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Updated mitochondrial phylogeny of Pteriomorph and Heterodont Bivalvia, including deep-sea chemosymbiotic *Bathymodiolus* mussels, vesicomyid clams and the thyasirid clam *Conchocele* cf. *bisecta*

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ABSTRACT

The mitochondrial genomes of bivalves have often been used for comparative genomics and for resolving phylogenetic relationships. More than 100 bivalve complete mitochondrial genomes have been sequenced to date. However, few mitochondrial genomes have been reported for deep-sea chemosymbiotic bivalves, which belong to the subclasses Pteriomorphia and Heterodonta. In the present study, we sequenced the mitochondrial genomes of eight deep-sea chemosymbiotic bivalve species: three species of *Bathymodiolus* mussels (*B. japonicus*, *B. platifrons*, and *B. septemdierum*), four species of vesicomyid clams (*Abyssogena mariana*, *A. phaseoliformis*, *Isorropodon fossajaponicum*, and *Phreagena okutanii*, all of which were formerly classified in the genus *Calyptogena*), and one species of thyasirid clam (*Conchocele* cf. *bisecta*). With a few exceptions, these mitochondrial genomes contained genes that are typical of metazoans: 13 protein-coding genes, two rRNA genes, and 22 tRNA genes. The major non-coding region with a high A + T content of each genome, which contained tandem repeats and hairpins, was hypothesized to function as a control region. The phylogenetic trees of Pteriomorphia and Heterodonta were reconstructed based on the concatenated sequences of 14 shared genes. *Bathymodiolus* formed a monophyletic clade with asymbiotic Mytilidae mussels, the vesicomyid clams formed a monophyly that was sister to the Veneridae, and *C.* cf. *bisecta* branched basally in the Heterodonta. It is known that the gene orders of mitochondrial genomes vary among bivalves. To examine whether gene order variation exhibits phylogenetic signals, tree topologies based on the minimum number of gene rearrangements were reconstructed for two clades (superfamily Tellinoidea, which includes the Psammobiidae, Semelidae, Solecurtidae, and Tellinidae; and the clade comprising the Myidae, Mactridae, Arcticidae, Vesicomyidae, and Veneridae) with high statistical support in sequence-based phylogenies. The resulting tree topologies were almost identical to those of the sequence-based trees. Our present findings suggest that the evolution of bivalves could be precisely traced back through the analysis of mitochondrial genomes, and that such an analysis could contribute to understanding bivalve evolution and diversity.

1. Introduction

Along with morphological classification, molecular phylogenetic analyses are essential for evaluating the diversity of metazoans. The usefulness of evolutionary studies has stimulated the sequencing of many mitochondrial genomes (e.g., Inoue et al., 2003; Lavrov et al., 2008; Iwasaki et al., 2013). The mitochondrial genomes of metazoans are generally circular and contain 37 canonical genes: 13 protein genes (*atp6*, *atp8*, *cox1–3*, *cob*, *nad1–6*, and *nad4L*), two rRNA genes (*rrnL* and *rrnS*), and 22 tRNA genes (single genes for 18 amino acids and two each for leucine and serine). In addition, the gene arrangements of vertebrate

Abbreviations: A + T, adenosine and thymidine; *atp6* and *8*, ATP synthase Fo subunit 6 and 8; bp, base pairs; BP, bootstrap probability; *cob*, cytochrome *b*; *cox1–3*, cytochrome *c* oxidase subunits I-III; dNTPs, deoxynucleotides; L1, $tRNA^{Leu1}$ (anticodon: UAA); L2, $tRNA^{Leu2}$ (anticodon: UAG); ML, maximum likelihood; *nad1–6* and *nad4L*, NADH dehydrogenase subunits 1–6 and 4L; NCR, non-coding region; PCR, polymerase chain reaction; PP, posterior probability; *rrnL* and *rrnS*, large and small subunits ribosomal RNA; S1, $tRNA^{Ser1}$ (anticodon: UCU or GCU); S2, $tRNA^{Ser2}$ (anticodon: UGA); tRNA, transfer RNA.

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Table 1
Characteristics of deep-sea bivalve specimens used in the present study, compliant with the MIxS standard.

