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MITOGENOME REPORT

The mitogenomes of two species of sea pigs, *Scotoplanes clarki* and *Protelpidia murrayi* (Elasipodida: Holothuroidea: Echinodermata)

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ABSTRACT
Mitochondrial genomes of *Scotoplanes clarki* and *Protelpidia murrayi* are presented, each with 13 protein coding genes, two rRNA genes, and 22 tRNA genes. That of *Scotoplanes clarki* has 15,909 base pairs and that of *Protelpidia murrayi* is 15,896 base pairs. There is a suspected tandem repeat region of undetermined length in the assemblies of both *Scotoplanes clarki* and *Protelpidia murrayi*. The gene order of both mitogenomes is identical to that of other Elasipodida. Phylogenetic analysis revealed that *Protelpidia murrayi* lies within the *Scotoplanes* clade, suggesting that *Scotoplanes* is paraphyletic. Sequencing of the type species, *Scotoplanes globosa*, is needed to confirm if *Protelpidia murrayi* should be renamed as *Scotoplanes murrayi*.

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KEYWORDS
Sea cucumber; Elpidiidae; phylogeny; deep sea; taxonomy

Introduction

Elpidiidae (Holothuroidea, Elasipodida) consists of 13 genera (WoRMS 2024), including two that together form a group colloquially known as sea pigs, *Scotoplanes* (Théel, 1882) and *Protelpidia* (Gebruk, 1983). Members of Elpidiidae are benthic detritivores that may form large populations on the seafloor, which have been found to play an important role in the movement and recycling of organic carbon in deep-sea ecosystems, known as bioturbation (Huffard et al. 2016). Furthermore, sea pigs have been found to act as a shelter for juvenile decapods from predators on abyssal plains (Barry et al. 2017) and are a known host of parasitic eulimid gastropods (Takano et al. 2018). Prior to recent genetic sequencing advancements (Miller et al. 2017; Mongiardino Koch et al. 2023), analysis of microscopic calcium carbonate ossicles found in the body wall of sea cucumbers was often the primary method to distinguish species, as they exhibit diversity that is often consistent within defined species. Gebruk (1983) analyzed ossicle morphology of the sea pig *Scotoplanes murrayi* Théel, 1879 and erected the genus *Protelpidia* for it, renaming it as *Protelpidia murrayi* (Théel, 1879). *Protelpidia* was proposed as a sister group to a *Scotoplanes* clade, with *Protelpidia murrayi* as the only known species in *Protelpidia* (Gebruk, 1983). To date, the genetic data publicly available for the sea pig genera *Scotoplanes* and *Protelpidia* is limited, with only three mitochondrial genomes for *Scotoplanes* published as *Scotoplanes* sp. (Takano et al. 2019), all likely the same species and based on the geographical locality represent *Scotoplanes theeli* Ohshima, 1915, and no mitochondrial genomes for *Protelpidia*. Sequencing additional mitochondrial genomes from confidently identified voucher specimens within *Scotoplanes* and *Protelpidia* is essential to further understand the relationship between sea pigs and their placement within Elasipodida.

In this study, we present the first mitochondrial genomes of the sea pig species *Scotoplanes clarki* Hansen, 1975 (Figure 1A) and *Protelpidia murrayi* (Figure 1B) to expand the database of available mitochondrial genome data for *Scotoplanes* and gain insight into the phylogenetic relationship between the genera *Protelpidia* and *Scotoplanes*.

Materials and methods

A single *Protelpidia murrayi* specimen was collected from off the South Sandwich Islands, South Atlantic Ocean (59.386° S 27.313° W), trawled at a depth of 403–501 m and recovered dead. A single *Scotoplanes clarki* specimen was collected from the Pescadero Basin, Gulf of California, Mexico (23.9596° N 108.863° W) at a depth of 3676 m and was dead on recovery. Both specimens were fixed in 95% ethanol and deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (https://sioapps.ucsd.edu/collections/bi/, contact: Greg Rouse, grouse@ucsd.edu) under the vouchers SIO-BIC E5110 for *Protelpidia murrayi* and SIO-BIC E6794 for *Scotoplanes clarki*.

