

# Environmental DNA reveals protist biodiversity in mangrove sediments of Java and Madura, Indonesia

MIRZA HANIF AL FALAH<sup>1,6</sup>, TRI RETNANINGSIH SOEPROBOWATI<sup>2,6,\*</sup>, HADIYANTO HADIYANTO<sup>3</sup>,  
AULIA RAHIM<sup>1,6</sup>, JUMARI JUMARI<sup>2,6</sup>, LILIH KHOTIMPERWATI<sup>2,6</sup>, NI KADEK DITA CAHYANI<sup>2,6</sup>,  
MARTA WOJEWÓDKA-PRZYBYŁ<sup>4,6</sup>, ŁUKASZ KRUSZEWSKI<sup>4,6</sup>, PAWEŁ RYDELEK<sup>5,6</sup>

<sup>1</sup>Doctoral Program in Environmental Science, Postgraduate School, Universitas Diponegoro. Jl. Imam Barjo, Semarang 50241, Central Java, Indonesia

<sup>2</sup>Department of Biology, Faculty of Science and Mathematics, Universitas Diponegoro. Jl. Prof. Jacob Rais, Semarang 50275, Central Java, Indonesia.

Tel.: +62-24-7474754, \*email: trsoeprowati@live.undip.ac.id

<sup>3</sup>Department of Chemical Engineering, Faculty of Engineering, Universitas Diponegoro. Jl. Prof. Soedarto, Semarang 50275, Central Java, Indonesia

<sup>4</sup>Institute of Geological Sciences, Polish Academy of Sciences. Twarda 51/55 St., Warsaw PL00-818, Poland

<sup>5</sup>Faculty of Geology, University of Warsaw. ul. Żwirki i Wigury 93 02-089 Warsaw, Poland

<sup>6</sup>Cluster for Paleolimnology (CPalim), Universitas Diponegoro. Jl. Prof. Soedarto, Semarang 50275, Central Java, Indonesia

Manuscript received: 20 May 2025. Revision accepted: 15 October 2025.

**Abstract.** *Al Falah MH, Soeprowati TR, Hadiyanto H, Rahim A, Jumari J, Khotimperwati L, Cahyani NKD, Wojewódka-Przybył M, Kruszewski Ł, Rydelek P. 2025. Environmental DNA reveals protist biodiversity in mangrove sediments of Java and Madura, Indonesia. Biodiversitas 26: 5211-5225.* Mangrove ecosystems provide critical ecological services and harbor diverse microbial communities; however, the diversity of protists and their ecological roles in these habitats remains understudied. This research investigated the protist community composition in mangrove sediments from seven sites across the Java and Madura Islands, Indonesia, using environmental DNA (eDNA) metabarcoding targeting the 18S rRNA gene. Sediment samples were analyzed via Illumina MiSeq sequencing, with taxonomic assignment performed using the PR2 database. Five major protist supergroups were detected, exhibiting significant site-specific variations in community structure. Additionally, a wide range of taxa at the genus and species levels, including microalgae, vegetation, fish, insects, amoebae, protists, nematodes, fungi, and merostomata, were identified. The detection of parasitic and potentially pathogenic taxa, such as Exobasidiomycetes and *Malassezia globosa*, highlights potential ecological risks in mangrove sediments. The presence of diverse protists and fungi indicates active nutrient cycling and ecosystem resilience. Water quality supports the ecological function of mangrove habitats, with Total Dissolved Solids influencing the abundance of *Malassezia globosa* and *Tetrahymena*. This study demonstrates that eDNA metabarcoding is a novel method for revealing hidden biodiversity that is often missed by traditional methods. These findings provide crucial baseline data for the conservation and sustainable management of mangrove habitats, contributing to informed restoration and environmental monitoring efforts that protect vital ecosystem services in coastal regions.

**Keywords:** 18s rRNA, bioindicator, environmental DNA, Java, Madura, mangrove

## INTRODUCTION

Mangrove ecosystems, situated at the dynamic interface between land and sea, provide indispensable ecological services and socioeconomic benefits. These coastal forests serve as natural bulwarks, protecting shorelines from erosion and the growing impacts of extreme weather events, thereby enhancing coastal resilience (Ripple et al. 2017; Lincoln et al. 2022). Functioning as vital nurseries and refuges for a rich diversity of species, they support local fisheries that form the economic backbone of many coastal communities. Furthermore, mangroves are global powerhouses for carbon sequestration and nutrient cycling, which are crucial in maintaining regional environmental balance (Friess et al. 2019; Walker et al. 2022). Despite their immense value, these ecosystems face an unprecedented global decline. A confluence of pressures, including climate change-driven sea-level rise, intensive coastal development, pollution, and unsustainable resource extraction, is accelerating their degradation, as well as the biodiversity and human livelihoods that depend on them.

The foundation of mangrove ecosystem health and function lies within its unique sediments, harboring distinct physicochemical conditions characterized by high salinity, fluctuating redox states, and abundant organic matter. These conditions cultivate highly specialized and diverse microbial communities that drive critical biogeochemical processes. While extensive research has illuminated the roles of bacteria, fungi, and archaea within these sediment microbiomes (Basak et al. 2016; Xiao et al. 2021; John et al. 2024), a significant knowledge gap persists regarding protists. This diverse and functionally pivotal group of unicellular eukaryotes remains profoundly understudied, particularly within the tropical mangroves of Southeast Asia, which are recognized as global hotspots of biodiversity (Li et al. 2022; Wan 2024).

Protists play fundamental ecological roles that are integral to the health and stability of sediments. This vast group encompasses phototrophic microalgae, which contribute to primary production (Tréguer et al. 2018), and heterotrophic protozoa, which serve as key predators of bacteria and smaller eukaryotes. These protozoa enhance nutrient recycling and energy transfer through microbial

food webs through grazing activities, thereby shaping community structure and bolstering ecosystem resilience (Geisen and Bonkowski 2018; Shi et al. 2021). However, recent findings have revealed an alarming dominance of parasitic taxa in some mangrove sediments, occasionally exceeding 50% of the relative abundance of the protist community (Liu et al. 2024a). The prevalence of parasites raises critical ecological and health concerns, as they may negatively impact mangrove flora and fauna and pose potential risks to human health. Since protist community composition is tightly linked to local physicochemical factors, these assemblages are highly sensitive bioindicators of environmental quality and ecological integrity.

Fahma et al. (2024) and Nabila et al. (2024) found that intestinal parasite-rich protists (Trichomonadidae) dominate the sediments of Balekambang Lake, reflecting the influence of agriculture. For comparison, in the mangrove ecosystem in China, Liu et al. (2024b) documented widespread parasitic protist prevalence in mangrove sediments across Chinese provinces, found *Chloroflexi*, playing an essential role in the degradation of terrestrial-derived organic carbon, and Wang et al. (2023) used eDNA to monitor meiofauna, marine nematodes, and pathogens within mangrove sediments. Additionally, some eDNA research using 18s rRNA reveals different conditions, for example, in Makassar, Indonesia (Basyuni 2025), where protists are the most abundant kingdom and can serve as an environmental biomonitoring. In the Philippines mangrove, the exploration of fish biodiversity using 12s was investigated by Naputo et al. (2024), who identified 89 species, and Balatero et al. (2025) revealed 29 species. The eDNA approach also revealed the benthic foraminiferal community in the Sundarbans mangrove ecosystem (Mandal et al. 2025). Marinchel et al. (2024) found heterogeneity of the protist community in the Mediterranean Sea of Italy.

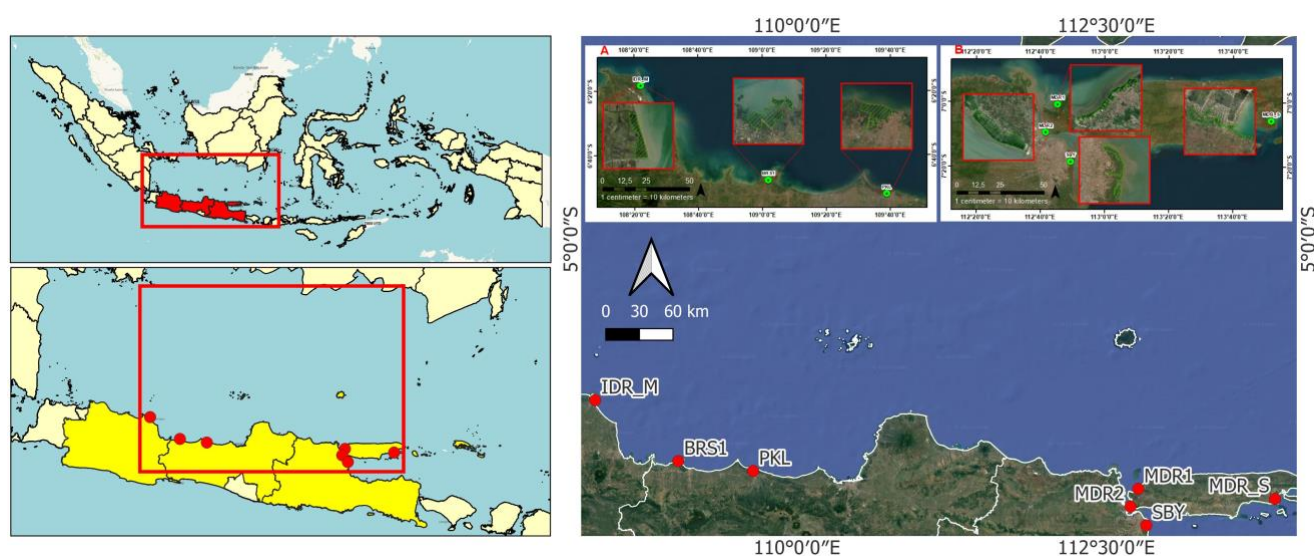
Despite these advances, a detailed investigation into the fine-scale taxonomic and functional diversity of protist



communities across different mangrove ecosystems in Southeast Asia is still lacking. Most regional eDNA studies have prioritized fish or other macro-eukaryotes, leaving the ecological drivers of protist community structure poorly understood. The present study comprehensively characterizes protist communities from selected mangrove areas using high-throughput 18S rRNA gene sequencing to address this critical gap. We aim to elucidate their taxonomic composition, identify potential bioindicator taxa, and provide foundational data to enhance our understanding of protist-mediated ecological processes. The findings are intended to guide science-based conservation and management strategies, ultimately supporting the long-term health and resilience of these vital coastal ecosystems.

