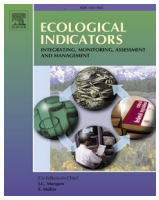
Ecological Indicators 150 (2023) 110223

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Environmental DNA metabarcoding of intertidal meiofauna sheds light on its potential for habitat discovery

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ARTICLE INFO

Keywords:
Sediment DNA
Marine biodiversity
Nematoda
Acrobeloides
Environmental variables

ABSTRACT

Benthic meiofauna is a fundamental component of food webs and nutrient exchange of marine ecosystems. However, its diversity pattern and ecology of intertidal meiofauna remain poorly understood because the studies are often constrained by morphology-based species identification. Environmental DNA metabarcoding is a powerful tool to overcome this limitation. In the present study, we assessed the intertidal meiofauna diversity and their correlations with environmental variables using eDNA metabarcoding approach with both 18S rRNA and two COI markers. The 18S rRNA marker suggested Nematoda (32.1%), Arthropoda (10.5%), and Cercozoa (8.0%) were the three most abundant phyla while COI primers show strong biased towards either Arthropoda or Nematoda and generated inconsistent results when using different reference databases. The correlation analysis showed that most examined environmental factors were strongly associated with community separation, but only salinity, the content of clay, and pheophorbide were found to be significant. The eDNA metabarcoding recovered a terrestrial nematode belonging to the genus *Acrobeloides* in a marine-related environment. Further resampling and laboratory experiments confirmed this species is tolerant to high salinity concentrations, suggesting eDNA metabarcoding recovery is consistent with the laboratory experiment. This result demonstrated eDNA metabarcoding can be a promising tool for high-throughput new habitat discovery in meiofauna.

1. Introduction

Intertidal zones are transitional habitats typified by tidal actions and extreme salinity variations, harboring different associated biological communities adapted to intermediate salinity regimes (Spruzen et al., 2008; McLachlan and Defeo, 2018). Of those distinct organisms, meiofauna is small animals between 45 and 500 μm, regarded as a fundamental component of food webs and nutrient exchange (Giere, 2008; Snelgrove, 1997), particularly serving as a trophic linkage between bacteria and macrofauna (Coull, 1999; Giere, 2008; Schratzberger and Ingels, 2018). Additionally, the meiofauna may also provide a potentially huge resource for assessing ecosystem health (Baird and Hajibabaei, 2012).

Despite their ecological significance, the study of meiofauna diversity and ecology is largely impeded by traditional morphology-based species identification. Their microscopically small size and diverse taxa require specialists for proper extraction and identification, thus consuming substantial time, labor, and cost. As a result, researchers typically focus on few specific taxonomic groups (Urban-Malinga et al., 2005; Xuan et al., 2007), limiting the ability to gain in-depth knowledge of diversity and spatial patterns at a local and global scale. Consequently, autecological information is largely missing for the majority of meiofauna taxa. In recent years, environmental DNA (eDNA) metabarcoding has demonstrated advantages in profiling biodiversity in complex environmental samples (Deiner et al., 2017; Leasi et al., 2018) and has been widely used in assessing the biodiversity of marine benthos (Fonseca et al., 2010; Brannock et al., 2014; Sinniger et al., 2016; Atienza et al., 2020; Steyaert et al., 2020). This molecular approach allows the simultaneous identification of multiple species without taxonomic experts and largely improves the taxonomic resolution as compared to the morphology-based methods. Many research reported that eDNA metabarcoding not only revolutionizes the way to uncover unknown diversity of marine metazoan, but also offers us great promise to explore the community response to environmental stressors or change

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https://doi.org/10.1016/j.ecolind.2023.110223
Received 24 December 2022; Received in revised form 4 April 2023; Accepted 5 April 2023
Available online 14 April 2023

in an accurate, effective, and high-throughput way (Fonseca et al., 2010; Lallias et al., 2014; Leasi et al., 2018). However, eDNA metabarcoding is far less commonly used to explore the correlations between intertidal meiofauna communities with environmental variables (Fonseca et al., 2010; Lallias et al. 2014; Fais et al., 2020b; Bellisario et al., 2021). Among the few available studies, they mainly focused on the effect of salinity and sediment granulometry (Fonseca, et al., 2010, 2014; Fais et al., 2020b; Bellisario, et al., 2021), while the effects of other environmental variables like pH, water content, chlorophyll, and pheophorbide were rarely explored. More importantly, these meiofauna eDNA metabarcoding studies typically focused on species inventory and distribution pattern, nevertheless, none of them extend the application of eDNA metabarcoding to possible habitat discovery and prediction.

In this study, we applied eDNA metabarcoding approach to study the intertidal meiofauna along two locations of eastern China's coastline, aiming to elucidate (1) their diversity and distribution patterns; (2) the effect of environmental variables on the community composition; (3) examine whether the eDNA metabarcoding recovery corresponds to their actual habitat.

2. Material and methods

2.1. Sample collection and processing

A total of fifty-six benthic samples were collected during October 2020 from four localities (N4, N8, N13, N16) of Nantong and 20 localities (L1–L20) of Lianyungang, the two coastal mudflats in eastern China (Fig. S1). The details of the sampling sites are given in Table S1. In the four localities (N4, N8, N13, N16) of Nantong coastal zone, sediment eDNA samples were collected from the high-, middle-, and low-tide zones along 3 transects (transect a, b, c) in each locality, which was perpendicular to the coastal line. Each transect was 100 m apart from the other. In the 20 localities of Lianyungang, samples were collected from the low-tide zones. We sampled sediment eDNA by inserting a 30 mm diameter corer to a depth of 50 mm. Four replicates were collected and pooled as one sample and then stored in the lucifungal glass tube. All the eDNA samples were kept on packs of ice until frozen at −20 ℃. Before the collection, the corers were submerged in a 30% bleach solution for 12 h and repeatedly washed by 99% ethanol and sterile deionized water, and between collections, plastic gloves and corers were changed to prevent the cross contamination.

