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Insights into structural features and phylogenetic implications of the complete mitochondrial genome of Fasin rainbow fish (Melanotaenia fasinensis)

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Abstract

The Fasin Rainbow fish, scientifically known as *Melanotaenia fasinensis*, is highly sought after by aquarium enthusiasts due to its vibrant colors and adaptability to artificial aquatic environments. This species is endemic to the karst landscape of the Bird's Head region in Papua, Indonesia, and belongs to the Melanotaeniidae family. Discovered relatively recently in 2010, it has been designated as endangered by the International Union for Conservation of Nature (IUCN) in 2021. However, there is currently insufficient data regarding its phylogenetic positioning. To address this gap, our study employed next-generation sequencing to analyze the entire mitochondrial genome of *M. fasinensis*. The mitochondrial genome consists of 13 protein-coding genes, 22 transfer RNA genes, and two ribosomal RNA genes, with a total length of 16,731 base pairs. The base composition of the mitogenome revealed percentages of 27.76% adenine (A), 27.34% thymine (T), 16.15% guanine (G), and 28.75% cytosine (C). Our phylogenetic analysis, based on nucleotide sequences mitogenome, indicated that *M. fasinensis* occupies a relatively basal position within the Melanotaenia genus. This study provides valuable molecular insights for further exploration of phylogeography and evolutionary history, not only for *M. fasinensis* but also for other members of the Melanotaenia genus.

1. Introduction

The ornamental fish industry in Indonesia, particularly in the freshwater sector, exhibits significant potential for growth and development. Freshwater ornamental fish, which are indigenous to Indonesia, serve as a valuable fishery commodity with substantial export value, thereby making a significant contribution to the nation's foreign exchange earnings. The export value of freshwater ornamental fish in Indonesia surpasses that of marine ornamental fish, accounting for approximately 80.63% and 19.37%, respectively [1, 2]. At present, Indonesia holds a significant share of approximately 20% of the global market for ornamental fish. The production of these fish is primarily derived from natural catches, which account for approximately 95% of the total, while cultivated products contribute only 5% [1, 2].

Rainbow fish, scientifically known as Melanotaeniidae, are a popular choice for freshwater ornamental fish among aquarists due to their vibrant and visually appealing coloration, as well as their capacity to flourish in captive environments [3, 4], making them of significant economic value. The Fasin rainbowfish, *Melanotaenia fasinensis* Kadarusman, Sudarto, Paradis & Pouyaud 2010 belongs to the family of Melanotaeniidae, known as endemic species, has a small size, usually less than 10 cm, and possesses bright coloration and one of the popular ornamental fish. The species is known only in the type locality of the Fasin River, near Ween Village, West Papua Province, Indonesia [3, 4].

In recent years, several partial mitochondrial genes have been sequenced and used for phylogenetic analysis of Melanotaeniidae involving some Melanotaenia species. Furthermore, the partial gene sequences used in previous investigations not really clear to resolve systematic and phylogenetic relationships within the genus Melanotaenia species [3, 5].Therefore, genetic relationships within Melanotaenia remained lack information and indicated the requirement of additional informative sites from longer DNA sequences such as mitochondrial genome (mitogenomes) [6]. The mitogenome is generally considered as an excellent molecular marker for phylogenetic analyses among fish taxa [7, 8]. Most teleost mitogenomes are circular in shape, 15

to 19 kb in size and encode 37 genes that comprise 13 protein-coding genes (PCGs), 2 ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs) and two non-coding regions (control region (CR), and origin of the light strand (OL) [9, 10]. A typical teleost mitogenome is characterized by conserved gene content, small genome size, maternal inheritance, rapid evolutionary rate, great copy numbers and lack of intermolecular genetic recombination [11–13]. The systematic analysis and comparison of the mitogenome and its distinct features allow this molecule to be extensively used for species identification, studying population genetics, evolutionary relationships and phylogeography in many teleosts [14].

So far, about 87 taxonomically accepted species [15]. Of these, only 7 species have complete mitochondrial DNA sequences deposited in the GenBank and relatively used to understand the evolutionary relationships within melanotaeniid fishes. Therefore, phylogenetic relationships of this species to other Melanotaenia species are still unknown. *M. fasinensis* has recently been classified as endangered by the International Union for the Conservation of Nature [16]. The population of rainbow ornamental fish is facing a significant threat due to the extensive freshwater fishing practices in rivers, which involve the use of pesticides, encroachment, and the conversion of forests into plantation areas. In addition, due to its restricted habitat in the karst formations, significant economic worth as an ornamental fish, and insufficient genetic research conducted thus far, efforts should be made to study this species e.g., to evaluate the phylogenetic placement of this species within melanotaeniid fishes. Hence, we sequenced and characterized the complete mitogenome of Assamese *M. fasinensis* using next generation sequencing (NGS) technology and compared it with other Melanotaenia mitogenomes, analyzing genome organization, codon usage patterns, tRNA secondary structure and strand asymmetry. Additionally, we reconstructed phylogenetic trees based on PCG sequences to confirm the taxonomic position of *M. fasinensis* and its relationship among Melanotaeniidae. The primary objective of this investigation was to obtain the whole mitochondrial genome of *M. fasinensis* by using high-throughput sequencing technology and to evaluate its phylogenetic relationship among other species in the genus *Melanotaenia*. The present study reflects the inaugural genomic investigation of the species. The current study will also significantly contribute to genome references for the family Melanotaeniidae.

2. Materials and methods

2.1. Specimen collection, identification and DNA extraction

Live specimens of *M. fasinensis* (Fig. 1) were collected from the Fasin River near Ween Village, District Sawiat, South Sorong, West Papua Province, Indonesia. Specimens were caught by the cast net on Thursday, March 2, 2023. Geographic coordinate position of the sampling site was 01^{o}14’18.0” N, 131^{o}58’31.3 E. Samples were preserved in a solution of 95% ethanol and stored at a temperature of -80^{0}C. Local permits, acquired from Akademi Perikanan Sorong (APSOR) in West Papua, allowed for the capture of specimens. We followed the International Union for Conservation of Nature's guidelines, as outlined in the IUCN Red List Data's Guidelines for Proper Utilization (Version 4.0) (IUCN, 2022), when conducting research involving endangered species. The genomic DNA was extracted from fin clip samples with the DNAeasy Blood and Tissue Kits (Qiagen, Germany). The extracted DNA quality was assessed using 0.8% agarose gel. The DNA

concentration was measured using the Qubit™ dsDNA HS Assay Kit and the QubitVR 2.0 fluorometer (Life Technologies, United States).

2.2. Mitogenome sequencing and assembly

High-quality genomic DNA was used to construct a DNA library. For the Nanopore library, a total of about 8 µg of genomic DNA (gDNA) was subjected to size selection (targeting 50 kb) using a Blue Pippin instrument (Sage Science, Beverly, MA). The further processing of the gDNA was carried out utilizing the Ligation Sequencing 1D Kit (SQK-LSK109, ONT, UK), following the guidelines provided by the manufacturer. The library was prepared and sequenced using the Flow Cells FLO-PRO002 on the ONT PromethION next generation sequencer manufactured by ONT in the United Kingdom. The coverage plot for studies utilizing the high-throughput DNA sequencing technology of the ONT PromethION sequencer is available in Supplementary 1. This process took place at the Genomic Laboratory, National Research and Innovation Agency, Cibinong, Indonesia. The fast5 files were subjected to base calling using the ONT Albacore program (v0.8.4). The readings that passed the filter and exhibited relatively greater quality were selected for future downstream studies. *M. fasinensis* mitochondrial genome coverage Depth Map showed in figure S1.

