www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online ISSN 1308-5301 Print

*Research article/Araştırma makalesi*
*DOI: 10.46309/biodicon.2021.963174*
*14/3 (2021) 431-441*

# Phylogenetic analysis for endemic *Fritillaria baskilensis* Behçet (Liliaceae): Evidence from cpDNA "*trn*" sequences

Pelin YİLMAZ SANCAR [*1], İrmak İCEN TASKİN[2], Murat KURSAT[3] and Omer MUNZUROĞLU[4]
ORCID: 0000-0002-6134-622X; 0000-0002-1612-0563; 0000-0002-0861-4213; 0000-0002-6134-622X

[1] Department of Biology, Faculty of Science, Firat University, 23119, Elazig, Turkey
[2] Department of Molecular Biology and Genetics, Faculty of Science, Inonu University, 44280, Malatya, Turkey
[3] Department of Biology, Faculty of Arts and Science, Bitlis Eren University, 13000, Bitlis, Turkey
[4] Department of Bioengineering, Faculty of Engineering, Firat University, 23119, Elazig, Turkey

**Abstract**

*Fritillaria baskilensis* Behçet., which was first collected from Elazig - Baskil district in 1998 and introduced to the scientific world as a new species, is one of Turkey's endemic inverted tulip species. This study aims to determine the systematic relationships between *Fritillaria baskilensis* and other *Fritillaria* species using molecular techniques. Also; As a result of this study, we tried to determine for the first time in which subgenus the endemic species in question will take place.

After the DNA isolation with the CTAB method, the *trn*T-*trn*L3' region of 5 samples was amplified by using *trn*a and *trn*d primers, and a total of 27 *Fritillaria* species were analyzed together with 2 outgroups in the MEGA program, together with other sequence information obtained from NCBI. While our analyzes are consistent with the literature, the fact that *Fritillaria baskilensis* is in the same branch as members of the subgenus *Fritillaria* suggests that *F. baskilensis* is included in this subgenus.

This study is very important as it is the first molecular-based study using sequence information from *Fritillaria baskilensis* populations, and the new "*trn*" gene region haplotypes of the mentioned species were determined by us for the first time and added to the GenBank database.

**Key words:** *Fritillaria*, endemic, Turkey, cpDNA, *trn*T-*trn*L3'

---------- * ----------

## Endemik *Fritillaria baskilensis* Behçet (Liliaceae) için filogenetik analiz: cpDNA "*trn*" dizilerinden kanıtlarla

**Özet**

İlk defa 1998 yılında Elazığ'ın Baskil ilçesinden toplanan ve bilim dünyasına yeni tür olarak kazandırılan *Fritillaria baskilensis* Behçet Türkiye'nin endemik ters lale türlerinden biridir. Bu çalışmanın amacı *Fritillaria baskilensis*' in diğer *Fritillaria* türleri ile aralarındaki sistematik ilişkilerin moleküler teknikler kullanarak belirlenmesidir. Ayrıca; söz konusu endemik türün hangi altcinste yer alacağı da bu çalışmanın sonucu olarak ilk defa tarafımızca tespit edilmeye çalışılmıştır.

CTAB metodu ile manuel olarak gerçekleştirilen DNA izolasyonu sonrası *trn*a ve *trn*d primerleri kullanılarak 5 örneğe ait *trn*T-*trn*L3' bölgesi çoğaltılmış, NCBI' dan elde edilen diğer sekans bilgileri ile birlikte toplam 27 *Fritillaria* türü 2 dış grup ile birlikte MEGA programında analiz edilmiştir. Analizlerimiz literatürle uyumluluk gösterirken, *F. baskilensis*' in *Fritillaria* altcinsi üyeleri ile aynı kolda yer alması *F. baskilensis*'in bu altcinste yer aldığı fikrini düşündürmektedir.

Bu çalışma *F. baskilensis* populasyonlarına ait sekans bilgileri kullanılarak yapılmış ilk moleküler tabanlı çalışma olması açısından oldukça önemlidir ve sözkonusu türe ait yeni "*trn*" gen bölgesi haplotipleri ilk defa tarafımızca tespit edilerek GenBank veritabanına kazandırılmıştır.