2.2. Measurements of environmental variables

The environmental properties of the interstitial water including water temperature, pH, salinity, and the properties of the sediment including water content, chlorophyll (reflects phytoplankton concentration), pheophorbide (reflects marine detritus), sediment particle diameter, sediment composition, and organic content were measured from the high-tide, middle-tide, and low-tide zones at each site. Water temperature, pH, salinity were measured using U-10 multiparameter water quality detector (HORIBA Ltd., Japan). The Chlorophyll and pheophorbide were measured by fluorospectro photometer Turner 7200 (Turner Designs, USA). Sediment particle diameter and sediment composition were measured by Mastersizer 3000 particle size analyzer (Malvern Panalytical, Britain), and organic content was measured by Euro Vector EA3000 automatic element analyzer (Euro Vector, Italy). All the environmental measurements and analyses followed standard methods from the Specifications for the oceanographic survey (GB/T 12763.4–2007) and the Specifications for marine monitoring (GB 17378.1–2007). The detailed measurements of environmental variables were given in Table S2.

2.3. eDNA extraction, amplification, and sequencing

Each sediment sample was completely blended using a beating homogenizer (30 times/min, 5 min), and four replicates of 0.25 g were subsampled for the DNA extraction. The total genomic DNA (gDNA) was extracted using DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). The universal metazoan primers SSU_F04/SSU_R22 (GCT TGT CTC AAA GAT TAA GCC; GCC TGC TGC CTT CCT TGG A) (Blaxter et al., 1998) were used to amplify 18S rRNA, and mlCOlintF/jgHCO2198 (GGW ACW GGW TGA ACW GTW TAY CCY CC; TAN ACY TCN GGR TGN CCR AAR AAY CA (MJ-COI) (Leray et al., 2013) and JB3/JB4.5 (JB-COI) (CCT TTG GGC ATC CNG ARG TNT AT; ACC TAA ACT TAR WAC RTA RTG AAA ATG) (Bowles et al., 1992) were used to amplify COI gene. For 18S rRNA, three PCR replicates were conducted with the conditions: 35 cycles of 94 ℃ for 30 s, 54 ℃ for 30 s, 72 ℃ for 30 s, and a final extension at 72 ℃ for 5 min. Similar conditions were applied to COI, except for a 52 °C annealing temperature and a final extension at 65 °C for 5 min. The replicates were pooled and DNA concentrations were measured by Qubit Fluorometer (Invitrogen, CA, USA). The libraries were built with NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs) and sequenced on a HiSeq 2500 platform (2 × 250 bp reads) at Novogene (Beijing, China).

2.4. Bioinformatics and statistics analysis

The bioinformatical part of the metabarcoding analysis was performed with QIIME2 (Bolyen et al., 2019). Demultiplexing and primer removal were implemented by q2-cutadapt (Martin, 2011). Reads were quality controlled by q2-quality-filter (Bokulich et al., 2013) and merged, dereplicated, stripped from chimeras and clustered to operational taxonomic units (OTUs) with a threshold of 99% for 18S rRNA marker and 97% for COI markers by q2-vsearch (Rognes et al., 2016). Taxonomy was assigned using the SILVA (SSU Ref NR 99, v138.1) (Thompson et al., 1994) for the 18S rRNA dataset. Both BOLD (Ratnasingham and Hebert, 2007) and NCBI reference databases were used for COI annotation. Sequences prior to classifier training were processed by RESCRIPt (Robeson et al. 2021). All sequences assigned to organelles, fungi, and OTUs counted less than 5 times and found in less than 2 samples, as well as completely unassigned to certain taxonomy sequences were removed. Diversity analysis was performed in two parts, i.e. all-included datasets and Nematoda-only datasets (the most abundant phylum). We performed a redundancy analysis (RDA) in the package "vegan" in R (Oksanen et al., 2013) to further test and visualize the relationships between the environmental variables and community composition. Environmental variables obtained in the study were correlated with the top 50 abundant species using Spearman's rank correlation implemented in linkET package in R.

2.5. The nematode extraction and salinity tolerance test

The nematode *Acrobeloides* sp. was used to validate if the eDNA metabarcoding recovery corresponds to their actual habitat. This species was selected as it is one of few culturable nematodes, and unexpected in the tidal zone or marine environments (typical terrestrial species) but recovered by eDNA metabarcoding. We resampled sediment where this species recovered by eDNA metabarcoding and the Baermann tray were used to extract nematodes. To confirm the species identification and improve resolution, the 18S rRNA and D2–D3 domain of 28S rRNA was amplified using primer SSU_F04/SSU_R22 and D2A/D3B (De Ley et al., 2005), respectively. The extracted nematodes were maintained in a 1% agar plate supplied with *E.coli* OP50. For the test, the nematodes were washed and the 50 individuals of 4th stage juveniles (J4) were placed on 1% agar water medium containing NaCl at different concentrations (0, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700 mM) with three (24 h survival rate test) or eight (generation time test) replicates for each of treatment. The 200 μl bacteria *E.coli* OP50 were added to the medium as a food resource and the petri dishes were placed in an incubator at 24℃. The model organism *Caenorhabditis elegans* N2 strain was used as a reference to compare the tolerance.

3. Results

3.1. Meiofauna community composition and distribution pattern

The Illumina sequencing of 18S rRNA, MJ-COI, and JB-COI markers generated a total of 3937559, 4615762, and 13499419 paired-end raw reads for 56 samples, respectively. After the clustering and filtering step, 2247, 1792 and 382 unique OTUs were retained for these markers. For the 18S rRNA marker, Nematoda was predominant at the phylum level comprising about 32.1% (on average by samples) of taxa, followed by Arthropoda (10.5%) and Cercozoa (8.0%). At the order level, the three most abundant were Monhysterida (9.5%), followed by Chromadorida (7.4%) and Enoplida (7.4%). All of them were nematodes. Apart from nematodes, Podocopida (Arthropoda), Mytiloida (Mollusca), and Rhabdocoela (Platyhelminthes) also showed great richness (Fig. 1A). While the MJ-COI based metabarcoding revealed a predominant Arthropoda with nearly no Nematoda recovery when annotated by BOLD (Fig. S2A), but a more diverse composition with Arthropoda (57.2%), Mollusca (18.6%) and Nematoda (10.1%) as the three most abundant phyla when using the NCBI reference database (Fig. 1B). We further checked the Arthropoda OTUs annotated by BOLD, and found that a considerable proportion of them were nematodes being misidentified due to a lack of nematode reference sequences. Nevertheless, for JB-COI marker, Nematoda appeared to be predominant (80.8%), followed by Arthropoda (15.1%) and Mollusca (2.1%) (Fig. 1C) when annotated by NCBI. Conversely, this marker generated similar predominant Arthropoda when using the BOLD database (Fig. S2B). Most of the obtained OTUs of the two COI markers were rarely assigned to species level using both BOLD and NCBI databases. Therefore only the 18S rRNA dataset was retained for downstream analyses.

