TROPICAL LIFE SCIENCES RESEARCH

Characterization of the Complete Mitochondrial Genome of Silver Tigerfish (*Datnioides polota* Hamilton, 1822) from South Sumatra, Indonesia, Revealing Its Phylogenetic Position within the Genus *Datnioides*

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Highlights

- First complete mitogenome of South Sumatra *Datnioides polota* was characterized using Oxford Nanopore Technologies.
- The 16,496-bp mitogenome contains 13 protein-coding genes, 22 tRNAs, 2 rRNAs, and a control region.
- Mitogenomic phylogeny places *D. polota* close to *D. microlepis*, *D. undecimradiatus*, and *D. pulcher*.

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EARLY VIEW

Characterization of the Complete Mitochondrial Genome of Silver Tigerfish (*Datnioides polota* Hamilton, 1822) from South Sumatra, Indonesia, Revealing Its Phylogenetic Position within the Genus *Datnioides*

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Running head: The Complete Mitochondrial Genome of Silver Tigerfish

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Abstract: The silver tigerfish (*Datnioides polota* Hamilton, 1822) is an indigenous species of Indonesia, distributed in South Sumatra from tributaries to the Musi River. It is also found in the Kapuas River, Kalimantan. Genetic studies on the genus *Datnioides*, particularly those involving complete mitochondrial genome analyses, have primarily focused on species outside

Indonesia. This study aimed to generate and characterize the complete mitochondrial genome of silver tigerfish from South Sumatra, Indonesia, using whole-genome sequencing based on Oxford Nanopore Technologies and to clarify its phylogenetic position within the genus *Datnioides*. The first mitochondrial genome of *D. polota* species from South Sumatra, Indonesia, has been successfully sequenced using the next-generation sequencing platform of Oxford Nanopore Technologies (ONT). The sequence has a length of 16,496 bp and consists of 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs), and a control region (D-loop). The total nucleotide base composition of the mitochondrial genome contained 29.09% adenine, 25.59% thymine, 15.37% guanine, and 29.95% cytosine, with a GC content of 45%. Phylogenetic analysis based on mitochondrial genome sequences showed that silver tigerfish from South Sumatra had a closer relationship with three species within the *Datnioides* genus, including Indonesian tigerfish (*D. microlepis*), Mekong tiger perch (*D. undecimradiatus*), and Siamese tigerfish (*D. pulcher*), while exhibiting a more distant relationship with Campbell's tigerfish (*D.* campbelli) from New Guinea. The complete mitochondrial genome generated in this study provides an important genetic reference resource for *D. polota*, supporting species identification, phylogenetic assessment, and future population genetic studies. These mitogenomic data may contribute to the sustainable management of silver tigerfish in Indonesia, with relevance to domestication, selective breeding, aquaculture development, and conservation planning.

Keywords: Complete Mitochondrial Genome, Sequencing, Silver Tigerfish, *Datnioides*

INTRODUCTION

The silver tigerfish (*Datnioides polota* Hamilton, 1822) is an indigenous species of Indonesian fish known locally as "Ringau" and commercially as "Datz Silver" (Zamroni et al., 2023). This fish species inhabits brackish water to freshwater environments, typically occurring along river currents and extending downstream to estuarine regions (Habib & Islam, 2021). The genus *Datnioides*, belonging to the order Lobotiformes, comprises five globally recognized species. Three of them are found in Indonesia, including Campbell's tigerfish (*D. campbelli*), which is endemic to Papua, Indonesian tigerfish (*D. microlepis*), and silver tigerfish (*D. polota*), both of which are distributed in Sumatra and Kalimantan (Ahmad, 2020b; Zhou et al., 2022; Zamroni et al., 2023). The remaining two species, the Siamese tigerfish (*D. pulcher*) and the Mekong tigerfish (*D. undecimradiatus*), occupy the Mekong River, which flows through China, Myanmar, Laos, Thailand, Cambodia, and Vietnam. In addition, the Siamese tigerfish is also found in the Chao Phraya River, Thailand (Wang et al., 2016; Sun et al., 2020; Zhengwei et al., 2020).

The silver tigerfish is distributed across South Asia to Southeast Asia, specifically ranging from eastern India and Bangladesh eastward through Indochina and Indonesia to New Guinea, according to the IUCN (Ahmad, 2020a; Habib & Islam, 2021). In Vietnam, silver tigerfish are found in the middle to lower reaches of the Hau River, particularly from Can Tho City to Soc Trang Province. This fish is commonly caught using trawl nets from the river. The Hau River is part of the Mekong Delta, also known as the Nine Dragons River Delta, located in the southwestern region of Vietnam (Ut et al., 2020). Silver tigerfish are also found in the Cua Tieu River, Tien Giang Province, Southern Vietnam, which remains part of the Mekong Delta (Dong *et al.*, 2022). In Indonesia, it is distributed in South Sumatra along the Musi River and the Kapuas River in Kalimantan (Ridho, 2021; Zamroni et al., 2023).

