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Where Meiofauna? An Assessment of Interstitial Fauna at a Belgian Beach

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Abstract: Meiofauna are frequently overlooked in biodiversity assessments, resulting in a lack of understanding regarding their current status, the potential impact of anthropogenic activities, and climate change. This study on the intertidal zone of the Small Beach of Ostend marks a new effort to characterize meiofaunal communities along the Belgian coast. Sampling was carried out on five separate occasions throughout the year, with abiotic data collected during each event. Collected specimens were sorted according to their taxonomic group, resulting in a retrieval of 1742 organisms. Among these, Platyhelminthes and Nematoda were most abundant. Through metabarcoding of the 18S ribosomal region, a biodiversity assessment was conducted, yielding a total of 106 Amplicon Sequence Variants (ASVs). After filtering out rare reads, 65 metazoan ASVs were retained: 18 representing Platyhelminthes, 16 Nematoda, 15 Copepoda, 12 Polychaeta, and 4 Acoela. Identification of the ASVs through blasting generated 23 unique species-level identifications. The highest species richness was observed among Proseriata and Nematoda, each comprising six different species. Additionally, four different species of Polychaeta and Copepoda, two species of Acoela, and one species of Rhabdocoela were identified. Compared to findings on similar beaches along the Belgian coast from about 40 years ago, the meiofaunal communities on this beach exhibit an overall low species richness. Finding fewer and other species might be linked to the potential impact of beach nourishments, human trampling, and climate change. However, confirming this hypothesis requires future research.

Keywords: biodiversity; Ostend; metabarcoding; 18S rDNA; invertebrates

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1. Introduction

A group that is often neglected in biodiversity assessments is meiofauna, a highly diverse and abundant assemblage of animals with an important role in global ecosystems [1–4]. Meiofauna measure roughly between 0.045 and 1 mm in size, but no fixed size definition exists [5–7]. These organisms typically reside in the space between sediment grains [1,8]. Meiofauna encompass representatives of almost all major animal phyla—as many as 24 of 35 animal phyla have meiobenthic representatives living amongst meiofauna—and can be exceptionally abundant and diverse [6,8]. Their high species richness and rapid

response to environmental change make them promising targets for ecological and biomonitoring studies, which is particularly important in times of high anthropogenic pressure and climate change [9–11].

Meiofaunal activities modify a series of physical, chemical, and biological sediment properties. They often do so simultaneously by, for example, displacing sediment grains during burrow construction and displacing organic matter and microorganisms within the sediment matrix during feeding. These modifications can directly or indirectly, and positively or negatively, affect various ecosystem services including sediment stabilization, biogeochemical (nutrient) cycling, waste removal, and food web dynamics, at various spatial and temporal scales [8,12,13].

Although several studies have examined specific meiofaunal taxa or local zonation patterns along parts of the Belgian coast, meiofaunal communities along the Belgian coast as a whole remain poorly characterized [14,15]. In particular, "soft-bodied" meiofauna are often overlooked because of the difficulties associated with their extraction and identification after fixation [16–25]. For instance, biodiversity assessments of sandy beaches often report nematodes and copepods as prime components of meiofaunal communities, while there is no mention of soft-bodied taxa [20–23]. An example can be found in the final report of the Agentschap Maritieme Dienstverlening en Kust (MDK) *Studie over de impact van zandsuppleties op het ecosysteem* [17]. This report provides information about the beach ecosystem of the Belgian coast and the ecological effects of beach nourishments, and proposes possibilities for future research. The report states that the meiofauna of sandy beaches is dominated by Nematoda and Harpacticoida (Copepoda) [17,21]. However, once again, the information in the report of the MDK only focuses on hard-bodied meiofauna—i.e., Polychaeta, Copepoda, and Nematoda—while soft-bodied meiofauna—i.e., turbellarian flatworms—are neglected [17]. The report also states that on Flemish beaches, species diversity is higher in the lower intertidal zone, while organism densities in this zone are lower compared to the high intertidal zone [17,26]. However, as the report only provides information about hard-bodied groups and does not provide any data about soft-bodied organisms, their statement about "meiofaunal densities" is biased towards hard-bodied meiofauna [17]. Furthermore, expertise in meiofauna taxonomy is limited by a number of factors, including the large number of unknown species (Linnean shortfall) and the lack of researchers in the field, combined with the lack of studies on individual meiofaunal taxa (Prestonian shortfall) [3,27–29].

To improve the poor characterization of the meiofaunal communities along the Belgian coast, the primary aim of this study is to document the diversity and abundance of all major meiofaunal groups present in this area, beginning with a pilot assessment on the Small Beach of Ostend. The meiofaunal communities from the intertidal zone of this sheltered, heavily visited beach of Ostend will serve as a case study. Abiotic data, including weather conditions (wind and temperature), water level, and an estimate of algal biomass on the beach, will be collected monthly at a specific location (coordinates) and time. This time indicates the start of the sampling event. Also, to ensure that both soft-bodied and hard-bodied meiofauna are included, soft-body-friendly collection methods were employed, addressing a common oversight in previous studies.

This study serves as a pilot for future perspectives, for which we aim to discover a clear connection between meiofaunal distribution patterns, abundance, diversity, the potential impact of anthropogenic activities (including beach nourishments), and climate change. Many Belgian and foreign tourists visit the Belgian coast every year [30]. For instance, in 2023 alone, 2.2 million tourists visited the coastal communities [31], with Ostend being the most popular of them, with 442,000 arrivals [31]. We already know that people on beaches, more specifically human trampling, has an effect on meiofaunal

communities [32]. However, the potential impact of other anthropogenic activities, such as beach nourishment, remains unknown. Meiofauna also have the potential to serve as indicators of environmental change. However, to use them effectively, we must first identify which organisms are present and gain a deeper understanding of how shifts in community composition and interactions affect ecosystem function [33]. Therefore, in a first phase, it is essential to assess meiofaunal communities across various beaches along the Belgian coast, considering seasonal variations and abiotic factors that may influence these communities. In the second phase, the current meiofaunal communities can be compared across sites and with historical data, such as the data provided by Dr. P.J. (RSZA), to evaluate long-term changes.

