Complete mitochondrial genome of Cultellus attenuatus and its phylogenetic implications

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Short Report

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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 GenBank. The mitochondrial genome of *C. attenuatus* is 16888 bp 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 *Sinonovacula constricta* from the same family were not rearranged, but those of six other species from different families were rearranged to different degrees. The largest noncoding region of *C. attenuatus* is 1173 bp in length and has an A + T content of 68.24%, located between nad2 and trnK. The results of phylogenetic analysis show that *C. attenuates* and *S. constricta* belonging to Cultellidae cluster into one branch while two species of Solenidae (*Solen grandis* and *Solen strictus*) cluster as their sister taxa. In conclusion, we used the mitochondrial genome data to demonstrate the closest relationship between *C. attenuatus* and *S. constricta* in Heterodonta. These data not only contribute to the understanding of the phylogenetic relationship of Heterodonta but also serve as a resource for the development of genetic markers in aquaculture.

Introduction

Most animals' mitochondrial DNA is a closed circular molecule independent of 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 conserved in replication, featuring matrilineal inheritance, no rearrangement and high substitution rates [2]. Many characteristics make mitochondrial genes valuable and have become a powerful source in genetic evolution, phylogeographic analysis, and species identification [3–5].

In bivalve mollusks, *Mytilus edulis* was the first to obtain complete mitochondrial genetic information [6], and mitochondrial genes were subsequently obtained in other categories, including oysters [7], scallops [8], clams [9] and razor shells [10]. Although mitochondrial genetic resources of bivalves are increasingly being developed, they are limited to only some species with high economic value. For niche classes, molecular information is scarce [11]. In the species of the Cultellidae family, only the mitochondrial genes of *Sinonovacula constricta* have been determined, while those of the other species, including *Cultellus attenuates*, have not been determined, which has been very unfavorable for 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 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 increasingly popular among Chinese consumers [15]. In this way, the high market value of *C. attenuatus* gradually increases its artificial catching, while the quantity of wild resources decreases [14]. In

China, artificial breeding of *C. attenuatus* has been carried out in an attempt to restore diminishing wild resources by introducing hatchery-produced seeds. The study of mitochondrial genes can be applied to produce genetic markers that can monitor the restoration of aquatic animal stocks, which is quite helpful for the restoration and conservation of wild populations [3,16–17]. Therefore, it is necessary to obtain the mitochondrial gene sequence of *C. attenuates*. However, no relevant reports have been reported thus 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 Heterodonta. The data not only contribute to our understanding of 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

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

PCR and DNA sequencing

PCR amplification of the cox1 and rrnL genes was performed with the primer sets 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, 0.2 mM of each dNTP, 1 μM of each primer, 1.5 mM of $MgCl_2$ and 50 ng of genomic DNA. The PCR cycle was as follows: 94 ºC for 2 min, then 35 cycles of 94 ºC for 30 s, 40 s at 52 ºC and 1 min at 72 ºC, followed by a final extension at 72 ºC 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, 0.4 μM of each primer and 50 ng of genomic DNA. The PCR conditions were as follows: 94 ºC for 2 min, 30 cycles of 94 ºC for 20 s, 1 min/kb at 68 ºC and a final extension at 72 ºC 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/). Transfer RNA was identified by tRNAscan-SE v.1.21 [18] and DOGMA [19] using the invertebrate mitochondrial genetic code. CGView was used to map the whole mitochondrial genome circle [20].

Phylogenetic analysis

To clearly show the phylogenetic relationship of Heterodonta, the mitochondrial gene sequences of 18 species from Heterodonta were obtained from Genbank. *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 neighbor-joining (NJ), and 1000 bootstraps were used for estimation.

