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Research Article

Phylogeographic Structure of Five-Toed Jerboas of the Genus *Scarturus* (Dipodoidea and Allactaginae) With Taxonomic Clarification in Türkiye

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Understanding the genetic structure and phylogeographic patterns of *Scarturus* species is crucial for accurately delineating their taxonomic status and informing conservation strategies. This study explores the genetic differentiation of *Scarturus williamsi*, *S. aulacotis*, and *S. elater* species complex across Turkish populations by analyzing mitochondrial (*Cytb*, *12S rRNA*, and *16S rRNA*) and nuclear (*IRBP*) gene sequences. Our phylogenetic analyses have firmly established the monophyly and distinct species status of *S. williamsi* and *S. aulacotis* (formerly known as *S. euphraticus*), challenging previous subspecies classifications. Within *S. williamsi*, we identified five distinct lineages from *Cytb* sequences, illustrating a complex population structure shaped by geographical and ecological factors. Notably, the Niğde population emerged as a unique and ancient lineage, likely influenced by historical isolation. Our findings further indicate that *S. aulacotis* encompasses two divergent lineages, one spanning Syrian samples and the other Turkish and Iranian samples, both now classified under the revised taxonomy of *S. aulacotis*. Analysis of the *S. elater* species complex unveiled three distinct subclades, with the Turkish population aligning closely with Iranian and Armenian samples, identified as *S. indicus aralychensis* within the *S. indicus* superspecies.

Keywords: intraspecific variation; molecular phylogeny; *Scarturus aulacotis*; *Scarturus elater*; *Scarturus williamsi*; Türkiye

1. Introduction

The genus *Scarturus* Gloger, 1841, which includes both four- and five-toed jerboas, is a pivotal group within the subfamily Allactaginae. These nocturnal rodents, adapted to semidesert and steppe environments, utilize their elongated hind legs for rapid movement across sandy and gravelly terrains. As omnivores, they feed on a diverse diet of plant material, seeds, insects, and larvae. The distribution range of the genus *Scarturus* extends from North Africa to Western Asia, covering the Anatolian Peninsula, the Caucasus, and Central Asia [1].

Recent taxonomic revisions by Michaux and Shenbrot [1] list seven species within the genus *Scarturus*: *S. williamsi*, *S. aulacotis*, *S. euphraticus*, *S. hotsoni*, *S. elater*, *S. vinogradovi*, and *S. tetradactylus*. In Anatolia, three species—*S. elater*, *S. aulacotis* (previously recognized as *S. euphraticus*), and *S. williamsi*—are found in ecologically distinct regions [1–3]. *Scarturus williamsi* predominates in western, central, and northern Anatolia; *S. aulacotis*, which includes populations previously classified as *S. euphraticus*, is found in southern Anatolia; and *S. elater* is localized to specific areas in Iğdır.

Scarturus williamsi [4], occurring throughout central, eastern, and western Anatolia, shows variable subspecies classifications, historically based on morphological and coloration differences. These include *S. williamsi laticeps* [5] west of the Ceyhan River, *S. williamsi shmidti* [6] in northern Eastern Anatolia, and *S. williamsi williamsi* [4] around Van

and Iğdır [7]. The taxonomic delineations between *S. euphraticus* and *S. williamsi* have been subject to considerable debate; Atallah and Harrison [8] considered *S. williamsi* a subspecies of *S. euphraticus*, whereas Çolak, E. Kıvanç, and N. Yiğit [9] upgraded it to species status following detailed morphological, morphometric, and phallic analyses. Further, Çolak and Yiğit [10] identified a new subspecies, *S. e. kivanci*, in southeast Anatolia, while another subspecies, *S. e. caprimulga* [11], has been reported from Afghanistan. Molecular studies by Kryštufek et al. [12] discerned two clades of *S. euphraticus*: one encompassing specimens from Harran (Türkiye) and Iran and another from Syria. Recent revisions by Michaux and Shenbrot [1] and further genetic data by Lebedev et al. [3] have updated the taxonomic status of *S. euphraticus* to *S. aulacotis*.

Scarturus elater, the smallest species in the genus, is broadly distributed across Asia but is only documented in Aralık (Iğdır) within Türkiye. The recognized subspecies include *S. e. elater* Lichtenstein, 1825 (type locality: Kazakhstan), *S. e. indicus* Gray, 1842 (type locality: Afghanistan), *S. e. caucasicus* Nehring, 1900 (type locality: Azerbaijan), and *S. e. aralychensis* Satunin, 1901 (type locality: Aralık, Türkiye). However, recent studies by Bannikova et al. [13] suggest that *S. elater* is part of a complex of cryptic lineages, with distinct subgroups such as *S. indica* and *S. aralychensis*, indicating a need for taxonomic reevaluation of these subspecies.

Prior research on the *Scarturus* genus in Türkiye has primarily focused on morphological and karyological characteristics [7–11, 14–21]. Despite the extensive morphological and karyological insights into the genus *Scarturus*, molecular studies remain remarkably limited and have not comprehensively addressed the taxonomic ambiguities within the Turkish populations of *Scarturus* species [3, 12, 13, 22]. Critical unresolved issues include subspecies differentiation within *S. williamsi*, the validation of *S. e. aralychensis*, its phylogeographic relationships with other *S. elater* populations in nearby regions, and the taxonomic status of *S. aulacotis* and *S. williamsi* in Türkiye. These uncertainties hinder our understanding of the evolutionary relationships and ecological adaptations within the genus. Our study aims to address these critical gaps by utilizing advanced molecular techniques, including analyses of *Cytochrome b*, *12S rRNA*, *16S rRNA*, and *IRBP* genes. By elucidating the genetic structure and phylogenetic relationships among *Scarturus* species, this research seeks to clarify taxonomic classifications and enhance the framework for biodiversity conservation in semiarid and arid landscapes.

2. Materials and Methods

2.1. Sample Collection and DNA Extraction. In this study, we analyzed gene regions *Cytb*, *12S rRNA*, *16S rRNA*, and *IRBP* to investigate the phylogenetic relationships and taxonomic status of three *Scarturus* species identified in Türkiye: *S. williamsi*, *S. aulacotis*, and *S. elater*. We sequenced a total of 60 new *Scarturus* specimens, collected from 20 different locations across Türkiye (Figure 1). Additionally, our analysis included 34 samples representing various *Scarturus* species.

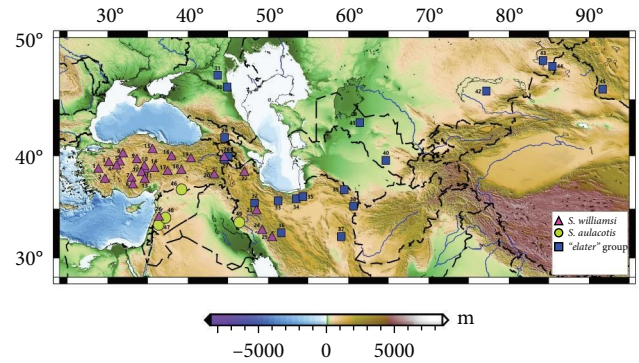


FIGURE 1: Sampling localities for the three *Scarturus* species analyzed in this study. Different symbols represent each species: magenta triangles for *Scarturus williamsi*, blue squares for the *"elater"* group, and green circles for *Scarturus aulacotis*. Numbers assigned to each locality correspond to detailed information available in Table S1.