About 40 years ago, Dr. Jouk studied (free-living) Platyhelminthes ('Turbellaria') from sandy beaches along the Belgian coast and adjacent areas [34,35]. He conducted various studies beginning in the 1980s, providing a detailed overview of their species composition, diversity, distribution, and ecology [34–37]. Until now, no studies like Dr. Jouk's—examining both hard- and soft-bodied meiofauna along the entire Belgian coast—have been conducted again. As a result, we lack up-to-date knowledge on the current status of Platyhelminthes or any other meiofaunal group in this area—i.e., the Belgian coast. Much may have changed over the past 40 years: species that were very abundant then could be absent on the beaches now, and diversity and species richness may have changed dramatically. Armonies [38] conducted a similar study on the tidal inlets of the northern Wadden Sea. In this study, the species composition of selected taxa of the small benthos is compared to a study performed about 35 years before, using the same methods and sampling sites as in the past [38]. The site-by-site comparison of the species spectrum between 1982, 1984, and 2018 suggests very different communities [38]. This proves that, although research on meiofaunal biodiversity along the Belgian coast has been conducted in the past, our current knowledge remains very limited. In a future study, this is what we plan to do along the Belgian Coast as well, by comparing future findings from our studies with the findings of Dr. Jouk from about 40 years ago [35].

Even though research on meiofaunal abundance and diversity on the Belgian coast has been conducted in the past, our knowledge remains limited, highlighting the importance of additional studies like ours. This pilot study aims to document the diversity and abundance of all major meiofaunal groups, including both hard- and soft-bodied meiofauna, starting with an initial assessment at the Small Beach of Ostend. By providing a baseline for further investigations into meiofaunal biodiversity and ecosystem health along the Belgian coast, this study will lay the groundwork for future research.

2. Materials and Methods

2.1. Sampling and Identification of Meiofauna

Sampling took place in the intertidal zone of the Small Beach of Ostend (Figure 1). As indicated by the name, this beach is part of the beach of Ostend, one of the major coastal communities of Belgium [27]. The Small Beach is located between the Westerstaketsel and the Western Strekdam (51.2362532° N, 2.9186196° E). It is about 400 m long and, on average, 130 m wide [30]. Because of its demarcated location and many visitors every year, the Small Beach of Ostend was selected as a case study for this pilot research.

Meiofaunal samples were collected once a month, starting in late summer 2022 and continuing until mid-winter 2022. Five sampling campaigns were conducted, beginning with a pilot sampling in July (12 July 2022), followed by official samplings in August (30 August 2022), September (30 September 2022), November (12 November 2022), and December (10 December 2022). The pilot sampling served as a first exploration of the study

locality and to check whether the methodology to collect meiofauna was still valid for this location. Its results are not included.

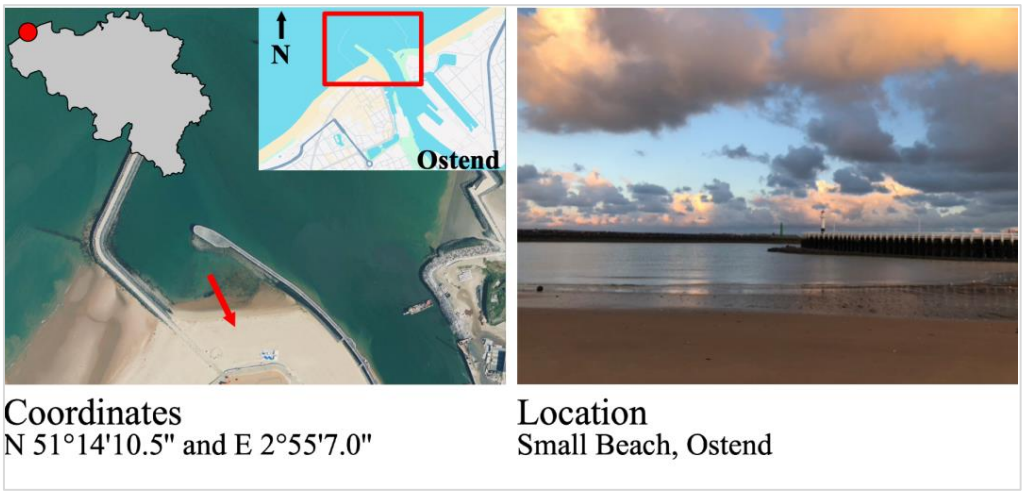

Figure 1. Sampling location of the Small Beach, Ostend. The Small Beach is located between the Westerstaketsel and the Western Strekdam (51.2362532° N, 2.9186196° E). The beach itself is about 400 m long and, on average, 130 m wide. The red dot shows the location of Ostend on the map of Belgium. The red box provides a zoomed-in view of the city of Ostend, highlighting the area where the Small Beach is located. The red arrow indicates the exact location on the Small Beach of Ostend where sand samples were collected for meiofaunal analysis.

During the first official sampling in August, six sand samples were collected, beginning at the low water line and progressing inland at five-meter intervals. Each subsequent sample was taken five meters further inland than the previous one—i.e., at 0 m, 5 m, 10 m, 15 m, 20 m, and 25 m from the sea. Upon processing the August samples, it became apparent that a higher concentration of meiofaunal organisms was recorded in the samples collected farthest away from the sea. Consequently, in the subsequent three sampling sessions, the decision was made to increase the number of samples from six to eight, including samples from a greater distance from the sea—i.e., 0 m, 5 m, 10 m, 15 m, 20 m, 25 m, 30 m, and 35 m from the sea. All samples were collected during low tide with limited exposure to the waves. Sampling date and time were selected to maintain a maximum water-level difference of 0.15 m. Location coordinates, time, weather conditions (wind and temperature), water level, and an estimate of the percentage of algae covering the sampling area of the beach were recorded for each sampling (Table 1). For each sample, a 500 mL jar was filled with sand by scraping off the top layer of sand to a depth of approximately 20 cm. To maximize meiofaunal diversity and abundance in the samples, collection was focused on areas with coarse sand and high organic matter, where meiofauna were expected to be most abundant [15,39]. This approach helped avoid collecting "empty" samples. Additionally, reduced zones, characterized by oxygen-depleted, blackened sand, were deliberately avoided. Also, while organic matter-rich areas were targeted for meiofauna collection, including large organic debris in the samples was avoided, as it could decompose over time and affect sample integrity. It is important to note that due to the limited number of data points available, formal statistical analysis to assess trends in meiofaunal abundance and diversity is not feasible at this stage. Nonetheless, we can discuss some observed patterns based on the available data.

Table 1. Circumstances during the different sampling campaigns from August until December. All samples were taken at the same location: The Small Beach of Ostend (51.2362532° N, 2.9186196° E). "Time" indicates the start of each sampling event. Algae: + + indicates more than 50% of the sampling area of the beach was covered in algae, + − indicates less than 50% of the sampling area of the beach was covered in algae, and 0 indicates there were no algae present on the beach.

