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Research Article

Revealing Mucilage Event-Linked Community Composition in the Sea of Marmara from eDNA Metabarcoding Data

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ABSTRACT

Mucilage events are among the most conspicuous phenomena in marine ecosystems and present numerous challenges in determining the composition of communities associated with them. To overcome this problem, we used environmental DNA (eDNA) metabarcoding approaches to reveal the species-level resolution of community composition. Mucilaginous aggregates were sampled at six collecting sites during a novel mucilage event (autumn 2021–summer 2022) in the Sea of Marmara, Türkiye. A wide range of plankton community compositions was detected in mucilage samples. eDNA metabarcoding was effective in predicting the community composition of mucilage, which is composed of a wide variety of organisms from mucilaginous aggregates.

Keywords: eDNA, metabarcoding, mucilage, the Sea of Marmara, molecular ecology, biomonitoring

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INTRODUCTION

Marine ecosystems have been facing a greater impact from human activities than that at any other time in history (Berry et al., 2019), driven by a combination of natural factors and hydrological conditions (Mecozzi et al., 2012). These activities, particularly industrialization (Balint et al., 2018), have had negative consequences such as exploitation, pollution, and habitat loss in marine ecosystems (Lotze et al., 2006). Furthermore, intense industrial activities have caused unprecedented changes in ecosystem functions (Cardinale et al., 2012; Smith, 2003) across the world in this era of anthropogenic degradation (Boussarie et al., 2018; Danovaro, Umani & Pusceddu, 2009). As a result of these impacts, one remarkable event is the occurrence of marine mucilage formation that has been reported over the past 200 years, with an increasing trend in frequency for over the past 40 years (Kovač et al., 2023, Faganeli et al., 2010; Turk et al., 2010; Danovaro et al., 2009). Recently in Türkiye, a eutrophication-linked event was also captured by the Landsat 8 – OLI satellite and shared on the official website of NASA with the title "Blooms in the Sea of Marmara" in the "images day" category in May 2015 (NASA Earth Observatory, 2015) in the Sea of Marmara (SoM). Subsequently, between autumn 2020 and summer 2021, a significant event of mucilage formation was captured by the satellites of Sentinel-2 and Worldview-3 (Tuzcu Kokal, Olgun, & Musaoglu, 2022), which was unprecedented in terms of the amount and duration of observation for the SoM.

Several studies (Fuks et al., 2005; MacKenzie, Sims, Beuzenberg & Gillespie, 2002) have indicated that mucilage phenomenon often begins in regions with stratified water columns. The SoM has also a two-layered water column, where the upper layer is low-salinity Black Sea water, and the lower layer is high-salinity Mediterranean Sea water (Ünlülata, Oğuz, Latif & Özsoy, 1990) with a boundary between them known as a pycnocline (Beşiktepe et al., 1994). The ecosystem of the SoM, which is composed of biological components from these seas, is unique (İşinibilir Okyar, Üstün & Orun, 2015).

Subsequently, the Istanbul (Bosporus) and Çanakkale (Dardanelles) straits have been established as a result of bidirectional dual water-mass exchange events, and studies have indicated that the last occurrence of this event was during the Holocene period at the end of the Würm Glaciation (Çağatay et al., 2009).

In addition to the stratified water columns of the SoM (Çağatay et al., 2015), the effects of anthropogenic activities on the coastal cities of the SoM are of significant concern (Aksu, Balkis, Taşkin & Erşan, 2011) and should be thoroughly investigated (Burak et al., 2009) in terms of mucilage formation. As a nearly enclosed intercontinental basin, the SoM (size ~ 70 × 250 km) (Albayrak, Balkis, Zenetos, Kurun & Erşan, 2006) contains straits that act as biological corridors (Demirel et al., 2023), making it an important region in several manners. To understand the key elements of the SoM that initiate mucilage formations, it is crucial to explore the following points, among others: (i) the SoM is an inland sea between the Anatolian and Thrace peninsulas (Wong, Lüdmann, Ulug & Görür, 1995); (ii) Istanbul, situated on the coast of SoM, is one of the most populous cities in the world with a significant impact on the region's anthropogenic activities (Karaca, 2013); (iii) the region is home to 20% of Türkiye's population and 87% of Türkiye's coastal population (Algan, Balkıs, Çağatay, & Sarı, 2004); (iv) industrial activities in the region have caused significant environmental harm, primarily affecting the coastal and shelf areas of the SoM (Korkmaz et al., 2022); (v) the historical peninsula of Istanbul, including the Yenikapı port and the Golden Horn Estuary, as well as well-delineated polluted coastal inlets such as Erdek (Balkıs & Çağatay, 2001) and Izmit Bay have typically been significant in human settlement (Lotze et al., 2006) throughout history (Onar et al., 2013; Algan et al., 2004); (vi) intense industrial activities responsible for the majority of anthropogenic discharges to the SoM continue to operate in these areas (Demirel et al., 2023); (vii) a high volume of tanker traffic carrying oil, thousands of vessels per day, poses a constant threat to the SoM ecosystem (Albayrak et al., 2006); and (viii) the metropolitan city of Istanbul, with a population of 16 million in 2023 (Türkiye İstatistik Kurumu, 2023), has been an attraction for people for centuries and is facing ongoing challenges related to waste management and pollution (Güneralp et al., 2021). Moreover, Yaşar (2001) reported that Izmit Bay receives untreated domestic waste from two million inhabitants living around its shores, combined with solid and liquid waste discharge from 300 large industrial plants, contributing to pollution in the bay. Furthermore, as emphasized in studies conducted by Okay et al. (Okay et al., 2001; Okay et al., 1998; Okay et al., 1996), Izmit Bay faces challenges in dealing with toxicity, heavy ship traffic, and petroleum refineries, which supply >30% of Turkey's demand, located on the northeastern coast of the bay.

Marine biofilms are colonized primarily by surface-associated (Salta et al., 2013) marine organisms (Dang & Lovell, 2016), which are determined by source type, planktonic activities (Gram et al., 2002), and competition between organisms (Bosch, 2013). Extracellular particles, such as DNA, are released by marine organisms, and can be utilized as a supply of nutrients (including carbon, nitrogen, and phosphorus), which are essential for growth and biofilm development (Finkel & Kolter, 2001) of bacterial communities (Das et al., 2013). Surface-associated/attached communities of microorganisms (Muhammad et al., 2020), zooplankton feces, or feeding structures contain oil compounds, and mucus-rich particles formed by bacteria, which contribute to the formation of marine mucilage (Burd et al., 2020). Zooplankton (e.g., copepods and amphipods) as consumers of marine mucilage or oil provide for the sinking of oil into the seafloor (Almeda, Connelly, & Buskey, 2016; Schwing et al., 2015; Almeda et al., 2014; Fisher et al., 2014; Montagna et al., 2013; Mitra et al., 2012; Conover 1971) and also contribute to the production of marine oil snow with their fecal pellets (Burd et al., 2020). The dynamic and heterogeneous community structure of marine biofilms makes it challenging to model and investigate. Fungi secrete extracellular polymeric substances (Metzger et al., 2009), similar to those secreted by bacteria and phytoplankton, and particularly diatoms (Wotton, 2004). These substances act as a "glue" that holds the different components of marine mucilage together (Burd et al., 2020). Furthermore, the reflection of key environmental factors of a substratum by biofilms has vital implications for larval settlement of marine invertebrates (Dobretsov, 2010).

