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Utility of partial 26S rDNA sequences in DNA-based identification of endemic plants

Endemik bitkilerin filogenetik tanımlaması için kısmi 26S rDNA dizilerinin kullanımının değerlendirilmesi

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ABSTRACT

Endemic and rare plant taxa are critical components of biodiversity, playing key roles in ecosystem stability and resilience. However, these species are increasingly threatened by habitat loss, climate change, and anthropogenic pressures. Molecular approaches, particularly DNA barcoding, have become essential for accurate species identification and the assessment of genetic diversity. In this study, we evaluated the effectiveness of the partial 26S rDNA region as a DNA barcode marker for the molecular identification of 30 endemic and rare plant species from Kars Province, Türkiye. To our knowledge, this is the first application of the 26S rDNA region in these taxa. Sequence analyses revealed no exact matches in the GenBank database, indicating potential novelty and underscoring the scarcity of reference data. A total of 42 variable sites were identified across the sequences, and phylogenetic analyses largely clustered the species in accordance with their taxonomic families. Importantly, 30 novel barcode sequences were generated and submitted to public databases, offering valuable resources for future taxonomic, phylogenetic, and conservation-oriented studies. This work demonstrates the utility of partial 26S rDNA sequences for the molecular characterization of understudied endemic plants and provides a foundational step toward enhancing biodiversity documentation and conservation efforts in Türkiye.

ÖZ

Endemik bitki türleri, biyolojik çeşitliliğin hayati bileşenleridir ve ekosistem sürekliliğine ve direncine katkıda bulunurlar. Ancak bu türlerin birçoğu habitat tahribatı, iklim değişikliği ve insan faaliyetleri nedeniyle artan tehditlerle karşı karşıyadır. Moleküler yaklaşımlar, özellikle DNA barkodlama, endemik bitkilerin genetik çeşitliliğini tanımlamak ve anlamak için gerekli hale gelmiştir. Bu çalışmada, Kars ilinden 30 endemik bitki türünün filogenetik ilişkileri 26S rDNA gen bölgesi kullanılarak araştırılmıştır. Genomik DNA, modifiye CTAB yöntemi kullanılarak izole edilmiş ve 26S rDNA bölgesi bu türler için ilk kez başarılı bir şekilde çoğaltılmış ve dizilenmiştir. Dizi karşılaştırmaları, GenBank veri tabanında tam eşleşme göstermemiştir ve yakın akraba taksonlarla hizalama göstermiştir. *Allium czelghauricum* ve *Fritillaria michailovskyi*'nin kayda değer genetik farklılaşma sergilediği ve farklı evrimsel geçmişlere işaret eden toplam 42 değişken bölge tespit edilmiştir. Filogenetik analiz çoğu türü taksonomik ailelerine göre gruplandırmış, ancak muhtemelen taksonomik tutarsızlıklar veya yakınsak evrim nedeniyle bazı beklenmedik gruplanmalar gözlenmiştir. Bu çalışmanın sonuçları, Türkiye'nin endemik florasını anlamak için değerli genetik veriler sağlamakta ve biyoçeşitliliğin korunması için bir temel sunmaktadır.

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1. INTRODUCTION

Endemic species, restricted to specific geographic regions and absent elsewhere, constitute unique and vulnerable components of biodiversity. Their conservation is crucial for maintaining ecosystem resilience, stability, and functionality (Myers et al., 2000; Vellend et al., 2013). Endemic plants often fulfill specialized ecological roles and are particularly susceptible to threats such as habitat loss, climate change, and other environmental pressures. Molecular

identification techniques, particularly DNA barcoding, have become indispensable tools for the accurate identification and conservation of endemic plant taxa (CBOL, 2009). By enabling precise monitoring, these methods allow conservationists to develop targeted strategies for the preservation and sustainable management of vulnerable species. Furthermore, molecular data provide valuable insights into the biogeography of endemic plants, shedding light on their historical distribution patterns, migration routes, and ecological relationships (Chase et al., 2005; Shaw et al., 2005; Sgro et al., 2011). In addition, molecular identification supports the effective management and utilization of endemic plants as critical genetic resources of regional biodiversity (Hebert et al., 2003; Hollingsworth et al., 2016).

The 26S rDNA gene region, located in the nuclear genome of eukaryotes such as plants, animals, and fungi, encodes an important part of the 26S ribosomal RNA, which is a key component of the large ribosomal subunit (Gerbi, 1985; Kuzoff et al., 1998). Because of its essential role in protein synthesis, this region has become a focus of scientific research. Researchers use the 26S rDNA sequence, along with other molecular markers, to study plant systematics, taxonomy, and historical biogeography (Linder et al., 2000; Soltis et al., 2001; Markos and Baldwin, 2022). The 26S rDNA region is highly conserved across different plant groups, reflecting the importance of ribosomal RNA in cell function (Alvarez and Wendel, 2004). However, it also shows enough variation to help resolve relationships at different taxonomic levels, from genera to families. Its conserved nature and broad applicability make it a valuable tool for phylogenetic studies across many organisms. The moderate rate of sequence variation allows researchers to detect small genetic differences and build reliable phylogenetic trees (Baldwin et al., 1995). These trees contribute to our understanding of species' historical distributions (Markos and Baldwin, 2002). In addition, sequence differences in the 26S rDNA region can be used to distinguish between closely related species.

