MITOCHONDRIAL DNA PART B: RESOURCES
2023, VOL. 8, NO. 9, 985–988
https://doi.org/10.1080/23802359.2023.2241696

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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome of a marine polychaete, *Prionospio* cf. *japonica* (Annelida: Spionidae)

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ABSTRACT

Prionospio Malmgren 1867 is one of the abundant genera of the family Spionidae Grube, 1850. Despite its rich diversity, information on their complete mitochondrial genome has remained unknown. In this study, we determined the complete mitochondrial genome of a spionid polychaete, *Prionospio* cf. *japonica* Okuda 1935. The specimen was collected from the fine sand in the intertidal zone of South Korea. The mitogenome consists of 15,267 base pairs, harboring 13 protein-coding genes (PCGs), 22 transfer RNAs, and two ribosomal RNAs. The maximum-likelihood phylogenetic tree based on the 11 PCGs showed that *Prionospio* cf. *japonica* grouped with other spionid polychaetes and formed a monophyletic group. Also, the mtDNA of *P.* cf. *japonica* was more closely related to that of non-polydorin spionid, *Marenzelleria neglecta*, than polydorin spionids. The molecular data will be valuable for studying evolutionary relationships among annelids.

ARTICLE HISTORY
Received 30 November 2022
Accepted 21 July 2023

KEYWORDS
Prionospio cf. *japonica*; illumina sequencing; mitogenome; mtDNA; phylogeny; Korea

1. Introduction

Prionospio Malmgren 1867 is one of the most diverse polychaetous annelid groups belonging to the family Spionidae Grube, 1850. More than 100 species are currently known worldwide (Read and Fauchald 2022). Among them, *P. japonica* Okuda 1935 was originally described in the brackish water of lakes on the southern coast of Japan. This species has been reported by several authors in Northeast Asia, including China, Japan, and Korea (Imajima and Hartman 1964; Paik 1975, 1982; Zhou and Li 2009; Lee et al. 2020). It is morphologically characterized by having four pairs of apinnate branchiae (first branchiae distinctly long), prostomium truncates anteriorly with a small median peak, and hooded hooks with four to five pairs of small teeth above the main fang (Okuda 1935). Recently, Lee et al. (2020) determined the mitochondrial and nuclear gene fragments of *P. japonica* based on the specimens collected from the Yellow Sea, Korea. Later, Abe and Sato-Okoshi (2021) provided the molecular information of *P. japonica* collected from Sendai Bay, Japan.

Complete mitogenome sequences provide valuable evolutionary information (Chen et al. 2016). To date, the complete mitogenome sequences of five spionid species, *Boccardiella hamata* (Webster 1879) (Lee et al. 2021), *Marenzelleria neglecta* (Sikorski and Bick 2004; Gastineau et al. 2019), *Polydora brevipalpa* Zachs, 1933, *Polydora websteri* Hartman in Loosanoff & Engle, 1943 (Ye et al. 2021), and *Polydora hoplura* (Claparède 1868; Lee and Lee 2022) have been reported. In this study, we determined the complete

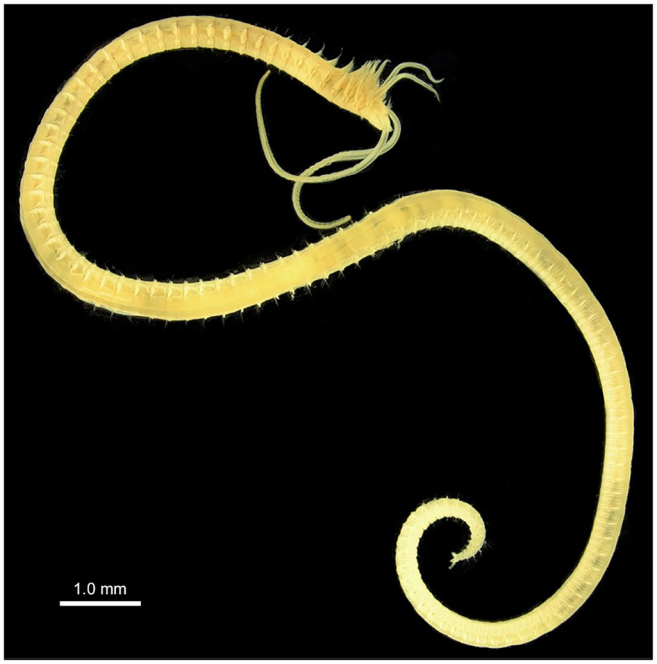

Figure 1. Image of *Prionospio* cf. *japonica* Okuda 1935 (sample collected from the Yellow Sea of Korea in January 2021; photo by Geon Hyeok Lee).

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2023.2241696.

mitogenome sequence of *P.* cf. *japonica* based on a Korean specimen.

