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MITOGENOME REPORT

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Complete mitochondrial genome and phylogenetic analysis of *Cirriformia tentaculata* (Annelida, Polychaeta, Cirratulidae) from Weihai, Shandong, China

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ABSTRACT

Cirriformia species usually inhabit intertidal zones and deep-sea sediments. Their accurate identification has proven to be challenging. Here, we present the complete mitochondrial genome of one *Cirriformia tentaculata*_Montagu 1808 specimen collected from China. The total length of the complete mitochondrial sequence of *C. tentaculata* is 15,516 bp and consists of 13 protein-coding genes (PCGs), 23 tRNA genes, two rRNA genes, and an A + T rich region (64.20%). All PCGs begin with the typical ATN start codon, except for *cox1*, which uses TTG. TAA or TAG serve as termination codons for twelve PCGs, while *nad5* terminates with an incomplete codon, T. The phylogenetic tree revealed a close relationship between *C. tentaculata* in this study, and *Cirriformia* cf. *tentaculata* and *Timarete posteria* from Korea. The information will assist in the future identification and understanding of this species and offers a novel point of reference for identifying *Cirriformia* species, and phylogenetic studies.

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Mitogenome; phylogeny analysis; Cirratulidae; *Cirriformia tentaculata*

Introduction

Cirratulidae is one of the important taxa of Terebellida, comprising 22 genera and 387 species. The genus *Cirriformia* is a relatively small taxon within the family Cirratulidae. Worms of the genus *Cirriformia* are segmented bristle-bearing worms, and are found in a variety of habitats, particularly intertidal areas and deep-sea sediments (George 1964). Certain species of Cirratulid worms can survive in polluted environments that are contaminated by organic substances and have therefore been recognized as bioindicators of marine environmental pollution (Çinar 2007). Due to morphological similarity and some degree of variation, it is arduous to ensure the accuracy of species identification in the genus *Cirriformia* by morphology alone. Moreover, a limited number of molecular biology studies have been undertaken on the *Cirriformia* species to date (Rousset et al. 2004; Magalhães et al. 2014; Cowart et al. 2015; Lobo et al. 2016; Weidhase et al. 2016; Grosse et al. 2020; Struck et al. 2023). Here, we sequenced the mitogenomes of *Cirriformia tentaculata*_Montagu 1808 from China (Figure 1). This species was collected in the culture pond of *Apostichopus japonicus*_Selenka 1867 and considered as a new enemy of *A. japonicus*. This study presents a comprehensive analysis of the structural details of the mitochondrial genome of *C. tentaculata* and its phylogenetic relationship with other Polychaeta species, which would provide a reference for further research on its classification status and resource conservation.

Materials and methods

The samples were collected from a sea cucumber culture pond in Weihai, Shandong Province, China (36°57′14.1″N, 122°06′29.7″E) in 2023. The specimen was deposited into Fishery Barcode Database of China under voucher number Ysfri-Z0121 (Prof. Shufang Liu is the contact person: liusf@ysfri.ac.cn). The collected samples were stored in 95% ethanol to maintain their genomic integrity. Total genomic DNA was extracted from the entire body using a DNeasy tissue kit (Qiagen, Beijing, China) following the manufacturer's protocols. After DNA isolation, 1 μg of purified DNA was fragmented to 500 bp using the Covaris M220 system, used to construct short-insert libraries according to the manufacturer's instructions (TruSeq™ Nano DNA Sample Prep Kit, Illumina), and then sequenced on an Illumina NovaSeq 6000 platform (BIOZERON Co., Ltd, Shanghai, China) with 150 bp paired-end reads length. Mitochondrial genome assembly was performed using GetOrganelle v1.7.5 software (https://github.com/Kinggerm/GetOrganelle) (Jin et al. 2020).

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Figure 1. Reference image of *Cirriformia tentaculata* taken by Hongjing Zhao.

