Journal of Animal & Plant Sciences, 35(2): 2025, Page: 403-414
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2025.2.0034

ASSESSMENT OF LADYBIRD BEETLE SPECIES DIVERSITY IN SWAT DISTRICT USING MORPHOMETRIC AND DNA BARCODING TECHNIQUES

H. Jamal[1], S.U. Rehman[*2] and K. Osmonaliev[2].

[1]Department of Entomology Abdul Wali Khan University Mardan Pakistan.
[2]Faculty of Medicine, Ala-Too International University Bishkek Kyrgyzstan.
[*]Corresponding Author Email: shafeeur.rehman@alatoo.edu.kg
ORIC ID: https://orcid.org/my-orcid?orcid=0000-0001-5238-5211

ABSTRACT

This study uses morphometric and molecular analyses to explore the diversity of the Coccinellidae family in Swat District, Khyber Pakhtunkhwa, Pakistan. A total of 365 ladybird beetle specimens were collected from various tehsils, leading to the identification of ten species across nine genera, including *Coccinella transversalis*, *Coccinella septempunctata*, *Menochilus sexmaculata*, *Illeis confusa*, *Oenopia mimica*, *Hippodamia variegata*, *Propylea dissecta*, *Exochomus nigripennis*, *Anegleis cardoni*, and *Epilachna convexa*. DNA barcoding of ten selected genotypes using the universal CO1 primer produced high-quality sequences for six genotypes. The CO1 sequences of *C. transversalis* showed 99% identity with its species (*C. transversalis*, accession number KX052276.1) and related genera, while *C. septempunctata* exhibited 99% similarity with its species. Similarly, *M. sexmaculata* demonstrated 99% identity with *Pristonema sp.* (accession number KR485427.1), and *H. variegata*, *P. dissecta*, and *E. nigripennis* showed 99% resemblance with *Hippodamia sp.* (MT622588.1), *Coelophora inaqdualis* (MF280617.1), and *Halyzia sp.* (MH510775.1), respectively. Ladybird beetles play a critical ecological role as natural predators of agricultural pests, making them integral to biological pest control and sustainable agriculture. This study highlights the rich diversity of Coccinellidae in the Swat District and provides valuable morphological and molecular data, contributing to Pakistan's faunal records and global genetic repositories. The findings emphasize the ecological importance of ladybird beetles and underscore the need for continued biodiversity research in this region to support conservation and pest management efforts.

Keywords: Ladybird Beetles, Coccinellidae, Morphometric Analysis, DNA Barcoding Swat District

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Published first online February 22, 2025 Published final April 28, 2025

INTRODUCTION

Ladybird beetles belonging to the family Coccinellidae and order Coleoptera are tiny insects ranging from 1 to 9 mm in size (Majerus, 2016; Ali *et al.,* 2023; Musa *et al.,* 2024). Their bodies are typically round but can also be oval, elongated, or flattened, with colors varying from reddish to black, straw, and orange (Erdoğan, 2023). These beetles inhabit diverse terrestrial ecosystems, including forests, grasslands, agro-ecosystems, and tundras, and are found from plains to mountainous regions (Sahayaraj and Hassan, 2023). The family Coccinellidae is divided into six subfamilies: *Sticholotidinae*, *Coccinellinae, Scymninae, Chilocorinae, Coccidulinae*, and *Epilachininae*. Notably, the Phytophagous (feeding on plants) and *Epilachininae* subfamilies include significant pests of crops like potatoes, brinjal, melons, tomato, and bitter gourd (Iqbal *et al.,* 2024). However, these beetles are crucial as natural predators of agricultural pests and contribute to pest regulation (Singh *et al.,* 2024).

Despite their ecological importance, ladybird beetles face threats from extensive pesticide use, which harms beneficial insects and causes environmental pollution (Khan *et al.,* 2023). With over 6,000 species across 490 genera reported globally (Ali *et al.,* 2023) and 91 species documented in Pakistan (Hanif *et al.,* 2024), exploring and conserving these beetles is crucial. DNA barcoding, introduced by Hebert *et al.* in 2003 (Chac and Thinh, 2023), is an effective tool for species identification and has been extensively applied to insects, including beetles. The cytochrome oxidase I (CO1) gene, animal barcode, is beneficial due to its low variability and effectiveness in distinguishing closely related species (Elyasigorji *et al.,* 2023).

The current study aims to sequence the CO1 gene to assess phylogenetic relationships among six ladybird beetle species from Swat District, Khyber Pakhtunkhwa, Pakistan, by employing DNA barcoding and phylogenetic analysis methods, this study uniquely combines morphometric and molecular approaches to document the diversity of Coccinellidae in Swat District, Pakistan. It provides the first DNA barcoding-based identification of ladybird beetles in the region. It significantly contributes to biodiversity records and highlights the ecological importance of these beneficial insects in sustainable agriculture.

MATERIALS AND METHODS

Study Area: Queen Elizabeth II famously described Swat as the "East Switzerland," situated between 34° 34' and 35° 55' north latitude and 72° 08' and 72° 50' east longitude. This picturesque valley spans approximately 8,150 square kilometers and has a population of 2,309,570, according to the 2017 report. Swat District is divided into seven Tehsils: Babuzai, Barikot, Matta, Khwaza Khela, Charbagh, Kabal, and Bahrain. It contains 65 Union Councils, comprising 56 rural and 9 urban areas. The valley is renowned for its diverse natural beauty, including rivers, high mountains, plains, green meadows, lakes, ponds, and waterfalls. Popular tourist spots such as Malam Jabba, Kalam, Marguzar, and Bahrain attract visitors from both Pakistan and abroad year-round (Figure 1).

