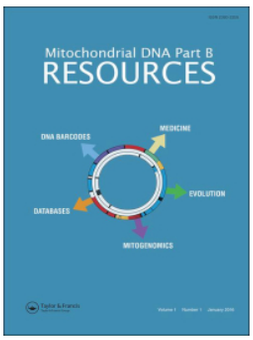

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MITOGENOME ANNOUNCEMENT

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Complete mitochondrial genome of a livebearing freshwater fish (Cyprinodontiformes: Poeciliidae): *Poecilia parae*

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ABSTRACT

Members of the fish family Poeciliidae (livebearing 'tooth-carps') have historically been used as models in medical research, behavior ecology, and biological control. This group of primarily freshwater fishes is highly tolerant to environmental factors such as salinity and warm temperatures and includes some invasive species. Here, we present the mitochondrial genome of *Poecilia parae*. A representative of this species was obtained from Suriname. The complete mitochondrial genome was sequenced using Oxford Nanopore technology and is 16,559 bp long. The genome contains 13 protein-coding genes, two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs), and one control region (D-loop). Phylogenetic analysis yielded topologies similar to those previously published. The data generated here will be useful in future studies of comparative biology and those utilizing environmental DNA (eDNA).

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Poecilia parae; Oxford Nanopore; mitochondrial genome; Poeciliidae

Introduction

For many decades, livebearing fishes of the family Poeciliidae have been valuable models for research in evolutionary ecology and comparative biology. Specifically, the Guppy (*Poecilia reticulata*) and Southern Platyfish (*Xiphophorus maculatus*) have served as indicator taxa and as models for behavioral ecology, life history evolution, and cancer biology (Schartl 2014; Reznick et al. 2017; Goldberg et al. 2019; Gomes-Silva et al. 2020). Poeciliids, including some invasive species, can use a wide range of habitats because they are successful colonizers and have high thermal and salinity tolerances (Meffe and Snelson 1989). Here, we present the mitochondrial genome of a lesser-known species with close phylogenetic affinity to *P. reticulata*, *P. parae*. We anticipate that the mitogenome presented here will aid future research in comparative biology and will be useful for noninvasive investigations of watersheds using environmental DNA (eDNA).

Poecilia parae (Eigenmann, 1894) occupies a geographic range from Guyana to northern Brazil (Figure 1). *Poecilia parae* is a novel model system for the study of sex chromosome evolution and sexual polymorphism (Metzger et al. 2021; Sandkam et al. 2021). The International Union for Conservation of Nature (IUCN) has not evaluated the conservation status of *P. parae*. Congeners of *Poecilia* in the genus *Xiphophorus* are important models for the study of sexual dimorphism, sex chromosome evolution, and carcinogenesis (Schartl 1990; Woolcock et al. 2006; Schartl and Walter 2016). While many studies have examined the evolutionary history of Poeciliids in the contexts of ornamentation and sexual selection, few have used complete mitochondrial data (Morris et al. 2001; Cui et al. 2013; Kang et al. 2013; Goldberg et al. 2019; Méndez-Janovitz et al. 2019; Metzger et al. 2021; Sandkam et al. 2021).

Figure 1. Representative photograph of *Poecilia parae* (blue melanzona morph).

