

DNA barcoding reveals genetic divergence and phylogenetic relationships of *Scymnus* species (Coleoptera: Coccinellidae) from Uzbekistan

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Abstract

Ladybird beetles of the genus *Scymnus* (Coleoptera: Coccinellidae) are key predators in agroecosystems, but species-level identification remains challenging due to high morphological similarity among closely related taxa. Molecular data for Central Asian populations are scarce, limiting regional representation in global barcode libraries. Here, we analyzed mitochondrial cytochrome c oxidase subunit I (COI) sequences to investigate phylogenetic relationships and genetic divergence of four *Scymnus* species from Uzbekistan (*S. rubromaculatus*, *S. frontalis*, *S. subvillosus*, and *S. apetzii*). A total of 32 mitochondrial COI sequences (503 bp) were analyzed, comprising 31 sequences representing four species of *Scymnus* (16 newly generated from Uzbekistan and 15 recovered from GenBank) and one

GenBank sequence of *Axinoscymnus cardilobus* used as the outgroup. Phylogenetic reconstruction using Maximum Likelihood and Bayesian Inference recovered distinct COI lineage clusters corresponding to the four initial morphology-based taxonomic assignments. The mean intraspecific K2P divergence was 0.00051 in *S. rubromaculatus*, 0.00362 in *S. frontalis*, 0.00811 in *S. apetzi*, and 0.03446 in *S. subvillosus*. The four newly generated *S. subvillosus* sequences from Uzbekistan were identical, whereas the elevated intraspecific divergence in the complete dataset was associated with publicly available records. The interspecific distances ranged from 0.11383 to 0.17778. Although the maximum intraspecific distance (0.09085) remained below the minimum interspecific distance (0.11383), the resulting separation should be regarded descriptive because the highly divergent public *S. subvillosus* records could not be independently taxonomically verified. These findings support the utility of COI to discriminate among the examined sequence groups, while highlighting the need for voucher-based morphological verification and broader multilocus sampling.

Keywords

Scymnus, DNA barcoding, mitochondrial COI, phylogenetic analysis, Coccinellidae, Uzbekistan

Introduction

Ladybird beetles (Coleoptera: Coccinellidae) are among the most important predatory insect groups in terrestrial ecosystems and contribute substantially to the natural regulation of agricultural pests. Most species prey on aphids, scale insects, mites, and other soft-bodied arthropods, thus reducing pest abundance in both cultivated and natural habitats (Obrycki et al. 2009). Owing to their ecological and economic importance, coccinellids have been widely investigated in studies of biodiversity, trophic interactions, and biological control. Several species of *Scymnus* have also been evaluated as predators of aphids and adelgids, highlighting their potential importance in biological control programs (Obrycki et al. 2009; Limbu et al. 2015, 2016; Rosagro et al. 2020). Their effectiveness as natural enemies has also increased interest in understanding species diversity, ecological specialization, and evolutionary relationships within the family.

Despite extensive ecological research, species delimitation within several coccinellid groups remains problematic due to high morphological similarity among closely related taxa. This challenge is particularly evident in small-bodied genera, where diagnostic characters such as coloration, pubescence, elytral markings, and body shape often overlap among species (Huang et al. 2020; Peng et al. 2025). The genus *Scymnus* Kugelann, 1794 represents one of the most taxonomically complex groups within Coccinellidae. Many species exhibit conservative external morphology and limited diagnostic differentiation, increasing the risk of misidentification when only morphological characters are used (Iqbal et al. 2024; Peng et al. 2025). Recent Scymnini taxonomic studies have demonstrated that reliable species delimitation frequently requires detailed morphological comparison, including examination of genital characters (Poorani 2015; Rashid et al. 2017; Poorani & Thanigairaj 2023; Poorani et al. 2024). In addition, variation associated with sex, developmen-

tal stage, or geographic population can further obscure species boundaries. These limitations highlight the need for complementary molecular approaches in the taxonomy of *Scymnus*.

Mitochondrial cytochrome c oxidase subunit I (COI) has become one of the most widely used molecular markers for insect identification and phylogenetic studies. COI-based DNA barcoding has proven effective in distinguishing closely related taxa, testing species monophyly, detecting cryptic diversity, and assessing genetic differentiation between populations (Hebert & Gregory 2005; Valentini et al. 2009). In Coccinellidae, COI datasets have substantially improved species-level resolution and clarified phylogenetic relationships across multiple genera (Huang et al., 2020; Iqbal et al., 2024). Broader phylogenetic studies have further demonstrated the value of integrating molecular and morphological evidence for resolving relationships within Coccinellidae (Magro et al. 2010; Seago et al. 2011). Although molecular studies of *Scymnus* have increased in recent years, available barcode data are still concentrated mainly in Europe, China, Pakistan, and other parts of East and South Asia (Elekcioglu 2020; Huang et al. 2020; Peng et al. 2025). The high identification success of COI barcoding has also been demonstrated in a broad regional assemblage of beetles (Pentinsaari et al. 2014). On the contrary, Central Asian populations remain poorly represented in global genetic databases.

Uzbekistan occupies a biogeographically important position between arid lowlands, desert ecosystems, foothill zones, and mountain systems, creating diverse ecological conditions for insect assemblages. *Scymnus* species are widespread in these habitats and play an important role in the regulation of pest populations in alfalfa fields, orchards, and vegetable agroecosystems. Among them, *Scymnus rubromaculatus*, *S. frontalis*, *S. subvillosus* and *S. apetzii* are frequently encountered and are considered ecologically significant predators in agricultural landscapes. However, previous studies in Uzbekistan and neighboring regions have focused mainly on faunistic records, distributional observations and morphological descriptions, while molecular evidence remains extremely limited (Gafurova et al. 2025). As a result, phylogenetic relationships, genetic divergence, and population-level variation within regional *Scymnus* taxa remain insufficiently understood.

The absence of representative COI datasets from Central Asia limits accurate species identification and reduces the phylogeographic resolution of Palearctic *Scymnus* populations. This gap also constrains comparative biodiversity studies and weakens the development of molecular reference libraries for ecologically important predatory beetles. Expanding barcode coverage for Central Asian taxa is therefore important not only for regional taxonomy, but also for broader studies of evolutionary diversity and biological control.

The present study investigates mitochondrial COI sequences assigned to four species of *Scymnus* from Uzbekistan to characterize genetic divergence and COI lineage patterns within the available dataset. Specifically, the study aims to: (i) examine whether the analyzed COI sequences form distinct mitochondrial lineage clusters corresponding to the initial morphology-based taxonomic assignments; (ii)

compare intraspecific and interspecific genetic divergence patterns; and (iii) expand the available COI reference data for *Scymnus* from Uzbekistan. Because the dataset includes only four *Scymnus* taxa and a single mitochondrial marker, the analyses are not intended as a comprehensive species-delimitation test or as a reconstruction of deeper evolutionary relationships within the genus.

Materials and methods

Sampling and specimen collection

This study examined four species of the genus *Scymnus* collected from different regions of Uzbekistan: *Scymnus rubromaculatus* (Goeze, 1777), *S. frontalis* (Fabricius, 1787), *S. subvillosus* (Goeze, 1777) and *S. apetzi* (Mulsant, 1846) (Fig. 1). A total of 20 adult specimens were morphologically examined. The phylogenetic data set comprised 32 COI sequences, including 31 sequences representing the four focal species of *Scymnus* (16 newly generated from Uzbekistan and 15 recovered from GenBank) and one GenBank sequence of *Axinoscymnus cardilobus* used as the out-group.

