Enoplida Nematode Diversity in the Pacific Clarion-Clipperton Fracture Zone (Postprint)

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Abstract

A total of 26 Enoplida nematode individuals were detected in deep-sea sediment samples collected from four stations in the Clarion-Clipperton fracture zone (CCZ) of the deep Pacific Ocean. Integrating morphological and molecular biological methods, we identified six families and eight genera of Enoplida nematodes, with Oxystominidae being the most abundant (57.7% of the total), followed by Anticomidae (19.2%), Phanodermatidae (7.7%), Oncholaimidae (7.7%), Ironidae (3.8%), and Enchelidiidae (3.8%). The family and genus composition was similar to that of nematodes from adjacent stations sampled during the same period, whereas the abundance proportions differed. Molecular methods yielded 16 nematode rRNA gene sequences, which exhibited 94%-99% similarity to existing sequences in the GenBank database, enabling identification to the family level and most genera (84.6%). DNA barcoding results showed high consistency with morphological identification, indicating that molecular barcoding technology is an effective tool for deep-sea nematode identification. Phylogenetic analysis based on 18S and 28S rRNA gene sequences revealed that phylogenetic trees constructed using different methods had essentially consistent branch topologies; Oncholaimidae and Enchelidiidae clustered together, as did Phanodermatidae and Anticomidae, indicating relatively close genetic relationships.

Full Text

Preamble

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Nematode (Enoplida) Diversity in Sediment Samples Collected from the Clarion-Clipperton Fracture Zone

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Abstract

We investigated nematode diversity in deep-sea sediment samples collected from four stations in the Clarion-Clipperton Fracture Zone (CCZ) of the Pacific Ocean. By integrating morphological and molecular approaches, we identified 26 Enoplida nematodes. The Oxyostominiidae was the most abundant family, representing 57.7% of the total, followed by Anticomidae (19.2%), Phanodermatidae (7.7%), Oncholaimidae (7.7%), Ironidae (3.8%), and Enchelidiidae (3.8%). Community composition at the family and genus levels was similar to that observed at adjacent sites during the same sampling period, though abundance patterns differed. Molecular analysis yielded 16 rRNA gene sequences with 94–99% similarity to existing GenBank entries. Based on BLAST results, all sequences could be identified to the family level, and 84.6% could be identified to the genus level. Morphological and molecular results showed high consistency, indicating that molecular barcoding is an effective tool for deep-sea nematode identification. Phylogenetic trees constructed from 18S and 28S rRNA gene sequences showed similar topological structures: species from Oncholaimidae and Enchelidiidae clustered together, while those from Phanodermatidae and Anticomidae formed another cluster, revealing close genetic relationships among these families.

Keywords: Enoplida; diversity; phylogeny; Clarion-Clipperton Fracture Zone

Introduction

Marine free-living nematodes (Phylum Nematoda) are widespread and abundant in marine sediments, often representing 70–90% of benthic metazoans. However, marine nematode taxonomy remains severely underdeveloped, with only approximately 4,000 free-living marine nematode species described to date. Traditional morphological identification is time-consuming, expensive, and challenging because many marine nematodes are small and exhibit similar morphological characters. Molecular technology, or "DNA barcoding," offers a fast and objective approach to species identification. Phylogenetic analysis based on small and large subunit ribosomal DNA (SSU rDNA and LSU rDNA) genes provides a powerful tool for clarifying evolutionary relationships among nematode taxa.

