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Molecular Characterization and Phylogenetic Relationships of *Blaps* Species from the Central Kyzylkum Based on Mitochondrial COI Sequences (Coleoptera: Tenebrionidae)

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Abstract The genus *Blaps* Fabricius, 1775 comprises more than 200 species of darkling beetles adapted to desert and semi-desert environments. However, molecular data for several *Blaps* species occurring in the Central Kyzylkum Desert remain limited. In this study, three *Blaps* species collected from the Central Kyzylkum Desert - *Blaps deplanata*, *Blaps holconata*, and *Blaps titanus* - were molecularly characterized based on a 589-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene, and their phylogenetic relationships were evaluated. Genomic DNA was extracted from adult beetle specimens, and the target COI fragment was amplified by polymerase chain reaction (PCR) and sequenced. Pairwise comparisons revealed 12 nucleotide differences between *B. deplanata* and *B. holconata*, 13 between *B. deplanata* and *B. titanus*, and 17 between *B. holconata* and *B. titanus*. The greatest genetic divergence was observed in comparisons with *Blaps lethifera*: 109 nucleotide differences (18.5%) between *B. deplanata* and *B. lethifera*, and 107 differences (18.1%) between *B. titanus* and *B. lethifera*. Phylogenetic analysis indicated that the three *Blaps* species from the Central Kyzylkum clustered together with *B. lethifera* within two major clades. The newly generated COI sequences were deposited in GenBank under accession numbers PX712921 (*B. holconata*), PX712922 (*B. titanus*), and PX712923 (*B. deplanata*). These findings enrich the available molecular data for *Blaps* species occurring in the Central Kyzylkum and confirm the usefulness of the COI marker for molecular species identification and preliminary phylogenetic inference. Because this study was based on a single mitochondrial marker and a limited number of specimens, future studies incorporating larger sample sizes and additional molecular markers will provide a more comprehensive understanding of the evolutionary relationships among *Blaps* species.

Keywords *Blaps*, Tenebrionidae, Central Kyzylkum Desert, Uzbekistan, COI, DNA barcoding, Molecular phylogeny, GenBank

1. Introduction

Darkling beetles (Coleoptera: Tenebrionidae) represent one of the most diverse groups of insects in terms of ecology and morphology. They are particularly abundant in desert and semi-desert ecosystems, where they exhibit remarkable adaptations to arid environments. Consequently, Tenebrionidae constitute an important model group for studies of desert biodiversity, ecology, adaptation, and biogeography.

The genus *Blaps* Fabricius, 1775 is among the most species-rich genera within the family Tenebrionidae, currently comprising more than 200 described species according to the latest taxonomic revision [10]. Members of this genus are distributed mainly throughout the Palearctic region and exhibit particularly high diversity in arid habitats. Species identification has traditionally relied on external morphological characters and genital structures. However, morphological similarity among closely related species and variation in diagnostic characters may complicate reliable identification. Therefore, integrating morphological and molecular evidence has become increasingly important in the systematics of *Blaps* [5,8,10].

The mitochondrial cytochrome c oxidase subunit I (COI) gene is one of the most widely used molecular markers in animal DNA barcoding. Some regions of this gene are highly conserved, allowing efficient primer binding, whereas other regions evolve rapidly and enable discrimination among closely related species [1]. Although COI-based analyses cannot fully replace morphological studies because a single molecular marker cannot completely reconstruct evolutionary history, the COI marker remains an effective tool for species identification and preliminary phylogenetic assessment.

Previous studies have demonstrated the importance of integrating molecular and morphological data when

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investigating phylogenetic relationships within the genus *Blaps* [5,8,10]. Nevertheless, molecular information for several Tenebrionidae species occurring in Uzbekistan and Central Asia remains scarce. Therefore, newly generated COI sequences from the Central Kyzylkum will contribute to expanding existing molecular databases and clarifying the phylogenetic relationships of *Blaps* species in the region. The objective of this study was to obtain partial COI sequences of *B. deplanata*, *B. holconata*, and *B. titanus* collected from the Central Kyzylkum Desert, compare them with COI sequences of selected *Blaps* species available in GenBank, evaluate nucleotide divergence, and infer their phylogenetic relationships.

