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Complete Mitogenome and Phylogenetic Analysis of a Marine Ray-Finned Fish, *Alcichthys elongatus* (Perciformes: Cottidae)

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Abstract: *Alcichthys elongatus* is the only species in the genus, and this work is the first to provide a comprehensive mitogenome analysis of this species. The *A. elongatus* mitogenome was 16,712 bp long, with biased A + T content (52.33%), and featured thirteen protein-coding genes (PCGs), twenty-two tRNAs, two rRNAs, and the control region (D-loop). The H strand encoded twenty-eight genes (twelve PCGs, fourteen tRNA, and two rRNA) and the control region, whereas the L strand encoded the remaining nine genes (*ND6* and eight tRNA). Except for *COXI*, which started with GTG, all PCG sequences started with ATG and ended with TAA (*ND4L*, *ND5*, *COXI*, *ATP8*) or TAG (*ND1*, *ND6*) termination codons, with some (*ND2*, *ND3*, *ND4*, *COXII*, *COXIII*, *ATP6*, *Cytb*) having an incomplete termination codon. Except for tRNA-serine-1 (*trnS*), the majority of the tRNAs exhibited characteristic cloverleaf secondary structures. Based on 13 PCGs, phylogenetic analysis placed *A. elongatus* in the same clade as *Icelus spatula*. This genomic data will be useful for species identification, phylogenetic analysis, and population genetics.

Keywords: *Alcichthys elongatus*; Perciformes; Cottidae; mitochondrial genome; phylogenetic analysis; ray-finned fish; marine sculpin

Key Contribution: This study presents a comprehensive investigation of the mitochondrial genome of *A. elongatus*, providing publicly accessible genetic data for future research pertaining to fish species.

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1. Introduction

The Cottidae (Cottiodei, Perciformes), which has 275 known species in 70 genera, is one of the most diversified fish families found across the globe [1]. Initially, fishes of this family were grouped into a phylogenetic diagram based on morphological data, but this approach was ineffective since the species share so many similar morphological characters. Subsequently, internal and external morphological characters were combined to solve taxonomic difficulties [2]. Additionally, phylogenetic relationships were conducted using molecular markers (mitochondrial or nuclear genes) [3], although many questions about the relationships between genera and species still persist.

Within Cottidae, Elongated sculpin *Alcichthys elongatus* (Steindachner, 1881) belongs to the monospecific genus *Alcichthys* and is distributed in the northwestern Pacific Ocean

including the Sea of Okhotsk and Japan [4–6]. *A. elongatus* is a marine, demersal, and low boreal fish that dwells on rocky reefs at depths of up to 253 m [6–8]. Few studies on the molecular features of *A. elongatus* have been published. A complete mitochondrial or nuclear genome sequence has not yet been published; only a few gene sequences are available in the National Center for Biotechnology Information (NCBI) GenBank, including *COI*, *Cytb*, rRNA (12S and 16S), and tRNA-Val genes of the mitochondrial genome and recombination activating protein 1 (*RAG1*) gene of the nuclear genome, and these genes were used for evolutionary and phylogenetic studies [3,9]. With the progress in genetic studies of biodiversity and systematics, the determination of how fish evolved by studying their complete mitochondrial genomes has developed quickly [10]. Mitochondrial genome analysis can often help us understand adaptive divergence and speciation [11].

The objective of this study was to generate the complete mitogenome of *A. elongatus* and to characterize its genomic features to advance the construction of a phylogenetic tree. The complete mitochondrial genomic data of *A. elongatus* will be a valuable genomic resource for future studies on resolving the phylogenetic relationship and evolutionary history of the family Cottidae.

2. Materials and Methods

2.1. Sample Collection and DNA Isolation

A. elongatus fish samples were collected from the coastal waters of the East Sea (Pohang, South Korea; 36°6′43.5″ N, 129°26′32.4″ E) and deposited at the Department of Marine Biology, Pukyong National University, Busan, South Korea (Prof. Jin-Koo Kim, taengko@pknu.ac.kr) under voucher number PKU-50558 (Figure 1). Muscle tissues were used for total genomic DNA extraction according to the DNeasy Blood and Tissue Kit's (Qiagen, Germany) instructions. The quality (concentration) of extracted DNA was determined by a NanoDrop D1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and preserved at −4 °C for further analysis.

Figure 1. *Alcichthys elongatus* (Photo by Jin-Koo Kim), a marine ray-finned fish captured from the East Sea (coast of Pohang, South Korea). The body has a creamy-brown color with dorsal spines (9–10), dorsal soft rays (14–17), and anal soft rays (13–16).

