Crocus biflorus subsp. weldenii	.	.	.	.	.	.	.	.	.
Crocus biflorus subsp. melantherus	.	.	.	.	.	.	.	.	.
Crocus biflorus subsp. biflorus	.	A	.	.	.	.	.	.	.
Crocus biflorus subsp. fibroannulatus	A	.	.	A	G	.	C	.	.
Crocus biflorus subsp. albocoronatus	.	A	.	A	.	.	C	.	.
Crocus biflorus subsp. wattiorum	.	.	.	.	.	.	.	.	.
Crocus biflorus subsp. atrospermus	.	.	.	.	.	.	.	.	.
Crocus biflorus subsp. artvinensis	.	.	.	A	.	T	C	.	.
Crocus cf. biflorus subsp. tauri	.	.	A	A	G	.	C	.	.
Crocus biflorus subsp. nubigena	.	.	.	.	.	.	.	.	.
Crocus biflorus subsp. caelestis	.	.	.	.	.	.	C	.	.
Crocus biflorus subsp. yataganensis	.	.	.	.	.	.	.	.	.
Crocus biflorus subsp. caricus	.	.	.	.	.	.	.	C	.
Crocus biflorus subsp. crewei	.	.	.	.	.	.	.	.	.
Crocus biflorus subsp. pseudonubigena	.	.	.	A	.	.	C	.	T
Crocus biflorus subsp. adamii	.	.	.	A	G	.	C	.	.

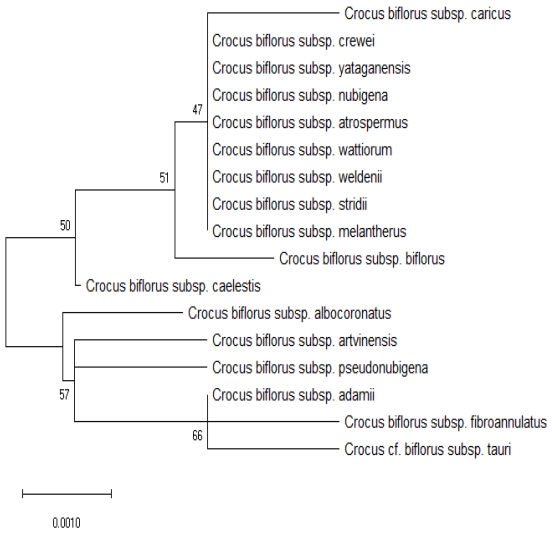

Figure 4 Neighbor-Joining dendrogram provided from *trnL-trnF IGS*

Table 5

The comparisons of all studied DNA regions.

DNA regions	Taxon (number)	Alignment lenght (bp)	Variable site	Transitional substitutions (%)	Transversional substitutions (%)	Transition/Transversion rate Purines (k_1)	Pyrimidines (k_2)	Overall (R)	Nucleotide freq. (%) A+T/U	G+C
ITS1-5.8S rRNA-ITS2	16	604	62	75.72	24.28	3.73	8.84	2.89	37.42	62.58
psbA-trnH IGS	9	603	6	70.39	29.61	2.30	7.12	2.22	62.41	37.59
rpoC1	16	575	8	91.83	8.17	30.85	13.60	11.13	58.08	41.97
trnL-trnF IGS	17	670	9	79.77	20.23	8.45	7.21	3.65	65.08	34.92
Total	58									

4. CONCLUSIONS

In the comparisons of sequence informations according to species identification abilities, it can be said that the region belonging to *ITS1-5.8S rRNA gene-ITS2* is more efficient among the DNA regions examined for *Crocus* taxa. This region phylogenetically separated the taxa in two groups, besides it identified all taxa studied. Furthermore, variable sites that was important in species identification and phylogenetic relationships among taxa was observed in highest rate on *ITS1-5.8S rRNA gene-ITS2*.