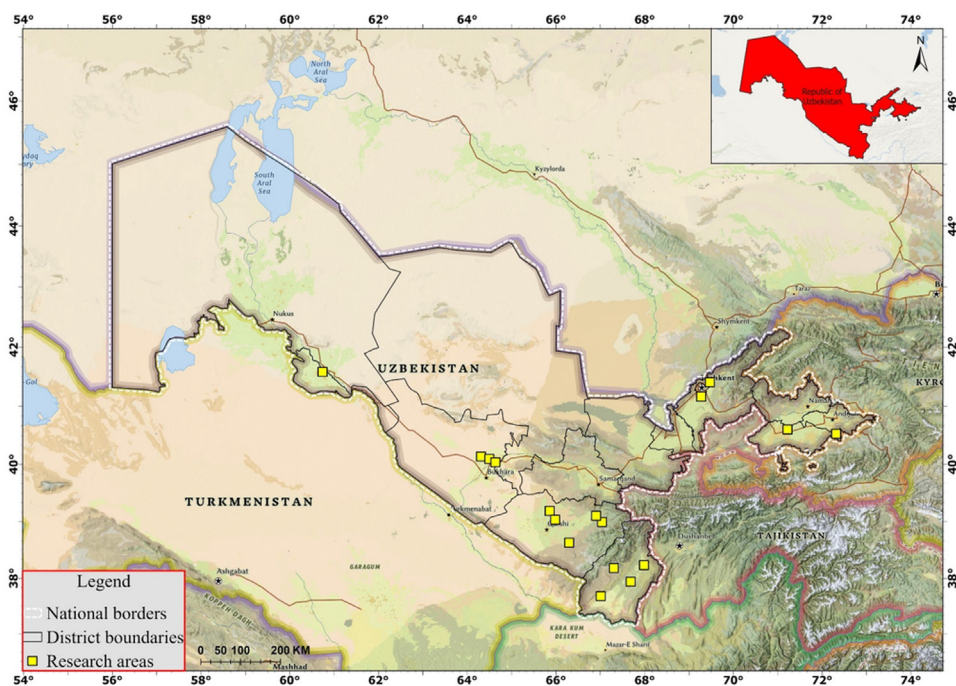


Figure 1. Geographic distribution of the studied *Scymnus* species (*S. rubromaculatus*, *S. frontalis*, *S. subvillosus*, and *S. apetzi*) in Uzbekistan. The sampling localities were mapped in ArcGIS Pro.

The sampling was carried out in multiple localities in Uzbekistan, including Kashkadaryo, Bukhara, Fergana, Andijan, Jizzakh, Tashkent and Surkhandarya regions. These collections were intended to obtain geographically distributed material for the four focal taxa rather than to provide exhaustive geographic sampling of their ranges. Complete specimen-level metadata linking each newly generated GenBank accession to its exact collection location, coordinates, collection date, habitat, collector, and physical voucher / repository record were not available during the present manuscript revision. Therefore, missing specimen-level information was not inferred or reconstructed. The available geographic information is reported below at the level supported by the original collection records, while the GenBank accession numbers of the newly generated sequences are provided in Table 1.

The documented sampling locations ranged from 191 to 1000 m above sea level, between 38.728935°N and 41.406616°N and 64.323156°E and 69.469544°E. The samples were collected in agricultural fields, foothill habitats, and semi-natural vegetation associated with agroecosystems. Geographic coordinates and elevation were recorded for collection locations using a handheld GPS device. Where specimen-level collection or voucher metadata could not be independently verified during manuscript revision, these fields are explicitly reported as unavailable rather than inferred.

Morphological observations

The initial species assignments were based on external morphological characteristics using published diagnostic descriptions and taxonomic treatments for Coccinellidae and Scymnini (Canepari 2011; Ali et al. 2015; Peng et al. 2023; Iqbal et al. 2024). The characters examined included body size and shape, dorsal coloration, pronotal and elytral patterns, pubescence, and the distribution of setae. The identifications were also compared with named reference material named found in the entomological collection of the Institute of Zoology, Uzbekistan Academy of Sciences.

Unique physical voucher identifiers, sample sex, and records of genitalic examination could not be independently verified for the specimens associated with the newly generated COI sequences during the present manuscript revision. The isolate identifiers reported in Table 1 were obtained from the corresponding GenBank records and are therefore treated strictly as sequence identifiers rather than as verified physical voucher codes. The initial species assignments were based on external morphology, published taxonomic treatments, dorsal habitus characteristics, and comparison with the reference material. However, because traceable voucher information and genital documentation were unavailable for independent reassessment, these assignments cannot be regarded as a complete integrative taxonomic verification. Accordingly, throughout this study, the molecular results are interpreted primarily as concordance between the initial morphology-based species assignments and the recovered COI lineages. Future voucher-based studies should link each sequence to a uniquely numbered specimen deposited in a recognized entomological

collection and include diagnostic genital examination where required for reliable species identification.

The examined species exhibited distinct morphological characteristics. Adults of *Scymnus frontalis* measured 2.8–3.4 mm in body length and were characterized by a predominantly black dorsal coloration with one or two yellow spots on the elytra. *Scymnus subvillosus* showed a smaller body size (1.8 to 2.5 mm), an elongated oval body shape, yellowish brown coloration, and dense fine punctation. *Scymnus rubromaculatus* measured approximately 2 to 2.5 mm in length and possessed characteristic reddish markings on the elytra. *Scymnus apetzi* ranged from 2 to 3 mm in body length and had a single reddish spot on each elytron (Fig. 2). The external morphological characters used for the initial species assignments are summarized in Suppl. material 1: Table S1.

Table 1. Data set of COI sequence used for phylogenetic and genetic divergence analyses of *Scymnus* species. The dataset includes newly generated sequences from Uzbekistan together with homologous sequences retrieved from GenBank, as well as the outgroup taxon used for phylogenetic reconstruction

Species	n	GenBank Accession	Source	Country
<i>A. cardilobus</i>	1	EU392430	GenBank	–
<i>S. rubromaculatus</i>	8	KR486694	GenBank	–
		HQ953858	GenBank	–
		KM451437	GenBank	–
		MG059584	GenBank	–
		PX963222	This study	Uzbekistan
		PZ235504	This study	Uzbekistan
		PZ235503	This study	Uzbekistan
		PZ235430	This study	Uzbekistan
<i>S. subvillosus</i>	8	GU073957	GenBank	–
		MK802037	GenBank	–
		ON980768	GenBank	–
		ON980769	GenBank	–
		PX963223	This study	Uzbekistan
		PZ235428	This study	Uzbekistan
		PZ235427	This study	Uzbekistan
		PZ235429	This study	Uzbekistan
<i>S. apetzi</i>	8	KM439621	GenBank	Unknown
		KM441259	GenBank	Unknown
		HQ953922	GenBank	Unknown
		HQ953923	GenBank	Unknown

Species	n	GenBank Accession	Source	Country
<i>S. frontalis</i>	7	PX963224	This study	Uzbekistan
		PZ235505	This study	Uzbekistan
		PZ235506	This study	Uzbekistan
		PZ235507	This study	Uzbekistan
		KU907730	GenBank	Unknown
		MZ659820	GenBank	Unknown
		KJ966166	GenBank	Unknown
		PZ241090	This study	Uzbekistan
		PZ241091	This study	Uzbekistan
		PZ241089	This study	Uzbekistan
		PX963228	This study	Uzbekistan

Note: Isolate identifiers associated with the newly generated sequences reproduce the information available in the corresponding GenBank records. They are sequence identifiers and not independently verified physical voucher codes. Sex, genital examination records, and unique depository information could not be verified for these samples.

