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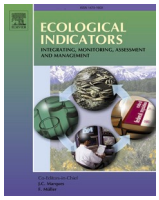

Environmental DNA metabarcoding shows potential for monitoring meiofauna and marine nematodes diversity in mangrove ecosystems in China

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ABSTRACT

Mangrove ecosystems, located at the vital interface between land and sea, exhibit exceptionally high biodiversity. Meiofauna are key components in the food webs and nutrient cycles of mangrove ecosystems. However, the diversity of intertidal meiofauna is not well understood, primarily due to the constraints of morphological species identification. Environmental DNA metabarcoding offers a potent solution to these challenges. In this study, the community diversity of meiofauna and marine nematodes were investigated using eDNA metabarcoding and traditional morphological methods in the mangrove from Quanzhou Bay, Fujian Province, China. The findings showed that eDNA metabarcoding using the COI gene marker detected 19 meiofaunal groups, while morphological method identified only 4 groups, with copepoda and marine nematodes being recognized by both methods. Regarding abundance, copepoda (relative abundance: 81.01%) was the most dominant group identified by eDNA metabarcoding, whereas marine nematodes (abundance: 92.03%) was the most dominant group identified by the morphological method. The marine nematodes from five families and six genera were detected by eDNA metabarcoding, but those in morphological method were from 15 families and 23 genera. *Daptonema* and *Terschellingia* were common in both methods. In terms of abundance via eDNA metabarcoding, *Daptonema* had the highest relative abundance (85.71%), followed by *Neochromadora* (7.59%). In morphological method, *Daptonema* was also prominent (abundance of 15.95%), followed by *Admirandus* (12.36%). In conclusion, eDNA metabarcoding offers distinct advantages in identifying meiofaunal groups but exhibits notable discrepancies in abundance estimates compared to morphological method. It shows promise in monitoring marine nematodes diversity, with both methods highlighting *Daptonema* as the most abundant genus. The appropriate specific primers, a comprehensive database, and the definitive correlation between individual counts and sequences are crucial for the further widespread implementation of eDNA metabarcoding in monitoring meiofauna and marine nematodes diversity.

1. Introduction

Meiofauna is a type of benthos that can pass through a 0.5 mm pore size sieve and is blocked by a 0.042 mm pore size sieve during sorting, primarily indicating multicellular metazoans (Higgins and Thiel, 1988). Meiofauna significantly contribute to carbon transfer and material cycling by consuming dissolved organic carbon, ingesting primary producers, and bacteria (van der Heijden et al., 2020; Schratzberger et al., 2018; Wang et al., 2020). Characterized by straightforward field sampling, considerable species richness, brief life cycles (averaging 3–5 generations annually), high reproductive and productive capacities, low mortality, a permanent benthic presence, and lacking a pelagic phase in their lifecycle, meiofauna distributions are intricately linked to their benthic habitats (Austen, 1989; Thiermann et al., 2000). Within the 34 recognized phyla of benthos, at least 22 phyla are classified as meiofauna, with free-living marine nematodes (hereinafter referred to as marine nematodes) frequently predominating (Heip et al., 1992a). Representing the most extensive, numerous, and diverse group within

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meiofauna, marine nematodes usually make up more than 60 % of the total population of meiofauna (Heip et al., 1985b). In energy-dissipative coastal areas rich in organic matter, meiofauna, predominantly marine nematodes, are intimately connected to higher trophic levels and are vital to the marine micro-food web (Menn, 2002). These connections situate marine nematodes centrally in the entire benthic food web, creating essential links among macrofauna, waterfowl, detritus, and microorganisms (Woodward et al., 2005). Shifts in the diversity of marine nematodes can precisely indicate the health and ecological conditions of mangrove wetland ecosystems. The diversity indices and alterations in their community distribution patterns serve as effective tools for environmental monitoring (Patrício et al., 2012; Hua et al., 2021). Precise monitoring of the present status of meiofauna and marine nematodes diversity is essential to accurately reflect environmental conditions.

Historically, the monitoring of diversity among meiofauna and marine nematodes in mangrove wetlands has predominantly utilized traditional morphological identification method, which have substantially restricted their ecological and diversity research (Wang et al., 2023). Such morphological method exhibit significant drawbacks, including the complexities and prolonged processes of sample sorting and identification, intensive use of human resources and time, and difficulties in differentiating morphologically similar species closely related, requiring high levels of expertise from identifiers (Hopkins et al., 2002; Bucklin et al., 2011). As a result, researchers tend to concentrate on particular taxonomic groups, which curtails the comprehensive understanding of the diversity and spatial distribution of meiofauna across regional and global dimensions (Xuan et al., 2007; Malinga et al., 2005). Thus, the development of a swift, precise, and standardized molecular identification approach, building on morphological method, has emerged as a widely accepted standard in the fields of taxonomy and diversity studies.

Environmental DNA (eDNA) comprises a complex mixture of genomic DNA from living cells, extracellular DNA, and DNA external to the organism, derived from the degradation of organic substances and biological secretions (Torti et al., 2015). Extracellular DNA is abundantly present in marine sediments, often representing 50 % to 90 % of the total DNA content (Corinaldesi et al., 2018; Dell'Anno et al., 2005). DNA barcoding coupled with high throughput sequencing makes eDNA metabarcoding significant advantages in monitoring the biodiversity from environmental samples (Leasi et al., 2018; Deiner et al., 2017), evolving into a groundbreaking method for biodiversity detection with extensive potential in biological monitoring (Yoccoz, 2012). In recent years, eDNA metabarcoding has been applied successfully to assess biodiversity, including fish (Ruan et al., 2022; Deng et al., 2024), aquatic plants (Zeng et al., 2024; Prieto et al., 2024), plankton (Ji et al., 2022; Chen et al., 2024), and microorganisms (Banchi et al., 2020; Djurhuus et al., 2020). For benthic invertebrate, this method has been mainly used to reveal and estimate macrofauna diversity (Steyaert et al., 2020; Atienza et al., 2020; Ji et al., 2021). Limited studies have been reported on using eDNA metabarcoding to monitor the diversity of meiofauna and marine nematodes, especially in mangrove wetlands. For example, Wang et al. (2023) assessed meiofauna and nematodes diversity in the coastal mudflats along the eastern coast of China (Wang et al., 2023). Bellisario et al. (2021) obtained meiofaunal diversity and identified hierarchical spatial structures within the meiofaunal communities in the estuaries of the North-Western Iberian Peninsula (Bellisario et al., 2021). Overall, eDNA metabarcoding is still in its infancy for assessing the diversity of meiofauna and marine nematodes. Compared with coastal mudflats, estuaries, and other intertidal ecosystems, mangroves possess complex and unique habitat characteristics that support distinct meiofaunal and marine nematodes communities (Song et al., 2022). Therefore, the eDNA metabarcoding deserves to be evaluated for surveying the diversity of meiofauna and marine nematodes in mangrove wetlands.