Date	Time	Water Level	Wind	Temperature	Algae	Other
August 30 August 2022	10:13 h	Low tide 0.24 m	NE 16 km/h	Actual 19 °C Apparent 18 °C	+ +	/
September 30 September 2022	11:10 h	Low tide 0.25 m	SW 21 km/h	Actual 13 °C Apparent 13 °C	+ −	Seal on beach
November 12 November 2022	09:27 h	Low tide 0.39 m	SE 15 km/h	Actual 8 °C Apparent 6 °C	0	Many seabirds
December 10 December 2022	08:40 h	Low tide 0.37 m	W 13 km/h	Actual 1 °C Apparent −2 °C	+ −	Beach is frozen

After each sampling event, samples were transported to the laboratory at campus Diepenbeek (Hasselt University) and left overnight. This allowed for oxygen depletion in the lower layers, prompting meiofauna to migrate toward the surface, thereby facilitating their extraction. Live meiofauna were extracted using the $MgCl_2$ method, as illustrated in Figure 2 [40]. Collected specimens were sorted morphologically under a stereomicroscope according to their taxonomic group, with Platyhelminthes divided into two major taxonomic groups; Rhabdocoela and Proseriata. This yielded pooled samples of Proseriata, Rhabdocoela, Acoela, Copepoda, Nematoda, Polychaeta, and Isopoda. In case taxonomic identification was not clear under the stereomicroscope, organisms were examined under a Leica DM2500 LED microscope and photo-vouchered using the manufacturer's software LAS X v3.6. Pooled samples were fixed in liquid nitrogen and stored in the −80 °C freezer for metabarcoding.

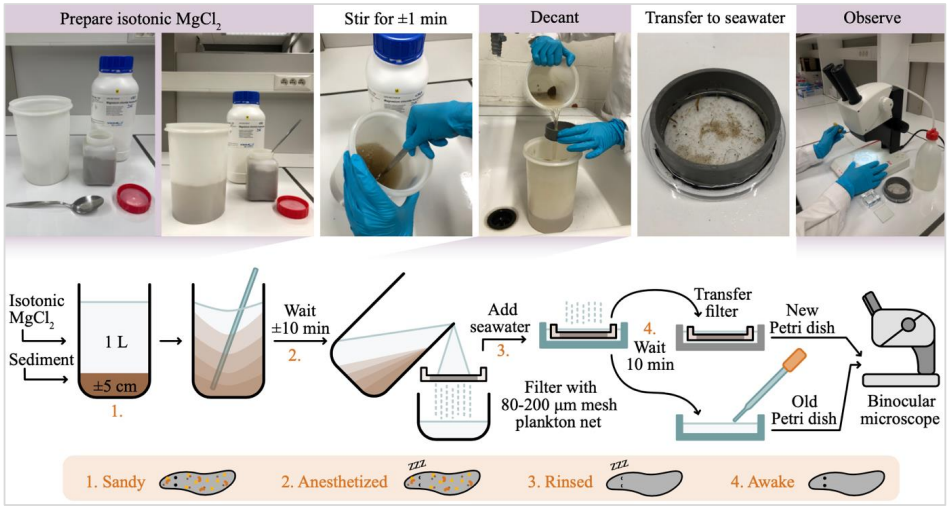

Figure 2. $MgCl_2$ extraction method protocol. An Isotonic $MgCl_2$-solution was prepared, as described by Schockaert [40]. Salinity was checked using a refractometer. Subsequently, the top layer of sand (±5 cm) was added to the solution and stirred firmly for about 1 min. Reduced pieces of sand, recognizable by their black color, were avoided. Next, the solution was left alone for about 10 min. After this, the sand

was stirred gently and decanted twice: first through a net with a 200 µm mesh and secondly through a net with an 80 µm mesh. The nets were transferred to a Petri dish containing some seawater and left there for another 10 min. After that, the nets were transferred to a new Petri dish and the first Petri dishes were ready to be observed with a binocular (stereo)microscope.

2.2. DNA Extractions, Library Preparation, and Sequencing

Due to limited time, logical constraints, and a very high abundance of some taxa, only a subset of organisms identified during the morphological part of this study was selected for molecular sequencing. For instance, although a large number of nematodes were detected morphologically, only a portion of these were included in the molecular sequencing.

DNA extractions were performed following a salting-out protocol [41]. First, the pooled samples, consisting of multiple whole organisms, were submerged in 195 µL TNES buffer and 5 µL proteinase K, and short-spinned to remove droplets. Samples were incubated at 55 °C overnight to ensure lysis was completed. Next, (Invitrogen™, Waltham, MA, USA) yeast tRNA, 65 µL 5M NaCl and 290 µL 96% EtOH were added. The samples were stored at −20 °C for at least one hour and then centrifuged (spinned down) for 15 min at 18,000 rcf. The supernatant was removed and replaced with 1 mL chilled 70% EtOH, and spinned 5 min again at 18,000 rcf. This ethanol rinse was repeated one more time, after which the supernatant was removed. The tube was then left uncapped to allow the pellet to dry, after which elution buffer (0.1X TE with 0.02% Tween™ 20 Surfact-Amps™ Detergent Solution) could be added. DNA was then resuspended at 4 °C overnight. DNA concentrations were evaluated on a Qubit 2.0 fluorometer. One sample (S7; Isopoda, November) did not digest during the DNA extraction and was therefore excluded from downstream work. DNA concentrations of bulk extractions are listed in Table A1.

After, amplicon libraries for Illumina (Eindhoven, The Netherlands) MiSeq Sequencing were prepared. For this preparation, the manufacturer's guidelines of Illumina were followed, and the PCR products Q5® of New England Biolabs (Ipswich, MA, USA) were used. Two primer pairs were selected for metabarcoding, targeting the 18S ribosomal RNA (small subunit) and the cytochrome c oxidase subunit I (COI) [11], using the SSU-F04/(SSU)R22mod primer set for 18S and the mICOIintF/LoboR1 primer set for COI, respectively [42–45].