The Kars province is home to an exceptionally rich floral diversity, comprising 16% of Turkey's flora with 1,615 plant species, 71 of which are endemic (Uyanık et al., 2013; Güneş and Özba, 2014). This region, located in the Caucasus region of Turkey, lies at the intersection of the Irano-Turanian, Euro-Siberian, and Mediterranean phytogeographic regions (Güneş and Özba, 2014). However, the area faces challenges such as excessive and unmanaged grazing, as well as land clearance activities, which pose a significant threat to the continuity of various species, particularly endemic ones (Ekim et al., 2000). Uncovering and preserving the genetic diversity that enables species to adapt to changing environmental conditions is crucial for safeguarding biodiversity. Among the 71 endemic species, 12 are specifically localized around Lake Çıldır, the Allahuekber Mountains, and the Sarıkamış forests, areas designated as Important Plant Areas (IPA) in Turkey (Güneş and Özba, 2014; Özhatay, 2006). Assigning molecular identities to endemic plants plays a vital role in the conservation and sustainable development of biological diversity in our country.

In this study, we aimed to assign molecular identities to the endemic plant species of Kars province. The phylogenetic relationships of 30 endemic plant species were analyzed using the 26S rDNA gene region. By examining both intra- and inter-species variations, we aimed to gain a deeper understanding of their genetic relatedness. The genetic data generated in this study can serve as a foundation for future research focused on the conservation of endemic species and biodiversity. Molecular identification of these endemic plants may play a crucial role in enhancing efforts to understand, conserve, and manage the unique plant diversity of the Kars region.

2. MATERIALS AND METHODS

2.1. Sampling and genomic DNA extraction

The plant samples collected were numbered, with the necessary field records and locality information documented, before being pressed and dried according to herbarium techniques. The Flora of Turkey (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000) was used as the primary reference for identifying these samples. The plant samples collected from each population in sufficient quantities for the study were numbered, dried according to herbarium techniques, and converted into herbarium specimens. These were then stored at the Van Yüzüncü Yıl University Herbarium (VANF) and assigned log numbers. Leaf samples were systematically collected from various locations within

Kars province, representing 18 distinct endemic plant species (Table 1). Due to habitat loss, which has led to the disappearance of some species from their natural habitats, leaf samples of 12 endemic plant species were obtained from the Van Yüzüncü Yıl University Herbarium. Nuclear DNA extraction from leaf tissues was conducted using the modified CTAB DNA isolation method devised by Kistler (2012). To assess DNA concentrations and quality, readings at 230 nm, 260 nm, and 280 nm were obtained using the Biodrop lLite 7141 V.1.0.4 spectrophotometer. The 26S rDNA forward and reverse primer sequences, specifically 5'-ttcccaaacaacccgactc-3' and 5'-gccgtccgaattgtagtctg-3' (Alvarez and Wendel, 2004), were employed for the PCR reaction. The reaction mixture, totaling 20 µl, comprised 4 µl HOT FIREPol Blend Master Mix (Solis BioDyne, Tartu, Estonia), 0.5 µl of 200 nM forward and reverse primers, 5 µl of template DNA (diluted to 10 ng), and 10 µl of water. The PCR protocol included an initial cycle at 95 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 58 °C (Ta) for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 min. After PCR, electrophoresis was performed on 3% agarose gels at 90V for 30 min. PCR products demonstrating the desired amplification were subjected to purification and sequencing at BM Labosis (Çankaya, Ankara). Chromatogram data visualization, BLAST search (Altschul et al., 1990), and CLUSTAL alignment (Thompson et al., 1994) were executed using MEGA 11 Software (Kumar et al., 2018). MEGA 11 software facilitated the computation of essential phylogenetic parameters, identifying DNA polymorphism among the endemic plant species. BLAST analysis compared the 26SrDNA sequences of the 30 endemic plant species with sequences of closely related species from the NCBI database (NCBI, 2024). These analyses assessed the correspondence between the acquired sequences and previously studied sequences of the same species or closely related species. The use of 26S rDNA sequences in phylogenetic analyses allows the identification of relatedness relationships among endemic species and helps to detect genetic divergence among morphologically similar species. To explain relatedness similarities and relationships among endemic species, the Neighbor Joining Method (Saitou and Nei, 1987) was used to construct a phylogenetic tree (Tajima and Nei, 1984).