The mangrove wetland ecosystem, situated at the land-sea interface, provides substantial ecosystem services, including biodiversity preservation, pollutant filtration, habitat provision, and nutrient biogeochemical cycling for endangered species (Erséus, 2002; Pinto et al., 2013). As an integral part of the "blue carbon" ecosystems, these wetlands are crucial for carbon sequestration and storage (Houghton et al., 1999; Falkowski et al., 2000). The Fujian Quanzhou Bay Estuary Wetland Nature Reserve covers a total of 7,065.31 ha, with 305.89 ha allocated to artificially restored mangrove wetlands, marking the largest continuous mangrove area in Fujian Province. The Quanzhou Bay mangrove wetland is predominantly composed of *Kandelia obovata*, *Avicennia marina*, *Aegiceras corniculatum*, *Bruguiera gymnorhiza*, and *Rhizophora stylosa*. Among them, *A. marina* and *A. corniculatum* also signify the northern boundary of their natural distribution in China (Hu et al., 2023; Chu et al., 2024). Although the area was previously threatened by the invasion of *Spartina alterniflora*, it was successfully eradicated in September 2022, and a variety of mangrove species (*K. obovata*, *A. marina*, *A. corniculatum*, *B. gymnorhiza*, and *R. stylosa*) were replanted. The unique ecological environment of the Quanzhou Bay mangrove wetland make it a highly representative study area. Therefore, we used this mangrove wetland as a representative area to evaluate monitoring methods for the diversity of meiofauna and marine nematodes, providing valuable reference for the monitoring of these two groups in other mangrove wetlands.

Due to the limitations of morphological method, such as the complex sorting process and the difficulty distinguishing between closely related species during identification, biodiversity may be underestimated (Hu et al., 2023). However, eDNA metabarcoding directly collects and processes environmental samples, reducing the loss of target organisms that can occur during morphological method detection. Meanwhile, its high sensitivity facilitates the efficient recovery of biological communities, especially for groups that are difficult to identify and have low abundance. Therefore, we predict that eDNA metabarcoding will be able to detect more meiofauna and marine nematodes groups. In this study, centered on the Quanzhou Bay Estuary Wetland Nature Reserve, both eDNA metabarcoding and morphological methods were utilized to examine the meiofauna and marine nematodes diversity within the reserve. The objective of this research is to elucidate the effectiveness and practicality of eDNA metabarcoding for monitoring the diversity of meiofauna and marine nematodes in mangrove wetlands. These results will advance the development of monitoring methods for the diversity of meiofauna and marine nematodes in mangrove wetlands, contributing to the efficient and accurate assessment of the diversity of these two groups.

2. Materials and methods

2.1. Study area and sampling stations

The study area is located within the experimental zone of the Quanzhou Bay Estuary Wetland Nature Reserve in Fujian Province, China. In December 2022, six stations A, B, C, D, E and F were arranged in the Quanzhou Bay mangrove wetland, according to the different tidal levels. Stations A and B were located at high tide levels, stations C and F were at mid tide levels, and stations D and E were at low tide levels (Fig. 1). The latitude and longitude of each sampling station are provided in Table S1.

2.2. Traditional morphological method

Locations with consistent sediment types and no disturbance were selected for sediment sample collection. A sampling tube with an inner diameter of 2.9 cm was used, and three core samples were collected at each station at a depth of 5 cm. After collecting the samples, they were fixed with DMSO-EDTA-Salt-Solution (referred to as DESS: DMSO-salt solution made of 20 % DMSO, 0.25 M sodium-EDTA, and NaCl to saturation, pH 7.5) and stored at room temperature before being taken

