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MITOGENOME REPORT

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The first complete mitochondrial genome of *Mylabris sibirica* (Coleoptera: Meloidae) and its phylogenetic analysis

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ABSTRACT

The complete mitochondrial genome of *Mylabris sibirica* Fischer von Waldheim, 1823 was sequenced and characterized. The mitogenome is 15,794 bp long with 37 annotated genes, comprising 13 protein-coding genes, 22 transfer RNA genes, and two ribosomal RNA genes. The phylogenetic analysis based on the mitochondrial genome sequences revealed that *M. sibirica* clustered with *M. quadripunctata*. This study presents the complete mitochondrial genome of *M. sibirica* for the first time, which could be beneficial for systematic studies on Mylabrini.

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Mylabris sibirica; complete mitochondrial genome; phylogenetic relationship

Introduction

Mylabrini is the most speciose tribe of Meloidae, with approximately 750 described species assigned to 13 genera (Bologna et al. 2018). *M. sibirica* belongs to Mylabrini and is mainly distributed in China, Kazakhstan, Kyrgyzstan, Russia, and Ukraine (Li et al. 2020). Figure 1 shows a photograph of a captured adult of *M. sibirica*. As of June 2024, complete mitogenomes of 15 species in Mylabrini can be found in the NCBI database. This study presents the first complete sequence of the mitogenome of *M. sibirica*, which would contribute its phylogenetic relationships within the Mylabrini.

Materials and methods

M. sibirica were collected in Wuchuan County, Hohhot City, Inner Mongolia Autonomous Region, China (41.18°N, 110.99°E) in August 2023 and brought back to the laboratory. The samples were identified based on the following taxonomic features: the lower side of the dorsal lobe of the tarsal claw is smooth; the middle part of the dorsal plate of the prothorax is concave; the elytra are yellow with black spots, and the end spot does not cover the end of the elytra; and the body and the black plate of the elytra do not have a bluish-green metallic luster (Li 2010; Wang et al. 2010; Pan et al. 2011). The insect specimen was stored at the Museum of Baotou Teachers College (specimen no. BA850315, Contact: Yunpeng Liu, 472426573@qq.com).

We used the TIANamp Genomic DNA kit (TIANGEN, Beijing, China) to extract total genomic DNA from an *M. sibirica* adult following the manufacturer's procedure, with the gut removed. The library was constructed using TIANSeq Fast DNA Library Kit (Illumina, San Diego, CA), with an insert size of 250 bp. The library was sequenced using pair-end protocol and HiSeq 2000 platform (Illumina, San Diego, CA) with a read length of 150 bp. A total of 3.37 GB (22.48 million reads) raw data were filtered using fastp (Chen et al. 2018). The mitogenome was assembled using Mitobim 1.9 (Hahn et al. 2013) with the mitogenome of *M. aulica* (KX161860) as a reference. The circular genome was confirmed by an overlapping sequence (117 bp) at the anterior and posterior of the assembled genome. Then, the mitochondrial genome was annotated using MitoZ 2.3 (Meng et al. 2019). The drawing of the mitogenome map was completed by Proksee (Grant et al. 2023). The coverage depth map was generated using the method from the previous study (Ni et al. 2023) and showed the average depth 146.66× (Supplementary Figure S1). Primers (F14721: 5′TCCCTACCCCCATAGTCGG3′; R15719: 5′GGGGGTCGTTTCAACTCATAG3′) were designed for amplifying the low depth fragment from 14,721 to 15,719 bp. The PCR product was sequenced using Sanger sequencing by Sangon Biotech Company (Shanghai, China). The sequencing results were aligned with the previously assembled mitogenome to verify the mitogenome assembly (Supplementary Figure S2).