Table 1. Information on 30 endemic plant species from Kars province (* indicates endemic plants obtained from Van Yüzüncü Yıl University Herbarium)

Endemic plant taxa	Distrubution	Endemism	Family
Onosma nigricaulis Riedl	North East Anatolia, Kars	Local Endemic	Boraginaceae
Onosma isaurica Boiss. & Heldr.	North East Anatolia, Sarıkamış	Endemic	Boraginaceae
Nonea karsensis Popov	North East Anatolia, Kars	Local endemic	Boraginaceae
Anchusa leptophylla Roem. & Schult. subsp. *incana* (Ledeb.) D.F.Chamb.	North and Central Anatolia, Kars	Endemic	Boraginaceae
**Galinsoga parviflora* Cav.	Whole Anatolia, Kars	Endemic	Asteraceae
Hieracium sarykamyschense Üksip	North East Anatolia, Sarıkamış	Endemic	Asteraceae
Tragopogon aureus Boiss.	North Anatolia, Kars	Endemic	Asteraceae
Corydalis oppositifolia DC.	North, South, East Anatolia, Sarıkamış	Endemic	Papaveraceae
Papaver triniifolium Boiss.	North East and South Anatolia, Çıldır	Endemic	Papaveraceae
Rosa pisiformis (Christ) Sosn.	North East Anatolia, Kars	Endemic	Rosaceae
Lathyrus karsianus P.H. Davis	North East Anatolia, Sarıkamış	Local Endemic	Fabaceaea
Astragalus globosus Vahl	North Anatolia, Kısır Mountain	Endemic	Fabaceae
**Hedysarum aucheri* Boiss.	North and Central Anatolia, Kars	Endemic	Fabaceae
**Hedysarum nitidum* Willd.	North East Anatolia, Kars	Endemic	Fabaceae
Lamium galactophyllum Boiss. & Reuter	North East Anatolia, Selim	Endemic	Lamiaceae
Salvia rosifolia Sm.	North East Anatolia, Kağızman	Endemic	Lamiaceae
**Stachys cretica* L. subsp. *anatolica* Rech. f.	West, Central, North Anatolia, Kars	Endemic	Lamiaceae
**Phlomis armeniaca* Willd	North and Central Anatolia, Kars	Endemic	Lamiaceae
Allium czelghauricum Bordz.	North East Anatolia, Göle	Local Endemic	Amaryllidaceae
Trinia scabra Boiss. & Noë	North East and South Anatolia, Çıldır	Endemic	Apiaceae
Pastinaca armena Fisch. & C.A.Mey. subsp. *dentata* (Freyn et Sint.) Chamberlain	North East Anatolia, Arpaçay	Endemic	Apiaceae
**Gypsophila brachypetala* Trautv.	North East Anatolia, Kars	Endemic	Caryophyllaceae

**Gypsophila eriocalyx* Boiss.	North and Central Anatolia, Kars	Endemic	Caryophyllaceae
**Cerastium armeniacum* Gren	North East Anatolia, Kars	Endemic	
**Cerastium gnaphalodes* Fenzl	North East and Central Anatolia, Kars	Endemic	Caryophyllaceae
Vincetoxicum coskuncelebianus Makbul & Güven	North East Anatolia, Çıldır, Taşbaşı village	Local Endemic	Apocynaceae
Fritillaria michailovskyi Fomin	North East Anatolia, Sarıkamış	Endemic	Liliaceae
**Draba orientalis Karabacak & Behçet*	East Anatolia, Kars	Endemic	Brassicaceae
**Draba bruniifolia* Steven subsp. *armeniaca* Coode & Cullen	Whole Anatolia, Kars	Endemic	Brassicaceae
**Rumex gracilescens* Rech.f.	North and Central Anatolia, Kars	Endemic	Polygonaceae

3. RESULTS

A large amount and good quality of genomic DNA were obtained from 30 endemic plant species with the help of the modified CTAB method from Kistler and Shapiro (2011). The 26S rDNA region of 30 endemic plant taxa collected and studied from Kars province was successfully amplified and sequenced for the first time. The aligned length of the 26S rDNA region was found to be about 150 base pairs for all endemic species. The 30 endemic and rare taxa analyzed did not have previously published 26S rDNA sequences available in the NCBI GenBank database (NCBI, 2024). Therefore, direct BLAST match with reference sequences from the same species could not be performed. Instead, the sequences obtained from these endemic plants were exhibited a BLAST match with sequences of closely related species at the genus or family level. As a result, exact matches through BLAST searches could not be established. Accession codes for sequences obtained from the 26S rDNA region were taken and deposited on GenBank database (NCBI, 2024) (Table 2).

