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Research Article

Molecular Characterization of Scarab Beetles (Coleoptera: Scarabaeidae) from the Malakand Division, Pakistan

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ABSTRACT

Dung beetles (Coleoptera: Scarabaeidae) play a pivotal role in agroecosystem functioning by promoting nutrient recycling, enhancing soil aeration, and suppressing dung-breeding pests. Despite their ecological significance, molecular data on regional scarab beetle fauna in Pakistan remain limited. This study provides a comprehensive molecular characterization of scarab beetles from Malakand division, Khyber Pakhtunkhwa, Pakistan, integrating traditional morphological identification with mitochondrial Cytochrome c Oxidase subunit I (COI) DNA barcoding. Specimens representing eight species, *Onitis falcatus*, *O. philemon*, *Digitonthophagus gazella*, *Onthophagus nuchicornis*, *Copris lunaris*, *Catharsius molossus*, and *Oniticellus cinctus*, were collected using standardized dung-baited pitfall traps and manual sampling. COI sequences were generated, submitted to GenBank, and analyzed using the Maximum Likelihood method under the Kimura 2-Parameter model with 1,000 bootstrap replicates. Phylogenetic analyses showed strong concordance between morphological and molecular identifications, with all species resolved as well-supported monophyletic clades. Intraspecific genetic divergence was generally low, whereas interspecific divergence was substantial, confirming clear taxonomic boundaries. Some taxa exhibited evidence of geographic structuring, suggesting the influence of regional isolation on genetic differentiation. This study establishes a reliable COI barcode reference library for scarab beetles of Malakand division and highlights the importance of molecular tools in biodiversity assessment, conservation planning, and sustainable agroecosystem management.

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Introduction

Dung beetles (Coleoptera: Scarabaeidae) are among the most ecologically important insects in terrestrial ecosystems, particularly in agricultural and pastoral landscapes (Kumar et al., 2018). Their ecological importance primarily stems from their feeding and nesting behavior, which involves the removal, consumption, and burial of animal dung (Chandra and

Avedisian, 1991). Through these activities, dung beetles play a vital role in nutrient cycling by returning organic matter and essential nutrients, such as nitrogen and phosphorus, to the soil. This process enhances soil fertility, improves soil structure and aeration, and increases water infiltration, thereby contributing to healthier and more productive agroecosystems. Moreover, dung burial reduces surface dung accumulation, limiting breeding sites for dung-associated pests such as flies and parasitic nematodes. Consequently, dung beetles indirectly suppress pest populations and reduce parasite transmission in livestock systems, leading to improved animal health and a reduced dependence on chemical pest control measures (Folmer et al., 1994). These ecosystem services closely align with the core objectives of plant protection, sustainable agriculture, and environmentally friendly farming practices (Halffter and Matthews, 1966).

Globally, Scarabaeidae is one of the largest and most diverse beetle families, comprising approximately 27,800 described species distributed across a wide range of habitats. Scarab beetles exhibit remarkable diversity in body size, coloration, morphology, behavior, and ecological function (Herbert et al., 2003a, b). Within this family, dung beetles constitute a functionally significant group that has been extensively studied worldwide for their ecological roles, bioindicator potential, and evolutionary relationships (Kimura, 1980). Despite their importance, scarab beetle diversity remains poorly documented in many developing countries, including Pakistan (Kumar et al., 2018). Pakistan harbors a rich and diverse scarab beetle fauna due to its varied climate, topography, and agro-ecological zones (Losey and Vaughan, 2006). Existing studies in the country have largely relied on traditional morphological identification based on external characters such as body shape, coloration, genitalia, and setal patterns (Nichols et al., 2008). Although morphological taxonomy has long served as the foundation of insect systematics, it has notable limitations when applied to scarab beetles. Many species exhibit pronounced phenotypic plasticity influenced by environmental conditions, as well as sexual dimorphism, which can complicate accurate identification (Shah et al., 2021). Moreover, the presence of cryptic species complexes, morphologically similar but genetically distinct taxa, poses a major challenge to reliable species delimitation using morphology alone (Ratnasingam and Hebert, 2007). These constraints underscore the need for integrative taxonomic approaches that combine morphological and molecular data (Shah et al., 2021).

DNA barcoding has emerged as a powerful and widely accepted tool for species identification and taxonomic validation in insects (Slade et al., 2011). This approach typically utilizes a standardized fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, which has proven effective in distinguishing closely related species due to its high interspecific variability and relatively low intraspecific divergence (Svensson and Larson, 2008). Beyond species identification, DNA barcoding supports phylogenetic inference, detection of cryptic diversity, and assessment of genetic divergence among populations (Tarasov et al., 2016). Globally, COI-based barcoding has been successfully applied to scarab beetles, substantially improving taxonomic resolution and contributing to large-scale biodiversity databases such as the Barcode of Life Data System (BOLD). However, molecular data on Pakistani scarab beetles remain extremely limited (Zhang, 2018). Only a small number of COI sequences from Pakistan are currently available in international databases, creating a significant knowledge gap regarding the genetic diversity and phylogenetic relationships of the regional scarab fauna (Tarasov et al., 2016). This lack of molecular reference data hampers accurate species identification, restricts comparative studies, and limits the effective application of scarab beetles in biodiversity monitoring and agroecosystem management (Slade et al., 2011).