Despite numerous studies having emphasized the presence of mucilage in the SoM through traditional marine surveillance programs (Toklu-Alicli, Ozdelice & Durmus et al., 2021; Balkıs-Ozdelice, Durmuş, & Balci, 2021; Toklu-Alicli, Polat & Balkıs-Ozdelice et al., 2020; Tas, Kus & Yilmaz, 2020; Ergul et al., 2021, İşinibilir-Okyar et al., 2015; Yilmaz, 2015; Altiok & Kayişoğlu, 2015; Balkis et al., 2011; Tüfekçi et al., 2010) such as "continuous plankton recording" and/or "trawl," (Zingone et al., 2021), these methods are not always easy to use or cost-effective (Zaiko et al., 2015; Barbour, 1999; Jennings & Kaiser, 1998) to monitor the biodiversity (Thomsen et al., 2016). Nevertheless, emerging DNA-based tools, such as environmental DNA (eDNA) metabarcoding, are cost-effective, nature-friendly, and widely adopted and promising for future applications (Zaiko, Samuiloviene & Ardura, 2015; Pochon et al., 2013; Wood et al., 2013; Darling & Mahon, 2011). Mucilage phenomena have been one of the most conspicuous in terms of characterization (Pompei et al., 2003), and their characterization involves several difficulties. However, eDNA analysis (Ogram et al.,1987) is a molecular tool that can overcome some of the abovementioned difficulties (Genitsaris, Stefanidou & Sommer, 2019, Zaiko et al., 2015; Del Campo et al., 2014; Mächler et al., 2014; Bik et al., 2012) to ensure effective biomonitoring. The emerging science of eDNA (Kelly et al., 2014) refers to the genetic remnants of life that can be obtained from a wide range of environmental samples (Taberlet, 2012). This method can provide detailed taxonomic resolution even when the samples are bulk mixtures of organisms obtained via plankton tows (Berry et al., 2019; Taberlet et al., 2018; Thomsen & Willerslev, 2015).

In this study, we applied eDNA metabarcoding approaches to explore the poorly studied biodiversity of marine mucilage/snow using a molecular approach. We built on the study of Doğan et al. (in press) and going to the species-level characterization, we attempt to help understand the possible linkages between the components, including eukaryotic organisms (algae, fungi, and animalia), and community composition of mucilage samples, using the CO1 gene, collected from selected stations in the SoM during the novel 2020–2021 mucilage event, as mentioned earlier.

MATERIALS AND METHODS

During the Marmara Sea and North Aegean Sea Expedition (in June 2021), three replicates of mucilage samples were obtained from six stations (M1–M6) (Figure 1), targeting 0–1 m of the surface seawater layer in the water column, via Yunus-S Research/Vessel (R/V). We followed and modified the protocol outlined by Buxton et al. (2021) and collected three samples of mucilage using negative field controls, according to Keskin (2014), at each station.

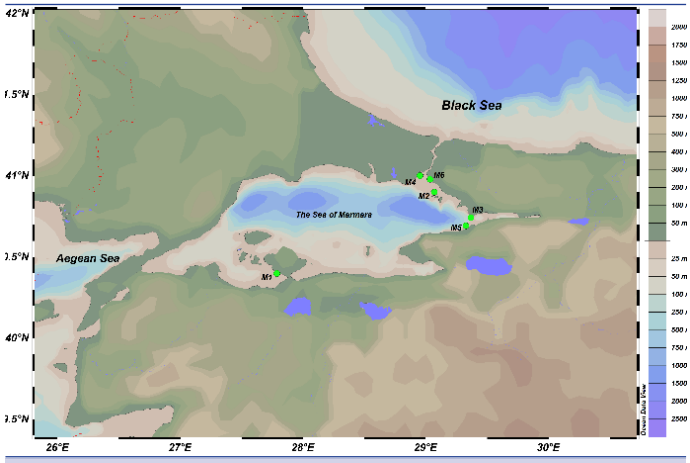

Figure 1. Study area in the Sea of Marmara, (M) indicating the location of the six sampling sites: M1, M2, M3, M4, M5, and M6, as the Bay of Erdek, Kınalıada, Bay of İzmit, Yenikapı, Çınarcık, and Kalamış, respectively.

DNA extraction was performed using the DNeasy Plant Pro Kit-Qiagen (Germany) according to the manufacturer's instructions. The primer pairs of CO1, as described elsewhere (Leray, et al., 2013), were used for the initial PCR assay. The index primers were incorporated into the second PCR assay based on their specific locations and genes. The PCR products were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA). The concentration of the pool was determined and validated using the KAPA Library Quantification qPCR kit (Roche, Germany). The pool was then sequenced on an Illumina platform (Illumina, USA) with paired reads (2 × 150 bp) (Gen Era Diagnostics Inc., Türkiye).

Following the sequencing of NovaSeq, S4, 2 × 150 bp read length service, the raw sequences were received as demultiplexed fastq files and processed using The Advanced Pipeline for Simple yet Comprehensive Analyses of DNA metabarcoding data (the APSCALE graphical user interface) "apscale_gui" pipeline v1.2.0 (https://github.com/TillMacher/apscale_gui) (Buchner, Macher & Lesse 2022), which is based on VSEARCH (Rognes, Flouri, Nichols, Quince & Mahe, 2016) and cutadapt (Martin, 2011). This module consisted of demultiplexing, paired-end merging, primer trimming, quality & length filtering, dereplication & pooling, Operational Taxonomic Units (OTU) clustering, denoising (ESVs), chimera removal, LULU filtering, and remapping steps.

The analysis commenced with the adapter primer trimming stage, as the raw data had already been demultiplexed by the sequencing company. The early stages of the analysis pipeline comprised the deletion of primer sequences and tags by cutadapt (Martin, 2011) and the evaluation of the quality of each read based on specific per-base quality and read length thresholds. The pooled and dereplicated reads were clustered into OTUs based on the similarity threshold (97%) and denoised into "Exact Sequence Variants" (ESVs). Chimeras were automatically detected and removed from the OTUs and ESVs using the vsearch -uchime_denovo command. OTUs and ESVs were mapped against the dereplicated files. The LULU filtering algorithm (Frøslev et al., 2017) was used to reduce the number of erroneous OTUs/ESVs and achieve more realistic biodiversity metrics. Finally, the OTUs and ESVs were remapped to the sequences of each sample, and read tables were generated. A taxonomic assignment was performed using the BOLD system with the BOLDigger module (Buchner & Leese, 2020) and Midori2 (MIDORI2_UNIQ_NUC_GB257_CO1_BLAST) in the local blast module. The final taxonomy table was generated using the "JAMP filter" option (Elbrecht, 2022). During this process, ambiguous records were flagged along with their respective situation.

The TaxonTableTools (TTT) v1.4.8 (Macher, Beermann & Leese, 2021) was used for downstream analyses. A taxon table was generated using the taxon table converter module, with the taxonomy and read tables generated in previous analyses serving as input. The replicates were merged, considering of the consistency of the OTUs present in each replicate. Subsequently, progress was made in the taxon table filtering module, where taxa exhibiting <85% similarity were eliminated. The read threshold was set at 0.01%. Negative control subtraction was performed as the final step.

The assigned taxonomy of each OTU in the taxon table was validated against the Global Biodiversity Information Facility (GBIF) database (https://www.gbif.org/). Consequently, any spelling errors were corrected, and the synonyms for each respective taxon were examined and updated automatically. The reads were processed by normalization and metadata were generated to facilitate the execution of downstream analyses. Basic statistics (read count, OTU number, taxonomic resolution, and richness) were generated. Venn diagrams were generated by comparing the BOLD and Midori taxon tables at each taxonomic level, ranging from OTU numbers to phyla. Rarefaction analyses were performed using both all-in-one and per-taxon approaches with the aim of determining the number of species and/or OTUs that separated among each respective taxonomic group. The species-level site occupancy was calculated using a heatmap representation of presence/absence of data. Per-taxon statistical analyses were conducted to retrieve the count of reads, number of OTUs, and species. Parallel categorical analyses were performed to determine the samples that were represented by species and/or higher taxonomic levels. The distribution of designated taxa was confirmed by cross-referencing with the GBIF using an Application Programming Interface (API). At the phylum and species levels, read proportions were calculated and plotted using heat maps, bar charts, and pie charts. Krona charts were gener-

ated to display both single sample and combined data points (Supplementary Data).