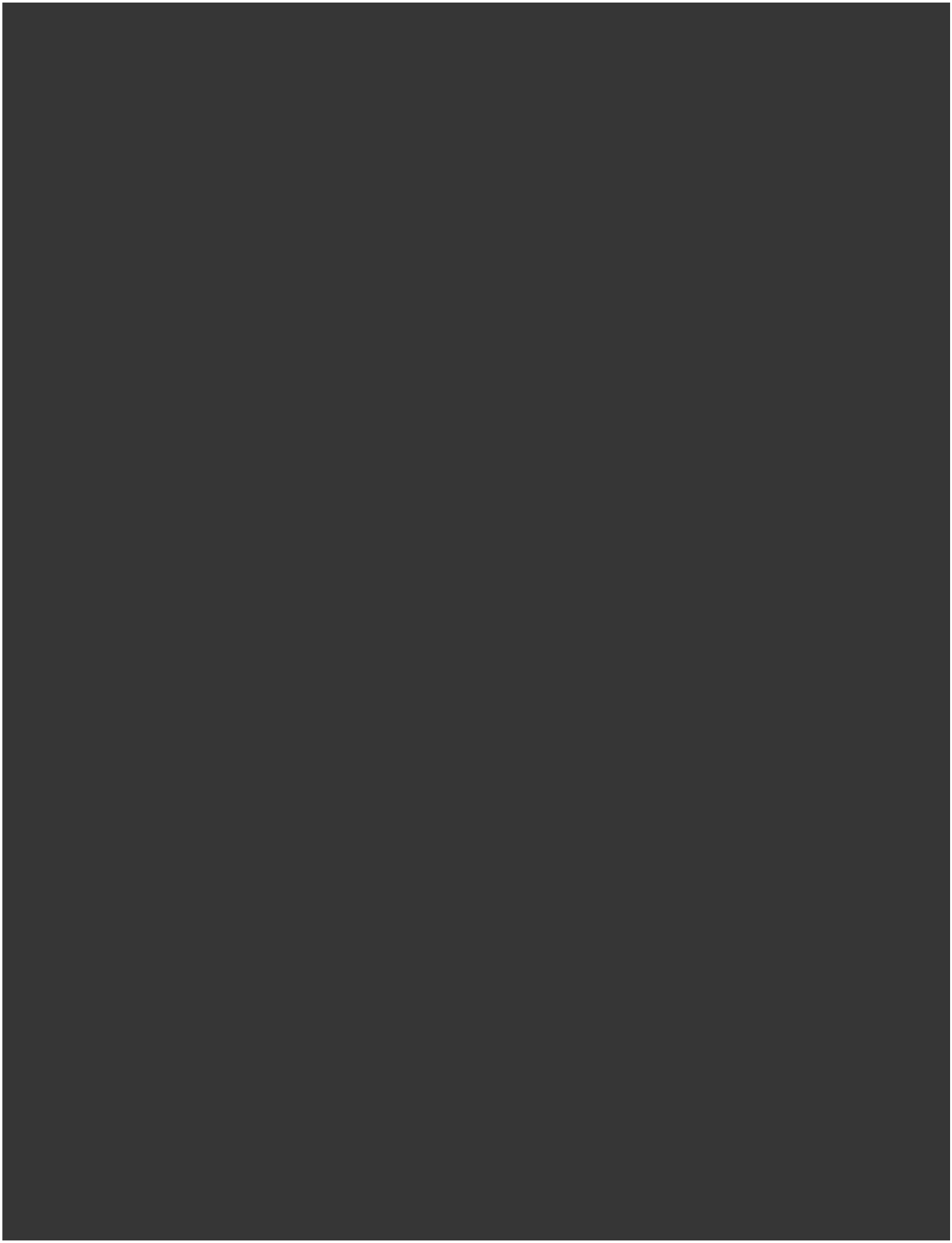
Results And Discussion

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 was within the normal range. In addition, four overlaps were detected in the mitochondrial genome, with sizes of 1 bp, 6 bp, 1 bp and 3 bp 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 was 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 376 bp shorter than that of *S. constricta* in the same family [22] and 104 bp longer than that of *S. grandis* in different families [10].

