Research Article GU J Sci 39(3): 1676-1690 (2026) DOI: 10.35378/gujs.1908682

Gazi University

Journal of Science

http://dergipark.org.tr/gujs

Preliminary Phylogenetic Analysis and DNA Barcoding of Endemic and Threatened *Silene* Species in Türkiye using *ITS* and *rps16* Intron Markers

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Highlights

- First molecular characterization of endangered Turkish *Silene* species.
- Identification of unique diagnostic genetic markers.
- Robust phylogenetic resolution through multilocus analysis.

Article Info

Received: 13 Mar 2026
Accepted: 01 Jul 2026

Keywords

Endemic
Molecular phylogeny
Silene
Türkiye

Abstract

The genus *Silene* (Caryophyllaceae) is one of the most species-rich groups in Türkiye, with 180 recognized taxa, 63 of which are endemic. Despite their ecological and conservation importance, the phylogenetic relationships of many endemic and threatened taxa remain unresolved. Here, we investigated twelve Turkish *Silene* taxa, including four endemics, using nuclear ribosomal *ITS* and chloroplast *rps16* intron sequences. The concatenated dataset (1517 bp) contained 108 polymorphic sites and provided strong phylogenetic signal. Bayesian analyses revealed two well-supported clades: (i) Lasiostemones, Sclerocalycine, and Nanosilene; and (ii) Spergulifoliae and Auriculatae. Notable markers included a unique 38-bp insertion in *S. argaea* and several section-specific substitutions. This study represents the first integrated DNA barcoding and phylogenetic analysis of endangered Turkish *Silene* taxa, highlighting the utility of combined *ITS* and *rps16* intron markers for species identification and resolving phylogenetic relationships, while providing a genetic baseline for the conservation of these rare taxa.

1. INTRODUCTION

The order Caryophyllales Juss. ex Bercht. and J. Presl. comprises approximately 12.500 species across 39 families. Within this order, Caryophyllaceae Juss. is among the most species-rich families, containing nearly 3.000 species in about 100 genera, with its main distribution in the Holarctic region and centers of diversity in the Mediterranean and Irano-Turanian phytogeographic zones [1]. Molecular evidence strongly supports the monophyly of Caryophyllaceae [2]. Based on Bittrich [3], the family is divided into three subfamilies: Alsinoideae, Caryophylloideae, and Paronychioideae. Caryophylloideae is further divided into three tribes: Caryophylleae, Drypideae, and Sileneae. However, infra-familial classification remains only partly resolved, and the delimitation of some genera and species is still uncertain [4, 5]. Several genera within the family present significant taxonomic and nomenclatural challenges, with *Silene* L. being particularly notable [6].

The genus *Silene* (tribe Sileneae, subfamily Caryophylloideae) is among the largest genera of flowering plants, comprising roughly 850 taxa worldwide [1]. In Türkiye, the Flora of Turkey and the East Aegean Islands (1965–1985, with supplements in 1988 and 2000) documented 145 *Silene* taxa [7-9], of which 63 (43%) were endemic. More recent revisions have increased the recognized diversity to 180 taxa [10].

Morphological traits such as stamen and carpel number, petal and seed morphology, indumentum type, and anthophore length are traditionally used to identify *Silene* taxa [11]. However, these characters often vary

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with environmental conditions, leading to potential misidentification at the genus or subgenus level. Consequently, molecular approaches have become essential for delineating species boundaries and clarifying phylogenetic relationships [11-14]. In Türkiye, numerous studies have investigated *Silene* species using morphological, anatomical, karyological, and cytological traits [15-23]. However, molecular and phylogenetic data remain scarce [24-26]. When traditional taxonomic approaches fail to provide sufficient resolution, molecular data offer crucial insights for understanding species boundaries and phylogenetic relationships [27-31]. DNA barcoding has been widely applied to accelerate and improve plant species identification [32-36]. In this context, the internal transcribed spacer (*ITS*) region of nuclear ribosomal DNA is recognized as a powerful universal barcode for diagnostic species-level identification [28, 34, 37-43]. Beyond its barcoding utility, *ITS* sequence data also provide critical data for broader phylogenetic analyses, offering high resolution for defining larger clades and generic boundaries [32, 33, 44]. The ribosomal protein subunit 16 (*rps16*) intron was selected as a second marker to complement *ITS*. Compared to *ITS*, *rps16* intron evolves more slowly, making it particularly useful for resolving deeper phylogenetic relationships [45]. This region is also favored in phylogenetic studies because it is relatively easy to amplify and provides sufficient sequence variation to generate informative characters [5, 40, 46-49].