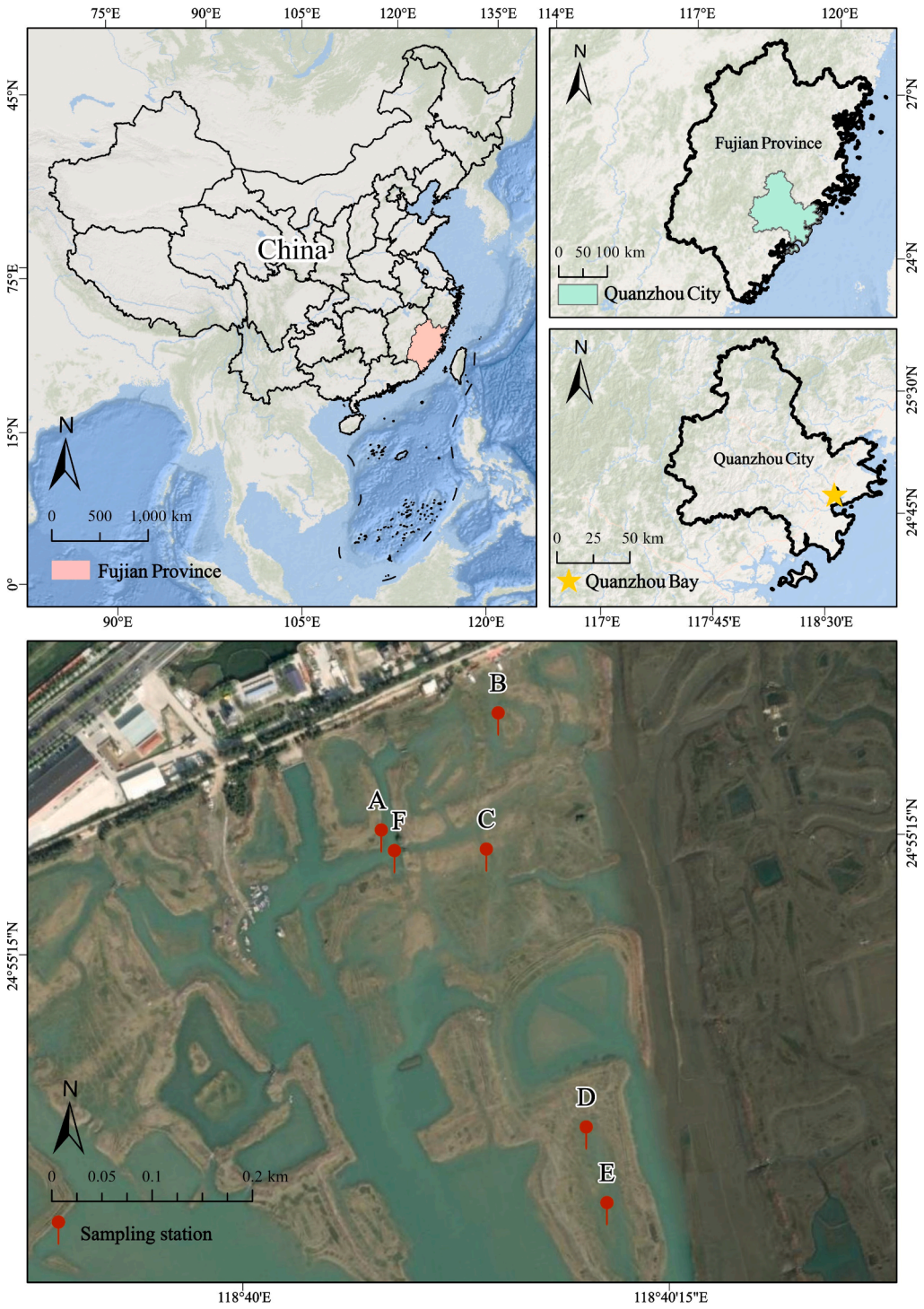

Fig.1. Study area and sampling station map in mangrove wetland of Quanzhou Bay.

back to the laboratory for sample sorting (Seutin et al., 1991).

The indoor sorting of meiofauna and mounted methods of marine nematodes have been described in the literature (Higgins and Thiel, 1988; Somerfield and Warwick, 1996; Platt and Warwick, 1983a, 1988b; Warwick et al., 1998). Each sediment sample was placed on a separation device consisting of a 500 μm upper screen and a 42 μm screen below. It was then slowly washed with filtered tap water until most of the clay and silt were removed. The retained sample was rinsed in a sieve with Ludox-TM silicone solution with a density of 1.15 g/mL into a centrifuge tube. The sample was then centrifuged twice at a speed of 4000 r/min for 10 min each time. The meiofauna extracted from the supernatant were transferred to Petri dishes with equal-width parallel lines. Under a dissecting microscope (Nikon SMZ800, Tokyo, Japan), the meiofauna were classified and counted by group. The marine nematodes were picked out into a special glass container containing a mixture of alcohol, glycerol, and water (V alcohol: V glycerol: V water = 1:1:18). After placing the glass container in a drying oven for one week, the marine nematodes were picked out onto a cover glass slide with an appropriate amount of mixture. Identification was conducted under a differential interference microscope (Nikon ECLI-PSE-80i).

2.3. eDNA metabarcoding

2.3.1. eDNA sampling

Samples were obtained following a standardized method from the sediments collected by sampling frame (measuring 30 cm × 30 cm in length and width, and 30 cm in depth). The surficial sediments (approximately the first 3 cm) were instantly put into 4 mL sterile centrifuge tubes (Beyotime Biotechnology, Shanghai, China) and stored in liquid nitrogen. The sterile centrifuge tubes remained closed until the moment of sampling and were closed immediately after sampling. The sampling frame was sterilized in an autoclave before sampling, rinsed with sterile water per sampling for each station and disinfected using sodium hypochlorite between each new station. The field crews wore sterile gloves to collect and handle samples and gloves were replaced after collecting each sample to minimize the risk of cross-site contamination. All samples were stored at −80°C until DNA extraction.

2.3.2. DNA extraction and PCR amplification

Genomic DNA was extracted from sediment samples using CretMag™ Power Soil DNA Kit (CretBiotech, China) according to manufacturer's instructions. The DNA extract was checked on 1 % agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). The COI gene were amplified with primer pairs (Leray et al., 2013) mlCOIintF (5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′) and jgHCO2198 (5′-TANACYTCNGGRTGNCCRAARAAYCA-3′) by an A200 PCR thermocycler (A200, LongGene, China). The PCR amplification of COI gene was performed as follows: initial denaturation at 94 ℃ for 2 min, followed by 30 cycles of denaturing at 94 ℃ for 30 s, annealing at 55 ℃ for 30 s and extension at 72 ℃ for 45 s, and single extension at 72 ℃ for 10 min, and end at 4 ℃. The PCR mixtures contain 2 x ES Taq MasterMix (Dye) 25 μL, forward primer (10 μM) 2 μL, reverse primer (10 μM) 2 μL, template DNA 10 ng, and finally ddH_2O up to 50 μL. PCR reactions were performed in triplicate. The PCR product was extracted from 2 % agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, USA).

2.3.2.1. Illumina novaseq sequencing. Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Baiaoweifan Biotechnology Co., Ltd. (Wuhan, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database BioProject ID: PRJNA1171633 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1171633).

2.3.2.2. Processing of sequencing data. The raw COI gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0 (Chen et al., 2018) and merged by FLASH version 1.2.7 (Magoč et al., 2011) with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of < 20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatches in primer matching.

Operational taxonomic units (OTUs) with 97 % similarity cutoff were clustered using UPARSE version 7.1 (Edgar, 2013; Stackebrandt et al., 1994), and chimeric sequences were identified and removed. The taxonomic categories of each OTU representative sequence were assigned using *blastn*, and taxonomic categories were predicted using the Nucleotide Sequence Database (NT) with a confidence threshold of 0.7 (Brandt et al., 2021).

2.4. Data processing and analysis

The data was processed and analyzed using the software programs ArcGIS pro 2.8, Excel 2016, SPSS 22, and Primer 6.0. Sampling station map was drawn using ArcGIS pro 2.8. The bar chart of species relative abundance was created in Excel 2016. A one-way ANOVA was performed in SPSS 22 to determine whether there were significant differences in the abundance of meiofauna or marine nematodes across sampling stations using traditional morphological method. The diversity indices data were analyzed in Primer 6.0 with DIVERSE: Species richness index (d), Evenness index (J'), Shannon-Wiener index (H'), and Simpson index ($1\text{-}\lambda$). The formulas are as follows:

$d = (S - 1)/\ln N$,

$J' = H'/\ln S$,

$H' = -\sum (N_i/N) \ln (N_i/N)$,

$1\text{-}\lambda = 1 - \sum (N_i/N)$,

where S is the number of species at each station, N is the total number of individuals, and Ni is the number of individuals of the ith genus (i from 1 to S).