DNA was extracted from tissue of both specimens using the Zymo Research DNA-Tissue Miniprep kit (Zymo Research, Irvine, CA). Library preparation was performed by Novogene (en.novogene.com/) and sequenced using Illumina Novaseq6000 (Illumina, San Diego, CA) to produce paired-end reads with

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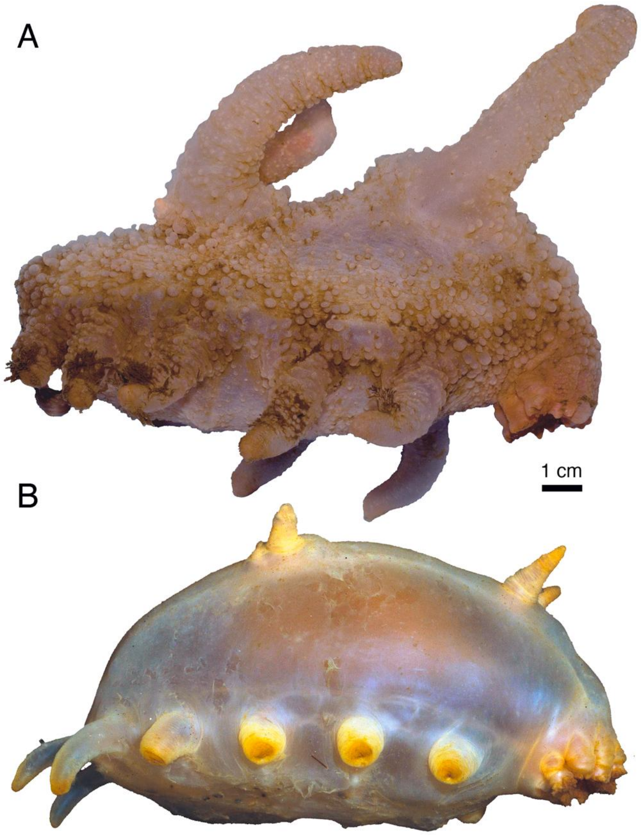

Figure 1. (A) Lateral photo of *Scotoplanes clarki* (SIO-BIC E6794) taken by Greg Rouse. (B) Lateral view of *Protelpidia murrayi* (SIO-BIC E5110) taken by Greg Rouse.

lengths of 150 base pairs (bp). The total number of paired-end read pairs obtained was 6,916,773 for *Scotoplanes clarki* and 12,550,144 for *Protelpidia murrayi*. Raw sequence reads were trimmed for Illumina TruSeq3 adapters using Trimmomatic v. 0.39 (Bolger et al. 2014). Reads less than 36 bp in length after adapter trimming were removed, resulting in approximately 98% of raw reads retained for *Scotoplanes clarki* and approximately 97% of raw reads retained for *Protelpidia murrayi*. The retained reads were assembled into circularized mitogenomes using NOVOPlasty v. 4.3.4 (Dierckxsens et al. 2017). The seed used for each assembly was the gene for cytochrome *c* oxidase subunit 1 (*CO1*) obtained from the same specimens using Sanger sequencing with holothurian *CO1* primers. The average coverage of the *Scotoplanes clarki* and *Protelpidia murrayi* assemblies reported by NOVOPlasty was 81× and 73×, respectively. The reads were mapped back to the assembly using the BWA-MEM algorithm of BWA v. 0.7.15 (Li 2013) and SAMtools v. 1.17 (Danecek et al. 2021) was used to calculate depth of coverage to create a coverage map (Figure S1). The resulting mitochondrial genomes were annotated using the MITOS WebServer (Bernt et al. 2013). The annotated assemblies were manually edited and finalized in Geneious Prime 11.1.5 (Biomatters Ltd., Auckland, New Zealand).