Table 1
Organization of the mitochondrial genome of *Cultellus attenuates*

Feature	Position	Length (bp)	Initiation condon	Stop condon	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	



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 63 bp to 67 bp, and all of them can be folded into typical secondary structures. The two ribosomal RNA genes include rrnL and rrnS — the former is 1237 bp, located between nad6 and atp6, and the latter is 827 bp located between trnM and cox3 (Table 1). The content of A + T in rrnL was 69.68%, which was the highest of all protein-coding genes and rRNA genes. The A + T content of rrnS was 66.38%, which was 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 toward T and G. The contents of AT skew and GC skew of rrnS are 0.056 and 0.209, respectively, showing a bias toward A and G, which is different from other genes in the whole mitochondrial genes (Table 2).

Noncoding regions

The mitochondrial genome of the whole *C. attenuates* contains 24 noncoding regions, ranging in size from 2 bp -1173 bp, with a total length of totaling 1917 bp, accounting for 11.35% of the entire mitochondrial genome. Four of the 24 noncoding regions were larger, with sizes greater than or equal to 100 bp, which were 103 bp (trnP-nad4), 149 bp (trnF-cox1), 100 bp (trnM-rrnS) and 1173 bp (nad2-trnK). The largest noncoding region (NCR) is 1173 bp in length and has an A + T content of 68.24%, located between nad2 and trnK. In addition, in the largest NCR, a sequence (15884 bp -15974 bp) 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 a 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 the other 6 species of different families (Table 3), indicating the highly rearranged gene order in bivalves.

Table 3
The comparison of noncoding regions (NCRs) within the eight mitochondrial genomes of Heterodonta

Species	No. of NCR	Total length (bp)	Proportion of the mt genome (%)	Largest NCR		
				Lenth (bp)	A + T %	Location
Paphia euglypta	28	2431	13.04	1774	65.80	nad4 l - trnI
Acanthocardia tuberculata	24	1751	9.60	1103	59.11	trnaM - trnaH
Hiatella arctica	30	2558	14.05	614	66.10	trnaA - atp8
Solen grandis	27	1699	10.12	435	66.44	trnaN - trnaP
Sinonovacula constricta	25	2134	12.39	1492	66.89	nad2 - trnK
Cultellus attenuates	24	1917	11.35	1173	68.24	nad2 - trnK
Lucinella divaricata	31	3825	20.20	1050	69.24	trnaC - trnaL2
Solecurtus divaricatus	22	1160	6.93	775	65.81	rrnS - trnM

Gene arrangement

One species was selected from six families and one superfamily of Heterodonta, and its mitochondrial gene was compared with the gene arrangement of *C. attenuates* (Fig. 2). The mitochondrial genes of *S. constricta* and *C. attenuates* were 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 *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 nad4 l-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, an increasingly number of researchers have conducted phylogenetic research on species through mitochondrial genes, but to date, there has been relatively little information about the mitochondrial genome of heterodonts [10, 11]. In this study, the phylogenetic tree was reconstructed through the amino acid sequence of the mitochondrial protein gene of heterodont bivalves (Fig. 3). Two species of Lucinidae clustered into a single clade, and other species clustered into a large clade containing three small clades, similar to previous studies [11, 26]. *Paphia euglypta*, *Venerupis philippinarum*, *Meritrix meretrix* and *Meritrix petechialis* clustered together, supporting their genetic relationship within Veneridae [26]. *Moerella iridescens*, *Sanguinolaria diphos*, *Sanguinolaria olivacea*, *Semele scaba* and *Solecurtus divaricatus* belonging to the superfamily Tellinoidea clustered together, consistent with previous studies [22]. The new finding of this study is that *C. attenuates* had the closest relationship with *S. constricta*, which further determines that *C. attenuates* and *S. constricta* belong to the same family (Cultellidae) [12]. *S. grandis* and *Solen strictus* had a close relationship, with *C. attenuates* and *S. constricta* being the sister taxa.