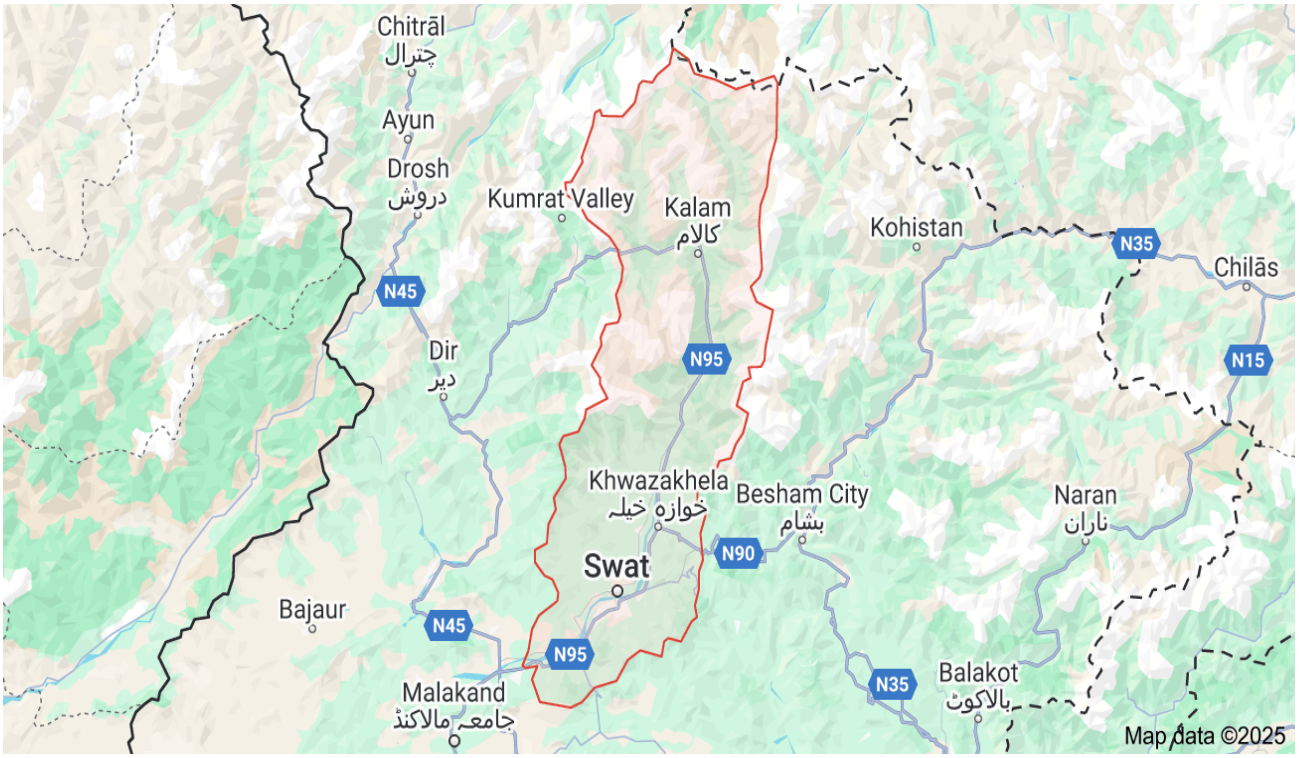

Figure 1: Map of Swat District, Khyber Pakhtunkhwa, the line shows the district's boundary.

Sample Collection and Preservation: The study was conducted across all six Tehsils of District Swat, Khyber Pakhtunkhwa, Pakistan from September 2022 to March 2023. Beetle samples were collected from various habitats, including fields, gardens, and other plant-rich environments, using spot-catching and pneumatic networks. The collected samples were carefully preserved for further analysis. Dead specimens were fixed on setup plates, labeled with detailed collection information (date, collector, region, latitude, and longitude), and stored appropriately. Larger specimens were pinned, while smaller beetles were glued to white paper. To prevent fungal contamination, all samples were stored in a deep freezer at -21°C for two days. Afterward, they were transferred to collection boxes containing naphthalene balls to deter pests.

Morphological Identification: Morphological identification was carried out at the Molecular Biology Lab, Centre for Animal Sciences and Fisheries, University of Swat. Beetles were identified using taxonomic literature and identification keys (Gimmel and Johnston, 2023). Specimens were examined under a stereo microscope (CZM6) equipped with a 10X eyepiece. Key taxonomic features were illustrated using a camera Lucida attached to a stereoscopic microscope (Olympus SZM-16). Morphometric measurements, including body length, width, head length, head width, elytra length, and elytra width, were recorded using a millimeter scale and a digital caliper (Mitutoyo Absolute

Digimatic Caliper 6”-150mm LCD, Model 500-171). Additional characteristics such as body shape, color, and markings were also documented. Morphological data were analyzed using morphometric analysis in Past Ver. 1.34 software to compare physical traits and classify species.

DNA Extraction: DNA extraction was performed at the Molecular Biology Lab, Centre for Animal Sciences and Fisheries, University of Swat. Dried ladybird beetle specimens were used for DNA extraction, following a modified protocol of the Qiagen DNA extraction kit. One leg from each sample was isolated and placed in a 1.5 mL microcentrifuge tube. The leg was cut into small pieces using dissection scissors, and 60 µL of PBS buffer, 40 µL of proteinase K, and 200 µL of ATL solution (without ethanol) were added. The mixture was vortexed thoroughly and incubated at 60°C for 4 hours to facilitate lysis. After incubation, 200 µL of buffer AL and 200 µL of 99.9% ethanol were added, bringing the total volume to 700 µL. The mixture was vortexed again, and the supernatant was transferred to a DNeasy spin column. The column was washed with 500 µL of buffer AW1 and centrifuged at ≥6000 x g (8000 rpm) for 1 minute. The column was then placed in a new 2 mL collection tube, and 500 µL of buffer AW2 was added, followed by centrifugation at 14,000 rpm for 3 minutes. The flow-through was discarded, and the spin column was transferred to a new, labeled 1.5 mL microcentrifuge tube. Finally, 100 µL of buffer AE was added to the column, which was incubated at room temperature for 10 minutes and centrifuged at ≥6000 x g (8000 rpm) for 1 minute to elute the DNA. The extracted DNA was stored at -20°C for further use.

Primer Design and PCR: Conserved COI gene sequences were downloaded from the NCBI database, and primers were designed using Primer3. PCR amplification of the COI gene was performed with a 25 µL master mix: 2.5 µL MgCl2, 2.5 µL 10x buffer, 3 µL dNTPs, 12 µL dH2O, 2 µL primers, 1 µL Taq polymerase, and 2 µL template DNA.

The CO1 gene was carried out using specific forward (GGTCAACAAATCATAAAGATATTGG) and reverse (TAAACTTCAGGGTGACCAAAAAATCA) primers under optimized reaction conditions. The process began with an initial denaturation at 94°C for 4 minutes to separate the DNA into single strands. This was followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 45°C for 45 seconds to allow the primers to bind to their complementary sequences, and extension at 72°C for 45 seconds, during which Taq polymerase synthesized new DNA strands complementary to the template. After completing the cycles, a final extension at 72°C for 10 minutes was performed to ensure the full elongation of any incomplete strands. The reaction was then held at 10°C to preserve the amplified DNA. These conditions enabled the efficient amplification of the CO1 gene for molecular identification and analysis through DNA sequencing.

Agarose Gel Electrophoresis and Sequencing: PCR products were verified using 1.5% agarose gel electrophoresis. Cleaned PCR products of the COI gene were sent to Macrogen Inc., Korea, for sequencing. Phylogenetic analysis of the successful sequences was conducted for sequencing. The successful sequences were phylogenetically analyzed by aligning them with NCBI sequences using BLAST.

RESULTS

A total of 365 Coccinellidae samples were collected from seven Tehsils of District Swat (Matta, Babuzai, Bahrain, Kabal, Charbagh, Khwaza Khela, and Barikot) between June and November 2022. The beetles were identified using taxonomic keys and were classified into 10 species across 9 genera*: Cheilomenes, Oenopia, Exochomus, Coccinella, Hippodamia, Illeis, Propylea, Anegleis*, and *Epilachna, within the sub-families Chilocorinae, Epilachininae,* and *Coccinellinae* (Table 1 and Figure 2). The morphometric analysis of collected ladybird beetle species reveals notable body size and proportions variations. *Coccinella septempunctata* is the largest species, with a body length of 6.0 mm and a width of 3.8 mm, while Oenopia mimica is the smallest, with a body length of 3.4 mm and a width of 2.0 mm. *Coccinella transversalis* has the most prominent head dimensions (3.0 mm length and width), while *Exochomus nigripennis* and *Illeis confusa* have the smallest head widths at 1.9 mm and 2.0 mm, respectively. Elytra length varies from 3.5 mm (*Illeis confusa* and *Propylea dissecta*) to 6.0 mm (*Coccinella septempunctata*), while the widest elytra were observed in Anegleis cardoni (2.9 mm). Overall, these species demonstrate significant diversity in morphometric characteristics, reflecting their adaptations to different ecological niches (Table 2).

