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Original Research Articles

Exploring the Complete Mitochondrial Genome of *Schizothorax biddulphi* via PacBio HiFi Sequencing: Phylogenetic Insights and Molecular Evolutionary Implications

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This study presents the first complete and fully assembled mitochondrial genome (mitogenome) of *Schizothorax biddulphi* derived from PacBio HiFi sequencing technology, providing novel insights into its phylogenetic relationships within the Cyprinidae family. The mitogenome of *S. biddulphi* exhibits a canonical structure typical of teleost fishes, comprising 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and a control region (D-loop). Most PCGs initiate with the standard ATG codon, and termination is achieved through TAA, TAG, or truncated T stop codons, suggesting a potential influence of selection pressure on these regions. Comparative genomic analysis indicates high conservation of gene order and nucleotide composition with other *Schizothorax* species. Phylogenetic analyses using both maximum likelihood (ML) and Bayesian inference (BI) methods reveal that *S. biddulphi* and *Schizothorax eurystomus* form a well-supported clade, indicating a close phylogenetic relationship between these two species. These results contribute novel insights into the phylogenetic relationships within the genus *Schizothorax* and underscore the evolutionary divergence of *S. biddulphi* from other congeneric species. The newly characterized mitogenome enhances the available genetic resources and offers a valuable reference for future phylogenomic studies. This research provides a foundation for understanding the evolutionary dynamics driving diversification and adaptive radiation within *Schizothorax*. Furthermore, it underscores the significance of mitogenomics in elucidating the ecological adaptations and biological success of these freshwater fishes.

INTRODUCTION

Mitochondria, ubiquitous in nearly all eukaryotic organisms, serve pivotal roles in regulating energy metabolism, apoptosis, aging, and various diseases, rendering them indispensable cellular components.[1] Mitochondrial DNA (mtDNA) emerges as a valuable molecular marker for systematic inquiries, owing to its straightforward structure, accelerated evolutionary pace, copious copies, and straightforward isolation techniques. These attributes render mtDNA a convenient and efficient tool for scrutinizing genetic relationships and delineating phylogenetic frameworks.[2] Mitochondrial genomes, essential in molecular biology research, furnish critical insights into evolutionary connections, population histories, and genetic variations.[3] Widely utilized in species delineation, classification, and phylogenetic analyses, they unveil species phylogenetic interrelations and aid in constructing evolutionary lineages within a genus.[4] Furthermore, mitochondrial genomes facilitate investigations into gene flow, migration routes, and genetic diversity among species.[5]

Schizothorax, also known as snowtrout or Schizothoracins, belongs to the genus of ray-finned fish in the Cyprinidae family.[6] These fish predominantly inhabit the freshwater systems of Asia, especially in high-altitude areas like the Himalayas, Tibetan Plateau, and nearby regions.[7] Adapted to cold and high-altitude conditions,

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Figure 1. The sample image of *S. biddulphi*, taken by Shengao Chen

species of *Schizothorax* play a crucial role in maintaining the ecological balance of their habitats. Among the species, *Schizothorax biddulphi* is particularly noteworthy.[8] It flourishes in the mountainous river systems of Asia, characterized by a spindle-shaped body, small mouth, and unique teeth.

However, *S. biddulphi* is at risk due to increasing human activities,[9] habitat degradation,[10] the effects of climate change, and emerging pathogen like *Acinetobacter lwoffii*[11] marking it as a species needing urgent conservation efforts.[12] Current research focuses on its ecology, conservation biology, and genetics to better understand its habitat requirements, reproductive behaviors, and population dynamics.[8,13-15] These studies are essential for developing effective conservation strategies and managing its genetic diversity for future preservation.

This study presents a comprehensive analysis of the mitogenome of *S. biddulphi*. We have successfully assembled the complete mitogenome of *S. biddulphi* using Hifi sequencing technology. This research significantly contributes to the phylogenetic understanding of *Schizothorax*, providing novel insights into the genus and facilitating taxonomic revisions, especially regarding species relationships within this diverse genus. This achievement not only enhances our understanding of *S. biddulphi*'s genetic composition but also provides valuable insights into the phylogenetic relationships within the broader family Cyprinidae.

MATERIALS AND METHODS

SAMPLE AND SEQUENCE

The fish species featured in this study were collected from Weigan River in Tarim River basin (41°38 ‘25 "N, 81°26’ 42" E) of Xinjiang. China, and are housed at Tarim University (Collection No. 20230816006; for inquiries, contact Shengao Chen, shengao@taru.edu.cn) (**Figure 1**).

Genomic DNA extraction was performed using the TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). The HiFi Library was prepared following the manufacturer's protocol. Initially, a 15 µg sample was selected and the SMRTbell® Express Template Preparation Kit v2 was employed to construct the SMRTbell library. Subsequently, small DNA fragments were eliminated using BluePippin. The SMRTbell template was then annealed with a sequence primer, and the resulting complex was bound by DNA polymerase. The library underwent sequencing on the Sequel II sequencing platform (Pacific Biosciences of California, Inc., Menlo Park, CA, USA). CCS (v.6.4.0) software was utilized to generate the HiFi reads.

