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Preliminary Insights into the Phylogeny of *Colchicum* Species

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ABSTRACT

Colchicum has played a vital role in the field of medicine due to its inherent properties, with its importance extending from the past to the present. DNA barcoding is an effective tool for the identification and conservation of plant species. This study tested the application of the *18S rRNA* gene for the phylogenetic construction of *Colchicum* species. The phylogenetic UPGMA (unweighted pair group method with arithmetic mean) tree and STRUCTURE analysis were conducted to assess the consistency of the results for 33 *Colchicum* specimens and one outgroup species, utilizing the *18S rRNA* partial gene. The results of the study showed that DNA barcoding using *18S rRNA* was an effective method for identifying *Colchicum* species, with 97.76% of the tested sequences being successfully determined. This study contributes to the field of phylogenetic analysis of *Colchicum* species by illustrating the utility of the *18S rRNA* gene for phylogenetic construction, underscoring its significance in advancing our understanding of plant genetics. The nucleotide sequences obtained for species identification in this study were uploaded to the barcode of life data system (BOLD), contributing to internationally accessible genetic data.

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Colchicum Türlerinin Filogenisine İlişkin Ön Bilgiler

ÖZET

Colchicum, sahip olduğu doğal özellikleri nedeniyle tıp alanında önemli bir rol oynamış olup, önemi geçmişten günümüze kadar uzanmaktadır. DNA barkodlama, bitki türlerinin tanımlanması ve korunması için etkili bir araçtır. Bu çalışma, *Colchicum* türlerinin filogenetik yapısının oluşturulmasında *18S rRNA* geninin uygulamasını test etmiştir. 33 *Colchicum* örneği ve bir dış grup türü kullanılarak, *18S rRNA* kısmi geni ile filogenetik UPGMA (aritmetik ortalama ile ağırlıksız çift grup yöntemi) ağacı ve STRUCTURE analizi yapılmıştır. Çalışmanın sonuçları, *18S rRNA* kullanılarak yapılan DNA barkodlamanın *Colchicum* türlerinin tanımlanmasında etkili bir yöntem olduğunu ve test edilen dizilerin %97.76'sının başarılı bir şekilde belirlendiğini göstermiştir. Bu çalışma, *18S rRNA* geninin filogenetik yapı oluşturmadaki faydasını göstererek *Colchicum* türlerinin filogenetik analizine katkıda bulunmakta ve bitki genetiğini anlama konusunda ilerlememize olanak sağlamaktadır. Tür tanımlaması için elde edilen nükleotid dizileri, uluslararası erişilebilir genetik verilere katkıda bulunmak amacıyla yaşam barkodu veri sistemine (BOLD) yüklenmiştir.

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INTRODUCTION

Colchicum, a genus of herbaceous plants belonging to the Liliaceae family, is commonly utilized both as an ornamental species and for its medicinal properties, primarily due to the presence of alkaloids. In today's medical field, it assumes a vital role in addressing many global diseases, whether used in isolation or conjunction with different pharmaceuticals (Toplan et

al., 2016). In addition to alkaloids, *Colchicum* species also include flavonoids, phenolic acids, tannins, and fatty acids (Hailu et al., 2021). *Colchicum* species face several challenges in terms of conservation. The loss of their habitats due to urbanization and land development, as well as agricultural activities, poses a significant threat to their survival. Additionally, over-harvesting for medicinal purposes has led to a decline in wild populations, and illegal trade further jeopardizes rare and endangered *Colchicum* species. Climate change also poses a significant threat to their habitats, with disruptions in precipitation patterns and extreme weather events affecting their survival (Chandra, 2016; Rather et al., 2022).

Beyond the preservation of biodiversity, accurate taxonomic identification carries considerable significance in the domain of herbal or traditional medicine (Techen et al., 2014). In response to the challenges posed by traditional taxonomy, various genomic methods have been suggested. Among these, DNA barcoding, initially introduced by Hebert et al. (2003) has proven highly effective in both the determination of existing species and the discovery of unknown ones. DNA barcoding employs short, uniform, and variable DNA regions (<1000 bp) to assess and classify species (Savolainen et al., 2005). Due to its simplicity and greater accuracy compared to the difficulties and subjectivity involved in morphology-based taxonomy classification, DNA barcoding is growing in popularity (Kress, 2017). While the *cytochrome c oxidase 1* (*CO1*) gene in the mitochondrial genome is widely accepted as a universal barcode marker for animals, an equivalent universal barcode for plants remains to be identified (Kress & Erickson, 2008). In the realm of plant life, the employment of DNA barcoding is not ideally suited due to its slow progression and limited variation. As a result, the search for acceptable plant barcode regions has shifted to the nuclear and chloroplast genomes, which have higher substitution rates (Hollingsworth et al., 2011). Due to their ability to distinguish between different plant species, various potential regions, including *matK*, *rbcL*, *trnH-psbA*, *trnL-F*, *ITS*, *18S rRNA*, and *5S rRNA*, have been extensively studied (Mishra et al., 2016).

