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Complete mitochondrial genome of the Starhead Topminnow *Fundulus dispar* (Cyprinodontiformes: Fundulidae)

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ABSTRACT

Topminnows of the Teleost genus *Fundulus* serve as model organisms in ecotoxicology because of their broad physiological tolerance and propensity to breed in captivity. This research has been primarily limited to intraspecific comparisons, due to incomplete understanding of the evolutionary history of the genus, which is necessary for use of phylogenetic comparative methods. Interspecific relationships of topminnows remain unresolved, despite recent advances in mitochondrial and nuclear genome sequencing. Specifically, interrelationships of a group containing the starhead topminnows (*Fundulus blairae*, *F. dispar*, *F. escambiae*, *F. lineolatus*, and *F. nottii*) typically yield low node support values. Here, we present the first annotated mitochondrial genome of the Starhead Topminnow (*F. dispar*) and provide a phylogenetic hypothesis for starhead topminnows within the genus *Fundulus*. DNA was isolated from a specimen of *F. dispar* collected in Kentucky, USA. The circular genome is 16,564 bp long and contains 13 protein-coding genes, two ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA), and one control region (D-loop). Our phylogenetic analysis supports a sister relationship between *F. dispar* and a group containing *F. notatus* and *F. olivaceus*. This data helps to resolve the phylogenetic placement of starhead topminnows.

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Introduction

Members of the fish family Fundulidae have been used as field models to answer questions about interactions with the environment because of their ability to survive along thermal and osmotic gradients (Whitehead 2009). These unique qualities have placed the genus *Fundulus* as a priority group for generating genomic data (Burnett et al. 2007; Johnson et al. 2020; Drown et al. 2023). While interesting questions have been answered about targeted *Fundulus* taxa, there are knowledge gaps within the genus for lesser-studied fishes; a complete understanding of the taxonomic placement of all *Fundulus* would help advance the toolbox of the genus as a model organism (Burnett et al. 2007). The phylogenetic placement of the starhead topminnow group has not been consistently resolved (Bernardi and Powers 1995; Kreiser 2001; Whitehead 2010; Ghedotti and Davis 2013; Cashner et al. 2020). Results of phylogenetic analyses of nuclear DNA sequence data support the *F. notatus* species group (*F. notatus*, *F. olivaceus*, and *F. euryzonus*) as a sister taxon to the starhead topminnow group (Whitehead 2010). Additional studies have hypothesized phylogenetic relationships in the family Fundulidae but have not included the starhead

topminnows (Bernardi et al. 2007; Duvernell et al. 2007; Whitehead 2009; Nunez and Oleksiak 2016). Mitogenomes have not been annotated for any of the starhead topminnows (*F. dispar*, *F. blairae*, *F. lineolatus*, *F. escambiae*, and *F. nottii*). Here we use the first complete mitochondrial genome of *Fundulus dispar* (Agassiz 1854), the Starhead Topminnow, (Cyprinodontiformes: Fundulidae) to resolve placement of the starhead topminnows in Fundulidae.

Materials and methods

A male specimen of *Fundulus dispar* was captured alive in the Running Slough system in Kentucky, USA (36°31'50.0"N, 89°18'09.2"W); it was anesthetized using clove oil following Institutional Animal Care and Use Committee (IACUC; PROTO201900195) and scientific collecting protocols (2000231040720-133637; Figure 1). Three other species of *Fundulus* co-occur with *F. dispar*: *F. notatus*, *F. olivaceus*, and *F. chrysotus* (Boschung and Mayden 2004). The specimen of *F. dispar* was distinguished from congeners using the following characteristics. *Fundulus notatus* and *F. olivaceus* differ from *F. dispar* in the presence of a strong lateral stripe on

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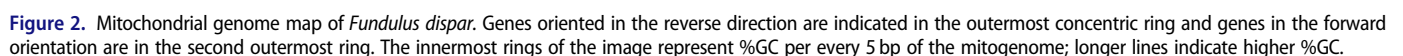
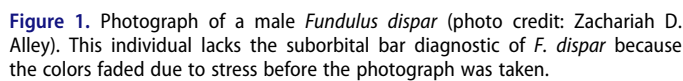
 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/23802359.2024.2327564>.

