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The complete mitochondrial genome of the alvinocaridid shrimp *Shinkaicaris leurokolos* (Decapoda, Caridea): Insight into the mitochondrial genetic basis of deep-sea hydrothermal vent adaptation in the shrimp

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ABSTRACT

Deep-sea hydrothermal vent is one of the most extreme environments on Earth with low oxygen and high levels of toxins. Decapod species from the family Alvinocarididae have colonized and successfully adapted to this extremely harsh environment. Mitochondria plays a vital role in oxygen usage and energy metabolism, thus it may be under selection in the adaptive evolution of the hydrothermal vent shrimps. In this study, the mitochondrial genome (mitogenome) of alvinocaridid shrimp *Shinkaicaris leurokolos* (Kikuchi & Hashimoto, 2000) was determined through Illumina sequencing. The mitogenome of *S. leurokolos* was 15,903 bp in length, containing 13 protein-coding genes, 2 rRNAs, and 22 tRNAs. The gene order and orientation were identical to those of sequenced alvinocaridids. It has the longest concatenated sequences of protein-coding genes, tRNAs and shortest pooled rRNAs among the alvinocaridids. The control regions (CRs) of alvinocaridid were significantly longer ($p < 0.01$) than those of the other caridaen. The alignment of the alvinocaridid CRs revealed two conserved sequence blocks (CSBs), and each of the CSBs included a noncanonical open reading frame (ORF), which may be involved in adjusting mitochondrial energy metabolism to adapt to the hydrothermal environment. Phylogenetic analysis supported that the deep-sea hydrothermal vent shrimps may have originated from those living in shallow area. Positive selection analysis reveals the evidence of adaptive change in the mitogenome of Alvinocarididae. Thirty potentially important adaptive residues were identified, which were located in *atp6*, *cox1*, *cox3*, *cytb* and *nad1–5*. This study explores the mitochondrial genetic basis of hydrothermal vent adaptation in alvinocaridid for the first time, and provides valuable clues regarding the adaptation.

1. Introduction

The discovery of hydrothermal vents in 1977 at 2500 m depth on the Galápagos Spreading Centre (Corliss et al., 1979) has stimulated an increasing research effort examining the diversity, ecology, physiology, and biogeography of vent organisms (Martin et al., 2008). These hydrothermal vents are considered as extremely harsh environment given the high pressure, high temperature (up to 390 °C), low oxygen levels, and the chemical toxicity of the fluids (H_2S, CH_4 and various heavy metals) (Van Dover, 2000). Nevertheless, with the exception of chemoautotrophic bacteria that oxidize hydrogen sulfide emitted from vents, it is surprising that a number of specialized macro-faunas (e.g. vestimentiferan tube worms, vesicomyid and bathymodiolin bivalves, provannid gastropods, bythograeid and galatheid crabs, and bresiliid shrimps) have also been observed in these vents with dramatically high densities (Van Dover, 2000; Little and Vrijenhoek, 2003; Yang et al., 2008). Therefore, vent fauna is well adapted to this extremely harsh environment compared with marine species in coastal environments.

The organisms living in hydrothermal vents generally display biochemical and physiological adaptations (Van Dover et al., 2002; Milius, 2006) that are of interest for both fundamental and applied sciences (Ki et al., 2009). However, relatively little attention has been devoted to the study of molecular mechanisms of adaptation to deep-sea hydrothermal vents environment. The extreme environment of vents has the potential to affect mitogenome, as well as the metabolic processes of vent faunas, because several components of the oxidative pathway are encoded by mitochondrial genes (Ki et al., 2009). It is therefore interesting and necessary to investigate the unusual mitogenomic features and the key mitochondrial genes for energy metabolic pathways to understand the adaptive molecular mechanisms of the organisms living

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in the hydrothermal vent environment.

During the past few decades, mitochondrial DNA (mtDNA) sequences have been extensively used in studies of comparative and evolutionary genomics, phylogeography, population genetics, species identification, and phylogenetic relationships, owing to the abundance of mitochondria in cells, lack of recombination, maternal inheritance, absence of introns, and higher evolutionary rates (Moritz and Brown, 1987; Boore and Brown, 1998; Gissi et al., 2008; Gvoždík et al., 2010; Sun et al., 2016). The mitogenome contains 13 energy pathway protein-coding genes (*atp6*, *atp8*, *cox1–3*, *cytb*, *nad1–6* and *nad4l*), which are all key subunits of complexes involved in the oxidative phosphorylation (OXPHOS) machinery: seven subunits of ND, three subunits of COX, one subunit of the cytochrome bc_1 complex and two subunits of ATPase (Das, 2006). Therefore, mitochondria play a vital role in oxygen usage and energy metabolism providing up to 95% cell energy through OXPHOS (Das, 2006). Recently, the unique evolutionary modeling of mitogenome in extremely harsh environment had already come to the attention of researchers. Yang et al. (2008) found a novel mitochondrial gene structure and an incomplete tRNA suite (19 tRNAs versus 22 tRNAs in typical metazoans) in the mitogenome of the hydrothermal vent crab *Shinkaia crosnieri* Baba & Williams, 1998. In the mitogenome of deep-sea giant isopod *Bathynomus* sp., only 18 tRNAs were detected and 10 genes were inverted when compared to the pancrustacean ground pattern (Shen et al., 2017). Despite strong functional constraints, mtDNA may be subject to positive directional selection in response to pressures of extreme environments (Tomasco and Lessa, 2011). In recent studies, mtDNA analysis have displayed evidence of adaptive evolution in mitochondrial genes, such as the NADH dehydrogenase genes of Tibetan horses, high-altitude snub-nosed monkeys and deep sea anemone (Xu et al., 2007; Yu et al., 2011; Zhang et al., 2017), the cytochrome *c* oxidase genes of plateau pika and Tibetan antelope (Xu et al., 2005; Luo et al., 2008), the cytochrome *b* gene of high-altitude alpaca (da Fonseca et al., 2008), and the ATP synthase genes of the artiodactylian tribe Caprini and deep sea anemones (Hassanin et al., 2009; Zhang et al., 2017).