2.3. Mitogenome annotation, visualization, and comparative analysis

Raw nanopore reads were assembled by using Flye v2.9.2 [17]. Mitochondrial genome was then extracted from assembled reads by using GetOrganelle v1.7.7.0 (2). To reduce the error rate of assembled mitochondrial genome, we then polished the mitochondrial genome two rounds by using NextPolish [18]. The contigs were compiled and consisted of a combination of sequences derived from both organellar and nuclear genomes. Mitochondrial contigs were found through the utilization of similarity searches conducted by BLASTN 2.13.0 against the NCBI nucleotide collection (nt) database. MitoFish (http://mitofish.aori.u-tokyo.ac.jp/) was used to annotate the visualized mitochondrial genome [19, 20]. PCGs, rRNA genes were further confirmed manually using CLC Genomics Workbench 7.5.2 by aligning the new mitogenome with closely related Melanotaenia mitogenome [21]. The tRNAScan-SE server version 2.0 was used to identify the 22 tRNAs as well as reconfirm their secondary structures [22]. To ensure the correct reading frame, nucleotide sequences of the 13 PCGs were translated with MEGA XI [23] on the basis of the vertebrate mitochondrial genetic code. Similarly, CSBs were also identified by comparing the recognition sites with other Melanotaenia species in the control region of *M. fasinensis*. The circular map of the *C. meladerma* mitogenome was illustrated by the online server MitoAnnotator [19, 20, 24]. The secondary structure of the putative origin of light strand replication was predicted by the Mfold web server [25]. The nucleotide composition and the relative synonymous codon usage (RSCU) were calculated using MEGA XI [23]. The bias of nucleotide composition was measured as AT skew = (A − T)/(A + T) and GC skew = (G − C)/(G + C), respectively. Finally, the complete annotated mitogenome was submitted to the NCBI database.

2.4. Phylogenetic analyses to determine the phylogenetic position of *M. fasinensis*.

We conducted phylogenetic analyses based on 21 mitochondrial genome sequences highly similar to *M. fasinensis* (Table S1). Two families, Atherinidae and Isonidae, were used as out-groups. We aligned

mitogenome sequences using the MUSCLE program with the default settings in MEGA11 [23]. Phylogenetic analysis was performed using the Maximum Likelihood (ML) with 1000 bootstrap replicates. The length of consensus sequences, amount of variable sites, Kimura 2-Parameter (K2P) distance, and Ts/Tv ratios were calculated by the software MEGA11 [23].

3. Results

3.1. Genome structure, organization and composition

The total length of the mitochondrial genome of *M. fasinensis* was found to be 16,731 base pairs (bp) as reported in GenBank (accession number: OR879116). There are 13 genes that code for proteins, 22 genes that code for transfer RNA, 2 genes that code for ribosomal RNA, and a noncoding regulatory region called the D-loop in the mitochondria genome (Fig. 2, Table 1). The *M. fasinensis* mitogenome sequence is larger than other Melanotaenia mitogenomes ranging from 1,6487 bp in *M. lacustris* to 1,6731 in *M. fasinensis* (Table 2). Among them, 28 genes (12 PCGs, two rRNAs, and 14 tRNAs) were located on the heavy strand (H strand) and others (ND6 and 8 tRNAs) were located on the light strand (L strand) (Table 1). The comprehensive mitochondrial genome of M. fasinensis exhibited a base composition of 27.76% for adenine (A), 27.34% for thymine (T), 16.15% for guanine (G), and 28.75% for cytosine (C), with an A + T content of 55.10%. Furthermore, the A + T content of *M. fasinensis* was similar than that of other Melanotania genus mt genomes (Table 2).

Overall, the complete mitogenome displayed a preference for A + T content, comprising 54.56% (Table 2). The highest A + T content was observed in the control region (CR) at 61.92%, followed by transfer RNA sequences (tRNAs) at 55.75%, protein-coding genes (PCGs) at 54.4%, and ribosomal RNA sequences (rRNAs) at 54.42%. Similarly, in the comparison of mitogenomes among 7 Melanotaenia species, including *M. fasinensis*, they showed relatively similar nucleotide compositions to each other (Table 2).

In the newly sequenced mitogenome of *M. fasinensis*, the AT and GC skew were 0.008 and − 0.310 respectively. The AT skew of other representative Melanotaenia species varied from 0.021 (*M. praecox*) to 0.028 (*M.australis*) and GC skew varied from − 0.310 (*M. australis*, *M. splendida*, *M. fluviatilis*) to -0.299 (*M. boesmani*) (Table 2). The weakly positive AT skew in most of the genes indicates the occurrence of more Adenine (A)s Thymine (T)s than Thymine (T)s in the complete mitogenome of Melanotaenia species. With respect to the absolute value, the GC skew was always lower than the AT skew in all surveyed mitogenome of Melanotaenia species (Table 2).

Table 1
List of annotated mitochondrial genes of *M. fasinensis* and its characteristic features.

Name	Position		Length (bp)	Amino Acids	Start codon	Stop codon	Anticodon	Space (+) Overlap (-)[a]	Stand[b]
	From	To							
tRNA-Phe	1	68	68					0	H
12S RNA	69	1013	947					0	H
tRNA-Val	1014	1084						0	H
16S RNA	1085	2755						0	H
tRNA-Leu	2756	2829					UAA	0	H
ND1	2830	3804		324	ATG	TAG		4	H
tRNA-Ile	3809	3878						8	H
tRNA-Gln	3887	3959						-1	L
tRNA-Met	3959	4027						0	H
ND2	4028	4399		123	ATG	TAA		0	H
tRNA-Trp	4400	4471						0	H
tRNA-Ala	4472	4540						1	L
tRNA-Asn	4542	4614						0	L
O_L	4615	4648						0	H
tRNA-Cys	4649	4715						-4	L
tRNA-Tyr	4812	4879						68	L
COXI	4948	6498		516	GTG	TAA		11	H
tRNA-Ser	6510	6579					UGA	4	L
tRNA-Asp	6584	6654						4	H
COXII	6659	7349		230	ATG	T--		0	H

Name	Position		Length (bp)	Amino Acids	Start codon	Stop codon	Anticodon	Space (+) Overlap (-)[a]	Stand[b]
tRNA-Lys	7350	7422						1	H
ATPase8	7424	7591		55	ATG	TAG		-10	H
ATPase6	7582	8264		227	GTG	TA-		0	H
COXIII	8265	9049		261	ATG	TA-		0	H
tRNA-Gly	9050	9121						0	H
ND3	9122	9472		116	ATG	TAG		0	H
tRNA-Arg	9473	9541						0	H
ND4L	9542	9838		98	ATG	TAA		-7	H
ND4	9832	11214		460	ATG	TAA		0	H
CR1	11215	11924						0	H
tRNA-His	11925	11994						0	H
tRNA-Ser	11995	12062					GCU	6	H
tRNA-Leu	12066	12137					UAG	0	H
ND5	12138	13976		612	ATG	TAA		-4	H
ND6	13973	14494		173	ATG	TAA		0	L
tRNA-Glu	14495	14563						6	L
Cytb	14570	15709		379	ATG	TAA		1	H
tRNA-Thr	15711	15782						0	H
tRNA-Pro	15783	15851						0	L
CR2	15852	16731	880						H

a Negative value indicates the overlapping sequences between adjacent genes.

b H: heavy strand; L: light strand.