Malakand division, located in northwestern Pakistan, is a biologically diverse region characterized by a wide range of altitudes, climatic conditions, and agricultural practices. The area encompasses valleys, foothills, and mountainous landscapes that support diverse livestock systems and crop production (Uddin et al., 2022). These heterogeneous agro-ecological zones provide suitable habitats for a broad range of dung beetle species. Nevertheless, Malakand division has not yet been subjected to comprehensive molecular studies of scarab beetles, leaving its dung beetle diversity largely unexplored at the genetic level (Shah and Shah, 2022). The present study aims to address these gaps by conducting a molecular investigation of common scarab beetle species from Malakand division using COI-based DNA barcoding. Specifically, the objectives are to: (1) molecularly characterize representative scarab beetle species collected from different agro-ecological zones of

the region; (2) assess genetic divergence and phylogenetic relationships among the recorded taxa; and (3) establish a regional DNA barcode reference library. This barcode library will provide a valuable resource for future taxonomic research, biodiversity conservation initiatives, and sustainable agroecosystem management in Pakistan. By integrating molecular tools with classical taxonomy, the study will contribute to a more accurate understanding of scarab beetle diversity and support evidence-based conservation and agricultural strategies (Zhang, 2018).

Materials and Methods

Study area and sampling design

The present study was conducted in the Malakand division, located in the northwestern region of Khyber Pakhtunkhwa province, Pakistan. The area is characterized by diverse topography, ranging from lowland valleys to hilly and mountainous terrains, with elevations spanning approximately 700 to over 2,500 m above sea level. The region experiences a subtropical to temperate climate, with warm summers, mild to cold winters, and moderate to high seasonal rainfall. These environmental conditions, together with extensive agricultural activities and livestock rearing, provide suitable habitats for a diverse assemblage of dung beetles.

Specimen sampling was carried out across multiple localities within the division, including agricultural fields, grazing lands, pasture areas, and semi-natural habitats adjacent to farmlands. Sampling was conducted over a seven-month period (March-September) during 2019-2021, corresponding to the peak seasonal activity of dung beetles in the region. To ensure representative coverage, multiple sampling sites were selected within each locality, and collections were performed at regular intervals throughout the study period.

Collection methods

Dung beetles were collected using a combination of dung-baited pitfall traps and manual sampling. Pitfall traps were constructed from plastic containers (approximately 10-12 cm in diameter and 12-15 cm in depth) and buried with their rims flush with the soil surface. Fresh cattle dung was used as bait and was either placed directly inside the trap or suspended above it using fine mesh to attract beetles while minimizing dilution by rainwater. At each sampling site, traps were deployed for 24-48 h, depending on weather conditions and site accessibility, and were inspected regularly to minimize specimen damage and predation.

In addition to pitfall trapping, manual collection was performed by hand-picking beetles from freshly deposited cattle dung pats during nighttime, when beetle activity was highest. Collected specimens were preserved in labeled vials containing 95% ethanol for molecular analyses, while selected specimens were preserved dry for detailed morphological examination. A total of 48 specimens were collected, representing approximately 6-8 individuals per species to capture intraspecific variation.

Morphological identification and voucher deposition

Preliminary species identification was conducted based on external morphological characters using standard taxonomic keys and relevant literature for the family Scarabaeidae. Diagnostic characters examined included body size and shape, coloration, punctation patterns, elytral striation, pronotal structure, leg morphology, and male genitalia where necessary. Identification referenced classical and contemporary taxonomic treatments and keys such as The Fauna of British India (Arrow, 1931) for Scarabaeoidea morphology and species delineation, the multilingual key to Scarabaeinae genera and subgenera (Vaz-de-Mello et al., 2011), and regional species lists and identification accounts of dung beetles in Pakistan and adjacent regions (Abbas et al., 2024). Male genitalia terminology and comparative morphology were interpreted following established morphological standards in Scarabaeinae systematics (Medina et al., 2013). Specimens were examined under a stereomicroscope to ensure precise observation of key diagnostic features.

All identified specimens were assigned unique voucher numbers and deposited in the Department of Zoology Insect Collection, University of Swabi, Pakistan, to ensure long-term preservation and accessibility for future reference. Voucher accession numbers ranged from UOS-SCB-001 to UOS-SCB-048. Each specimen was labeled with collection locality, date, habitat type, and collector information.

DNA extraction

For molecular analyses, genomic DNA was extracted from one or two legs of each specimen to preserve diagnostic morphological features. DNA extraction was performed using the Qiagen DNeasy Blood & Tissue Kit following the manufacturer's protocol. Tissue samples were incubated with Proteinase K in lysis buffer at 56°C until complete digestion, followed by purification using

spin-column technology. DNA was eluted in 50-100 µl of elution buffer and stored at -20°C until further analysis.