Although several mitochondrial genome sequences are available for species within the genus *Datnioides*, mitogenomic information representing Indonesian populations with silver tigerfish (*D. polota*) remains limited. Previous genetic studies on *Datnioides* have mainly reported complete mitochondrial genomes for related species, such as Indonesian tigerfish (*D. microlepis*), Mekong tigerfish (*D. undecimradiatus*), and Campbell's tigerfish (*D. campbelli*) (Wang et al., 2016; Sun et al., 2020; Zhou et al., 2022). In addition, an existing *D. polota* mitochondrial reference sequence is available in NCBI; however, detailed mitogenome characterization of Indonesian *D. polota*, particularly from South Sumatra, remains insufficient. This represents an important knowledge gap because population-specific mitochondrial genome data can provide genetic reference resources for species identification, phylogenetic comparison, and future population genetic studies.

Several sequencing approaches have been used for mitochondrial genome characterization, including Sanger sequencing, Illumina short-read sequencing, and third-generation long-read sequencing. Sanger sequencing is highly accurate but requires primer walking or long-PCR strategies, which can be time-consuming for complete mitochondrial genome assembly. Illumina short-read sequencing provides high per-base accuracy and high throughput, but short reads may be less effective for resolving repetitive regions, long structural regions, or highly AT-rich control regions. In contrast, Oxford Nanopore Technologies (ONT) sequencing can generate long reads that may span large genomic regions and facilitate the rapid assembly of complete mitochondrial genomes (Wang et al., 2021; De Vivo et al., 2022; Szakállas et al., 2024). Previous studies have shown that nanopore long-read sequencing can successfully assemble complete mitochondrial genomes and support mitophylogenomic or species-identification studies (Baeza & Bejarano, 2022; Baeza et al., 2025). However, ONT sequencing also has limitations, particularly a higher raw-read error rate compared with short-

read platforms, potential insertion/deletion errors in homopolymer regions, and the need for high-quality DNA, sufficient coverage, and careful bioinformatic validation (Baeza & Bejarano, 2022; De Vivo et al., 2022; Zheng et al., 2023).

Therefore, this study aimed to generate and characterize the complete mitochondrial genome of silver tigerfish from South Sumatra, Indonesia, using whole-genome sequencing based on Oxford Nanopore Technologies, and to determine its phylogenetic position within the genus *Datnioides*. The resulting mitogenome provides a population-level genetic reference for Indonesian *D. polota* and contributes to future studies on phylogeography, genetic diversity, conservation, and sustainable management.

MATERIALS AND METHODS

Fish and tissue collection

Fish with a total length of 16.2 cm and a body weight of 69.2 g were captured by a local fisherman from the Musi River in Palembang Province, South Sumatra, Indonesia, and subsequently transported to the laboratory. Standard morphological analysis, including external morphological and meristic traits, was performed to confirm species identification (Hayuningtyas et. al., 2026). The caudal fin tissue was collected and preserved in 90% ethanol until DNA extraction. Before tissue collection, the fish was anesthetized using 0.4 mL/L 2-phenoxyethanol to minimize stress and ensure ethical handling (Felix et al. 2013). The use of experimental animals and anesthetics to minimize fish stress was approved by the Animal Ethics Committee under approval No. 169/KE.02/SK/07/2024. The specimen of silver tigerfish has been deposited at the Zoological Museum, National Research and Innovation Agency, Indonesia and is registered under the number of MZB.fish.26995.

DNA Extraction and Whole Genome Sequencing (WGS)

The DNA genome was extracted from the fin tissue using DNeasy Blood & Tissue Kits (Qiagen) following the manufacturer's instructions (Marnis et al., 2024). DNA quality and quantity were measured using a nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Spectrophotometric measurements revealed a DNA concentration of 239 ng/µL and a purity of 1.99 (A260/A280). Accurate DNA concentration was also performed using the QubitTM dsDNA HS Assay Kit and QubitVR 2.0 fluorometer (Life Technologies, United States). DNA genome integrity was evaluated on a 1.5% agarose gel at 1x TBE using 0.01% Safe DNA dye solution visualized with a UVITEC Gel Documentation System. Genomic

DNA was placed in 1.5 mL microtubes and stored at -20°C until further analysis. DNA integrity was also visually clear, showing no fragmentation or contamination. Therefore, the high-quality genome obtained was used for Whole Genome Sequencing (WGS).