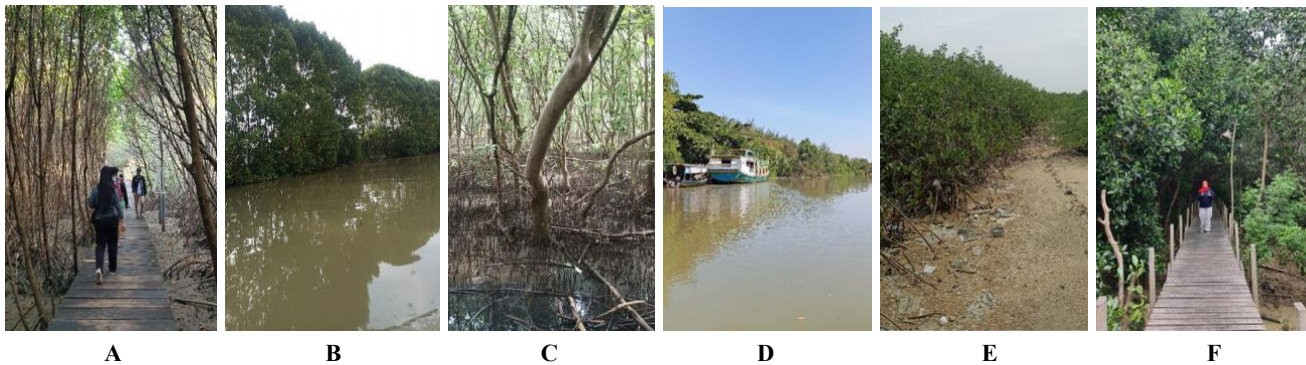
## MATERIALS AND METHODS

### Study area

Fieldwork was conducted in January and February 2024, with seven mangrove locations selected using purposive sampling. The selection criteria were based on variations in environmental characteristics and community usage surrounding each sampling site (Prahardika and Styawan 2020). This research examines the mangrove ecosystem along the north coast of Java Island and Madura Island, including: (i) Indramayu: 6°17'47.2"S 108°22'07.5"E, (ii) Brebes: 6°47'17.3"S 109°02'24.8"E, (iii) Pekalongan: 6°52'07.1"S 109°38'47.3"E, (iv) Surabaya: 7°18'26.3"S 112°49'26.5"E, (v) Kedatim: 7°05'36.4"S 113°51'54.2"E, (vi) Bangkalan: 112°41'44.5"E 7°00'44.0"S 112°45'32.1"E, (vii) Tanjung: 7°09'19.9"S 112°41'44.5"E (Figure 1). Each site is marked according to its primary ecological function, such as a natural coastal barrier or an ecotourism area, providing context for environmental and community-based differences.



**Figure 1.** Map of mangrove sampling locations on Java and Madura Islands, Indonesia. This map illustrates the seven sites selected for sediment sampling, highlighting their geographic distribution along the north coast of Java and Madura, Indonesia. IDR\_M: Karangsong, Indramayu, BRS1: Pandansari, Brebes, PKL: Mulyo Asri, Pekalongan, SBY: Wonorejo, Surabaya, MDR\_S: Kedatim, Madura, MDR1: Bangkalan, Madura, MDR2: Tanjung, Madura. : Mangrove ecosystem area, : Sampling location



**Figure 2.** Mangrove ecosystem conditions and functions as barriers can be found in: A. Brebes, B. Pekalongan, C. Indramayu, E. Tajungan and Bangkalan. Meanwhile, ecotourism functions can be found in: A. Brebes, D. Surabaya, and F. Kedadim. All locations were in bad condition, with much home/industrial waste

These areas serve various ecological and rehabilitation functions. On Java Island, mangroves play a crucial role in buffering coastal abrasion, particularly in the areas of Brebes, Pemalang, Kendal, and Demak, where muddy beaches characterize the coastline (Mahendra et al. 2017). In contrast, the Surabaya mangrove area is managed primarily for river normalization and pollution reduction, given its proximity to the Surabaya City area. Additionally, it holds significant potential for ecotourism (Azizah et al. 2020). Similarly, the Kedadim (MDR\_S) mangrove ecosystem supports ecotourism and is an important fish spawning ground. Bangkalan (MDR1) and Tajungan (MDR2), located on the island of Madura, function as key fishing zones.

According to the Ministry of Environment and Forestry of Indonesia (Ministry of Environment and Forestry of Indonesia 2017), the country has coastline spanning 95,181 km<sup>2</sup> and, as of 2015, a mangrove area of 3,489,140.68 ha. Of this, 1,671,140.75 ha are in good condition, while the others are classified as degraded. In Indonesia, mangrove ecosystems are often cultivated as natural coastal barriers, supporting fisheries and ecotourism, and contributing to the livelihoods of surrounding communities (Prihadi et al. 2018). In this study, the condition of analyzed mangrove ecosystems falls into two main categories: natural barriers and ecotourism sites (Figure 2).

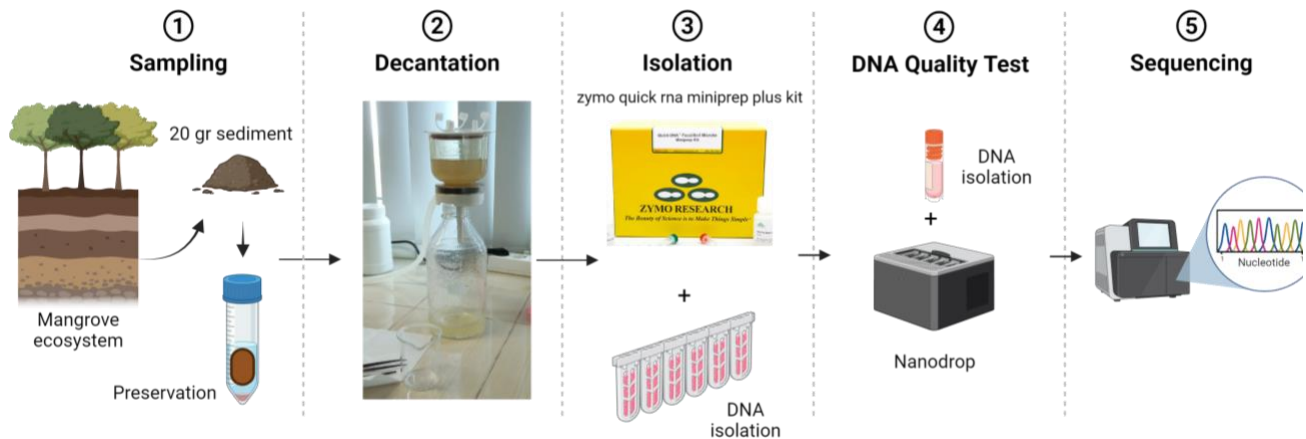
#### **Sediment collection, decantation, and water quality**

Approximately 20 g of surface sediment samples (5 cm depth) were collected using a sterile spatula and placed into a 15 mL Falcon tube containing 10 mL of 96% ethanol for preservation. A sterile disposable hand glove must be used when taking the sediment into the Falcon tube to avoid contamination. Every site collects three replications of soil samples in the decantation process. The samples were then stored in a cool box at 4°C to preserve their integrity during transport to Universitas Diponegoro, Semarang. Soil samples pose challenges for DNA extraction due to their complex composition. When targeting microorganisms, DNA extraction yields sufficient genetic material for library preparation. However, sediment samples, including metazoans, often require additional processing for eukaryotic organisms. This may involve a decantation process— separating solids from solutions — or a multi-stage filtration system to improve DNA recovery (Cahyani et al. 2024a). The

decantation method used in this study follows a standardized approach to separating sediment particles from the solution, building upon previous research methodologies (Cahyani et al. 2024b). The process began by placing approximately two-thirds of the total sediment sample into a 1 L Erlenmeyer flask and adding 400 mL of distilled water. The mixture was then shaken for 2 minutes to ensure proper dispersion of the particles. Following initial sediment suspension, the solution undergoes sequential filtration using a 500 µm Nectex filter. Any retained sediment was discarded, while the filtered solution was shaken for 30 seconds, then filtered using a 100 µm Nectex filter, and the process was repeated twice. The final solution was filtered through a Nectex 45 µm filter to refine the sample further and stored for 24 hours. A final filtration step, using a vacuum pump and a 2.5 µm Whatman filter, ensures optimal separation of particulate matter. Water quality measurements are made using a Horiba water checker U50 with three replicates for each site.

#### **DNA extraction and quantitative measurements**

The samples were prepared according to the isolation protocol to obtain pure DNA, free from other materials. DNA isolation was carried out using the Zymo Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit (Zymo Research, Germany), chosen for its efficiency and ability to yield high DNA concentrations (Pambudi et al. 2021). Cell lysis was performed using the ZR Bashing Bead Lysis Tubes kit, which involved bead-beating. These beads effectively disrupt cell walls in sediment samples, facilitating physical DNA extraction. The DNA yield and purity were then assessed by measuring absorbance at 260/280nm using a Thermo Scientific™ NanoDrop 2000C Spectrophotometer. The quality of DNA purity will be retested at Genetics Science to ensure it meets quality control standards prior to the sequencing stage. The steps taken there include: i) Genomic Quantification by Nanodrop & Qubit, ii) Initial quantification and purity observation with Nanodrop 2000 (Thermo Scientific), iii) PCR amplification with Phusion™ Plus PCR Master Mix (F631S), iv) PCR and product PCR visualization with Agarose Gel Electrophoresis, and v) Accurate quantification with Qubit dsDNA HS Assay Kits (Thermo Scientific).



**Figure 3.** The eDNA method starts from: 1. Collecting sediment from seven sites, 2. Decantation to get the separate sediment from the solution, 3. DNA isolation using an eDNA kit, 4. DNA quality test using a nanodrop, and 5. DNA sequencing in the PT. Genetika Science Indonesia facility

### DNA sequencing

Samples with a 2 ng/ $\mu$ L concentration were sent to the sequencing facility (PT. Genetika Science Indonesia) for PCR amplification and sequencing. The analysis was conducted using the amplicon-based approach with the Next Generation Sequencing (NGS) Illumina platform by Novogene Co., Ltd. in Singapore. The 18s rRNA gene was targeted using specific primers: the forward primer TAREuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3'), and reverse primer TAREukREV3 (5'-ACTTTCGTTCTTGATYRA-3') with the V4 region, which has a universal complementary sequence to eukaryotic DNA (Maritz et al. 2017). Sequencing was performed using the Illumina MiSeq platform to obtain raw sequencing data in FASTQ format. Details for sampling until DNA sequencing are shown in Figure 3.

### Data analysis

The data analysis was conducted using the GALAXY program (<https://usegalaxy.org>) for initial data processing and R-STUDIO software for data visualization. The GALAXY platform, developed by the "Galaxy Project" community, provides an automated framework for bioinformatics analysis (Saif et al. 2020). In this program, raw sequencing data in FASTQ format were subjected to a quality check using FASTQC. Adapters and primers were trimmed using DADA2, and unwanted sequences were removed. The cleaned sequences were merged and classified into Amplicon Sequence Variants (ASVs). Taxonomic classification was performed by aligning sequences against the PR2 database (<https://pr2-database.org>), which generated an OTU (Operational Taxonomic Unit) table and a taxonomy file. Analysis of alpha diversity and visualization of protist taxonomy is carried out using R-STUDIO V2024.12.1+563, while visualization of alpha diversity analysis is carried out using Origin 2024B.