MITOGENOME ASSEMBLY AND ANNOTATION

We constructed the mitochondrial genome of *S. biddulphi* by employing the MitoHiFi pipeline (https://github.com/marcelauliano/MitoHiFi), which utilized PacBio HiFi reads and was guided by a reference sequence. The initial reference sequence used was from *Schizothorax lissolabiatus* (NCBI: NC_027162.1). Following the assembly of the nuclear genome, we employed BLAST+ (Camacho et al. 2009) to identify similarities between the mitochondrial assembly and the nuclear genome. To exclude nuclear DNA contamination (NUMTs), we compared the mitochondrial assembly against the nuclear genome using BLAST+ with a 99% sequence identity threshold. Nuclear contigs and scaffolds with more than 99% identity to the nuclear genome were discarded. To quantify sequencing depth and validate the assembly, HiFi reads were mapped back to the final circular mitogenome using minimap2 with the HiFi preset (-ax map-hifi), and alignments were sorted and indexed with samtools. Per-base depth was computed with samtools depth.

To validate the mitochondrial assembly, we compared it to the reference mitogenomes of related species using BLAST+ and Mitoz.[16] The annotated genes in the assembled mitogenome of *S. biddulphi* showed high identity with the corresponding genes in related species, confirming the accuracy of the assembly. To visualize the organization of the genomic features, circular maps of the *S. biddulphi* mitochondrial genome were created using OGDraw (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html, accessed on 3 May 2024).[17] These maps show the locations of all genes, tRNAs, and rRNAs, offering a detailed view of the mitochondrial structure.

ASSESSMENT OF SEQUENCE PROPERTIES

The nucleotide composition, codon usage, and relative synonymous codon usage (RSCU) of the *S. biddulphi* mitogenome were analyzed using CodonW.[18] This shed light on the nucleotide makeup and codon preferences of the mitogenome. Nucleotide diversity (Pi) and Ka/Ks ratios for the 13 mitochondrial protein-coding genes (PCGs) in Cyprinidae were calculated using DnaSP in order to assess genetic variation patterns.[19] Sliding window analyses of the PCGs were also conducted in DnaSP using 100 bp windows with 25 bp steps in order to examine diversity within PCGs. Additionally, genetic distances were estimated using the Kimura-2 parameter (K2P) model in MEGA in order to determine evolutionary relationships. Combining codon usage analysis, Pi, Ka/Ks ratios, and K2P distances enabled us to obtain comprehensive insights into the mitogenomic diversity and evolution of *Schizothorax*.

PHYLOGENETIC ANALYSES

To determine phylogenetic relationships within *Schizothorax*, the 13 concatenated mitochondrial PCGs from *S. biddulphi* and other *Schizothorax* species (Supplement Table 1) were aligned using MAFFT.[20] Bayesian inference employed MrBayes with two independent MCMC runs of 200 million generations, sampling every 1000 generations until convergence.[21] The first 10% of trees were discarded as burn-in before computing a consensus tree. The bootstraps and posterior probabilities provided statistical support to the evaluation of topology robustness. Bayesian approaches enabled a robust phylogenomic assessment of the evolutionary relationships in *Schizothorax* to be performed. The phylogenetic tree was visualized with Figtree (v1.4.4), with bootstrap support values and posterior probabilities indicated on the branches.

RESULTS

GENOMIC ORGANIZATION AND NUCLEOTIDE COMPOSITION

The mitogenome of *S. biddulphi* was fully assembled as a 16,558 bp circular molecule (**Figure 2**), with no gaps or missing regions identified. The assembly was supported by high coverage depth, ranging from 339× to 902×, and 100% of the genome was covered at least 10× (**Figure S1**). Mapping the HiFi reads back to the assembled genome confirmed uniform coverage across the entire mitogenome, ensuring its completeness. Additionally, the mitogenome was validated by comparing it to known mitochondrial genomes from closely related species, further confirming the absence of any fragmented or missing regions. The average sequencing depth was 607.89×, reinforcing the robustness and accuracy of the assembly. The analysis of its nucleotide composition revealed 28.14% A, 25.64% C, 18.81% G, and 28.41% T, reflecting an AT bias (56.55%) consistent with that of other Cyprinidae species. The structure of the *S. biddulphi* mitogenome follows the typical organization found in other *Schizothorax* species, with 13 protein-coding genes, 22 tRNAs, and 2 rRNAs arranged in a typical pattern (**Table 1**). The shortest tRNAs were tRNA^{Phe}, tRNA^{Cys}, and tRNA^{Ser} at 69 bp, while the longest were tRNA^{Leu}, tRNA^{Asn}, and tRNA^{Leu} at 74 bp. The 896 bp control region lies between tRNA^{Pro} and tRNA^{Phe}.

ANALYSIS OF MITOCHONDRIAL PROTEIN-CODING GENES

The *S. biddulphi* mitogenome contains a typical set of 28 genes—9 encoded on the L-strand (nad6, trnQ, trnA, trnN, trnC, trnY, trnS, trnE, and trnP) and 19 on the H-strand (atp6, atp8, cox1-3, cob, nad1-5, l-rRNA, s-rRNA, trnD-G, trnH-M, trnR-V, and trnW). The conservation of gene composition and arrangement indicates a shared evolutionary history and suggests that these genes can contribute to phylogenetic resolution at the family level of Cyprinidae species (**Figure 3**). The observed similarities in gene order and orientation between this study and previous investigations imply the potential utility of these genes in future phylogenetic studies within the family Cyprinidae.