PCR amplification and sequencing

A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). Polymerase chain reaction (PCR) was performed in a total volume of 25 µl containing template DNA, PCR buffer, $MgCl_2$, dNTPs, primers, and Taq DNA polymerase.

Thermal cycling conditions consisted of an initial denaturation at 94°C for 3 min; followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 45 sec, and extension at 72°C for 1 min; with a final extension at 72°C for 10 min. PCR products were visualized on 1.5% agarose gels stained with ethidium bromide to confirm successful amplification. Successfully amplified products were purified and subjected to bidirectional Sanger sequencing at a commercial sequencing facility.

Sequence editing and phylogenetic analysis

Raw chromatograms were examined, edited, and assembled using Geneious version 6.1.8. Low-quality regions and ambiguous bases were trimmed, and high-quality consensus sequences were generated. Sequence alignment was performed using the integrated alignment tools in Geneious. Species identity was verified by comparison with reference sequences available in GenBank and the Barcode of Life Data System (BOLD) using BLAST searches.

Phylogenetic analyses were conducted in MEGA version 12 using the Maximum Likelihood (ML) method under the Kimura 2-Parameter (K2P) substitution model. The robustness of inferred phylogenetic relationships was assessed using 1,000 bootstrap replicates.

Data deposition

All newly generated COI sequences were submitted to the GenBank database and assigned accession numbers PX945229, PX945230, PX955226, PX945340, PX945224, PX945220, and PX945222. These sequences contribute to the development of a regional DNA barcode reference library for scarab beetles from Pakistan.

Results

Phylogenetic analysis of *Onitis falcatus* based on the COX1 gene

The evolutionary relationships of *Onitis falcatus* were inferred using the Maximum Likelihood (ML) method under the Kimura 2-Parameter (K2P) model implemented in MEGA. The analysis included 35 mitochondrial COX1 sequences, yielding a final alignment of 446 nucleotide positions after pairwise deletion of ambiguous sites.

The optimal tree topology had a log-likelihood value of −5362.352. Among the analyzed sites, 46 were conserved, whereas 374 were variable, including 363 parsimony-informative and 11 singleton sites. Initial trees were generated using the Neighbor-Joining and BioNJ algorithms, and node support was assessed through bootstrap resampling. High bootstrap values (>90%) confirmed the robustness of the inferred phylogenetic relationships (Figure 1). The Pakistani specimen from Malakand division clustered tightly with previously reported Pakistani sequences (MT169779, MN906909), exhibiting zero intraspecific genetic divergence (K2P = 0.000). This finding indicates strong genetic homogeneity and supports the morphological identification. In contrast, interspecific divergence between *O. falcatus* and *Catharsius molossus* exceeded 1.7, reflecting deep evolutionary separation and clear genus-level monophyly.

Phylogenetic analysis of *O. philemon*

The ML phylogeny of *O. philemon* was reconstructed using 28 COX1 sequences with a final alignment length of 445 bp and a log-likelihood value of −3602.836. The dataset comprised 314 variable sites, of which 297 were parsimony-informative, providing strong phylogenetic resolution.

The Pakistani specimen formed a well-supported monophyletic clade, clearly separated from *Gymnopleurus* and *Onthophagus* species. Interspecific genetic distances exceeded 3.5, confirming distinct taxonomic boundaries. A high transition/transversion bias (R = 3.525) suggests an accelerated mitochondrial substitution rate. Bootstrap support values above 85% across major nodes indicate stable evolutionary relationships (Figure 2).

Phylogenetic analysis of *Digitonthophagus gazelle*

The ML tree of *D. gazella* was reconstructed from 27 COX1 sequences with a final alignment of 658 bp and a log-likelihood value of −3970.365. Bootstrap support was estimated using 200 replicates.

Specimens from Pakistan, Australia, Canada, Kenya, and the USA formed a single, strongly supported monophyletic clade. Low intraspecific divergence values (K2P = 0.060–0.078) indicate minimal geographic structuring, consistent with recent global dispersal likely facilitated by anthropogenic activities. High bootstrap support (>95%) confirms genetic homogeneity across continents (Figure 3).

Phylogenetic analysis of *Onthophagus gazelle*

The ML phylogeny of *O. gazella* was inferred from 31 COX1 sequences (634 bp), yielding a log-likelihood value of −4322.539. The dataset contained 217 variable and 187 parsimony-informative sites.

The Pakistani specimen formed a distinct clade clearly separated from *C. molossus*, despite moderate interspecific divergence (K2P = 0.198–0.210). These results demonstrate clear species-level differentiation among ecologically overlapping dung beetles. The transition/transversion bias (R = 0.89) reflects typical mitochondrial substitution patterns within Scarabaeidae (Figure 4).