All 16 released mitogenomes of Mylabrini species (Table 1) were used to construct phylogenetic trees to infer the taxonomic position of *M. sibirica*. The phylogenetic trees were rooted by *Megetra punctata* (belongs to the tribe Eupomphini), which was selected based on the following rationale: Eupomphini and Mylabrini are sister groups by López-Estrada et al. (2022). The nucleotide sequences of the 13 PCGs of each species were extracted from these

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Figure 1. Photograph of *Mylabris sibirica* (taken by Jinxing Guo), which was collected by Xu Long and Mengjiao Liu in Wuchuan County, China. This is another sample collected alongside the sequenced sample.

Table 1. Mitochondrial genome sequences with GenBank accession numbers used in this study.

Species	GenBank no.	Size (bp)	Source
Actenodia billbergi	OK360631	15,660	Unpublished
Croscherichia paykulli	OK360635	15,658	Unpublished
Hycleus chodschenticus	KT808466	16,257	Yuan et al. (2016)
Hycleus cichorii	MF491388	15,847	Wu et al. (2018)
Hycleus marcipoli	KX161857	15,923	Du et al. (2017)
Hycleus phaleratus	KX161858	16,003	Du et al. (2017)
Hycleus scutellatus	MN207126	16,035	Mora et al. (2022)
Megetra punctata	OK360640	15,640	López-Estrada et al. (2022)
Mylabris aulica	KX161860	15,758	Du et al. (2017)
Mylabris calida	MT880604	15,149	Jiang et al. (2020)
Mylabris hingstoni	OP080610	15,689	Cheng et al. (2023)
Mylabris longiventris	OP080609	15,685	Cheng et al. (2023)
Mylabris mongolica	OK638152	15,034	Song et al. (2022)
Mylabris phalerata	OM161968	16,108	Unpublished
Mylabris przewalskyi	OP080608	15,692	Cheng et al. (2023)
Mylabris quadripunctata	OK360641	16,683	López-Estrada et al. (2022)
Mylabris sibirica	OR582428	15,794	This study

mitogenomes. Sequences were aligned using ClustalW, trimmed and concatenated in MEGA11 (Tamura et al. 2021). The ModelFinder (Kalyaanamoorthy et al. 2017) was used to calculate partition models for IQ-Tree and MrBayes (Supplementary Table S1). The maximum-likelihood (ML) analysis was performed using IQ-tree 2.0.7 (Minh et al. 2020) with 1000 bootstrap replicates. The Bayesian inference (BI) analysis was performed using MrBayes 3.2 (Ronquist et al. 2012), with 5,000,000 generations and chain sampling every 1000 generations. The burn-in value was set to 25%. The average standard deviation of split frequencies was less than 0.01 (0.002568). Finally, the two phylogenetic trees were visualized and illustrated using iTOL (Letunic and Bork 2021).

Results

The complete mitochondrial genome of *M. sibirica* is 15,794 bp long and contains 13 PCGs, 22 tRNAs, two rRNAs, and a control region (Figure 2). The overall base composition of the mitogenome was: A 35.9%, T 33.8%, C 18.3%, and G 12.0%. ATN (six ATT, six ATG, and one ATA) codons were predicted to be the initiator for 13 PCGs. Most of the termination codons in PCGs were predicted to be TAA or TAG, while *nad4* contains an incomplete codon T(aa). The tRNAs ranged in length from 58 bp (*trnS* (ucu)) to 71 bp (*trnK*). The lengths of *rrnL* and *rrnS* in *M. sibirica* are 1274 and 822 bp.

Since both the ML and BI trees exhibited a congruent topological structure, the posterior probabilities derived from the BI analysis were integrated into the ML tree (Figure 3). The phylogenetic trees based on mitogenomes strongly supported that *M. sibirica* is the sister relationship of *M. quadripunctata*, with a bootstrap support value of 100% and a posterior probability value of 1.