The area-wise collection of ladybird beetles from District Swat highlights significant variation in species distribution. *Coccinella septempunctata* was the most abundant species across all regions, with the highest numbers recorded in Kabal (56) and Matta (52), indicating these areas as hotspots for this species. *Coccinella transversalis* was also relatively common, particularly in Matta (19) and Kabal (13). Other species such as *Menochilus sexmaculata* and *Hippodamia variegata* showed moderate representation, while species like *Illeis confusa*, *Oenopia mimica*, and *Propylea dissecta* were sparsely distributed across all areas. Rare species such as *Exochomus nigripennis*, *Anegleis cardoni*, and *Epilachna convexa* were only occasionally observed, with very low numbers, particularly in Barikot, Babuzai, and Matta. This distribution reflects a diverse but unevenly distributed ladybird beetle population, with certain regions like Matta and Kabal supporting more extraordinary species richness and abundance (Table 3). The statistical analysis of species distribution across six categories in the study area reveals distinct patterns of dominance and diversity. The highest species abundance was recorded in Matta (25.2%), followed by Kabal (22.7%), indicating these areas as biodiversity hotspots. Conversely, Charbagh had the lowest abundance (8.5%), suggesting a less diverse ecological composition. The Simpson Dominance Index (R1 = 0.1883) indicates low dominance, implying that no single category overwhelmingly dominates the community. The Shannon Entropy value (R2 = 1.7254) suggests moderate diversity, reflecting a relatively even species distribution among the categories. These metrics highlight the ecological variability within the study area, with certain regions supporting richer and more balanced species assemblages (Table 4).

Table 1: The body coloration of a ladybird beetle consists of a brightly colored dorsal surface, typically red or orange with black spots, a pale ventral surface, a dark pronotum, a black head often with white or yellow markings, black or dark brown legs and antennae, and colorful elytra that vary in color and pattern.

Name of species	Colour of head	Pronotum	Scutellum	Elytra	Body ventral side
Coccinella transversalis	Dark	Black, anterior horizontally dim yellow	Dark	Dim creamy to dark reddish	Brownish dark
Coccinella septempunctata	Dark	Anterolaterally Dark orange-yellow	Dark	Dull reddish to yellow brown	Black
Menochilus sexmaculata	Yellowish brown	Reddish to orang yellow, taking v-shaped black grades in the middle near the posterior border	Dark	Reddish yellow to normally orange	Dark brown
Illeis confuse	Creamy	Creamy white with two black spots on the center	White creamy	Creamy without spots	Creamy brownish
Oenopia mimica	Black	Black, anterior laterally orange	Dark	bright yellow	Brown black
Hippodamia variegate	Brown	Yellow white	Brownish black	Orange-yellow with thirteen dark spots	Dark
Propylea dissecta	Yellow bright near orange	Yellow dark	Dark	Yellow bright to red	Brown dark
Exochomus nigripennis	Black	Pronotum yellow	Black	Black without spots	Dark brown
Anegleis cardoni	Light creamy	Yellow black	Dark	Yellow pale through dark marks	Light yellow
Epilachna convexa	Red-brown	Red-brown with three black spots	Black	Red-brown with ten black spots	Dark red brown

Table 2: Morphometric characters, of the collected lady bird beetle species.

S. N	Names of Species	Length of body (mm)	Width of body (mm)	Length of head (mm)	Width of head (mm)	Length of elytra (mm)	Width of elytra (mm)
1	*C. transversalis*	3.6	3.6	3.0	3.0	5.6	3.3
2	*C. septempunctata*	6.0	3.8	1.9	2.9	6.0	3.2
3	*M. sexmaculata*	3.6	2.9	2.0	2.6	4.0	2.6
4	*I. confuse*	4.3	2.6	1.8	2.0	3.5	2.5
5	*O. mimica*	3.4	2.0	2.2	2.2	3.8	2.5
6	*E. nigripennis*	3.5	2.6	1.8	1.9	4.6	2.7
7	*H. variegate*	4.5	2.8	2.4	2.2	4.6	2.5
8	*P. dissecta*	4.5	3.4	2.2	2.3	3.5	2.5
9	*A. cardoni*	5.1	4.1	2.1	3.1	5.1	2.9
10	*E. convexa*	5.3	3.2	2.0	2.6	4.0	2.6