Phylogenetic analysis of *O. nuchicornis*

The ML phylogeny of *O. nuchicornis* was reconstructed using 30 COX1 sequences with a final alignment of 658 bp and a log-likelihood value of −6578.355. The dataset included 266 variable sites and 204 parsimony-informative sites.

Pakistani specimens clustered with European and Asian congeners, showing moderate genetic divergence (K2P = 0.114–0.148). This pattern suggests regional differentiation within the species complex. Bootstrap values exceeding 80% for major nodes confirm the robustness of the phylogenetic structure (Figure 5).

Phylogenetic analysis of *Copris lunaris*

The phylogeny of *C. lunaris* was inferred from 30 COX1 sequences (658 bp) with a log-likelihood value of −3517.83. Pakistani specimens clustered closely with German sequences, exhibiting zero intraspecific divergence (K2P = 0.000).

Sister-group relationships between *C. lunaris* and *C. tripartitus* were resolved with moderate interspecific divergence (K2P ≈ 0.093–0.098). Strong bootstrap support (>90%) confirmed genus-level monophyly, indicating evolutionary stability and limited intraspecific variation (Figure 6).

Phylogenetic analysis of *C. molossus*

The ML phylogeny of *C. molossus* was reconstructed from 27 COX1 sequences (596 bp) with a log-likelihood value of −5879.442. The dataset contained 427 parsimony-informative sites, indicating strong phylogenetic resolution.

Pakistani specimens clustered with Chinese sequences (K2P = 0.000), suggesting a shared evolutionary lineage. Interspecific divergence from *Onitis* species exceeded 1.3, reflecting deep evolutionary separation. The transition/transversion bias (R = 0.84) indicates moderate substitution asymmetry (Figure 7).

Phylogenetic analysis of *Oniticellus cinctus*

The ML phylogeny of *O. cinctus* was inferred from 40 COX1 sequences (434 bp) with a log-likelihood value of −4245.319. The dataset comprised 326 variable and 298 parsimony-informative sites.

Pakistani specimens clustered with African and Asian taxa, forming a well-supported monophyletic clade. High interspecific divergence (>4.0) from *Onthophagus* species suggests strong genus-level isolation and possible cryptic diversification. A high transition/transversion bias (R = 3.863) indicates accelerated mitochondrial evolution within this lineage (Figure 8).

Combined phylogenetic analysis of Scarabaeidae

The combined ML phylogeny integrating all analyzed Scarabaeidae taxa resolved eight well-supported monophyletic species-level clades, fully consistent with morphological identifications. Deep nodes separating the genera *Onitis*, *Onthophagus*, *Copris*, *Catharsius*, and *Oniticellus* confirm strong evolutionary structuring within the family.

The tree reveals contrasting evolutionary patterns, ranging from globally homogeneous species such as *D. gazella* to geographically structured taxa such as *O. cinctus*. Overall, the results demonstrate the robustness of COX1 barcoding for molecular taxonomy, species delimitation, and evolutionary inference of dung beetles in Pakistan (Figure 9).

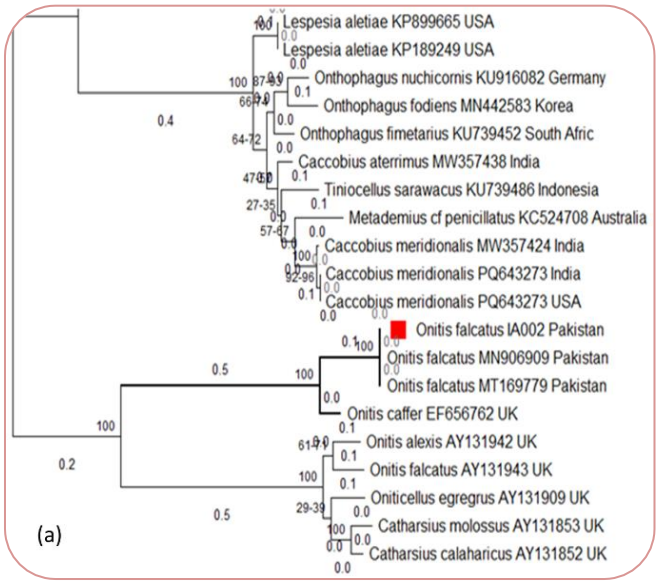

Figure 1. Maximum Likelihood phylogenetic tree of *O. falcatus* inferred from mitochondrial COX1 sequences under the Kimura 2-Parameter model.