For all gene regions, *Dipus sagitta* and *Jaculus jaculus* were utilized as outgroups. The sequences generated in this study are available on GenBank under the following accession numbers: *Cytb* (MG255325−MG255411 and PQ064096−PQ064104), *12S rRNA* (PQ068758−PQ068812), *16S rRNA* (PQ066042−PQ066097), and *IRBP* (PQ041892−PQ041934 and PQ063997−PQ064009). Detailed information regarding all specimens used in this study is provided in Table S1.

Genomic DNA was extracted from ear tissue samples using the methods described by Rogers and Bendich [23] and Gaubert and Zanatello [24]. The DNA concentration and purity were assessed using a NanoDrop ND–1000 spectrophotometer.

2.2. PCR Amplification and Sequencing. To amplify the *Cytb* gene, we utilized two overlapping segments. The first segment was amplified using the universal primers L14724 [25] and H15154 [26], and the second segment was amplified with primers L15162 [27] and H15752 [28]. The *12S rRNA* region was amplified using primers L1091 and H1478 [29], while the *16S rRNA* region was amplified with primers L2513 and H2714 [30]. For the IRBP gene region, we used primers F25dip and R701dip as described by Lebedev et al. [31]. The PCR protocols for each gene region, including cycling conditions are detailed in Table S2, following the procedure outlined by [32]. The PCR products were visualized on an agarose gel and sequences were subsequently converted to FASTA format using FinchTV software (https://digitalworldbiology.com/FinchTV). Sequence editing was performed using BioEdit version 7.2.5 [33]. Alignment of the sequences was conducted using the MUSCLE algorithm implemented in MEGAX software [34].

2.3. Phylogenetic and Genetic Diversity Analyses. Phylogenetic trees for each gene region were constructed using maximum likelihood (ML) methods with IQ-TREE version 1.6 [35]. The reliability of these trees was assessed using the ultrafast bootstrap method with 1000 replications (UFBoot; [36]). Bayesian inference (BI) analyses were conducted using MrBayes version 3.2 [37]. We selected the best substitution model for each gene region under the Bayesian information

TABLE 1: Comparative Kimura 2-Parameter (K2P) genetic distances and their standard deviations across gene regions between *Scarturus* species.

Species		Genes							
		Cytb		*12S rRNA*		*16S rRNA*		*IRBP*	
		K2P (%)	St. Dev.	K2P (%)	St. Dev.	K2P (%)	St. Dev.	K2P (%)	St. Dev.
Scarturus williamsi	*Scarturus aulacotis*	15.0	1.2	5.39	1.1	1.12	0.7	0.8	0.2
Scarturus williamsi	*"elater"* group	14.8	1.1	7.75	1.4	3.75	1.4	1.4	0.4
Scarturus aulacotis	*"elater"* group	16.9	1.2	8.4	1.4	3.1	1.3	1.2	0.2

criterion (BIC) using MEGAX software [34]. The selection of specific models was based on their ability to best accommodate the nucleotide substitution patterns observed in each dataset. Specifically, $GTR + G + I$ was chosen for *Cytb* due to its complex evolutionary patterns, $T92 + G$ for *12S rRNA* and *IRBP* gene regions as they fit simpler substitution models, and $K2 + G + I$ for *16S rRNA* to account for both transitions and transversions with rate heterogeneity and invariant sites. In addition to the individual gene analyses, a concatenated phylogenetic analysis was performed using the combined mtDNA sequences (*Cytb*, *12S rRNA*, and *16S rRNA*) for specimens with overlapping data. This concatenated dataset was analyzed using the same ML and BI methods to assess the overall phylogenetic relationships among *Scarturus* species with greater resolution.

Genetic differentiation between and within species was quantified using the Kimura two-parameter (K2P) model in MEGAX, chosen for its effectiveness in handling interspecific divergences. Number of haplotypes (h), haplotype diversity (Hd) and nucleotide diversities (P_i), and parsimony informative sites (I) within each species were analyzed using DnaSP version 6 [38]. Relationships among haplotypes were visualized through median-joining networks constructed using Network version 10.2 [39], enabling a detailed exploration of the genetic structure within and between species.

3. Results

3.1. Genetic Structure of Scarturus Species in Türkiye. In this study, we analyzed the genetic structure of *Scarturus* species in Turkish populations using four gene regions: *Cytb* (921 bp), *12S rRNA* (429 bp), *16S rRNA* (193 bp), and *IRBP* (510 bp; Table S3). The results, including the number of variable sites, parsimony informative sites, Hd, and nucleotide diversity (Pi%) for each gene region, are summarized in Table S3. Additionally, detailed information such as GenBank accession numbers, localities, and museum numbers for all samples analyzed is provided in Table S1.

For *S. williamsi*, we examined 53 specimens, identifying 157 variable sites in the *Cytb* region, with 151 being parsimony informative. The Hd and nucleotide diversity (Pi%) for *Cytb* were 1.00 and 2.6, respectively. In *S. aulacotis* ($N = 13$), we found 165 variable sites in *Cytb*, with 159 being parsimony informative, yielding Hd of 1.00 and Pi% of 5.1. Analysis of *S. elater* sequences ($N = 21$) revealed 220 variations in *Cytb*, with 202 being parsimony informative, and Hd of 0.92 and Pi% of 8.0.

Analysis of the *12S rRNA* gene region in *S. williamsi* ($N = 42$) revealed 16 variable sites, all of which were parsimony informative, with Hd of 0.84 and Pi% of 0.5. In *S. aulacotis* ($N = 8$), we observed one variable site in the *12S rRNA* region, with Hd of 0.42 and Pi% of 0.1. For *S. elater* ($N = 5$), there were two mutations in the *12S rRNA* region, both parsimony informative, resulting in Hd of 0.7 and Pi% of 0.2.

In the *16S rRNA* region (193 bp), *S. williamsi* showed only two parsimony informative sites, with Hd of 0.13 and Pi% of 0.1. For *S. aulacotis*, this gene region revealed only one non-informative mutation, resulting in both haplotype and nucleotide diversity of zero. No mutations were found in the 16S rRNA gene region of the S. elater population in Türkiye.

For the *IRBP* gene region (510 bp), *S. williamsi* had 19 mutation sites, all parsimony informative, with Hd of 0.72 and Pi% of 0.3. In *S. aulacotis*, we identified seven informative mutations, resulting in Hd of 0.84 and Pi% of 0.3. Analysis of the *IRBP* gene region from S. elater revealed 17 informative variations, with Hd of 0.88 and Pi% of 0.8.

3.2. Molecular Diversity and Phylogenetic Relationships of Scarturus Species. Phylogenetic analysis of mtDNA gene regions (*Cytb*, *12S rRNA*, and *16S rRNA*) confirmed the monophyletic nature of *S. williamsi* and *S. aulacotis* populations, supported by both ML and BI analyses. The reliability values from UFBoot/BI for these analyses are high for *Cytb* and *12S rRNA* (99/1.00 and 93/1.00, respectively) but lower for *16S rRNA* (51/–), indicating variable support across gene regions. Additionally, *S. elater* was positioned as a sister species to all other *Scarturus* taxa based on mtDNA analyses (*Cytb*: 100/1.00, *12S rRNA*: 100/1.00, and *16S rRNA*: 51/–). However, analyses using the *IRBP* gene region provided weak support for the monophyletic relationship between *S. aulacotis* and *S. elater* (UFBoot: 70 and BI: 0.55), contrasting with the strong support for *S. williamsi* (UFBoot: 100 and BI: 1.00). This suggests varying levels of phylogenetic signal across different gene regions.