WGS analysis was performed using NGS services at E-Science Services (ELSA) at the Genomics Laboratory, National Research and Innovation Agency (BRIN), Indonesia. DNA library was synthesized from a genome DNA sample using kit type SQK-LSK114, Oxford Nanopore Technologies (ONT), following the manufacturer's procedures (Marnis et al., 2024). Each library was run using an FLO-PRO114M flow cell for 20 hours and 45 minutes, following the manufacturer's instructions. The DNA library was then sequenced on a PromethION PC24B329 sequencer (Nanopore, Oxford, UK), with 30x coverage (±16 Gb) reads. Software parameters used were default unless otherwise specified. Sequencing was run directly with default settings on MinKNOW core version 5.9.12, Bream version 7.9.8 with configuration 5.9.18, and Dorado 7.3.11. Run settings were set to a minimum read length of 200 bp, unmodified basecalling with a high-accuracy model, 400 bps, and a Min Q score of 9. Output data were stored as FASTQ output data, which automatically saves one file every 10 minutes.

Mitochondrial genome annotation

The quality of the genome sequencing data was assessed using NanoPlot version 1.43.0 (De Coster et al., 2018). Assembly and annotation of the complete mitochondrial genome were carried out using Geneious Prime version 2026.0.1 (Kearse et al., 2012). Annotation was performed by comparing the assembled mitochondrial genome with reference sequences of silver tigerfish available in the NCBI database (Accession No. MZ930122.1). Further, circular mitochondrial genome was visualized using MitoAnnotator (Mitochondrial Genome Database of Fish, MitoFish) (Iwasaki et al., 2013). The AT-skew and GC-skew nucleotide composition biases were obtained using MEGA 11 based on the following formulas: AT-skew = (A-T)/(A+T) and GC-skew = (G-C)/(G+C) (Gao et al., 2024). Amino acid composition and relative synonym codon usage (RSCU) calculations were analyzed using Ezcodon on the EZmito web server (http://ezmito.unisi.it).

Phylogenetic Analysis

Phylogenetic analysis was performed by comparing the assembled complete mitochondrial genome of Indonesian tigerfish (Accession No. OP242183) with the complete mitochondrial genomes of other species within the genus *Datnioides*, including Mekong tigerfish (NC_030259), Siamese tigerfish (NC_071872), and Campbell's tigerfish (MZ930121) (Wang

et al., 2016; Sun et al., 2020; Zhou et al., 2022). Additionally, the tripletail (*Lobotes surinamensis*, Accession No. PP032980) from the family Lobotidae was included for a broader taxonomic comparison. The mitochondrial genome of the broadbanded velvetchin (*Hapalogenys analis*, Accession No. MT561534) was used as an outgroup to root the phylogenetic tree (Gao et al., 2024). The phylogenetic tree was constructed in Geneious Prime using the neighbor-joining (NJ) method based on the Tamura-Nei genetic distance model (La Rosa et al., 2013).

RESULTS

Whole-genome sequencing of the silver tigerfish caused approximately 16 Gbp of raw reads. From these reads, the complete mitochondrial genome was successfully assembled, resulting in a sequence of 16,496 bp in length. The assembled mitogenome has been deposited in the NCBI GenBank database under accession number PV696079. The assembled mitogenome consisted of two ribosomal RNA genes (12S and 16S), 22 transfer RNA (*trnF*, *trnV*, *trnL*, *trnI*, *trnQ*, *trnM*, *trnW*, *trnA*, *trnN*, *trnC*, *trnY*, *trnS*, *trnD*, *trnK*, *trnG*, *trnR*, *trnH*, *trnS*, *trnL*, *trnE*, *trnT*, *trnP*), 13 protein-coding genes (*atp6, atp8, nd1, nd2, nd3, nd4, nd4l, nd5, nd6, cox1, cox2, cox3,* and *cytb*), and one non-coding control region (*D-loop*) (Fig. 1). The nucleotide composition was 29.09% adenine (A), 25.59% thymine (T), 15.37% guanine (G), and 29.95% cytosine (C), resulting in a total GC content of 45.32%.

