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MITOGENOME REPORT

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The mitochondrial genome of the deep-sea pyramid urchin *Echinocrepis rostrata* (Echinoidea: Holasteroida: Pourtalesiidae)

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ABSTRACT

We present the mitochondrial genome of the deep-sea, epibenthic, irregular echinoid *Echinocrepis rostrata*, representing the first sequenced mitogenome of the order Holasteroida. The length of the complete *E. rostrata* mitochondrial genome is 15,716 base pairs, and its GC content is 34.87%. It contains 13 protein-coding genes, two rRNA genes, and 22 tRNA genes, whose order is identical to that of all other available echinoid mitogenomes. Phylogenetic analysis of available mitochondrial genomes, based on all coding loci, places *E. rostrata* as the sister group to spatangoids (heart urchins).

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Introduction

The genus *Echinocrepis* A. Agassiz, 1881 contains two species of irregular echinoids characterized by a uniquely high and pyramidal test (i.e. the main structure of the echinoid skeleton, which is generally either spherical or flattened in shape). The genus is part of the family Pourtalesiidae A. Agassiz, 1881 (Kroh and Mooi 2023), which includes exclusively deep sea (bathyal to abyssal) taxa that feed by plowing through the upper layers of sediment and ingesting detritus (Schultz 2017). *Echinocrepis cuneata* A. Agassiz 1878 is known only from two specimens collected in Antarctic waters by the H.M.S *Challenger* expeditions (Agassiz 1878). The remaining species, *Echinocrepis rostrata* Mironov 1973 (Figure 1A), is much better known, and is found across the North Pacific, from the Kamchatka Peninsula to the northwest of the Channel Islands in California, where it inhabits waters 3,315–5,020 m deep (Mironov 1973; Schultz 2017). *E. rostrata* is considered a deep-sea 'bulldozer' species, whose bioturbating activity plays an important role in determining the fate of the particulate organic carbon (POC) that reaches the ocean bottom. Its abundance and feeding ecology fluctuate with POC influx (Vardaro et al. 2009; Miguez-Salas et al. 2022) potentially affecting sediment geochemistry, nutrient availability in benthic ecosystems, and rates of carbon sequestration. Distinct color morphotypes of the species are known, yet molecular barcoding suggests these belong to a single species (Vardaro 2010).

Materials & methods

A specimen of *E. rostrata* was collected from the North Pacific Ocean (40.771504° N, 127.548792° W) during research expedition TN-403 by remotely operated vehicle (ROV) *Jason* aboard R/V *Thomas G. Thompson* (https://www.rvdata.us/search/cruise/TN403). No ethical approval was required for sampling. The specimen was collected on June 7th, 2022 from a depth of 3,232 m, slightly shallower than the reported depth range for the species. Upon arrival to the vessel, gonadal material was dissected, placed in 95% ethanol, and kept refrigerated. Ethanol-preserved test fragments and internal tissues are deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (SIO-BIC; https://scripps.ucsd.edu/benthic-invertebrate-collection, contact: Greg Rouse, grouse@ucsd.edu) under voucher SIO-BIC E11460.

DNA was extracted using the Zymo Research DNA-Tissue Miniprep kit (Zymo Research, Irvine, CA) and sequenced on the Illumina Novaseq 6000 platform (Illumina, San Diego, CA) following library preparation by Novogene (en.novogene.com). Species identity was confirmed by comparing the COX1 sequence (extracted from the assembled mitogenome) with publicly available data.

Genomic raw reads were trimmed or excluded using quality scores with Trimmomatic v0.39 (Bolger et al. 2014) under default settings. Mitogenome assembly was performed with MitoFinder v1.4 (Allio et al. 2020). The Echinoderm and Flatworm Mitochondrial Code (NCBI; transl_table = 9) was specified for the translation of protein-coding genes (PCGs).