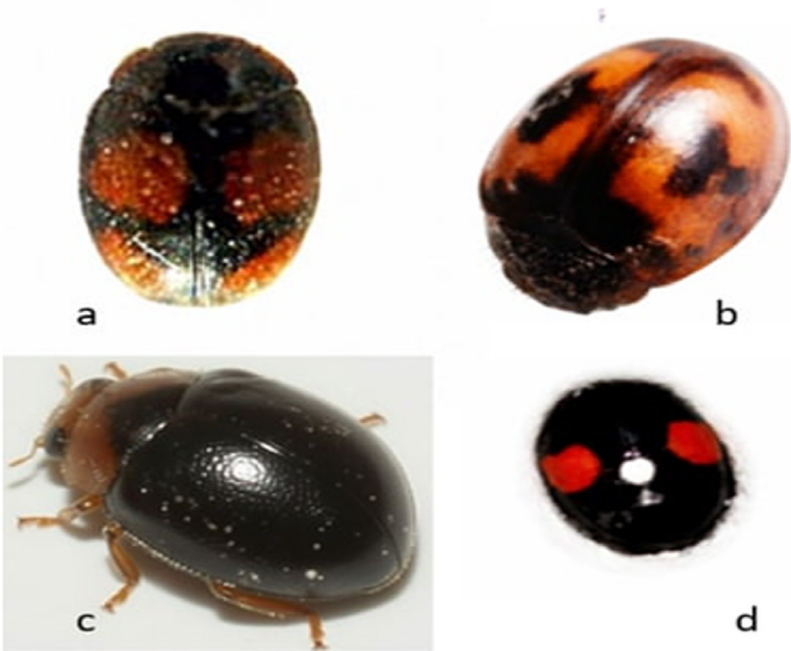


Figure 2. Dorsal habitus of the four examined *Scymnus* species: (a) *S. frontalis*, (b) *S. subvillosus*, (c) *S. rubromaculatus*, and (d) *S. apetzii*. Images document the external morphology used in the initial species assignments; genital preparations were not available for independent verification.

DNA extraction, PCR amplification, and sequencing

For molecular analyzes, genomic DNA was extracted from adult specimens of *Scymnus frontalis*, *S. apetzi*, *S. subvillosus*, and *S. rubromaculatus*. A single leg from each ethanol-preserved specimen was used to minimize damage to voucher material. Before extraction, specimens preserved in 70% ethanol were briefly air dried on sterile filter paper for approximately 10–15 min to remove residual ethanol.

Genomic DNA extraction followed the protocol of Ivanova et al. (2006) with minor modifications. Individual tissue samples were transferred to 1.5 ml microcentrifuge tubes containing 100 µl of lysis buffer composed of 0.5 M Tris-HCl (pH 8.5), 2 M KCl, 1 M MgCl₂, NP-40, 50% sucrose, distilled autoclaved water, and proteinase K (10 mg/ml). The concentrations listed above reproduce the values documented in the available laboratory record. Because the original record did not distinguish whether these values represented stock concentrations or final working concentrations in the lysis mixture, they are reported here without reinterpretation. The samples were incubated at 65 °C for 1–2 h to facilitate tissue digestion, followed by enzyme inactivation at 92 °C for 10 min. The DNA was stored at –20 °C until further analysis. DNA concentration and purity were evaluated using a NanoDrop spectrophotometer.

Fragments of the mitochondrial cytochrome c oxidase subunit I (COI) gene were amplified using modified Folmer primers: ClepFolF (5'–ATTCAACCAATCAT-AAAGATATTGG–3') and ClepFolR (5'–TAAACTTCTGGATGTCCAAAAAATCA–3'). Polymerase chain reaction (PCR) amplifications were performed in a nominal reaction volume of 10 µl. According to the laboratory record, the documented reaction mixture contained 7.1 µl sterile distilled water, 1.0 µl 10× PCR buffer, 0.2 µl dNTP mixture, 0.25 µl of each forward and reverse primer and 0.2 µl Taq DNA polymerase. The laboratory record did not specify the volume of template DNA or the stock/final concentrations of dNTPs, primers, and Taq DNA polymerase; therefore, these details are not reconstructed here. Negative controls lacking template DNA were included in all PCR runs to monitor possible contamination.

Thermal cycling conditions consisted of an initial denaturation step at 95 °C for 2 min, followed by 35 amplification cycles of 93 °C for 20 s, 52 °C for 45 s, and 72 °C for 2 min, with a final extension step at 72 °C for 10 min. The amplified PCR products were visualized on 1.5% agarose gels stained with DNA dye under ultraviolet illumination to confirm the successful amplification and fragment integrity.

Detailed laboratory records specifying sequencing direction, sequencing chemistry, sequencing instrument, sequencing facility, and chromatogram processing workflow were not available during the revision of the present manuscript. Therefore, these methodological details could not be reliably reconstructed and are not inferred here. The newly generated COI sequences used in subsequent analyses were deposited in GenBank under the accession numbers listed in Table 1.

Bioinformatic and phylogenetic analyses

Newly generated mitochondrial COI sequences were combined with homologous sequences retrieved from the NCBI GenBank database for comparative analyses. Multiple sequence alignment was performed in MAFFT v7 (Katoh et al. 2002) using default alignment settings. The resulting alignments were visually inspected and manually refined in MEGA X (Kumar et al. 2018) to ensure positional consistency and eliminate potential alignment artifacts.

Sequence quality assessment was conducted prior to downstream analyses. Only sequences with high read quality were retained, allowing a maximum of two ambiguous nucleotide positions per sequence and an average Phred quality score of ≥ 20 . The final dataset consisted of 32 aligned COI sequences of 503 bp, comprising 31 sequences of the four focal *Scymnus* species and one sequence of *Axinoscymnus cardilobus* used as the outgroup.

Phylogenetic analyses were conducted using both Maximum Likelihood (ML) and Bayesian inference (BI) approaches to evaluate mitochondrial COI lineage clustering within the available dataset. COI is widely used in DNA barcoding because it can provide informative sequence variation among closely related insect taxa (Hebert et al. 2003; Hajibabaei et al. 2007). However, in the present study, the marker is used to evaluate lineage clustering and genetic divergence among the examined sequence groups rather than to infer comprehensive species boundaries or deeper phylogenetic relationships within *Scymnus*.

Maximum likelihood analyses were conducted in IQ-TREE v2.1.4 (Minh et al. 2020). The optimal nucleotide substitution model was selected using ModelFinder, which identified the General Time Reversible (GTR) model as the best-fitting evolutionary model for the dataset. Statistical support for tree topology was evaluated using 1000 ultra-fast bootstrap (UFBoot) replicates and 1000 SH-like approximate likelihood ratio test (SH-aLRT) replicates. The COI alignment was analyzed as a single, unpartitioned dataset. Codon-position-specific partitioning and explicit comparison of alternative partitioning schemes were not performed in the original analysis. Consequently, the resulting topology and support values are interpreted conservatively, particularly for deeper nodes, because substitution patterns may differ among COI codon positions.