Table 2
Nucleotide composition and skewness of mitogenome in 8 Melanotaenia species and 2 species outgroups

Species	Accession Number	Length (bp)	A%	T%	G%	C%	A + T %	AT Skew	GC Skew
Complete mitochondrial genome									
Melanotaenia fasinensis	OR879116.1	16,731	27.76	27.34	16.16	28.75	55.10	0.008	-0.280
Melanotaenia lacustris	NC_004385.1	16,487	27.83	26.38	15.83	29.95	54.22	0.026	-0.308
Melanotaenia boesemani	NC_028208.1	16,493	27.88	26.47	15.74	29.92	55.35	0.022	-0.299
Melanotaenia australis	NC_030170.1	16,530	27.79	26.66	15.9	29.66	54.45	0.028	-0.310
Melanotaenia splendida	NC_028280.1	16,528	28.02	26.61	15.69	29.68	54.63	0.027	-0.310
Melanotaenia parkinsoni	NC_026904.1	16,529	28.08	26.55	15.66	29.71	54.63	0.015	-0.297
Melanotaenia fluviatilis	NC_081030.1	16,524	27.52	26.69	16.09	29.70	54.21	0.026	-0.310
Melanotaenia praecox	NC_030253.1	16,536	27.51	26.34	16.18	29.97	53.85	0.021	-0.302
Protein Coding genes (PCG)									
Melanotaenia fasinensis	OR879116.1	10,757	24.90	29.41	16.05	29.64	54.31	-0.083	-0.297
Melanotaenia lacustris	NC_004385.1	11,427	25.35	27.99	15.23	31.43	53.34	-0.049	-0.347
Melanotaenia boesemani	NC_028208.1	11,427	25.28	28.22	15.28	31.22	53.50	-0.055	-0.343
Melanotaenia australis	NC_030170.1	11,427	25.12	28.54	15.43	30.91	53.66	-0.064	-0.334
Melanotaenia splendida	NC_028280.1	11,427	25.51	28.30	15.14	31.05	53.81	-0.052	-0.344
Melanotaenia parkinsoni	NC_026904.1	11,427	25.62	28.25	15.08	31.05	53.87	-0.049	-0.346
Melanotaenia fluviatilis	NC_081030.1	11,427	24.94	28.36	15.53	31.16	53.85	-0.064	-0.335
Melanotaenia praecox	NC_030253.1	11,427	24.82	28.18	15.78	31.22	53.00	-0.063	-0.329

Species	Accession Number	Length (bp)	A%	T%	G%	C%	A + T %	AT Skew	GC Skew
tRNA genes									
Melanotaenia fasinensis	OR879116.1	1,549	28.28	27.63	22.72	21.37	55.91	0.012	0.031
Melanotaenia lacustris	NC_004385.1	1,550	28.32	27.48	22.97	21.23	55.81	0.015	0.039
Melanotaenia boesemani	NC_028208.1	1,549	28.41	27.44	22.85	21.30	55.84	0.017	0.036
Melanotaenia australis	NC_030170.1	1,549	28,21	27.50	23.05	21.24	55.71	0.013	0.041
Melanotaenia splendida	NC_028280.1	1,548	28.29	27.58	23.06	21.06	55.88	0.013	0.045
Melanotaenia parkinsoni	NC_026904.1	1,548	28.23	27.52	23.13	21.12	55.75	0.013	0.045
Melanotaenia fluviatilis	NC_081030.1	1,549	28.08	27.30	22.98	21.62	55.38	0.014	0.030
Melanotaenia praecox	NC_030253.1	1,552	28.35	27.38	22.68	21.59	55.73	0.017	0.025
rRNA genes									
Melanotaenia fasinensis	OR879116.1	2,616	32.22	22.40	20.83	24.54	54.63	0.180	-0.082
Melanotaenia lacustris	NC_004385.1	2,622	32.07	22.35	20.90	24.71	54.42	0.179	-0.084
Melanotaenia boesemani	NC_028208.1	2,626	32.03	22.39	20.75	24.83	54.42	0.177	-0.089
Melanotaenia australis	NC_030170.1	2,622	31,77	22.39	20.94	24.90	54.16	0.173	-0.087
Melanotaenia splendida	NC_028280.1	2,621	32.13	22.43	20.83	24.61	54.56	0.178	-0.083
Melanotaenia parkinsoni	NC_026904.1	2,622	32.23	22.35	20.75	24.68	54.58	0.181	-0.086
Melanotaenia fluviatilis	NC_081030.1	2,621	31.97	22.36	21.18	24.49	54.33	0.177	-0.073
Melanotaenia praecox	NC_030253.1	2,625	31.81	22.44	21.03	24.72	54.25	0.173	-0.081
Control Regions (CR)									
Melanotaenia fasinensis	OR879116.1	1,590	30.00	30.75	14.72	24.53	60.75	-0.012	-0.250

Species	Accession Number	Length (bp)	A%	T%	G%	C%	A + T %	AT Skew	GC Skew
Melanotaenia lacustris	NC_004385.1	830	31.57	32.41	14.94	21.08	63.98	-0.013	-0.171
Melanotaenia boesemani	NC_028208.1	833	31.93	31.81	14.77	21.49	63.75	0.002	-0.185
Melanotaenia australis	NC_030170.1	873	32.65	31.73	14.32	21.31	64.38	0.014	-0.196
Melanotaenia splendida	NC_028280.1	874	32.04	32.49	14.53	20.94	64.53	-0.007	-0.181
Melanotaenia parkinsoni	NC_026904.1	874	31.81	32.38	14.53	21.28	64.19	-0.009	-0.188
Melanotaenia fluviatilis	NC_081030.1	871	31.11	33.52	14.81	20.55	64.64	-0.037	-0.162
Melanotaenia praecox	NC_030253.1	880	31.93	29.77	15.45	22.84	61.70	0.035	-0.193

3.2. Overlapping and intergenic spacer regions

The complete mitogenome of *M. fasinensis* contained six overlapping sequences totaling 26 bp in length (Table 1). These sequences exhibited varying lengths, spanning from 1 bp to 10 bp, with the most extensive overlap observed on the H strand between ATP8 and ATP6 (10 bp), as well as ND4L and ND4 (7 bp).

In addition, short noncoding intergenic spacers across the newly sequenced mitogenome with a total length of 93 bp and variation in the number of overlapping sequences was 5 with a total length of 29 bp (Table 1). The longest spacer (68 bp) was situated between tRNA-Try and COXI gene on the L strand. The comparative analysis exhibited variation in the number of overlapping sequences ranging from 4 (*M. praecox*) to 7 (*M. fluviatilis*) with a length variation of length variation ranging 9 bp to 30 bp in other Melanotaenia species (Table S2). Additionally, the longest intergenic spacer (69 bp) was observed between tRNA-Gly and ND3 of *M. boesemani*, while 11 bp were found between tRNA-Try and COXI of *M. australis*, *M. lacustris*, *M. parkinsoni*, and *M. splendida*. Conversely, 9 bp separated tRNA-Try and COXI of *M. praecox*, and only 4 bp were present between tRNA-Asp and COXII of *M. fluviatilis*, indicating greater diversity in both location and intergenic nucleotide lengths compared to the overlaps (Table S2).

