



Mitochondrial Genome Characteristics and Implications for Biodiversity of Tropical Freshwater Fish *Hyphessobrycon columbianus* in the Minjiang River

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ABSTRACT

We evaluated the mitochondrial genome characteristics of tropical freshwater fish *Hyphessobrycon columbianus*, analyzed the phylogenetic relationships among Characidae species, and explored the taxonomic status and potential biodiversity conservation measures. The mitochondrial genome sequence of *H. columbianus* collected in Fuzhou, Fujian, China was obtained by high-throughput sequencing after morphological identification. Phylogenetic trees were constructed using the Bayesian inference and maximum likelihood methods. The mitochondrial genome sequence of *H. columbianus* (GenBank ID: OP613012) has a total length of 16,645 bp, and encodes 37 genes, including 13 protein-coding genes, 22 transfer RNA genes, two ribosomal RNA genes, and an AT-rich control region. The A, T, G, and C contents in the entire mitochondrial genome of *H. columbianus* are 29%, 29.7%, 15.9%, and 25.4%, respectively, indicating significant AT bias. The mitochondrial genome characteristics of *H. columbianus* are consistent with those of most known fish species. At the family classification level, the trees obtained using the two methods revealed similar tree topologies. *H. columbianus* and *H. elachys* converged into one branch. The complete mitochondrial genome of *H. columbianus* disclosed in this study provides a molecular biology reference for exploring the mitochondrial whole genomes of other *Hyphessobrycon* species, and provide basic data for the later biodiversity investigation in the Minjiang region.

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Authors' Contribution

QZ, JZ, and XC designed the study. JZ and XC executed experimental work. JZ and XC analyzed the data. QZ wrote the paper. QZ provided the laboratory equipment. QZ supervised the research.

Key words

Complete mitochondrial genome, Phylogeny, *Hyphessobrycon columbianus*

INTRODUCTION

Mitochondria are double-membrane organelles found in eukaryotic cells. The biochemical reactions that occur within mitochondria provide energy for normal cellular activities; therefore, mitochondria are known as the power workshop of the cell (Osellame *et al.*, 2012). Mitochondria are semi-autonomous organelles that, in addition to being regulated by the nuclear genome, possess their own genetic material and genetic system (Nass and Nass, 1963). In fish, the mitochondrial genome is a covalently closed circular double-stranded DNA molecule, generally 14–21 kb in length and stably encoding 37 genes, including 13 protein-coding genes (PCGs), 22 transfer (t) RNA-coding genes, and two ribosomal (r)RNA coding genes, as well as an AT-rich non-coding control region

(Cameron, 2014). Compared to the nuclear genome, the mitochondrial genome has a relatively small molecular weight, maternal inheritance, fast evolution rate, and no introns (Zimmer and Wen, 2015). It is widely used as an effective molecular marker in species identification, systematic geography, population genetics, and phylogenetic biology research (Sun *et al.*, 2021).

Hyphessobrycon columbianus, which belongs to the order Characiformes and family Characidae, is an omnivorous fish. It is distributed in the Acadi River drainage (Caribbean slope of Colombia) and drainages in San Blas province (Southeastern Panama) in South America (Garcia-Alzate *et al.*, 2010). It is large in size, with a maximum length of 7 cm. Its initial body colors are dark gray and bright silver, with shiny and opaque scales and transparent or yellow fins. After coloring, male *H. columbianus* fish have a blue-green metallic reflection on the body surface, and their fins turn red and the tail fins deep red. Because of their colorful appearance, they are known as red-tail dream lamp. Because of its relatively large body size, it has a significant advantage in terms of height and weight compared to light fish and therefore often wins in combative behavior (Lima and Moreira, 2003).

At present, the classification of Characidae is uncertain. We used next-generation sequencing

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technology to sequence the complete mitochondrial genome of *H. columbianus*, which was then analyzed for its basic structural composition and annotated. Our aim was to provide a basis and reference for mitochondrial genome mining in other *Hyphessobrycon* species and novel molecular evidence for the systematic evolution and biodiversity of *Hyphessobrycon* in Characidae.

