Characteristics of Complete Mitochondrial Genomes Reveals a Potential Division of Genus *Modiolus* into Three Genera

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Research Article

Keywords: *Modiolus modulaides*, mitogenome, Mytilidae, phylogeny

Posted Date: January 31st, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1287793/v1>

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Abstract

Backgrounds Mussels have been one of the most abundant and highly evolved marine biological groups with high level of genetic differentiation between and within species. The yellow-banded horse mussel, *Modiolus modulaides* (Röding, 1798), is an important economic species in Philippines and an endangered species in Japan, with no whole mitogenome characterized.

Methods and Results In this study, we sequenced the whole mitochondrial genome of *Modiolus modulaides* (Röding, 1798), a medium-sized mussel collected from Guangxi, China. Result shows a 15,422 bp closed-circular DNA molecule, including 12 protein-coding genes, 22 transfer RNA genes and two ribosomal RNA genes. The genome composition is obviously A+T biased (62.5%), with 23.1% A, 39.4% T, 25.4% G and 12.0% C, making AT-skew and GC-skew being -0.26 and 0.36, respectively. Phylogenetic analysis of *Modiolus modulaides* and other 36 mussel species based on 12 protein-coding genes and two ribosomal RNAs of mitogenomes shows that *Modiolus modulaides* is most closely related with *Modiolus philippinum* (Hanley, 1843).

Conclusions A hypothesis that the current genus *Modiolus* should be separated three distinctive genera was raised based on morphological and phylogenetic characteristics, as well as gene patterns of mitogenomes. These results put forward new insight of phylogenetic relationship of *Modiolus* species and will help to further study the taxonomy and phylogeny of Mytilidae.

Introduction

Mitochondrial DNA (mtDNA) exhibits special characteristics that differ from the nuclear genome, such as rapid evolution, maternal inheritance, and lack of recombination [1], and has been an efficient tool and widely used in molluscan evolutionary and phylogenetic analyses [2, 3]. Molluscan mtDNA is nearly a closed circular molecule, always containing 13 protein-coding genes (NADH dehydrogenase subunits 1–6 and 4L, ATPase subunits six and eight, cytochrome oxidase subunits 1-3, and cytochrome b), 22 transfer RNA genes and two ribosomal RNAs (rrnS and rrnL) [4, 5], with missing ATPase subunits eight, variable tRNAs and extra rrnS in some species [6–8]. Different mtDNA composition would result in functional diversity, as mtDNA carries genetic information essential to mitochondrial function [9], thus it's interesting to explore the difference of mtDNA related to adaptive evolution. Nonetheless, the gene organization and arrangement of mitochondrial protein-coding genes in closely related species are relatively conservative [10] and can be used to analyze phylogenetic relationships between and above genera [11]. Though development of sequencing technology and the establishment of mitochondrial genome database provides convenience and comprehensive information for molluscan phylogeny studies [12], quantity of current mitogenomes seems far from adequate in comparison with enormous amounts of Mollusca species.

As one of the most abundant and highly evolved groups in Mollusca, mussels (Bivalvia, Pteriomorphia, Mytilida) exhibit a highly variable morphological characteristics and adapt to multiple habitats from fresh

water, intertidal zone, to deep sea, resulting in diversity of mitogenomes [13, 14]. To date, mitochondrial genomes of only 42 mussel species belonging to 18 genera of 9 subfamilies are available from NCBI. The yellow-banded horse mussel, *Modiolus modulaides* (Röding, 1798), known as an important economic species in Philippines [15] and an endangered species in Japan [16], is widely distributed in tropical, subtropical and temperate regions of Indo-West Pacific [17]. However, synonymic as *Musculus modulaides* (Röding, 1798), *Modiolus metcalfei* (Hanley, 1843), *Modiola triangulum* (Koch in Philippi, 1847), etc., taxonomic and phylogenetic status of this species remains in dispute. Lack of whole mitogenome of *Modiolus modulaides* limits further studies in taxonomy, utilization and protection.

Only five complete mtDNAs of the *Modiolus* species have been previously characterized. We here sequenced the whole mitochondrial genome of *Modiolus modulaides*, aiming to: (i) show the mitogenomic organization of *Modiolus modulaides*; (ii) compare the mitogenomic characteristics of *Modiolus* species; and (iii) analyze the phylogenetic relationship of Mytilidae species based on mitogenomic characteristics.

Materials And Methods

Sample collection, DNA extraction and Sequencing

The specimen of *Modiolus modulaides* was collected from Guangxi Zhuang Autonomous Region, China (108°4'25.02"N, 21°32'1.46"E) in May 2010, stored in 95% alcohol, and deposited in the Marine Biological Museum, Chinese Academy of Sciences with the Specimen code IOCAS_museum_4-02-1450). The specimen was identified according to morphological characteristics. Total genomic DNA was extracted from the adductor muscle using TIANamp Marine Animals DNA kit (DP324-03, TIANGEN BIOTECH Co., Ltd, Beijing, China) following the manufacturer's protocols. Genomic DNA adopted the Whole Genome Shotgun strategy to construct the library, and used the Next Generation Sequencing to sequence based on Illumina NovaSeq sequencing platform (Qingdao Insight Exbio Technology co., Ltd, Qingdao, China).