Barcoding efforts focus on the region comprising the V4 hypervariable domain of the *18S rRNA* gene, which is roughly 400 base pairs (bp) in length and is highly variable, making it useful for species-level identification. They are widely used markers for plants concerning their discrimination capacity, and due to their conserved nature, and their widespread availability across plant taxa (Srivastava et al., 2016). The first *18S rRNA* sequences of angiosperms were of maize (Messing et al., 1984), rice (Takaiwa et al., 1984), soybean (Eckenrode et al., 1985), and Poaceae (Hamby & Zimmer, 1988). Also, the study that provides a comprehensive comparison of the *18S RNA* and *rbcL* sequences of various plant species (Nickrent & Soltis, 1995) and concludes that *18S rDNA* sequences provide an adequate dataset for conducting phylogenetic analyses at more comprehensive taxonomic levels within angiosperms (Soltis et al., 1997) provided a framework for future studies on selecting the *18rRNA* gene for phylogenetic analysis and DNA barcoding in plants.

UPGMA (unweighted pair group method with arithmetic mean) is a widely used method in phylogenetic analysis for constructing dendrograms based on genetic distance. The utility of the UPGMA method in phylogenetic and population structure analyses has been demonstrated in various studies, underscoring its relevance for diverse plant taxa (Demirel et al., 2022; Karakan et al., 2024). In this preliminary study, the UPGMA method was employed to construct the phylogenetic relationships among 33 *Colchicum* specimens using nucleotide sequences from the *18S rRNA* gene region, and STRUCTURE analysis was used to validate the phylogenetic tree data with population structure. The *18S rRNA* gene is chosen for phylogenetic studies due to its highly conserved nature, which allows for the comparison of evolutionary relationships across diverse taxa. Its relatively slow rate of evolution provides stable and reliable markers for inferring deeper evolutionary lineages and relationships among species.

MATERIAL and METHOD

Plant material

In this preliminary study, three individuals from each of the 11 *Colchicum* species were collected to provide an initial insight into the genetic diversity within the genus. Detailed information on the specimens, including the season of collection, is provided in Table 1. All specimens were supplied by the Republic of Türkiye Ministry of Agriculture and Forestry, Ataturk Horticultural Central Research Institute in Yalova. As an outgroup, *Androcymbium dregei* (obtained from GenBank with the accession number: JQ405011.1) from the Colchicaceae family was used. The choice of *Androcymbium dregei* as an outgroup was based on its phylogenetic distance within the family, providing a robust reference for comparative analysis.

DNA extraction, PCR amplification, and sequencing

Genomic DNA from leaf tissue was extracted using the CTAB method developed by Doyle & Doyle (1987). Partial *18S rRNA* gene amplifications by the polymerase chain reaction (PCR) were performed using 2 ng total genomic DNA as a template in a 25 µL reaction mixture, consisting of PCR buffer (1 X), 4 mmol/L $MgCl_2$, 0.8 mmol/L each primer, 0,2 mmol/L each dNTP, and 0.5 U Taq DNA polymerase. Primers designed using Primer3, flanking the *18S rRNA* gene

regions, are as follows:*18S rRNA* Forward: 5'- ATT GTG ACG GAG CCG GGC GAG G -3', *18S rRNA* Reverse: 5'- CGG AGT TTG GTG GGG ACG TAC C -3'; The PCR program employed in this study followed these steps: an initial denaturation step at 94°C for 3 minutes, followed by 39 cycles consisting of denaturation at 94°C for 1 minute, annealing at 53°C for 1 minute, and extension at 72°C for 1 minute. The process concluded with a final extension at 72 °C for 10 minutes. The PCR amplifications were carried out on a Veriti™ 96-Well Fast Thermal Cycler. The PCR products were visualized using electrophoresis on agarose gel (1.5%).

Table 1. Genotype codes and seasonal information of 11 *Colchicum* species were used in this study.
Çizelge 1. Çalışmada kullanılan 11 Colchicum türünün genotip kodları ve mevsimsel bilgileri.