The DNA trimming process using DADA2 (Callahan et al. 2016) is employed to detect and correct amplicon sequences generated by the Illumina platform. This process includes quality control steps that filter out phiX reads and

chimeric sequences. PhiX is a commercially used single-stranded DNA (ssDNA) virus employed as a positive control during sequencing. If primer trimming is required before running DADA2, the tool "cutadapt" can be used. Subsequently, within DADA2, the parameters "p-trim-left-f" and "p-trim-left-r" should be set to zero (Cahyani et al. 2024b).

To summarize the taxonomic composition of each sample, we used the *phyloseq* package (McMurdie and Holmes 2013) in R Studio. Stacked bar plots illustrating taxonomic composition and sequence abundance were generated with ggplot2 (Wickham 2019) based on the total abundance observed in each sample. The Shannon-Wiener and Simpson diversity indices are classical ecological metrics commonly used in eDNA biodiversity studies to assess species diversity and dominance in environmental samples. In our analysis, these indices were calculated automatically in RStudio using the *vegan* package, which implements the standard ecological formulas for these indices based on species abundance data (Wang et al. 2024).

## RESULTS AND DISCUSSION

### Identification of protist communities

The bioinformatics analysis yielded 63,440-107,334 sequencing reads (Figure 4). Across the seven sampling locations, 109 Amplicon Sequence Variants (ASVs) were identified.

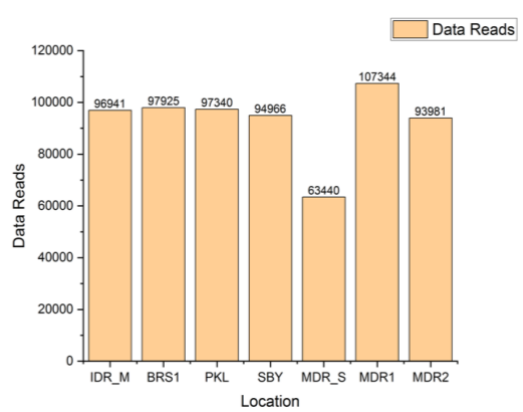
Based on the read abundance, the taxonomic composition reveals slight variations among the seven samples (Figure 5). Overall, analysis revealed the presence of five supergroups: Amoebozoa, Archaeplastida, Eukaryota\_X, Obazoa, and TSAR. Obazoa was present across all research locations, whereas Eukaryota\_X was exclusively found in MDR2.

An analysis of family composition in each location revealed variations in taxa abundance (Figure 6). Ostracoda was the most abundant group in sample BRS1, accounting for 26.57% of the total composition, while the highest overall abundance was observed in sample IDR\_M. In

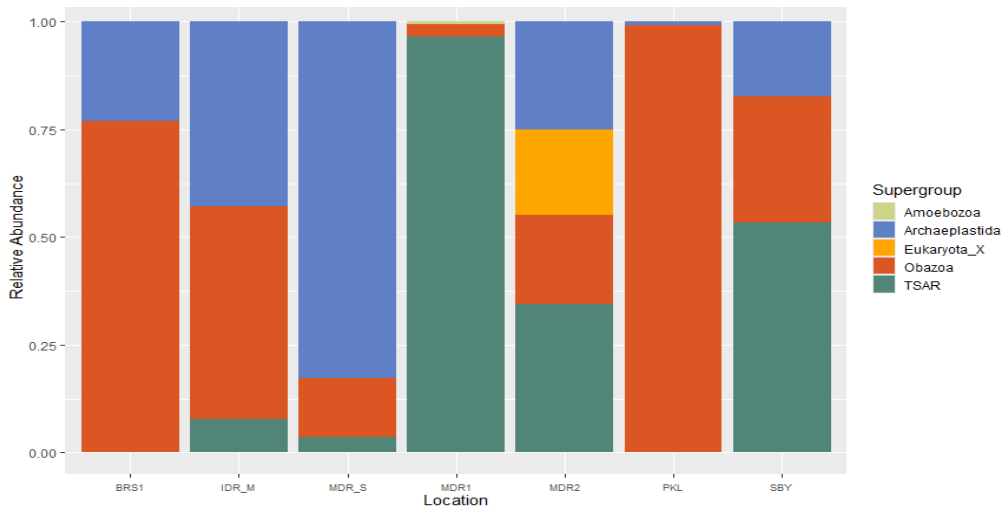
contrast, different patterns emerged in the three samples from Madura. Chlorellales (48.36%) was the dominant group in MDR\_S and represented 22.37% of the composition in MDR 2. Meanwhile, Tetrahymenida, which accounted for 77.72%, increased to 87.16% in MDR1. Additionally, Chaetocerotaceae was most abundant in SBY, making up 24.30% of the total composition.

Figure 6 presents the relative abundance of dominant protist families at each site. Notable patterns include the prevalence of Ostracoda in Brebes (BRS1), Chlorellales in Kedatim (MDR\_S) and Tajungan (MDR2), Tetrahymenida in Bangkalan (MDR1) and Tajungan (MDR2), and Chaetocerotaceae in Surabaya (SBY). The results highlight spatial variation in community structure.

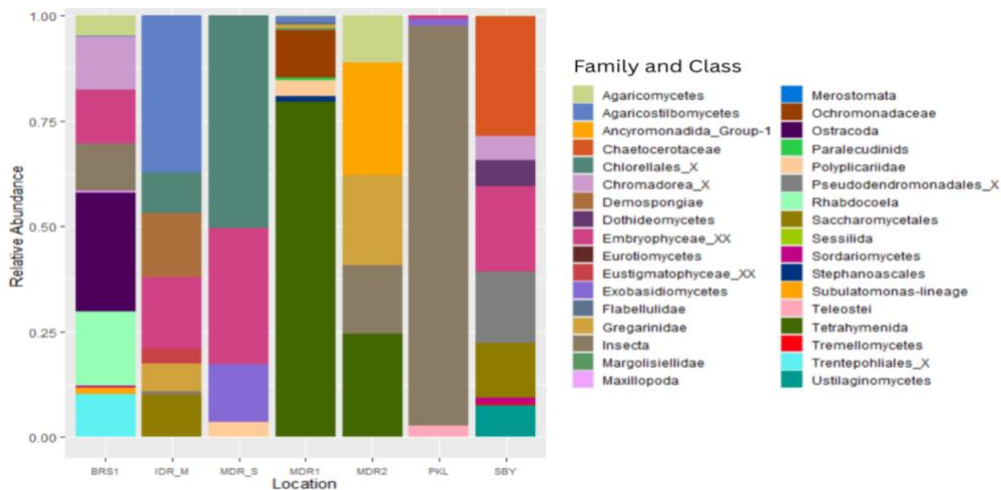
The eDNA analysis allowed identification at both the genus and species levels. In this study, 33 genera and 19 species were identified (Table 1).



**Figure 4.** Sequencing read counts from eDNA samples across mangrove sites. This bar plot displays the total number of high-quality sequencing reads obtained from each sediment sample using the Illumina MiSeq platform. The variation in read counts among sites reflects differences in DNA yield and sequencing depth, ensuring robust coverage for community analysis



**Figure 5.** Relative abundance of protist supergroups detected by eDNA metabarcoding. Stacked bar plots show the proportional composition of five major protist supergroups—Amoebzoa, Archaeplastida, Eukaryota\_X, Obazoa, and TSAR—across all sampling sites. The analysis reveals distinct community profiles, with Obazoa present at all locations and Eukaryota\_X most detected in MDR2



**Figure 6.** Family and class-level composition of protist communities in mangrove sediments

**Table 1.** Distribution and ecological roles of identified protist species across sampling sites. The table lists all genera and species detected through eDNA analysis, indicating their presence at each location. Ecological roles (such as primary producer, decomposer, or parasite) and potential impacts on mangrove ecosystem health are summarized, with site-specific bioindicator taxa highlighted

Genus	Species	Research location						
		BRS1	IDR_M	MDR_S	MDR1	MDR2	PKL	SBY
Amoebozoa								
<i>Paraflabellula</i>								+
Archaeplastida								
Microalgae								
<i>Picochlorum</i>	<i>Picochlorum</i> sp.		+	+				
<i>Trentepohlia</i>	<i>Trentepohlia</i> sp.	+						
Vegetation								
<i>Embryophyceae_XXX</i>	<i>Embryophyceae_XXX</i> sp.			+				
<i>Populus</i>	<i>Populus trichocarpa</i>		+					
<i>Rehmannia</i>	<i>Rehmannia glutinosa</i>							+
Eukaryota_X								
Protist								
<i>Ancyromonadida_Group-1_X</i>		+						
Obazoa								
Fish								
<i>Salmo</i>	<i>Salmo salar</i>							+
Insecta								
<i>Liposcelis</i>		+						
<i>Zatania</i>	<i>Zatania albimaculata</i>						+	
Protist								
<i>Subulatomonas-lineage</i>	<i>Subulatomonas-lineage_X</i> sp.	+						
Nematoda								
<i>Sabatieria</i>		+						
<i>Chromadorea_XX</i>	<i>Chromadorea_XX</i> sp.	+						
<i>Metachromadora</i>	<i>Metachromadora</i> sp.	+						
Fungi								
<i>Aspergillus</i>							+	
<i>Coprinopsis</i>							+	
<i>Kellermania</i>								+
<i>Trametes</i>		+						
<i>Candida</i>	<i>Candida wouanorum</i>				+			
<i>Exobasidiomycetes_X</i>	<i>Exobasidiomycetes_X</i> sp.							+
<i>Malassezia</i>	<i>Malassezia globosa</i>			+				
<i>Sporisorium</i>	<i>Sporisorium scitamineum</i>							+
<i>Sterigmatomyces</i>	<i>Sterigmatomyces halophilus</i>	+	+		+			
Merostomata								
<i>Tachypleus</i>		+						
TSAR								
Microalgae								
<i>Chaetoceros</i>								+
<i>Nannochloropsis</i>			+					
Protist								
<i>Leidyana1</i>			+				+	
<i>Spumella</i>					+			
<i>Tetrahymena</i>					+	+		
<i>Gregarina3</i>	<i>Gregarina3</i> sp.				+			
<i>Paralecudina</i>	<i>Paralecudina</i> sp.				+			
<i>Polyplacium</i>	<i>Polyplacium</i> sp.			+	+			
<i>Pseudodendromonadales_XX</i>	<i>Pseudodendromonadales_XX</i> sp.							+

Note: (+) explain why those species appear

### Diversity of the protist communities

Based on the Shannon-Wiener Diversity Index and Simpson dominance index, IDR\_M exhibited the highest diversity value, indicating a moderate to good level of ecosystem stability. Similar conditions were also observed in BRS1, SBY, MDR2, and MDR\_S. In contrast, MDR1 and PKL showed low diversity values, suggesting poor

ecosystem stability. This instability was further supported by a high Simpson dominance index (Figure 7), indicating the beginning of species dominance.