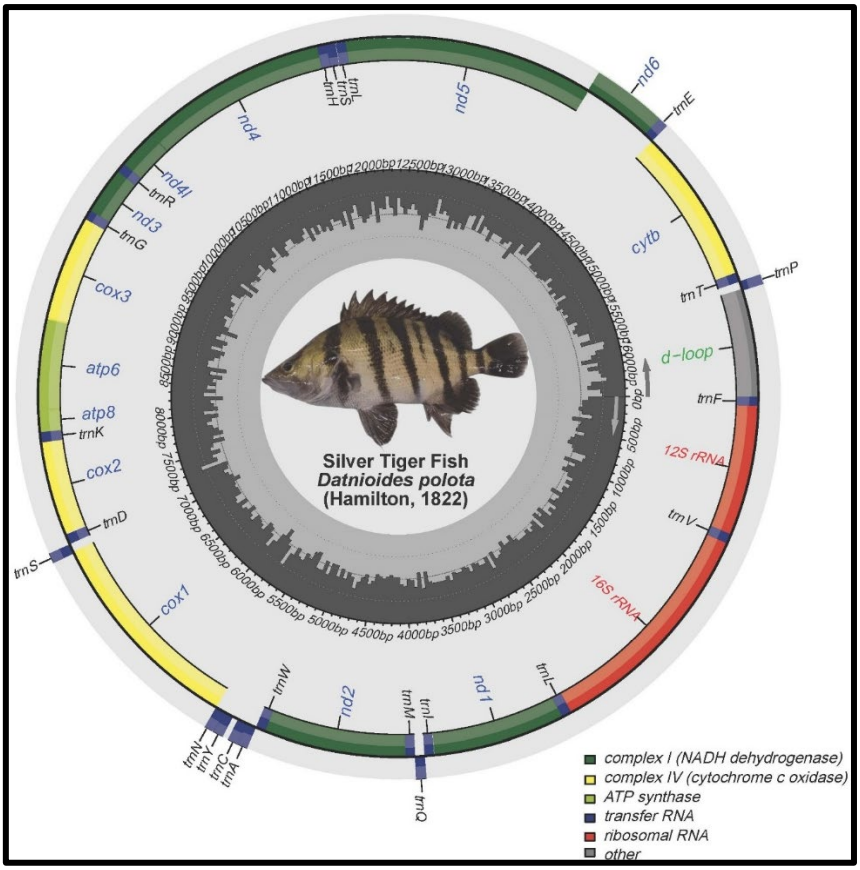


Figure 1: Gene map of the complete mitochondrial genome of silver tiger fish. Genes located on the heavy strand are displayed on the inner ring of the circle, whereas genes on the light strand are shown on the outer ring. The map includes 13 protein-coding genes (PCGs), 22 transfer RNA (tRNAs), 2 ribosomal RNA (rRNA), and a control region (*d-loops*). The darker and longer segments on the inner circle represent regions with higher GC content.

The characteristics of the silver tigerfish mitochondrial genome are summarized in Table 1. The base sizes of ribosomal RNA were 962 bp (*12srRNA*) and 1704 bp (*16srRNA*). The size of the 13 protein-coding genes (PCG) was the shortest in the *atpase8* gene (168 bp) and the longest in the *nd5* gene (1839 bp). Transfer RNA genes had a base size ranging from 68 to 75 bp. A total of 15 intergenic spacers (IGS) were identified, functioning as separators between genes, with lengths ranging from 1 to 42 bp, and some regions also exhibited overlapping bases. The GC content of individual genes varied between 31% and 57%, reflecting differences in base composition among genes. In addition, the number of amino acids encoded by the genes ranged from 19 to 600. The serine tRNA (*trnS*) was encoded with anticodons UGA and GCU, while the leucine tRNA (*trnL*) contained anticodons UAA and UAG. Most protein-coding genes (PCGs) initiated with the standard start codon ATG; however, *cox1* and *atp6* began with alternative start codons GTG and TTG, respectively. Among the 13 PCGs, not all terminated with complete stop codons, such as TAA or TTA; some ended with

an incomplete stop codon (e.g., T--) or lacked a clear termination codon. Based on strand orientation, nine genes were located on the light strand (L-strand), including eight tRNA genes and one PCG (*nd6*), while the remaining 29 genes were encoded on the heavy strand (H-strand), comprising 14 tRNAs, 12 PCGs, two rRNAs, and the control region (D-loop), positioned between *trnF* and *trnP*.

Table 1. Characteristics of the complete mitochondrial genome of silver tigerfish from South Sumatra.