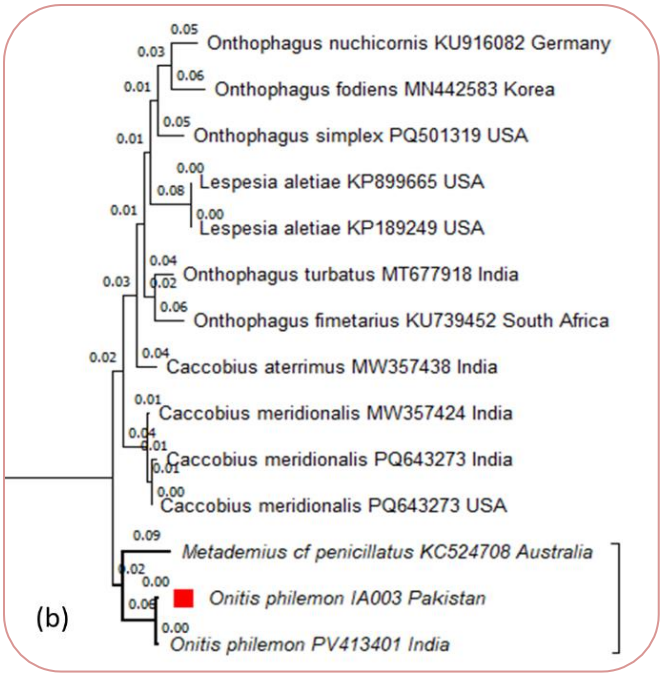

Figure 2. Maximum Likelihood phylogenetic tree of *O. philemon* based on COX1 gene sequences showing species-level divergence and clade support.

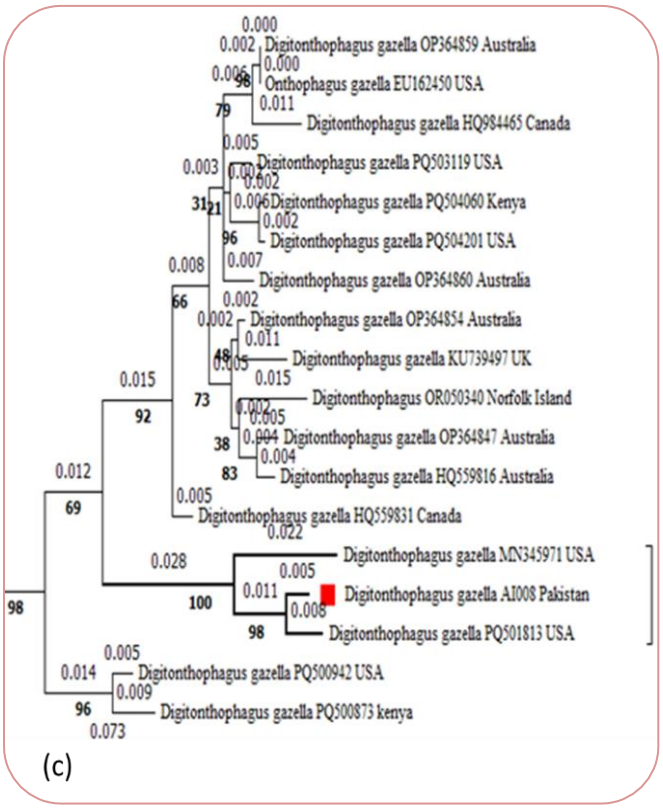

Figure 3. Maximum Likelihood phylogeny of *D. gazella* reconstructed from COX1 sequences illustrating global genetic homogeneity.

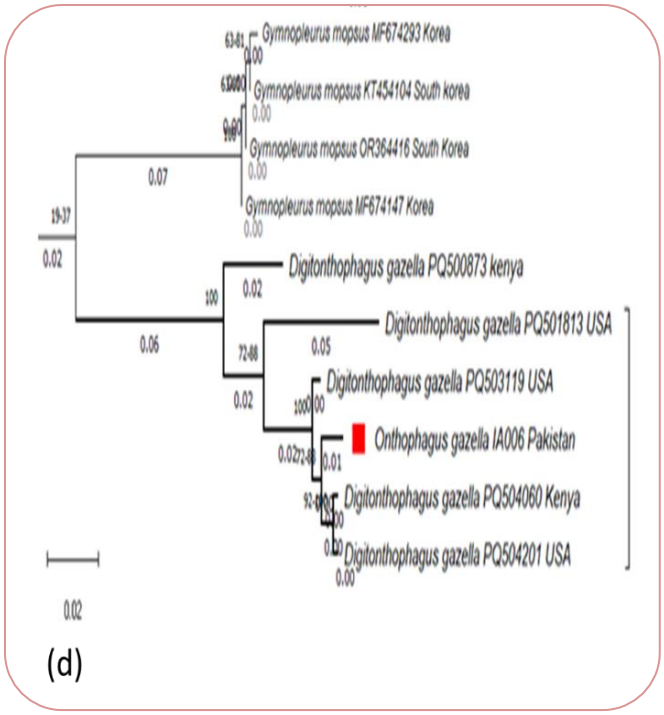

Figure 4. Maximum Likelihood phylogenetic tree of *O. gazella* based on COX1 gene sequences demonstrating species differentiation within Scarabaeidae.

