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MITOGENOME REPORT

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The complete mitochondrial genome of *Triplophysa erythraea* (Huang et al. 2019) (Cypriniformes, Nemacheilidae)

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ABSTRACT

In this study, the complete mitochondrial genome of *Triplophysa erythraea* was determined for the first time. Results showed the mitogenome was 16 565 bp, including 2 ribosomal RNA genes, 22 transfer RNA genes, 13 protein-coding genes and 2 non-coding regions. The overall base composition of its mitochondrial genome was 28.9% A, 25.2% T, 28.7% C and 17.2% G. Phylogenetic tree revealed that *Triplophysa erythraea* had the closest relationship with *Triplophysa xiangxiensis*, which was also found in Xiangxi Tujia and Miao Nationality Autonomous Prefecture, Hunan province. In general, this study provided valuable information for conservation genetics analyses of *Triplophysa erythraea* and further displayed the evolution of species within the genus *Triplophysa*.

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Cypriniformes; phylogenetics; mitochondrial genome; *Triplophysa erythraea*

Introduction

Triplophysa erythraea (Huang et al. 2019) is a blindfish found in Dalong cave, Huayuan County, Hunan Province, Central south China, belonging to the genus *Triplophysa* (Nemacheilidae, Cypriniformes), most of which is distributed in the Tibetan Plateau and adjacent areas (Jacobsen et al. 2017). Previous researches indicated *Triplophysa erythraea* is a demersal carnivorous fish living in the underground river where a mean water temperature and pH of 13.5 °C and 6.0, respectively. As a typical cavefish, it was eyes absent and scaleless, but presented a highly developed lateral line and barbel (Huang et al. 2019). *Triplophysa erythraea* can be recognized for its transparent body surface and visible red blood vessels, live adults were bright red while larvae were reddish white (Figure 1). It can be distinguished from other *Triplophysa* for special external characteristics, such as the distance between fins, smooth lip, absent pigments and long outer rostral barbel.

Mitochondrial genome was the most widely applied molecular marker in the study of fish taxonomy, genetic diversity and phylogeny, and was prevailing in fish population genetics (Lord et al. 2012). Despite mitochondrial *cytochrome b* (*cyt b*) gene sequencing of this species was carried out previously (Yan 2017), the complete mitochondrial genome sequence of *Triplophysa erythraea* was still not publicly available, which caused an obstacle to carrying out more genetic studies on this species. Therefore, we aimed to sequence the whole genome of *Triplophysa erythraea* and analyze the evolutionary relationship within the genus

Figure 1. Specimens of *Triplophysa erythraea* photographed separately from the back(left) and abdomen(right) (photographed by Yude Wang).

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Triplophysa based on 39 complete mitochondrial genomes of *Triplophysa*, which could provide basic information for further phylogenetic studies of *Triplophysa erythraea* and enrich the genetic biodiversity of *Triplophysa*.

Materials and methods

In this study, we have presented the complete mitochondrial genome of *Triplophysa erythraea*. The specimen was obtained in a living state in Dalong cave (N28°16′25.11″, E109° 28′57.18″, 563 m a. s. l.), Huayuan County, Hunan Province, Central south China in November 2023. We conducted anesthetic treatment to the specimen by ingesting MS-222(Sigma-Aldrich, St.Louis, MO, USA), and finally deposited it in the State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University (https://lifescience.hunnu.edu.cn/, Yude Wang, 461669793@qq.com) under voucher number 42703.The specimen of *Triplophysa erythraea* were preserved immediately after death, the muscle tissue taken from it was deposited in −80 °C environment at first and were used to extract DNA soon.