MATERIALS AND METHODS

Sample collection

This study aimed to investigate the impact of ornamental fish on biodiversity conservation. *H. columbianus* was collected in January 2023 at the Fuzhou Flower and Bird Market in Cangshan, Fuzhou, Fujian, China, and was also found for the Minjiang River basin in Fujian, China. The collected samples were placed in a 10-mL centrifuge tube containing 95% ethanol, transferred to the laboratory, and stored at -20°C until use. We referred to relevant books for the morphological identification of the collected samples.

DNA extraction and sequencing

Total DNA was extracted from the pectoral fin muscle tissue of *H. columbianus* based on the magnetic bead method using an Animal Genome DNA Extraction Kit [Bioengineering (Shanghai) Co., Ltd.]. The integrity of the total DNA was assessed using 1% agarose gel electrophoresis, and DNA quality was evaluated using a NanoDrop 2000 instrument (Thermo). The qualified samples were sent to Bioengineering (Shanghai) Co., Ltd. for sequencing using an the NovaSeq6000 platform (Illumina, USA) for second-generation sequencing and assembly.

Data analysis

The spliced complete mitochondrial genome was uploaded to the MITOS web server (<http://mitos2.bioinf.uni-leipzig.de/index.py>). Preliminary annotation was conducted according to [Bernt *et al.* \(2013\)](#). The 13 PCGs were annotated using the ORF website (http://www.bioinformatics.org/sms2/orf_find.html). The genome positions of tRNAs and rRNAs were determined using the RNAfold website (<http://rna.tbi.univie.ac.at/cgi-bin/rnawebsite/rnafold.cgi>). MEGA-X was used to analyze the base composition and codon usage, and base composition bias was calculated using the formulas $\text{AT skew} = (A - T)/(A + T)$ and $\text{GC skew} = (G - C)/(G + C)$. The secondary structures of the tRNAs were predicted using MITOS and tRNAscan-SE ([Lowe and Eddy, 1997](#)). The secondary structure of rRNAs were predicted using MITOS ([Bernt *et al.*, 2013](#)) and compared with those of

nearby species. Based on the mitochondrial genomes of 39 fish species belonging to 19 genera and 10 subfamilies of Characidae, phylogenetic trees were constructed using the Bayesian inference (BI) and maximum likelihood (ML) methods, using *Oliotius oligolepis* (Cyprinidae) ([Sun *et al.*, 2023](#)) as an outgroup. The 13 PCGs were aligned using PhyloSuite v1.2.1 ([Zhang *et al.*, 2020](#)) in MAFFT and then, the sequences were trimmed using Gblocks ([Talavera and Castresana, 2007](#)). After concatenation, the optimal model of sequence evolution was determined using Model Finder ([Kalyaanamoorthy *et al.*, 2017](#)). In Bayesian inference, four Markov chain Monte Carlo algorithms were run for 1,000,000 generations, with samples taken every 100 generations and 25% of aging samples being discarded. The confidence level of each branch node in the maximum likelihood phylogenetic tree was determined using the ultrafast bootstrap approach, with 10,000 repeated samples.