### Water quality measurement

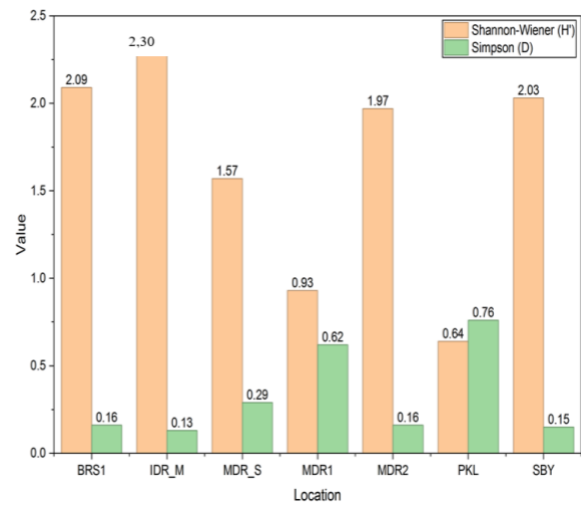
The water quality measurements showed that temperature levels across all sites stayed within the normal range for

estuarine environments. The pH levels tended to be alkaline. Dissolved Oxygen (DO) concentrations at MDR\_S, PKL, and SBY were below 5 mg/L, falling short of the standards set by the Indonesian Ministry of Environment and Forestry. Salinity levels were generally within the typical range for mangrove ecosystems, reaching 34 ppt. Elevated Total Dissolved Solids (TDS) readings indicated a high mineral content; however, TDS levels at all sites remained within safe limits for aquatic life and suitable for consumption. These results suggest that, apart from localized low DO in some areas, the water quality parameters support the ecological health of the mangrove habitats studied (Figure 8).

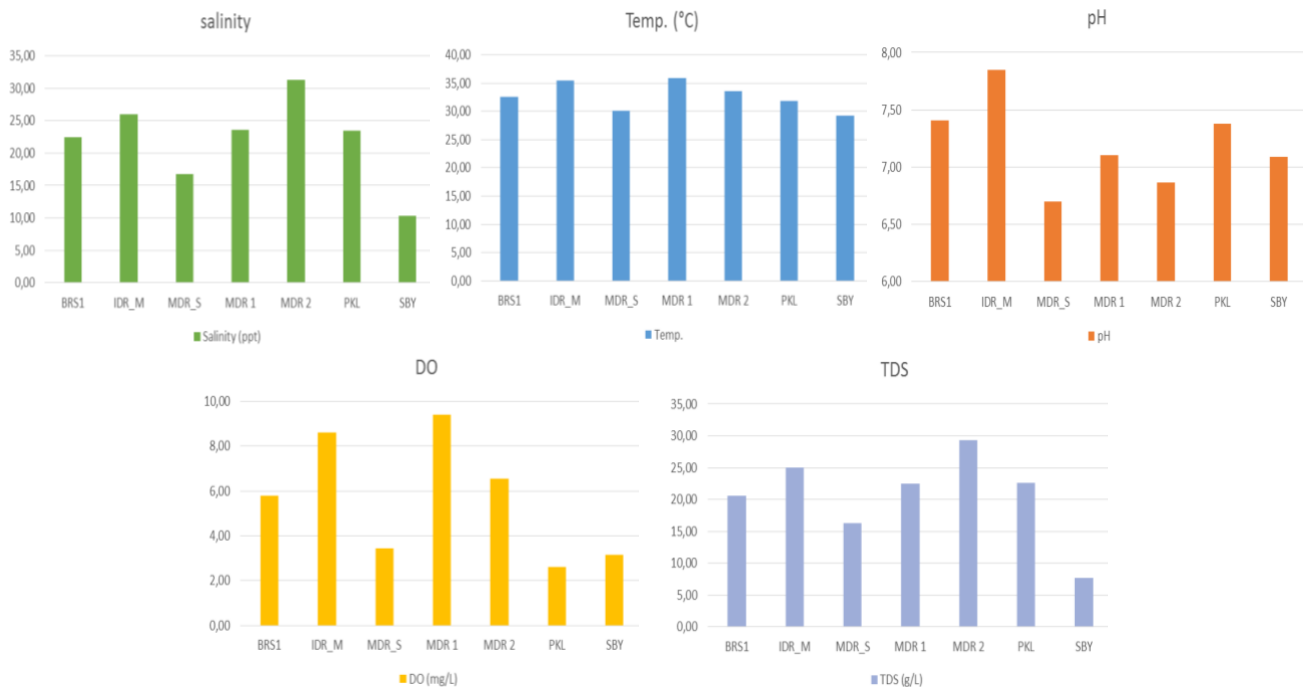
**nMDS analysis between water quality parameters and protist composition**

The NMDS plot shows species distributions along environmental gradients, with TDS being the only statistically significant variable ( $p < 0.05$ ), represented by a directional arrow (Figure 9). Species groups are enclosed within ellipses, suggesting potential ecological clusters. It shows that TDS is the key environmental gradient. The TDS vector points rightward and slightly downward, indicating a positive correlation with species in that direction. Species in the opposite direction (e.g., *Chaetoceros*, *Sporisorium scitamineum*, *Pseudodendromonadales*, *Rehmannia glutinosa*) are associated with lower TDS levels, likely reflecting freshwater or less mineralised environments. Species closer to the direction of the arrow (e.g., *Coprinopsis*, *Zatania albimaculata*, *Populus*

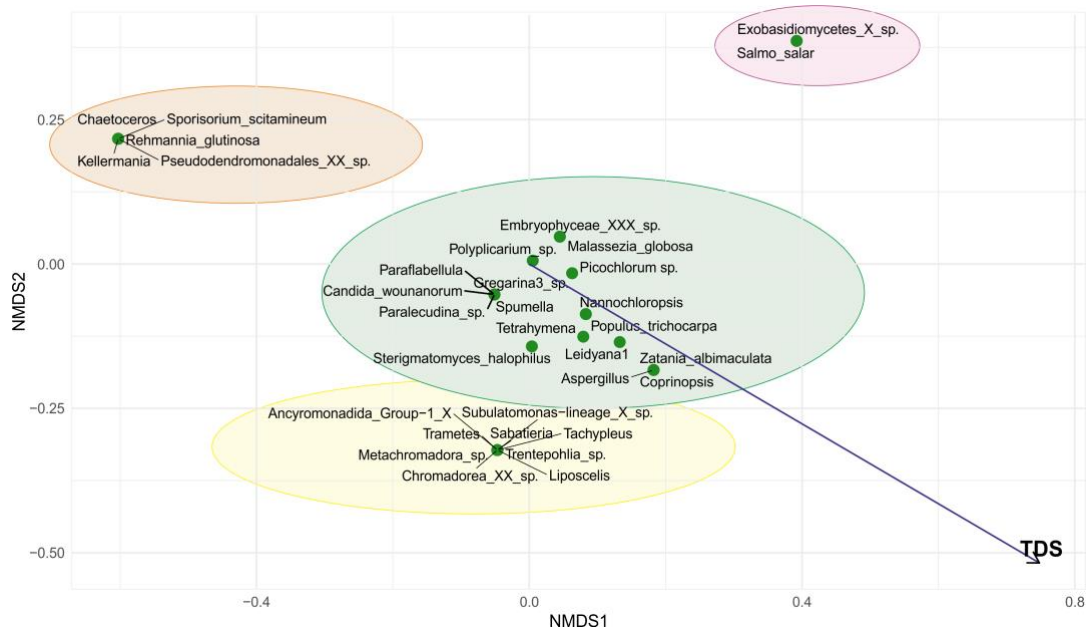
*trichocarpa*) are more closely associated with higher TDS levels, indicating environments richer in dissolved substances.



**Figure 7.** Diversity indices of protist communities in mangrove sediments. Bar plots illustrate each site's Shannon-Wiener Diversity Index (orange) and Simpson dominance index (green). Higher Shannon values indicate greater species diversity and ecosystem stability, while higher Simpson values reflect dominance by a few taxa. Indramayu (IDR\_M) exhibited the highest diversity, whereas Bangkalan (MDR1) and Pekalongan (PKL) showed lower diversity, suggesting environmental stress at these sites



**Figure 8.** Water quality measurement shows that all parameters were within normal conditions



**Figure 9.** Non-metric Multidimensional Scaling metric. Green cluster (center): Includes various protists, algae, and yeasts—species with broader ecological tolerance, likely found in intermediate TDS conditions. Orange cluster (upper left): This cluster consists of species favoring low TDS environments, possibly characteristic of freshwater or oligotrophic systems. Purple cluster (upper right): Includes *Exobasidiomycetes* and *Salmo salar*, potentially reflecting unique or isolated ecological niches. Yellow cluster (lower left): Contains more terrestrial or mesohaline species, which TDS may less influence

## Discussion

### *Protists diversity and ecological impacts*

The eukaryotic community within the sediment plays a vital role in maintaining ecosystem health. Eukaryotic groups, such as arthropods, apicomplexans, and diatoms, are commonly used as bioindicators because of their quick responses to water quality changes. These organisms are crucial for nutrient cycling, providing habitat, and serving as a food source for other species (B-Béres et al. 2023). They are valuable indicators for reconstructing the historical conditions of aquatic ecosystems (paleolimnology, paleoceanography). Furthermore, eukaryotic communities in the sediment are identified and quantified through microscopic analysis of their morphology and abundance. However, despite the large number of estimated species, our understanding of this diverse biodiversity remains limited (Lejzerowicz et al. 2021). To overcome these limitations, molecular techniques such as DNA metabarcoding have proven effective in detecting previously unidentified eukaryotic taxa and enhancing our understanding of their distribution patterns (Juhel et al. 2022). The eDNA metabarcoding technique enables the identification and quantification of species, allowing for the prediction of taxonomic dominance within an ecosystem (Beng and Corlett 2020; Andriyono et al. 2021; Garcia-Vazquez et al. 2021; Saccò et al. 2022; Cahyani et al. 2024b).

Numerous studies have demonstrated the effectiveness of the 18s rRNA gene as a marker for single-cell eukaryotes, including diatoms (Pawłowski et al. 2016; Bailet et al. 2020), marine protists in Indonesian waters (Cahyani 2021), fish (Kumar et al. 2022), coral-Zoothantella (Shinzato et al. 2018), and fungi (Quandt et al. 2023). The V4 region of the 18s rRNA gene is particularly promising for identifying

diatoms and other protist sequences (Zimmermann et al. 2015). However, challenges persist in selecting genetic markers, and the availability of comprehensive reference databases hinders accurate identification. This study analyses the diversity composition recorded through sedimentary DNA. Variations in diversity across locations suggest that environmental factors uniquely shape each mangrove ecosystem. Ríos-Castro et al. (2021) identified four eukaryotic groups using the 18s method in their research on the Spanish coast. Eukaryotic community dynamics are influenced by abiotic and biotic factors, including predation, sunlight exposure, nutrient availability, and temperature (Pramanik and Bhattacharyya 2023). Additionally, wind-driven upwelling and downwelling, caused by fluctuations in water column stratification and mixing (Odic et al. 2022), drive the seasonal succession of small planktonic eukaryotes in surface waters (Hernández-Ruiz et al. 2018). The study site examined by Ríos-Castro et al. (2021) is strongly influenced by upwelling dynamics, particularly during the summer months, when deep oceanic currents penetrate the area.