In this study, we investigated the phylogenetic relationships of *Silene* taxa that are endemic, endangered, or critically endangered in Türkiye, focusing specifically on the unique flora of the Van basin (Eastern Anatolia). Specifically, we aimed to (i) examine historical biogeography, (ii) estimate genetic divergence, and (iii) clarify phylogenetic relationships among these regional Turkish *Silene* taxa. Phylogenetic analyses, combined with morphological observations, provide a framework for necessary taxonomic revisions. Our findings not only enhance understanding of *Silene* phylogeny but also highlight the importance of molecular data for resolving relationships in endemic and threatened plant groups. Finally, we compared the efficacy of *ITS* and *rps16* intron markers, offering insights relevant to broader phylogenetic studies in Caryophyllaceae.

2. MATERIAL METHOD

2.1. Plant Material

Leaf samples of the investigated taxa were collected between March and October of 2023–2024 from Van and surrounding regions (Table 1). Specimens were identified according to the *Flora of Turkey and the East Aegean Islands* and relevant literature [7-10]. Fresh leaves were dried in silica gel and transported to the laboratory for molecular analysis. In total, twelve *Silene* taxa were studied. Based on the IUCN Red List categories, these included one Critically Endangered (CR), four Near Threatened (NT), six Least Concern (LC), and one Data Deficient (DD) species. Alongside the 22 newly generated sequences, additional *ITS* (22 accessions) and *rps16* intron (14 accessions) sequences were retrieved from GenBank to increase taxon sampling, genetic diversity, and to better resolve the phylogenetic placement of Turkish taxa. Detailed information regarding the GenBank accession numbers for all retrieved sequences is provided in the Appendix/Supplementary Data. *ITS* (MT153596) and *rps16* intron (Z83154) sequences of *Agrostemma githago* L. were included as outgroup references (Supplementary Data, Appendix).

2.2. DNA Isolation, PCR Amplification, Sequencing, Sequence Alignment and Phylogenetic Analyses

Genomic DNA was extracted from dried leaf tissue using a modified cetyltrimethylammonium bromide (CTAB) method [50], with minor optimizations to improve yield and purity. DNA concentration and quality were assessed using a NanoDrop 2000c spectrophotometer (Thermo Scientific) and confirmed by 0.7% agarose gel electrophoresis. Samples were diluted to a working concentration of 10 ng/µL for polymerase chain reaction (PCR).

The nuclear ribosomal *ITS* region was amplified using primers ITS1F (5′-CTT GGT CAT TTA GAG GAA GTAA-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [51]. The chloroplast *rps16* intron was amplified with primers rps16-F (5′-GTG GTA GAA AGC AAC GTG CGA CTT-3′) and rps16-R2R (5′-TCG GGA TCG AAC ATC AAT TGC AAC-3′) [46]. PCR reactions (25 µL total) contained: 2.5 µL of

10× buffer, 2.5 µL $MgCl_2$ (25 mmol/L), 1 µL dNTPs (10 mmol/L; 0.8 µL for *rps16*), 1.5 µL of each primer (10 µmol/L), 2–3 µL template DNA (~10 ng), 0.2 µL Taq DNA polymerase (2.5 U/µL), and nuclease-free water to volume. Thermal cycling conditions were: initial denaturation at 95 °C for 3 min; 30 cycles of 94 °C for 1 min, annealing at 54 °C (52 °C for *rps16* intron) for 30 s, and extension at 72 °C for 1 min; followed by a final extension at 72 °C for 5 min. Products were visualized on 1% agarose gels, and successfully amplified fragments were purified and sequenced bidirectionally with an ABI 3730XL automated sequencer (Applied Biosystems, CA, USA).

Raw chromatograms were edited to correct base-calling errors in FinchTV v1.4.0 (Geospiza, Denver, USA). Sequences were aligned using ClustalW implemented in MEGA v7.0 [52], and consensus sequences were generated for each taxa. Identity was confirmed by BLAST searches (NCBI), selecting hits with the highest similarity and lowest E-values. Newly obtained sequences were submitted to GenBank (accessions in Table 1). Additional homologous *ITS* and *rps16* intron sequences were retrieved from GenBank and incorporated into the dataset to increase sampling and genetic diversity.

To characterize sequence variation, genetic distance, conserved sites, parsimony-informative sites, and variable sites were calculated in MEGA v7.0. The best-fit nucleotide substitution model was identified using the "Find Best DNA Model" function, and the Tamura-3 parameter model with gamma distribution (T92 + G) was selected for tree construction. Phylogenetic trees were visualized using FigTree v1.4.3 [53] and MEGA v7.0. Bayesian inference (BI) was conducted in MrBayes v3.0 [54]. Analyses ran for 5 million generations for *ITS* and *rps16* intron datasets, and 2 million generations for the concatenated dataset. Four Metropolis-coupled Markov chains were sampled every 1000 generations in two parallel runs, with the first 25% of trees discarded as burn-in to ensure convergence.