2. Materials and Methods

Study Area and Specimen Collection

Adult specimens of *Blaps deplanata*, *B. holconata*, and *B. titanus* were collected during field surveys conducted in the Central Kyzylkum Desert, Uzbekistan. Specimens were initially identified based on external morphological characteristics and preserved in 70% ethanol until molecular analysis.

DNA Extraction

Genomic DNA was extracted from the legs and antennae of adult beetles using the GeneJET Genomic DNA Purification Kit according to the manufacturer's instructions. Extracted DNA was stored at - 20 ℃ until polymerase chain reaction (PCR) amplification.

PCR Amplification of the Mitochondrial COI Gene

A fragment of the mitochondrial COI gene was amplified by polymerase chain reaction (PCR). Amplification was performed using Sileks reagents in a Touchgene Gradient thermal cycler (United Kingdom). The reaction mixture was prepared according to the manufacturer's recommendations.

Thermal cycling conditions included an initial denaturation at 95 ℃ for 3 min, followed by 45 cycles of denaturation at 93 ℃ for 20 s, annealing at 52 ℃ for 30 s, and extension at 72 ℃ for 2 min, with a final extension at 72 ℃ for 10 min. The PCR products were separated by electrophoresis on a 2% agarose gel prepared in 1× TAE buffer and visualized under ultraviolet light.

Purification and Sequencing

Amplified COI fragments were purified from agarose gels using Sileks M reagents according to the manufacturer's protocol. Purified PCR products were sequenced using ABI PRISM® BigDye™ Terminator v3.1 chemistry on an ABI PRISM 3100-Avant automated DNA sequencer (Applied Biosystems). Raw nucleotide sequences were inspected using Chromas v1.45 and prepared for subsequent bioinformatic analyses.

Sequence Processing and Phylogenetic Analysis

The obtained nucleotide sequences were examined and edited using Chromas v1.45. Additional COI sequences of *Blaps* species were retrieved from the GenBank database. Multiple sequence alignment was performed using MAFFT v7 [3]. Maximum-likelihood phylogenetic analysis was conducted using IQ-TREE v1.6.12 with 1,000 ultrafast bootstrap replicates [2,6]. The phylogenetic tree was visualized using iTOL v6.6 [4]. Pairwise genetic divergence percentages were calculated by dividing the observed number of nucleotide differences by the alignment length of 589 bp.

3. Results and Discussion

Characteristics of COI Sequences

PCR amplification and sequencing successfully generated a 589-bp fragment of the mitochondrial COI gene for each studied species. These sequences were used for comparative and phylogenetic analyses. The COI sequence of *B. lethifera* (GenBank accession KM447337) was included for pairwise comparisons.

Pairwise Nucleotide Differences

Analysis of the COI fragment revealed nucleotide differences among the studied species. *B. deplanata* differed from *B. holconata* by 12 nucleotide positions and from *B. titanus* by 13 positions. A total of 17 nucleotide differences were observed between *B. holconata* and *B. titanus*. Comparisons with *B. lethifera* revealed considerably greater divergence: 109 nucleotide differences between *B. deplanata* and *B. lethifera*, and 107 differences between *B. titanus* and *B. lethifera* (Table 1).

Table 1. Pairwise nucleotide differences among Blaps species based on the 589-bp COI fragment

Species 1	Species 2	Number of differences (n)	Difference (%)
B. deplanata	*B. holconata*	12	2.0
B. deplanata	*B. titanus*	13	2.2
B. holconata	*B. titanus*	17	2.9
B. deplanata	*B. lethifera*	109	18.5
B. titanus	*B. lethifera*	107	18.1

Deposition in GenBank

The newly generated partial COI sequences were deposited in the NCBI GenBank database under accession numbers PX712921 (*B. holconata*), PX712922 (*B. titanus*), and PX712923 (*B. deplanata*). These sequences are publicly available through GenBank.

