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STRUCTURE AND EVOLUTION OF THE COMPLETE MITOCHONDRIAL GENOME OF THE FRESHWATER DRUM, *APLODINOTUS GRUNNIENS* (ACTINOPTERYGII: PERCIFORMES: SCIAENIDAE)

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Background. Overfishing and habitat degradation caused a decline of populations of many fish species belonging to the speciose family Sciaenidae. A reliable taxonomic framework is a prerequisite for implementing effective stock management and conservation measures, but phylogeny and taxonomy of the Sciaenidae remain poorly resolved. As traditionally used morphological and single gene-based molecular markers carry a too limited phylogenetic signal for the task, mitochondrial phylogenomics may be a more suitable tool. The freshwater drum, *Aplodinotus grunniens* Rafinesque, 1819, is one of the few Sciaenidae species that live in freshwater habitats, which makes it an important model for studying the phylogeny and evolution of Sciaenidae.

Material and methods. We sequenced and characterized its mitogenome, and reconstructed the phylogeny of Sciaenidae using mitogenomes of 28 species.

Results. The architecture of the mitogenome (16487 bp in length) is standard for this family, and three typical elements were identified in the control region: extended termination associated sequences, central conserved region, and conserved sequence block. Poor availability of sciaenid mitogenomes (especially those belonging to different lineages) prevented us from resolving the phylogeny of this family with confidence. Notably, our results indicate that *Larimichthys* and *Collichthys* species may belong to a single genus, and we suspect that the mitogenome of *Chrysochir aureus* (Richardson, 1846) has been misidentified taxonomically, and urge its resequencing.

Conclusion. The sequencing of additional mitogenomes belonging to non-represented and poorly represented lineages is needed to facilitate the understanding of phylogeny and taxonomy of Sciaenidae.

Keywords: freshwater drum, mitogenome, control region, phylogenetics, Sciaenidae

INTRODUCTION

The freshwater drum, *Aplodinotus grunniens* Rafinesque, 1819, one of the most widely distributed freshwater fish in North America (Boschung and Mayden 2004), is one of the few Sciaenidae species (and the only North-American sciaenid species) that live in freshwater for the entirety of their lifespan. Thus, this species may be an important model to study the phylogeny and evolution of the Sciaenidae, especially the regional biogeographic patterns of marine and freshwater varieties. The freshwater drum has good breeding prospects in China (Zhou 2005), and it is the only natural host required for the artificial breeding of pink heelsplitter (*Potamilus alatus*) in China (Wen et al. 2018). Because of this, in 2016, we introduced a batch of freshwater drum fry from the United States for artificial domestication research and established a

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stable population in the Freshwater Fisheries Research Center in Wuxi. Although many aspects of the freshwater drum biology have received ample scientific attention, including the morphology and growth (Rypel 2007), diet (Jacquemin et al. 2013), reproductive biology (Swedberg and Walburg 1970), behaviour (Rypel and Mitchell 2007), and carcass composition (Zhou 2005), the sequence of its mitochondrial genome remains unavailable.

The family Sciaenidae (Perciformes), to which the freshwater drum belongs, currently comprising over 280 species in more than 60 genera (Froese et al. 2019), is one of the most important fish families in the world's capture fisheries and aquaculture (Anonymous 2016). However, overfishing and habitat degradation resulted in a worrying level of the population decline of a number of sciaenid species, so the IUCN-Species Survival Commission identified the entire family as a conservation priority (Anonymous 2018). A reliable taxonomic framework is a prerequisite for implementing effective stock management and conservation measures (Cariani et al. 2017). The phylogeny of the Sciaenidae has been studied using morphological features (mostly relying on otolith and swim bladder morphology), but these have major limitations, that have been discussed at length before (Sasaki 1989), as well as several different single gene-based molecular markers (Lakra et al. 2009, Cheng et al. 2012, Ma et al. 2012, Lo et al. 2017). However, due to high species richness and wide distribution of (predominantly) marine species, resolution provided by small molecular markers is likely to be too low for this problem, so taxonomy and phylogeny of this family remain only partially resolved (Barbosa et al. 2014, Xu et al. 2014, Lo et al. 2015, 2017, Silva et al. 2018). This indicates that a molecular marker with a higher resolution may be needed to resolve the evolutionary history of this speciose family. Indeed, the most important recent advances in the understanding of the historical biogeography of the Sciaenidae were achieved using a combined mitonuclear set of six concatenated genes (Lo et al. 2015) and a set of complete mitochondrial (mt) genomic sequences (Xu et al. 2014).

Mt genomes, which usually contain 12–13 protein-coding genes (PCGs), provide much higher phylogenetic resolution than single-gene markers, so they are becoming an increasingly popular tool for resolving phylogenetic debates (Der Sarkissian et al. 2015, Lan et al. 2017, Bourguignon et al. 2018, Zou et al. 2018). Although a number of studies relied on this approach to study the phylogeny of the Sciaenidae (or selected sciaenid taxa) (Cheng et al. 2010, 2012, Xu et al. 2015, Zhao et al. 2015, Lin et al. 2017, Wang et al. 2017, Yang et al. 2018), the resolution of the mitochondrial phylogenomics approach is still hampered by the limited number of mt genomes available for this family.

Therefore, the objective of this study was to sequence and characterize the mitochondrial genome sequence of the freshwater drum, and use the sequence to study its evolutionary history and the taxonomy of the family Sciaenidae. For the latter, we constructed the phylogenetic tree of the Sciaenidae, based on 28 complete mitochondrial genomes of species belonging to this family. We discuss the phylogenetic position of the freshwater drum within the Sciaenidae, suggest some new viewpoints on the taxonomy of the Sciaenidae, and provide an important reference for future studies of the taxonomy and evolution of the Sciaenidae.