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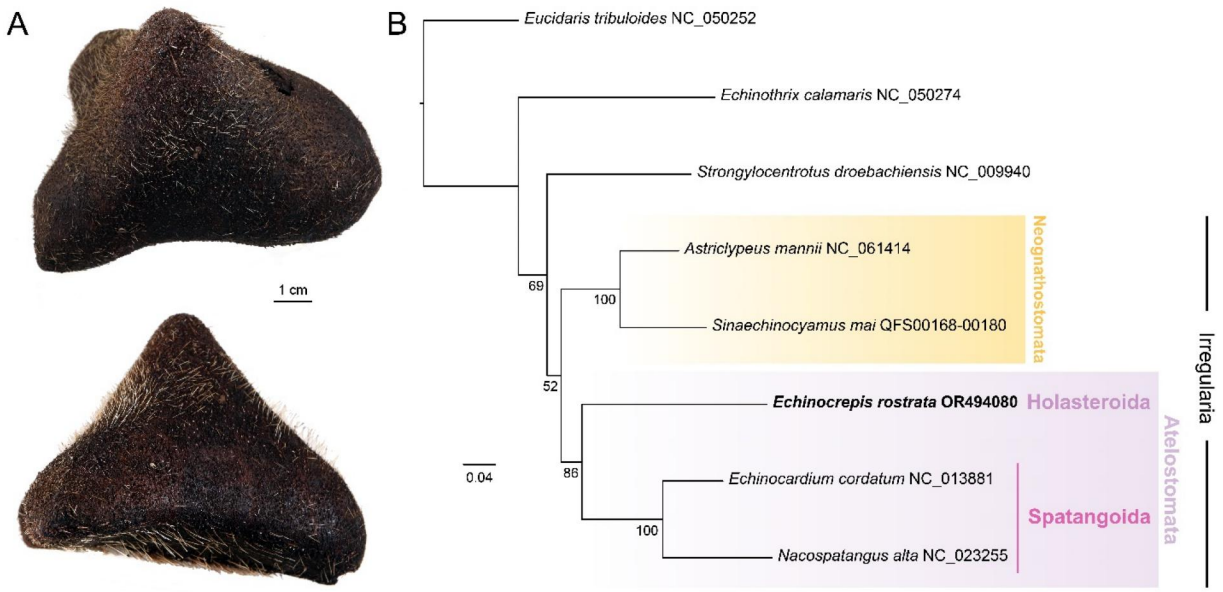

Figure 1. **A.** Aboral (top) and lateral (bottom) views of *Echinocrepis rostrata* (SIO-BIC E4015). Anterior margin toward the left. Photo by Greg Rouse. **B.** Phylogenetic position of *E. rostrata* (highlighted; accession number OR494080) among major echinoid clades using partitioned maximum likelihood inference with an amino acid dataset including all 13 mitochondrial protein-coding loci. Node numbers correspond to ultrafast bootstrap support values. The following sequences were used: *Eucidaris tribuloides* NC_050252 (unpublished), *Echinothrix calamaris* NC_050274 (Wakayama et al. 2019), *Strongylocentrotus droebachensis* NC_009940 (unpublished), *Astriclypeus mannii* NC_061414 (Shin et al. 2022), *Sinaechinocyamus mai* QFS00168 – GQF00180 (Lin et al. 2020), *Echinocerdium cordatum* NC_013881 (Perseke et al. 2010), and *Nacospatangus alta* NC_023255 (unpublished).

Complete records for all echinoid mitogenomes publicly available on NCBI's RefSeq (downloaded May 18th, 2023) were provided as a reference file during assembly (listed in Table S1). The assembled mitogenome was annotated using the integrated MitoFinder pipeline with MEGAHIT v1.2.9 (Li et al. 2016) and ARWEN v1.2 (Laslett and Canbäck 2008), and a second annotation was obtained using the MITOS Web server (Bernt et al. 2013). Coverage was estimated by mapping trimmed reads to the assembled contig using BBMap (part of BBTools v35.34, Bushnell 2014). Assemblies and annotations were imported into Geneious v11.1.5 (Kearse et al. 2012), which was used to circularize the mitogenome and manually edit annotations by comparing against the aforementioned echinoid mitogenomes.