Item/species	*Bathymodiolus japonicus*	*Bathymodiolus platifrons*	*Bathymodiolus septemdierum*	*Abyssogena mariana*
Investigation				
Submitted to INSDC databank	AP014560 (DDBJ)	AP014561 (DDBJ)	AP014562 (DDBJ)	LC126311 (DDBJ)
Investigation type	Organelle	Organelle	Organelle	Organelle
Project name	Mitochondrial genome sequencing	Mitochondrial genome sequencing	Mitochondrial genome sequencing	Mitochondrial genome sequencing
Environment				
Latitude and longitude	+35.0158 + 139.2222	+35.0158 + 139.2222	+32.1039 + 139.8692	+11.6569 + 143.0490
Depth (m)	−861	−861	−1228	−5633
Geographic location name	Japan: Off Hatsushima, Sagami Bay	Japan: Off Hatsushima, Sagami Bay	Japan: Izu-Bonin Arc, Myojin knoll	USA: Mariana trench
Collection date	2010-05-12	2010-05-12	2010-05-15	2013-09-09
Biome	Marine benthic biome (ENVO:01000024)	Marine benthic biome (ENVO:01000024)	Marine benthic biome (ENVO:01000024)	Marine benthic biome (ENVO:01000024)
Feature	Cold seep (ENVO:01000263)	Cold seep (ENVO:01000263)	Hydrothermal vent (ENVO:01000122)	Cold seep (ENVO:01000263)
Material	Sea water (ENVO:01002149)	Sea water (ENVO:01002149)	Sea water (ENVO:01002149)	Sea water (ENVO:01002149)
MIGS/MIMS/MIMARKS extension				
Environmental package	Water	Water	Water	Water
Nucleic acid sequence source				
Isolation and growth condition	Missing	Missing	Missing	Missing
Sample collection device or method	ROV (Remotely Operated Vehicle) *Hyper-Dolphin* suction sampler	ROV (Remotely Operated Vehicle) *Hyper-Dolphin* suction sampler	ROV (Remotely Operated Vehicle) *Hyper-Dolphin* suction sampler	DSV (Deep Submergence Vehicle) *Shinkai 6500* suction sampler
Sample material processing	Frozen specimen	Frozen specimen	Frozen specimen	Frozen specimen
Sequencing				
Nucleotide acid extraction	https://www.qiagen.com/us/products/catalog/sample-technologies/dna-sample-technologies/genomic-dna/dneasy-blood-and-tissue-kit	https://www.qiagen.com/us/products/catalog/sample-technologies/dna-sample-technologies/genomic-dna/dneasy-blood-and-tissue-kit	https://www.qiagen.com/us/products/catalog/sample-technologies/gdna-sample-technologies/genomic-dna/dneasy-blood-and-tissue-kit	https://www.qiagen.com/us/products/catalog/sample-technologies/dna-sample-technologies/genomic-dna/dneasy-blood-and-tissue-kit
Sequencing method	Sanger	Sanger	Sanger	Sanger
Assembly	Sequencher 4.10.1	Sequencher 4.10.1	Sequencher 4.10.1	Sequencher 4.10.1

mitochondrial genomes are identical, with only a few exceptions (Boore, 1999; Inoue et al., 2003). However, the gene orders of invertebrate mitochondrial genomes are not as conserved and are markedly diverse in mollusks (Serb and Lydeard, 2003).

Among mollusks, bivalves are the second largest group, after Gastropoda. They inhabit a wide range of environments, including freshwater, shallow-sea, continental shelves and the deep-sea. To date, >100 complete mitochondrial genomes of bivalves have been sequenced. However, the taxonomic distribution of the genomes sequenced is biased, and a broad range of taxon sampling is still needed to accurately estimate the phylogenetic relationships of the group (Heath et al., 2008). Unfortunately, a significant fraction of the bivalve families and genera, for which mitochondrial genome sequences are needed, inhabit environments that are difficult to access, thus complicating their analyses.

Bivalves dwelling in or near deep-sea hydrothermal vents and seeps are among such "missing" taxa. In deep-sea hydrothermal vents and seeps, bivalves are the most dominant invertebrate taxon and usually harbor chemoautotrophic bacteria in the epithelial cells of their gills (Dubilier et al., 2008). Most of the bivalves inhabiting these environments belong to the subclasses Pteriomorphia or Heterodonta. For instance, *Bathymodiolus* mussels belong to the subclass Pteriomorphia, whereas clams of the Vesicomyidae, Thyasiridae, and Lucinidae belong to the subclass Heterodonta (Dubilier et al., 2008). Among these deep-sea chemosymbiotic bivalves, the complete mitochondrial genomes of *Calyptogena magnifica* Boss and Turner, 1980, (Vesicomyidae; Liu et al., 2015), *Lucinella divaricata* Linnaeus, 1758 (Lucinidae; Accession No. NC_013275), and *Loripes lacteus* Linnaeus, 1758 (Lucinidae; Accession No. NC_013271) have already been sequenced. However, the mitochondrial genomes of *Bathymodiolus* mussels and thyasirid clams, both of which are ecologically important to deep-sea chemosynthetic communities, have yet to be reported.

In the present study, we newly sequenced eight mitochondrial genomes of deep-sea chemosymbiotic bivalve species: three species of *Bathymodiolus* mussels (*B. japonicas* Hashimoto and Okutani, 1994, *B. platifrons* Hashimoto and Okutani, 1994, and *B. septemdierum* Hashimoto and Okutani, 1994), four species of vesicomyid clams (*Abyssogena mariana* Okutani et al., 2013, *A. phaseoliformis* Métivier et al., 1986, *Isorropodon fossajaponicum* Okutani et al., 2000, and *Phreagena okutanii* Kojima and Ohta, 1997, all of which were formerly classified in the genus *Calyptogena*), and one species of thyasirid clam (*Conchocele* cf. *bisecta* Conrad, 1849). The mitochondrial genomes of these chemosymbiotic bivalves, along with publicly available mitochondrial genomes of Pteriomorphia (34 spp.) and Heterodonta (35 spp.), were used to reconstruct updated phylogenetic trees of both Pteriomorphia and Heterodonta. In addition, to test whether the gene order arrangement of mitochondrial genomes was useful for estimating the phylogenetic relationships of bivalves, we compared the trees based on sequence data and on gene arrangements. On the basis of our findings, we discuss the phylogenetic positions of deep-sea chemosymbiotic bivalves.

2. Materials and methods

2.1. Sample collection and DNA extraction

Bathymodiolus, vesicomyid, and *C.* cf. *bisecta* specimens were collected from seeps and hydrothermal vents, using the Deep Submergence Vehicle (DSV) *Shinkai 6500* or the Remotely Operated Vehicle (ROV) *Hyper-Dolphin* of Japan Agency for Marine-Earth Science and Technology, and stored at −80 °C until use (Table 1). After thawing the bivalve samples, the mantle tissue was dissected and cut into small pieces on ice. Total genomic DNA was purified from ~25 mg mantle tissue, using a DNeasy Tissue Kit (Qiagen, Hilden, Germany),