**Anahtar kelimeler:** *Fritillaria*, endemik, Turkiye, cpDNA, *trn*T-*trn*L3'

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +904242370000; Fax.: +904242370000; E-mail:peyilmaz@firat.edu.tr
 Received: 08.08.2021; Published: 15.12.2021 BioDiCon. 988-080821

## 1. Introduction

Liliaceae is an important family of mostly geophytic plants represented by about 250 genera and 3500 species in the world [1]. The Liliaceae family, which has 44 genera and 426 species in Turkey, is cosmopolitan and shows natural distribution mostly in tropical and temperate regions [2]. Medicinal species from Liliaceae include important ornamental plants and some species are used as foodstuffs. Liliaceae is a family with a high endemism rate [3]. In our country (Turkey), 227 species of the family are endemic. Liliaceae family members can be used as ornamental plants (tulips, hyacinths, lilies, and inverted tulips) and vegetables (onions, leeks, and garlic), as well as medicinal plants due to the chemicals they contain [4].

Species belonging to the genus *Fritillaria* are found in very different habitats such as limestone rocks, stony areas, in-field, field edges, stony fields, meadows, wet meadows, roadsides, *Pinus* L., *Juniperus* L., *Cedrus* Link. forests, deciduous *Quercus* L. forests, forest clearings, *Quercus coccifera* L. scrubs, thickets, mountainous steppe, Umbelliferae and *Astragalus* L. steppes, serpentine areas, rocky and sandy areas near the sea, places where snow has just melted away, and loose slopes. They adapt very well to similar cultural areas. It spreads from sea level to 3500 m. and flowering time is between February and July [5].

The *Fritillaria* genus, which is expressed in a total of 165 taxa, 7 subgenus, and 2 sections in the world, spreads in Europe, the Middle East, Central Asia, and the west of North America [6]. The genus *Fritillaria* is represented by 48 taxa in Turkey, 27 of these taxa are endemic and the endemism rate is 36.53%. This high rate shows that the Flora of Turkey may be the center of the genetic diversity of this genus. The species belonging to the genus *Fritillaria* are of high agricultural and economic importance [7, 8]. Species belonging to the genus *Fritillaria* show a wide variation in terms of their morphological features and physiological adaptation to the environment, and as a result of this variation, they are widely used as ornamental bulbous plants [9]. Only 2 species of *Fritillaria, F. imperialis* and *F. persica* are allowed to trade in Turkey, and their commercial value is quite high [10]. In addition, some *Fritillaria* species are used as antitussives and expectorants in traditional Chinese medicine [11].

Plants belonging to the genus *Fritillaria* spread between 1200-2000 m altitudes. *Fritillaria baskilensis*, which was first collected from Elazig - Baskil district in 1998 and introduced as a new species to the scientific world, is one of Turkey's endemic inverted tulip species [12]. This is the only known habitat for the type sample from this population so far. No other records have been found until today. The plant forms natural populations only in the lower parts of two hills in Selil Mountain in Kuluşağı village of Baskil district Elazığ province in Turkey, and a single or several individuals naturally grow together in an area of 20 decares in total [13].

This study aims to determine the systematic relationships between Endemic *Fritillaria baskilensis* (Liliaceae) and other *Fritillaria* species using molecular techniques. Also; As a result of this study, we will try to determine for the first time, which subgenus the endemic species in question will be. This study is very important as it is the first molecular-based study to be conducted using sequence information from endemic *Fritillaria baskilensis* populations, and the new "*trn*" gene region haplotypes of the mentioned species were determined by us for the first time and added to the GenBank database.

## 2. Materials and methods

### *2.1. Plant material*

In this study, the Turkish endemic populations belonging to *Fritillaria baskilensis*, which grows only in the Baskil district of Elazig, were used. Plant samples were collected from their natural habitats by Kursat and Munzuroğlu during their vegetative period between March and May 2015 [13]. The localities where the samples were collected are given in Table 1. In addition, the flowering state and general view of the plant are shown in Figure 1.