No	Gene	Position		IGS (bp)	Size (bp)	GC content (%)	Composition (%)				Amino Acid	Codon			Strand
		Start	**End**				**A**	**T**	**G**	**C**		**Start**	**Stop**	**Anti**	
1	*trnF*	1	68	0	68	41	36	23	19	22	22	-	-	-	H
2	*12S rRNA*	69	1030	0	962	48	29	23	21	27	301	-	-	-	H
3	*trnV*	1031	1104	0	74	48	25	27	21	27	20	-	-	-	H
4	*16S rRNA*	1105	2808	0	1704	44	34	22	19	25	540	-	-	-	H
5	*trnL*	2809	2882	0	74	51	27	22	22	29	24	-	-	UAA	H
6	***nd1***	2883	3857	4	**975**	44	27	29	12	32	316	ATG	TAA	-	H
7	*trnI*	3862	3931	0	70	49	28	23	25	24	22	-	-	-	H
8	*trnQ*	3932	4001	0	71	44	25	31	28	16	19	-	-	-	L
9	*trnM*	4002	4072	0	72	39	29	32	16	23	22	-	-	-	H
10	***nd2***	4073	5118	0	**1046**	47	27	26	10	37	337	ATG	TTA	-	H
11	*trnW*	5119	5190	0	72	54	29	17	25	29	23	-	-	-	H
12	*trnA*	5191	5259	1	69	40	24	36	26	14	22	-	-	-	L
13	*trnN*	5261	5333	42	73	47	19	34	28	19	22	-	-	-	L
14	*trnC*	5376	5444	0	69	51	20	29	31	20	23	-	-	-	L
15	*trnY*	5445	5514	1	70	48	21	31	30	18	22	-	-	-	L
16	***cox1***	5516	7075	-9	**1560**	46	25	29	18	28	498	GTG	---	-	H
17	*trnS*	7067	7137	3	71	48	21	31	29	19	23	-	-	UGA	L
18	*trnD*	7141	7213	8	73	48	24	28	27	21	24	-	-	-	H
19	***cox2***	7222	7912	0	**691**	42	29	29	15	27	226	ATG	T--	-	H
20	*trnK*	7913	7987	1	75	47	28	25	21	26	22	-	-	-	H
21	***atp8***	7989	8156	-10	**168**	44	29	27	11	33	52	ATG	TAA	-	H
22	***atp6***	8147	8829	0	**683**	46	25	29	14	32	221	TTG	TA-	-	H
23	***cox3***	8830	9614	0	**785**	46	24	30	16	30	254	ATG	TA-	-	H
24	*trnG*	9615	9686	0	72	31	38	31	15	16	23	-	-	-	H
25	***nd3***	9687	10035	0	**349**	48	21	31	13	35	112	ATG	T--	-	H
26	*trnR*	10036	10104	0	69	49	27	24	23	26	21	-	-	-	H
27	***nd4l***	10105	10401	-7	**297**	48	23	29	14	34	97	ATG	TAA	-	H
28	***nd4***	10395	11775	0	**1381**	46	27	27	13	33	442	ATG	T--	-	H
29	*trnH*	11776	11843	0	68	34	35	31	17	17	20	-	-	-	H

No	Gene	Position		IGS (bp)	Size (bp)	GC content (%)	Composition (%)				Amino Acid	Codon			Strand
		Start	**End**				**A**	**T**	**G**	**C**		**Start**	**Stop**	**Anti**	
30	*trnS*	11844	11910	4	67	57	20	23	26	31	22	-	-	GCU	H
31	*trnL*	11915	11987	0	73	43	32	25	20	23	22	-	-	UAG	H
32	***nd5***	11988	13826	-4	**1839**	44	29	27	12	32	600	ATG	TAA	-	H
33	***nd6***	13823	14344`	2	**522**	42	15	43	32	10	172	ATG	TAA	-	L
34	*trnE*	14347	14415	5	69	41	21	38	27	14	23	-	-	-	L
35	***Cytb***	14421	15561	0	**1141**	44	25	31	13	31	369	ATG	TT-	-	H
36	*trnT*	15562	15631	-1	70	45	25	30	24	21	21	-	-	-	H
37	*trnP*	15631	15699	0	69	31	30	39	21	10	20	-	-	-	L
38	*d-loop*	15700	16496	0	797	-	-	-	-	-	-	-	-	-	H

Note: Positive values in the IGS/overlap column indicate intergenic spacers between adjacent genes, whereas negative values indicate gene overlaps. A value of 0 indicates that adjacent genes are continuous, with no spacer or overlap. Bold text: 13 protein-coding genes (PCGs).

Gene boundary analysis identified both intergenic spacers and overlapping regions between adjacent mitochondrial genes. Nine intergenic spacers were detected, ranging from 1 to 42 bp, and were located between *nd1–trnI* (4 bp*), trnW–trnA* (1 bp), *trnA–trnN* (42 bp), *trnY–cox1* (1 bp), *trnS–trnD* (3 bp), *trnD–cox2* (8 bp), *trnK–atp8* (1 bp), *nd6–trnE* (2 bp), and *trnE–cytb* (5 bp). The longest intergenic spacer was found between *trnA* and *trnN*. In addition, five gene overlaps were identified, ranging from 1 to 10 bp, including *cox1–trnS* (9 bp), *atp8–atp6* (10 bp), *nd4l–nd4* (7 bp), *nd5–nd6* (4 bp), and *trnT–trnP* (1 bp). The longest overlapping region occurred between *atp8* and *atp6*.