Table 2. GenBank BLAST match results of 26S rDNA gene region for the studied 30 endemic plant taxa

Endemic Species	Product Length (bp)	Aligned Species	Accession Number	Newly Deposited Accession Number	Coverage (%)	e-Value	Identity (%)
Draba brunifolia	150	*Draba incana*	OY755217.1	PV1382298	100	9e-73	100
Draba orientalis	150	*Draba incana*	OY755217.1	PV1382299	100	3e-69	100
Cerastium gnaphalodes	150	*Agrostemma githago*	OY288254.1	PV1382294	100	0	99.32
Stachys cretica subsp. anatolica	150	*Stachys palustris*	MT610963.1	PV1382292	100	3e-70	99.32
Phlomis armeniaca	150	*Phlomis herba*	OR290891.1	PV1382293	100	8e-73	100
Galinsago parviflora	150	*Cirsium vulgare*	MT610929.1	PV1382302	100	2e-73	100
Hedysarum aucheri	150	*Astragalus canadensis*	MT610924.1	PV1382300	100	6e-74	100
Gypsophila brachypetala	150	*Agrostemma githago*	OY288244.1	PV1382297	97	1e-71	100
Hedysarum nitidum	150	*Astragalus canadensis*	MT610924.1	PV1382301	100	6e-74	100
Gypsophila eriocalyx	150	*Silene latifolia*	MT610955.1	PV1382296	100	1e-71	99.33
Cerastium armeniacum	150	*Agrostemma githago*	OY288260.1	PV1382295	100	6e-70	99.32
Onosma nigricaulis	150	*Echium plantagineum*	OL580770.1	PP344709	98	1e-68	100
Onosma isaurica	150	*Echium plantagineum*	OL580770.1	PP344702	98	1e-68	100
Tragopogon aureus	150	*Tragopogon dubius*	KT179725.1	PP344700	97	9e-78	100
Corydalis oppositifolia subsp. Oppositifolia	150	*Corydalis wilsonii*	LN610850.1	PP344701	100	2e-74	100
Rosa pisiformis	150	*Rosa chinensis*	XR_002934681	PP344705	98	6e-72	100
Lathyrus karsianus	150	*Lathyrus decaphyllus*	KT459234.1	PP344703	98	2e-72	99.32
Astragalus globosus	150	*Astragalus canadensis*	MT610924.1	PP344707	98	2e-70	99.32
Lamium galactophyllum	150	*Ballata nigra*	ON685391.1	PP344704	100	1e-69	100
Salvia rosifolia	150	*Salvia carduaceae*	MK257800.1	PP344699	100	3e-73	100
Allium czelghauricum	150	*Allium altaicum*	MK049255.1	PP344712	94	7e-57	96.48
Papaver triniifolium	150	*Papaver somniferum*	XR_003342571.1	PP344710	100	5e-68	99.33
Pastinaca armena	150	*Zizia aurea*	MT610976.1	PP344711	100	1e-69	100
Vincetoxicum coskuncelebianus	150	*Asclepias tuberosa*	KY860923.1	PP344708	100	2e-66	98.66
Fritillaria michailovskyi	150	*Lilium michauxii*	AF205126.1	PP344706	96	3e-65	99.31
Trinia scabra	150	*Zizia aurea*	MT610976.1	PQ824973	99	1e-72	100
Rumex gracilescens	150	*Rumex sanguineus*	MT937131.1	PV138303	99	1e-64	94.63
Nonea karsensis	150	*Nonea vesirica*	OL580769.1	PQ824975	97	2e-74	98.63
Hieracium sarykamychense	150	*Carthamus rhaponticoides*	OR674047.1	PQ824974	98	2e-71	100
Anchusa leptophylla subsp. incana	150	*Pentaglottis sempervirens*	OZ078321.1	PQ824976	98	5e-68	98.64