All GenBank available (accessed November 13, 2023) Elasipodida mitochondrial genomes were used: MH208310.1 (=NC_040968.1) *Benthodytes marianensis* (Mu et al. 2018), KF915304 *Peniagone* sp. YYH-2013 (unpublished), LC416624 *Scotoplanes sp*. TT-2017 (Takano et al. 2019), LC416626 *Scotoplanes sp*. H8 (Takano et al. 2019), and LC416625 *Scotoplanes sp*. H5 (Takano et al. 2019). The outgroup Holothuriida mitochondrial genome used was FN562582 *Holothuria forskali* (Perseke et al. 2010) The 13 protein coding genes were translated into amino acid sequences in Mesquite v. 3.81 (Maddison and Maddison 2023) and aligned separately using MAFFT v. 7 with the G-INS-i alignment method (Katoh and Standley 2013). The alignments were loaded into raxmlGUI v. 2.0.1 (Edler et al. 2021) and ModelTest-NG (Darriba et al. 2020) was used to determine the best-fit amino acid substitution model by AIC for amino acid sequences encoded by each gene (Figure S2). A partitioned maximum likelihood analysis using RAxML-NG v. 1.1.0 (Kozlov et al. 2019) was performed with *Holothuria forskali* Delle Chiaje, 1824 as an outgroup, 10 runs, and 1,000 bootstrap replicates.

Results

The mitochondrial genome of *Scotoplanes clarki* was found to be 15,909 bp with a GC content of 26.79% (Figure 2A). The mitochondrial genome of *Protelpidia murrayi* was 15,896 bp with a GC content of 27.07% (Figure 2A). Each mitochondrial genome included 13 protein-coding genes, two rRNA genes, and 22 tRNA genes (Figure 2). A large spike in read coverage in both mitochondrial genomes was present at approximately the 8000–9000 bp region (Figure S1). This region did not overlap any genes or other mitochondrial genome features The phylogenetic analysis showed *Protelpidia murrayi* grouped with *Scotoplanes clarki*, forming a clade sister to the other three *Scotoplanes* sp. mitogenomes (Figure 3).

Discussion and conclusion

The mitochondrial gene order for both species was identical to all six Elasipodida mitochondrial genomes available on GenBank (accessed November 13, 2023) (Mu et al. 2018; Takano et al. 2019). The read coverage spike in both *Scotoplanes clarki* and *Protelipida murrayi* likely indicates a tandem repeat region between genes *NAD5* and *NAD6*. Long-read sequencing methods would likely be necessary to resolve the repeats within this region with confidence.

The placement of *Protelpidia murrayi* within a highly supported *Scotoplanes* clade suggests that *Scotoplanes* is a paraphyletic group with respect to *Protelpidia*. More extensive sequencing of Elasipodida, including the type species of *Scotoplanes*, *Scotoplanes globosa*, is necessary to further investigate the molecular relationships within the sea pig genera *Scotoplanes* and *Protelpidia*. The paraphyletic status of *Scotoplanes* and the grouping of *Scotoplanes clarki* with *Protelpidia murrayi* may suggest that the erection of the genus *Protelpidia* was unjustified and leaves *Scotoplanes* paraphyletic. Sequencing the mitochondrial genome of the type species, *Scotoplanes globosa,* and analysis of the resulting phylogenetic placement is necessary to fully determine if *Protelpidia murrayi* should be renamed as *Scotoplanes murrayi*.

