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The mitochondrial genome organization of the rice frog, *Fejervarya limnocharis* (Amphibia: Anura): a new gene order in the vertebrate mtDNA

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Abstract

The mitochondrial DNA of the rice frog, *Fejervarya limnocharis* (Amphibia, Anura), was obtained using long-and-accurate polymerase chain reaction (LA-PCR) combining with subcloning method. The complete nucleotide sequence (17,717 bp) of mitochondrial genome was determined subsequently. This mitochondrial genome is characterized by four distinctive features: the translocation of ND5 gene, a cluster of rearranged tRNA genes (tRNA^{Thr}, tRNA^{Pro}, tRNA^{Leu} ^(CUN)), a tandem duplication of tRNA^{Met} gene, and eight large 89-bp tandem repeats in the control region, as well as three short noncoding regions containing two repeated motifs existing in the gene cluster of ND5/tRNA^{Thr}/tRNA^{Pro}/tRNA^{Leu}/tRNA^{Phe}. The tandem duplication of gene regions followed by deletions of supernumerary genes can be invoked to explain the shuffling of tRNA^{Met} and a cluster of tRNA and ND5 genes, as observed in this study. Both ND5 gene translocation and tandem duplication of tRNA^{Met} were first observed in the vertebrate mitochondrial genomes.

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Keywords: Fejervarya limnocharis; Rice frog; Complete mtDNA sequence; Unique ND5 gene order

1. Introduction

The content of the mitochondrial genome, including 13 protein-coding genes, two rRNA genes, and 22 tRNA genes, as well as a control region, is highly conserved in vertebrates, with only a few exceptions. To date, complete mitochondrial

genomes have been determined in 321 species of vertebrates, but among these, there are only six species of amphibians: the frogs *Xenopus laevis* (Roe et al., 1985) and *Rana nigromaculata* (Sumida et al., 2001), the caecilian *Typhlonectes natans* (Zardoya and Meyer, 2000), and the salamanders *Mertensiella luschani* (Zardoya et al., 2003), *Ranodon sibiricus* (Zhang et al., 2003a), and *Andrias davidianus* (Zhang et al., 2003b). Characteristic descriptions of these vertebrate mitochondrial genomes show that few of them either bear pseudogenes of tRNA or even lost certain individual genes (Macey et al., 1998; Kumazawa et al., 1998).

Although gene order is also highly conserved in most of vertebrate mtDNAs, it is found that 81 out of 321 known mitochondrial genomes possess a rearranged gene order, and many of these rearrangements involve tRNA genes only.

Abbreviations: ATP6, ATPase subunit 6; ATP8, ATPase subunit 8; COI-III, cytochrome c oxidase subunit I–III; Cyt b, cytochrome b; D-loop, displacement loop; H-strand, heavy strand; L-strand, light strand; LA-PCR, long-and-accurate polymerase chain reaction; ND1-6, 4L, NADH dehydrogenase subunit 1–6, 4L; PCR, polymerase chain reaction; tRNA, transfer ribonucleic acid.

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Translocation of protein coding genes is infrequently observed in vertebrates with the exception of Cyt *b* in a sea lamprey and a sphenodontid lizard and ND6 in birds and two species of fish. Moreover, the translocations of long gene cluster containing both protein genes and tRNA genes were also observed in two eels (see review by Zhong et al., in press).

The rice frog, *Fejervarya limnocharis*, is a species widely distributed in the area from temperate to tropical Asia, and its taxonomic status is still controversial (Fei et al., 2002). To figure out this problem, in this paper, we investigate the geographic variation of the rice frog by analyzing its mitochondrial genome. A rice frog specimen was collected from east China, and its mitochondrial genome was sequenced using the LA-PCR combined with subcloning techniques. A new gene order for vertebrates was identified that composes of translocation of ND5, a tandem duplication of tRNA^{Met}, and a cluster of rearranged tRNA genes.