It can be stated that although other DNA regions examined (*psbA-trnH IGS, rpoC1* and *trnL-trnF IGS*) clearly separated some taxa from each other, these regions were insufficient in the separation and identification of all taxa studied. Variable sites expressing the substitutions based on the sequence informations are the most important characters in the evaluation of taxa phylogenetically. However, these regions showing the sequence changes among the taxa were observed on several nucleotides. In other words, it can be stated that the sequence informations belonging to *psbA-trnH IGS*, *rpoC1* and *trnL-trnF IGS* were highly preserved for *C. biflorus* taxa.

Recent molecular studies show that taxa belonging to *C. biflorus subspecies* were not grouped together, even occur in distinct clades [9, 10]. In other words, it is observed that subspecies status of the genus for *C. biflorus* is incorrect and can not be maintained any more [2].

Harpke et al. (2016) [2] updated the Mathew's study (1982) [1] which present the nineteen subspecies of *Crocus biflorus* in Turkey and they stated as a result of this study that all subspecies of *C. biflorus* ranged from Balkan Peninsula to Caucasus and Iran represent the independent lineages and should be treated at species level. Similarly, Addam et al. (2019) [19] states as a result of the studies based on morphological and molecular genetic in the *Crocus* genus that the most of the subspecies must be categorized as species because of their genetic divergences. This opinion is still controversial among scientists and

it has not completely resolved as in the number of taxa.

All of them make necessary the phylogenetically evaluation of *C. biflorus* taxa and the examination of their subspecies status in more detail.

For these reasons, in this study, *ITS1-5.8S rRNA-ITS2* belonging to nuclear DNA and three regions (*psbA-trnH IGS*, *rpoC1* and *trnL-trnF IGS*) belonging to plastid DNA were used to understand the taxonomy of *C. biflorus subspecies* and to contribute the solution of the still existing problems. Moreover, the discrimination abilities of each DNA regions examined and nucleotide substitutions were analysed for *C .biflorus* taxa. Although the different DNA regions and their combinations are very important for effective phylogenetic analysis, some barcoding regions are not enough for species identification and discrimination on the dendrogram prepared to show phlogenetic relationships. This study results could provide important data for further studies in the selection of usefull regions, in addition to undestanding of taxonomic relationships of *C. biflorus subspecies*.

Appendix

ITS1-5.8S rRNA-ITS2:

HE663991, HE663973, HE664018, HE663980, HE663975, HE664004, HE663976, HE664016, HE663972, HE664014, HE663978, HE664003, HE663969, LN864707, HE664017, HE664013

psbA-trnH IGS:

EU110184, EU110183, EU110150, EU110202, EU110195, EU110140, EU110134, EU110185, EU110129

rpoC1:

EU110612, EU110523, EU110533, EU110539, EU110544, EU110550, EU110605, EU110595, EU110637, EU110527, EU110530, EU110593, EU110522, EU110594, EU110524, EU110560

trnL-trnF IGS:

HE864180, HE864177, HE864182, HE864185, HE864208, HE864210, HE864257, HE864275, HE864227, HE864220, HE864183, HE864211, HE864207, HE864174, HE864165, HE864212, HE864198

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The Declaration of Conflict of Interest/ Common Interest

No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval

This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

REFERENCES

[1] B. Mathew, ‘‘The *Crocus*. A revision of the genus *Crocus*,’’ Timber Press, London, 224 pp, 1982.

[2] D. Harpke, H. Kerndorff, E. Pasche, and L. Peruzzi, ‘‘Neotypification of the name *Crocus biflorus* Mill. (Iridaceae) and its consequences in the taxonomy of the genus,’’ Phytotaxa, vol. 260, no. 2, pp. 131–143, 2016.

[3] R.B. Saxena, ‘‘Botany taxonomy and cytology of *Crocus* series *Reticulati*,’’ World Journal of Pharmaceutical and Medical Research, vol. 2, no. 3, pp. 53–73, 2016.

[4] F. Roma-Marzio, D. Harpke, and L. Peruzzi, ‘‘Rediscovery of *Crocus biflorus* var. *estriatus* (Iridaceae) and its taxonomic characterisation,’’ Italian Botanist, vol. 6, pp. 23−30, 2018.