2.2. Whole Genome Sequencing

The Illumina Platform (Illumina Inc., San Diego, CA, USA) was used to sequence the genome of *A. elongatus*. The Macrogen Company (Daejeon, South Korea) took part in the library preparation and sequencing procedures. The TrueSeq® Nano DNA Kit (San Diego, CA, USA) was used to produce the DNA libraries in accordance with the manufacturer's instructions, and the Illumina HiSeq 2500 (Illumina) was used for paired-end, 150 bp mode of sequencing. To achieve clean reads, raw data first passed quality control before moving on to subsequent processing. Trimmomatic [12] was used to remove adapter sequences and low-quality reads (phred quality score (%) Q20 = over 20 and Q30 = over 30) in order to reduce biases in analysis. In the *A. elongatus* library, 144,607,336 total raw reads (GC = 42.05%, Q20 = 96.82%, and Q30 = 92.82%) and 120,226,836 total filtered reads (GC = 41.54%, Q20 = 99.18%, and Q30 = 97.13%) were generated. The trimmed reads were randomly sampled in order to assemble the mitochondrial genome. In this case, only

sampled reads were used for de novo assembly. The quality of the generated sequencing reads was assessed using FastQC v0.11.5 (Babraham Institute, Bioinformatics) [13]. The high-quality reads of the mitochondrial genome were de novo assembled using several *k*-mers [14] and the SPAdes v3.13.0 software [15]. After the complete genome was assembled, BLAST analysis was carried out to identify contig containing mitogenome sequences in the NCBI database.

2.3. Mitogenome Assembly and Annotation

The contig was annotated using the MitoFish (http://mitofish.aori.u-tokyo.ac.jp/, accessed on 1 July 2023) [16] and MITOS (http://mitos.bioinf.uni-leipzig.de/index.py, accessed on 1 July 2023) [17] pipeline with genetic code 2 (Vertebrates code). Predicted open reading frames (ORFs) were manually examined, and the final annotations were verified using ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/, accessed on 1 July 2023). By using a BLAST homology search in the NCBI database, protein-coding genes (PCGs) were manually verified against previously reported mitogenomes of Cottidae members [18]. Transfer RNAs (tRNAs) were identified using tRNAscan-SE v2.0 (http://lowelab.ucsc.edu/tRNAscan-SE/, accessed on 1 July 2023) with default parameters (Genetic code: Vertebrate Mito) [19], and tRNA secondary structure was predicted and confirmed using ARWEN [20]. The assembled contig was analyzed for identification by querying BlastN [21] and comparing the sizes with previously published Cottidae mitogenomes. A physical map of the mitogenome of *A. elongatus* (NCBI GenBank accession number: OR288162.1) was generated using OGDRAW v.1.3.1 (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html, accessed on 1 July 2023) [22]. The nucleotide compositions of mitogenomes were calculated in MEGA11 v.11.2.8 [23]. Codon usage of PCGs was determined using Sequence Manipulation Suite (SMS) tool (http://www.bioinformatics.org/sms2/codon_usage.html, accessed on 1 July 2023) with genetic code 2 [24]. The composition of the skew analysis was calculated using formulae: AT-skew = (A − T)/(A + T) and GC-skew = (G − C)/(G + C) [25]. The intergenic spacers between the genes and the overlapping regions were calculated manually.

2.4. Phylogenetic Tree Construction

A total of 31 mitogenomes belonging to the Order Perciformes were chosen (Table 1) for phylogenetic tree study within Family Cottidae. Among these, *A. elongatus* (in this study) and other 28 mitogenomes were from Family Cottidae, which served as the in-group, while mitogenomes from the Scorpaenidae (*Scorpaena neglecta* (ON109388.1)) and Stichaeidae family (*Chirolophis wui* (OP388414)), were utilized as the outgroups for performing phylogenetic tree construction. The selected sequences used in this study were downloaded from the NCBI database. The phylogenetic analysis used a series of concatenated nucleotide sequences from 13 PCG datasets. These datasets were arranged in the specific order of *nad1*, *nad2*, *cox1*, *cox2*, *atp8*, *atp6*, *cox3*, *nad3*, *nad4L*, *nad4*, *nad5*, *nad6*, and *cytb*. The process of multiple sequence alignment was executed in the MEGA11, using the ClustalW algorithm [26]. Subsequently, a phylogenetic tree was generated utilizing the maximum likelihood (ML) method [27]. ML analysis was performed using default parameters in the Tamura-Nei model with 1000 bootstrap replications [23].

Table 1. Nucleotide composition of the complete mitogenomes from members of Order Perciformes.

