Sequencing of the complete mitochondrial genomes of eight freshwater snail species

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Abstract

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Protocol

Samples collection

Step 1.

At every location(

1. Lake Dianchi in Kunming city, Yunnan province, SW corner of China (south subtropical climate zone).
2. Zhangwei River, near Anyang city, Henan province, central China (warm temperate climate zone).
3. Amur River, about 1 km northwest of Fuyuan city, Heilongjiang province, NE corner of China (middle temperate climate zone).

) we have collected benthic organisms along 50 meters of shore using hand-held nets. After selecting all of the viviparid snails found in the sample, the remaining organisms were returned into the water. Snails were transported alive to our lab in Wuhan, where they were taxonomically determined based on their morphological features.

PCR amplification and sequencing

Step 2.

The total DNA was extracted from the foot muscle using Mollusc DNA Kit (Omega, USA) according to the manufacturer's recommended protocol. Publicly available sequences were used to determine the parts of *COX1*, *12S, 16S*, *Cytb* and *COX3* genes conserved across different caenogastropod species, which were then used to design five primer pairs (Supplementary Table S1) for amplification and sequencing of fragments of these genes in all eight species. Based on these newly sequenced fragments, species-specific primers were designed to amplify the complete mitogenome by long-range PCR. The PCR reaction constituted a 50 µL volume with 1 U of KOD FX polymerase (Toyobo, Japan), 2.5 µL (approximately 100 ng) of DNA, 25 µL 2x PCR buffer for KOD FX, 10 µL of 2mM dNTPs, and 1.5 µL of each 10 pmol primer. PCR amplification was performed under the following procedure: denaturation at 94°C for 2 min, followed by 35 cycles of 10 s at 98°C, with the annealing temperature (Tm) adjusted to suit the specific primer (generally at Tm - 5 °C), extension time set to 1 min per Kb of the expected product size (1 to 5 min in total), and the final 5 min extension at 72°C. The PCR

products were resolved by electrophoresis on 1.0% agarose gel and sequenced by Tsingke company (Wuhan, China) using primer walking strategy.

Sequence analysis

Step 3.

The sequencing results were subjected to quality control involving checking the ab1 sequencing pictures. Sequences were BLASTed to confirm that the amplicon is the actual target sequence. Mitogenomes were then assembled manually in a stepwise manner with the help of DNAstar 5.0 software and annotated in Geneious program , using two available viviparid mitogenome sequences (*Bellamya quadrata*, NC_031850 and *Cipangopaludina cathayensis*, NC_025577) as references. Protein-coding genes (PCGs) were determined by finding the ORFs (employing codon table 5) and checking the nucleotide alignments against the reference genomes in Geneious. Transfer RNA genes were identified using both the results of ARWEN and MITOS web server analyses. Similarly, rRNAs (12S and 16S) were identified using MITOS and alignments to closely related references in Geneious. Base composition was computed using MEGA 5. Tables with statistics for mitogenomes were generated using a newly developed GUI-based program, MitoTool (https://github.com/dongzhang0725/MitoTool). Rearrangement events in the mitogenomes and pairwise comparisons of gene orders were analysed by CREx web tool utilizing breakpoint dissimilarity measurement. The annotated mitogenome sequences were deposited in GenBank (see Table 1 for accession numbers).