Sequence assembly, annotation and analyses

A5-miseq v20150522 and SPAdesv3.9.0 were used for De Novo assembly of high-quality data. The complete mitochondrial genome sequence was uploaded to the MITOS2 web server (<http://mitos2.bioinf.uni-leipzig.de/index.py>) for functional annotation. The genetic code selection was set to 05, and the other settings were set according to the default parameters. The boundaries of protein-coding genes (PCGs) were determined by ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder>). The transfer RNA genes (tRNA) were identified using tRNAscan-SE with the default search mode and the invertebrate mitochondrial genetic code source, and the two ribosomal RNA genes (rRNA) were identified by sequence comparison with previously reported *Modiolus* rRNA genes. The mitochondrial genome circle map was drawn by CGView Server.

Nucleotide base composition was calculated using MEGA 7.0. The following formula were used to calculate AT-skew and GC-skew: $AT-skew = (A - T) / (A + T)$ and $GC-skew = (G - C) / (G + C)$, in which A, T, G

and C represented the frequencies of the four bases. Codon usage for each PCG was estimated using the invertebrate mitochondrial code in the web server Codon Usage (http://www.bioinformatics.org/sms2/codon_usage.html). Values of nonsynonymous substitutions per nonsynonymous site to the number of synonymous substitutions per synonymous site by pairwise comparison between *Modiolus modulaides* and other species of *Modiolus* were calculated by DnaSP v5.

Phylogenetic analysis

The complete mitogenome of *Modiolus modulaides* and other 46 Mytilidae mitochondrial genome sequences available from GenBank (Table 1) were included to estimate phylogenetic relationships, with *Crassostrea gigas* (Ostreidae) and *Anadara sativa* (Arcidae) being outgroups. The nucleotide sequences of 12 PCGs and two rRNA genes of all mitogenomes were aligned using MAFFT v7.313. The nucleotide sequences of 12 PCGs was then imported into MACSE v2.03 for optimization. Gblocks v0.91b was used with default settings to obtain conserved regions of the aligned sequences. The best-fit substitution model for the data set were detected using ModelFinder. Phylogenetic relationships were reported using maximum likelihood (ML) with IQ-TREE v3.2.6 and Bayesian inference (BI) by MrBayes v3.2.6. The ML tree consisted of 5,000 bootstrap replicates and automatic algorithm. The BI analyses were performed using 4 parallel Markov chain Monte Carlo (MCMC) chains for 2,000,000 generations, sampling every 100 generations and discarding the first 25% generations as burn-in. The phylogenetic trees were viewed and edited by iTOL (<https://itol.embl.de/>).

Results

Mitochondrial genome characterization

The mtDNA of *Modiolus modulaies* is a closed circular DNA molecule with a length of 15,422 bp (GenBank: OL853493). It contains 36 genes, including 12 PCGs, 2 rRNA genes and 22 tRNA genes (Table 2). With the exception of trnT, all of these genes are encoded on the heavy strand (H strand). The nucleotide formation of the complete mitogenome is equal to 23.1% A, 39.4% T, 25.4% G and 12.0% C. The AT content and GC content are 62.5% and 37.4%, respectively. A significant AT bias was detected. AT-skew of mitochondrial genome of *Modiolus modulaies* is negative and GC-skew is positive, yielding -0.26 and 0.36, respectively. There are five base overlaps between adjacent genes, ranging in size from 1 bp to 8 bp, summing to a full 16 bp gene overlap. And the longest base overlap locates between rrnL and trnS2, whereas the smallest one is between cob and nad4l. Additionally, the mtDNA of *Modiolus modulaides* contains 1209 bp intergenic spacer regions at 27 locations ranging from 1 bp to 478 bp. The longest intergenic spacer lies between trnI and nad2.

PCGs and codon usage

The overall length of the PCGs is 10,873 bp, and it occupies 74.9% of the whole mitochondrial genome of *Modiolus modulaides*. The base composition of PCGs was 21.3% A, 40.52% T, 25.75% G and 12.43% C,

yielding an AT content of 61.82%. The 12 PCGs are NADH dehydrogenase subunit 1–6 and 4L (nad1–6 and nad4l), cytochrome c oxidase subunit I–III (cox 1–3), cytochrome b (Cytb) and one ATPase subunits six (atp6), which are all encoded on heavy strand. The size of 12 PCGs ranged from 276 bp (nad4l) to 1695 bp (nad5). Seven PCGs start with ATG, except for gene nad6 (TTG), nad4 and cox3 (ATT) and atp6 (GTG), while six PCGs (cox1, nad3, nad6, cob, nad5, cox2) stop with TAA and five PCGs (atp6, nad4, nad2, nad4l, nad1) stop with TAG. The cox3 of *Modiolus modulaides* has incomplete stop codon, which is T for TAA. Each amino acid is encoded by at least two codons in the PCGs of *Modiolus modulaides*. The most frequently used codons are TTT (Phe, N = 339 times used, 6.598%). CGC (Arg, N = 10 times used, 0.195%) and ACC (Thr, N = 10 times used, 0.195%) are the least frequently used codons. The most frequent amino acids in the PCGs of *Modiolus modulaides* is Leucine and the least used amino acid is Histidine, which is consistent with other five *Modiolus* species. The predominant amino acids (with frequency above 6%) are Leucine, Serine, Valine, Phenylalanine (Fig. 1a). The amino acid codons ended with A/T are more frequent than those terminated with G/C, reflecting that a high A+T content occurred. According to the ratio of nonsynonymous (Ka) to synonymous (Ks) substitutions, we confirmed that average values of all the protein-coding genes in the mitogenomes of *Modiolus* species are less than 1, indicating purification selection pressures, with only Ka/Ks values of atp6 and nad6 are higher than 0.25 (Fig. 1b).

