GENOME NOTE

The complete mitogenome sequence of clam (*Corbicula fluminea*) [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

The global invasion of the genus *Corbicula* has caused serious ecological and economic problems. The species of *Corbicula fluminea* stands out amongst the greater part of freshwater invaders around the world. Here, we sequenced the entire mitogenome of the *Corbicula fluminea*, which were gathered from Dongting Lake, in the Hunan province of central China. The circular genome is 17,073 bp long. The raw reads were obtained from the platform of Illumina HiSeq 2500, and assembled by the MITObim method followed by alignments to related species. The entire dataset was deposited at the NCBI Short Read Archive via accession number SRR14692229 and NCBI GenBank with accession number MZ231034.1.

Keywords

Corbicula fluminea; mitochondrial genome; phylogenetic analysis

This article is included in the **Genomics and Genetics** gateway.

Open Peer Review

Approval Status

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1. Xiaotong Wang , Ludong University, Yantai, China		
2. Arsalan Emami-Khoiyi , University of Johannesburg, Johannesburg, South Africa		
Any reports and responses or comments on the article can be found at the end of the article.		

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Author roles: Luo Q: Data Curation, Formal Analysis, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; Pi J: Investigation, Resources; Tang Y: Investigation, Resources; Zeng C: Methodology, Software, Validation; Li D: Funding Acquisition, Project Administration, Supervision, Validation, Writing – Review & Editing

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Introduction

Clams of the genus *Corbicula* are currently spread worldwide, and they cause great ecological threats and tremendous impacts in the ecological system (Counts 1981; Gomes *et al.* 2016; Peñarrubia *et al.* 2017; Hünicken *et al.* 2019; Douglass *et al.* 2020). However, their taxonomic status and systematic status are still unclear (Wang *et al.* 2018; Haponski 2019; Bodon *et al.* 2020; Ramli *et al.* 2020). *Corbicula fluminea* is an important component of Asian freshwater macrobiota and has always been consumed as food in East Asia (Wang *et al.* 2014; López-Soriano *et al.* 2018; Zhang *et al.* 2019; Sano *et al.* 2020). In this work, the complete mitochondrial genome of *Corbicula fluminea* from Dongting Lake was sequenced, and the phylogenetic relationships among Venerida were investigated. These results could contribute to distinguishing the taxonomic placement and systematic status of genus *Corbicula* in further studies.

Methods

The specimens (*Corbicula fluminea*) used in this work was collected from Dongting Lake (geographic location: 28° 46'45"N, 112°41'22"E), in Hunan province, China in August 2018.

After sampling, the living specimens were stored in College of Animal Science and Technology, Hunan Agricultural University, the voucher number is DT1808-118.

Figure 1. Circular maps of the mitochondrial genome of *Corbicula fluminea* from Dongting Lake, including genes encoding proteins, ribosomes and transporters.

To obtain the total genomic DNA, the 40mg frozen muscle of the foot was derived from the specimen. DNA was obtained by DNeasy™ Blood & Tissue extraction kit (Qiagen, Hilden, Germany). The library was conducted by Berry Genomics Co. Ltd (Beijing, China) according to the Illumina TruSeq Nano DNA library Prep Kit and the manufacturer's recommendations. DNA fragments were selectively enriched using Illumina PCR Primer (F: 5'-AATGATACG GCGACCACCGAGA-3' and R: 5'-CAAGCAGAAGACGGCATACGAGT-3') Cocktail in a PCR reaction with the following cycling conditions: 95°C for 3 minutes, followed by 8 cycles of 98°C for 20 seconds, 60°C for 15 seconds and 72°C for 30 seconds, with a final extension step at 72°C for 5 minutes. The raw reads of the mitogenome sequence were obtained from the platform of Illumina HiSeq 2500. The adaptors and low sequencing qualities reads (N bases exceeding 10% and more than 50% of phred quality score ≤ 5) were trimmed and reduced using the program Trimmomatic v 0.38.0 (Bolger *et al.* 2014).

The sequence reads of untrimmed high-quality were assembled by the program MITObim (Hahn *et al.* 2013). The annotation was performed using MITOS WebServer (Bernt *et al.* 2013) for the entire mitogenome of *Corbicula fluminea* from Dongting Lake, and adjusted manually in Geneious Prime v2020.2.2 based on the published mitogenomes of Venerida species.

