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MITOGENOME REPORT

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The complete mitochondrial genome of *Triplophysa grahami* Regan 1906 (Cypriniformes: Nemacheilidae) and phylogenetic analysis

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ABSTRACT

Triplophysa grahami Regan 1906 is a member of the family Nemacheilidae, Cypriniformes, and native loach in Yunnan. In this study, the complete mitochondrial genome (mitogenome) of *T. grahami* Regan 1906 was firstly reported and analyzed. The mitogenome of *T. grahami* Regan 1906 is 16,566 bp in length, including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and one control region (D-loop). The arrangement and orientation of protein coding genes and RNAs in *T. grahami* Regan 1906 are identical to other species of Nemacheilidae. The base composition of *T. grahami* Regan 1906 mitogenome was 29.25% A, 28.55% T, 25.03% C, and 17.17% G. The phylogenetic analysis based on the mitogenome showed that *T. grahami* Regan 1906 belongs to the clade of genus *Triplophysa* and the monophyly of *Triplophysa* is identified. This study contributed valuable genetic data for *T. grahami* Regan 1906 and explored the phylogenetic relationships in Nemacheilidae.

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Introduction

Nemacheilus grahami (Regan) was a former name for *Triplophysa grahami* Regan 1906, which belongs to Cypriniformes, Nemacheilidae, *Triplophysa* and is endemic to the basin of Jinsha River and Dianchi Lake in Yunnan Province (Chen 2013). The genus of *Triplophysa* inhabits mainly in the spaces between stones and floating grasses in slow streams (Li et al. 2007; Liu et al. 2019; Luo et al. 2023). The morphological features of *T. grahami* Regan 1906 were described as: mouth terminal; arcuate back; head height equals head width; six branched anal-fin; eye diameter is less than or equal to interorbital distance (Bashir et al. 2016; Ren et al. 2018).

A lot of species of Nemacheilidae have been identified with complete mitogenome, including some plateau and cave-dwelling loaches (Liu et al. 2017; Wu et al. 2018; Luo et al. 2023). But the molecular data of species in genus *Triplophysa* were sparsely reported. In previous research, the 652 Mb genome of *T. tibetana* Regan 1905 was assembled by using PacBio and the Hi-C technique to lay a solid foundation for further investigation into the mechanisms of environmental adaptation of endemic fishes in the Tibetan Plateau (Yang et al. 2019), meanwhile, intense stresses caused by high-altitude environments resulted in noticeable genetic adaptions with lipid metabolism and immune response gene families expanded were also detected in genome of *T. bleekeri* Sauvage & Dabry de Thiersant 1874 (Yuan et al. 2020). In addition, adaptation to hypoxia (such as duplicated HIF-α, EGLN1, and PPARA candidate genes involved in adaptation to hypoxia) in *Triplophysa* also been identified by using comprehensive transcriptome analysis (Wang et al. 2015).

In last decades, the genus of *Triplophysa* were species with fantastic features, such as blind cave species (Wu et al. 2018; Huang et al. 2019), but unfortunately, we have nothing about *T. grahami* Regan 1906, except for its morphological feature and distribution. To elucidate the phylogenetic history of taxonomic status and phylogeny of *T. grahami* Regan 1906, we first determined its mitogenome and constructed a ML phylogenetic tree to provide data support and molecular evidence for the phylogenetic relationship of *T. grahami* Regan 1906 with other species in Nemacheilidae. We believe that the genome data could help us understanding of environmental adaptation and genetic diversity of *Triplophysa* and provide valuable genetic resources for future studies on the evolution and conservation of high-altitude fish species.

Materials and methods

Specimen collection and preservation

The biological specimens of *T. grahami* in this study were sampled from the Muyang river (N: 25°13′10″, E: 102°49′42″), Kunming, Yunnan, China. The ethanol-soaked specimen of *T. grahami* at Yunnan Institute of Fishery Sciences Research

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(contact person: Junjie Wu, wujunjie2007@yeah.net) with the voucher number KMGYQ-01. The specimen of *T. grahami* Regan 1906 was identified by morphological feature according to previous description (Bashir et al. 2016; Ren et al. 2018), and descriptive image was taken by Junjie Wu (corresponding author), which was first published (Figure 1).