Ribosomal RNAs and transfer RNAs

The rrnS and rrnL of *Modiolus modulaides* are 771 bp and 1144 bp, respectively. The rrnL genes of *Modiolus* species are uniformly arranged between trnF and trnS, but the rrnS genes are arranged between different genes (Fig. 1c). The rrnS of both *Modiolus modulaides* and *Modiolus philipinarum* is located between trnS and trnM, while between trnK and atp6 in *Modiolus nipponicus*, and between trnK and trnQ in other three species (Fig. 1c). The rrnS and rrnL exhibit a AT-skew -0.087 and -0.088, respectively. The mitogenome of *Modiolus modulaides* has 22 tRNAs of 1441 bp, ranging from 60 bp (trnS2) to 70 bp (trnI and trnQ) (Table 2), of which trnL and trnS have one replication. The number of tRNAs in *Modiolus modulaides* is exactly identical to other *Modiolus* species except *Modiolus comptus* since it has two copies of trnQ. All tRNAs have a typical clover structure and are encoded in H-chain, except that trnS (TGA) lacks the entire dihydrouridine (DHU) arm and trnT is encoded in L-chain. Difference of quantity and arrangement of tRNA occurs clockwise between nad1 and nad4 within *Modiolus*, along with the extra atp8 in *Modiolus modiolus* (Fig. 1c).

Phylogenetic analysis

The topological structure of ML and BI phylogenetic tree constructed by 14 mt-genes (12 PCGs and 2 rRNAs) is almost consistent, except for the position of *Bathymodiolus japonicus* and *Bathymodiolus childressi*, with most branches being highly supported, i.e., bootstrap support=100, posterior probability=1 (Fig. 2). Our result of the phylogenetic trees support that all Mytilidae species are divided into two clades, of which clade1 includes the subfamilies of Mytilinae, Musculinae, Arcuatulinae, Septiferinae, Brachidontinae, and Mytiliseptinae and clade 2 includes the subfamilies of Bathymodiolinae, Modiolinae and Arcuatulinae (*Limnoperna fortunei*). However, the phylogenetic relationships are not in accordance with the existing subfamily-level classification that several species that were commonly supposed to

belong to Mytilinae actually should occupy different positions. Genera *Perna* and *Mytella* are closely related with *Arcuatula*, and genera both *Semimytilus* and *Perna perna* are sister to genera from subfamily Brachidontinae. *Limnoperna fortunei* is more closely related with Modiolinae and Bathymodiolinae species than other Arcuatulinae species.

Within genus *Modiolus*, the phylogenetic trees shows that three groupings are highly supported and can be easily recognized. *Modiolus modulaides* is the most closely related with *Modiolus philippinarum*, making up to grouping 1, which is then gathers with grouping 2 (*Modiolus comptus* + *Modiolus nipponicus*). Grouping 3 (*Modiolus kurilensis* + *Modiolus modiolus*) has further genetic distance with the other two groupings.

Discussion

Characteristics of mitogenomes of Mytilidae

The constitution of protein-coding genes of *Modiolus modulaies* is in accordance with most known Mytilidae mitogenome, except for additional gene *atp8* in *Mytilus*, *Bathymodiolus*, *Brachidontes exustus* [18, 19], and even *Modiolus modiolus* from the same genus [20]. Song et al. [21] hypothesized that the absence of gene *atp8* resulted from the adaptive evolution of environment, but it was detected in both fresh water and seawater bivalves. An anthropogenic factor might be the false annotation of the protein-coding gene due to the structural properties (such as short length) and extreme variability of *atp8* among bivalves [22]. Boore & Brown [23] proposed that gene rearrangements are infrequent and generally remain unchanged during many years of evolutionlong periods of evolutionary time, thus retaining the signal of ancient common ancestor. Nonetheless, the constitution and arrangement of tRNAs is more variable within *Modiolus*, which presents questions about the previous taxonomy of *Modiolus*. Here, based on the considerable translocation of tRNA, we think new genus and subgenus should be raised from the previous *Modiolus*. In addition, we find the replication and translocation of tRNA genes occurs clockwise between *nad1* and *nad4* within *Modiolus*, which makes it a highly active region. Duplication of tRNA is common in mollusks, for example, *Mytilus edulis*, *Muilus galloprovincialis* and *Mutilus trossulus* have a second *trnM* [24, 25], and *Mutilus trossulus* has an additional copy of *trnQ* [25]. The secondary structure of *trnS2* lacks a DHU arm, forming a simple loop, which is conserved across metazoans [26]. The missing of the DHU arm in *trnS2* can be functional in the structural compensation mechanism between other arms [27]. There are five base overlaps between adjacent genes in the mitogenome of *Modiolus modulaides*, possibly promoting the miniaturization of mitochondrial genome in response to the natural selection [28].