To amplify the target gene regions for metabarcoding, an amplicon PCR was performed using Q5® Hot Start High-Fidelity DNA polymerase (M0493) [46] with some slight adaptations. A PCR mastermix was prepared in a single batch for 103 reactions, with an additional 10% excess to account for pipetting variability, resulting in a total mastermix volume of 2719.2 µL. This mastermix contained 566.5 µL of 5 × Q5 Reaction Buffer, 56.7 µL of 10 mM dNTPs, 70.8 µL of 10 µM forward primer, 70.8 µL of 10 µM reverse primer, 28.3 µL of Q5 Hot Start DNA Polymerase (2 U/µL), 566.5 µL of 5 × Q5 High GC Enhancer, and 1359.6 µL of nuclease-free water. After preparation, the mastermix was vortexed, briefly centrifuged, and kept on ice. The mix was then distributed into the required wells of a 96-well plate, which was placed into an ISOFREEZE cooling rack, with 24 µL of mastermix added to each well designated for sequencing. Subsequently, 1 µL of DNA template was added to each well containing mastermix, bringing the total reaction volume per well to 25 µL. To assess potential contamination, a negative control sample containing nuclease-free water instead of DNA template was also included. This control sample was processed alongside the biological samples to detect possible contamination introduced during extraction and/or sequencing. If amplicon sequence variants (ASVs) were detected in the control, corresponding sequences found in the biological samples were eliminated from the dataset. Additionally, the control sample served as a quality check to ensure the reliability

of the sequencing and bioinformatics pipeline. The control DNA was visible during gel electrophoresis, but after sequencing (see further), it was determined that the control DNA did not contain any ASVs with significant reads. All reactions were briefly spun down before thermocycling. The thermocycling protocol began with an initial denaturation at 98 °C for 3 min. This was followed by 30 cycles consisting of denaturation at 98 °C for 10 s, annealing at the primer-specific annealing temperature for 30 s, and extension at 72 °C for 30 s. A final extension step was performed at 72 °C for 7 min. After completion, the reaction was held at a temperature between 4 and 12 °C until further processing. PCR products were assessed using 1.5% agarose gel electrophoresis. This gel electrophoresis revealed that the chosen COI primer pair did not amplify any of the collected samples, even after several optimization steps were undertaken. As the COI primers did not yield sufficient results for any of the collected samples, downstream sequencing was performed only for ribosomal regions.

After amplicon PCR, index PCR was carried out to attach dual indices and Illumina adapter sequences to each sample. The index PCR was also performed using Q5® Hot Start High-Fidelity DNA Polymerase (M0493) [46] with some slight adaptations. A PCR mastermix was prepared in bulk for 62 reactions with an additional 10% volume to compensate for pipetting loss, resulting in a total mastermix volume of 1023 µL. The mastermix consisted of 341 µL of 5 × Q5 Reaction Buffer, 34.1 µL of 10 mM dNTPs, 17.1 µL of Q5 Hot Start DNA Polymerase (2 U/µL), and 630.9 µL of nuclease-free water. After vortexing and brief centrifugation, the mastermix was kept on ice. Following preparation, the mastermix was distributed into the necessary wells of a 96-well plate, which was placed into an ISOFREEZE cooling block, with 15 µL added per well designated for sequencing. To each well containing mastermix, 5 µL of purified amplicon product from the first (amplicon) PCR was added, along with 2.5 µL of Nextera XT Index Primer 1 (N7xx) and 2.5 µL of Nextera XT Index Primer 2 (S5xx), bringing the final reaction volume to 25 µL. The thermocycling protocol was identical to the protocol of the amplicon PCR with the only difference being that the initial denaturation at 98 °C for 3 min was followed by 18 cycles consisting of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. This was followed by a final extension step at 72 °C for 7 min.

After index PCR, the resulting PCR products were subsequently purified and pooled equimolarly for downstream Illumina MiSeq sequencing. Sequencing was carried out on an in-house MiSeq machine (Illumina, Eindhoven, The Netherlands), using the MiSeq Reagent Kits v3 (2 × 300 bp; Illumina, Eindhoven, The Netherlands) and 15% Phix spike in. The demultiplexing was run on the Miseq instrument using default settings. After sequencing, downstream analysis was performed in DADA2, following the guidelines of DADA2 for big data [47]. First, primers were removed from the raw amplicon sequencing data via the R-package cutadapt v2.9 [48]. Next, readings were processed using DADA2 v1.22.0 to identify exact amplicon sequence variants (ASVs) [47]. These ASVs represent unique DNA sequences defined at 100% sequence identity, meaning that even a single nucleotide difference results in a distinct ASV. Based on the quality profiles of the reads, we truncated the forward reads at position 240 and the reverse reads at position 160. The maximum number of expected errors was set to 2, and the number of ambiguous nucleotides to 0. Standard parameters were used in all subsequent steps (error rate learning, sample inference, merging, and chimera removal). Unique ASVs were aligned against the SILVA v132 dada2 formatted 18S 'train set' [49]. The R-package phyloseq v1.38.0 [50] was used to merge the ASV-feature table, taxonomy table, and table containing the metadata. ASVs with a low relative abundance were critically assessed. A threshold of >0.1% relative abundance was applied, corresponding to a minimum of 9.6 reads. Any ASV with fewer than 9.6 reads was considered rare and subsequently filtered out.

3. Results

3.1. Morphological Results

In the following section, the results of a total of four sampling efforts in Ostend are summarized. The data from the pilot sampling in July are not included since this was not an official sampling effort. Table 1 (Section 2) lists the recorded environmental conditions during the different samplings.

After $MgCl_2$-decantation and morphological identification, a total of 1742 organisms were retrieved. Figure 3 displays the variation in meiofaunal communities along the intertidal zone. All data underlying this figure can be found in Table A2. During the sampling event in August, only six samples were taken, resulting in no data for sample points 7 and 8. The highest total abundance occurred in samples 5 and 6—i.e., at 20 m and 25 m from the sea—with a total of 303 and 454 organisms across all sampling months, respectively. Among the taxonomic groups, Platyhelminthes (Proseriata and Rhabdocoela) reached their highest abundance in sample 6, with a total of 277 organisms across all sampling months. Acoela were most abundant in samples 6 and 7, with a total of 23 organisms. Nematoda showed their highest abundance in sample 5, with 109 organisms recorded. Polychaeta reached their peak abundance in sample 7, totaling 68 organisms. Copepoda were most numerous in sample 6, with a total of 75 organisms, and Isopoda exhibited the lowest overall abundance, with a maximum of only three individuals recorded in sample 3 across all sampling months. In contrast to total abundance, the highest diversity—measured as the number of different taxa—was observed in samples 3, 4, and 5, located 10 m, 15 m, and 20 m from the low water line, respectively. In these samples, all six taxonomic groups—i.e., Platyhelminthes, Acoela, Nematoda, Polychaeta, Copepoda, and Isopoda—were recorded, indicating a greater taxonomic richness in this mid-intertidal zone.