Illumina sequencing, assembly, and annotation

The genomic DNA of *T. grahami* Regan 1906 was extracted from muscle tissue and purified in TIANamp Genomic DNA Kit (TIANGEN Co., Ltd., Beijing, China) by following manual's protocol. After DNA isolation, 0.8–1 μg of purified DNA was fragmented to 500 bp was used to construct short-insert libraries according to the manufacturer's instructions (TruSeq™ Nano DNA Sample Prep Kit, Illumina, San Diego, CA) and sequenced in HiSeq X platform (Wright et al. 2017) (Tsingke Co., Ltd., Beijing, China). The paired-end fastq files were assembled in MitoZ 3.6 (Meng et al. 2019). The functional annotation of mitochondrial genome was analyzed in MITOS online server (Bernt et al. 2013; Galaxy 2022).

Phylogenetic analysis

A total of 59 mitochondrial genomes of Nemacheilidae were aligned in MAFFT with default parameters (Katoh and Standley 2013), 39 members belonged to genus *Triplophysa*, all of species information and references related to published NCBI records were listed in legend of Figure 2. After alignment, the sequences of 13 PCGs were extracted and linked end to end in PhyloSuite (Zhang et al. 2020). The best-fit partitioned substitution model was evaluated in ModelFinder (Kalyaanamoorthy et al. 2017), the default criterion BIC results were carried in IQ-TREE for estimating the fast maximum-likelihood phylogeny (Nguyen et al. 2015). Two members of *Misgurnus* were chosen for outgroups.

Results

Mitochondrial characterization

The complete mitochondrial DNA sequence of *T. grahami* Regan 1906 was 16,566 bp in length with mean depth of 840.15× (Figure S1), including 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and a putative control region (D-loop) (Figure 3). Most of PCGs began with regular initiation codon (ATG) except for COX1, which was GTG. The complete stop codons were identified in six PCGs (ND5, ND4L, ATP6, ATP8, COX1, and ND1), others were ended with 'T' or 'TA'. The base composition was 29.25% A, 28.55% T, 25.03% C, and 17.17% G. Most of the genes located in the heavy strand (H-strand), except for ND6 and eight tRNAs (tRNA-Gln, tRNA-Ala, tRNA-Asn, tRNA-Cys, tRNA-Tyr, tRNA-Ser, tRNA-Glu, and tRNA-Pro). In genus of *Triplophysa*, the nucleotide similarity blast search shows that the mitochondrial sequences of *T. grahami* Regan 1906 was 89.05% to *T. leptosoma* Herzenstein 1888, 88.98% to *T. scleroptera* Herzenstein 1888, and 88.87% to *T. markehenensis* Zhu & Wu, 1981.

Phylogenetic analysis

After ModelFinder analysis, five partition group and best substitute model were established. In detail, GTR + F + I + R3 was best for complex of ATP6 and ND3, GTR + F + R4 was best for complex of ATP8, COX1, COX2, COX3, and ND4L, GTR + F + I + R4 best for complex of CYTB, ND4, and ND5, TIM3 + F + R5 was best for ND2, and TN + F + I + R3 was best for ND6. The maximum-likelihood phylogenetic tree was constructed in IQ-TREE by performing ultrafast bootstrap method with 5000 duplications. The ML tree showed that all members of genus *Triplophysa* were clustered together, which was monophyletic and sister to clade of genus *Barbatula*. *T. grahami* Regan 1906 was phylogenetically inside the clade of genus *Triplophysa*, and a discrete phylogeny of which was shown (Figure 2).

Discussion and conclusions

In this study, we first sequenced and published the mitochondrial genome of *T. grahami* Regan 1906, and an usual mitogenome structure was reported, which has the same orientation and gene order with other Nemacheilidae species (Wang et al. 2012; Chen et al. 2016; Jing et al. 2016; Yan and Luo 2016; Yang et al. 2020; Zhao et al. 2023).

Figure 1. A reference image of *T. grahami* Regan 1906 sequenced in this work, collected by Junjie Wu in Muyang River (N: 25°13′10″, E: 102°49′42″), Kunming, Yunnan, China. The reference image was photographed by Junjie Wu.