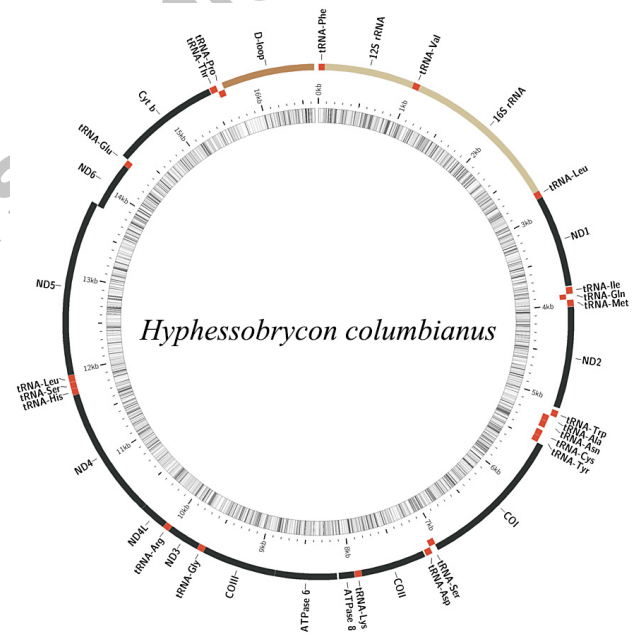


Fig. 1. Structure of the mitochondrial genome of *Hyphessobrycon columbianus*.

RESULTS

The total length of the mitochondrial genome of *H. columbianus* is 16,645 bp ([Fig. 1](#), [Table I](#)). The genome comprises 37 genes, including 13 PDGs, 22 tRNA genes, and two rRNA genes, and one AT-rich region and forms a closed double-stranded circular structure. Among the 37 genes, one PCG (*nad6*) and eight tRNA genes (*trnQ*, *trnA*, *trnN*, *trnC*, *trnY*, *trnS2*, *trnE*, and *trnP*) are located on the L-chain, whereas the remaining 28 genes are located

Table I. Annotation of the mitochondrial genome of *Hyphessobrycon columbianus*.

Gene	Position		Size (bp)	Intergenic nucleotides	Codon		Strand
	From	To			Start	Stop	
<i>trnF (gaa)</i>	1	68	68	0			H
<i>rrnS</i>	69	1018	950	0			H
<i>trnV (tac)</i>	1019	1090	72	0			H
<i>rrnL</i>	1091	2753	1663	0			H
<i>trnL2 (taa)</i>	2754	2828	75	0			H
<i>nad1</i>	2829	3797	969	10	ATG	TAA	H
<i>trnI (gat)</i>	3808	3879	72	-1			H
<i>trnQ (ttg)</i>	3879	3934	56	7			L
<i>trnM (cat)</i>	3942	4013	72	1			H
<i>nad2</i>	4015	5079	1065	31	ATG	TAA	H
<i>trnW (tca)</i>	5111	5180	70	6			H
<i>trnA (tgc)</i>	5187	5255	69	1			L
<i>trnN (gtt)</i>	5257	5329	73	31			L
<i>trnC (gca)</i>	5361	5427	67	-1			L
<i>trnY (gta)</i>	5427	5497	71	1			L
<i>cox1</i>	5499	7052	1554	-9	GTG	AGG	H
<i>trnS2 (tga)</i>	7044	7115	72	1			L
<i>trnD (gtc)</i>	7117	7185	69	15			H
<i>cox2</i>	7201	7888	688	0	ATG	T	H
<i>trnK (ttt)</i>	7889	7961	73	1			H
<i>atp8</i>	7963	8130	168	17	ATG	TAG	H
<i>atp6</i>	8148	8802	655	0	ATG	T	H
<i>cox3</i>	8803	9586	784	0	ATG	T	H
<i>trnG (tcc)</i>	9587	9657	71	0			H
<i>nad3</i>	9658	10006	349	0	ATG	T	H
<i>trnR (tcg)</i>	10007	10076	70	0			H
<i>nad4L</i>	10077	10373	297	-7	ATG	TAA	H
<i>nad4</i>	10367	11747	1381	0	ATG	T	H
<i>trnH (gtg)</i>	11748	11816	69	0			H
<i>trnS1 (gct)</i>	11817	11884	68	1			H
<i>trnL1 (tag)</i>	11886	11958	73	0			H
<i>nad5</i>	11959	13806	1848	-16	ATG	AGG	H
<i>nad6</i>	13791	14306	516	0	ATG	TAG	L
<i>trnE (ttc)</i>	14307	14374	68	3			L
<i>cytb</i>	14378	15511	1134	6	ATG	TAA	H
<i>trnT (tgt)</i>	15518	15591	74	-2			H
<i>trnP (tgg)</i>	15590	15658	69	-1			L
control region	15658	16645	988				H