Phylogenetic Relationships

The maximum-likelihood phylogenetic tree separated the analyzed *Blaps* sequences into two major clades. The first clade comprised *B. deplanata*, *B. titanus*, *B. holconata*, and *B. lethifera*, and was strongly supported with a bootstrap value of 99%. The second clade consisted of *B. verrucosa*, *B. caucasica*, and *B. subalpina*, supported by an 82% bootstrap value. Within this clade, *B. verrucosa* and *B. subalpina* each formed distinct clusters with conspecific sequences supported by 100% bootstrap values, whereas the phylogenetic

placement of *B. caucasica* received relatively weak support (53%).

Discussion

The partial COI sequences successfully discriminated the three *Blaps* species studied from the Central Kyzylkum. Specifically, *B. deplanata* and *B. holconata* differed by 12 nucleotide substitutions, whereas *B. holconata* and *B. titanus* differed by 17 substitutions. These results indicate that the molecular data are consistent with species identifications based on morphology. However, because only a limited number of specimens were analyzed for each species, the present results do not fully represent intraspecific genetic variation. Therefore, COI-based findings should be interpreted together with morphological evidence and, when possible, additional molecular markers.

Although COI barcoding has proven highly effective for species identification, combining COI with morphological characters and additional molecular data generally provides more reliable taxonomic conclusions [1]. Such an integrative approach is particularly important for *Blaps*, as previous studies have demonstrated that combining morphological and molecular evidence improves the reconstruction of evolutionary relationships within the genus [5,8]. The substantial genetic divergence observed between *B. lethifera* and the Central Kyzylkum species indicates a relatively distant mitochondrial relationship. Conversely, the comparatively low genetic divergence among *B. deplanata*, *B. holconata*, and *B. titanus* suggests a closer evolutionary relationship based on the analyzed COI fragment.

Phylogenetic analysis placed *B. deplanata*, *B. holconata*, *B. titanus*, and *B. lethifera* within a well-supported clade. Previous studies have likewise shown that the COI marker is useful for distinguishing morphologically similar *Blaps* species [5]. The second clade contained *B. verrucosa*, *B. caucasica*, and *B. subalpina*. The placement of *B. caucasica* within this clade received only moderate bootstrap support (53%), indicating relatively low confidence for this node.

The three newly generated COI sequences presented here expand the available molecular data for *Blaps* species inhabiting the Central Kyzylkum Desert. Future studies incorporating a larger number of specimens and additional molecular markers will allow a more comprehensive assessment of phylogenetic relationships within the genus.

4. Conclusions

A 589-bp fragment of the mitochondrial COI gene was successfully obtained from specimens of *Blaps deplanata*, *B. holconata*, and *B. titanus* collected in the Central Kyzylkum Desert. Pairwise comparisons revealed nucleotide differences among the studied species, while maximum-likelihood analysis placed them together with *B. lethifera* in a strongly supported clade. The newly deposited GenBank sequences (PX712921, PX712922, and PX712923) enrich the available molecular resources for *Blaps* species from the Central Kyzylkum. The results demonstrate that the COI marker is effective for molecular identification and preliminary phylogenetic analysis of *Blaps* species. Future studies based on larger sample sizes and additional molecular markers will provide a more comprehensive understanding of the phylogenetic relationships within the genus.

Data Availability

The COI sequences generated in this study are available in the GenBank database under accession numbers PX712921 (*Blaps holconata*), PX712922 (*Blaps titanus*), and PX712923 (*Blaps deplanata*). For comparative analyses, the COI sequence of *Blaps lethifera* (GenBank accession KM447337) was retrieved from the GenBank database.

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