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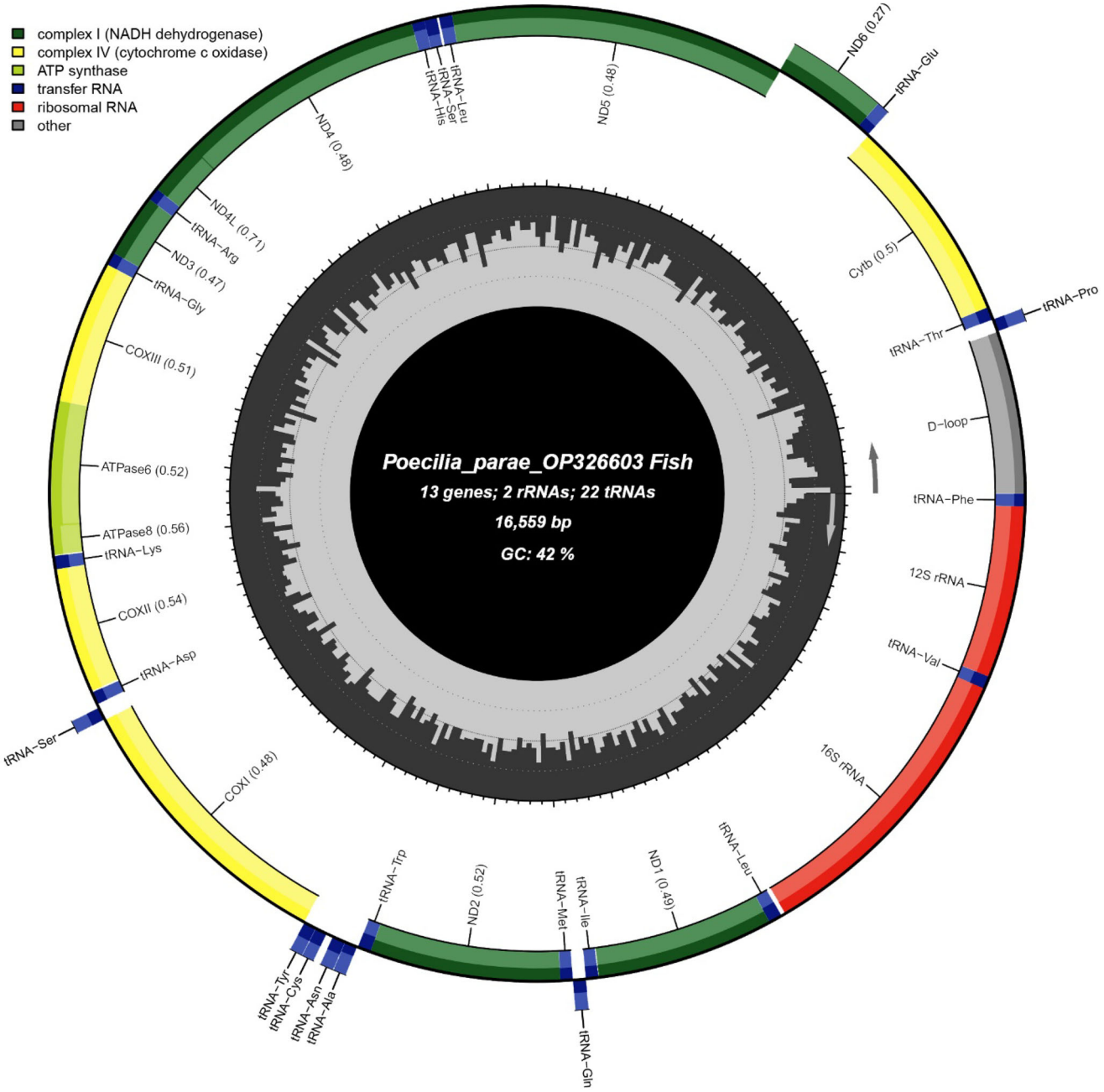

Figure 2. Mitochondrial genome map of *Poecilia parae*. The innermost circle of the image represents %GC per every 5 bp of the mitogenome; longer lines indicate higher %GC.

Materials and methods

An aquarium trade specimen of *Poecilia parae* (blue melanzona morph) was obtained from Suriname (5°51′36″N, 55°7′48″W). The preserved specimen was deposited in the University of West Alabama Zoological Collection (https://www.uwa.edu/, kaylafast0@gmail.com) under voucher number AR20090201:03. Whole genomic DNA was extracted from the pectoral fin using the DNeasy Blood and Tissue Kit following the manufacturer's instructions (QIAGEN, Hilden, Germany). DNA quality was confirmed by gel electrophoresis using a 1.5% agarose gel stained with ethidium bromide. The quantity of DNA was determined using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Purified DNA was stored at 4 °C.

The sequencing library was prepared using the Oxford Nanopore Ligation Sequencing Kit and loaded onto a Flongle flow cell following the manufacturer's instructions (Oxford Nanopore, Oxford, UK). Sequencing was performed on a MinION device using the Flongle adapter and monitored with MinKNOW software v.22.08.9 (Figure S1; Oxford Nanopore, Oxford, UK). Basecalling was done in Guppy v.6.2.11 using the high-accuracy basecalling model and reads filtered to a minimum qscore = 9. Reads were assembled using Geneious Prime v.2022.2.2 under the Medium/Fast sensitivity setting and iterative fine-tuning. The *P. reticulata* mitochondrial genome (KJ460033) was selected as a reference sequence. A consensus sequence was generated using a strict 50% threshold and then checked by eye and ambiguous base calls resolved in BioEdit v.7.2.5 (Hall 1999; Hall and Alzohairy 2011). The genome was