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the flanks in both males and females; *F. dispar* lacks these scattered spots and instead has thin, organized rows of spots. In addition, *F. dispar* possesses dark, suborbital, triangular bars in wild, reproductive adults, while the other three species lack this bar.

Sequencing by ligation was performed using Oxford Nanopore Technology on a MinION paired with a Flongle adapter (Oxford Nanopore, Oxford, UK). A DNA library for sequencing was prepared using a Ligation Sequencing Kit according to the manufacturer's instructions (Oxford Nanopore, Oxford, UK). Sequencing was completed with



MinKNOW v.21.02 and basecalled in Guppy v.4.4.2 under the high-accuracy basecalling model. Reads were filtered using Geneious Prime v.2021.1 according to the default quality standards for the software, and mitochondrial reads were isolated from nuclear reads by mapping to a *Fundulus olivaceus* (AP006776) reference sequence. Assembly was performed using Medium/Fast sensitivity and iterative fine-tuning (see Figure S1 for read depth). Aligned reads were combined into a consensus sequence. Genome annotation was performed in MitoAnnotator v.3.65 (Iwasaki et al. 2013; Sato et al. 2018). Protein coding gene start and stop codons were verified in Geneious Prime. The annotated mitochondrial genome is openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov> (MZ286764). We confirmed species identification of the specimen by performing an NCBI BLAST search using the *cytb* gene as the query (Altschul et al. 1990). The best match was to *F. dispar* with a percent identity of 99.8 (GQ119707.1; Whitehead 2010). In phylogenetic analysis, mitogenomes from all available members of the family Fundulidae were

used. An outgroup taxon closely related to the family Fundulidae (*Cyprinodon variegatus*) was chosen based on previous usage in literature and the results of an NCBI BLAST search (Whitehead 2009; Ghedotti and Davis 2013). Concatenated protein coding sequences were aligned with the MAFFT server v.7 (Katoh et al. 2002; Katoh and Standley 2013). A maximum likelihood phylogenetic tree was reconstructed in a partitioned analysis using IQ-TREE v.2.1.2 on the CIPRES Science Gateway (Miller et al. 2010; Nguyen et al. 2015). The substitution model and evolutionary rate of each protein coding sequence was selected under the edge-proportional partition model based on BIC scores (Table S1). The analysis was run with 1,000 bootstrap replications.

Results

The circular, mitochondrial genome of *F. dispar* is 16,564 bp long. It is composed of 13 protein-coding genes, two ribosomal RNAs (rRNA), 22 transfer RNAs (tRNA), and one control