Name	Accession Number	Size (bp)	In Percentage						AT-Skew	GC-Skew	Ref.
			G	A	T	C	A + T	G + C			
Alcichthys elongatus	OR288162	16,712	17.48	26.43	25.90	30.14	52.33	47.62	0.0101	−0.2659	This study
Argyrocottus zanderi	NC_057483	16,608	17.09	26.99	26.47	29.44	53.46	46.53	0.0097	−0.2654	[28]
Batrachocottus baicalensis	MT527180	16,523	17.53	26.47	25.87	30.13	52.33	47.67	0.0115	−0.2644	[29]
Batrachocottus multiradiatus	MT527181	16,532	17.51	26.34	25.96	30.19	52.30	47.70	0.0073	−0.2658	[29]
Batrachocottus nikolskii	MT527182	16,535	17.41	26.42	26.01	30.17	52.42	47.58	0.0078	−0.2682	[29]
Batrachocottus talievi	MT527183	16,530	17.41	26.38	26.04	30.16	52.43	47.57	0.0065	−0.2680	[29]
Comephorus baikalensis	MF346885	16,538	17.17	26.74	26.05	30.00	52.79	47.18	0.0131	−0.2720	[30]
Comephorus dybowskii	NC_036149	16,527	17.20	26.73	26.19	29.88	52.92	47.08	0.0102	−0.2693	[30]
Cottiusculus nihonkaiensis	NC_045245	16,612	17.44	26.32	24.75	31.50	51.07	48.93	0.0307	−0.2873	[31]
Cottocomephorus grewingki	MW732165	16,590	17.15	27.13	26.60	29.10	53.73	46.24	0.0099	−0.2584	[32]
Cottocomephorus inermis	MW732163	16,510	17.14	27.10	26.58	29.17	53.68	46.31	0.0097	−0.2598	[32]
Cottus koreanus	NC_063951	16,558	17.62	26.48	26.02	29.89	52.49	47.51	0.0088	−0.2583	-
Cottus marginatus	NC_066924	16,603	16.68	27.28	26.10	29.93	53.39	46.61	0.0221	−0.2843	-
Cottus princeps	NC_066915	16,561	16.32	27.83	26.44	29.41	54.27	45.73	0.0256	−0.2862	-
Cottus reinii	NC_004404	16,561	17.63	26.30	25.78	30.28	52.09	47.91	0.0100	−0.2640	[33]
Enophrys bison	NC_066929	16,888	16.88	27.19	26.69	29.23	53.88	46.12	0.0092	−0.2678	-
Enophrys diceraus	NC_022147	16,976	16.65	27.53	27.19	28.64	54.71	45.29	0.0062	−0.2647	[34]
Gymnocanthus herzensteini	NC_034651	16,691	17.46	26.54	25.92	30.01	52.46	47.47	0.0118	−0.2644	[35]
Gymnocanthus intermedius	NC_034650	16,639	17.65	26.40	25.52	30.42	51.92	48.06	0.0169	−0.2657	[35]
Gymnocanthus tricuspis	NC_045927	16,570	17.36	26.74	25.76	30.14	52.49	47.51	0.0187	−0.2691	[36]
Icelus spatula	NC_027587	16,384	17.43	26.43	26.03	30.04	52.46	47.47	0.0076	−0.2656	[37]
Megalocottus platycephalus	MK936041	16,673	17.14	27.03	26.53	29.29	53.57	46.43	0.0093	−0.2617	[38]
Mesocottus haitej	NC_022181	16,527	17.35	26.64	26.12	29.88	52.76	47.24	0.0099	−0.2653	-
Myoxocephalus jaok	NC_045875	16,653	16.89	27.08	26.61	29.43	53.68	46.32	0.0088	−0.2707	[39]
Myoxocephalus quadricornis	NC_053359	16,736	17.42	26.83	26.42	29.33	53.25	46.75	0.0077	−0.2548	[40]
Myoxocephalus scorpius	NC_042186	16,626	16.83	27.22	26.78	29.18	53.99	46.01	0.0081	−0.2684	[41]
Paracottus knerii	MW732164	16,550	17.43	26.62	26.04	29.92	52.65	47.35	0.0110	−0.2638	[32]
Porocottus allisi	NC_057484	16,369	17.44	26.15	25.24	31.17	51.39	48.61	0.0177	−0.2825	[28]
Procottus major	MW732167	16,512	17.14	26.94	26.18	29.74	53.12	46.88	0.0143	−0.2688	[32]
Scorpaena neglecta	ON109388	17,202	17.45	28.36	26.40	27.79	54.76	45.24	0.0358	−0.2286	[42]
Chirolophis wui	OP388414	16,522	18.28	25.52	28.53	27.67	54.05	45.95	−0.0556	−0.2043	[43]

3. Results and Discussion

3.1. Genome Size and Organization

The mitogenome of *A. elongatus* was assembled into a circular DNA molecule measuring 16,712 bp in length (GenBank: OR288162; Figure 2, Table 2). This length falls within the range of other mitogenomes belonging to the Family Cottidae, which vary from 16,369 bp (*Porocottus allisi*, NC_057484) [28] to 18,374 bp (*Clinocottus analis*, NC_013828) (Table 1). The analysis of the complete mitogenome sequence of *A. elongatus* by nucleotide BLAST indicated high sequence similarities with closely related species, namely *Icelus spatula* NC_027587 (89.63%) [37], *Gymnocanthus intermedius* NC_034650.1 (89.08%), *G. herzensteini* NC_034651 (89.37%), *G. tricuspis* NC_045927 (89.24%) [36], *Enophrys diceraus* NC_022147 (88.70%) [34], and *E. bison* NC_066929 (87.82%). The mitochondrial genome of *A. elongatus* comprised two rRNA genes, thirteen PCGs, twenty-two tRNA genes, and a D-loop region. The heavy strand (H-strand) contained fourteen tRNA, twelve PCGs, two rRNA genes, and the D-loop, whereas the light strand (L-strand) had eight tRNA and one PCG (*ND6*) (Table 2). These features of *A. elongatus* were identical to those of other Cottidae mitogenomes [28,34,36,37] and could be regarded as effective markers for authentication at the genus and species level.