3. RESULTS

This study represents the first application of *ITS* and *rps16* intron sequencing for the molecular identification of *Silene* taxa that are endemic, endangered, or critically endangered in Türkiye. For each sample, sequences from both regions were generated and deposited in the NCBI GenBank database (accession numbers listed in Table 1). The concatenated dataset comprised approximately 1517 bp in total, including 640 bp from the *ITS* region and 877 bp from the *rps16* intron. Phylogenetic analysis of this combined dataset identified 108 polymorphic sites, providing strong resolution for reconstructing taxonomic relationships among the studied taxa.

Table 1. *Silene taxa included in this study, with section, collection number, locality, IUCN conservation status, voucher number, and GenBank accession numbers for ITS and rps16 intron sequences*

Section	*Silene* taxon	Collection VANF #	Locality * Endemic and IUCN Categories	Voucher #	Accession # *ITS*	Accession # *rps16* intron
Lasiostemones Boiss.	*S. longipetala* Vent	16312	1 DD	F15696	PV535643	-
Sclerocalycinae (Boiss.) Schischk.	*S. armena* Boiss. var. *armena*	16314	2 LC	F15718	PV535638	PV540767
	S. sclerophylla Chowdhuri	10073	3 LC	LFM3340	PV535639	PV540768
	S. cartilaginea Hub.-Mor.	16316	4 Endemic; NT	F497	PV535640	PV540769
Spergulifoliae Boiss.	*S. stenobotrys* Boiss. & Hausskn.	16317	5 NT	B54466b	-	PV540772
	S. muradica Schischk.	16313	6 LC	F15697	PV535641	PV540770
	S. argaea Fisch. & C.A.Mey.	16318	7 Endemic; LC	F5598	PV535644	PV540773

Auriculatae (Boiss.) Schischk.	*S. araratica* Schischk. subsp. *araratica*	16319	8 NT	B6308	PV535634	PV540763
	S. araratica Schischk. subsp. *davisii*	4545	9 NT	MA1442	PV535635	PV540764
	S. brevicaulis Boiss.	16320	10 Endemic; LC	F694	PV535636	PV540765
	S. lucida Chowdhuri subsp. *lucida*	14063	11 LC	I1329	PV535637	PV540766
Nanosilene Otth	*S. acaulis* (L.) L. subsp. *vanensis* Özgökçe & Kit Tan	16311	12 Endemic; CR	F15640	PV535642	PV540771

*** Locality information of the investigated taxa**

1. B9 Van: Özalp, East of Aşkın Village, Kuştepe, steppe, 2200 m, 25.VI.2024, *16312* (VANF).
2. B9 Van: Özalp, east of Eğribelen village, steppe, 2146 m, 11.VII.2024*, 16314* (VANF).
3. B9 Muş: Malazgirt, Kırımkaya village, steppe, 2095 m, 01.VIII.2007*, 10073* (VANF),
4. B9 Van: Özalp, from Özalp to Saray roadside, steppe, 2050 m, 08.VIII.1996*, 16316* (VANF), Endemic.
5. B7 Elazığ: Baskil, Between Şahindere Konak villages, steppe, 1600 m, 01.VII.1996, *16317* (VANF).
6. B9 Van: Erciş, above Ulupamir village, steppe, 2000 m, 25.VI.2024*, 16313* (VANF).
7. B10 Van: Özalp, Lower Balçıklı Village, steppe, 2148 m, 11.VIII.1997, *16318* (VANF), Endemic.
8. B9 Bitlis: Adilcevaz, east of Mount Süphan, rocky, 3300 m, 22.VI.2000, *16319* (VANF).
9. B9 Van: Gürpınar, Güzelsu (Hoşap), Güzeldere Pass, rocky, 2400 m, 24.VI.2001*, 4545* (VANF).
10. B10 Van: Özalp, surroundings Sarıköy village, steppe, 2100 m, 25.VI.2024*, 16320* (VANF), Endemic.
11. B9 Van: Gürpınar, between Cavitalan Umut village, Karavin hill, rocky, 2800 m, 21.VI.2008, *14063* (VANF).
12. B9 Van: Özalp, Mount Ahtadağ, southeast of Kalecik village near Özalp, steppe, 2600 m, 20.V.2024*,16311* (VANF), Endemic.

The aligned *ITS* dataset for the studied *Silene* taxa comprised 640 characters, of which 572 were constant and 68 were variable (31 in *ITS1* and 37 in *ITS2*). Sequence lengths ranged from 634 bp (*S. argaea, S. brevicaulis, S. araratica,* and *S. lucida*) to 639 bp (*S. longipetala, S. sclerophylla, S. cartilaginea,* and *S. armena*). Variability was concentrated in the *ITS2* subregion (37 variations), while the 5.8S region showed no variation. No insertions or deletions (indels) were observed in the 5.8S region; however, several single- or two-nucleotide indels were detected in both *ITS1* and *ITS2*. Most nucleotide variations were single substitutions, generally section-specific and occasionally taxon-specific, such as the distinct markers for *S. argaea* and *S. sclerophylla*, which are consistent with the topology of the phylogenetic tree presented in Figure 1. Representative diagnostic molecular markers (single nucleotide polymorphisms; SNPs and indels) were also summarized in Supplementary Data (Table S1).

