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The Complete Mitogenomes of Two Species of Snakehead Fish (Perciformes: Channidae): Genome Characterization and Phylogenetic Analysis

Tangjun Xu [1], Wenwen Zhang [2], Yao Li [3], Jiachen Wang [1], Yawen Bai [1] and Hongyi Liu [1,*]

[1] Co-Innovation Center for Sustainable Forestry in Southern China, College of Life Sciences, Nanjing Forestry University, Nanjing 210037, China; xutangjun@njfu.edu.cn (T.X.); wangjiachen@njfu.edu.cn (J.W.); baiyawen@njfu.edu.cn (Y.B.)

[2] Research Center for Biodiversity Conservation and Biosafety/State Environmental Protection Scientific Observation and Research Station for Ecological Environment of Wuyi Mountains/Biodiversity Comprehensive Observation Station for Wuyi Mountains/State Environmental Protection Key Laboratory on Biosafety, Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment of China, Nanjing 210042, China; zhangww0224@126.com

[3] College of Ecology and Environment, Nanjing Forestry University, 159 Longpan Road, Nanjing 210037, China; liyaolisantu@njfu.edu.cn

* Correspondence: hongyi_liu@njfu.edu.cn

Abstract: Channidae (snakehead fish) is a family of medium-to-large freshwater carnivorous fish and contain the genus, *Channa*. Here, the complete mitogenomes of two *Channa* fish were determined and comparatively analyzed with the mitogenomes of 16 other Channidae fish species. The two newly sequenced complete mitogenomes were circular DNA molecules with sizes of 16,953 bp (*Channa burmanica*; OP954106) and 16,897 bp (*Channa aurantimaculata*; OQ134162). The mitogenomes were composed of 37 genes and one D-loop region. Positive AT skews and negative GC skews were found in the mitogenomes. Most protein-coding genes (PCGs) started with the conventional start codon, ATG; however, the sequence of the stop codon was variable. There was no obvious difference in relative synonymous codon usage among the two mitogenomes, and the two species shared a similar number of codon usage of mitogenomic PCGs, which was also similar to the mean values for the other 15 species of *Channa*. All Ka/Ks values were <1; *cox1* had the lowest value, and *atp8* had the highest. All of the tRNAs were typical clover structures, except trnS1. Phylogenetic analysis showed that *C. burmanica* and *C. aurantimaculata* shared a close relationship and that they were also closely related to *C. gachua*. These findings enrich the gene database of Channidae species, clarify the mitochondrial genome structure of the two species, and provide basic data for invasive biological surveillance in the future.

Keywords: Channidae; mitochondrial genome; phylogeny; mitochondrial structure; *Channa burmanica*; *Channa aurantimaculata*

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1. Introduction

Channidae, a family of Anabantiformes, is a family of medium-to-large carnivorous freshwater fish [1], which are also known as snakehead fish. They are characterized by peculiar morphological features, such as elongated cylindrical bodies, long and entirely soft-rayed dorsal and anal fins, a large mouth with well-developed teeth on both upper and lower jaws, and an accessory air-breathing apparatus known as the suprabranchial organ [2]. They have flattened heads and possess large scales on their heads, and their eyes are located in the dorsoventral position on the anterior part of the head. They are highly adaptable and can breathe with the help of the suprabranchial organ in the event of a lack of oxygen or being out of water [3]. At present, according to the information published in the Integrated Taxonomic Information System (https://itis.gov/), Channidae is divided

into the genera *Channa* and *Parachanna*. *Channa* contains 53 species that are distributed across Asia [4–10], while *Parachanna* contains only 3 species that are distributed across Africa [11]. Previously, the classification of snakeheads was mainly based on morphological characteristics. However, because individuals vary significantly in color patterns during development, the different sizes and colors have resulted in juveniles and adults of the same species being described as distinct species [11]. The naming and classification of the Channidae are therefore confusing. There are over 100 nominal species of Channidae [12], but only 56 have been admitted. The vast majority of the remaining names are considered to be synonyms of the old names [13].

With the increasing globalization of trade, the threat of invasive alien species continues to increase, which can impact biodiversity and ecosystem functions. The wide-ranging diet, level of parental care, and fierce character give snakehead fish a high probability of becoming an invasive species [14]. Some snakehead fish are popular as food or ornamental fish in their native habitats. However, when snakehead fish were introduced to areas outside their natural range, they became highly invasive [11]. For example, *Channa argus*, an important commercial fish used as food in China [15], known for its fast growth, high meat content with few bone spurs, and tolerance to water pollution and diseases [15], has established several populations in the eastern United States, threatening local ecosystems [16]. In China, in addition to the native snakehead fish species, some exotic species have also been introduced as ornamental fish. A study in Shanghai has shown that non-native snakehead fish have invaded local waters [17]. Despite the attention snakeheads have received, there are substantial difficulties for accurate species identification. Moreover, they vary in their ecological requirements and potential invasive ability [18]. In order to prevent possible species invasion, and to better understand this kind of species, snakehead fish accurate identification and classification are very necessary.

It is difficult to identify snakehead fish by morphology because of the great difference in color patterns during ontogeny and the large number of species. Molecular identification can help solve this dilemma. The mitochondrion is an elementary eukaryotic organelle that exists in most eukaryotic cells. Mitochondria possess mitochondrial DNA (mtDNA), which has a closed circular double-stranded structure and self-replicates semi-conservatively [19,20]. In general, the mitogenome of Osteichthyes is a circular, double-stranded molecule, 16 to 23 kb in size, typically containing a standard set of 13 protein-coding genes (PCGs), two ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and one displacement loop (D-loop) region [21]. Owing to their maternal inheritance, high copy number, high mutation rate, relatively rapid evolutionary rate, and lack of genetic recombination, mitogenomes have been valuable and extremely popular markers in molecular ecology, evolutionary biology, population genetics, animal phylogenetic studies, and species identification [15]. Although some mitochondrial genes (such as *cox1*, *cytb,* and *rrnL*) have been widely used for phylogenetic analysis and species identification [13,16], partial mitochondrial sequences provide only limited information, missing information on gene rearrangement, genetic code changes, replication, and transcriptional regulation patterns. The complete mitochondrial genome sequence can provide higher resolution and sensitivity for the study of evolutionary relationships. In recent years, some molecular phylogenetic studies have solved the relationships between some of the species within the snakehead fish family. Ruber et al. divided the Asian genus *Channa* into eight distinct species groups (Argus, Asiatica, Gachua, Lucius, Marulius, Micropeltes, Punctata, and Striata groups) [12]. Wang et al. determined the phylogenetic relationships of five *Channa* species (*C. andrao*, *C. bleheri*, *C. ornatipinnis*, *C. pulchra*, and *C. stewartii*) and found three new pairs of sisters (*C. andrao* + *C. bleheri*, *C. ornatipinnis* + *C. pulchra*, and *C. stewartia* + *C. gachua*) [22]. However, this work still needs to be improved.

In this study, we determined the complete mitogenomes of two *Channa* species (*C. burmanica* and *C. aurantimaculata*), which are common and popular ornamental fish. We analyzed their mitochondrial genome, including the genome size, nucleotide composition, codon usage, and selective pressure on 13 protein-coding genes (PCGs) and compared

them with the mitogenomes of 16 other Channidae species. The purpose of this study was to provide new data for the characterization of mitochondrial genomes in the Channidae family, to further refine the phylogenetic relationships between the snakehead fish groups, and to provide reliable molecular data for species invasion monitoring in the future.