on the H-chain. There are seven gene overlaps in the mitochondrial genome of *H. columbianus*, totaling 37 bp, with the longest overlap of 16 bp being between *nad5* and *nad6*. There are 15 gene intervals, totaling 132 bp. The A, T, G, and C contents in the complete mitochondrial genome of *H. columbianus* are 29%, 29.7%, 15.9%, and 25.4%, respectively. The AT content is 58.7% (Table II), and the GC content is 41.3%, indicating significant AT bias. AT and GC skewness are -0.012 and -0.230 , respectively, indicating that the T base content in the mitochondrial genome is higher than that of A, and the C content is higher than that of G.

Table II. Nucleotide composition of the complete mitochondrial genome of *Hyphessobrycon columbianus*.

Re-gions	Size (bp)	A	T	G	C	AT (%)	GC (%)	AT skew	GC skew
<i>atp6</i>	655	25.6	36.3	13.9	24.1	61.9	38	-0.172	-0.269
<i>atp8</i>	168	36.3	28.6	10.1	25	64.9	35.1	0.119	-0.424
<i>cox1</i>	1554	24.2	32.4	18.7	24.8	56.6	43.5	-0.144	-0.141
<i>cox2</i>	688	28.2	30.1	15.6	26.2	58.3	41.8	-0.032	-0.254
<i>cox3</i>	784	24.4	31.9	17.2	26.5	56.3	43.7	-0.134	-0.213
<i>cytb</i>	1134	26.6	34.3	13.8	25.2	60.9	39	-0.126	-0.291
<i>nad1</i>	969	28	30.8	14.2	27	58.8	41.2	-0.047	-0.31
<i>nad2</i>	1065	27	30.1	14.3	28.5	57.1	42.8	-0.054	-0.333
<i>nad3</i>	349	22.3	38.4	15.2	24.1	60.7	39.3	-0.264	-0.226
<i>nad4</i>	1381	28.1	31.3	14	26.6	59.4	40.6	-0.054	-0.312
<i>nad4L</i>	297	21.9	35.7	15.5	26.9	57.6	42.4	-0.24	-0.27
<i>nad5</i>	1848	29.4	31.8	12.9	25.8	61.2	38.7	-0.039	-0.332
<i>nad6</i>	516	16.5	39.1	30.8	13.6	55.6	44.4	-0.408	0.389
<i>rrnL</i>	1663	35.1	22.1	19.2	23.6	57.2	42.8	0.228	-0.101
<i>rrnS</i>	950	32.7	21.4	20.6	25.3	54.1	45.9	0.21	-0.101
Full genome	16645	29	29.7	15.9	25.4	58.7	41.3	-0.012	-0.23

The total length of the PCG sequences in the mitochondrial genome of *H. columbianus* is 11,408 bp, accounting for approximately 68.54% of the total mitochondrial genome sequence. The *nad5* gene is the longest (1,848 bp), whereas the *atp8* gene is the shortest (168 bp). The AT content of the 13 PCGs is greater than the GC content. Except for *cox1*, which starts with GTG, all PCGs start with the standard codon ATG. The *cox1* and *nad6* genes terminate with AGG, whereas *cox2*, *atp6*, *cox3*, *nad3*, and *nad4* terminate with T, and the remaining termination codons are TAA/TAG. There are a total of 60 different codons, among which the most frequently used are AUU (Ile, 208 times), UUA (Leu, 2,181 times), CUU (Leu, 1,164 times), UUU (Phe, 161 times), CUA (Leu, 1,139 times), and AUA (Met, 127 times), all entirely composed of A or U (Fig. 2).