The *S. biddulphi* mitogenome consists of 3,794 codons across 13 protein-coding genes (**Figure 5**). Analysis of codon usage reveals important information about gene expression, mRNA stability, and evolutionary relationships. The codon usage in the *S. biddulphi* mitogenome revealed a strong bias towards specific codons. The most frequent amino acids encoded were Leucine, Serine, Alanine, Arginine, Threonine, and Isoleucine, while Cysteine was the least represented (**Table S2**). The Ka/Ks ratio (ω) were calculated using the DnaSP software,[19] applying the default settings for codon-based analysis. The ratios were interpreted to detect the relative evolutionary pressure on the protein-coding genes. In this study, 12 protein-coding genes showed Ka/Ks values below 1, ranging from 0.012 to 0.147, except for nad1, which had a value of 1.89 (**Figure 4**). This suggests that purifying selection has significantly influenced most genes, while nad1 has undergone positive selection. Notably, the nad4L and cob genes, which encode components of the respiratory chain—nad4L being part of Complex I and cytochrome b (cob) being part of Complex III—displayed the lowest Ka/Ks ratios (0.012 and 0.018, respectively). These findings indicate stronger evolutionary constraints and a higher degree of functional constraint on these genes. Given that mitochondrial DNA encodes critical components of the respiratory chain and is vital for mitochondrial inheritance, it is particularly prone to the accumulation of deleterious mutations.

The nad2 gene displayed the highest nucleotide diversity with a π value of 0.086, followed by nad6 (0.079), nad1 (0.073), and cob (0.068). Conversely, the genes cox2 (0.034) and atp8 (0.024) showed the lowest nucleotide diversity values. To further explore the genetic distances among these sequences, mean genetic distances were calculated. Reflecting the patterns of nucleotide diversity, nad2, nad1, and cob exhibited higher genetic distances of 0.10, 0.08, and 0.08 respectively, indicating greater sequence divergence. In contrast, *cox2, cox3,* and *atp8* had lower genetic distances of 0.04, 0.04, and 0.03 respectively, suggesting less divergence.

Nucleotide diversity (π) quantifies the average differences between two randomly selected sequences within a gene or genomic region, serving as a critical genetic parameter that measures the level of genetic variation or diversity within a population. Higher π values indicate more diversity in the nucleotide sequences of a specific region, thus

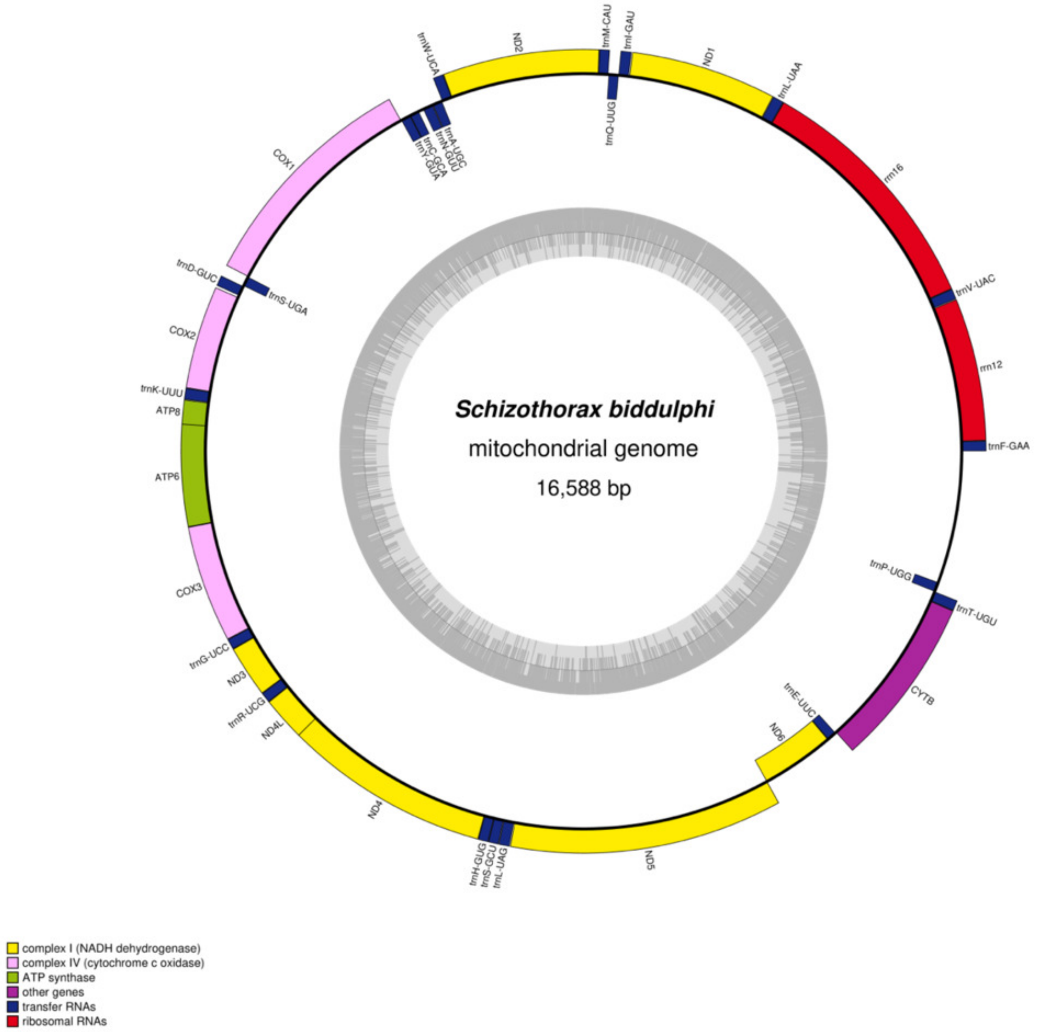

Figure 2. A circular map of the *S. biddulphi* mitochondrial genome is shown, with the outer circle denoting the heavy (H) strand and the inner circle denoting the light (L) strand. The inner gray circle illustrates the GC and AT content distribution, where darker regions indicate higher GC content and lighter regions indicate higher AT content.

allowing researchers to assess the extent of genetic variation present.

