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Research paper

Complete mitochondrial genome of the first deep-sea spongicolid shrimp *Spongiocaris panglao* (Decapoda: Stenopodidea): Novel gene arrangement and the phylogenetic position and origin of Stenopodidea

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ABSTRACT

Stenopodidea Claus, 1872 (Crustacea: Decapoda) is one of the major groups of decapods crustaceans. Hitherto, only one complete mitochondrial genome (mitogenome) from the family Stenopodidae is available for the infraorder Stenopodidea. Here, we determined the complete mitogenome of *Spongiocaris panglao* de Grave and Saito, 2016 using Illumina sequencing, representing the first species from the family Spongicolidae. The 15,909 bp genome is a circular molecule and consists of 13 protein-coding genes (PCGs), 2 ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes and one control region. Although the overall genome organization is typical for metazoans, the mitogenome of *S. panglao* shows some derived characters. A + T content of 77.42% in *S. pamglao* mitogenome is second-highest among the dacapods described to date. The *trnR* gene exhibit modified secondary structure with the TψC loop completely missing, which might be a putative autapomorphy of *S. pamglao* mitogenome. Compared with the shallow-water stenopodidean species *S. hispidus*, the control region of *S. pamglao* exhibits three characteristics: larger size, higher A + T content, and more tandem repeat sequences. The gene order exhibited difference from the ancestral mitogenome pattern of the Pancrustacea, with 5 tRNA genes rearrangement. The result from BI was agreed with most morphological characters and molecular evidences, revealing that Stenopodidea and Reptantia had the closest relationship, as the sister group of Caridea. Still, the alternative hypothesis supported from ML topology cannot be completely rejected based on the current data. Estimated times revealed that the two stenopodideans families Stenopodidae and Spongicolidae diverged from each other around 122 Mya. The divergence time of spongicolid shrimp is in good agreement with the origin of their hexactinellid hosts (78–144 Mya).

1. Introduction

The typical metazoan mitochondrial genome (mitogenome) is a covalently closed circular molecule, encoding 37 genes: 13 protein genes, 22 transfer RNAs and two ribosomal RNAs and a control region (CR) including sites for the initiation of transcription and replication (Boore, 1999). The small genomes, typically ranging in size from 14 to 20 kb, in combination with a generally conserved gene content and relatively high evolutionary rate, has extensively used in population genetics, species identification, phylogenetic relationships at various taxonomic levels and comparative and evolutionary genomics studies (Moritz and Brown, 1987; Curole and Kocher, 1999; Hebert et al., 2002; Gissi et al., 2008), because it carries not only individual and combined mt genes but also gene order.

Mitochondrial gene arrangements seem seldom to have changed in some phyla of animal. Most vertebrate mitogenomes have highly conserved gene orders, from fishes to mammals (Boore, 1999; Satoh et al., 2016), indicating that no rearrangements have occurred for > 500 million years across diverse clades of vertebrates. In contrast, invertebrates display accelerated gene rearrangement evens within

Abbreviations: atp6, ATPase subunit 6 genes; *Cytb*, cytochrome *b* gene; *cox1-3*, cytochrome *c* oxidase subunitsI-III genes; CR, control region; mtDNA, mitochondrial DNA; PCG, protein coding gene; rRNAs, ribosomal RNA; tRNA, transfer RNA; RSCU, relative synonymous codon usage; AIC, Akaike information criterion; ML, Maximum Likelihood; BI, Bayesian inference; MCMC, Markov chain Monte Carlo; TDRL, Tandem duplication-random loss

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groups at many taxonomic levels (Dowton et al., 2002; Hassanin et al., 2005; Cameron et al., 2007). Many crustaceans such as copepods also show amount of variation in mitochondrial gene orders (Machida et al., 2002; Ki et al., 2009), whereas the gene arrangement has been highly conserved among penaeoid and carideans shrimp (Sun et al., 2017). According to the types of genes rearranged, genome rearrangements can be characterized in two aspects: minor rearrangements (tRNAs only) and major rearrangements (protein-coding and rRNA genes) (Cameron et al., 2007). Gene arrangement has been shown to be very powerful characters to investigate evolution of organisms and of their genomes, and the rapidity of rearrangement within a lineage determines the level at which rearrangements are likely to be phylogenetically informative (Boore and Brown, 1998; Serb and Lydeard, 2003; Boore et al., 2004).