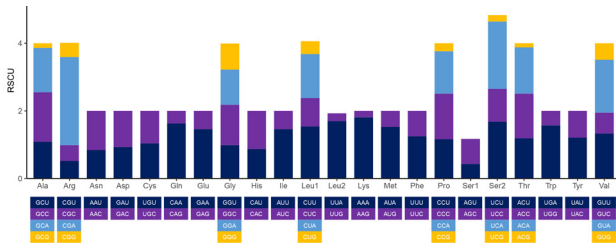


Fig. 2. Codon usage in protein-coding genes in the mitochondrial genome of *Hypessobrycon columbianus*.

The genome annotation results showed that all 22 tRNA genes have lengths ranging from 56 to 75 bp. There are two rRNA genes in the mitochondrial genome of *H.*

columbianus. The *rrnL* (1,363 bp) gene is located between *trnV* and *trnL2*, whereas the *rrnS* (950 bp) gene is located between the *trnF* and *trnV* regions, both of which have a high AT content. Based on the tandem sequences of the 13 PCGs in the mitochondrial genome. Using the BI and ML methods, the phylogenetic relationships of 40 fish species were analyzed, using *Oliotius oligolepis* (ON864407) as an outgroup. BI trees (Fig. 3) and ML trees (Fig. 4) were constructed. The two phylogenetic analysis methods yielded similar tree topologies, with *H. columbianus* and *H. elachys* converging into one branch, and the 39 Characidae 39 species being well separated from the outgroup. However, the clustering of species within the family was relatively chaotic.

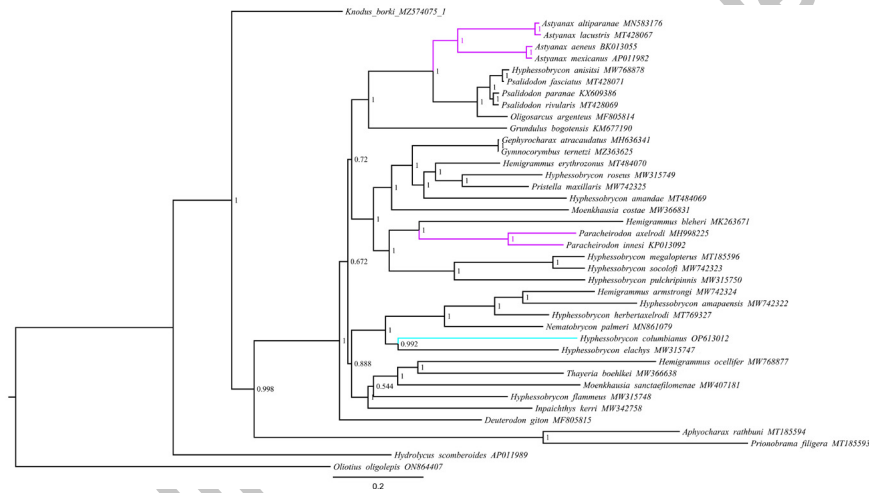


Fig. 3. Bayesian phylogenetic tree of Characidae based on the sequences of the 13 protein-coding genes in the mitochondrial genome.

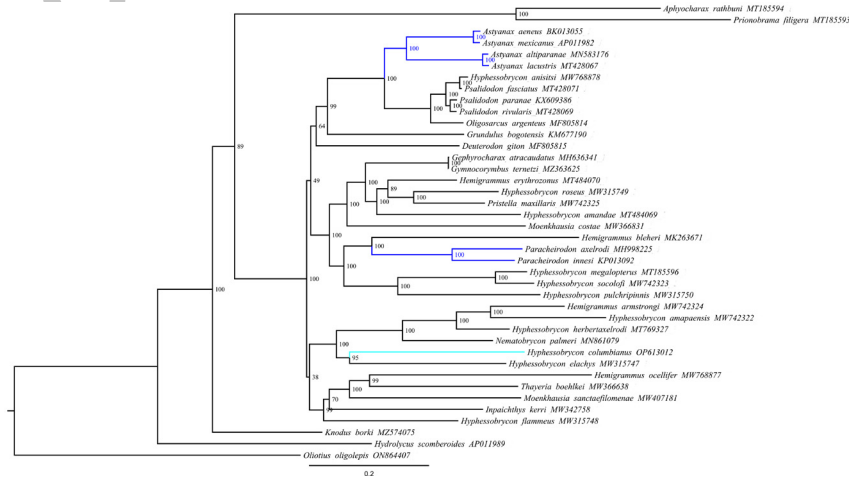


Fig. 4. Maximum likelihood phylogenetic tree of Characidae based on the sequences of the 13 protein-coding genes in the mitochondrial genome.