This study provide insights into the genetic diversity and sequence divergence in protein-coding genes among Cyprinidae mitogenomes. The identification of genes with high nucleotide diversity and genetic distances, such as nad2, nad1, and cob, suggests that these genes may be subjected to selective pressures or evolutionary forces that contribute to their higher variability. Exploring the functional roles of these genes and their evolutionary implications in Cyprinidae would enhance our understanding of genetic diversity and adaptation in this family.

PHYLOGENETIC ANALYSES

To ensure robust phylogenetic analysis, our dataset was expanded to 16 mitogenomes. This included 29 from *Schizothorax* as the focal family and *Aspiorhynchus laticeps* as outgroups. These reference mitogenomes were retrieved from the NCBI RefSeq database, with data updated as of 17 June 2024.Phylogenetic relationships were investigated using both maximum likelihood (ML). *S. biddulphi* showed close affinity to *Schizothorax eurystomus*, in accordance with a prior study performed using morphometric data. Our study provides the first complete *Schizothorax* phylogenetic analysis, addressing this gap. *S. biddulphi*'s real taxonomy status provides insights into Schizothoracinae evolution, highlighting the need for further analyses with complementary datasets.

The phylogenetic tree illustrates the evolutionary relationships among various species within the genus *Schizothorax* and related genera. *A. laticeps* and *S. biddulphi* (NC_017873) appear as distinct lineages with minimal genetic distance. The genus *Schizothorax* is primarily com-

Table 1. Features Annotated in the *S. biddulphi* Mitochondrial Genome

Locus	Start	Stop	Size (bp)	Start Coding	Stop Coding	Strand
$tRNA^{Phe}$	1	69	69			H
12S rRNA	69	1024	956			H
$tRNA^{Val}$	1024	1096	73			H
16S rRNA	1097	2784	1688			H
$tRNA^{Leu}$	2784	2857	74			H
nad1	2816	3829	1014	ATG	TAA	H
$tRNA^{Ile}$	3831	3901	71			H
$tRNA^{Gln}$	3900	3971	72			L
$tRNA^{Met}$	3970	4039	70			H
nad2	4039	5082	1045	ATG	T	H
$tRNA^{Trp}$	5084	5156	73			H
$tRNA^{Ala}$	5160	5229	70			L
$tRNA^{Asn}$	5230	5303	74			L
$tRNA^{Cys}$	5338	5406	69			L
$tRNA^{Tyr}$	5406	5476	71			L
cox1	5468	7027	1560	GTG	TAA	H
$tRNA^{Ser}$	7030	7101	72			L
$tRNA^{Asp}$	7103	7175	73			H
cox2	7181	7872	692	ATG	T	H
$tRNA^{Lys}$	7872	7944	73			H
atp8	7945	8113	169	ATG	TAA	H
atp6	8103	8787	685	ATG	TAA	H
cox3	8786	9572	787	ATG	T	H
$tRNA^{Gly}$	9571	9641	71			H
nad3	9641	9992	352	ATG	TAG	H
$tRNA^{Arg}$	9990	10,059	70			H
nad4l	10,059	10,356	298	ATG	TAA	H
nad4	10,349	11,730	1382	ATG	TAG	H
$tRNA^{His}$	11,730	11,799	70			H
$tRNA^{Ser}$	11,799	11,867	69			H
$tRNA^{Leu}$	11,875	11,948	74			H
nad5	11,948	13,787	1840	ATG	TAG	H
nad6	13,783	14,305	523	ATG	TAA	L
$tRNA^{Glu}$	14,306	14,375	70			L
cob	14,380	15,521	1142	ATG	T	H
$tRNA^{Thr}$	15,521	15,593	73			H
$tRNA^{Pro}$	15,593	15,663	71			L

posed of two major clades (Figure 6). The first major clade includes species such as *S. biddulphi* (this study) and *Schizothorax eurystomus*, which are closely related with high bootstrap support, along with *Schizothorax pseudoaksaiensis*. Within this clade, there is a sub-clade consisting of *Schizothorax curvilabiatus*, *Schizothorax oconnori*, *Schizothorax wangchiachii*, and *Schizothorax waltoni*, all showing high genetic affinity. Additionally, *Schizothorax macropogon* is part of this group, along with *Schizothorax integrilabiatus*, *Schizothorax molesworthi*, and *Schizothorax plagiostomus*. Another cluster includes *Schizothorax esocinus*, *Schizothorax labiatus*, *Schizothorax richardsonii*, and *Schizothorax progastus*. *Schizothorax nepalensis* is a distinct lineage within this clade, indicating significant genetic divergence. The second major clade encompasses species such as *Schizothorax chongi* and *Schizothorax kozlovi*, which are highly supported. This clade also includes *Schizothorax davidi*, *Schizothorax grahami*, and *Schizothorax prenanti*. Additionally, *Schizothorax griseus* and *Schizothorax sinensis* are closely related to *Schizothorax lissolabiata* and *Schizothorax taliensis*. The clade of *Schizothorax gongshanensis* and *Schizothorax lantsangensis* is also part of this major group and is highly supported.