2. Materials and methods

A single adult *Prionospio* sample (Figure 1) was collected from fine sand in the intertidal zone at Eurwang-dong, Incheon, South Korea (37°24′20.8"N 126°24′49.4"E) using a 500 μm-mesh sieve. Morphological observations were conducted using a stereomicroscope (Leica, Wetzlar, Germany). Species identification was performed based on morphological characteristics. A voucher specimen was deposited at the National Institute of Biological Resources in South Korea (deposit number: NIBRIV0000876631; Url: https://www.nibr.go.kr; Contact person: Min Seock Do, viper@korea.kr). Mitochondria were isolated from live individuals, using the Qproteome Mitochondria Isolation Kit (Qiagen). Mitochondrial DNA (mtDNA) was extracted from the mitochondria using a LaboPass Tissue Mini (Cosmo GENETECH, Seoul, South Korea). Whole-genome amplification (WGA) of the extracted mtDNA was performed using the REPLI-g Mitochondrial DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The amplified mtDNA was sequenced using Illumina HiSeq 4000 (Macrogen, Seoul, Korea), and assembled using Geneious 8.1.9 (Biomatters, Auckland, New Zealand) and NovoPlasty v. 2.7.1 (Dierckxsens et al. 2017). The read coverage depth map, assembled using the Geneious program, is presented in Supplementary Figure 1. The complete mitogenome was annotated using the MITOS Webserver (Bernt et al. 2013). The extracted mtDNA was deposited in the DNA collection of the National Institute of Biological Resources in South Korea (deposit number: NIBRGR0000645180). The selection of protein-coding genes (PCGs) was conducted using DAMBE 7.0.35 (Xia 2017). DAMBE 7.0.35 was used to determine whether the molecular data is suitable for phylogenetic analysis. A maximum-likelihood tree was constructed using IQ-TREE with the GTR + F + R4 model with 1,000 ultrafast bootstrap replicates (Kalyaanamoorthy et al. 2017; Hoang et al. 2018). Errantia was used as an outgroup taxon (*Perinereis aibuhitensis*). The mitogenome sequence obtained was registered in GenBank (GenBank accession number ON470577).

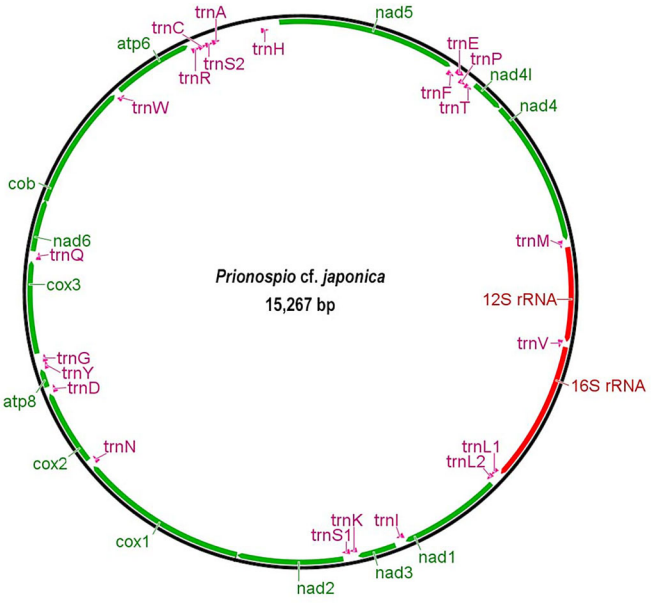

Figure 2. Mitochondrial genome map of *Prionospio* cf. *japonica* Okuda 1935.

3. Results and discussion

3.1. Species identification

The most striking characteristics of the Korean specimen are having four pairs of apinnate branchiae (first branchiae distinctly long), prostomium truncates anteriorly with a small median peak, and hooded hooks with four to five pairs of small teeth above the main fang. In this respect, the Korean specimen agrees well with the original description of *P. japonica* (Okuda 1935). However, the pairwise genetic distance of gene fragments between the materials from Korea and *P. japonica* from Japan was identical in 18S rDNA but

