The mitochondrial genome of the deep-sea axiid shrimp, *Eiconaxius baja* (Decapoda: Axiidae)

Ian V. Hughes, Avery S. Hiley and Greg W. Rouse

Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA, USA

**ABSTRACT**

Here, we present the first mitochondrial genome of *Eiconaxius baja*. The mitogenome contains 13 protein-coding genes (PCGs), two rRNA genes, and 22 tRNA genes. The total length of the complete *E. baja* mitochondrial genome is 16,212 base pairs, and the GC content is 26.82%. The gene order is consistent with that of *Eiconaxius serratus*, and most other members of Axiidea. Phylogenetic analysis based on 13 PCGs places *E. baja* sister to *E. serratus* within Axiidae.

The axiid shrimp genus *Eiconaxius* Bate, 1888 comprises more than 30 species found in deep oceans worldwide, often in association with hexactinellid sponges (Komai and Tsuchida 2012; Poore 2018; WoRMS 2022). The classification of *Eiconaxius* has been debated, including being placed in its own family Eiconaxiidae Sakai and Ohta 2005. However, molecular phylogenies and morphological evidence provide support for placing *Eiconaxius* in Axiidae Huxley, 1879 (Tsang et al. 2008; Kou et al. 2020). Although Axiidae was traditionally placed within Thalassindea Latreille, 1831, molecular phylogenies have challenged the validity of this taxon, and instead position Axiidae within Axiidea de Saint Laurent, 1979 (Tsang et al. 2008; Lin et al. 2012; Tan et al. 2017; WoRMS 2022). Of the 371 accepted species in Axiidea (WoRMS 2022), 11 have published mitochondrial genomes available in GenBank to date. Further molecular data will be useful for confirming the taxonomic placement of *Eiconaxius* and for resolving the relationships among species in the genus.

*Eiconaxius baja* Kensley, 1996 is an eastern Pacific species distributed from northern Baja California to the Channel Islands off California. To date, no genetic data have been available for *E. baja*, and its position within Axiidea remains unknown. A pair of *E. baja* individuals, resident in a glass sponge (Farreidae Gray, 1872), were collected at 500–1000 m depth on the slope of the San Juan Seamount (33.0391°N 121.0052°W) in October 2020. No ethical approval was required for collection (IACUC, UC San Diego, La Jolla, CA). The specimens were preserved in 95% ethanol and deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (https://siopapps.ucsd.edu/collections/bi/, contact: Greg Rouse, grouse@ucsd.edu), under the voucher SIO-BIC C14467. The objects of this study are to provide the first published genetic data for *Eiconaxius baja* and to further help resolve phylogenetic relationships within Axiidea.

DNA was extracted from tissue of the female specimen using the Zymo Research DNA-Tissue Miniprep kit (Zymo Research, Irvine, CA), following the manufacturer’s protocol. Extracted DNA was sequenced on the Illumina Novaseq6000 150 base pairs (bp) platform (Illumina, San Diego, CA) following library preparation by Novogene (en.novogene.com/), generating 7,993,403 paired-end raw reads of 150 bp each.

Sequence reads were trimmed (leading and trailing low quality or N bases below quality 3 were removed; reads were scanned with a four-base wide sliding window and deleted when the average quality per base dropped below 15; and reads under 36 bp long were dropped) and cleaned of adapters using Trimmomatic v. 0.39 (Bolger et al. 2014). A single mitochondrial genome with an average coverage of 59.83x was assembled from 7,853,206 paired-end reads using MitoFinder v. 1.4 (Allio et al. 2020), with The Invertebrate Mitochondrial Code (NCBI; transl_table = 5) specified as the organism genetic code used for translation of the 13 protein-coding genes (PCGs). Complete records for all RefSeq Decapoda Latreille, 1802 mitogenomes publicly available on NCBI were used as the reference file for MitoFinder. The assembly was annotated using the integrated MitoFinder pipeline with MEGAHIT v. 1.2.9 (Li et al. 2016) and ARWEN v. 1.2 (Laslett and Canbäck 2008) parameters, as well as the MITOS Web server (Bernt et al. 2013). Furthermore, a single circular mitochondrial genome was assembled using MITObim v. 1.9.1 (Hahn et al. 2013) and annotated using the MITOS Web server (Bernt et al. 2013); the MITObim assembly resulted in an additional 232 bp to the final contig. Both annotated assemblies were modified using Geneious 11.1.5 (http://www.geneious.com, Kearse et al. 2012). The final mitochondrial genome contains 16,212 bp and includes 13 PCGs,
two rRNA genes, and 22 tRNA genes. The GC content is 26.82%. The length of the control region (CR) varies among available Axiidea mitochondrial genomes, and ranges between 546 bp (Neaxius glyptocercus (von Martens, 1868)) and 2036 bp (Filhollianassa ceramica (Fulton & Grant, 1906)). In the E. baja mitogenome, the largest non-coding sequence (766 bp) was located between the trnI and trnQ genes, which is consistent with most other members of Axiidea, and may represent the CR.

The mitochondrial gene order of E. baja was identical to E. serratus and nine of the 11 Axiidea representatives for which data were available. The mitochondrial gene order of Trypaea australiensis Dana, 1852 and F. ceramicificera differ from E. baja and other members of Axiidea in that for these two taxa, trnaD, CR, and trnaI are located upstream of rrnS, and the PCG nad1 is positioned after rnl (Kou et al. 2020).