The mitogenome of *Modiolus modulaies* exhibits an evident AT bias, identical to all known Mytilidae species [29, 30]. This is also reflected in the differential codon usage patterns and amino acid compositions. The initiation codon ATG is a typical start codon in metazoan [31] as well as in *Modiolus*

modulaides. The incomplete stop codon of *cox3* here in *Modiolus modulaides* is presumed to be completed by post-translational polyadenylation [32]. We found that the frequency of amino acid usage is conserved in *Modiolus* species, strongly correlates with GC content and perhaps, expression level of genes [33]. As mitochondria are energy producing organelles that play a central role in amino acid homeostasis [34], the homogeneous amino acid usage may be a sign of congener similarities in environmental suitability. The Ka/Ks (nonsynonymous/synonymous substitution rates) ratio was commonly used to estimate the effect of selection pressure on the diversity molecular evolution of related species [35]. Result indicates that gene *atp6* of *Modiolus modulaides* is under the least purification selection pressure and suffered a rapid, recent evolution [36]. It can provide valuable information into various adaptive mechanisms [37]. Gene *atp6* has the highest Ka/Ks value and receives the least purification selection. Meanwhile, continuous purification selection of *cox1* genes means the genes are strongly constrained by evolution to maintain their functional stability and thus often used in species identification [16].

Phylogenetic relationships in Mytilidae

The recognition of subfamilies in the family Mytilidae or genera in the subfamily remains inconsistent in different taxonomic systems [11]. The two-clades model of subfamilies in Mytilidae based on mitochondrial characters was also proposed by Lee et al. [3], though detailed division was different from our study. The phylogenetic result in this study strongly recommends that genus *Perna* (including *Perna canaliculus* and *Perna viridis*) should be rearranged into subfamily Arcuatulinae, as it is closest related with *Mytella strigata* from Arcuatulinae, which can be supported by the similar shell morphology as well [38, 39]. Additionally, the sister relationship between *Perna* and *Arcuatula* species were proposed by recent phylogenetic studies based on not only anatomical but also molecular features [3, 11, 14]. The genus *Semimytilus* under subfamily Mytilinae was described for the first time by Soot-Ryen [40] to include *Mytilus algosus* and only this one *Semimytilus* species (synonymised as *Semimytilus patagonicus*) has been identified till now [41]. And no one doubted the subfamily status of this species before. Here in our study and research of Lubosny et al. [42], *Semimytilus* is clustered with *Perumytilus* from subfamily Brachidontinae and then sister to *Mytilisepta* from subfamily Mytiliseptinae. Therefore, the genus *Semimytilus* is more likely to belong to subfamily Brachidontinae than Mytilinae based on the phylogenetic relationship in this study, though more morphological, anatomical data and molecular evidence should be considered. Subfamily Mytiliseptinae was firstly proposed by Morton et al. [43] to contain *Mytilisepta* species. But we insist that *Mytilisepta* should be retained within the *Brachidontes* as *Mytilisepta keenae* is placed among representatives of the Brachidontinae. As for genus *Perna*, Lee. et al. [3] argued the same manifestation in this study that *Perna perna* was not clustered with two congeneric species, *Perna canaliculus* and *Perna viridis*, but with *Brachidontes* species from the subfamily Brachidontinae instead, with no unanimous conclusion. We agree with a polyphyletic model for *Perna* genus and that the current taxonomy of this genus may be questionable [44].

Within clade 2, Modiolinae is clustered with Bathymodiolinae, implying their close evolutionary relationships, and then sister to a freshwater mussel *Limnoperna fortunei*, which agrees with previous

studies [11, 45], but contradicts phylogenetic models based on 18S and anatomical characteristics [14, 46]. The gene arrangements also shows that the Modiolinae and Bathymodiolinae are closely related [3]. *Modiolus* is a large and diverse genus and no recent review is available [47]. As previous studies proposed, subfamily Modiolinae was polyphyletic [11, 46]. Interestingly, our phylogenetic tree shows more complicated relationships among three *Modiolus* species that three groupings could be easily recognized. Although the same protein-coding gene pattern is shared by all six species, the differences in tRNA arrangement cannot be neglected, as gene order is rather highly conserved at lower taxonomic levels in Bivalvia [1]. Consequently, based on the phylogenetic relationships and corresponding to the considerable translocation of tRNA, we harbor the idea that the current genus *Modiolus* should be separated into three genera, including species of (*Modiolus modulaides* + *Modiolus philippinarum*), (*Modiolus comptus* + *Modiolus nipponicus*) and (*Modiolus kurilensis* + *Modiolus modiolus*), respectively. Morphological characteristics summarized by Wang [48] provided evidences for this hypothesis. Both *Modiolus comptus* and *Modiolus nipponicus* are small or medium sized, while the other four species are bigger, with adult shells up to 80 mm or more. The dorsal horn of *Modiolus modulaides* and *Modiolus kurilensis* is obvious and high, and the hair is very thin, making them easily confused. Besides, both *Modiolus philippinarum* and *Modiolus modiolus* have low dorsal angle and thick hair. The common features within groupings are easily observed and concluded, supporting the three genera hypothesis.