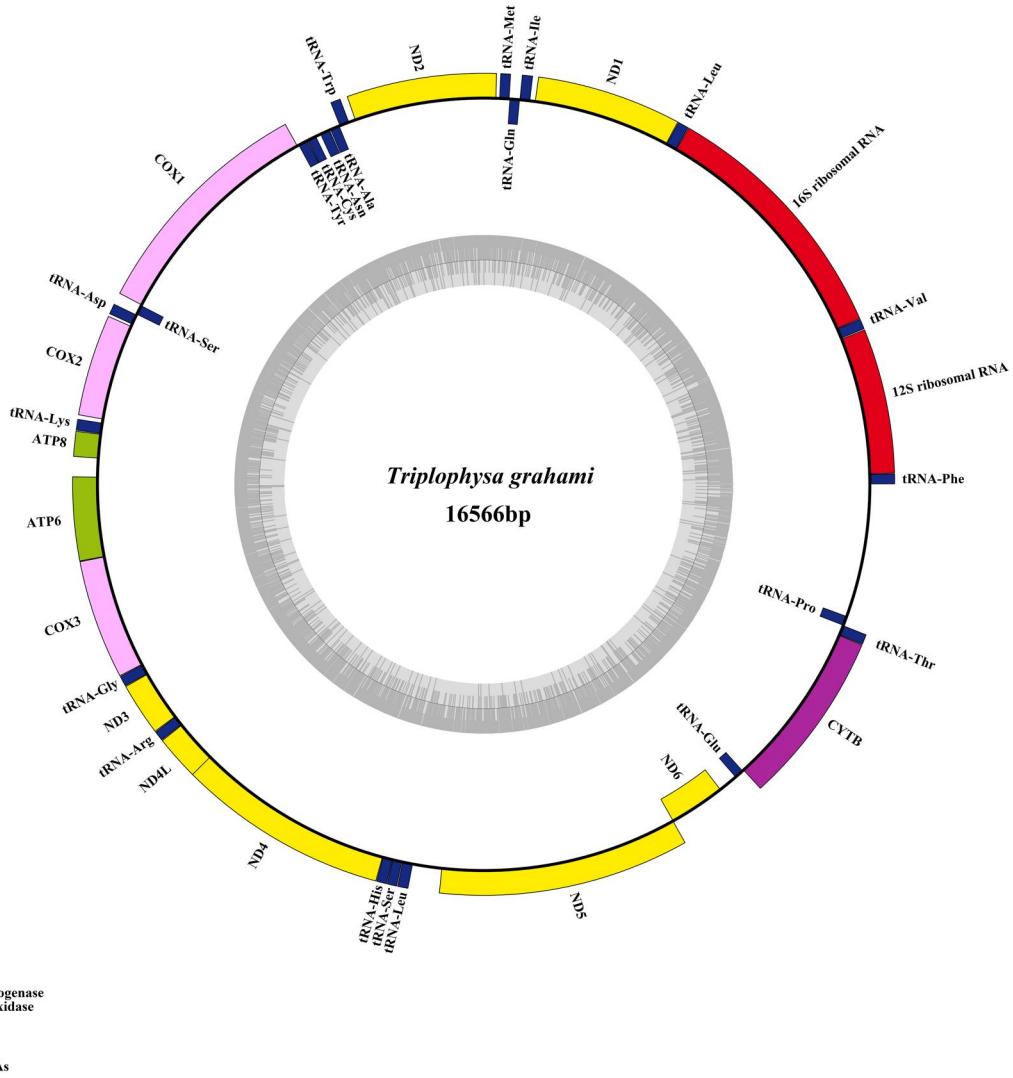

Figure 2. Phylogenetic tree of *T. grahami* Regan 1906 and other 60 species based on the complete mitochondrial sequences from NCBI. *Misgurnus anguillicaudatus* Cantor 1842 and *Misgurnus mizolepis* Günther 1888 were treated as outgroups. The bootstrap values were marker near the nodes. Accession numbers for each species were shown after the name of the species. The *T. grahami* generated in this study (PP114297) was labeled in black outline. The reference information of related sequences: NC 027192 NC 022858 , NC 040302 , NC 046753 , NC 025321 , NC 056344 , NC 073134, NC 068250, NC 073140 (Luo et al. 2023), NC 031581 , NC 073141 (Luo et al. 2023), KT 213584–KT 213602 (Wang et al. 2016), NC 024597 (Que et al. 2016a), NC 056365 , NC 018774 , NC 027189 (Wang, Song, et al. 2023), NC 037924 (Feng, Tang, et al. 2019), NC 029423 (Lei et al. 2016), NC 077583 , NC 073586 (Wang, Luo, et al. 2023), NC 030521 (Jing et al. 2016), NC 072346 (Zhao et al. 2023), NC 058005 (Yang et al. 2020), NC 025632 , NC 019587 (Wang et al. 2012), KY 851112 (Feng, Zhou, et al. 2019), NC 024611 (Chen et al. 2016), NC 032692 (Yan and Luo 2016), NC 017890 (Li et al. 2013), NC 030483 (Wang et al. 2019), NC 029492 (Wang et al. 2017), NC 073129–NC 073145 (Luo et al. 2023), NC 011209 , and NC 038151 .