Diversity analyses were conducted by computing both the alpha and beta diversity measures. Specifically, we determined alpha diversity by calculating the number of OTUs per sample, and for beta diversity, we computed Jaccard distances between samples. The taxon table was used to generate a taxon list that incorporated all recognized taxa and diminished redundancy. In cases where numerous OTUs were assigned to the species level, the maximum, average, and minimum genetic distances between OTUs were calculated to discern highly diverse or cryptic species.

RESULTS AND DISCUSSION

Analyzing the CO1-based community composition within mucilage samples obtained from collection sites

Following the sequencing process, the raw reads underwent processing, resulting in the trimming of 3,078,342 M reads, with an average of 1,669,203 and 1,284,649 reads that successfully passed the quality-filtering step. Subsequently, the preprocessing steps were clustered into 1657 OTUs for further analysis. Of these, 429 records were found as "No Match," 172 records were found as "blank," and the remaining 1056 records were assigned to OTUs. After curation (merging of replicates and subtraction of negative controls), 431 OTUs (629,535 reads) with a similarity of ≥85% to the reference sequence remained and were selected for downstream analyses. Among the records of the 431 OTUs, 22 were ambiguous. The average sequence length was 118 bp, with a minimum length of 100 bp and a maximum length of 147 bp. A total of 34 phyla, 78 classes, 157 orders, 106 families, 65 genera, and 57 species, all of which were unique to each taxonomic level, were observed against the Midori2 database.

When we compared our basic statistics in both Midori2 and BOLD databases, 22 phyla were detected as shared between the two databases, including Amoebozoa, Arthropoda, Ascomycota, Bacillariophyta, Bryozoa, Cnidaria, Echinodermata, Mollusca, Porifera, Rhodophyta, and Rotifera. In addition, Midori2 identified 10 phyla, including Bigyra, Cercozoa, Choanoflagellata, and Evosea, and BOLD identified 5 phyla, including Heterokontophyta, Onychophora, and Zygomycota. At the class level, the 39 taxonomic groups that were detected by both Midori2 and BOLD databases and were the most abundant based on OTUs and species were Anthozoa, Bivalvia, Bacillariophyceae, Copepoda, Dinophyceae, Gastropoda, Hydrozoa, Rhodophyta, Scyphozoa, and Staurozoa. The two databases under consideration shared 214 OTUs (Figure 2a) and 23 species (Figure 2b), whereas Midori2 detected an additional 32 species not found in BOLD, and BOLD detected 16 species not found in Midori2. Moreover, Midori2 detected 217 OTUs not present in BOLD, whereas BOLD detected 143 OTUs not present in Midori2 (Figure 2). The analysis of the two databases revealed that a significant overlap exists between the two, with approximately 20% of OTUs (Figure 3a) and species (Figure 3b) being shared between them.

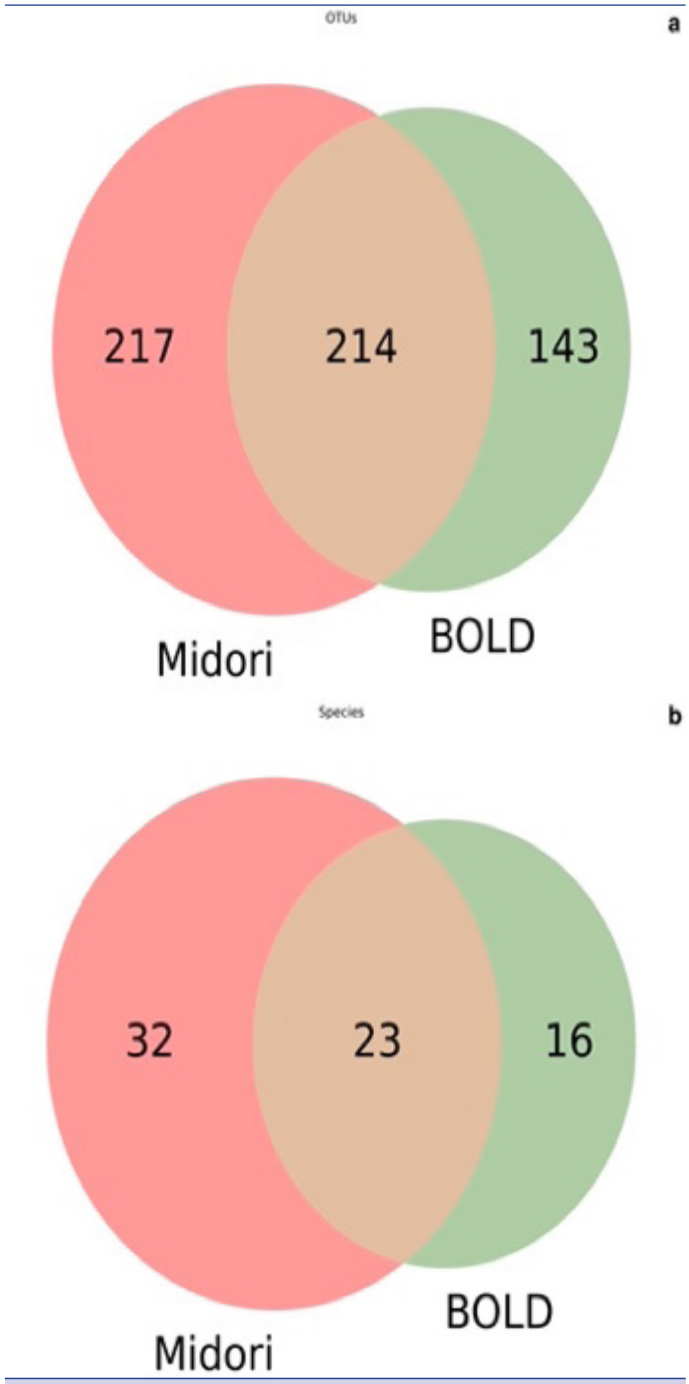

Figure 2. A comparison of Midori2 and BOLD databases focusing on (a) OTU and (b) species in each database.

The relative abundance and distribution proportions of the orders across different samples were investigated by comparing the BOLD and Midori2 databases. Amphipoda, Caenogastropoda, Ceramiales, Cheliostomatida, Corallinales, Diplostraca, Gastrochaenida, Perciformes, Sessila, Stauromedusae, and Thalassiosirales correlated in both databases (Figure 4).