For the *rps16* intron dataset of the Turkish *Silene* taxa, the aligned matrix contained 877 characters, with 792 constant and 40 variable. Sequence lengths varied from 796 bp in *S. acaulis* subsp. *vanensis* to 844 bp in *S. argaea*. Compared with the *ITS* dataset, the *rps16* intron region exhibited a lower substitution density (5% vs. 11%), indicating that it is more conserved overall. Several SNPs served as diagnostic markers useful for phylogenetic differentiation among sections. For instance, Sect. Sclerocalycine displayed distinct substitutions, including a T→C substitution at position 93, A→G transitions at positions 247 and 449, C→A transversions at positions 404, 450, and 687, a C→T transition at position 423, and a T→G transversion at position 677. *Silene acaulis* subsp. *vanensis* was uniquely characterized by substitutions such as a G→A transition at position 247, a T→G transversion at position 254, a T→C transition at position 269, an A→G transition at position 479, and a C→G transversion at position 550.

The first section of the *rps16* intron sequence was relatively conserved, whereas the second section showed substantial variability, characterized by frequent insertions and deletions (indels) of varying lengths and abundant mononucleotide repeats, especially poly-T tracts. Several phylogenetically informative indels were identified, including a five-nucleotide insertion (AAGAT) at positions 450–456, a 21-nucleotide insertion (TGCTAATCATCAATCAATAAA) at positions 572–594, and a six-nucleotide deletion (ACCTAT) at positions 635–642. These markers proved critical for distinguishing species within Sect. Sclerocalycine. Additionally, in *S. argaea*, a unique 38-bp insertion (TGAGGAAAGAAAAGAAAGGGCTTAATTTTAATTCATTT) at positions 718–757 provided a key diagnostic marker for species-level identification, while contributing an informative character that supports its phylogenetic divergence (Supplementary Data, Table S2).

Bayesian inference analyses were performed on the concatenated dataset as well as on the individual *ITS* and *rps16* intron markers (Figure 1; Supplementary Figures S1 and S2). Analyses of single markers produced relatively well-resolved tree topologies, though clade support varied from low to high. In contrast, the combined dataset yielded a more robust phylogeny, with major clades receiving strong support and inter-clade relationships being consistently well resolved (Figure 1).