Total DNA of *Triplophysa erythraea* was extracted from the muscle tissue using a tissue genome DNA extraction kit (Tiangen, Beijing, China). Sequencing library was generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA), the sequencing library was then paired-end sequenced using Nextera Index Kit adapter primer (Illumina). DNA quality of this sample was evaluated to ensure the validity, results showed that the quantity of raw bases and clean

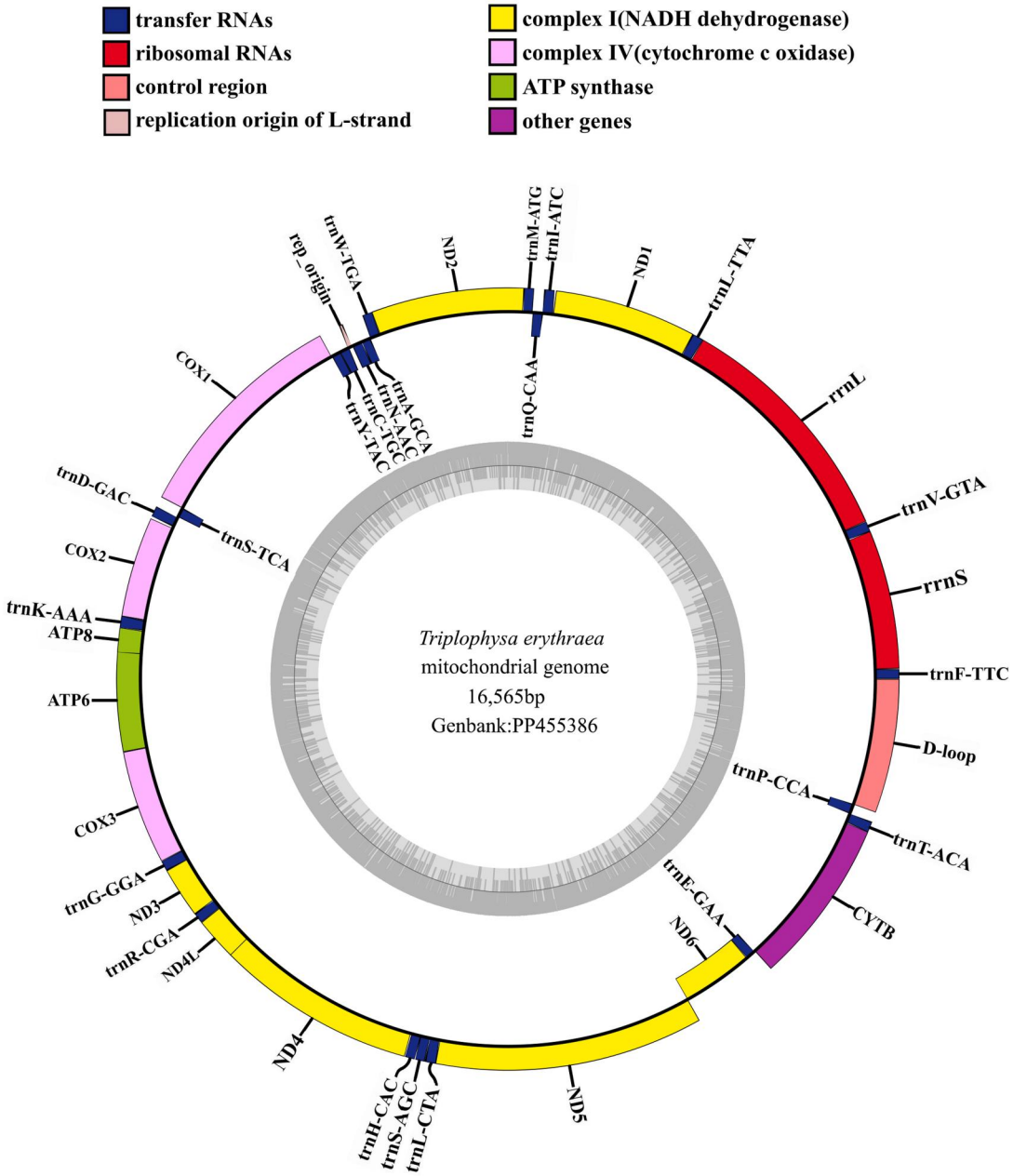

Figure 2. Mitochondrial genome map of the *Triplophysa erythraea* (GenBank: PP455386).
Genes inside the loop represent that the transcription direction is counterclockwise, while the genes outside the loop are the opposite. Different functional genes are marked with different colors. The built-in gray histogram shows the GC content of the genome, and the intermediate gray line is a 50% threshold line.