Declarations

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Consent for publication

All authors have read and approved the final manuscript.

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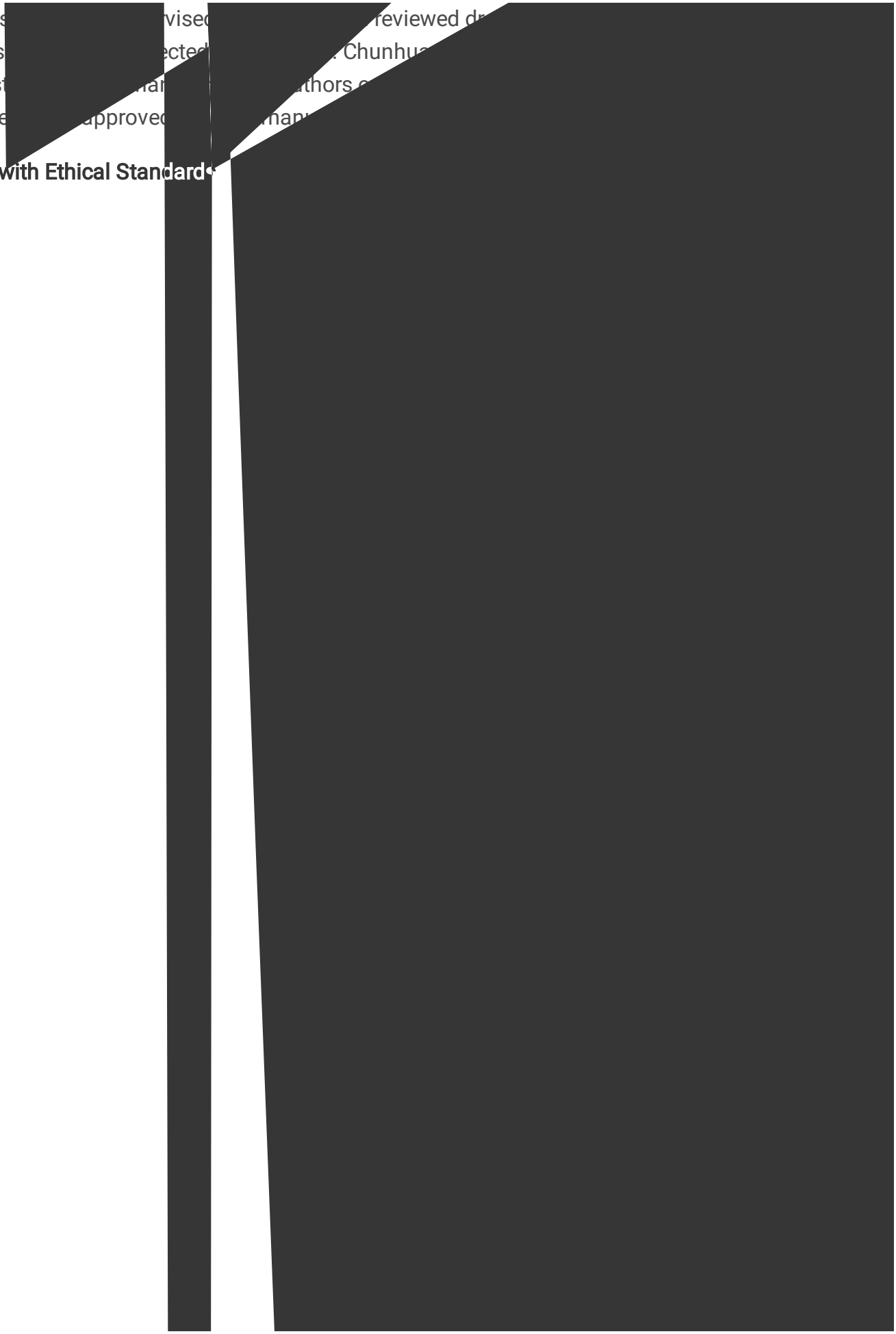