2. Materials and Methods

2.1. Sample Collection and Identification

Specimens of *C. burmanica* and *C. aurantimaculata* were collected from the Qiqiaoweng pet market in Jiangsu Province, China. The specimens were identified based on the morphological characteristics described by FishBase (https://www.fishbase.de/). Only one individual per species was used for sequencing. Fresh tissues were dissected from fins and stored at −20 °C. Genomic DNA was extracted using a DNAiso reagent (Takara, Beijing, China). All remaining samples were stored in a cryogenic freezer at −80 °C at the Zoology Laboratory of Nanjing Forestry University.

2.2. Sequence Analysis and Assembly and Mitochondrial Genome Annotation

The library of mitogenome DNA was sequenced using the Illumina platform (Personal, Shanghai, China). The mitogenomes of *C. stewartii* (accession: OP402840) and *C. bleheri* (accession: OP186040) were used as templates for the two *Channa* species. Sequence contigs were assembled and trimmed using the medium sensitivity/fast option in the Geneious Prime 2021 software (https://www.geneious.com). Consensus sequences were constructed in Geneious Prime using a 99% base call threshold. The complete mitogenomes of *C. burmanica* and *C. aurantimaculata* were obtained.

MITOS WebServer (http://mitos.bioinf.uni-leipzig.de/index.py) was used for sequence annotation [23], and all parts of the sequence were confirmed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 1 December 2022)). The two species were confirmed by BLASTn searches of the *cox1* gene against GenBank, with top hits showing 100% identity in *C. burmanica* (GenBank Accession: MF496705) and *C. aurantimaculata* (GenBank Accession: EU342193), respectively. The tRNA and rRNA secondary structures were described by ViennaRNA Web Services (http://rna.tbi.univie.ac.at/ (accessed on 1 December 2022)) [24]. Circular gene maps were generated using the CGView Server (https://paulstothard.github.io/cgview/ (accessed on 1 December 2022)). MEGA11 was used to determine the base composition, the relative synonymous codon usage (RSCU) of each codon, and the nonsynonymous (Ka) and synonymous substitutions (Ks) [25]. Nucleotide compositional skewness was calculated according to the following formulas: AT skew = (A − T)/(A + T); GC skew = (G − C)/(G + C) [26].

2.3. Phylogenetic Analyses

The phylogenetic relationships were constructed using 17 *Channa* mitogenomes with a *Parachanna* mitogenome as an outgroup. Details of the species used in this study are listed in Table 1. Bayesian inference (BI) and maximum likelihood (ML) methods were used for phylogenetic analyses with the nucleotide sequences of the 13 PCGs. MAFFT v.7.313 was used to align all PCGs, and the best substitution model was identified by ModelFinder. Phylogenetic analysis was performed using PhyloSuite v.1.2.2 [27]. The BI method was used to construct a tree using the software MrBayes v.3.2.6 based on the model GTR + I + G4 (2 parallel runs, 1000 sample frequency, 10 million generations), and the initial 25% of the sampled data were discarded as burn-in [28]. The ML tree was constructed using IQ-TREE based on the model GTR + F + R4 for 100,000 ultrafast bootstraps [29]. The phylogenetic trees were visualized and edited using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/ (accessed on 2 December 2022)).

Table 1. List of the mitogenomes analyzed in this study.

Family	Species	Accession No.	Length (bp)
Channa	*Channa diplogramma*	MG986721.1	16,571
	Channa lucius	MF804538.1	16,570
	Channa marulius	KF420268.1	16,569
	Channa micropeltes	KX129904.1	16,567
	Channa gachua	MK371068.1	16,561
	Channa argus	MG751766.1	16,558
	Channa maculata	KC823606.1	16,559
	Channa asiatica	KJ930190.1	16,550
	Channa striata	KX177965.1	16,509
	Channa punctata	MK007075.1	16,409
	Channa ornatipinnis	OP271694.1	16,866
	Channa pulchra	OP271693.1	16,895
	Channa stewartii	OP402840.1	16,765
	Channa andrao	OP402839.1	16,729
	Channa bleheri	OP186040.1	16,714
	Channa burmanica	OP954106.1	16,953
	Channa aurantimaculata	OQ134162.1	16,897
Parachanna	*Parachanna insignis*	AP006042.1	16,607

3. Results and Discussion

3.1. Mitogenome Organization and Structure

The complete mitogenomes of *C. burmanica* and *C. aurantimaculata* were found to be 16,953 bp and 16,897 bp in size, respectively. Of these, the complete mitogenome of *C. burmanica* has the longest length, and *C. aurantimaculata* has the second longest. Both mitogenomes contained 37 typical mitochondrial genes (13 PCGs, 22 tRNAs, and two rRNAs) and one control region. Two rRNAs, 12 PCGs, and 14 tRNAs were found to be encoded on the major strand (J-chain), whereas the remaining genes (8 tRNAs and 1 PCG) were located on the minor strand (N-chain) (Figure 1). The gene arrangement and size of the two Channidae mitogenomes were typical of the Channidae and are highly conserved [22].

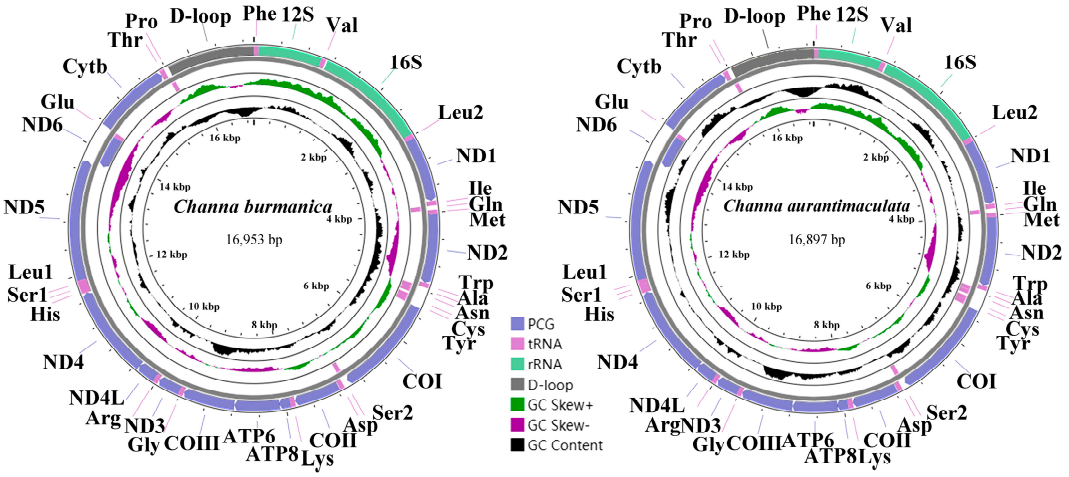

Figure 1. Gene map of the two sequenced mitogenomes of *Channa* species. Genes encoded by the J-chain are shown outside the circle, and those encoded by the N-chain are shown inside the circle. Different gene types are shown as filled boxes in different colors.

Both genomes had nine gene overlaps. The size of each overlap in both genomes was the same, except for the overlap between *trnI* and *trnQ*, which was three in *C. burmanica* and one in *C. aurantimaculata*. All overlap lengths ranged from 1 to 10 bp, and the longest overlap occurred between *atp8* and *atp6* (Table 2). Unusually, no overlap between tRNA and

protein gene sequences was found in a previous study of five species of snakehead fish [22]. In this study, the complete mitogenomes of both *C. burmanica* and *C. aurantimaculata* have overlaps between tRNA and protein gene sequences. The overlaps were between *ND2* and *trnW* and between *cox3* and *trnG*. Both genomes had twelve intergenic spacers. The intergenic regions occur at 12 gene junctions, with the longest intergenic spacer between *trnN* and *trnC* (38 bp). This situation was similar to the genomes of other species of Channa [22].