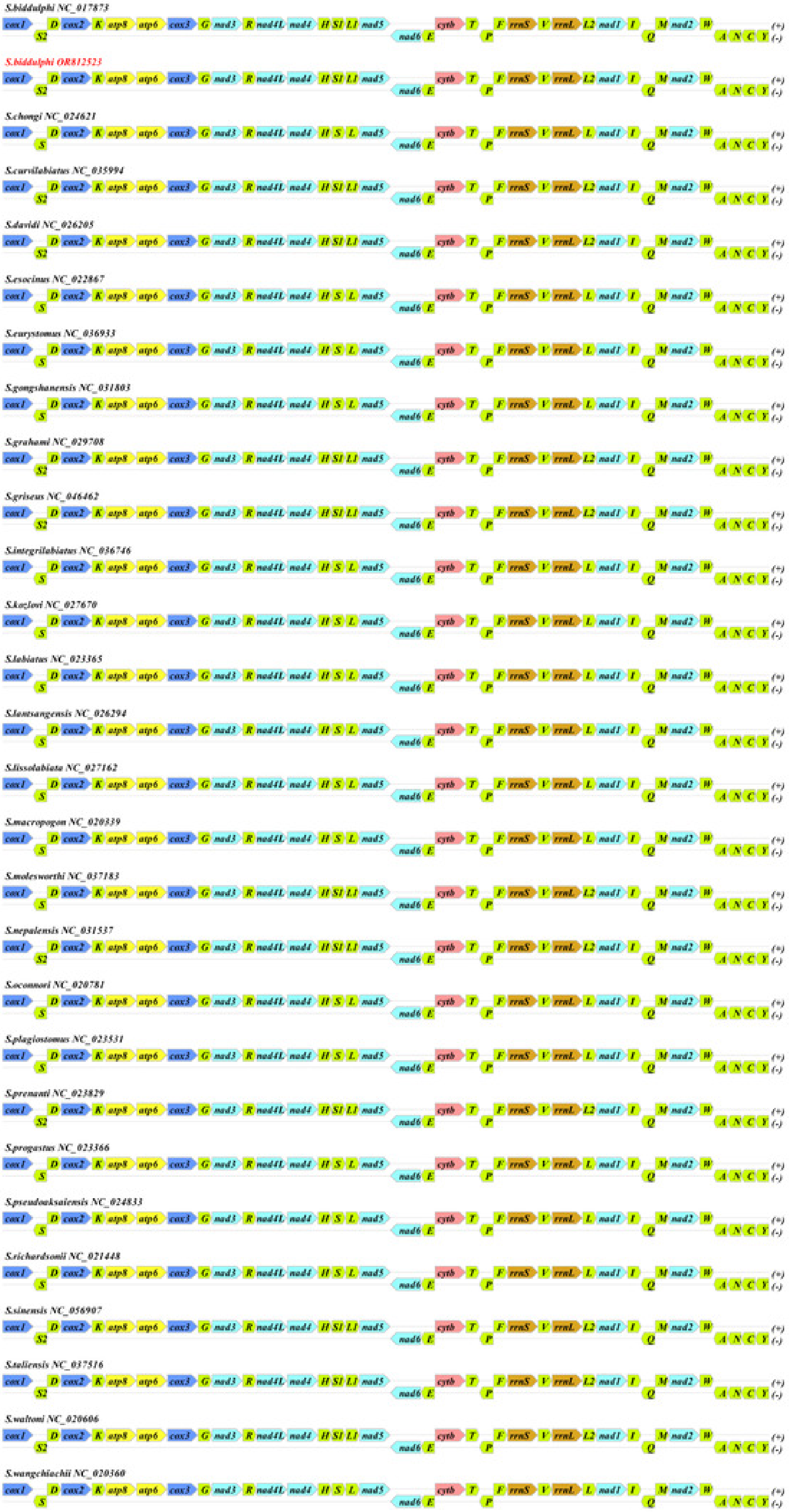
S.biddulphi NC_017873
S.biddulphi OR812523
S.chongi NC_024621
S.curvilabiatus NC_035994
S.davidi NC_026205
S.esocinus NC_022867
S.eurystomus NC_036933
S.gongshanensis NC_031803
S.grahami NC_029708
S.griseus NC_046462
S.integrilabiatus NC_036746
S.kozlovi NC_027670
S.labiatus NC_023365
S.lantsangensis NC_026294
S.lissolabiata NC_027162
S.macropogon NC_020339
S.molesworthi NC_037183
S.nepalensis NC_031537
S.oconnori NC_020781
S.plagiostomus NC_023531
S.prenanti NC_023829
S.progastus NC_023366
S.pseudoaksaiensis NC_024833
S.richardsonii NC_021448
S.sinensis NC_056907
S.taliensis NC_037516
S.waltoni NC_020606
S.wangchiachii NC_020360
cox1
S2
D
cox2
K
atp8
atp6
cox3
G
nad3
R
nad4L
nad4
H
S1
L1
nad5
nad6
E
cytb
T
P
F
rrnS
V
rrnL
L2
nad1
I
Q
M
nad2
W
A
N
C
Y
(+)
(-)