Microscopic pictures of collected organisms representing the different taxonomic groups are displayed in Figure 4A. Figure 4B displays the total number of organisms collected per sampling effort, organized according to their taxonomic group. In August, only six samples were taken, while eight samples were taken during the other sampling periods (September, November, and December). To facilitate direct comparisons across all sampling periods, the organism counts from August were adjusted to correspond to an eight-sample dataset. Since only six samples were collected in August, the total number of organisms recorded for that period was divided by six to obtain an average per sample and then multiplied by eight to estimate the expected count for an eight-sample set.

The report of the MDK highlights that the zonation patterns of several species are seasonal [17,51,52], implying that the presence of certain organisms may vary across different sampling periods [53]. Indeed, Figure 4B illustrates fluctuations in specimen numbers across the various sampling sessions, and thus, months. The highest overall abundance of organisms was observed in August, the warmest month, whereas the lowest count was recorded in December, the coldest month. This pattern is well exemplified by Acoela, which had 47 specimens (corrected for eight samples) recorded in August, decreasing to 30 in September, 18 in November, and only 9 in December, mirroring the gradual decline in environmental temperature (Table 1, Section 2). Similarly, Nematoda displayed its highest abundance in August, while Polychaeta peaked in November. Copepods reached their maximum abundance in December, and Isopoda were most abundant in September and November. Among all taxonomic groups, Platyhelminthes were the most frequently observed, with their highest numbers recorded in December, consisting primarily of proseriates. Notably, only one specimen of Rhabdocoela was observed in December, and no other platyhelminth groups were detected during this study. In contrast, Isopoda were the least frequently encountered organisms throughout the sampling period.

Figure 3. Variation in meiofaunal communities along the intertidal zone. Samples were collected at multiple time points (August, September, November, and December), showing seasonal variations in species composition, and at different distances from the sea (0 m to 35 m), demonstrating spatial variation. The taxonomic groups represented include Platyhelminthes (Proseriata and Rhabdocoela), Acoela, Nematoda, Polychaeta, Copepoda, and Isopoda, with total counts varying across sampling positions and months. During the sampling event in August, only six samples were taken, resulting in no data for sample points 7 and 8. This is indicated in the figure by "NA".

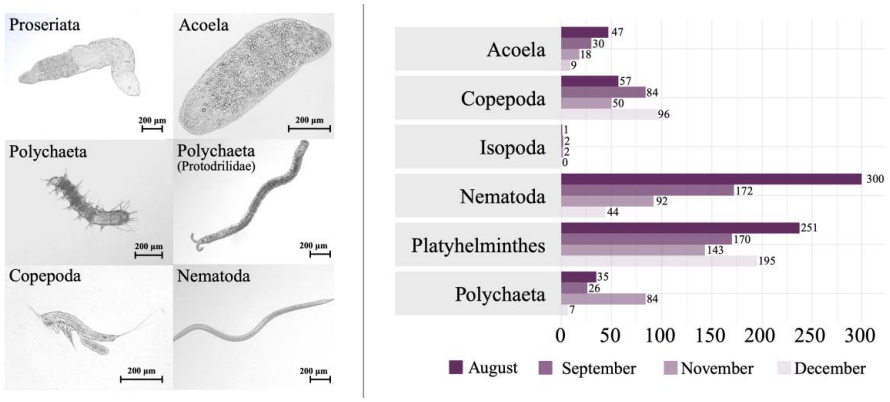

Figure 4. Collected specimens from each sampling effort, organized according to their taxonomic group. (**A**) Microscopic pictures taken from some organisms of different taxonomic groups. (**B**) Total number of organisms collected per sampling effort (August, September, November, December), organized according to their taxonomic group. The number of organisms counted in August were corrected for eight samples, in order to easily compare all different sampling efforts.

3.2. Molecular Results

Figure 5 shows the number of different species per taxon for all samplings from September until December based on SSU-metabarcoding. A total of 106 Amplicon Sequence Variants (ASVs) were identified, 77 of which were assigned to Metazoa. Tables A3–A9 show the full list of ASVs per taxa. After filtering out rare (insignificant) reads, 65 metazoan ASVs were retained: 12 representing Polychaeta, 17 Proseriata (Platyhelminthes), 15 Copepoda, 4 Acoela, 16 Nematoda, and 1 representing Rhabdocoela (Platyhelminthes). Identification of the ASVs through blasting yielded 23 unique species identifications (Figure 5). The highest species richness was observed among Proseriata and Nematoda, each comprising six different species. Additionally, four different species of Polychaeta and Copepoda, two species of Acoela, and one species of Rhabdocoela were identified.

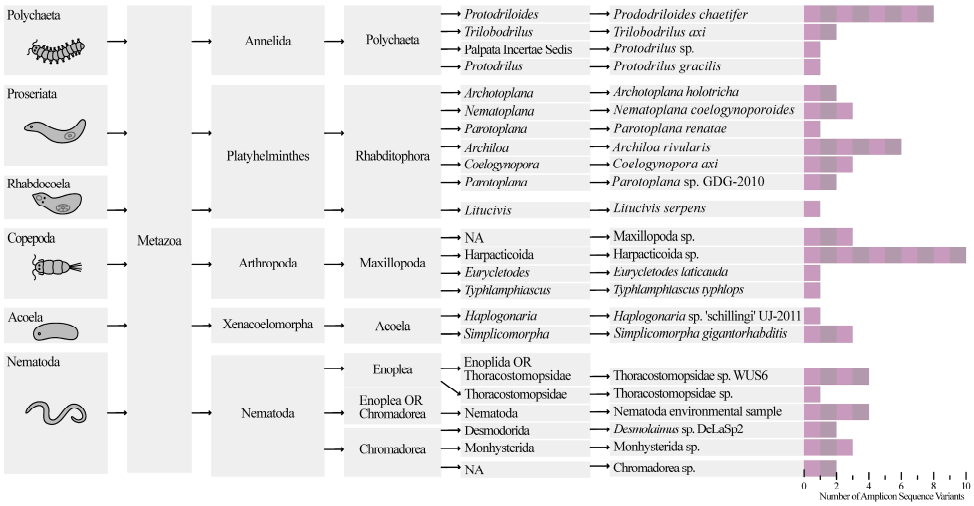

Figure 5. Preliminary biodiversity assessment of the Small Beach of Ostend based on metabarcoding of the 18S ribosomal region. A total of 106 Amplicon Sequence Variants (ASVs) were identified, including 77 metazoan reads. After filtering out rare reads, 65 metazoan ASVs were retained. These reads belonged to Polychaeta, Proseriata, Rhabdocoela, Copepoda, Acoela, and Nematoda. A total of twenty-three different species were identified: six different species of Proseriata and Nematoda, four different species of Polychaeta and Copepoda, two different species of Acoela, and one species of Rhabdocoela. After sequencing, the guidelines of DADA2 for big data were followed. Taxonomy was blasted against the SILVA v132 dada2 formatted 18S 'train set'.