GC and AT skew values of the assembled mitochondrial genome are presented in Fig. 2. The *nd6* gene exhibited the highest GC skew (0.52), while the lowest was recorded in the *nd2* gene (-0.56). For AT skew, the highest value (0.08) was observed in the *nd2* and *atp8* genes, whereas the lowest value (-0.45) occurred in the *nd6* gene, which is the only protein-coding gene located on the light strand (L-strand).

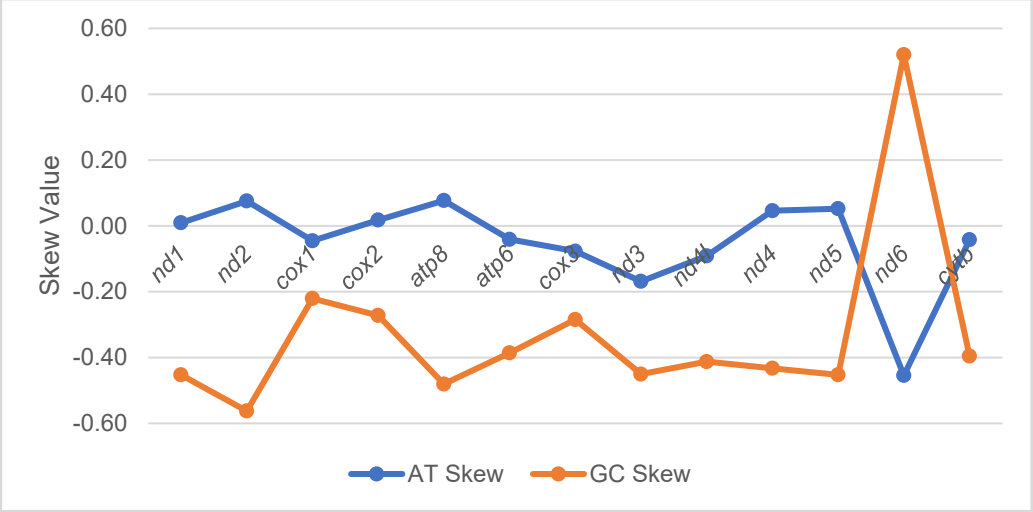


Figure 2: Graphical representation of AT and GC skew for all 13 protein-coding genes in silver tigerfish (*D. polota*) mitogenome.

To further characterize the protein-coding genes of the *D. polota* mitogenome, amino acid composition and codon usage patterns were analyzed across all 13 mitochondrial protein-coding genes. This analysis was performed to identify the dominant amino acids encoded by the mitogenome and to compare codon representation among genes located on the heavy and light strands. Leucine (Leu) was the most frequently encoded amino acid, followed by serine (Ser). Leucine was encoded by five to six codons, with four codons, CTA, CTT, TTA, and TTG, consistently present, whereas CTC and CTG occurred variably among genes. Among the 12 protein-coding genes located on the heavy strand, the number of amino acid types ranged from 17 to 20, while the nd6 gene on the light strand contained 17 amino acid types. The lowest amino acid representation varied among genes: aspartic acid (Asp) was the least abundant in *nd2* and *nd4l,* lysine (Lys) was least represented in *nd1, cox2,* and *nd5*, and cysteine (Cys) was least represented in *atp6, atp8, cox1, cox3, nd3,* and *nd4*. In nd6, the lowest amino acid counts were shared among cysteine, asparagine, and aspartic acid. The most complete leucine codon representation, involving all six leucine codon types, was observed in *nd2, nd4l,* and *cytb.* These amino acid frequency patterns are shown in Figure 3 and detailed further in Supplementary 1.