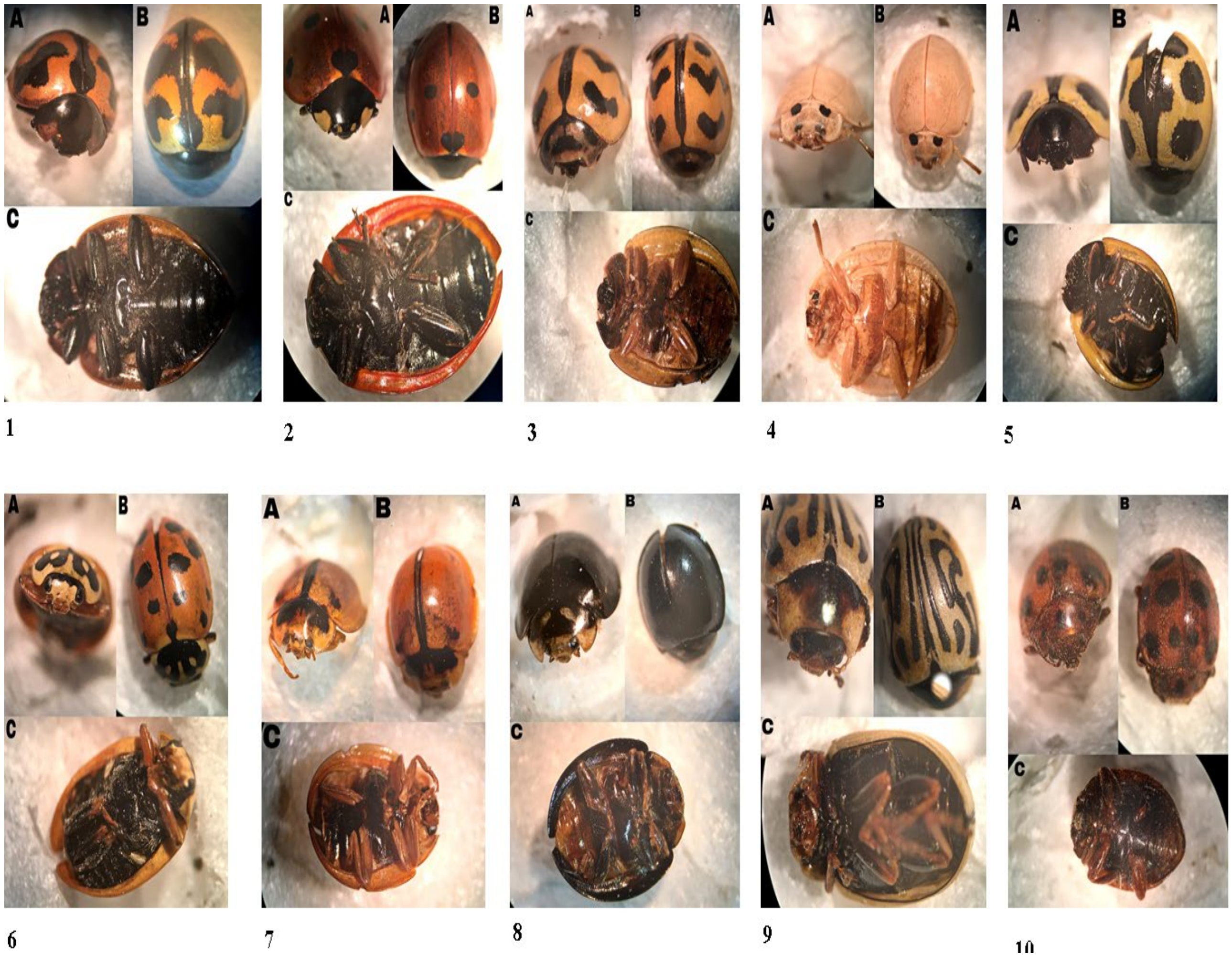

Figure 2: (1). *Coccinella transversalis* (a) (Head) (b) dorsal view(c) ventral view. (2). *Coccinella septempunctata* (a) Head (b) dorsal view(c) ventral view. (3). *Menochilus sexmaculata* (a) Head (b) dorsal view(c) ventral view. (4). *Illeis confusa* (a) Head (b) dorsal view(c) ventral view. (5). *Oenopia mimica* (a) Head (b) dorsal view(c) ventral view. (6). *Hippodamia variegata* (a) Head (b) dorsal view(c) ventral view. (7). *Propylea dissecta* (a) Head (b) dorsal view(c) ventral view. (8). *Exochomus nigripennis* (a) Head (b) dorsal view(c) ventral view. (9). *Anegleis cardoni* (a) Head (b) dorsal view(c) ventral view. (10). *Epilachna convexa* (a) Head (b) dorsal view(c) ventral view.

Table 3: Area-wise collection of ladybird beetle from district swat

S. No	Species	Barikot	Babozai	Charbagh	Khwaza Khela	Matta	Kabal
1	***Coccinella transversalis***	6	3	2	9	19	13
2	***Coccinella septempunctata***	49	30	21	28	52	56
3	***Menochilus sexmaculata***	2	1	1	2	5	2
4	***Illeis confuse***	1	1	0	1	2	1
5	***Oenopia mimica***	1	2	1	1	3	1
6	***Hippodamia variegate***	4	2	1	3	5	3
7	***Propylea dissecta***	1	2	1	1	1	1
8	***Exochomus nigripennis***	0	0	0	1	1	0
9	***Anegleis Cardoni***	1	1	1	0	1	1
10	***Epilachna convexa***	0	0	0	0	1	0

Table 4: Statistical analysis of the collected species from study area

6 Categories/Classes					
No.	**Category**	**Value**	**X**	**x^2**	**-x ln(x)**
1	Barikot	68	18.6%	0.035	0.313
2	Babuzai	43	11.8%	0.014	0.252
3	Charbagh	31	8.5%	0.007	0.209
4	Khwazakhila	48	13.2%	0.017	0.267
5	Matta	92	25.2%	0.064	0.347
6	Kabal	83	22.7%	0.052	0.337
R1	Simpson Dominance			0.1883	
R2	Shannon Entropy				1.7254

MOLECULAR ANALYSIS

DNA Extraction: Good quality DNA was extracted from the collected samples of ladybird beetle by using a Qiagen kit, as shown in figure 3.

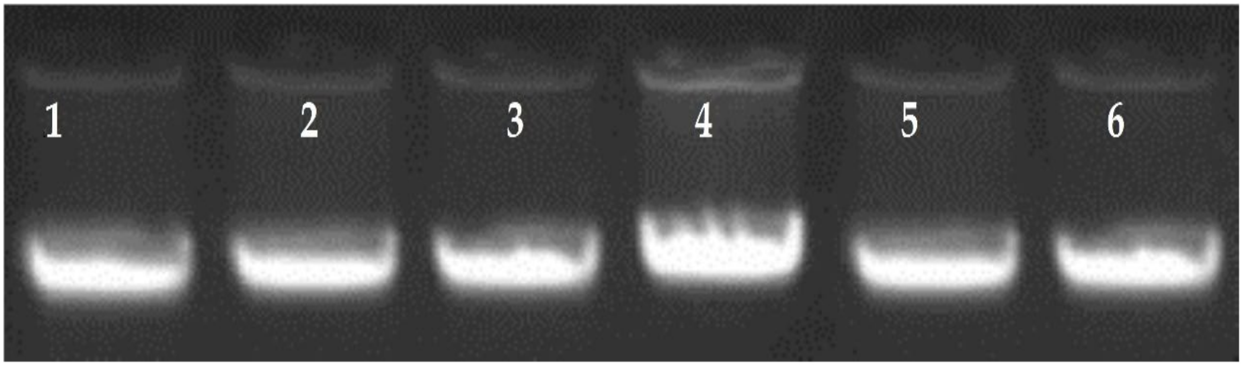

Figure 3. Extracted DNA from the selected samples of ladybird beetle

Polymerase chain reaction: The CO1 gene was further amplified in the genome of collected ladybird beetle samples. After PCR amplification, the selected fragments were confirmed by Agarose gel electrophoresis, as shown in Figure 4.

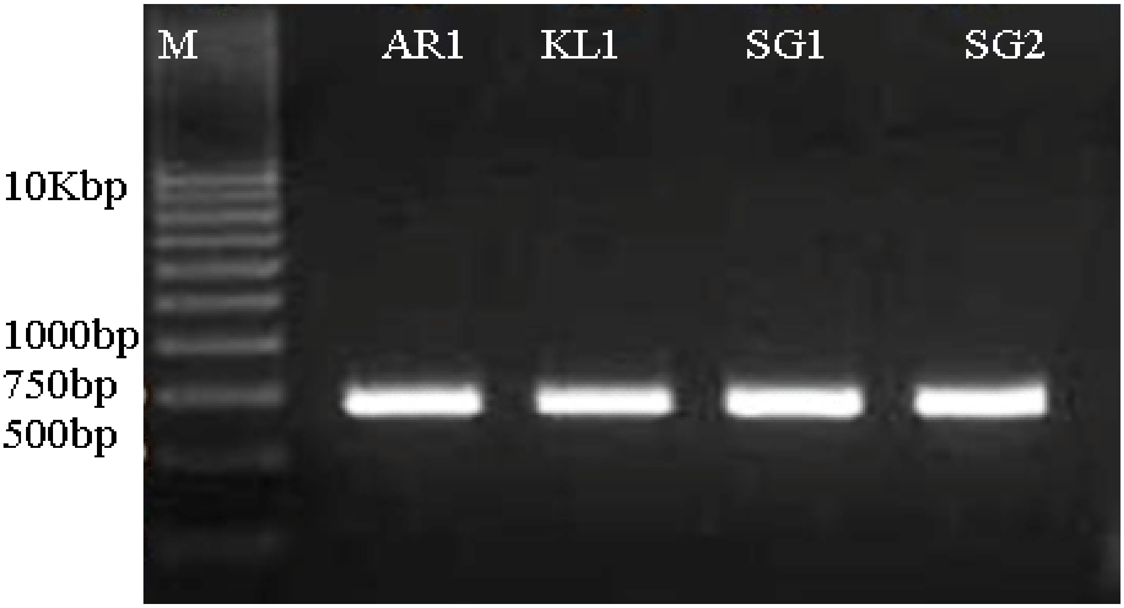

Figure 4. Polymerase chain reaction result

Nucleotide Sequence Analysis of Various Coccinellidae Genotypes: The nucleotide sequence analysis of the CO1 gene from various Coccinellidae genotypes revealed significant genetic diversity and phylogenetic relationships within the family**.**