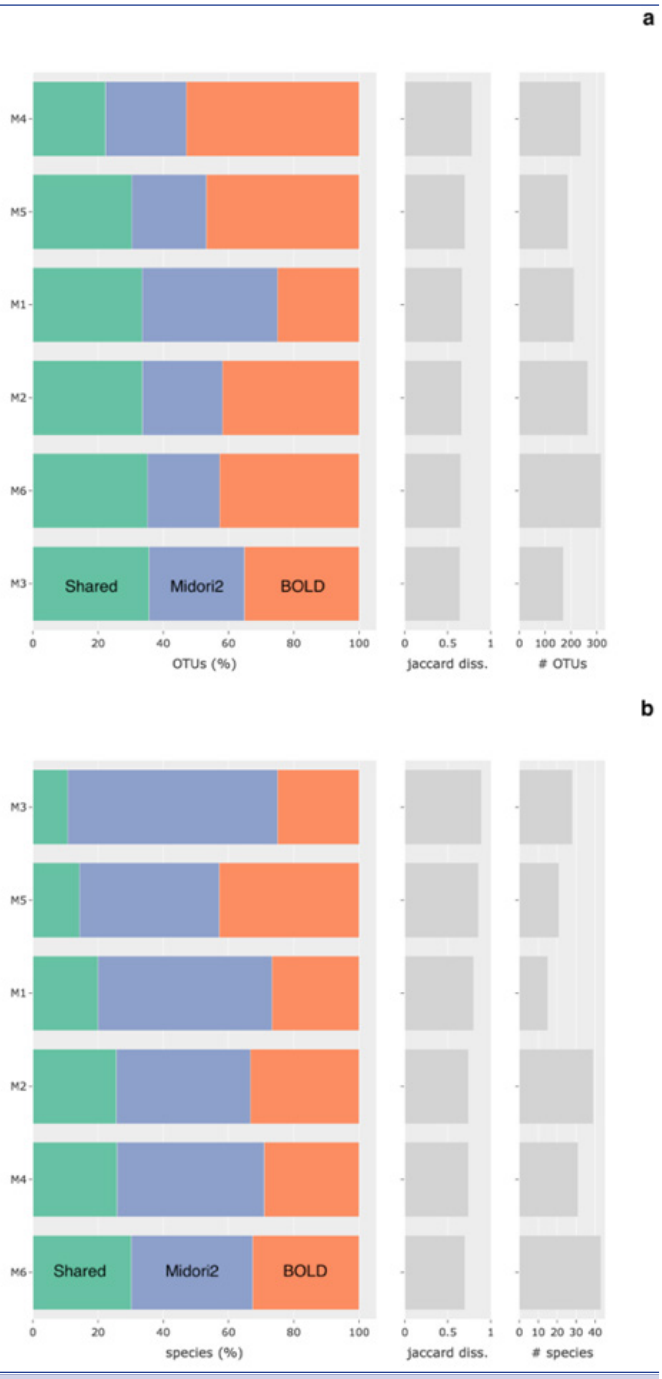

Figure 3. (a) OTUs and (b) species sources between Midori2 and BOLD databases.

At the species level, taxonomic richness retrieved from BOLD and Midori2 databases correlated per phylum. Arthropoda, Rhodophyta, Mollusca, and Ochrophyta were the most prominent phyla (Figure 5).

By selecting the top 10 phyla containing the most OTUs, the sample-based rarefaction analysis demonstrated that 15/52 species observed in the first sample subsequently increased to 41/52 in the all-in-one method (Figure 6a). Testing the species and OTUs to separate among phyla (Figure 6b) revealed that the phylum Ochrophyta reached eight species (8/8) in the second sample, which flattened in subsequent samples. The phylum Mollusca reached five species in the third sample and ended with seven (7/7) species in the second sample. The phylum Cnidaria reached two species (2/2) in the third sample and remained constant throughout the samples (Figure 6b).

The M6 sample exhibited the greatest degree of alpha diversity among all samples, whereas the M3 and M5 samples were limited to fewer than 115 OTUs/30 species each (Figure 7). The Kalamış (M6) sample had 181 OTUs (Figure 7a) (29 OTUs at species level) and 23 species (Figure 7b), following the M1 sample with 159 OTUs. The M3 and M5 samples exhibited the lowest degree of alpha diversity among all samples, as evidenced by the presence of 111 and 100 OTUs, respectively. Despite the comparatively small number of OTUs in M1 and M5 samples, the number of reads at these samples was substantial, with 140 k reads recorded in the M1 sample and 108 k reads in the M5 sample. Compared with the M1 sample, which had a low number of OTUs at the total (111) and species level (13) and 33 k reads, the M2 sample exhibited a higher number of OTUs of 26 at the species level and 121 k reads (Figure 7).

A total of 87 OTUs were identified in the phylum Arthropoda, 73 OTUs were identified in the phylum Ochrophyta, 46 OTUs were identified in the in the phylum Rhodophyta, and 41 OTUs were identified in the in the phylum Mollusca. Conversely, the remaining 27 phyla were represented by fewer than three species each. Various taxonomic groups such as Amoebozoa (27), Chlorophyta (7), Dinoflagellata (4) from Protozoa, Cnidaria (14), Porifera (6), and Echinodermata (3) from Animalia showed the presence of OTUs. Overall, 87 OTUs of the phylum Arthropoda were assigned to 15 distinct species, 73 OTUs of the phylum Ochrophyta were assigned to species, 41 OTUs of the phylum Mollusca were assigned to six species, and 46 OTUs of the phylum Rhodophyta were assigned to five species. Despite comprising the most numbers of OTUs, the phylum Arthropoda exhibited a relatively low proportion of reads by <4%. In contrast, members of the phylum Cnidaria (Figure 8a), including *Aurelia aurita*, exhibited a dominant presence, accounting for >40% of all reads (for samples with >97% similarity) (Figure 8b). Members of Ascomycota, including *Cladosporium allicinum*, accounted for 9% of the reads, followed by the members of Mollusca, such as *Bittium reticulatum*, accounting for 5% of the reads, whereas the coccolithophore *Emiliania huxleyi* (3%) was also represented.

Among the identified Arthropoda OTUs, the class Insecta consisted of the most abundant OTUs, with 56 OTUs comprising >60% of the OTUs. Despite this, only seven species were assigned to this class. In contrast, the class Copepoda that included five OTUs was well represented by three distinct species and comprised >80% of Arthropoda reads. The calanoids *Paracalanus parvus* (83% reads of Copepoda) and *Pseudocalanus elongatus* (13% reads of Copepoda) were dominant among copepod species, with low read representation by the cyclopoid *Oithona similis* (1% reads of Copepoda and 56% of Cyclopoida), with 100% similarity. Furthermore, the maxillopods *Amphibalanus amphitrite* (96% reads of Maxillopoda) and *Amphibalanus impro-*