bases we obtained were 5.65 G and 5.54 G. Q20, Q30 of the sample were separately 97.85% and 94.79%. Moreover, the GC content of whole bases was 39.78%. SPAdes (v3.14.1) was subsequently used to the *de novo* assembly of mitogenome while MITOS (http://mitos.bioinf.uni-leipzig.de/index.py) was used to annotate and predict the protein-coding genes, tRNA genes and rRNA genes of the mitochondrial genome. Finally, we adopted IQTREE(IQTREE Web Server: Fast and accurate phylogenetic trees under maximum likelihood (univie.ac.at)) to perform molecular evolution analysis of 39 mitochondrial DNA obtained from GenBank (Trifinopoulos et al. 2016), including 38 sequences of *Triplophysa* and 1 sequence of *Aborichthys* (Cypriniformes, Nemacheilidae) (Li et al. 2021), the latter was set as the outer group. Result was generated using the maximum likelihood (ML) method with 1000 bootstrap and was adjusted with MEGA11(Koichiro et al. 2021).

Results

The complete mitogenome of *Triplophysa erythraea* was 16565 bp in size with 2 rRNA genes (12S rRNA and 16S rRNA), 13 mRNA genes, 22 tRNA genes and 2 non-coding regions (Figure 2). The complete mitogenome sequence had been submitted to NCBI, GenBank accession of the sequence is PP455386. Among 37 coding genes, most were encoded on heavy strand while *ND6* and 8 tRNA genes (*tRNA-Gln, -Ala, -Asn, -Cys, -Tyr, -Ser1, -Glu, -Pro*) were on the light strand (Table 1). Average sequencing depth of this mitogenome was 567.64 X (Supplementary Figure 1). The total base composition was 30.53% A, 26.82% C, 16.44% G, 26.21% T.

All protein-coding genes except *COX1* gene started with the standard ATG codon, and *COX1* gene uses GTG as the starting codon. The termination codons of protein-coding genes are diverse, including TAA, TAG and T. The 12S rRNA (950 bp) and 16S rRNA (1674 bp) genes were separated by the *tRNA-Val* gene. The 22 tRNA genes ranged from 67 bp (*tRNA-Cys*) to 76 bp (*tRNA-Lys*). Between *tRNA-Asn* and *tRNA-Cys* was a 30 bp sequence identified as the origin of L-strand replication (OL). The length of D-loop is 919 bp, which is located between *tRNA-Phe* and *tRNA-Pro*. Phylogenetic analysis showed that genetic relationship between *Triplophysa erythraea* and *Triplophysa xiangxiensis* (Teleostei, Cypriniformes, Nemacheilidae, Triplophysa) (Xue et al. 2017) was the closest (Figure 3). The two species were both blindfish and discovered in Xiangxi Tujia and Miao Nationality Autonomous Prefecture, in Dalong cave and Feihu cave, separately (Yao et al. 2012). *Triplophysa yarkandensis* was mainly distributed in the Tarim River, Xinjiang province, China (Chen et al. 2020), it formed the longest distance from *Triplophysa erythraea* within the same genus. Apart from *Triplophysa yarkandensis* and *Triplophysa labiate*, most species can form a clade, which reflected the regional and genetic discrepancy between different species.

Table 1. Characteristics of the mitochondrial genome of *Triplophysa erythraea*.