Declarations

Funding

This work was supported by the National Key R&D Program of China (2019YFD0902100, 2019YFD0900802, 2019YFD0901303); the Biological Resources Programme, Chinese Academy of Sciences (KFJ-BRP-017-40); and the National Natural Science Foundation of China (Nos. 42006080, 41906083).

Author contributions

Hou-Mei Li and Pei-Zhen Ma conducted the investigation, data curation, formal analysis and original draft. Yu-Meng Liu helped carrying out the data analysis. Cui Li, Hai-Yan Wang and Cheng-Hua Li reviewed and edited the manuscript. Zhen Zhang was in charge of the methodology and funding provision. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval

Not applicable

Consent to participate

Not applicable

Consent to publish

Not applicable

References

1. Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Res* 27(8):1767–1780. <https://doi.org/10.1093/nar/27.8.1767>
2. Ozawa G, Shimamura S, Takaki Y et al (2017) Updated mitochondrial phylogeny of Pteriomorph and Heterodont Bivalvia, including deep-sea chemosymbiotic *Bathymodiolus* mussels, vesicomysid clams and the thyasirid clam *Conchocele* cf. *bisecta*. *Mar Genomics* 31,: 43-52. <https://doi.org/10.1016/j.margen.2016.09.003>
3. Lee Y, Kwak H, Shin J et al (2019) A mitochondrial genome phylogeny of Mytilidae (Bivalvia: Mytilida). *Mol Phylogenet Evol* 139:106533. <https://doi.org/10.1016/j.ympev.2019.106533>
4. Curole JP, Kocher TD (1999) Mitogenomics: digging deeper with complete mitochondrial genomes. *Trends Ecol Evol* 14(10):394–398. [https://doi.org/10.1016/s0169-5347\(99\)01660-2](https://doi.org/10.1016/s0169-5347(99)01660-2)
5. Wang Y, Yang Y, Liu HY et al (2021) Phylogeny of Veneridae (Bivalvia) based on mitochondrial genomes. *Zool Scr* 00(1):1–13. <https://doi.org/10.1111/zsc.12454>
6. Breton S, Stewart DT, Hoeh WR (2010) Characterization of a mitochondrial ORF from the gender-associated mtDNAs of *Mytilus* spp. (Bivalvia: Mytilidae): identification of the "missing" ATPase 8 gene. *Mar Genomics* 3(1):11–18. <https://doi.org/10.1016/j.margen.2010.01.001>
7. Ren JF, Shen X, Jiang F et al (2010) The mitochondrial genomes of two scallops, *Argopecten irradians* and *Chlamys farreri* (Mollusca: Bivalvia): the most highly rearranged gene order in the family Pectinidae. *J Mol Evol* 70(1):57–68. <https://doi.org/10.1007/s00239-009-9308-4>
8. Ren JF, Xiao L, Zhang GF et al (2009) "Tandem duplication-random loss" is not a real feature of oyster mitochondrial genomes. *BMC Genom* 10:84. <https://doi.org/10.1186/1471-2164-10-84>
9. Hutchiso CA, Newbold JE, Potter SS et al (1974) Maternal inheritance of mammalian mitochondrial DNA. *Nature* 251(5475):536–538. <https://doi.org/10.1038/251536a0>
10. Boutet I, Jollivet D, Shillito B et al (2009) Molecular identification of differentially regulated genes in the hydrothermal-vent species *Bathymodiolus thermophilus* and *Paralvinella pandorae* in response to temperature. *BMC Genom* 10(1):222. <https://doi.org/10.1186/1471-2164-10-222>
11. Liu J, Liu HL, Zhang HB (2018) Phylogeny and evolutionary radiation of the marine mussels (Bivalvia: Mytilidae) based on mitochondrial and nuclear genes. *Mol Phylogenet Evol* 126,:233–240. <https://doi.org/10.1016/j.ympev.2018.04.019>