The order Enoplida represents one of the most important groups of marine nematodes, with many enoplids serving as active predators that play crucial ecological roles in marine environments. Here we report the isolation of 26 Enoplida nematodes from sediment samples collected at four sites in the Clarion-Clipperton Fracture Zone of the Pacific Ocean. Specimens were preserved in DESS solution (20% dimethyl sulphoxide, 0.25 mol/L disodium EDTA pH 8.0, saturated with NaCl) immediately after collection. Each sediment sample was rinsed through a 38- m sieve using filtered seawater, and nematodes were extracted using the Ludox flotation method. Nematodes were placed on temporary slides and observed using a Leica DM5500 microscope. After image capture, each specimen was washed, cut into several pieces, transferred into micro-centrifuge tubes, and digested with Proteinase K. A series of frozen specimens was subsequently thawed and subjected to PCR amplification of the 18S rRNA gene and D3 expansion segments of the 28S rRNA gene. Sequences were analyzed and compared with published data from GenBank via BLAST searches. Phylogenetic trees were constructed using neighbor-joining, maximum likelihood, and maximum parsimony methods with the MEGA5 program package, after multiple alignment of the data by CLUSTAL W.

Methods

Sample Collection and Preservation

Sediment samples were collected from four stations in the Pacific CCZ using a Box-Corer (BC) and Multi-Corer (MC) in August–September 2013 . Samples were immediately fixed in DESS solution (20% DMSO, 0.25 M disodium EDTA pH 8.0, saturated with NaCl) to preserve morphology and extractable DNA.

Nematode Extraction and Morphological Identification

Sediments were rinsed through a 38- m mesh using filtered seawater and extracted via Ludox flotation (density 1.18 g/mL). Nematodes were transferred to temporary slides and observed under a Leica DM5500 microscope using differential interference contrast and phase-contrast modes. Morphological identification followed Gerlach, Riemann, and Lorenzen' s methods [18–20]. To prevent genomic degradation, DNA was extracted immediately after observation [21]. Nematodes were removed from slides, washed with sterile water, cut into several pieces, and transferred to 0.5 mL microcentrifuge tubes containing 20 L worm lysis buffer (WLB: 50 mmol/L KCl, 1% gelatin, 10 mmol/L Tris-Cl pH 8.2, 2.5 mmol/L $MgCl$, 0.45% (v/v) Tween 20, 60 g/mL proteinase K). Samples were incubated at 65°C for 1 minute, then 95°C for 15 minutes to remove proteins. After cooling to room temperature, the supernatant was used as PCR template.

Molecular Analysis

Two gene fragments were amplified: the 18S rRNA gene (~990 bp) and the D3 expansion segment of the 28S rRNA gene (~260 bp). The 18S rRNA gene was amplified using primers 988F (5 -CTCAAAGATTAA GCCATGC-3) and

1912R (5 -TTTACGGTCAGAACTAGGG-3) [22]. The 28S rRNA D3 region was amplified using primers D3a (5 -GACCCGTCTTGAAACACGGA-3) and D3b (5 -TCGGAAGGAACCAGCTACTA-3). PCR reactions (50 L) contained 34.5 L ddH O, 2 L each primer (10 M), 4 L dNTPs (2.5 mmol/L), 0.5 L Ex Taq polymerase (Takara, 5 U/ L), 5 L 10× Ex buffer, and 2 L DNA template. Cycling conditions were: 98°C for 30 s, followed by 35 cycles of 98°C for 10 s (49°C annealing for 18S primers; 54°C for D3 primers), 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were detected by 1% agarose gel electrophoresis. Products were cloned into pMD19-T vectors, and positive clones were sequenced by biotechnology companies.

Phylogenetic Analysis

Sequences were downloaded from GenBank and aligned using CLUSTAL W [23] implemented in MEGA5. Phylogenetic trees were constructed using neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods. For NJ, the Kimura 2-parameter model was used; for ML, the G+I model was applied. Bootstrap values were calculated with 1,000 replicates.