Gene	Start position	Stop position	Intergenic length	Start code	Stop code	Size(bp)	Strand
tRNA-Phe(GAA)	1	69	0			69	H
12sRNA	70	1019	0			950	H
tRNA-Val(UAC)	1020	1091	0			72	H
16sRNA	1092	2765	0			1674	H
tRNA-Leu(UAA)	2766	2840	0			75	H
ND1	2841	3815	0	ATG	TAA	975	H
tRNA-Ile(GAU)	3822	3893	6			72	H
tRNA-Gln(UUG)	3892	3962	−2			71	L
tRNA-Met(CAU)	3964	4032	1			69	H
ND2	4033	5077	0	ATG	TAG	1045	H
tRNA-Trp(UCA)	5078	5147	0			70	H
tRNA-Ala(UGC)	5150	5218	2			69	L
tRNA-Asn(GUU)	5220	5292	1			73	L
OL	5293	5322	0			30	H
tRNA-Cys(GCA)	5323	5389	0			67	L
tRNA-Tyr(GUA)	5390	5457	0			68	L
Cox1	5459	7009	1	GTG	TAA	1551	H
tRNA-Ser1(UGA)	7010	7080	0			71	L
tRNA-Asp(GUC)	7082	7154	1			73	H
Cox2	7168	7858	13	ATG	T–	691	H
tRNA-Lys(UUU)	7859	7934	0			76	H
ATP8	7936	8103	1	ATG	TAA	168	H
ATP6	8094	8777	−10	ATG	TAA	684	H
Cox3	8777	9560	−1	ATG	TA-	784	H
tRNA-Gly2(UCC)	9561	9633	0			73	H
ND3	9634	9982	0	ATG	TAG	349	H
tRNA-Arg(UCG)	9983	10052	0			70	H
ND4L	10053	10349	0	ATG	TAA	297	H
ND4	10343	11721	−7	ATG	TA-	1379	H
tRNA-His(GUG)	11725	11793	3			69	H
tRNA-Ser2(GCU)	11794	11860	0			67	H
tRNA-Leu2(UAG)	11862	11934	1			73	H
ND5	11935	13773	0	ATG	TAA	1839	H
ND6	13770	14291	−4	ATG	TAA	522	L
tRNA-Glu(UUC)	14292	14360	0			69	L
Cytb	14366	15506	5	ATG	T–	1141	H
tRNA-Thr(UGU)	15507	15577	0			71	H
tRNA-Pro(UGG)	15576	15646	−2			71	L
D-loop	15647	16565	0			919	H