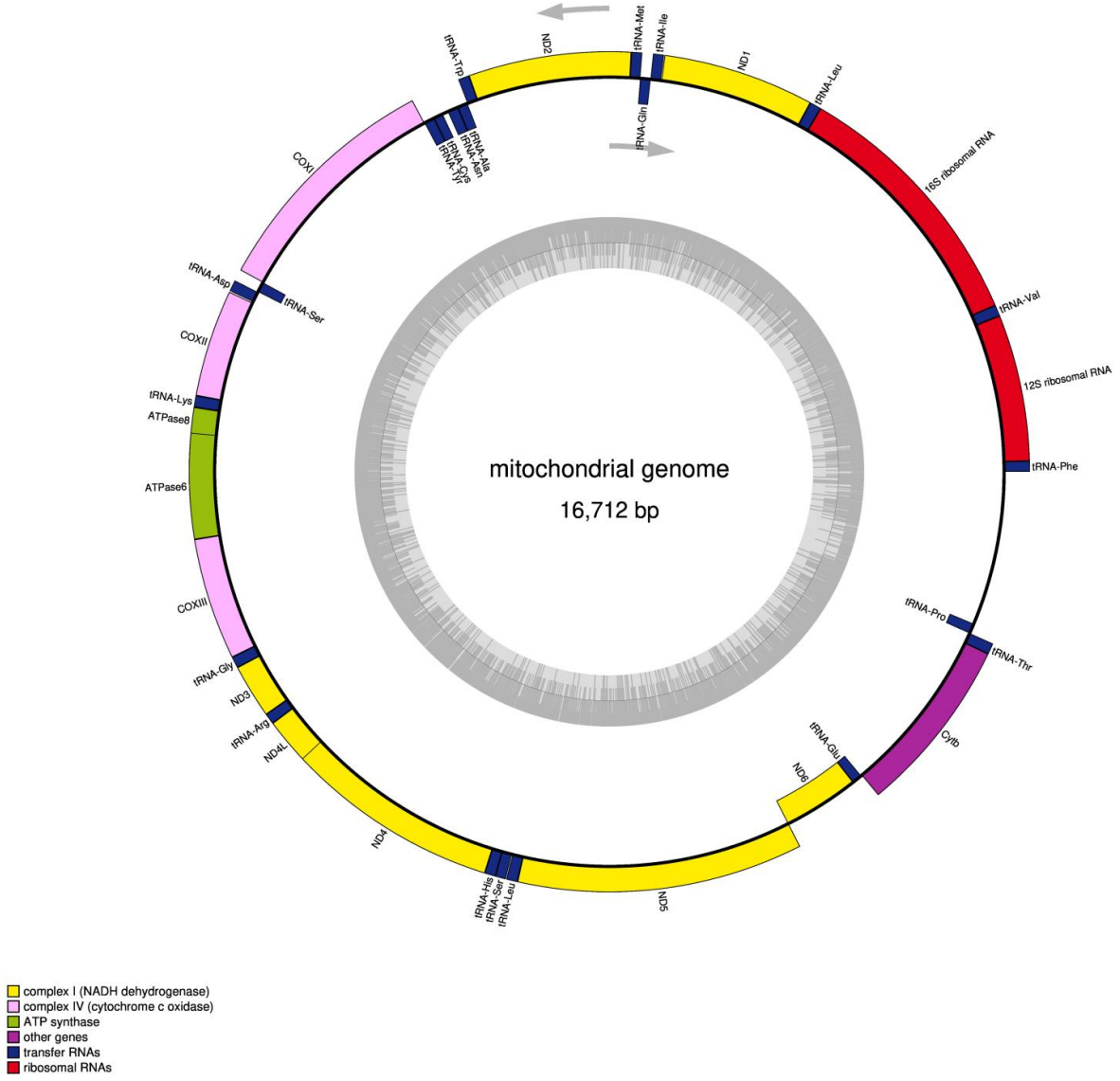

Figure 2. The circular mitogenome of *A. elongatus*. The direction of the arrow denotes the orientation of the genes, and the various colors denote the grouping of functional genes along with their abbreviations.

Table 2. Sequence characteristics of *A. elongatus* mitogenome.