The composition and diversity of protist communities in mangrove sediments are closely linked to key water quality parameters, including pH, Dissolved Oxygen (DO), salinity, and Total Dissolved Solids (TDS). In this study, sites with higher protist diversity, such as Indramayu (IDR\_M), generally exhibited moderate to optimal water quality conditions, including neutral to slightly alkaline pH, adequate DO, and balanced salinity and TDS levels. These conditions support a diverse range of protist taxa, including phototrophic groups such as Chlorellales and various heterotrophic and parasitic protists, reflecting a stable and resilient ecosystem. This finding is consistent with recent

research demonstrating that protist richness and community structure are strongly influenced by physicochemical gradients, particularly salinity and nutrient availability, in freshwater and brackish environments (da Silva and Fernandes 2023).

Conversely, sites such as Bangkalan (MDR1) and Pekalongan (PKL), which showed lower protist diversity and higher dominance of specific taxa (e.g., Tetrahymenida), were characterized by suboptimal water quality, notably low DO levels and signs of organic pollution. Low DO can limit the metabolic activity and survival of protist taxa, favoring those adapted to hypoxic or polluted conditions, such as certain ciliates and parasitic forms. High TDS and altered pH can further influence the bioavailability of metals and nutrients, shaping protist assemblages by selecting for tolerant or opportunistic species. These patterns highlight the role of protists as sensitive bioindicators of environmental stress and water quality degradation in mangrove sediments (Febriansyah et al. 2023; El-Tohamy et al. 2024).

*Trentepohlia* is a filamentous green alga belonging to the class Trebouxiophyceae in Chlorophyta. Unlike most green corticolous algae, which appear green, *Trentepohlia* exhibit orange, yellow, or red hues due to a high concentration of carotenoids in their cells, making them easily recognizable in the field. All *Trentepohlia* species produce a high total carotenoid content, which protects them from ultraviolet light and high irradiance (Saraphol et al. 2021). Additionally, *Trentepohlia* can serve as bioindicators of atmospheric metallic pollution (García-Florentino et al. 2018) and are commonly found in humid habitats worldwide (Rindi et al. 2018). Their presence in mangroves may reflect air and water quality and contribute to primary production and microhabitat complexity.

As the most commonly found family in BRS1, Ostracoda is a group of microscopic crustaceans known for their excellent preservation in sediment (Vázquez-García et al. 2021). They inhabit lacustrine, estuarine, and marine environments (Horne et al. 2022) and feed on detritus, diatoms, foraminifers, and microscopic polychaete worms. Ostracods thrive best in habitats with muddy sands, silts, algae, and seagrass (Szlauek-Lukaszewska and Pešić 2020). In the Indo-Australian region, some commonly encountered genera include *Hemicytherura*, *Loxoconcha*, *Parakrithella* (Hussain et al. 2021). Their community composition and abundance respond rapidly to changes in salinity, organic matter, and pollution, making them effective for monitoring anthropogenic impacts and ecosystem health (Schmitz et al. 2025). Their abundance can reflect the impact of human activities (Ruiz et al. 2005) and geochemical changes in their carapace (Ruiz et al. 2013). Overall, because BRS1 functions as a fish pond and ecotourism site, it is easy to find ostracods, as some species can provide information about tide levels.

*Tetrahymena*, a genus within the family Tetrahymenida, was found to dominate in MDR1. These free-living, unicellular ciliate protozoa belong to the phylum Ciliophora and are commonly found in freshwater environments, where they play crucial ecological roles as decomposers and predators of bacteria (Gupta et al. 2019). *Tetrahymena*

are widely used as model organisms in toxicological and environmental research (Chasapis 2019). Their ability to reduce the growth rate of harmful algae and toxins suggests that MDR1 may be subject to contamination. Additionally, as key consumers in the food chain, *Tetrahymena* acts as a bioaccumulator of pollutants (Maurya and Pandey 2020). Based on the condition of MDR1, the environment can be polluted due to waste; this is related to community awareness in protecting the mangrove ecosystem. Mongabay reported in 2019 that the mangroves in Madura were in danger, with most of the root cause being a lack of community awareness; on the other hand, they found that some areas had been converted into shrimp ponds.

*Chaetoceros* is one of the most diversified genera of planktonic diatoms (Lu et al. 2024). *Chaetoceros* are characterized by their cells and setae, which contain multiple chloroplasts and are widely distributed (Kooistra et al. 2022). These species are highly prevalent in marine ecosystems, with certain members, such as *Chaetoceros atlanticus* and *Chaetoceros danicus*, capable of triggering blooms (Helleren 2016). Furthermore, their rigid, spiky setae can cause mechanical damage to the gills of fish and other economically significant organisms, leading to financial losses (Fryxell and Medlin 1981; Lassus et al. 2016). The presence of this genus is often indicative of soluble pollution in aquatic environments, as *Chaetoceros* can act as a reducing agent. Moreover, *Chaetoceros tenuissimus* exhibits heightened sensitivity to various toxic chemicals, making it a valuable indicator for assessing water quality (Pastorino et al. 2022).

Parasites have been identified within the fungal groups, including the genus *Kellermania* (Minnis et al. 2012) and *Exobasidiomycetes\_x\_sp.* (Wang et al. 2015), both can cause plant diseases by infecting leaves, tissues, and flowers. In humans, *Malassezia globosa* is known to cause dandruff (Dawson 2007; Torres et al. 2024), while species from the genus *Malassezia* have also been linked to acne (Hamdino et al. 2022). Parasitic protists have also been identified, such as *Leidyana1*, which can infect grasshoppers (Odle et al. 2024). Detecting parasitic and potentially pathogenic protists, including *Exobasidiomycetes* and *Malassezia globosa*, in sites with elevated TDS and lower DO highlights the ecological risks of declining water quality. Recent studies have demonstrated that mangrove sediments can serve as environmental hotspots for pathogenic protists, with parasitic taxa occasionally dominating the community and posing risks to both the ecosystem and human health (Liu et al. 2024a). The strong association between protist community composition and water quality parameters observed in this study supports the use of eDNA-based protist monitoring as an effective tool for early detection of ecological disturbances, pollution, and emerging pathogenic threats in mangrove ecosystems. Integrating protist bioindicators with routine water quality assessments will enhance the capacity for timely management interventions and long-term conservation of these critical coastal habitats. The summary of protists that can serve as bioindicators in this research, along with the methods for controlling them, is presented in Table 2.

**Table 2.** List of protists that can be environmental indicators in the mangrove ecosystem

Species/Group	Ecological role/Impact	Source/Indicator	Management/Control
Ostracoda	Bioturbation, nutrient cycling, bioindicator	Natural, sensitive to sediment quality	Maintain sediment quality, reduce pollution
<i>Tetrahymena</i>	Decomposer, bacterial predator, pollution indicator	Organic pollution, eutrophication	Reduce waste input, raise awareness
<i>Chaetoceros</i>	Primary producer, can form blooms, bioindicator	Nutrient enrichment, water quality	Control nutrient input, monitor blooms
<i>Chlorellales</i>	Primary producer, sediment stabilizer	Nutrient availability	Maintain balanced nutrients
<i>Malassezia globosa</i>	Human pathogen, pollution indicator	Wastewater contamination	Improve wastewater treatment
<i>Exobasidiomycetes/ Kellermania</i>	Plant parasite, disease risk	Plant tissue infection	Monitor plant health, reduce stressors
<i>Leidyana</i>	Insect parasite affects the food web	Insect hosts	Monitor insects, maintain balance
<i>Trentepohlia</i>	Bioindicator, primary producer	Air/water pollution, humidity	Monitor pollution, preserve habitat

### Water quality factor for protists' habitat

The NMDS (Non-metric Multidimensional Scaling) plot provides a visual summary of the relationships between protist, fungal, and micro-eukaryote communities in mangrove sediments, highlighting how environmental gradients, especially TDS (Total Dissolved Solids), influence community structure. The distinct clustering of taxa indicates that specific environmental conditions, such as mineral content and organic matter, play a significant role in shaping the distribution of these organisms. For example, the central green cluster contains diverse taxa like *Tetrahymena*, *Malassezia globosa*, and *Nannochloropsis*, suggesting these species are well-adapted to environments with moderate TDS and mixed organic inputs. This aligns with recent studies demonstrating that environmental heterogeneity, including salinity and nutrient levels, drives the spatial structuring of microbial and protist communities in mangrove sediments (Ho et al. 2023; Liu et al. 2024b).

The strong vector for TDS in the NMDS plot underscores its significance as an environmental driver. Taxa positioned along this gradient, such as *M. globosa* and *Tetrahymena*, are likely more abundant in sites with higher mineral and solute concentrations. Elevated TDS can result from both natural processes and anthropogenic activities, such as runoff or pollution, which can influence the bioavailability of nutrients and metals. Recent research has shown that TDS, along with other factors such as salinity and organic content, can significantly impact the diversity and function of sediment microbial communities, thereby affecting ecosystem processes like nutrient cycling and organic matter decomposition (Liu et al. 2024b; Spedicato et al. 2024). Potential pathogens, such as *M. globosa*, further highlight the role of mangrove sediments as hotspots for environmental pathogens, with consequences for both ecosystem and human health (Liu et al. 2024a).

Separating other clusters, such as those containing *Chaetoceros* and *Exobasidiomycetes\_X\_sp.*, indicates that some taxa are more specialized, occupying unique environmental niches less influenced by TDS. This pattern suggests that while particular species are generalists capable of thriving across various conditions, others are specialists with narrower ecological requirements. Such differentiation is consistent with findings that environmental filtering selects for specific community assemblages in mangrove

sediments (Spedicato et al. 2024). Understanding these patterns is crucial for effective ecosystem management, as shifts in environmental parameters can rapidly alter community composition, potentially enhancing the prevalence of harmful or invasive species and impacting overall ecosystem resilience (Liu et al. 2024b; Zhang et al. 2024).

Detecting parasitic and pathogenic taxa, such as *Exobasidiomycetes* and *M. globosa*, highlights the potential ecological risks in these ecosystems and underscores the importance of eDNA for early warning and bioindicator development. Our findings confirm that eDNA metabarcoding is a sensitive, noninvasive, and scalable approach for monitoring microbial biodiversity and ecosystem health in mangrove environments. It can detect both dominant and cryptic taxa that are often missed by traditional methods. Compared with Cahyani et al. (2024a), who used the Cytochrome Oxidase Subunit I (COI) gene unit from two sampling locations, the communities were dominated by Chordata, Bacillariophyta, and arthropods. Evidence of environmental contamination was found because *Homo sapiens* DNA was detected. In another region, Liu et al. (2024a) found a 59,67% abundance of protist communities. Microbial communities (bacteria, fungi, and archaea) positively influence parasitic communities, including both horizontal and vertical-scale interactions. Additionally, sulfate and salinity had the most significant impact on the protistan community. The method provides comprehensive insights into genetic structure and is cost-effective for biodiversity research and understanding dynamic ecosystem changes.