Discussion and conclusions

The circular mitochondrial genome was 15,794 bp in length, containing 37 genes, including 13 PCGs, 22 tRNAs, and two rRNAs. The phylogenetic analysis based on mitogenomes supported that *M. sibirica* was sister to *M. quadripunctata*. The phylogenetic results of the genus *Mylabris* are consistent with those of previous studies (López-Estrada et al. 2022; Mora et al. 2022; Cheng et al. 2023), except for the additions of *M. sibirica* and *M. phalerata* in the present study. Cheng et al. (2023) posited that *Mylabris* is monophyletic, citing the erroneous identification of *M. calida* by Jiang et al. (2020). However, the phylogenetic tree with the addition of these two *Mylabris* species suggests that the genus *Mylabris* is polyphyletic. To accurately ascertain whether *Mylabris* is monophyletic or polyphyletic, more molecular data of correctly identified Meloid species are needed. The complete mitogenome data of *M. sibirica* will contribute to future studies on the molecular phylogeny of the Mylabrini.

Ethical approval

The material involved in the article does not involve ethical conflicts. This species is neither endangered on the CITES catalogue nor collected from a natural reserve, so it did not need specific permissions or licenses. All collection and sequencing work was strictly executed under local legislation and related laboratory regulations to protect wild resources.

Author contributions

JG: conceptualization, methodology, formal analysis, and writing – original draft. KW: conceptualization, writing – review and editing. XL and ML: conceptualization, sample collection, and modified the article. CD: study design, funding acquisition, and writing – review and editing. ZM: conceptualization, funding acquisition, project administration, and writing – review and editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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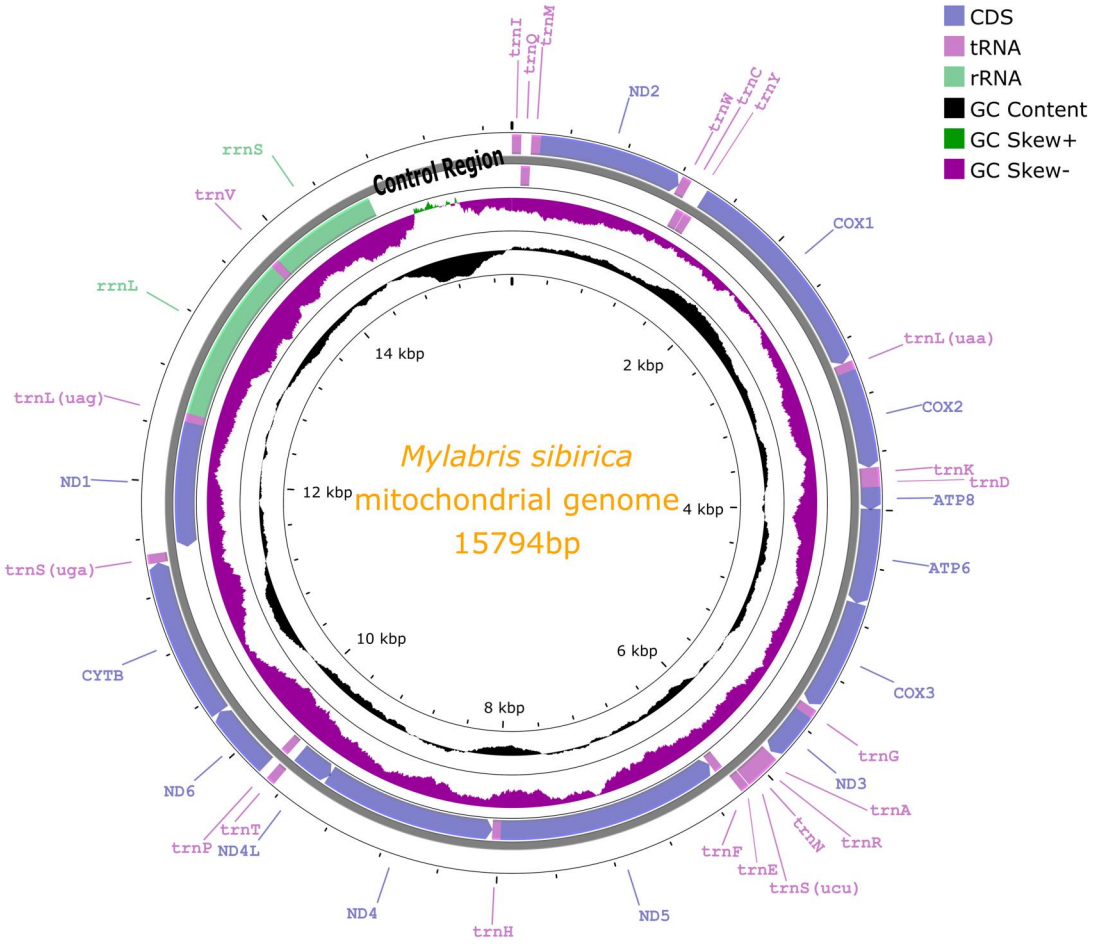