MATERIALS AND METHODS

Sample source and genomic DNA extraction. Five freshwater drum specimens (body length = 15.18 cm; age = 1 year old) were randomly selected in July 2017 from a batch of fry introduced a year earlier from the United States by the Freshwater Fisheries Research Center of the Chinese Academy of Fishery Sciences. We cut off a small fragment of caudal fins from live specimens, washed the collected clips with sterile water 2–3 times, and stored in absolute ethanol at –20°C. Before the DNA extraction, approximately 50 mg of (each) fin clip was cut with sterile scissors and again rinsed in sterile water. The extraction was performed using DNA Rapid Extraction Kit (Beijing Aidlab Biotechnologies) according to the kit manual. The DNA integrity was determined by agarose gel electrophoresis and a NANODROP 2000 (Thermo Scientific) spectrophotometer (OD 260/280 value). The DNA was diluted to a concentration of about 100 ng · μL^{-1}, then split into vials and stored in –20°C. This study was approved by the Animal Care and Use Committee of the Nanjing Agricultural University (Nanjing, China). The handling of fishes was conducted in accordance with the Guide for the Care and Use of Experimental Animals of China.

Primer design, LA-PCR amplification, and sequencing. Primers (Table 1) were designed according to the mt genomic sequences of closely related species: *Bahaba taipingensis* (Herre, 1932) (JX232404), *Sciaenops ocellatus* (Linnaeus, 1766) (JQ286004), *Argyrosomus amoyensis* (Bleeker, 1863) (KM257863 and KU738606, the latter being nominally labelled as "*Nibea miichthioides* Chu, Lo et Wu, 1963", a junior synonym of *A. amoyensis*), *Argyrosomus japonicus* (Temminck et Schlegel, 1843) (KT184692), and *Miichthys miiuy* (Basilewsky, 1855) (HM447240). The long PCR (LA-PCR) amplification was performed using the standard LA Taq polymerase (Takara). The PCR conditions were as follows: initial denaturation at 94°C for 2 min, then 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min · kb^{-1}, followed by the final extension at 72°C for 10 min. The total volume for PCR and LA-PCR was 50 µL, of which Takara LATaq (5 U · μL^{-1}) was 0.5 µL, 10 · LATaq Buffer II (Mg^{2+}) was 5 µL, dNTP mixture (2.5 mM) was 8 µL, template was 60 ng, and the total volume was then made up with distilled water. The final concentration of the forward and reverse primers was 0.2~1.0 µM, and that of $MgCl_2$ was 2.0 mM. The PCR products were purified using AidQuick Gel Extraction Kit (AidLab), and sequenced directly, or if needed first cloned into a pMD18-T vector (Takara, JAP) and then sequenced, by the dideoxynucleotide procedure, using an ABI 3730 automatic sequencer (Sanger sequencing) with the same set of primers.

Table 1

Primers used for the PCR amplification of the mitochondrial genome of *Aplodinotus grunniens*

Gene/region	Primer name	Sequence (5′–3′)	Length [bp]
12S-16S	YUF1	GACACCTTGCTTTGCCACAC	2475
	YUR1	CGTACTAGAAAAGATCATGGC	
16S-tRNA-Cys	YUF2	GAGCCCATATCGACAAGAGG	2870
	YUR2	CTGAAGAAGTAGGCTAGCGC	
tRNA-Ala-NAD4	YUF3	CTAACCCACATCTTCTGTATGC	5513
	YUR3	CGCTAAAGGCTATGATGAGG	
NAD4-NAD5	YUF4	CAGGCTGAACCTTTCTTAGCC	1538
	YUR4	GTTGAGAGTTGTGAAGATGG	
NAD5	YUF5	GCTCCTAAAGGATAACAGCTC	1370
	YUR5	CTACTCGAAGACTATAGATG	
12S-COX1	YUF6	GCCTAGCCCTCACAGGCACC	3766
	YUR6	GTGTTCTTTCTAACCACTC	

Mitochondrial genome assembly and annotation. Mt genome was assembled and annotated largely as described before (Zou et al. 2017, Zhang et al. 2018). Briefly, sequenced fragments were quality-proofed by visually inspecting the electropherograms and queried against the GenBank using BLAST to confirm that the amplicon is the target sequence. The complete mt genome sequence was assembled from the sequenced fragments using DNAstar software (Burland 2000). We made sure that the overlaps between sequences were identical, the genome circular, and that no numts (Hazkani-Covo et al. 2010) were incorporated. ORFs for PCGs were located using DNAstar, and manually fine-tuned via a comparison with available sciaenid orthologs using BLAST and BLASTx. tRNAscan (Schattner et al. 2005) and ARWEN (Laslett and Canbäck 2008) were used to identify tRNAs. PhyloSuite (Zhang et al. 2020) was used to parse and extract the mt genome annotated in a Microsoft Word document, as well as to create the files for submission to the GenBank (Accession number MG599474).

Mitochondrial genome characterization. The Mtviz tool (Bernt et al. 2019) was used to map the architecture of the mitochondrial genome. The total length and base composition were analysed using DNAStar's Editseq 7.1 tool. Tandem Repeats Finder (Benson 1999) was used to search for the long-segment tandem repeats contained in the control region, and the repeated sequences were then manually analysed and refined. D-loop sequences were aligned using MAFFT (Katoh and Standley 2013), and specific motifs using visual comparison in Mega X (Kumar et al. 2018). The homing sequences of conserved sequence blocks CSB-F and CSB1 were used as the boundaries to discriminate TAS, CD, and CSB,.