The classification of meiofauna was mainly conducted with reference to the Introduction to the Study of Meiofauna (Higgins and Thiel, 1988). The taxonomic identification of marine nematodes was mainly based on (i) Freeliving marine nematodes: Part I. British enoplids (Platt and Warwick, 1983a); (ii) Freeliving marine nematodes: Part II. British chromadorids (Platt and Warwick, 1988b); (iii) Freeliving marine nematodes: Part III. Monhysterida (Warwick et al., 1998).

3. Results

3.1. Diversity of meiofauna

3.1.1. Composition of meiofauna

The eDNA metabarcoding produced a total of 570,055 sequences. The number of valid sequences per station sample ranged from 4,122 to 63,740, with an average of 19,166.8 sequences. The rarefaction curves indicate that the sequence counts for all station samples approached saturation, suggesting that the sequencing data volume can cover the majority of groups. (Fig. S1).

The eDNA metabarcoding identified 19 meiofauna groups, including copepoda, rotifera, marine nematodes, amphipoda, bivalvia, nemertea, isopoda, polychaeta, cnidaria, gastrotricha, pycnogonida, tardigrada, holothuroidea, bryozoa, sipuncula, ostracoda, tanaidacea, ciliophora, and kinorhyncha.

The traditional morphological method identified four meiofauna groups: marine nematodes, copepoda, oligochaeta, and turbellaria. Both methods jointly identified two groups of meiofauna: copepoda and marine nematodes. There were 17 groups identified by eDNA metabarcoding which were not detected by morphological method. Among the meiofauna groups identified by morphological method, only copepoda and marine nematodes were detectable using eDNA metabarcoding, while oligochaeta and turbellaria were not identified by this method (Fig. 2a).

3.1.2. Abundance of meiofauna

The eDNA metabarcoding results showed that copepoda had the highest relative abundance at 81.01 %, followed by rotifera at 5.61 % and marine nematodes at 3.29 %. At stations A, B, and C, copepoda and rotifera were the dominant groups, with relative abundances of 12.81 % and 31.85 %, 96.57 % and 1.22 %, and 29.58 % and 25.88 %, respectively. At stations D and F, rotifera and marine nematodes were the dominant groups, with relative abundances of 26.25 % and 18.01 %, and 20.92 % and 23.62 %. At station E, copepoda and marine nematodes were the dominant groups, with relative abundances of 86.10 % and

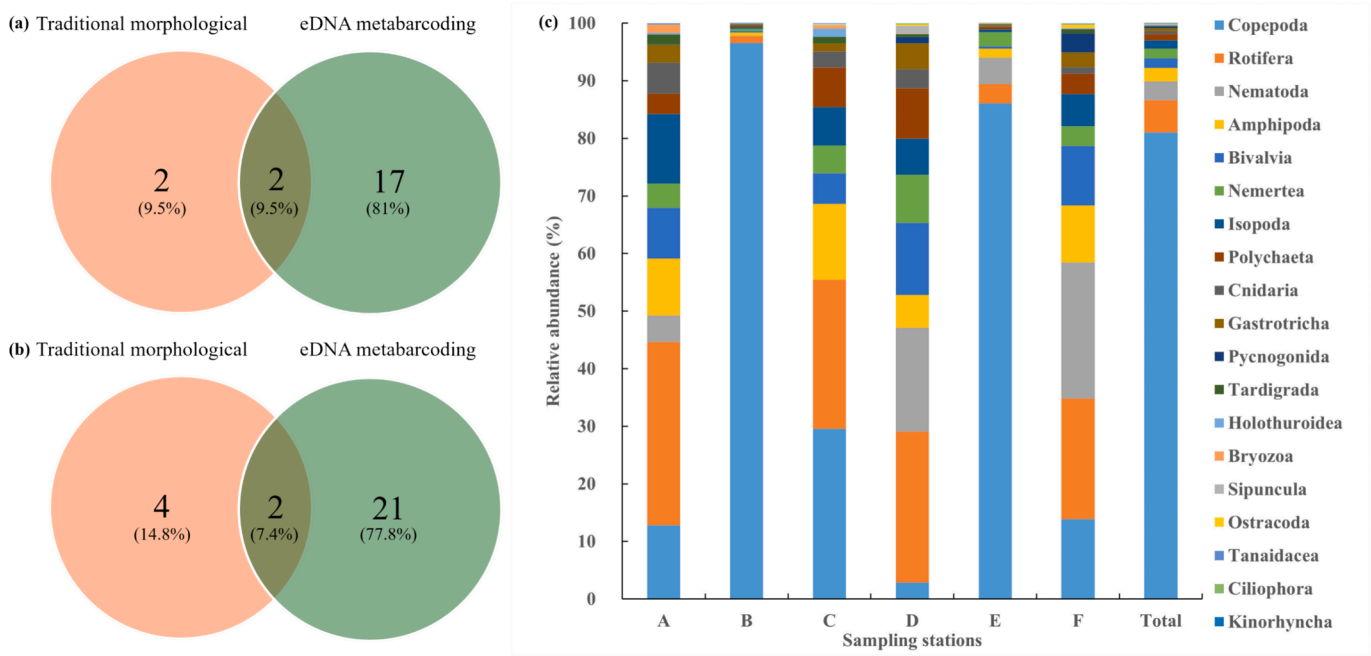

Fig.2. Venn diagram of meiofauna (a) and marine nematodes (b) composition using eDNA metabarcoding and traditional morphological method. Meiofauna relative abundance using eDNA metabarcoding (c).

4.62 % (Fig. 2c).

The traditional morphological method revealed that marine nematodes were the dominant group, constituting 92.03 % of the total meiofauna abundance. Copepoda, oligochaeta, and turbellaria followed, with abundances of 3.84 %, 3.11 %, and 1.01 %, respectively. At all stations, marine nematodes were the primary dominant group, with abundances of 90.38 %, 96.23 %, 75.16 %, 91.74 %, 96.68 %, and 96.42 % (Table 1). One-way ANOVA showed no significant differences in meiofauna abundance among the stations ($F = 0.625$, $df = 5$, $p > 0.05$).