3.3. Protein-coding genes

The collective length of protein-coding genes (PCGs) within the entire mitochondrial genome accounted for 10,757 bp, representing 64.29% of the total length. The 13 protein-coding genes within the mitochondria have a common initiation codon, ATG. The nucleotide sequence 'TAA', which serves as a complete stop codon, was observed in the genes ND2, COXI, ND4L, ND4, ND5, ND6, and Cytb. Additionally, an incomplete stop codon represented by 'T––' was identified in the genes COXII, COXIII, and ATPase 6. Among the protein-coding genes examined, it was observed that the ND5 gene had the greatest length, measuring 1,839 base pairs (bp), while the ATPse8 gene displayed the smallest length, measuring 168 bp (Table 1).

The collective A + T content of the 13 PCGs in *M. fasinensis* was calculated at 54.31%, with values ranging from 51.85% for ND4L to 57.16% for COXII (Table 3). Furthermore, to assess the extent of base bias across all PCGs, base skews were determined. The AT skew and GC skew values for all 13 PCGs of *M. fasinensis* are depicted in Fig. 3. Notably, twelve out of thirteen PCGs exhibited significant negative GC skewness, with a deviation observed in the ND6 region, consistent with patterns seen in most teleost fishes. Similar to other Melanotaenia mitogenomes, the majority of these PCGs initiated with the ATG codon, except for COX I, which commenced with GTG.

Regarding stop codons, eight protein-coding genes (PCGs) were terminated by the standard codons (TAA and TAG), whereas the remaining genes terminated with truncated codons (TA and T) (Table 1 and Table S3). The occurrence of these incomplete termination codons is potentially widespread in Melanotaenia mitogenomes and is thought to be offset by post-transcriptional polyadenylation mechanisms [6, 21, 26–29].

Table 3
Composition of protein-coding genes in *M. fasinensis*

Protein-coding genes	Length (bp)	A	T	G	C	A%	T%	G%	C%	A + T %
Total of PCGs	10757	2678	3164	1727	3188	24.90	29.41	16.05	29.64	54.31
ND1	975	242	283	144	306	24.82	29.03	14.77	31.38	53.85
ND2	372	104	89	49	130	27.96	23.92	13.17	34.95	51.88
COXI	1551	379	468	280	424	24.44	30.17	18.05	27.34	54.61
COXII	691	195	200	110	186	28.22	28.94	15.92	26.92	57.16
ATPase8	168	45	46	24	53	26.79	27.38	14.29	31.55	54.17
ATPase6	683	149	212	101	221	21.82	31.04	14.79	32.36	52.86
COXIII	785	205	215	129	236	26.11	27.39	16.43	30.06	53.50
ND3	351	73	114	58	106	20.80	32.48	16.52	30.20	53.28
ND4L	297	64	90	41	102	21.55	30.30	13.80	34.34	51.85
ND4	1383	357	391	195	440	25.81	28.27	14.10	31.81	54.09
ND5	1839	492	522	246	579	26.75	28.38	13.38	31.48	55.14
ND6	522	82	190	188	62	15.71	36.40	36.02	11.88	52.11
Cyt-b	1140	291	344	162	343	25.53	30.18	14.21	30.09	55.70

The relative synonymous codon usage (RSCU) values of PCGs in *M. fasinensis* (Fig. 4) were summarized along with those other Melanotaenia fish (Fig. S1). *M. fasinensis* encoded a total of 3611 amino acids in its PCGs. Analysis of codon usage patterns in these genes indicated that codons for Leucine (11.58%), Alanin (9.50%), Thr (7.70%), Ile (7.31%), and Serine (6.62%) were the most frequently employed, while Cys was less prevalent (0.72%). The distribution of amino acids and their relative frequencies in *M. fasinensis* corresponded with those observed in the mitogenome of other Melanotaenia species. Significantly, the third

position of the predominant codons (CUA-Leu1, AUU-Ile, CUU-leu2, CUC-leu2, and GCC-Ala) exhibited a preference for A and T, a pattern in line with earlier findings in Melanotaenia fish species (Fig. 4, Figure S2).

3.4. Transfer and ribosomal RNA genes

M. fasinensis possessed a full set of 22 tRNAs, otaling 1,549 base pairs. The lengths of these tRNAs varied individually, ranging from 68 bp for tRNA-Phe to 73 bp for tRNA-Gln, tRNA-Asp, and tRNA-Lys (Table 1). Among these tRNAs, 15 genes were situated on the H-strand, while the remainder were located on the L-strand (Table 1). The anticodons found in all tRNAs within the M. fasinensis mitogenome were consistent with those typically observed in most teleost species. Generally, there was a direct match between codons and anticodons. However, serine was represented by two different anticodons (UGA, GCU), and leucine was represented by UAG and UAA in *M. fasinensis*.

The tRNA secondary structures were depicted in Fig. 5. Apart from tRNASer 2, most tRNAs were predicted to adopt canonical cloverleaf secondary structures. Numerous non-complementary base pairs were identified throughout the stems of these tRNAs. The tRNASer2 found in M. fasinensis did not possess an identifiable DHU stem and loop, which is a characteristic observed in other fish mitogenomes The tRNASer2 identified in *M. fasinensis* lacked a recognizable DHU stem and loop or these abnormal tRNAs to function similarly to normal tRNAs. As in other fishes, *M. fasinensis* also had two rRNAs. Like other fishes. The 16S large ribosomal gene spanned 1,671 base pairs and was situated between tRNAVal and tRNALeu. Conversely, the 12S small ribosomal gene measured 945 base pairs and resided between tRNAPhe and tRNAVal (Table 1). Both ribosomal RNAs were positioned on the H-strand and were separated by tRNAVal.

3.5. Non coding regions

As in most vertebrates, the OL of *M. fasinensis* was found within a cluster of five tRNA genes (Trp, Ala, Asn, Cys, and Tyr) between tRNAAsn and tRNACys (Table 1). The length of the putative origin of light strand replication was 33 bp. The region had the ability to fold into a stable stem-loop secondary structure, with a stem formed by 8 paired nucleotides and a loop of 13 nucleotides.

Melanotaenia genus, *M. fasinensis* possesses two control regions (CRs): one situated between trnT and ND6 (designated as CR1) and the other positioned between trnT and trnF (designated as CR2). The CR stands out as the longest non-coding segment within vertebrate mitochondrial DNA, typically hosting crucial elements essential for replication and transcription processes [14, 30, 31]. Two identical CRs, CR1 measuring 710 bp in length were found between ND4 and tRNA-His (Table S6). Additionally, CR2, situated between tRNAPro and tRNAPhe and spanning approximately 880 base pairs. In addition, At the 5' end of the CR, a domain with termination-associated sequences was observed, featuring three TACAT motifs and two corresponding palindrome sequences, ATGTA. Additionally, a conserved sequence block (CSB-2) was detected at the end of the CR (Table S4). However, unlike most other fishes, the central conserved sequence block (CSB) domain and the CSB-1 and CSB-3 could not be recognized in *M. fasinensis*. Moreover, the general organization of the CR was similar to that reported for other Melanotaenia fishes [6, 21, 26]. The A + T content, AT skew and GC skew in CR were 60.75%, -0.012 and − 0.250 respectively, which was also nearly consistent with the findings of previous reports on other Melanotaenias studied here (Table S2)

3.7. Phylogeny

The mitogenome arrangement of M. fasinensis closely resembles that of various species within the Melanotaenia genus, such as *M. lacustris*, *M. boesemani*, *M. australis*, *M. splendida*, *M. Parkinson*i, *M. fluviatilis*, and *M. praecox*, as well as species from the Glossolepis and Iriatherina genera. Similarities were observed, ranging from 88.66–83.64%.