To further refine the phylogenetic relationships, we performed a concatenated analysis of the mtDNA sequences (*Cytb*, *12S rRNA*, and *16S rRNA*) for specimens with overlapping data (Figure 2). This combined analysis corroborated the initial findings, confirming the monophyletic status of *S. williamsi* and *S. aulacotis* with support values of UFBoot: 89 and BI: 0.89 for both clades. The phylogenetic structure within *S. williamsi* revealed a notable difference between the *Cytb* and concatenated trees. In the *Cytb* tree, sublineages W1–W4 formed a monophyletic group distinct from W5, indicating an early divergence of the Niğde (Kemerhisar)

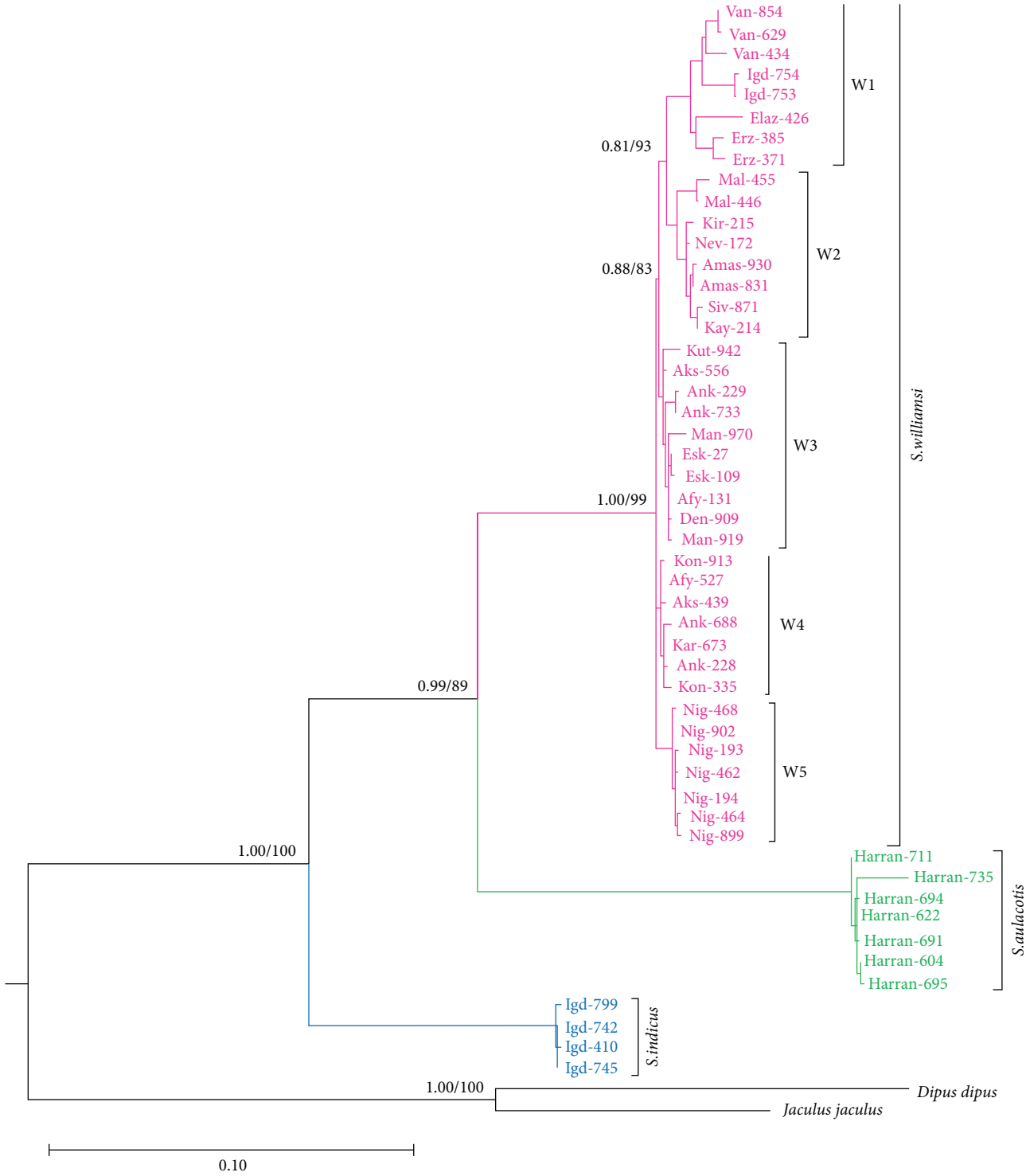


FIGURE 2: Concatenated maximum likelihood (ML) phylogenetic tree of *Scarturus* species based on combined mtDNA gene regions (*Cytb*, *12S rRNA*, and *16S rRNA*) using specimens with overlapping sequence data. Bootstrap support values (UFBoot) and Bayesian inference (BI) posterior probabilities are indicated at each node.

lineage. However, in the concatenated tree, sublineages W1–W3 grouped together as a clade, while W4 and W5 formed a separate monophyletic clade, suggesting a closer genetic relationship between these two sublineages. This discrepancy underscores the complex evolutionary history of *S. williamsi* and highlights the importance of using multiple gene regions for robust phylogenetic inferences.

Genetic distance values calculated using the K2P model varied significantly, ranging from 16.9% between *S. aulacotis* and *S. elater* for *Cytb* to as low as 0.8% between *S. williamsi*

and *S. aulacotis* for *IRBP*. Within-species genetic distances also showed considerable variation, from 8.7% within *S. elater* for *Cytb* to 0% within *S. elater* and *S. aulacotis* for *16S rRNA*, with detailed values provided in Table 1.

Phylogenetic analysis of *Cytb* sequences delineated five distinct sublineages (W1–W5) within *Scarturus williamsi*, highlighting geographic patterns of differentiation (Figure 3). Notably, sublineages W1 and W2 include populations from Eastern Anatolia, comprising subspecies *S. w. shmidti* and *S. w. williamsi*, along with specimens from Iran and Azerbaijan. The W2 sublineage is specific to *S. w. laticeps* from Eastern Anatolia. In contrast, samples from Western Anatolia and specific Central Anatolian samples formed sublineages W3 and W4, respectively, with a unique sublineage W5 emerging from Niğde (Kemerhisar) in the southern margin of Central Anatolian Plateaus.

While *12S rRNA* sequences also identified sublineages within *S. williamsi*, they did not show the same clear clustering as *Cytb* sequences, indicating a lower phylogenetic resolution for this marker (Figure 4). The *16S rRNA* and *IRBP* gene regions did not support these sublineages, highlighting the importance of selecting appropriate molecular markers for resolving phylogenetic relationships (Figures 5 and 6).

