Complete mitochondrial genome of *Cultellus attenuatus* and its phylogenetic implications

Haikun Li (✉ 1157474258@qq.com)

Ocean University of China Fisheries College

Ruihai Yu

Ocean University of China Fisheries College

Peizhen Ma

Institute of Oceanology Chinese Academy of Sciences

Chunhua Li

Ocean University of China Fisheries College

Short Report

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Complete mitochondrial genome of *Cultellus attenuatus* and its phylogenetic implications

1 Haikun Li¹, Ruihai Yu¹, Peizhen Ma², Chunhua Li¹

2 ¹Key Laboratory of Mariculture, Ministry of Education, Ocean University
3 of China, 5 Yushan Road, Qingdao 266003, China

4 ²Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road,
5 Qingdao 266071, China

6 Corresponding author

7 Ruihai Yu, Key Laboratory of Mariculture, Ministry of Education, Ocean
8 University of China, Qingdao 266003, China.

9 Email: yuruihai@ouc.edu.cn

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Abstract

The complete mitochondrial genome of *Cultellus attenuates*, a new aquaculture species, was sequenced and compared with mitogenomes from seven species of Heterodonta bivalve mollusk in the gene bank. The mitochondrial genome of *C. attenuatus* is 16888bp in length and contains 36 genes, including 12 protein-coding genes, 2 ribosomal RNAs and 22 transfer RNAs, and all genes are encoded on the same strand. In comparison with *C. attenuates*, the mitochondrial genes of the *Sinonovacula constricta* from the same family were not rearranged, but those of six other species from different family were rearranged to different degrees. The largest non-coding region of *C. attenuatus* is 1173bp in length and with the A + T content of 68.24%, located between nad2 and trnK. The results of phylogenetic analysis show that the *C. attenuates* and the *S. constricta* belonging to Cultellidae cluster into one branch while two species of Solenidae (*Solen grandis* and *Solen strictus*) are clustering as their sister taxon. These data not only contribute to the understanding of the phylogenetic relationship of the Heterodonta, but also serve as a resource for the development of the genetic markers in aquaculture.

Keywords *Cultellus attenuates* · Mitochondrial gene · Phylogeny · Heterodonta

Introduction

Most animals' mitochondrial DNA is a closed circular molecule independent of the nuclear DNA, ranging in size from 14 to 17 kb. It contains 37 genes including 13 proteins, 2 ribosomal RNAs, and 22 transfer RNAs^[1]. The mitochondrial genes are quite conservative in replication, featuring matrilineal inheritance, no rearrangement and high substitution rates^[2]. Many characteristics make mitochondrial genes in value and have become a powerful source in genetic evolution, phylogeographic analysis, and species identification^[3-5].

In bivalve mollusk, *Mytilus edulis* was the first to obtain complete mitochondrial genetic information^[6], and subsequently mitochondrial genes were obtained in other categories, including oysters^[7], scallops^[8], clam^[9] and razor shells^[10]. Although mitochondrial genetic resources of bivalve are increasingly being developed, it is only limited to some species with high economic value. For niche classes, the molecular information is scarce^[11]. In the species of Cultellidae family, only the mitochondrial genes of *Sinonovacula constricta* have been determined while those of the other species including *Cultellus attenuatus* have been not, which has been very unfavorable for the phylogenetic research in this family^[12,13].

C. attenuatus mainly inhabits the shallow sea area of the subtidal zones, and can be found in Japan, South Korea, the Philippines and in China

(mainly from Liaoning in the north to Taiwan in the south)^[14]. *C. attenuatus* has high edible value due to its nutritional composition of low fat and high protein, which makes it more and more popular among Chinese consumers ^[15]. In this way, the high market value of *C. attenuatus* has makes its artificial catching gradually increase while, the quantity of wild resources ones decrease^[14]. In china, artificial breeding of *C. attenuatus* has been carried out in an attempt to restore the diminishing wild resources by introducing hatchery-produced seed. The study of mitochondrial gene can be applied to produce genetic markers that can monitor the restoration of aquatic animal stock, which is quite helpful for the restoration and conservation of wild population ^[3,16-17]. Therefore, it is necessary to obtain the mitochondrial gene sequence of *C. attenuates*. However, no relevant reports have been reported so far. In this study, we obtained and analyzed the entire mitochondrial gene of *C. attenuates*, and compared the mitochondrial gene with other family species from the Heterodonta. The data not only contribute to our understanding of the phylogenetic status, but also serve as a source for the development of useful genetic markers in aquaculture.