Phylogenetic and comparative analyses. The complete mitochondrial genome sequences of 27 species of the family Sciaenidae were retrieved from GenBank, along with three species belonging to three closely related families from the Perciformes order as outgroups: *Acanthopagrus schlegelii* (Bleeker, 1854) of the family Sparidae, *Siniperca chuatsi* (Basilewsky, 1855) of the family Percichthyidae, and *Hapalogenys nigripinnis* (Temminck et Schlegel, 1843) (the valid name of the GenBank entry NC_014404: *Hapalogenys nitens*) of the family Haemulidae. PhyloSuite was used to batch-download all selected mitogenomes from the GenBank, re-annotate ambiguously annotated tRNA genes with the help of ARWEN, and extract genomic features. To assess the impacts of different algorithms and mutational saturation, we conducted phylogenetic analyses on sequences using both nucleotide (NUC dataset) and amino acid (AAs dataset) sequences of all 13 concatenated PCGs, and two different algorithms: Bayesian Inference (BI) using MrBayes 3.2.6 (Ronquist et al. 2012), and Maximum Likelihood (ML) using IQ-TREE (Trifinopoulos et al. 2016). Including data extraction, all steps for phylogenetic analyses were conducted in the Flowchart mode of PhyloSuite, with help of several plug-in programs integrated into it: sequences were aligned in batches with MAFFT using '--auto' strategy and codon alignment mode; poorly aligned segments were removed from the alignments with Gblocks (Talavera and Castresana 2007) using the default PhyloSuite settings; aligned genes were concatenated using PhyloSuite; ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit evolutionary model using the BIC criterion; ML phylogenetic inference was performed with 1000 bootstrap replicates; and BI analysis was performed with default settings (burnin = 0.25), $5 \cdot 10^6$ generations, sampling every 1000 generations, where the stationarity was considered to be reached when the mean standard deviation of split frequencies was < 0.01, ESS (estimated sample size) value > 200, and PSRF (potential scale reduction factor) approached 1. All analyses were conducted using the corresponding selected best-fit models: NUC = GTR + I + G, and AAs = mtVer + F + R4. Phylograms and gene orders were visualized in iTOL (Letunic and Bork 2007) using dataset files generated by PhyloSuite.

RESULTS AND DISCUSSION

Characteristics of the mitochondrial genome. The length of the mitochondrial genome of the freshwater drum was 16487 bp. It contained 13 protein-coding genes (total length 11439 bp), 2 rRNA genes (total length 2650

bp), 22 tRNA genes (total length 1549 bp), and several non-coding regions (NCR) (Fig. 1, Table 2). Nine genes (8 tRNAs and *nad6*) were located on the minus (–) strand, and the remaining 28 genes were located on the plus (+) strand. There were 11 gene overlaps in the genome, the size of which ranged from 1 to 10 bp, adding up to a total of 33 bp. Non-coding regions included a control region (817 bp), an origin of the ± strand replication (OL) (36 bp), and intergenic bases adding up to a total of 29 bp. The whole mitochondrial genome of the freshwater drum had an AT content of 53.6% and a GC content of 46.4%. This is relatively similar to the base composition of most other Sciaenidae species (52%–56%), with the exception of species in the genus *Johnius*, which have a base composition different from other sciaenids (Fonseca et al. 2014, Xu et al. 2015, Yang et al. 2018).

All 13 mitochondrial protein-coding genes used ATG as the start codon. The most common termination codon was TAA (6 genes); *cox1* used AGA; *nad3*, *nad5*, and *nad6* used TAG; and *cox2*, *nad4*, and *cytb* used an incomplete termination codon T-- (Table 2). All these characteristics are common for sciaenid mt genomes (Cui et al. 2009, Liu et al. 2010, Cheng et al. 2012, Zhao et al. 2015, Sun et al. 2017, Yang et al. 2018).

Origin of the + strand replication (OL). The OL was located between *tRNA-Asn* and *tRNA-Cys* genes. Its secondary structure exhibited a large hairpin loop with a stem length of 10 bp and a loop length of 12 bp. It had a very high A base content (58%), and a low T content (8%), which is similar to the A bias of the OL loop region reported in *Epinephelus akaara* (Temminck et Schlegel, 1842) (see Zhuang et al. 2009). Intriguingly, *Larimichthys crocea* (Richardson, 1846) and *Larimichthys polyactis* (Bleeker, 1877) of the same family both exhibited G bias (Liu et al. 2010).

Control region: structural characteristics. The control region was 817 bp in length and located between *tRNA-Pro* and *tRNA-Phe* genes, and it exhibited an A + T bias of 62.8% (A = 32.8%, T = 30%, C = 22.2%, G = 15.1%), both of which features are common in this family of fishes (Cheng et al. 2012, Zhao et al. 2015, Yang et al. 2018). In vertebrates, mitochondrial control regions are usually divided into a typical tripartite structure comprised of the extended termination association sequence (TAS), central conserved domain (CD), and conserved sequence block (CSB) (Sbisà et al. 1997). We compared the control region of the freshwater drum to the homologous sequences of other sciaenid fish species (Tables 3 and 4) and identified the TAS (250 bp), CD (362 bp), and CSB (205 bp) regions.