In the phylogenetic trees derived from *Cytb* sequences, distinct clustering patterns are observed within *S. aulacotis* and *S. elater* species (Figure 3). The Syrian (Euph 1) and the Türkiye–Iran (Euph 2) populations of *S. aulacotis* form distinct sublineages. Furthermore, Turkish and Iranian samples of *S. aulacotis* remain grouped within the same sublineage, with strong support (BI: 1.00 and UFBoot: 100). *Scarturus elater* displays three sublineages: the Caspian–Central Asia population (E1 and E2) and the Türkiye–Iran–Armenia population (E3). The clustering of Iranian and Turkish samples shows relatively low reliability (BI: 0.62 and UFBoot: 88) in separate branches. Due to the absence of *12S rRNA* and *16S rRNA* gene sequences from diverse geographic distributions of *S. aulacotis* and *S. elater*, the phylogenetic analyses for these regions were restricted to sequences obtained in this study. Consequently, these analyses did not reveal any differentiation among the samples (Figures 4 and 5). In the phylogenetic trees generated from *IRBP* sequences, the Turkish population of *S. elater* aligns closely with Iranian samples (Figure 6).

The median-joining trees for the genus *Scarturus* revealed haplotype relationships both within and between species that were similar to those observed in the phylogenetic trees. However, the network patterns exhibited by *Scarturus* species varied depending on the analyzed gene sequences. The *Cytb* sequences showed the highest number of intra- and interspecific mutations, presenting a complex mutational network pattern (Figure 3). This suggests a high genetic variability within this gene region, possibly reflecting diverse evolutionary histories or strong adaptive differences among populations. Conversely, the *IRBP* gene sequences manifest a simpler, star-like pattern particularly in *S. williamsi* (Figure 6), suggesting a more recent common ancestry or lower evolutionary pressures on this gene. Furthermore, the networks derived from *12S rRNA* and *16S rRNA* sequences were reciprocally monophyletic, that these gene regions are more stable and less variable, making them good markers for clearly distinguishing between species or major lineages (Figure 4).

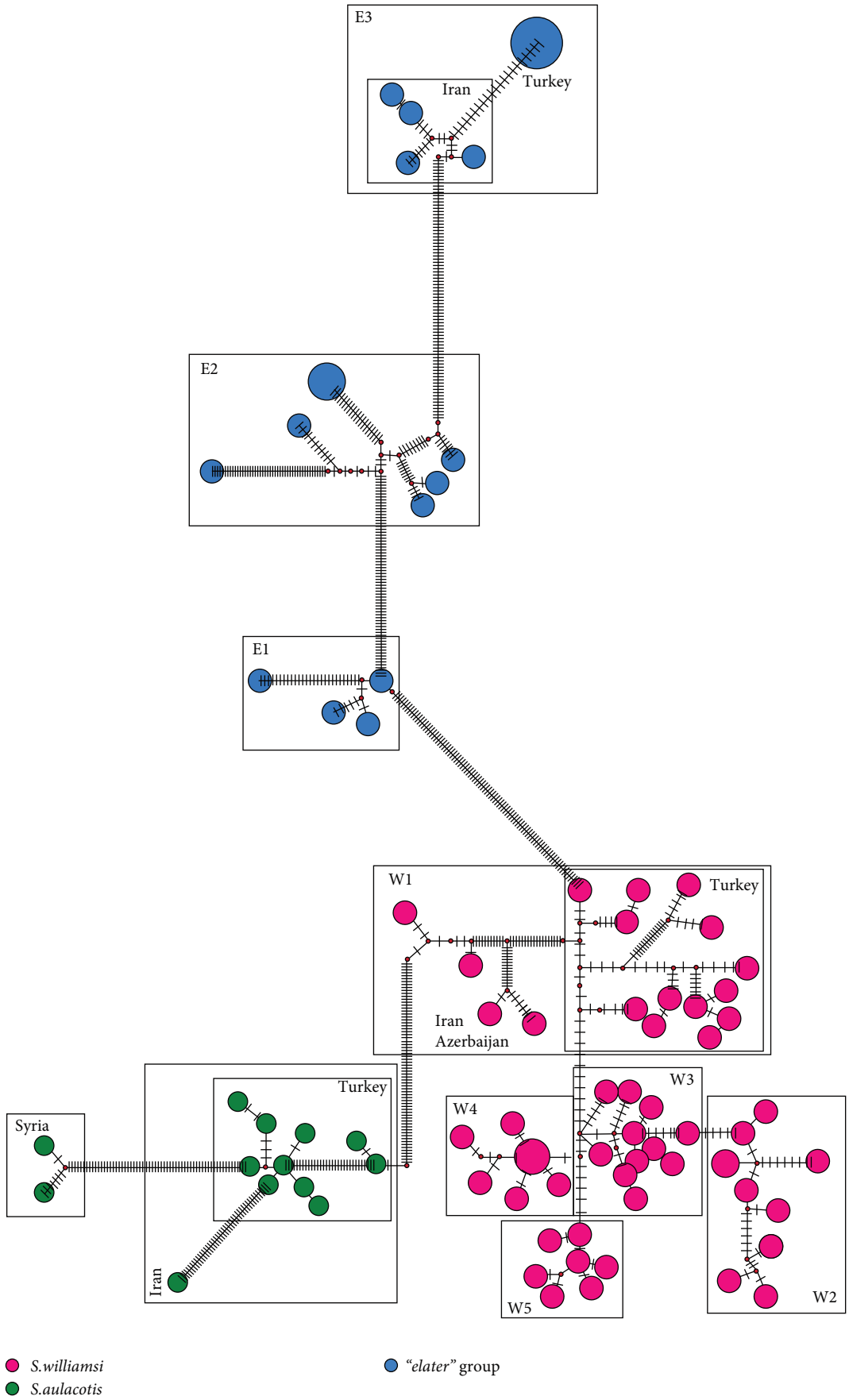
4. Discussion

4.1. Genetic Differentiation in S. williamsi. Our study confirmed the validity of three species within the specimens of the genus *Scarturus* distributed across Türkiye and neighboring regions, including Syria, Iran, Georgia, Uzbekistan, and Kazakhstan. Notably, *S. williamsi* exhibited a complex population structure. Although three subspecies have been recognized based on morphological features [7], their taxonomic statuses and validity could not be confirmed by genetic data [12]. Our findings identified five distinct lineages within the *S. williamsi* population based on the *Cytb* phylogenetic tree (Figure 3). However, phylogenetic trees constructed from *12S rRNA*, *16S rRNA*, and *IRBP* sequences did not support these lineages, which may be attributed to the higher number of informative sites and greater genetic variability in the *Cytb* gene region, particularly for closely related taxa [40, 41]. The *Cytb* gene region has been widely used in numerous studies for its ability to delineate genetic relationships effectively within the family Dipodidae [12, 42–47]. In contrast, nuclear markers have typically been used to differentiate higher taxa within the Dipodidae superfamily [31, 48]. In our analysis, the *IRBP* gene region clearly differentiated the three *Scarturus* species. Meanwhile, the resolution of the *16S rRNA* was limited due to its shorter sequence length. Additionally, while the *12S rRNA* analysis was effective at distinguishing between *Scarturus* species, it provided insufficient support for intraspecific variations within *S. williamsi*, unlike the *Cytb* sequences.

One of these lineages, comprising *S. williamsi*, is predominantly found in Eastern Anatolia (Figure 1). The highest genetic distance among *S. williamsi* lineages occurs between W1—which includes specimens previously classified as *S. w. shmidti* and *S. w. williamsi*—and the other lineages (W2–W5), with a distance of 3.6% (K2P; Table S4). Although *S. w. shmidti* and *S. w. williamsi* were initially recognized as distinct subspecies based on morphological characteristics [7], our phylogenetic analyses show them clustering together. This implies that the East Anatolian lineage of *S. williamsi* does not constitute a distinct subspecies, indicating a homogeneous genetic structure within the species.

