

MITOGENOME ANNOUNCEMENT

**Complete mitochondrial genome of the sheepshead minnow
Cyprinodon variegatus (Cyprinodontiformes: Cyprinodontidae)**

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Abstract

The complete mitochondrial genome of the sheepshead minnow (*Cyprinodon variegatus*) with the length of 16,498 bp was determined using long PCR and Sanger sequencing. The mitogenome contains 13 protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes and a D-loop region of 831 bp in length. All protein-coding genes except NADH dehydrogenase subunit 6 (*nd6*) are encoded on the heavy strand (H-strand). ATG acts as the start codon for all protein-coding genes except cytochrome c oxidase subunit I (*col*), which has GTG as a start codon. Fourteen tRNA genes and both rRNA genes are also encoded on the H-strand, as observed in other Actinopterygian fishes. This complete mitogenome sequence will facilitate studies on adaptation of *C. variegatus* to local variation in temperature, salinity and chemical pollution conditions, as well as inform studies of genetic introgression by *C. variegatus* into other *Cyprinodon* species that have imperiled protection status.

Keywords

Introgression, metabolic rate, mitogenome, mtDNA, pupfish, toxicology

History

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The sheepshead minnow *Cyprinodon variegatus* (Lacépède 1803) is a small killifish (Cyprinodontiformes), which inhabits freshwater, brackish and marine environments of the Eastern coasts of North America and Central America as well as islands of the Caribbean Sea region. This species exhibits remarkable capabilities for salinity and thermal tolerance (Bennett & Beiting, 1997; Nordlie, 1985), it has been a model organism for studying how salinity, temperature, and environmental hypoxia influence metabolism (Barton & Barton, 1987; Haney & Nordlie, 1997; Jordan et al., 1993; Nordlie et al., 1991; Peterson, 1990) and for mechanistic investigations of osmo- and ion-regulation (Karnaky et al., 1976). *Cyprinodon variegatus* is also a model teleost fish for studying the toxic and endocrine-disrupting effects of environmental chemical pollutants (Folmar et al., 2000; Heitmuller et al., 1981; Karels et al., 2003).

Here, we report the complete mitochondrial genome of *C. variegatus* (Genbank accession no. KR061357). A female *C. variegatus* (*variegatus* subspecies) was collected on 12 May 2011, from an estuary near Charleston, South Carolina, USA (32°44'54.05"N 79°54'06.06"W). Genomic DNA was extracted from liver tissue (DNeasy Blood and Tissue Kit; Qiagen, Valencia, CA) and amplified (GoTaq® Long PCR Master Mix, Promega Corp., Madison, WI) using primers designed for *C. rubrofluvialtilis* (EF442803) to generate overlapping sequence

products of 6,112 to 10,731 bps in length, which were then Sanger sequenced (MCLab, South San Francisco, CA) and assembled (Sequencher v5, Gene Codes Corp., Ann Arbor, MI).

The complete mitochondrial genome of *C. variegatus* is 16,498 bp in length and encodes 13 protein-coding genes, 22 tRNA genes and the 12S and 16S rRNA genes. Genome synteny adheres to the arrangement typical for vertebrate mitogenomes, and all genes are encoded on the heavy strand (H-strand) except the *nd6* protein-coding gene and the following tRNAs: *tRNA^{Pro}*, *tRNA^{Glu}*, *tRNA^{Ser}*, *tRNA^{Tyr}*, *tRNA^{Cys}*, *tRNA^{Asn}*, *tRNA^{Ala}* and *tRNA^{Gln}*. The nucleotide composition of the H-strand is 26.20% A, 27.25% T, 17.03% G, and 29.52% C, and the combined G + C content (46.55%) is similar to mitogenomes of congeners *C. rubrofluvialtilis* (46.53%; EF442803) and *C. nevadensis pectoralis* (47.05%; KP064222). Phylogenetic analysis using the complete mitogenome nucleotide sequence confirmed its identity within the Cyprinodontidae clade of Cyprinodontiformes (Figure 1), and estimates of evolutionary change are 0.049 base substitutions per site compared to the mitogenome of *C. rubrofluvialtilis*, and 0.068 substitutions per site relative to *C. n. pectoralis* (Maximum Composite Likelihood model, Tamura et al., 2011).

The availability of this complete mitogenome for *C. variegatus* has the potential to advance research into how environmental parameters including temperature, salinity and dissolved oxygen regulate cellular respiration in fish. This mitogenome will also facilitate assessments of genetic introgression by *C. variegatus* into imperiled *Cyprinodon* species inhabiting deserts of the southwestern USA and northern Mexico (Echelle & Echelle, 1997).