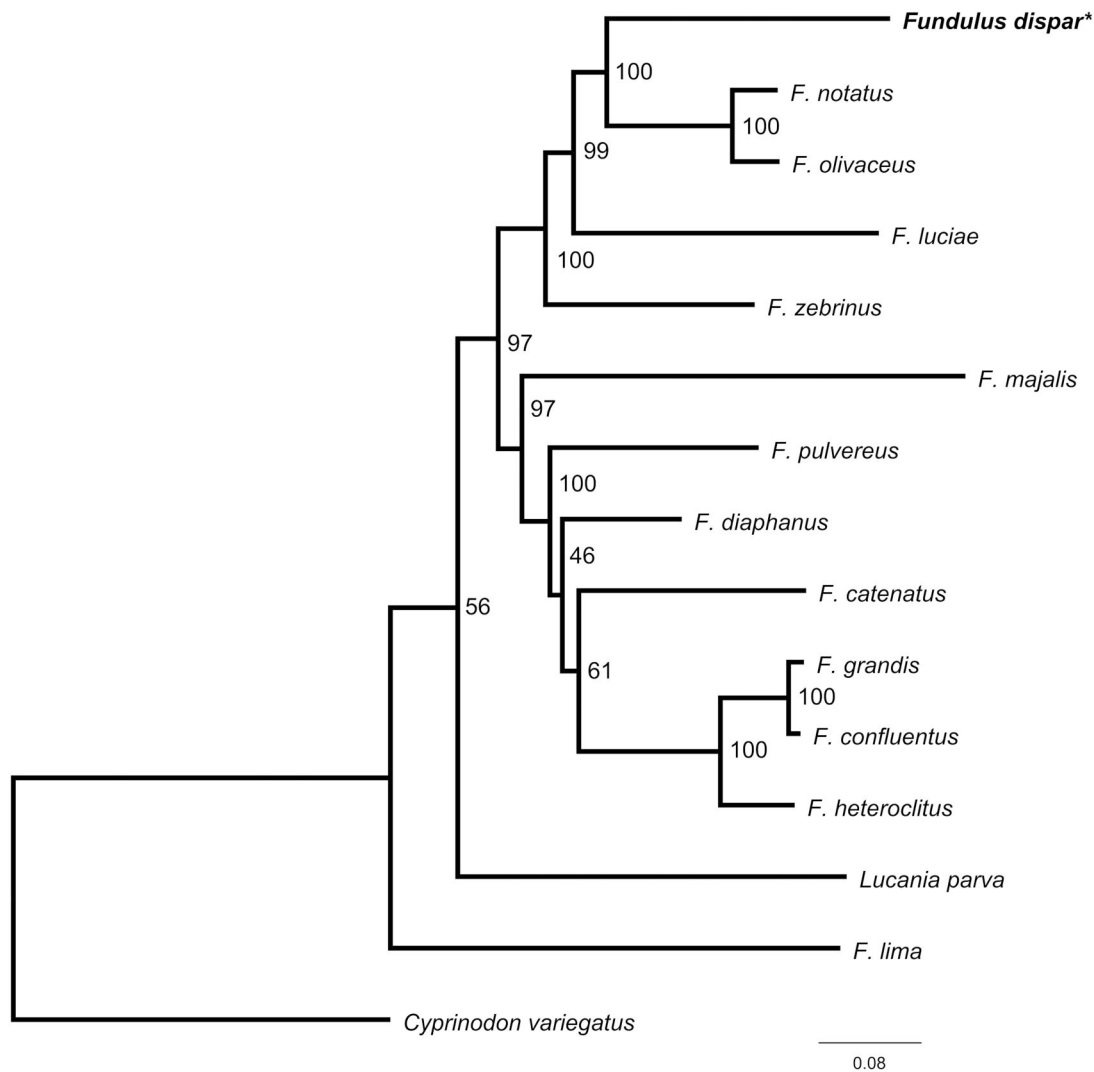


Figure 3. Maximum likelihood phylogeny reconstructed using concatenated mitochondrial coding sequences and 1,000 bootstrap replicates. Substitution models for each partition are in Table S1. The following sequences were used: *Fundulus dispar*-MZ286764, *F. notatus*-KP013106 (unpublished), *F. olivaceus*-AP006776 (Setiamarga et al. 2008), *F. luciae*-OR546168 (unpublished), *F. zebrinus*-MW300328 (unpublished), *F. majalis*-OR582709 (unpublished), *F. pulvereus*-OR546223 (unpublished), *F. diaphanus*-FJ445394 (Whitehead 2009), *F. catenatus*-OR552045 (unpublished), *F. grandis*-FJ445396 (Whitehead 2009), *F. confluentus*-OP035105 (unpublished), *F. heteroclitus*-FJ445402 (Whitehead 2009), *Lucania parva*-OP056801 (unpublished), *F. lima*-MW033979 (unpublished), and *Cyprinodon variegatus*-KT288182 (unpublished). Bootstrap values are indicated on nodes. The scale bar represents the number of nucleotide substitutions per site. The sequence generated in this study is written in bold font and marked with an asterisk.

region (D-loop) (Figure 2). The mitogenome included 29 forward and nine reverse gene orientations. Nucleotide composition is as follows: 28.0% A, 25.2% C, 15.9% G, 30.8% T and 0.1% ambiguous bases (i.e. Y and N). Ten protein-coding genes use the start codon ATG (*nd2*, *nd4l*, *nd6*, *nd5*, *atp8*, *cytb*, *cox3*, *cox2*, *nd1*, *nd4*), two use GTG (*atp6*, *cox1*), and one ATA (*nd3*). Six protein-coding genes (*nd1*, *cox1*, *atp8*, *nd4l*, *nd5*, *nd6*) end with the complete TAA stop codon and seven (*nd2*, *cox2*, *atp6*, *cox3*, *nd3*, *nd4*, *cytb*) end with an incomplete stop codon which is completed by the addition of 3' A residues. The maximum likelihood phylogenetic tree (Figure 3) recovered a group containing *F. notatus* and *F. olivaceus* as the sister species to *F. dispar*. The relationship between the genus *Fundulus* and *Lucania parva* is not well resolved as there is low bootstrap support for this group.