In phylogenetic analysis, a total of 59 members of Nemacheilidae were involved. In our analysis, a ML tree with strong bootstrap support value was obtained (most of bootstrap value of nodes $\geq$95), the diverse loaches of Nemacheilid formed a monophyletic group, which comprised of eight genera: *Schistura*, *Homatula*, *Barbatula*, *Traccatichthys*, *Oreonectes*, *Yunnanilus*, *Troglonectes*, and *Triplophysa*. *Triplophysa* was the largest group and closely allied to *Barbatula* and *Lefua*, identical to previous description (Wang et al. 2017). Meanwhile, our phylogenetic tree confirmed a monophyletic relationship in *Triplophysa*, identical results were found in Feng and Wang's studies (Feng, Tang, et al. 2019; Feng, Zhou, et al. 2019; Wu et al. 2020).

Interestingly, the discrete phylogeny of *Triplophysa* was identified, we found some species of *Triplophysa* clustered with species distribution, for example, *T. labiata* Kessler 1874, *T. dorsalis* Kessler 1872, *T. tenuis* Day 1877, and *T. bombifrons* Herzenstein 1888 were grouped, which mainly distributed in Xinjiang Province, Tarim River (Lei et al. 2016; Wang, Luo, et al. 2023; Wang, Song, et al. 2023). But some other species in *Triplophysa* clustered without distribution and river system also have been identified, for example, *T. siluroides* Herzenstein 1888 clustered with *T. pseudostenura* He, Zhang & Song 2012, *T. siluroides* Herzenstein 1888, *T. siluroides* Herzenstein 1888 distributes in Tao River, Yellow River basin (Chen et al. 2016), and *T. pseudostenura* He, Zhang & Song 2012 distributes in Yalong river, Sichuan, Yangtze River basin. Similarity to *T. grahami* Regan 1906 and other species distributes only in Yunnan Province (*T. jianchuanensis*, *T. nanpanjiangensis* Zhu & Cao 1988, and *T. nujiangensa* Chen, Cui & Yang 2004), species in Tibetan Plateau (*T. stewarti* Hora 1922, *T. stenura* Herzenstein 1888, and *T. stoliczkai* Steindachner