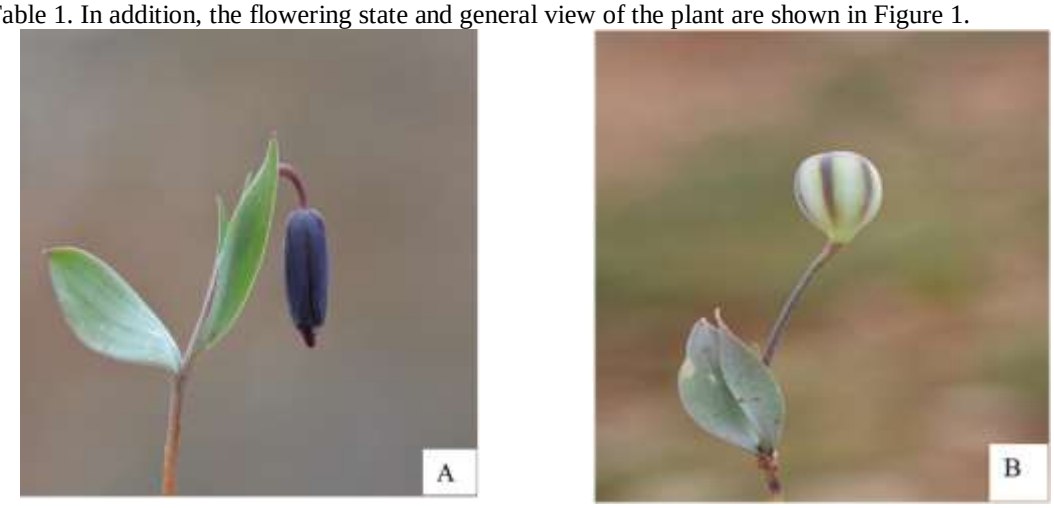


Figure 1: *Fritillaria baskilensis* general view (A. Flowering view B. Fruity view)

Table 1. The examined individuals of the *F. baskilensis* used in the study and detailed localities information

| **Taxon name** | **Detailed localities of populations** |
|---|---|
| *Fritillaria baskilensis 1* | B7 Elazığ: Baskil, Kuluşağı 2 population |
| *Fritillaria baskilensis 2* | B7 Elazig: Baskil, Şahindere village, Aliağa hamlet (Marh and Munzuroğlu populations) |
| *Fritillaria baskilensis 3* | B7 Elazig: Baskil, Hasan Mountain (Haroğlu) population |
| *Fritillaria baskilensis 4* | B7 Elazig: Baskil, Kuluşağı 1 population (The first identified population of the species) |
| *Fritillaria baskilensis 5* | B7 Elazığ: Harput, Göllübağ population |

*2.2. DNA isolation, PCR, and Sequence analysis*

For DNA isolation, leaf tissue that was collected from the field and kept at -20C for molecular studies was used. DNA isolation was performed manually, using a modified CTAB method [14]. The concentrations of the isolated DNAs were adjusted to 20 ng/µl by measuring with a nanodrop spectrophotometer. Stock DNA was stored at -20°C.

In the PCR stage, 2 consecutive regions were amplified with a single primer pair to be used in phylogenetic analysis. The sequence of the primer set for the *trn*T-*trn*L3'(*trn*a-*trn*d) region is given in Table 2. [15]. For PCR studies 10 µL of buffer, 3 µL of MgCl2, 1 µL of dNTP, from each primer 0.50 µL (forward and reverse), 0.50 µL Taq polymerase, approximately 6 ng (2.70 µL) template DNA was mixed with dH2O and the final concentration was adjusted to 50 µL, and the PCR device was repeated for 30 cycles. The content of the PCR cycle was adjusted as follows; Initial denaturation at 95°C for 2 minutes, denaturation at 95°C for 1 minute, annealing at 55°C for 40 seconds (*trn*T-*trn*L3' region), extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes. The resulting PCR products were observed on 1% agarose gel.

Table 2: The base sequences of the primers used [15]

| **Primers** | **Sequence (5'-3')** |
|---|---|
| ***trn*a (Forward):** | 5' CAT TAC AAA TGC GAT GCT CT 3' |
| ***trn*d (Reverse):** | 5' GGG GAT AGA GGA CTT GAA C 3' |

The PCR products obtained for sequence analysis were sent to Macrogene. Purification and sequencing were done by Macrogen. Two-way scanning was performed for each sample. Chromatogram data including sequence results were evaluated using Finch TV version 1.4.

Values such as intra- and inter-species variable regions, genetic distance, nucleotide diversity, molecular diversity parameters for each dataset were calculated using the MEGA program Version X, and phylogenetic analyzes were performed [16]. GenBank Accession numbers were obtained by registering with NCBI (Table 3).

## 3. Results

In this study, the Turkish endemic populations belonging to *Fritillaria baskilensis*, which grows only in the Baskil district of Elazig, were used. Sequence information of two different "*trn*" regions of chloroplast DNA, which are the continuation of each other, in all individuals examined from populations of *Fritillaria* baskilensis were used to determine the systematic relationship of this species. Among the two-way reading sequences sent from Macrogen, the best quality ones were selected using Version 1 of the Finch TV program. Then, the sequences were aligned using the "Multiple Alignment Blast System" of Automatic sequencing systems in the MEGA program. Noticeable differences are fixed manually. As a result of the scans made on NCBI, 12 different species belonging to the genus *Fritillaria* were included in our analyzes and the phylogenetic relationship between 29 species, including outgroups, was examined [17-21]. The GenBank accession numbers of the individuals examined and the samples included in our analysis from NCBI are given in Table 3.