Table 2. General features of the mitogenomes. *Channa burmanica* is in front, *Channa aurantimaculata* is behind.

Gene	Location		Intergenic Nucleotides	Size	Codon		Stand
	From	To			Start	Stop	
tRNA-Phe	1/1	69/69	0/0	69/69			H
12S rRNA	70/70	1017/1017	0/0	948/948			H
tRNA-Val	1018/1018	1089/1089	0/0	72/72			H
16S rRNA	1113/1113	2768/2765	23/23	1656/1653			H
tRNA-Leu2	2769/2766	2842/2839	0/0	74/74			H
ND1	2843/2840	3817/3814	0/0	975/975	ATG/ATG	TAA/TAA	H
tRNA-Ile	3822/3819	3891/3888	4/4	70/70			H
tRNA-Gln	3889/3888	3959/3958	−3/−1	71/71			L
tRNA-Met	3959/3958	4028/4027	−1/−1	70/70			H
ND2	4029/4028	5075/5074	0/0	1047/1047	ATG/ATG	TAA/TAA	H
tRNA-Trp	5075/5074	5144/5143	−1/−1	70/70			H
tRNA-Ala	5146/5145	5214/5213	1/1	69/69			L
tRNA-Asn	5216/5215	5288/5287	1/1	73/73			L
tRNA-Cys	5327/5326	5391/5390	38/38	65/65			L
tRNA-Tyr	5392/5391	5461/5460	0/0	70/70			L
COI	5463/5462	7004/7003	1/1	1542/1542	GTG/GTG	TAA/TAA	H
tRNA-Ser2	7013/7012	7083/7082	8/8	71/71			L
tRNA-Asp	7086/7086	7157/7157	2/2	72/72			H
COII	7165/7165	7855/7855	7/7	691/691	ATG/ATG	T/T	H
tRNA-Lys	7856/7856	7930/7930	0/0	75/75			H
ATP8	7932/7932	8099/8099	1/1	168/168	ATG/ATG	TAA/TAA	H
ATP6	8090/8090	8773/8773	−10/−10	684/684	ATG/ATG	TAA/TAA	H
COIII	8773/8773	9558/9558	−1/−1	786/786	ATG/ATG	TAA/TAA	H
tRNA-Gly	9558/9558	9626/9626	−1/−1	69/69			H
ND3	9627/9627	9975/9975	0/0	349/349	ATA/ATA	T/T	H
tRNA-Arg	9976/9976	10,044/10,044	0/0	69/69			H
ND4L	10,045/10,045	10,341/10,341	0/0	297/297	ATG/ATG	TAA/TAA	H
ND4	10,335/10,335	11,715/11,715	−7/−7	1381/1381	ATG/ATG	T/T	H
tRNA-His	11,716/11,716	11,784/11,784	0/0	69/69			H
tRNA-Ser1	11,785/11,785	11,852/11,852	0/0	68/68			H
tRNA-Leu1	11,855/11,855	11,927/11,927	2/2	73/73			H
ND5	11,928/11,928	13,766/13,766	0/0	1839/1839	ATG/ATG	TAA/TAA	H
ND6	13,763/13,763	14,284/14,284	−4/−4	522/522	ATG/ATG	TAG/TAG	L
tRNA-Glu	14,285/14,285	14,353/14,353	0/0	69/69			L
Cyt b	14,358/14,358	15,498/15,498	4/4	1141/1141	ATG/ATG	T/T	H
tRNA-Thr	15,499/15,499	15,570/15,570	0/0	72/72			H
tRNA-Pro	15,570/15,570	15,639/15,639	−1/−1	70/70			L
D-loop	15,640/15,640	16,953/16,897	0/0	1314/1258			

However, *C. burmanica* and *C. aurantimaculata* had the longest mitogenomes in known *Channa*. Their lengths of PCGs, tRNAs and rRNAs were similar to other species of *Channa*. The difference in total length arises from the non-coding region. Bilaterian animals possess a large non-coding region referred to as the "control region", or "D-loop" [30]. Most of the size variation among animal mitogenomes is due to differences in the length of non-coding regions [30]. The A + T content of *C. burmanica* and *C. aurantimaculata* was 53.67% and 55.68%, respectively. The A + T content in the Channidae mitogenomes ranged from 51.43%

to 55.75%, as shown in the mitochondrial genomes of other fish [31]. The A + T content of PCGs, tRNAs, and rRNAs showed similar features to the complete mitogenome; however, the A + T content of the D-loop (60.00–70.03%) was higher than that of the other parts of the mitogenome. The AT skew was positive, while the GC skew was negative (Table 3). All Channidae mitogenomes have similar characteristics in these aspects.

Table 3. Base compositions of the complete genomes, PCGs, rRNAs, tRNAs, and D-loop regions of the 18 Channidae mitogenomes.

Species	Whole Mitogenome		AT Skew	GC Skew	PCGs		tRNAs		rRNAs		D-Loop	
	Size (bp)	AT (%)			Size (bp)	AT (%)	Size (bp)	AT (%)	Size (bp)	AT (%)	Size (bp)	AT (%)
C. burmanica	16,953	53.67	0.030	−0.312	11,422	52.50	1550	55.16	2604	52.69	1314	65.14
C. aurantimaculata	16,897	55.68	0.024	−0.315	11,420	54.40	1550	56.97	2601	53.94	1258	70.03
C. diplogramma	16,571	53.53	0.102	−0.337	11,430	52.35	1557	54.66	2627	54.05	918	65.14
C. lucius	16,570	54.00	0.084	−0.335	11,429	52.70	1560	55.13	2633	54.58	909	67.55
C. marulius	16,569	52.75	0.077	−0.325	11,430	51.56	1556	55.21	2631	54.24	915	60.00
C. micropeltes	16,567	53.34	0.102	−0.333	11,430	52.32	1558	55.13	2602	53.65	921	63.52
C. gachua	16,561	55.16	0.028	−0.306	11,429	54.72	1548	55.36	2629	54.43	908	63.66
C. argus	16,558	51.43	0.059	−0.301	11,426	49.91	1556	54.37	2632	53.12	907	61.30
C. maculata	16,559	51.86	0.041	−0.360	11,426	51.30	1558	55.39	2631	54.12	908	63.66
C. asiatica	16,550	55.75	0.055	−0.310	11,426	55.30	1555	56.01	2636	54.74	896	63.73
C. striata	16,509	54.98	0.055	−0.296	11,422	54.31	1554	55.47	2625	55.01	870	63.56
C. punctata	16,409	53.67	0.026	−0.308	11,428	52.63	1549	54.74	2623	54.02	774	66.41
C. ornatipinnis	16,866	53.71	0.035	−0.336	11,412	52.83	1547	55.07	2624	53.24	1244	62.14
C. pulchra	16,895	53.90	0.045	−0.350	11,411	52.97	1550	54.39	2630	53.54	1263	63.66
C. stewartii	16,765	53.30	0.017	−0.297	11,420	51.78	1550	55.81	2625	53.14	1126	66.43
C. andrao	16,729	56.55	0.022	−0.307	11,420	56.26	1550	56.52	2628	55.06	1086	63.90
C. bleheri	16,714	53.08	0.012	−0.302	11,420	51.74	1549	55.78	2620	53.59	1081	63.09
P. insignis	16,607	52.88	0.058	−0.325	11,426	51.98	1552	54.96	2660	53.08	923	61.00

3.2. PCGs and Codon Usage

The total lengths of the 13 PCGs of *C. burmanica* and *C. aurantimaculata* were 11,422 bp and 11,420 bp, respectively, which were similar to the other species in Channidae (Table 3). The lengths of individual PCGs ranged from 168 bp of *atp8* to 1839 bp of *nad5*. All PCGs were located on the J-chain, except *nad6*, which was located on the N-chain. Most PCGs used the conventional start codon, ATG, except for *cox1* and *nad3*, which started with GTG and ATA, respectively. Eight PCGs ended with the conventional stop codon, TAA, while *nad6* ended with TAG and the other four PCGs (*cox2*, *nad3*, *nad4*, and *cytb*) had incomplete stop codons (T). According to previous studies, incomplete stop codons are commonly observed across fish mitogenomes and may be related to post-transcriptional modification [32,33].