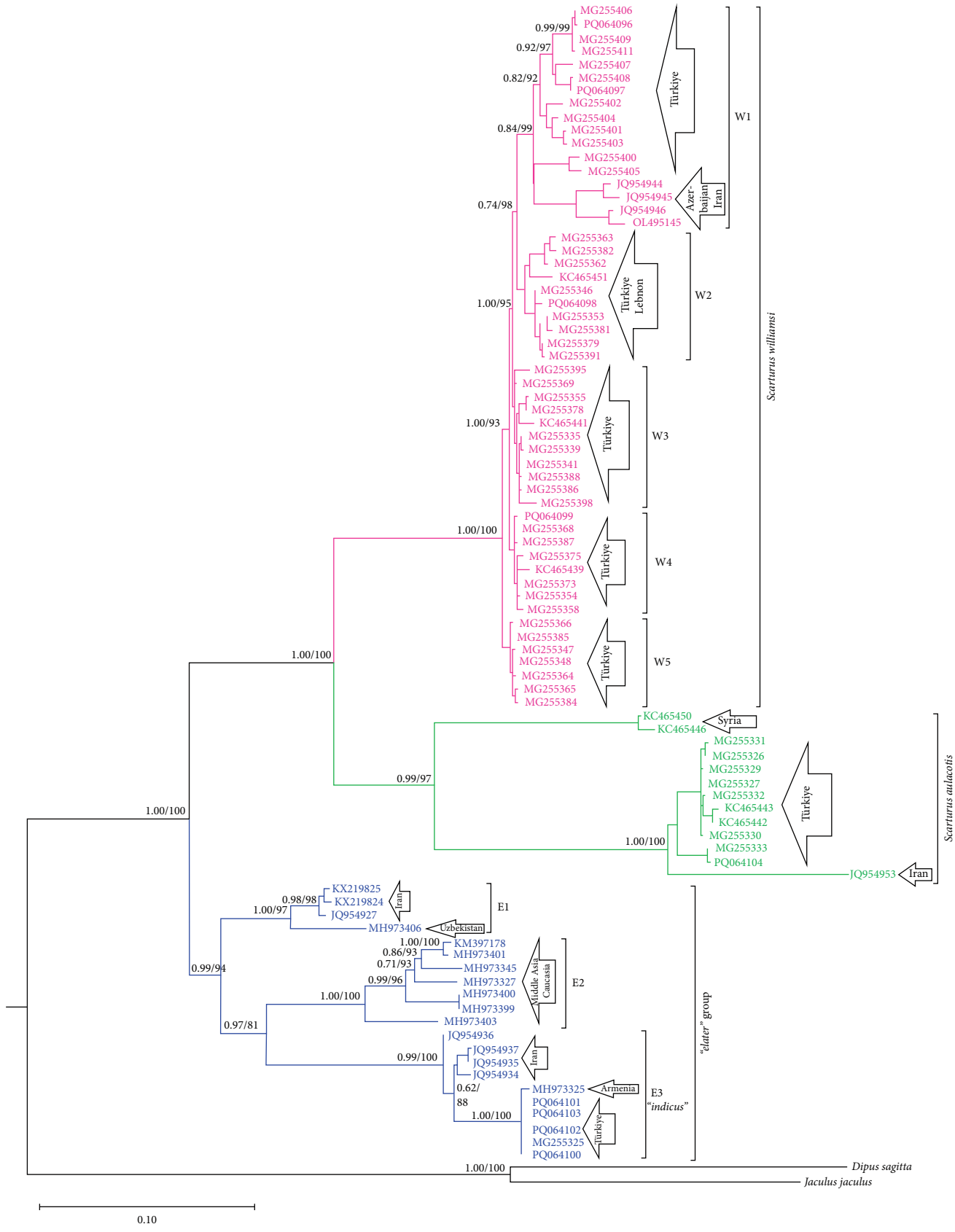
Additionally, the clustering of specimens from northwestern Iran, Azerbaijan, and eastern Türkiye within the same clade suggests that similar habitat preferences across these regions play a significant role in the genetic clustering of these geographically separated populations of *S. williamsi*. Specifically, *S. williamsi* thrives in environments with sparse vegetation, which supports their rapid bipedal movement and provides easy access to burrows, facilitating efficient movement and adequate food access [17, 46, 49, 50].

Phylogenetic analysis of *Cytb* sequences revealed that the *S. williamsi* specimens from Niğde (Kemerhisar) form a distinct group (W5). This group is genetically distinct, clustering separately from other *S. williamsi* lineages in eastern, central, and western Anatolia, with a notable genetic distance (K2P = 2.6%). The basal position of W5 in the phylogenetic



(a)

FIGURE 3: Continued.



(b)

FIGURE 3: Phylogenetic relationships and haplotype networks of *Scarturus* species based on the *Cytb* gene region. (a) Median-joining network displaying the haplotype relationships within and between *Scarturus* species. Each circle represents a distinct haplotype, with circle size proportional to haplotype frequency. Lines between circles represent mutational steps. (b) Maximum likelihood (ML) phylogenetic tree illustrating the genetic relationships among *Scarturus* species. Bootstrap support (BS) values and Bayesian inference (BI) probabilities are provided at each node to indicate the robustness of the clade support.