Discussion

The arrangement and number of genes in the mitochondrial genome of *F. dispar* followed other closely related taxa. We show strong bootstrap support for a sister relationship between *F. dispar* and a group comprised of *F. notatus* and *F. olivaceus*. This lends further evidence to that in literature suggesting the starhead topminnows are sister to the *F. notatus* species group. The genus *Fundulus* is not fully resolved, including the relationship with *Lucania parva*. Others have suggested a non-neutral divergence of *Fundulus* mitochondrial genes as an explanation for the lack of resolution (Parenti 1981; Wiley 1986; Bernardi 1997; Whitehead 2010). This mitogenome and phylogenetic analysis provide an opportunity to conduct further studies on evolutionary selection in this group of fishes and to address further taxonomic resolution with closely related taxa.

Acknowledgements

The photograph of *Fundulus dispar* was provided by co-author Zachariah D. Alley.

Ethical approval

All research followed protocols approved and promoted by the University of West Alabama and Mississippi State University. Work was conducted following the Institutional Animal Care and Use Committee (IACUC) protocols (PROTO201900195). This project was performed in adherence with ARRIVE guidelines (<https://arriveguidelines.org/arrive-guidelines>).

Authors' contributions

The research project was designed by MWS with contributions from all authors. The specimen was collected by ZDA; DNA extraction and sequencing were performed by JDL; annotation was completed by JDL, KMF, and MWS. The paper was written by KMF with contributions from all authors. All authors give final approval of this version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure statement

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

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Data availability statement

The data that support the findings of this study are openly available in GenBank of NCBI at <https://www.ncbi.nlm.nih.gov>, reference number MZ286764. The associated BioProject, SRA, and BioSample numbers are PRJNA742674, SRR19860324, SRR27304809, and SAMN29365559.

References

- Agassiz L. 1854. Notice of a collection of fishes from the southern bend of the Tennessee River, Alabama. *Am JSci Arts*. 17:297–308. 353–365.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol*. 215(3):403–410. doi:10.1016/S0022-2836(05)80360-2.
- Bernardi. 1997. Molecular phylogeny of the Fundulidae (Teleostei, Cyprinodontiformes) based on the cytochrome *b* gene. In: Kocher TD and Stepien CA, editors. *Molecular systematics of fishes* (p. 189–197. New York: Academic Press.
- Bernardi G, Powers DA. 1995. Phylogenetic relationships among nine species from the genus *Fundulus* (Cyprinodontiformes, Fundulidae) inferred from sequences of the cytochrome *b* gene. *Copeia*. 1995(2): 469–473. doi:10.2307/1446912.
- Bernardi G, Ruiz-Campos G, Camarena-Rosales F. 2007. Genetic isolation and evolutionary history of oases populations of the Baja California killifish, *Fundulus lima*. *Conserv Genet*. 8(3):547–554. doi:10.1007/s10592-006-9190-1.
- Boschung HT, Mayden RL. 2004. *Fishes of Alabama*. Washington, DC: Smithsonian Books.
- Burnett KG, Bain LJ, Baldwin WS, Callard GV, Cohen S, Di Giulio RT, Evans DH, Gómez-Chiarri M, Hahn ME, Hoover CA, et al. 2007. *Fundulus* as the premier teleost model in environmental biology: opportunities for new insights using genomics. *Comp Biochem Physiol Part D Genomics Proteomics*. 2(4):257–286. doi:10.1016/j.cbd.2007.09.001.
- Cashner RC, Schaefer J, Warren ML, Jr, Echelle AA, Galvez F, Ghedotti MJ. 2020. *Fundulidae: topminnows*. In: ML Warren, Jr. and BM Burr, with AA Echelle, BR Kuhajda, and ST Ross, editors. *Freshwater Fishes of North America*. Baltimore, MD: Johns Hopkins University Press; p. 549–608.
- Drown MK, Oleksiak MF, Crawford DL. 2023. Trans-acting genotypes associated with mRNA expression affect metabolic and thermal tolerance traits. *Genome Biol Evol*. 15(7):evad123. doi:10.1093/gbe/evad123.
- Duvernell DD, Schaefer JF, Hancks DC, Fonoti JA, Ravanelli AM. 2007. Hybridization and reproductive isolation among syntopic populations of the topminnows *Fundulus notatus* and *F. olivaceus*. *J Evol Biol*. 20(1):152–164. doi:10.1111/j.1420-9101.2006.01213.x.
- Ghedotti MJ, Davis MP. 2013. Phylogeny, classification, and evolution of salinity tolerance of the North American Topminnows and Killifishes, Family Fundulidae (Teleostei: Cyprinodontiformes). *Fieldiana Life Earth Sci*. 7:1–65. doi:10.3158/2158-5520-12.7.1.