The investigation of benthic protists faces challenges due to the various processes that influence sediment dynamics. The seafloor plays a significant role in shaping communities, influenced by continuous surface resuspension. This phenomenon may vary based on surface waves, storm impacts, shipping activities, and trawling, with effects that differ in intensity depending on depth (Giannakourou et al. 2005; Sanchez-Vidal et al. 2012). Consequently, the sedimentation rates, mechanical resuspension, and the interactions between benthic and pelagic habitats render the evaluation of dynamics near sediments complex (Cibic et al. 2022). Furthermore, it has been demonstrated that factors such as currents, horizontal flow speed, upwelling, and depth affect the dispersal, transport, and sinking rates

of eDNA particles, living microorganisms, and resting stages, which may be found in sediments located far from their source (Harrison et al. 2019; Nooteboom et al. 2019; Nguyen et al. 2023).

When applying eDNA metabarcoding on sediments, it is important to recognize that sediments form a complex matrix. They accumulate both extra- and intracellular DNA, making it difficult to distinguish between living and dead organisms (Pawlowski et al. 2022). Additionally, when eDNA settles on the seafloor, sediments can absorb it, protecting it from degradation (Romanowski et al. 1991). Decay times vary greatly, from days to thousands of years, depending on sediment structure and various abiotic and biotic factors (Harrison et al. 2019; Ellegaard et al. 2020; Pawlowski et al. 2022). This was not a concern in our study, as we focused on identifying benthic organisms. The long-lasting presence of eDNA in sediments may add uncertainty to reconstructing living benthic communities or using this method for monitoring purposes (Harrison et al. 2019).

It is important to note that, although our findings may be subject to certain biases, the resulting errors are systematic and do not undermine the relative comparisons between samples and locations. Consequently, the key findings of our study are solid, especially regarding the identified alterations in benthic protist community composition across the seven mangrove areas. The diversity detected through sedimentary eDNA varied between research locations, indicating that each mangrove ecosystem is uniquely shaped by its environmental factors. Notably, using the 18S rRNA gene proved effective for capturing a broad spectrum of single-cell eukaryotes, protists, and fungi. However, the accuracy of identification continues to be challenged by the limited availability of reference databases and the selection of genetic markers.

In conclusion, the study revealed that the ability of eDNA to survey multiple taxonomic groups simultaneously makes it a powerful and minimally invasive approach for biodiversity monitoring in mangroves. Findings such as the detection of both well-known and rare taxa signal the method's sensitivity. At the same time, the observed location-specific variation underscores the influence of abiotic factors, such as nutrient levels, sunlight, temperature, and hydrodynamics, on community composition. This approach enables a more nuanced understanding of ecosystem health, local stressors, and ongoing changes in these vulnerable coastal habitats.

eDNA methods should be integrated into routine monitoring programs for effective mangrove restoration and conservation. Establishing standardized sampling and processing protocols will improve the comparability of data between sites and over time. Building and updating comprehensive reference libraries for regional mangrove biota will also enable more precise taxonomic assignments. Combining eDNA results with environmental data (e.g., salinity, sediment quality, and anthropogenic pressures) will facilitate the early detection of biodiversity loss or invasive species, guiding targeted restoration and adaptive management. As eDNA techniques advance, their application offers an efficient, scalable, and cost-effective pathway for safeguarding the complex web of life found in mangrove

ecosystems and ensuring their essential ecological services endure into the future.

## ACKNOWLEDGEMENTS

The authors sincerely thank the Cluster for Paleolimnology and the Biodiversity Lab for their invaluable support. Thanks to the Polish Academy of Science and Warsaw University for making this paper more insightful. Thanks to the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for the research scholarship grant. This research was funded by the Ministry of Education, Culture, Research, and Technology, Republic of Indonesia, in the *Program Magister menuju Doktor untuk Sarjana Unggul (PMDSU)* program through Universitas Diponegoro, with contract number 345-43/UN7.D2/PP/V/2024 and *Peningkatan Kualitas Publikasi Internasional (PKPI)* Program from PMDSU with contract number 250.6/E4.4/KU/2024.

## REFERENCES

- Andriyono S, Alam MJ, Kim H-W. 2021. Marine fish detection by environmental DNA (eDNA) metabarcoding approach in the Pelabuhan Ratu Bay, Indonesia. *Intl J Adv Sci Eng Inf Technol* 11 (2): 729-737. DOI: 10.18517/ijaseit.11.2.9528.
- Azizah N, Rianto B, Lubis L. 2020. Pengembangan ekowisata mangrove Wonorejo di Surabaya. *Aplikasi Administrasi: Media Analisa Masalah Administrasi* 23 (2): 33-42. DOI: 10.30649/aamama.v23i2.119. [Indonesian]
- Baillet B, Apothéloz-Perret-Gentil L, Baričević A, Chonova T, Franc A, Frigerio J, Kelly M, Mora D, Pfannkuchen M, Proft S, Ramon M, Vasselon V, Zimmermann J, Kahlert M. 2020. Diatom DNA metabarcoding for ecological assessment: Comparison among bioinformatics pipelines used in six European countries reveals the need for standardization. *Sci Total Environ* 745: 140948. DOI: 10.1016/j.scitotenv.2020.140948.
- Balatero TP, Molina Z, Dalayap RM, Dechavez R, Manubag JJ, Sumaya NH, Peña JD, Tabugo SR. 2025. eDNA metabarcoding in mangrove ecosystems for fish conservation and stock assessment of *Sardinops sagax melanostictus* in the Philippines. *Biodiversitas* 26 (1): 347-357. DOI: 10.13057/biodiv/d260134.
- Basak P, Pramanik A, Sengupta S, Nag S, Bhattacharyya A, Roy D, Pattanayak R, Ghosh A, Chattopadhyay D, Bhattacharyya M. 2016. Bacterial diversity assessment of pristine mangrove microbial community from Dhulibhashani, Sundarbans using 16S rRNA gene tag sequencing. *Genom Data* 7: 76-78. DOI: 10.1016/j.gdata.2015.11.030.
- Basyuni M. 2025. Data set for manuscript: eDNA metabarcoding reveals rich microbial eukaryote communities in tropical mangrove forest of Makassar, Indonesia [Data set]. Figshare. DOI: 10.6084/m9.figshare.29421434.v2.
- B-Béres V, Stenger-Kovács C, Buczkó K, Padišák J, Selmečzy GB, Lengyel E, Tapolczai K. 2023. Ecosystem services provided by freshwater and marine diatoms. *Hydrobiologia* 850: 2707-2733. DOI: 10.1007/s10750-022-04984-9.
- Beng KC, Corlett RT. 2020. Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodivers Conserv* 29: 2089-2121. DOI: 10.1007/s10531-020-01980-0.
- Cahyani NKD, Jumari J, Hariyati R, Rahadian R, Rahim A, Zega ES, Kholifah RWN, Barber PH, Putro SP, Soeprobawati TR. 2024a. Utilizing environmental DNA to identify eukaryotic diversity in mangrove sediments at Demak, Central Java, Indonesia. *Ilmu Kelautan: Indones J Mar Sci* 2: 543-556. DOI: 10.14710/ik.ijms.29.4.543-556.
- Cahyani NKD, Malik MDA, Soeprobawati TR, Anggoro AW, Syakal ER. 2024b. Metode Pengambilan Sampel eDNA dan Analisisnya. Undip Press, Semarang. [Indonesian]