No	Species	Genotype Code	Season
1		C4803A	
2	*C. balansae* Planchon	C4810	Autumn
3		C4821	
4		C0730	
5	*C. baytopiorum* C.D. Brickell	C0740	Late-autumn
6		C0748	
7		C4819	
8	*C. macrophyllum* B.L. Burtt	C4822	Autumn
9		C4823	
10		C0605	
11	*C. bivonae* Guss.	C1412	Autumn
12		C1703	
13		C0749	
14	*C. boissieri* Orph.	C0751	Autumn
15		C3503	
16		C3304	
17	*C. cilicicum* (Boiss.) Dammer	C5103	Autumn
18		C5104	
19		C2710	
20	*C. polyphyllum* Boiss. & Heldr.	C3108	Autumn
21		C3308	
22		C0101	
23	*C. serpentinum* Woron. ex Miscz.	C4227	Winter
24		C4601	
25		C2802	
26	*C. speciosum* Steven	C3701	Autumn
27		C5301	
28		C0733	
29	*C. stevenii* Kunth	C0735	Late-autumn
30		C0765	
31		C2003	
32	*C. burttii* Meikle	C4801	Winter
33		C4814	

Purification of PCR products was carried out by the 'ChargeSwitch-Pro PCR Clean-up Purification' protocol. Cycle sequencing reactions were performed using the "Big Dye-Kit Standard" protocol. DNA sequencing was performed with the "ABI 310 Genetic Analyzer" sequencing system.

Sequence analysis

After the sequencing step, the chromatograms acquired were subjected to further examination through Geneious Prime version 2023.2.1. To ensure the accuracy of each sequence, a comprehensive examination of the peaks corresponding to individual nucleotides was conducted, followed by the generation of a consensus sequence after eliminating segments of lower quality. The molecular taxonomic identity of the resulting consensus sequence was authenticated using the nucleotide blast tool (BLASTN - basic local alignment search tool-nucleotide) available at the National Center for Biotechnology Information (NCBI). This tool assesses nucleotide/protein sequences by comparing them to sequence databases,

determining identities based on percentage similarity, and considering the E-value.

The phylogenetic analysis was performed in the "CLC Genomics Workbench 23.0.2" program by the "UPGMA" procedure, using the "Kimura 80" model of evolution with confidence measured through 1000 replicates of bootstrap.

For population structure analysis based on *18S rRNA* sequence information, the determination of SNPs was first performed in the "Mega 6" program. STRUCTURE analysis was conducted using the software application "STRUCTURE (version 2.3.4)" to categorize the genotypes based on the approach proposed by Pritchard et al. (2000). During this analysis, various models (K=1-10) were tested after 100,000 iterations of the markov chain monte carlo (MCMC) algorithm, along with customized statistical methods, to identify the most suitable population count. In addition, each model was performed 10 times with 10.000 length burning period. To calculate the ΔK value of the populations, "STRUCTURE HARVESTER" (Earl & Vonholdt, 2012) software was used, and the number of subpopulations was identified. For the K and ΔK values obtained for this purpose, the data determines the optimum K value based on the probability value reached (Evanno et al., 2005). The genotypes that have a probability ≥0.80 are classified as 'pure lineage', whereas those with a probability ≤0.80 are classified as 'mixed ancestry'.

RESULTS

PCR amplification and sequencing

The PCR products obtained from the amplification of the *18S rRNA* gene region were analyzed by agarose gel electrophoresis (Figure 1). Clear bands of approximately 200 bp were observed for all samples. These results indicate that the target gene region was successfully amplified and that the PCR conditions used in the study were reliable.

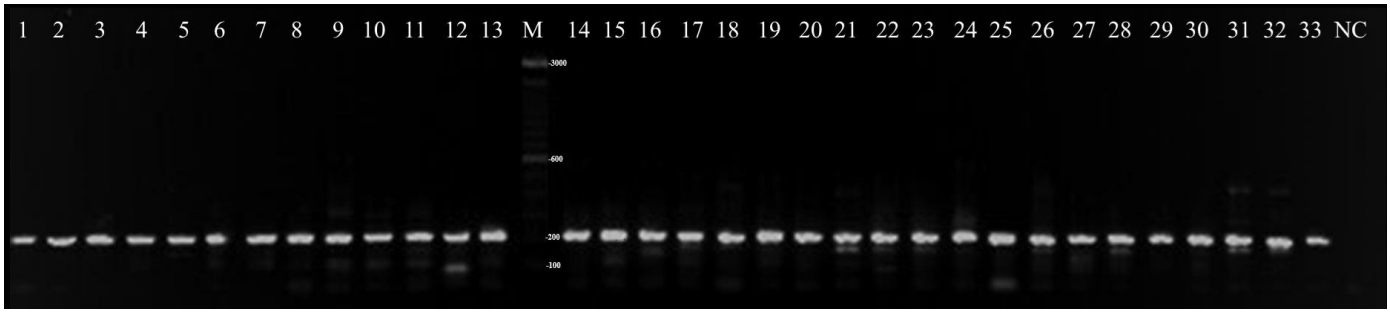

Figure 1. Agarose gel electrophoresis of PCR products for the *18S rRNA* gene region of *Colchicum* specimens (1-33). M: molecular weight marker (100-3000 bp); NC: negative control.