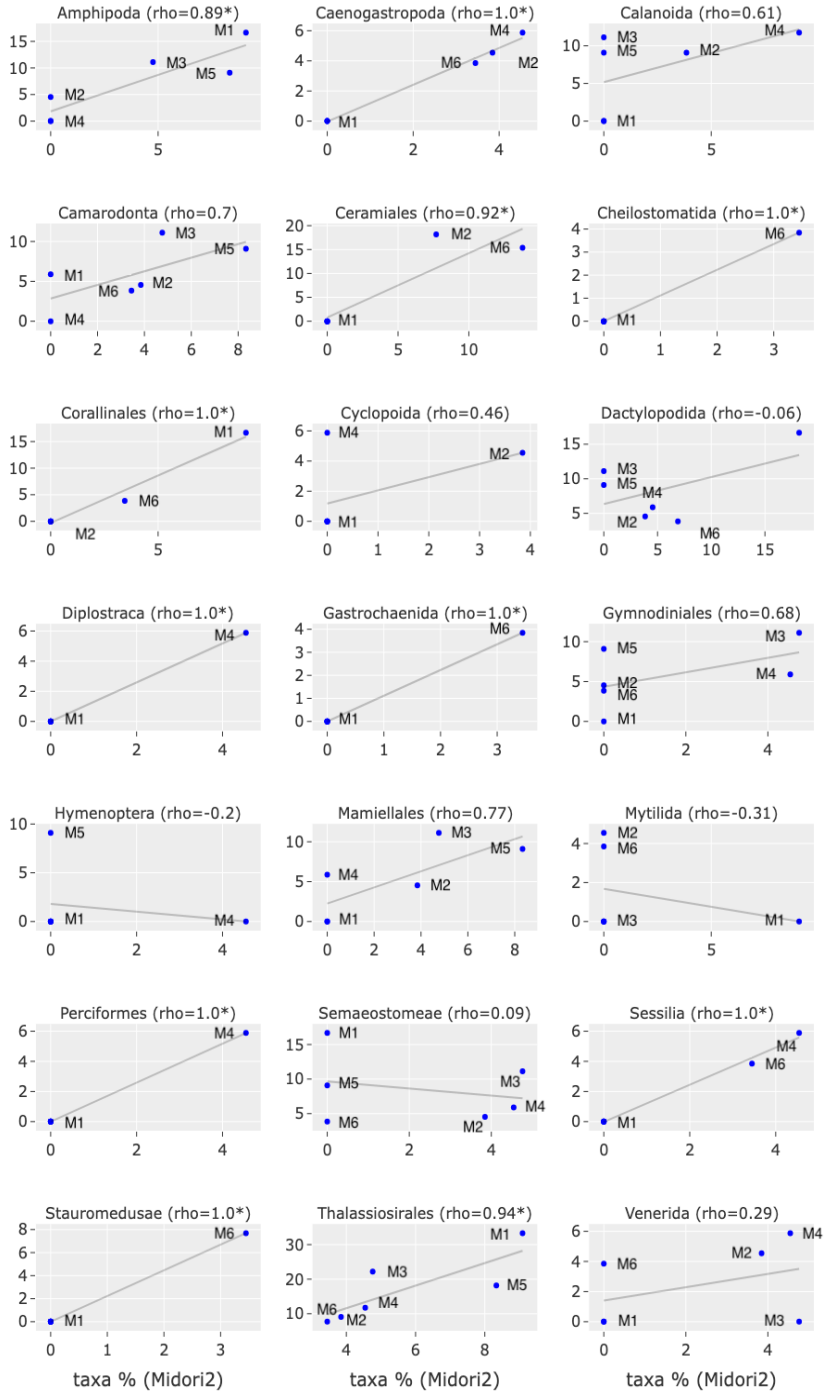

Figure 4. Taxon proportions, pairwise analysis of samples (including >98% similarity): rho (Spearman's rank correlation coefficient), samples marked with an asterisk indicate a positive monotonic relationship (they display consistency in the same direction).

visus (4% reads of Maxillopoda) and the branchiopod *Pleopsis polyphemoides* were represented by three OTUs with 100% similarity and comprised 10% reads of arthropods. Remarkably, nine OTUs were classified as ambiguous and subsequently assigned to the same species, viz., *Lucilia caesar*, which belongs to the order Diptera.

A total of 73 OTUs were detected within the phylum Ochrophyta. In particular, 12 OTUs belonged to the order Ectocarpales, and 16 OTUs were assigned to the order Naviculales within the phylum Ochrophyta. Among the phylum Rhodophyta, 25 OTUs were identified within the order Ceramiales, and the species-level match was well represented by the following species: *Apoglos-*

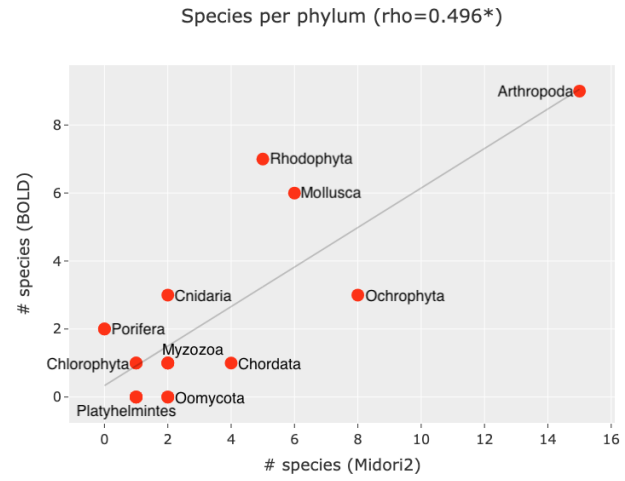

Figure 5. Comparisons of taxonomic richness across each database (BOLD and Midori2) based on phylum level.

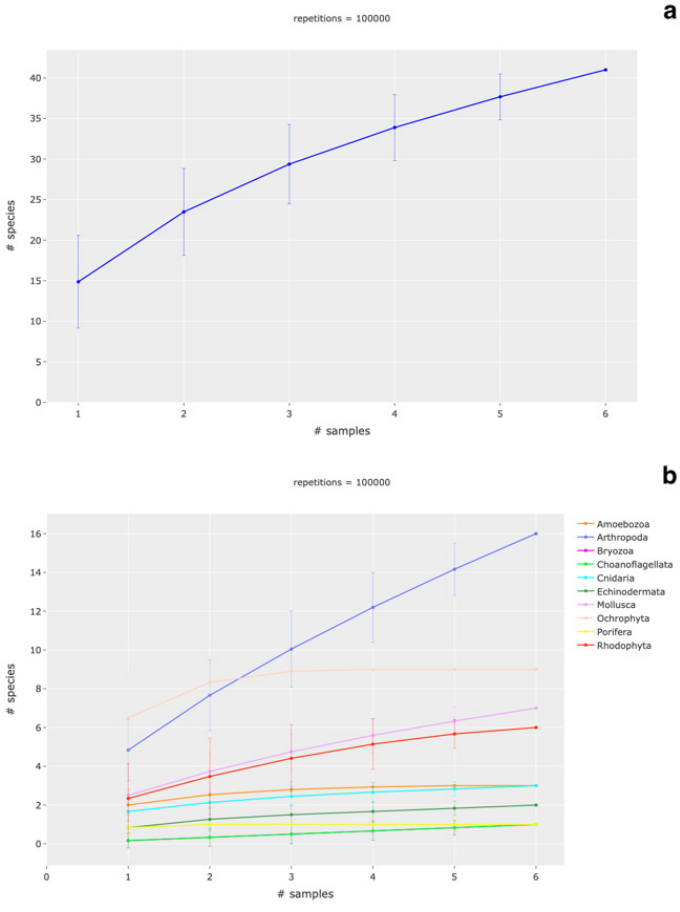

Figure 6. The use of sample-based rarefaction was examined through the application of both all-in-one (a) and per-taxon (b) approaches (100,000 repetitions) in top 10 phyla.