Termination-associated sequence (TASes). The TAS is believed to act as a signal for the termination of + strand elongation in vertebrates (Sbisà et al. 1997). In most fishes, the conserved TAS motif is TACAT, including its complementary palindrome ATGTA (Cheng et al. 2012, Zhao et al. 2015). The extended TAS of freshwater drum contained four TACAT motifs, the first of which was followed by ATGTA, with an AT interval between them. Apart from *Miichthys miiuy*, *Siniperca chuatsi* (both 4 TACAT repeats), and *Acanthopagrus schlegelii* (5 TACAT repeats), other species included in our dataset had less than 4 TACAT motifs (Table 4). We did not identify the ACAT motif in *Johnius grypotus* (Richardson, 1846), whereas *Sciaenops ocellatus* and *Larimichthys crocea* lacked the ATGTA motif. Intriguingly, the two conspecific *Argyrosomus amoyensis* mitogenomes exhibited different numbers of TACAT motifs (3 and 2). This is likely to be a reflection of the generally fast evolution of the D-loop, and an indication of functional redundancy of multiple TACAT motifs.

Central conserved region (CD). Although the CD region in mammals generally contains five blocks (CSB-B to CSB-F) (Sbisà et al. 1997), fishes mostly possess only three: CSB-D, CSB-E, and CSB-F (Lee et al. 1995, Zhao et al. 2015). All three motifs were successfully identified in the CD of the freshwater drum, as well as in the majority of other (13) species included in our dataset (Table 3). The sequence alignment revealed that CSB-D and CSB-F sequences were relatively conserved, whereas the CSB-E sequence was very variable (Table 3). This is consistent with the results of the comparative sequence analysis of Bagridae and Botiinae (see Zhang et al. 2003, Tang et al. 2005). *Acanthopagrus schlegelii* lacked the CSB-F sequence, only CSB-D was identified in *Johnius grypotus*, only CSB-E was identified in *Larimichthys crocea*, *L. polyactis*, and *Collichthys niveatus* Jordan et Starks, 1906, and none of these three motifs were identified in the CD of *Collichthys lucidus* (Richardson, 1844) (Table 3).

The three motifs of freshwater drum were identical to those of *Chrysochir aureus*, and very similar to those of *Argyrosomus amoyensis*, *Miichthys miiuy*, *Nibea albiflora* (Richardson, 1846), *Siniperca chuatsi* (only 1 bp difference), *Bahaba taipingensis*, and *Atrobucca nibe* (Jordan et Thompson, 1911) (2 bp difference). It is noteworthy that there was a significant rearrangement in the mitochondrial architecture of *Johnius grypotus* and *Johnius belangerii* (Cuvier, 1830) of Sciaenidae: the control region (D-Loop) was located between *tRNA-Pro* and *tRNA-Leu* genes, and the typical CSB-F and CSB-E sequences were not found. There was a 10 bp difference in the CSB-D sequence between freshwater drum and *J. grypotus*, and the CSB-D was not recognized in *J. belangerii*.

Conserved sequence block (CSB). Within the CSB region, associated with the initiation of mitochondrial DNA replication (Cheng et al. 2012), we identified three motifs (CSB-1, CSB-2, and CSB-3) via the comparison with CSB sequences of related species (Table 4). Among the studied Sciaenidae species, all three motifs were identified in all species, except for *Johnius grypotus*, where CSB-2 and CSB-3 motifs could not be identified. With the exception of *Acanthopagrus schlegelii*, the structure of the CSB-2 motif was conserved: TAAA or TAGA, followed by two symmetrical C-base tandem repeats of 6–8 bp separated by a TA interval (see Table 4). The freshwater drum shared an identical CSB-2 motif with *Argyrosomus amoyensis*, *Dendrophysa russelii* (Cuvier, 1829), *Atrobucca nibe*, *Hapalogenys nigripinnis* (Temminck et Schlegel, 1843) (the valid name of the GenBank entry NC_014404: *H. nitens*) and *Siniperca chuatsi*. CSB-1 and

CSB-3 motifs were comparatively variable, with *Bahaba taipingensis* exhibiting the highest similarity: an identical CSB-3, and a three-bp difference in the CSB-1 sequence.

Phylogenetic analysis and gene order. The mt genome of freshwater drum did not exhibit any gene rearrangements compared to the mt genomes of the majority of sciaenid species: apart from the six species belonging to the genus *Johnius*, the gene orders of mitochondrial genomes of the other 22 species were identical (Fig. 2). Mitochondrial genomes of the six *Johnius* species showed different degrees of tRNA rearrangement (and duplication) in the *cyb-nad1* box: a duplication and transposition of *trnP* and *trnF* in

Table 2

Organization and features of the mitochondrial genome of *Aplodinotus grunniens*

Gene	Position		Size [bp]	Codon		Anti-codon	Strand	IGR
	Start	End		Start	Stop			
tRNA-Phe	1	68	68			TTC	+	
12S	69	1020	952				+	
tRNA-Val	1021	1092	72			GTA	+	
16S	1093	2790	1698				+	
tRNA-Leu	2791	2864	74			TTA	+	
nad1	2865	3839	975	ATG	TAA		+	
tRNA-Ile	3844	3913	70			ATC	+	4
tRNA-Gln	3913	3983	71			CAA	–	–1
tRNA-Met	3983	4051	69			ATG	+	–1
nad2	4052	5098	1047	ATG	TAA		+	
tRNA-Trp	5098	5168	71			TGA	+	–1
tRNA-Ala	5170	5238	69			GCA	–	1
tRNA-Asn	5242	5314	73			AAC	–	3
tRNA-Cys	5351	5416	66			TGC	–	36
tRNA-Tyr	5417	5486	70			TAC	–	
cox1	5488	7044	1557	ATG	AGA		+	1
tRNA-Ser	7040	7110	71			TCA	–	–5
tRNA-Asp	7114	7182	69			GAC	+	3
cox2	7190	7880	691	ATG	T		+	7
tRNA-Lys	7881	7955	75			AAA	+	
atp8	7957	8124	168	ATG	TAA		+	1
atp6	8115	8798	684	ATG	TAA		+	–10
cox3	8798	9583	786	ATG	TAA		+	–1
tRNA-Gly	9583	9653	71			GGA	+	–1
nad3	9654	10004	351	ATG	TAG		+	
tRNA-Arg	10003	10071	69			CGA	+	–2
nad4L	10072	10368	297	ATG	TAA		+	
nad4	10362	11742	1381	ATG	T		+	–7
tRNA-His	11743	11811	69			CAC	+	
tRNA-Ser	11812	11879	68			AGC	+	
tRNA-Leu	11885	11957	73			CTA	+	5
nad5	11958	13796	1839	ATG	TAG		+	
nad6	13793	14314	522	ATG	TAG		–	–4
tRNA-Glu	14315	14383	69			GAA	–	
CYTB	14388	15528	1141	ATG	T		+	4
tRNA-Thr	15529	15600	72			ACA	+	
tRNA-Pro	15601	15670	70			CCA	–	
D-Loop	15671	16487	817					