The final alignment contained 136 distinct site patterns, including 123 parsimony-informative sites and 41 singleton sites. Base composition analyses detected no significant compositional heterogeneity among sequences. The resulting ML tree showed a log-likelihood score of -1985.391 . The optimized nucleotide frequencies were estimated as $A = 0.300$, $C = 0.162$, $G = 0.152$, and $T = 0.386$.

Bayesian phylogenetic reconstruction was performed in MrBayes v3.2.7a using two independent Markov chain Monte Carlo (MCMC) runs, each consisting of four chains and 5,000,000 generations. The trees were sampled every 500 generations and the first 25% of sampled trees were discarded as burns before generating a majority-rule consensus tree of majority rules. The original MrBayes diagnostic

output files were not available for independent re-assessment during the current manuscript revision. Consequently, numerical convergence diagnostics, including the average standard deviation of split frequencies, potential scale reduction factors (PSRF), effective sample sizes (ESS) and likelihood-trace assessments, could not be retrospectively reported. The 25% burn-in used in the original analysis is therefore reported as an analytical setting rather than as a threshold independently validated during the present revision. Therefore, Bayesian posterior probabilities should be interpreted with appropriate caution.

The COI sequence of *Axinoscymnus cardilobus* (GenBank accession EU392430) was selected as the outgroup for tree rooting. The final phylogenetic trees were visualized and annotated using iTOL v6.6 (Letunic & Bork 2024).

Genetic divergence was estimated using the Kimura 2-parameter (K2P) model. Distance analyzes included the 31 sequences representing the four focal *Scymnus* species; the outgroup sequence of *Axinoscymnus cardilobus* was excluded. Ambiguous and missing nucleotide positions were handled using pairwise deletion. Comparisons between zero distances between identical sequences were retained when calculating mean intraspecific distances, standard deviations, and minimum and maximum values. Summary statistics for intraspecific and interspecific K2P distances are presented in Table 2.

To evaluate the elevated divergence detected in *S. subvillosus*, a sensitivity analysis was performed by comparing three datasets: (i) all eight *S. subvillosus* sequences, (ii) the four newly generated Uzbekistan sequences, and (iii) the four publicly available GenBank sequences. This analysis was used to determine whether the observed intraspecific divergence was associated primarily with the newly generated or publicly available records. No population structure or geographic-differentiation tests were performed.

Table 2. Summary of pairwise intraspecific K2P distances and nearest interspecific neighbors among the 31 focal *Scymnus* COI sequences. Comparisons between zero distances between identical sequences were retained. The outgroup was excluded

Species	n	Pairwise comparisons	Mean intraspecific	SD	Minimum	Maximum	Nearest-neighbour species	Minimum interspecific K2P
<i>S. rubromaculatus</i>	8	28	0.00051	0.00089	0.00000	0.00214	<i>S. apetzi</i>	0.14340
<i>S. subvillosus</i>	8	28	0.03446	0.03671	0.00000	0.09085	<i>S. frontalis</i>	0.13478
<i>S. frontalis</i>	7	21	0.00362	0.00361	0.00000	0.00800	<i>S. apetzi</i>	0.11383
<i>S. apetzi</i>	8	28	0.00811	0.01032	0.00000	0.02642	<i>S. frontalis</i>	0.11383

Results

Phylogenetic reconstruction

Phylogenetic analyzes were performed using 32 mitochondrial COI sequences representing four species of *Scymnus* and one outgroup taxon (Fig. 3). The aligned dataset comprised 503 bp per sequence and showed a high level of overall sequence quality suitable for phylogenetic reconstruction. The final alignment contained 136 distinct site patterns, including 123 parsimony-informative sites, 41 singleton sites, and 339 conserved nucleotide positions.

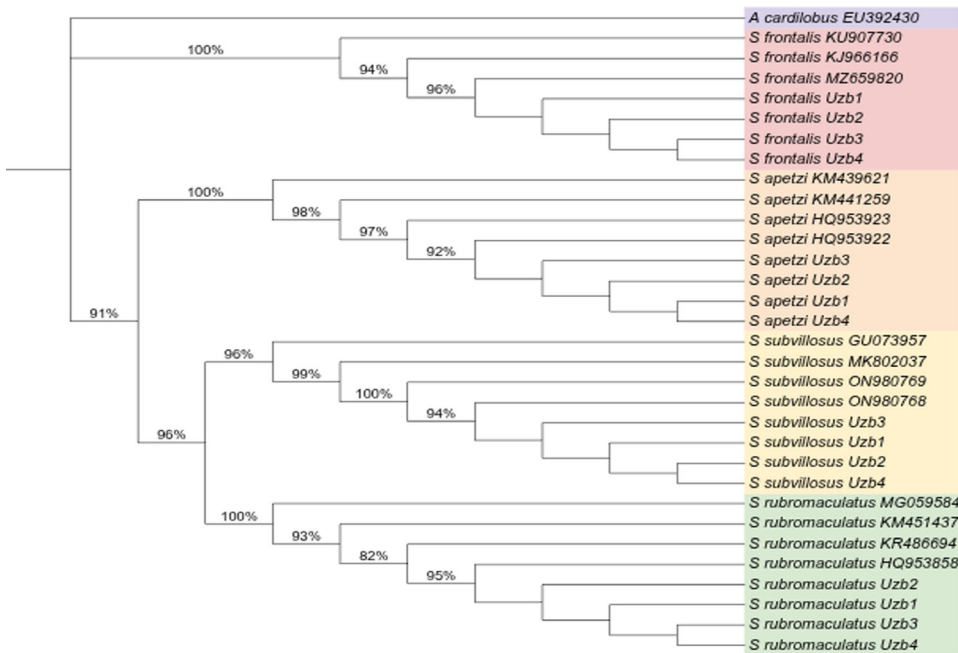


Figure 3. Maximum Likelihood (ML) phylogenetic tree of *Scymnus* species based on the mitochondrial COI gene. The tree was reconstructed in IQ-TREE v2.1.4 using the GTR model. Node values indicate phylogenetic support based on 1,000 ultrafast bootstrap (UF-Boot) replicates. Colored sectors indicate monophyletic clusters. *A. cardilobus* (EU392430) was used as the outgroup.

The proportion of gaps and ambiguous nucleotides in the dataset was low (0.63%), indicating minimal alignment uncertainty and high sequence reliability. Nucleotide composition analysis indicated no significant compositional heterogeneity between sequences based on the compositional χ^2 test ($p > 0.05$). The original analysis output containing the exact χ^2 statistic was not available for independent reassessment during manuscript revision; therefore, an exact χ^2 value is not reported here.

The number of informative sites and the low proportion of ambiguous positions provided a sequence variation suitable for comparing COI lineage patterns within the present data set. However, these characteristics do not establish resolution of species boundaries or deeper phylogenetic relationships within *Scymnus*. The absence of significant compositional heterogeneity reduced one potential source of bias associated with unequal nucleotide frequencies among the sequences analyzed.