Materials and methods

Sample collection and DNA extraction

The lived *C. attenuatus* were collected from Bohai Bay (Dongying City, Shandong Province, China). The total genomic DNA was extracted from adductor muscle of an individual *C. attenuatus* by a DNA extraction kit.

PCR and DNA sequencing

The PCR amplification of *cox1* and *rrnL* gene was performed with the primer set of LCO1490/HCO2198 and *rrnLAR-L/rrnLAR-H*^[13], respectively. PCR was performed in 50 µl volumes containing 0.5 units Taq polymerase (Takara), 1 × PCR buffer, 0.2mM of each dNTP, 1 µM of each primer, 1.5 mM of MgCl₂ and 50 ng of genomic DNA. The PCR cycle was as follows: 94°C for 2 min, then 35 cycles of 94°C for 30s, 40 s at 52°C and 1 min at 72°C, followed by a final extension at 72°C for 5 min.

Based on the above two sequences, two sets of Long-PCR primers LB-L/LB-H and LC-L/LC-L were used for long-PCR amplification^[13].

Long-PCR was performed in 25 µL volumes containing 1 × GC buffer I, 1.25 U of LATaq (Takara), 0.5 mM of each dNTP, 0.4µM of each primer and 50 ng of genomic DNA. The PCR conditions were as follows: 94°C for 2 min, 30 cycles of 94°C for 20s, 1 min/kb at 68°C and a final extension at 72°C for 10 min. The PCR products were sequenced by Personal Biotechnology Co. Ltd. Qingdao.

Analysis of sequence data

The protein-coding and ribosomal RNA genes were identified by their similarity to published gene sequences through BLAST searches (<http://www.ncbi.nlm.nih.gov/BLAST/>). The transfer RNA were identified by tRNAscan-SE v.1.21^[18] and DOGMA^[19] using invertebrate mitochondrial genetic code. CGView was used to map the whole mitochondrial genome circle^[20].

Phylogenetic analysis

To clearly show the phylogenetic relationship of the Heterodonta, the mitochondrial gene sequences of 18 species from the Heterodonta were obtained from the gene bank. The *Chlamys farreri* and *Mimachlamys nobilis* were used as distinct outgroups. The amino acid sequences of 12 proteins (except atp8) were adopted for phylogenetic analysis. The amino acid sequences of 12 mitochondrial proteins were aligned by MEAG 7.0.^[21] The phylogenetic relationships of heterodont bivalves were reconstructed by the Neighbor-Joining (NJ), and 1000 bootstraps were used to estimate.

Results and discussion

Genome organization and nucleotide composition

The mitochondrial genome of *C. attenuates* is 16,888 bp in length and contains 12 protein-coding genes, 22 transfer RNA genes, and 2 ribosomal RNA genes (Fig. 1). Compared with the genome size of the

sequenced mollusk mtDNAs, the mitochondrial genome size is within the normal range. Besides, four overlaps were detected in mitochondrial genome, with the size of 1bp, 6bp, 1bp and 3bp respectively, among which the overlap between trnE and trnS2 was the largest (Table 1). Of the whole mitogenome of *C. attenuates*, its A+T content is 66.46% (Table 2), comparable to that of *S. constricta* (67.00%) and *Solen grandis* (64.84%). In addition, the mitochondrial gene length of *C. attenuates* is 376bp shorter than that of *S. constricta* in the same family^[22], while 104bp longer than that of *S. grandis* in the different family^[10].