Group	Group of Genes	Gene	Three Letter Code	Sequence		Size (bp)	Strand	No. of Amino Acids	Start Codon	Stop Codon	Anti-Codon	Intergenic Nucleotides *
				Start	End							
PCGs	NADH dehydrogenase subunit	*ND1*	-	2852	3826	975	H	324	ATG	TAG	-	4
		ND2	-	4039	5084	1046	H	348	ATG	TA-	-	0
		ND3	-	9645	9993	349	H	116	ATG	T--	-	0
		ND4L	-	10,063	10,359	297	H	93	ATG	TAA	-	−7
		ND4	-	10,353	11,733	1381	H	460	ATG	T--	-	0
		ND5	-	11,948	13,786	1839	H	613	ATG	TAA	-	−4
		ND6	-	13,783	14,304	522	L	174	ATG	TAG	-	0
	Cytochrome c oxidase subunit	*COXI*	-	5475	7025	1551	H	516	GTG	TAA	-	0
		COXII	-	7180	7870	691	H	230	ATG	T--	-	0
		COXIII	-	8787	9571	785	H	261	ATG	TA-	-	0
	ATP synthase subunit	*ATP8*	-	7946	8113	168	H	55	ATG	TAA	-	−10
		ATP6	-	8104	8786	683	H	227	ATG	TA-	-	0
	Cytochrome b	*Cytb*	-	14,379	15,519	1141	H	380	ATG	T--	-	0
RNAs	Transfer RNA genes	*trnF*	Phe	1	68	68	H	-	-	-	GAA	0
		trnV	Val	1012	1083	72	H	-	-	-	TAC	0
		trnL	Leu	2778	2851	74	H	-	-	-	TAA	0
		trnI	Ile	3831	3900	70	H	-	-	-	GAT	−1
		trnQ	Gln	3900	3970	71	L	-	-	-	TTG	−1
		trnM	Met	3970	4038	69	H	-	-	-	CAT	0
		trnW	Trp	5085	5155	71	H	-	-	-	TCA	1
		trnA	Ala	5157	5225	69	L	-	-	-	TGC	1
		trnN	Asn	5227	5299	73	L	-	-	-	GTT	38
		trnC	Cys	5338	5403	66	L	-	-	-	GCA	0
		trnY	Tyr	5404	5473	70	L	-	-	-	GTA	1
		trnS	Ser	7026	7096	71	L	-	-	-	TGA	3
		trnD	Asp	7100	7172	73	H	-	-	-	GTC	7
		trnK	Lys	7871	7944	74	H	-	-	-	TTT	1
		trnG	Gly	9572	9644	73	H	-	-	-	TCC	0
		trnR	Arg	9994	10,062	69	H	-	-	-	TCG	0
		trnH	His	11,734	11,802	69	H	-	-	-	GTG	0
		trnS	Ser	11,803	11,870	68	H	-	-	-	GCT	4
		trnL	Leu	11,875	11,947	73	H	-	-	-	TAG	0
		trnE	Glu	14,305	14,373	69	L	-	-	-	TTC	5
		trnT	Thr	15,520	15,591	72	H	-	-	-	TGT	−1
		trnP	Pro	15,591	15,660	70	L	-	-	-	TGG	0
	12S rRNA	*rrnS*	-	69	1011	943	H	-	-	-	-	0
	16S rRNA	*rrnL*	-	1084	2777	1694	H	-	-	-	-	0
D-loop	Control region	-	-	15,661	16,712	1052	H	-	-	-	-	0

Notes: * The number of nucleotides between the given and previous gene, with a negative value indicating an overlap; H and L indicate that the genes are transcribed on the heavy and light strands, respectively.

The nucleotide compositions of A, T, G, and C were determined to be 26.43%, 25.90%, 17.48%, and 30.14%, respectively. These findings indicate a biased A + T composition of 52.33%, which is consistent with the nucleotide composition seen in other members of Family Cottidae, as shown in Table 1. The observed positive AT-skew (0.0101) in this research is consistent with the other fish species that were used. This skew indicates a higher abundance of adenine (A) nucleotides compared with thymine (T) nucleotides.

3.2. Protein Coding Genes

The PCG region constituted 68.38% of the *A. elongatus* mitogenome, covering a length of 11,428 bp. The *ND5* gene was longest among the PCGs, covering a total of 1839 bp. On the other hand, the *ATP8* gene was the shortest PCG, consisting of just 168 bp. Each PCG was started by a standard ATG codon, with the exception of *COXI*, which was started by a GTG codon (Table 2). Previous studies have shown comparable findings in other species of Perciformes [28–42]. In the mitogenome of *A. elongatus*, we observed that four of the thirteen PCGs (*ND4L*, *ND5*, *COXI*, *ATP8*) used a standard TAA termination codon, which is commonly observed in the mitogenomes of Order Perciformes [28–45]. On the other hand, *ND1* and *ND6* genes terminated with the codon TAG, *ND2*, *COXIII*, and *ATP6* genes ended with the codon TA, and *ND3*, *ND4*, *COXII*, and *Cytb* genes terminated with a single T (Table 2). The incomplete termination codons may be completed to TAA by RNA processing by the addition of a poly-A tail [44].

The genome of *A. elongatus* encodes a total of 3800 amino acids within its PCGs (Table 3). The amino acid composition of the PCGs in *A. elongatus* revealed that leucine (17.45%), alanine (9.47%), and threonine (7.97%) were the most frequently occurring amino acids. On the other hand, glutamate/glutamine (2.63%), aspartate (1.92%), and cysteine (0.63%) were the least abundant amino acids (Table 3). Similar codon usage patterns were noticed in other members of the Family Cottidae [28–41].

Table 3. Codon usage in the mitochondrial PCGs of *A. elongatus*.