- Iwasaki W, Fukunaga T, Isagozawa R, Yamada K, Maeda Y, Satoh TP, Sado T, Mabuchi K, Takeshima H, Miya M, et al. 2013. MitoFish and MitoAnnotator: a mitochondrial genome database of fish with an accurate and automatic annotation pipeline. *Mol Biol Evol.* 30(11): 2531–2540. doi:10.1093/molbev/mst141.
- Johnson LK, Sahasrabudhe R, Gill JA, Roach JL, Froenicke L, Brown CT, Whitehead A. 2020. Draft genome assemblies using sequencing reads from Oxford Nanopore Technology and Illumina platforms for four species of North American *Fundulus* killifish. *Gigascience.* 9(6):1–8. doi: 10.1093/gigascience/giaa067.
- Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30(14):3059–3066. doi:10.1093/nar/gkf436.
- Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7. Improvements in performance and usability. *Mol Biol Evol.* 30(4):772–780. doi:10.1093/molbev/mst010.
- Kreiser B. 2001. Mitochondrial cytochrome *b* sequences support recognition of two cryptic species of plains killifish, *Fundulus zebrinus* and *Fundulus kansae*. *American Midland Naturalist.* 146(1):199–209. doi:10.1674/0003-0031(2001)146[0199:MCBSSR]2.0.CO;2.
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. 2010 Gateway Computing Environments Workshop (GCE). 1:1–8. doi:10.1109/GCE.2010.5676129.
- Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a Fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol.* 32(1):268–274. doi:10.1093/molbev/msu300.
- Nunez JCB, Oleksiak MF. 2016. A cost-effective approach to sequence hundreds of complete mitochondrial genomes. *PLOS ONE.* 11(8): e0160958. doi:10.1371/journal.pone.0160958.
- Parenti LR. 1981. A phylogenetic and biogeographic analysis of Cyprinodontiform fishes Teleostei, Atherinomorpha. *Bull A Mus Nat Hist.* 168:335–557.
- Sato Y, Miya M, Fukunaga T, Sado T, Iwasaki W. 2018. MitoFish and MiFish Pipeline: A mitochondrial genome database of fish with an analysis pipeline for environmental DNA metabarcoding. *Mol Bio Evol.* 35(6):1553–1555. doi:10.1093/molbev/msy074.
- Setiamarga DHE, Miya M, Yamanoue Y, Mabuchi K, Satoh TP, Inoue JG, Nishida M. 2008. Interrelationships of Atherinomorpha (medakas, flyingfishes, killifishes, silversides, and their relatives): The first evidence based on whole mitogenome sequences. *Mol Phylogenet Evol.* 49(2):598–605. doi:10.1016/j.ympev.2008.08.008.
- Whitehead A. 2009. Comparative mitochondrial genomics within and among species of killifish. *BMC Evol Biol.* 9(1):11. doi:10.1186/1471-2148-9-11.
- Whitehead A. 2010. The evolutionary radiation of diverse osmotolerant physiologies in killifish (*Fundulus* Sp.). *Evolution.* 64(7):2070–2085. doi: 10.1111/j.1558-5646.2010.00957.x.
- Wiley EO. 1986. A Study of the evolutionary relationships of *Fundulus* topminnows (Teleostei: Fundulidae). *Am Zool.* 26(1):121–130. doi:10.1093/icb/26.1.121.