Genotype Analysis: *Coccinella transversalis* (Ct), CO1 gene sequencing showed 99% similarity and 87% query coverage with accession KF54347.1 and 99% identity with 95% query coverage for accession KX052276.1. Phylogenetic analysis using Maximum Neighbor Joining (NJ) revealed two main clades. Genotype Ct clustered in Clade I with 99% bootstrap support. The second clade included outliers like Micraspis discolor (Figure 5).

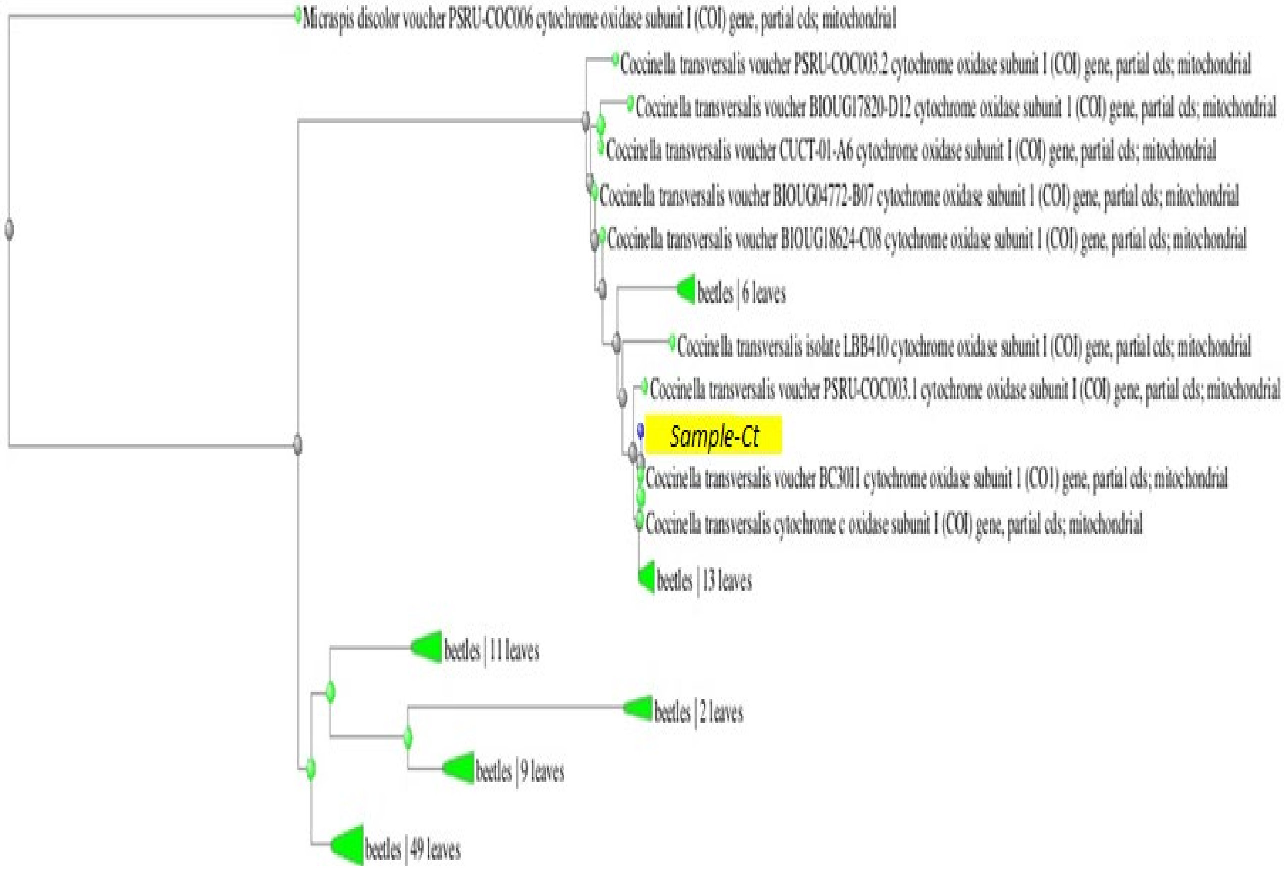

Figure 5. Phylogenetic tree based on the homology of *Coccinella transversalis* with available sequences in NCBI using the CO1 gene with maximum neighbor joining method.

Coccinella septempunctata (Cs), The CO1 gene sequence had 99% resemblance and 98% query coverage with accession MH020505.1 and 99% resemblance with 94% query coverage for accession KR485427.1. Phylogenetic analysis placed genotype Cs in Clade I with 99% bootstrap support, with the second clade also consisting of similar genera and species (Figure 6).

Menochilus sexmaculata (Ms), CO1 gene sequencing showed 99% similarity and 98% query coverage with *Pristonema* sp (accession KR485427.1) and 84% identity with 85% query coverage for *Aphidecta obliterate* (accession KJ967193.1). The phylogenetic tree showed three clades: genotype Ms clustered in Clade I with 99% bootstrap support, while Clades II and III included other related species (Figure 7).

Hippodamia variegata (Hv), CO1 gene analysis revealed 99% similarity and 98% query coverage with Hippodamia sp (accession MT622588.1) and 84% identity with 86% query coverage for Ceratomgilla ulkei (accession HM432924.1). The phylogenetic tree was divided into two major clades, with genotype Hv clustered in Clade I with 99% bootstrap support (Figure 8).

Propylea dissecta (Pd), The CO1 gene sequence showed 99% identity and 95% query coverage with *Coelophora inaqdualis* (accession MF280617.1) and 85% identity with 86% query coverage for *Australoneda bourgeois* (accession MF280606.1). Phylogenetic analysis revealed three clades, with genotype Pd clustering in Clade I with 99% bootstrap support (Figure 9).

Figure 6. Phylogenetic tree based on the homology of *Coccinella septempunctata* with available sequences in NCBI using the CO1 gene with maximum neighbor joining method.