Amino Acid	Codon	Number	%	Fraction	Amino Acid	Codon	Number	%	Fraction
Ala	GCG	12	0.316	0.03	Asn	AAT	30	0.789	0.27
	GCA	75	1.974	0.21		AAC	83	2.184	0.73
	GCT	70	1.842	0.19	Pro	CCG	8	0.211	0.04
	GCC	203	5.342	0.56		CCA	35	0.921	0.16
Cys	TGT	10	0.263	0.42		CCT	54	1.421	0.25
	TGC	14	0.368	0.58		CCC	121	3.184	0.56
Asp	GAT	23	0.605	0.32	Gln	CAG	26	0.684	0.26
	GAC	50	1.316	0.68		CAA	74	1.947	0.74
Glu	GAG	30	0.789	0.30	Arg	CGG	15	0.395	0.20
	GAA	70	1.842	0.70		CGA	28	0.737	0.37
Phe	TTT	122	3.211	0.53		CGT	13	0.342	0.17
	TTC	107	2.816	0.47		CGC	20	0.526	0.26
Gly	GGG	68	1.789	0.27	Ser	AGT	11	0.289	0.04
	GGA	54	1.421	0.22		AGC	48	1.263	0.19
	GGT	35	0.921	0.14		TCG	15	0.395	0.06
	GGC	92	2.421	0.37		TCA	44	1.158	0.18
His	CAT	25	0.658	0.24		TCT	52	1.368	0.21
	CAC	81	2.132	0.76		TCC	78	2.053	0.31
Ile	ATT	127	3.342	0.49	Thr	ACG	27	0.711	0.09
	ATC	132	3.474	0.51		ACA	78	2.053	0.26
Lys	AAG	14	0.368	0.20		ACT	54	1.421	0.18
	AAA	57	1.500	0.80		ACC	144	3.789	0.48

Table 3. *Cont.*

Amino Acid	Codon	Number	%	Fraction	Amino Acid	Codon	Number	%	Fraction
Leu	TTG	33	0.868	0.05	Val	GTG	34	0.895	0.15
	TTA	74	1.947	0.11		GTA	68	1.789	0.29
	CTG	58	1.526	0.09		GTT	61	1.605	0.26
	CTA	165	4.342	0.25		GTC	68	1.789	0.29
	CTT	152	4.000	0.23	Trp	TGG	27	0.711	0.23
	CTC	181	4.763	0.27		TGA	93	2.447	0.78
Met	ATG	74	19.47	0.50	Tyr	TAT	27	0.711	0.25
	ATA	74	19.47	0.50		TAC	82	2.158	0.75

3.3. Transfer RNA and Ribosomal RNA Genes

The current study found a total of 22 tRNA in the mitogenome of *A. elongatus*. These tRNAs exhibited a characteristic complement structure, with lengths ranging from 66 bp for *trnC* to 74 bp for *trnL* and *trnK* (Table 2). Among these tRNAs, leucine (TAA, TAG) and serine (TGA, GCT) were represented by two tRNA forms each, while the other amino acids had a single tRNA gene. The cumulative length of all tRNA was determined to be 1554 bp, accounting for about 9.30% of the whole mitogenome. A total of fourteen tRNA genes were identified on the H strand, whereas the remaining tRNA genes were found on the L strand. All tRNAs fold into typical cloverleaf secondary structures with the exception of the serine tRNA (*trnS*, (GCT)), which lacks the dihydrouridine (DHU) arm (Figure 3). The DHU arm of this tRNA was a large loop instead of the conserved stem and loop structure. In the typical secondary structure of the tRNA genes, it was noted that seventeen (tRNA: Leu (TAA), Ile, Gln, Met, Trp, Ala, Asn, Cys, Tyr, Ser (TGA), Asp, Gly, Arg, His, Ser (GCT), Glu, Pro) showed the presence of at least one G-T mismatch, which formed a weak bond. Three T-T mismatches were noticed, with two found in the amino acid (AA) arm of the tRNA (Gln, Met), and one in the TΨC arm of tRNA-Glu (Figure 3). The presence of the mismatched base pairs seen in tRNA sequences may be corrected by the RNA-editing process, which has been extensively studied in vertebrate mitogenomes [45]. Overall, the secondary structure of the tRNA in *A. elongatus* exhibited the normal Watson–Crick pairing seen in vertebrate mitogenomes [46].

The mitochondrial genome of *A. elongatus* contains two rRNA genes, namely 12S rRNA (943 bp) and 16S rRNA (1694 bp), (Table 2). The combined size of 2637 bp corresponds to about 15.78% of the whole mitogenome. Both of these genes are encoded on the H strand. The 12S rRNA and 16S rRNA genes are separated by the tRNA-Val gene, and these genes are situated between the tRNA-Phe and tRNA-Leu (TAA) genes. The above-mentioned characteristics were consistent with the typical perciform mitogenomes [28–42].

3.4. Overlapping and Intergenic Spacer Regions

There were six gene boundaries where 1–10 bp of overlapping bases occurred between adjacent genes. The longest overlapping region between *ATP8* and *ATP6* was 10 bp (Table 2), which has been reported in many other perciform mitogenomes [34]. Moreover, *A. elongatus* mitogenome intergenic spacers occurred across nine locations and ranged from 1 to 38 bp, a total of 61 bp; the longest intergenic spacer region (38 bp) was between tRNA-Asn and tRNA-Cys (Table 2).