3.1.3. Alpha diversity of meiofauna

The eDNA metabarcoding results showed that the maximum values for the Species richness index (*d*), Evenness index (J'), Shannon-Wiener index (H'), and Simpson index ($1-\lambda$) were observed at Station D, while the minimum values were observed at Station B (Fig. 3a). The traditional morphological method indicated that the maximum values for the Evenness index (J'), Shannon-Wiener index (H'), and Simpson index ($1-\lambda$) were observed at Station C, while the maximum for the Species richness index (*d*) was observed at Station F. Additionally, the minimum values for the Evenness index (J') and Shannon-Wiener index (H') were observed at Station F, while the Species richness index (*d*) was lowest at Station B and the Simpson index ($1-\lambda$) was lowest at Station E (Fig. 3b).

Table 1
Meiofauna abundance (individual/10 cm^2) and proportion (%) using traditional morphological method.

Group Station	Marine nematode	Copepoda	Oligochaeta	Turbellaria	Total
A	118.59 ± 5.00 (90.38 %)	1.01 ± 0.71 (0.77 %)	7.07 ± 4.28 (5.38 %)	4.54 ± 5.00 (3.46 %)	131.21 ± 3.57
B	167.54 ± 42.82 (96.23 %)	4.79 ± 2.50 (2.75 %)	1.77 ± 0.36 (1.01 %)	0.00 ± 0.00 (0.00 %)	174.11 ± 40.68
C	59.55 ± 6.42 (75.16 %)	17.41 ± 22.48 (21.97 %)	1.01 ± 0.00 (1.27 %)	1.26 ± 1.78 (1.59 %)	79.23 ± 27.12
D	50.47 ± 28.55 (91.74 %)	0.25 ± 0.36 (0.46 %)	4.29 ± 6.07 (7.80 %)	0.00 ± 0.00 (0.00 %)	55.01 ± 34.97
E	110.27 ± 119.54 (96.68 %)	0.25 ± 0.36 (0.22 %)	3.53 ± 5.00 (3.10 %)	0.00 ± 0.00 (0.00 %)	114.05 ± 124.18
F	67.88 ± 19.63 (96.42 %)	0.25 ± 0.36 (0.36 %)	1.77 ± 1.07 (2.51 %)	0.50 ± 0.71 (0.72 %)	70.40 ± 21.77
Mean	95.42 ± 36.99 (92.03 %)	4.00 ± 4.46 (3.84 %)	3.24 ± 2.80 (3.11 %)	1.05 ± 1.25 (1.01 %)	104.00 ± 42.05

3.2. Diversity of marine nematodes

3.2.1. Composition of marine nematodes

The eDNA metabarcoding identified marine nematodes from 5 families 6 genera: *Daptonema*, *Neochromadora*, *Stilbonema*, *Desmodora*, *Adoncholaimus*, and *Terschellingia*. The traditional morphological method identified marine nematodes from 15 families 23 genera, including *Daptonema*, *Admirandus*, *Parodontophora*, *Ptycholaimellus*, *Terschellingia*, *Anoplostoma*, *Sabatieria*, *Halalaimus*, *Sphaerolaimus*, *Metachromadora*, *Hopperia*, *Oxystomina*, *Haliplectus*, *Halichoanolaimus*, *Pseudochromadora*, *Anticyathus*, *Micoletzkyia*, *Tripyloides*, *Parasphaerolaimus*, *Trissonchulus*, *Marylynnia*, *Paragnomoxyala*, and *Theristus*. Both methods identified 2 genera of marine nematodes in common: *Daptonema* and *Terschellingia*. 4 genera of marine nematodes identified by the eDNA metabarcoding were not detected by the morphological method, only *Daptonema* and *Terschellingia* identified by morphological method were recognized by the eDNA metabarcoding, while the remaining 21 genera were not detected by this method (Fig. 2b).

3.2.2. Abundance of marine nematodes

The eDNA metabarcoding results showed that *Daptonema* had the highest relative abundance at 85.71 %, followed by *Neochromadora* and *Stilbonema* at 7.59 % and 3.02 %, respectively. At station A, *Stilbonema* was the dominant genus, with a relative abundance of 56.08 %. At station B, *Desmodora* dominated with a relative abundance of 65.71 %. No marine nematodes were found at station C. *Daptonema* was the dominant genus at stations D, E, and F, with relative abundances of 89.68 %, 83.57 %, and 96.31 %, respectively (Fig. 4a).

The traditional morphological method indicated that *Daptonema* was

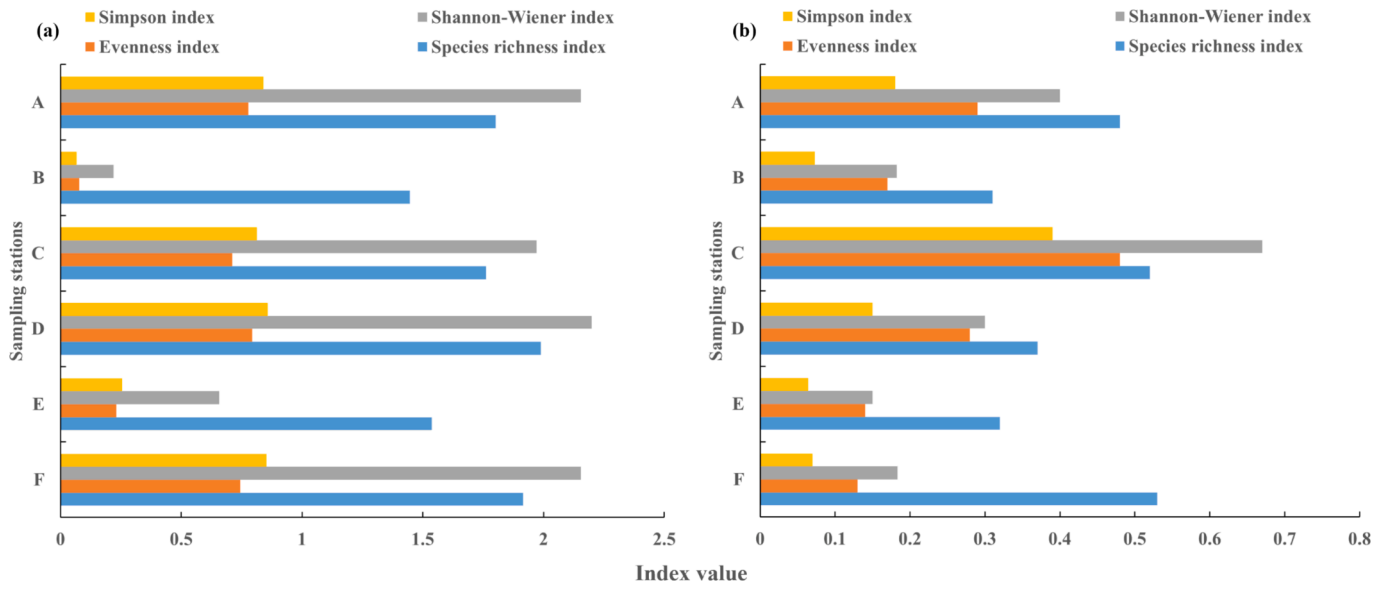

Fig.3. Meiofauna biodiversity index using eDNA metabarcoding (a) and traditional morphological method (b).