Onosma nigricaulis Riedl (Boraginaceae) and *O. isaurica* Boiss. & Heldr. (Boraginaceae) shared the same sequence as *Echium plantagineum* (OL580770.1). *Cerastium gnaphalodes* Fenz (Caryophylaceae), *C. armeniacum* Gren (Caryophylaceae), and *Gypsophila brachypetala* Trautv. (Caryophylaceae) aligned with *Agrostemma githago* L. (Caryophylaceae) (OY288260.1) with 99.32% and 100% identity, respectively, while *G. eriocalyx* Boiss. (Caryophylaceae) showed a BLAST match with *Silene latifolia* Poir. (Caryophylaceae) with 99.33% identity. *Lamium galactophyllum* Boiss. & Reuter (Lamiaceae) showed the highest alignment with *Ballota nigra* L. (Lamiaceae) (ON685391.1). *Pastinaca armena* Fisch. & C.A.Mey. subsp. *dentata* (Freyn et Sint.) Chamberlain (Apiaceae) and *Trinia scabra* Boiss. & Noë (Apiaceae) exhibited the highest alignment with *Zizia aurea* (L.) W.D.J.Koch (Apiaceae) (MT610976.1), both with 100% identity. *Fritillaria michailovskyi* Fomin (Liliaceae) showed a BLAST match with *Lilium michauxii* Poir (Liliaceae)(AF205126.1) with 99.31% identity. *Hedysarum aucheri* Boiss. (Fabaceae) and *H. nitidum* Willd (Fabaceae) showed 100% sequence similarity with *Astragalus canadensis* L. (Fabaceae). The 26S rDNA sequences of *Tragopogon aureus* Boiss (Asteraceae), *Corydalis oppositifolia* DC. (Papaveraceae), *Rosa pisiformis* (Christ) Sosn. (Rosaceae), *Lathyrus karsianus* P.H. Davis (Fabaceae), *Salvia rosifolia* SM (Lamiaceae), *A. globosus* Vahl (Fabaceae), *Allium czelghauricum* Bordz (Amaryllidaceae), *Stachys cretica* subsp. *anatolica* Rech.f. (Lamiaceae), *Rumex gracilescens* Rech. f., (Polygonaceae), Nonea karsensis Popov (Boraginaceae), *Phlomis armeniaca* Willd (Lamiaceae), and *Papaver triniifolium* Boiss. (Papaveraceae) corresponded with sequences of members of the same genera. *R. gracilescens* exhibited the lowest sequence similarity, aligning with *R. sanguineus* L. (Polygonaceae) (MT937131.1) with 94.63% identity. *Galinsoga parviflora* Cav. (Asteraceae), *Hieracium sarykamychense* Üksip (Asteraceae), and *Anchusa leptophylla* Roem. & Schult. subsp. *incana* (Ledeb.) D.F.Chamb. (Boraginaceae) indicated a BLAST match with different genera within their respective families. When comparing the 26S rDNA sequences of 30 endemic plant taxa, 42 variable sites were observed (Figure 1, Figure 2).

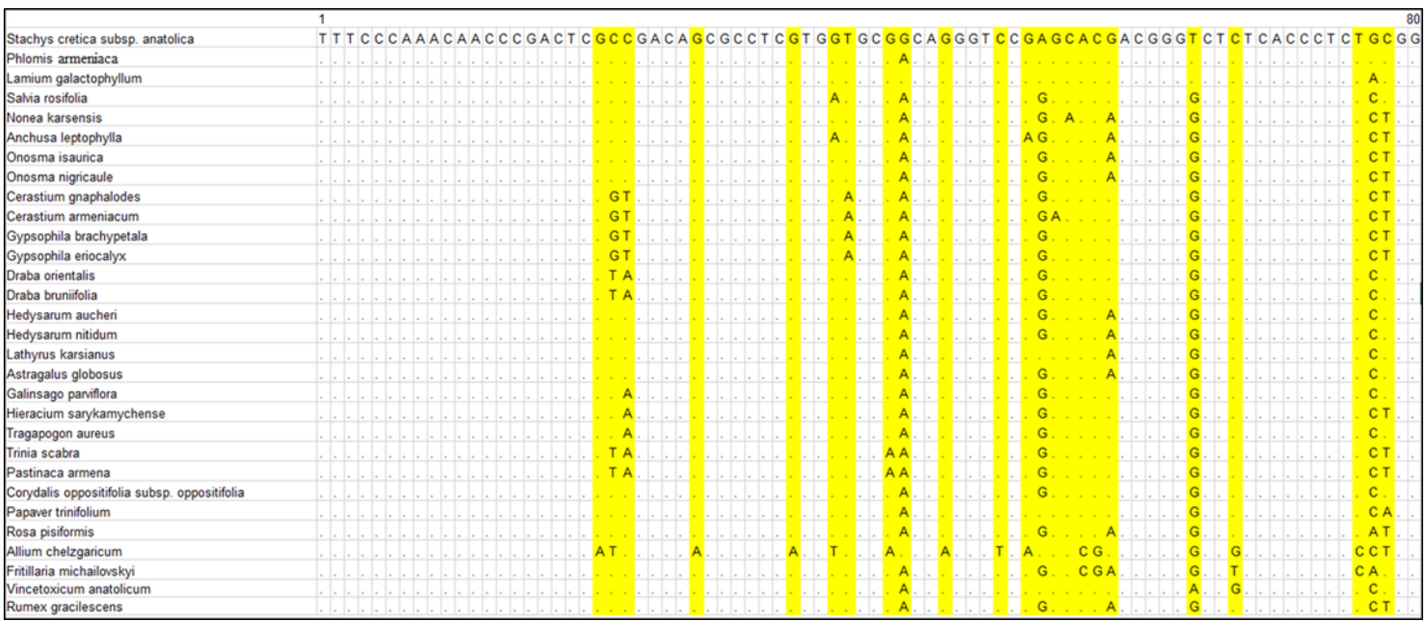

Figure 1. Multiple alignments of 1 to 80 bp of 26S rDNA gene sequence for the studied 30 endemic plant taxa