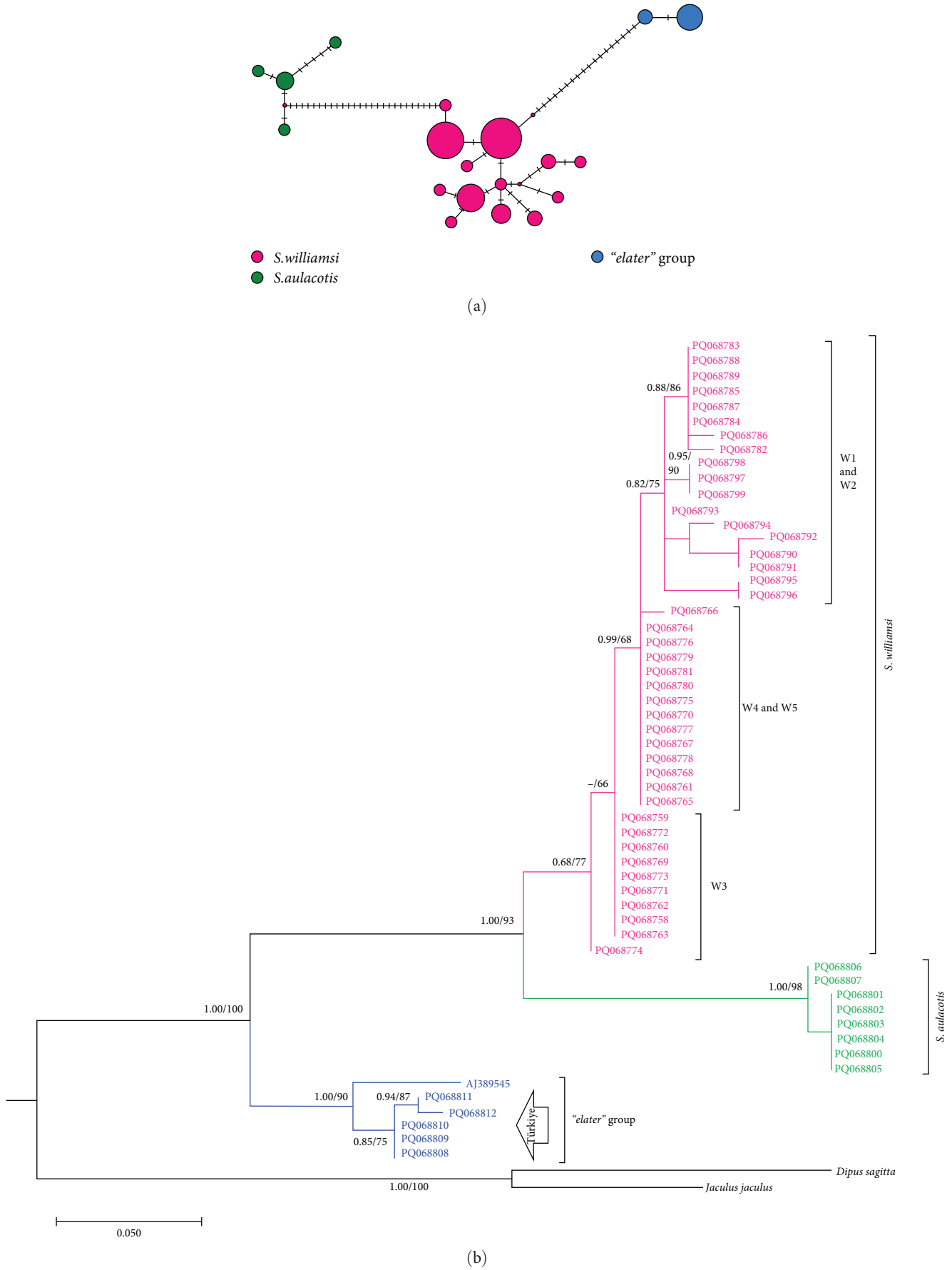


FIGURE 4: Phylogenetic relationships and haplotype networks of *Scarturus* species based on the *12S rRNA* gene region. (a) Median-joining network displaying the haplotype relationships within and between *Scarturus* species. Each circle represents a distinct haplotype, with circle

size proportional to haplotype frequency. Lines between circles represent mutational steps. (b) Maximum likelihood (ML) phylogenetic tree illustrating the genetic relationships among *Scarturus* species. Bootstrap support (BS) values and Bayesian inference (BI) probabilities are provided at each node to indicate the robustness of the clade support.

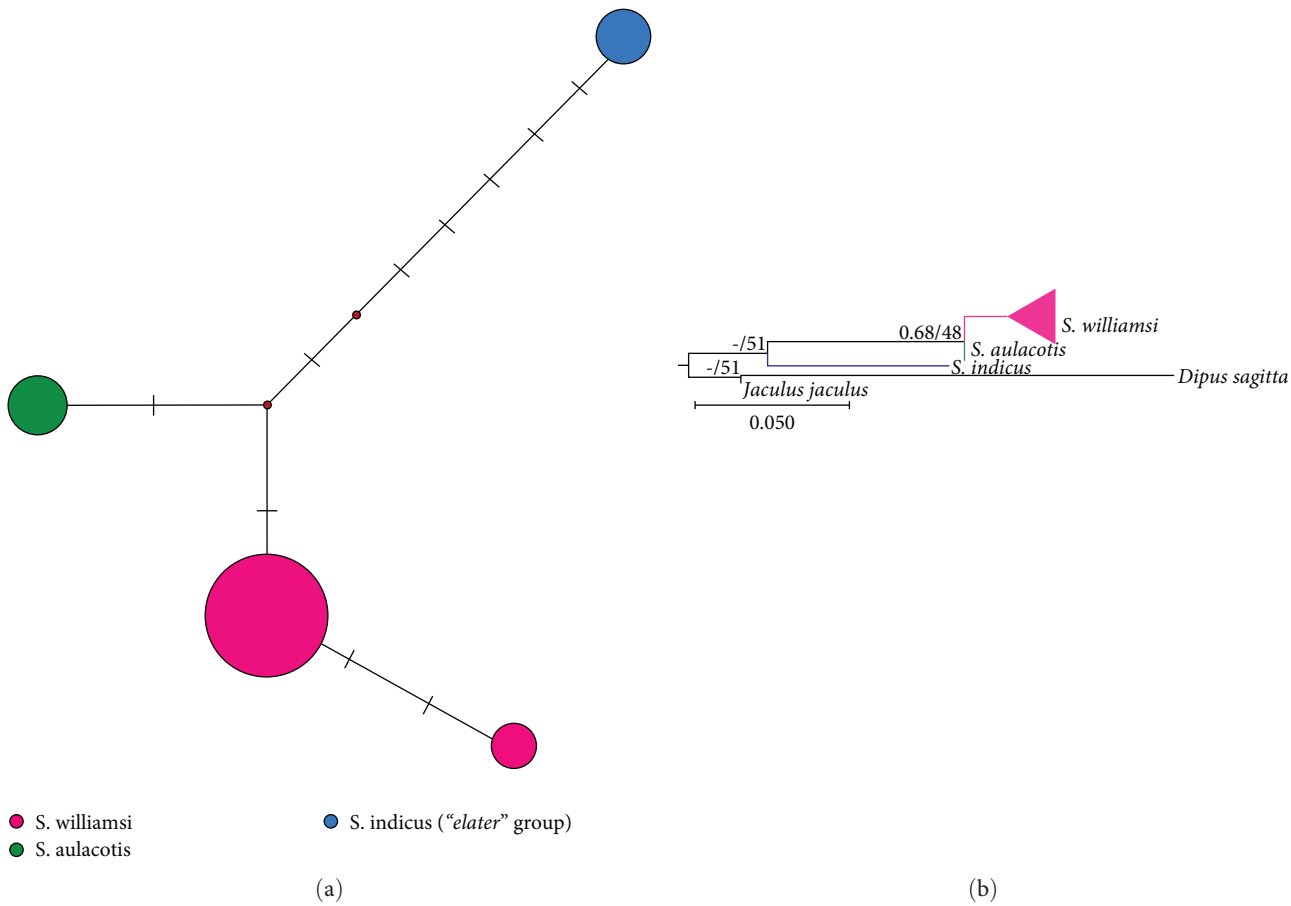
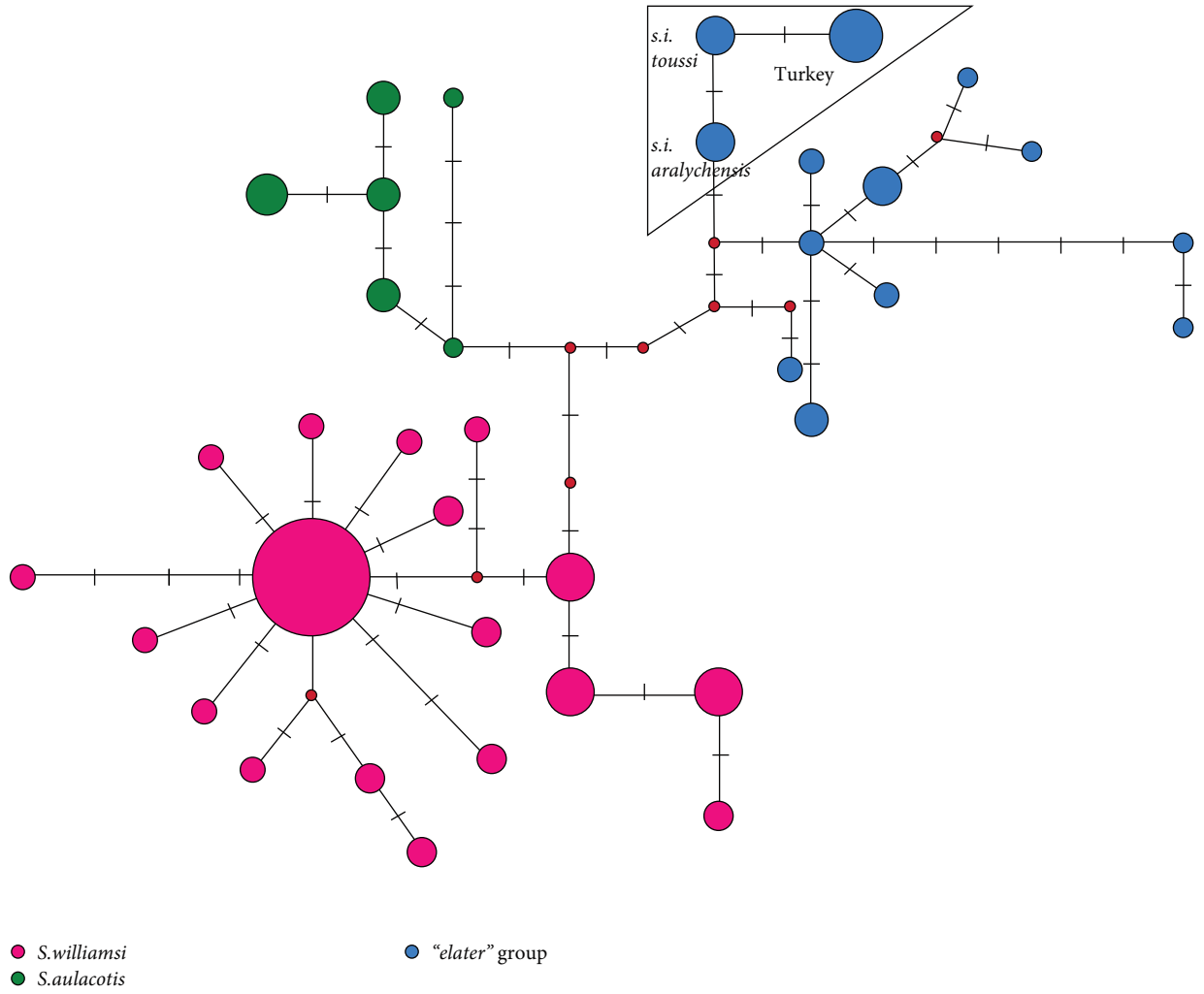