DNA sequences of *trn* regions of 29 individuals (2 outgroup) belonging to the genus *Fritillaria* were statistically analyzed. By evaluating the sequences of *trn* regions of the individuals studied, both separately and together, conserved regions (C), regions with variation (V), parsimony-informative regions (Pi), single base pairs (S), nucleotide composition, homologous base pairs (ii), transitional base pairs (si), transverse base pairs (sv) and R-value (si / sv) were calculated and the obtained values are given in Table 4. These parameters determine the distribution of individuals in the phylogenetic tree and thus give us information about their phylogenetic relationships.

Table 3. GenBank accession numbers of the individuals examined and the samples included in our analysis from NCBI [17-21]

| **Taxa of the genus *Fritillaria*** | **GenBank Accesion Numbers** |
|---|---|
| *F. baskilensis 1* | MZ327256 |
| *F. baskilensis 2* | MZ327257 |
| *F. baskilensis 3* | MZ327258 |
| *F. baskilensis 4* | MZ327259 |
| *F. baskilensis 5* | MZ327260 |
| *F. aurea* | JQ289553 |
| *F. caucasica* | JQ289557 |
| *F. imperialis 1* | JQ327135 |
| *F. imperialis 2* | JQ289551 |
| *F. imperialis 3* | KM435201 |
| *F. crassifolia ssp. kurdica* | JQ289552.1 |
| *F. crassifolia ssp. kurdica 2* | JQ289552.2 |
| *F. latifolia* | JQ289555 |
| *F. michailovskyi 1* | JQ289554 |
| *F. michailovskyi 2* | JQ327136 |
| *F. michailovskyi 3* | JQ327137 |
| *F. minuta* | JQ289556 |
| *F. karelinii* | MG211821 |
| *F. cirrhosa* | MH593346 |
| *F. usuriensis* | MH593369 |
| *F. thunbergii* | MH593362 |
| *F. tortifolia* | NC037214 |
| *F. verticillata* | NC037217 |
| *F. meleagroides* | MF947710 |
| *F. davidii* | NC045895 |
| *F. maximowiczii* | NC045894 |
| *Lilium lancifolium* (outgroup) | MH177880 |
| *Lilium davidii* (outgroup) | EU597205 |

Table 4. Molecular diversity parameters obtained from sequences of the "*trn*" regions of examined individuals.

| **Parameters of Molecular Diversity** | ***trn*T-*trn*L3' Region** |
|---|---|
| Total individuals | 29 |
| Total band Length | ~ 570 |
| The ratio of G-C base pair (%) | 33.6 |
| Conserved regions (C) | 547 |
| Variation regions (V) | 22 |
| Single parts (S) | 2 |
| Parsimony informative regions (Pi) | 20 |
| Homologous base pairs (ii) | 552 |
| Transitional base pairs (si) | 2 |
| Transversional base pairs (sv) | 2 |
| R value (si/sv) | 0.7 |

When Table 4 is examined, it is seen that individuals are not very different in terms of the ratio of T, C, A, and G bases. At the same time, when the average values of the base content of the individuals were calculated, it was seen that the A-T ratio was 66.4% and the G-C ratio was 33.6%, that is, the A-T base pair was richer than the G-C base pair.

To determine the phylogenetic relationships between both the *F. baskilensis* species we studied and the individuals belonging to other *Fritillaria* taxa whose sequences we obtained from NCBI, a total of 29 individuals, 2 of which were outgroups, were examined in the Mega program.

Then, using the Best DNA / Protein step in the Models menu of this program, the methods that can best express the phylogenetic relationship between individuals were determined, and the lowest BIC (Bayesian Information Criterion) value in the given list of methods was found in the K2 + G (Kimura-3-parameter) method. According to K2+G, it was determined that phylogenetic trees can be drawn using any of the methods such as Maximum Parsimony, Neighbor-Joining, UPGMA, and Maximum Likelihood to see phylogenetic relationships between individuals.