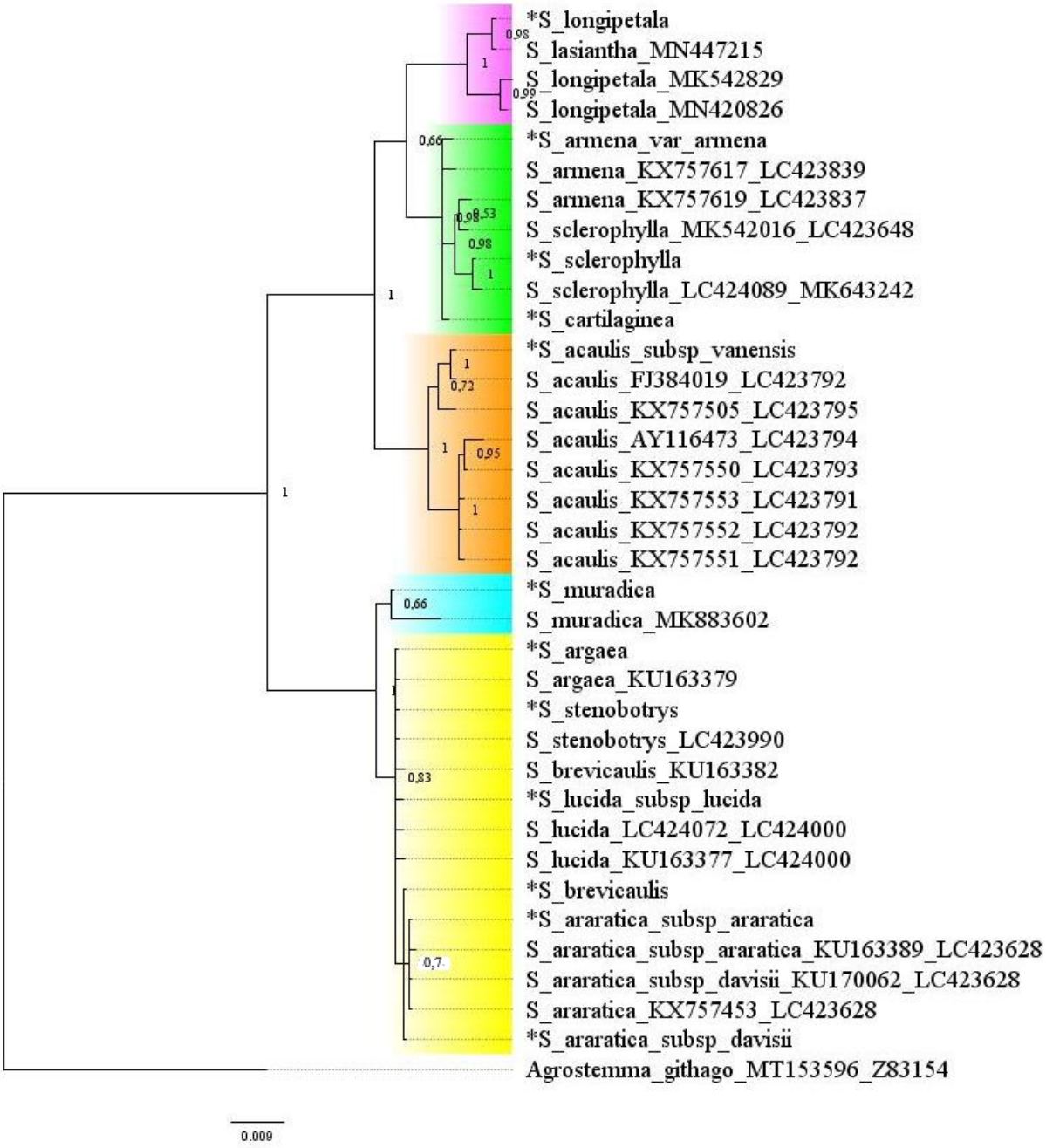

Figure 1. *50% majority-rule consensus tree derived from the Bayesian Inference analysis of the concatenated ITS and rps16 intron datasets for selected endemic, endangered, or critically endangered Silene taxa from Türkiye. Asterisk (*) denotes taxa sequenced in this study. Posterior probabilities (PP) is indicated above the branches. Subclade colors represent different sections within the genus: magenta for Sect. Lasiostemones, green for Sect. Sclerocalycine, orange for Nanosilene, cyan for Sect. Spergulifoliae, and yellow for Sect. Auriculatae*

The phylogenetic tree was constructed using a concatenated dataset of 36 taxa (35 *Silene* taxa and 1 *Agrostemma* outgroup). Of the 35 *Silene* taxa, 12 were newly sequenced in this study, while 23 were retrieved from GenBank. The analysis recovered two primary clades, both strongly supported (PP = 1.0; Figure 1). The first clade comprised three well-defined subclades, each corresponding to a distinct section within *Silene*: Sect. Lasiostemones (magenta), Sect. Sclerocalycine (green), and Sect. Nanosilene (orange). Posterior probability support was consistently high (PP ≈ 1.0), indicating robust resolution of relationships among these sections. Within the Lasiostemones subclade, the studied *S. longipetala* clustered closely with *S. lasiantha* (MN447215), followed by additional database representatives. *Silene lasiantha* was included

in the analysis due to its strong sequence homology with *S. longipetala*. In the Sclerocalycine subclade, the studied taxa *S. armena* var. *armena*, *S. sclerophylla*, and *S. cartilaginea* grouped with their respective representatives, with the exception of *S. cartilaginea*, for which no sequence data were available in public databases. These taxa were phylogenetically distinct from one another due to substitutions in the *ITS* and *rps16* intron regions. Notably, the *S. armena* var. *armena* sample analyzed in this study was positioned more distantly from previously published *S. armena* sequences, likely reflecting nucleotide variation between the datasets. This divergence likely highlights significant intraspecific genetic variation driven by the geographic isolation of high-altitude populations in Eastern Anatolia, rather than morphological misidentification. Such genetic distances are well-documented when localized endemic populations are compared with broader public database accessions. The third subclade contained the studied *S. acaulis* subsp. *vanensis* and its representatives, all of which clustered within Sect. Nanosilene (orange; Figure 1).
The second major clade consisted of two subclades corresponding to Sect. Spergulifoliae (cyan) and Sect. Auriculatae (yellow). The Spergulifoliae subclade included *S. muradica* and its database representatives, supported by moderate posterior probability (PP = 0.66; Figure 1). The second subclade comprised taxa belonging to Sect. Auriculatae (Figure 1).

4. DISCUSSION

The present study represents the first comprehensive phylogenetic analysis of twelve *Silene* taxa that are endemic, endangered, or critically endangered in Türkiye. Species boundaries and phylogenetic relationships were evaluated using nuclear (*ITS*) and chloroplast (*rps16* intron) markers. The concatenated phylogenetic tree revealed two well-supported major clades: the first comprising sections Lasiostemones, Sclerocalycine, and Nanosilene, and the second comprising sections Spergulifoliae and Auriculatae. These results indicate that each of the studied sections is monophyletic.