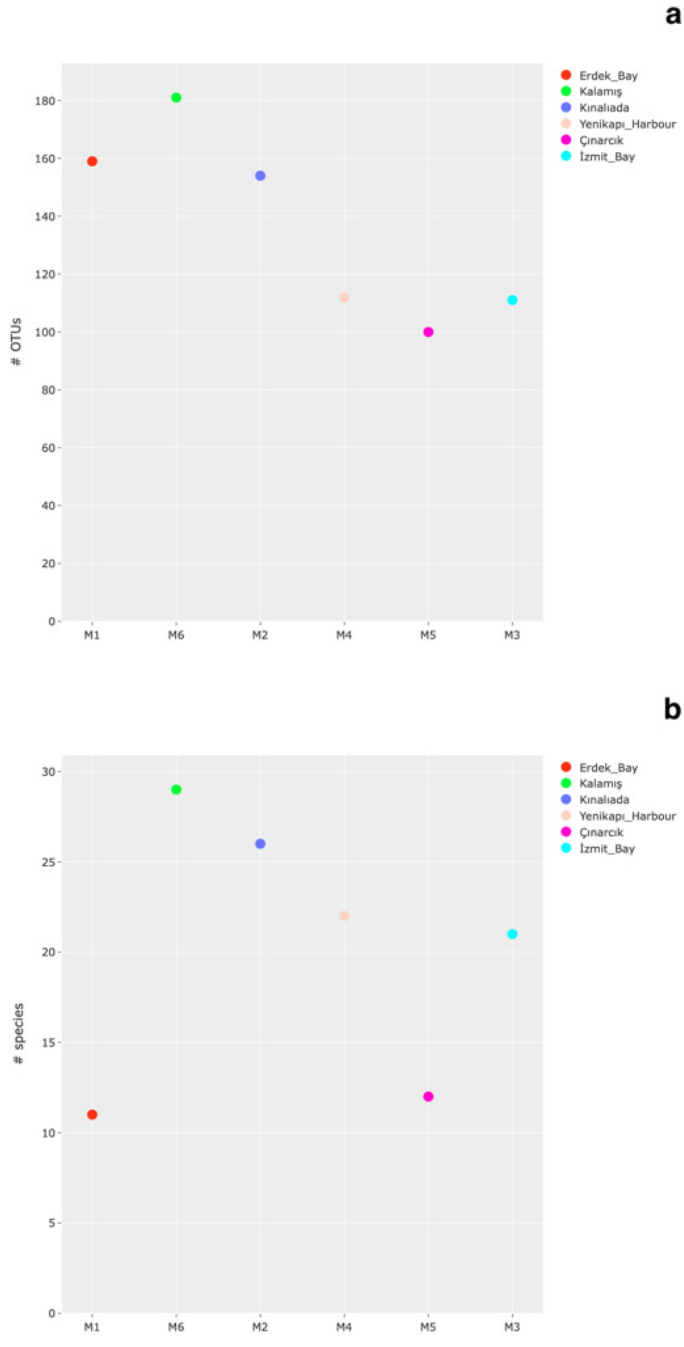

Figure 7. Alpha diversity, comprising (a) the number of OTUs and (b) the number of species per sample.

sum ruscifolium (19% of Rhodophyta reads), *Polysiphonia morrowii*, *Dasysiphonia japonica*, and *Pterothamnion crispum*, all of which exhibited 100% similarity. Furthermore, 11 OTUs were identified within the order Bacillariales that exhibited a remarkable abundance of reads within the phylum Ochrophyta, which comprised three OTUs identified for Cylindrotheca and two OTUs identified for the genus *Nitzschia*. The order Thalassiosirales comprised three OTUs, which included a two-species-level representation of *Skeletonema pseudocostatum* (1% of all reads)

Figure 8. The proportions of reads for (a) each phylum (including all data) and (b) species (for samples with >97% similarity) in a heat map and bar graph among samples.

(observed among all mucilage samples) and *Skeletonema* sp., with 100% similarity. Although taxonomic assignment was 100% similar to the genus *Skeletonema* due to the multiple species hits (*Skeletonema dohrnii*, *Skeletonema marinoi*, and *Skeletonema costatum*), these OTUs corresponded to a single genus (*Skeletonema*). Nevertheless, despite the presence of 12 OTUs in the order Ectocarpales, no OTUs were assigned to species-level representation. The order Lithodesmiales was represented by *Ditylum brightwellii*. The order Chaetocerotales comprised two OTUs that were assigned to *Chaetoceros socialis* with 100% similarity. The order Rhizosoleniales was represented by two OTUs, both of which were assigned to the family Rhizosoleniaceae.

Among the protists, 27 OTUs were identified within the phylum Amoebozoa, exhibiting a high similarity of 85%–93% to the class Discosea. In addition, two OTUs and one taxa *Squamamoeba japonica* were well represented at the species level, with 100% similarity. Four OTUs and two species were identified within Dinoflagellata as follows: *Pfiesteria piscicida* belonged to the order Peridiniales, and *Cochlodinium polykrikoides* and *Gyrodinium instriatum* belonged to the order Gymnodiniales. Although five OTUs were assigned to the Bigyra class, no species were identified within this assignment. The coccolithophore *Emiliania huxleyi* (3% of all reads) that belonged to the phylum Haptophyta was represented by a single OTU. A total of 49 OTUs were identified within Fungi, of which 42 were present in both Oomycota and Basidiomycota, with 22 and 18, respectively, and 9 OTUs were exclusive to Ascomycota. Although Fungi exhibited a relatively high representation, only three OTUs could match at the species level, with their similarity exceeding 100%. These OTUs belonged to the following species: *Cladosporium allicinum* (9% of all reads and 95% of Ascomycota reads), *Aspergillus puulaauensis*, *Globisporangium spinosum* (Oomycota), and *Anisolpidium ectocarpii* (Basidiomycota).

An analysis of the phylum Porifera revealed six OTUs, with five of these belonging to the Demospongiae class and the remaining one belonging to the Homoscleromorpha class. The Demospongiae class was represented by four different orders in Midori2 and *Halisarca desqueyrouxae* with 99% similarity, which belongs to the Halisarcidae family in the BOLD database. A total of 12 OTUs were identified in the phylum Cnidaria, of which 5 were assigned to the Hydrozoa class, and two species were identified. One of the OTUs matched with the Hydractiniidae family with 93% similarity, and three of them were assigned to the Staurozoa class, with one matched with the Lucernariidae family at 91% similarity and another matched with the order Stauromedusae at

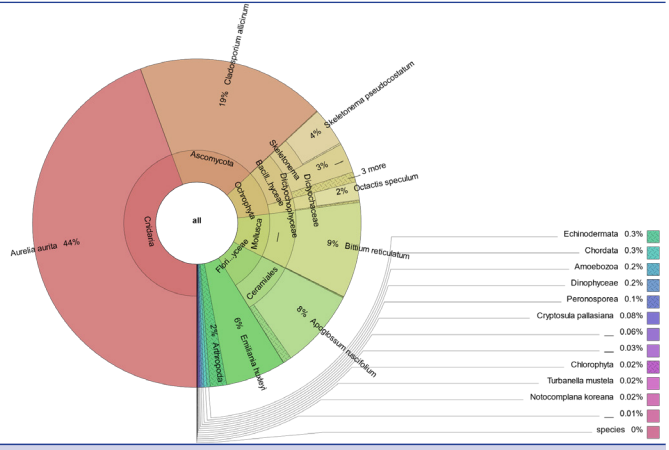

Figure 9. Krona charts based on read proportions (>97% similarity) when sampling locations were merged.

85% similarity. Furthermore, two of them matched with *Haliclystus inabai* and *Calvadosia cruciformis* species with 100% similarity. OTU_2 has been categorized as a member of the class Scyphozoa with 100% similarity to the moon jellyfish *Aurelia aurita*, along with the remarkable high number of reads (99% of Cnidaria) (Figure 9) assigned to this taxon. An analysis of the phylum Mollusca revealed the presence of 41 OTUs. The classes Bivalvia and Gastropoda comprised eight and 32 OTUs, respectively. Multiple species, including *Chamelea gallina* (18% of Bivalvia), *Mytilus edulis* (16% of Bivalvia), *Rocellaria dubia* (3% of Bivalvia), and *Spisula subtruncata* (2% of Bivalvia), exhibited 100% similarity to their corresponding OTUs belonging to the Bivalvia class. Remarkably, two distinct OTUs (OTUs_470 and OTUs_1926) were identified in the same species, *Chamelea gallina*. Due to the possibility of assigning multiple genera in both *Venus verrucosa* and *Chamelea gallina* of OTU_1926, this situation of OTU was considered ambiguous. Despite this ambiguity, the OTU was assigned as *Chamelea gallina* because of its dominance during the taxonomic assignment process. In addition, six of these species matched with the order Littorinimorpha within the class Gastropoda (6% of all and 98% of Mollusca) and had a high number of reads. The classes Neogastropoda and Stylommatophora each contained three and two OTUs, respectively. Both the green sea slug *Elysia viridis* and the sea snail *Bittium reticulatum* (>5% of all, and 85% of Gastropoda reads) (Figure 9) were assigned to their respective OTUs with 100% similarity. The phylum Echinodermata yielded three OTUs, with two of these belonging to the class Echinoidea. In particular, two of these OTUs (OTU_131 and OTU_852) were assigned to the same species (*Paracentrotus lividus*), exhibiting 100% similarity.