2. Materials and methods

2.1. Sampling, mtDNA isolation, LA-PCR, and sequencing

Mitochondrial DNA was isolated from a fresh liver of F. limnocharis sampled from Yancheng, China. The DNA fragments of 12S and 16S rRNA genes were amplified respectively from purified mtDNA using two pairs of highly conserved primers (Kocher et al., 1989; Simon et al., 1994) and subsequently sequenced. Based on acquired sequences information and the homologous of other vertebrate mtDNAs, three pairs of primers (sequences are available upon request) were designed to amplify and sequence mtDNA fractions (12S rRNA, ND1, and ND4). Then based on the sequence data obtained in this study, two pairs of LA-PCR primers ND1-L (5-GAAAGTTAGGGTTCTCCT-TGATAGGGAGGC-3') and ND4-H (5'-TGTGGCTGACG-GAAGATATAGCAATGAGGG-3'), ND4-L (5'-CAAAGCGCAATATACATGATAATTGCCCATGG-3') and 12S-H (5'-TCCTCACTGGTGTGCTGAGACTTGCAT GTG-3') were designed to amplify large fragments ranging from tRNA^{IIe} to ND4 and from ND4 to 12S rRNA genes, respectively. LA-PCR products were cleaved with restriction enzymes. One EcoRI restricted fragment (1.6 kb), two HindIII/XbaI fragments (2.28 and 0.8 kb), and four HindIII/PstI fragments (0.45, 0.38, 0.9, and 0.84 kb) were cloned into *E. coli* pGEM-3zf⁺ vector. Both strands of these clones were sequenced on an ABI PRISM[™] 310 Genetic Analyzer (Perkin Elmer) or LI-COR DNA sequencer 4200 using the primer walking strategy. The gaps were amplified and sequenced using newly designed primers, and each segment overlapped the next contig by 80–120 bp.

2.2. Analyses of sequence data and structure of RNAs

Nucleotide sequences were analyzed using the software Lasergene version 5.0. The locations of 13 protein-coding

genes and 2 rRNA genes were determined by comparison with homologous sequences of other amphibian mtDNA. The tRNA genes were also identified by their cloverleaf secondary structure and anticodon sequences presumed using DNASIS (Ver. 2.5, Hitachi Software Engineering). The complete mitochondrial genome sequence of *F. limnocharis* reported here was deposited in GenBank under the accession number AY158705.

3. Results

3.1. Genome content and organization

The complete mtDNA sequence of *F. limnocharis* is 17,717 bp in length containing 13 protein-coding genes, 2 rRNA genes and 23 tRNAs genes (including an extra copy of tRNA^{Met}), and noncoding regions (including the control region). As found in other vertebrates, most of these genes are coded on the H-strand except for ND6 and 8 tRNA genes. Base composition of L-strand is as follow: A: 28.1%; C: 26.8%; G: 15.5%; T: 29.9%, agreeing with the mitochondrial genomes of other amphibians.

The mitochondrial genome organization of *F. limnocharis* is shown in Fig. 1, indicating a unique position of ND5 gene and a new gene order of tRNA^{Thr}/tRNA^{Pro}/tRNA^{Leu} (^{CUN)}. The ND5 gene of *F. limnocharis* is translocated to the 3 end of control region, which is accompanied with the relocation of tRNA^{Thr}, tRNA^{Pro}, and tRNA^{Leu} (^{CUN)} at the downstream of ND5, giving an order of D-loop/ND5/

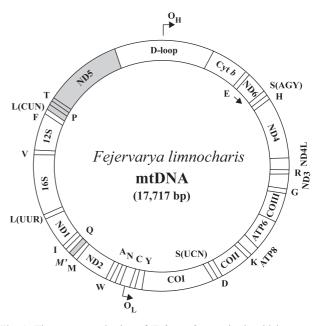


Fig. 1. The gene organization of *F. limnocharis* mitochondrial genome. tRNA genes are denoted by the single-letter amino acid code. All proteincoding genes are H-strand-encoded with the exception of ND6 indicated by an arrow. OH and OL represent the replication origins of H- and L-strands, respectively. *M'* shows the extra copy of tRNA^{Met}. Shadows are depicted to indicate a unique gene order in vertebrates.

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