There was no significant difference in the RSCU between the two species (Figure 2). The most frequently used codons are CGA and UGA. In contrast, the codons ACG and GCG are rarely used. The most commonly used codons are composed of A or T, and the rarely used ones are composed of C or G. This indicates that the RSCU value is positively correlated with the AT bias of the PCGs.

We found that the two species shared a similar number of codon usage of mitogenomic PCGs, which was also similar to the mean values for the other 15 species of *Channa* (Figure 3). Codons encoding Ile, Ala, and Leu1 were the most frequent, whereas those encoding Met, Cys, and Ser1 were the rarest. Among these, Leu1 showed the highest codon usage, which was presumed to play an important role in maintaining protein activity [34].

All Ka/Ks values were <1, indicating that all the PCGs evolved under purifying selection [35] (Figure 4). The value of *atp8* was the highest of the 13 PCGs, which showed greater amino acid diversity since it was under the least selection pressure [36]. The *cox1* gene had the lowest average Ka/Ks value, indicating that it was the most slowly evolving gene. Researchers believe that drastic selection pressure applied to *cox1* has led to this result [36]. These results were consistent with recent findings and may be a general rule for the evolutionary rate of mitochondrial protein genes in *Channa* [22].

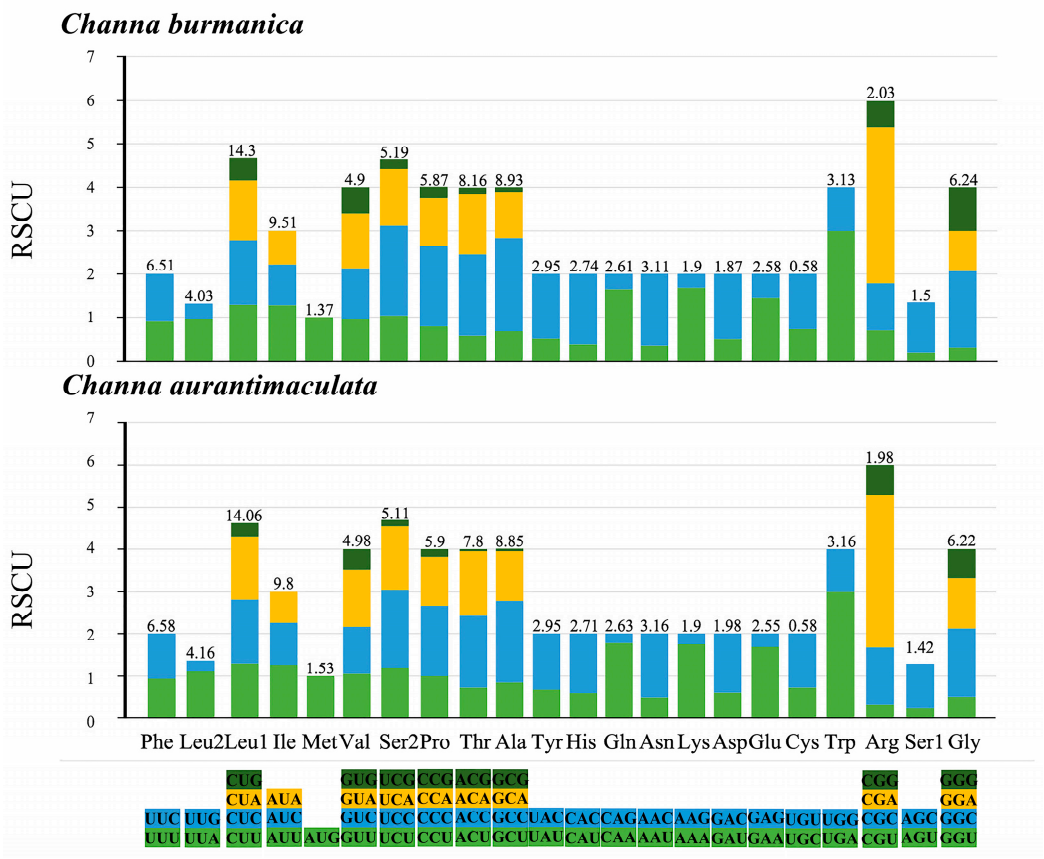

Figure 2. The codon distribution and RSCU of the mitogenomes of the two *Channa* species.

Although *cox1* may be the most conserved mitochondrial protein gene of *Channa*, some studies have shown that sequence divergences at *cox1* regularly enable the discrimination of closely allied species in all animal phyla except the Cnidaria [37]. In fact, *cox1* is the most commonly used molecular marker for species identification and discovery [38]. Because *atp8* has the fastest evolutionary rate, *atp8* may be used as a suitable molecular marker for population genetic diversity [39].

3.3. Transfer RNAs, Ribosomal RNAs, and Displacement Loop Region

Similar to previous reports of other Channidae mitogenomes, the size and order of the arrangement of the transfer RNAs in *C. burmanica* and *C. aurantimaculata* mitogenomes were conserved [22]. The total length of the tRNAs in both mitogenomes was 1550 bp. The tRNA gene size ranged from 65 bp (*trnC*) to 75 bp (*trnK*) (Table 2). Some base pairs are not the classic bonds of A-U and C-G in the tRNA secondary structure. All tRNAs were of the standard cloverleaf structure, except trnS1. Only trnS1 could not fold into the typical cloverleaf structure; trnS1 showed a loss of the pseudo uracil (TΨC) arm, which was replaced with a simple loop (Figure 5).

The total length of the rRNAs in mitogenomes of *C. burmanica* was 2604 bp, and in mitogenomes of *C. aurantimaculata*, it was 2601 bp. The AT% was 52.69% and 53.94%. The *rrnS* was located between *trnF* and *trnV*, and the *rrnL* was located between *trnV* and *trnL2*. They do not overlap with the genes before and after. Previous research has suggested that, compared with *rrnL*, *rrnS* is more highly conserved in Channidae mitogenomes [22]. This situation was also observed in the current study. The length of *rrnS* in both mitogenomes was 948 bp, whereas *rrnL* was 1656 bp in length in *C. burmanica* and 1653 bp in *C. aurantimaculata*. In addition, the rrna secondary structures of the two species predicted by the software also showed that the secondary structures of rrnL of the two species were similar. However, the secondary structures of rrnS of the two species had great difference (Figure 6).