4. Discussion

4.1. Morphologically Observed Trends in Meiofaunal Diversity and Abundance

The final report of the MDK, *Studie over de impact van zandsuppleties op het ecosysteem*, provides a baseline for the state of Belgian beaches before the start of this project [17]. The report of the MDK, however, focuses solely on hard-bodied meiofauna—i.e., Polycheata, Copepoda, and Nematoda—highlighting the importance of our present study, which also includes soft-bodied organisms. According to the report of the MDK, meiofaunal diversity on Flemish beaches is higher in the lower intertidal zone, whereas organism densities (abundances) are greater in the high intertidal zone [17,26]. Our findings on the Small Beach align with the MDK report, as the highest meiofaunal abundance was recorded in samples 5 and 6, located higher in the intertidal zone at 20 m and 25 m from the low-water line. Species richness, on the other hand, was greatest in samples 3, 4, and 5, which are located closer to the low water line, specifically at 5 m, 10 m, and 15 m from the sea. Additionally, this pattern aligns with findings from other beaches with slow drainage and a high-water table, where meiofauna tends to be concentrated in the upper tidal zone [54].

The observed trends in this present study concerning meiofaunal abundance and diversity, illustrated by fluctuations in specimen counts across different sampling months, may be attributed to the seasonal zonation patterns of certain organisms, as suggested by the final report of the MDK. Another explanation could be predator–prey relationships or incompatibility among different species, preventing them from coexisting in high numbers [51,52]. To gain a better understanding of these patterns, further investigation is required to explore the underlying ecological factors influencing the observed variations in abundance among different taxa.

Finally, a group worth discussing here is Rhabdocoela. A previous sampling effort at the Small Beach of Ostend performed by members of our research group in 2019 retrieved these organisms in high numbers. However, our new sampling efforts only retrieved a single species, represented by one specimen, of Rhabdocoela in December. SSU-metabarcoding identified this species as *Litucivis serpens* Ax and Heller, 1970. *Litucivis serpens* has not yet been recorded on the Small Beach of Ostend, but this species has been found on the Belgian east coast (Bredene–Dutch border) [55]. A possible explanation for this decrease could be the recent supplementation works performed on the Small Beach of Ostend (last nourishment before this research: February 2022). Supplementation works on the beaches of Ostend are carried out on behalf of the MDK and are used for beach nourishments. A thin layer of sand (<30 cm) is scraped from the beach using a variety of heavy machinery. The impact of these nourishments might explain the lower number of rhabdocoels observed in this study compared to the research conducted four years ago, when no supplementation works had been carried out. Another explanation could be that meiofaunal communities suffer from human passage. Martínez et al. [32] suggest that the effect of the presence of people could primarily be attributed to trampling. Other potential effects might be indirectly related to the human presence, such as the amount of sunscreen and fecal-related bacteria entering the water, which are likely proportional to the number of tourists [32]. It is important to monitor meiofaunal communities because similar impacts may be affecting other taxa as well.

4.2. Molecular Findings: Varying Amplification Yields

For this study, two primer pairs were selected, targeting 18S (SSU-F04; (SSU) R22mod) and COI (mICOIintF; LoboR1) [11]. However, only the 18S primers yielded sufficient results, while the COI primers, which were successful in other studies [11], failed for all samples in this instance. This failure may be attributed to the higher substitution rate of COI compared to 18S [56], causing greater sequence divergence among organisms in

the samples. Indeed, a recent study comparing COI and 18S rRNA genes for identifying marine nematodes demonstrates that 18S is more readily amplified compared to COI, with amplification success rates of 57% versus 43%, and sequencing success rates of 61% versus 39% [57]. For platyhelminths specifically, Vanhove et al. [58] also demonstrated how high molecular variability and contamination problems limit the possibilities for barcoding using standard COI-based protocols.

4.3. Diversity Assessed by 18S Metabarcoding

Tables A3–A9 show all metazoan ASVs identified through metabarcoding of the 18S ribosomal region. After filtering out rare reads, 65 significant reads remained, belonging to Acoela, Proseriata, Rhabdocoela, Nematoda, Copepoda, and Polychaeta. This sequencing effort also revealed traces of some additional organisms, belonging to one of the following taxa: Nemertea, Macrostomida, and Gastropoda. However, there were no significant ASVs for either of these taxa.

Reads were processed into exact ASVs, generated using 100% sequence similarity, via DADA2 v1.22.0 [43]. Taxonomy was blasted against the SILVA v132 dada2 formatted 18S 'train set', clustered at 99% similarity [49]. However, meiofaunal taxonomy is constrained by several factors, including a high number of unknown species, limited expertise in the field, and a paucity of studies on individual meiofaunal taxa. Consequently, meiofaunal species databases are relatively underdeveloped. While ASVs can be matched to the most plausible species, these identifications may be subject to uncertainty. Therefore, morphological analysis remains essential for accurate species identification in meiofaunal research [59].