12. Liu FY, Li YL, Yu HW et al (2021) MolluscDB: an integrated functional and evolutionary genomics database for the hyper-diverse animal phylum Mollusca. *Nucleic Acids Res* 49(D1):D988–D997. <https://doi.org/10.1093/nar/gkaa918>
13. Sun J, Zhang Y, Xu T et al (2017) Adaptation to deep-sea chemosynthetic environments as revealed by mussel genomes. *Nat Ecol Evol* 1(5):121. <https://doi.org/10.1038/s41559-017-0121>
14. Morton B (2015) Evolution and adaptive radiation in the Mytiloidea (Bivalvia): clues from the pericardial-posterior byssal retractor musculature complex. *Molluscan Res* 35(4):227–245. <https://doi.org/10.1080/13235818.2015.1053167>
15. Tumanda MI, Yap HT, McManus LT et al (1997) Growth, mortality and recruitment pattern of the brown mussel, *Modiolus metcalfei* (Bivalvia: Mytilacea), in Panguil Bay, Southern Philippines. *Aquaculture* 154(3-4): 233-245. [https://doi.org/10.1016/S0044-8486\(97\)00049-5](https://doi.org/10.1016/S0044-8486(97)00049-5)
16. Matsumoto M (2003) Phylogenetic analysis of the subclass Pteriomorphia (Bivalvia) from mtDNA COI sequences. *Mol Phylogenet Evol* 27(3):429–440. [https://doi.org/10.1016/S1055-7903\(03\)00013-7](https://doi.org/10.1016/S1055-7903(03)00013-7)
17. Uba KIN, Monteclaro HM (2020) Habitat characteristics of the horse mussel *Modiolus modulaides* (Röding 1798) in Iloilo, Philippines. *Philipp J Sci* 149(3–a):977–987
18. Bennett KF, Bailey AW, Brambert DJ et al (2016) The F type mitochondrial genome of the scorched mussel: *Brachidontes exustus*. *Mitochondrial DNA Part A* 27(2):1501–1502. <https://doi.org/10.3109/19401736.2014.953111>. Mytiloidea, Mytilidae
19. Hoffmann RJ, Boore JL, Brown WM (1992) A novel mitochondrial genome organization for the blue mussel, *Mytilus edulis*. *Genetics* 131(2):397–412. <https://doi.org/10.1093/genetics/131.2.397>
20. Robicheau BM, Breton S, Stewart DT (2017) Sequence motifs associated with paternal transmission of mitochondrial DNA in the horse mussel, *Modiolus modiolus*. *Gene* 605:32–42. Bivalvia: Mytilidae <https://doi.org/10.1016/j.gene.2016.12.025>
21. Song WT, Gao XG, Li YF et al (2009) Comparison of mitochondrial genomes of bivalves. *Hereditas* 31(11):1127–1134
22. Gaitán-Espitia JD, Quintero-Galvis JF, Mesas A et al (2016) Mitogenomics of southern hemisphere blue mussels (Bivalvia: Pteriomorphia): Insights into the evolutionary characteristics of the *Mytilus edulis* complex. *Sci Rep* 6:26853. <https://doi.org/10.1038/srep26853>
23. Boore J, Lavrov DV, Brown WM (1998) Gene translocation links insects and crustaceans. *Nature* 392(6677):667–668. <https://doi.org/10.1038/33577>
24. Mizi A, Zouros E, Moschonas N et al (2005) The complete maternal and paternal mitochondrial genomes of the Mediterranean mussel *Mytilus galloprovincialis*: implications for the doubly uniparental inheritance mode of mtDNA. *Mol Biol Evol* 22(4):952–967. <https://doi.org/10.1093/molbev/msi079>
25. Breton S, Burger G, Stewart DT et al (2006) Comparative analysis of gender-associated complete mitochondrial genomes in marine mussels (*Mytilus* spp.). *Genetics* 172(2):1107–1119. <https://doi.org/10.1534/genetics.105.047159>

26. Juehling F, Puetz J, Bernt M et al (2012) Improved systematic tRNA gene annotation allows new insights into the evolution of mitochondrial tRNA structures and into the mechanisms of mitochondrial genome rearrangements. *Nucleic Acids Res* 40(7):2833–2845.
<https://doi.org/10.1093/nar/gkr1131>
27. Steinberg S, Cedergren R (1994) Structural compensation in atypical mitochondrial tRNAs. *Nat Struct Mol Biol* 1(8):507–510. <https://doi.org/10.1038/nsb0894-507>
28. Boyce TM, Zwick ME, Aquadro CF (1989) Mitochondrial DNA in the bark weevils: size, structure and heteroplasmy. *Genetics* 123(4):825–836. <https://doi.org/10.1093/genetics/123.4.825>
29. Zhang Z, Ma PZ, Hu LS et al (2019) The complete mitochondrial genome of a marine mussel, *Modiolus comptus* (Mollusca: Mytilidae), and its phylogenetic implication. *Mitochondrial DNA Part B* 4(2):4057–4058. <https://doi.org/10.1080/23802359.2019.1688728>
30. Lubośny M, Śmietanka B, Przyłucka A et al (2020) Highly divergent mitogenomes of *Geukensia demissa* (Bivalvia, Mytilidae) with extreme AT content. *J Zoolog Syst Evol Res* 00(2):1–10.
<https://doi.org/10.1111/jzs.12354>
31. Wang QQ, Zhang ZQ, Tang GH (2016) The mitochondrial genome of *Atrijuglans hetaohei* Yang (Lepidoptera: Gelechioidea) and related phylogenetic analyses. *Gene* 581(1):66–74.
<https://doi.org/10.1016/j.gene.2016.01.027>
32. Yong HS, Chua KO, Song SL et al (2021) Complete mitochondrial genome of *Dacus vijaysegarani* and phylogenetic relationships with congeners and other tephritid fruit flies (Insecta: Diptera). *Mol Biol Rep* 48(8):6047–6056. <https://doi.org/10.1007/s11033-021-06608-2>
33. Schaber J, Risper C, Wernegreen J et al (2005) Gene expression levels influence amino acid usage and evolutionary rates in endosymbiotic bacteria. *Gene* 352:109–117.
<https://doi.org/10.1016/j.gene.2005.04.003>
34. Johnson MA, Vidoni S, Durigon R et al (2014) Amino acid starvation has opposite effects on mitochondrial and cytosolic protein synthesis. *PLoS ONE* 9(4):e93597.
<https://doi.org/10.1371/journal.pone.0093597>
35. Hurst LD (2002) The *Ka/Ks* ratio: diagnosing the form of sequence evolution. *Trends Genet* 18(9):486–487. [https://doi.org/10.1016/s0168-9525\(02\)02722-1](https://doi.org/10.1016/s0168-9525(02)02722-1)
36. Navarro A, Barton NH (2003) Chromosomal speciation and molecular divergence - accelerated evolution in rearranged chromosomes. *Science* 300(5617):321–324.
<https://doi.org/10.1126/science.1080600>
37. Zhang J, Xie PH, Lascoux M et al (2013) Rapidly evolving genes and stress adaptation of two desert poplars, *Populus euphratica* and *P. pruinosa*. *PLoS One* 8(6): e66370.
<https://doi.org/10.1371/journal.pone.0066370>
38. Lim JY, Tay TS, Lim CS et al (2018) *Mytella strigata* (Bivalvia: Mytilidae): an alien mussel recently introduced to Singapore and spreading rapidly. *Molluscan Res* 38(3):170–186.
<https://doi.org/10.1080/13235818.2018.1423858>