IGR = intergenic region, where negative numbers indicate overlaps.

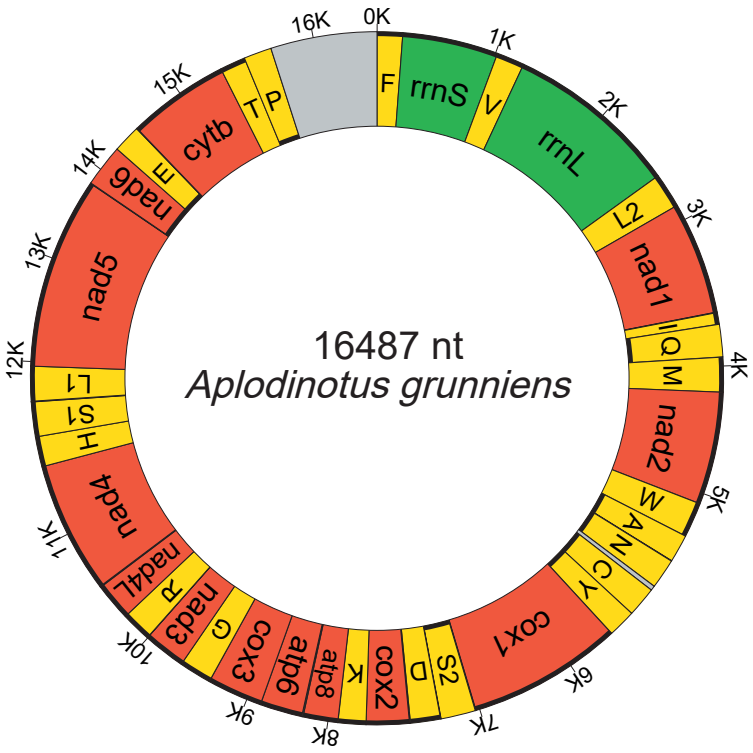

Fig. 1. Arrangement of genes in the mitochondrial genome of *Aplodinotus grunniens*; protein-coding genes are red, rRNAs green, tRNAs yellow, and non-coding regions are grey; tRNA genes are abbreviated to a one-letter amino acid code; thick black line indicates the strand on which the gene is encoded (H = outer, L = inner)

Johnius amblycephalus (Bleeker, 1855); a duplication and transposition of *trnF* and a transposition of *trnP* in *Johnius distinctus* (Tanaka, 1916); a duplication and transposition of *trnF* and a transposition of *trnV* in *Johnius carouna* (Cuvier, 1830); a duplication and transposition of *trnL2* and the loss of *trnF* (assuming the loss is not an annotation artefact) in *J. grypotus*; a transposition of *trnF* and *trnV* in *J. belangerii*; and a duplication and transposition of *trnV* in *Johnius trewavasae* Sasaki, 1992. The unique gene order of this genus was reported before, which led the authors to propose that *Johnius* is the most ancient genus within the family Sciaenidae (see Xu et al. 2015).

A phylogenetic tree was reconstructed using all 13 mitochondrial PCGs of 28 available species of the family Sciaenidae, with three species from three other percomorph families (Percichthyidae, Sparidae, and Haemulidae) used as outgroups. All four analyses (2 datasets × two algorithms -BI and ML) produced identical topologies of the Sciaenidae (Fig. 2), with only a minor rearrangement in the topology of two outgroup species (*Hapalogenys nigripinnis* and *Siniperca chuatsi*) between the amino acid dataset and nucleotide dataset. Although the overall topology of the phylogram was generally congruent with results of previous, both morphology and molecular data-based, studies (Zhu et al. 1963, Ma et al. 2012, Sun et al. 2017), several features deserve to be discussed in more detail. Traditional morphology-based division of the family Sciaenidae into subfamilies (Zhu et al. 1963, Sasaki 1989) has been questioned by many subsequent molecular data-based studies, and a recent combined mitonuclear dataset-based study proposed a general subdivision into 11 lineages (Lo et al. 2015). In that study, *Aplodinotus* was assigned to a separate lineage, which clustered at the base of the largest Sciaenidae clade, comprised of 22 genera, provisionally named Lineage 11. Although our results appear to imply that freshwater drum is the sister-group to all other Sciaenidae species, this is merely a consequence of poor availability of sciaenid mitogenomes belonging to different lineages; apart from *Sciaenops ocellatus* and *A. grunniens*, all species included in our dataset belong to the Lineage 11. In comparison to the results of Lo et al. (2015), our topology differs only in the position of *S. ocellatus*, which clustered with other species belonging to the Lineage 11. Along with *Micropogonias*, this genus was nominally assigned to Lineage 1 by Lo et al. However, it is not unlikely that this is an artefact caused by a limited availability of sciaenid mt genomes, so this result should be confirmed by an analysis comprising mitogenomes of all lineages before any conclusions can be made with confidence.