- Cahyani NKD. 2021. Delineating macro and micro marine biodiversity in the Coral Triangle using autonomous reef monitoring structures and DNA metabarcoding. [Dissertation]. University of California, Los Angeles.
- Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 13: 581-583. DOI: 10.1038/nmeth.3869.
- Chasapis CT. 2019. Preliminary results from structural systems biology approach in *Tetrahymena thermophila* reveal novel perspectives for this toxicological model. *Arch Microbiol* 201 (1): 51-59. DOI: 10.1007/s00203-018-1571-6.
- Cibic T, Baldassarre L, Cerino F, Comici C, Fornasaro D, Kralj M, Gianini M. 2022. Benthic and pelagic contributions to primary production: Experimental insights from the gulf of trieste (Northern adriatic sea). *Front Mar Sci* 9: 877935. DOI: 10.3389/fmars.2022.877935.
- Da Silva VC, Fernandes N. 2023. Protist taxonomic and functional diversity in aquatic ecosystems of the Brazilian Atlantic Forest. *PeerJ* 11: e15762. DOI: 10.7717/peerj.15762.
- Dawson Jr TL. 2007. *Malassezia globosa* and *restricta*: Breakthrough understanding of the etiology and treatment of dandruff and seborrheic dermatitis through whole-genome analysis. *J Invest Dermatol Symp Proc* 12 (2): 15-19. DOI: 10.1038/sj.jidsymp.5650049.
- Ellegaard M, Clokie MRJ, Czepionka T, Frisch D, Godhe A, Kremp A, Letarov A, McGenity TJ, Ribeiro S, Anderson NJ. 2020. Dead or alive: Sediment DNA archives as tools for tracking aquatic evolution and adaptation. *Commun Biol* 3 (1): 169. DOI: 10.1038/s42003-020-0899-z.
- El-Tohamy WS, Taher ME, Ghoneim AM, Hopcroft RR. 2024. Protozoan communities serve as a strong indicator of water quality in the Nile River. *Sci Rep* 14 (1): 16382. DOI: 10.1038/s41598-024-66583-z.
- Fahma TLN, Cahyani NKD, Jumari, Hariyati R, Soeprbowati TR. 2024. Environmental DNA approach to identify protists community in sediment of Balekambang Lake, Indonesia, using 18s rRNA gene. In: Haddout S, Priya K, Hogue AM (eds). *Proceedings of The 2nd International Conference on Climate Change and Ocean Renewable Energy (CCORE 2022)*. Springer Proceedings in Earth and Environmental Sciences. Springer, Cham. DOI: 10.1007/978-3-031-71555-6\_25.
- Febriansyah SC, Hakim L, Saptoyo S, Retnaningdyah C. 2023. Phytoplankton diversity as a bioindicator of water quality mangrove ecosystems in Clungup Mangrove Conservation, Kondang Merak and Sempu Island, Malang Regency. *J Trop Biodivers Biotechnol* 8 (1): jttb73002. DOI: 10.22146/jttb.73002.
- Friess DA, Rogers K, Lovelock CE, Krauss KW, Hamilton SE, Lee SY, Lucas R, Primavera J, Rajkaran A, Shi S. 2019. The state of the world's mangrove forests: Past, present, and future. *Ann Rev Environ Resour* 44: 89-115. DOI: 10.1146/annurev-environ-101718-033302.
- Fryxell GA, Medlin LK. 1981. Chain forming diatoms, evidence of parallel evolution in *Chaetoceros*. *Cryptogam Algol* 1: 3-29. DOI: 10.5962/p.309011.
- García-Florentino C, Maguregui M, Morillas H, Marcaida I, Salcedo I, Madariaga JM. 2018. *Trentepohlia* algae biofilms as bioindicator of atmospheric metal pollution. *Sci Total Environ* 626: 441-450. DOI: 10.1016/j.scitotenv.2018.01.096.
- García-Vázquez E, Georges O, Fernandez S, Ardura A. 2021. eDNA metabarcoding of small plankton samples to detect fish larvae and their preys from Atlantic and Pacific waters. *Sci Rep* 11 (1): 7224. DOI: 10.1038/s41598-021-86731-z.
- Geisen S, Bonkowski M. 2018. Methodological advances to study the diversity of soil protists and their functioning in soil food webs. *Appl Soil Ecol* 123: 328-333. DOI: 10.1016/j.apsoil.2017.05.021.
- Giannakourou A, Orlova TY, Assimakopoulou G, Pagou K. 2005. Dinoflagellate cysts in recent marine sediments from Theraikos Gulf, Greece: Effects of resuspension events on vertical cyst distribution. *Cont Shelf Res* 25: 2585-2596. DOI: 10.1016/j.csr.2005.08.003.
- Gupta GS, Kansara K, Shah H, Rathod R, Valecha D, Gogisetty S, Joshi P, Kumar A. 2019. Impact of humic acid on the fate and toxicity of titanium dioxide nanoparticles in *Tetrahymena pyriformis* and zebrafish embryos. *Nanoscale Adv* 1: 219-227. DOI: 10.1039/c8na00053k.
- Hamdino M, Saudy AA, El-Shahed LH, Taha M. 2022. Identification of *Malassezia* species isolated from some *Malassezia* associated skin diseases. *J Mycol Med* 32 (4): 101301. DOI: 10.1016/j.mycmed.2022.101301.
- Harrison JB, Sunday JM, Rogers SM. 2019. Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proc Biol Sci* 286 (1915): 20191409. DOI: 10.1098/rspb.2019.1409.
- Helleren S. 2016. The diatom *Chaetoceros* spp. as a Potential Contributing Factor to Fish Mortality Events in Cockburn Sound, November 2015. Dalcon Environmental Report, Western Australia.
- Hernández-Ruiz M, Barber-Lluch E, Prieto A, Álvarez-Salgado XA, Logares R, Teira E. 2018. Seasonal succession of small planktonic eukaryotes inhabiting surface waters of a coastal upwelling system. *Environ Microbiol* 20 (8): 2955-2973. DOI: 10.1111/1462-2920.14313.
- Ho CT, Tatsuya U, Nguyen SG, Nguyen T-H, Dinh ST, Le ST, Pham T-M-H. 2023. Seasonal change of sediment microbial communities and methane emission in young and old mangrove forests in Xuan Thuy National Park. *J Microbiol Biotechnol* 34 (3): 580-588. DOI: 10.4014/jmb.2311.11050.
- Horne DJ, Cabral MC, Fatela F, Radl M. 2022. Salt marsh ostracods on European Atlantic and North Sea coasts: Aspects of macroecology, palaeoecology, biogeography, macroevolution and conservation. *Mar Micropaleontol* 174: 101975. DOI: 10.1016/j.marmicro.2021.101975.
- Hussain SM, Nazeer MN, Radhakrishnan K, Rajkumar A, Sivapriya V. 2021. Mangrove Ostracoda species fluctuations, habitual adaptation, and its environmental implications—A review. In: Kumaran N, Damodara P (eds). *Holocene Climate Change and Environment*. Elsevier, New York. DOI: 10.1016/B978-0-323-90085-0.00008-5.
- John E, Paul NM, Selven S. 2024. Metagenomic analysis of bacterial diversity in the surface water, top soil and sediment from the Mangrove ecosystem of Panangad, India. *Ecol Genet Genom* 30: 100225. DOI: 10.1016/j.egg.2024.100225.
- Juhel J-B, Marques V, Utama RS, Vimono IB, Sugeha HY, Kadarusman K, Cochet C, Dejean T, Hoey A, Mouillot D, Hocdé R, Pouyaud L. 2022. Estimating the extended and hidden species diversity from environmental DNA in hyper-diverse regions. *Ecography* 2022 (10): e06299. DOI: 10.1111/ecog.06299.
- Kooistra WHCF, Kusber W-H, Hernández-Becerril DU, Montresor M, Sarno D. 2022. The type species of the diatom genus *Chaetoceros*. *Diatom Res* 37 (2): 81-88. DOI: 10.1080/0269249X.2022.2066182.
- Kumar G, Reaume AM, Farrell E, Gaither MR. 2022. Comparing eDNA metabarcoding primers for assessing fish communities in a biodiverse estuary. *PLoS One* 17: e0266720. DOI: 10.1371/journal.pone.0266720.
- Lassus P, Nicolas C, Philipp H, Elisabeth N. 2016. *Toxic and Harmful Microalgae of the World Ocean*. In: Intergovernmental Oceanographic Commission of UNESCO. *IOC Manuals and Guides*, Denmark.
- Lejzerowicz F, Gooday AJ, Angeles IB, Cordier T, Morard R, Apothéloz-Perret-Gentil L, Lins L, Menot L, Brandt A, Levin LA, Arbizu PM, Smith CR, Pawlowski J. 2021. Eukaryotic biodiversity and spatial patterns in the clarion-clipperton zone and other abyssal regions: Insights from sediment DNA and RNA metabarcoding. *Front Mar Sci* 8: 671033. DOI: 10.3389/fmars.2021.671033.
- Li Y, Gao P, Sun X, Li B, Guo L, Yang R, Su X, Gao W, Xu Z, Yan G, Wang Q, Sun W. 2022. Primary succession changes the composition and functioning of the protist community on mine tailings, especially phototrophic protists. *ACS Environ Au* 2 (5): 396-408. DOI: 10.1021/acsenvironau.1c00066.
- Lincoln S, Andrews B, Birchenough SNR, Chowdhury P, Engelhard GH, Harrod O, Pinnegar JK, Townhill BL. 2022. Marine litter and climate change: Inextricably connected threats to the world's oceans. *Sci Total Environ* 837: 155709. DOI: 10.1016/j.scitotenv.2022.155709.
- Liu F, Ding J, Zeng J, Wang C, Wu B, Yan Q, He Z, Shu L. 2024a. Mangrove sediments are environmental hotspots for pathogenic protists. *J Hazard Mater* 467: 133643. DOI: 10.1016/j.jhazmat.2024.133643.
- Liu Y, Chen S, Liang J, Song J, Sun Y, Liao R, Liang M, Cao H, Chen X, Wu Y, Bei L, Pan Y, Yan B, Li Y, Tao Y, Bu R, Gong B. 2024b. Bacterial community structure and environmental driving factors in the surface sediments of six mangrove sites from Guangxi, China. *Microorganisms* 12: 2607. DOI: 10.3390/microorganisms12122607.
- Lu X, Zhai M, Lundholm N, Li Y. 2024. Taxonomy and phylogeny of the section *Chaetoceros* (Chaetocerotaceae, Bacillariophyta), with description of two new species. *J Ocean Limnol* 42: 1286-1311. DOI: 10.1007/s00343-023-3112-7.
- Mahendra IWY, Maulana E, Wulan TR, Rahmadana ADW, Putra AS. 2017. *Pemetaan Kawasan Rawan Abrasi di Provinsi Jawa Tengah Bagian Utara. Kepesisiran dan Kemaritiman Jawa Tengah, Volume II*. Badan Informasi Geospasial, Bogor. [Indonesian]
- Mandal A, Ghosh A, Kumar G, Bhadury P. 2025. Dataset of benthic foraminiferal community structure from sediment eDNA of Sundarbans mangrove ecosystem. *Data Brief* 60: 111554. DOI: 10.1016/j.dib.2025.111554.
- Marinich N, Casabianca S, Marchesini A, Vernesi C, Scardi M, Penna A. 2024. Structural variability of protist assemblages in surface