Şekil 1. Colchicum örneklerinin (1-33) 18S rRNA gen bölgesine ait PCR ürünlerinin agaroz jel elektroforezi. M: moleküler ağırlık markerı (100-3000 bp); NC: negatif kontrol.

The sequence information for 33 *Colchicum* specimens is given in Appendix 1. The lengths of the *18S rRNA* sequences used for the analyses ranged from 131 to 145 bp. GC content is 52.5%. The identification of species was carried out through nucleotide blast, where the highest similarity score reached 97.76%, and the E value was minimal at 8e-56. The sequencing data has been registered in the BOLD data system under the CSDB project code (www.boldsystems.org).

Before performing the phylogenetic analyses, a multiple sequence alignment was conducted to assess the genetic variation among the *Colchicum* species, as shown in Figure 2. This figure presents the multiple sequence alignment of various *Colchicum* species, highlighting the nucleotide variations within the *18S rRNA* region. This alignment provides insights into the genetic diversity among the species and supports the efficacy of *18S rRNA* as a suitable barcode for species identification.

Phylogenetic analysis

UPGMA tree based on *18S rRNA* sequence data identified 33 *Colchicum* specimens, and each was well supported with high bootstrap values (50%-100%). The phylogenetic tree (Figure 3) was grouped by seasonal state, with each group represented by a specific color. Additionally, to distinguish individual species, the nodes were color-coded, with each species assigned a unique color.

The phylogenetic tree provided data for the classification of *Colchicum* species into two primary groups, labeled as Group I and Group II, with two subgroups within each major group. IA-IB and IIA-IIB, respectively (Figure 3). The IA subgroup contains *C. boissieri* Orph., *C. cilicicum*; the IB subgroup contains *C. bivonae* Guss.; the IIA subgroup contains *C. polyphyllum* Boiss. & Heldr.; the IIB group is divided into two branches: IIC and IID. The IIC subgroup contains *C. baytopiorum* C.D. Brickell, *C. stevenii;* the IID group is also divided into two branches: The IIE and IIF. The IIE subgroup contains *C. burttii*, *C. serpentinum* Woron. ex Miscz.; the IIF subgroup contains *C. speciosum* Steven, *C. macrophyllum* B.L. Burtt, *C. balansae* Planchon.

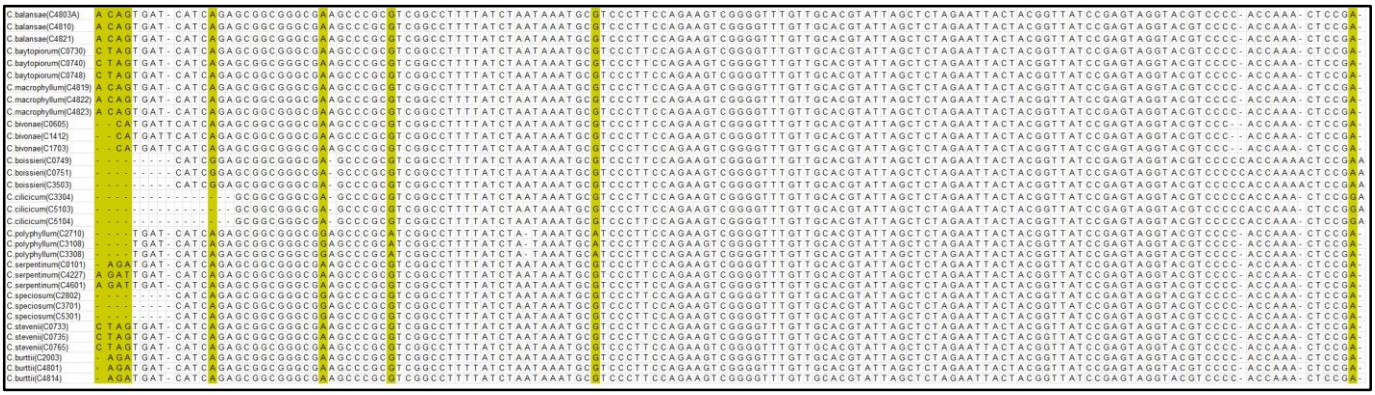

Figure 2. Sequence alignment of *Colchicum* species based on *18S rRNA* region. The alignment shows conserved regions shaded, with nucleotide variations marked in yellow.

Şekil 2. 18S rRNA bölgesine dayalı Colchicum türlerinin dizi hizalaması. Hizalamada korunan bölgeler gölgeli olarak gösterilmiş olup, nükleotid varyasyonları sarı ile işaretlenmiştir.