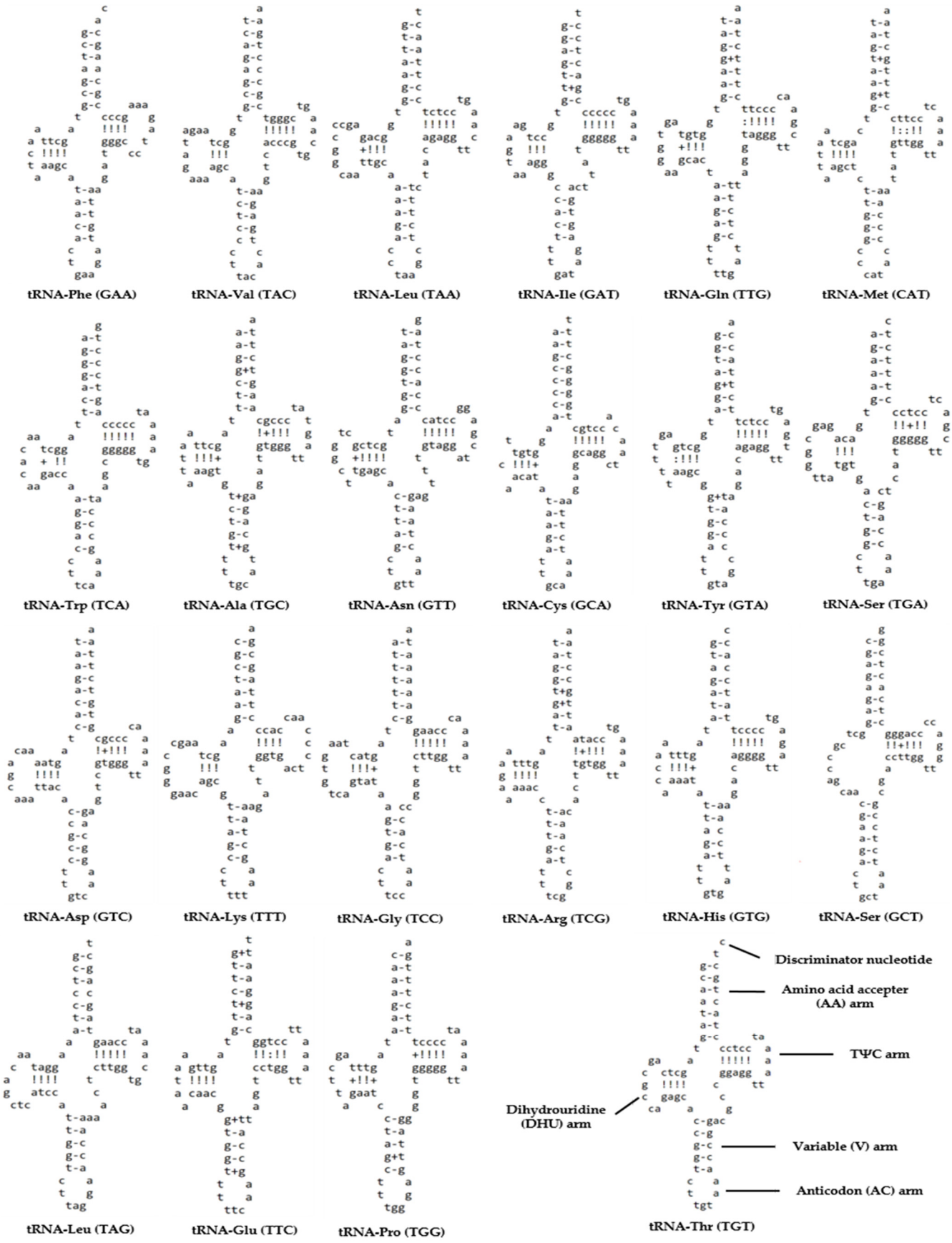

Figure 3. Predicted secondary structure for 22 tRNA genes in the mitogenome of *A. elongatus*. Watson–Crick and GT bonds are illustrated as "-" and "+", respectively.

3.5. Phylogenetic Relationship

To better understand the phylogenetic relationships within the Family Cottidae, a maximum likelihood approach was used. A dataset of 32 species was utilized, whereby the concatenated nucleic acid sequences of 13 PCGs were analyzed to construct the phylogenetic tree (Figure 4). In previous studies, the determination of phylogenetic relationships was based on the analysis of partial mitogenome sequences, mostly focusing on *COX1* or 16S rRNA genes [3,9]. The mitogenome of *A. elongatus* in this study clustered with *I. spatula* (NC_027587) (Figure 1). A phylogenetic analysis based on selected cottid species showed two main clades, with nine genera grouped together in one clade (*Cottiusculus*, *Gymnocanthus*, *Alcichthys*, *Icelus*, *Enophrys*, *Myoxocephalus*, *Megalocottus*, *Argyrocottus*, *Porocottus*) and seven genera grouped together in the other clade (*Mesocottus*, *Cottus*, *Cottocomephorus*, *Procottus*, *Paracottus*, *Batrachocottus*, *Comephorus*). Recent phylogenetic studies based on mitochondrial PCGs [40] and complete genomes [28,38] of cottid species found a similar topology. In order to enhance an understanding of the evolutionary relationships among species within the Perciformes order, it is necessary to examine the mitogenomes of more species within this taxonomic group.