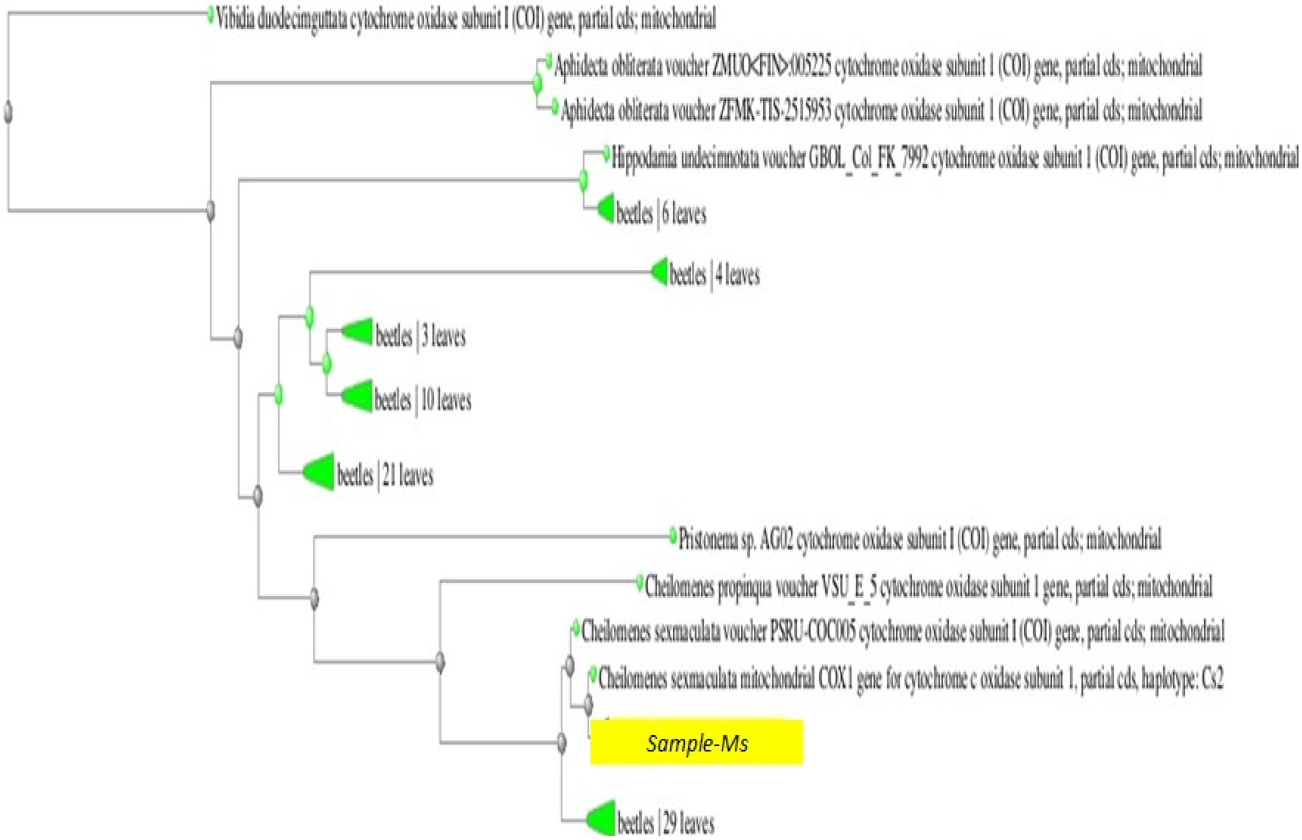

Figure 7. Phylogenetic tree based on the homology of *Menochilus sexmaculata* with available sequences in NCBI using the CO1 gene with maximum neighbor-joining method.

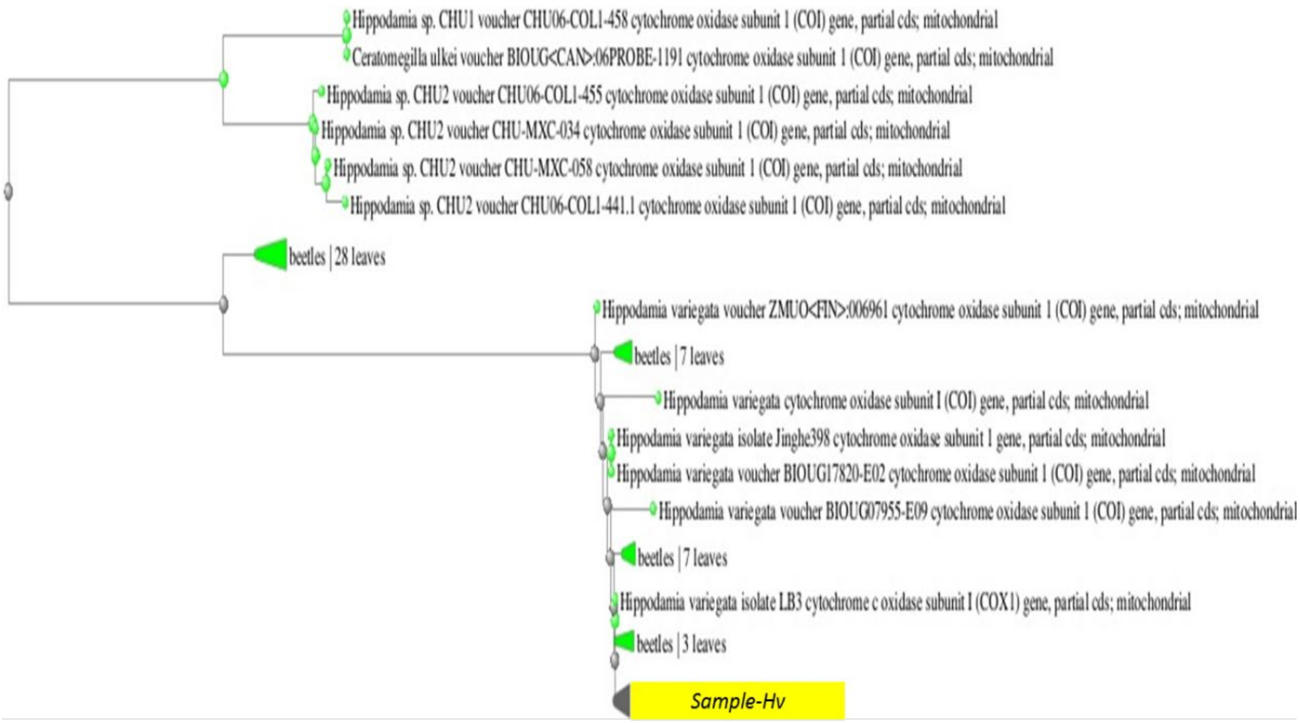

Figure 8. Phylogenetic tree based on the homology of *Hippodamia variegata* with available sequences in NCBI using the CO1 gene with maximum neighbor joining method.