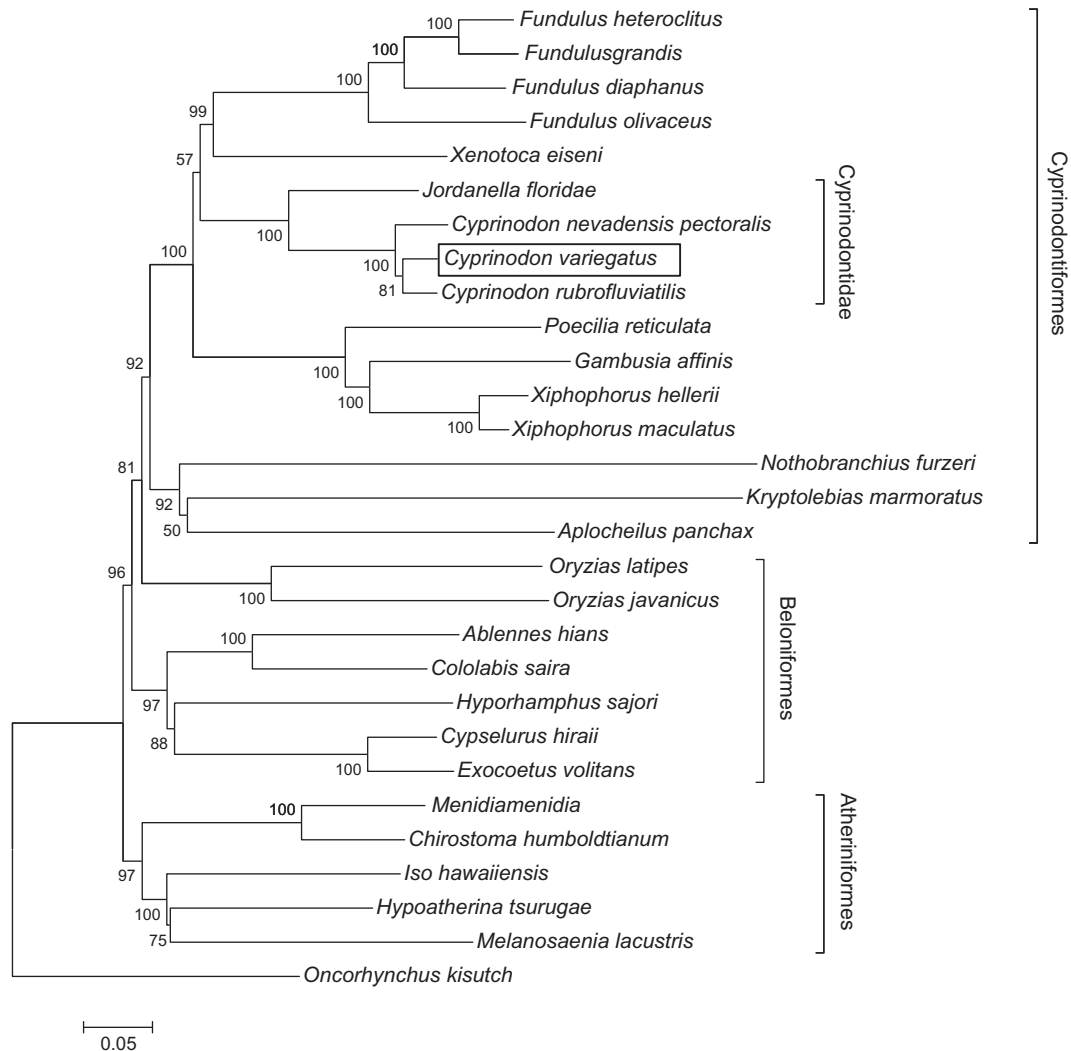


Figure 1. Maximum likelihood tree of the mitogenomes of *C. variegatus* (KR061357), other Cyprinodontiformes fishes, and select Belontiiform and Atheriniform fishes. Complete mitogenome nucleotide sequences for each taxa (excepted *J. floridae*, AP006778, which lacks the D-loop control region sequence) were aligned using ClustalX, and a phylogenetic tree was constructed using a Tamura-Nei Model with partial deletion of gaps (50% cutoff) using MEGA v5 (Tamura et al., 2011). Coho salmon (*O. kisutch*, EF126369; Salmoniformes) was used as the outgroup. Bootstrap values (1000 replicates) are indicated at each node. Belontiiform and Atheriniform orders were selected for comparison based on the phylogenies provided by Betancur-R et al. (2013). Note that order Belontiiformes is not monophyletic in the phylogeny shown here. Genbank accession nos. for component taxa are as follows: *F. heteroclitus* (FJ445402), *F. grandis* (FJ445396), *F. diaphanus* (FJ445394), *F. olivaceus* (AP006776), *X. eiseni* (AP006777), *C. n. pectoralis* (KP064222), *C. rubrofluviatilis* (EF442803), *P. reticulata* (KJ460033), *G. affinis* (AP004422), *X. hellerii* (FJ226476), *X. maculatus* (AP005982), *N. furzeri* (EU650204), *K. marmoratus* (AF283503), *A. panchax* (AB373005), *O. latipes* (AP004421), *O. javanicus* (AB498067), *A. hians* (AB373007), *C. saira* (AP002932), *H. sajori* (AB370892), *C. hiraii* (AB182653), *E. volitans* (AP002933), *M. menidia* (AB370893), *C. humboldtianum* (KJ921739), *I. hawaiiensis* (AB373006), *H. tsurugae* (AP004420), *M. lacustris* (AP004419).

Declaration of interest

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