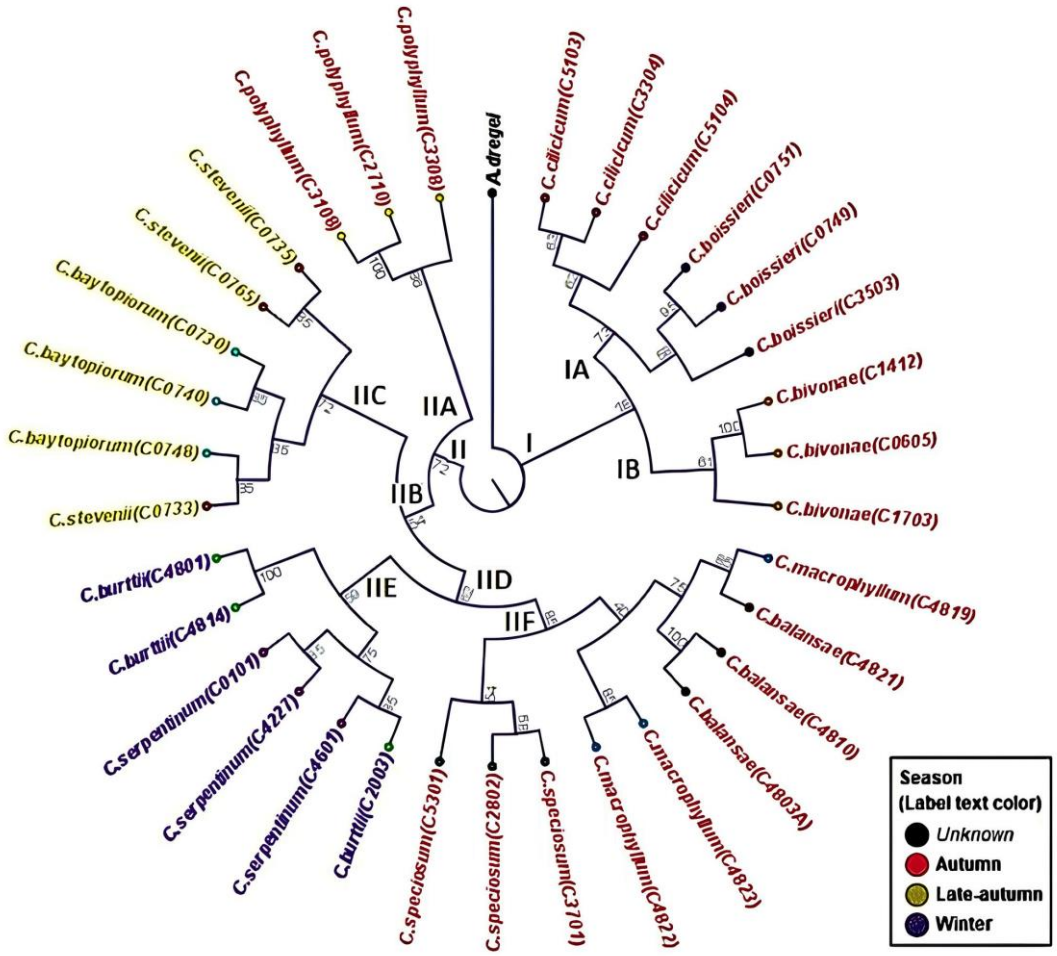

Figure 3. Phylogenetic tree of 33 *Colchicum* specimens constructed using UPGMA cluster analysis based on *18S rRNA* sequences. *Androcymbium dregei* from Colchicaceae was used as the outgroup. The color-coded labels indicate the flowering season of each specimen (Autumn, Late-autumn, Winter, and Unknown). Bootstrap values are provided at the nodes to support the clustering.

Şekil 3. 33 Colchicum örneğinin 18S rRNA dizilimlerine dayalı olarak UPGMA kümeleme analizi kullanılarak oluşturulan filogenetik ağacı. Colchicaceae familyasından Androcymbium dregei dış grup olarak kullanılmıştır. Etiketlerde kullanılan renkler, her bir örneğin çiçeklenme mevsimini (Sonbahar, Geç sonbahar, Kış ve Bilinmeyen) göstermektedir. Düğümler üzerinde yer alan bootstrap değerleri kümelenme desteğini göstermektedir.

Structure analysis

The model displaying the greatest ΔK value was chosen as the optimal model for characterizing the population. A genetic identity threshold of ≥ 0.80 was adopted to distinguish genotypes as subpopulation individuals within the selected best model (K). Genotypes falling below this threshold were not included in any specific subpopulation and were regarded as genetically mixed individuals (Fukunaga et al., 2005).