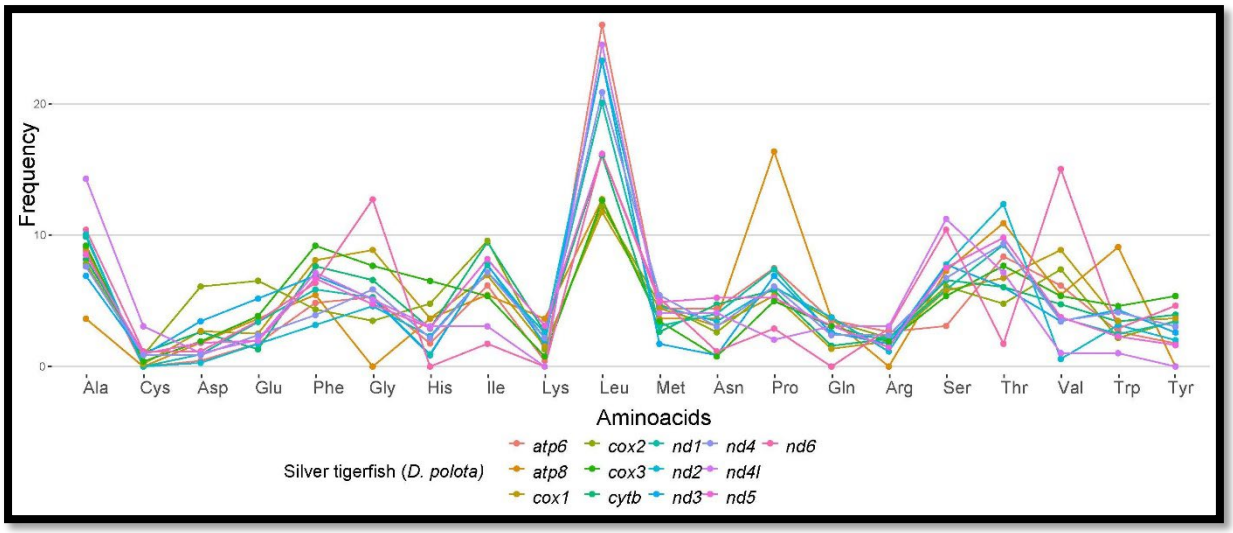


Figure 3: Amino acid frequency of complete mitogenome for all 13 protein-coding genes in the silver tigerfish (*D. polota*).

Phylogenetic analysis based on complete mitochondrial genome sequences revealed that the South Sumatra silver tigerfish clustered closely with three other species within the genus *Datnioides* (Indonesian tigerfish, Mekong tigerfish, and Siamese tigerfish). In contrast, it exhibited a more distant relationship with Campbell's tigerfish. Additionally, the outgroup species *Hapalogenys analis* showed greater similarity to *Lobotes surinamensis* than to any species within the genus *Datnioides* (Fig. 4).

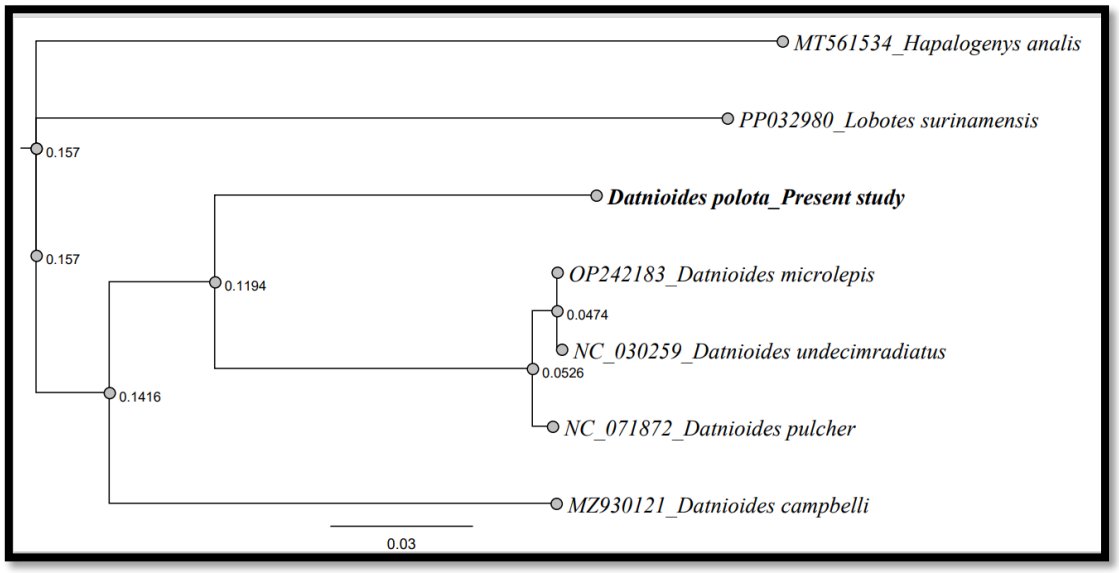


Figure 4. The phylogenetic tree of tigerfish species was constructed using the neighbor-joining (NJ) method based on complete mitochondrial genome sequences. The cladogram depicts the evolutionary relationships among species in the genus *Datnioides*, with *Lobotes*

surinamensis representing a related taxon from the same family, and *Hapalogenys analis* used as the outgroup. Bootstrap support values from 1,000 replicates are shown at each node.