In a future study, we aim to use meiofauna as a bioindicator for ecosystem health along the Belgian coast. However, before this can be achieved, it is essential to first assess the current meiofaunal communities in the region. Once this baseline is established, we can compare these contemporary communities with those documented in a historical study conducted by Dr. Jouk in the 1980s [35]. To demonstrate the feasibility and potential value of such a comparison, we have already conducted a preliminary analysis, comparing the meiofaunal communities identified through metabarcoding with those reported in the historical study. In our present study, six proseriate species were identified: *Archiloa rivularis* de Beauchamp, 1910; *Archotoplana holotricha* Ax, 1956; *Coelogynopora axi* Sopott, 1972; *Nematoplana coelogynoporoides* Meixner, 1938; *Parotoplana renatae* Ax, 1956; and *Parotoplana* sp. GDG-2010. *Archotoplana holotricha* and *Archiloa rivularis* were not found in the assessment conducted in the 1980s. *Nematoplana coelogynoporoides* had not previously been recorded in Ostend but has been observed at multiple other sampling locations along the Belgian coast [35]. *Parotoplana renatae* was not found in Ostend during the research by Jouk [35], but *Parotoplana papii* was found back then. *Coelogynopora axi* was found by Jouk [35] during his research, but only in Knokke and 'Het Zwin'. This species could also have spread to the Small Beach of Ostend. For Rhabdocoela, only a single species was identified: *Litucivis serpens*. This species was also recorded during the 1980s sampling effort, but only in 'Het Zwin' and Zeebrugge, never in Ostend [35]. For Acoela, two different species were identified: *Haplogonaria* sp. 'schillingi' UJ-2011 and *Simplicomorpha gigantorhabdis*. Neither species was recorded in the research from 40 years ago [35]. Conversely, *Paratomella rubra*, a species commonly encountered in the historical study [35], was not detected in the current research. This initial comparison suggests that valuable insights can be gained from examining long-term meiofaunal community changes, supporting the use of meiofauna as an indicator of ecosystem health.

5. Conclusions

The primary goal of this study was to document the biodiversity and abundance of the major meiofaunal groups on the Belgian coast, specifically on the Small Beach of Ostend, through a combination of morphological and molecular methods. A morphological assessment yielded abundance data, and 18S ribosomal RNA metabarcoding was used to assess meiofaunal diversity. However, it is important to note that, given that metabarcoding studies depend heavily on available databases, it remains crucial to invest in alpha taxonomical research and continued development of sequence databases. The now available data suggest a potential seasonal trend in meiofaunal communities, which requires statistical validation from a more comprehensive dataset in future work. Sediment temperature, pH, oxygen concentration, organic matter content, sand grain size, and salinity were not examined in this study. However, these are all recognized as important factors shaping meiofaunal habitats and will be considered in future investigations. The present study indicates that meiofaunal communities are not thriving on the studied beach, with an overall lower species richness and abundance. However, to fully understand the interrelationships between meiofaunal abundance, diversity, and environmental factors, additional data points are required. In particular, we encourage future researchers to investigate the impacts of ongoing beach nourishments, human activity, and anthropogenic climate change on meiofaunal communities.

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Conflicts of Interest: Author Emma Van de Reydt was employed by the company Emma Van de Reydt BV. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A

Table A1. Results of Qubit measurement from all the pooled samples kept from September, November, and December. The pooled Isopoda sample of November (S7) did not dissolve during DNA extractions and was excluded from downstream work.

Sample Number	Specimen	Month	Concentration (ng/µL)
0	Control	/	<0.50
1	Polychaeta Protodrilidae	September	23.2
2	Acoela	September	29.0
3	Copepoda	September	25.6
4	Proseriata	September	23.8
5	Polychaeta	September	21.0
6	Nematoda	September	25.2
7	Isopoda	November	/
8	Polychaeta Protodrilidae	November	19.8
9	Proseriata	November	7.82
10	Copepoda	November	22.2
11	Nematoda	November	6.68
12	Rhabdocoela	November	23.0
13	Acoela	November	18.0
14	Polychaeta	November	21.0
15	Proseriata	December	20.6
16	Polychaeta Protodrilidae	December	1.55
17	Nematoda	December	17.0
18	Rhabdocoela	December	17.4
19	Copepoda	December	16.1

Table A2. Detailed meiofaunal abundance data across sampling locations and months. This table presents the total abundance of meiofaunal organisms identified at different distances from the sea (0 m to 35 m) and across four sampling months (August, September, November, and December). The data include taxonomic group-specific abundances, highlighting seasonal and spatial variations in community composition. Due to logistical constraints, only six samples were taken during the August sampling event, resulting in no data for sample points 7 and 8 for this month. This is indicated in the table by "NA."

Sample Number	Taxonomic Group	August	September	November	December	All Months Together
1	Platyheminthes	1	1	3	3	8
	Acoela	0	0	0	0	0
	Nematoda	4	14	7	2	27
	Polychaeta	0	0	3	0	3
	Copepoda	0	0	0	1	1
	Isopoda	0	0	0	0	0

Table A2. *Cont.*

Sample Number	Taxonomic Group	August	September	November	December	All Months Together
2	Platyheminthes	1	8	8	5	22
	Acoela	3	2	2	0	7
	Nematoda	18	23	15	18	74
	Polychaeta	18	8	3	5	34
	Copepoda	0	1	8	17	26
	Isopoda	0	0	0	0	0
3	Platyheminthes	2	7	5	8	22
	Acoela	1	4	2	1	8
	Nematoda	29	19	13	13	74
	Polychaeta	0	0	9	1	10
	Copepoda	2	6	12	8	28
	Isopoda	0	1	2	0	3
4	Platyheminthes	1	13	4	10	28
	Acoela	9	4	0	1	14
	Nematoda	53	20	24	8	105
	Polychaeta	2	1	2	1	6
	Copepoda	9	5	5	4	23
	Isopoda	0	1	0	0	1
5	Platyheminthes	41	20	13	40	114
	Acoela	8	7	0	2	17
	Nematoda	71	14	24	0	109
	Polychaeta	6	5	4	0	15
	Copepoda	24	1	13	9	47
	Isopoda	1	0	0	0	1
6	Platyheminthes	142	23	63	49	277
	Acoela	14	2	5	2	23
	Nematoda	50	24	2	1	77
	Polychaeta	0	0	2	0	2
	Copepoda	8	13	9	45	75
	Isopoda	0	0	0	0	0
7	Platyheminthes	NA	55	35	59	149
	Acoela	NA	11	9	3	23
	Nematoda	NA	30	2	1	33
	Polychaeta	NA	7	61	0	68
	Copepoda	NA	6	3	12	21
	Isopoda	NA	0	0	0	0

Table A2. *Cont.*

Sample Number	Taxonomic Group	August	September	November	December	All Months Together
8	Platyheminthes	NA	43	12	21	76
	Acoela	NA	0	0	0	0
	Nematoda	NA	28	5	1	34
	Polychaeta	NA	5	0	0	5
	Copepoda	NA	34	0	0	34
	Isopoda	NA	0	0	0	0

Table A3. Metazoan ASVs belonging to Polychaeta identified through metabarcoding of the 18S ribosomal region. Rare reads are marked in red and were excluded from the results.