Figure 2. The circular structure of the mitogenome of *Mylabris sibirica*. Genes outside the circle are encoded on the heavy strand and genes inside the circle are encoded on the light stand. Plots of GC skew and content utilized a window size of 500 and reflect GC skew/content on a scale from 0 to 1, with the middle line representing 0.5. Positive and negative skew are indicated by values above and below the midpoint, respectively.

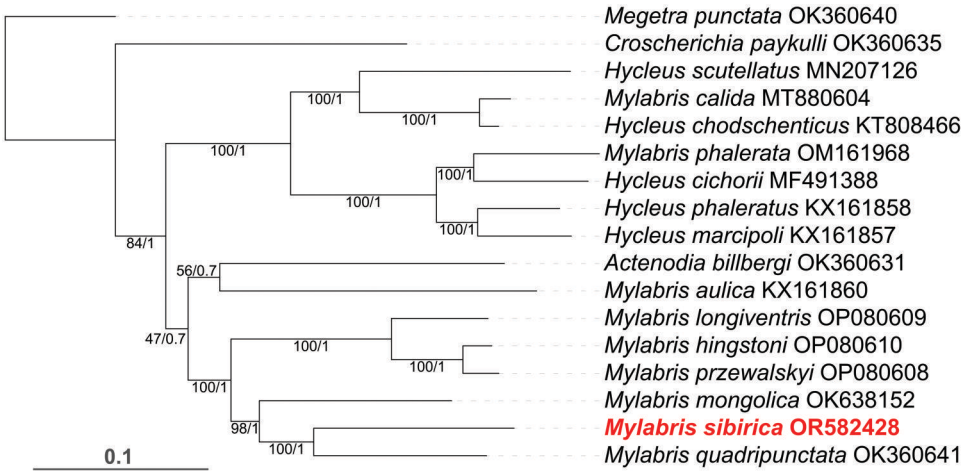

Figure 3. The ML tree and BI tree of Mylabrini were constructed using the nucleotide sequences of 13 PCGs from 17 species. The same topological structure was shared by both trees, with the Bayesian posterior probabilities integrated into the ML tree. Here, we used ML trees for result presentation. The scale bar represents the evolutionary distance, with a unit length of 0.1. Numbers on the branches indicate the ML bootstraps/Bayesian posterior probabilities. The *M. sibirica* in the study was labeled in red color. The GenBank accession numbers of all species are shown in the figure, and citations are given in Table 1.

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Data availability statement

The data that support the findings of this study are openly available in NCBI (National Center for Biotechnology Information) at https://www.ncbi.nlm.nih.gov/, reference number OR582428. The associated BioProject, BioSample, and SRA numbers are PRJNA1066990, SAMN39512926, and SRR27665205, respectively. The low-depth region was sequenced using Sanger sequencing. The result ab1 file was

converted to fastq format and uploaded to the SRA database under the SRA number SRR30500859.

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