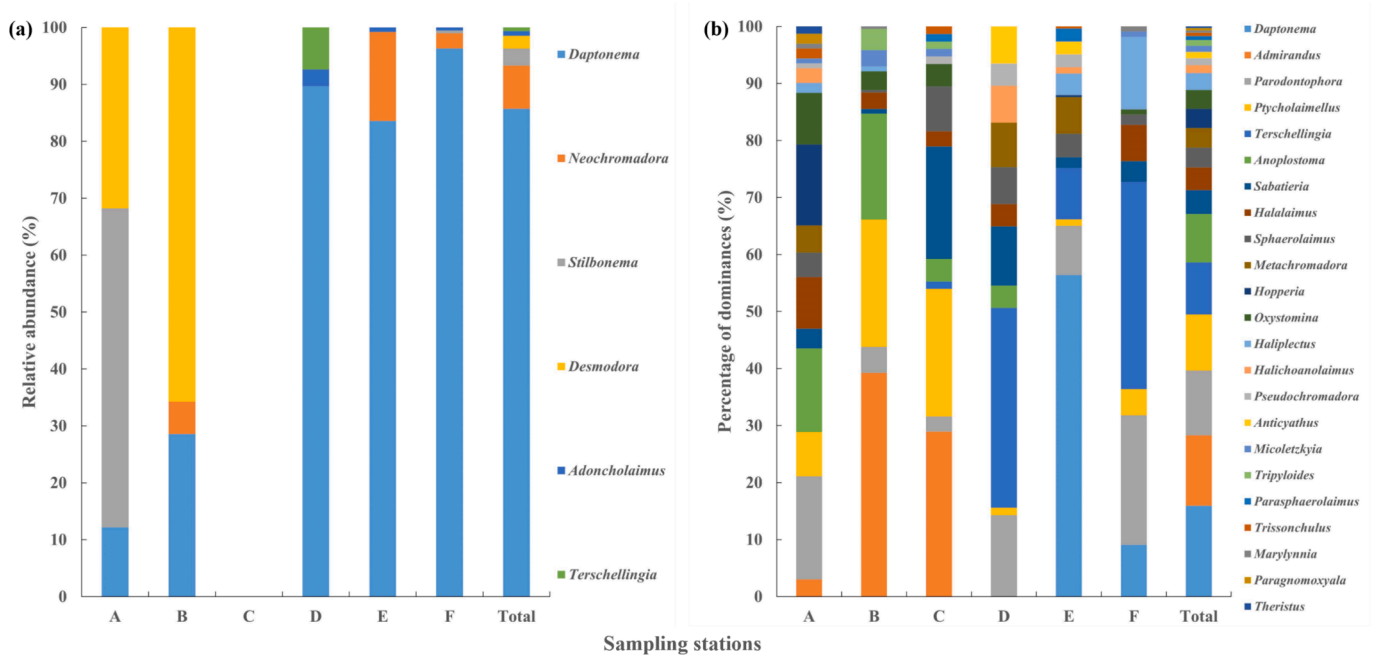

Fig.4. Marine nematodes relative abundance using eDNA metabarcoding (a) and percentage of dominances using traditional morphological method (b).

the most dominant genus, with a dominance of 15.95 %. Five other dominant genera (quantity percentage ≥ 5 %) included *Admirandus*, *Parodontophora*, *Ptycholaimellus*, *Terschellingia*, and *Anoplostoma*, with dominances of 12.36 %, 11.37 %, 9.77 %, 9.17 %, and 8.47 %, respectively. One-way ANOVA showed no significant differences in marine nematodes abundance among the stations ($F = 0.604$, $df = 5$, $p > 0.05$). At station A, *Parodontophora* exhibited the highest dominance of 18.10 %. At stations B and C, *Admirandus* exhibited the highest dominances of 39.26 % and 28.95 %, respectively. At stations D and F, *Terschellingia* exhibited the highest dominances of 35.06 % and 36.36 %, respectively. At station E, *Daptonema* was the most dominant genus, with a dominance of 56.39 % (Fig. 4b; Table 2).

Table 2
Dominances percentage of marine nematodes dominant genera using traditional morphological method at each station (%).

Station Genera	A	B	C	D	E	F
Daptonema	–	–	–	–	56.39	10.00
Admirandus	3.02	39.26	28.95	–	–	–
Parodontophora	18.10	4.55	2.63	14.29	8.65	22.73
Ptycholaimellus	7.76	22.31	22.37	1.30	1.13	4.55
Terschellingia	–	–	1.32	35.06	9.02	36.36
Anoplostoma	14.66	18.60	3.95	3.90	–	–

3.2.3. *Alpha diversity of marine nematodes*

The eDNA metabarcoding results showed that the maximum values

for the Evenness index (J'), Shannon-Wiener index (H'), and Simpson index ($1-\lambda$) were observed at Station D, while the maximum values for the Species richness index (d) were observed at Station B. Except for Station C, where no marine nematodes were identified, the minimum values for the Evenness index (J'), Shannon-Wiener index (H'), and Simpson index ($1-\lambda$) were observed at Station F, while the minimum Species richness index (d) was observed at Station E (Fig. 5a). The traditional morphological method indicated that the maximum values for the Species richness index (d), Evenness index (J'), Shannon-Wiener index (H'), and Simpson index ($1-\lambda$) were observed at Station A. The minimum values for the Evenness index (J'), Shannon-Wiener index (H'), and Simpson index ($1-\lambda$) were observed at Station B, while the minimum Species richness index (d) was observed at Station F (Fig. 5b).

4. Discussion

4.1. eDNA metabarcoding's capability for assessing meiofauna diversity

The meiofauna diversity in Quanzhou Bay Mangrove Wetland Nature Reserve has been investigated in recent years, however, the diversity monitoring was conducted using traditional morphological method (Hu et al., 2023). Morphological method, typically limited to certain life stages or genders of meiofauna, often fails to accurately recognize many individuals. eDNA metabarcoding circumvents these issues, providing rapid, high-resolution taxonomic analysis of complex environmental samples or mixed species (Taberlet et al., 2012).