FIGURE 5: Phylogenetic relationships and haplotype networks of *Scarturus* species based on the *16S rRNA* gene region. (a) Median-joining network displaying the haplotype relationships within and between *Scarturus* species. Each circle represents a distinct haplotype, with circle size proportional to haplotype frequency. Lines between circles represent mutational steps. (b) Maximum likelihood (ML) phylogenetic tree illustrating the genetic relationships among *Scarturus* species. Bootstrap support (BS) values and Bayesian inference (BI) probabilities are provided at each node to indicate the robustness of the clade support.

tree suggests an early divergence from the other lineages. Moreover, the monophyly of both the W5 lineage and other lineages indicates a common evolutionary origin for each group, emphasizing the uniqueness of the Niğde (Kemerhisar) lineage within the phylogenetic framework of *S. williamsi*. The arid terrain of Niğde (Kemerhisar), along with its potentially unique environmental conditions, may have acted as a refugium, preserving an ancient genetic lineage of *S. williamsi*. Despite its phylogenetic distinction and early divergence, the Niğde (Kemerhisar) population exhibits lower nucleotide diversity (Pi = 0.25%) compared to other lineages (W1: 2.92%, W2: 1.43%, W3: 0.77%, and W4: 0.45%). Furthermore, the concatenated analysis of mtDNA sequences reinforced the distinctiveness of the Niğde (Kemerhisar) lineage, with W4 and W5 forming a monophyletic group that is separate from the remaining *S. williamsi* lineages (W1–W3). Our phylogenetic analysis reveals that the distinct clade of *S. williamsi* from Niğde (Kemerhisar) is likely influenced by the Pleistocene lakes previously described by Altın, Ouahabi, and Fagel [51]. These lakes formed isolated refugia that promoted genetic differentiation by limiting gene flow and amplifying genetic drift among local populations. Additionally, the ongoing salinization, driven by rising temperatures in this region, may impose further ecological stress influencing the current genetic structure of the *S. williamsi* populations. Together, these historical and ongoing environmental pressures offer a plausible explanation for the early divergence and unique genetic identity of the Niğde (Kemerhisar) clade within our phylogenetic framework.

4.2. Distinction Between S. williamsi and S. aulacotis. Formerly, *S. williamsi* was defined as a subspecies of *S. euphraticus* [8, 15, 52]. Çolak, Kivanc, and Yigit [9] reported that *S. williamsi* and *S. euphraticus* are two distinct species based on the morphological data. This was also corroborated by



(a)

FIGURE 6: Continued.