Phylogenetic trees were constructed using maximum likelihood, incorporating 11 species of Melanotaenia, as well as families Atherinomorus and Isonidae as the outgroup. This analysis unveiled the existence of two primary clades, labeled as clades I and II (Fig. 6, Table S1). The result indicated a high level of agreement among the ML trees in their overall structure and exhibited strong support values. The phylogenetic tree analysis indicated that all species of the Melanotaeniidae genera clustered together on the same branch. Furthermore, the intergeneric and interspecific taxonomic positions were explicit and clear. Phylogenetically, the Melanotaeniidae are closer to the family Isonidae than to the Atherinomorus family. The phylogenetic position of *M. fasinensis* is relatively in the basal position in the genus Melanotaenia. The result from this study would offer a comprehensive understanding of the evolutionary correlation between *M. fasinensis* and other rainbowfish species residing in the region of West Papua. The incorporation of more taxa from the genus Melanotaenia in the phylogenetic analyses on future may help to better understand the phylogenetic relationships and evolutionary history among species in this genus.

4. Discussion

In the present study, we found that the *M. fasinensis* mitogenome sequence is larger than other Melanotaenia mitogenomes. Within this group, 28 genes, comprising 12 protein-PCGs, two rRNAs, and 14 tRNAs, were situated on the heavy strand (H strand), while the remaining genes, including ND6 and 8 tRNAs, were positioned on the light strand (L strand). Like in other vertebrates, the majority of genes were encoded on the H strand, with the exception of ND6 and eight tRNA genes. Additionally, all genes exhibited similar lengths to those found in other bony fishes [32]. In addition, the gene arrangement in both L and H strand were canonically identical and consistent with other Melanotaenia genus [21, 29]. Genes located on the L-strand exhibited a notable preference for thymine (T) in the codon wobble position, while adenine (A) or cytosine (C) ending codons were overrepresented in genes on the H-strand [33].

The A + T content of *M. fasinensis* closely resembled that of mitochondrial genomes in other species within the Melanotania genus. The A + T content observed in the L-strand closely resembled that of other teleost mitochondrial genomes [30, 34]. This suggested that the mitochondrial genome of *M. fasinensis* displayed the typical arrangement found in both teleosts and vertebrates. Genes encoded on the L-strand exhibited a notable preference for thymine in the codon wobble position, while adenine or cytosine ending codons were more prevalent in the genes on the H-strand. This strand-specific bias is believed to stem from asymmetrical directional mutation pressure [33].

The predominance of Adenine over Thymine in most genes of the complete mitogenome of Melanotaenia species is indicated by a weakly positive AT skew. Additionally, the absolute value of GC skew consistently remained lower than that of AT skew across all surveyed mitogenomes of Melanotaenia species. This discrepancy may arise from strand asymmetry, suggesting a strand compositional bias and a potential

violation of Chargaff's second parity rule. This could be the result of strand asymmetry (strand compositional bias) due to the violation of Chargaff's second parity rule [7, 35].

The overlapping of nucleotides between adjacent genes is a common characteristic found in teleost mitogenomes, which aids in compacting the mitogenomes [30, 36]. The overlapping regions between ND4L/ND4 and ATP8/ATP6 were identified as common features found in Melanotaenia species [6, 21, 26–29] and other teleosts [37–40]. In addition, in this study showed that greater diversity in both location and intergenic nucleotide lengths compared to the overlaps. The same phenomenon has been observed in other teleost organisms [7, 14, 37, 41, 42].

In the majority of Melanotaenia species, a consistent trait is evident: ATP8 ranks as the shortest while ND5 stands as the longest among PCGs [6, 21, 26–29], This pattern mirrors a common occurrence observed in various other teleost fish species [43–46]. As with other Melanotaenia mitogenomes, the majority of protein-coding genes (PCGs) in this study were found to commence with the ATG codon, with the exception of COX I, which initiated with GTG. Across fish mitogenomes, conventional start codons are predominantly utilized for these genes, while alternate start codons are rare within the Melanotaenia genus, a trend also observed in teleosts and other eukaryotic organisms [33, 47, 48]. Concerning stop codons, eight protein-coding genes (PCGs) concluded with standard codons (TAA and TAG), while the rest ended with truncated codons (TA and T). Incomplete termination codons are likely widespread in Melanotaenia mitogenomes, potentially compensated for by post-transcriptional polyadenylation mechanisms [6, 21, 26–29].

The anticodons present in all tRNAs in the *M. fasinensis* mitogenome were in line with those commonly seen in the majority of teleost species. Overall, there was a direct correspondence between codons and anticodons. Nevertheless, serine was encoded by two distinct anticodons (UGA, GCU), and leucine was encoded by UAG and UAA in M. *fasinensis*. It was common for teleost mitogenomes to have multiple tRNAs recognizing different anticodons [7, 45].

Several non-matching base pairs were detected within the stems of these tRNAs. These mismatches observed in tRNA sequences appeared to be a common occurrence in teleost mitochondrial tRNA genes [49, 50]. It's probable that the mismatches present in the stems of these tRNAs underwent modifications via post-transcriptional editing mechanisms [51, 52]. The tRNASer2 found in *M. fasinensis* did not possess an identifiable DHU stem and loop, which is a characteristic observed in other fish mitogenomes [8, 53]. The tRNASer2 found in M. fasinensis did not feature a recognizable DHU stem and loop, a feature observed in other fish mitogenomes [8, 53] or these atypical tRNAs operate in a manner akin to regular tRNAs. They may necessitate coevolved interacting factors or post-transcriptional RNA editing [7, 33].

As in other fishes, *M. fasinensis* also had two rRNAs. Like other fishes. The 16S large ribosomal gene was situated between tRNAVal and tRNALeu. Conversely, the 12S small ribosomal gene resided between tRNAPhe and tRNAVal. Both ribosomal RNAs were positioned on the H-strand and were separated by tRNAVal, which aligns with a typical pattern found in most vertebrates [13, 54, 55]

The CR, also known as the control region, stands out as the largest non-coding segment within vertebrate mtDNA, commonly hosting vital elements essential for both replication and transcription functions [12, 56, 57]. Unlike its counterparts within the Melanotaenia genus, *M. fasinensis* is unique in having two control

regions (CRs). The CR stands out as the longest non-coding segment within vertebrate mitochondrial DNA, typically hosting crucial elements essential for replication and transcription processes [14, 30, 31]. CR1, spanning a length of 710 base pairs, was identified between ND4 and tRNA-His refer to Table S6. However, it lacked typical features associated with control regions, such as conserved sequence blocks (CSBs) as identified by [58], or termination-associated sequences (TASs) as identified by [59]. The result of this study align with the earlier documented findings concerning bony fish [60]. Furthermore, CR2, positioned between tRNAPro and tRNAPhe, spans approximately 880 base pairs. At the 5' end of the CR, a region containing termination-associated sequences was noted, comprising three TACAT motifs and two corresponding palindrome sequences, ATGTA. Additionally, a conserved sequence block (CSB-2) was identified at the termination of the CR. This block plays a role in positioning RNA polymerase during transcription and priming replication [58]. However, unlike most other fishes, the central conserved sequence block (CSB) domain and the CSB-1 and CSB-3 could not be recognized in M. fasinensis. This study also aligns with previous reports on other teleost mitogenomes, noting that only certain portions of CSB in fish were detected [58, 59]. Moreover, the general organization of the CR was similar to that reported for other Melanotaenia fishes [6, 21, 32]. The A + T content, AT skew, and GC skew observed in the control region (CR) were almost consistent with the results reported in previous studies on other Melanotaenia species examined in this study [6, 21, 26, 32]

The mitochondrial genome arrangement of *M. fasinensis* closely resembled that of all species within the Melanotaenia species [6, 21, 26, 28, 29], Glossolepis [26], and Iriatherina genera [32, 61]. Based on whole mitogenomic data, 11 species of Melanotaenia were analyzed using maximum likelihood analyses, revealing the presence of two major clades. Moreover, from a phylogenetic perspective, the Melanotaeniidae family shares a closer evolutionary relationship with the Isonidae family than with the Atherinomorus family. This finding aligns with the results of a prior molecular study that relied on partial mitochondrial DNA cytb gene analysis [62].