Within the first clade (Figure 1), *S. longipetala* and *S. lasiantha* formed a small cluster, suggesting recent divergence or possible cryptic speciation within Sect. Lasiostemones. This relationship was supported only by the *ITS* region, as the *rps16* intron sequence for *S. longipetala* could not be amplified. More complex patterns were observed within Sect. Sclerocalycine. The studied *S. armena* was positioned more distantly from database representatives, likely reflecting intraspecific variation or potential misidentification in public datasets. In contrast, *S. acaulis* subsp. *vanensis* grouped with its database representatives, corresponding to Sect. Nanosilene. Considerable sequence variation within *S. acaulis* produced subclusters even within the same clade, and its phylogenetic separation was supported by unique substitutions and indels in both *ITS* and *rps16* intron regions.

In the second clade (Figure 1), Sect. Auriculatae showed the greatest phylogenetic dispersion. *S. araratica, S. argaea, S. brevicaulis,* and *S. lucida* formed a subclade, but their phylogenetic separation was unclear, suggesting high genetic similarity and potential hybridization. *S. muradica* clustered with its database representatives and was clearly separated from Sect. Auriculatae.

The *ITS* region exhibited greater variability than the *rps16* intron and was therefore more informative for resolving relationships among *Silene* species. Nonetheless, *rps16* intron also provided valuable insights, particularly for deeper divergences. Several single nucleotide polymorphisms and indels in this region distinguished species of Sect. Sclerocalycine. A notable example was *S. argaea*, which contained a unique 38-bp insertion in the *rps16* intron that serves as a diagnostic marker for species identification and phylogenetic separation. The combined dataset offered superior resolution compared to individual markers, demonstrating the value of a multilocus approach for taxa with complex phylogenetic histories.

Türkiye, with approximately 14,000 plant taxa (3,500 endemic), represents one of the global hotspots of biodiversity, harboring nearly 75% of Europe's plant species [55]. This exceptional diversity highlights the importance of clarifying the phylogenetic relationships of endemic and endangered *Silene* species, as such studies contribute not only to systematic understanding but also to regional conservation efforts.

The findings of this study are valuable for clarifying the boundaries of endemic *Silene* species and for informing systematic and conservation efforts. DNA sequences from both markers were deposited in the

NCBI database, creating a comprehensive reference for future phylogenetic studies on *Silene* and related genera. Importantly, missing barcodes for several locally endemic taxa were uploaded, ensuring that their genetic resources are now recorded. Incorporating newly generated sequences with existing data will help resolve remaining uncertainties and refine sectional classifications within the genus.

In conclusion, the *ITS* region serves as a reliable DNA barcode for species identification, while its sequence data provide valuable molecular characters to elucidate phylogenetic relationships within *Silene*. This study provides the first DNA barcoding framework for *Silene* species that are endemic, endangered, or critically endangered in Türkiye. The analysis of twelve taxa has yielded important insights; however, additional molecular markers will be essential to further explore and validate other candidate barcoding regions. Furthermore, while this study provides a crucial localized genetic baseline for the high-risk endemics strictly restricted to the Van region, it is limited in terms of nationwide generalizability. Therefore, future investigations incorporating a larger sample size from across all geographic regions of Türkiye and utilizing additional hypervariable plastid markers like *matK* or *trnH-psbA* will be essential to provide a comprehensive country-wide phylogenetic revision of the genus *Silene.*

ACKNOWLEDGEMENT

This study was financially supported by Van Yüzüncü Yıl University, Van, Türkiye, through the Scientific Research Project Foundation under project number FBA-2024-10834.

DECLARATION OF ARTIFICIAL INTELLIGENCE (AI) USE

Generative AI agents or large language models (LLMs) were used only to improve clarity and quality of the English language presentation of the manuscript.

CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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SUPPLEMENTARY DATA

Appendix*: *Silene specimens retrieved from NCBI datase

The *Silene* Taxa Included in the ITS Region Analysis

Silene lasiantha (MN447215), *S. longipetala* (MK542829, MN420826), *S. muradica* (MK883602), *S. armena* (KX757617, KX757619), *S. sclerophylla* (MK542016, LC424089), *S. argaea* (KU163379), *S. brevicaulis* (KU163382), *S. araratica subsp araratica* (KU163389), *S. araratica subsp davisii* (KU170062), *S. araratica* (KX757453), *S. lucida* (LC424072, KU163377), *S. acaulis* (KX757505, FJ384019, AY116473, KX757553, KX757550, KX757552, KX757551) ve *Agrostemma githago* (MT153596)

The *Silene* Taxa Included in the *rps*16 Intron Region Analysis

S. stenobotrys (LC423990), *S. araratica* (LC423628), *S. lucida* (LC424000), *S. acaulis* (LC423792, LC423795, LC423794, LC423791, LC423793), *S. armena* (LC423839, LC423837, LC423633), *S. sclerophylla* (MK643242, LC424021, LC423648), *Agrostemma githago* (Z83154)