39. Alves FAdS, Beasley CR, Hoeh WR et al (2012) Detection of mitochondrial DNA heteroplasmy suggests a doubly uniparental inheritance pattern in the mussel *Mytella charruana*. *Revista Brasileira de Biociências* 10(2):176–185
40. Soot-Ryen T (1955) A report on the family Mytilidae (Pelecypoda). *Allan Hancock Pacif Exped* 20:1–174
41. Signorelli J, Pastorino G (2021) *Semimytilus patagonicus* (d'Orbigny, 1842) (Bivalvia: Mytilidae) revealed as the valid name with priority over *Semimytilus algosus*. *Archiv für Molluskenkunde* 1850(1):55–63. <https://doi.org/10.1127/arch.moll/150/055-063>. Gould
42. Lubosny M, Smietanka B, Przylucka A et al (2020) Highly divergent mitogenomes of *Geukensia demissa* (Bivalvia, Mytilidae) with extreme AT content. *J Zoolog Syst Evol Res* 58(2):571–580. <https://doi.org/10.1111/jzs.12354>
43. Morton B, Leung PTY, Wei JH et al (2020) A morphological and genetic comparison of *Septifer bilocularis*, *Mytilisepta virgata* and *Brachidontes variabilis* (Bivalvia: Mytiloidea) from Hong Kong and erection of the Mytiliseptiferinae sub-fam. nov. *Reg Stud Reg Sci* 34:100981. <https://doi.org/10.1016/j.rsma.2019.100981>
44. Uliano-Silva M, Americo JA, Costa I et al (2016) The complete mitochondrial genome of the golden mussel *Limnoperna fortunei* and comparative mitogenomics of Mytilidae. *Gene* 577(2):202–208. <https://doi.org/10.1016/j.gene.2015.11.043>
45. Samadi S, Quéméré E, Lorion J et al (2007) Molecular phylogeny in mytilids supports the wooden steps to deep-sea vents hypothesis. *C R Biol* 330(5):446–456. <https://doi.org/10.1016/j.crv.2007.04.001>
46. Distel DL (2000) Phylogenetic relationships among Mytilidae (Bivalvia): 18S rRNA data suggest convergence in mytilid body plans. *Mol Phylogenet Evol* 15(1):25–33. <https://doi.org/10.1006/mpev.1999.0733>
47. Huber M (2010) Compendium of bivalves. A full-color guide to 3,300 of the World's Marine Bivalves. A status on Bivalvia after 250 years of research. ConchBooks, Hackenheim
48. Wang ZR (1997) Fauna sinica phylum molludca order mytiloida. Science Press, Beijing

Tables

Table 1

Complete mitochondrial genomes used for phylogenetic analysis in this study, with 39 species including *Crassostres gigas* and *Anadara sativa* as outgroups, and 47 sequences in total. Female-lineage and male-lineage mitogenomes are marked by “-F” and “-M” respectively following the scientific names.

Species	Subfamily	Length (bp)	GenBank accession number
<i>Bathymodiolus japonicus</i>	Bathymodiolinae	17510	AP014560
<i>Bathymodiolus septemdierum</i>	Bathymodiolinae	17069	AP014562
<i>Bathymodiolus</i> sp.5 South	Bathymodiolinae	18376	MT916740
<i>Bathymodiolus aduloides</i>	Bathymodiolinae	17243	MT916741
<i>Bathymodiolus azoricus</i>	Bathymodiolinae	17598	MT916742
<i>Bathymodiolus brooksi</i>	Bathymodiolinae	17728	MT916743
<i>Bathymodiolus childressi</i>	Bathymodiolinae	17637	MT916744
<i>Bathymodiolus marisindicus</i>	Bathymodiolinae	17138	MT916745
<i>Bathymodiolus securiformis</i>	Bathymodiolinae	17199	KY270857
<i>Gigantidas haimaensis</i>	Bathymodiolinae	18283	MT916746
<i>Gigantidas platifrons</i>	Bathymodiolinae	17653	AP014561
<i>Modiolus modiolus</i>	Modiolinae	15816	KX821782
<i>Modiolus kurilensis</i>	Modiolinae	16210	KY242717
<i>Modiolus philippinarum</i>	Modiolinae	16389	KY705073
<i>Modiolus modulaides</i>	Modiolinae	15422	This study
<i>Modiolus comptus</i>	Modiolinae	15591	MN602036
<i>Modiolus nipponicus</i>	Modiolinae	15638	MK721547
<i>Mytilus edulis</i>	Mytilinae	16740	AY484747
<i>Mytilus galloprovincialis</i> -F	Mytilinae	16744	AY497292
<i>Mytilus galloprovincialis</i> -M	Mytilinae	17671	AY363687
<i>Mytilus trossulus</i>	Mytilinae	18652	AY823625
<i>Mytilus trossulus</i> -M	Mytilinae	16601	GQ438250
<i>Mytilus chilensis</i> -F	Mytilinae	16748	KT966847
<i>Mytilus chilensis</i>	Mytilinae	16765	KP100300
<i>Mytilus unguiculatus</i>	Mytilinae	16642	KJ577549
<i>Mytilus californianus</i> -F	Mytilinae	16729	JX486124
<i>Mytilus californianus</i> -M	Mytilinae	16122	JX486123