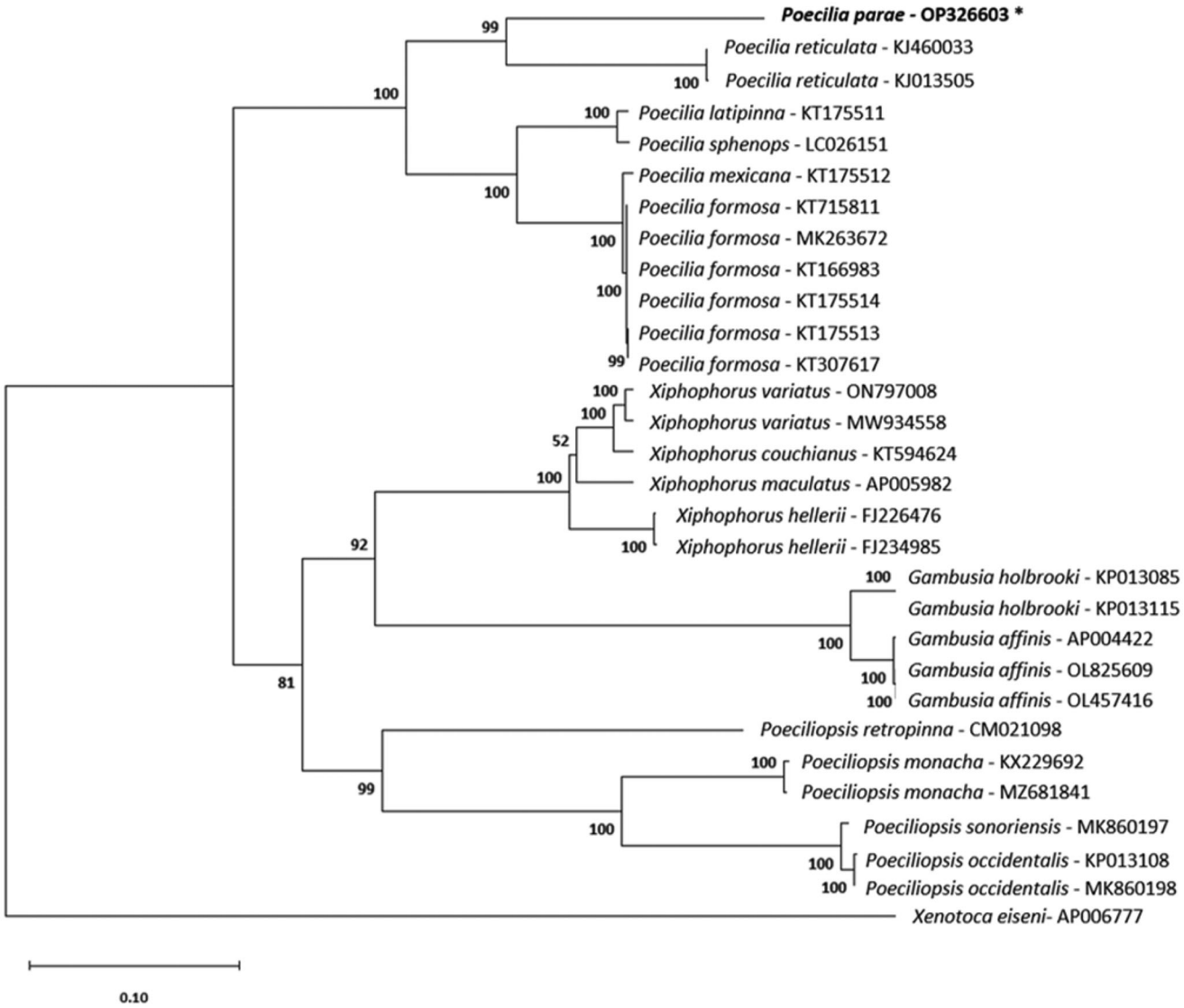

Figure 3. Maximum-likelihood phylogeny reconstructed using mitochondrial protein-coding sequences under the GTR + G+I model and 1000 bootstrap replicates. The following sequences were used: AP005982 (Miya et al. 2003), KT594624 (Zhang et al. 2016), MW934558 (Eastis et al. 2021), ON797008, FJ226476 (Bai et al. 2009), FJ234985 (Bai et al. 2009), CM021098 (van Kruistum et al. 2020), KJ013505 (Kong et al. 2016), KJ460033 Künstner et al. (2016), OP326603 (this study), KT166983 (Dang et al. 2016), KT175513, KT307617 (Sung et al. 2016), KT715811, MK263672, KT175514, KT175512, KT175511, LC026151 (Jiang et al. 2016), KX229692 (Jeon et al. 2016), MZ681841, MK860197 (Mateos et al. 2019), KP013108, MK860198 (Mateos et al. 2019), OL825609, OL457416, AP004422 (Miya et al. 2003), KP013085, KP013115, and AP006777 (Setiamarga et al. 2008). Numbers on nodes are bootstrap support values. The sequence generated in this study is written in bold font and marked with an asterisk.

annotated in MitoAnnotator v.3.75 (Iwasaki et al. 2013; Sato et al. 2018). The presence of appropriate start and stop codons in protein coding genes was confirmed and internal stop codons resolved in MEGA11: Molecular Evolutionary Genetics Analysis v.11.0.10 (Tamura et al. 2021). The annotated mitochondrial genome is openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov (OP326603). Congeneric species were identified using NCBI BLAST (Altschul et al. 1990; Miya et al. 2003; Setiamarga et al. 2008; Bai et al. 2009; Dang et al. 2016; Jeon et al. 2016; Jiang et al. 2016; Kong et al. 2016; Künstner et al. 2016; Sung et al. 2016; Zhang et al. 2016; Mateos et al. 2019; van Kruistum et al. 2020; Eastis et al. 2021). Concatenated protein-coding sequences from the congener mitochondrial genomes and a *Xenotoca eiseni* outgroup were aligned using the MAFFT server v.7 before phylogenetic analysis (Katoh et al. 2002; Katoh and Standley 2013). Model selection and evolutionary analysis by the maximum-likelihood method were performed in MEGA11. A maximum-likelihood phylogenetic tree was reconstructed using the general time reversible model with gamma and invariable sites allowed and 1000 bootstrap replications.

Results