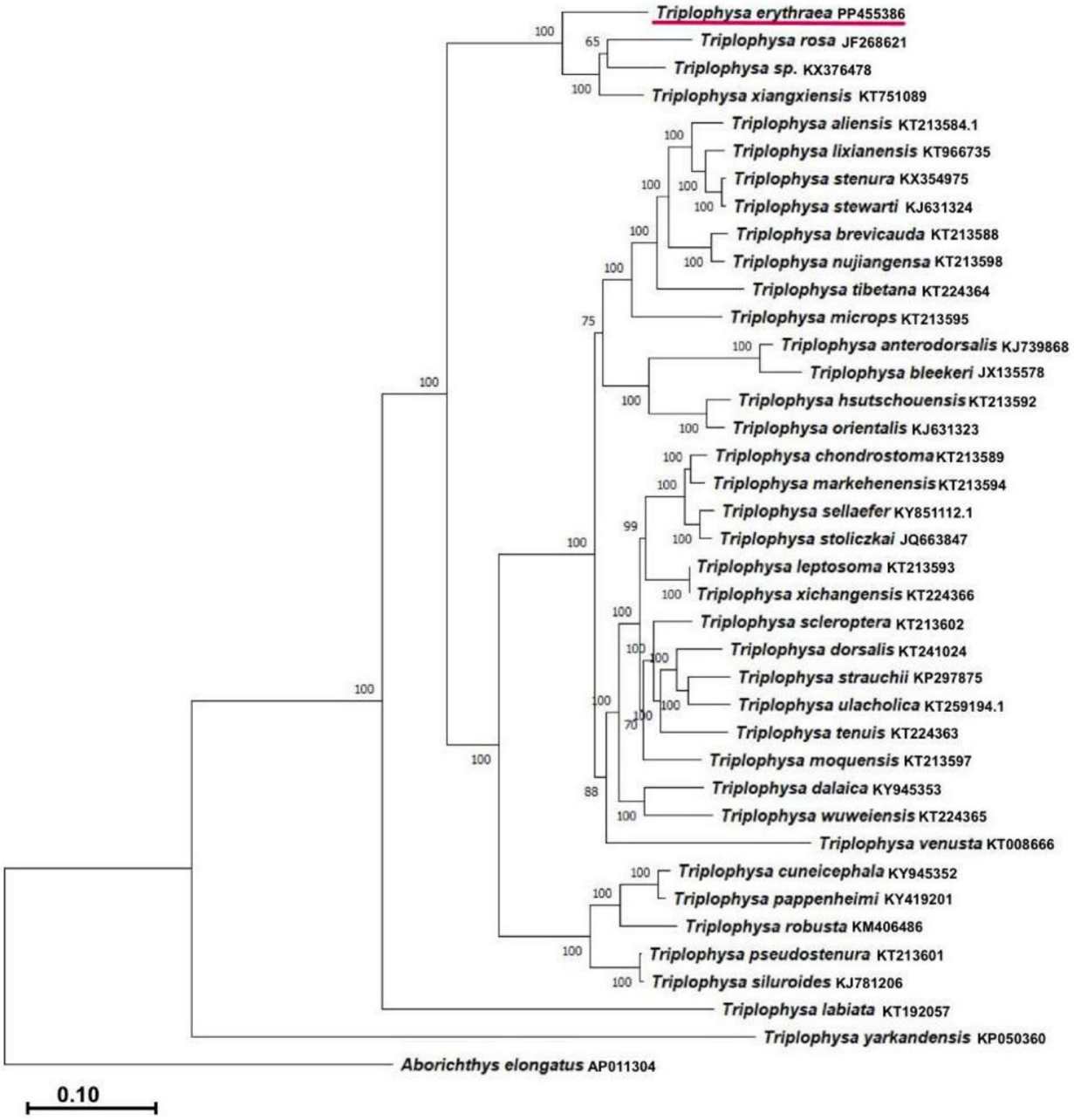

Figure 3. Phylogenetic tree was constructed using the Maximum-likelihood method based on whole mitogenomes of *Triplophysa erythraea* and other 38 closely related organisms. GenBank numbers and references for sequences used to establish this phylogenetic tree were showed in Supplemental Table S1. The last one(*Aborichthys elongatus*) served as the outgroup. *Triplophysa erythraea* (PP455386) in this study was marked with a red line.

Discussion and conclusion

The research described the first complete mitogenome of *Triplophysa erythraea*, providing novel molecular data for its genetic and evolutionary studies. Results revealed that the complete mitogenome of *Triplophysa erythraea* was 16565 bp in length and this species shared the same gene composition and structural arrangement of mitogenome with other Cobitidae fish (Li et al. 2012). A previous study found that the *Triplophysa erythraea* and *Triplophysa lewangensis* had higher similarity (90%) than *Triplophysa xiangxiensis* and *Triplophysa rosa* by the NCBI Blast analysis of the mitochondrial *cyt b* gene. In this study, the phylogenetic analysis showed that *Triplophysa erythraea* had closer genetic relationship with *Triplophysa xiangxiensis* and *Triplophysa rosa*, which is corresponding with the phylogenetic results analyzed with NJ method based on the *Cox1*, *ND5* and *Cytb* genes (Li et al. 2021), this suggested that the developmental processes between them may be more similar (Wang et al. 2016). We think these data would contribute to the genetic conservation of *Triplophysa erythraea* and phylogenetic relationships among *Triplophysa.*

Ethical approval

Fish researchers were certified under a professional training course for laboratory animal practitioners held by the Institute of Experimental Animals, Hunan Province, China (Certificate No.4263). The experiment

strictly followed the Measures of Hunan Province on Administration of Laboratory Animals and was approved by Biomedical Research Ethics Committee of Hunan Normal University. Approval from the Science and Technology Bureau of China and the Department of Wildlife Administration is not required for the experiments since the fish we used is neither listed as first- or second-class state protection level.

Authors' contributions

The manuscript includes the contribution of all authors. Yude Wang designed this study and collected the muscle tissue; Hanbo Liu prepared the DNA sample and assembled the genome; Xiaoyi Dong analyzed the whole mitochondrial genome and drafted the manuscript. The final manuscript was reviewed and approved by all authors.

Disclosure statement

The authors declare no potential competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at [https://www.ncbi.nlm.nih.gov] (https://www.ncbi.nlm.nih.gov/) under the accession no. PP455386. The associated BioProject, SRA, and BioSample numbers are SAMN40932187, SRR28682665, and PRJNA1099222 respectively.

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