The MITOS (http://mitos.bioinf.uni-leipzig.de/index.py) online software was used to forecast the mitochondrial genome for coding proteins, tRNA, and rRNA genes (Bernt et al. 2013). Sample genomes were visualized in a circular plot using the software CGView (https://proksee.ca/) (Stothard et al. 2019). AT skew and GC skew were calculated as follows: AT skew = (A-T)/(A + T) and GC skew = (G-C)/(G + C) (Hassanin et al. 2005). The base composition of the mitochondrial genome was calculated using MEGA 11. A phylogenetic tree was constructed for the mitochondrial genome sequences of 17 Polychaeta species using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model in MEGA 11.

Results

A total of approximately 11.16 Gb clean data were generated with 98.55% exhibited high quality (>Q20). The complete

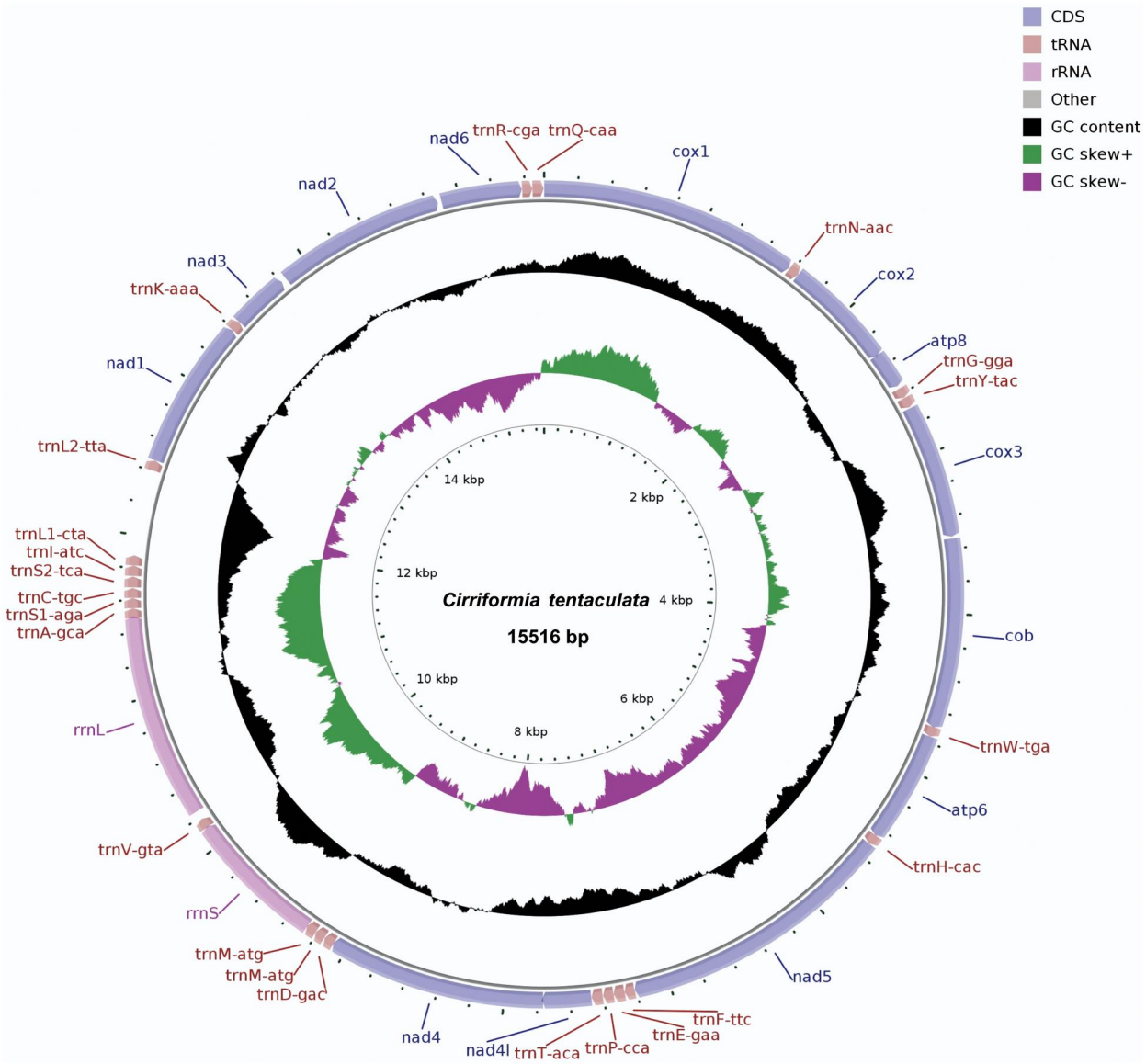

Figure 2. Mitogenome map of *Cirriformia tentaculata* generated in this study. The outer circle displays the positional coordinates of genes, ncRNAs, and other genomic components, each with its corresponding name. The inner circle indicates the GC skew and genomic GC content. Green illustrates GC skew + and purple represents GC skew-. black indicates GC content, with peaks protruding outwards signifying that the GC content of the region is higher than the average GC content of the whole genome, and vice versa.