Maximum Likelihood, Neighbor-Joining, UPGMA, and Maximum Parsimony methods were applied separately, but it was decided that the Maximum Parsimony method was the method that best showed the evolutionary and

phylogenetic relationships between the individuals studied. In the phylogenetic tree drawing, the *trn* region of the chloroplast genome was evaluated together using the X version of the Mega program [16]. In the Maximum Parsimony method, 100 bootstrap values were entered and phylogenetic trees were obtained for 29 individuals in total, and comments were made according to the most useful tree (Figure 2).

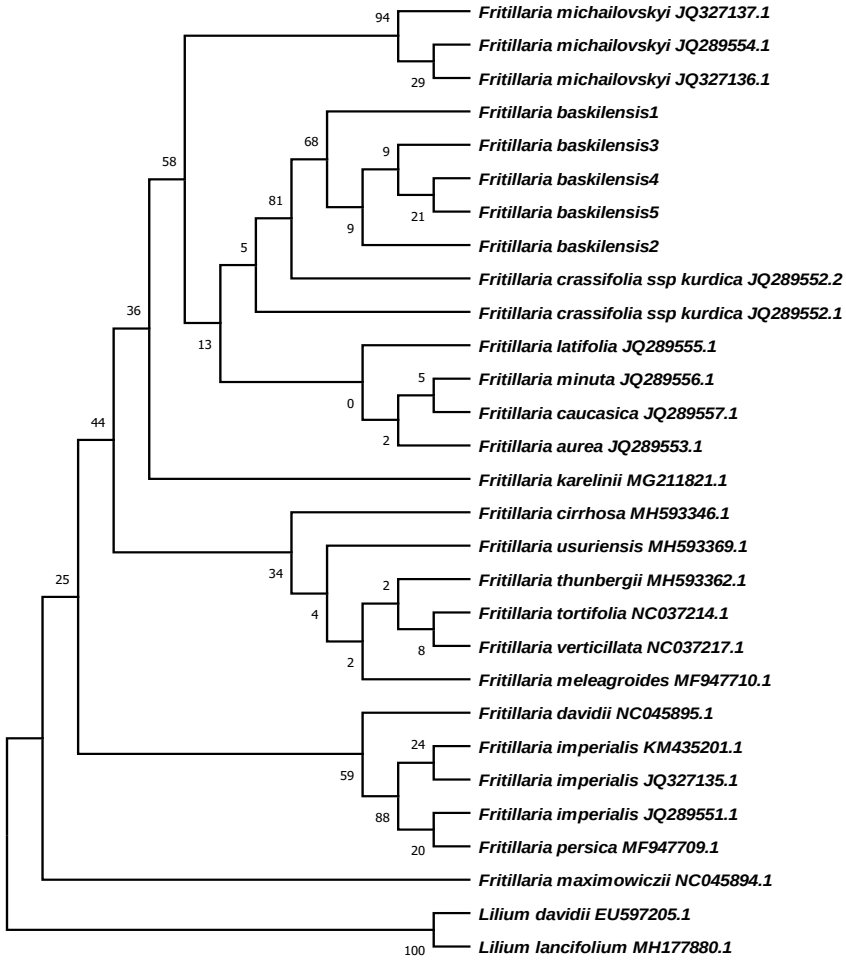


Figure 2: Maximum Parsimony tree obtained from the sequences of the "*trn*" region

## 4. Discussion

The genus *Fritillaria* is considered to be a monophyletic genus, the sister group of *Lilium* and the largest member of this tribe, in the tribe Lilieae [22,23]. The most accepted classification of the *Fritillaria* genus, which has been subjected to many different classifications until today, is the classification made by Rix. Rix conducted extensive studies on *Fritillaria* in 1975 and 1979 [24,25]. In the list of *Fritillaria* species, *Fritillaria* is classified into subgenus, sections, and series [6]. Describing 8 subgenera in total, Rix used sections to further group the subgenus. Rix reorganized both *Rhinopetalum* and *Korolkowia* as subgenus, renamed *Eufritillaria* as *Fritillaria*, and added the subgenus *Davidii* and *Japonica*. The largest subgenus of the *Fritillaria* genus, which is characterized by a total of 8 subgenera, is *Fritillaria*, while *Theresia*, *Korolkowia*, and *Davidii* are defined as monotypic as they contain only one species. The subgenus, which has the most species in our country, is the *Fritillaria* subgenus, and the species belonging to the *Rhinopetalum*, *Liliorhiza*, *Korolkowia*, *Davidii*, and *Japonica* subgenus are not found in our country [6-22].