Results

Morphological Identification

We isolated 26 Enoplida nematodes from the four stations. Chinese nomenclature followed reference [24]. The Oxyostominiidae was the dominant family (57.7%), including *Oxyostomina* and *Halalaimus* (unidentified to genus). Anticomidae comprised 19.2% of nematodes, all identified as *Anticoma*. Phanodermatidae (7.7%) included *Phanodermopsis* and *Crenopharynx*. Oncholaimidae (7.7%) included *Viscosia* and *Syringolaimus*. Ironidae (3.4%) was represented by *Bathyeurystomina*. The box-corer and multi-corer stations shared Oxyostominiidae, Anticomidae, Phanodermatidae, and Oncholaimidae. Oxyostominiidae was the dominant group at the box-corer stations, while Anticomidae dominated at the multi-corer stations. Ironidae and Syringolaimidae were only found at the multi-corer station [Figure 1: see original paper].

shows the number of gene fragments obtained. Body length of Enoplida nematodes generally ranged from 0.2 mm to 1.8 mm, with the longest being *Viscosia* sp. at 1.8 mm. Morphometric measurements are summarized in .

Molecular Data

We obtained 16 sequences each of 18S rRNA and 28S rRNA genes, which have been submitted to GenBank (accession numbers KR0811950–KR0811965). The 18S rRNA gene fragments were ~991 bp with lower variable region proportion than conserved region, showing high conservation. The 28S rRNA gene fragments were ~306 bp with higher variability. Sequence composition analysis showed 27.7% variable sites in 18S and 35.6% in 28S .

BLAST comparisons revealed 94–99% similarity with GenBank sequences. All sequences could be identified to family level, and 84.6% to genus level. Some 18S rRNA sequences lacked close matches in the database, indicating that nucleotide sequences for some species have not yet been deposited.

Phylogenetic Analysis

Phylogenetic trees constructed using NJ, MP, and ML methods based on 28S rRNA gene sequences showed consistent branching patterns. Oncholaimidae and Enchelidiidae clustered together, while Phanodermatidae and Anticomidae formed another cluster, indicating close genetic relationships within these groups [Figure 2: see original paper].

Discussion

This study focused on Enoplida nematodes in the CCZ, with Oxyostominiidae being most abundant and Ironidae least abundant. *Halalaimus* was the dominant genus, consistent with Lambshhead et al.' s findings [14]. Notably, Phanodermatidae nematodes (*Phanodermopsis* and *Crenopharynx*) were rare in previous CCZ studies [14–15], suggesting that some nematode taxa remain undiscovered and that CCZ Enoplida diversity requires further investigation.

Molecular barcoding results showed high consistency with morphological identification. The 94–99% similarity between our sequences and GenBank data demonstrates that barcoding is an effective tool for rapid identification of marine free-living nematodes. The 18S rRNA gene' s high conservation makes it suitable for higher-level taxonomy but limits resolution at lower taxonomic levels. The 28S rRNA gene shows higher variability, making it valuable for distinguishing genera and species [11].

Our phylogenetic analysis supports previous findings that Oncholaimoidea (including Oncholaimidae and Enchelidiidae) is monophyletic [1, 26]. The close relationship between Phanodermatidae and Anticomidae suggests they may belong to Enoploidea. The unstable topology for Oxyostominiidae indicates it may be paraphyletic, requiring further study.

For effective application of DNA barcoding to marine nematodes, we recommend: (1) creating a comprehensive barcode database combining morphological and molecular data; (2) developing universal primers or new genetic markers; and (3) improving morphological analysis through techniques like video capture, SEM, confocal imaging, and 3D reconstruction [28–29]. Integrating morphological and molecular approaches will enhance understanding of nematode biodiversity and refine classification systems.

Conclusion

At the family level in the CCZ, Oxyostominiidae was most abundant and Ironidae least abundant, with *Halalaimus* as the dominant genus. Phanodermatidae, rarely reported in previous CCZ studies, suggests undiscovered diversity requiring systematic investigation. Molecular barcoding showed high consistency with morphological identification, proving effective for deep-sea nematode identification. Phylogenetic trees constructed using different methods showed consistent branching patterns, with Oncholaimidae/Enchelidiidae and Phanodermatidae/Anticomidae forming distinct clusters that reflect close genetic relationships.

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Note: Figure translations are in progress. See original paper for figures.