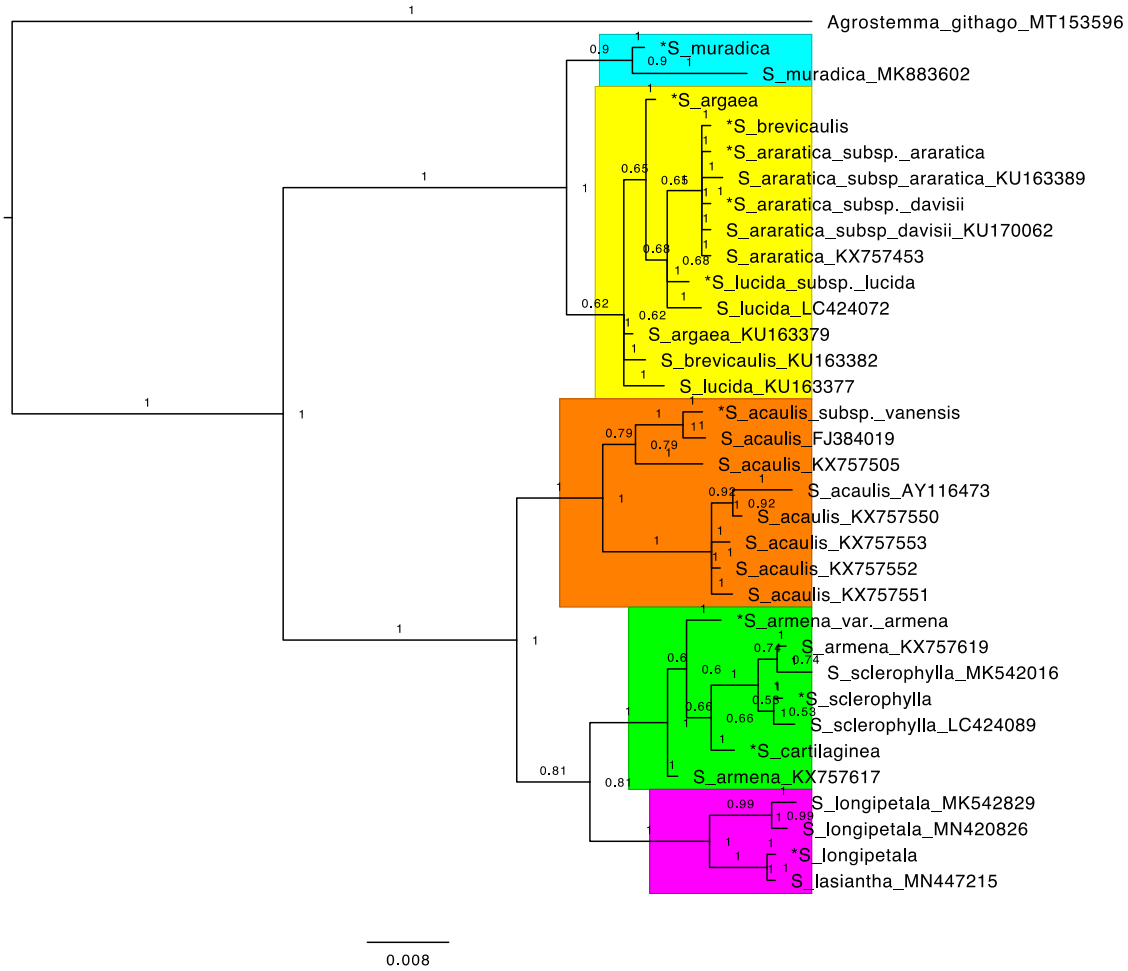

Figure S1. *50% majority-rule consensus tree derived from the Bayesian Inference analysis of the ITS region for selected endemic, endangered, or critically endangered Silene species from Türkiye. Asterisk (*) denotes taxa sequenced in this study. Posterior probabilities (PP) is indicated above the branches. Subclade colors represent different sections within the genus: magenta for Sect. Lasiostemones, green for Sect. Sclerocalycine, orange for Nanosilene, cyan for Sect. Spergulifoliae, and yellow for Sect. Auriculatae*