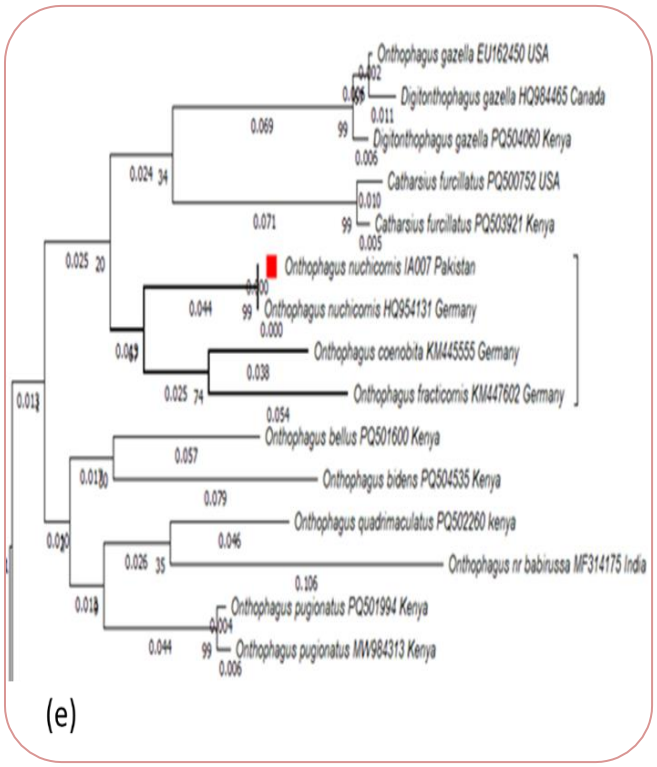

Figure 5. Maximum Likelihood phylogeny of *O. nuchicornis* inferred from COX1 sequences showing regional genetic structuring.

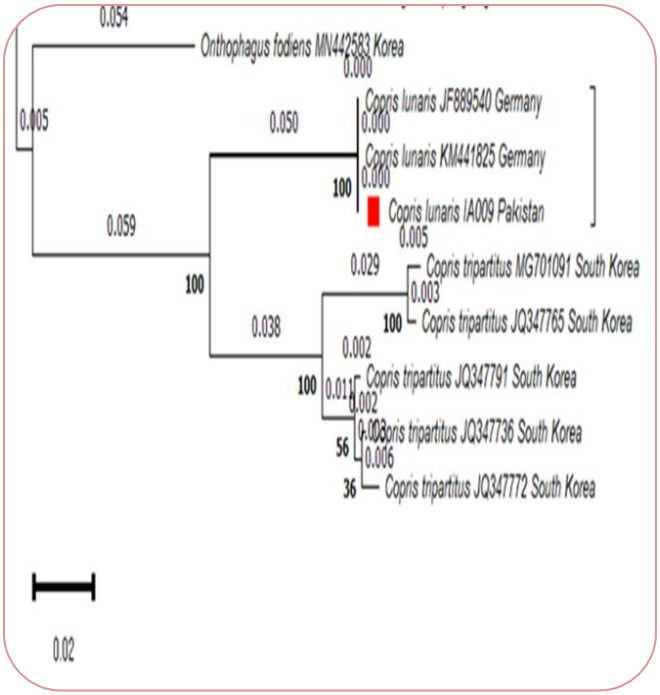

Figure 6. Maximum Likelihood phylogenetic tree of *C. lunaris* based on COX1 sequences highlighting sister-group relationships and genus-level monophyly.

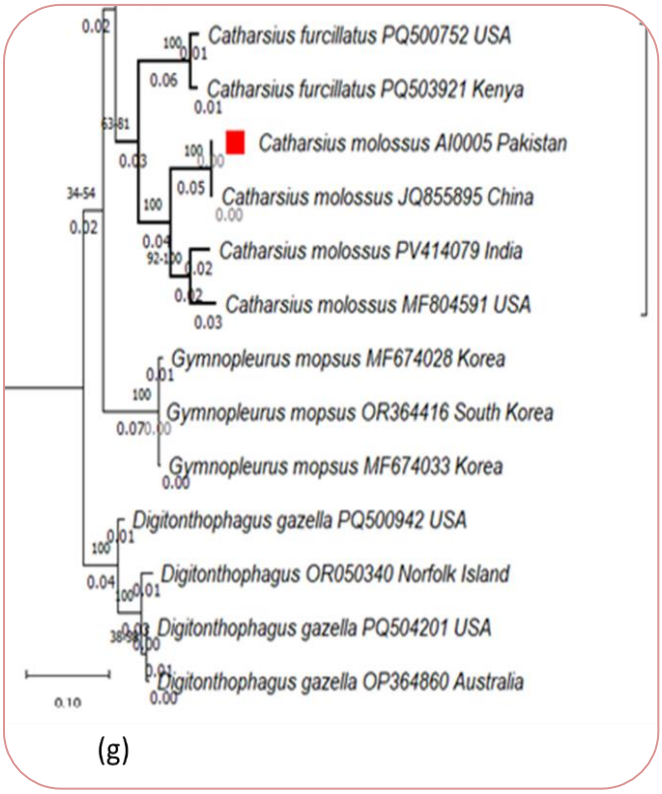

Figure 7. Maximum Likelihood phylogeny of *C. molossus* reconstructed from mitochondrial COX1 sequences indicating deep intergeneric divergence.