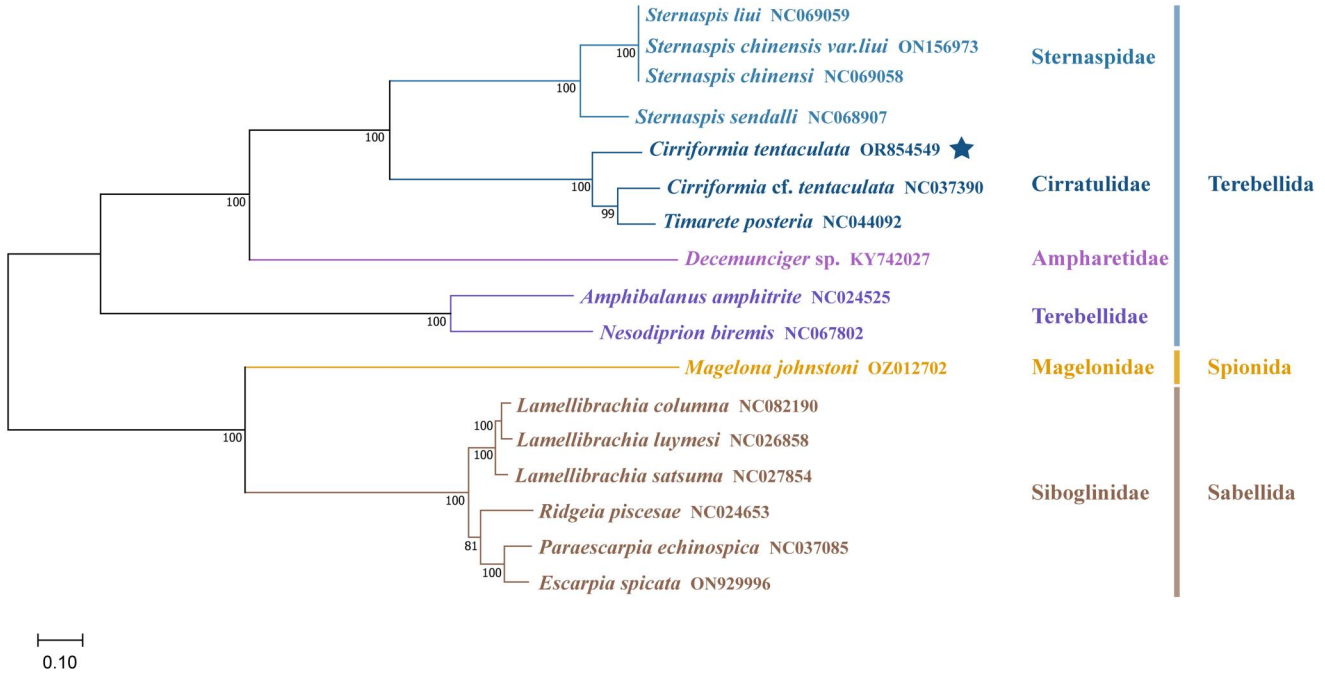

Figure 3. Maximum likelihood phylogenetic tree based on *Cirriformia tentaculata* mitogenome obtained from this investigation (marked with an asterisk), as well as 16 other polychaeta mitogenomes from NCBI. The accession numbers are given next to the species name and the corresponding references are listed as below: NC069059, ON156973, NC069058, NC068907, OR854549 (this study), NC037390 (Choi et al. 2017), NC044092 (Kim et al. 2019), KY742027 (Bernardino et al. 2017), NC024525 (Shen et al. 2015), NC067802 (Niu et al. 2022), OZ012702, NC082190 (McCowin et al. 2023), NC026858 (Li et al. 2015), NC027854, NC024653 (Jumin et al. 2016), NC037085 (Sun et al. 2018), ON929996 (McCowin et al. 2023). The branches are labeled with bootstrap values from 1000 replications, while distinct colors represent different tribes.

mitogenome of *C. tentaculata* (GenBank accession number OR854549) is 15,516 bp in length, containing 13 protein coding genes (PCGs), 23 transfer RNAs (tRNAs), and 2 ribosomal RNAs (rRNAs), which constituted 72.67%, 9.42% and 13.67%, correspondingly, of the complete mitochondrial length (Figure 2). The mitogenome read coverage depth map are shown in Supplementary Figure S1. The nucleotide composition of the base pairs found in the sequence was 31.36% A, 32.84% T, 12.55% G, and 23.25% C. The A + T content was significantly higher (64.20%) than the G + C content (35.8%). The AT-skew and GC-skew values in the whole mitochondrial genome were −0.0231 and −0.2989, respectively, suggesting a preference for T and C bases throughout the genome. Nevertheless, the GC-skew is more pronounced than the AT-skew, with the AT-skew being negligible.

The 13 PCGs discovered in this sequence contained three start codons: TTG, ATA, and ATG. TTG served as the initiatory codon for the *cox1*, while ATA acted as the initiation codon for the *atp8*, *cob*, and *nad6*. Nine PCGs (*cox2*, *cox3*, *atp6*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4l*, and *nad5*) began with the ATG start codon. The *atp6* utilized TAG as its termination codon, and the *nad5* concluded with an incomplete T codon. As for the remaining 11 PCGs, they all had TAA as their termination codon. Within the tRNAs, the length varied between 61–68 bp, with an overall length totaling 1461 bp. Except for tRNA-R and tRNA-S1, which lacked the DHU arm, all other tRNAs had the typical cloverleaf-shaped structure.