DISCUSSION

The complete mitochondrial genome of silver tigerfish from South Sumatra was 16,496 bp in length and contained the typical vertebrate mitochondrial gene set, including 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and a control region. This organization is consistent with the conserved structure of most animal mitochondrial genomes, which generally contain 37 genes arranged in a compact circular molecule (Boore, 1999; Taanman, 1999). The same gene composition has also been reported in other *Datnioides* species, including *D. microlepis* and *D. campbelli* (Wang et al., 2016; Zhou et al., 2022). Therefore, the mitogenome of South Sumatra *D. polota* indicates that the general mitochondrial genome architecture is conserved within the genus *Datnioides*. The small differences in mitochondrial genome length among *Datnioides* species are likely associated with variation in non-coding regions, intergenic spacers, or gene overlaps rather than major differences in gene content or gene order.

The nucleotide composition and strand-specific patterns observed in the South Sumatra *D. polota* mitogenome also provide useful evolutionary information. Most protein-coding genes were located on the heavy strand, whereas *nd6* was the only protein-coding gene located on the light strand. This arrangement is commonly observed in vertebrate mitochondrial genomes and reflects the conserved organization of mitochondrial genes (Boore, 1999; Fonseca et al., 2014). The distinct AT- and GC-skew pattern of *nd6* compared with other protein-coding genes may be associated with strand-specific mutational pressure and asymmetric mitochondrial DNA replication (Fonseca et al., 2014; Satoh et al., 2016). Such strand bias is biologically important because it can influence nucleotide composition, codon usage, and the evolutionary rate of mitochondrial genes (Broughton et al., 2001; Satoh et al., 2016).

Codon usage analysis showed that leucine and serine were the most frequently encoded amino acids among the mitochondrial protein-coding genes. Similar codon usage patterns have been reported in other fish mitogenomes, suggesting that codon usage bias may be shaped by nucleotide composition and functional constraints in mitochondrial oxidative phosphorylation genes (Broughton et al., 2001; Satoh et al., 2016). Because mitochondrial protein-coding genes encode key subunits of oxidative phosphorylation complexes, conserved codon usage patterns may reflect selective constraints on mitochondrial energy metabolism (Taanman, 1999). However, because the present study analyzed a single individual, further sampling from additional Indonesian populations is needed to determine whether the observed

nucleotide composition and codon usage patterns are consistent across the broader geographic range of *D. polota*.

The phylogenetic analysis placed *D. polota* close to *D. microlepis, D. undecimradiatus,* and *D. pulcher*, whereas *D. campbelli* was positioned more distantly. This topology suggests that the South Sumatra *D. polota* shares a closer maternal evolutionary relationship with Southeast Asian and Sundaic *Datnioides* lineages than with *D. campbelli* from New Guinea. This pattern is relevant because *Datnioides* species show geographically structured distributions across Southeast Asia and New Guinea (Wang et al., 2016; Sun et al., 2020; Zhou et al., 2022). The placement of *D. polota* within the *Datnioides* clade contributes to a better understanding of the evolutionary divergence within the genus and provides additional molecular evidence for comparative studies of tigerfish diversity.

The mitogenome reported here is also significant as a genetic reference resource for Indonesian silver tigerfish. Complete mitochondrial genome data are widely used in species identification, phylogenetic reconstruction, and evolutionary studies because mitochondrial DNA is maternally inherited, relatively compact, and informative for tracing lineage divergence (Boore, 1999; Xing et al., 2025). For *Datnioides*, which includes economically valuable ornamental fishes, the availability of population-level mitogenomic data can support future studies on phylogeography, population structure, and genetic monitoring. Such information is relevant for sustainable management, conservation planning, domestication, and aquaculture development. Nevertheless, mitochondrial data represent only the maternal lineage; therefore, future studies should combine mitogenomic data with nuclear markers and broader population sampling to provide a more comprehensive understanding of genetic diversity and management units in *D. polota*.

CONCLUSION

This study provides the first complete mitochondrial genome characterization of silver tigerfish from the South Sumatra population of Indonesia using Oxford Nanopore Technologies-based whole-genome sequencing, adding a population-level mitogenomic reference for *D. polota*. Phylogenetic analysis based on the neighbor-joining method successfully resolved its evolutionary positioning, demonstrating that the silver tigerfish from a well-supported monophyletic clade with the Indonesian tigerfish, Mekong tigerfish, and Siamese tigerfish, highlighting their close genetic affinity. The genomic information generated here contributes to the growing mitochondrial database and serves as a valuable resource for future studies in

phylogeography, evolutionary biology, and the sustainable management of *Datnioides* species.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. Animals were treated according to applicable guidelines in Indonesia. All required permissions and authorisation were obtained.

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AUTHORS' CONTRIBUTIONS

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Huria Marnis: Visualization, validation data, writing–original draft & editing.
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