In this study, the population structure of the 33 *Colchicum* specimens with the peak of delta K was observed at K = 3, indicating the presence of three main populations (clusters, Q1–Q3) (Figure 4a). 33 *Colchicum* specimens have membership coefficients of 0.80 and higher and are therefore likely to be pure. The classification of accessions into populations based on this model-based structure is shown in Figure 4b. The STRUCTURE analysis corroborated the findings of the dendrogram analysis, revealing the presence of three distinct clusters within the population. Out of the 33 specimens, there were 3 genotypes in the first subpopulation (19, 20, 21), 12 genotypes in the second subpopulation (28, 6, 9, 1, 5, 29, 4, 3, 30, 8, 2, 7), and 6 genotypes in the third subpopulation (13, 14, 15, 16, 17, 18). The first subpopulation consists of 10 genotypes (11, 31, 33, 22, 32, 12, 10, 27, 26, 25) and is a combination of genotypes from both the first and second populations. The two remaining genotypes in the second subpopulation, 23 and 24, are a combination of the genotypes from the first and second populations. The combination of genotypes from both the first and second populations includes 10 genotypes (11, 31, 33, 22, 32, 12, 10, 27, 26, 25), while the remaining two genotypes, 23 and 24, are also a blend of genotypes from the first and second populations.

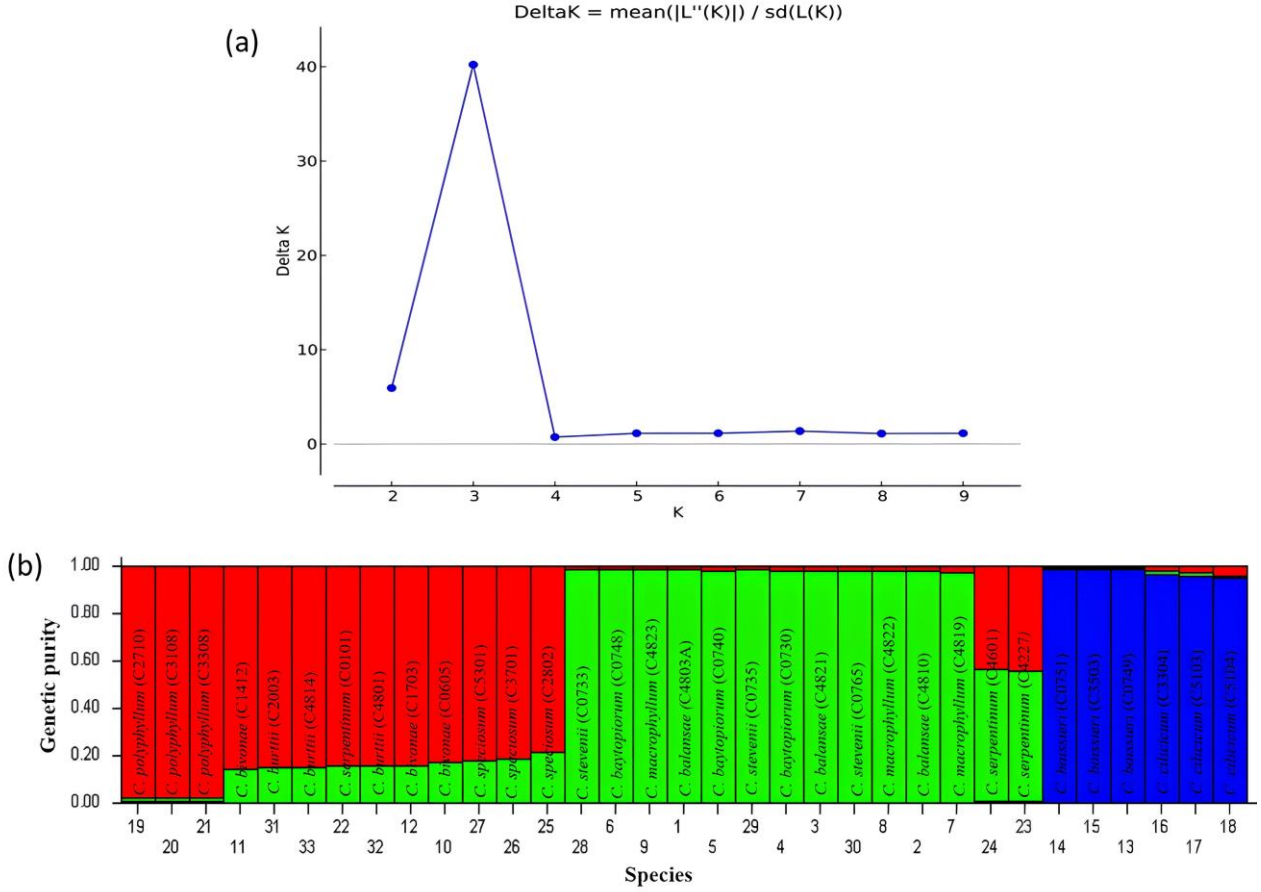

Figure 4. Results of the clustering analysis performed using STRUCTURE Version 2.3.4 (a) Delta K values for various assumed population numbers (K) in the STRUCTURE analysis, and (b) The Q-plot illustrating the clustering of 33 *Colchicum* specimens at the K = 3 clustering level using the set of discriminative single-nucleotide polymorphisms (SNPs). Different subpopulations are represented by distinct colors (first subpopulation: red, second subpopulation: green, third subpopulation: blue).