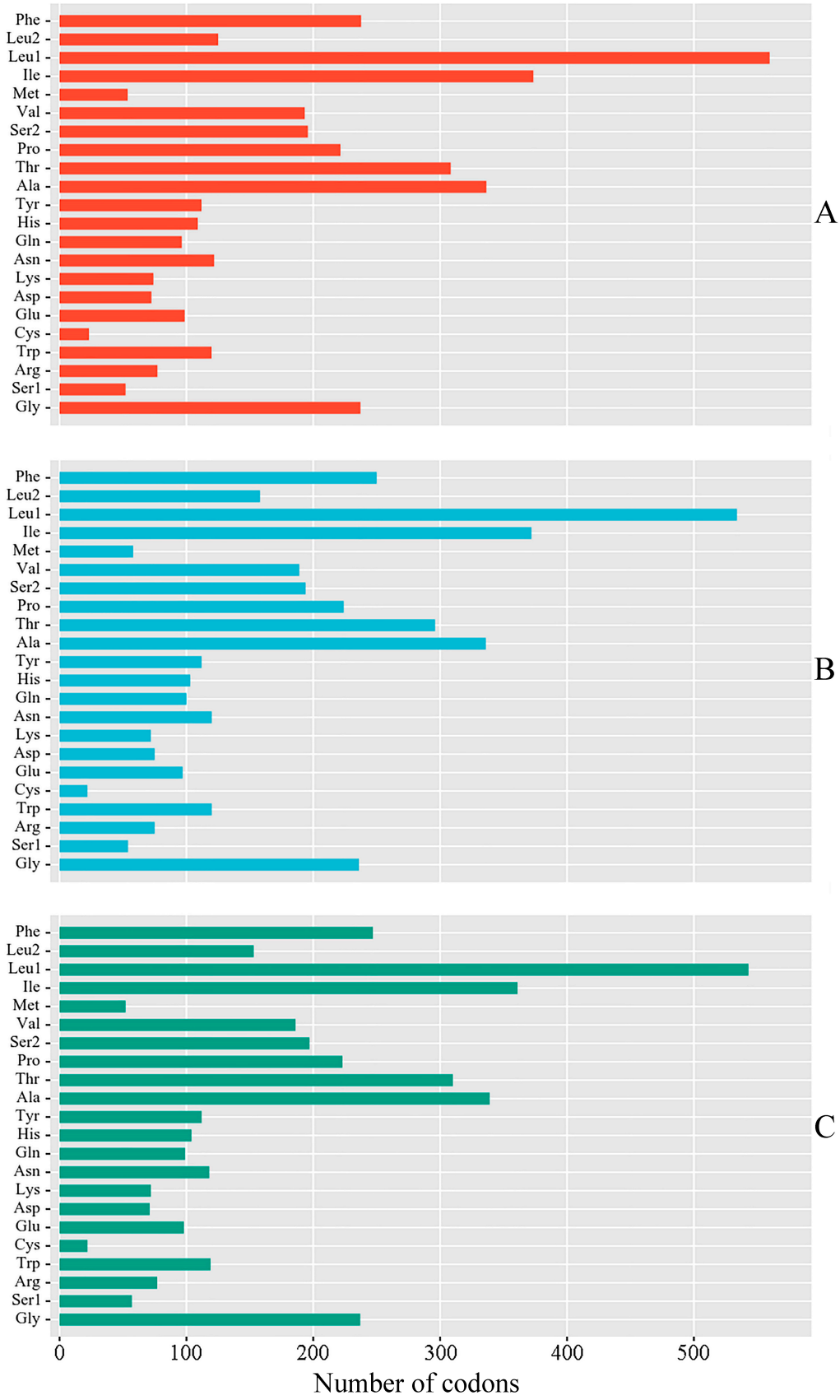

Figure 3. The number of amino acids coded in mitogenomes of the *Channa* species. (**A**) Mean value of amino acid number of 15 *Channa* mitogenomes. (**B**) Amino acid number of *C. aurantimaculata* mitogenome. (**C**) Amino acid number of *C. burmanica* mitogenome.

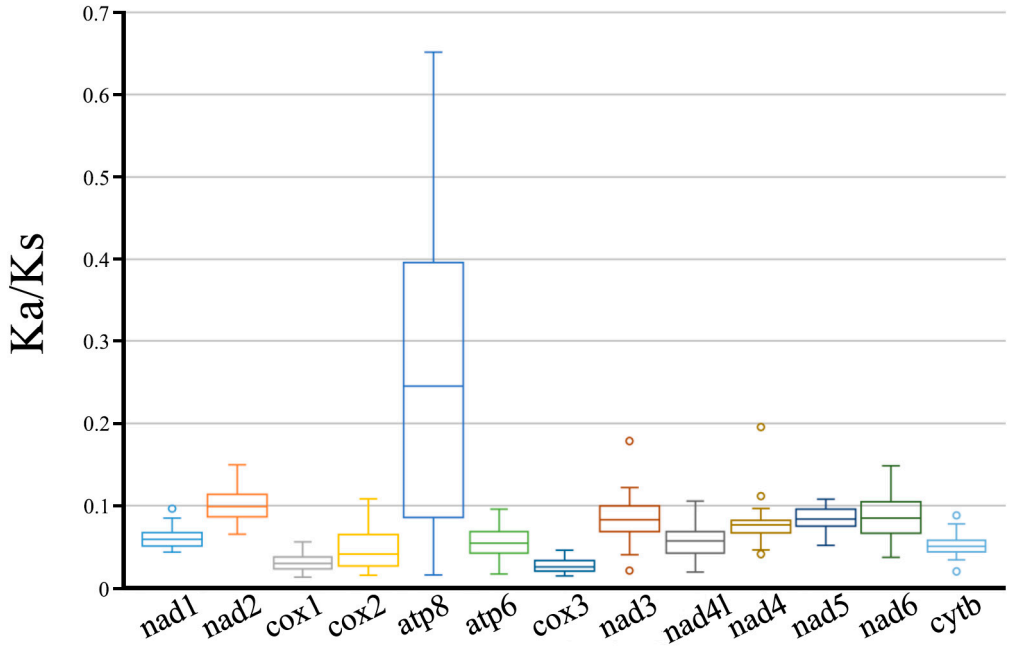

Figure 4. Ka/Ks values for the 13 PCGs of 17 *Channa* mitogenomes.

The D-loop region of all species of Channidae currently reported is located between *trnP* and *trnF* in the mitogenomes, and we found this to also be true for *C. burmanica* and *C. aurantimaculata*. The size of D-loop was 1314 bp in *C. burmanica* and 1258 bp in *C. aurantimaculata*. The A + T content of the D-loop was 65.14% in *C. burmanica* and 70.03% in *C. aurantimaculata*, much higher than that of the whole genome, PCGs, rRNAs, or tRNAs. This region showed great variability between the species of Channidae. The D-loop region is the most rapidly evolving part of the mitogenome [40].

3.4. Phylogenetic Relationships within the Genus Channa

As shown in Figure 7, the trees generated by BI and ML had identical topology and nodal support. The two species in this study showed a close relationship. *C. burmanica* first clustered into one branch with *C. stewartii* and then with *C. aurantimaculata*. The two species also showed a close relationship with *C. andrao* and *C. bleheri*. The existence of distinct phylogenetic groups has been proposed by the putative *C. gachua* species assemblage described by Britz [41], which contains *C. orientalis*, *C. gachua*, *C. bleheri*, *C. burmanica*, *C. barca*, *C. aurantimaculata*, and *C. stewartii*. The current study partly supported this point. However, according to the phylogenetic analysis, *C. burmanica* and *C. aurantimaculata* show a close relationship. The adult sizes of these two species differ greatly. *C. burmanica* is one of several genus members that lacks pelvic fins, while *C. aurantimaculata* has pelvic fin. *C. asiatica*, which also lacks pelvic fins, was slightly farther in the phylogenetic context from *C. burmanica*. Thus, some studies suggest that the lack of a pelvic fin is hypothesized to occur independently in the evolution of Channidae [12,42]. In the future, more research is needed to clarify the mechanisms behind these phenomena.