Polychaeta					
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloides chaetifer*
Polychaeta	Metazoa	Annelida	Polychaeta	*Trilobodrilus*	*Trilobodrilus axi*
Polychaeta	Metazoa	Annelida	Polychaeta	*Trilobodrilus*	*Trilobodrilus axi*
Polychaeta	Metazoa	Annelida	Polychaeta	Palpata incertae sedis	*Protodrilus* sp.
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodrilus*	*Protodrilus gracilis*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloidessymbioticus*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodriloides*	*Protodriloidessymbioticus*
Polychaeta	Metazoa	Annelida	Polychaeta	*Protodrilus*	*Protodriluscorderoi*

Table A4. Metazoan ASVs belonging to Copepoda identified through metabarcoding of the 18S ribosomal region. Rare reads are marked in red and were excluded from the results. "NA" indicates that no taxonomic assignment could be made at that specific rank.

Copepoda					
Copepoda	Metazoa	Arthropoda	Maxillopoda	NA	Maxillopoda sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	NA	Maxillopoda sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	NA	Maxillopoda sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.

Table A4. *Cont.*

Copepoda					
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	Harpacticoida	Harpacticoida sp.
Copepoda	Metazoa	Arthropoda	Maxillopoda	*Eurycletodes*	*Eurycletodes laticauda*
Copepoda	Metazoa	Arthropoda	Maxillopoda	*Typhlamphiascus*	*Typhlamphiascus typhlops*
Copepoda	Metazoa	Arthropoda	Insecta	*Frieseomelitta*	*Frieseomelittavaria*

Table A5. Metazoan ASVs belonging to Proseriata identified through metabarcoding of the 18S ribosomal region. Rare reads are marked in red and were excluded from the results.

Proseriata					
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archotoplana*	*Archotoplana holotricha*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archotoplana*	*Archotoplana holotricha*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Nematoplana*	*Nematoplana coelogynoporoides*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Nematoplana*	*Nematoplana coelogynoporoides*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Nematoplana*	*Nematoplana coelogynoporoides*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Parotoplana*	*Parotoplana renatae*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archiloa*	*Archiloa rivularis*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archiloa*	*Archiloa rivularis*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archiloa*	*Archiloa rivularis*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archiloa*	*Archiloa rivularis*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archiloa*	*Archiloa rivularis*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Archiloa*	*Archiloa rivularis*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Coelogynopora*	*Coelogynopora axi*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Coelogynopora*	*Coelogynopora axi*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Coelogynopora*	*Coelogynopora axi*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Parotoplana*	*Parotoplana* sp. GDG-2010
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Parotoplana*	*Parotoplana* sp. GDG-2010
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Cirrifera*	*Cirriferasopottehlersae*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	*Cirrifera*	*Cirriferasopottehlersae*
Proseriata	Metazoa	Platyhelminthes	Rhabditophora	Proseriata	*Proseriata* sp.

Table A6. Metazoan ASVs belonging to Rhabdocoela identified through metabarcoding of the 18S ribosomal region. There were no insignificant reads for this taxon.

Rhabdocoela					
Rhabdocoela	Metazoa	Platyhelminthes	Rhabditophora	*Litucivis*	*Litucivis serpens*

Table A7. Metazoan ASVs belonging to Acoela identified through metabarcoding of the 18S ribosomal region. Rare reads are marked in red and were excluded from the results.

Acoela					
Acoela	Metazoa	Xenacoelomorpha	Acoela	*Haplogonaria*	*Haplogonaria* sp. 'schillingi' UJ-2011
Acoela	Metazoa	Xenacoelomorpha	Acoela	*Simplicomorpha*	*Simplicomorpha gigantorhabditis*
Acoela	Metazoa	Xenacoelomorpha	Acoela	*Simplicomorpha*	*Simplicomorpha gigantorhabditis*
Acoela	Metazoa	Xenacoelomorpha	Acoela	*Simplicomorpha*	*Simplicomorpha gigantorhabditis*
Acoela	Metazoa	Xenacoelomorpha	Acoela	*Atriofronta*	*Atriofronta polyvacuola*

Table A8. Metazoan ASVs belonging to Nematoda identified through metabarcoding of the 18S ribosomal region. Rare reads are marked in red and were excluded from the results.

Nematoda					
Nematoda	Metazoa	Nematoda	Enoplea	Enoplida	*Thoracostomopsidae* sp. WUS6
Nematoda	Metazoa	Nematoda	Enoplea	Enoplida	*Thoracostomopsidae* sp. WUS6
Nematoda	Metazoa	Nematoda	Enoplea	Thoracostomopsidae	*Thoracostomopsidae* sp. WUS6
Nematoda	Metazoa	Nematoda	Enoplea	Enoplida	*Thoracostomopsidae* sp. WUS6
Nematoda	Metazoa	Nematoda	Enoplea	Thoracostomopsidae	*Thoracostomopsidae* sp.
Nematoda	Metazoa	Nematoda	Chromadorea	Nematoda	Nematoda environmental sample
Nematoda	Metazoa	Nematoda	Enoplea	Nematoda	Nematoda environmental sample
Nematoda	Metazoa	Nematoda	Chromadorea	Nematoda	Nematoda environmental sample
Nematoda	Metazoa	Nematoda	Enoplea	Nematoda	Nematoda environmental sample
Nematoda	Metazoa	Nematoda	Chromadorea	Desmodorida	*Desmolaimus* sp. DeLaSp2
Nematoda	Metazoa	Nematoda	Chromadorea	Desmolaimus	*Desmolaimus* sp. DeLaSp2
Nematoda	Metazoa	Nematoda	Chromadorea	Monhysterida	*Monhysterida* sp.
Nematoda	Metazoa	Nematoda	Chromadorea	Monhysterida	*Monhysterida* sp.
Nematoda	Metazoa	Nematoda	Chromadorea	Monhysterida	*Monhysterida* sp.
Nematoda	Metazoa	Nematoda	Chromadorea	NA	*Chromadorea* sp.
Nematoda	Metazoa	Nematoda	Chromadorea	NA	*Chromadorea* sp.
Nematoda	Metazoa	Nematoda	Enoplea	Enoplida	Uncultured eukaryote

Table A9. Other metazoan ASVs identified through metabarcoding of the 18S ribosomal region. All these reads are rare (marked in red) and were excluded from the results.

Other					
Nemertea	Metazoa	Nemertea	Anopla	Nemertean	*Nemertean* sp. 2 SA-2011
Macrostomida	Metazoa	Platyhelminthes	Rhabditophora	*Myozonaria*	*Myozonaria fissipara*
Gastropoda	Metazoa	Mollusca	Gastropoda	Heterobranchia	*Heterobranchia* sp.

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