Maximum Likelihood phylogenetic reconstruction was performed in IQ-TREE v2.1.4 using the General Time Reversible (GTR) substitution model selected by ModelFinder as the best-fitting evolutionary model for the dataset. Nodal support was evaluated using 1000 ultrafast bootstrap (UFBoot) replicates and 1000 SH-like approximate likelihood ratio tests (SH-aLRT). Bayesian Inference analyzes generated a 50% majority-rule consensus tree with posterior probability (PP) values used to estimate branch support.

The outgroup taxon, *Axinoscymnus cardilobus* (GenBank accession EU392430), was clearly separated from all examined *Scymnus* taxa with maximal statistical support (UFBoot = 100%; SH-aLRT = 100%; PP = 1.00). However, because the phylogeny was rooted using a single outgroup sequence, the inferred root position should be regarded provisional and interpreted with caution (Fig. 4).

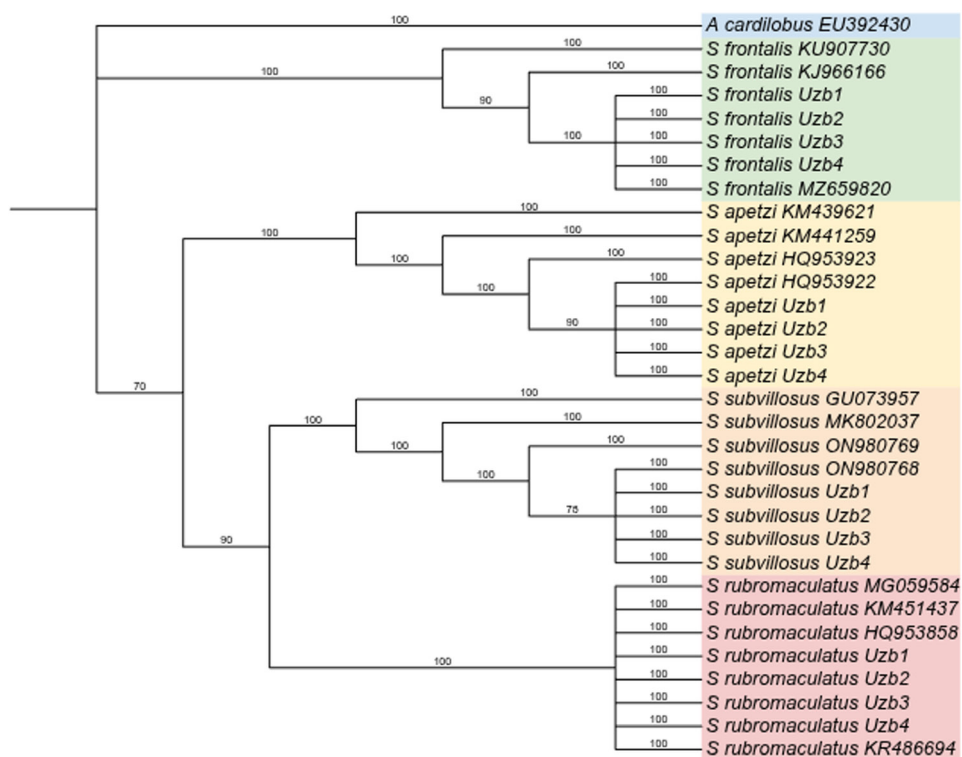


Figure 4. Bayesian 50% majority rule consensus tree of *Scymnus* species based on mitochondrial COI sequences. Posterior probability (PP) values are shown at each node. Newly generated sequences from Uzbekistan are indicated by the label 'Uzb'.

All four focal taxa formed distinct COI lineage clusters in both analyses. Because the numerical support values reported in the original text could not be independently reconciled with the values displayed in the phylogenetic figures during manuscript revision, clade-specific support values are not repeated in the text here. The support values displayed directly on the corresponding phylogenetic trees should be regarded as the graphical representation of the analyses.

The COI-based analyses recovered distinct lineage clusters corresponding to the four initial morphology-based taxonomic assignments within the present dataset. The lowest interspecific COI distance was observed between the sequences assigned to *S. apetzii* and *S. frontalis* (minimum K2P = 0.11383). However, because the analysis is based on a 503-bp fragment of a single mitochondrial marker, four focal *Scymnus* taxa and a single outgroup sequence, the deeper branching pattern is not interpreted here as evidence of broader evolutionary relationships within *Scymnus*. Robust assessment of deeper phylogenetic relationships would require denser taxon sampling together with independent nuclear markers.

Branch-length patterns differed among the examined taxa. *Scymnus rubromaculatus* displayed very short internal branches, consistent with low pairwise COI divergence. In contrast, *S. subvillosus* exhibited longer internal branches in the complete dataset. However, the four newly generated Uzbekistan sequences were identical, whereas the elevated divergence was associated with publicly available GenBank records. Because locality information was unavailable for several public records and no population genetic analyses were conducted, no inference was made about geographic structure or population subdivision.

The broadly similar clustering recovered by ML and BI indicates consistency in the placement of the four focal COI lineage groups within the present dataset. Because clade-specific numerical support values could not be independently reconciled with phylogenetic figures during revision, no additional interpretation of support magnitude is made here. Within the limits of the present taxon sampling, COI provides useful barcode-level information for distinguishing recovered mitochondrial lineage clusters, but the analyses do not resolve deeper evolutionary relationships within *Scymnus* or Coccinellidae.

Genetic divergence analysis

Genetic divergence among the examined *Scymnus* species was assessed using mitochondrial COI sequences under the Kimura 2-parameter (K2P) model. The distance analysis included 31 aligned COI sequences representing the four focal *Scymnus* species; the outgroup sequence of *Axinoscymnus cardilobus* was excluded. Pairwise genetic distances were calculated among all sequences to evaluate both intra- and interspecific variation. Summary statistics for pairwise K2P distances are presented in Table 2, with the corresponding machine-readable data provided in Suppl. material 2: Tables S2a–S2c, while divergence patterns among taxa are visualized in the hierarchical heatmap shown in Fig. 5.

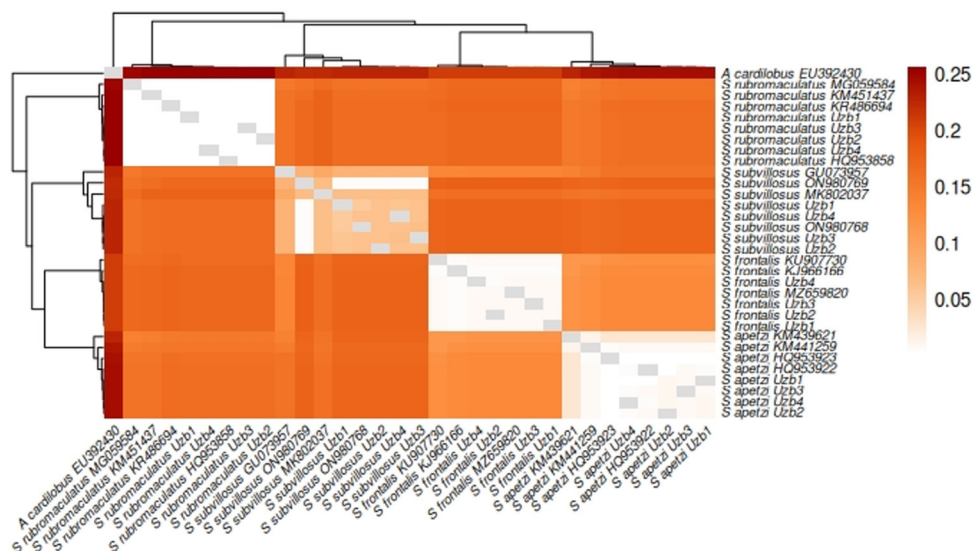


Figure 5. Heatmap of pairwise K2P distances among all 32 analyzed COI sequences, including *Axinoscymnus cardilobus* as the outgroup. The outgroup was excluded from the summary analyses of intraspecific and interspecific divergence.