The fine distribution map was plotted for the top 50 abundant species against the 56 sampling sites (all-in datasets Fig. S3, Nematoda-only datasets Fig. 2). In general, most recovered taxa were typically marine or sediment species, coinciding with their expected habitat. One exception is nematode *Acrobeloides thornei,* a typical terrestrial nematode that has never been reported from the tidal zone or marine environment (Fig. 2). Given the fact that this species was detected in eight independent locations, the chance for terrestrial contamination is rather

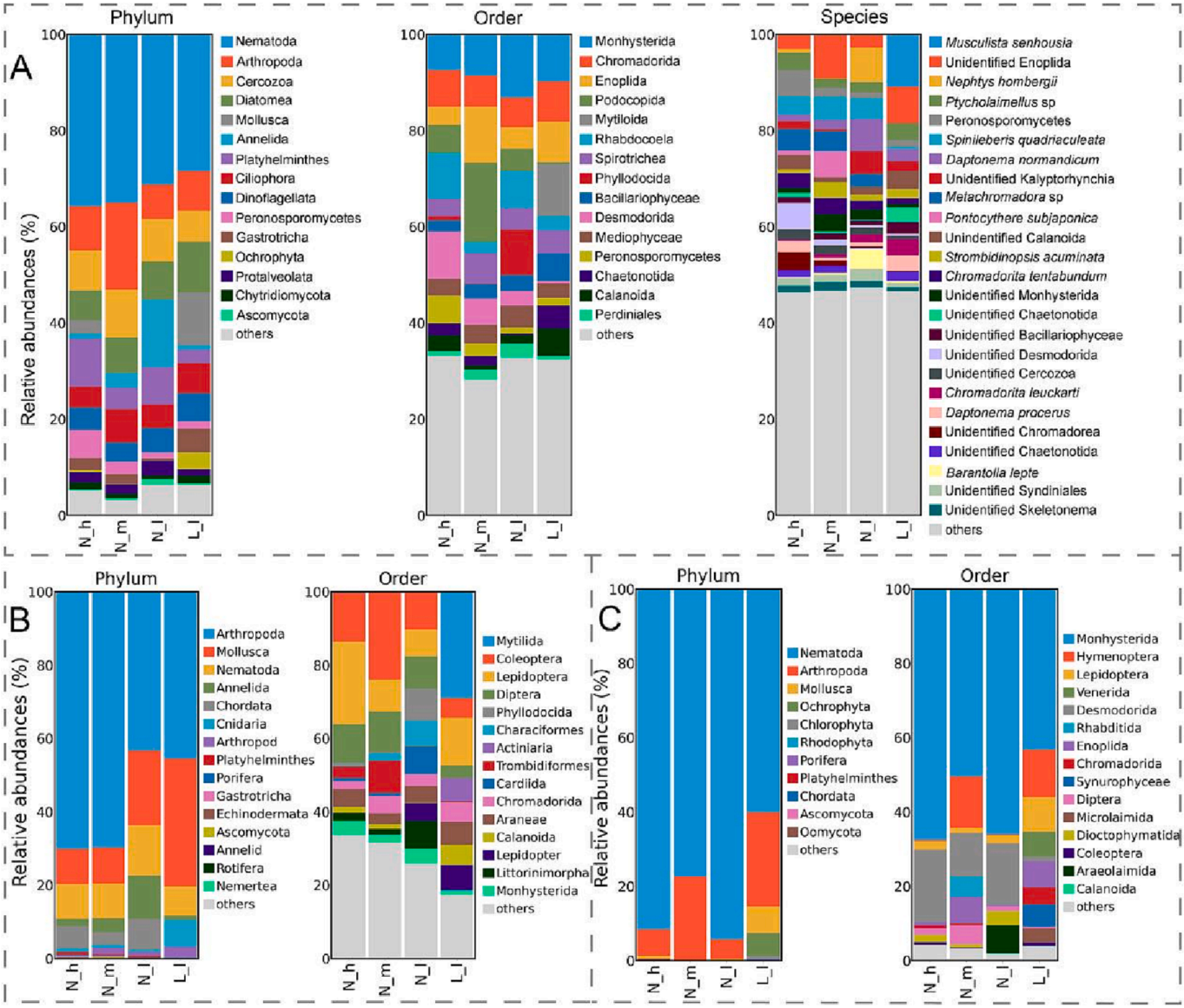

Fig. 1. Community composition and relative abundance of recovered eukaryotes at two coastal locations revealed by different markers. A: 18S rRNA. B: MJ-COI marker annotated by NCBI. C: JB-COI marker annotated by NCBI. Abbreviations for samples: L-l = Lianyungang low-tide zones; N-h = Nantong high-tide zones; N-m = Nantong middle-tide zones; N-l: Nantong low-tide zones.