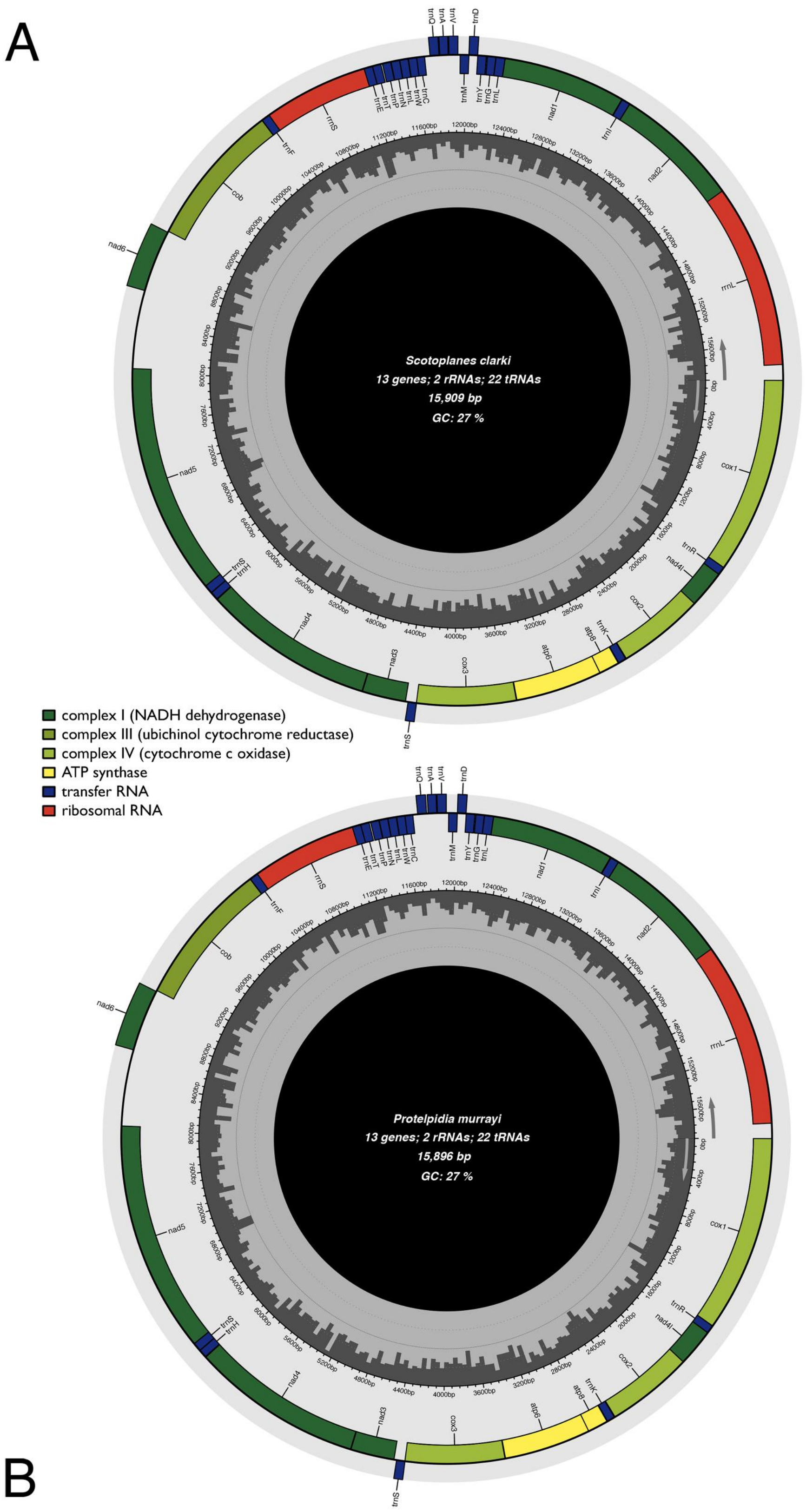

Figure 2. Mitochondrial genome maps of (A) *Scotoplanes clarki* (SIO-BIC E6794) and (B) *Protelpidia murrayi* (SIO-BIC E5110) produced using Chloroplot (Zheng et al. 2020). The arrangement of features is shown on the outermost ring, with the direction of transcription being clockwise on the outside of the ring and counterclockwise on the inside of the ring, as indicated by the arrow. GC content is represented by the histogram around the center of each map.