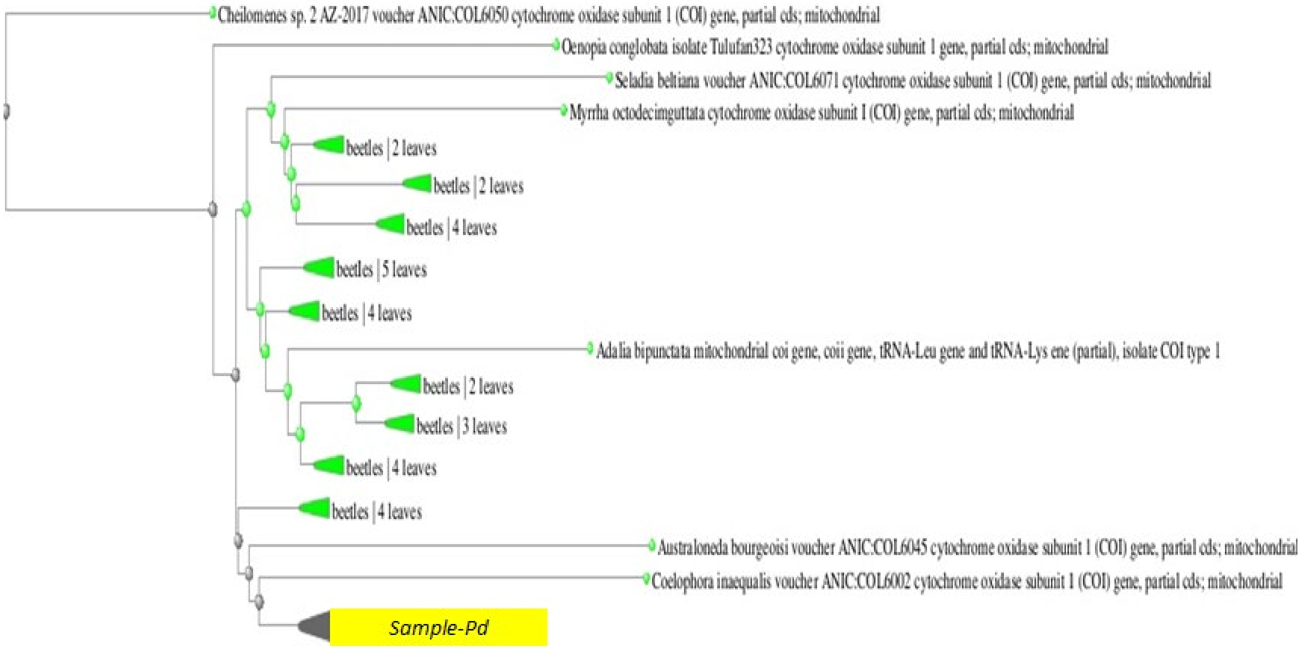

Figure 9. Phylogenetic tree based on the homology of *Propylea dissecta* with available sequences in NCBI using the CO1 gene with maximum neighbor joining method.

Exochomus nigripennis (En), CO1 gene sequencing showed 99% identity and 94% query coverage with *Halyzia* sp (accession MH5107751) and 84% identity with 86% query coverage for *Halyzia sedecimguttata* (accession KU911356.1). The phylogenetic tree was divided into two major clades, with genotype En clustering in Clade I with 99% bootstrap support (Figure 10).

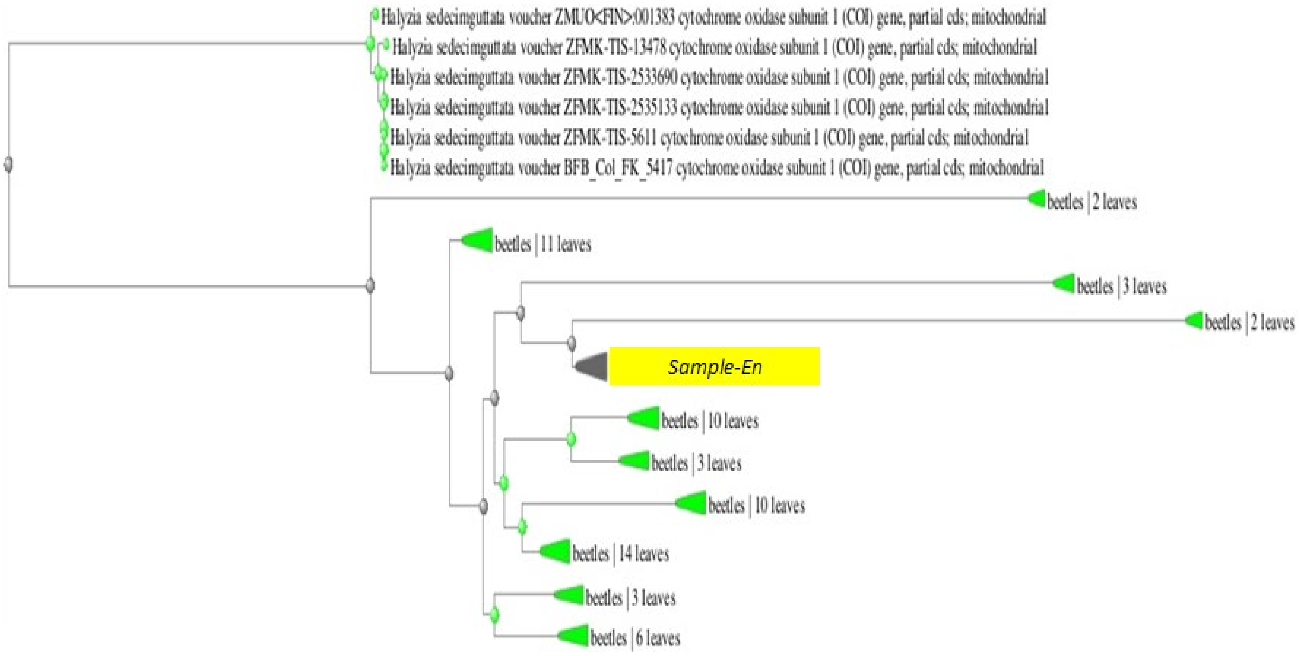

Figure 10. Phylogenetic tree based on the homology of *Exochomus nigripennis* with available sequences in NCBI using the CO1 gene with maximum neighbor joining method.

DISCUSSION

This study integrates morphological and molecular analyses to document and clarify the taxonomic relationships of Coccinellidae species in the Swat District, with a specific focus on CO1 gene-based DNA barcoding. Previous research on ladybird beetles has heavily relied on morphology, often limited by subjectivity and the availability of experts. Recent advancements in molecular techniques, particularly DNA barcoding, have proven effective in resolving species identification challenges, particularly in diverse and underexplored regions like Swat (Kim *et al.,* 2012; Wang *et al.,* 2018; Mukherjee *et al.,* 2024). The molecular analysis in this study revealed high-resolution phylogenetic clustering that validated morphological identifications. Species such as *Coccinella septempunctata* and *Coccinella transversalis* exhibited strong bootstrap support and close clustering with reference sequences, confirming their taxonomic identities. Similarly, other species, including *Hippodamia variegata* and *Cheilomenes sexmaculata*, demonstrated robust alignment with previously reported CO1 sequences, consistent with the findings of Wang *et al.* (2018) and Mukherjee *et al*. (2024).