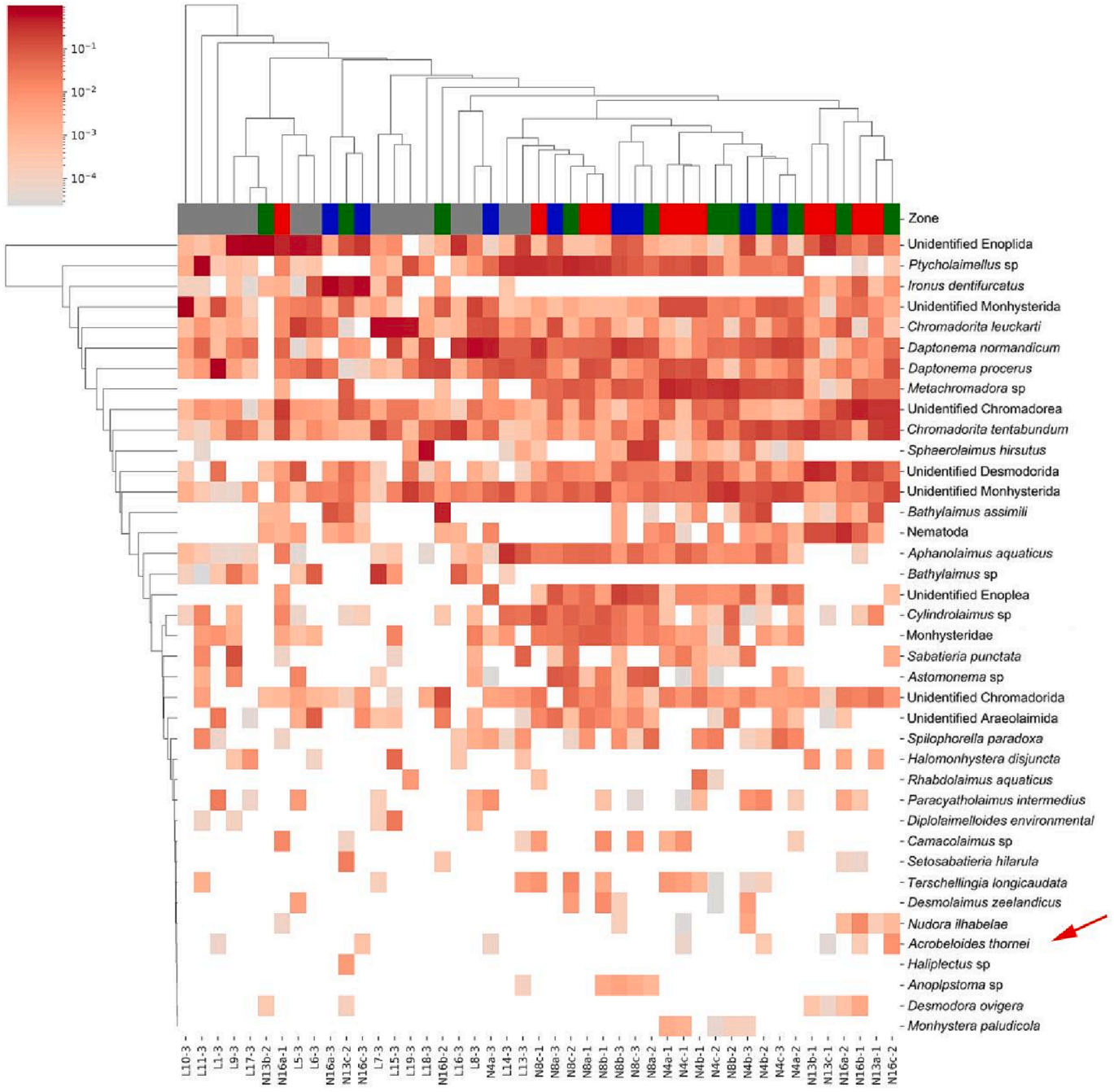

Fig. 2. The 18S rRNA based heatmap of the top 50 abundant nematode species across sampling sites. The OTUs were assigned to order or class level when species-level annotation was not possible. The abundance is shown on a logarithmic scale. The dark and light color represents the relatively higher and lower abundance, respectively. The arrow pointed to the terrestrial nematode *Acrobeloides thornei.*

low. Moreover, the most common sediment-dwelling nematode genera *Ptycholaimellus*, *Daptonema*, and *Chromadorita* were found heavily represented and widely spread across the sites by eDNA metabarcoding (Fig. 2). *Arcuatula senhousia* is a well-known mollusk invasive species living in shallow subtidal zones. The heatmap showed it has high abundance in the low-tide zone while being largely absent in middle- and high-tide zones (Fig. S3).

3.2. The impact of environmental variables on meiofauna diversity

The RDA analysis was applied to investigate correlations between environmental variables and meiofauna community composition (Fig. 3). The first two axes (RDA1 and RDA2) explained approx. 21.9% of the total variances. Within these explained variance, most environmental factors were strongly associated with community separation in RDA2 axis, but only salinity ($r^2 = 0.22$, $p = 0.045$), the content of clay ($r^2 = 0.23$, $p = 0.021$), and pheophorbide ($r^2 = 0.28$, $p = 0.028$) were significant (Table S3). The salinity, water temperature, and median particle diameter were negatively correlated with community separation, while pheophorbide, organic matter, water content, pH value, and fine sand proportion were positively associated with community separation.

A total of 34 out of the 50 recovered taxa significantly correlated to at least one environmental variable in all-included datasets (Fig. 4). The salinity was significantly correlated with the most taxa (18), followed by pheophorbide (12) and proportion of fine sand (nine), while pH and