Figure 3. Gene order of the *Schizothorax* mitochondrial genomes

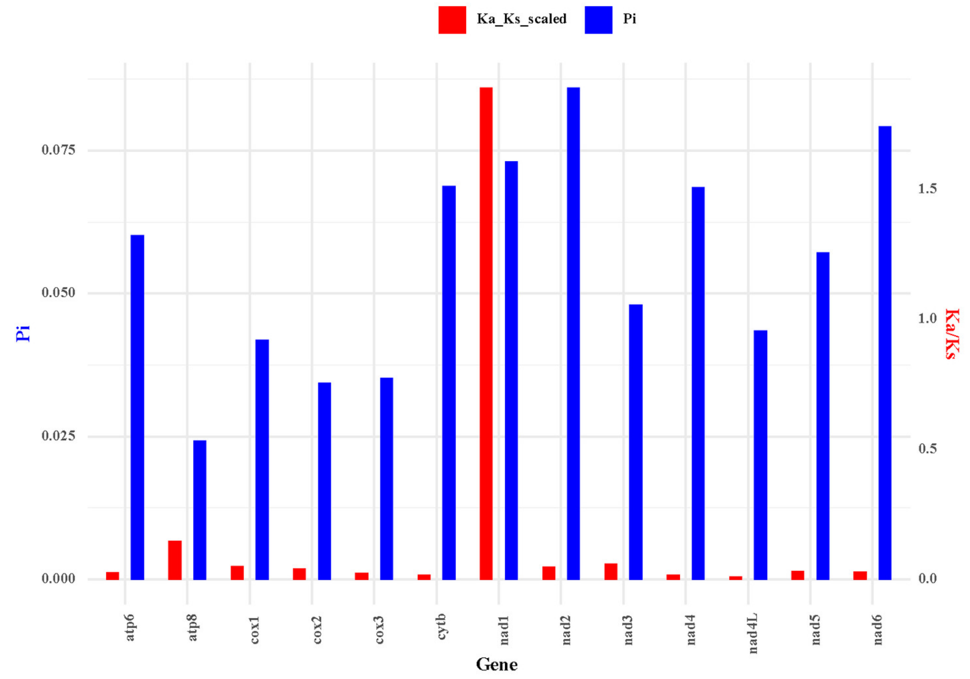

Figure 4. Genetic diversity and evolutionary dynamics of mitogenomes in this study

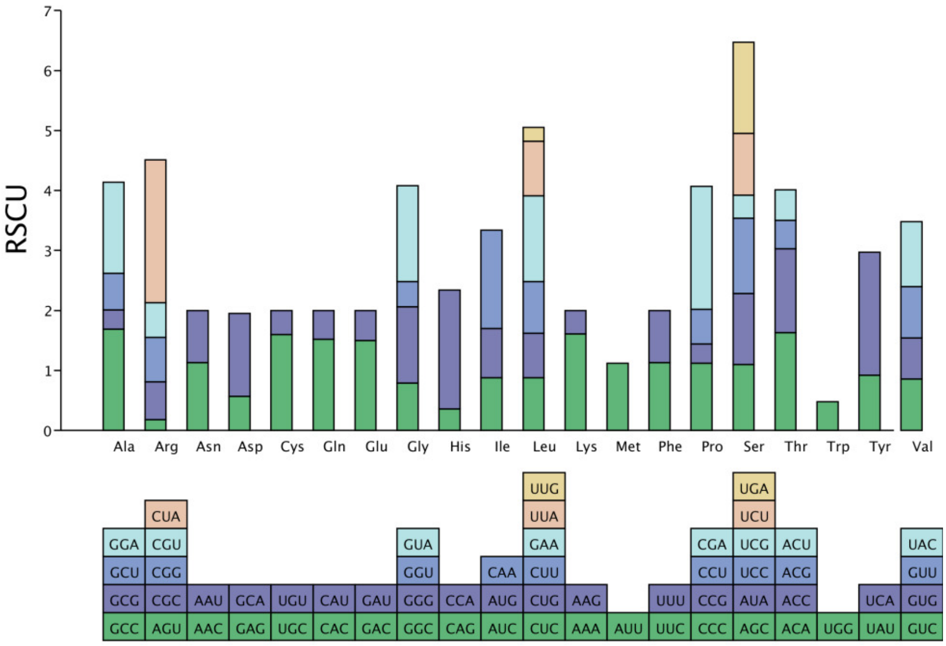

Figure 5. Codon Usage in *S. biddulphi* Mitochondrial Protein-Coding Genes

DISCUSSION

The characterization of the *S. biddulphi* mitogenome revealed typical features including AT bias and conserved RNAs and genes, highlighting their functional significance. Variations among *Schizothorax* species point to a complex interplay between conservation and adaptation. Further investigation of these variations will provide deeper insights into mitogenomic diversity and evolution in *Schizothorax*. The conservation of gene composition and arrangement indicates a shared evolutionary history and suggests that these genes can contribute to phylogenetic resolution at