Protein-coding genes and codon usage

The mitochondria of *C. attenuates* contain 12 protein-coding genes, which, range in size from 291 bp to 1761 bp and, with a total length of 11470bp, accounting for 67.92% of the total length of the genes (Table 1). There are two starting codons in these 12 protein-coding genes, ATG for 7 protein-coding genes (cox2, cox3, cob, atp6, nad2, nad3 and nad6), ATT for 3 protein-coding genes (cox1, nad4 and nad5) and ATA for 2 protein-coding genes (nad41 and nad1), which differs from the initial codon composition of the protein-coding genes in mtDNA of *S. grandis*. Except for cox2, all the other 11 protein-coding genes have complete termination codons, including TAA (N=5) and TAG (N=6). The A+T content of all the protein-coding genes exceeds 60%, indicating that the protein-coding genes prefer AT.

Of the entire mitochondrial genome, the values of AT skew and GT skew of the entire mitochondrial genome are -0.291 and 0.368, respectively, suggesting that the entire mitochondrial gene is biased towards T and G, a phenomenon also found in some other bivalves^[7-8,23]. All the 12 protein-coding genes are similar to the whole genome, with negative AT skew and positive GT skew, showing a bias towards T and G (Table 2).

Ribosomal RNAs and transfer RNAs

Similar to most bivalves, the mitochondrial genomes of *C. attenuates* contain 22 transfer RNA genes and 2 ribosomal RNA genes. The size of 22 tRNA genes varies from 63bp to 67bp, and all of them can be folded into typical secondary structures. The two ribosomal RNA genes include *rrnL* and *rrnS* — the former is 1237bp, located between *nad6* and *atp6*, and the latter is 827bp located between *trnM* and *cox3* (Table 1). The content of A+T in *rrnL* is 69.68%, which is the highest in all protein coding genes and rRNA genes. The A+T content of *rrnS* is 66.38%, which is slightly lower than the average level of the whole mitochondrial genome. The contents of AT skew and GC skew of *rrnL* are -0.063 and 0.285 respectively, which is similar to the protein coding gene and has a bias towards T and G. The contents of AT skew and GC skew of *rrnS* are 0.056 and 0.209 respectively, showing a bias towards A and G, which is different from other genes in the whole mitochondrial genes (Table 2).

Non-coding regions

The mitochondrial genome of the whole *C. attenuates* contains 24 non-coding regions, ranging in size from 2bp -1173bp, with a total length of totaling 1917bp, accounting for 11.35% of the entire mitochondrial genome. 4 of the 24 non-coding regions are larger, with the size greater than or equal to 100bp, which are respectively 103bp (trnP-nad4), 149bp (trnF-cox1), 100bp (trnM-rrnS) and 1173bp (nad2-trnK). The largest non-coding region (NCR) is 1173bp in length and with the A + T content of 68.24%, located between nad2 and trnK. Besides, in the largest NCR, a sequence (15884bp -15974bp) with low A+T content (53.85%) was found, which is considered to be the origin of L-strand replication. The remaining sequence of the largest NCR is 914 bp in length, with an AT content of 69.80%, and is identified as a putative control region. Compared with the largest NCR of the *C. attenuates*, the largest NCR of *S. constricta* in the same family is at the same position (nad2 – trnK) with slightly larger size (1492 bp) and lower A+T content (66.89%)^[22]. The largest NCR positions and sizes of *C. attenuates* varied considerably compared with other 6 species of different families (Table 3), indicating the highly rearranged gene order in bivalves.

Gene arrangement

One species was respectively selected from six families and one superfamily of the Heterodonta and its mitochondrial gene was compared with the gene arrangement of *C. attenuates* (Fig. 2). It is found that the mitochondrial genes of the *S. constricta* and *C. attenuates* are exactly in the same order. For mitochondrial genes, species in different families have a large degree of rearrangement, while species in the same family tend to have a small degree of rearrangement, and species in the same genus even have almost no rearrangement ^[8,24]. Therefore, the mitochondrial genes of the *S. constricta* and *C. attenuates* were not rearranged, further confirming the closer relationship between the two species ^[12]. The same phenomenon was also found in this comparison of mitochondrial gene arrangement. There were only three gene blocks, nad5-cob, rrnL-atp6-rrnS-cox3 and nad4l-nad4, shared between *S. grandis* and *C. attenuates*. However, the remaining five species from different families shared fewer gene blocks, indicating a greater rearrangement. This enormous gene rearrangement in bivalves is associated with single-stranded coding, because double-stranded coding tends to inhibit gene rearrangement compared to single-stranded coding ^[25].