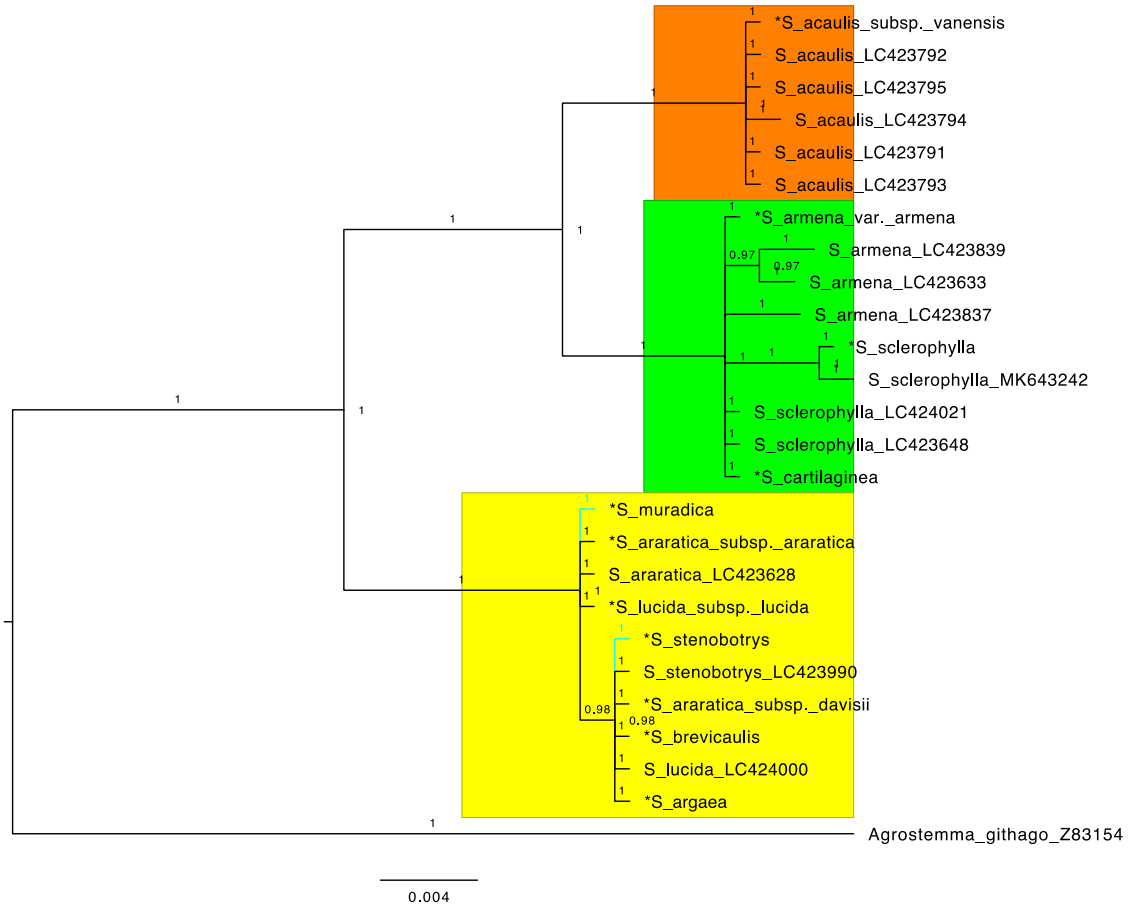

Figure S2. *50% majority-rule consensus tree derived from the Bayesian Inference analysis of the rps16 intron region for selected endemic, endangered, or critically endangered Silene species from Türkiye. Asterisk (*) denotes taxa sequenced in this study. Posterior probabilities (PP) is indicated above the branches. Subclade colors represent different sections within the genus: magenta for Sect. Lasiostemones, green for Sect. Sclerocalycine, orange for Nanosilene, cyan for Sect. Spergulifoliae, and yellow for Sect. Auriculatae*

Table S1. *Representative diagnostic molecular markers (SNPs) identified in the ITS sequences of the studied Silene taxa. Asterisk (*) denotes the position of the variable site within the alignment of the 12 studied taxa*

Taxon	**Position (bp)***	**Variation**	
S. acaulis	32	SNP	G → A
	40		C, T→ G
	68		T → G
	88		C → A
	125		A → C
	408		A, T → C
S. muradica	109	SNP	C → T
	203		C → T
	205		T → C
S. longipetala	492	SNP	T → C
	525		C → T
	538		G → T
	549		C → T
	554		G → A
	560		C → T
S. armena armena	162	SNP	A → T
S. sclerophylla	176		T → C
S. cartilaginea	476		T → A

Table S2. *Selected diagnostic molecular markers (SNPs and indels) identified in the rps16 intron sequences of the studied Silene taxa. Asterisk (*) denotes the position of the variable site within the alignment of the 12 studied taxa*

Taxon	**Position (bp)***	**Variation**	
S. acaulis	247	SNP	A → G
	254		G → T
	276		C → T
	479		G → A
	550		G → C
	680		G → A
	815		A, - → T
	820		- → T
S. sclerophylla	275		A → G
	324		A → C
	510		G → A
	511		G → T
S. argaea	718–757	Indel	TGAGGAAAGAAAAGAAAGGGCTTAATTTTAATTCATTT
S. cartilaginea,	450–456	Indel	AAGAT
S. armena armena,	572–594		TGCTAATCATCAATCAATAAA
S. sclerophylla			