[5] M.J. Bagur, G.L.A. Salinas, A.M. Jiménez-Monreal, S. Chaouqi, S. Llorens, M. Martínez-Tomé, and G.L. Alonso, ‘‘Saffron: An Old Medicinal Plant and a Potential Novel Functional Food,’’ Molecules, vol. 23, no. 1, 2018.

[6] R.G. Harrison and E.L. Larson, ‘‘Hybridization, introgression, and the nature of species boundaries,’’ J. Hered., vol. 105, pp. 795–809, 2014.

[7] H. Kerndorff, E. Pasche, and D. Harpke, ‘‘The Genus *Crocus* (Liliiflorae, Iridaceae): Taxonomical Problems and How to Determine a Species Nowadays?’’ Stapfia, vol. 105, pp. 42–50, 2016.

[8] O. Erol, D. Harpke, and H. Yıldırım, ‘‘A new *Crocus* L. (Iridaceae) species from SE Turkey, based on morphological and molecular data,’’ Phytotaxa, vol. 239, pp. 223–232, 2015.

[9] G. Petersen, O. Seberg, S. Thorsøe, T. Jørgensen, and B. Mathew, ‘‘A phylogeny of the genus *Crocus* (Iridaceae) based on sequence data from five plastid regions,’’ Taxon, vol. 57, no. 2, pp. 487–499, 2008.

[10] D. Harpke, S. Meng, T. Rutten, H. Kerndorff, and F.R. Blattner, ‘‘Phylogeny of *Crocus* (Iridaceae) based on one chloroplast and two nuclear loci: ancient hybridization and chromosome number evolution,’’ Molecular Phylogenetics and Evolution, vol. 66, pp. 617–627, 2013.

[11] D. Harpke, L. Peruzzi, H. Kerndorff, T. Karamplianis, T. Constantinides, N. RanĐelović, V. RanĐelović, M. Jušković, E. Pasche, and F.R. Blattner, ‘‘Phylogeny, geographic distribution and new taxonomic circumscription of the crocus reticulatus species group (Iridaceae),’’ Turk. J. Bot., vol. 38, no. 5, 2014.

[12] D. Harpke, A. Carta, G. Tomović, V. Ranđelović, N. Ranđelović, F.R. Blattner, and L. Peruzzi, ‘‘Phylogeny, karyotype evolution and taxonomy of Crocus series Verni (Iridaceae),’’ Plant Syst Evol., vol. 301, no. 1, pp. 309−325, 2015.

[13] H. Kerndorff, E. Pasche, and D. Harpke, ‘‘*Crocus isauricus* Siehe ex Bowles (Liliiflorae, Iridaceae) and its relatives,’’ Stapfia, vol. 101, pp. 3–14, 2014.

[14] H. Kerndorff, E. Pasche, and D. Harpke, ‘‘*Crocus lyciotauricus* Kerndorff & Pasche (Liliiflorae, Iridaceae) and its relatives,’’ Stapfia, vol. 103, pp. 67–80, 2015.

[15] O. Erol, D. Harpke, and A. Çiftçi, ‘‘*Crocus heilbronniorum*, a new Turkish species of Series *Lyciotauri* (Iridaceae),’’ Phytotaxa, vol. 298, no. 2, pp. 173−180, 2017.

[16] NCBI, National Centre of Biotechnology Information, https://www.ncbi.nlm.nih.gov/genbank.

[17] S. Kumar, G. Stecher, M. Li, C. Knyaz, and K. Tamura, ‘‘MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms,’’ Molecular Biology and Evolution, vol. 35, pp. 1547−1549, 2018.

[18] K. Tamura, M. Nei, and S. Kumar, ‘‘Prospects for inferring very large phylogenies by using the neighbor-joining method,’’ Proceedings of the National Academy

of Sciences (USA), vol. 101, pp. 11030−11035, 2004.

[19] K. Addam, M. Bou-Hamdan, N. Sabbagh, J. Takkoush, and K. Hout, ‘‘*Crocus baalbekensis* K. Addam & M. Bou Hamdan sp. Nov and its three forms (IRIDACEAE), new endemic species and forms from Lebanon, joined the Lebanese Flora,’’ MOJ Ecology & Environmental Sciences, vol. 4, no. 2, pp. 75−83, 2019.