This study aims to try to determine the systematic position of *F. baskilensis*, which is an endemic species and whose subgenus has not yet been determined, by making a phylogenetic analysis. In addition, molecular data about this endemic taxon will be brought to the literature by us for the first time. There are few studies at the molecular level on *Fritillaria* species that grow naturally in Turkey. In this study, phylogenetic analysis of 29 *Fritillaria* taxa (2 outgroup) based on the cpDNA *trn* region, (6 of which was by us), was performed. By adding the obtained new sequence data to the GenBank database, the chloroplast DNA *trn*T-*trn*L3' region of *F. baskilensis* was clarified by us.

Because of the reproducibility of the results, DNA sequencing-based strategies are widely used in identifying species and resolving phylogenetic relationships [26]. Therefore, both forward and reverse sequencing of our samples was performed, and the resulting data successfully confirmed each other. To clarify the systematic limits of this species,

the obtained sequences were compared with the closely related sequences in the database to obtain information about the phylogenetic position of the endemic taxon we studied. As a result of the scans made by NCBI, the cpDNA regions of many species could not be included in our research because parallel studies were not conducted with our studies. However, samples from every subgenus were included in the study as much as possible.

Our phylogenetic analysis revealed that the 27 *Fritillaria* taxa were divided into 2 main classes. The first class mostly includes species of the subgenus *Fritillaria*, which is the largest subgenus of the genus. The phylogenetic tree we obtained showed a separation, supporting the previous studies of the genus and its phylogeny. According to the phylogenetic tree, *F. maximowiczii*, a species belonging to the subgenus *Liliorhiza*, divided the tree into 2 (Figure 2.). In the literature, the North American origin *Liliorhiza* subgenus is separated from all other Eurasia origin subgenus (Figure 3.) [22].

Subgenus *Petilium* (*F. imperialis*) is in the same branch as the monotypic *Theresia* (*F. persica*), supporting the idea that the two subgenera are very close to each other. Subgenus *Davidii* with a group of subgenus *Fritillaria* members, on the other hand, is separated from the other group by being in the same arm with these two subgenera. Taxa belonging to Subgenus *Fritillaria* showed grouping among themselves, while one group appeared close to *Petilium*, *Theresia*, *Davidii*, and the other group were in the same branch with *Rhinopetalum*.

The taxa belonging to the genus *F. baskilensis*, which is the subject of this study, are in the same branches together with the *Fritillaria* taxa grouped with the *Rhinopetalum* subgenus. This gave us the idea that *F. baskilensis* may belong to the subgenus *Fritillaria*. High bootstrap values also supported our idea.

The development of molecular phylogenetic and cladistic analysis has allowed a better understanding of the infrageneric relationships of *Fritillaria* species. Early studies showed that the major infrageneric division is divided into two classes, North America (class A) and Eurasia (class B) based on biogeographic region. Class A overlapped most closely with the subgenus *Liliorhiza* [19].

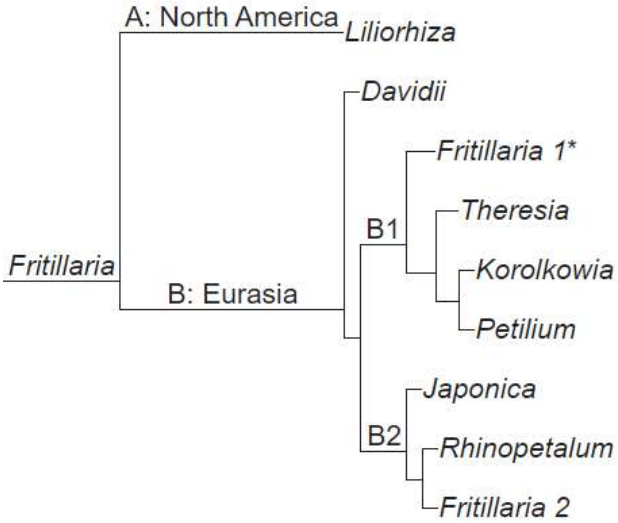


Figure 3: General classification of the subgenus *Fritillaria* [22]

Rønsted et al. (2005) conducted a study using an expanded pool of taxa of 37 species, including all subgenus and divisions classified by Rix and extensively analyzed the sequences of the *mat*K gene, *rpl*16 plastid gene, *trn*K, and nuclear ribosomal ITS. Their results confirmed the first division of *Fritillaria* taxa based on geography (Figure 3.) [22]. Class A corresponds to the California-based subgenus *Liliorhiza*, but some species are found in Western Asia. These Asian species form a clad with true North American species, suggesting redistribution after an Asian origin [22]. The taxa belonging to *Fritillaria*, the largest subgenus of class B, are divided into two groups and spread to different branches, suggesting that this subgenus is polyphyletic [15]. The phylogenetic tree we obtained showed a parallel split, confirming this study.