Mean pairwise intraspecific K2P distances were calculated in all comparisons, including zero-distance pairs between identical sequences. *Scymnus rubromaculatus* showed the lowest mean intraspecific divergence (0.00051 ± 0.00089 ; range = 0–0.00214), followed by *S. frontalis* (0.00362 ± 0.00361 ; range = 0–0.00800) and *S. apetzii* (0.00811 ± 0.01032 ; range = 0–0.02642). The highest mean intraspecific divergence was recorded for *S. subvillosus* (0.03446 ± 0.03671 ; range = 0 to 0.09085).

In the complete dataset, the maximum intraspecific K2P distance (0.09085) was lower than the minimum interspecific distance (0.11383), producing an apparent barcode gap of 0.02299. However, the maximum intraspecific value was derived from publicly available *S. subvillosus* sequences whose specimen identifications could not be independently verified in the present study (Fig. 6). Therefore, the barcode gap calculated from the combined dataset should be regarded as descriptive rather than as evidence of a validated species-delimitation threshold. In particular, the four newly generated *S. subvillosus* sequences from Uzbekistan were identical (K2P = 0), while the elevated intraspecific divergence was restricted to comparisons involving public GenBank records. A separate complete barcode gap estimate based exclusively on the newly generated sequences could not be recalculated during revision because the original aligned sequence matrix was unavailable for reanalysis.

The interspecific distances ranged from 0.11383 to 0.17778. The minimum interspecific distance occurred between *S. apetzii* KM439621 and *S. frontalis* KU907730 (K2P = 0.11383), while the maximum occurred between *S. subvillosus* ON980769

and *S. frontalis* MZ659820 ($K2P = 0.17778$). The mean distances for species-level comparisons ranged from 0.12839 between *S. apetzi* and *S. frontalis* to 0.16981 between *S. frontalis* and *S. subvillosus*.

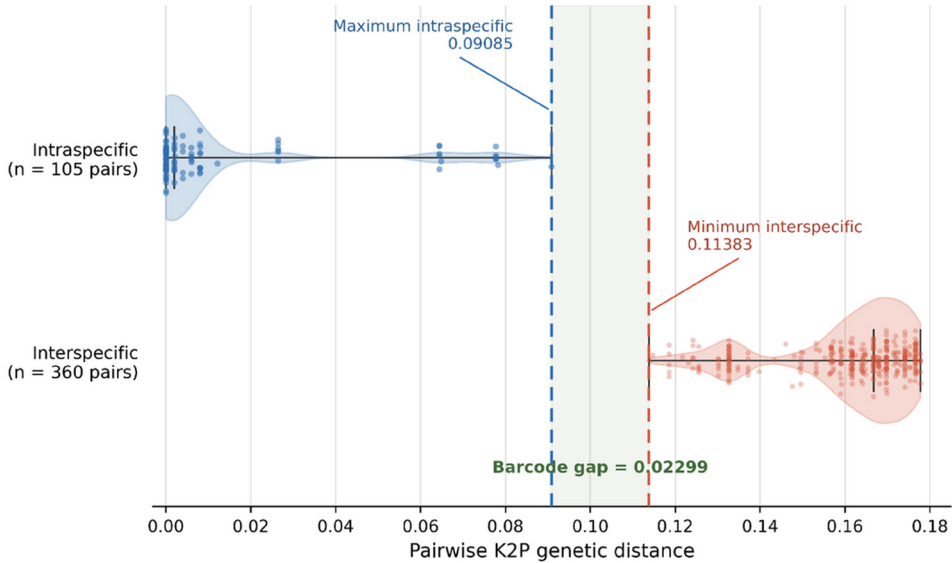


Figure 6. Distribution of pairwise intraspecific and interspecific Kimura 2-parameter (K2P) distances among 31 mitochondrial COI sequences representing four *Scymnus* taxa. Individual points represent pairwise comparisons. The colored lines indicate the maximum intraspecific distance (0.09085) and minimum interspecific distance (0.11383), and the shaded region indicates the separation between these values in the complete dataset. Because the maximum intraspecific distance was derived from unverified public records of *S. subvillosus*, this separation is treated as descriptive rather than as a validated species delimitation threshold. The outgroup was excluded from the distance analysis.

Discussion

The present study provides one of the first COI-based molecular assessments of selected *Scymnus* taxa from Uzbekistan and expands the available barcode representation of Central Asian Coccinellidae. Within the present dataset, sequences corresponding to the four initial morphology-based taxonomic assignments were recovered as distinct COI lineage clusters. The broadly similar clustering patterns obtained under maximum likelihood and Bayesian analyses indicate consistency between the two analytical approaches for focal sequences. However, given the limited taxon sampling, the use of a single mitochondrial marker, and the absence of complete voucher-based taxonomic verification, these results are interpreted as

barcode-level lineage concordance rather than as a comprehensive test of species boundaries or deeper phylogenetic relationships within *Scymnus*.

The recovered phylogenetic structure is generally consistent with previous molecular studies of Coccinellidae, where COI sequences successfully resolved closely related taxa despite relatively limited morphological differentiation (Huang et al. 2020; Iqbal et al. 2024; Pentinsaari et al. 2014). In *Scymnus*, external diagnostic characters are often subtle and partially overlapped, particularly among small-bodied species with similar pubescence patterns and coloration (Poorani 2015; Rashid et al. 2017; Poorani & Thanigairaj 2023; Poorani et al. 2024). Under such conditions, molecular markers can provide useful complementary evidence for evaluating morphology-based taxonomic assignments. In the present dataset, the recovery of distinct COI lineage clusters corresponding to the four initial morphology-based assignments demonstrates the concordance between the molecular clustering and the provisional identifications, but does not independently validate species boundaries.

The comparatively lower interspecific COI distances between *S. apetzii* and *S. frontalis* indicate a greater sequence similarity between these taxa within the analyzed data set. The mean K2P distance between these two species was 0.12839, while the minimum interspecific distance was 0.11383. However, this distance pattern alone does not establish a sister-group relationship, and the limited single-marker data set is insufficient to draw robust conclusions about deeper phylogenetic relatedness (Magro et al. 2010; Seago et al. 2011). The remaining pairs of species showed higher interspecific distances, consistent with a clear COI-based separation among the four examined taxa.