In this study, eDNA metabarcoding identified 19 meiofauna groups, significantly surpassing the 4 groups detected with morphological method. Regarding community composition, both methods consistently recognized only copepoda and marine nematodes. eDNA metabarcoding further identified 17 groups that are not observable through morphological method (Fig. 2a; Fig. 2c; Table 1). Lejzerowicz et al. (2015) reported that 163 species and 117 species were identified by eDNA metabarcoding and morphological method, respectively, demonstrating eDNA metabarcoding with superior capability in species identification (Lejzerowicz et al., 2015). Overall, our findings indicated that eDNA metabarcoding can detect more meiofaunal groups. Its efficiency in identification notably exceeds morphological method, establishing it as an essential tool for a thorough understanding of meiofauna. However, researchers may still face challenges when using eDNA metabarcoding to distinguish between macrofauna and meiofauna. Since it cannot directly determine the developmental stages of benthos, some groups may be classified as meiofauna during their larval stage but as macrofauna when they reach adulthood. In this study, we have made efforts to exclude groups classified as macrofauna or non-target taxa from the results. However, for researchers lacking morphological expertise, the likelihood of such errors or overestimations increases substantially. This limitation, arising from the inability to account for growth stages, is one of the reasons why eDNA metabarcoding can detect a greater diversity of groups.

Regarding abundance, eDNA metabarcoding recorded copepoda at the highest relative abundance (81.01 %), contrasting sharply with marine nematodes at only 3.29 % (Fig. 2c). Conversely, morphological method showed marine nematodes at a substantial 92.03 % abundance, while copepoda were much lower at 3.84 % (Table 1). Wang et al. (2023) monitored the meiofauna diversity in the intertidal zone from Nantong and Lianyungang City, China, and reported that a significant bias of mlCOIintF/jgHCO2198 primers (COI) towards arthropods, which exhibited a relative abundance of 57.2 %, with nematodes at a mere 10.1 %. Conversely, SSU_F04/SSU_R22 primers (18S) revealed nematodes as the predominant group at 32.1 %, followed by arthropods at 10.5 % (Wang et al. 2023). Additionally, Fais et al. (2020) indicated that variations in COI primers frequently lead to substantial shifts in community composition, whereas 18S rRNA primers generally maintain consistent community patterns despite different targeting sites (Fais et al., 2020). In addition, most studies employ only a single molecular marker and typically one primer pair (Fonseca et al., 2010; Guardiola et al., 2015; Nascimento et al., 2018), with few studies opting for both COI and 18S or other multiple primer pairs (Cowart et al., 2015; Cordier et al., 2019). Hence, the findings of this study suggest that eDNA metabarcoding offers a substantial advantage in tracking meiofaunal community composition, identifying a wider array of groups than morphological method. However, notable disparities exist in abundance between eDNA metabarcoding and morphological methods, with marine nematodes predominantly identified in the latter, and copepoda in metabarcoding. These variations likely stem from primer selection. Precise monitoring of marine nematodes' relative abundance is vital for accurately assessing meiofaunal diversity through eDNA metabarcoding.

4.2. eDNA metabarcoding shows potential in marine nematodes diversity monitoring

Research indicates that marine nematodes constitute one of the most

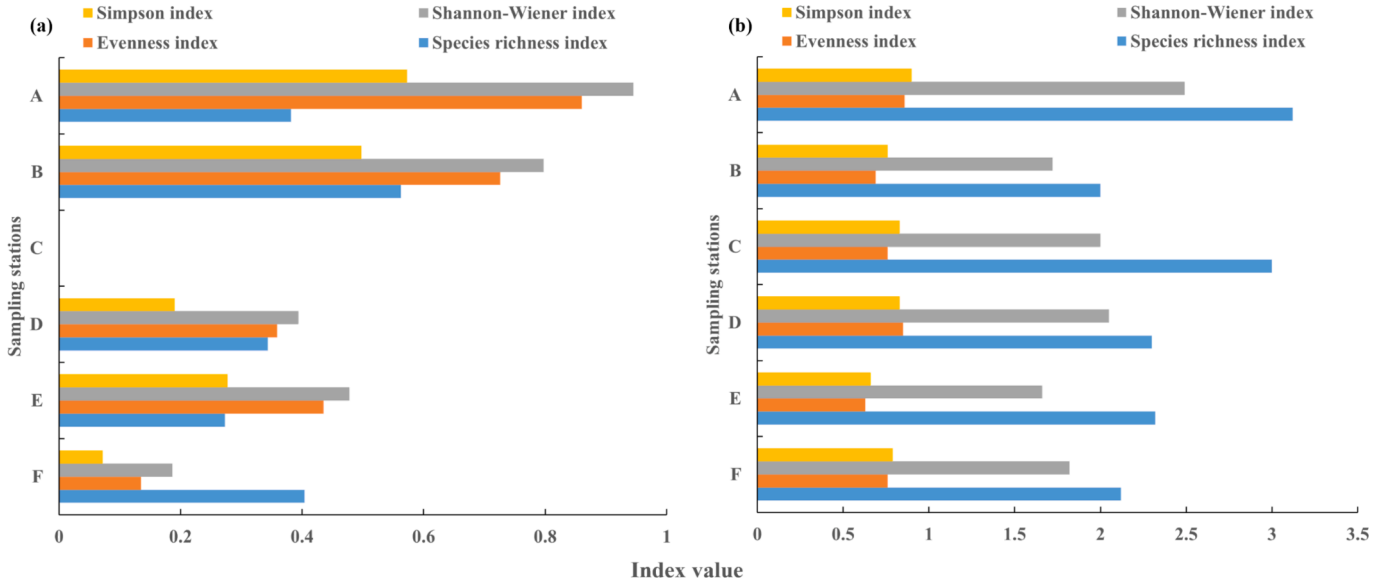

Fig.5. Marine nematodes biodiversity index using eDNA metabarcoding (a) and traditional morphological method (b).

abundant groups in intertidal benthic environments (Hu et al., 2023; Cai et al., 2020). Morphological identification of nematodes is labor-intensive, relying heavily on male specimens and posing substantial challenges (Zhao et al., 2020). eDNA metabarcoding offers a solution to these limitations, though its use in monitoring marine nematodes diversity has been scant (Tytgat et al., 2019; Macheriotou et al., 2019; Faria et al., 2018).