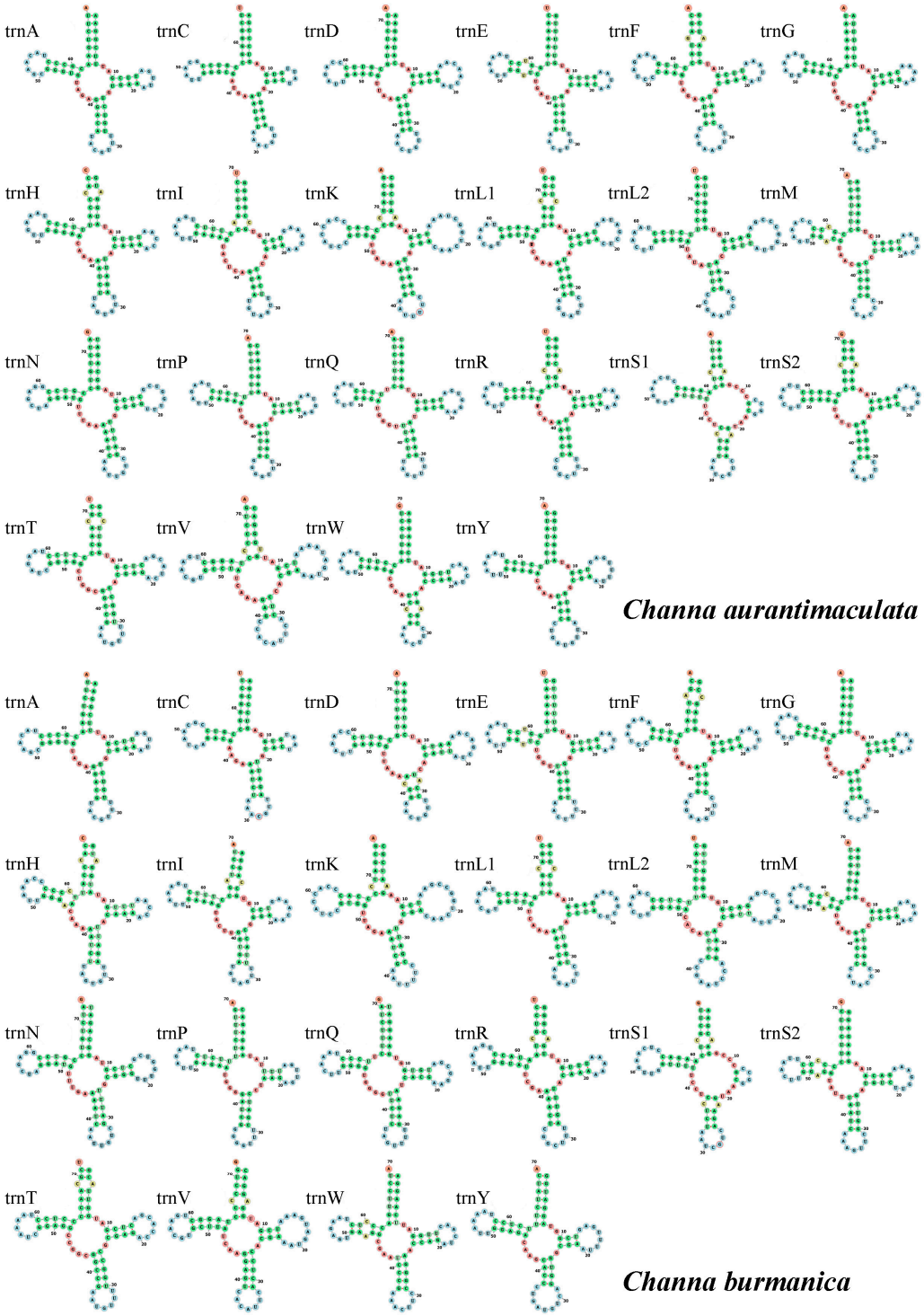

Figure 5. Secondary structures of the 22 transfer RNA of two *Channa* species.

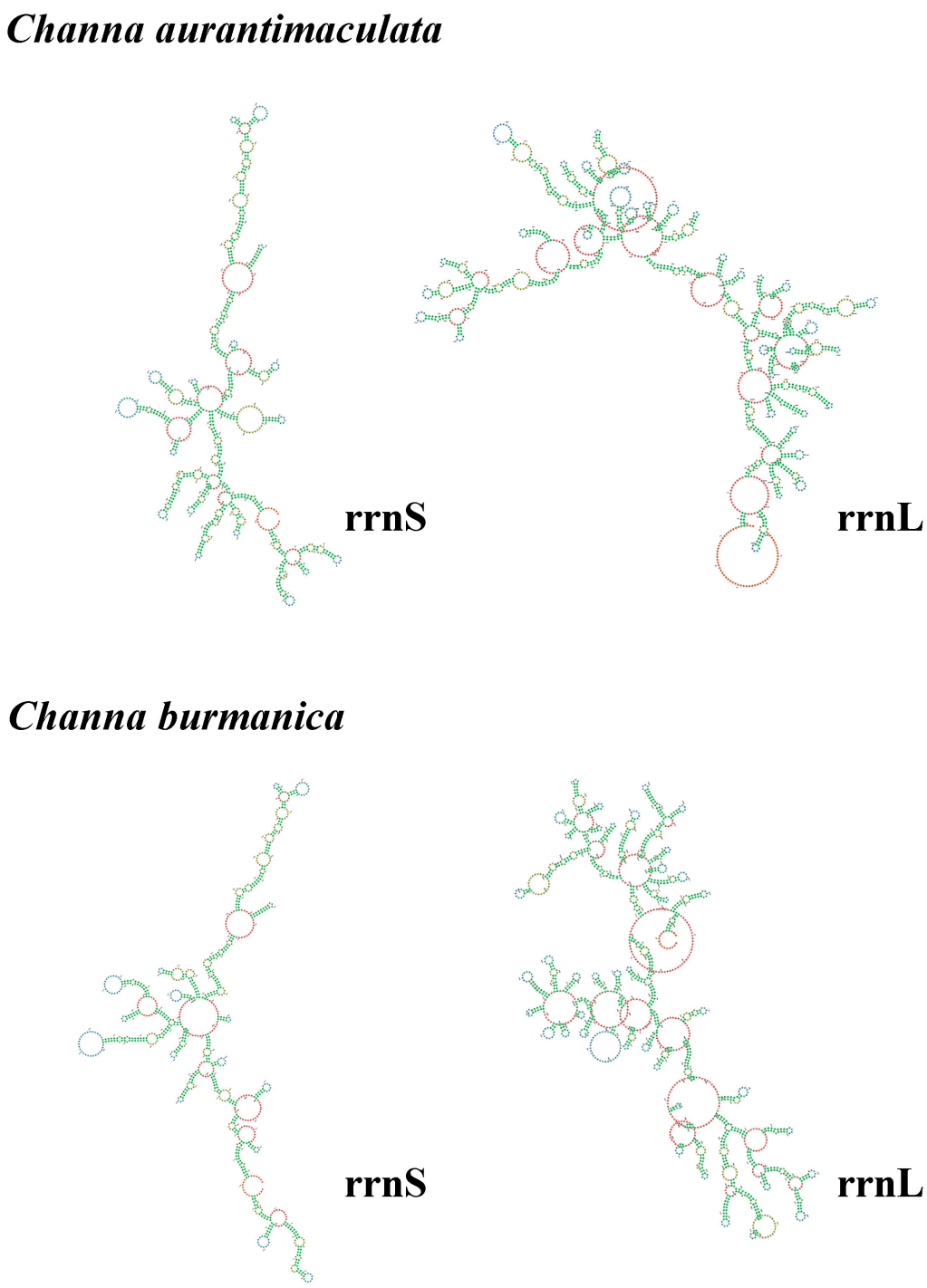

Figure 6. Secondary structures of the rRNA of two *Channa* species.