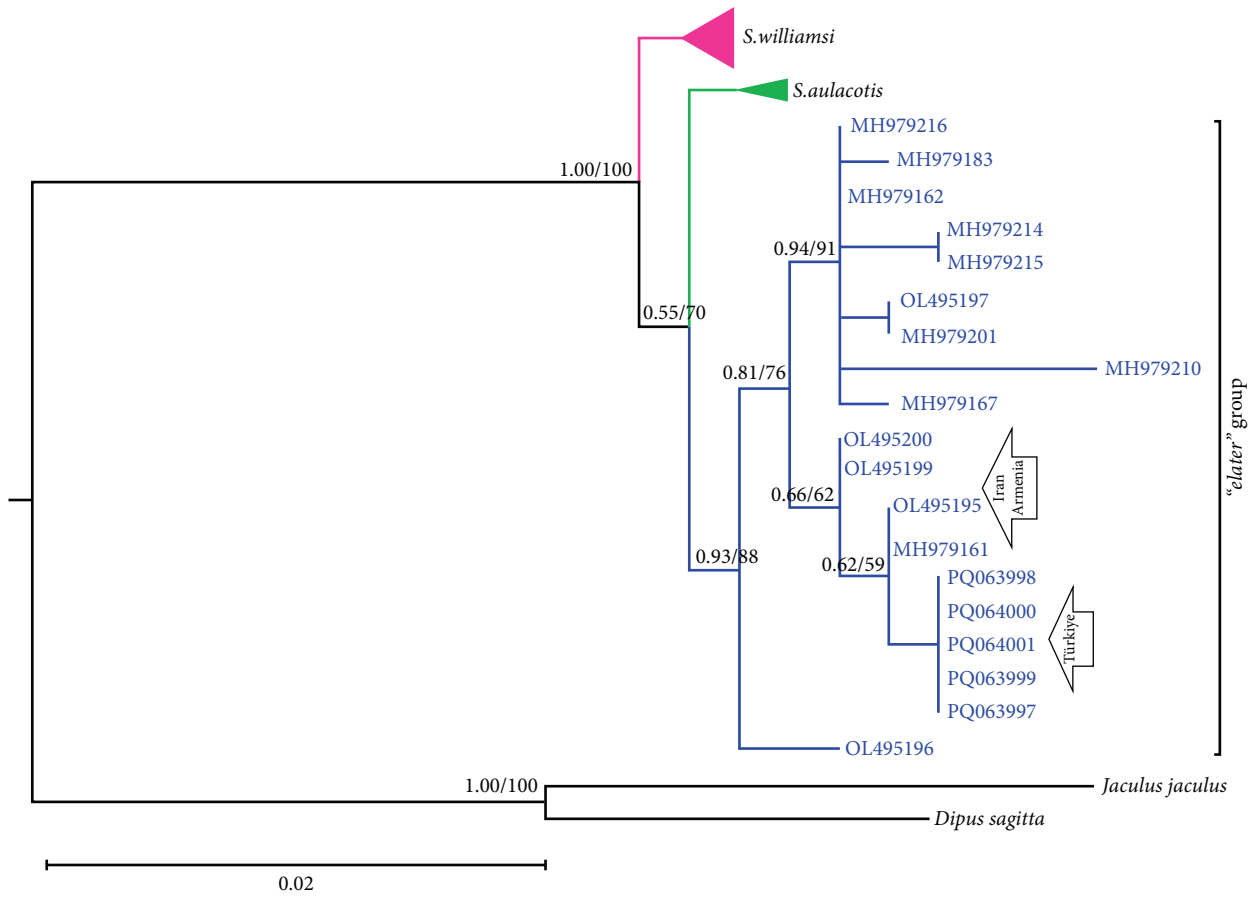


FIGURE 6: Phylogenetic relationships and haplotype networks of *Scarturus* species based on the *IRBP* gene region. (a) Median-joining network displaying the haplotype relationships within and between *Scarturus* species. Each circle represents a distinct haplotype, with circle size proportional to haplotype frequency. Lines between circles represent mutational steps. (b) Maximum likelihood (ML) phylogenetic tree illustrating the genetic relationships among *Scarturus* species. Bootstrap support (BS) values and Bayesian inference (BI) probabilities are provided at each node to indicate the robustness of the clade support.

Kryštufek et al. [12] using mtDNA sequence data from Turkish specimens of these two species. In this study, we used haplotypes from Turkish, Syrian, and Iranian *S. aulacotis* (previously known as *S. euphraticus*) and compared them with *S. williamsi* specimens from Türkiye. The phylogenetic trees (Figures 3–5) clearly demonstrate the monophyly of both *S. aulacotis* and *S. williamsi*, as well as their distinctiveness, confirming the species status proposed by Çolak, Kivanc, and Yigit [9] and validated by Kryštufek et al. [12]. Furthermore, the K2P distance values from *Cytb* (15.0%) and *12S rRNA* (5.39%) sequences between these species strongly suggest speciation [53] (Table 1). Due to low evolutionary rates, the *IRBP* sequences show lower genetic differentiation (K2P = 0.8%), indicating closer relatedness between *S. williamsi* and *S. aulacotis* than either is to *S. elater*. Similarly, *16S rRNA* sequences also supported lower genetic differentiation between *S. williamsi* and *S. aulacotis* (K2P = 1.12%) compared to that between *S. williamsi* and *S. elater* (K2P = 3.75%).

4.3. Genetic Differentiation in S. aulacotis. The intraspecific variation observed in the *Cytb* sequences of *S. aulacotis* (K2P = 5.6% and Pi = 5.1%) suggests distinct lineages within this taxon, possibly indicating cryptic subspeciation. This variation aligns with findings from Çolak and Yiğit [10], who identified distinct morphological traits in specimens from Urfa, Syria, and Jordan, previously referred to as *A. e. kivanci*. However, according to Michaux and Shenbrot [1] and corroborated by nuclear data from Lebedev et al. [3], what was once considered a separate subspecies, *A. e. kivanci*, is now junior synonym of *S. aulacotis*. Our analyses have led to the reclassification of what was traditionally identified as *S. euphraticus* samples from Türkiye and Iran, alongside the Syrian samples, within the taxonomic framework of S. *aulocotis*. Notably, within S. *aulacotis*, we have identified significant genetic divergence between the Syrian samples and the Türkiye–Iran samples, with a genetic distance of 13.8% (K2P; Table S4). This substantial divergence underscores the complexity within *S. aulocotis* and supports the existence of distinct evolutionary lineages within the species.

4.4. Taxonomic Implications of Scarturus elater. Historical taxonomic discussions on *S. elater* have varied, with Ellerman [11] and Ellerman and Morrison-Scott [14] suggesting

synonymy between the sublineages aralychensis and indica, while Corbet [15] associated the sublineage aralychensis with caucasicus. However, Çolak, Kıvanç, and Yiğit [16] confirmed the distinct presence of *S. e. aralychensis* in Türkiye. Furthermore, recent studies have proposed that specimens from Tehran represent distinct taxa, with Moshtaghi et al. [54] identifying *S. toussi* in Tehran, while Shenbrot [55] classified these specimens as *S. elater turkmeni*. Dianat et al. [56] further supported the distinct clade formation separate from *S. toussi*, highlighting the complex taxonomic structure within *S. elater*. Most recently, Bannikova et al. [13] and Lebedev et al. [3] have demonstrated that the samples from Iran and Armenia belong to the *S. indicus* superspecies, identifying them as *S. indicus aralychensis*.

Our phylogenetic analysis of the *"elater"* species complex, based on *Cytb* data, reveals significant genetic diversity, delineating the species into three distinct groups (Figure 3). The first group (E3) included the specimens from Aralık (Türkiye), Tehran (Iran), and Armenia. The second group (E2), consisting of specimens from South Khorasan (Iran) and Central Asia, forms a monophyletic group with E3, suggesting a recent common ancestry. The third group (E1) includes specimens from Uzbekistan, Torbat-Jam (Iran), and Golestan (Iran), indicating a distinct population within the *S. elater* species complex. The genetic divergence values between these groups, with K2P ranging from 11% to 12% (Table S4), suggest that these could represent different species rather than subspecies. Additionally, *IRBP* sequence analysis supports the formation of three subclades, albeit with low confidence; notably, the Turkish population clusters with Iranian and Armenian specimens (Figure 6). Given the significant genetic divergence observed, our findings strongly suggest that the Turkish population belongs to *S. i. aralychensis*.

Data Availability Statement

The NCBI GenBank accession numbers of the sequences used to support the findings of this study are included within the article (Table S1). The sequences will remain restricted until the manuscript is published.

Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*) The supporting information file includes additional data and tables that support the findings of this study: Table S1: locality, sampling, and genotype information of specimens used in this study. Table S2: PCR conditions for each gene region. Table S3: genetic variation and diversity metrics for *Scarturus* species across different gene regions (*n*: number of specimens, *S*: segregating sites, *h*: number of haplotypes, Hd: haplotype diversity, and Pi: nucleotide diversity). Table S4: the Kimura 2-parameter genetic distances and their standard deviations between *Scarturus* clades as inferred from phylogenetic trees.

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