Among other notable observations, the genus *Nibea* was rendered paraphyletic by *Chrysochir aureus* clustering within the *Nibea* clade. In the traditional morphological taxonomic system, *Nibea* and *Argyrosomus* genera were classified as the subfamily Argyrosominae (see Zhu et al. 1963), but this was later rejected (Meng et al. 2004, Lo et al. 2015, 2017). The taxonomic position of *Chrysochir aureus* has long been controversial: Zhu et al. (1963) classified it into the genus *Nibea*, then later it was reassigned to *Chrysochir* (subfamily Otolithesinae) (see Cheng and Zheng 1987,

Table 3

Comparison of control region motifs in freshwater drum and selected Sciaenidae (and Perciformes) representatives

Scientific name	Accession No.	CSB-F	CSB-E	CSB-D
Aplodinotus grunniens	MG599474	ATGTAATAAGAACCGACCAT	AGGGACAATTATCGTGGGGG	TATTCCTGGCATTTGGTTCCT
Argyrosomus amoyensis	NC_025937	ATGTAATAAGAACCGACCAT	AGGGACAAATATCGTGGGGG	TATTCCTGGCATTTGGTTCCT
"*Argyrosomus amoyensis*"	NC_029875	ATGTAGTAAGAACCGACCAT	AGGGACAAATATCGTGGGGG	TATTCCTGGCATTTGGTTCCT
Sciaenops ocellatus	NC_016867	ATGTAGTAAGAACCGACCAT	AGGGACAAAAATAGTGGGGG	TATTCCTGGCATCTGGTTCCT
Dendrophysa russelii	NC_017606	ATGCAATAAGAACCGACCAT	AGGGACAGTTATTGTGAGGG	TATTCCTGGCATTTGGCTCCT
Bahaba taipingensis	NC_018347	ATGTAATAAGAACCGACCAT	AGGGACAAGTATTGTGGGGG	TATTCCTGGCATTTGGTTCCT
Collichthys lucidus	NC_014350	—	—	—
Collichthys niveatus	NC_014263	—	AGGTTGGTGGGGG	—
Larimichthys crocea	NC_011710	—	AGGTTGGTGGGGG	—
Larimichthys polyactis	NC_013754	—	AGGTTGGTGGGGG	—
Otolithes ruber	NC_033909	ACCCAATAAGAACCGACCAT	AGGGACAAGTATTTGTGGGGG	TATTCCTGGCATTTGGTTCCT
Pennahia argentata	NC_015202	GCCCAATAAGAACCGACCAT	AGGGACAAATATTGTGGGGG	TATTCCTGGCATTTGGTTCCT
Protonibea diacanthus	NC_024573	ACCCAATAAGAACCGACCAT	AGGGACAAATCTTGTGGGGG	TATTCCTGGCATTTGGTTCCT
Miichthys miiuy	NC_014351	ATGTAGTAAGAACCGACCAT	AGGGACAATAATTGTGGGGG	TATTCCTGGCATTTGGTTCCT
Nibea albiflora	NC_015205	ATGTAATAAGAACCGACCAT	AGGGACAACTATTGTGGGGG	TATTCCTGGCATTTGGTTCCT
Chrysochir aureus	NC_016987	ATGTAATAAGAACCGACCAT	AGGGACAATTATCGTGGGGG	TATTCCTGGCATTTGGTTCCT
Atrobucca nibe	NC_035982	ATGTAATAAGAACCGACCAT	AGGGACAACTATCGTGGGGG	TATTCCTGGCATCTGGTTCCT
Johnius grypotus	NC_021130	—	—	TATTAATAAAGCTTATGTCCT
"*Hapalogenys nigripinnis*"	NC_014404	TTGCGGCGAGAACCGACCAT	—	TATTACTGGCATCTGGTTCCT
Acanthopagrus schlegelii	NC_018553	—	AGGGACAAAAATTGTGGGGG	TATTACTGGCATCTGGTTCCT
Siniperca chuatsi	JF972568	ATGTAGTAAGAACCGACCAT	AGG-ACAACCATTGTGGGGG	TATTCCTGGCATTTGGTTCCT

Names within quotation marks indicate mitogenomes that have incorrect names in the GenBank: the valid name for *Nibea miichthioides* (NC_029875) is *Argyrosomus amoyensis*, and for *Hapalogenys nitens* (NC_014404) the valid name is *Hapalogenys nigripinnis*.

Table 4

Mitochondrial control region (D-loop) length, number of motifs in the termination-associated sequences (TAS), and conserved sequence block (CSB) sequences of the freshwater drum and selected Sciaenidae (and Perciformes) representatives