DISCUSSION

The *H. columbianus* mitochondrial genome assembled in this study is 16,645 bp in size, and there is no evidence of gene rearrangement in the mitochondrial structure. The GC content is 41.3%, indicating significant AT bias. This finding is consistent with the preference of mitochondrial base composition for bases A and T in teleost fish (Consuegra *et al.*, 2015; Sun *et al.*, 2022). The G content is 15.9%, showing significant anti-G bias, consistent with research results in most fish. Among the 13 PCGs, *cox1* uses GTG as the start codon, whereas the remaining genes use ATG, which has the highest translation efficiency (Consuegra *et al.*, 2015). The appearance of incomplete termination codons such as T-- in PCG termination codons is because these genes are followed by a gene encoded on the same chain, allowing transcription to terminate without a complete codon (Hecht *et al.*, 2017). The presence of incomplete termination codons is common in fish mitochondrial genomes (Sun *et al.*, 2021) and may be due to the addition of a poly(A) tail during mRNA processing (Liu *et al.*, 2009). The AT skew and GC skew of PCGs were negative. Generally, the amplitude of AT tilt is smaller than that of GC tilt, and in many cases, it is not statistically significant. Here, AT skew was lower than GC skew (absolute value), which is in line with traditional preferences (Yu *et al.*, 2019). The maximum value of AT skew and minimum value of GC skew both occurred in *atp8*, and the AT/GC skeleton value of this gene fluctuated greatly. Nucleotide bias may be due to the balance between mutation and selection pressures during replication and transcription, providing a potential direction for gene replication (Touchon *et al.*, 2008). The *rrnS* and *rrnL* genes in the mitochondrial genome of *H. columbianus* are located between the H-chain *trnF* and *trnL2* genes, with *trnV* in the interval. The 12S rRNA gene is more conserved than the 16S rRNA gene (Satoh *et al.*, 2016). The D-loop region is located between *trnP* and *trnF*, similar to the arrangement in most other vertebrates.

Constructing phylogenetic trees based on mitochondrial genomes to determine the evolutionary relationships of species has become a common method for phylogenetic analysis. Miya *et al.* (2015) summarized the development mitochondrial genomes and their contribution to phylogenetics over the past 15 years, suggesting that mitochondrial genome analysis and high-throughput sequencing are becoming important methods for species classification. We constructed a phylogenetic tree based on the 13 PCGs and analyzed the phylogenetic relationships of *H. columbianus*, using *Oliotius oligolepis* as an outgroup. The ML and BI trees indicated that *H. columbianus* and *H. elachys* converge into one branch,

consistent with morphological findings. This study provides fundamental support for the investigation of fish biodiversity in the Minjiang River. Related fish have been found in both the Minjiang River and the flower and bird markets, indicating the widespread release of ornamental fish in the region.

CONCLUSIONS

We determined the complete mitochondrial genome of *H. columbianus*, analyzed its structural characteristics, and constructed phylogenetic trees using the ML and BI methods. The results suggested that the taxonomic status of *H. columbianus* is close to that of *H. elachys*. Our study provided molecular data for exploring the phylogenetic origin and biodiversity evolution of the genus *Hyphessobrycon*. The mitochondrial genome data provided in this study provide a reference for mining the mitochondrial genomes of other *Hyphessobrycon* species and novel molecular evidence for the systematic evolution of *Hyphessobrycon* in Characidae. This study provides fundamental support for the investigation of fish biodiversity in the Minjiang River.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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