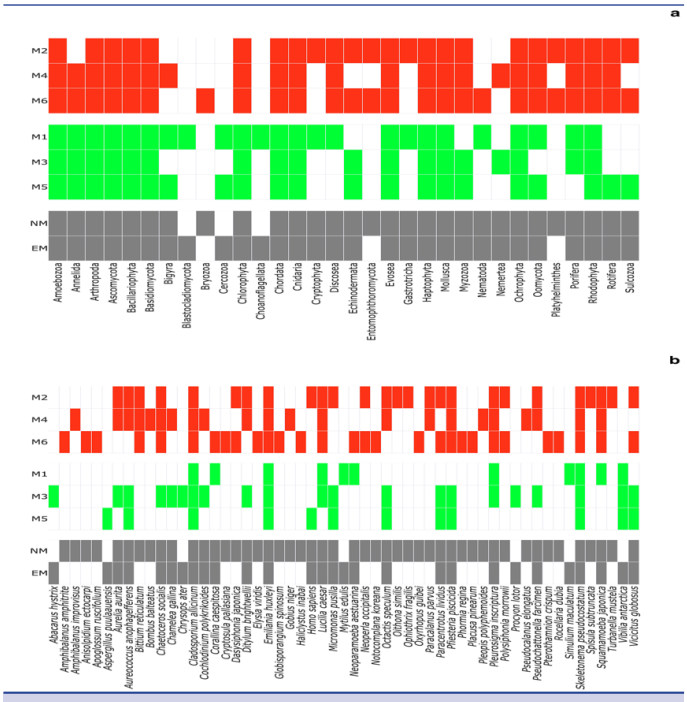

Figure 10. Site occupancy between both phylum (a) and species (b) levels (NM: Northeastern Marmara and EM: Eastern Marmara).

In most locations and regions of northeastern and eastern Marmara, all phyla were well represented, except for Bryozoa, Cercozoa, and Choanoflagellata (Figure 10a). Although Bryozoa were found only at the Kalamış sample in northeastern Marmara, members of Cercozoa were identified in Erdek Bay and Çınarcık in eastern Marmara, and Choanoflagellata members were found only in Erdek Bay (Figure 10b).

In our analysis of similarity test (Anosim), we detected a slight dissimilarity in the composition of OTUs among the regions (R = −0.3, indicating a mild dissimilarity) (Figure 11).

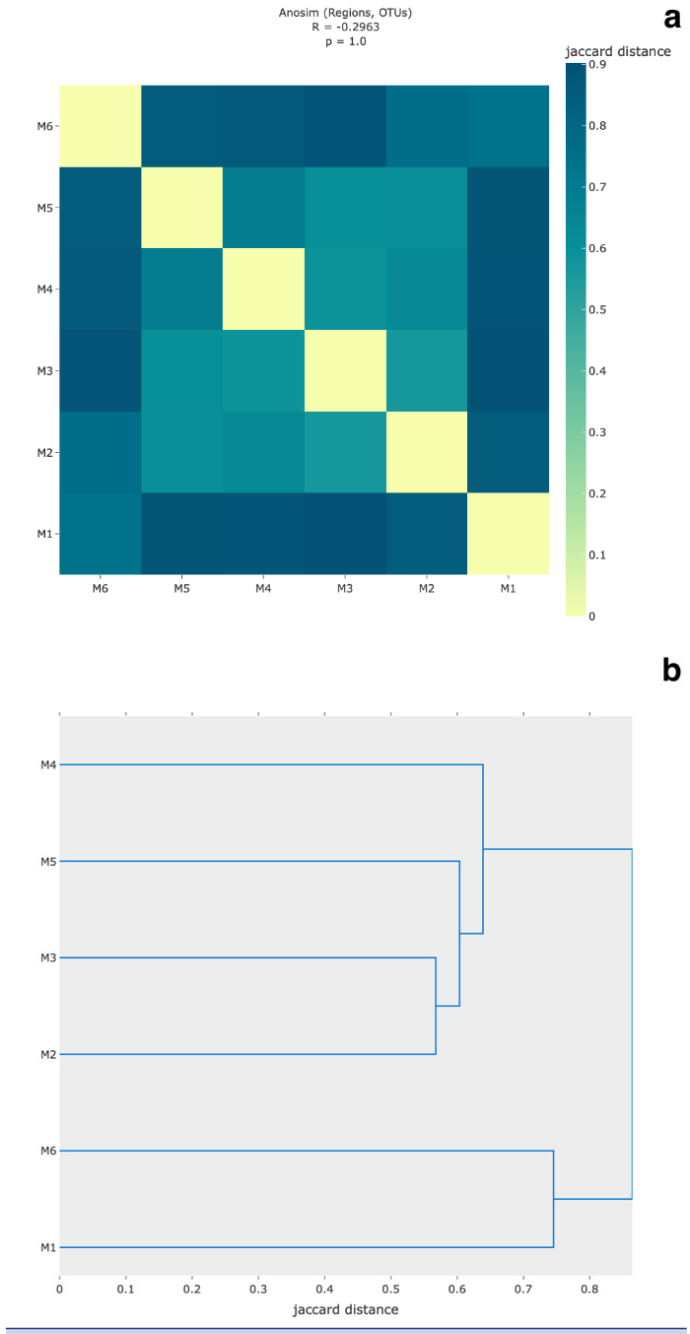

Figure 11. The beta diversity of samples, as determined by the Anosim, Jaccard distance.

In contrast to the representative phyla (Figure 12a), the absence/presence of species was not uniform across all regions (NM and EM), except for a few cosmopolitan organisms such as *Aurelia aurita*, *Aureococcus anophagefferens*, *Cladosporium allicinum*, *Corallina caespitosa*, *Emiliania huxleyi*, *Micromonas pusilla*, *Neoparamoeba aestuarina*, *Octactis speculum*, *Paracentrotus lividus*, *Cochlodinium polykrikoides*, *Gyrodinium instriatum*, *Pfiesteria piscicida*, *Pseudochattonella farcimen*, *Skeletonema pseudocostatum*, and *Vicicitus globosus* (Figure 12b).

This study represents one of the first extensive investigations to utilize metabarcoding approaches to analyze community composition, including protists, fungi, and animalia, associated with the mucilage events that occurred during the novel 2020–2021 mucilage event in the SoM. Results revealed the community composition of the mucilage and identified the predominant microorganisms thriving among samples.

Taxonomic assignment revealed that the mucilage samples were categorized into three orders of the main category and were rep-