Phylogenetic analysis

With the development of mitochondrial genome research, more and more researchers conduct phylogenetic research on species through

204 mitochondrial genes, but up to now, there has been relatively little
205 information about the mitochondrial genome of Heterodont^[10,11]. In this
206 study, the phylogenetic tree was reconstructed through the amino acid
207 sequence of mitochondrial protein gene of heterodont bivalves (Fig. 3).
208 Two species of Lucinidae clustered into a single clade, and other species
209 clustered into a large clade containing three small clades, similar to
210 previous studies^[11,26]. *Paphia euglypta*, *Venerupis philippinarum*,
211 *Meritrix meretrix* and *Meritrix petechialis* clustered together, supporting
212 their genetic relationship within Veneridae^[26]. *Moerella iridescens*,
213 *Sanguinolaria diplos*, *Sanguinolaria olivacea*, *Semele scaba* and
214 *Solecurtus divaricatus* belonging to the superfamily Tellinoidea clustered
215 together, consistent with previous studies^[22]. The new finding of this
216 study is that the *C. attenuates* had the closest relationship with *S.*
217 *constricta*, which further determines that *C. attenuates* and *S. constricta*
218 belong to the same family (Cultellidae)^[12]. *S. grandis* and *Solen strictus*
219 had a closely relationship, with the *C. attenuates* and *S. constricta* being
220 the sister taxon.

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Declarations

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Consent for publication

All authors read and approved the final manuscript.

Availability of data and material

The mitochondrial genome sequence is available from gene Banks (MW653805)

Code availability

There is no relevant code

Authors' contributions

Ruihai Yu designed and supervised the project, and reviewed drafts of the paper. Peizhen Ma provided technical assistance and collected the samples. Chunhua Li provided technical assistance. Haikun Li wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript before they read and approved the final manuscript.

Compliance with Ethical Standards

—Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

337 **—Ethics approval**

The present study was performed according to the standard operation procedures (SOPs) of the Guide for the Use of Experimental Animals of the Ocean University of China. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Ocean University of China.

338 **—Consent to participate**

339 This article does not contain any studies with human

Table 1 The organization of the mitochondrial genome of *Cultellus*

attenuates

Feature	Position	Length (bp)	Initiation codon	Stop codon	Anticodon	Intergenic nucleotide
trnK	1-66	66			TTT	64
cox2	131-968	838	ATG	T(AA)		74
trnY	1,043-1,106	64			GTA	9
nad4l	1,116-1,406	291	ATG	TAA		-1
trnG	1,406-1,472	67			TCC	8
trnP	1,481-1,546	66			TGG	103
nad4	1,650-3,020	1371	ATT	TAA		13
trnH	3,034-3,097	64			GTG	2
trnW	3,100-3,166	67			TCA	11
trnR	3,178-3,242	65			TCG	27
trnE	3,270-3,334	65			TTC	-6
trnS2	3,329-3,391	63			TGA	
nad3	3,392-3,757	366	ATG	TAG		-1
trnT	3,757-3,823	67			TGT	
trnI	3,824-3,890	67			GAT	5
trnD	3,896-3,962	67			GTC	
trnQ	3,963-4,029	67			TTG	25
trnC	4,055-4,120	66			GCA	25
trnA	4,146-4,210	65			TGC	28
trnF	4,239-4,302	64			GAA	149
cox1	4,452-6,140	1689	ATT	TAA		11
trnL2	6,152-6,218	67			TAA	-3
nad1	6,216-7,145	930	ATA	TAG		2
trnL1	7,148-7,214	67			TAG	10
trnV	7,225-7,289	65			TAC	12
trnN	7,302-7,367	66			GTT	
nad5	7,368-9,128	1761	ATT	TAG		38
cob	9,167-10,312	1146	ATG	TAG		7
nad6	10,320-10,850	531	ATG	TAG		
rrnL	10,851-12,087	1237				
atp6	12,088-12,786	699	ATG	TAA		15
trnM	12,802-12,867	66			CAT	100
rrnS	12,968-13,794	827				
cox3	13,795-14,583	789	ATG	TAG		6
trnS1	14,590-14,656	67			TCT	
nad2	14,657-15,715	1059	ATG	TAA		1173