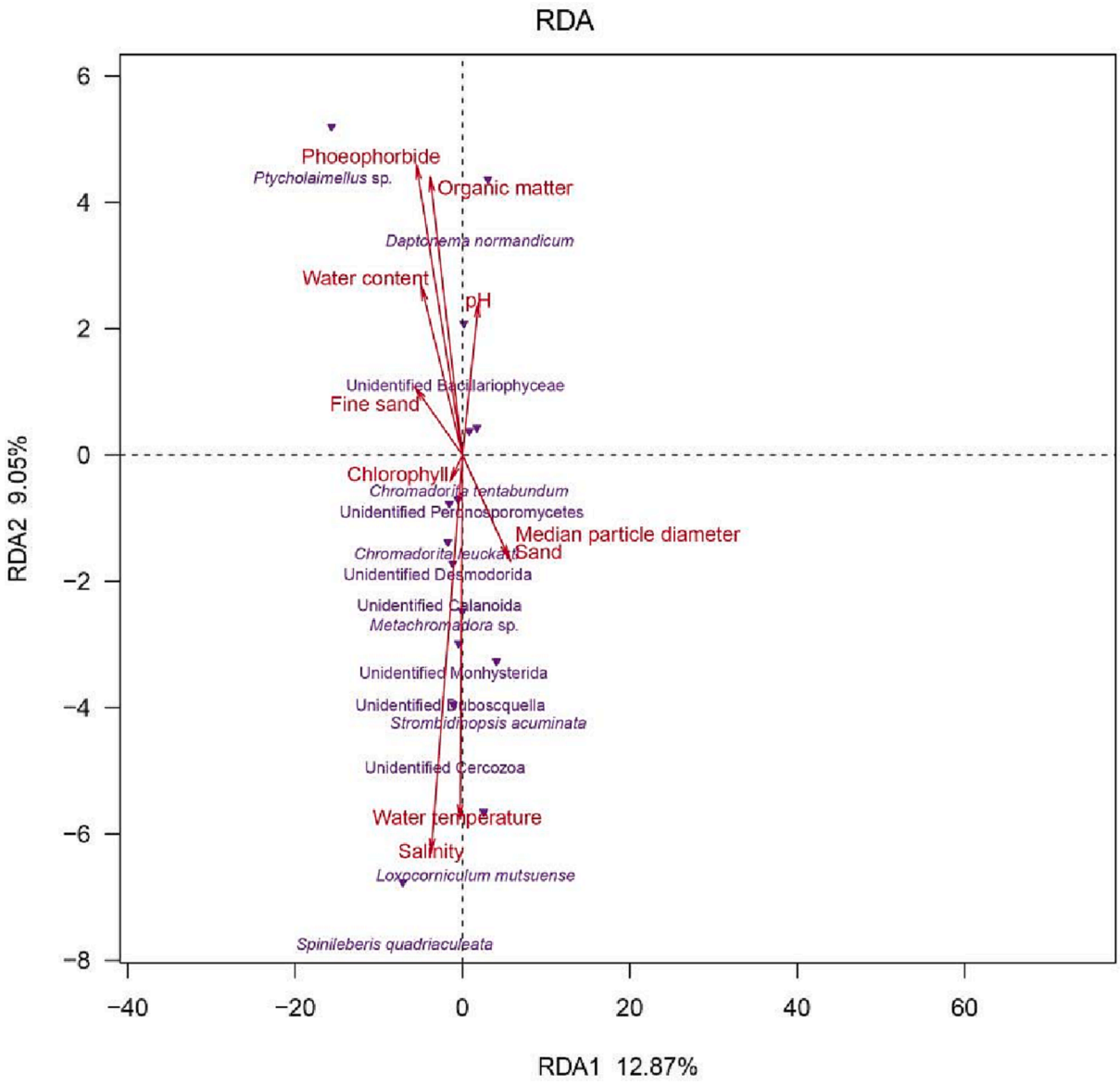

Fig. 3. Redundancy analysis diagrams for ordination of environmental variables and top 20 taxa recovered with 18S rRNA marker.

organic matter are only associated with three taxa. Few variables tend to have more negative correlations, *e.g.* content of clay (four out of six) and proportion of fine sand (six out of nine), and pheophorbide concentration (five out of 12), suggesting that higher levels of these factors can lead to decrease of these taxa. Conversely, water temperature (seven out of eight) and salinity (14 out of 18) have more positive impacts. In respect to each taxon, an unidentified Cercozoa was significantly affected by seven out of 11 examined environmental variables, followed by an ostracode (*Pontocythere subjaponica*), unidentified Calanoida and nematode (*Bathylaimus assimilis*) affected by six variables. Conversely, the cosmopolitan nematodes *Metachromadora* sp. and *Ptycholaimellus* sp. were only significantly correlated with two to three variables (Fig. 4).

3.3. The salinity tolerance test using Acrobeloides sp.

We validated if eDNA metabarcoding recovery is consistent with the biological observation by culturing nematode *Acrobeloides* sp. in laboratory conditions. We resampled five of eight eDNA positive locations and were able to extract *Acrobeloides* sp. from two locations. The morphological analysis suggested the recovered species belongs to the genus *Acrobeloides* (Fig. 5A–D). The amplified 18S rRNA sequence has the highest hits to *Acrobeloides thornei*, which was the same as that recovered in eDNA metabarcoding. However, 28S rRNA phylogeny based on 720 bp sequence placed newly obtained species as sister to an unidentified *Acrobeloides* (MW327029) rather than *A. thornei* (Fig. 5E). We opted to consider it as unidentified, as ~ 400 bp sequences in conserved 18S rRNA gene is unreliable in species-level identification.

We evaluated the salinity tolerance by counting the survival rate after NaCl exposure and generation time. The obtained *Acrobeloides* sp. has more than 80% survival rate at 300 mM NaCl after 24 h, and 2% of individuals can survive up to 550 mM. In comparison, the survival rate of reference *C. elegans* significantly drops when NaCl concentration is higher than 150 mM. None of these tested individuals were alive when salinity is higher than 400 mM (Fig. 5F). This suggested the recovered *Acrobeloides* sp. can well tolerant to high salinity. The generation time was tested on concentrations ranging from 0 to 300 mM NaCl. We demonstrated *Acrobeloides* sp. can complete its life cycle from 5 to 17 days under different salinity pressure, and the generation time was positively correlated to concentration (Fig. 5G). Under 300 mM NaCl, we observed an elevated percentage of juveniles turning to the dauer stage, while many of individuals can still regularly feed and reproduce.