5. Conclusion

The present study describes the complete mitogenome of rainbow fish (*Melanotaenia fasinensis*), which is 16,731 bp in length. The organization and gene content did not differ significantly from the already registered mitogenome of other Melanotaenia genus, a distinctive two control region was observed in *M. fasinensis*. This observed variation has a scope to form an important tool for the identification of Melanotaenia at the genus level. Phylogenetic analyses using mitogenome confirmed the phylogenetic position of *M. fasinensis* within the genus Melanotaenia, and occupying a basal position, and was clearly distinct from other Melanotaenia species. The comprehensive mitogenome data presented here will serve as a valuable resource for future investigations into molecular taxonomy, genetic diversity, and population structure of *M. fasinensis*. Additionally, it offers fresh perspectives that could enhance conservation strategies for this species.

Declarations

Declaration of competing interest

The authors report no conflicts of interest. The authors alone are responsible for doing the research and writing the paper.

Ethics statement

The study protocol received approval from the Ethical Clearance and Research Permits granted by the Directorate of Research and Innovation, BRIN (number: 070/KE.02/SK/04/2023).

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Author Contributions Statement

H.M: Conceptualization, designed the experiments, performed the experiments, analyzed the data, wrote the main manuscript, and funding acquisition. K.S: designed the experiments, performed the experiments, and analyzed the data. K.K: Collected samples, designed the experiments, and performed the experiments. I.C.C, R.A, and A.A.M: Analyzed the data. W.E.K and S.L: Designed the experiments. E.P.H, B.I, A.T.S, S.S, and I.I : Performed the experiments. All authors concur to assume responsibility for all facets of the research.

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Figures



Figure 1

Photograph of *Melanotaenia fasinensis*, male (top), female (bottom). Photo: Kadarusman.

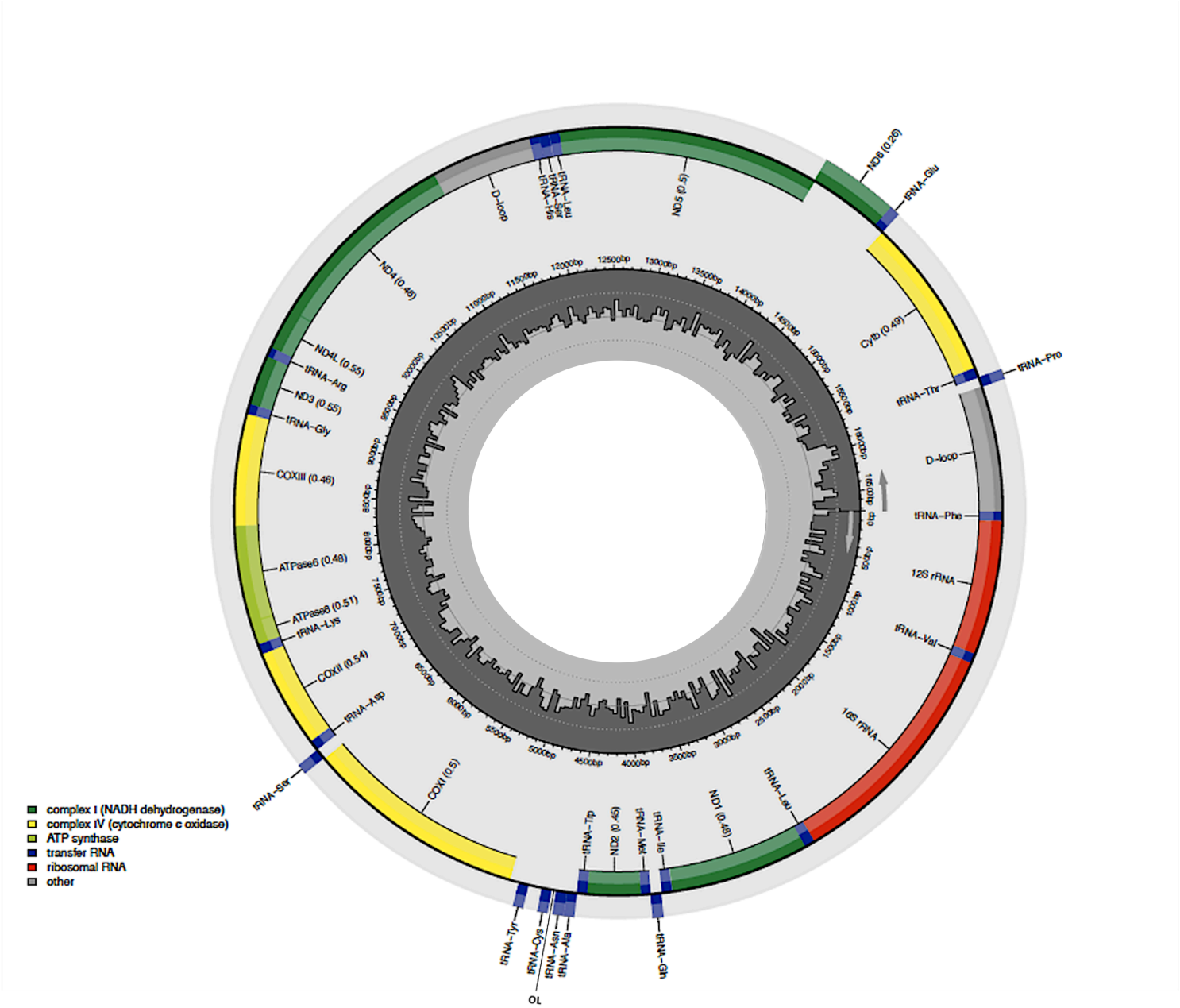


Figure 2

The circular mitochondrial genome map of *Melanotaenia fasinensis* in this study. Genes oriented in the reverse direction are depicted in the outermost concentric ring, while those in the forward orientation are situated in the second outermost ring. The innermost rings of the image illustrate the percentage of GC content for every 5 base pairs of the mitogenome, with longer lines indicating higher GC percentages.