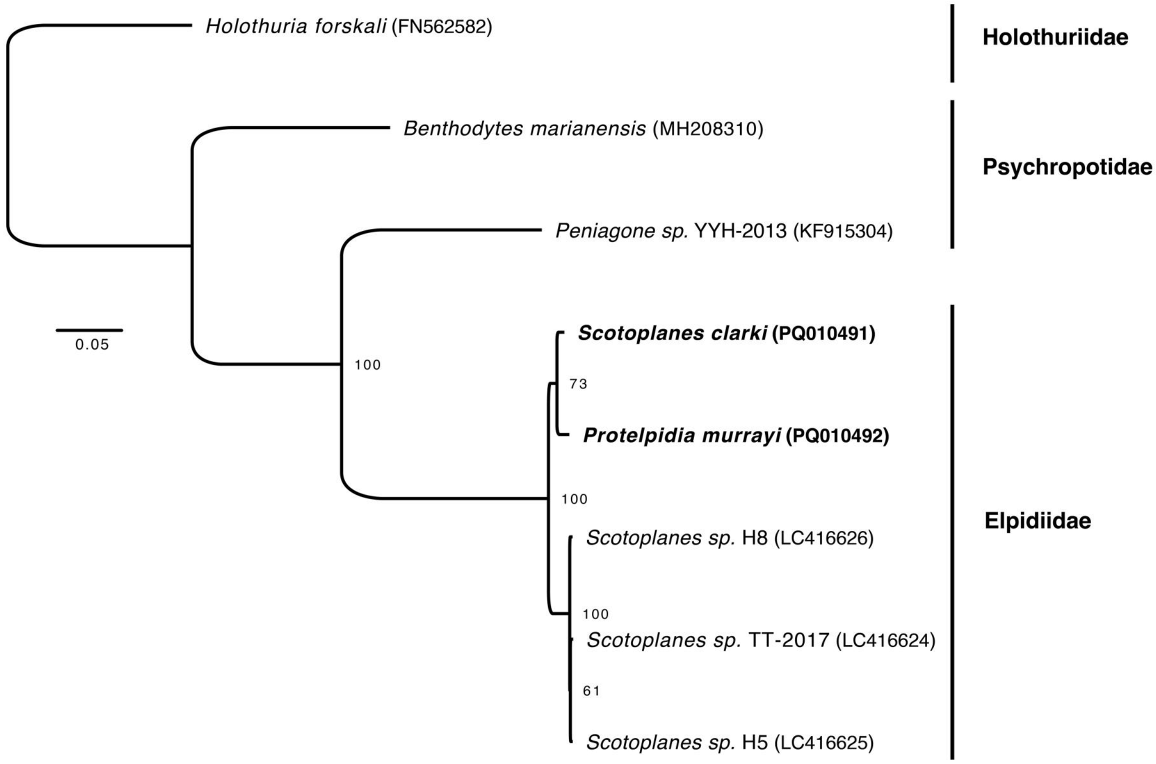

Figure 3. (A) Maximum likelihood tree based on amino acid sequences of 13 mitochondrial protein coding genes of *Scotoplanes clarki* (PQ010491), *Protelpidia murrayi* (PQ010492), and 6 additional holothuroids. Bootstrap values are indicated for each node. The following other mitogenomes were used: *Holothuria forskali* FN562582 (Perseke et al. 2010), *Scotoplanes* sp. LC416624, LC416625, LC416626 (Takano et al. 2019), *Benthodytes marianenesis* Li, Xiao, Zhang & Zhang, 2018 MH208310 (Mu et al. 2018), and *Peniagone* sp. KF915304 (Huo et al. unpublished).

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Author contributions

This study was funded and developed by GWR. The samples in this study were collected by GWR. CNM assembled and annotated the mitochondrial genome data. CNM and GWR performed phylogenetic analysis. The manuscript was drafted by CNM and revised by GWR. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

Disclosure statement

No potential competing interest was reported by the authors.

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Data availability statement

The complete mitochondrial genomes for *Scotoplanes clarki* and *Protelpidia murrayi* are available in NCBI GenBank (https://www.ncbi.nlm.nih.gov) under the accession numbers PQ010491 and PQ010492, respectively. The associated BioProject is PRJNA1136884. The BioSample and SRA accessions for *Scotoplanes clarki* are SAMN42595034 and SRR29886391, respectively. The BioSample and SRA accessions for *Protelpidia murrayi* are SAMN42652193 and SRR29895891, respectively.

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