Amino acid sequences for the 13 mitochondrial PCGs of *E. rostrata* were extracted and compiled with those of all four available irregular echinoid mitogenomes, as well as three regular echinoid outgroups. Individual genes were aligned using MAFFT v7.505 (Katoh and Standley 2013) and concatenated. A maximum likelihood tree was constructed with IQ-TREE v2.0.3 (Minh et al. 2020) using the best-fit partitioned model of evolution (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017), which merged loci into two partitions. One thousand replicates of ultrafast bootstrap (Hoang et al. 2018) were used to determine node support values.

Results

Percent identities of the obtained COX1 sequence to those published by Vardaro (2010) ranged between 99.5% and 100% (GenBank accession numbers GU228453-GU228465). This material was collected from Station M, West Pacific (35.16° N, 123.02° W), approximately 780 km south of the sampling locality of the specimen employed for mitogenome sequencing, and only 85 km away from the type locality of *E. rostrata* (Kroh and Mooi 2023), confirming the identity of the sequenced specimen.

A total of 13,376,922 paired-end reads of 150 base pairs (bp) were sequenced, of which 13,085,912 (97.8%) passed quality controls and were used for assembly. A single circular contig with an average coverage of 27.03x was obtained (Figure S1; 100 bp sliding window coverage range = 13.04–48.9). The mitochondrial genome of *E. rostrata* contains 15,716 bp and includes 13 PCGs, two rRNA genes, and 22 tRNA genes. The GC content is 34.87% and the control region (located between tRNA-Thr and tRNA-Pro) is 135 bp long. As in other echinoids, the ND4L coding sequence is likely initiated with an alternative codon (Quek et al. 2021). The mitochondrial gene order of *E. rostrata* (shown in Figure S2) is identical to that of all other known echinoid mitogenomes, which represents the ancestral gene order for Echinodermata (Perseke et al. 2010).

The result of the phylogenetic inference agrees with recent phylotranscriptomic studies (Mongiardino Koch et al. 2018, 2022), placing holasteroids as the sister-group to spatangoids (Figure 1B). Atelostomata, the clade defined by these two orders, is included within a monophyletic Irregularia, whose most closely-related lineage of regular echinoids is Echinacea (represented here by *Strongylocentrotus droebachiensis*).

Discussion

While a large number of echinoid mitogenomes are available, sequencing effort has mostly focused on regular echinoids that are used as model organisms in developmental and

genomic research. Data for irregular echinoids (sand dollars, heart urchins, and related groups) is much scarcer. The data presented here represent the first available mitogenome for a deep-sea irregular taxon, as well as the first for the order Holasteroida, within which pourtalesiid echinoids are placed. The structure of the mitogenome reinforces the known pattern of gene order conservation within Echinoidea. Phylogenetic results agree with the topologies of previous phylogenomic efforts, yet support values for deep nodes are low, including both Irregularia and the node directly subtending it, Carinacea (i.e. Irregularia + Echinacea). The fossil record of irregular echinoids stretches back to the Early Jurassic (Smith et al. 2006), while molecular estimates of divergence times place the origin of both aforementioned clades within the Triassic (Mongiardino Koch et al. 2022). Such ancient divergences might be beyond the reach of mitogenome datasets, suggesting that caution should be exerted when interpreting novel topologies obtained through such efforts (e.g. Shin et al. 2022; Sun et al. 2022).

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Author contributions

GWR and NMK conceived and designed the study. The mitochondrial genome data were sequenced, assembled, and annotated by ASH, MS, and NMK. The phylogenetic analysis was performed by NMK and MS. NMK and MS 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

No potential conflict of interest was reported by the author(s).

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Data availability statement

All molecular data reported here is available from NCBI (https://www.ncbi.nlm.nih.gov). The assembled mitogenome is available under accession number OR494080. Genomic raw reads are deposited under SRA number SRR25731399, part of BioProject PRJNA1008243. Associated BioSample number is SAMN37113738.

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