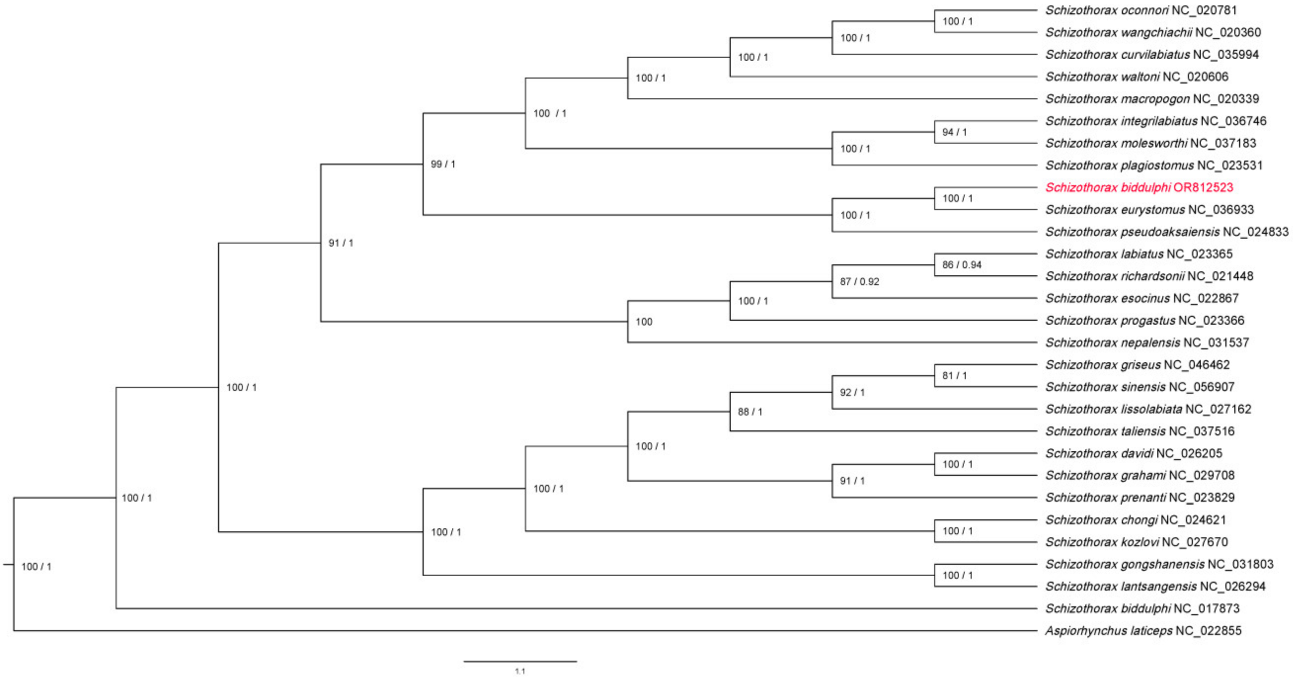

Figure 6. Phylogenetic Reconstruction of 29 Fish Mitogenomes Using 13 PCGs.

Note: *S. biddulphi* highlighted in red in this study

the family level of Cyprinidae species. The observed similarities in gene order and orientation between this study and previous investigations imply the potential utility of these genes in future phylogenetic studies within the family Cyprinidae.[22-31]

The Ka/Ks ratios indicate stronger evolutionary constraints and a higher degree of functional constraint on these genes. Given that mitochondrial DNA encodes critical components of the respiratory chain and is vital for mitochondrial inheritance, it is particularly prone to the accumulation of deleterious mutations. The robust purifying selection on the nad4L and cob genes aids in eliminating such mutations, making them ideal molecular markers for phylogenetic analysis. Based on these findings, it is reasonable to infer that these two genes can contribute to the phylogenetic resolution, providing insights into the evolutionary relationships and divergence patterns within this group.[32] Genes such as nad6, nad2, nad4, nad5, and cob exhibit higher nucleotide diversity and genetic distances. These genes may be under selective pressures linked to ecological factors such as oxygen availability or temperature fluctuations, potentially contributing to their functional importance in mitochondrial adaptation. These findings are critical for understanding the phylogenetic and conservation dynamics of *Schizothorax*.

Positive selection on the mitochondrial nad1 gene suggests an adaptive response to ecological or environmental pressures.[33,34] As a component of complex I in the mitochondrial respiratory chain, nad1 plays a critical role in cellular energy production by participating in oxidative phosphorylation. The selective advantage of positive selection in this gene may be linked to the organism's ability to cope with variable environmental stressors such as fluctuating oxygen levels, temperature extremes, or high metabolic demands in specific habitats.[33,35] For example, species living in environments where oxygen availability is low or temperature variability is high often exhibit adaptations in mitochondrial function to maintain efficient ATP production. In the case of *S. biddulphi*, positive selection on nad1 could be associated with metabolic adjustments that optimize energy production under such conditions. Mitochondrial genes, especially those involved in the electron transport chain, are known to undergo rapid adaptation in response to environmental stressors, contributing to an organism's overall fitness and survival.[34,36] Positive selection on the mitochondrial nad1 gene suggests a potential adaptive response, though this signal is preliminary and warrants further investigation in future studies.

Nucleotide diversity provided a critical parameter for quantifying the genetic variation within a population, representing the average differences between two randomly selected sequences within a gene or genomic region.[37] Higher π values indicate greater diversity in nucleotide sequences, providing insights into the extent of genetic variation in a given region. Our study revealed that genes such as nad6, nad2, nad4, nad5, and cob exhibit higher nucleotide diversity and genetic distances. These genes may be under selective pressures or evolutionary forces that contribute to their elevated variability. This variability could be linked to functional adaptations or specific ecological pressures acting on the Cyprinidae family. The identification of genes with significant nucleotide diversity highlights important areas for future research into the evolutionary dynamics of Cyprinidae. Understanding the functional roles of these genes, along with their evolutionary implications, will provide deeper insights into genetic diversity and adaptation within this family. This research could have broader implications for conservation efforts and species management strategies. The robust purifying selection on the nad4L and cob genes aids in eliminating such mutations, making them ideal molecular markers for phylogenetic analysis.

Our phylogenetic analysis provides the comprehensive and accurate phylogenetic tree for the *Schizothorax* genus, revealing important insights into its evolutionary relationships. The high nucleotide diversity observed in certain genes, such as nad1 and nad2, suggests that *S. biddulphi* may have evolved adaptive traits to environmental changes. These insights could inform conservation strategies by targeting these genes for monitoring and maintaining genetic diversity in the species, particularly in the face of ecological threats such as habitat loss and climate change.

Notably, the mitogenome labeled as *S. biddulphi* (NC_017873) in the previous study falls outside the principal Schizothorax clades, indicating that this sequence may require further re-examination.[13] Within *Schizothorax*, we also recover two well-supported clades: one comprising *S. curvilabiatus, S. oconnori, S. wangchiachii*, and *S. waltoni,* and a second including *S. chongi* and *S. kozlovi.* Together, these results support a revised placement of *S. biddulphi* and underscore the need to re-examine earlier taxonomic assignments using complementary datasets. This study underscores the need for further taxonomic revisions and additional analyses with complementary datasets to address these discrepancies, validating our findings as a more accurate representation of *Schizothorax* evolution.