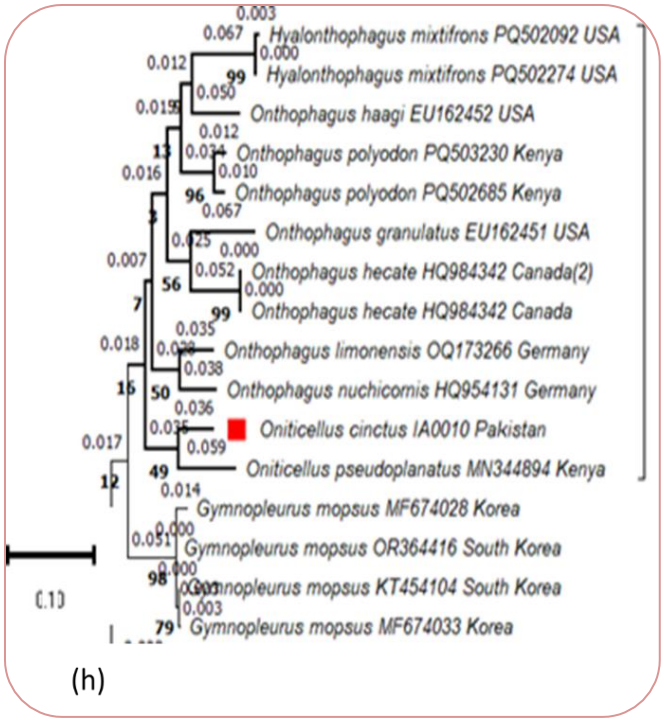

Figure 8. Maximum Likelihood phylogenetic tree of *O. cinctus* based on COX1 sequences demonstrating genus-level isolation and potential cryptic diversification.

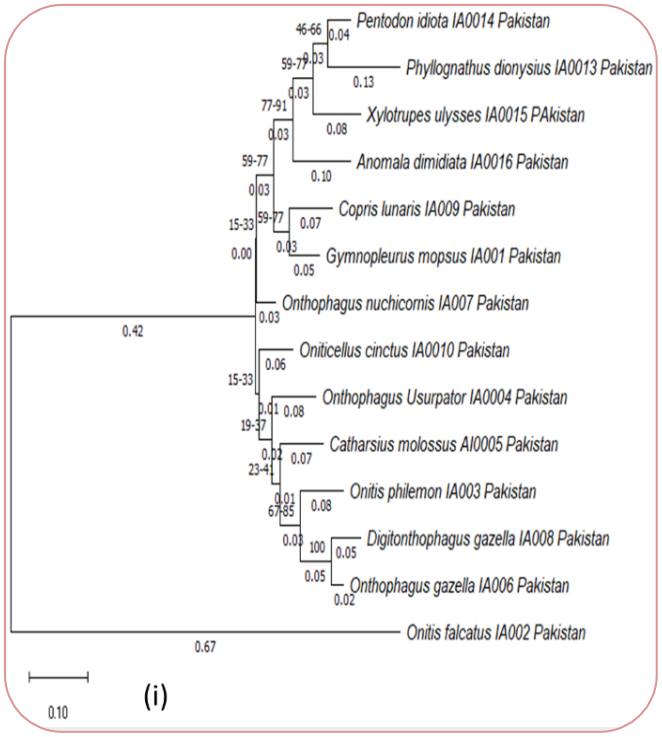

Figure 9. Combined Maximum Likelihood phylogenetic tree of selected Scarabaeidae species based on mitochondrial COX1 sequences showing eight well-supported monophyletic clades.

Discussion

The phylogenetic analysis of dung beetles collected from Malakand Division, Pakistan, was conducted using mitochondrial COI sequences. Statistical support for evolutionary relationships was evaluated through bootstrap analysis, with evolutionary distances estimated under the Kimura 2-Parameter (K2P) model (Kimura, 1980). An unrooted phylogenetic tree was reconstructed using the Neighbor-Joining (NJ) algorithm, a widely applied method in molecular systematics for DNA barcode-based phylogenetic inference.

The analysis included *Onthophagus gazeela* from Malakand division, which was examined for its phylogenetic placement in relation to morphologically defined species groups from different biogeographical regions. The obtained sequences showed 99% similarity with conspecific sequences reported from Punjab (Pakistan), India, Australia, Germany, the United States, and the United Kingdom. This represents the first DNA barcode record of *O. gazeela* from Malakand division. The phylogenetic tree strongly supported its species-level identification based on classical taxonomy, thereby validating the accuracy of molecular identification approaches (Bickel and Cranston, 2010).