The mean intraspecific divergence was low in *S. rubromaculatus*, *S. frontalis*, and *S. apetzii*, although identical sequences resulted in comparisons within each species. *Scymnus subvillosus* showed a higher divergence in the entire data set, with a maximum K2P distance of 0.09085 between GU073957 and MK802037. However, the four newly generated Uzbekistan sequences were identical, demonstrating that the elevated divergence was driven by variation among public GenBank records. Because geographic provenance was unavailable for several of these records, the observed pattern cannot be attributed to geographic isolation or population subdivision. Possible explanations include deep mitochondrial lineages, misidentification of one or more public records, or unrecognized cryptic diversity; resolving these alternatives will require voucher-based morphological verification, locality-resolved sampling, and additional molecular markers (Hebert & Gregory 2005; Hajibabaei et al. 2007; Huang et al. 2020).

In the complete dataset, the maximum intraspecific divergence (0.09085) remained below the minimum interspecific distance (0.11383), producing an apparent separation of 0.02299 between the two values. However, the maximum intraspecific divergence was determined by public records of *S. subvillosus* whose taxonomic identities could not be independently verified through voucher examination, genital morphology, or additional molecular markers. Consequently, this apparent barcode gap is treated as a descriptive feature of the combined data set rather than as

evidence of a validated species-delimitation threshold. The identical COI sequences obtained from the four newly generated Uzbekistan specimens of *S. subvillosus* further indicate that the elevated intraspecific divergence was associated with public records rather than with newly generated material. Barcode-gap patterns are widely used as an exploratory component of DNA barcoding, but their interpretation depends strongly on taxonomic coverage, sampling density, and the reliability of specimen identification (Hebert & Gregory 2005; Pentinsaari et al. 2014). The unusually deep intraspecific divergence observed in some beetle barcode datasets further supports a cautious interpretation of distance-based separation when taxonomic verification is incomplete (Pentinsaari et al. 2014).

From a regional perspective, the present study contributes new molecular data from an area that remains poorly represented in global barcode databases. Recent work on Uzbek Coccinellidae has substantially improved faunistic knowledge of the family, including records from the Ferghana Valley, but molecular barcode information for regional taxa remains comparatively limited (Gafurova et al. 2025). Most of the COI data sets available for *Scymnus* currently originate from Europe, East Asia, or South Asia, whereas Central Asian populations remain largely unsampled. Therefore, the expansion of the molecular coverage from Uzbekistan improves the phylogeographic representation of Palearctic *Scymnus* and provides an initial reference framework for future taxonomic, ecological, and conservation-related studies in the region.

Several limitations should also be considered when interpreting the results. The analyzes were based on a single mitochondrial marker and relatively limited sample numbers for each species. Although COI performs well for species-level identification, incorporation of additional nuclear markers and broader geographic sampling would improve resolution of deeper phylogenetic relationships and population-level processes (Hajibabaei et al. 2007; Magro et al. 2010; Seago et al. 2011). Additionally, formal analyzes of demographic history, gene flow, and haplotype structure were beyond the scope of the present study. Future investigations that integrate multi-locus datasets, expanded regional sampling, and population genetic approaches would provide a more detailed understanding of evolutionary diversification within the Central Asian *Scymnus*. An additional limitation concerns the morphological documentation of the newly sequenced material. Although initial species assignments were based on external characters, published taxonomic treatments, dorsal images, and comparison with named reference material, unique voucher identifiers, specimen sex, and genital examination records could not be verified during the present revision. Because genital morphology can be essential to distinguish closely related Scymnini, the molecular clustering recovered here should not be regarded as a substitute for complete integrative taxonomic verification (Poorani 2015; Rashid et al. 2017; Poorani & Thanigairaj 2023; Poorani et al. 2024). Future studies should link every sequence to a uniquely numbered physical voucher and include diagnostic genital images. The use of a single outgroup sequence represents an additional limi-

tation, and broader outgroup sampling from related Scymnini lineages would be required to test the stability of the inferred root position and deeper relationships.

Another analytical limitation is that the COI alignment was analyzed without codon-position partitioning. Because nucleotide substitution rates and evolutionary constraints can differ among the first, second, and third codon positions, explicit comparison of partitioned and unpartitioned models could influence estimates of topology and branch support. The absence of such model-partition comparisons should therefore be considered when interpreting the recovered relationships, especially deeper nodes. Future phylogenetic analyses should evaluate codon-partitioned schemes and alternative substitution models using broader taxon sampling and additional molecular markers.

Bayesian analysis is subject to additional reproducibility limitations because the original MCMC diagnostic output files were unavailable for re-assessment during manuscript revision. Thus, numerical convergence criteria such as the average standard deviation of split frequencies, PSRF values, effective sample sizes, and likelihood traces could not be independently verified. Although two independent runs with four chains and 5,000,000 generations were used in the original analysis, posterior probabilities, particularly those associated with deeper nodes, should be interpreted cautiously in the absence of independently verifiable convergence diagnostics.

Collectively, the results show that mitochondrial COI sequences provide useful barcode-level information to distinguish lineage clusters represented in the present dataset. The newly generated sequences from Uzbekistan increase the available molecular representation of Central Asian Coccinellidae and provide an initial reference for future voucher-based, multilocus, and more densely sampled investigations of *Scymnus* diversity in the region.

An additional limitation concerns the screening of newly generated COI sequences for potential nuclear mitochondrial pseudogenes (numts). Translation-based verification of the open reading frame, including explicit screening for premature stop codons and frame-shift mutations under the invertebrate mitochondrial genetic code, could not be independently repeated during the present revision because the original nucleotide sequence files were not available for reanalysis. Consequently, the possible presence of numts cannot be excluded solely on the basis of the analyses reported here. Future work should include explicit amino acid translation and open-reading-frame verification of all newly generated COI sequences before downstream phylogenetic and DNA-barcode analyses.

Data availability statement

The newly generated COI sequences produced in this study were deposited in the NCBI GenBank database under the accession numbers listed in Table 1. Machine-readable K2P summary data is provided in Suppl. material 2: Tables S2a–S2c. The

original analysis files required for the retrospective sequence level and convergence-diagnostic reassessment were not available during the present manuscript revision.

Conclusion

This study provides new mitochondrial COI reference sequences for four *Scymnus* taxa from Uzbekistan and expands the molecular representation of Central Asian Coccinellidae. Within the present dataset, COI-based analyzes consistently recovered distinct mitochondrial lineage groups corresponding to the initial morphology-based assignments of *S. rubromaculatus*, *S. frontalis*, *S. subvillosus*, and *S. apetzi*.

Genetic distance analyzes revealed marked differences in intraspecific variation between taxa examined. The four newly generated *S. subvillosus* sequences from Uzbekistan were identical, whereas the elevated intraspecific divergence in the combined data set was associated with publicly available records whose taxonomic identities could not be independently verified. Although the maximum intraspecific distance (0.09085) remained below the minimum interspecific distance (0.11383), the resulting apparent separation of 0.02299 is therefore regarded as a descriptive feature of the present data set rather than as a validated species delimitation threshold.

Overall, the results indicate that COI provides useful barcode-level information to distinguish mitochondrial lineage clusters represented in this data set. However, the limited taxon sampling, reliance on a single mitochondrial marker and a single outgroup, and incomplete voucher-based verification preclude broader conclusions regarding species boundaries or deeper evolutionary relationships within *Scymnus*. Future studies integrating traceable voucher specimens, diagnostic morphology, broader taxon and geographic sampling, and independent nuclear markers will be necessary to test these relationships more robustly.