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AUTHORS’ CONTRIBUTION

Conceptualization: Chengxin Wang, Site Luo; Methodology: Linghui Hu, Fangze Zi; Formal analysis and investigation: Huanhuan Wang, Wenxia Cai; Writing - original draft preparation: Chengxin Wang; Supervision: Yong Song, Bin Huo and Xiaotao Shi; Writing - review and editing: Shengao Chen.

COMPETING OF INTEREST – COPE

The authors declare no conflicts of interest.

ETHICAL CONDUCT APPROVAL – IACUC

All experimental protocols were approved by the Science and Technology Ethics Committee of Tarim University (approval code:2023027) and adhered to animal welfare laws, guidelines and policies.

INFORMED CONSENT STATEMENT

All authors and institutions have confirmed this manuscript for publication.

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/) under accession no OR812523. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1113635, SAMN41459639 , SRR29086388, respectively.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Mitochondrial genome sequences of Cyprinidae species from NCBI used in this study

Family	Genus	Organism	ID	Length	AT%
Cyprinidae	*Schizothorax*	*Schizothorax biddulphi*	NC_017873.1	16585	55.9
		Schizothorax macropogon	NC_020339.1	16588	55.3
		Schizothorax wangchiachii	NC_020360.1	16593	55.4
		Schizothorax waltoni	NC_020606.1	16589	55.3
		Schizothorax oconnori	NC_020781.1	16590	55.4
		Schizothorax richardsonii	NC_021448.1	16592	55.3
		Schizothorax esocinus	NC_022867.1	16583	55.2
		Schizothorax labiatus	NC_023365.1	16582	55.3
		Schizothorax progastus	NC_023366.1	16575	55.2
		Schizothorax plagiostomus	NC_023531.1	16576	55.9
		Schizothorax prenanti	NC_023829.1	16587	55
		Schizothorax chongi	NC_024621.1	16584	55
		Schizothorax pseudoaksaiensis	NC_024833.1	16582	55.6
		Schizothorax davidi	NC_026205.1	16576	54.9
		Schizothorax lantsangensis	NC_026294.1	16580	54.9
		Schizothorax lissolabiata	NC_027162.1	16583	54.9
		Schizothorax kozlovi	NC_027670.1	16585	55
		Schizothorax grahami	NC_029708.1	16584	54.9
		Schizothorax nepalensis	NC_031537.1	16589	55.4
		Schizothorax gongshanensis	NC_031803.1	16591	55.1
		Schizothorax curvilabiatus	NC_035994.1	16578	55.5
		Schizothorax integrilabiatus	NC_036746.1	16588	55.7
		Schizothorax eurystomus	NC_036933.1	16588	55.1
		Schizfothorax molesworthi	NC_037183.1	16585	55.7
		Schizothorax taliensis	NC_037516.1	16578	54.9
		Schizothorax griseus	NC_046462.1	16586	55.1
		Schizothorax sinensis	NC_056907.1	16571	55
		Schizothorax biddulphi	OR812523.1	16588	55.1
	Aspiorhynchus	*Aspiorhynchus laticeps*	NC_022855.1	16591	56

Supplementary Table 2. Amino acid composition and relative synonymous codon usage in the S. biddulphi mitogenome

Locus	Start	Stop	Size (bp)	Start Coding	Stop Coding	Strand
tRNA^{Phe}	1	69	69			H
12S rRNA	69	1024	956			H
tRNA^{Val}	1024	1096	73			H
16S rRNA	1097	2784	1688			H
tRNA^{Leu}	2784	2857	74			H
nad1	2816	3829	1014	ATG	TAA	H
tRNA^{Ile}	3831	3901	71			H
tRNA^{Gln}	3900	3971	72			L
tRNA^{Met}	3970	4039	70			H
nad2	4039	5082	1045	ATG	T	H
tRNA^{Trp}	5084	5156	73			H
tRNA^{Ala}	5160	5229	70			L
tRNA^{Asn}	5230	5303	74			L
tRNA^{Cys}	5338	5406	69			L
tRNA^{Tyr}	5406	5476	71			L
cox1	5468	7027	1560	GTG	TAA	H
tRNA^{Ser}	7030	7101	72			L
tRNA^{Asp}	7103	7175	73			H
cox2	7181	7872	692	ATG	T	H
tRNA^{Lys}	7872	7944	73			H
atp8	7945	8113	169	ATG	TAA	H
atp6	8103	8787	685	ATG	TAA	H
cox3	8786	9572	787	ATG	T	H
tRNA^{Gly}	9571	9641	71			H
nad3	9641	9992	352	ATG	TAG	H
tRNA^{Arg}	9990	10,059	70			H
nad4l	10,059	10,356	298	ATG	TAA	H
nad4	10,349	11,730	1382	ATG	TAG	H
tRNA^{His}	11,730	11,799	70			H
tRNA^{Ser}	11,799	11,867	69			H
tRNA^{Leu}	11,875	11,948	74			H
nad5	11,948	13,787	1840	ATG	TAG	H
nad6	13,783	14,305	523	ATG	TAA	L
tRNA^{Glu}	14,306	14,375	70			L
cob	14,380	15,521	1142	ATG	T	H
tRNA^{Thr}	15,521	15,593	73			H
tRNA^{Pro}	15,593	15,663	71			L