Maximum-Likelihood phylogenetic tree for the mitochondrial genome of 17 Polychaeta species was constructed (Figure 3). The results found that the Cirratulidae were in a distinct cluster, while *Cirriformia* cf. *tentaculata_* (Choi et al. 2017) and *Timarete posteria_* (Choi et al. 2018) from Korea formed a cohesive unit before joining with *C. tentaculata* from China in a cluster.

Discussion and conclusions

The study presents the initial comprehensive mitochondrial genome of *C. tentaculata*, which supplies the first genomic reference data for the genus *Cirriformia* in China. Even though *C. tentaculata*, *C.* cf. *tentaculata*, and *T. posteria* show a close relationship, the NCBI comparison reveals that they are distinct species. Accurately identifying *C. tentaculata* can be challenging due to the limited availability of publicly accessible mitochondrial sequences for this species. The results provide an important basis for the identification of *C. tentaculata* and enhance the existing *Cirriformia* mitochondrial genome database. The augmented species diversity facilitates molecular identification, furthers comprehension of the evolutionary status of Cirratulidae, and aids phylogenetic analyses.

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Ethical approval

All material was collected under the appropriate collection permits issued by the Institutional Animal Care and Use Committee from the Yellow Sea Fisheries Research Institute, CAFS (YSFRI-2024001).

Authors' contributions

XR, HZ, and JW gathered and classified the samples. HZ conducted data analysis and authored the manuscript, with contributions from JG. YW, ML, CY and QC developed and organized the project, and verified the final version for publication. The manuscript has been reviewed and approved by all authors.

Disclosure statement

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

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Data availability statement

The genomic sequencing data that underpin the conclusions of this investigation are openly accessible in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov/) under accession number OR854549. The associated BioProject, Biosample, and SRA numbers are PRJNA1059232, SAMN39201222, and SRR27392159, respectively.

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