Şekil 4. STRUCTURE Versiyon 2.3.4 kullanılarak gerçekleştirilen kümeleme analizi sonuçları (a) STRUCTURE analizinde varsayılan farklı popülasyon sayıları (K) için Delta K değerleri ve (b) Ayırt edici tek nükleotid polimorfizmleri (SNP'ler) kullanılarak K = 3 kümeleme düzeyinde 33 Colchicum örneğinin kümelenmesini gösteren Q-plot. Farklı alt popülasyonlar farklı renklerle temsil edilmiştir (birinci alt popülasyon: kırmızı, ikinci alt popülasyon: yeşil, üçüncü alt popülasyon: mavi).

DISCUSSION

As in this study, many studies have demonstrated the effectiveness of the *18S rRNA* gene in plant barcoding. Dong et al. (2003) used DNA sequences from the *5S rRNA* spacer, the *ITS* region, and the *18S rRNA* coding region to identify the phylogenetic relationships of ten Astragalus species generally found in China. Their research indicated the highest sequence similarity between *A. membranaceus* and *A. membranaceus* var. *mongholicus*, whereas other Astragalus species exhibited less significant genetic relationships. Safhi et al. (2022) demonstrated the efficiency of DNA barcoding using nuclear regions (*ITS* and *18S rRNA*) for the identification of *C. gileadensis* accessions. Aykut (2020) contributed to the field of DNA barcoding by highlighting the importance of using *rDNA* regions for identifying and evaluating relationships of taxonomically problematic species, specifically Quercus species in Türkiye. The study found that both regions include intergenic spacer (*IGS*), *5S rRNA* genes, and *ITS1*, *ITS2*, *18S rRNA*, *5.8S rRNA*, and *25S rRNA* genes, which are useful tools for DNA barcoding of *Quercus* species. Based on the study by Bae (2009), the region containing three genes, including the *18S rRNA* gene, of *Atractylodes japonica* Koidz was successfully amplified and sequenced, revealing a close phylogenetic relationship with other members of the Compositae family. Yang et al. (1999) estimated the phylogenetic relationships among nine genera in four tribes of the Brassicaceae family using nuclear ribosomal DNA sequences, including the *18S rRNA* gene, revealing distinct groupings and suggesting closer relationships between certain genera. Soltis et al. (2000) conducted a phylogenetic analysis of 560 angiosperms and seven outgroups using a combined data set that included the *18S rDNA* gene. Their study provided the most highly resolved and strongly supported topology for angiosperms to date.

So far, there has been no study to identify *Colchicum* species with *18S rRNA* gene region. However, there are identifications of *Colchicum* species in the literature by both morphological and molecular methods. In the differentiation of *Colchicum* species, it is seen that markers such as AFLP and RAPD and gene regions such as *ITS*, *trnl-trnH* are frequently used on a molecular basis. Several studies have investigated the identification of *Colchicum* species using molecular markers. Karakas et al. (2014) conducted molecular analyses of *Colchicum* species using AFLP molecular marker from Türkiye, contributing to a better understanding of genetic variations within the genus. Persson et al. (2011) analyzed the phylogenetic relationships among *Colchicum* species using *trnL-trnF IGS, trnL intron, trnH-psbA IGS, trnY-trnD IGS, atpB-rbcL IGS,* and *rps16 intron* plastid regions, morphological and chromosomal characteristics, and non-coding chloroplast DNA sequence data. Sahin et al. (2020) used the *trnL-trnF* chloroplast gene region and the *ITS* nuclear gene region to determine the phylogenetic relationships of 52 *Colchicum* species. Consistent with the research findings, the *ITS* and *trnL-trnF* analyses revealed a close relationship between *C. baytopiorum* C.D. Brickell, and *C. stevenii*. Also, the *trnL-trnF* phylogenetic tree supported the findings of Persson et al. (2011) that *C. balansae* Planchon and *C. macrophyllum* B.L. Burtt were close species in the same clade. Similarly, this analysis revealed the close relationship of these two species. Tuyel et al. (2020) assessed genetic variation patterns in 16 *Colchicum* species, utilizing RAPD markers and analyzing the chloroplast DNA sequence *trnL-trnF*. This study confirmed conclusions of Tuyel et al. (2020) about the relationship between *C. speciosum* Steven and *C. balansae* Planchon based on RAPD data. Gandhi et al. (2023) utilized DNA barcoding to accurately identify two varieties of *Colchicum*, specifically *Colchicum autumnale* L. and *Colchicum luteum* Baker. The DNA barcodes used in this study included the *rbcL* and *psbA-trnH* regions, which were effective in distinguishing between the two species. This method proved essential in differentiating the species, especially given the lack of prior literature on the DNA barcoding of *Colchicum luteum* Baker.