Scientific name	D-Loop [bp]	TAS		CSB		
		TACAT	ATGTA	CSB-1	CSB-2	CSB-3
Aplodinotus grunniens	817	4	1	ATATCTAGATATCACGTGCATAA	TAAACCCCCCTACCCCCC	TGTAAACCCCCCGTAAACA
Argyrosomus amoyensis	824	3	1	ATATAACAATATCAAGTGCATAA	TAAACCCCCCTACCCCCC	TGTAAACCCCCCGGAAACA
"*Argyrosomus amoyensis*"	824	2	1	ATATAGCAATATCAAGTGCATAG	TAAACCCCCCTACCCCCC	TGTAAACCCCCCGGAAACA
Sciaenops ocellatus	845	2	0	ATATTAGGATATCAAGTGCATAA	TAAACCCCCCCTACCCCCCC	CTGAAACCCCCCGGAAACA
Dendrophysa russelii	923	1	1	ATATATTGAATTCTAGTGCATAA	TAAACCCCCCTACCCCCC	TGCAAACCCCC-GAAAACA
Bahaba taipingensis	826	3	1	ATATTTGGATATCAAGTGCATAA	TAAACCCCCCCTACCCCCCC	TGTAAACCCCCCGTAAACA
Collichthys lucidus	771	2	1	ATTTACTGTATTCTAGTGCATAA	TAGACCCCCCCCCTACCCCCCCCC	ATTAAAACCCCTAAAAACA
Collichthys niveatus	799	2	2	ATATTTGGATATCAAGTGCATAA	TAGACCCCCCCCCTACCCCCCCCC	TACTAAAACCCATAAAACA
Otolithes ruber	838	1	1	ATATAAGGATATCAAGTGCATAA	TAAACCCCCCCTACCCCCCC	TGTAAACCCCCCCGTAAACA
Pennahia argentata	837	2	1	ATATTAGGATATCAAGTGCATAA	TAAACCCCCCCTACCCCCCC	TGTAAACCCCCCCGTAAACA
Larimichthys crocea	795	1	0	ATTTTAAGTATTCAAGTGCATAA	TAGACCCCCCCCCTACCCCCCCCC	TACTAAAACCCATAAAACA
Larimichthys polyactis	799	2	2	ATTTTAAGTATTCAAGTGCATAA	TAGACCCCCCCCCTACCCCCCCCC	TACTAAAACCCGTAAAACA
Protonibea diacanthus	821	1	1	ATAATAGGATATCATGTGCATAA	TAAACCCCCCCTACCCCCCC	TGTAAACCCCCCCGTAAACA
Miichthys miiuy	845	4	1	ATATTTGGATGTCAAGTGCATAA	TCAAACCCCCCCTACCCCCCC	TGCAAACCCCCCGTAAACA
Nibea albiflora	823	2	1	ATATTAGGATATCAAGTGCATAC	TAAACCCCCCCCTACCCCCCC	TGTAAACCCCCCCGTAAACA
Chrysochir aureus	822	2	1	ATATTAGGATATCAAGTGCATAA	TAAACCCCCCCTACCCCCCC	TGTAAACCCCCCGGAAACA
Atrobucca nibe	1159	2	1	ATATTAGGATATCATGTGCATAA	TAAACCCCCCTACCCCCC	TGCAAACCCCCCGGAAACA
Johnius grypotus	1247	0	3	TTCCTGAAATAAGTTAATCATAA	—	—
"*Hapalogenys nitens*"	789	2	1	ATTACATAGTCTCAAGAGCATAA	TAAACCCCCCTACCCCCC	TAGCTAAACCCCAAAAGCA
Acanthopagrus schlegelii	945	5	2	ACTTAAACTTATGAATTGCATAA	TAAACCCCCCCCCCCCCTAAACTCC	TGCAAACCCCTCAAAAACA
Siniperca chuatsi	834	4	1	ACACTTTCATCGACGCTGGCATAA	TAAACCCCCCTACCCCCC	TGAAAACCCCCCGGAAACA

Names in quotation marks indicate incorrect names in the GenBank: the valid name for *Nibea miichthioides* (NC_029875) is *Argyrosomus amoyensis*, and for *Hapalogenys nitens* (NC_014404) the valid name is *Hapalogenys nigripinnis*.