Decapoda is one of the most diverse groups of Crustacea. The distinctive morphology and ecological diversity of this group, as well as their economic importance, makes decapod crustacean extensively research subjects in all fields of biology (Martin and Davis, 2001; Bracken et al., 2009). Despite widespread focus on this group, phylogenetic relationships among the major lineages of decapods remain unsettled, particularly in the relative position of the infraorder Stenopodidea. Stenopodidea Claus, 1872 (Crustacea: Decapoda) is one of the major groups of decapods crustaceans found in both shallow warm water and deep-sea benthic marine habitats. Although this infraorder is rather small in diversity and numbers of species compared to other infraorders of decapod crustaceans, it represent a unique group of shrimp-like crustaceans that bear an interesting blend of “natant” and “reptant” characters, making them hard to exactly place in the taxonomic of Decapoda (Goy, 2010). Thus, testing hypotheses of the phylogenetic position of Stenopodidea in the order Decapoda could help clarify evolutionary patterns among decapods crustaceans. Over the last century, the systematic placement of the stenopodidean shrimps in relation to carideans, lobsters, crabs and anomurans based on morphological characters has been controversial (Burkenroad, 1963, 1981; Abele and Felgenhauer, 1986; Christoffersen, 1988). Recently, the molecular phylogenetic analyses also have attempted to investigate the phylogeny of Decapoda (Porter et al., 2005; Tsang et al., 2008; Bracken et al., 2009; Bracken et al., 2010), but the phylogenetic relationships between the infraorder Stenopodidea and the major lineages of decapods crustaceans remain ambiguous. A firmer decision must await the inclusion of powerful molecular tools and a broader representation of decapod infraorders. Compared to the shorter sequences of individual genes (e.g. *18S*, *16S*, *12S* and *cox1*), complete mitochondrial DNA sequences can be more phylogenetically informative, and provide multiple characteristics at the generic level (Boore et al., 2005; Boore, 2006; Lee et al., 2007). However, only one complete mitogenome sequence from the family Stenopodidae is available for species of the infraorder Stenopodidea in the NCBI database to date. And the mitogenomic phylogenetic analyses of Stenopodidea is poorly documented (Shi et al., 2012), possibly because of many lineages being rare in nature and difficult to collect. The complete mitochondrial DNA sequence from other family, Spongicolidae in particular, can contribute to a better understanding of the phylogenetic position of Stenopodidea within Decapoda.

In the current classification, the infraorder Stenopodidea includes 83 species and assigned to 12 genera in three families, the family Stenopodidae Claus, 1872 containing mainly free-living species, the family Spongicolidae Schram, 1986 containing sponge-associated species, and the new family Macromaxillocarididae Alvarez, Iliffe and Villabobos, 2006, which was created for a single cave-dwelling species *Macromaxillocaris bahamaensis* Alvarez, Iliffe and Villalobos, 2006 (Chen et al., 2016). The family Spongicolidae contains 34 described species of 6 genera: *Globospongicola* Komai and Saito, 2006, *Microprosthema* Stimpson, 1860, *Paraspongicola* de Saint Laurent and Cleva, 1981, *Spongicola* de Haan, 1844, *Spongicoloides* Hansen, 1908 and *Spongiocaris* Bruce and Baba, 1973. With the exception of *Microprosthema*, which comprises free-living species in shallow rocky reefs, all of the remaining shrimps of the other genera are primarily obligate symbionts with deep-sea hexactinellid sponges or octocorals, living in the atrium of hosts (Komai and Saito, 2006; Saito, 2008). The phylogenetic relationships within the family Spongicolidae were examined based on both morphological characters and molecular evidences from mitochondrial and nuclear genes (Saito and Takeda, 2003; Chen et al., 2016). But no study has been focus on the evolution of the symbiotic relationship between spongicolid shrimp and hexactinellid sponges.

In this study, we aim to (i) characterize the complete mitochondrial genome of *Spongiocaris panglao* de Grave and Saito, 2016, representing the first species from the family Spongicolidae with entire mitogenome sequenced; (ii) compare its mitochondrial gene content, especially gene arrangement with those of another stenopodidean shrimp; and attempt to (iii) explore the phylogenetic position of Stenopodidea in relation to other decapod crustaceans; and (iv) determine the divergence time of the major lineages of Decapoda and reveal the origin of the symbiotic between spongicolid shrimp and hexactinellid sponges.

2. Materials and method

2.1. Sampling and DNA extraction

Specimens of *S. panglao* was captured from Yap Seamount, Western Pacific (11°16′15.765″ N, 139°25′30.697″ E) at a depth of 1452.5 m using the remotely operated vehicle (ROV). Species-level morphological identification were abided by the key point of Komai et al. (2016). Specimen were immediately preserved in 95% ethanol until DNA extraction. Total genomic DNA was isolated using the DNeasy tissue kit (Qiagen) according to the manufacturer's instructions.

2.2. Illumina sequencing, mitogenome 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 clustering of the index-coded sample was performed on a cBot Cluster Generation System according to the manufacturer's instructions. After cluster generation, 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 de novo assembly in CLC Genomics Workbench v. 11.0.64. The mitogenome of *Stenopus hispidus* (JN399096) from the family Stenopodidae in the infraorder Stenopodidea was used as an initial reference for genome assemble. The contigs identified as mitogenome sequences were manually examined for repeats at the beginning and end of the sequence to establish a circular mtDNA.

Locations of the protein coding genes (PCGs) were determined 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 identified by their similarity of inferred sequences to those of other published crustacean mtDNAs by BLAST search (http://www.ncbi.nlm.nih.gov/BLAST).

The gene map of the *S. panglao* mitogenome was generated with the program CGView (Stothard and Wishart, 2005). The mitochondrial genome has been deposited in the GenBank database under the accession numbers MG812382.

2.3. Sequence analysis

The A + T content values and nucleotide frequencies were computed using Editseq program from DNASTAR. The GC and AT skews were calculated according to the formulae by Perna and Kocher (1995):