- sediments across Italian Mediterranean marine subregions. *Front Mar Sci* 11: 1427357. DOI: 10.3389/fmars.2024.1427357.
- Maritz JM, Rogers KH, Rock TM, Liu N, Joseph S, Land KM, Carlton JM. 2017. An 18S rRNA workflow for characterizing protists in sewage, with a focus on zoonotic trichomonads. *Microbial Ecol* 74 (4): 923. DOI: 10.1007/s00248-017-0996-9.
- Maurya R, Pandey AK. 2020. Importance of protozoa *Tetrahymena* in toxicological studies: A review. *Sci Total Environ* 741: 140058. DOI: 10.1016/j.scitotenv.2020.140058.
- McMurdie PJ, Holmes S. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8 (4): e61217. DOI: 10.1371/journal.pone.0061217.
- Ministry of Environment and Forestry of Indonesia. 2017. Miliki 23% Ekosistem Mangrove Dunia, Indonesia Tuan Rumah Konferensi Internasional Magrove 2017. SP. 58/HUMAS/PP/HMS.3/03/2017. Ministry of Environment and Forestry of Indonesia, Jakarta. [Indonesian]
- Minnis AM, Kennedy AH, Grenier DB, Palm ME, Rossman AY. 2012. Phylogeny and taxonomic revision of the Planistromellaceae including its coelomycetous anamorphs: Contributions towards a monograph of the genus *Kellermania*. *Persoonia* 29: 11-28. DOI: 10.3767/003158512X658766.
- Mongabay. 2019. <https://www.mongabay.co.id/2019/12/19/mangrove-madura-kritis-makin-terkikis/>, accessed 17 March 2024.
- Nabila ST, Soeprubowati TR, Cahyani NKD, Jumari J, Hariyati R, Hejnis H. 2024. Environmental DNA application to identify protozoan community in the sediment of Balekambang Lake, Dieng, Central Java. *Indones J Limnol* 5 (1): 39-48. DOI: 10.51264/inajl.v5i1.48.
- Naputo CFP, Isowa Y, Gerona-Daga ME, Artigas MD, Kajita T, Salmo III SG. 2024. Application of eDNA metabarcoding in the assessment of fish biodiversity in Philippine mangroves: Challenges and opportunities. *Reg Stud Mar Sci* 77: 103642. DOI: 10.1016/j.rsma.2024.103642.
- Nguyen N-L, Devendra D, Szymańska N, Greco M, Angeles IB, Weiner AKM, Ray JL, Cordier T, De Schepper S, Pawłowski J, Pawłowska J. 2023. Sedimentary ancient DNA: A new paleogenomic tool for reconstructing the history of marine ecosystems. *Front Mar Sci* 10: 1185435. DOI: 10.3389/fmars.2023.1185435.
- Nooteboom PD, Bijl PK, van Sebille E, von der Heydt AS, Dijkstra HA. 2019. Transport bias by ocean currents in sedimentary microplankton assemblages: Implications for paleoceanographic reconstructions. *Paleoceanogr Paleoclimatol* 34 (7): 1178-1194. DOI: 10.1029/2019PA003606.
- Odic R, Bensoussan N, Pinazo C, Taupier-Letage I, Rossi V. 2022. Sporadic wind-driven upwelling/downwelling and associated cooling/warming along Northwestern Mediterranean coastlines. *Continental Shelf Res* 250: 104843. DOI: 10.1016/j.csr.2022.104843.
- Odle E, Riewluang S, Ageishi K, Kajihara H, Wakeman KC. 2024. Pacific marine gregarines (Apicomplexa) shed light on biogeographic speciation patterns and novel diversity among early apicomplexans. *Eur J Protistol* 94: 126080. DOI: 10.1016/j.ejop.2024.126080.
- Pambudi AC, Bachtiar E, Yuniarti MS, Prasetya FS. 2021. Identifikasi komunitas diatom yang berasosiasi pada makroalga *Padina* sp. di Perairan Pulau Pramuka dan Semak Daun melalui pendekatan metagenomik. *J Fish Mar Res* 5 (1): 70-77. DOI: 10.21776/ub.jfmr.2021.005.01.11. [Indonesian]
- Pastorino P, Broccoli A, Anselmi S, Bagolin E, Prearo M, Barcelo D, Renzi M. 2022. The microalgae *Chaetoceros tenuissimus* exposed to contaminants of emerging concern: A potential alternative to standardized species for marine quality assessment. *Ecol Indic* 141 (1): 109075. DOI: 10.1016/j.ecolind.2022.109075.
- Pawłowski J, Bruce K, Panksep K et al. 2022. Environmental DNA metabarcoding for benthic monitoring: A review of sediment sampling and DNA extraction methods. *Sci Total Environ* 818: 151783. DOI: 10.1016/j.scitotenv.2021.151783.
- Pawłowski J, Lejzerowicz F, Apotheloz-Perret-Gentil L, Visco J, Esling P. 2016. Protist metabarcoding and environmental biomonitoring: Time for change. *Eur J Protistol* 55: 12-25. DOI: 10.1016/j.ejop.2016.02.003.
- Prahardika BA, Styawan WMLD. 2020. Studi keanekaragaman diatom epilitik serta potensinya sebagai bioindikator kualitas perairan sungai di Coban Tarzan Kabupaten Malang. *Biotropika J Trop Biol* 8 (2): 116-124. DOI: 10.21776/ub.biotropika.2020.008.02.07. [Indonesian]
- Pramanik A, Bhattacharyya M. 2023. Microbial community structure of the Sundarbans mangrove ecosystem. In: Das S, Dash HR (eds). *Microbial Diversity in the Genomic Era (Second Edition)*. Academic Press, New York. DOI: 10.1016/B978-0-443-13320-6.00008-1.
- Prihadi DJ, Riyantini I, Ismail MR. 2018. Pengelolaan kondisi kosistem mangrove dan daya dukung lingkungan kawasan wisata bahari mangrove di Karangsong Indramayu. *Jurnal Kelautan Nasional* 13: 53-64. DOI: 10.15578/jkn.v1i1.6748. [Indonesian]
- Quandt CA, Marino JA, Simmons DR, Davis WJ, Hassett BT, Picard KT, James TY. 2023. Evaluating the diversity of the enigmatic fungal phylum Cryptomycota across habitats using 18s rRNA metabarcoding. *Fungal Ecol* 64: 101248. DOI: 10.1016/j.funeco.2023.101248.
- Rindi F, Sikes J, Shapiro L. 2018. The identity of *Trentepohlia* algae (Chlorophyta: Trentepohliales) from Point Lobos State Reserve and the San Francisco Region, California. *Madroño* 65 (2): 89-95. DOI: 10.3120/0024-9637-65.2.89.
- Ríos-Castro R, Romero A, Aranguren R, Pallavicini A, Banchi E, Novoa B, Figueras A. 2021. High-throughput sequencing of environmental DNA as a tool for monitoring eukaryotic communities and potential pathogens in a coastal upwelling ecosystem. *Front Vet Sci* 8: 765606. DOI: 10.3389/fvets.2021.765606.
- Ripple WJ, Wolf C, Newsome TM et al. 2017. World scientists' warning to humanity: A second notice. *BioScience* 67 (12): 1026-1028. DOI: 10.1093/biosci/bix125.
- Romanowski G, Lorenz MG, Wackernagel W. 1991. Adsorption of plasmid DNA to mineral surfaces and protection against DNase I. *Appl Environ Microbiol* 57: 1057-1061. DOI: 10.1128/aem.57.4.1057-1061.1991.
- Ruiz F, Abad M, Bodergat A, Carbonel P, Rodríguez-Lázaro J, Yasuhara M. 2005. Marine and brackish-water ostracods as sentinels of anthropogenic impacts. *Earth-Sci Rev* 72 (1-2): 89-111. DOI: 10.1016/j.earscirev.2005.04.003.
- Ruiz F, Abad M, Bodergat A, Carbonel P, Rodríguez-Lázaro J, González-Regalado ML, Toscano A, Garcia EX, Prenda J. 2013. Freshwater ostracods as environmental tracers. *Intl J Environ Sci Technol* 10: 1115-1128. DOI: 10.1007/s13762-013-0249-5.
- Saccò M, Guzik MT, Van der Heyde M, Nevill P, Cooper SJB, Austin AD, Coates PJ, Allentoft ME, White NE. 2022. EDNA in subterranean ecosystems: Applications, technical aspects, and future prospects. *Sci Total Environ* 820: 153223. DOI: 10.1016/j.scitotenv.2022.153223.
- Saif R, Ejaz A, Mehmood T, Asif F, Alghanem SM, Ahmad TS. 2020. Introduction to Galaxy platform for NGS variant calling pipeline. *Adv Life Sci* 7 (3): 129-134. DOI: 10.62940/als.v7i3.809.
- Sanchez-Vidal A, Canals M, Calafat A, Lastras G, Pedrosa-Pàmies R, Menéndez M, Medina R, Company JB, Hereu B, Romero J, Alcoverro T. 2012. Impacts on the deep-sea ecosystem by a severe coastal storm. *PLoS One* 7 (1): e30395. DOI: 10.1371/journal.pone.0030395.
- Saraphol S, Vajrodanya S, Wongkantrakorn N, Sanevas N. 2021. Factors in culture media affecting the growth, and pigment contents of alga *Trentepohlia monilia*. *Appl Ecol Environ Res* 19 (3): 2443-2458. DOI: 10.15666/aer/1903\_24432458.
- Schmitz O, Alivernini M, Yasuhara M, Frenzel P. 2025. Ostracoda (Crustacea) as indicators of anthropogenic impacts - A review. *Earth-Sci Rev* 263: 105049. DOI: 10.1016/j.earscirev.2025.105049.
- Shi Y, Queller DC, Tian Y, Zhang Y, Yan Q, He Z, He Z, Wu C, Wang C, Shu L. 2021. The ecology and evolution of amoeba-bacterium interactions. *Appl Environ Microbiol* 87 (2): e01866-20. DOI: 10.1128/aem.01866-20.
- Shinzato C, Zayasu Y, Kanda M, Kawamitsu M, Satoh N, Yamashita H, Suzuki G. 2018. Using seawater to document coral-Zoothanthella diversity: A new approach to coral reef monitoring using environmental DNA. *Front Mar Sci* 5: 28. DOI: 10.3389/fmars.2018.00028.
- Spedicato A, Zeppilli D, Thouzeau G, Cuny P, Milton C, Sylvi L, Hubas C, Dirberg G, Jézéquel R, Barrière G, Michel LN, Bezerra TN, Michaud E. 2024. Deciphering environmental forcings in the distribution of meiofauna and nematodes in mangroves of the Atlantic-Caribbean-East Pacific and Indo-West Pacific regions. *Sci Total Environ* 930: 172612. DOI: 10.1016/j.scitotenv.2024.172612.
- Szlauer-Lukaszewska A, Pešić V. 2020. Habitat factors differentiating the occurrence of Ostracoda (Crustacea) in the floodplain of a small lowland River Krapiel (N-W Poland). *Knowl Manag Aquat Ecosyst* 421: 23. DOI: 10.1051/kmae/2020012.
- Torres M, Diaz-Ortiz J, Davis MG, Schwartz JR, Ramírez AMC. 2024. *Galleria mellonella* as a superficial model for *Malassezia globosa* and its treatment. *Access Microbiol* 6: 000745.v3. DOI: 10.1099/acmi.0.000745.v3.
- Tréguer P, Bowler C, Moriceau B, Dutkiewicz S, Gehlen M, Aumont O, Bittner L, Dugdale R, Finkel Z, Iudicone D, Jahn O, Guidi L, Lasbleiz M, Leblanc K, Levy M, Pondaven P. 2018. Influence of diatom

- diversity on the ocean biological carbon pump. *Nat Geosci* 11: 27-37. DOI: 10.1038/s41561-017-0028-x.
- Vázquez-García B, Ceolin D, Fauth G, Borghi L, Valle B, De Moraes Rios Netto A. 2021. Ostracods from the late Albian-early Cenomanian of the Sergipe-Alagoas Basin, Brazil: New taxonomic and biostratigraphic inferences. *J S Am Earth Sci* 108: 103169. DOI: 10.1016/j.jsames.2021.103169.
- Walker JE, Ankersen T, Barchiesi S, Meyer CK, Altieri AH, Osborne TZ, Angelini C. 2022. Governance and the mangrove commons: Advancing the cross-scale, nested framework for the global conservation and wise use of mangroves. *J Environ Manag* 312: 114823. DOI: 10.1016/j.jenvman.2022.114823.
- Wan KY. 2024. Biophysics of protist behaviour. *Curr Biol* 34 (20): R981-R986. DOI: 10.1016/j.cub.2024.07.002.
- Wang B, Jiao L, Ni L, Wang M, You P. 2024. Bridging the gap: The integration of eDNA techniques and traditional sampling in fish diversity analysis. *Front Mar Sci* 11: 1289589. DOI: 10.3389/fmars.2024.1289589.
- Wang M, Yergaliyev T, Sun C, Martinez JG, Wang B. 2023. Environmental DNA metabarcoding of intertidal meiofauna sheds light on its potential for habitat discovery. *Ecological Indicators* 150: 110223. DOI: 10.1016/j.ecolind.2023.110223.
- Wang Q-M, Begerow D, Groenewald M, Liu X-Z, Theelen B, Bai F-Y, Boekhout T. 2015. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. *Stud Mycol* 81: 55-83. DOI: 10.1016/j.simyco.2015.10.004.
- Wickham H, Averick M, Bryan J, Chang W, McGowan LDA, François R, et al. 2019. Welcome to the Tidyverse. *J Open Source Software* 4 (43): 1686. DOI: 10.21105/joss.01686.
- Xiao Y, He M, Xie J, Liu L, Zhang X. 2021. Effects of heavy metals and organic matter fractions on the fungal communities in mangrove sediments from Techeng Isle, South China. *Ecotoxicol Environ Saf* 222: 112545. DOI: 10.1016/j.ecoenv.2021.112545.
- Zhang K, Chen C, Long D, Wang S, Li J, Lin X. 2024. Unveiling sedimentary microbial communities: Restored mangrove wetlands show higher heterogeneity and network stability than natural ones. *Ecol Indic* 166: 112274. DOI: 10.1016/j.ecolind.2024.112274.
- Zimmermann J, Glöckner G, Jahn R, Enke N, Gemeinholzer B. 2015. Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. *Mol Ecol Resour* 15 (3): 526-542. DOI: 10.1111/1755-0998.12336.