Table 2 The base composition of mitochondrial genome, protein coding genes and ribosomal RNA genes

Region	A%	C%	G%	T%	A+T%	G+C%	AT skew	GC skew
Whole genome	23.54	10.61	22.94	42.91	66.46	33.54	-0.291	0.368
cox2	25.89	10.26	26.13	37.71	63.60	36.40	-0.186	0.436
nad4l	21.65	7.56	24.40	46.39	68.04	31.96	-0.364	0.527
nad4	18.75	8.17	24.07	49.02	67.76	32.24	-0.447	0.493
nad3	18.85	8.20	22.40	50.55	69.40	30.60	-0.457	0.464
cox1	20.25	12.97	23.92	42.87	63.11	36.89	-0.358	0.297
nad1	19.14	9.57	24.73	46.56	65.70	34.30	-0.417	0.442
nad5	18.57	10.39	23.74	47.30	65.87	34.13	-0.436	0.391
cob	20.68	12.13	21.38	45.81	66.49	33.51	-0.378	0.276
nad6	20.90	8.10	23.35	47.65	68.55	31.45	-0.390	0.485
rrnL	32.66	10.83	19.48	37.03	69.68	30.32	-0.063	0.285
atp6	20.89	10.87	21.17	47.07	67.95	32.05	-0.385	0.321
rrnS	35.07	13.30	20.31	31.32	66.38	33.62	0.056	0.209
cox3	21.04	12.67	23.70	42.59	63.62	36.38	-0.339	0.303
nad2	20.68	10.48	23.70	45.14	65.82	34.18	-0.372	0.387

Table 3 The comparison of non-coding regions (NCR) within the eight mitochondrial genomes of the Heterodonta

Species	No. of NCR	Total length (bp)	Proportion of the mt genome (%)	Largest NCR		
				Lenth (bp)	A+ T %	Location
<i>Paphia euglypta</i>	28	2431	13.04	1774	65.80	nad4L - trnI
<i>Acanthocardia tuberculata</i>	24	1751	9.60	1103	59.11	trnaM - trnaH
<i>Hiatella arctica</i>	30	2558	14.05	614	66.10	trnaA - atp8
<i>Solen grandis</i>	27	1699	10.12	435	66.44	trnaN - trnaP
<i>Sinonovacula constricta</i>	25	2134	12.39	1492	66.89	nad2 - trnK
<i>Cultellus attenuates</i>	24	1917	11.35	1173	68.24	nad2 - trnK
<i>Lucinella divaricata</i>	31	3825	20.20	1050	69.24	trnaC - trnaL2
<i>Solecurtus divaricatus</i>	22	1160	6.93	775	65.81	rrnS - trnM

Figure legend

Fig.1 The complete genome circle of mitochondria in *Cultellus attenuates*.

From inside to outside, the first circle represents the scale; the second circle represents the GC skew; the third circle represents GC content; the fourth and fifth circle represent the arrangement of protein-coding genes, tRNA genes and rRNA genes on the genome

Fig.2 The linear representation of the mitochondrial gene arrangement in eight species of the Heterodonta. The bars indicate identical gene blocks

Fig.3 The phylogenetic trees derived from Neighbour-Joining (NJ) of concatenated amino acid sequences of 12 protein-coding genes

340

341

342

Figures

Figure 1

The complete genome circle of mitochondria in *Cultellus attenuatus*. From inside to outside, the first circle represents the scale; the second circle represents the GC skew; the third circle represents GC content; the fourth and fifth circle represent the arrangement of protein-coding genes, tRNA genes and rRNA genes on the genome

Figure 2

The linear representation of the mitochondrial gene arrangement in eight species of the heterodonta. The bars indicate identical gene blocks

Figure 3

The phylogenetic trees derived from Neighbour-Joining (NJ) of concatenated amino acid sequences of 12 protein-coding genes