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Figures

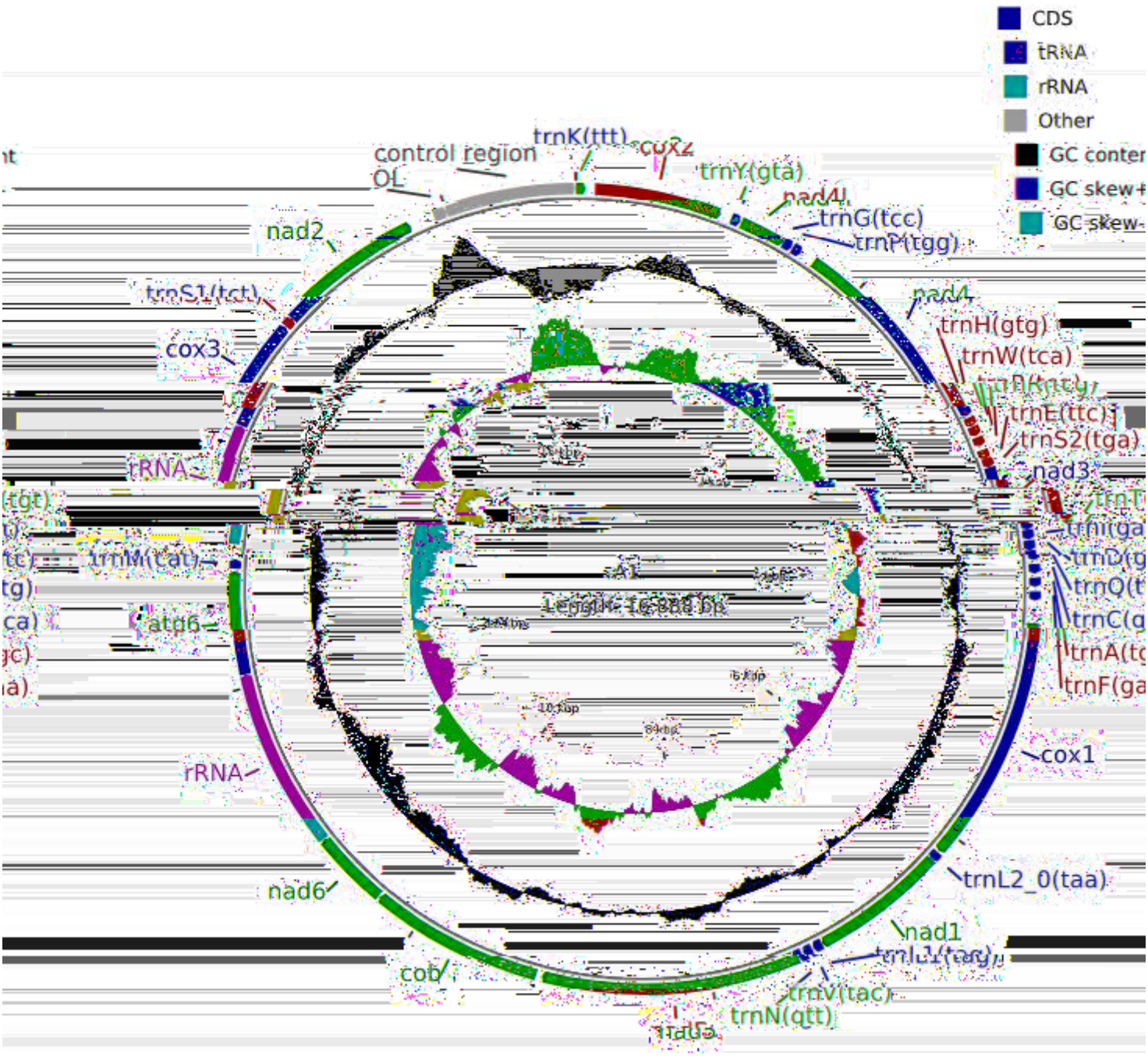

Figure 1

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; and the fourth and fifth circles represent the arrangement of protein-coding genes, tRNA genes and rRNA genes on the genome