The identification of species with high confidence underscores the utility of the CO1 gene for resolving taxonomic ambiguities in regions where morphological data alone may not suffice (Shum *et al.,* 2024). The current findings build upon earlier work by incorporating molecular data to provide a more comprehensive understanding of species diversity and distribution. Unlike earlier studies that primarily focused on morphology (Ullah *et al.,* 2012; Saeed *et al.,* 2016), the molecular approach employed here offers precise insights into evolutionary relationships and cryptic diversity. The integration of CO1 barcoding with traditional taxonomy has highlighted the genetic distinctiveness of species within the Coccinellidae family, enabling more accurate documentation. Moreover, the identification of unique haplotypes and rare species within the study area reflects the ecological importance and potential genetic uniqueness of Coccinellidae populations in Swat. These results align with global studies that emphasize the role of DNA barcoding in uncovering hidden biodiversity and contributing to conservation efforts (Kim *et al.,* 2012; Wang *et al.,* 2018; Mukherjee *et al.,* 2022). The study demonstrated high accuracy (approximately 90%) in species identification using K2P distance calculations and NJ tree construction, emphasizing the robustness of CO1 gene-based molecular taxonomy.

Conclusions: This study confirms significant interspecific variations among Coccinellidae species in Swat, with CO1 gene analysis providing precise taxonomic identification and reliable evolutionary relationships. The clustering of species like *Coccinella septempunctata* and *Coccinella transversalis* with high bootstrap support emphasizes the efficacy of DNA barcoding in resolving taxonomic ambiguities. These findings provide a valuable molecular and morphological baseline for future biodiversity and conservation studies. The results underscore the urgent need to study the ecological roles of these beneficial insects and to prioritize their conservation amidst ongoing environmental changes and habitat loss.

Authors' Contributions: Hazrat Jamal designed the study and conducted the experiment, and Shafee Ur Rehman wrote the manuscript. Kudaibergen Osmonaliev reviewed and edited the manuscript.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgment: The Authors are very grateful to the Dean Faculty of Medicine at Ala-Too International University for financial support.

REFERENCES

Ali, A., M. Yousuf and A.A. Khan (2023). First report of Coccinellid beetles (Coleoptera: Coccinellidae) from Gharana Wetland Conservation Reserve and connected agricultural fields in Jammu. J. Entomol. Acarol. Res. 55(1): 12255. https://doi.org/10.4081/jear.2023.12255

Ali, M.Y., T. Naseem, J.K. Holopainen, T. Liu, J. Zhang and F. Zhang (2023). Tritrophic interactions among arthropod natural enemies, herbivores and plants considering volatile blends at different scale levels. Cells. 12(2): 251. https://doi.org/10.3390/cells12020251

Chac, L.D and B.B. Thinh (2023). Species identification through DNA barcoding and its applications: a review. Biol. Bull. 50(6): 1143-56. https://doi.org/10.1134/S106235902360229X

Elyasigorji, Z., M. Izadpanah, F. Hadi and M. Zare (2023). Mitochondrial genes as strong molecular markers for species identification. The Nucleus. 66(1): 81-93. https://doi.org/10.1007/s13237-022-00393-4

Erdoğan, P. (2023). Harmful insects in some biofuel plants and their biology. In Biotechnology and Omics Approaches for Bioenergy Crops. Singapore: Springer Nature Singapore. 235-255 p. https://doi.org/10.1007/978-981-99-4954-0_11

Gimmel, M.L., M.A. Johnston and M.S. Caterino (2023). Annotated checklist of the beetles (Coleoptera) of the California Channel Islands. PeerJ. 11: e14793. https://doi.org/10.7717/peerj.14793

Hebert, P.D., A. Cywinska, S.L Ball and J.R. DeWaard (2003). Biological identifications through DNA barcodes. Proc. R. Soc. B. 270(1512): 313-321. https://doi.org/10.1098/rspb.2002.2218

Hanif, A., A. Manan, F. Rehman and I. Ali (2023). DNA Barcoding as A Tool for Taxonomic Identification of Ladybird Beetles (Coleoptera: Coccinellidae) from Pakistan: a review. PJMLS. 6(1): 39-46. https://doi.org/10.31580/pjmls.v6i1.1970

Iqbal, Z., R. Azad, X.S. Chen, X.L. Lin, Z. Zhou, X.M. Wang and R.E. Nie (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

Khan, B.A., M.A. Nadeem, H. Nawaz, M.M. Amin, G.H. Abbasi, M. Nadeem, M. Ali, M. Ameen, M.M. Javaid, R. Maqbool and M. Ikram (2023). Pesticides: impacts on agriculture productivity, environment, and management strategies. In Emerging contaminants and plants: Interactions, adaptations and remediation technologies. Cham: Springer International Publishing. 109-134 p. https://doi.org/10.1007/978-3-031-22269-6_5

Kim, M.J., X. Wan and I. Kim (2012). Complete mitochondrial genome of the seven-spotted lady beetle, Coccinella septempunctata (Coleoptera: Coccinellidae). Mitochondrial DNA. 23(3): 179-181. https://doi.org/10.3109/19401736.2012.668901

Musa, N.N., S.K.M. Hatta and S. Yaakop (2024). Diversity and assemblage patterns of ladybirds (Coccinellidae) in different crop management practices in Peninsular Malaysia. Arthropod Plant Interact. 1-18. https://doi.org/10.1007/s11829-024-10070-9

Majerus, M.E (2016). A natural history of ladybird beetles. Cambridge University Press. https://doi.org/10.1017/CBO9781316336960

Mukherjee, A., O. Kar, K. Mukherjee, D. Ghosh, D. Pramanik, A. Naskar and D. Banerjee (2024). DNA barcoding of ecologically important snail-killing flies (Diptera: Sciomyzidae: Sepedon) from India. Rec. zool. Surv. India. 231-240. https://doi.org/10.26515/rzsi/v124/i1S/2024/172735

Saeed, K.M., N.K. Khattak, M.F. Khan, F. Naz, A. Khan and N. Akhtar (2016). Ladybird Beetle (Coccinellidae; Coleoptera) of District Buner, Khyber Pakhtunkhwa-Pakistan. J. Appl. Environ. Biol. Sci. 6(2S): 58-78.

Sahayaraj, K and E. Hassan (2023). Predatory Insects Collection Methods. In Worldwide Predatory Insects in Agroecosystems. Singapore: Springer Nature Singapore.1-23 p. https://doi.org/10.1007/978-981-99-1000-7_2

Singh, V., S. Grewal, V. Goyal and V. Malik (2024). Taxonomic redescription of subfamily

Scymninae (Coleoptera: Coccinellidae) from Haryana, India. J. Appl. Nat. Sci. 16(2): 842-53. https://doi.org/10.31018/jans.v16i2.5461

Shum, P., M. Cusa, A. Prasetyo and S. Mariani (2024). Nanopore sequencing facilitates screening of diversity and provenance of seafood and marine wildlife. Food Cont. 161: 110382. https://doi.org/10.1016/j.foodcont.2024.110382

Ullah, R.F., H. Haq. M. Ahmad, K. Inayatullah, K. Saeed and S. Khan (2012). Morphological characteristics of ladybird beetles collected from District Dir Lower, Pakistan. Afr. J. Biotechnol. 11(37): 9149-9155.

Wang, Z.L., T.Z. Wang, H.F. Zhu, Z.Y. Wang and X.P. Yu (2018). DNA barcoding evaluation and implications for phylogenetic relationships in ladybird beetles (Coleoptera: Coccinellidae). Mitochondrial DNA Part A. 1-8. https://doi.org/10.1080/24701394.2018.1446950