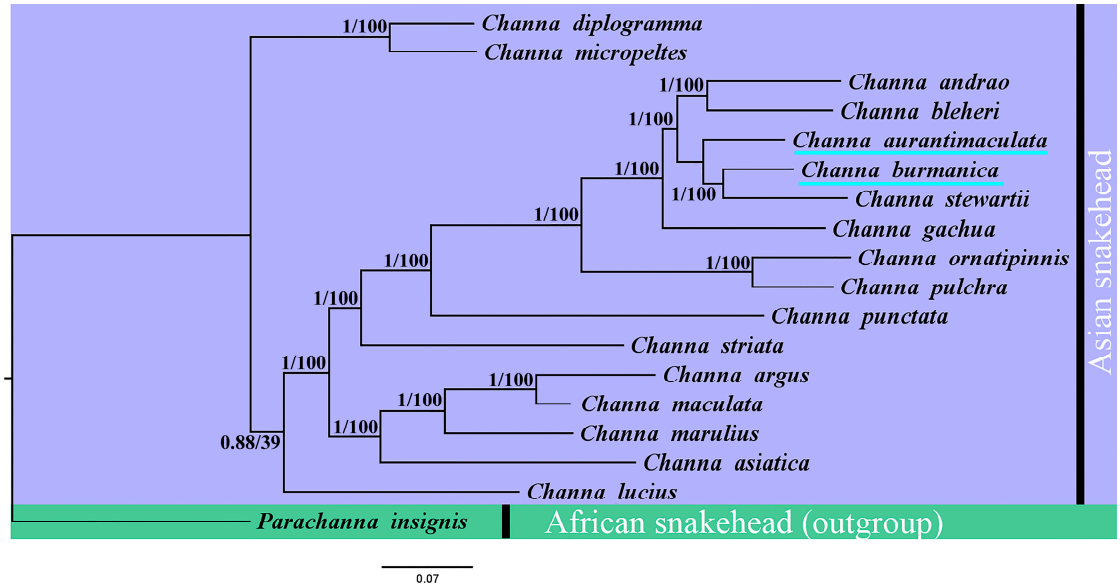

Figure 7. Phylogenetic tree based on 13 PCGs of 18 Channidae species. Numbers at nodes represent the posterior probability and bootstrap values for the BI and ML analyses, respectively. Underlines indicate sequences obtained in this study.

4. Conclusions

In this study, two mitochondrial genomes from *Channa* were sequenced and added to the existing data; we sequenced and analyzed the mitogenomes of *C. burmanica* and *C. aurantimaculata*. The two mitogenomes are conserved in genomic structure, base composition, and codon usage. All tRNAs were of the standard cloverleaf structure, except trnS1. *Cox1* is the most conserved mitochondrial protein gene of *Channa*, and *atp8* has the fastest evolutionary rate. We performed a phylogenetic analysis based on the sequences of thirteen PCGs genes. Our result showed that *C. burmanica* and *C. aurantimaculata* are closely related and supports the sister relationship between *C. burmanica* and *C. stewartii*. Our results provide a valuable resource for further phylogenetic and evolutionary analyses of the *Channa*. However, they have many morphological differences. In the future, the complex relationships among *Channa* fish should be elucidated based on comprehensive evidence including molecular characteristics, morphological characteristics, and geographical distribution.

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Data Availability Statement: The genome sequence data that support the findings of this study are openly available in GenBank of the NCBI at https://www.ncbi.nlm.nih.gov (accessed on 5 December 2022) under accession no. OP954106 and OQ134162.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Betancur, R.R.; Wiley, E.O.; Arratia, G.; Acero, A.; Bailly, N.; Miya, M.; Lecointre, G.; Ortí, G. Phylogenetic classification of bony fishes. *BMC Evol. Biol.* **2017**, *17*, 162. [CrossRef]
2. Bhat, A.A.; Haniffa, M.A.; Divya, P.R.; Gopalakrishnan, A.; Milton, M.J.; Kumar, R.; Paray, B.A. Molecular characterization of eight Indian Snakehead species (Pisces: *Perciformes channidae*) using RAPD markers. *Mol. Biol. Rep.* **2012**, *39*, 4267–4273. [CrossRef]
3. Duan, T.; Shi, C.; Zhou, J.; Lv, X.; Li, Y.; Luo, Y. How does the snakehead *Channa argus* survive in air? The combined roles of the suprabranchial chamber and physiological regulations during aerial respiration. *Biol. Open* **2018**, *7*, bio029223. [CrossRef]
4. Adamson, E.A.S.; Britz, R. The snakehead fish *Channa aurolineata* is a valid species (Teleostei: Channidae) distinct from Channa marulius. *Zootaxa* **2018**, *4514*, 542–552. [CrossRef]
5. Praveenraj, J.; Knight, J.D.M.; Kiruba-Sankar, R.; Halalludin, B.; Raymond, J.J.A.; Thakur, V.R. *Channa royi* (Teleostei: Channidae): A new species of snakehead from Andaman Islands, India. *Indian J. Fish.* **2018**, *65*, 1–14. [CrossRef]
6. Britz, R.; Anoop, V.; Dahanukar, N.; Raghavan, R. The subterranean *Aenigmachanna gollum*, a new genus and species of snakehead (Teleostei: Channidae) from Kerala, South India. *Zootaxa* **2019**, *4603*, 377–388. [CrossRef]
7. Britz, R.; Dahanukar, N.; Anoop, V.; Ali, A. Channa rara, a new species of snakehead fish from the Western Ghats region of Maharashtra, India (Teleostei: Labyrinthici: Channidae). *Zootaxa* **2019**, *4683*, 589–600. [CrossRef]
8. Praveenraj, J.; Uma, A.; Moulitharan, N.; Kannan, R. Channa brunnea, a new species of snakehead (Teleostei: Channidae) from West Bengal, India. *Zootaxa* **2019**, *4624*, 59–70. [CrossRef]
9. Praveenraj, J.; Uma, A.; Moulitharan, N.; Singh, S.G. A new species of dwarf channa (Teleostei: Channidae) from Meghalaya, Northeast India. *Copeia* **2019**, *107*, 61–71. [CrossRef]
10. Praveenraj, J.; Thackeray, T.; Singh, S.G.; Uma, A.; Moulitharan, N.; Mukhim, B.K. A New Species of Snakehead (Teleostei: Channidae) from East Khasi Hills, Meghalaya, Northeastern India. *Copeia* **2020**, *108*, 938–947. [CrossRef]
11. Conte-Grand, C.; Britz, R.; Dahanukar, N.; Raghavan, R.; Pethiyagoda, R.; Tan, H.H.; Hadiaty, R.K.; Yaakob, N.S.; Rüber, L. Barcoding snakeheads (Teleostei, Channidae) revisited: Discovering greater species diversity and resolving perpetuated taxonomic confusions. *PLoS ONE* **2017**, *12*, e0184017. [CrossRef]
12. Rüber, L.; Tan, H.H.; Britz, R. Snakehead (Teleostei: Channidae) diversity and the Eastern Himalaya biodiversity hotspot. *J. Zoöl. Syst. Evol. Res.* **2020**, *58*, 356–386. [CrossRef]
13. Sudasinghe, H.; Ranasinghe, R.H.T.; Pethiyagoda, R.; Meegaskumbura, M.; Britz, R. Genetic diversity and morphological stasis in the Ceylon Snakehead, *Channa orientalis*(Teleostei: Channidae). *Ichthyol. Res.* **2020**, *68*, 67–80. [CrossRef]
14. Landis, A.M.G.; Lapointe, N.W. First record of a northern snakehead (*Channa argus* cantor) nest in North America. *Northeast. Nat.* **2010**, *17*, 325–332. [CrossRef]
15. Liu, M.; Yin, J.; Han, J.; Ren, J.; Yang, S. *Channa argus* BMH from Baima Hu Lake: Sequencing and phylogenetic analysis of the mitochondrial genome. *Mitochondrial DNA Part B* **2020**, *5*, 2413–2415. [CrossRef]
16. Serrao, N.R.; Steinke, D.; Hanner, R.H. Calibrating snakehead diversity with DNA barcodes: Expanding taxonomic coverage to enable identification of potential and established invasive species. *PLoS ONE* **2014**, *9*, e99546. [CrossRef]
17. Yu, R.; Wu, Q.; Li, F.; Zhan, A.; Zhou, J.; Li, S. Risk Screening of invasive aquatic species and a survey of fish diversity using environmental dna metabarcoding analysis in shanghai. *Diversity* **2024**, *16*, 29. [CrossRef]
18. Herborg, L.-M.; Mandrak, N.E.; Cudmore, B.C.; MacIsaac, H.J. Comparative distribution and invasion risk of snakehead (Channidae) and Asian carp (Cyprinidae) species in North America. *Can. J. Fish. Aquat. Sci.* **2007**, *64*, 1723–1735. [CrossRef]
19. Prosdocimi, F.; de Carvalho, D.C.; de Almeida, R.N.; Beheregaray, L.B. The complete mitochondrial genome of two recently derived species of the fish genus Nannoperca (Perciformes, Percichthyidae). *Mol. Biol. Rep.* **2012**, *39*, 2767–2772. [CrossRef] [PubMed]
20. Paz, F.P.C.; Batista, J.d.S.; Porto, J.I.R. DNA Barcodes of Rosy Tetras and Allied Species (Characiformes: Characidae: Hyphessobrycon) from the Brazilian Amazon Basin. *PLoS ONE* **2014**, *9*, e98603. [CrossRef] [PubMed]
21. Iwasaki, W.; Fukunaga, T.; Isagozawa, R.; Yamada, K.; Maeda, Y.; Satoh, T.P.; Sado, T.; Mabuchi, K.; Takeshima, H.; Miya, M.; et al. MitoFish and MitoAnnotator: A Mitochondrial Genome Database of Fish with an Accurate and Automatic Annotation Pipeline. *Mol. Biol. Evol.* **2013**, *30*, 2531–2540. [CrossRef]
22. Wang, J.; Xu, W.; Liu, Y.; Bai, Y.; Liu, H. Comparative mitochondrial genomics and phylogenetics for species of the snakehead genus Channa Scopoli, 1777 (Perciformes: Channidae). *Gene* **2023**, *857*, 147186. [CrossRef]
23. Bernt, M.; Donath, A.; Jühling, F.; Externbrink, F.; Florentz, C.; Fritzsch, G.; Pütz, J.; Middendorf, M.; Stadler, P.F. MITOS: Improved de novo metazoan mitochondrial genome annotation. *Mol. Phylogenetics Evol.* **2013**, *69*, 313–319. [CrossRef]
24. Gruber, A.R.; Lorenz, R.; Bernhart, S.H.; Neuböck, R.; Hofacker, I.L. The Vienna RNA Websuite. *Nucleic Acids Res.* **2008**, *36*, W70–W74. [CrossRef]
25. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
26. Perna, N.T.; Kocher, T.D. Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *J. Mol. Evol.* **1995**, *41*, 353–358. [CrossRef]
27. Zhang, D.; Gao, F.; Jakovlić, I.; Zhou, H.; Zhang, J.; Li, W.X.; Wang, G.T. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Mol. Ecol. Resour.* **2020**, *20*, 348–355. [CrossRef]