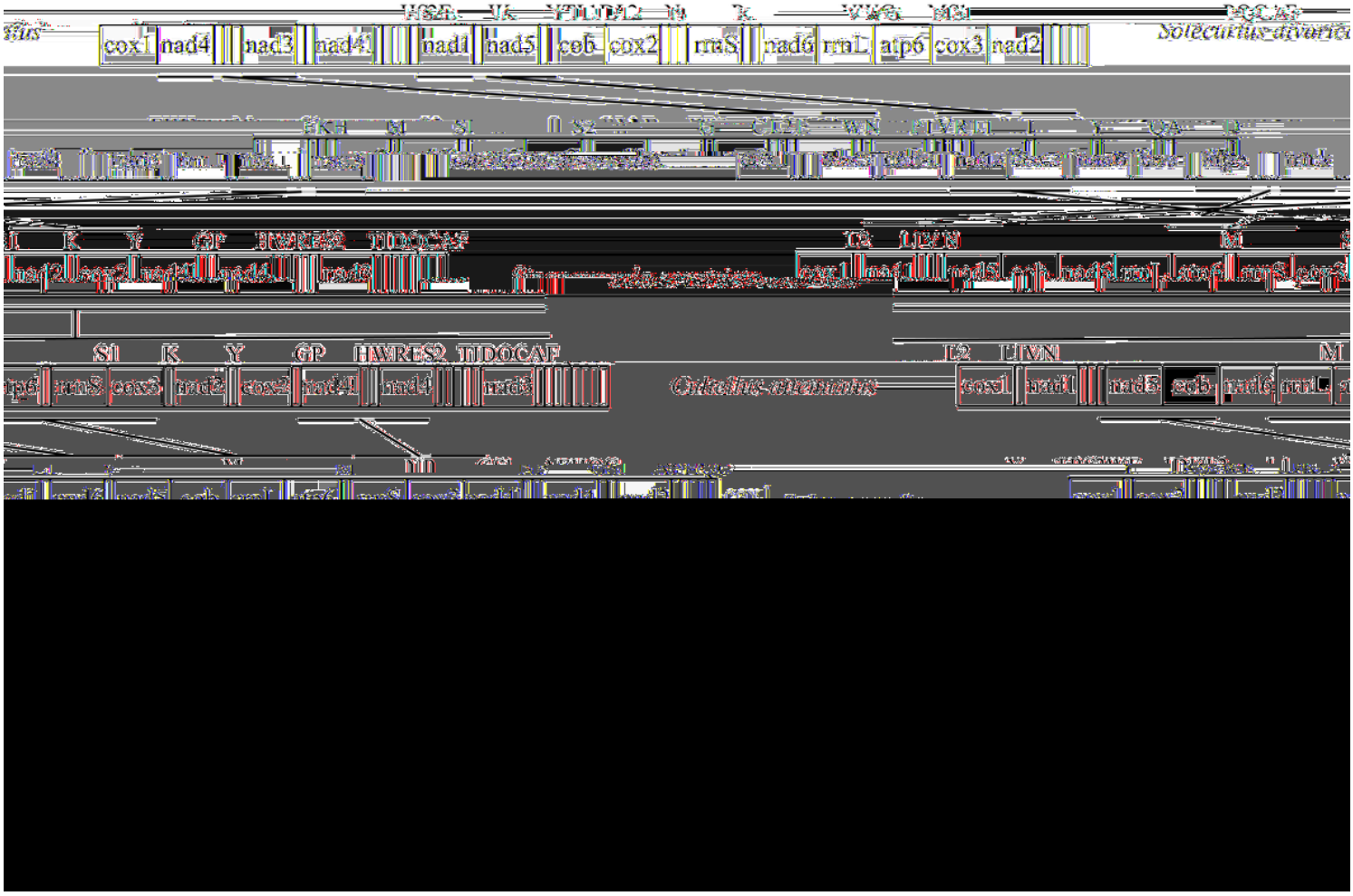

Figure 2

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

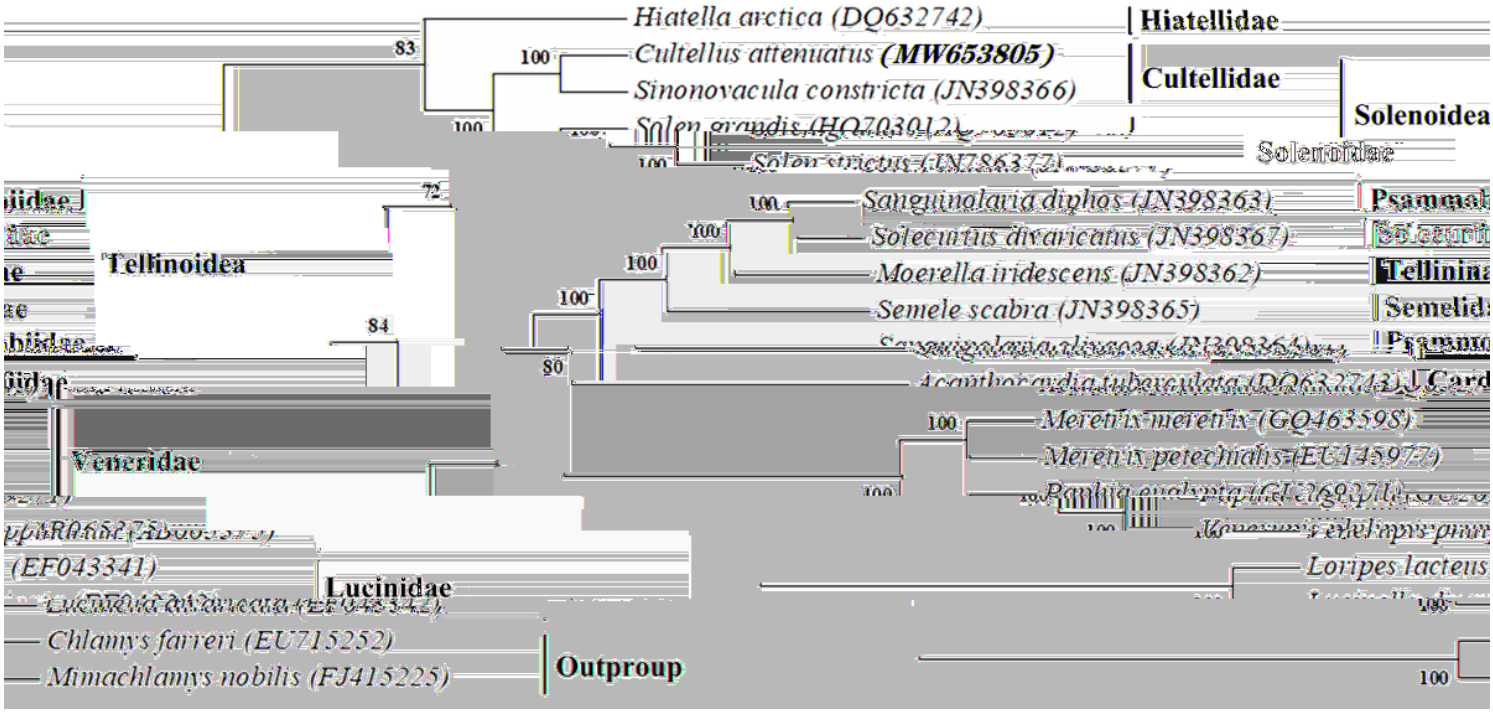

Figure 3

Phylogenetic trees derived from neighbour-joining (NJ) of concatenated amino acid sequences of 12 protein-coding genes