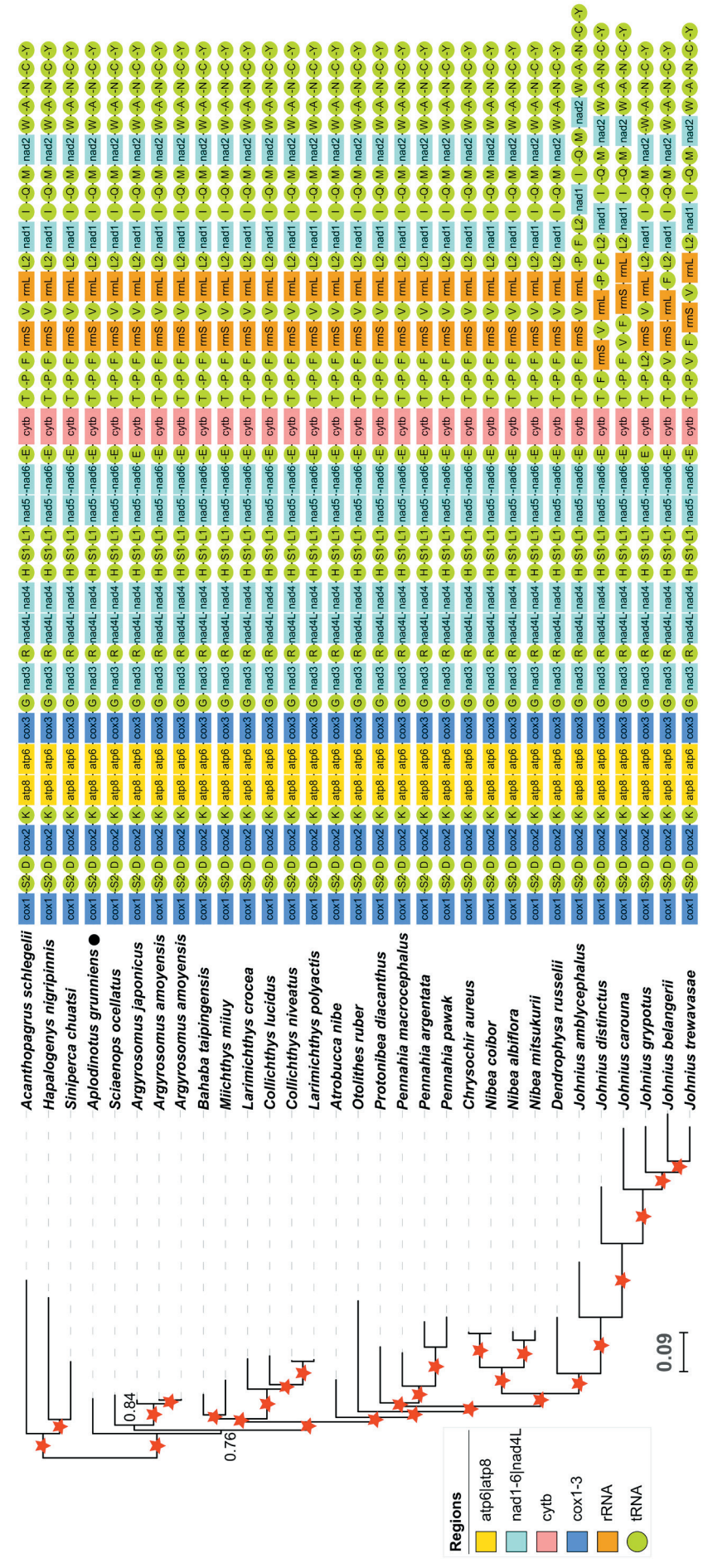

Fig. 2. Phylogenetic dendrogram and gene orders of 28 Sciaenidae mitochondrial genomes; star indicates that the statistical support is 1.0; the lower of the two *Argyrosomus amoyensis* mitogenomes is denominated under an invalid name, *Nibea miichthioides*, in the GenBank (NC_029875)

Sasaki 1989), but a more recent study proposed that it may belong to the genus *Otolithoides* (subfamily Bahabinae) (see Zhang et al. 2010). Although our results support the original taxonomic system proposed by Zhu et al. (1963), other recent studies do not support it (Lo et al. 2015, 2017). This made us suspect that this specimen may have been misidentified. There is no description of the identification of specimen in the original paper (Wang et al. 2017), and BOLD database (Ratnasingham and Hebert 2007) identification produced ambiguous results ("species level match could not be made, the queried specimen is likely to be one of the following: *Chrysochir aureus*, *Nibea coibor*, *Nibea* sp. WJC-2017, *Nibea chui*"). As both *C. aureus* hits in BOLD database are to this same mitogenome (additionally, the fact that the species name is misspelled in the title of, and throughout, the published paper also does not boost our confidence in proper identification of this species), we urge resequencing of the mitogenome of *C. aureus*, and ideally other species from this genus.

Larimichthys and *Collichthys* species clustered within a single clade, the topology of which indicated that they belong to a single genus. Another mitochondrial phylogenomic analysis produced a congruent topology (Cheng et al. 2012), but a combined mitonuclear dataset (*cox1* + *rag-1*) resolved the two genera as very closely related, but monophyletic (Lo et al. 2015, 2017). Although this could also be an artefact caused by a misidentified fish specimen, as both of these latter studies used a small number of species belonging to these two genera (only one *Collichthys* species in Lo et al. 2015), the phylogenetics of these two genera should be studied in detail using a dataset containing all recognized species and a sufficiently high-resolution marker.

Finally, our topology is in disagreement with the gene order-based hypothesis, outlined at the beginning of this section, that *Johnius* is the most ancient genus within this family (Xu et al. 2015). Molecular data are rather consistent in resolving this genus as the most derived sciaenid clade (Lo et al. 2015, 2017), which suggests exactly the opposite, that *Johnius* may be one of the youngest genera in this family. However, as species in the *Johnius* clade underwent an inversion of the control region (Fonseca et al. 2014), it is very likely that this inversion is the underlying cause for the unique base composition of the *Johnius* clade mitogenomes compared to other Sciaenidae (see Reyes et al. 1998, Hassanin et al. 2005, Fonseca et al. 2014). As mitochondrial architecture-driven mutational pressures can produce phylogenetic artefacts (Hassanin 2006, Zhang et al. 2019), the exact position of this genus within the Sciaenidae should be evaluated using nuclear (or combined morphonuclear) markers.

CONCLUSIONS

We characterised the mitochondrial genome of *Aplodinotus grunniens*, especially the elements in its control region. The mitochondrial control region is generally the fastest-evolving part of the mitochondrial genome in vertebrates (Lee et al. 1995), so it is very useful for aquaculture purposes and population-level studies, as it can be used to identify interspecies hybrids (Guo et al. 2003), and even different populations within a species (Wilkinson and Chapman 1991, McMillen-Jackson and Bert 2004). Although it is not without limitations, mitochondrial DNA has played a tremendously important role in our understanding of the diversity and interrelatedness of all life on earth (Rubinoff and Holland 2005). As we obtained a perfectly stable sciaenid topology using different algorithms and datasets, this is an indication that mitogenomes may be a suitable tool to establish a reliable phylogenetic framework for the Sciaenidae. However, as nuclear and mitogenomic data can produce different phylogenetic signals (Rubinoff and Holland 2005, Zhang et al. 2019), future studies should also test the signal from nuclear molecular data. We, therefore, urge the sequencing of additional sciaenid mt genomes, particularly those belonging to non-represented sciaenid lineages (Lo et al. 2015), to facilitate further progress in our understanding of the phylogeny and taxonomy of this fish family.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

WH and MX contributed to the experiment design, conducting experiments, interpretation of data, and writing of the manuscript; XP and GR contributed to experimental design and review and editing of the manuscript; ZB and JW contributed to data curation and formal analysis; CZ and SG contributed to conducting of experiments and methodology; HD contributed to review and editing of manuscript. All authors made substantial intellectual contributions to the work and are prepared to take accountability for it.

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