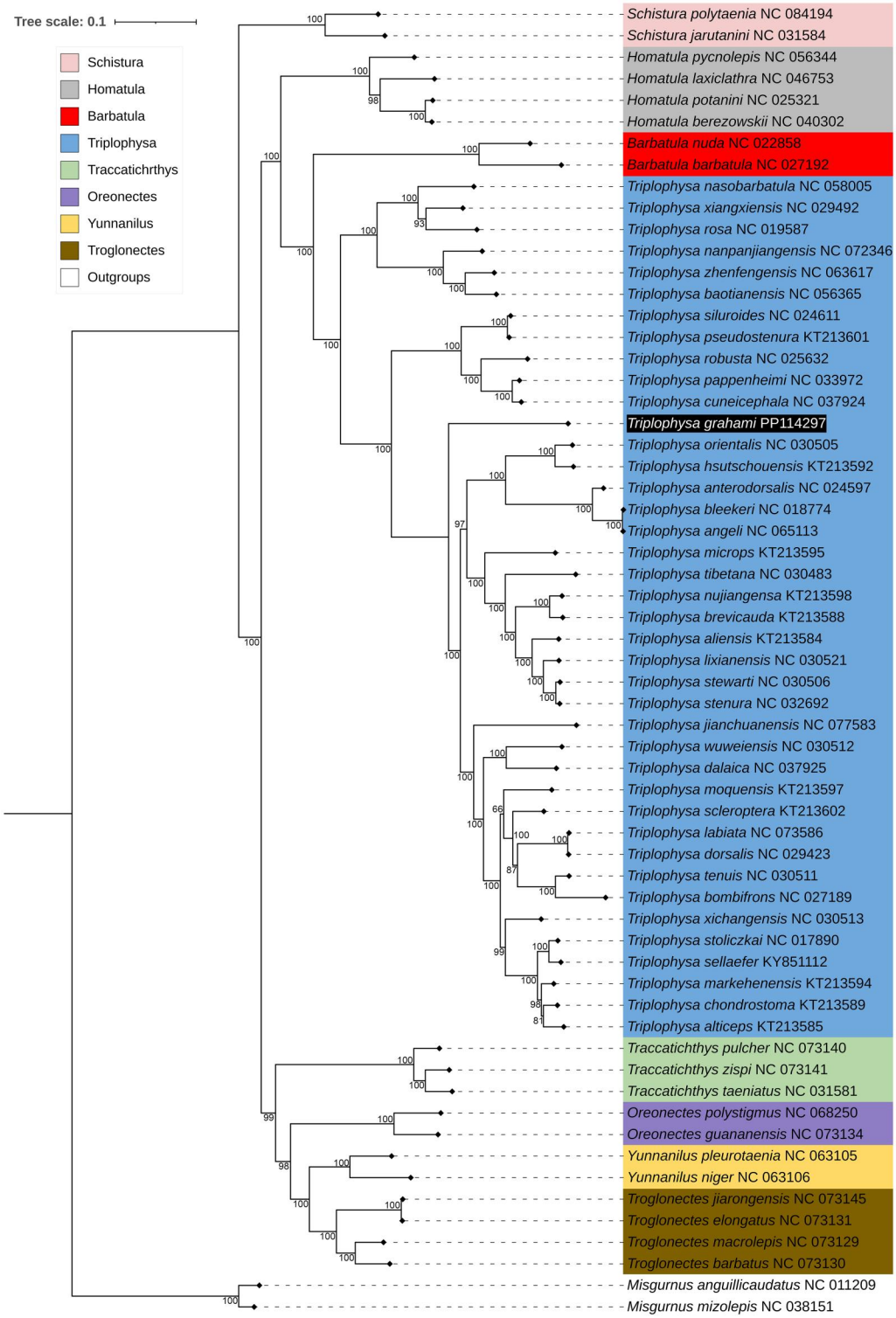

Figure 3. Complete mitochondrial genome map of *T. grahami* Regan 1906 (GenBank: PP114297). Encoded genes and RNAs were in different color, light, and heavy-strand were shown inner and outside of the circle, respectively.

1866). The similar phylogenetic relationship have been found in Wang's studies (Wang et al. 2016, 2017). *Triplophysa* lineage diverged approximately 23.5 Ma, which falls within the period of recent major uplifts of the Tibetan Plateau in the Early Miocene, and loaches adapted well to the severe conditions of the plateau by means of more accelerated evolutionary rates (Wang et al. 2016). We speculated that the species of *Triplophysa* distributes in Yunnan-Guizhou and Tibet plateau and their peripheral regions might have experienced the parapatric speciation events. During this high evolutionary rates stage, reproductive isolation has been establish rapidly, just similarity to species formation in sympatric Schizothoracine, and this sympatric adaptive ecological speciation has been first identified in La'angcuo Lake, a small glacier lake in the Tibetan Plateau (Chen et al. 2020), which could go some way toward explaining the evolutionary

origins within *Triplophysa* and other genera, but the further analysis should be needed.

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Author contributions

Mei Xu and Junjie Wu conceived this study and wrote the manuscript together. Mei Xu, Junjie Wu, Jian Zhang, and Jianyu Song carried out the experiments and analyzed the data. Junjie Wu took the photograph of specimen. Zifang Zhang, Jianyu Song, and Junjie Wu contributed to the collection of specimen of *T. grahami*, Zifang Zhang is responsible for the critical revision of professional knowledge content and final approval of the version to be published.

Ethical approval

Animals are in live and well cared for before and after the experiment. All experimental procedures involving animals were approved (animal protocol number: 202302) by the Ethics Committee and Animal Welfare of Yunnan Institute of Fishery Sciences Research. The sample in this study did not involve protected and endangered animals. Obtaining the sample was legal and the process of this research were in line with the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

Disclosure statement

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

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Data availability statement

The complete mitochondrial genome assembly data were available in GenBank database under the accession number PP114297. The associated BioProject, BioSample, and SRA numbers are PRJNA1073992, SAMN39848630, and SRX23556634, respectively.

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