<i>Perna viridis</i>	Mytilinae	16014	JQ970425
<i>Perna perna</i>	Mytilinae	18415	KM655841
<i>Perna canaliculus</i>	Mytilinae	16005	MG766134
<i>Semimytilus patagonicus</i> -F	Mytilinae	18113	MT026712
<i>Semimytilus patagonicus</i> -M	Mytilinae	24347	MT026713
<i>Crenomytilus grayanus</i>	Mytilinae	17582	MK721543
<i>Arcuatula senhousia</i> -F	Arcuatulinae	21557	GU001953
<i>Arcuatula senhousia</i> -M	Arcuatulinae	20612	GU001954
<i>Limnoperna fortunei</i>	Arcuatulinae	18145	KP756905
<i>Mytella strigata</i>	Arcuatulinae	16302	MT991018
<i>Brachidontes exustus</i>	Brachidontinae	16600	KM233636
<i>Geukensia demissa</i> -F	Brachidontinae	15838	MN449487
<i>Geukensia demissa</i> -M	Brachidontinae	15893	MN449488
<i>Perumytilus purpuratus</i> -M	Brachidontinae	16540	MH330331
<i>Perumytilus purpuratus</i> -F	Brachidontinae	16963	MH330332
<i>Mytilisepta keenae</i>	Mytiliseptinae	15902	MK721542
<i>Gregariella coralliophaga</i>	Musculinae	16273	MK721545
<i>Septifer bilocularis</i>	Septiferinae	16253	MK721549
<i>Crassostrea gigas</i>	—	18224	AF177226
<i>Anadara sativa</i>	—	48161	KF667521

Table 2

Genetic characteristics of the *Modiolus modulaides* mitogenome

Gene	Position (bp)		Size (bp)	Strand	Codon		Anticodon	Intergenic nucleotides (bp)
	From	To			Start	Stop		
cox1	1	1548	1548	H	ATG	TAA		8
trnK	1557	1623	67	H			TTT	9
trnY	1633	1696	64	H			GTA	331
trnP	2028	2094	67	H			TGG	-3
trnE	2092	2158	67	H			TTC	8
trnL1	2167	2232	66	H			TAG	1
nad3	2234	2590	357	H	ATG	TAA		26
trnC	2617	2681	65	H			GCA	2
trnL2	2684	2750	67	H			TAA	223
trnR	2974	3040	67	H			TCG	3
trnS1	3044	3107	64	H			GCT	10
rrnS	3118	3888	771	H				11
trnM	3900	3964	65	H			CAT	4
trnQ	3969	4038	70	H			TTG	8
atp6	4047	4778	732	H	GTG	TAG		6
trnV	4785	4845	61	H			TAC	2
nad4	4948	6152	1305	H	ATT	TAG		-2
trnN	6151	6216	66	H			GTT	3
cox3	6220	6985	766	H	ATT	T(AA)		10
trnF	6996	7060	65	H			GAA	0
rrnL	7061	8204	1144	H				-8
trnS2	8197	8256	60	H			TGA	2
trnD	8259	8325	67	H			GTC	33
nad6	8359	8817	459	H	TTG	TAA		10
trnI	8828	8897	70	H			GAT	478
nad2	9376	10350	975	H	ATT	TAG		0
trnW	10351	10416	66	H			TCA	8

trnG	10425	10491	67	H		TCC	4
trnT	10496	10558	63	L		TGT	1
cob	10560	11696	1137	H	ATG	TAA	-1
nad4l	11696	11971	276	H	ATG	TAG	-2
trnA	11970	12033	64	H		TGC	0
trnH	12034	12096	63	H		GTG	0
nad5	12097	13791	1695	H	ATG	TAA	1
cox2	13793	14497	705	H	ATG	TAA	3
nad1	14501	15418	918	H	ATG	TAG	4

Figures

Figure 1

Bayesian tree based on 12 protein-coding genes and 2 rRNAs showing phylogenetic relationships of 37 Mytilidae species, with *Crassostres gigas* and *Anadara sativa* being outgroups. Numbers near the nodes are branch support values (Bayesian posterior probabilities followed by ML bootstrap supports)