In addition, the 5S rDNA region sequences of 4 *Fritillaria* taxa were patented by Sucher et al. [28]. Türktaş et al. (2012) performed a phylogenetic analysis based on the cpDNA *trn*L-F region of 11 different *Fritillaria* species collected from their natural habitats. Their results showed that 11 *Fritillaria* taxa were grouped in 2 branches, strongly supporting the separation of *Fritillaria* subgenus from *Petilium* and *Theresia* subgenus, while the resolution within *Fritillaria* subgenus was not clear [8]. They argued that the phylogeny of the genus *Fritillaria* should be reanalyzed using more advanced techniques. In addition, Türktaş et al. stated that new hybrid species can be produced from *Fritillaria*, an ornamental plant, with DNA-based studies [8].

Metin et al. (2013) studied the genetic relationships of 12 *Fritillaria* taxa collected from different regions of Turkey with the AFLP method in their research on *Fritillaria*. Their results supported the classical classification of *Fritillaria* [29].

As a result of this study, original data were obtained for use in new scientific molecular studies, and new *trn*T-*trn*L3' region haplotypes of endemic *F. baskilensis* were provided for GenBank. In the phylogenetic evolutionary family tree we created, it is observed that the taxon *F. baskilensis* is very close to Subgenus *Fritillaria*, while the polyphyletic nature of this subgenus brings about the fact that further studies are needed to solve the systematic problems in between.

**Acknowledgment**

This study was financed within the scope of the "Baskil reverse tulip species protection action plan implementation activities project" supported by the Turkish Ministry of Forestry and Water Affairs, General Directorate of Nature Conservation and National Parks.

**References**

[1] Satil, F. & Akan, H. (2006). Anatomical Studies on Some Endemic and Rare Geophytes of Liliaceae Family. *Ekoloji*, 58, 21-27.

[2] Erik, S. & Tarıkahya, B. (2004). Türkiye Florası Üzerine. *Kebikeç*, 17, 139-163.

[3] Davis, P.H. (1965). Flora of Turkey and the East Aegean Islands. *Edinburgh University Press*, Edinburgh 11, 243-558.

[4] Heywood, V.D. (1978). Flowering Plants of the World. *Oxford University Press*, London.

[5] Kıran, Y. & Gedik, O. (2014). Karyological Study on Turkish Endemic *Fritillaria baskilensis* Behçet. (Liliaceae). *Turkish Journal of Science & Technology*, 9(2), 105-108.

[6] Rix, M., Frank, E., Webster, G. & Alpine Garden Society (Great Britain). (2001). *Fritillaria*: A revised classification : together with an updated list of species. England: *Fritillaria* Group of the Alpine Garden Society.

[7] Tekşen, M. & Zeki, A. (2008). *Fritillaria mughlae* (Liliaceae), a New Species from Turkey. *In Annales Botanici Fennici*, 45(2),141-147.

[8] Türktaş, M., Aslay, M., Kaya, E. & Ertuğrul, F. (2012). Molecular characterization of phylogenetic relationships in *Fritillaria* species inferred from chloroplast *trn*L-*trn*F sequences. *Turkish Journal of Biology*, 36, 552-560. https://doi:10.3906/biy-1201-30.

[9] Tekşen, M. & Zeki, A. (2011). The revision of the genus *Fritillaria* L.(Liliaceae) in the Mediterranean region (Turkey). *Turkish Journal of Botany*, 35, 447-478. https://doi:10.3906/bot-0812-9.

[10] Demir, A. (2019). Türkiye'de *Fritillaria imperialis*'in Ekonomik Değer Analizi. *Biological Diversity and Conservation*, 12(3), 103-110.

[11] Shujun, W., Wenyuan, G., Wei, J. & Peigen, X. (2006). Crystallography, morphology and thermal properties of starches from four different medicinal plants of *Fritillaria* species. *Food Chemistry*, 96, 591-596. https://doi:10.1016/j.foodchem.2005.03.014.

[12] Behçet, L. (1998). A new species of *Fritillaria* L.(Liliaceae) from east anatolia Turkey. *Bulletin of Pure and Applied Sciences*, 17,1-2.