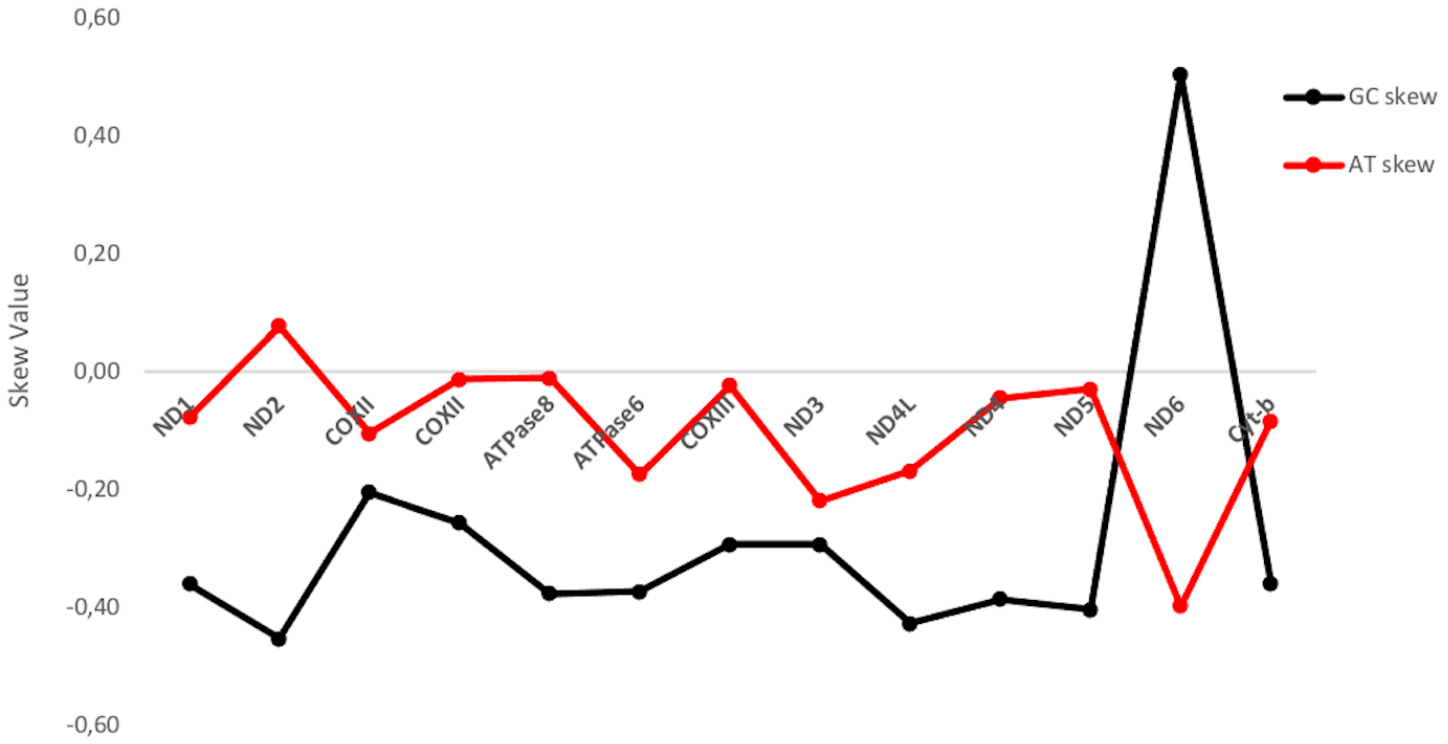


Figure 3

Graphical representation of AT and GC-skew in all the 13 protein-coding genes of *M. fasinensis* mitogenome.

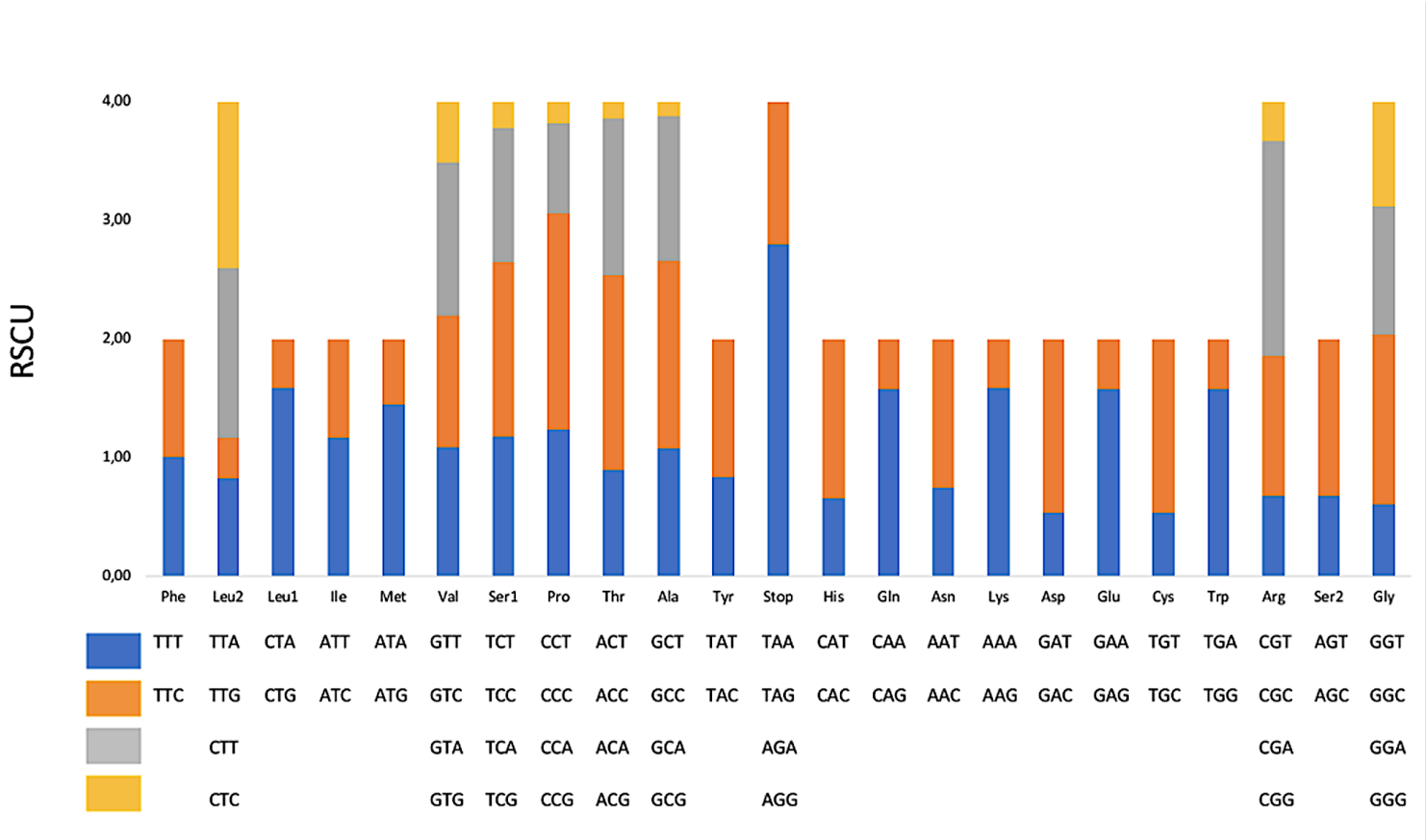
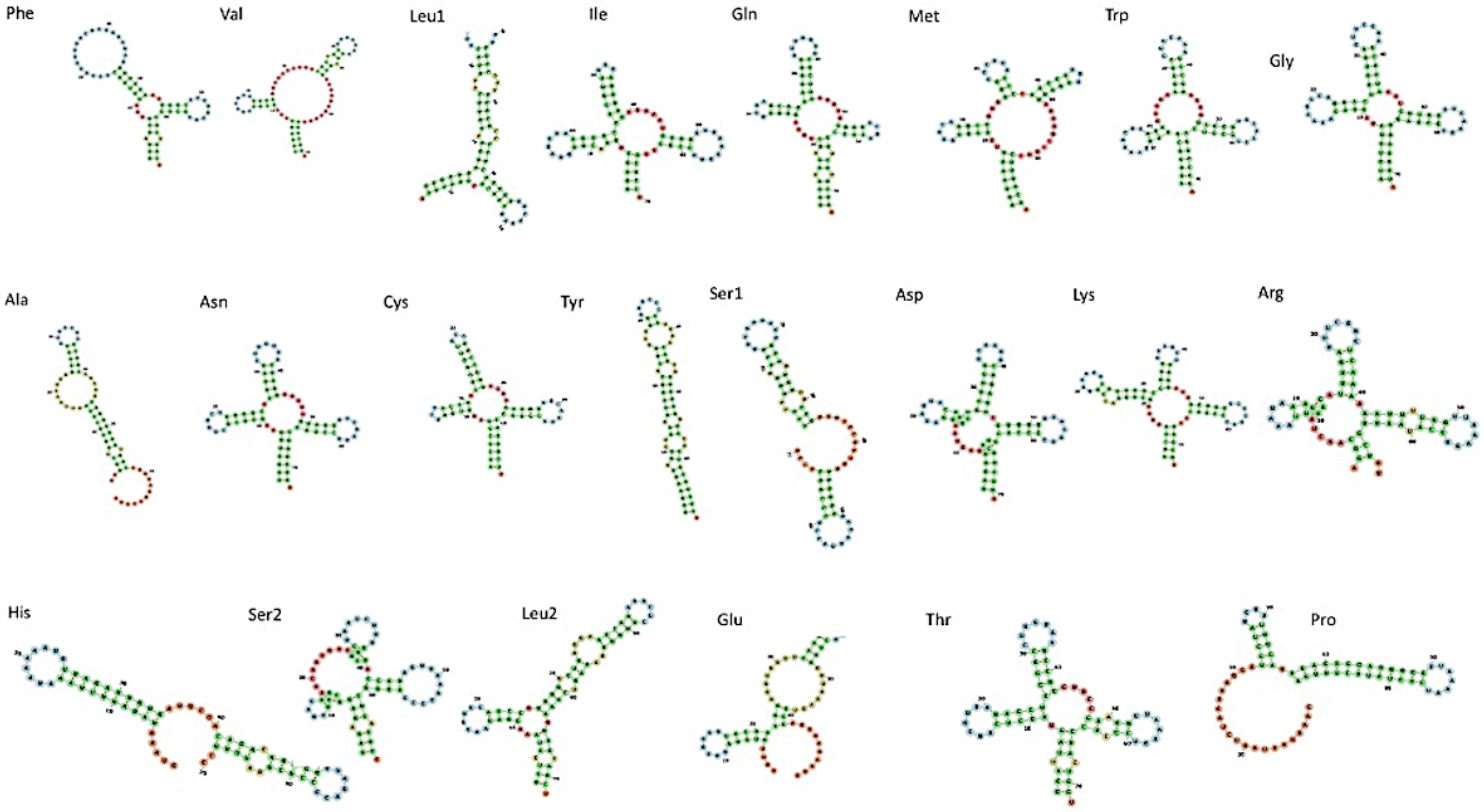