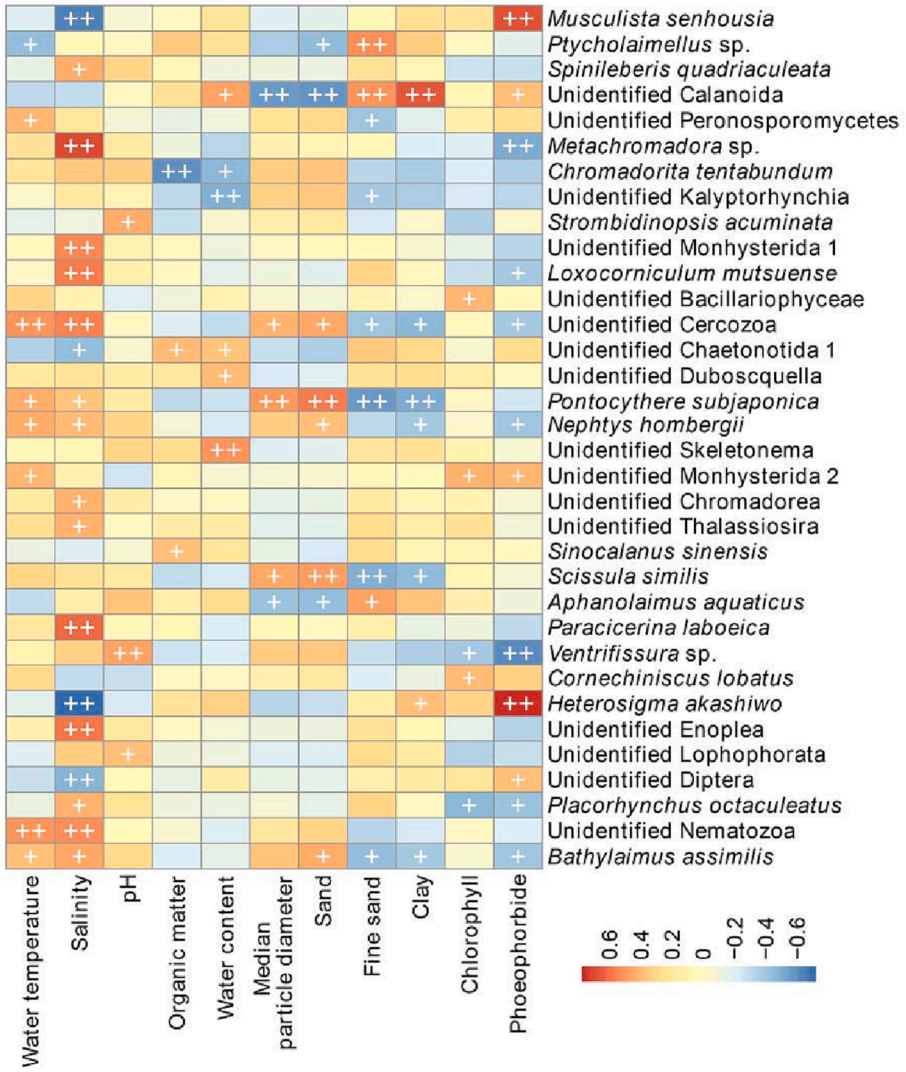

Fig. 4. Correlation heatmap analysis between top 50 abundant taxa and environmental variables using the 18S rRNA marker. No significant taxa were removed and taxa with at least one significant correlation were retained. The red color indicates positive correlations while the blue means negative correlations. The value on the block indicates the level of significance (++ indicates $p = 0.05$, + indicates $p = 0.01$) and was calculated by –log (p adj.) × sign (coef.). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

4.1. The eDNA metabarcoding approach of meiofauna

The study of meiofauna diversity is associated with a range of taxonomic challenges: the small size and fragility of organisms, convergent evolution, morphological conservatism (Derycke et al., 2005; Bhadury et al., 2008; Fontaneto et al., 2009), and developmental and sexual variations in morphology (Tautz et al., 2003; Lambshead, 2004; Blaxter et al., 2005). The eDNA metabarcoding approach circumvents such challenges as morphological identification and enable rapid taxa recovery from pooled samples with high taxonomic resolution (Taberlet et al., 2012). Consequently, there is a growing consensus that the future of biomonitoring lies with metabarcoding and the targeting of eDNA (Aylagas et al., 2018; Pawlowski et al., 2018). Although metabarcoding has received a great appeal for monitoring in a variety of aquatic habitats and functional or taxonomic groups (Chariton et al., 2015; Leray and Knowlton, 2015; Keeley et al., 2018), marine meiofauna, especially nematodes received limited attention in metabarcoding regardless of their dominance in marine ecosystems (Lambshead, 2004). Few pioneer works have demonstrated that metabarcoding could provide wide coverage and good resolution in determining marine nematode diversity (Dell'Anno et al., 2015; Avó et al., 2017; de Faria et al., 2018; Macheriotou et al., 2019; Tytgat et al., 2019). However, these studies involved either the use of Ludox or manual picking that requires a few hundred grams of sediment and is often time-consuming. Consequently, the transport of samples and extraction can be significantly constrained when dealing with large-scale biodiversity investigation. Practically, Ludox extraction was used to improve nematode recovery when applied with the marine primer set SSU_04F/SSU_22R. In the present study, instead of using Ludox extracted specimen, eDNA was directly obtained from sediment for diversity assessment. The amplification and sequencing yielded 32.1% OTUs in 18S rRNA assigned to the nematode. This result is similar to the 40–48.7% of Nematoda recovered when using the Ludox enrichment method (Fonseca et al., 2010, 2014), but far more efficient than similar sediment metabarcoding (primers NF1/18Sr2b) where only 1–3% reads were nematodes (Sapkota and Nicolaisen, 2015; Sikder and Vestergård, 2020). Such a result is promising as it demonstrated that metabarcoding of intertidal nematodes is feasible without prior specimen enrichment, supporting eDNA as a promising alternative for acquiring diversity data and monitoring the ecosystem in a large-scale, efficient, and cost-effective way. Notably, recent studies have demonstrated that the amount of sediment used for DNA extraction can affect biodiversity recovery (Nascimento et al., 2018; Fais et al., 2020b). The PowerSoil DNA isolation kits used in this study only accepts small volume of sample (0.2–0.5 g), which could potentially introduce biases. Alternatively, the PowerMax DNA isolation kit enables to process up to 10 g of sediment and may capture the eDNA more efficiently for meiofauna (Bellisario et al., 2021; Fais et al., 2020b; Nascimento et al., 2018). However, this kit costs four times than the