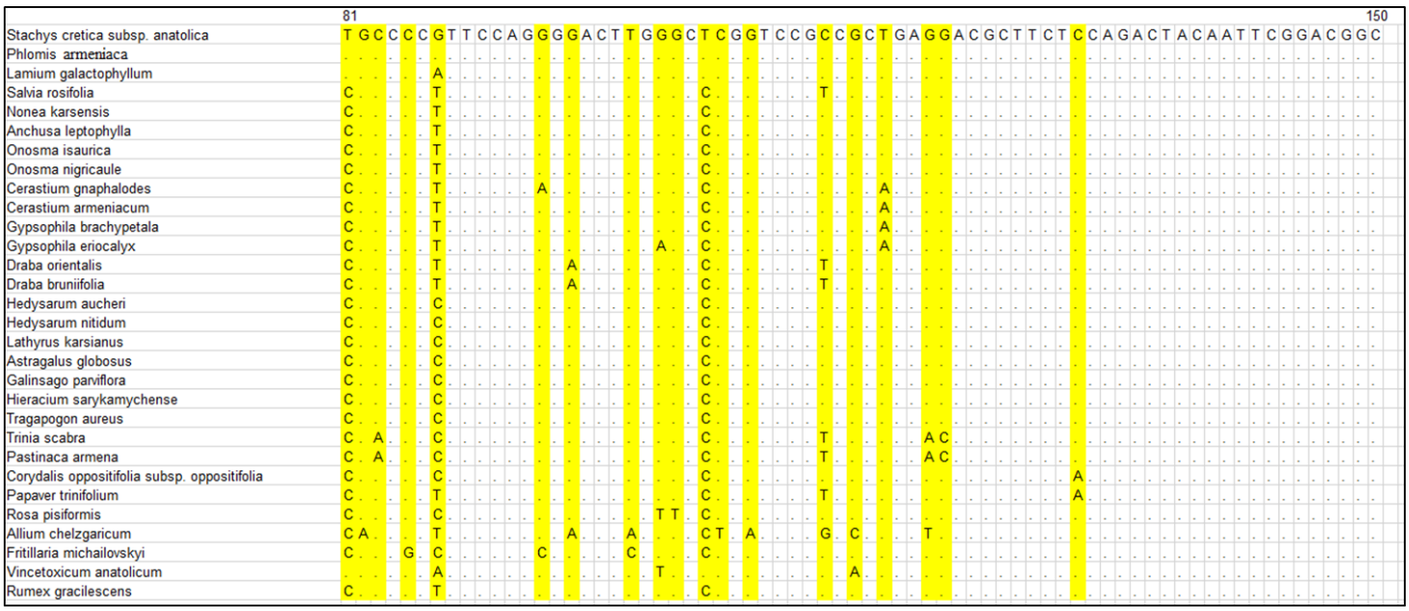

Figure 2. Multiple alignments of 80 to 150 bp of 26S rDNA gene sequence for the studied 30 endemic plant taxa

No variation was detected between *O. isaurica* and *O. nigricaulis*. *Nonea karsensis* exhibited one variable site, while *A. leptophylla* showed two variable sites when compared to Onosma species. A single variable site was observed at the 52 bp position among *L. karsianus*, *H. aucheri, H. nitidum*, and *A. globosus*, all members of the Fabaceae family. Within the Papaveraceae family, *C. oppositifolia* subsp. *oppositifolia* and *P. triniifolium* exhibited four nucleotide substitutions at the 52, 77, 86, and 112 bp positions. Among the Asteraceae family members, *Hieracium sarykamychense*, *Galinsoga parviflora*, and *T. aureus* differed by only one base pair at position 78. Although *Lamium galactophyllum*, *S. rosifolia*, *S. cretica* subsp. *anatolica*, and *Phlomis armeniaca* all belong to the Lamiaceae family, they differed at nine base positions in the 26S rDNA sequence.

Members of the Caryophyllaceae family exhibited three variable sites at the 54, 94, and 102 bp positions (Figure 1, Figure 2).

Although the studied species were positioned on separate branches of the phylogenetic tree, the genetic differentiation observed among closely related taxa was relatively low. Even though members of each family clustered together in distinct genetic groups, *Salvia rosifolia* was grouped with *P. triniifolium* and *C. oppositifolia* subsp. *oppositifolia* instead of with *L. galactophyllum*, *S. cretica* subsp. *anatolica*, and *P. armeniaca*. When analyzing the 30 endemic species, *A. czelghauricum* and *F. michailovskyi* were significantly distinct from the other endemic species (Figure 3).