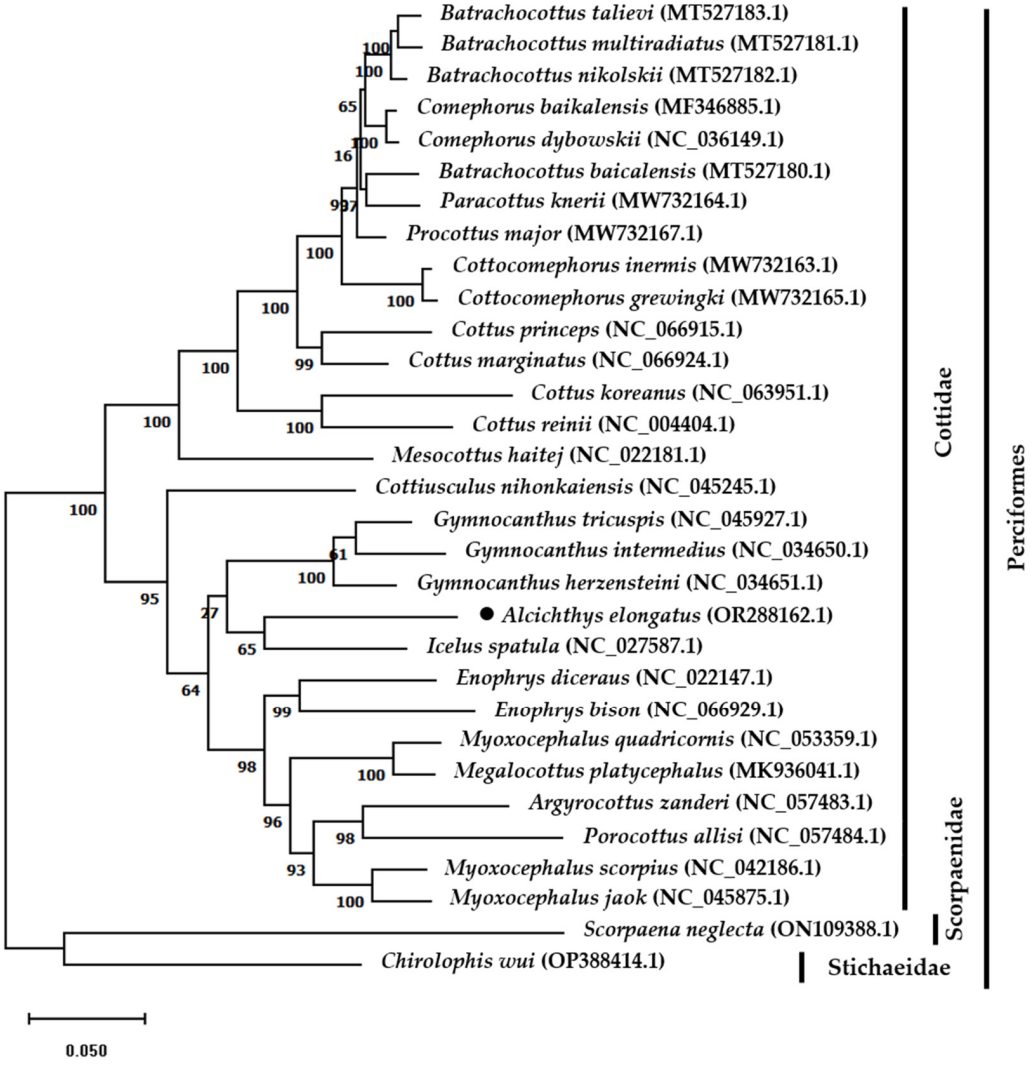

Figure 4. Maximum-likelihood (ML) tree constructed for a total of 29 species belonging to the Family Cottidae, with one representative from each of the Scorpaenidae and Stichaeidae. This phylogeny was constructed using the concatenated nucleotide sequences of 13 PCGs. The numbers on the branches represent ML bootstrap percentages (1000 replicates). For published sequences, NCBI GenBank accession numbers are included following the species name. This study analyzed *Alcichthys elongatus*.

4. Conclusions

The present study reports the complete mitogenome of *A. elongatus*, which includes 37 distinct mitochondrial genes that occur in fishes. The organizational structure of the mitogenome in *A. elongatus* closely resembled that of other members of the Family Cottidae. The sequenced mitogenome dataset from this work is a useful resource for future phylogenetic and evolutionary research. PCGs seem to better represent the evolution of a complete mitochondrial genome than rRNA genes. Therefore, it is necessary to perform phylogenetic reconstruction using PCGs in a greater number of species within the Family Cottidae to achieve a complete understanding of the evolution of this group.

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Institutional Review Board Statement: The sample used for this study was a dead body of fish and as per the animal experimental ethics in the Republic of Korea (Standard operating guideline; IACUC—Institutional Animal Care and Use Committee, Book no. 11-1543061-000457-01, effective from Dec. 2020), it does not need any approval from an Ethics Committee.

Informed Consent Statement: Not applicable.

Data Availability Statement: The complete mitochondrial genome sequence of *A. elongatus* and related data were deposited to the NCBI GenBank (https://www.ncbi.nlm.nih.gov/, accessed on 17 July 2023 and 4 August 2023). The complete mitogenome sequence data are available under GenBank number OR288162.1 and related data including BioProject, BioSample, and Sequence Read Archive (SRA) are available under numbers PRJNA1002095, SAMN36832317, and SRR25514326, respectively.

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