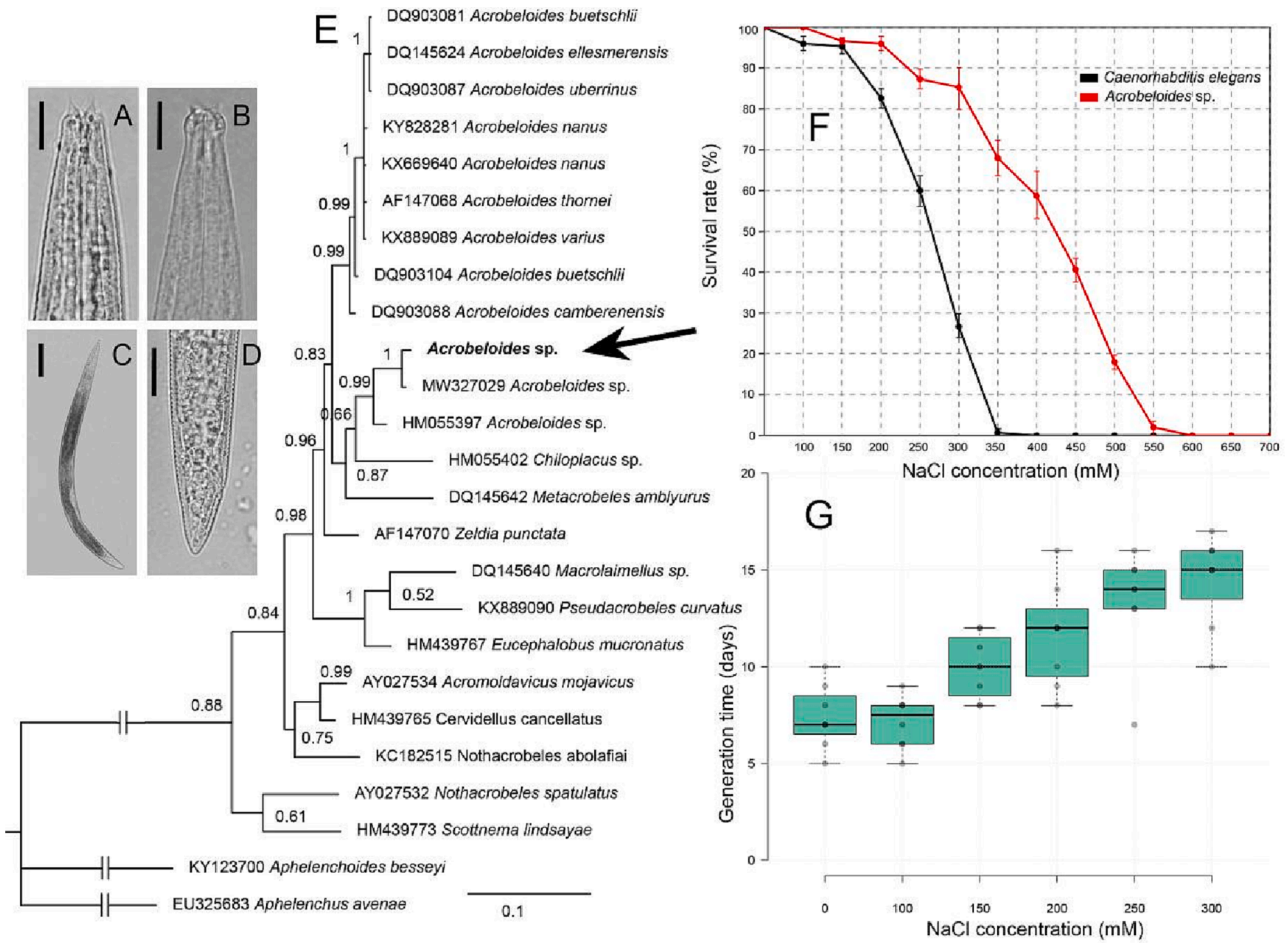

Fig. 5. The test of salinity tolerance for the recovered *Acrobeloides* sp. A–D: morphology of *Acrobeloides* sp. A: Head region of hermaphrodite; B: Head region of dauer juveniles; C: Hermaphrodite body habitus; D: Tail region; E: Bayesian phylogeny inferred from 28S rRNA. The newly obtained species was indicated in bold with an arrow. F: The 24 h survival rate of J4 under different NaCl concentrations; G: The generation time (from egg to gravid hermaphrodite) under different NaCl concentrations. All nematodes were cultured at 24°C.

price of PowerSoil kits, thus presenting a potential barrier to large-scale use (Pawlowski et al., 2021).

The accurate taxonomic annotation of eDNA metabarcoding highly relies on the completeness of the reference database. Unfortunately, the commonly used nematode sequence repository GenBank, EMBL, and BOLD includes numerous errors of various types, and lacks taxonomic coverage for many species (Holovachov, 2016; Qing et al., 2019), especially for COI marker (Ahmed et al., 2019). As a result, COI metabarcoding data from sediment eDNA typically contains a high number of unassignable reads (Atienza et al., 2020), and often produces inconsistent results (Hestetun et al., 2021). In the present study, we demonstrated the BOLD reference database is unsuitable for meiofauna COI metabarcoding because it introduces tremendous artifacts by misidentifying Nematoda taxa as Arthropoda. Hence, the construction of well-developed reference databases of meiofauna should be the priority to improve the taxa recovery and taxonomic assignment accuracy in eDNA-based biodiversity monitoring.