Amino acid sequences were selected for 13 mitochondrial PCGs of E. baja and 11 members of Axiidea, along with out-groups Upogebia major (De Haan, 1841 [in De Haan, 1833–1850]) and Shinkaia crosnieri Baba & Williams, 1998. Sequences of each PCG were aligned using MAFFT (Katoh and Standley 2013). The 13 PCG alignments were concatenated, and a maximum-likelihood tree was constructed with raxmlGUI v. 2.0.1 (Edler et al. 2021) using RaxML-NG with automatic partitions and substitution models (Figure 1). One thousand bootstrap replicate searches were used to determine node support values. These results support the monophyly of Axiidae, with the two species in Eiconaxius recovered as a clade that was sister to Calocaris macandreae Bell, 1846 [in Bell, 1844–1853].

Acknowledgements

We thank the captain and crew of the E/V Nautilus and the pilots of the ROVs Argus and Hercules for their invaluable assistance in sample collecting. We also thank the chief scientists Lisa Levin and Paul Jensen. Many thanks to Marina McCowin for DNA extraction and sample processing and to Charlotte Seid for accessioning the specimens into SIO-BIC.

Author contributions

GWR collected the samples, conceived, designed, and funded this study. The mitochondrial genome data were acquired assembled and annotated by ASH and IVH. The phylogenetic analysis and tree construction were performed by GWR and IVH. IVH drafted the manuscript with revisions from ASH and GWR. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Disclosure statement

The authors report no potential conflict of interest.

Funding

Ship time was provided by the Ocean Exploration Trust.
Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at [https://www.ncbi.nlm.nih.gov](https://www.ncbi.nlm.nih.gov) under the accession number OK448494. The associated BioProject, SRA, and Bio-Sample numbers PRJNA777625, SRX13074528, and SAMN22867214, respectively.

References

Allio R, Schomaker-Bastos A, Romiguier J, Prosdocimi F, Nabholz B, Delsuc F. 2020. MitoFinder: efficient automated large-scale extraction of mitogenomic data in target enrichment phylogenomics. Mol Ecol Resour. 20(4):892–905.

Bate C. 1888. Report on the Crustacea Macrura collected by the Challenger during the years 1873–76. Report on the scientific results of the voyage of HMS “Challenger” during the years 1873–76; (24): 1–157.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics. 30(15):2114–2120.

Edler D, Klein J, Antonelli A, Silvestro D. 2021. raxmlGUI 2.0: a graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol Evol. 12(2):373–377.

Hahn C, Bachmann L, Chevreux B. 2013. Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads – a baiting and iterative mapping approach. Nucleic Acids Res. 41(13):e129.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780.

Kease M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12):1647–1649.

Kensley B. 1996. New thalassinidean shrimp from the Pacific Ocean (Crustacea: Decapoda: Axiidae and Calocarididae). Bull Mar Sci. 59(3):469–489.

Komai T, Tsuchida S. 2012. Rediscovery and redescription of a sponge-associated axiid shrimp, *Eiconaxius acutifrons* Bate, 1888 (Crustacea: Decapoda: Axiidea). Zootaxa. 3393(1):27–40.

Kou Q, Xu P, Poore GC, Li X, Wang C. 2020. A new species of the deepsea sponge-associated genus *Eiconaxius* (Crustacea: Decapoda: Axiidae), with new insights into the distribution, speciation, and mitogenomic phylogeny of axiidean shrimps. Front Mar Sci. 7:469.

Laslett D, Canbäck B. 2008. ARWEN: a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics. 24(2):172–175.

Li D, Luo R, Liu CM, Leung CM, Ting HF, Sadakane K, Yamashita H, Lam TW. 2016. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods. 102:3–11.

Lin FJ, Liu Y, Sha Z, Tsang LM, Chu KH, Chan TY, Liu R, Cui Z. 2012. Evolution and phylogeny of the mud shrimps (Crustacea: Decapoda) revealed from complete mitochondrial genomes. BMC Genomics. 13(1):1–12.

Poore GC. 2018. Caribbean species of *Eiconaxius* (Decapoda: Axiidea: Axiidea). Zootaxa. 4524(1):139–146.

Sakai K, Ohita S. 2005. Some thalassiniid collections by R/V “Hakuhou-Maru” and R/V “Tansei-Maru”, University of Tokyo, in the Sulu Sea, Philippines, and in Sagami Bay and Suruga Bay, Japan, including two new species, one new genus, and one new family (Decapoda, Thalassinidea). Crustac. 78(1):67–93.

Tan MH, Gan HM, Lee YP, Poore GC, Austin CM. 2017. Digging deeper: new gene order rearrangements and distinct patterns of codons usage in mitochondrial genomes among shrimps from the Axiidea, Gebiidea and Caridea (Crustacea: Decapoda). PeerJ. 5:e2982.

Tsang LM, Lin FJ, Chu KH, Chan TY. 2008. Phylogeny of Thalassinidea (Crustacea, Decapoda) inferred from three rDNA sequences: implications for morphological evolution and superfam family classification. J Zool Syst Evol Res. 46(3):216–223.

WoRMS. 2022. *Eiconaxius* Bate, 1888; [accessed 2022 Apr 26]. [https://www.marinespecies.org/aphia.php?p=taxdetails&id=390862](https://www.marinespecies.org/aphia.php?p=taxdetails&id=390862)