The phylogenetic analysis of *Catharsius molossus* from Malakand division revealed noticeable nucleotide divergence at both intra- and interspecific levels. Asian populations clustered closely together, whereas sequences from Kenya and the United States exhibited comparatively greater divergence, likely reflecting long-term geographical isolation. These patterns indicate extended evolutionary separation among populations of *Catharsius*, potentially shaped by historical dispersal events, anthropogenic movement, and ecological pressures. Moreover, *C. molossus* showed clear phylogenetic separation from *Digitonthophagus gazella*, further emphasizing the role of geographic isolation in driving independent evolutionary trajectories (Cambefort, 1991).

Similarly, *Copris lunaris* specimens from Malakand division were compared with sequences from Germany and South Korea. The analysis demonstrated strong genetic conservation between the Malakand and European populations, with only limited nucleotide polymorphisms detected. In contrast, pronounced divergence was observed between *C. lunaris* and *C. tripartitus* from South Korea, highlighting the influence of geographic isolation on species differentiation. Phylogenetic reconstruction confirmed the taxonomic distinctiveness of *Copris* species, with outgroup taxa such as *D. gazella* providing a stable phylogenetic reference framework (Chandra et al., 2013).

D. gazella exhibited minimal intraspecific variation among populations from Malakand division, the United States, Australia, and Kenya, indicating a genetically cohesive and widely distributed species. Nevertheless, minor sequence differences among geographically distant populations suggest the potential influence of local adaptation and restricted gene flow. These findings reinforce the phylogenetic distinctiveness and evolutionary stability of *D. gazella* within the family Scarabaeidae (Cambefort, 1991).

The phylogenetic assessment of *Onitis* species, including *O. falcatus* and *O. philemon*, revealed both conserved and variable nucleotide regions within the COI gene. *O. falcatus* demonstrated high sequence similarity across geographically distant populations from Pakistan and the United Kingdom, whereas *O. caffer* and *O. alexis* exhibited substantial nucleotide substitutions, supporting their recognition as distinct species. In addition, *Caccobius* species from India showed considerable interspecific genetic divergence, indicating clear evolutionary separation from other dung beetle lineages. Collectively, these findings emphasize the importance of geographic isolation, dispersal history, and ecological adaptation in shaping evolutionary patterns within the genus *Onitis* and related taxa (Chandra et al., 2013).

Conclusion

This study presents the first comprehensive molecular and phylogenetic assessment of dung beetle species (Coleoptera: Scarabaeidae) from Malakand division, Khyber Pakhtunkhwa, Pakistan, using mitochondrial COI DNA barcoding. By integrating molecular data with traditional morphological identification, the study effectively addressed challenges associated with phenotypic plasticity, sexual dimorphism, and potential cryptic diversity within scarab beetles.

The strong concordance between morphological taxonomy and molecular phylogenetic results confirms the reliability and robustness of COI barcoding for accurate species delimitation in regional dung beetle fauna. Phylogenetic analyses demonstrated clear species-level clustering and well-supported evolutionary relationships among the examined taxa.

The first DNA barcode record of *Onthophagus gazeela*

from Malakand division expands the molecular reference database for Pakistani scarab beetles and confirms its close genetic affinity with conspecific populations from other biogeographical regions. Likewise, the observed genetic structuring in *Catharsius molossus*, *Copris lunaris*, *Digitonthophagus gazella*, and *Onitis* species highlights the influence of geographic isolation, ecological pressures, and dispersal history on population divergence and evolutionary trajectories. The low intraspecific variation detected in widely distributed species such as *D. gazella* suggests a cohesive global genetic structure, whereas localized differentiation in other taxa reflects regional evolutionary dynamics.

A key outcome of this research is the establishment of a regional COI barcode reference library for dung beetles of Malakand division. This molecular baseline will facilitate rapid and accurate species identification, strengthen biodiversity monitoring programs, and support the use of dung beetles as bioindicators in agroecosystem management. Furthermore, the findings reinforce the ecological importance of dung beetles in nutrient cycling, pest suppression, soil health maintenance, and sustainable agriculture.

Overall, this study contributes significantly to bridging the knowledge gap regarding the molecular diversity of Pakistani scarab beetles and highlights the value of integrative taxonomic approaches for biodiversity assessment. Future research should expand geographic sampling, increase specimen numbers, and incorporate additional molecular markers (e.g., nuclear genes) to further resolve population-level structure and evolutionary relationships. Such efforts will enhance conservation planning and promote sustainable agroecosystem management in Pakistan.

Authors' Contribution

IA conceptualized aspects of the study, conducted data collection and analysis, and prepared the initial draft of the manuscript. FA contributed to data collection and performed data analysis, and assisted in manuscript preparation. MSA provided academic guidance and critically revised the manuscript for intellectual content. KM carried out the literature review, contributed to data interpretation, and revised the manuscript. AS coordinated the fieldwork, supervised the study, and reviewed the manuscript. All authors read, revised, and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 13: Climate Action
SDG 15: Life on Land

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