The caridean family Alvinocarididae is comprised of 28 described species belonging to eight genera, living in the Atlantic, Pacific, and Indian Oceans. The alvinocaridid shrimps are endemic to deep-sea hydrothermal vent and cold seep environments, and constitute the predominant faunal biomass of various hydrothermal and cold seep ecosystems (Nye et al., 2012; Yahagi et al., 2014; Hernandez-Avila et al., 2015). Molecular evidence indicates that they were established during the late Cretaceous/Early Tertiary (Yang et al., 2012). Within this family, the genus *Shinkaicaris* is newly established, which is comprised of only one species *Shinkaicaris leurokolos* (Komai and Segonzac, 2005). Unlike many other alvinocaridid shrimps, inhabiting the peripheral area of hydrothermal vents, *S. leurokolos* inhabit the area near the vent (Yahagi et al., 2015). *S. leurokolos* are expected to tolerate higher temperatures. Therefore, it offers a more interesting organismal model of adaptation to extreme environmental stress. Although several complete mitogenomes of alvinocaridid shrimps have been sequenced in recent years, to date, no information on the mitogenome of *S. leurokolos* is available. Moreover, for deep-sea species from hydrothermal vent, little is known about their environmental adaptation mechanism at mitogenome level.

In the present study, we firstly sequenced the mitogenome of alvinocaridid shrimp *S. leurokolos*, the sole representative of the genus *Shinkaicaris*, sampled from hydrothermal vent chimney. Further, we compared the mitogenome with other available Alvinocarididae mitogenomes, and discussed mitogenomic gene structures and taxonomy of the alvinocaridid shrimp. Additionally, we performed phylogenetic analyses of caridean species based on 13 concatenated mitochondrial protein coding gene sequences. Finally, we evaluated the selective pressures operating on alvinocaridid mitogenome in the first attempt to understand the genetic basis of deep-sea hydrothermal vent adaptation in alvinocaridid shrimps.

2. Materials and method

2.1. Sampling and DNA extraction

Hydrothermal vent shrimp *S. leurokolos* was captured from hydrothermal vent chimney at a depth of 1582.7 m in Okinawa Island (127° 04′10.422″ E; 27° 14′56.509″ N) using the remotely operated vehicle (ROV). Specimens were immediately preserved in 95% ethanol until DNA extraction. Species-level morphological identification abided by the key point of Komai and Segonzac (2005). Total genomic DNA was isolated using the DNeasy tissue kit (Qiagen) according to the manufacturer's instructions.

2.2. Genome assembly and annotation

Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added to attribute sequences to the sample. The library preparations were sequenced on an Illumina HiSeq 2500 platform and paired-end reads were generated. The raw reads filtered with average quality value (lower than Q20) were excluded from further analysis. Clean data were then assembled using SOAP denovo (Li et al., 2010) with k-mer = 55. Then we blast contigs against the reference mitogenomes from species of the family Alvinocarididae shown in Table 1. The contigs identified as mitogenome sequences were manually examined for repeats at the beginning and end of the sequence to establish a circular mtDNA. The exact size and sequence of the mtDNA was verified by PCR followed by Sanger sequencing. The primers were designed with Primer Premier v5.0 software (Premier Biosoft International). The primer sequences used for PCR are presented in Supplementary file 1.

The protein coding genes were analyzed with ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) and BLASTx using the invertebrate mitochondrial genetic code. The positions of tRNA genes were determined by ARWEN (Laslett and Canback, 2008) and DOGMA (Wyman et al., 2004) using the invertebrate mitochondrial genetic code and the default search mode. The rRNA genes were confirmed by BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/) against the published gene sequences.

The complete mitochondrial DNA sequence was deposited in the GenBank database under the accession number MF627741. The gene map of the *S. leurokolos* mitogenome was generated with the program CGView (Stothard and Wishart, 2005).

2.3. Sequence analysis

The A + T content values were computed using Editseq program from DNASTAR. The skew in nucleotide composition was calculated by GC and AT skew, which were measured according to the formulae by Perna and Kocher (1995), AT skew = (A − T) / (A + T); GC skew = (G − C) / (G + C), where A, T, G and C are the occurrences of the four nucleotides. The frequencies of both codons and amino acids, and relative synonymous codon usage (RSCU) were calculated using MEGA 5 (Tamura et al., 2011). Pairwise divergence of the mitochondrial genes was calculated by MEGA 5 based on Kimura two-parameter (K2P) distance. The DNA polymorphism analysis of control region (CR) was performed by DnaSP version 5.10 (Librado and Rozas, 2009) with the sliding window of 50 bp and steps of 25 bp. Tandem Repeats Finder 4.0 (Benson, 1999) was used to search for the tandem repeat sequences. Prediction of potential secondary structure was performed by the online version of the Mfold software, version 3.2 (Zuker, 2003), with default settings. When more than one secondary structures were possible, the one with the lowest free energy score was used.