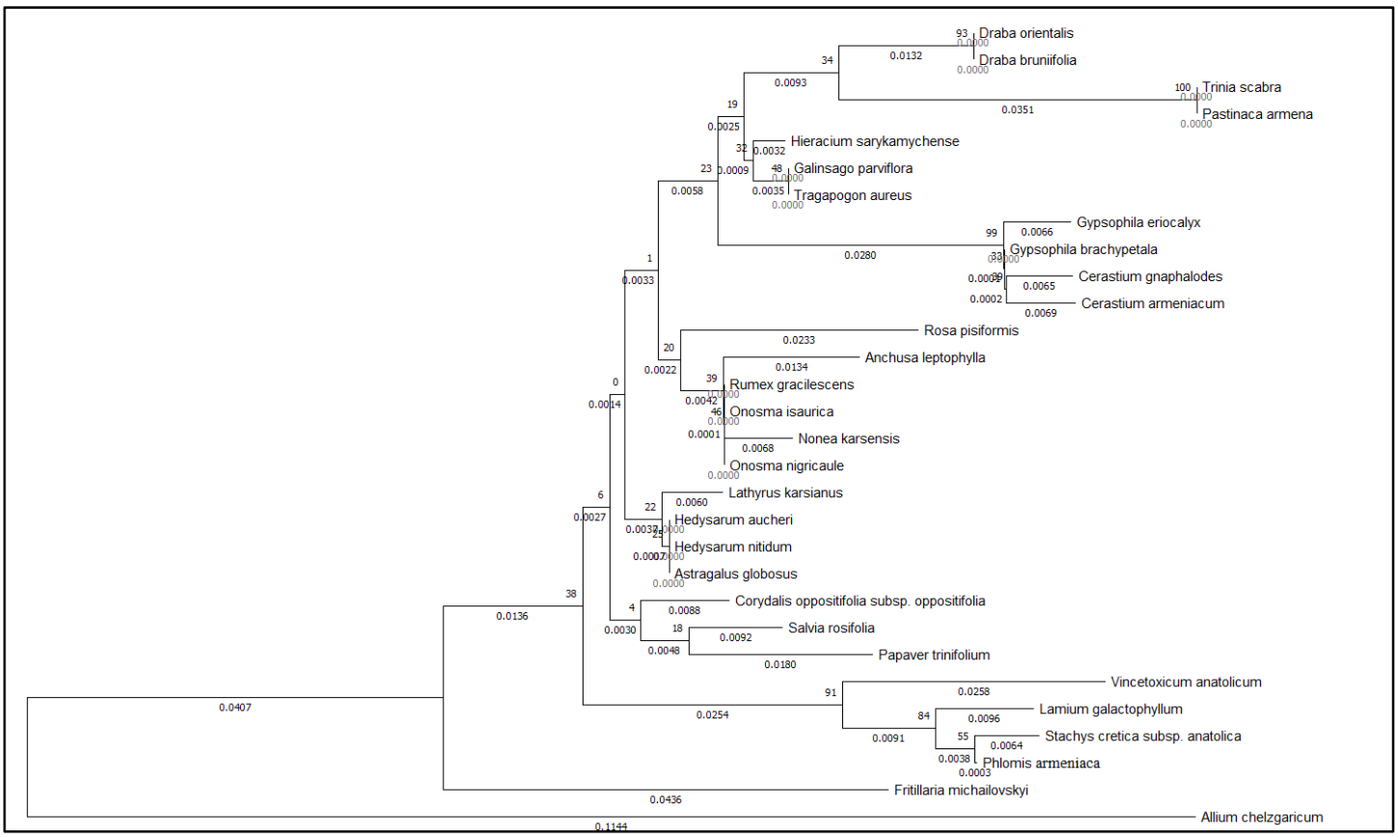

Figure 3. Phylogenetic tree based on the sequence of 26S rDNA gene region for 30 endemic plant taxa

4. DISCUSSION

This study presents the first detailed molecular analysis of the 26S rDNA gene region in 30 endemic plant species from Kars province, offering new insights into their genetic characteristics, including patterns of genetic differentiation, conserved and variable sites, and phylogenetic relationships that inform species delimitation and conservation strategies. The successful amplification and sequencing of the 26S rDNA region across these species revealed a notable level of genetic variation, with 42 variable sites identified. Genetic differentiation was more pronounced among species from distinct families, whereas closely related taxa displayed relatively conserved sequences, consistent with the typically low substitution rate of the 26S rDNA region. These findings provide valuable insights into the molecular diversity in this group of plants and contribute to our understanding of their relatedness (Soltis et al., 2001; Markos and Baldwin, 2002). Notably, varying levels of genetic differentiation were observed among the studied species, providing a clearer picture of the genetic diversity within these endemic taxa.