[13] Kursad, M. (2017). Baskil ters lalesi tür koruma eylem planı uygulama faaliyetleri projesi. *T.C. Orman ve Su işleri bakanlığı, Doğa koruma ve milli parklar genel müdürlüğü*.

[14] Doyle, J.J. & Doyle, J.L. (1987). A Rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin*, 19, 11-15. https://doi: 10.1007/978-3-642-83962-7_18.

[15] Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991). Universal primers amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, 17(5), 1105-1109. https:// dx.doi.org/10.1007/BF00037152.

[16] Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547-1549. https://doi: 10.1093/molbev/msy096.

[17] Do, H. & Kim, J.H. (2019). The implication of plastid transcriptome analysis in petaloid monocotyledons: A case study of *Lilium lancifolium* (Liliaceae, Liliales). *Scientific Reports*, 9**,**6662. https://doi.org/10.1038/s41598-019-43259-7.

[18] Bi, Y., Zhang, M.F., Xue, J., Dong, R., Du, Y.P. & Zhang, X.H. (2018). Chloroplast genomic resources for phylogeny and DNA barcoding: a case study on *Fritillaria*. *Scientific Reports*, 8(1), 1184. https://doi: 10.1038/s41598-018-19591-9.

[19] Chen, Q., Wu, X. & Zhang, D. (2020). Comparison of the abilities of universal, super, and specific DNA barcodes to discriminate among the original species of *Fritillariae cirrhosae* bulbus and its adulterants. *Plos One*, 15(2), e0229181. https://doi.org/10.1371/journal.pone.0229181.

[20] Yan, Li., Zhirong, Z. & Guanghui, L.V. (2017). The complete chloroplast genome of *Fritillaria yuminensis*, a rare and endangered species endemic to China. *Mitochondrial DNA*, Part B, 2(2), 913-914. https://doi: 10.1080/23802359.2017.1413315.

[21] Huang, J., Yu, Y., Liu, Y.M., Xie, D.F., He, X.J. & Zhou, S.D. (2020). Comparative Chloroplast Genomics of *Fritillaria* (Liliaceae), Inferences for Phylogenetic Relationships between *Fritillaria* and *Lilium* and Plastome Evolution. *Plants (Basel)*, 9(2), 133. https://doi:10.3390/plants9020133.

[22] Rønsted, N., Law, S., Thornton, H., Fay, M.F. & Chase, M.W. (2005). Molecular phylogenetic evidence for the monophyly of *Fritillaria* and *Lilium* (Liliaceae) and the infrageneric classification of *Fritillaria*. *Molecular Phylogenetics and Evolution*. 35(3), 509–527. https://doi.10.1.1.574.7908.

[23] Peruzzi, L., Innangi, M., Tatino, F. & Santangelo, A. (2017). *Fritillaria messanensis* subsp. *gracilis* (Liliaceae), a new record for the Italian Flora (S Italy). *Phytotaxa*, 307(2), 167. https://doi: 10.11646/phytotaxa.307.2.11.

[24] Rix, E.M. (1975). Notes on *Fritillaria* (Liliaceae) in the Eastern Mediterranean Region, III. *Kew Bulletin*: Kew Bulletin, 30(1),153-162. https://doi.org/10.2307/4102882.

[25] Rix, EM. (1979). Notes on *Fritillaria* (Liliaceae) in the Eastern Mediterranean Region: IV. *Kew Bulletin*: 33(4), 585-600. https://doi.org/10.2307/4109803.

[26] Khan, I.A. & Azim, M.K. (2011). Variations in intergenic spacer *rpl*20-*rps*12 of mango (*Mangifera indica*) chloroplast DNA: implications for cultivar identification and phylogenetic analysis. *Plant Systematics and Evolution*, 292, 249-255. https://doi.org/10.1007/s00606-011-0424-4.

[27] Fay, M.F. & Chase, M.W. (2000). Modern concepts of Liliaceae with a focus on the relationships of *Fritillaria*. *Curtis's Botanical Magazine*, 17(3), 146-149.

[28] Sucher, N.J., Tsim, K.W. & Ip, N.Y. (2003). *Fritillaria* species identification. US Patent 6.569.625. Washington, DC: US Patent and Trademark Office.

[29] Karakas M. O., Turktas, M., Aslay, M. & Kaya, E. (2013). Evaluation of the genetic relationship between *Fritillaria* species from Turkey's flora using fluorescent-based AFLP. *Turkish Journal of Biology*, 37, 273-279. https://doi:10.3906/biy-1207-62.