Apart from the reference database, a proper primer selection is also crucial. We observed a strong primer bias towards Arthropoda with MJ-COI primer while towards Nematoda with JB-COI primer. This result is comparable with previous studies using similar primers (Atienza et al., 2020; Castro et al., 2021). Different COI primers tend to lead to dramatic variations in community composition while 18S rRNA primers consistently recovered similar patterns of community assemblages regardless of targeting different loci (Fais et al., 2020b). Given COI gene is highly variable even at the order level, such bias is hard to overcome by designing a universal primer like 18S rRNA. Therefore, despite its low species-level resolution (Tang et al., 2012), 18S rRNA remains the most reliable marker in meiofauna metabarcoding to date. Alternatively, other genes should be introduced as possible markers (Belinky et al., 2012).

4.2. *Intertidal meiofauna composition and their correlations to the environmental variables*

While an equal or more dominant role was recovered for non-Nematoda groups in deep sea eukaryotic metabarcoding (Bik et al., 2012), our observation was consistent with earlier studies showing that nematodes are the most abundant metazoans in intertidal zones, both in the temperate and tropical environments (Fonseca et al., 2010; Hua et al., 2016; Cai et al., 2020). Few recovered species in the present study were important ecological indicators to assess environmental health. For instance, the high abundance of *Ptycholaimellus* sp. is an indicator of good ecological quality (EcoQ) (Moreno et al., 2011; Sahraeian et al., 2020), while the high abundance of *Daptonema* sp. in the low-tide zones in Nantong is an indicator of the poor ecological status of the area (Moreno et al., 2011).

Many abiotic factors have been reported to strongly affect the meiofauna community composition. For instance, salinity along with sediment grain size and tidal exposure are often considered as major drivers of nematode community patterns (Heip et al., 1985; Gheskiere et al., 2004; Vanaverbeke et al., 2011) where higher salinity can lead to

higher biodiversity (Broman et al., 2019). Earlier studies explored the influence of environmental variables limited to salinity and sediment granulometry (Fonseca et al., 2010, 2014; Fais et al., 2020a; Bellisario et al., 2021), and sediment granulometry emerged as a major driving factor of meiofauna diversity and community structure (Fonseca et al., 2010; Bellisario et al., 2021). Apart from these well-known factors, few other variables can also shape specific community. One example with significant impact is the pheophorbide. As a product of chlorophyll breakdown, pheophorbide reflects the level of marine detritus, and has been used as an indicator for macrofaunal grazing intensity in intertidal sediments (Ford and Honeywill, 2002). In the present study, we demonstrated pheophorbide is negatively related with many meiofauna taxa. However, the mechanism that drives this pattern remains unclear and studies are needed to clarify this.

The diversity of free-living marine nematodes is closely linked with the biogeochemical properties of the sediment (Vanaverbeke et al., 2011), while the positive dispersal capacities are limited (Derycke et al., 2013). Theoretically, sensitive species are expected to be constrained by the ecological factors in their favorable niches, whereas a cosmopolitan species are well adapted to various environments. This idea was supported by our eDNA metabarcoding results. The nematodes *Ptycholaimellus* sp., *Daptonema* spp., and *Metachromadora* sp. were considered as cosmopolitan species that are tolerant to inhospitable habitats (Moreno et al., 2011) or even to metal contamination (Heininger et al., 2007). Our environmental correlation analysis demonstrated that those environmental variables can affect these species, although only few were found significant.

4.3. eDNA metabarcoding as a promising tool for new habitat discovery

Assigning the range of species-specific habitat of meiofauna is practically challenging, basically because they are unculturable and tiny in size. The available knowledge were often based on the ecological survey of a specific nature environment, or from morphology-based assumptions, *e.g.* the feeding ecology of nematodes was mostly interpreted from the morphology of buccal cavity rather than experiment (Jensen, 1987 ; Moens and Vincx, 1997). These deficiencies reduced the reliability of the conclusion and limited the scale of meiofauna habitat discovery.

In the present study, our result pointed out that *Acrobeloides* can surprisingly survive in this marine-related environment, while this genus is typically a soil-dwelling bacterivore taxon (Bird and Ryder, 1993). Our further resampling along with morphology and molecular identification confirmed the presence of *Acrobeloides*. The further laboratory culture experiment revealed a similar life cycle as previously reported (Sohlenius, 1973), and confirmed this species can survive and reproduce at the salinity where it was isolated. Although *Acrobeloides* sp. is new to the marine-related environment, it was known to be predominant in soil with high salinity (Ṣalamún et al. 2014), indicating this genus was adapted to such an ecological environment. Since the osmotic tolerance in *C. elegans* can be enhanced after long exposure to high salinity concentration (Lamitina et al., 2004), we expect the upper limitation of salinity tolerance in *Acrobeloides* could be even higher than our observation in this study. This result notably expands our knowledge of its habitats and provides us with new insight into the marine-terrestrial transition in Nematoda. More importantly, our finding demonstrated that eDNA metabarcoding recovery consistent with the actual specie-specific habitat, hence supporting it as a promising tool for high-throughput new habitat discovery and prediction.

5. Conclusions

Our study suggested eDNA metabarcoding can circumvent the limits of the morphology-based method, allowing the rapid and large-scale detection and identification of meiofauna, which are small, cryptic, and poorly known, yet ecologically important. Apart from well-known salinity and sediment granulometry, few more environmental variables were found significantly influenced, *e.g.* content of clay and pheophorbide. The resampling confirmed the presence of a typical terrestrial nematode of *Acrobeloides* in the intertidal sediment and hence demonstrated sediment eDNA has the potential for the new habitat discovery and prediction. Future methodological studies are needed to validate this idea in terms of developing more detailed sampling regimes and a standardized analysis pipeline for a more reproducible result.

CRediT authorship contribution statement

Meng Wang: Investigation, Formal analysis, Writing – original draft. **Timur Yergaliyev:** Formal analysis, Visualization. **Changhai Sun:** Funding acquisition. **Joey Genevieve Martinez:** Writing – review & editing. **Beixin Wang:** Conceptualization, Resources, Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the Major social development projects of Jiangsu, China (grant numbers SBE2018710003). We also acknowledge support by the High Performance and Cloud Computing Group at the Zentrum für Datenverarbeitung of the University of Tübingen, the state of Baden-Württemberg through bwHPC, and the German Research Foundation (DFG) through grant no. INST 37/935-1FUGG.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110223.

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