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References

- Ali M, Perveen R, Khan KA, Ahmed K (2015) Tribe Scymnini (Coccinellidae: Coleoptera) from Sindh Province, Pakistan. *Journal of Insect Science* 15(1): 146. <https://doi.org/10.1093/jisesa/iev125>
- Canepari C (2011) Contribution to the knowledge of Coccinellidae of Sardinia (Coleoptera). *Conservazione Habitat Invertebrati* 5: 501–516.
- Elekcioglu NZ (2020) Species composition of Coccinellidae (Coleoptera) and their preys in Adana (Turkey) with observations on potential host medicinal and aromatic plants. *Applied Ecology and Environmental Research* 18(1): 369–388. https://doi.org/10.15666/aeer/1801_369388
- Gafurova ST, Kholmatov BR, Mirzaeva GS (2025) An annotated checklist of ladybeetle species (Coleoptera, Coccinellidae) of Ferghana Valley (Uzbekistan). *Acta Biologica Sibirica* 11: 171–195. <https://doi.org/10.5281/zenodo.14915341>
- Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007) DNA barcoding: How it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics* 23(4): 167–172. <https://doi.org/10.1016/j.tig.2007.02.001>
- Hebert PDN, Gregory TR (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology* 54(5): 852–859. <https://doi.org/10.1080/10635150500354886>
- Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270(1512): 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- Huang W, Xie X, Huo L, Liang X, Wang X, Chen X (2020) An integrative DNA barcoding framework of ladybird beetles (Coleoptera: Coccinellidae). *Scientific Reports* 10: 10063. <https://doi.org/10.1038/s41598-020-66874-1>
- Ivanova NV, Dewaard JR, Hebert PDN (2006) An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular Ecology Notes* 6(4): 998–1002. <https://doi.org/10.1111/j.1471-8286.2006.01428.x>
- Iqbal Z, Azad R, Chen X-S, Lin X-L, Zhou Z, Wang X-M, Nie R-E (2024) A new species of *Scymnus* (Coleoptera, Coccinellidae) from Pakistan with mitochondrial genome and its phylogenetic implications. *Insects* 15(5): 371. <https://doi.org/10.3390/insects15050371>
- Katoh K, Misawa K, Kuma K-i, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30(14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Letunic I, Bork P (2024) Interactive Tree of Life (iTOL) v6: Recent updates to the phylogenetic tree display and annotation tool. *Nucleic Acids Research* 52(W1): W78–W82. <https://doi.org/10.1093/nar/gkae268>
- Limbu S, Cassidy K, Keena M, Tobin P, Hoover K (2016) Host range specificity of *Scymnus camptodromus* (Coleoptera: Coccinellidae), a predator of hemlock woolly adel-

- gid (Hemiptera: Adelgidae). *Environmental Entomology* 45(1): 94–100. <https://doi.org/10.1093/ee/nvv174>
- Limbu S, Keena MA, Long D, Ostiguy N, Hoover K (2015) *Scymnus camptodromus* (Coleoptera: Coccinellidae) larval development and predation of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environmental Entomology* 44(1): 81–89. <https://doi.org/10.1093/ee/nvu006>
- Magro A, Lecompte E, Magne F, Hemptinne JL, Crouau-Roy B (2010) Phylogeny of ladybirds (Coleoptera: Coccinellidae): Are the subfamilies monophyletic? *Molecular Phylogenetics and Evolution* 54(3): 833–848. <https://doi.org/10.1016/j.ympev.2009.10.022>
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution* 37(5): 1530–1534. <https://doi.org/10.1093/molbev/msaa015>
- Obrycki JJ, Harwood JD, Kring TJ, O’Neil RJ (2009) Aphidophagy by Coccinellidae: Application of biological control in agroecosystems. *Biological Control* 51(2): 244–254. <https://doi.org/10.1016/j.biocontrol.2009.05.009>
- Peng F, Poorani J, Booth RG, Wang X, Chen X, Anuradha C (2023) *Slipinskiscymnus* gen. nov., a new ladybird genus of Scymnini (Coleoptera, Coccinellidae) and notes on the taxonomic status of *Keiscymnus* Sasaji. *Zootaxa* 5325(1): 97–115. <https://doi.org/10.11646/zootaxa.5325.1.6>
- Peng F, Tang M, Wang X, Chen X (2025) *Reniscymnus* gen. nov., a new genus of Scymnini (Coleoptera, Coccinellidae) from the Oriental region. *ZooKeys* 1226: 87–100. <https://doi.org/10.3897/zookeys.1226.130352>
- Pentinsaari M, Hebert PDN, Mutanen M (2014) Barcoding beetles: A regional survey of 1,872 species reveals high identification success and unusually deep interspecific divergences. *PLoS ONE* 9(9): e108651. <https://doi.org/10.1371/journal.pone.0108651>
- Poorani J (2015) Two new species of Scymnini (Coleoptera: Coccinellidae) from Karnataka, India. *Biodiversity Data Journal* 3: e5296. <https://doi.org/10.3897/BDJ.3.e5296>
- Poorani J, Thanigairaj R (2023) A new species of *Scymnus* Kugelann (Coleoptera: Coccinellidae) predatory on amla aphid, *Schoutedenia emblica* (Patel & Kulkarni) (Hemiptera: Aphididae), from India. *Zootaxa* 5239(3): 421–430. <https://doi.org/10.11646/zootaxa.5239.3.6>
- Poorani J, Anuradha C, Thanigairaj R, Prashina Mol P (2024) Coccinellid predators of mealybugs infesting banana in South India, including a new species and a new record of *Scymnus* Kugelann (Coleoptera: Coccinellidae), with notes on other natural enemies. *Zootaxa* 5419(4): 525–544. <https://doi.org/10.11646/zootaxa.5419.4.3>
- Rashid A, Chen X, Qiu B, Wang X (2017) A new species of the subgenus *Scymnus* from Pakistan (Coleoptera, Coccinellidae). *ZooKeys* 694: 31–39. <https://doi.org/10.3897/zookeys.694.12863>
- Rosagro RM, Borges I, Vieira V, Pons Solé G, Soares AO (2020) Evaluation of *Scymnus nubilus* (Coleoptera: Coccinellidae) as a biological control agent against *Aphis spiraeicola* and *Cinara juniperi* (Hemiptera: Aphididae). *Pest Management Science* 76(2): 818–826. <https://doi.org/10.1002/ps.5585>

Seago AE, Giorgi JA, Li J, Ślipiński A (2011) Phylogeny, classification, and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. *Molecular Phylogenetics and Evolution* 60(1): 137–151. <https://doi.org/10.1016/j.ympev.2011.03.015>

Valentini A, Pompanon F, Taberlet P (2009) DNA barcoding for ecologists. *Trends in Ecology & Evolution* 24(2): 110–117. <https://doi.org/10.1016/j.tree.2008.09.011>

Supplementary material 1

Table S1. External morphological characters used for the initial identification of the four species of *Scymnus*

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Data type: table

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Supplementary material 2

Table S2a. Summary of pairwise intraspecific K2P distances and nearest interspecific neighbours among the focal *Scymnus* COI sequences

Table S2b. K2P sensitivity-analysis results for *Scymnus subvillosus*

Table S2c. Summary of interspecific K2P distances among the focal *Scymnus* taxa

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Data type: table

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