Sahin et al. (2021) examined the floristic diversity of the *Colchicum* genus in Türkiye by analyzing 52 species based on 36 morphological traits using principal component analysis (PCA). Their study highlighted significant morphological differences, particularly in flowering time, perigon tube structure, and leaf characteristics, aiding in species characterization. They recommended the need for further investigation to gain a complete understanding of the biogeography and evolutionary aspects of these species. Sahin et al. (2021) found the same seasonal patterns in species grouping in their morphological characterization analysis utilizing the same samples as in this study. When comparing this study with Sahin et al. (2021) in terms of the relationship among species, it was determined that in both studies, *C. baytopiorum* C.D. Brickell and *C. stevenii*; *C. burttii* Meikle and *C. serpentinum* Woron. ex Miscz.; *C. speciosum* Steven and *C. balansae* Planchon were found to be closely related to each other. Furthermore, in both studies, *C. polyphyllum* Boiss. & Heldr. From autumn species were found to be closer to *C. baytopiorum* C.D. Brickell and *C. stevenii* Kunth species from late-autumn species. The findings demonstrated concurrence between the morphological and genetic identification approaches.

When comparing the morphological study by Düşen and Sümbül (2007) on *Colchicum* species with the

similarities observed in this study, *C. speciosum* Steven and *C. cilicicum* (Boiss.) Dammer as well as *C. balansae* Planchon and *C. cilicicum* (Boiss.) Dammer was identified as similar species in their research. However, in this study, *C. cilicicum* (Boiss.) Dammer is grouped distantly from *C. balansae* Planchon and *C. speciosum* Steven, while *C. balansae* Planchon and *C. speciosum* Steven are clustered on the same branch (IIF). Additionally, while Düşen and Sümbül (2007) classified *C. serpentinum* Woron. ex Miscz and *C. stevenii* as closely related species, this study finds them on the same main branch but in different sub-branches, with *C. serpentinum* Woron. ex Miscz in IIE and *C. stevenii* in IIC.

In this preliminary study, analysis of the complementary phylogenetic tree and STRUCTURE results revealed that each sample clustered within its respective species. Furthermore, species occurring in late autumn and winter formed distinct clusters corresponding to their seasonal groups. Notably, autumn species also displayed close clustering, except *C. polyphyllum* Boiss. & Heldr., which deviated from this pattern. In the STRUCTURE analysis, *C. polyphyllum* Boiss. & Heldr. (first subpopulation) was observed to form a distinct group within its species, independent of the other species. Within the phylogenetic tree, *C. cilicicum* (Boiss.) Dammer and *C. boissieri* Orph. are closely grouped in group 1A. This clustering is corroborated by the STRUCTURE analysis, which identifies these species within the third subpopulation. *C. stevenii* Kunth and *C. baytopiorum* C.D. Brickell are classified together in the IIC group on the phylogenetic tree and in the second subpopulation in the STRUCTURE analysis. Additionally, the STRUCTURE analysis, which assigns *C. balansae* Planchon and *C. macrophyllum* B.L. Burtt to the second subpopulation, supports the close grouping of these species within group IIF in the phylogenetic tree. This research also highlighted the close relationships among *C. stevenii* Kunth and *C. baytopiorum* C.D. Brickell; *C. balansae* Planchon, *C. speciosum* Steven, and *C. macrophyllum* B.L. Burtt; and *C. burttii* Meikle and *C. serpentinum* Woron. ex Miscz. These findings are consistent with previous studies on the identification of *Colchicum* species.

CONCLUSION

This study represents a preliminary effort to elucidate the phylogenetic relationships among *Colchicum* specimens using the *18S rRNA* gene region. While the findings provide valuable initial insights, they also highlight the need for further research incorporating additional molecular markers to enhance the robustness and accuracy of the phylogenetic analyses. Future studies should consider using multiple barcode genes, such as *matK*, and *rbcL*, to obtain a more comprehensive understanding of the genetic diversity and evolutionary relationships within the *Colchicum* genus. This approach will enable more detailed and reliable reconstructions of species relationships, ultimately contributing to a more nuanced understanding of the taxonomy and evolution of this important plant group.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors have declared no conflict of interest.

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