28. Ronquist, F.; Teslenko, M.; van der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef]
29. Nguyen, L.-T.; Schmidt, H.A.; Von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef] [PubMed]
30. Lavrov, D.V.; Pett, W. Animal mitochondrial DNA as we do not know it: Mt-Genome organization and evolution in nonbilaterian lineages. *Genome Biol. Evol.* **2016**, *8*, 2896–2913. [CrossRef] [PubMed]
31. Ruan, H.; Li, M.; Li, Z.; Huang, J.; Chen, W.; Sun, J.; Liu, L.; Zou, K. Comparative analysis of complete mitochondrial genomes of three *Gerres* fishes (Perciformes: Gerreidae) and primary exploration of their evolution history. *Int. J. Mol. Sci.* **2020**, *21*, 1874. [CrossRef]
32. Cooper, A.; Lalueza-Fox, C.; Anderson, S.G.; Rambaut, A.; Austin, J.J.; Ward, R. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature* **2001**, *409*, 704–707. [CrossRef]
33. Zhao, L.; Gao, T.; Lu, W. Complete mitochondrial DNA sequence of the endangered fish (Bahaba taipingensis): Mitogenome characterization and phylogenetic implications. *ZooKeys* **2015**, *546*, 181–195. [CrossRef]
34. Li, R.; Wang, G.; Wen, Z.-Y.; Zou, Y.-C.; Qin, C.-J.; Luo, Y.; Wang, J.; Chen, G.-H. Complete mitochondrial genome of a kind of snakehead fish Channa siamensis and its phylogenetic consideration. *Genes Genom.* **2019**, *41*, 147–157. [CrossRef]
35. Hughes, A.L.; Nei, M. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature* **1988**, *335*, 167–170. [CrossRef]
36. Hassanin, A.; Léger, N.; Deutsch, J. Evidence for multiple reversals of asymmetric mutational constraints during the evolution of the mitochondrial genome of metazoa, and consequences for phylogenetic inferences. *Syst. Biol.* **2005**, *54*, 277–298. [CrossRef]
37. Hebert, P.D.N.; Ratnasingham, S.; De Waard, J.R. Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. B Boil. Sci.* **2003**, *270* (Suppl. S1), S96–S99. [CrossRef]
38. Hebert, P.D.N.; Cywinska, A.; Ball, S.L.; Dewaard, J.R. Biological identifications through DNA barcodes. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **2003**, *270*, 313–321. [CrossRef]
39. Yu, J.; Liu, J.; Li, C.; Wu, W.; Feng, F.; Wang, Q.; Ying, X.; Qi, D.; Qi, G. Characterization of the complete mitochondrial genome of *Otus lettia*: Exploring the mitochondrial evolution and phylogeny of owls (Strigiformes). *Mitochondrial DNA Part B* **2021**, *6*, 3443–3451. [CrossRef]
40. Sun, C.-H.; Liu, H.-Y.; Xu, N.; Zhang, X.-L.; Zhang, Q.; Han, B.-P. Mitochondrial genome structures and phylogenetic analyses of two tropical characidae fishes. *Front. Genet.* **2021**, *12*, 627402. [CrossRef]
41. Britz, R. Channa ornatipinnis and C. pulchra, two new species of dwarf snakeheads from Myanmar (Teleostei: Channidae). *Ichthyol. Explor. Freshw.* **2008**, *18*, 335–344.
42. Li, X.; Musikasinthorn, P.; Kumazawa, Y. Molecular phylogenetic analyses of snakeheads (Perciformes: Channidae) using mitochondrial DNA sequences. *Ichthyol. Res.* **2006**, *53*, 148–159. [CrossRef]