Figure 4

The relative synonymous codon usage (RSCU) of the mitochondrial protein-coding genes of *M. fasinensis*.

Figure 5



Putative secondary structures for 22 tRNA genes in mitochondrial genome of *M. fasinensis*.

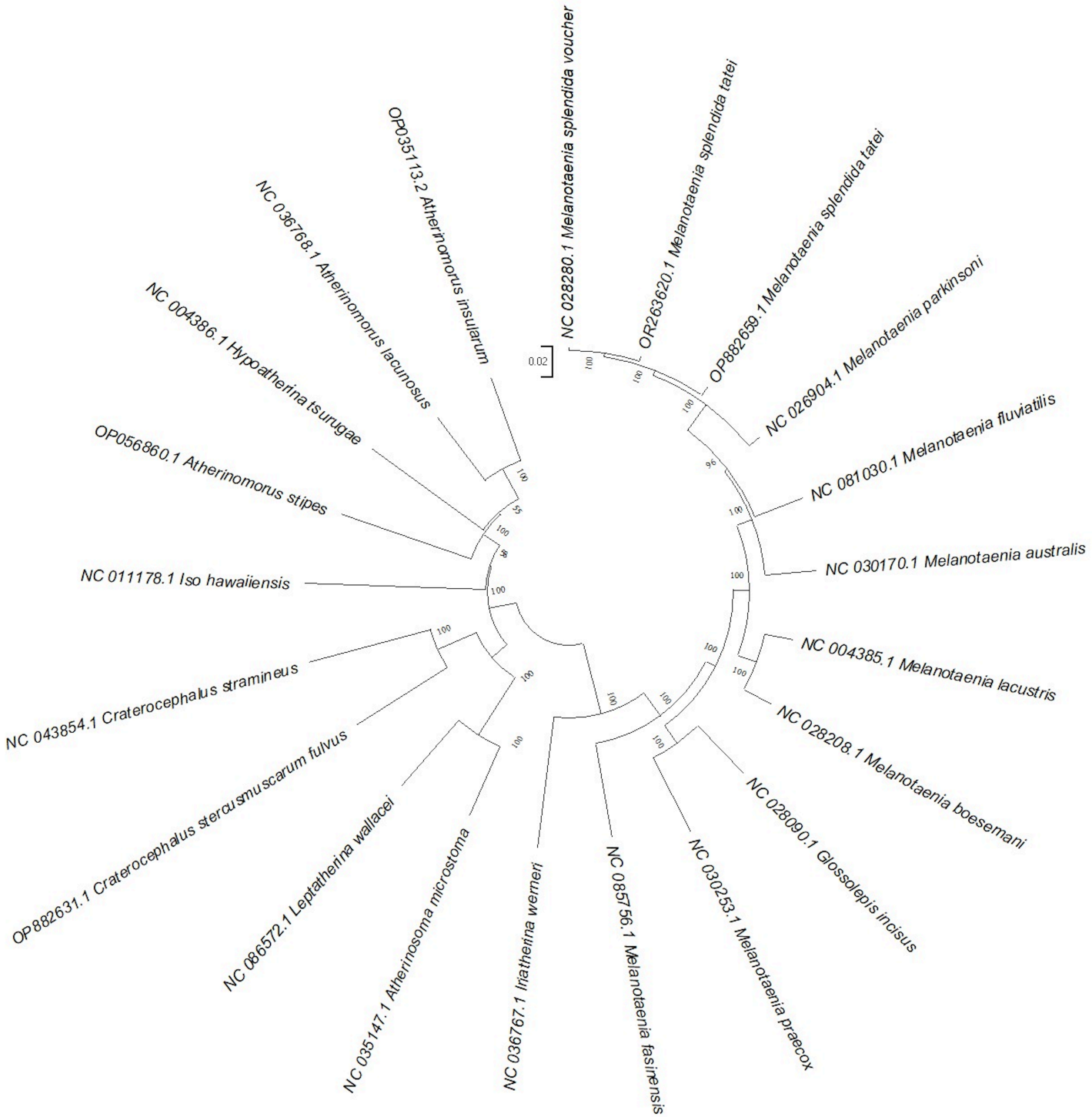


Figure 6

A maximum likelihood tree depicting the phylogenetic position of *M. fasinensis* among other 11 *Melanotaeniidae* families. Two species of families Atherinidae and Isonidaewere designated to be outgroups. The phylogenetic tree was constructed in MEGA software (version 11.0.13) using concatenated nucleotide sequences of mitogenome. Numbers at each node indicate the bootstrap support values by 1000 replications and shown only when they are larger than 50%. The scientific name and corresponding GenBank accession numbers were provided for each individual species.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.docx