The mitochondrial genome of *P. parae* is 16,559 bp long. The nucleotide composition of the *P. parae* mitochondrial genome is 29.70% A, 27.25% C, 14.80% G, and 28.26% T. The genome is circular, consisting of 13 protein-coding genes, two ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs), and one control region (D-loop; Figure 2). The *P. parae* mitochondrial genome contains 29 forward genes and nine reverse genes; all protein-coding genes use the start codon ATG. Seven protein-coding genes in the *P. parae* mitochondrial genome (ND1, COI, ATP8, ND4L, ND5, ND6, and CYTB) end

with the complete TAA stop codon and six (ND2, COII, ATP6, COIII, ND3, and ND4) end with an incomplete stop codon which is completed by the addition of 3′ A residues.

Discussion

Phylogenetic analysis using the maximum-likelihood method places the genera *Xiphophorus*, *Poecilia*, *Gambusia*, and *Poeciliopsis* each as monophyletic groups (Figure 3). Our data place *P. parae* as the sister group to *P. reticulata*, the Guppy. The phylogenetic tree topology of poeciliid genera is consistent with recent phylogenetic studies performed on whole poeciliid mitochondrial genomes (Pollux et al. 2014; Jeon et al. 2016; Eastis et al. 2021) and one-to-one orthologs (Mateos et al. 2019). Previous phylogenetic studies conducted with a more exhaustive sampling of *Poecilia* support the placement of *P. parae* (Pollux et al. 2014; Méndez-Janovitz et al. 2019; Metzger et al. 2021; Sandkam et al. 2021). A wider representation of *Poecilia* spp. in complete mitochondrial data will further resolve the positions of these taxa. The mitochondrial genome that we generated will be conducive to monitoring species presence using eDNA and aid in future research in comparative biology.

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Ethical approval

Ethical approval for research involving vertebrates in this study was provided by the Institutional Animal Care and Use Committee (IACUC) under IACUC ID: AMEND202000173/PROTO201900195.

Author contributions

The research project was designed by MWS with contributions from all authors. DNA extraction and sequencing were performed by AWR; annotation was completed by AWR and KMF. 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

The authors have no conflicts of interest to declare.

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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 OP326603. The associated BioProject, SRA, and BioSample numbers are PRJNA742674, SRR21296950, and SAMN30561436, respectively.

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