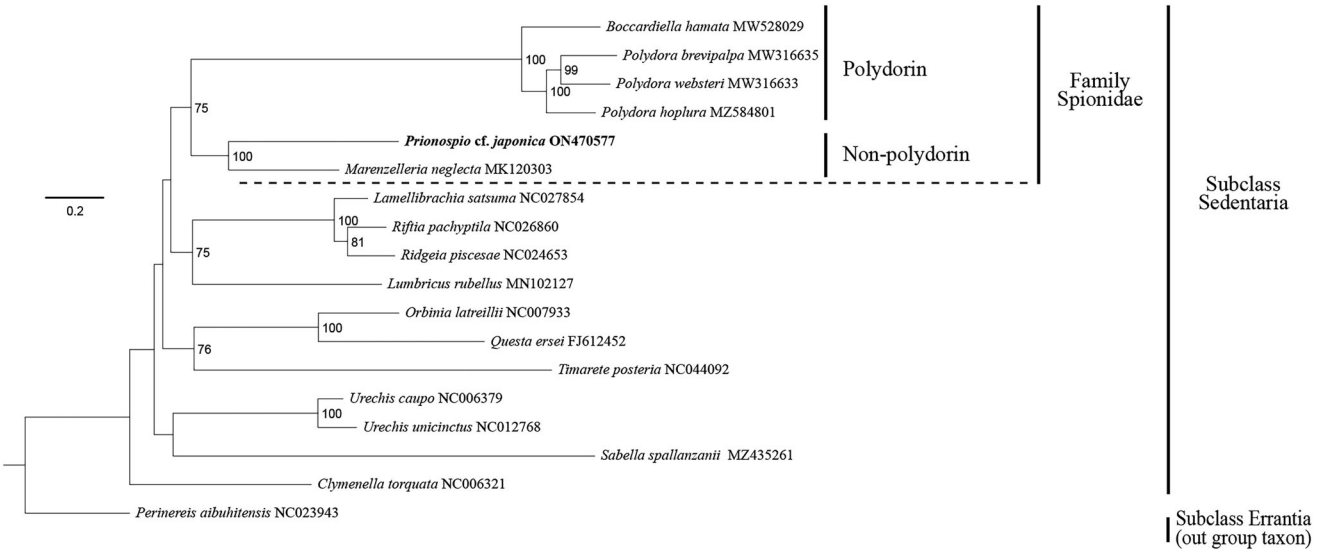

Figure 3. The consensus phylogenetic relationship of *Prionospio* cf. *japonica* (ON470577, in bold) and other annelids. *Perinereis aibuhitensis* was used as an outgroup for tree rooting. Bootstrap values of > 70% as percentage of 1000 bootstrap replicates are shown on each node.

showed 8.1% difference in 16S rDNA. The levels of inter-specific differentiation in 16S rRNA are comparable to those observed among other polychaete species, which usually exceed 5% (Wiklund et al. 2009; Álvarez-Campos et al. 2017; Meißner et al. 2017). This result indicated that these are different species. Nevertheless, no morphological differences were observed between the Korean and Japanese specimens (Okuda 1935). Therefore, the authors referred to the Korean samples as *P.* cf. *japonica* in this study.

3.2. Genomic analysis results

The newly determined complete mitogenome of *P.* cf. *japonica* was 15,267 bp in length and contained 13 PCGs, 22 transfer RNAs (tRNAs), two ribosomal RNAs (rRNAs), and one control region (AT-rich region) (Figure 2). In the segment between *nad6* and *nad5*, there is a total of 500 bp intergenic spacer that may correspond to the control region. The overall base composition of Korean *P.* cf. *japonica* is 32.4% A, 16.2% C, 16.2% G, and 35.3% T, revealing a G + C content of 32.4%.

3.3. Phylogenetic analysis

To infer the molecular phylogenetic relationships, a maximum-likelihood tree was constructed based on the concatenated nucleotide sequences of 11 protein-coding genes (*cox1*, *cox2*, *cox3*, *cytb*, *atp6*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4l*, and *nad5*) from 16 annelids, including the mtDNA sequence obtained in the present study. The concatenated mtDNA sequences of *P.* cf. *japonica* were grouped with those of the other five species of the family Spionidae (*Boccardiella hamata*, *Marenzelleria neglecta*, *Polydora brevipalpa*, *Polydora hoplura*, and *Polydora websteri*) (Figure 3). *Prionospio* cf. *japonica* was more closely related to *Marenzelleria neglecta*, one of the non-polydorin spionids (worms without modified spines in the fifth chaetiger), than to the other four polydorin spionids in the analysis (worms with modified spines in the fifth chaetiger).

4. Conclusion

The assembled mitogenome of *P.* cf. *japonica* was 15,267 bp in length and contained 13 PCGs, 22 transfer RNAs, two ribosomal RNAs, and one control region. The phylogenetic analysis of 11 protein-coding genes showed that the sequences of the Korean *P.* cf. *japonica* were grouped with those of the other species belonging to the family Spionidae. The newly determined complete mitogenome provides valuable data for further taxonomic and phylogenetic studies.

Ethical approval

This study did not involve endangered or protected species. All the experiments involving animal samples were approved by the animal care and use committee of Inha University (Incheon, South Korea).

Author's contributions

GH Lee contributed to the sampling in the field, identified worms, performed DNA experiments, analyzed the data, and wrote the manuscript. G-S Min contributed to the design and draft of the manuscript and revising the final version of the manuscript.

All authors agreed to be accountable for all aspects of the work.

Disclosure statement

The authors declare no conflicts of interest and are responsible for this study.

Funding

This study was supported by a research fund from Inha University (without a fund number).

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Data availability statement

The data supporting the findings of this study are openly available in the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/nuccore/ON470577) under accession no. ON470577. The associated BioProject, SRA, and Bio-Sample numbers were PRJNA823822, SRR18651217, and SAMN27361394, respectively.

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