In this paper, eDNA metabarcoding identified nematodes from 11 families 12 genera, in which 6 families and 6 genera being terrestrial nematodes. Excluding terrestrial nematodes, the marine nematodes accounted for 5 families 6 genera detected by eDNA metabarcoding, less than the 15 families 23 genera detected by morphological method (Fig. 4). Common genera identified by both methods were *Daptonema* and *Terschellingia*, whereas morphological method detected 21 groups not found by eDNA metabarcoding (Fig. 2b). Additionally, eDNA metabarcoding failed to detect dominant genera such as *Admirandus*, *Parodontophora*, *Ptycholaimellus*, and *Anoplostoma*, which were observed in morphological method (Fig. 4a; Table 2). The nematodes detected at station C were *Parastrongyloides*, *Heteroxynema*, *Strongyloides*, *Discocriconemella*, and *Ficophagus*, all of which are terrestrial genera. After filtering, no marine nematodes were annotated. Our morphological method revealed that the abundance of marine nematodes at station C was relatively low, particularly for groups such as *Halalaimus*, *Sphaerolaimus*, *Oxystomina*, *Micoletzkyia*, and *Trissonchulus*. Additionally, the COI primers used in this study may not be sensitive enough to detect these low-abundance marine nematodes in station C (Wang et al., 2023). These may explain why eDNA metabarcoding failed to detect these target groups. Notably, *Ptycholaimellus*, a widespread genus in mangrove wetlands globally (Nicholas et al., 1991; Mokievsky et al., 2011). Shih et al. (2022) identified a new species from *Ptycholaimellus* at the Luoyang River estuary in Quanzhou City, China, and added two COI gene sequences of *Ptycholaimellus luoyang* and *Ptycholaimellus adocius* to GenBank (Shih et al., 2022). The study area in this study was proximate to location from Shih's research, we recognized *Ptycholaimellus* as a prevalent genus through morphological method. However, eDNA metabarcoding did not detect this genus, likely due to the different COI primers used. The primers mlCOIintF/jgHCO2198 in this study differ from the JB3F/JB5R used by Shih et al., potentially resulting in non-binding to the COI region of *Ptycholaimellus*.

In addition to variations caused by primer selection, the accuracy of species annotation via eDNA metabarcoding heavily depends on the completeness and quality of the reference database. Tytgat et al. (2019) noted that many marine nematodes identified morphologically do not have representative sequences in reference databases. Specifically, 42 % of the 147 nematodes sequences obtained via metabarcoding were not classified at the genus level, and nine out of 18 genera identified morphologically were absent from the database (Tytgat et al., 2019). Zhou et al. (2022) identified two new species of the genus *Haliplectus* in the Shenzhen Mangrove Conservation Area, China, confirming the molecular identification through morphological traits and 18S rRNA gene analysis (Zhou et al., 2022). Chen et al. (2022a, 2023b) combined DNA barcoding with morphological method to discover two new species of *Anticyathus* and a new species of *Diplolaimelloides* in the mangrove wetlands of Futian, Shenzhen, and Shantou, contributing 39 sequences of 18S rRNA genes of *Anticyathus shenzhensis* to the GenBank database (Chen et al., 2022a, 2023b). These studies significantly enhance databases, demonstrating that the completeness of nematode databases is essential for effective marine nematodes monitoring using eDNA metabarcoding.

Regarding abundance, eDNA metabarcoding displayed the highest relative abundance for *Daptonema* at 85.71 %, followed by *Neochromadora* at 7.59 % (Fig. 4a). The morphological method also identified *Daptonema* as the most dominant, with a dominance of 15.95 %, followed by *Admirandus* at 12.36 % (Fig. 4b). Although *Daptonema* appeared most abundant in both methods, substantial differences in abundance value were observed. This discrepancy may partly result from the chaotic taxonomic classification of *Daptonema*, which includes a vast number of species. The NeMys database records 146 valid species of *Daptonema*, with many potentially misclassified, leading to numerous inaccurate sequences in nematode reference databases, thereby yielding incorrect results when these sequences are employed (Timchenko et al., 2024). Additionally, discrepancy in data analysis could partially explain the abundance difference from two methods. The abundance using morphological method are derived from the count of individuals identified per species, whereas which from eDNA metabarcoding are based on the number of sequences identified per species, the relationship between individual counts and sequences remained unclear (Lejzerowicz et al., 2015). Haddad et al. (2008) recommended developing specialized biological indices for high-throughput sequencing, aligning scores with the ecological characteristics of species. Nonetheless, data on abundance derived from high-throughput sequencing are influenced by various technical and biological biases, presenting ongoing challenges for quantitative analysis (Haddad et al., 2008). Moreover, we also contend that the primers, database, and discrepancy in data analysis constituted the primary determinants of the divergent variances between the two methods (Fig. 5). Consequently, this study illustrates that eDNA metabarcoding has promising capabilities for assessing the diversity of marine nematodes, with both approaches identifying the genera *Daptonema* as the most abundant. The appropriate specific primers, a comprehensive database, and the definite correlation between individual counts and sequences are crucial for the further widespread implementation of eDNA in monitoring marine nematodes diversity.

5. Conclusions

In this study, eDNA metabarcoding detected a wider diversity of meiofaunal groups, compared to those using the traditional morphological method. However, significant discrepancies in abundance were observed between the two methods. Fewer taxonomic groups were identified in marine nematodes using eDNA metabarcoding compared to the morphological method. Despite this, both methods consistently highlighted *Daptonema* as the most abundant genus, demonstrating the potential of eDNA metabarcoding for monitoring marine nematode diversity. These findings underscore the promise of eDNA metabarcoding in assessing meiofauna and marine nematodes diversity in mangrove wetlands. Currently, integrating eDNA metabarcoding with the morphological method is crucial for a more comprehensive assessment of meiofauna and marine nematode diversity in mangrove wetlands. Future research should focus on optimizing primers that can better detect meiofauna and marine nematodes, refining and expanding the database, and standardizing the relationship between individual counts and sequences to further advance eDNA metabarcoding.

CRediT authorship contribution statement

Mingcheng Hu: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Yuqing Guo:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Fenfen Ji:** Writing – review & editing, Validation, Methodology, Investigation, Funding acquisition, Conceptualization. **Yijia Shih:** Methodology, Investigation, Formal analysis. **Kai Liu:** Methodology, Investigation. **Xiangxiang Yi:** Investigation, Formal analysis. **Yuanxin Fan:** Investigation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2025.113134.

Data availability

Data will be made available on request.

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