To validate our data, the CDS sequences of 19 Venerida species (GenBank accession numbers are shown in Figure 2) were aligned. The phylogenetic analysis among Venerida was investigated using maximum-likelihood (ML) and Bayesian inference (BI) approaches. The ML tree was estimated by RAxML version 8.2.12 (Stamatakis 2014) and the BI tree was estimated by MrBayes version 3.2.7 (Ronquist *et al.* 2012), with GTR-GAMMA model. RAxML was analyzed with 1,000 bootstrap replicates (-m GTRGAMMAI -f a -x 1 -N 1 -p 1 -N 1000), 1 million generations Markov chain Monte Carlo iterations (nCat=4) were analysed and every 1,000 generations were sampled with the initial 10% of samples removed as "burn-in" in MrBayes. The average standard deviation of split frequencies (<0.01) was used to assess the convergence.

A phylogenetic relationship was estimated based on 13 protein-coding genes sequences from the mitogenome sequences of *Corbicula fluminea* from Dongting Lake and other 19 Venerida species (Figure 2). The study has provided a complete mitochondrial genome of *Corbicula fluminea* from Dongting Lake. As a phylogenetic tool, it could contribute to systematic and comparative analysis for resolving evolutionary relationships.

Figure 2. Phylogenetic relationships based on 13 mitochondrial protein-coding genes sequences inferred from RAxML and MrBayes. The nodal numbers indicate the bootstrap support values (left) and the posterior probability (right). Genbank accession numbers for the sequences are indicated next to the species names.

Results

The complete circular mitogenome of *Corbicula fluminea* from Dongting Lake (GenBank accession no. MZ231034.1) is 17,073 bp in total length. As other Venerida mitogenomes, 37 mitochondrial structural genes were found in the mitogenome of *Corbicula fluminea* from Dongting Lake, including 13 protein-coding genes, two ribosomal RNA genes (12S and 16S rRNA) and 22 transfer RNA genes (Figure 1). Both are encoded on the positive strand.

Data Availability

Underlying data

NCBI Gene: Complete mitochondrial genome of *Corbicula* sp. DT118, SRA

Accession number: SRR14692229

Accession number URL: <https://www.ncbi.nlm.nih.gov/sra/SRR14692229>

NCBI Gene: *Corbicula* sp. QL-2021 mitochondrion, complete genome

Accession number: MZ231034.1

Accession number URL: <https://www.ncbi.nlm.nih.gov/nucleotide/MZ231034.1>

Ethical approval

All the clams and experimental protocols involving experiments of this study were approved by the Animal Ethics Committee of Hunan Agricultural University, Hunan, China.

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Arsalan Emami-Khoyi

Centre for Ecological Genomics and Wildlife Conservation, Department of Zoology, University of Johannesburg, Johannesburg, South Africa

In my opinion, the paper contributes significantly to the knowledge base of this species. No major flaws in the analysis were noticed.

The authors need to better justify why this particular clam has been chosen for sequencing.

The authors need to clarify in which mode Mitobim has been used. Has a conserved template sequence from a closely related species been used as a starting seed?

a) The title needs to be changed to "**Asian**" clam (*Corbicula fluminea*).

b) Please note that ribosomal RNA are not genes.

c) Great "ecological threats" and "tremendous impacts" need to be clarified.

d) How were the Bayesian runs trace files evaluated for convergence? Why was the Effective Sample Size not reported?

Are the rationale for sequencing the genome and the species significance clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Partly

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 24 March 2022

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Xiaotong Wang

School of Agriculture, Ludong University, Yantai, China

This paper describes the assembly and annotation of a clam mitogenome. It's a brief note that provides some useful background about the species, *Corbicula fluminea*, and these data can contribute to distinguish the taxonomy and systematic status on the genus *Corbicula* in further studies.

The details of the analysis and the data is clear. I have 2 minor revisions:

1. Include version number of MITObim and MITOS.
2. The name of the species should be unified in the manuscript and the NCBI GenBank.

Are the rationale for sequencing the genome and the species significance clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Mollusc physiology and molecular biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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