Comparative analysis of the 26S rDNA sequences revealed that while some species exhibit a high degree of sequence conservation, others show significant divergence. For instance, *O. isaurica* and *O. nigricaulis* exhibited minimal variation, indicating a high degree of sequence conservation within these closely related species. The lack of sequence variation between *Onosma* species may reflect either recent divergence or the limited resolution of the 26S rDNA region for closely related taxa. Moreover, slow genetic divergence in these species may also be driven by ecological factors (Wang et al., 2023). Given that 26S rDNA is a relatively conserved nuclear marker, its ability to discriminate between recently diverged species is limited.

Conversely, within the Fabaceae family, subtle genetic differences were observed between *L. karsianum* and *A. globosus*, marked by a single variable site. These minor differences may reflect adaptation to different ecological

niches or other functional implications (Benton et al., 2015). Greater divergence was observed within the Papaveraceae family, particularly between *C. oppositifolia* subsp. *oppositifolia* and *P. triniifolium*, marked by four nucleotide substitutions. This increased divergence may reflect ecological factors that promote genetic differentiation between these species (Maia et al., 2014). Similarly, within the Lamiaceae family, nine variable sites were observed among *S. cretica* subsp. *anatolica, P. armeniaca, L. galactophyllum*, and *S. rosifolia*, underscoring the complexity of genetic differentiation even among taxonomically related species. This pattern challenges conventional expectations of intra-family homogeneity and highlights the need for a nuanced understanding of species-level variation (Benton et al., 2015; Heyduk et al., 2019).

The phylogenetic analysis of the 26S rDNA sequences revealed both expected and unexpected patterns of genetic clustering. As anticipated, species within the same family tended to cluster together, reflecting shared genetic background. However, the unexpected grouping of *S. rosifolia* with *C. oppositifolia* subsp. *oppositifolia* and *P. triniifolium* challenge conventional taxonomic expectations. This discrepancy may be indicative of variation or that transcend traditional taxonomic boundaries (Heyduk et al., 2019). Similar results have been reported in previous phylogenetic studies, where species from different families or genera cluster together due to shared ecological or functional adaptations (Maia et al., 2014; Heyduk et al., 2019). The striking finding in the phylogenetic analysis is the significant genetic differentiation observed in geophyte (Monocotyledonous) plants *A. czelghauricum* and *F. michailovskyi* compared to other endemic species. The distinctly separate placement of these species in the phylogenetic tree is due to the fact that these two taxa are in the Monocotyledonous Classis and all other taxa are in the Dicotyledonous Classis (Soltis et al., 2011; Judd et al., 2015). The high polymorphism detected in the 26S rDNA region for these species suggests the dynamic nature of their genomes, reflecting ongoing variation processes, potential gene flow or adaptive responses to environmental factors (Nieto Feliner and Rossello, 2007; Wang et al., 2023).

While the 26S rDNA region has proven valuable for elucidating the genetic relationships among these endemic taxa, the region may not offer sufficient resolution for distinguishing interfamilial relationships, particularly at deeper taxonomic levels. As highlighted in previous studies (Soltis et al., 2001; Markos and Baldwin, 2002), the conserved nature of this region limits its resolving power between distantly related families it is important to acknowledge its limitations in resolving relationships at lower taxonomic levels. The relatively slow evolutionary rate of the 26S rDNA region in certain lineages may lead to insufficient resolution for distinguishing closely related species, as seen in *O. isaurica* and *O. nigricaulis*. Furthermore, the multicopy nature of the 26S rDNA gene within the genome can pose challenges, such as paralogy or the presence of pseudogenes, complicating sequence alignment and interpretation (Benton et al., 2015). Faster-evolving genomic regions, such as the Internal Transcribed Spacer (ITS) and certain plastid loci in plant families often provide higher species-level resolution (Shaw et al., 2007; Pang et al., 2012; Nasrollahi et al., 2019). To enhance phylogenetic resolution, our findings should be complemented with these molecular markers.

Despite some limitations, this study provides valuable molecular data that enhance our understanding of the diversity and evolutionary history of endemic plants. Overall, our study provides the first molecular insights into the 26S rDNA characteristics of 30 endemic plant species from Kars province. The preliminary results lay the foundation for future genetic studies aimed at the conservation of these species and broader biodiversity efforts. The creation of 30 new genetic barcodes is a significant contribution, offering deeper insights into the distribution, diversity, and ecological dynamics of the endemic species in Kars. By incorporating these data into national and international genetic databases, we can strengthen collaboration on plant conservation efforts. Additionally, these genetic resources will be essential for future research on the ability of endemic species to adapt to environmental changes. In this way, the study not only helps protect Türkiye's unique plant species but also contributes to the global discussion on biodiversity conservation.

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