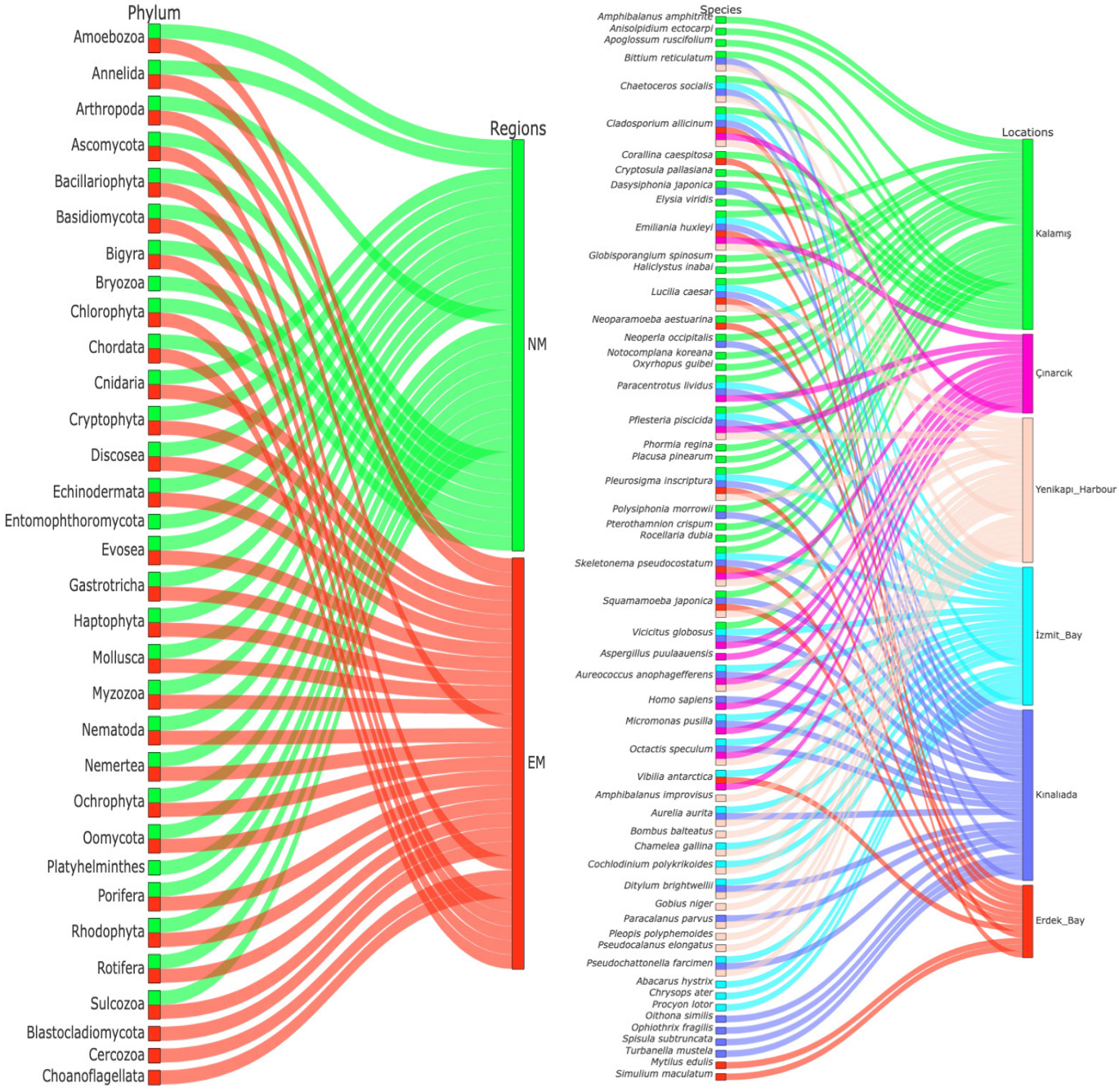

Figure 12. An examination of categories, particularly the taxonomic structure of mucilage samples, exploring the phyla (a) and (b) species compositions.

resented by unicellular eukaryotes (protists), fungi, and other multicellular eukaryotes. We detected a variety of groups, including harmful dinoflagellates such as *Cochlodinium polykrikoides* (Gobler, 2008), *Gyrodinium instriatum* (Nagasoe et al., 2006), and *Pfiesteria piscicida* (Burkholder & Glasgow Jr, 1997) that are implicated in the formation of red tides, which have the potential to cause fish death and fishery losses. Within the class Bacillariophyceae, some important groups that could have also contributed to the 2007–2008 mucilage event in the SoM, such as the genera *Cylindrotheca*, *Ditylum* (*Ditylum brightwellii*), and *Skeletonema* (*Skeletonema pseudocostatum*), were also detected.

The coccolithophores *Emiliania huxleyi* and *Dictyocha speculum*, which contribute to carbon and silica cycles, especially in fundamental mineral fluxes within the global ecosystems (Turley, 1991), were observed in our analyses. In the early 2000s, researchers recorded the occurrence of some *D. speculum* species for the first time in the SoM at relatively close stations and at depths with our sampling regime (Deniz, Taş & Koray, 2006), corroborating the findings of the two studies. The dataset has also revealed the presence of filamentous fungal species such as *Cladosporium allicinum* and *Aspergillus puulaauensis* as well as *Globisporangium spinosum* and *Anisolpidium ectocarpii* within the realm of Fungi, which release extracellular polymeric compounds similar to those released by bacteria and phytoplankton that serve as a glue in mucilage (Burd et al., 2020).

Some red algal species, including *Apoglossum ruscifolium*, *Corallina caespitosa*, and *Polysiphonia morrowii*, known for having orders that produce polysaccharides (Duarte, et al., 2004; Usov, 2011), were also observed. We also observed the presence of the bryozoan *Cryptosula* that depends on phytoplankton as a major food source, as well as some microspecies such as bivalves (*Chamelea gallina*, *Mytilus edulis*, *Rocellaria dubia*, and *Spisula subtruncata*) as consumers of mesozooplankton (Davenport, Smith, & Packer, 2000), gastropods (*Bittium reticulatum* and *Elysia viridis*), copepods (Calanoids: *Paracalanus parvus*, *Pseudocalanus elongatus*, and Cyclopoid: *Oithona similis*), the cladoceran *Pleopsis polyphemoides*, and the moon jellyfish *Aurelia aurita*. Among these, some taxa, including the abovementioned ones, were the most abundant during the 2018 mucilage event in the area (Okyar et al., 2015), and their (copepods and cladocerans) prevalence and dominance among zooplankton have been recorded in the northeastern SoM (Isinibilir et al., 2008). Chitinous zooplankton, such as copepods and their fecal pellets are recognized as hotspots for microbial activity. Specifically, copepods, such as *Paracalanus parvus*, *Pseudocalanus elongatus*, and *Oithona similis*, which play vital roles in the pelagic food web (Turner, 2004), and their documented mucilage-consuming habits, were also part of the animal composition. Moreover, the scyphozoan *Aurelia aurita*, a species associated with the major bacterial groups (Kos Kramar et al., 2019) and widely acknowledged as a significant player in marine ecosystems (Weiland-Bräuer et al., 2015), was observed. Studies (Brodeur et al., 2002; Sommer & Lengfellner, 2008) have demonstrated that *Aurelia aurita* can significantly influence ecosystem dynamics by affecting planktonic food web structure. By consuming ichthyoplankton, jellyfish exhibit predatory behavior and can potentially compete with fish (Purcell, 2005).

The phylum Mollusca has been well represented in animals that comprised certain groups such as Gastropoda (*Bittium reticulatum*) and Bivalvia, which are commonly documented in marine snow (Shanks & Walters, 1997). Specifically, the gastropod species *B. reticulatum* (12% reads of Animalia) was also previously demonstrated to be associated with eutrophication (Gacia et al., 2009) and biofilm formation (Castejón-Silvo & Terrados, 2017) (D'alelio et al., 2011). Moreover, some gastropod species are affected by the toxicity of algal species (Díaz, 2006) that also comprise nutrient sources (e.g., *Apoglossum ruscifolium* and *Dasysiphonia japonica*); these two algal species were also detected in our dataset.

CONCLUSION

The application of eDNA metabarcoding tools may provide a snapshot of the community composition of the factors that trigger mucilage formation events. Continuous attempts to gather data at a larger and more continuous timescale are crucial to improve our understanding of this phenomenon.

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Author contributions: Mİ, OD, and RB were responsible for devising the study's design. OD conducted the environmental DNA (eDNA) sampling, while ADÖ and OD managed the laboratory workflow and wet-lab process, respectively. OD performed bioinformatic analyses, visualization, and writing, whereas RB supervised the dry laboratory process. Mİ, OD, and RB composed the initial draft of the manuscript. Mİ supervised the project, administered it, and secured funding, while all authors contributed to the manuscript and approved the final version for publication.

Ethical approval: No animal testing was performed during this study.

Conflict of interest: The authors have no conflicts of interest to declare.

Sampling and field studies: All necessary permits for sampling has been obtained by the authors from the competent authorities.

Data availability: The raw sequencing data stored at TRUBA resources, as mentioned in the acknowledgments, are available upon request.

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